Eco-engineering for clarity

Clearing blue-green ponds and lakes in an urbanized area

Guido W.A.M. Waajen

Thesis committee

Promotor
Prof. Dr M. Scheffer
Professor of Aquatic Ecology and Water Quality Management
Wageningen University & Research

Co-promotor
Dr M.F.L.L.W. Lürling
Associate professor, Aquatic Ecology and Water Quality Management Group
Wageningen University & Research

Other members
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Dr B.M. Spears, Centre for Ecology & Hydrology, Edinburgh, United Kingdom

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CHAPTER 1

General introduction

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Eutrophication of water bodies is the process involving the enrichment by plant nutrient loadings. Natural eutrophication is often accelerated anthropogenically (Wetzel, 2001; Thornton et al., 2013) which is considered the primary water quality issue for most of the freshwater ecosystems in the world (Smith & Schindler, 2009). In Dutch freshwater ecosystems, high concentrations of the nutrients phosphorus (P) and nitrogen (N) are a major reason for insufficient water quality and around 55% of the monitoring locations nationwide do not meet the objectives (Unie van Waterschappen, 2014; Van Gaalen et al., 2015). Since World War II, many Dutch lakes became eutrophied by the runoff from agriculture and discharges of wastes from industry and households (Gulati & Van Donk, 2002). Striking effects of eutrophication in standing waters are the disappearance of submerged macrophytes and the increase of turbidity and phytoplankton biomass (Scheffer et al., 1993), often with a predominance of cyanobacteria (Watson et al., 1997; Smith et al., 1999). On a global scale the expansion of cyanobacteria is increasing rapidly since 1945, mainly due to the increase of nutrient influxes (Taranu et al., 2015). Climate change may intensify the effects of eutrophication and support cyanobacterial dominance (Paerl & Huisman, 2008; Kosten et al., 2012). Many cyanobacteria are known producers of potent toxins that can reach levels hazardous to humans and animals if ingested (Chorus et al., 2000; Van Apeldoorn et al., 2007). Besides the potential toxicity to humans, dogs, waterfowl and other animals, cyanobacterial blooms can cause fish kill due to anoxia, reduce biodiversity and cause unpleasant surface scums and malodors (Smith et al., 1999; Codd et al., 2005a; Smith & Schindler, 2009). As a consequence, eutrophication hampers the anthropogenic uses of the water bodies and may have negative economic impacts (Smith, 2003; Kashian et al., 2006; Steffensen, 2008). Water clarity and the absence of high concentrations of phytoplankton are strongly related to the perception of good water quality, both among the public and among experts (David, 1971; Rast & Thornton, 2005; Peeters et al., 2009; West et al., 2015), and are widely used as assessment criteria for water quality (Yang et al., 2008; Egan et al., 2009).

In The Netherlands, the implementation of the Service Water Pollution Act in 1970, of the European Water Framework Directive (WFD; Council of the European Union, 2000) and of the European Bathing Water Directive (BWD; Council of the European Union, 2006) have resulted in the increased attention for the maintenance of an acceptable water quality. A significant progress has been made in reducing the inflow of nutrients including improved wastewater treatments, reduced phosphate contents in detergents and measures in agriculture. These efforts have greatly contributed to the improvement of the water quality as is illustrated in Fig. 1.1 for the concentration of total phosphorus (TP) over time in the mouth of the river Dintel. This river, which is qualified as eutrophic (OECD, 1982) is the main discharge canal for rural and urbanized areas within the jurisdiction of the Water

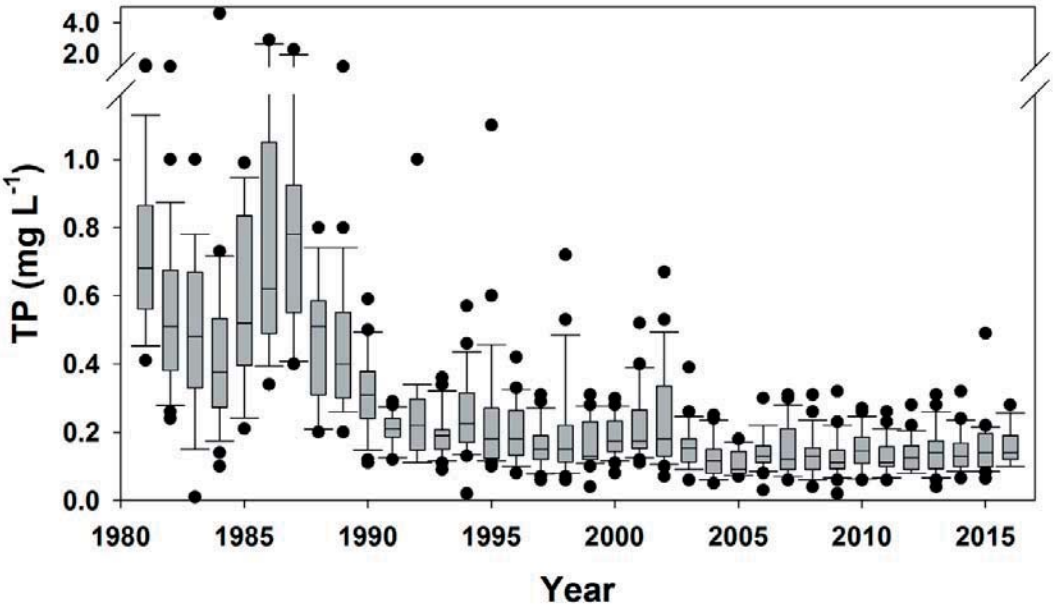


Fig. 1.1: Concentration of total phosphorus (TP, mg L^{-1}) in the river mouth of the Dintel (The Netherlands) during the period January 1981 – September 2016. Boxes indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles, dots indicate outliers (modified from Van der Wal & Waajen, 2010; data kindly provided by Water Authority Brabantse Delta).

Authority Brabantse Delta in the south of The Netherlands. Despite the many efforts to reduce eutrophication, over 60% of the large lakes in The Netherlands suffer from high concentrations of phytoplankton (Van Gaalen et al., 2015), while present-day improvement in water quality and reduction of eutrophication tends to be a slow process (Unie van Waterschappen, 2012), if present at all (Fig. 1.1). Driven by the WFD, the Dutch water managers' attention focuses on the large water bodies and in particular not on the smaller water bodies in urbanized areas. This leads to the situation in which there is a poor overview of the condition of urban water bodies with respect to eutrophication.

Within urban areas, standing freshwater ponds and small lakes (Fig. 1.2) are widespread and they provide ecological and societal services including recreational and cultural values, water retention, microclimate regulation and runoff treatment (Bolund & Hunhammar, 1999; Céréghino et al., 2008). Although urban ponds and small lakes can serve as a reservoir for biodiversity, high levels of anthropogenic stressors may result in low ecological values (Gledhill et al., 2008; Noble & Hassall, 2015). Cyanobacterial blooms seem to occur regularly and are wide spread in ponds and small lakes (Willame et al., 2005; Rahman & Jewel, 2008), but little information is available on the frequency and intensity of such blooms in urbanized areas in The Netherlands. Nevertheless, the societal need for safe and aesthetically acceptable water is high (Steffensen, 2008), as urban ponds and lakes enable easy public contact with surface water (Birch & McCaskie, 1999). Recreational activities as swimming and angling imply the risk of ingestion of cyanobacterial material. Recent studies indicate the occurrence of toxic cyanobacterial blooms in Dutch urban ponds (Faassen et al., 2009; Lürling & Faassen, 2012), but the access of the public to these waters during cyanobacterial blooms is usually not restricted. Water managers are in urgent

Fig. 1.2: Urban ponds in the city of Breda (The Netherlands).

need for effective management tools to reduce cyanobacterial blooms, which spurred the development of a wealth of end-of-pipe techniques claiming to be effective (Jančula & Maršálek, 2011; Chapter 8). These claims are often not proven or are refutable (e.g., Lürling et al., 2009). In contrast to the services they provide, there has been little scientific attention to support the water managers who are responsible for the management of the urban ponds and small lakes (Céréghino et al., 2008; Downing, 2010; Hassall, 2014). In daily practice of water management, the reduction of cyanobacterial blooms in urban ponds and lakes proves to be arduous.

Methods to reduce the risk of cyanobacterial blooms and mitigate their negative effects can be divided into public-oriented, symptom-oriented and source-oriented methods, although sharp boundaries between these categories are lacking and there is some overlap. Public-oriented methods comprise press releases, information panels and warning signs at the waterfront (Fig. 1.3). They intend to reduce the risk of public contact with cyanobacterial material, but these

Fig. 1.3: Warning signs at the waterfront during cyanobacterial blooms.

methods do not address the cyanobacterial blooms themselves. Although public information on cyanobacterial blooms in official bathing sites is regulated in national protocols for monitoring and assessment (e.g., Ibelings et al., 2012), a standardized procedure for monitoring, assessment and public information on cyanobacterial blooms in other urban water bodies is lacking.

Symptom-oriented methods intend to suppress the proliferation of cyanobacteria or destroy the blooms. These curative methods are end-of-pipe solutions and, although often claimed to be end-all solutions, they have been applied with varying successes. They are used as alternatives or supplements to source-oriented methods and they realize symptom relief rather than eutrophication relief. The symptom-oriented methods can be divided into mechanical, chemical and biological methods. Examples of mechanical methods are flushing in order to realize a physical washout of cyanobacteria at a rate that exceeds their growth rate (Welch & Patmond, 1980; Cooke et al., 2005), and the skimming of accumulations of cyanobacteria aggregated in scums (Atkins et al., 2001). Another example is the sinking of intact cells with coagulant and ballast (Pan et al., 2011a; Lürling & Van Oosterhout, 2013). Chemical symptom-oriented methods are the use of algaecides such as hydrogen peroxide (Matthijs et al., 2012), toxic metals as copper and silver or herbicides (Jančula & Maršalek, 2011). Biological methods to control algal populations are fishstock management to promote large filter-feeding zooplankton (Meijer et al., 1999) and the stocking of herbivorous mussels to increase the grazing on phytoplankton (Reeders & Bij de Vaate, 1990; Dionisio Pires et al., 2005; Fig. 1.4).

Fig. 1.4: Construction of a reef, using crates overgrown with juvenile herbivorous mussels (*Dreissena rostriformis bugensis*) in an urban pond in the city of Breda (The Netherlands, 2 April 2013).

Source-oriented methods target the reduction of phytoplankton biomass by limitation of nutrients, which is considered the best approach to reduce algal nuisance on the long-term. The probability of high phytoplankton biomass concentrations increases with increasing nutrient concentrations (OECD, 1982) and the 'nutrient load – lake response' concept is considered an extremely useful diagnostic management tool for assessing the

likely response of a lake to nutrient load reduction (Rast & Thornton, 2005). As the concept can provide useful guidelines for improvements, site-specific information is needed for effective management strategies (Eviner & Hawkes, 2008). A prerequisite for successful lake rehabilitation is knowledge of the lake and its catchment before designing and conducting an intervention (Søndergaard et al., 2012). The source-oriented approach focuses on the restriction of external nutrient inputs to the lake and of the internal nutrient recycling. Reducing the trophic state is considered to be the only way to realize positive long-term effects in reducing cyanobacterial nuisance (Scheffer, 2004; Cooke et al., 2005; Jeppesen et al., 2007a; Jančula & Maršalek, 2011; Jeppesen et al., 2012; Taranu et al., 2015). In addition to this, food web interventions and reconstruction of the water body can enhance the efficacy of the source-oriented approach. However, the approach is time-consuming and the required reductions of nutrient inputs are not always feasible in the real world (Jančula & Maršalek, 2011). Consequently, in nutrient-rich waterbodies such as many urban ponds and small lakes, recovery of the water quality should probably be considered as a management approach, rather than a 'once and for all' solution (Søndergaard et al., 2007). For shallow waterbodies (< ~5 m water depth), the framework for the approach is provided in the shallow lakes theory (Scheffer, 1989; Scheffer et al., 1993; Scheffer & Van Nes, 2007). Temperate shallow lakes can be in contrasting alternative stable states in relation to the nutrient loading: a clear state with abundant opportunities for submerged macrophytes and a turbid phytoplankton dominated state with few submerged macrophytes. When critical conditions are reached, an abrupt and non-linear shift from one state to the other is likely to occur, showing a hysteresis curve in which the water body changes from clear to turbid at a different nutrient load than it changes back from turbid to clear (Fig. 1.5). Each state is stabilized by self-reinforcing feedback mechanisms resulting in a different response of the lake to the increase and decrease of the nutrient loading, related to the water body's resilience to absorb changes without shifting to the other state (Holling, 1973). At low nutrient levels, the water body will be in a clear water state (Fig. 1.6).

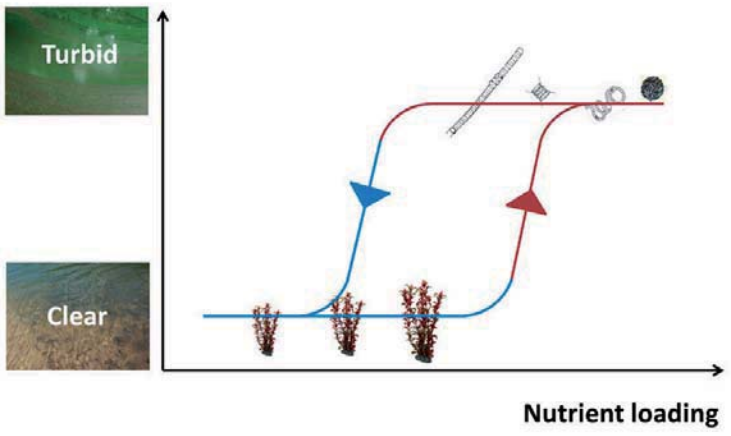


Fig. 1.5: Phytoplankton biomass in relation to the nutrient loading in shallow lakes (modified from Hosper, 1989).

Fig. 1.6: Clear water state in an urban pond in the city of Breda (The Netherlands, 10 August 2012).

Increased nutrient loading results in the stimulation of the growth of submerged macrophytes while the water body remains in the clear state. Aquatic vegetation enhances water clarity by the absorption of nutrients, by shading, by allelopathy and by reduction of sediment resuspension and the provision of shelter for herbivorous zooplankton and piscivorous fish (Scheffer, 2004; Scheffer & Van Nes, 2007). However, if the nutrient level is raised further, the predation pressure by a growing fish community on zooplankton and on periphyton grazing macro invertebrates increases, resulting in reduced light availability for submerged macrophytes, a diminishing plant biomass and increase of the phytoplankton biomass (Scheffer, 2004). If a critical value is exceeded, this process causes a rather abrupt switch to the turbid phytoplankton dominated state (Fig. 1.5; Scheffer et al., 1993). This turbid state is often dominated by cyanobacteria (Smith et al., 1999; Fig. 1.7), provided that the flush rate is < ~18% of the volume of the water body per day (Scheffer et al., 1997). The

Fig. 1.7: Turbid state with cyanobacterial dominance (*Microcystis, Anabaena*), peri-urban Lake Groote Melanen in the city of Bergen op Zoom (The Netherlands, 13 July 2010).

decline and disappearance of the aquatic vegetation supports a fish community dominated by benthivorous and planktivorous fish as bream (*Abramis brama*). The abundance of these fish reinforces the turbidity by sediment turbation and resuspension and it promotes a high phytoplankton biomass through control of the herbivorous zooplankton (Scheffer, 2004; Scheffer & Van Nes, 2007). When reducing the nutrient loading, self-stabilizing mechanisms keep the water body in the turbid state and cyanobacteria can maintain a high biomass due to their P efficiency (Janse, 2005). The return to the clear water state by reduction of the nutrient loading is impeded by the reduced zooplankton grazing pressure on phytoplankton due to fish predation and poor edibility of cyanobacteria, by the benthivorous feeding behaviour of fish causing sediment bed disturbance and reduced light availability, and by the ability of phytoplankton to increase the nutrient utilization efficiency under reduced nutrient availability (Janse, 2005). Eventually, the phytoplankton biomass will be reduced when the nutrient loading drops below a critical value and the water body shifts to the clear state with macrophytes (Fig. 1.5). In addition to these impeding mechanisms, the sediment P-release may delay the water body's response to a reduced nutrient loading for a considerable time (Welch & Cooke, 2005). The sediment P-release can be the legacy of former external loadings and it needs attention when striving for improvements of the water quality. Over a range of intermediate nutrient loadings, two alternative states exist: the clear state with macrophytes and the turbid phytoplankton dominated state (Fig. 1.5; Scheffer & Van Nes, 2007). In this range of intermediate loadings, additional measures targeting turbidity can force a turbid water body into the clear state.

Deep temperate lakes (> 6 m water depth; Wetzel, 2001), subject to seasonal thermal stratification, often lack the stabilizing effects of submerged macrophytes and benthivorous fish. Nevertheless, the clear water and turbid states are similar (Folke et al., 2004) and are determined by factors as the morphometry of the lake and the nutrient recycling (Carpenter, 2005). When nutrient loading is high, a large phytoplankton biomass in the epilimnion can develop. In the hypolimnion anoxic conditions (< 2 mg O_2 L^{-1}; Nürnberg, 1996) will develop, due to the rain into this stratum of organic matter synthesized in the epilimnion, its decomposition and the oxygen demand of the sediment (Wetzel, 2001). In these anoxic conditions ironbound P will be released from the sediment into the hypolimnion (Cooke et al., 2005). This P will spread throughout the water body at turnover events, fuelling phytoplankton production in the next growing season. As such, P-release from anoxic sediments can increase the phytoplankton production, which in turn leads to a higher detritus settling and oxygen demand. A larger area of sediment will be exposed to anoxic water, and the sediment P content increases due to increased sedimentation. The internal P-load is a self-reinforcing process increasing eutrophication (Nürnberg & Peters, 1984; Nürnberg, 1996). When reducing the external nutrient load, the recycling of P within the lake from sediments enriched by high external loads in the past, impedes the return to a situation with a low phytoplankton biomass (Carpenter, 2005). Vollenweider (1976) defined the boundary between the oligotrophic and mesotrophic state as the permissible

P-load, and the boundary between the mesotrophic and eutrophic state as the excessive P-load. Below the permissible load, the lake's P-load is small as are the likelihood for anoxic conditions in the hypolimnion and for phytoplankton blooms. Oligotrophic conditions are usually stable with low sediment P-release, thereby limiting the phytoplankton biomass in the clear water state (Carpenter, 2005). Above the excessive P-load, the lake's nutrient load exceeds its ability to assimilate the P-load without producing algal blooms (Rast & Thornton, 2005). In this eutrophic turbid state cyanobacterial blooms are likely to occur (Smith, 2003). Between the oligotrophic and eutrophic conditions an intermediate mesotrophic transition zone exists (Rast & Thornton, 2005), characterized by either a stable clear water state or a stable turbid state (Carpenter, 2005).

For producing a phytoplankton bloom, the excessive availability of all nutrients is required. In terms of eutrophication, P and N are the main nutrients. Despite the debate on the role of P relative to N in eutrophication (Paerl et al., 2014), in reducing eutrophication and rehabilitating lakes and ponds the focus can be on P as the key nutrient (OECD, 1982; Schindler et al., 2008). Dictated by Liebig's law of the minimum, only reduction in one nutrient is needed for the control of phytoplankton biomass. Based on the Redfield ratio (Redfield, 1958), P limitation is stoichiometrically the most efficient. In addition, P reduction is the most feasible as the bioavailability of P can be reduced through formation of poor to insoluble salts (with metals as Al and Fe; Cooke et al., 2005) while immobilizations for N are more difficult to realize. Furthermore, atmospheric deposition of N is substantial (Carpenter et al., 1998), while the reduction of N inputs may favor N-fixing cyanobacteria (Schindler et al., 2008). Golterman (1975) already stated: "*It is not important whether phosphate is currently the limiting factor or not, or even that it has ever been so; it is the only essential element that can easily be made to limit algal growth*." A strong P reduction will render N reduction meaningless. Experiments and whole lake applications targeting only P show a regime shift from a eutrophic turbid state to an oligo-mesotrophic clear water state with abundant submerged macrophytes and a substantial accompanying decrease of cyanobacterial blooms (Lürling & Van Oosterhout, 2013; Chapters 3, 4, and 5). Although considerable efforts have been made, the positive long-term effects of many lake rehabilitations are questionable due to insufficient reduction of the external and internal P-loadings, enhanced by high planktivorous fish biomass and by absence of stable submerged macrophyte communities (Gulati & Van Donk, 2002; Søndergaard et al., 2007). As such, often the combination of measures will be needed to become effective. In 2008, the Dutch Foundation for Applied Water Research published the report 'From clear to turbid… and back again' (Jaarsma et al., 2008). This report provided a framework for Dutch water managers in their efforts to mitigate the negative effects of high P concentrations in standing waters. The framework was based on ecological theory and pointed towards potential solutions for the eutrophication problem. Nevertheless, the way from potential solutions towards the practical application of effective measures is often complex. It has been suggested that, in addition to measures targeting the reduction of external nutrient inputs, recovery of the water quality can be

enhanced by the combined application of physico-chemical and biological methods, although the scientific evidence for this is poor (Jeppesen et al., 2012). To increase the knowledge of the applicability and efficacy of ecological (or eco-) engineering methods targeting the clear water state, a study based on site-specific diagnostics was executed from 2009 to 2012 (Lürling et al., 2012). The approach was elaborated and applied in the rehabilitation of small city lakes and ponds and extended with biomanipulation using freshwater mussels. This thesis finalizes the results from the studies, including experiments, whole-lake applications and long-term monitoring on efficacy, providing a road map for water managers for the rehabilitation of eutrophied urban lakes and ponds.

0bjectives and outline of the thesis

In their attempts to reduce cyanobacterial nuisance in urban freshwater lakes and ponds, Dutch water authorities and their associates (e.g., municipalities, recreation entrepreneurs, angling associations) apply different methods, mostly public- and symptom-oriented. The source-oriented approach is often neglected, indicated as not feasible or at best partially conducted. Despite the urgent need among water managers and stakeholders for the effective structural reduction of cyanobacterial nuisances, a systematic approach underpinned by diagnostics and supported by monitoring is mostly lacking. The contemporary approach can be characterized as 'trial and error', often resulting in failures, vague results or at best short-term positive effects. This thesis aims to provide insight in the applicability and efficacy of promising eco-engineering methods to reduce cyanobacterial nuisance in standing urban waters targeting the clear water state, and to present a guideline for water managers to rehabilitate such eutrophied water bodies. Diagnostics were conducted to underpin the selection of treatments at specific sites, with attention for the external and internal P-loadings. Experiments on relevant spatial scales were done to select effective measure combinations. Whole-lake applications were executed and monitored for efficacy, and results were confronted with the outcome of the diagnostics and experiments. The study sites were situated in the urbanized area of the province of North Brabant in the south of The Netherlands. They comprised of standing city waters, ponds and small lakes (< 7 ha) in or nearby residential areas.

To provide insight into the extent and potential causes of cyanobacterial blooms in standing city waters, *Chapter 2* gives the results of a survey in the province of North Brabant. Many urban waters suffer regularly from cyanobacterial blooms and four out of five locations registered for blooms concern urban ponds and small lakes. Three ponds were selected for detailed study and showed highly toxic microcystin concentrations in scums. These ponds were highly eutrophic and had high fish biomasses, dominated by carp.

Chapter 3 describes the results of site-specific diagnostics for two urban ponds, including the water- and P-budgets. Each pond was divided in six compartments (300-400 m^2; 210-700 m^3) to test different treatments. The treatments consisted of different combinations of measures and were based on the results of the diagnostics. The efficacy

of the treatments in the compartments was monitored for two years. It was shown that reduction of the external and internal P-loading combined with biomanipulation on fish was effective in realizing a stable clear water state. Lanthanum modified bentonite (LMB) can be an attractive alternative to dredging. At the end of the two-year experimental period, the compartments were removed and in both ponds restoration measures were executed. The efficacy of the restoration measures was monitored during a subsequent three-year period. The results showed that rehabilitation, based on the results of diagnostics, realized a long-term clear water state, while not taking the results of diagnostics into account was not successful.

The restoration of urban Lake Kleine Melanen is dealt with in *Chapter 4*. The restoration was based on a site-specific diagnostic system analysis, including the water- and P-budget. An enclosure experiment was conducted to select an effective in-lake treatment for the reduction of the sediment P-release. In-lake measures were implemented. The reduction of the external P-load however could hardly be limited and this reduction did not meet the recommendations of the diagnostics. Although subsequent monitoring showed some improvement of the water quality, the clear water state was not realized yet and the future reduction of the external P-load is a prerequisite for further improvement.

Cyanobacterial blooms regularly hampered the recreational function of Lake De Kuil. As described in *Chapter 5*, diagnostics showed that the blooms were mainly fueled by sediment P-release. To reduce the sediment P-release and subsequently the risk of cyanobacterial blooms, LMB was applied. The application of LMB was combined with a low dose flocculant (Flock & Lock), meant to strip the water column of phytoplankton and particulate and dissolved P. After the treatment, the trophic state of the lake shifted abruptly from hypertrophic/eutrophic to mesotrophic, which state was maintained for at least six subsequent years, while cyanobacterial blooms decreased substantially.

In *Chapter 6*, the development of the macroinvertebrate community of Lake De Kuil is described up to one year following the application of LMB and flocculant. A microcosm experiment with artificial macroinvertebrate communities supported the in situ research. The Flock & Lock treatment of the lake had a short-term adverse effect on the macroinvertebrate community. One year after the treatment, an increase in macroinvertebrate density and taxa-richness was observed.

Chapter 7 deals with the bioavailability of lanthanum (La) from LMB in in situ applications. It was shown that La accumulates in the macrophyte *Elodea nuttallii*, in chironomid larvae and in fish, up to at least two years (macrophyte, chironomids, fish) and five years (fish) following LMB application. No ecotoxicological effects were observed and human health risks are considered negligible.

Eutrophication control, as the route to long-term mitigation of harmful cyanobacterial blooms, is not always feasible or might become effective only after a long period. In such cases, curative measures might provide a short-term symptom relief. Several proposed end-of-pipe measures are reviewed in *Chapter 8*. Limited support could be given

for the efficacy of many curative measures, while the proposed underlying mechanisms were considered doubtful.

Chapter 9 focuses on the use of the freshwater quagga mussel *Dreissena rostriformis bugensis* as a promising tool to reduce phytoplankton biomass by its grazing. An enclosure experiment showed that the introduction of quagga mussels in a hypertrophic urban pond reduced phytoplankton biomass including cyanobacteria and induced a clear water state.

In conclusion, *Chapter 10* integrates and discusses the results of the previous chapters and provides a road map for water managers towards the reduction of cyanobacterial nuisance in eutrophic standing urban waters.

City pond with cyanobacterial surface scum (Breda, The Netherlands, 18 June 2013)

CHAPTER 2

Eutrophic urban ponds suffer from cyanobacterial blooms: Dutch examples

This chapter is based on:
Eutrophic urban ponds suffer from cyanobacterial blooms: Dutch examples. Waajen, G.W.A.M., E.J. Faassen & M. Lürling, 2014. Environmental Science and Pollution Research 21: 9983-9994. DOI 10.1007/s11356-014-2948-y.

Abstract

Ponds play an important role in urban areas. However, cyanobacterial blooms counteract the societal need for a good water quality and pose serious health risks for citizens and pets. To provide insight into the extent and possible causes of cyanobacterial problems in urban ponds, we conducted a survey on cyanobacterial blooms and studied three ponds in detail. Among 3,500 urban ponds in the urbanized Dutch province of North Brabant, 125 showed cyanobacterial blooms in the period 2009-2012. This covered 79 % of all locations registered for cyanobacterial blooms, despite the fact that urban ponds comprise only 11% of the area of surface water in North Brabant. Dominant bloom forming genera in urban ponds were *Microcystis*, *Anabaena* and *Planktothrix*. In the three ponds selected for further study, the microcystin concentration of the water peaked at 77 µg L^{-1} and in scums at 64,000 µg L^{-1}, which is considered highly toxic. Microcystin-RR and microcystin-LR were the most prevalent variants in these waters and in scums. Cyanobacterial chlorophyll-*a* peaked in August with concentrations up to 962 µg L^{-1} outside of scums. The ponds were highly eutrophic with mean total phosphorus concentrations between 0.16 and 0.44 mg L^{-1} and the sediments were rich in potential releasable phosphorus. High fish stocks dominated by carp lead to bioturbation, which also favors blooms. As urban ponds in North Brabant, and likely in other regions, regularly suffer from cyanobacterial blooms and citizens may easily have contact with the water and may ingest cyanobacterial material during recreational activities, particularly swimming, control of health risk is of importance. Monitoring of cyanobacteria and cyanobacterial toxins in urban ponds is a first step to control health risks. Mitigation strategies should focus on external sources of eutrophication and consider the effect of sediment P-release and bioturbation by fish.

Introduction

Ponds are important freshwater resources (Oertli et al., 2009) and worldwide there are hundreds of millions of them (Downing et al., 2006). In urban areas, ponds contribute to biodiversity, but also provide societal benefits such as micro climate regulation, rainwater drainage, recreation and cultural values (Bolund & Hunhammar, 1999; Robitu et al., 2006; Gledhill et al., 2008; Downing, 2010; Gledhill & James, 2012). Because most urban ponds are small, shallow and stagnant, the effect of anthropogenic disturbances on these ponds can be large (Brönmark & Hansson, 2002). Anthropogenic eutrophication is considered a major water quality issue in urban ponds (Klapwijk, 1988; Roijackers et al., 1998; Smith & Schindler, 2009). Main cause of eutrophication in urban ponds is nutrient loading, caused by for example sewage overflow, street dirt and bird droppings (Scherer et al., 1995; Stoianov et al., 2000; Waschbusch et al., 2000). In addition to the external nutrient loading, the sediment can play an important role in eutrophication as an internal source of nutrients (Søndergaard et al., 1999). Furthermore, a dense fish stock can aggravate eutrophication effects (Meijer et al., 1999; Peretyatko et al., 2009). Eutrophication can result in cyanobacterial blooms, which in turn can cause hypoxia, fish kills and high turbidity (Fastner et al., 1999; Paerl et al., 2001; Scheffer, 2004). Moreover, many cyanobacteria can produce potent toxins (Van Apeldoorn et al., 2007) which can reach levels hazardous to humans if ingested (Chorus et al., 2000). Globally, the most frequently reported and best known group of toxins are microcystins, which are produced by common genera such as *Microcystis*, *Anabaena*, *Nostoc* and *Planktothrix* (Carmichael, 2001; Zurawell et al., 2005; Van Apeldoorn et al., 2007).

Toxin producing cyanobacteria frequently bloom in lakes and reservoirs throughout Europe (Chorus 2001; Mankiewicz et al. 2005; Willame et al., 2005; Mooney et al., 2010). In many countries the use of these waters for recreation is therefore regulated during cyanobacterial blooms (Chorus, 2012). Urban ponds also enable public contact with surface water with a possibility of ingesting cyanobacterial material during activities as swimming and playing by children, possibly also angling and boating. Also pets, for example dogs, may ingest cyanobacterial material which poses a risk to their welfare. It is considered essential that the water quality of these ponds is maintained at a safe and aesthetically acceptable level (Birch & McCaskie, 1999; Steffensen, 2008). Despite the fact that recent studies indicate that toxic cyanobacterial blooms occur in urban ponds (Faassen et al., 2009; Lürling & Faassen, 2012), the access to these waters is usually not restricted and specific information, surveillance and control are generally lacking. There is little information on the frequency and intensity of cyanobacterial blooms in urban ponds. This study therefore aims to improve our understanding of the occurrence of cyanobacterial blooms in urban ponds. In this we focus on the southern part of The Netherlands as an example of an urbanized area. Furthermore, we sought to determine the occurrence of microcystins and identify the possible causes of cyanobacterial blooms in urban ponds. To achieve this, we surveyed the occurrence of cyanobacterial blooms in the southern part of The Netherlands, and we studied three ponds in detail.

Materials & methods

Survey of locations with cyanobacterial blooms

The province of North Brabant, in the southern part of The Netherlands, was selected as research area. In the period 2009-2012 cyanobacterial blooms were reported by three regional water authorities in this province (Brabantse Delta, De Dommel, Aa en Maas). As the water authorities do not operate an area-wide monitoring program for cyanobacterial blooms, we used an overview of registered reportings. In this, potential cyanobacterial blooms were reported after a field observation by the water authorities' field staff (turbid water with lumps and floating scums) and by complaints on blooms by citizens, anglers and farmers. When a potential cyanobacterial bloom was reported, an additional water sample (1 L, without scums) was taken by the water authorities to confirm whether it was a cyanobacterial bloom and to gain insight in the bloom forming taxa. Locations in the jurisdiction of water authority Brabantse Delta (73 samples) were considered to have a cyanobacterial bloom when additional semi-quantitative microscopic investigation confirmed the abundant presence of at least one of the genera *Microcystis*, *Anabaena*, *Aphanizomenon, Planktothrix* and *Oscillatoria*. For the additional microscopy, 100 mL of water were filtered through a 0.45 µm membrane filter (Whatman MicroPlus-21STL) and the material gathered on the filter was microscopically investigated (magnification 40X). Several fields of view (FOV) were investigated and each FOV had to show cyanobacteria to confirm a bloom. During 2013 a separate investigation showed a strong correlation (r^2 = 0.63, n = 73, unpublished data) between this type of results of microscopy and the concentration of cyanobacterial chlorophyll-*a* (using a FluoroProbe, bbe Moldaenke GmbH, Schwentinental, Germany; Catherine et al. 2012). Water authorities De Dommel and Aa en Maas (in total 188 samples) used a different procedure to confirm a cyanobacterial bloom. They reported a bloom when ≥50,000 cells or filaments mL^{-1} (Utermöhl technique; since 2011 modified according to Lo et al. (2011)) of at least one of the genera *Microcystis*, *Anabaena*, *Aphanizomenon*, *Planktothrix* and *Woronichinia* were present.

The reportings give an impression of the extent of blooms rather than a quantification. Additionally in 55 water bodies designated for recreation and under according surveillance (including seven urban ponds), blooms were registered by the three water authorities when the cyanobacterial chlorophyll-*a* concentration during the period April until September exceeded 12.5 µg L^{-1} in a two- or four-weekly monitoring program (using a FluoroProbe) or when the biovolume of *Microcystis*, *Anabaena*, *Aphanizomenon*, *Planktothrix* and *Woronichinia* (determined by microscopy) exceeded 2.5 mm^3 L^{-1}. These concentrations indicate the initial alert level for bathing water according to the current Dutch cyanobacteria protocol (Ibelings et al., 2012). Topographical maps (Top10N scale 1:10,000, and the Large Scale Base Map of The Netherlands GBKN) and aerial photographs (2012, resolution 10 cm) were used to determine the total number and surface area of urban ponds in North Brabant.

Selection of ponds

We selected three urban ponds for a more detailed study on the intensity and causes of the cyanobacterial problems. The ponds are spread over the province of North Brabant, one in each of the jurisdictions of the three water authorities, and they are representative for urban ponds in this part of the country: they are manmade, have a limited surface area (< 1 ha), are shallow and contain water year round. The ponds are located in the cities of Dongen, Eindhoven and Heesch and are characterized in Table 2.1 (Fig. 2.1).

Table 2.1: Characteristics for ponds Dongen, Eindhoven and Heesch.

	Pond Dongen	Pond Eindhoven	Pond Heesch
Coordinate North	51° 37’ 48.00’’	51° 48’ 96.57’’	51° 41’ 43.70’’
Coordinate East	4° 56’ 27.30’’	5° 47’ 65.31’’	5° 32’ 10.50’’
Area (m^2)	2500	6500	1600
Mean water depth (m)	0.7	1.5	1.0
Age of pond (y)	55	20	40
Discharges	Mixed sewer overflow (1970-2000)	Rainwater drainage (1994-present)	Mixed sewer overflow (1974-2009)
Average number of water birds during sampling events	19	17	10

Fig. 2.1: From left to right pond Dongen (1 September 2008), pond Eindhoven (29 July 2009) and pond Heesch (28 July 2009).

The three ponds regularly show cyanobacterial blooms. Pond Dongen is an isolated pond without connection to other surface waters. The pond is characterized by infiltration. During dry periods, the water level is maintained by the supply of pumped groundwater. During wet periods, a surplus of water can be discharged through a one-way discharge connection with the local sewer system. The water level fluctuated in the period 2009-2012 with plus or minus 0.26 m compared to the mean water level. The pond received discharges from a mixed sewage overflow (domestic water and rainwater drainage) from 1970 until 2000. From 2000, no sewage water could enter pond Dongen. Pond Eindhoven is connected to watercourses, which drain the excess of water from the pond. There is neither significant seepage nor infiltration. The major inflow comes from the rainwater drainage system in

the adjacent residential area. The water level of pond Eindhoven fluctuated in the period 2009-2012 with plus or minus 0.26 m compared to the mean water level. Pond Heesch is a small isolated pond without connections to other surface water. The pond is strongly influenced by seepage during wet periods and by infiltration during dry periods, resulting in fluctuations in water level of plus or minus 0.50 m compared to the mean water level. The pond received discharges from a mixed sewage overflow from 1974 until 2009. Ponds Dongen and Eindhoven have never been dredged. Pond Heesch has last been dredged in the year 2000 and following upon our survey underwent thorough restoration in December 2009. All three ponds are used for angling. The edges of the ponds are hard, submerged macrophytes are lacking and residents intensively feed waterfowl, mainly malards (*Anas platyrhynchos*).

Water sampling and analysis

All three ponds were sampled only a few (1 – 4) times in 2006 – 2008 and biweekly in the period March 2009 – August 2009. In each pond, dissolved oxygen concentration and saturation (Oxyguard, Birkerød, Denmark), conductivity (WTW-Cond 330i, WTW GmbH, Weilheim, Germany), pH (WTW-pH320, WTW GmbH, Weilheim, Germany) and water temperatures were measured at water depth 0.2 m and Secchi depths were determined. Two-litre water samples were taken from the ponds with a perspex sampling tube at water depth 0-1 m. From these samples, total- and cyanobacterial chlorophyll-*a* concentrations were measured using a PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany). Turbidity was measured using a Hach 2100P Turbidity meter (Hach Company, Loveland, USA). When a cyanobacterial floating scum was present, a scum sample was taken as grab sample in a glass sampling bottle.

Total phosphorus (TP) and total nitrogen (TN) were analyzed in unfiltered samples by a Skalar SAN+ segmented flow analyzer (Skalar Analytical BV, Breda, The Netherlands) following the Dutch standard protocols (NNI, 1986, 1990). After filtration through a 0.45 µm membrane filter (Whatman, NC45) nitrite, nitrate, ammonium and ortho-phosphate were analyzed (Skalar SAN+ segmented flow analyzer, NNI 1986, 1990, 1997). Filters with seston and scum samples were stored at -20 °C until extraction for microcystin (MC) analysis. The frozen filters with seston were extracted as described in Lürling & Faassen (2013).

Scum materials were prepared for MC analysis by freeze-drying. Aliquots of 5 mg freeze-dried material were transferred to 2 mL Eppendorf vials. MCs were extracted three times at 60°C in 0.5 mL 75% methanol-25% Millipore water (v/v). Extracts were dried in a Speedvac (Thermo Scientific Savant SPD121P, Waltham, USA) and reconstituted in 600 µL methanol. The reconstituted samples were transferred to 2 mL Eppendorf vials with a cellulose-acetate filter (0.2 µm, Grace Davison Discovery Science, Deerfield, USA) and centrifuged for 5 min at 16,000 × g (VWR Galaxy 16DH, Radnor, USA). Filtrates were transferred to amber glass vials before analysis.

MC analysis was performed as described in Lürling & Faassen (2013). In short, samples were analyzed for eight MC variants (dm-7-MC-RR, MC-RR, MC-YR, dm-7-MC-LR, MC-LR, MC-LY, MC-LW and MC-LF) by LC-MS/MS. The LC-MS/MS analysis was performed on an Agilent 1200 LC and an Agilent 6410A QQQ (Agilent Technologies, Santa Clara, USA). The compounds were separated on an Agilent Eclipse XDB-C18 4.6 × 150 mm, 5 µm column by Millipore water with a gradient of 0.1% formic acid and acetonitrile with 0.1% formic acid. The LC-MS/MS was operated in positive mode with an ESI source. For each compound, two transitions were monitored in MRM mode. We extended the data range for variables of pond Heesch as reported for July and August 2009 by Lürling & Faassen (2012).

Sediment sampling and analysis

In each pond sediment cores were taken from different sites at approximately 1 m water depth (pond Dongen four cores, ponds Eindhoven and Heesch each three cores) using a Uwitec Core sampler (Uwitec, Mondsee, Austria). In pond Eindhoven, six additional cores were collected along a transect in the deepest part (approximately 2 m water depth) of the pond. The top 5 cm of each sediment core was homogenized, where after a subsample was subjected to sequential P extraction using H_2O, bicarbonate/dithionite (BD, 0.11 M), NaOH (1 M), HCl (0.5 M) and persulfate ($K_2S_20_8$) as subsequent extractants (Psenner et al., 1984; Hupfer et al., 1995). In each fraction TP and, after filtration through a 0.45 µm membrane filter (Whatman, NC45), soluble reactive P (SRP) were determined using a Skalar segmented flow analyzer with the UV/persulfate destruction integrated in the system. Nonreactive P was calculated as the difference between SRP and TP. The mobile P pool was estimated from the content of the H_2O-P, BD-P and NaOH-NRP fractions (Schauser et al., 2006). Sediments were also subjected to persulfate oxidation digestions for TP analysis.

Fish sampling

The fish community was sampled on April 6th 2009 in pond Heesch, on April 7th 2009 in pond Dongen and on April 8th 2009 in pond Eindhoven by a professional fishery company (Visserijbedrijf P. Kalkman, Moordrecht, The Netherlands). The samplings were performed first by seine-haul fishing (75 m net, 8 to 12 mm mesh size), and followed by electrofishing (5 kW) along the banks. In each pond, the species composition was determined. The biomasses were determined as fresh weight by weighing all the caught fishes per species on an industrial balance. A known factor was used to correct for the efficiency of the capture rigs that were used (STOWA, 2002).

Results

Survey of cyanobacterial presence

In the period 2009-2012, a total of 158 different locations with cyanobacterial blooms were recorded in the province of North Brabant (Fig. 2.2), 125 of which were urban ponds including seven designated bathing waters. The total number of urban ponds in North Brabant is 3,473.

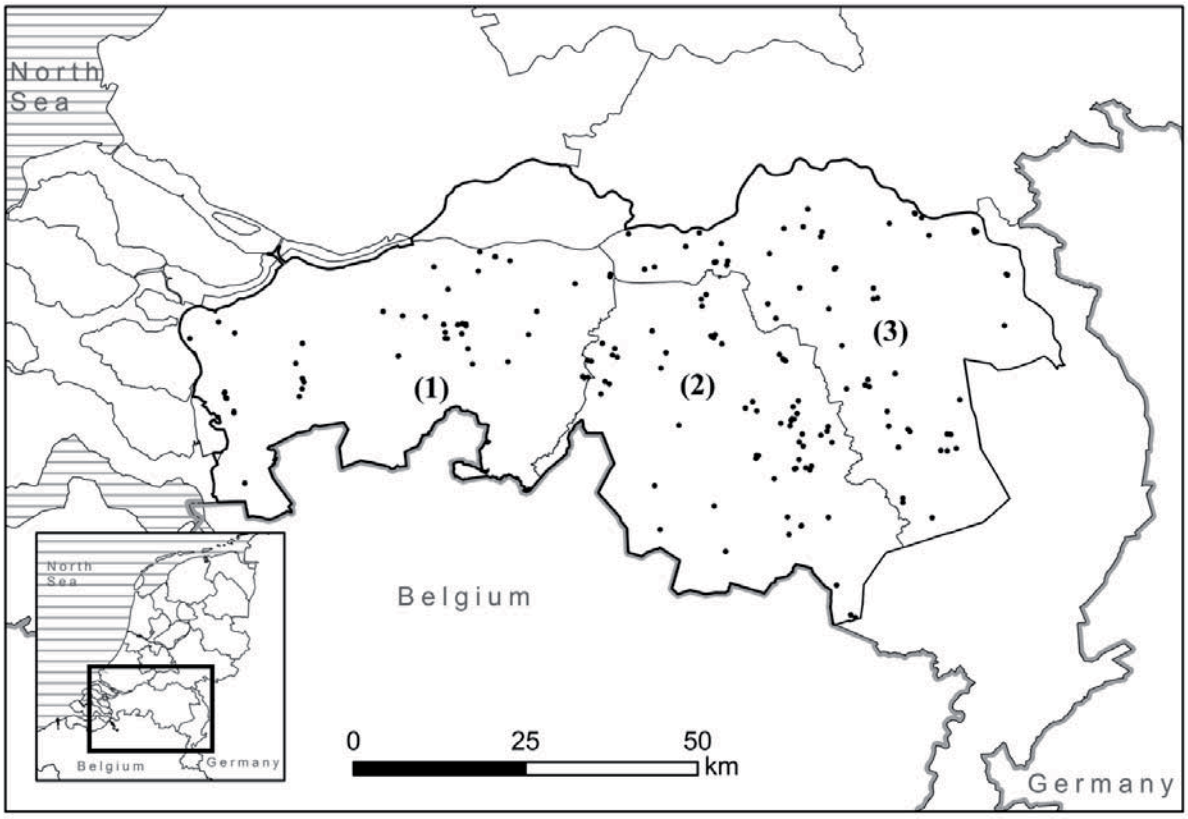


Fig. 2.2: Locations with reported cyanobacterial blooms during the period 2009-2012 in the province of North Brabant, The Netherlands. Water authorities: 1 = Brabantse Delta, 2 = De Dommel, 3 = Aa en Maas.

The total area covered by urban ponds in the province is 956 ha and the total area of the surface water is 8,663 ha. Over 80% of the blooms in ponds were reported in the months July, August and September, while the most abundant cyanobacterial genera differ per bloom and *Microcystis*, *Anabaena* and *Planktothrix* dominating most often (Table 2.2).

Table 2.2: Dominant genera in cyanobacterial blooms in urban ponds (2009-2012) for three water authorities (as % of all reported blooms in urban ponds per water authority).

	water authority Brabantse Delta	water authority De Dommel	water authority Aa en Maas
Anabaena	26	26	22
Aphanizomenon	15	13	7
Microcystis	36	17	30
Planktothrix	22	26	24
Woronichinia	1	19	17

Cyanobacterial presence and microcystin concentrations

In pond Dongen, chlorophyll-*a* concentrations increased from March to August 2009. In July and August, the dominant species *Microcystis aeruginosa* formed a surface scum. In August 2009, the cyanobacterial chlorophyll-*a* concentration reached 962 µg L^{-1} (Fig. 2.3). MC concentrations in the water column in July 22nd and August 26th 2009 were 56.7 and 48.5 µg L^{-1} with MC-RR and MC-LR as the dominant variants (Fig. 2.4).

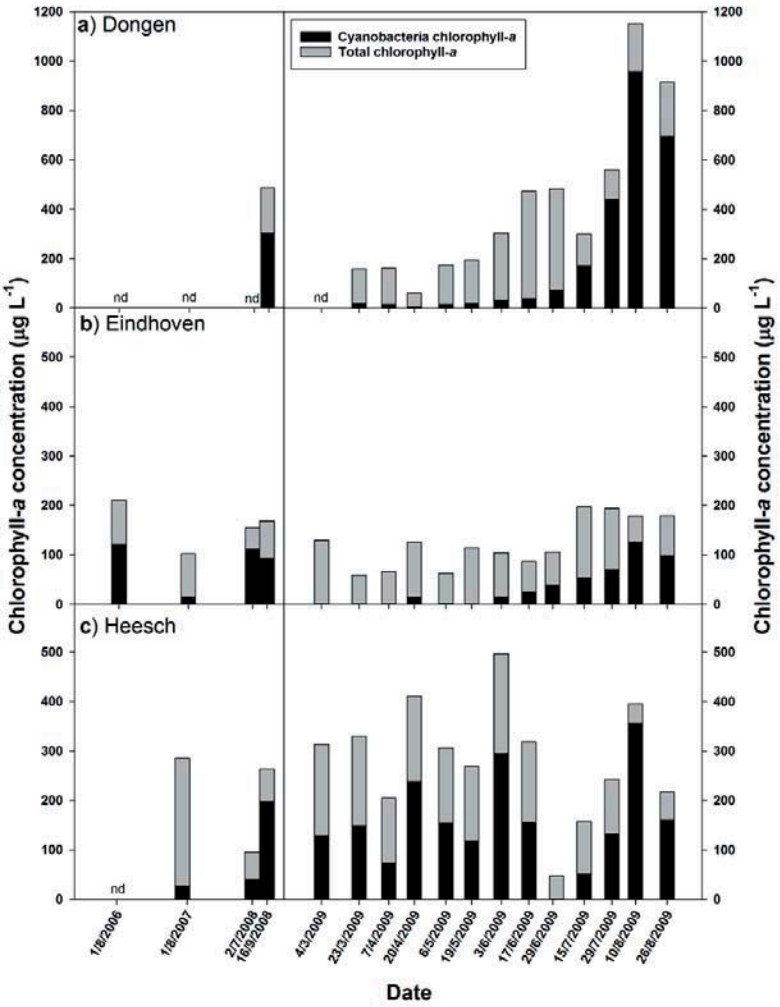


Fig. 2.3: Cyanobacteria (black bars) and eukaryote algae (grey bars) chlorophyll-*a* concentrations (µg L^{-1}) in the water from pond Dongen (a), pond Eindhoven (b) and pond Heesch (c) in 2008 and 2009 (nd = not detected).

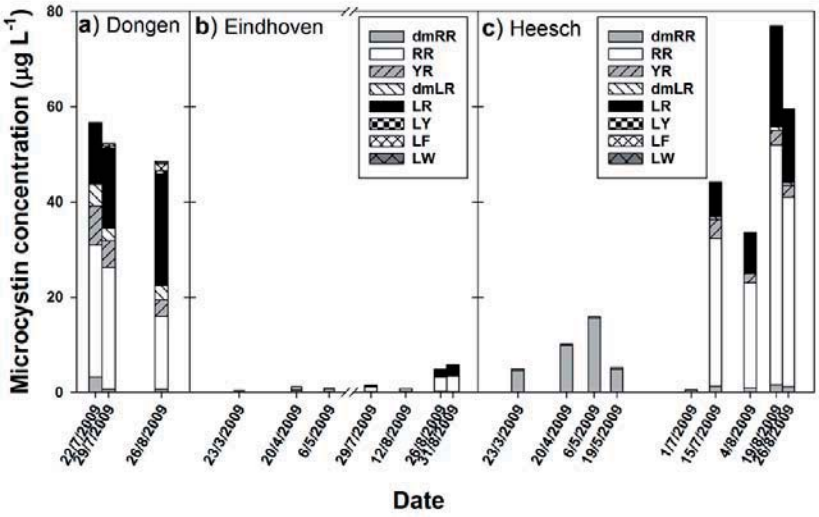


Fig. 2.4: Concentrations of eight microcystin variants (µg L^{-1}) in the water from pond Dongen (a), pond Eindhoven (b) and pond Heesch (c; partly modified from Lürling & Faassen, 2012) in spring and summer 2009.

In pond Eindhoven, maximum chlorophyll-*a* concentrations in the water column remained well below 250 µg L^{-1} during the research period (Fig. 2.3). Cyanobacterial chlorophyll-*a* concentrations reached approximately 100 µg L^{-1} during the summers of 2006, 2008 and 2009 (Fig. 2.3) and surface scums were formed every year. The scum of July 2009 consisted of *Aphanizomenon flos-aquae, Anabaena flos-aquae, M. aeruginosa* and *M. flos-aquae.* MC concentrations in the water column increased from 0.3 µg L^{-1} on March 23rd to 5.8 µg L^{-1} on August 31st 2009 (Fig. 2.4). The surface scums contained concentrations between 1,500 and 7,400 µg MC L^{-1} (Fig. 2.5).

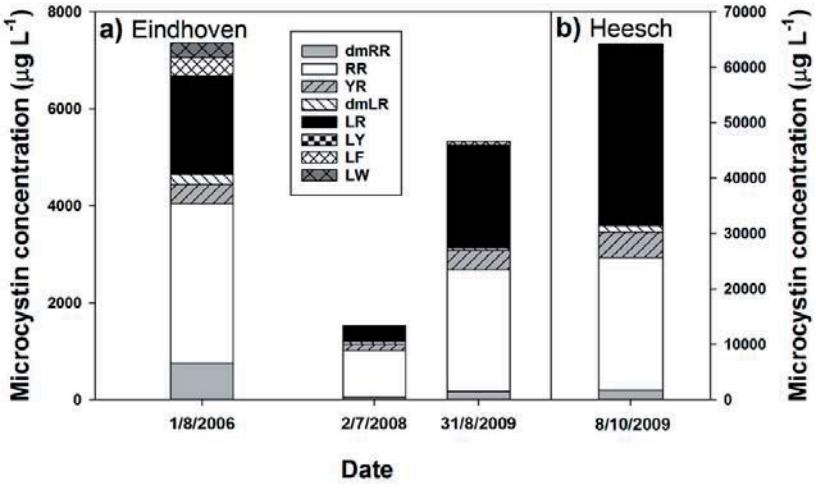


Fig. 2.5: Concentrations of eight microcystin variants (µg L^{-1}) in surface scums from pond Eindhoven (a; 2006, 2008, 2009) and pond Heesch (b; 2009).

In pond Heesch, chlorophyll-*a* concentrations showed no obvious trend between March and August 2009 with a mean chlorophyll-*a* concentration of 283 µg L^{-1}, of which 57% was cyanobacterial chlorophyll-*a* (Fig. 2.3). From March to May 2009, a *Pseudoanabaena* species dominated this pond. MC concentrations increased from 4.8 µg L^{-1} in March to 15.8 µg L^{-1} in the beginning of May 2009, where after MC concentrations declined to 0.4 µg L^{-1} on July 1st. During this period only the MC variants dm-7-MC-RR and MC-RR were detected, with dm-7-MC-RR being the most prominent variant (Fig. 2.4). In July 2009, *M. aeruginosa* and *Woronichinia naegeliana* became dominant and MC concentrations increased rapidly to 77 µg L^{-1} on August 19th 2009. MC-RR was the most dominant variant, followed by MC-LR (Fig. 2.4). The scum sample of pond Heesch from August 10th 2009 was dominated by *M. aeruginosa* and contained 64,000 µg MC L^{-1}. Also in this sample, MC-RR and MC-LR were the most abundant variants (Fig. 2.5).

Water quality

Table 2.3 shows that all three ponds are hypertrophic with mean TP concentrations ranging from 0.16 to 0.44 mg P L^{-1}. In 2009 maximum concentrations reached 0.82 mg P L^{-1} in pond Dongen, 0.35 mg P L^{-1} in pond Eindhoven and 1.90 mg P L^{-1} in pond Heesch. The minimum TP concentrations in 2009 were 0.12 mg P L^{-1} in pond Dongen and pond Heesch and 0.05 mg P L^{-1} in pond Eindhoven. The mean ortho-phosphate concentrations varied between 23 and 50 µg P L^{-1}, whereas the maximum concentrations in 2009 reached 114 µg P L^{-1} in pond Dongen, 220 µg P L^{-1} in pond Eindhoven and 131 µg P L^{-1} in pond Heesch. The minimum

Table 2.3: Overview of water quality variables presented as means of all samples (± 1 SD) determined in three urban ponds. Research periods: pond Dongen: September 2008, March until August 2009; pond Eindhoven: August 2006, August 2007, July + September 2008, March until August 2009; pond Heesch: July 2007, July + September 2008, March until August 2009

Water quality variable	Pond Dongen	Pond Eindhoven	Pond Heesch
Total phosphorus (mg P L^{-1})	0.37 (± 0.24)	0.16 (± 0.10)	0.44 (± 0.46)
Ortho-phosphate (µg P L^{-1})	23 (± 30)	24 (± 53)	50 (± 33)
Secchi depth (cm)	22 (± 9)	30 (± 9)	22 (± 9)
Turbidity (NTU)	92 (± 54)	44 (± 17)	81 (± 53)
pH	6.82 (± 0.86)	8.25 (± 0.45)	7.69 (± 0.68)
Total Nitrogen (mg N L^{-1})	3.27 (± 1.87)	1.40 (± 0.68)	2.55 (± 1.03)
Nitrite+nitrate (mg N L^{-1})	0.09 (± 0.04)	0.07 (± 0.07)	0.43 (± 0.34)
Ammonium (mg N L^{-1})	1.31 (± 1.14)	0.10 (± 0.10)	0.22 (± 0.48)
O_2 (mg L^{-1})	6.5 (± 2.5)	9.5 (± 2.8)	9.99 (± 3.7)
O_2 (%)	68 (± 25)	102 (± 27)	105 (± 41)
EC (µS cm^{-1})	450 (± 48)	331 (± 74)	320 (± 22)

ortho-phosphate concentrations in 2009 were 2 µg P L^{-1} in pond Dongen and pond Heesch and 10 µg P L^{-1} in pond Eindhoven. All three ponds were turbid and mean Secchi depths varied between 22 and 30 cm. Pond Dongen had on average the lowest pH value that ranged from 5.14 immediately after the supply of groundwater to 8.51 during a cyanobacterial bloom. The pH values in pond Eindhoven ranged between 7.34 and 8.93, and in pond Heesch between 6.76 and 8.97. The mean TN concentrations varied between 1.40 and 3.27 mg N L^{-1} (Table 2.3), while the minimum TN concentrations in 2009 were 0.32 mg N L^{-1} in pond Dongen, 0.30 mg N L^{-1} in pond Eindhoven and 0.81 mg N L^{-1} in pond Heesch.

Sediment quality

The mean mass fraction of total sediment P for pond Dongen was 0.26 mg g^{-1} dry sediment (DW). In pond Eindhoven, the sediment contained 0.43 mg P g^{-1} DW at 1 m depth and 1.11 mg P g^{-1} DW at 2 m depth. The P content of pond Heesch was lowest with 0.11 mg P g^{-1} DW (Table 2.4). Sediment digestion yielded similar TP amounts. P-fractionation indicated that the potentially releasable P was on average 42% of the sediment P pool in pond Dongen, varied from 10% to 50% in pond Eindhoven, and was 28% in pond Heesch (Table 2.4).

Table 2.4: Mean total sediment P (mg g^{-1} DW) and potential releasable sediment P (mg g^{-1} DW) in shallow sediments (~1 m water depth) in cores from pond Dongen, pond Eindhoven and pond Heesch, and deeper sediment (~2 m water depth) from pond Eindhoven. Figures in parentheses indicate ±1 SD.

	P content (mg g^{-1} DW)			
	pond Dongen	pond Eindhoven		pond Heesch
Water depth	~1m	~1m	~2m	~1m
Total sediment P	0.26 (0.12)	0.43 (0.10)	1.11 (0.08)	0.11 (0.04)
Potential releasable sediment P	0.11 (0.07)	0.04 (0.01)	0.55 (0.06)	0.03 (0.02)

Fish stock

All three ponds were heavily stocked with fish (> 900 kg ha^{-1}, Table 2.5). In all three ponds, the fish community was dominated by carp (*Cyprinus carpio*). Carp and Gibel carp (*Carassius gauratus gibelio*) made up 84% of the fish biomass of pond Dongen, 77% of pond Eindhoven and 85% of pond Heesch (Table 2.5).

Table 2.5: Fish community composition and biomass of the species (fresh weight FW kg ha^{-1}) in the three selected urban ponds in April 2009 (Kalkman, 2009a,b,c).

		Fish biomass (kg ha^{-1} FW)		
Species		**pond Dongen**	**pond Eindhoven**	**pond Heesch**
Abramis bjoerkna	Silver bream	1.8		
Abramis brama	Bream	22.3	81.7	3.4
Anarhichas lupus	Catfish			47.1
Carassius auratus gibelio	Gibel carp	16.3	265.4	218.0
Cyprinus carpio	Carp	1109.9	450.6	1011.5
Esox lucius	Pike		20.9	
Gobio gobio	Gudgeon			0.1
Hypophthalmichthys molitrix	Silver carp			124.6
Lepomis gibbosus	Sunfish			0.8
Leuciscus idus	Ide			0.4
Perca fluviatilis	Perch		3.7	2.6
Rutilus rutilus	Roach	178.1	94.6	30.2
Scardinius erythrophthalmus	Rudd	2.9	1.7	5.0
Tinca tinca	Tench		8.5	
	Total:	**1331**	**927**	**1444**

Discussion

Occurrence of cyanobacterial blooms

Many water bodies in the Dutch province of North Brabant suffer from cyanobacterial blooms and, with blooms in 125 urban ponds out of a total of 158 reported sites, blooms are reported more frequently in urban ponds than in other types of water bodies. As urban ponds represent only 11% of the total area of fresh surface water within the province, the high proportion of reported blooms in urban ponds is in contrast to their limited area. Because a structural monitoring program on blooms is lacking, we suspect that the actual frequency of cyanobacterial blooms in urban ponds is underestimated in our study. Indeed, observations by the authors indicate that blooms occur in more urban ponds than reported by the public and field staff.

Dominant cyanobacteria genera

Over 70% of the registered blooms in urban ponds were dominated by the genera *Microcystis*, *Anabaena* or *Planktothrix*, genera which tend to dominate in ponds also in neighbouring countries (Willame et al., 2005). These three genera are frequent in nutrient enriched lakes (Wetzel, 2001). The ponds that were investigated in detail showed high nutrient levels of water and sediment, a prerequisite for cyanobacterial blooms. Dominance of *Microcystis* is associated with thermal stratification and the genus is considered to be sensitive to mixing

(Reynolds et al., 2002). Our results show that many of the shallow urban ponds provide a suitable habitat for *Microcystis*. In addition to high nutrient levels, urban ponds are often surrounded by buildings, trees and shrubs which provide wind-sheltered situations. Low wind speeds favor stratification, which in turn favors algal blooms (Condie & Webster, 2001). *Planktothrix agardhii* is reported for mixed situations, while *Anabaena* spp. occur both under mixed and stratified conditions (Reynolds et al., 2002; Padisák et al., 2009). As species are favored with different needs regarding the mixing of the water, we conclude that there is a variety in environmental conditions in urban ponds, which supports functionally different cyanobacteria.

Fish stock

Beside the nutrient status and mixing conditions, the high fish community of urban ponds with a high abundance of carp (*C. carpio*), will be of influence on the phytoplankton: sediment resuspension by bottom dwelling species such as carp causes high turbidity of the water (Scheffer et al., 1993). Many cyanobacteria (e.g., *Microcystis*) are adapted to low light situations as they show an adaptive buoyancy (Reynolds et al., 2002; Visser et al., 2005). Secondly, in the presence of fish, small zooplankton is favored (Meijer et al., 1990) which, in turn, is less effective in controlling large sized cyanobacterial colonies (Gliwicz et al., 1990). Being less vulnerable to grazing, cyanobacteria can be enhanced.

Microcystins

The cyanobacteria we found dominantly in the urban ponds are known as potential MC producers (Carmichael, 2001; Van Apeldoorn et al., 2007). MCs are potent inhibitors of protein phosphatases and tumor promoters (Kuiper-Goodman et al., 1999; Zurawell et al., 2005). They have been implicated in human fatalities (Pouria et al., 1998) and in animal mortalities, for example in otters (Miller et al., 2010), turtles (Nasri et al., 2008), dogs (Lürling & Faassen, 2013) and birds (Matsunaga et al., 1999). The highest MC concentration found outside of scums in the water column of our three selected ponds was 77 µg L^{-1}, which is similar to most of the highest values recently reported from a survey in 86 Dutch surface waters (Faassen & Lürling, 2013). Although the concentrations did not exceed 100 *µ*g L^{-1}, which has been suggested a safe concentration for a single intake (Fromme et al., 2000), the ponds showed considerable spatial heterogeneity in cyanobacterial density. Ponds Eindhoven and Heesch had local surface scums that contained MC concentrations from 1,500 µg L^{-1} to 64,000 µg L^{-1} (Fig. 2.5; Lürling & Faassen, 2012). If a child would ingest less than one mL of such a scum, the suggested no adverse effect level of 25 µg MC for a single intake by children (Fromme et al., 2000) would already be exceeded. These high MC concentrations are similar to MC concentrations found in other ponds, e.g., 14,000 µg L^{-1} in a pond in Deurne (The Netherlands) (Faassen & Lürling, 2013), 38,000 µg L^{-1} in a pond in Eke (Belgium) (Descy et al., 2011) and 77,000 µg L^{-1} in a pond in Kluisbergen (Belgium) (Van Gremberghe et al., 2007). As the urban ponds are intensively used, and citizens (often

children) are exposed to pond water through particularly swimming without surveillance and possibly also through angling and boating, we conclude that cyanobacterial blooms in urban ponds are a threat to citizen's health if cyanobacterial material is ingested.

All eight MC variants included in the analysis were detected albeit not always simultaneously. In the blooms and scums, the variants MC-RR and MC-LR were the most abundant MCs, which were also observed in other studies (Mankiewicz et al., 2005; Willame et al., 2005; Mazur-Marzec et al., 2008). The variant dm-7-MC-RR and to a far less extent MC-RR were found to co-occur with almost complete dominance of *Pseudoanabaena* in pond Heesch at that time (some Scenedesmaceae co-occurred). In general *Pseudoanabaena* is not often listed as a MC producer (e.g., Van Apeldoorn et al., 2007), but there are a few studies that have shown *Pseudoanabaena* can produce MCs (Oudra et al., 2001; Teneva et al., 2009). Also this study suggests *Pseudoanabaena* might produce MCs, but solid proof can only be obtained from cultured isolates. Inasmuch as the MC-profile deviated from usually encountered in Dutch surface waters when *Planktothrix* is present (always consist of 15-30% of dm-7-MC-LR), *Anabaena* is dominant (virtually no dm-7-MC-RR and 50-80% MC-RR) or *Microcystis/Woronichinia* are present (MC-LR dominating) and the detection limit of the MC analysis is low enough to detect these other variants (Lürling & Faassen, 2013), the possibility that some of these 'usual suspects' - in such low abundance that they were not detected by microscopy - were responsible for the MC profile in spring in pond Heesch is highly unlikely. The seston MC-profile in pond Heesch completely shifted in summer towards one in which MC-RR, MC-LR and MC-YR were most abundant (Fig. 2.4). These MC variants are most frequently encountered in many water bodies (Sivonen & Jones, 1999; Graham et al., 2010; Faassen & Lürling, 2013), particularly when *Microcystis* is the chief MC-producer in them. In about a quarter of the samples in the latter study, also the variants MC-LW and MC-LF were detected that might be more toxic than MC-LR, the variant that is mostly used for risk assessments (Fischer et al. 2010; Vesterkvist et al., 2012). Also in the current study, we found these two variants in several samples from pond Dongen (Fig. 2.4) and in a surface scum in pond Eindhoven (Fig. 2.5). Although in the latter situation these variants made up only 9.4 % of the total MC-pool, using a toxicity conversion factor (Faassen & Lürling, 2013), they probably contributed at least 65% to the overall toxicity.

Implications for water management

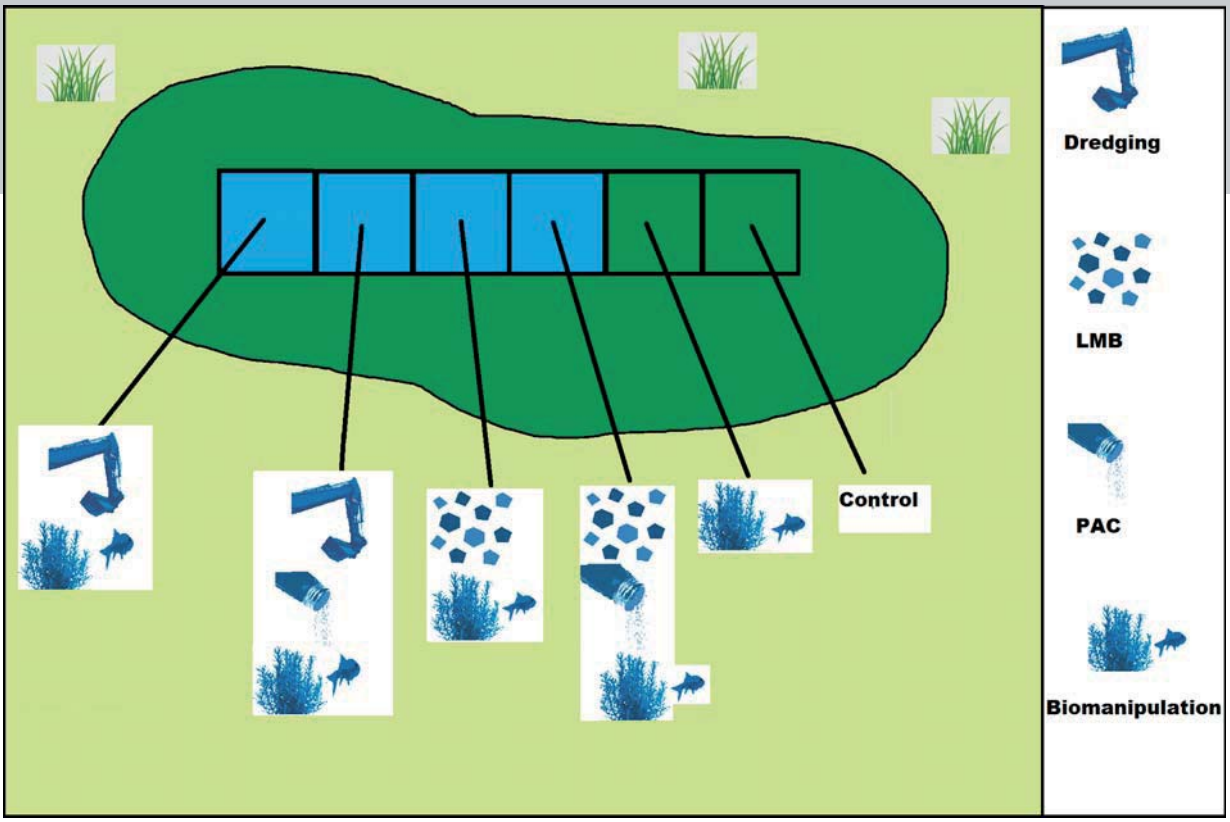
To minimize the risk of human exposure to cyanobacterial toxins in urban ponds, cyanobacterial blooms should be controlled. Urban ponds often receive high nutrient loads (Scherer et al., 1995; Stoianov et al., 2000; Waschbusch et al., 2000). Furthermore, most urban ponds are shallow, stagnant, and small with a mean area of 2,750 m^2 as determined in this study for North Brabant. These conditions favor cyanobacterial growth (Huisman et al., 2004; Paerl & Huisman, 2008). Controlling cyanobacterial blooms in a specific pond firstly requires a thorough system analysis that identifies the most important causes of the bloom. TP-concentrations well above 0.1 mg P L^{-1} and concentrations of chlorophyll-*a* rarely

below 100 µg L^{-1} show these ponds to be hypertrophic. Nutrient levels hardly seem to be limiting for phytoplankton biomass. Sediment concentrations of TP reflect they were in a similar range as in other eutrophic waters (Esten & Wagner, 2010; Zhou et al., 2008; Hill & Robinson, 2012). Furthermore, the dense, carp dominated fish stocks keep the ponds turbid by resuspending the sediment, thereby preventing submerged macrophytes to establish (Cline et al., 1994; Meijer et al., 1999; Zambrano & Hinojosa, 1999; Persson & Svensson, 2006; Roozen et al., 2007). This indicates that for this type of ponds, mitigation strategies should not only focus on external sources of eutrophication, but should also consider the effect of sediment P-release and bioturbation by fish.

Furthermore, we recommend that the toxicity of urban ponds is monitored. Despite their important role, ponds generally receive less attention from freshwater biologists (Céréghino et al., 2008; Oertli et al., 2009) and water managers (Boix et al., 2012) than other water types. This is also the case in The Netherlands, where the water authorities, following the European Water Framework Directive (WFD; Council of the European Union, 2000), primarily focus on large water bodies. An appropriate approach would start with the development and application of a monitoring program based on a uniform set of assessment criteria. Furthermore, when potential toxin producing cyanobacteria are found to be abundant, it is recommended to screen for the toxins that could be produced by the present species. At present, most water authorities focus only on concentrations of cyanobacteria and on the toxin MC-LR. Our study underpins the importance of identifying different MC variants in water samples, but also highlights that in surface accumulations MC concentrations might pose a risk to human health and animal welfare. Furthermore, other cyanobacterial toxins have also been detected in Dutch surface waters (Faassen et al., 2009; Kosten et al., 2011), sometimes at levels toxic to dogs (Faassen et al., 2012).

Conclusions

We conclude that many urban ponds in the Dutch province of North Brabant, and likely in other regions as well (Stoianov et al., 2000; Ibelings et al., 2012; Faassen & Lürling, 2013), suffer from cyanobacterial blooms. Because these blooms can be highly toxic, they can threaten citizen's health if ingested. Therefore, eutrophication control and reducing cyanobacterial blooms in urban ponds should be of importance to water managers. Dependent on the local uses and interests involved, their relevance can be similar to that of lakes and streams for which eutrophication control has become an important topic in the WFD.


Control
Dredging
LMB
PAC
Biomanipulation

CHAPTER 3

Geo-engineering in two urban ponds to control eutrophication

This chapter is based on:
Geo-engineering experiments in two urban ponds to control eutrophication. Waajen, G., F. van Oosterhout, G. Douglas & M. Lürling, 2016. Water Research 97: 69-82. http://dx.doi.org/10.1016/j.watres.2015.11.070

and

Diagnosis guided rehabilitation of eutrophic urban ponds in The Netherlands. Lürling, M., F. van Oosterhout, B. Engels & G. Waajen, *submitted*.

Abstract

Many urban ponds experience detrimental algal blooms as the result of eutrophication. During a two-year field experiment, the efficacy of five in situ treatments to mitigate eutrophication effects in urban ponds was studied. The treatments targeted the sediment phosphorus release and were intended to switch the ponds from a turbid phytoplankton-dominated state to a clear-water state with a low phytoplankton biomass. Two eutrophic urban ponds were each divided into six compartments (300-400 m^2; 210-700 m^3). In each pond the following treatments were tested: dredging in combination with biomanipulation (involving fish biomass control and the introduction of macrophytes) with and without the addition of the flocculant polyaluminiumchloride, interception and reduction of sediment phosphorus release with lanthanum-modified bentonite (Phoslock®) in combination with biomanipulation with and without polyaluminiumchloride; biomanipulation alone; and a control. Trial results support the hypothesis that the combination of biomanipulation and measures targeting the sediment phosphorus release can be effective in reducing the phytoplankton biomass and establishing and maintaining a clear-water state, provided the external phosphorus loading is limited. During the experimental period dredging combined with biomanipulation showed mean chlorophyll-*a* concentrations of 5.3 and 6.2 µg L^{-1}, compared to 268.9 and 52.4 µg L^{-1} in the control compartments. Lanthanum-modified bentonite can be an effective alternative to dredging and in combination with biomanipulation it showed mean chlorophyll-*a* concentrations of 5.9 and 7.6 µg L^{-1}. Biomanipulation alone did not establish a clear-water state or only during a limited period. As the two experimental sites differed in their reaction to the treatments, it is important to choose the most promising treatment depending on site-specific characteristics. At the end of the field experiment, the compartments were removed and rehabilitation measures were executed in both ponds. The efficacy of the measures was monitored during a subsequent period of three years (2012 – 2014). At one of both ponds (Dongen) the rehabilitation was based on the results of diagnostics, while at the other pond (Eindhoven) rehabilitation consisted of in-pond measures alone and the external P-loading continued despite the diagnostics recommended otherwise. The mean chlorophyll-*a* concentration in pond Dongen dropped significantly form 500 µg L^{-1} in 2009, 286 µg L^{-1} and 200 µg L^{-1} in the control compartment in 2010 and 2011 respectively, to 16 µg L^{-1} during the period 2012 – 2014. In pond Eindhoven the mean chlorophyll-*a* concentration was 90 µg L^{-1} before rehabilitation (2009 – 2011) and 46 µg L^{-1} after rehabilitation (2012 – 2014) and no significant difference could be shown. The results are in line with expectations based on the outcome of the diagnostics. In recovering the water quality status of urban ponds, continuing attention is required to the concurrent reduction of external phosphorus loading and the maintenance of an appropriate fish community.

Introduction

Most of the world's lakes are small and generally in the size class 0.1 to 1 ha (Downing et al., 2006). In urban areas, such small lakes or ponds are important components of the living environment. Through their ornamental function and recreational opportunities, they enhance the quality of urban life. Urban ponds provide the most important public contact with surface waters (Birch & McCaskie, 1999) and the need for safe and aesthetically acceptable water is critical in modern societies (Steffensen, 2008). Urban ponds also play a role in water retention and as a recipient of sewer overflows. Many urban ponds suffer from eutrophication with severe impacts on water quality and on the aquatic ecosystem (Brönmark & Hansson, 2002; Grimm et al., 2008). Eutrophication and the consequential growth of excessive, sometimes toxic phytoplankton biomass is a major water quality issue (Smith & Schindler, 2009).

In many eutrophic fresh waters, the submerged macrophytes disappear with a transition to a turbid, phytoplankton-dominated state (Scheffer et al., 1993) often with a predominance of cyanobacteria (Watson et al., 1997; Smith et al., 1999). Cyanobacterial blooms can cause fish kills, are potentially toxic to humans, dogs, waterfowl and other animals, reduce biodiversity, and can cause unpleasant surface scums and malodors (Smith et al., 1999; Codd et al., 2005a; Smith & Schindler, 2009). Cyanobacterial nuisance is a widespread phenomenon in eutrophic urban ponds in The Netherlands (Chapter 2) and many other countries (e.g., Fastner et al., 1999; Willame et al., 2005; Rahman & Jewel, 2008).

To mitigate eutrophication and hence blooms of cyanobacteria, external phosphorus (P) sources need to be reduced and, depending on the P-loading history of the water body and the societal acceptable time for recovery, also the internal P-source needs to be addressed (Gulati & Van Donk, 2002; Søndergaard et al., 2007; Schindler et al., 2008). Eutrophication mitigation should aim to reduce the P-loading below a critical threshold in order to realize a clear water state (Janse et al., 2008) and mitigate cyanobacterial nuisance. Ideally, water managers could select from a number of effective treatments to mitigate cyanobacterial nuisance in urban ponds, improve the water quality and promote the growth of submerged macrophytes, based on a thorough system analysis. However, in many systems repeated maintenance measures are needed, including deliberate manipulation of in-lake/pond processes to enhance recovery, also known as geo-engineering (Spears et al., 2013a; MacKay et al., 2014).

Removal of sediment can be an effective in-lake/pond measure in eutrophication control (Peterson, 1982; Brouwer et al., 2002) and is often conducted, but the costs in Dutch urban ponds are high and vary between € 25 and € 60 per m^3 of in situ sediment, including transport and treatment costs. An attractive alternative to sediment dredging which might reduce the costs, is the in situ capping of sediments with an active barrier capable of capturing nutrients released from the pore water and minimizing their release to the water column (Hart et al., 2003; Drábková, 2007; Gibbs et al., 2011). A promising

active capping agent is the lanthanum-modified bentonite clay (LMB) Phoslock® developed by CSIRO Australia (Douglas, 2002). Lanthanum is effective over a wide pH range in binding P (Douglas et al., 2004) and shows a very high P removal over the pH range 5 to 9 (Peterson et al., 1976; Ross et al., 2008). Importantly, the P-binding capacity of lanthanum is not affected by anoxia (Douglas et al., 2004; Ross et al., 2008; Gibbs et al., 2011; Copetti et al, 2016). These characteristics make LMB a promising agent in reducing the sediment P-release, although humic substances can reduce its efficacy (Douglas et al., 2000; Lürling et al., 2014b; Dithmer et al., 2016a). Several studies have shown that the LMB is highly efficient at both stripping soluble reactive phosphorus (SRP) from the water column and in intercepting P released from the sediments once settled where it forms a reactive capping (Robb et al., 2003; Akhurst et al. 2004; Douglas et al., 2008; Ross et al., 2008; Egemose et al., 2010; Gibbs et al., 2011; Van Oosterhout & Lürling, 2013). LMB has also been applied successfully in combination with a low dose flocculant polyaluminiumchloride (PAC) or iron chloride to two stratifying lakes in The Netherlands shifting them from a eutrophic state, dominated by cyanobacteria, to an oligo-mesotrophic clear water system (Lürling & Van Oosterhout, 2013; Chapter 5). Little, however, is known about the effectiveness of the LMB in shallow eutrophic urban ponds in comparison with sediment removal.

To compare the effectiveness of dredging to that of chemical P inactivation by the LMB (with and without PAC as flocculant), we conducted a field experiment in compartments in two hypereutrophic urban ponds during 2009-2011. Both ponds had a history of P-loading. Excessive fish stocks (often dominated by carp: *Cyprinus carpio carpio*; Chapter 2) are known to keep urban ponds turbid via sediment resuspension, hence preventing submerged macrophyte establishment (Cline et al., 1994; Meijer et al., 1999; Zambrano & Hinojosa, 1999; Persson & Svensson, 2006; Roozen et al., 2007). As both ponds studied were excessively stocked with carp, both sediment dredging and sediment capping were deemed ineffective without a reduction of fish stocks. Hence, the compartments were standardized with respect to their fish stock and biomanipulation, which involved a reduction in fish stock plus introduction of macrophytes. This was also tested as an individual treatment. We hypothesized that the combination of biomanipulation and measures targeting the internal P-load would be effective to create and maintain a clear water state without high phytoplankton biomass. The field experiment was supported by a laboratory assay focusing on the binding efficacy of LMB in which we hypothesized that a higher LMB concentration as well as the addition of the flocculant PAC would enhance the stripping of SRP from the water column. At the end of the field experiment, the compartments were removed, both ponds were reconstructed and mitigating measures were executed. The effect of these measures was monitored during a subsequent period of three years.

Material and methods

Study sites

Pond Dongen is located in the urban area of the city Dongen (The Netherlands, N 51° 37' 48.00''/E 4° 56' 27.30'') and has an area of 2500 m^2. The water depth is approximately 0.7 m and the pond has a ~0.7 m accumulated soft sediment layer on a sandy base. Until 2000, the pond received the discharge from a mixed sewer overflow. The pond has a history of cyanobacterial blooms (*Microcystis* sp., *Planktothrix* sp., *Anabaena* sp.) and scum formation. Prior to the research, submerged macrophytes were absent in the pond. The initial fish biomass was 1313 kg fish ha^{-1} (Table 3.1) as was determined on 7 April 2009 by combined seine netting (mesh size 8 – 12 mm) and electro fishing (5 kW; Kalkman, 2009b). The pond is used for angling and consists of two sections connected by culverts. In August 2009, the pond was divided into eight rectangular compartments by wooden sheet pilings (Figs. 3.1 and 3.2).

Table 3.1: Initial fish stock of pond Dongen and pond Eindhoven (fresh weight kg ha^{-1}). Percentages are given in parentheses.

	Pond Dongen		Pond Eindhoven	
Fish species	**Fish stock kg ha^{-1}**	**%**	**Fish stock kg ha^{-1}**	**%**
Carp (*Cyprinus carpio carpio*)	1109.9	(83)	450.6	(49)
Roach (*Rutilus rutilus*)	178.1	(13)	94.6	(10)
Bream (*Abramis brama*)	22.3	(2)	81.7	(9)
Gibel carp (*Carassius gibelio*)	16.3	(1)	265.4	(29)
Rudd (*Scardinius erythrophthalmus*)	2.9	(<1)	1.7	(<1)
White bream (*Blicca bjoerkna*)	1.8	(<1)		
Pike (*Esox lucius*)			20.9	(2)
Tench (*Tinca tinca*)			8.5	(1)
Perch (*Perca fluviatilis*)			3.7	(<1)

Each compartment had a surface area of approximately 300 m^2 and six compartments were used in this study (ranging 210-420 m^3). The pond is not connected to other surface water sources and is hydrostatically maintained by groundwater infiltration (Supplementary information, Appendix 3.A). In dry periods, however, the water level is maintained through the supply of pumped groundwater. During very wet periods, excess water can be discharged through the sewer, but this did not happen during the experiment. During the experiment, the external P-loading of the compartments was reduced as angling and the use of bait were prohibited and the feeding of water birds with associated faecal input was discouraged.

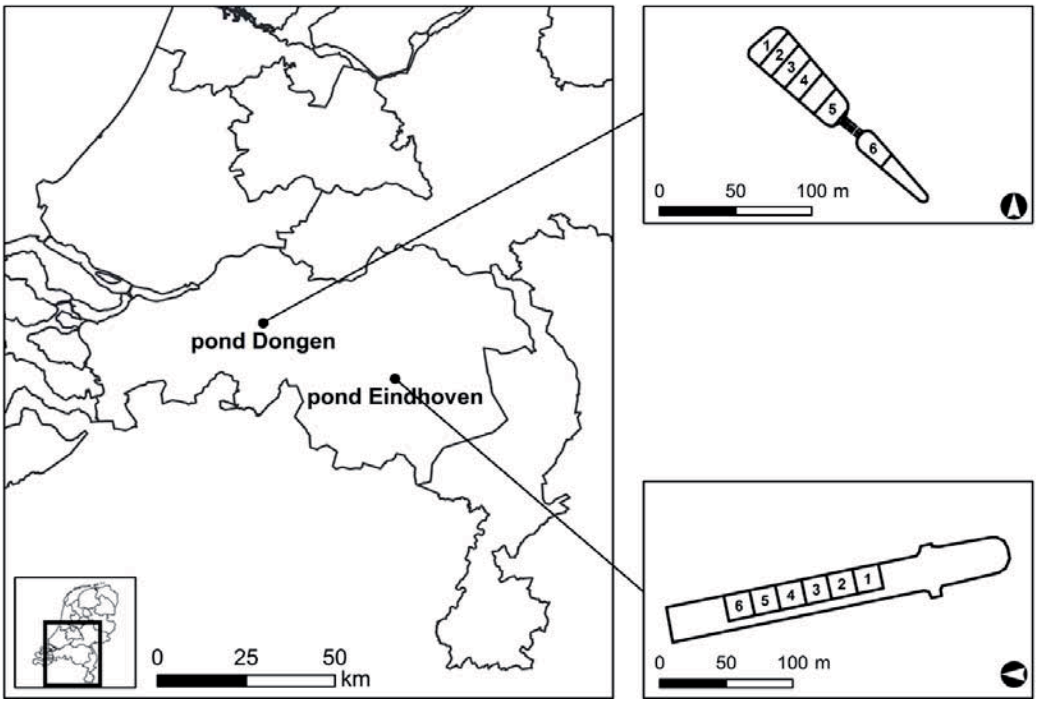


Fig. 3.1: Location of ponds Dongen and Eindhoven in The Netherlands (left panel) and schematic drawings of both ponds with compartments (right panels). Treatments: 1=dredging+biomanipulation (DB), 2=dredging+PAC+biomanipulation (DPB), 3=LMB+biomanipulation (LB), 4=LMB+PAC+biomanipulation (LPB), 5=biomanipulation (B), 6=control (C).

Fig. 3.2: Aerial view of the compartments at pond Dongen (left panel) and pond Eindhoven (right panel). Photographs by L. Turlings, 11 August 2010.

Pond Eindhoven is located in the north of the city Eindhoven (The Netherlands, N 51°48'96.57''/ E 5°47'65.31'') and has an area of ~7000 m^2. The average water depth is 1.5 m and the pond has a ~0.4 m accumulated soft sediment layer on a sandy base. A steep lawn containing substantial dog faeces surrounds the pond. This pond receives rainwater from impervious surfaces which enters the pond at several points. The pond has a history of cyanobacterial blooms (*Microcystis* sp., *Planktothrix* sp., *Anabaena* sp.) and scum formation. Macrophytes were absent in the pond and the fish stock consisted of 927 kg fish ha^{-1} (Table 3.1) as was determined on 8 April 2009 by combined seine netting (mesh size 8 – 12 mm) and electro fishing (5 kW; Kalkman, 2009c). The pond is used for angling and

other recreational activities. In August 2009, six compartments (400 m^2, 600-700 m^3) were constructed in the pond using steel dam barriers (Fig. 3.1, Fig. 3.2). During the experiment, the external P-loading of the compartments was reduced as angling and the use of bait were prohibited, the feeding of water birds was discouraged and the compartments had no direct inflow of surface water.

The external P-loading for pond Dongen was 2.7 mg P m^{-2} d^{-1} and for pond Eindhoven was 22.4 mg P m^{-2} d^{-1} (Table 3.2; Supplementary information, Appendix 3.A), while the critical P-loading thresholds as determined with the PCLake Metamodel (Mooij et al., 2010; PBL, 2015) was 0.6-1.5 mg P m^{-2} d^{-1} and 1.0-3.1 mg P m^{-2} d^{-1}, respectively. Information on the water quality of both ponds before the construction of the compartments is given in Supplementary Information, Appendix 3.B.

Table 3.2: Phosphorus (P) loads and critical phosphorus (P) loads (mg P m^{-2} d^{-1}) of pond Dongen and pond Eindhoven.

	P-load (mg P m^{-2} d^{-1})	
Source	**Pond Dongen**	**Pond Eindhoven**
Deposition	0.07	0.11
Runoff	1.12	4.69
Water birds	0.14	0.03
Feeding ducks	0.52	0.00
Feeding fish (bait)	0.42	0.44
Leaf litter	0.04	0.00
Rainwater discharge	0.00	17.16
Pumped groundwater	0.40	0.00
Sediment*	5.42	1.66
Total	**8.13**	**24.09**
	Critical P-load (mg P m^{-2} d^{-1})	
Clear to turbid	1.5	3.1
Turbid to clear	0.6	1.0

*At Dongen: in control compartment. At Eindhoven: in pond outside compartments.

Laboratory assay and compartment experiment

A laboratory assay was conducted to investigate the binding efficacy of LMB with and without the flocculant PAC. According to the supplier of the LMB, removal of P (as phosphate) by lanthanum has a molar ratio of 1:1, meaning that 100 g of LMB is needed to bind 1 gram of P, based on the assumption of 5% lanthanum in the LMB (http://www.phoslock.eu/en/phoslock/about-phoslock/; accessed 10 February 2016) and assuming that the lanthanum totally binds to phosphate (Ross et al., 2008). The assay was executed in 125 mL glass test tubes containing 100 mL of a SRP medium (0.72 mg P L^{-1} in nanopure water) and lasted 24 h. LMB was added as a slurry to the tubes in ratios 0:1 (control), 100:1, 200:1, 400:1 and 800:1.

A second series of tubes additionally received PAC with a concentration of 1 mg Al L^{-1} before adding the LMB. Samples were taken at time intervals of 0 – 1 – 2 – 4 – 6 – 8 – 24 h. After filtration through a 0.45 µm membrane filter (Whatman NC45), the SRP concentration of the samples was determined using a Skalar SAN+ segmented-flow analyzer (Skalar Analytical BV, Breda, The Netherlands) following the Dutch standard protocol (NNI, 1986). The assay was conducted in triplicate.

In the compartments of ponds Dongen and Eindhoven (Fig. 3.1) the following treatments were implemented in the field experiment:

1. dredging + biomanipulation (DB)
2. dredging + PAC+ biomanipulation (DPB)
3. LMB + biomanipulation (LB)
4. LMB + PAC + biomanipulation (LPB)
5. biomanipulation (B)
6. control (C).

The biomanipulation consisted of fish stock control and the introduction of submerged macrophytes (compartments DB, DPB, LB, LPB, B; Fig. 3.1). The original fish were removed in July 2009 (Dongen, before the construction of the compartments) and in August 2009 (Eindhoven, after the construction of the compartments but before the other measures were executed). Fish were removed by repeated seine netting combined with electro fishing (Dongen) and repeated seine netting (Eindhoven) until no more fish were caught. Restocking of the fish was carried out on 28th September 2009 in Eindhoven, while in Dongen restocking was initiated in July and August 2010 (all compartments, except carp in compartment C) and completed in January 2011 (carp in compartment C). The compartments DB, DPB, LB, LPB and B in Eindhoven received a mean fish stock of 128.0 kg ha^{-1} (SE 4.9) and in Dongen 86.0 kg ha^{-1} (SE 3.2). These stockings consisted of approximately 52 weight-% roach and 48 weight-% pike in Eindhoven and 60 weight-% roach and 40 weight-% pike in Dongen (Table 3.3). These densities are low for these waters and expected to exert no negative effect on water transparency (Meijer et al., 1999). The control compartments received a fish stock resembling the original fish densities: 655 kg ha^{-1} in Eindhoven and 927 kg ha^{-1} in Dongen (Table 3.3). Locally collected submerged macrophytes (*Elodea nuttallii* and *Myriophyllum heterophyllum*) were introduced as part of the biomanipulation, spread broadly and regularly by hand at the surface of the compartments. These species were selected because of their ability to respond rapidly after fish stock reduction (Strand & Weisner, 2001) and abundant availability. Using this method of introduction, the macrophyte establishment was expected to be easy, assuming that good water quality had been attained. Compartments DB, DPB, LB, LPB and B each received 1 kg fresh weight *Elodea* on 28 April 2010 (Dongen) and 29 April 2010 (Eindhoven) and 1 kg *Myriophyllum* on 29 March 2010 (Dongen) and 31 March 2010 (Eindhoven). The flocculant AquaPAC39 (polyaluminiumchloride, $Al_n(OH)_mCl_{3n-m}$, ρ = 1.37 kg L^{-1}, 8.9 % Al, 21.0% Cl; PAC) and the LMB (5% La) were supplied by Phoslock Europe GmbH (Ottersberg, Germany).

Table 3.3: Initial fish stock (fresh weight kg ha^{-1}) in the compartments at pond Dongen and pond Eindhoven. Treatments: DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation, B = biomanipulation, C = control.

	Pond Dongen						Pond Eindhoven					
	Fish stock (kg ha^{-1}) in compartments						Fish stock (kg ha^{-1}) in compartments					
Fish species	**DB**	**DPB**	**LB**	**LPB**	**B**	**C**	**DB**	**DPB**	**LB**	**LPB**	**B**	**C**
Carp						626.7						290.0
Roach	46.7	50.0	40.0	53.3	60.0	250.0	67.5	60.0	57.5	80.0	70.0	77.5
Bream						46.7						30.0
Gibel carp												247.5
White bream												3.3
Pike	33.3	33.3	40.0	36.7	36.7		67.5	57.5	70.0	62.5	47.5	7.5
Perch												2.5

Dredging (compartments DB, DPB) was conducted in August 2009 while the compartments were full of water. The sediment was removed down to the hard sandy substrate. The LMB was applied to compartments LB and LPB at Eindhoven on 2 and 3 September 2009 at 1.13 kg LMB m^{-2} and at Dongen on 4 September 2009 at 0.75 kg LMB m^{-2}. These dosages were tailored to match the concentration of potentially bioavailable P in the upper 5 cm of the sediment (as determined by sequential P extraction cf. Psenner et al., 1984) and to the total P (TP) concentration of the pond water prior to the application.

Dredging activities resuspend fine particles and liberate SRP from interstitial water (Peterson, 1982). The application of PAC in dredged compartments (compartment 2) was aimed at reducing the concentration of suspended particles and SRP. The application of LMB to the surface of the water column increases the concentration of suspended solids and temporarily reduces clarity. Adding PAC after the application of LMB in compartment LPB was aimed at more rapidly clearing the water in combination with the complimentary ability of the PAC for permanent P fixation. Shortly before the field application, the PAC dose for optimal flocculation was experimentally determined using glass jar tests. On site 1 L glass jars were filled with 800 mL surface water. To these jars 0, 1, 2, 3 or 4 mL of a 100 times diluted PAC solution was added, stirred briefly with a glass rod and formation of flocs was visually observed. These additions correspond to doses of 0, 1.45, 2.89, 4.34, 5.78 mg Al L^{-1}. In pond Eindhoven the 1 mL addition appeared sufficient, in pond Dongen the 2 mL addition gave good flocculation. With an estimated water volume of 195 m^3 (0.65 x 300) in Dongen and 532 m^3 (1.33 x 400) in Eindhoven the selected doses came to 565 and 772 g Al or 4890 and 6680 mL PAC respectively. In Eindhoven 16.3 mL PAC m^{-2} was dosed and in Dongen 16.7 mL PAC m^{-2} (~2 g Al m^{-2}). As dissolved PAC is acidic, 1.2 kg hydrated lime was added in powdered form, $Ca(OH)_2$, to compartment LPB at Dongen to maintain the pH between 6.5 and 7 for optimal flocculation and to mitigate the lanthanum displacement by aluminium

from the LMB matrix. Compartments DPB at Dongen and DPB and LPB at Eindhoven did not receive hydrated lime as the pH during PAC application stayed above 6.5.

Whole-pond rehabilitation

At the end of the compartment experiment, the compartments were removed and mitigating measures were executed in both ponds during autumn 2011 – spring 2012 (Table 3.4, Fig. 3.3).

Table 3.4: Measures implemented at pond Dongen and pond Eindhoven (autumn 2011 – spring 2012).

Pond Dongen	Pond Eindhoven
Dredging	Dredging and deepening
Creating soft banks	Creating soft banks
Planting macrophytes	Planting macrophytes
Prohibition dog outlet	Inform citizens dog outlet
Inform citizens feeding ducks/fish	Inform citizens feeding ducks/fish
No carp, less baiting	Less carp, less baiting
Reduction of pumped groundwater, fluctuations of water level	---
Fish stock manipulation	Fish stock manipulation

Fig. 3.3: Sediment removal at pond Dongen (10 November 2011).

The rehabilitation measures at pond Dongen were based on the P-loads and critical P-loads (Table 3.2) and on the results of the compartment experiment, in contrast to the rehabilitation of pond Eindhoven due to agreements between the water authority and the community of Eindhoven prior to the experiment. First, all fish were removed from the ponds by a professional fishing company (Visserijbedrijf P. Kalkman, Moordrecht, The Netherlands). Preceding the dismantling of the compartments at pond Dongen, fish were removed on

September 16th 2011 by seine-haul fishing using a 35 m net (mesh size 8 – 12 mm) that was pulled twice through each compartment. At pond Eindhoven, after the dismantling of the compartments a net was constructed on October 11th 2011 that split the pond in two parts. Fish were removed by combined seine haul fishing (twice in each part with a 225 m net with 8-12 mm mesh) and followed by electrofishing (5 kW) along the banks. On December 8th 2011, when the water level was lowered for the planned dredging and deepening of pond Eindhoven, the remaining fish were removed by four trawls and electrofishing. The fish stock manipulation implied restocking of 90 kg fish ha^{-1} in pond Dongen and 77 kg fish ha^{-1} in pond Eindhoven (Table 3.5).

Table 3.5: Fish restocked (fresh weight kg ha^{-1}) at pond Dongen and pond Eindhoven as one of the mitigating measures after fish removal.

	Fish stock (kg ha^{-1})	
	Pond Dongen	Pond Eindhoven
Bleak	16	
Perch	16	
Pike	6	31
Roach	24	46
Rudd	12	
Tench	16	
Total	90	77

Water quality sampling and analysis

From 31 August 2009 until 23 September 2011 the compartments (Dongen, Eindhoven) were sampled for surface water quality every two weeks. We selected Secchi depth (m), chlorophyll-*a* (µg L^{-1}) and TP (mg L^{-1}) as the prime water quality monitoring variables. Secchi depth was determined using a 20 cm diameter black-and-white quadrant Secchi disc. Two-liter water samples were taken with a sampling tube (whole water column integrated sample). Water samples were brought to the laboratory where chlorophyll-*a* concentrations were measured using a PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany) calibrated against the Dutch standard for the spectrophotometric analysis of chlorophyll-*a* (NNI, 2006b). The PHYTO-PAM phytoplankton analyzer differentiates cyanobacteria, green algae and diatoms. In addition chlorophyll-*a* concentrations were determined by spectrophometric analysis following the Dutch standard procedure (NNI, 2006b). Turbidity was measured with a Hach 2100P Turbidity meter. Unfiltered samples were analyzed for TP, while glass-fibre filtered (Whatman GF/C) samples were analyzed for SRP using ICP-MS in the Chemical–Biological Soil Laboratory of the Department of Soil Sciences (Wageningen University). The detection limits were 6 µg L^{-1} for TP and 1 µg L^{-1} for SRP.

On 6/8 October 2010, 28 June 2011 and 23/24 August 2011, macrophyte coverage was determined in the compartments by use of a throwing rake to collect samples. During the course of the experiment, the fish stock in the compartments in Eindhoven and Dongen was determined on 8 and 12 April 2011 respectively, using electrofishing (5 kW). All fish were identified, weighed and their lengths were measured. At the end of the experimental period in 2011, prior to dismantling the compartments, all macrophytes (Eindhoven 1 September 2011 and Dongen 12 September 2011) and fish (Eindhoven 6 September 2011 and Dongen 16 September 2011) were harvested. Macrophytes were harvested using a mowing boat and after drainage of excess water, weighed on a weighing bridge (± 20 kg). Fish were harvested using combined electrofishing (5 kW) and trawl fishing. The fish collection was done by a professional fishing company (Visserijbedrijf P. Kalkman, Moordrecht, The Netherlands).

To estimate the sediment P-release three replicate sediment cores were taken in March 2011 using a Uwitech core sampler from each compartment in Eindhoven and Dongen. The cores were transported to the laboratory, where the overlying water was gently syphoned off and replaced by oxygen free nanopure water, representing a worst case but realistic situation for the ponds. The cores were incubated for a week under anoxic conditions in darkness (20°C). To estimate the P-release, the SRP concentration in the overlying water was measured after filtration through a 0.45 µm membrane filter (Whatman NC45) using a Skalar SAN+ segmented-flow analyzer following the Dutch standard protocol (NNI 1986).

Fig. 3.4: Pond Dongen after reconstruction (25 July 2014).

After the removal of the compartments (Fig. 3.4), each pond was sampled monthly during October 2011 – December 2014. At location, Secchi depth was determined and water samples were taken for analysis of turbidity and concentrations of chlorophyll-*a*, TP and SRP according to the research of water quality variables described for the compartments.

Data analysis

A One Way ANOVA and post hoc Tukey test were used to analyze the results from the laboratory assay. In case homogeneity of variance was violated (Levene's test $P < 0.05$), Welch's ANOVA was used followed by Games-Howell post hoc test. The analyses were done in SPSS Statistics 21 (IBM). Sediment P-release in the sediment cores from the compartments was analyzed by a Kruskal-Wallis One Way Analysis of Variance on Ranks because the distribution of the data was not normal according to the Shapiro-Wilk Normality Test ($P < 0.05$). The analyses were done in SigmaPlot version 12.5 (Systat Software, Inc.). Classification of the ecological status of surface waters has been implemented according to the Water Framework Directive (Council of the European Union, 2000; Van der Molen et al., 2013). This classification was used as a reference for determination of the ecological status of the compartments based on chlorophyll-*a* and TP concentrations.

Inasmuch as most differences in water quality variables were to be expected during the summer season, values of each summer half year (April – September) during the period 2009 - 2014 were compared running repeated measures ANOVAs using the program SigmaPlot version 13 (Systat Software, Inc.). In case normality test (Shapiro-Wilk) or equal variance test (Brown-Forsythe) failed (i.e., $P < 0.05$) the data were log-transformed. To distinguish among years that differed from each other, all pairwise multiple comparison procedures were employed (Tukey test; $P < 0.05$). In case log-transformation could not produce normally distributed data, a Mann-Whitney rank sum test was run to investigate differences between years. In pond Dongen, the entire pond was compartmented so in the before (prior to 2012) – after (from 2012) analysis the control compartment (C) was included as representative for the pond before rehabilitation.

Results

Laboratory assay

At each measurement (1 – 24 h), the SRP concentrations of the several LMB treatments differed significantly from the non-treated control tubes (0 mg LMB L^{-1}; $P < 0.0005$). At each measurement (1 – 24 h) the SRP concentration was significantly lower at a higher LMB concentration (Fig. 3.5A). In the LMB + PAC treatments (Fig. 3.5B), the SRP concentrations were significantly lower than in the comparable exclusively LMB treatments ($P \leq 0.035$), except for the LMB treatment 800:1 at Time = 24 h ($P = 0.066$).

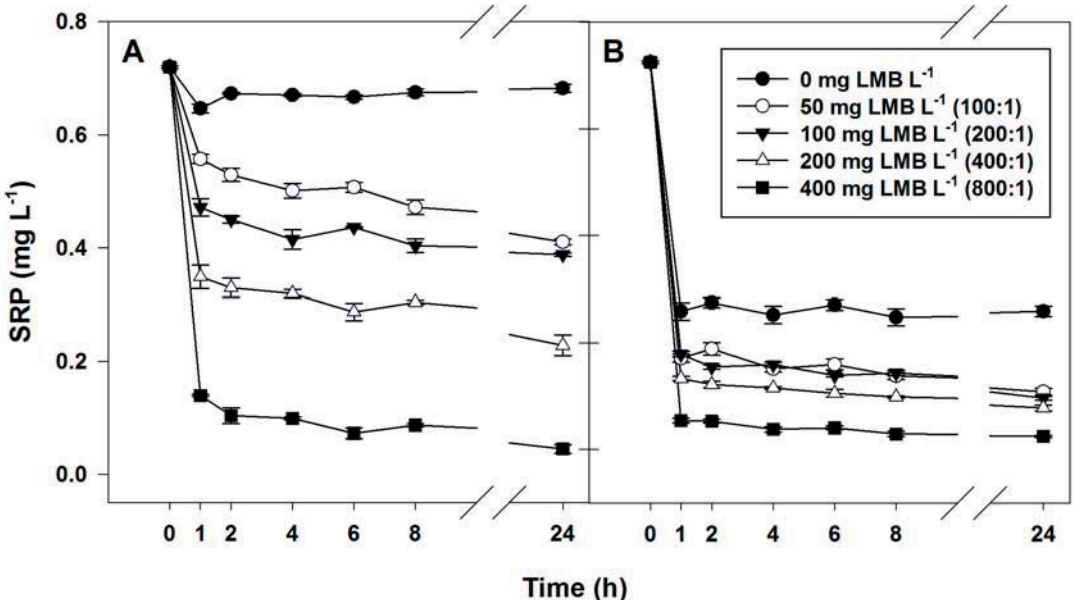


Fig. 3.5: Course of the soluble reactive phosphorus (SRP) concentration at different LMB doses (Panel A) and LMB + PAC (1 mg Al L^{-1}) (Panel B) during a 24 h laboratory assay.

Compartment experiment

Water clarity

At Dongen, a majority of the Secchi depth recordings in the compartments DB, DPB, LB and LPB reached the sediment. This resulted in the greatest Secchi depths in the dredged compartments DB and DPB, because they were the deepest. The control compartment at Dongen remained substantially more turbid with a median Secchi depth of 0.25 m, while the B and both the LMB treated compartments (LB, LPB) had twice the Secchi depth (Fig. 3.6A; Table 3.6). At Eindhoven, the dredged (DB, DPB) and LMB treated compartments (LB, LPB) gradually became similar, with the greatest Secchi depths recorded during the second year (Oct 2010 – Aug 2011; fig. 3.6B periods 3 and 4). In compartments B and C, the Secchi depths remained low (Fig. 3.6B). The median Secchi depths in compartments B and C were about half of those in the dredged (DB, DPB) and LMB compartments (LB, LPB; Table 3.6). Similarly, turbidity was highest in compartments B and C at Eindhoven and lowest in the four either dredged or LMB treated compartments (DB, DPB, LB, LPB; Table 3.6). At Dongen, the highest turbidity was measured in compartment C, but the turbidity in compartment B was similar to the other treatments (Table 3.6). Turbidity responded differently between treatments at each location. Differences in turbidity response were also observed between both ponds when comparing the same treatment (Table 3.6). At Dongen, the lowest turbidities were measured in compartment DPB. Apart from the controls, the highest turbidities were measured at Dongen in compartment LPB and at Eindhoven in compartment B (Table 3.6).

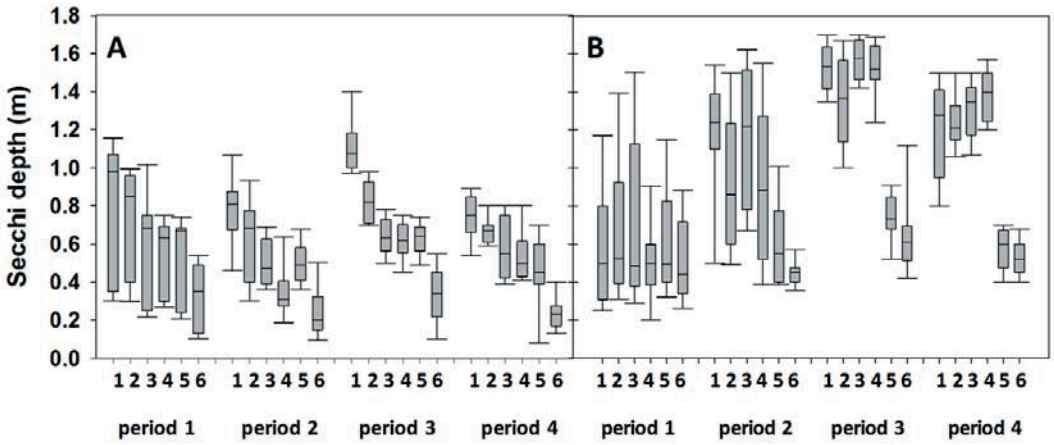


Fig. 3.6: Secchi depths (m) in compartments at pond Dongen (Panel A) and pond Eindhoven (Panel B). X-axes indicate different treatments during 4 periods. Treatments: DB (1) = dredging+biomanipulation, DPB (2) = dredging+PAC+biomanipulation, LB (3) = LMB+biomanipulation, LPB (4) = LMB+PAC+biomanipulation, B (5) = biomanipulation, C (6) = control. Periods Dongen: 1 = 9 September 2009 - 24 March 2010, 2 = 7 April 2010 - 22 September 2010, 3 = 4 October 2010 – 29 March 2011, 4 = 26 April 2011 – 30 August 2011. Periods Eindhoven: 1 = 31 August 2009 - 24 March 2010, 2 = 7 April 2010 - 22 September 2010, 3 = 4 October 2010 – 29 March 2011, 4 = 26 April 2011 – 30 August 2011. Boxes indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles.

Table 3.6: Median Secchi depth (m), median turbidity (NTU) and median concentrations total and cyanobacterial chlorophyll-*a*, total phosphorus (TP) and soluble reactive phosphorus (SRP) (µg L^{-1}) in compartments of pond Dongen and pond Eindhoven (31 August 2009-20 August 2011). Treatments: DB=dredging+biomanipulation, DPB=dredging+PAC+biomanipulation, LB=LMB+biomanipulation, LPB=LMB+PAC+biomanipulation, B= biomanipulation, C=control.

	Pond Dongen						Pond Eindhoven					
	compartments						compartments					
	DB	DPB	LB	LPB	B	C	DB	DPB	LB	LPB	B	C
Secchi depth (m)	0.85	0.70	0.58	0.50	0.55	0.25	1.20	1.00	1.29	1.06	0.58	0.50
Turbidity (NTU)	8.1	5.8	8.4	14.2	8.8	45.5	5.0	5.3	3.7	7.0	10.6	19.2
Total chlorophyll-*a* (µg L^{-1})	5.3	3.4	5.9	9.2	5.0	268.9	6.2	19.7	7.6	13.3	44.5	52.4
Cyanobacterial chlorophyll-*a* (µg L^{-1})	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.4	2.1	2.0	6.5
TP (µg L^{-1})	39	46	57	71	53	448	92	73	28	43	90	88
SRP (µg L^{-1})	14	12	15	14	20	47	29	22	17	13	19	19

Chlorophyll-*a*

At Dongen, a sharp drop in the chlorophyll-*a* concentrations occurred during construction in some of the compartments, which is reflected in large differences in total chlorophyll-*a* concentrations on 31 August 2009 ranging from 11.4 µg L^{-1} in compartment B to 493.3 µg L^{-1} in compartment C (Table 3.7). At Eindhoven the chlorophyll-*a* concentrations on 31 August 2009 ranged from 61.2 µg L^{-1} in compartment B to 103.6 µg L^{-1} in compartment DB

(Table 3.7) and were hardly influenced by the construction works. The compartments at Dongen were constructed with wooden sheet pilings, whereas at Eindhoven compartments were constructed with steel dam barriers. A controlled leaching experiment revealed no significant effect of leachates from the wooden sheet pilings on growth of phytoplankton (Supplementary information, Appendix 3.C).

Table 3.7: Chlorophyll-*a* concentrations (µg L^{-1}) in compartments at pond Dongen and pond Eindhoven on 31 August 2009. Treatments: DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation, B = biomanipulation, C = control.

	Pond Dongen						**Pond Eindhoven**					
	compartments						**compartments**					
	DB	DPB	LB	LPB	B	C	DB	DPB	LB	LPB	B	C
Chlorophyll-*a* (µg L^{-1})	198.1	86.5	61.5	13.7	11.4	493.3	103.6	90.8	69.1	71.0	61.2	98.7

At Dongen, during the course of the experiment, chlorophyll-*a* concentrations were higher in the control compartment (C) compared to the others (Fig. 3.7A), although during spring 2010 in compartments DB and LPB chlorophyll-*a* concentrations were also intermittently high, reaching peaks of 107 µg L^{-1} on 14 June 2010 (compartment DB) and 345 µg L^{-1} on 19 May 2010. Here illegal fish stocking in spring 2010 (*see* Results section, Fish communities) most probably contributed to the phenomena. After the removal of illegally stocked fish on 12 May and 4 June 2010 and the introduction of the intended fish stock largely in July and August 2010, the chlorophyll-*a* concentrations in all treated compartments were substantially lower than in the control (Fig. 3.7A periods 3 and 4, Table 3.6).

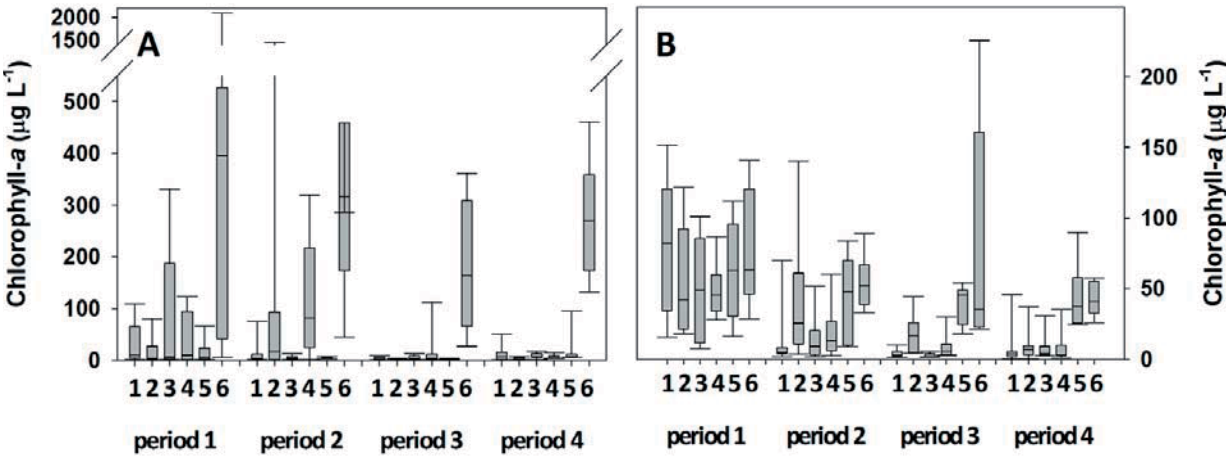


Fig. 3.7: Chlorophyll-*a* concentrations (µg L^{-1}) in compartments at pond Dongen (Panel A) and pond Eindhoven (Panel B). X-axes indicate different treatments during 4 periods. Treatments: DB (1) = dredging+biomanipulation, DPB (2) = dredging+PAC+biomanipulation, LB (3) = LMB+biomanipulation, LPB (4) = LMB+PAC+biomanipulation, B (5) = biomanipulation, C (6) = control. Periods Dongen: 1 = 9 September 2009 - 24 March 2010, 2 = 7 April 2010 - 22 September 2010, 3 = 4 October 2010 – 29 March 2011, 4 = 26 April 2011 – 30 August 2011. Periods Eindhoven: 1 = 31 August 2009 - 24 March 2010, 2 = 7 April 2010 - 22 September 2010, 3 = 4 October 2010 – 29 March 2011, 4 = 26 April 2011 – 30 August 2011. Boxes indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles.

Similar to Dongen, chlorophyll-*a* concentrations at Eindhoven remained highest in compartment C (Fig. 7B, Table 3.6). At Eindhoven, chlorophyll-*a* concentrations in all compartments declined in Autumn 2009, rose again in March 2010, after which during periods 2, 3 and 4 median chlorophyll-*a* concentrations in compartments B and C remained higher than in the others (Fig. 3.7B). This difference was particularly pronounced during the second year (2010/2011). At Eindhoven, the median chlorophyll-*a* concentrations over the entire experimental period were lower in the dredged compartments (DB, DPB) and LMB treated compartments (LB, LPB) than in the biomanipulated compartment (B), which was lower than in the control (C; Table 3.6).

Phosphorus

Both TP and SRP concentrations were highest in the control at Dongen (Fig. 3.8A; Table 3.6). A difference in TP concentrations was already present at the start of the experiment and remained throughout the experimental period, where in treatments TP concentrations further declined during autumn and winter 2010/2011, but gradually increased during summer 2011 (Fig. 3.8A). Considering that 90% of the water supply during summer consists of pumped groundwater containing substantial (81±72 µg L^{-1}) TP and that the residence time of the compartments during summer is 22-45 days implies that the observed increase of the TP concentrations was likely due to the addition of groundwater. The TP concentration was occasionally higher in the LPB compartment (Fig. 3.8A). TP remained considerably lower in treated compartments compared to the control (C), with the exception of compartment B at the end of the experiment (Fig. 3.8A). Here, complete die-off of the macrophytes caused

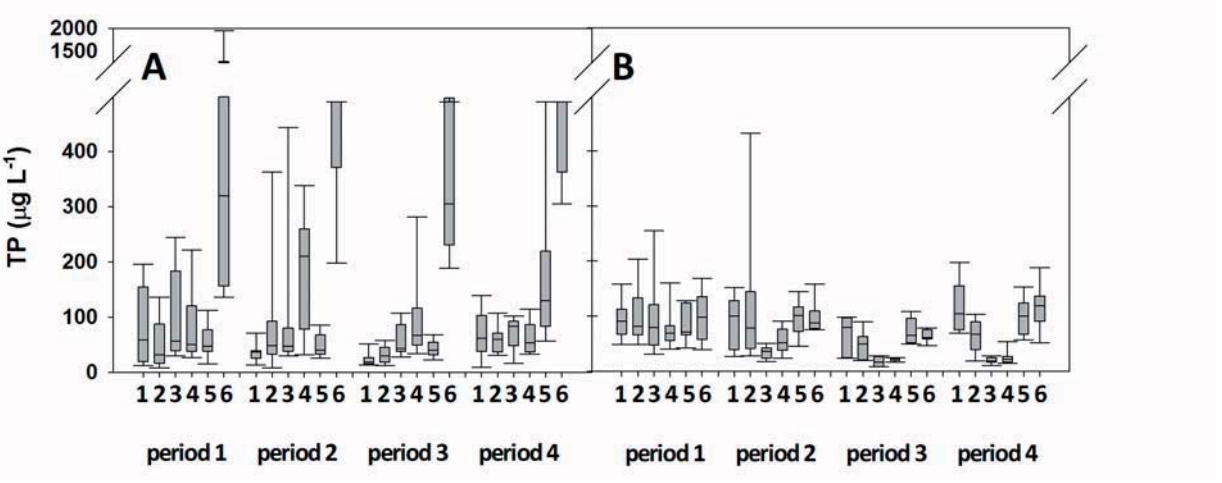


Fig. 3.8: Total phosphorus (TP) concentrations (µg L^{-1}) in compartments at pond Dongen (Panel A) and pond Eindhoven (Panel B). X-axes indicate different treatments during 4 periods. Treatments: DB (1) = dredging+biomanipulation, DPB (2) = dredging+PAC+biomanipulation, LB (3) = LMB+biomanipulation, LPB (4) = LMB+PAC+biomanipulation, B (5) = biomanipulation, C (6) = control. Periods Dongen: 1 = 9 September 2009 - 24 March 2010, 2 = 7 April 2010 - 22 September 2010, 3 = 4 October 2010 – 29 March 2011, 4 = 26 April 2011 – 30 August 2011. Periods Eindhoven: 1 = 31 August 2009 - 24 March 2010, 2 = 7 April 2010 - 22 September 2010, 3 = 4 October 2010 – 29 March 2011, 4 = 26 April 2011 – 30 August 2011. Boxes indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles.

anoxia (*see* Results section, Macrophytes), not only leading to a fish kill (*see* Results section, Fish communities) and malodor, but also to an increase in water column TP concentration of which 66% was SRP (i.e. 520 µg L^{-1}).

At Eindhoven, only the LB and LPB compartments had lower TP concentrations (Fig. 3.8B). These compartments also had the lowest SRP concentrations (Table 3.6). The median TP concentrations in the LB and LPB treated compartments were 41% to 70% lower than in the other treatments and in the control, respectively (Table 3.6).

Water quality classification

In both 2010 and 2011, mean summer (June 1st – September 30th) chlorophyll-*a* and TP concentrations (Fig. 3.9) in the control compartment at Dongen indicated bad water quality

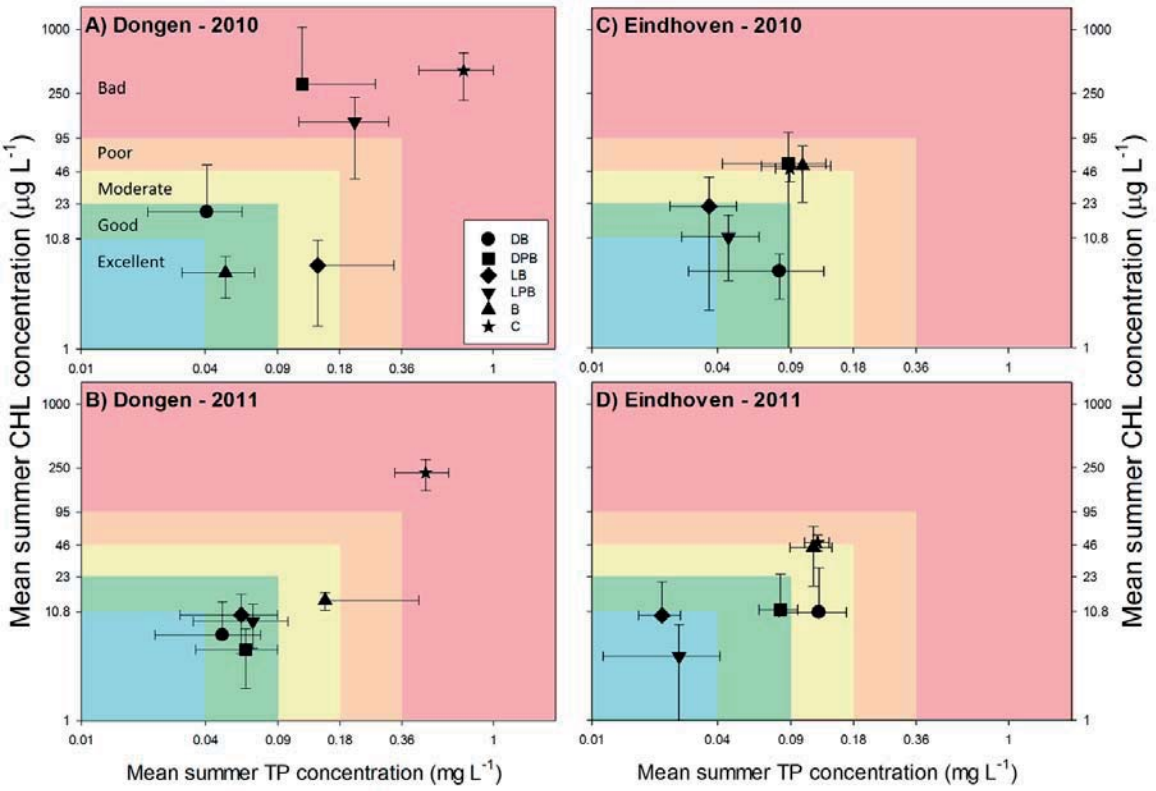


Fig. 3.9: Mean summer (June 1st – September 30th) chlorophyll-*a* (CHL) concentrations versus mean summer total phosphorus (TP) concentrations for the different treatments in compartments at pond Dongen (Panel A: 2010; Panel B: 2011) and pond Eindhoven (Panel C: 2010; Panel D: 2011). Error bars indicate 1 SD. The differently coloured planes indicate the corresponding WFD classifications for water type M11 (blue = excellent status, green = good, yellow = moderate, orange = poor/insufficient and red = bad status). Treatments: DB = dredging+biomanipulation, DPB = dredging+PAC+biomanipulation, LB = LMB+biomanipulation, LPB = LMB+PAC+biomanipulation , B = Biomanipulation, C = Control.

as classified by the WFD classification (Van der Molen et al., 2013). In 2010, the DPB and LPB compartments had bad quality category (Fig. 3.9A), however, both improved to a good status in 2011 where they were grouped with the DB and the LB treated compartments (Fig. 3.9B). Compartment B in Dongen that could be classified as good in 2010 (Fig. 3.9A), degraded to a moderate quality in 2011, mostly because of the increased TP concentration (Fig. 3.8A; Fig.

3.9B). At Eindhoven, the water quality in compartments B and C could be classified as poor to moderate in both summers. Both dredged compartments (DB, DPB) at Eindhoven were moderate to good in 2011, while the status of both LMB treated compartments (LB, LPB) improved to excellent that year (Fig. 3.9C, D).

Sediment P-release

In all cores SRP was released from the sediment to the overlying water. At Dongen, the release in the cores from compartment C was higher than in the other compartments (Fig. 3.10). At Eindhoven SRP-release in the cores from compartment C was similar to, or slightly lower than the DB, DPB and B compartments (Fig. 3.10). The SRP-release from the cores from the control compartment (C) at Eindhoven was lower than those at Dongen (Fig. 3.10). The SRP-release in the cores from Dongen differed among treatments (H_5 = 11.6; P = 0.042), with a greater release from the control core than from the LPB treatment (Fig. 3.10). The SRP-release in the cores from Eindhoven differed among treatments ($F_{5,17}$ = 5.10; P = 0.010) and the release from the cores from compartment DB was greater than from the LB and LPB compartments.

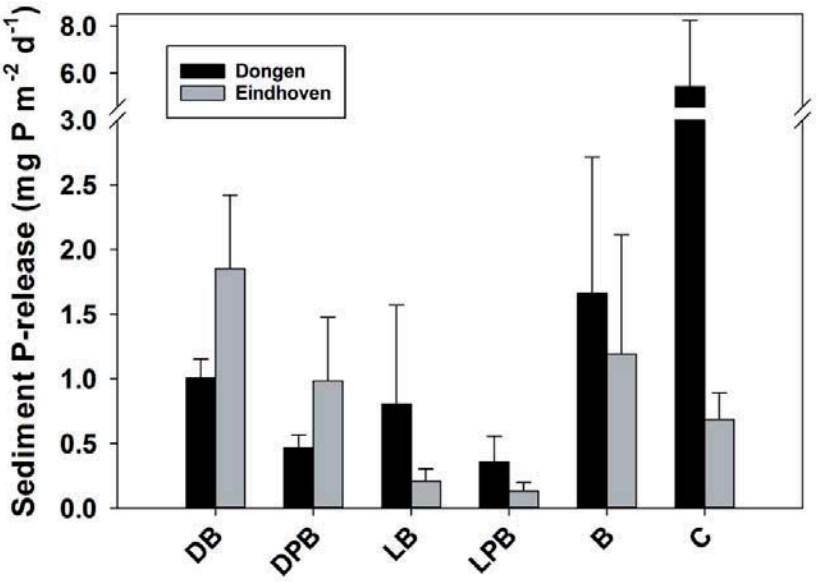


Fig. 3.10: Sediment P-release (mg P m^{-2} d^{-1}) in cores from compartments at pond Dongen (black bars) and pond Eindhoven (grey bars). Treatments: DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation, B = biomanipulation, C = control. Error bars indicate 1 SD (n = 3).

Macrophytes

After their introduction, *Elodea* and *Myriophyllum* established in all compartments except the controls, but *Elodea* dominated all macrophyte communities (Fig. 3.11). At Dongen, on 8 October 2010 after one season, coverage was high in the DB (85%), DPB (75%), LB (100%) and B (86%) compartments, but coverage was lower in the LPB (16%) compartment and absent in the control (Fig. 3.11). At Eindhoven, on 8 October 2010 macrophyte coverage was 99% in the DB compartment, 81% in DPB, 91% in LB and 45% in LPB, whereas coverage was only 11% in the B compartment and 0% in C. On 23 August 2011, after the second season,

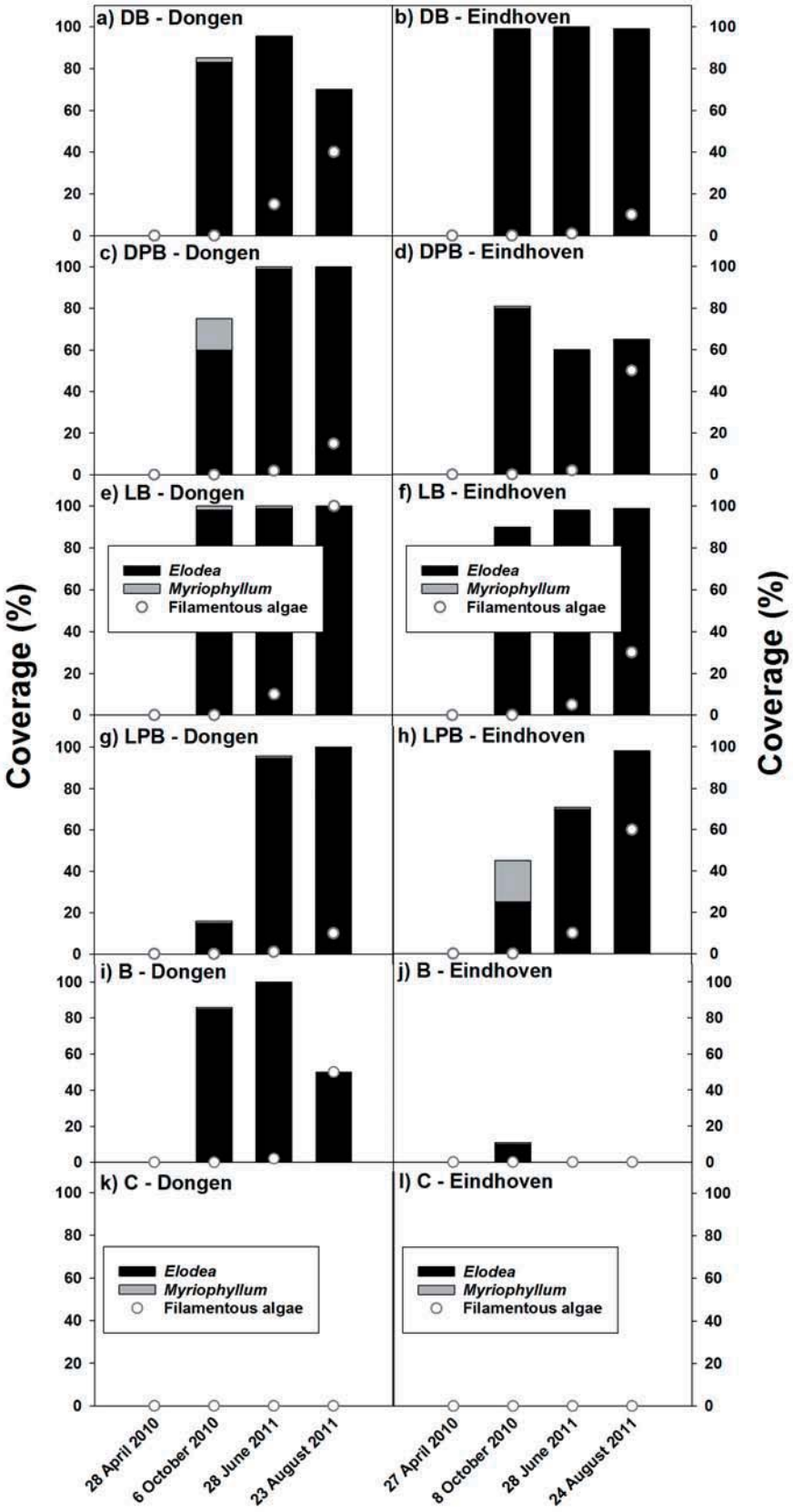


Fig. 3.11: Coverage (%) of macrophytes and filamentous macro algae in compartments at pond Dongen and pond Eindhoven on 4 dates. Treatments: DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation, B = biomanipulation, C = control.

the macrophyte coverage at Dongen was 70% in compartment DB, 100% in compartments DPB, LB and LPB, 50% in B and 0% in C. In July 2011 the macrophytes started to die in compartment B at Dongen and in August a complete die-off and disappearance of the macrophytes was observed in this compartment resulting in an O_2 concentration in the B compartment of 0.7 mg L^{-1} on 30 August 2010. At Eindhoven on 24 August 2011 the macrophyte coverage was 99% in compartment DB, 65% in DPB, 99% in LB, 98% in LPB and 0% in compartments B and C (Fig. 3.11). Compared to the coverage in the B compartment at Dongen of 86% after one season on 8 October 2010, coverage in the B compartment at Eindhoven was only 11%. At the end of the second season, the macrophytes had disappeared from the B compartments at both locations despite a 100% coverage at Dongen at the start of the second season on 28 June 2011. In the second season at Dongen and Eindhoven, filamentous macro algae occurred in the treatments where macrophytes had established, but not in compartments C (Dongen and Eindhoven) and compartment B (Eindhoven; Fig. 3.11). At the end of the experiment, two to five times more plant biomass was harvested at Dongen than in the comparable treatments at Eindhoven (Table 3.8).

Table 3.8: Weight (fresh weight kg m^{-2}) of harvested plant material from the compartments at pond Dongen and pond Eindhoven in September 2011. Treatments: DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation, B = biomanipulation, C = control.

	Fresh weight of harvested plant material (kg m^{-2})	
Treatment	Dongen	Eindhoven
DB	4.80	2.15
DPB	6.27	2.30
LB	6.47	1.25
LPB	8.47	3.15
B	0.03	0
C	0	0

Fish communities

During the construction of the compartments, all fish were removed in 2009 by intensive and repeated fishery. In the compartments at Dongen, controlled restocking started in July 2010. However, in May 2010 many large fish (carp and bream) were observed in the compartments, probably as the result of uncontrolled restocking that was supported by visual observations by local residents and by the professional fishing company Kalkman. The uncontrolled stocked fish were removed on May 12 and a second removal of uncontrolled stocked fish was undertaken on June 4 2010 (Kalkman, 2010a and 2010b). A total of 44 carp (20-40 cm) were removed from the DB compartment, 6 from the DPB compartment, 13 from the LB compartment, 6 from the LPB compartment, 4 from the B and 9 from the C compartment, while from the LPB compartment 5 bream (*Abramis brama*; 44-47 cm)

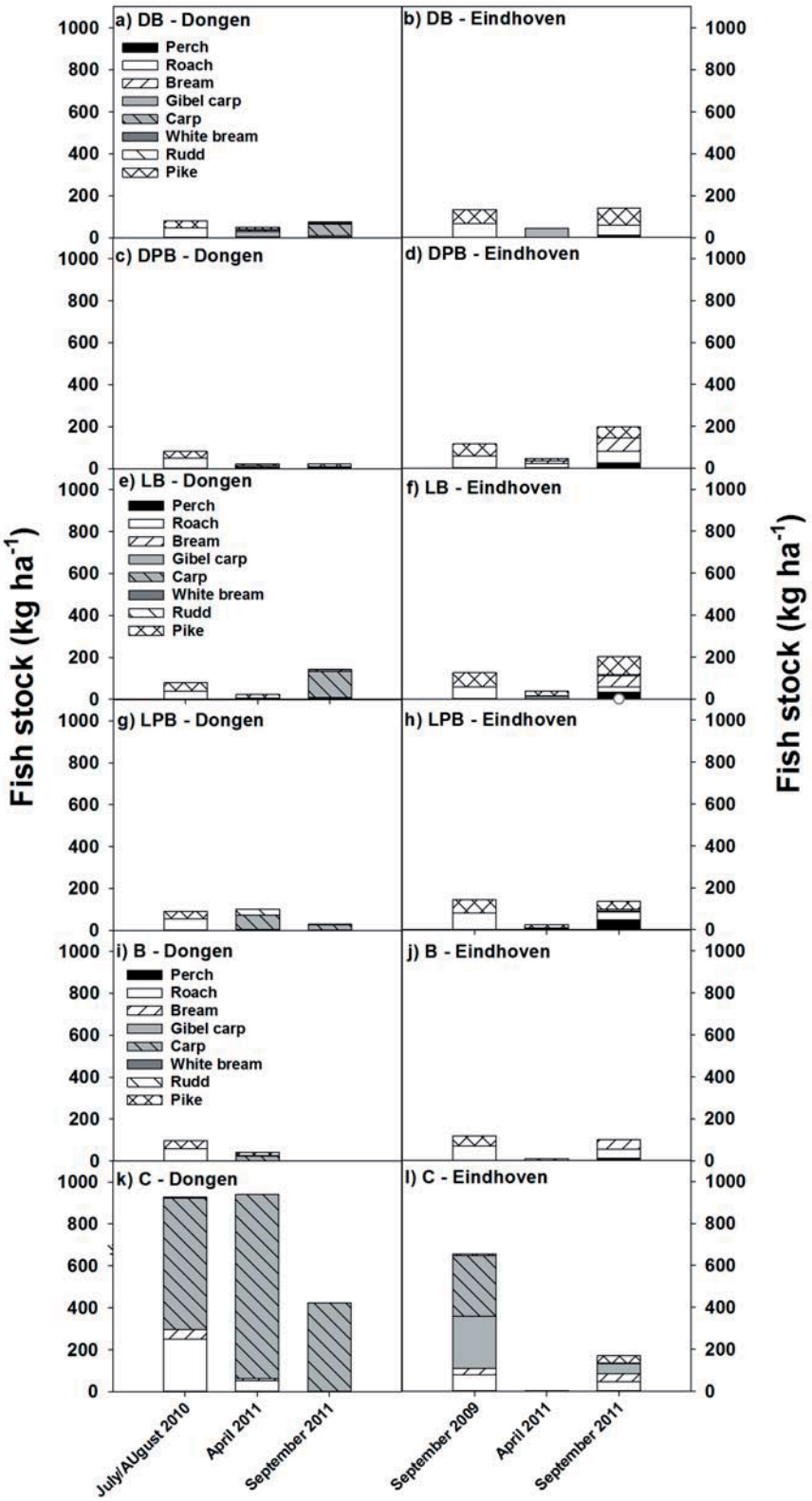


Fig. 3.12: Fish stock (fresh weight kg ha^{-1}) in compartments at pond Dongen and pond Eindhoven on three dates. Treatments: DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation, B = biomanipulation, C = control.

were also removed. Eight months after restocking with roach and pike the fish biomass in the treatments had declined to 30-60% of the stocked biomass (Fig. 3.12a, c, e, i) with

the exception of the LPB compartment, where the biomass had increased by 11% which was mostly due to again illegal carp stocking that constituted 70% of the total biomass (Fig. 3.12g). The fish biomass in the control compartment was still similar to the restocked biomass after eight months, but declined towards the end of the experiment to 50% of the restocked biomass (Fig. 3.12k) and being entirely comprised of carp. In the DB and the LB compartments fish biomass increased over summer 2011 (Fig. 3.12a, e), remained similar in the DPB compartment (Fig. 3.12c) and was reduced in the LPB compartment (Fig. 3.12g). In the B compartment, all fish died after the plant die-off which was accompanied by anoxia. The fish communities in the treatments showed a shift in biomass from the stocked roach and pike towards illegally introduced carp. For instance, in the DB, LB and LPB compartments at the end of the experiment 75, 86 and 89% of the fish biomass was carp (Fig. 3.12a, e, g). The abundant vegetation and wooden debris in the compartments at Eindhoven hampered fish sampling, which is reflected in the lower fish biomass caught in April 2011 as compared to the stocked biomass in September 2009 (Fig. 3.12b, d, f, h, j, l). At the end of the experiment, fish biomass in the control had declined to 25% of the stocked biomass and none of the stocked carp (50-54 cm) was retrieved (Fig. 3.12l). In the treatment compartments, fish biomass decreased by 15% in the B compartment (Fig. 3.12j), remained similar in the DB and LPB compartments (Fig. 3.12b, h) and had increased by 60% in the LB (Fig. 3.12f) compartment and by 70% in the DPB compartment (Fig. 3.12d). In addition to stocked roach and pike, 2 to 10 bream (10-38 cm) and 44 to 201 perch (*Perca fluviatilis*; 8-15 cm) were caught in the different compartments (Fig. 3.12b, d, f, h, j, l).

Whole-pond rehabilitation

Water clarity

The results of the compartment experiment pointed out that LMB could be an attractive alternative to dredging at both sites. Nevertheless, at Dongen as well as at Eindhoven dredging was chosen as the measure to reduce the sediment P-release. The reason for this was that the water managers at both sites wanted to increase water depth. Both sites had never been dredged before and a considerable amount of mud had accumulated (Fig. 3.3) reducing water depth substantially.

At Dongen, the Secchi depth increased (Fig. 3.13A), from a mean of 0.28 m before rehabilitation measures to 0.57 m after rehabilitation. Bottom sight was recorded in 13% of the measurements before rehabilitation, which increased to 54% after rehabilitation. Secchi depths in the summer period (April – September) differed among years, where a Tukey post hoc test showed that Secchi depths in 2009 and 2011 were significantly lower than those in 2013 and 2014.The increased water clarity at pond Dongen is also reflected in the turbidity that was reduced (Fig. 3.13C), from a mean of 61 NTU before to 8 NTU after the pond rehabilitation. Summer period turbidity before rehabilitation was significantly higher than after rehabilitation ($F_{5, 48}$ = 26.9, $P < 0.001$; Tukey $P < 0.05$).

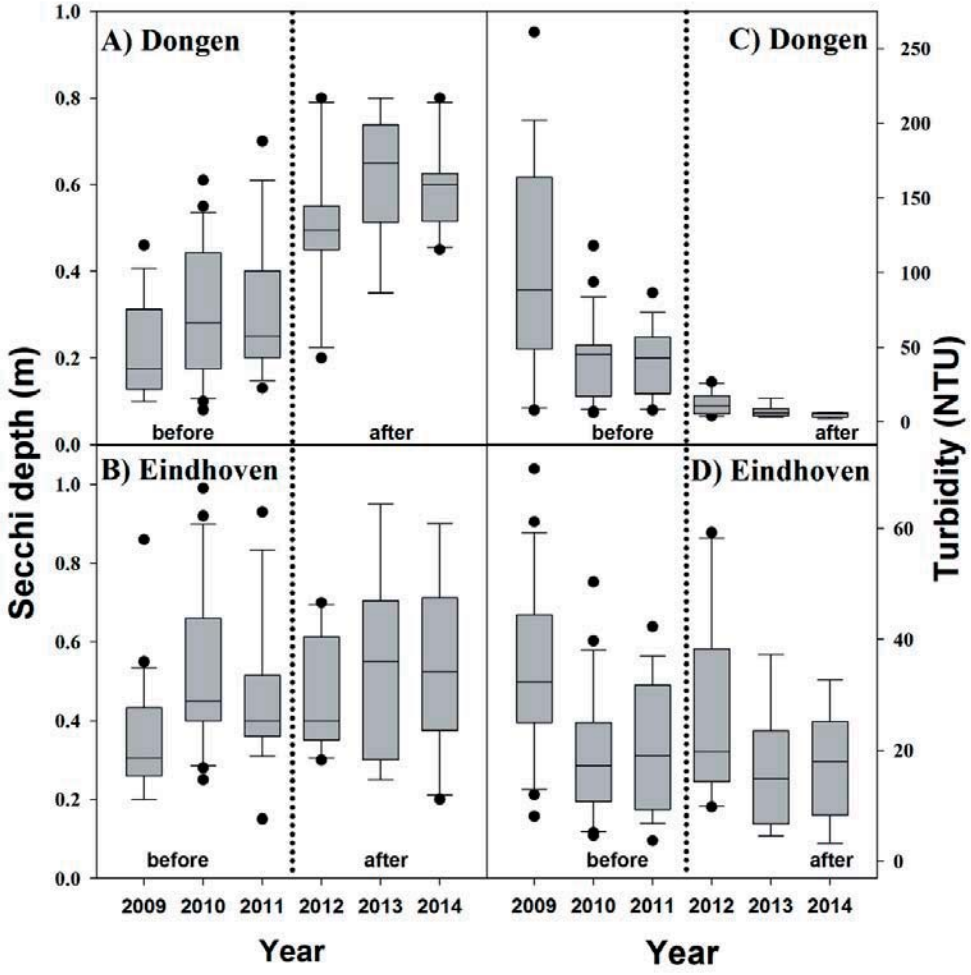


Fig. 3.13: Secchi depth (m) at pond Dongen (panel A) and pond Eindhoven (panel B) over the period 2009 – 2014 before and after pond rehabilitation (indicated by vertical dotted lines). Likewise, turbidity (NTU) at pond Dongen (panel C) and pond Eindhoven (panel D).

At Eindhoven, the mean Secchi depth differed significantly between various years ($F_{5, 54} = 1.54$, $P < 0.001$), but only marginally increased (Fig. 3.13B) from a mean of 0.45 m before rehabilitation to 0.50 m after. Likewise, turbidity was only slightly reduced after rehabilitation (Fig. 3.13D), from a mean of 25 NTU before to 20 NTU after. Secchi depth and turbidity only in 2009 differed significantly (Tukey $P < 0.05$) from the other years (2010-2014).

Chlorophyll-*a*

Cyanobacterial chlorophyll-*a* concentrations dropped in pond Dongen from a mean of 200 µg L^{-1} in 2009 to 14 and 15 µg L^{-1} in the control compartment in 2010 and 2011, respectively. After dismantling of the compartments and the execution of the rehabilitation measures (Table 3.4), the cyanobacterial chlorophyll-*a* concentrations further declined (Fig. 3.14A) to on average 11 µg l^{-1} in 2012 and < 1 µg L^{-1} in 2013 and 2014. The higher cyanobacterial chlorophyll-*a* concentration at Dongen in 2012 compared to 2013 and 2014 was caused by one short explosion of cyanobacteria in beginning August 2012 reaching a value of 113 µg L^{-1} (as indicated by the outlier in Fig. 3.14A). Total chlorophyll-*a* concentrations at pond Dongen declined (Fig. 3.14C) from a mean of 500 µg L^{-1} in 2009 to 286 and 200 µg L^{-1} in the control

compartment in 2010 and 2011, respectively. However, total chlorophyll-*a* concentrations dropped further to a mean of 16 µg L^{-1} in the three years after the rehabilitation measures had been done, which difference before and after rehabilitation is significant (t = 3.50; df = 4; P = 0.025).

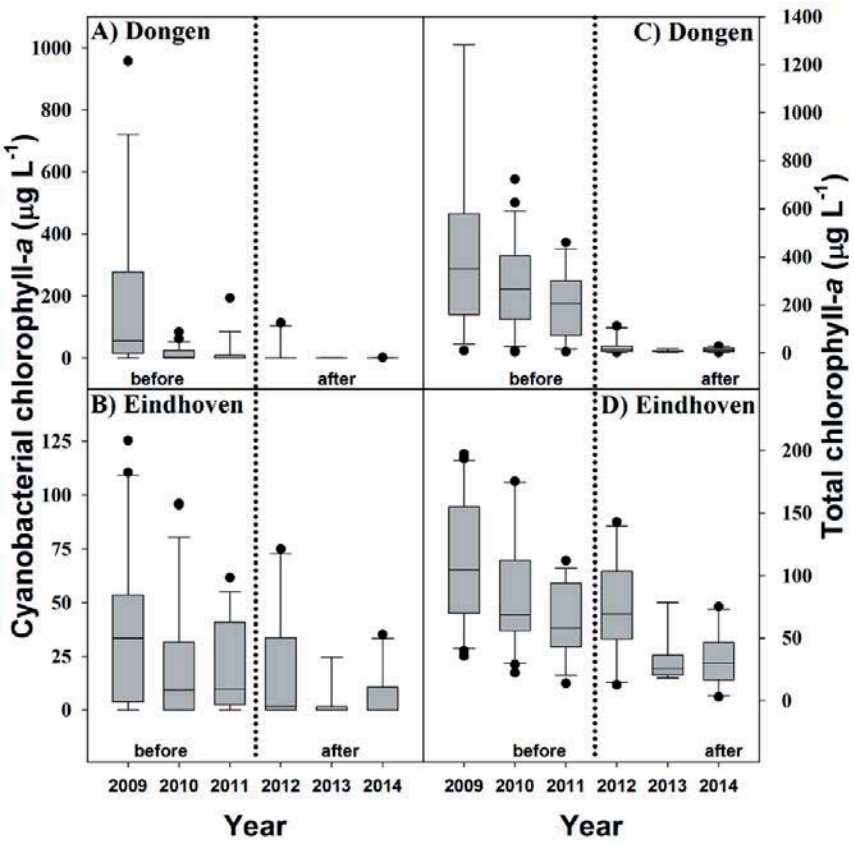


Fig. 3.14: Cyanobacterial chlorophyll-*a* concentrations (µg L^{-1}) at pond Dongen (panel A) and pond Eindhoven (panel B) over the period 2009 – 2014 before and after pond rehabilitation (indicated by vertical dotted lines). Likewise, the total chlorophyll-*a* concentrations (µg L^{-1}) at pond Dongen (panel C) and Eindhoven (panel D).

At pond Eindhoven the mean cyanobacterial chlorophyll-*a* concentration in the three years before rehabilitation measures were done was 26 µg L^{-1}, in the three years after it was on average 9 µg L^{-1} (Fig. 3.14C) and no significant difference could be shown before and after rehabilitation (P = 0.100). The total chlorophyll-*a* concentration at Eindhoven in the three years before rehabilitation was on average 90 µg L^{-1}, while in the three years after rehabilitation it was on average 46 µg L^{-1} and no significant difference between total chlorophyll-*a* concentration before and after could be shown (t = 2.22; df = 4; P = 0.091; Fig. 3.14C).

Phosphorus

At pond Dongen, the rehabilitation measures caused a significant decrease of the mean TP concentration from 512 µg L^{-1} in the three years before to 34 µg L^{-1} in the three years after implementation of the measures (P < 0.001; Fig. 3.15A). At Eindhoven, the measures caused hardly a decrease in TP concentration (Fig. 3.15B). The mean TP concentration was 95 µg L^{-1} before performing rehabilitation and 83 µg L^{-1} in the three years after.

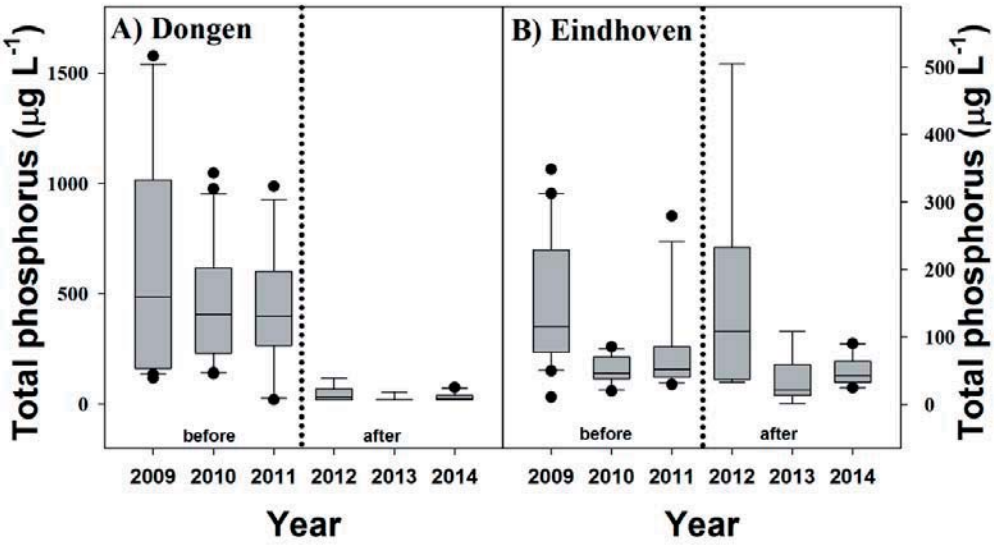


Fig. 3.15: Total phosphorus concentrations (µg L^{-1}) at pond Dongen (panel A) and pond Eindhoven (panel B) over the period 2009 – 2014 before and after pond rehabilitation (indicated by vertical dotted lines).

Discussion

Compartment experiment and laboratory assay

The compartment experiment undertaken in this study demonstrates that a combination of measures reducing internal P-loading with biomanipulation can improve water quality, and realize and maintain a clear water state in urban ponds without high phytoplankton biomass (Fig. 3.6, Fig. 3.7, Table 3.6). Biomanipulation as a single measure was shown only to be effective for a limited period or was not effective at all. The external P-loads differed between the studied ponds (Table 3.2). At Dongen, surface runoff, the feeding of waterfowl and pumped groundwater were major contributors (Table 3.2). At Eindhoven, discharge of rainwater from a separated sewer system was the most important contributor (Table 3.2), as nutrients collected during the runoff from the impervious surfaces polluted the presumed clean rainwater. During the experiment, the external P-loads were reduced to the critical range (Table 3.2). In the control compartments, the turbid state persisted, necessitating implementation of in situ treatments to improve water quality. In situ treatments targeting the internal P-release, if effective, become important for the restoration and maintenance of an improved water quality.

In The Netherlands, restoration measures complemented by biomanipulation on fish have generally enhanced lake recovery (Gulati & Van Donk, 2002). For instance, without a reduction in fish biomass, dredging or iron chloride addition had little effect on water quality (Van Donk et al., 1994). The initial fish stocks of 1212 kg ha^{-1} at Dongen and 927 kg ha^{-1} at Eindhoven, predominantly comprised of carp (~50 - > 80%; Table 3.1), kept the waters in a turbid state via sediment resuspension, thereby preventing submerged macrophyte establishment (Cline et al., 1994; Meijer et al., 1999; Zambrano & Hinojosa, 1999; Persson & Svensson, 2006; Roozen et al., 2007). Positive correlations between carp biomass and

turbidity, chlorophyll-*a* and TP concentrations exist for other systems (Chumchal et al., 2005), and demonstrate the importance of carp reduction prior to implementing other management initiatives. In addition to carp control, biomanipulation comprising the reduction of other benthivorous and zooplanktivorous fish species, such as bream and roach, often supplemented by piscivore stocking can also yield benefits in water quality (Søndergaard et al., 2007).

Biomanipulation alone at Dongen only had a temporary positive effect on water quality while at Eindhoven no improvement was observed (Fig. 3.6, Fig. 3.7) indicating a retarding effect of continued sediment P-release on recovery. Possible causes for the plant die-off in the B compartment at Dongen are impact of fish, and a negative effect of periphyton on macrophyte growth by covering and shading the plants (Scheffer, 2004). In addition, the abundant nutrient and organic matter remaining in the sediment may have led to increased phytotoxic hydrogen sulfide production (Lamers et al., 2013). The gaseous H_2S usually is by far the most abundant S species (60-95%) and prevails over ionic HS^- and S^{2-} in freshwater systems (Lamers et al., 2013). We found indirect evidence for the formation of gaseous H_2S as the concentration of S declined from 32 mg L^{-1} end of June 2011 towards 10 mg L^{-1} end of August 2011, while in all other compartments S remained rather stable between 30 and 40 mg L^{-1} (data not shown). Differences in water clarity, phytoplankton biomass, TP and macrophytes between the B compartments at Dongen and Eindhoven reflect that the B compartment at Dongen was only restocked with fish in summer 2010 and had remained without fish for almost a year. In addition, the shallower water of the B compartment at Dongen (~0.7 m) compared to Eindhoven (~1.5 m) favored submerged macrophytes (Chambers & Kalff, 1985). A combination of biomanipulation with measures that also reduce internal P-loading may be the most beneficial to water quality.

However, uncontrolled fish stocking is of concern in urban ponds. It was observed, not only during spring 2010 at Dongen, but also at the end of the field experiment when many carp and bream were retrieved that had not been restocked in the compartments at either Dongen and Eindhoven, except in the controls. In addition, goldfish or gibel carp (*Carassius gibelio*) were caught at Dongen in compartments DB and LB and at Eindhoven in compartment DB despite having not been restocked (Fig. 3.12). Also rudd (*Scardinius erythrophthalmus*) were caught at Dongen in compartments DB, DPB, LB, LPB and B and at Eindhoven in compartments LB, LPB and C despite having not been restocked. Although fish removal is suggested as a method to improve water quality of eutrophic urban ponds (De Backer et al., 2012; Teissier et al., 2012), longevity can be undermined by fish recolonization. Likewise, in shallow lakes fish removal can improve water quality (e.g., Meijer et al., 1999), although for a long-term effect the removal should be repeated (Søndergaard et al., 2007, 2008).

Despite the TP concentration in compartment B at Eindhoven being below 300 µg L^{-1} and even below 100 µg L^{-1}, thresholds at which biomanipulation can be effective in eutrophic urban ponds (De Backer et al., 2012) and shallow lakes (Hansson et al., 1998), the

biomanipulation treatment at both experimental sites did not yield improved water quality. In contrast, in an experiment with 25 x 25 m compartments in Lake Breukeleveen (The Netherlands) reduction of the fish stock to 20 kg ha^{-1} improved water quality and gave rise to abundant submerged vegetation, even while fish stock in the lake maintained turbidity (Van Donk et al., 1994). In this study, however, the fish stock reduction in compartment B at Eindhoven to 118 kg ha^{-1} and a further decline to 100 kg ha^{-1} at the end of the field experiment was insufficient to induce macrophyte growth, although at such fish biomass, submerged macrophytes may flourish (Van de Bund & Van Donk, 2002). The findings of Van de Bund & Van Donk (2002) indicate that fish stock densities as low as 118 kg ha^{-1} may be sufficient to improve water quality. It seems unlikely, however, that a lower fish stock can be realized and maintained in urban ponds, where uncontrolled fish recolonization may occur. This concern necessitates thorough communication with anglers, local residents and other stakeholders when restoring eutrophied urban ponds.

The high coverage of macrophytes observed in the B compartment at Dongen was expected to stabilize the clear water state in the compartment, although long-term effects of macrophyte reestablishment are less clear (De Backer et al., 2012). The submerged vegetation collapsed in August 2011 and this resulted in anoxic conditions, a fish kill and malodor. At this time, SRP and TP concentrations rapidly increased due to anoxic conditions promoting redox-sensitive P-release from the sediment (Smolders et al., 2006) and from the decomposition of macrophyte biomass (Barko & Smart, 1980). Because the compartments at Dongen were dismantled shortly after the collapse of the vegetation in the B compartment, no response of the high nutrient concentrations on algal growth could be observed.

In both ponds, the four compartments subject to biomanipulation and subsequently dredged or treated with LMB (with or without PAC) showed increased water clarity and less phytoplankton biomass than the controls. Dredging of P enriched sediment can be effective in eutrophication control (Cooke et al., 2005; Peterson, 1982; Van Wichelen et al., 2007). However, this will be the case when the majority of the stored P is being removed, not when freshly exposed sediment may release P to the overlying water (Geurts et al., 2010). For instance, at Eindhoven, the dredged compartments (DB, DPB) showed similar Secchi depths and macrophyte coverages as the LMB treated compartments (LB, LPB), but the water quality classification using TP and chlorophyll-*a* indicated that the dredged compartments ranked only moderate to good, whereas the LMB treated compartments attained an excellent water quality rating (Fig. 3.9). This reflects the substantially reduced TP concentration in the LMB treated compartments (LB, LPB) and the sustained SRP-release from the sediments in the dredged compartments (Fig. 3.10). Pond Eindhoven was created in the 1990's on former fertilized agricultural lands. Exposing such soils to water can lead to a substantial internal P-loading (Pant & Reddy, 2003) and consequently high phytoplankton biomass (Papadimitriou et al., 2013).

Dredging is considered a standard management response in many Dutch urban ponds, but it is expensive (Lürling & Faassen, 2012) which supports the need for other more

cost-effective measures. This is illustrated by the costs for dredging in the compartment study, which are ~3 times higher than the costs of LMB application (Table 3.9). The primary function of LMB is to intercept and absorb P-released from the sediment, and remove SRP from the overlying water as was shown in the laboratory assay (Fig. 3.5A; Douglas, 2002; Robb et al., 2003; Douglas et al., 2008; Egemose et al., 2010; Gibbs et al., 2011; Van Oosterhout & Lürling, 2013). At Dongen SRP and TP reductions in LMB treatments (LB, LPB) were comparable to reductions in treatments with dredging (DB, DPB). At Eindhoven, both LMB treatments showed a larger decrease in SRP and TP than dredging (Table 3.6). The LPB compartment at Eindhoven resulted in a 65% reduction in total chlorophyll-*a* concentration, which is comparable to the 58% reduction in a flocculation and sinking assay that was conducted with water from compartments 2, 3 and 4 from Eindhoven and addition of LMB and PAC (Supplementary Information, Appendix 3.D). Hence, geo-engineering using LMB constitutes a potentially viable alternative to dredging for water quality objectives in urban ponds when combined with biomanipulation, provided the external P-load is low.

Table 3.9: Costs (€ m^{-2}) of measures in compartments at pond Dongen and pond Eindhoven.

Measure	Dongen	Eindhoven
Dredging	11.79	9.03
LMB	3.37	3.37
PAC	0.76	0.76
Biomanipulation (fish stock + macrophytes)	1.54	1.47

The present compartment study, lasting two years, showed that LMB was able to reduce TP. Since macrophyte coverage, Secchi depths, turbidity and phytoplankton biomass were similar to the dredged compartments, the decline in TP most likely can be attributed to the P binding capacity of the LMB. Also in controlled laboratory experiments, a gradual decline of the P concentration was observed instead of a rapid depletion of most P from the water, which was explained by slow diffusion of phosphates to active sites (Lürling et al., 2014b; Fig. 3.5A). Consequently, the longer term efficacy of LMB may be larger than observed in short-term experiments lasting hours (Fig. 3.5A) to weeks (Lürling et al., 2014b), as evident from the results of the LMB treated compartments (LB, LPB) at Eindhoven. In situ, lanthanum will not only bind with phosphates, but also with other oxyanions, in particular carbonate (CO_3^{2-}; Johannesson & Lyons, 1994), which might further delay the response. The efficacy of the treatments over the long-term (> two years) was not a part of the compartment experiment. To gain insight in the efficacy and cost effectiveness of treatments in the long-term, it is important for water management that future applications are accompanied by monitoring, by evaluation of results and by dissemination of the findings. The flocculation of phytoplankton observed in the in situ treatment was comparable to the flocculation in a

laboratory assay (Supplementary information, Appendix 3.D, Fig. 3.D.1). The addition of PAC alone flocculated the phytoplankton. It is likely that flocculation of cyanobacteria and other suspended particles with clay substantially reduces cell buoyancy. Importantly, the reduction of turbidity and improved light climate achieved by PAC addition provide a good basis for the growth of submerged macrophytes. The application of PAC to the compartments was intended when they had a high density of suspended solids, i.e. directly after dredging or application of the LMB. However, due to a logistical delay, dredging was done several days before the application of PAC and inadvertently applied to the compartments before the LMB. Hence, the experiment did not reveal the full extent of the potential water clarifying properties of PAC in these compartments. As PAC in the compartments was applied at the end of the growing season on 2 and 3 September 2009, the instantaneous water clearing effect of the flocculant was not a necessity to promote macrophyte establishment and growth. The combination of PAC and LMB was applied to Lake Rauwbraken in April 2008 (Lürling & Van Oosterhout, 2013) and $FeCl_3$ as flocculant and LMB to Lake De Kuil in May 2009 (Chapter 5). Both lakes showed an effective suppression of developing cyanobacterial blooms, increased transparency and expansion of macrophytes. In the 24 h laboratory assay, the combination of LMB and PAC reduced the SRP instantly compared to the sole LMB application (Fig. 3.5). In the long-term compartment experiment however, the additional advantage of PAC to bind SRP (Lopata & Gawronska, 2008) was not detected (Table 3.6). On this basis, the combination of a flocculant with a solid phase P sorbent such as PAC with LMB, is advisable only early in the growing season to open a window of opportunity for submerged macrophyte establishment, or in ponds with perennial blooms; otherwise a solid phase P sorbent may suffice.

Despite the uncontrolled fish stocking and controlled pumped groundwater, no other known modifications than the treatments were imposed onto the compartments at Dongen and Eindhoven. Although unknown factors might potentially have influenced the results, all dredged and LMB treated compartments (DB, DPB, LB, LPB) in both ponds resulted in increased water column clarity and the establishment of submerged macrophytes. However, the differences between the two locations exemplify the importance of site-specific analyses to assist in choosing the most promising management measures. The external P-loads prior to the start of the experiment were by themselves sufficient to maintain turbid waters, which was enhanced by internal P-loading. This stresses the need not only to decrease the internal load, but also to minimize external P inputs as part of the management approach. At Dongen, the external P input can be minimized by reducing pumped groundwater, restoring of banks and reducing runoff, reducing of the number of water birds and by prohibiting the feeding of birds and fish. At Eindhoven, the external P input, especially from the separated sewer system and runoff, needs to be tackled to bring the external loading in the range where alternative stable states exist and additional in situ measures can become effective. After the reduction of the external P inputs, LMB can be an attractive alternative to dredging at both sites.

Whole-pond rehabilitation

The water and P-balances that were made for ponds Dongen and Eindhoven clearly revealed different major water sources: pumped groundwater at Dongen and rainwater discharge at Eindhoven. SRP and TP concentrations in the latter were comparable to what has been measured in stormwater sewer outfalls elsewhere (Bannerman et al., 1993) and the rainwater discharge turned out by far the main source of P for pond Eindhoven (Table 3.2). At Dongen, the total of the external P-sources yielded a P-load that was less than half of the sediment P-release. At Eindhoven, the sediment P-release was about one third of the sediment P-release at Dongen and was not a dominant part of the total P input.

Considering the outcome of the diagnostics, based on the P-loads and critical P-loads the implemented measures for pond Dongen (Table 3.4) were plausible. The measures intended to lower the external P-load to the range where alternative stable states may exist, to reduce the sediment P-release and to implement biomanipulation intended for further reduction of the in-pond P concentration (Benndorf, 1987) and shift the water to the clear water state (Janse et al., 2008).

The high external P-loading at pond Eindhoven was virtually unaffected by the rehabilitation measures, resulting in a P-load which was still about ten times the critical P-load after the mitigating measures had been executed. Despite recommendations based on the diagnostics to tackle the external P-sources first, the water manager and municipality kept up to their previous agreement to focus on in-pond measures only. After the measures were taken, water quality strongly improved at pond Dongen for at least three years. The improvements are in line with the expectations based on the system analysis and the findings in compartment DB. As the external P-loads were reduced, removal of sediment (Fig. 3.3) was expected to reduce the sediment P-release and in-pond measures were expected to further promote clear water and submerged macrophytes. The water clarity at pond Dongen improved considerably after rehabilitation, which seemed due to less nutrients available for phytoplankton and less resuspension of the sediment by fish. Removal of bottom resuspending fish such as carp may also have led to consolidation of the sediment (Lin & Wu, 2013). The incidence of bottom sight strongly increased at Dongen, which may have further benefitted macrophytes as a strong positive feedback exists between water clarity and macrophyte abundance in shallow waters (Scheffer et al., 1993). Macrophytes were introduced and visible at Dongen, but their abundance was not quantified. These macrophytes may not only have further stabilized the sediment (Madsen et al., 2001), but could also have provided refuge to zooplankton (Moss, 1990). While reduction of the nutrient loading is essential to move the water body towards conditions where a clear water state can be realized, stimulation of submerged macrophytes seems crucial in stabilizing the clear state and preventing or delaying the effect of fish recolonization (De Backer et al., 2012). The human vector in fish recolonization should not be underestimated (Copp et al., 2005) and it is important to include the neighbourhood and local angling associations in the rehabilitation process.

In pond Eindhoven, water clarity was not improved and consequently no growth of the introduced macrophyte *Elodea nuttallii* occurred, as it clearly did not develop in the pond. The main reason for a lack of response in this pond is thought to be that the external P-load remained high. The removal of sediment, additional deepening of the pond and reduction of the fish stock did not result in an improved water quality and reduced cyanobacterial nuisance. Indeed, summer cyanobacterial chlorophyll-*a* concentrations in the years after the measures were lower (31 ± 10 µg L^{-1}) than before (70 ± 22 µg L^{-1}), but the water manager still had to issue warnings in the summers of 2012 and 2014 because cyanobacteria were blooming periodically and accumulating at the water surface. Also the TP concentration did not drop at Eindhoven, while in pond Dongen TP was clearly lower after rehabilitation (Fig. 3.15). TN concentrations at Eindhoven were in the range where macrophytes could grow (Olsen et al., 2015) and were similar to those at Dongen after rehabilitation ($F_{5,55}$ = 1.63; P = 0.173; data not shown) and seem not the reason for absence of macrophytes at Eindhoven. Although measured Secchi depths were similar ($F_{5,56}$ = 2.29; P = 0.063), turbidity at Eindhoven was significantly higher ($F_{5,49}$ = 12.5; P < 0.001) than at pond Dongen. Hence, the frequent bottom sights and much clearer water favored submerged macrophytes at pond Dongen, while the deeper and more turbid water at pond Eindhoven hampered growth of introduced macrophytes (Chambers & Kalff, 1985). The rehabilitation measures had no influence on oxygen levels and pH in both ponds (data not shown).

The study underpins that a diagnostic system analysis should be the start of any lake and pond rehabilitation to elucidate the sources underlying the problem and enabling tailor made solutions with the highest chance for success. Combining measures that together act on both the nutrient load and water turbidity are recommended in rapid eutrophication abatement. Furthermore, this study strengthens that rainwater drainage should not be viewed as non-polluting and that mitigating measures will only be effective when the system analysis has revealed its feasibility. Including the stakeholders is pivotal to ensure prolonged success. Maintenance of the urban ponds is a shared responsibility of all stakeholders that can prevent degradation of the water quality and prolong delivery of the services of the pond to society and therewith to the quality of urban life.

Conclusions

Based on the results of the study it can be concluded that:

- A diagnostic system analysis based on P-loading and critical P-loads should be the start of any lake or pond rehabilitation project.
- The differences between the two experimental sites underpin the importance of a site-specific diagnostic system analysis to select the most promising mitigating measures.
- A combination of measures targeting the internal P-load and biomanipulation can be effective in creating and maintaining a clear water state in urban ponds without high phytoplankton biomass, provided the external P-load is limited.
- Geo-engineering using LMB to control the internal P-load can be an alternative to dredging in urban ponds.
- The use of LMB is sufficient and additional advantage of the flocculant PAC was not shown.
- Commitment of water managers, communities, residents, members of angling associations and other stakeholders is indispensable for controlling eutrophication in urban ponds.

Supplementary information to Chapter 3

Appendix 3.A: Water balances and external P-loads of ponds Dongen and Eindhoven

Introduction

The diagnostic water system analysis for each pond consisted of the water balance and the P-loading budget for the year 2010, in the hypothetical situation without compartments. The external P-loading was confronted with the critical P-loads for each pond.

Water balances

Water balance calculations for each pond for the year 2010 were based on the general equation (1) given by Nõges (2005):

$I + P – E – O \pm \Delta V = 0$ (1)

in which I = Inflow (surface runoff, groundwater, surface water), P = Precipitation onto the water surface, E = Evaporation from the water surface, O = Outflow, ΔV = Change in storage during the period in question; all parameters in m^3 $year^{-1}$.

Outflow through the sewer did not occur in pond Dongen in 2010. More specific the water balance equation for pond Dongen is:

$P + SR + PG – E - \Delta V – R = 0$ (2)

in which SR = Surface Runoff, PG = Pumped Groundwater, R = Residual (resultant of seepage and infiltration).

The water balance equation for pond Eindhoven is:

$P + SR + I – E - \Delta V – R = 0$ (3)

in which I = inflow from the separated sewer system in the surrounding neighbourhood, R = Residual (outflow downstreams over a weir).

For pond Dongen, surface runoff from adjacent areas was expected to occur when precipitation was > 5 mm d^{-1} and was estimated at 40% of the remaining precipitation (modified from Van der Velden, 2010) and adapted to the steepness of the slopes of the lawns and the soil type sand surrounding the pond. Precipitation data for pond Dongen were used from the precipitation radar of the Royal Dutch Meteorological Institute (information per km^2). For pond Eindhoven, the surrounding lawns were steeper and surface runoff was based on field observations, expected to occur when precipitation was > 2.3 mm d^{-1} and was estimated at 67% of the remaining precipitation. Precipitation data for pond Eindhoven were used from the meteorological station at Eindhoven Airport, at a distance of 7 km from pond Eindhoven. Evaporation data were available from the meteorological station at Gilze-Rijen Airport (7 km distance from pond Dongen) and Eindhoven Airport (pond Eindhoven). Evaporation data are recalculated to open water evaporation using the relation between reference evapotranspiration according to Makkink and the open water evaporation according to Penman (Hooghart & Lablans, 1988). Storage is the change of water volume between the first day of 2010 and the last day of 2010. As the residual term for pond

Dongen is mainly positive, the outflow is larger than the inflow which can be explained by the infiltration of pond water. During the period when the pump is used in pond Dongen, the residual term is about 8 times as high as average. Most probably the groundwater level under the pond is lowered during the pumping, which increases the infiltration. Pond Eindhoven has a 30 cm thick layer of impermeable clay in the subsoil which prevents infiltration. The separated sewer system which discharges on pond Eindhoven, delivers 100% discharge of rainwater collected from impervious surfaces to the pond when precipitation is > 1 mm d^{-1}. From unpaved surfaces, 50% of rainwater is discharged to the pond when precipitation is > 5 mm d^{-1} (Cultuurtechnische Vereniging, 2000). Outflow of pond Eindhoven is the residual item of the water balance. The water balances for ponds Dongen and Eindhoven are given in Table 3.A.1.

Table 3.A.1: Water balances for ponds Dongen and Eindhoven (situation 2010), m^3 $year^{-1}$. Residual term in Dongen is due to infiltration. Residual term in Eindhoven is outflow over a weir.

IN	Dongen	Eindhoven
Precipitation on open water	2045	5155
Surface runoff	733	3674
Pumped groundwater	4550	--
Inflow separated sewer	--	124,378
OUT		
Evaporation from open water	1840	4784
Change in storage	281	0
Residual	5207	128,423

External P-loads

The external P-load is given for the ponds in an average year which is based on the hydrological situation of the year 2010 and indicating the morphological situation before the construction of the compartments. The internal P-loads represents the situation before the construction of the compartments. The P-loads are:

• Precipitation on open water. Data for the TP concentration of rainwater were used from Stolk (2001, for pond Dongen meteorological station Gilze-Rijen and for pond Eindhoven meteorological station Vredepeel). It was assumed that SRP is 50% of TP in rainwater (Buijsman, 1989), resulting in TP concentrations in rainwater for pond Dongen of 0.03 mg L^{-1} and for pond Eindhoven of 0.05 mg L^{-1}. Dry deposition for TP was neglected. The annual precipitation (2010) for pond Dongen was 818 mm and for pond Eindhoven was 793 mm. P-loads were determined on an annual basis by multiplication of the P concentration by the quantity of this source. Resulting P-loads due to precipitation on open water are 0.07 mg P m^{-2} d^{-1} (Dongen) and 0.11 mg P m^{-2} d^{-1} (Eindhoven).

• Surface runoff. Runoff from the adjacent slopes of each pond was intercepted in a plastic collecting channel (length 3 m) between slope and pond, during four storm events

in July, August and September 2011. Samples were taken from the collecting channel and were analyzed for nutrients. At Dongen, the channel was situated between the slope and compartment 5. At Eindhoven, the channel was situated between the slope and the pond outside of the compartments. Alongside the compartments at Dongen and Eindhoven there was a fence with a 2 m wide strip of rough vegetation between slopes and compartments. It is assumed that the fence and rough vegetation reduced the P-load due to runoff by 50% (Uusi-Kämppä et al., 1998; Søvik et al., 2012). Due to the uniform land use over time and the uniformity of the catchment, the results of these measurements were regarded to be representative for runoff in the whole catchment of the pond for a longer period. Results of the individual measurements are given in Table 3.A.2. P-loads were determined on an annual basis by multiplication of the P concentration by the quantity of this source resulting in 0.56 mg P m^{-2} d^{-1} (Dongen) and 4.69 mg P m^{-2} d^{-1} (Eindhoven). Taking into account a 50% reduction of the P-load due to the fence and the strip with rough vegetation along the compartments, the P-load in the compartments at Eindhoven due to runoff is 2.35 mg P m^{-2} d^{-1}, while the P-load due to runoff at Dongen in a situation without compartments is 1.12 mg P m^{-2} d^{-1}.

Table 3.A.2: Concentrations (mg L^{-1}) of total phosphorus (TP), soluble reactive phosphorus (SRP), total nitrogen (TN) and nitrate (NO_3^-) in surface runoff at ponds Dongen and Eindhoven during four storm events in summer 2011. Storm events Dongen: 1 = 14 July, 2 = 15 July, 3 = 26 July, 4 = 23 August 2011. Storm events Eindhoven: 1 = 21 July, 2 = 16 August, 3 = 24 August, 4 = 5 September 2011. Analyses by Aquon Laboratory, The Netherlands.

	Dongen				Eindhoven			
Storm event	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
TP (mg L^{-1})	0.50	0.54	0.78	0.95	7.50	1.20	1.80	1.60
SRP (mg L^{-1})	0.30	0.32	0.43	0.67	2.80	0.80	1.30	1.00
TN (mg L^{-1})	3.88	4.17	6.26	4.45	47.70	5.39	4.40	7.70
NO_3^- (mg N L^{-1})	0.26	0.06	0.24	0.42	0.30	0.56	0.26	1.80

• Water birds. The number and species of water birds (mainly mallards, *Anas platyrhynchos*) were counted during sampling dates in 2010 (Table 3.A.3). The P-load caused by the birds were calculated with the food intake and dropping models of Hahn et al. (2007, 2008) and Waterbirds 1.1, Table 3.A.3.

• Feeding of water birds. Feeding of water birds was observed in pond Dongen, not in pond Eindhoven. Water birds in pond Dongen, mostly mallards (*Anas platyrhynchos*), are being fed with bread by residents. We assumed that 50% of the added bread is eaten by mallards and that a mallard has a maximum daily bread consumption of 50 g (Aalderink et al., 2009). The part of the bread that is consumed is included in the dropping model and runoff. The part of the bread which is not consumed gives an additional P-load. The P content of bread is based on Wolos (1992; 1.74 g P kg^{-1}), Aalderink et al. (2009; 4 g P kg^{-1}) and RIVM (2013;

whole meal 2.00 g P kg^{-1}, white water based bread 0.84 g P kg^{-1}) with a mean of 2.15 g P kg^{-1}. The resulting P-load from the feeding of water birds is 0.52 mg P m^{-2} d^{-1} (Dongen).

Table 3.A.3: Mean number of water birds per day on pond Dongen and Eindhoven per season and the resulting P-load (mg P m^{-2} d^{-1}, situation 2010). The number of observations is given in parentheses.

	Dongen		Eindhoven	
season	**Number of birds d^{-1}**	**P-load (mg m^{-2} d^{-1})**	**Number of birds d^{-1}**	**P-load (mg m^{-2} d^{-1})**
Autumn/winter	12.14 (7)	0.113	7.00 (6)	0.028
Spring	8.75 (4)	0.022	1.25 (4)	0.001
Summer	13.55 (11)	0	6.91 (11)	0
Total		0.135		0.029

• Feeding fish (bait). Anglers cause a P-load by the use of baits. The amount of bait is based on field observations, on information provided by the local angling association for pond Dongen and on Van Emmerik & Peters (2009):

- o Bait used during angling competitions 100 kg y^{-1} (Dongen);
- o Outside competitions: 200 fishing days y^{-1} (summer) with 2 anglers d^{-1} (Dongen) and 5 anglers d^{-1} (Eindhoven);
- o Outside competitions: 60% of anglers use bait, 1 kg bait $angler^{-1}$ d^{-1}. Because in pond Dongen, there is only angling by children, the amount of bait used outside competitions was set at 50%: 60% of anglers use bait, 0.5 kg bait $angler^{-1}$ d^{-1};
- o Bait: 50% bread, 50% Dutch crispbakes. P content of bread is 2.15 g P kg^{-1} (see 'Feeding of water birds'). P content of Dutch crispbakes is 1.30 g P kg^{-1} (RIVM, 2013).

The resulting P-load from bait is 0.42 mg P m^{-2} d^{-1} (Dongen) and 0.44 mg P m^{-2} d^{-1} (Eindhoven).

• Litter fall. The P-load caused by litter falling from trees and shrubs on the slopes, is included in the P-load caused by runoff. Leaf litter falling from branches directly over open water (determined from aerial photographs) gives an additional P-load, which is only relevant for pond Dongen. The average litter fall is set at 442 g DW m^{-1} y^{-1} (Liu et al., 2004), with an average P content of 0.22% (Dorney, 1985). The area of overhanging branches over pond Dongen is 33 m^2 resulting in a P-load on the pond of 0.04 mg P m^{-2} d^{-1}.

• Rainwater discharge. On five locations where water from the separated sewer system can be discharged on pond Eindhoven, water samples were taken from the discharging water and analyzed for nutrients (Table 3.A.4). The mean TP concentration is 0.33 mg L^{-1} which was regarded to be representative for a longer period. The P-load was determined on an annual basis by multiplication of the mean P concentration by the quantity of this source resulting in 17.16 mg P m^{-2} d^{-1}.

Table 3.A.4: Concentrations (mg L^{-1}) of total phosphorus (TP), soluble reactive phosphorus (SRP), total nitrogen (TN) and nitrate (NO_3^-) in water from the separated sewer system before discharge on pond Eindhoven on five locations (a-b-c-d-e) during four storm events in summer 2011. Storm events Eindhoven: 1 = 21 July, 2 = 16 August, 3 = 24 August, 4 = 5 September 2011. Analyses by Aquon Laboratory, The Netherlands.

		a	b	c	d	e
	Storm event					
TP (mg L^{-1})	1	0.33	0.24	0.47	1.00	0.45
	2	0.26	0.22	0.12	0.30	0.13
	3	0.26	0.26	0.12	0.49	0.10
	4	0.40	--	0.17	0.56	0.34
SRP (mg L^{-1})	1	0.06	0.02	0.27	0.49	0.25
	2	<0.01	<0.01	0.03	0.16	0.08
	3	<0.01	<0.01	0.03	0.24	0.03
	4	0.03	--	0.02	0.19	0.11
TN (mg L^{-1})	1	3.50	3.00	1.80	3.90	2.20
	2	2.30	2.60	1.08	1.73	1.39
	3	3.80	3.20	1.07	4.25	1.52
	4	10.66	--	1.50	3.34	2.48
NO_3^- (mg N L^{-1})	1	0.50	0.30	<0.05	<0.05	<0.05
	2	<0.05	<0.05	0.14	0.21	0.37
	3	<0.05	<0.05	0.07	0.60	0.69
	4	0.34	--	0.09	0.28	0.43

• Pumped groundwater. Pumped groundwater was collected in Dongen three times during 2010 and twice during 2011. TP concentrations for groundwater and surface runoff were measured according the analytical method for surface water. The results of the measurements were regarded to be representative for a longer period. Results of the individual measurements are given in Table 3.A.5. The P-load for pond Dongen was determined on an annual basis by multiplication of the P concentration by the quantity of this source resulting in 0.40 mg P m^{-2} d^{-1}.

Table 3.A.5: Concentrations of total phosphorus (TP, mg L^{-1}), soluble reactive phosphorus (SRP, µg L^{-1}), ammonium (NH_3, mg N L^{-1}) and nitrate+ nitrite (NO_3^-+NO_2^-, mg N L^{-1}) in groundwater during pumping events in pond Dongen. Pumping events: 1 = 30 June 2010, 2 = 7 July 2010, 3 = 28 July 2010, 4 = 29 March 2011, 5 = 31 May 2011. Analyses by the laboratory of Wageningen University, The Netherlands.

	Dongen				
	1	2	3	4	5
TP (mg L^{-1})	0.05	0.02	0.08	0.20	0.06
SRP (µg L^{-1})	14.2	2.7	3.7	5.2	5.51
NH_3 (mg N L^{-1})	1.32	1.30	1.44	1.90	0.75
NO_3^-+NO_2^- (mg N L^{-1})	0.01	0.04	0.01	0.06	0.01

Appendix 3.B: Water quality of ponds Dongen and Eindhoven before construction of compartments

The water quality of ponds Dongen and Eindhoven was investigated prior to the installation of the compartments during the period March - July 2009. The results are given in Tables 3.B.1 and 3.B.2.

Table 3.B.1: Water quality of pond Dongen at individual sampling events, 23 March – 1 July 2009.

	23 Mar	7 Apr	20 Apr	6 May	19 May	3 Jun	17 Jun	1 Jul
pH	7.03	7.28	5.73	6.50	6.39	7.02	6.02	5.14
EC (µS cm^{-1})	399	412	424	445	465	510	478	542
NTU	---	47.2	35.3	51.1	57.5	50.6	74.2	88.4
Temperature (°C)	10.0	14.5	16.1	14.1	16.9	20.7	19.4	24.1
Chlorophyll-*a* (µg L^{-1})	159.4	162.1	60.0	173.3	191.2	302.9	472.5	438.3
Cyanobacterial chlorophyll-*a* (µg L^{-1})	19.6	14.9	3.6	15.5	18.5	31.0	39.7	72.5
Suspended solids (mg L^{-1})	56.0	72.5	35.3	52.0	86.7	104.0	148.7	112.0
TP (mg L^{-1})	0.57	0.14	0.26	0.15	0.12	0.18	0.21	0.14
SRP (µg L^{-1})	113.8	6.3	9.8	3.4	3.5	2.3	12.8	11.5
NO_3 + NO_2 (mg N L^{-1})	0.05	0.06	0.05	0.05	0.06	0.07	0.06	0.08
NH_4 (mg N L^{-1})	0.08	0.09	0.24	2.06	2.38	2.61	1.12	1.39
TN (mg N L^{-1})	0.36	0.32	0.92	4.07	4.15	4.29	3.15	2.57
Filterable S (mg S L^{-1})	---	---	---	---	---	57.5	55.4	63.4
O_2 (mg L^{-1})	10.8	5.5	4.5	3.9	4.2	7.9	8.5	7.0
Chloride (mg L^{-1})	40.1	42.3	40.4	42.6	43.1	49.8	46.3	50.7
Filterable Al (µg L^{-1})	3.63	1.84	6.17	6.09	3.14	2.85	1.35	8.3
Total Al (µg L^{-1})	287	353	207	286	399	---	---	---
Filterable Fe (µg L^{-1})	88.6	40.1	143	125	113	88.6	31.3	193
Total Fe (µg L^{-1})	2615	3264	2051	3322	4474	---	---	---
Filterable La (µg L^{-1})	0.29	0.04	0.16	0.05	0.04	0.23	0.09	0.25
Total La (µg L^{-1})	2.11	0.59	0.54	0.51	0.39	---	---	---

Table 3.B.2: Water quality of pond Eindhoven at individual sampling events, 3 March – 29 June 2009.

	4 Mar	23 Mar	7 Apr	20 Apr	6 May	19 May	3 Jun	17 Jun	29 Jun
pH	8.67	8.31	8.06	8.35	7.69	8.63	8.38	7.34	7.83
EC (µS cm^{-1})	356	386	419	386	415	414	402	383	366
NTU	16.6	24.9	25.3	28.3	37.1	51.4	37.2	44.5	32.3
Temperature (°C)	6.5	9.4	14.0	17.0	14.9	17.5	20.5	18.6	23.7
Chlorophyll-*a* (µg L^{-1})	---	58.7	66.1	125.5	62.5	114.8	104.2	86.2	105.5
Cyanobacterial chlorophyll-*a* (µg L^{-1})	---	0.0	1.0	14.6	1.4	0.0	15.0	24.8	38.5
Suspended solids (mg L^{-1})	---	36.7	30.5	33.3	41.7	59.6	43.2	51.5	32.8
TP (mg L^{-1})	0.05	0.13	0.35	0.15	0.31	0.08	0.10	0.12	0.09
SRP (µg L^{-1})	10.9	220.4	3.7	2.0	7.0	3.8	2.3	45.5	8.6
NO_3 + NO_2 (mg N L^{-1})	<0.01	0.09	0.05	0.21	0.05	0.05	0.06	0.02	0.04
NH_3 (mg N L^{-1})	0.27	0.31	0.07	0.12	0.31	0.09	0.01	0.03	0.02
TN (mg N L^{-1})	0.30	0.72	0.83	0.90	1.41	1.86	1.45	2.13	1.64
Filterable S (mg S L^{-1})	---	---	---	---	---	---	7.99	7.75	6.96
O_2 (mg L^{-1})	15.3	11.2	10.3	10.3	4.7	10.2	8.2	5.7	8.6
Chloride (mg L^{-1})	---	41.8	46.5	43.4	44.8	45.5	46.5	44.5	46.2
Filterable Al (µg L^{-1})	2.21	8.23	70.60	10.20	104	35.20	18.40	17.40	3.69
Total Al (µg L^{-1})	211	531	---	448	925	1149	454	886	223
Filterable Fe (µg L^{-1})	40.70	77.60	164	49.00	201	125	65.20	67.70	24.70
Total Fe (µg L^{-1})	620	1026	---	1030	1452	1581	1065	1769	685
Filterable La (µg L^{-1})	0.03	0.13	0.12	0.32	0.26	0.25	0.36	0.39	0.07
Total La (µg L^{-1})	0.20	1.54	---	0.52	0.88	1.00	0.64	1.23	0.44

Appendix 3.C: Experiment on the leaching of phytoplankton inhibiting compounds from the wooden sheet pilings in pond Dongen

Introduction

In pond Dongen, the compartments were separated by wooden sheet pilings (Fig. 3.C.1). During construction of the compartments a sharp drop of the chlorophyll-*a* concentrations occurred in several compartments. Leaching of substances from wood can limit the growth of algae (Taylor et al., 1996; Kamaya et al., 2003; Yang et al., 2009a). A leaching experiment was conducted to investigate whether the leaching of phytoplankton inhibiting compounds from the construction material could explain the fast reduction in chlorophyll-*a* concentrations.

Fig. 3.C.1: Construction of compartments separated by wooden sheet pilings in pond Dongen (28 July 2009).

Material and methods

From a piece of wood that had been used to construct the sheet pilings, fresh sawdust was collected. The sawdust was brought in 100 mL algal growth WC-medium in Erlenmeyer flasks at concentrations of 0, 0.1, 1, 10 and 100 g sawdust L^{-1} and placed on a shaker in dark at 24° C for 48 h. After 48 h the medium was filtered over 0.45 µm (membrane filter). The absorption spectrum of the filtrates was determined from 350 to 750 nm (Beckman Coulter Du730 Life Sciences UV/VIS spectrophotometer; 5 cm cuvet, 1 nm interval). The filtrates were used for the growth of inocula of *Scenedesmus obliquus* (39 µg chlorophyll-*a* L^{-1} and 2×10^6 μm^3 mL^{-1}). From each concentration, 2.5 mL was brought in a 24-welled culture plate, in fourfold, and incubated for 72 h (24° C, continuous light of ~140 µmol quanta m^{-2} s^{-1}, shaken at 100 rpm). After 72 h incubation, the chlorophyll-*a* concentrations were determined and the photo system II efficiency using a PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany), as well as the number of particles, biovolume and mean particle volume using a CASY® cell counter (Schärfe System Gmbh., Germany). We

hypothesized that substances from wooden sawdust limited the growth of the green alga *S. obliquus*.

Results

Sawdust had a significant effect on the chlorophyll-*a* concentrations of cultures of the alga *S. obliquus* ($F_{4,15}$ = 19.5; P < 0.001); Fig. 3.C.2. A post-hoc Tukey test revealed two homogenous groups: 1) controls (0), 0.1 and 1 g sawdust L^{-1}, and 2) 10 and 100 g sawdust L^{-1} incubations. The chlorophyll-*a* concentrations in the second group were significantly lower than in the first group (Fig. 3.C.2).

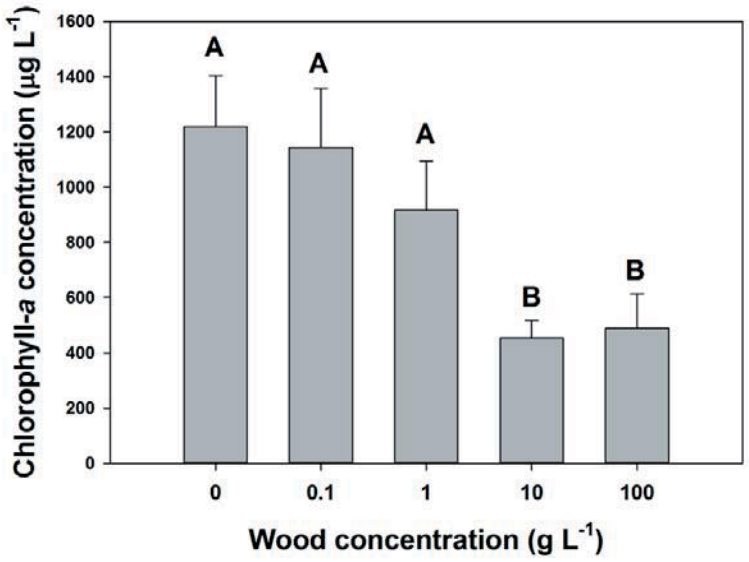


Fig. 3.C.2: Chlorophyll-*a* concentrations (µg L^{-1}) of the green alga *Scenedesmus obliquus* in filtrates of sawdust (wood, g L^{-1}) incubations of wooden sheet pilings used for the compartments in pond Dongen. Different capitals indicate significantly different homogenous groups (Tukey test, P < 0.05). Error bars indicate 1 SD (n=4).

In addition, the growth (d^{-1}) of the green alga was influenced by the filtrate medium with different concentrations of sawdust (Table 3.C.1).

Table 3.C.1: Growth (d^{-1}) of *Scenedesmus obliquus* in filtrates of sawdust incubations from wooden sheet pilings in pond Dongen (wood, g L^{-1}), based on increase of chlorophyll-*a* (µg L^{-1}), number of particles (n mL^{-1}) and biovolume (µm^3 mL^{-1}), inclusive ANOVA results and homogenous groups (A,B) from Tukey-tests.

	Growth (d^{-1})		
Wood concentration (g L^{-1})	**Chlorophyll-*a***	**Number of particles**	**Biovolume**
0	1.21 A	1.39 A	1.58 AB
0.1	1.19 A	1.36 A	1.59 AB
1	1.11 A	1.30 A	1.55 AB
10	0.86 B	1.11 B	1.44 A
100	0.88 B	1.14 B	1.68 B
One-way ANOVA	$F_{4,15}$ = 26.6	$F_{4,15}$ = 24.2	$F_{4,15}$ = 5.74
	P < 0.001	P < 0.001	P = 0.005

The mean particle volume of the green alga *S. obliquus* increased in filtrates based on higher concentrations of sawdust (Fig. 3.C.3).

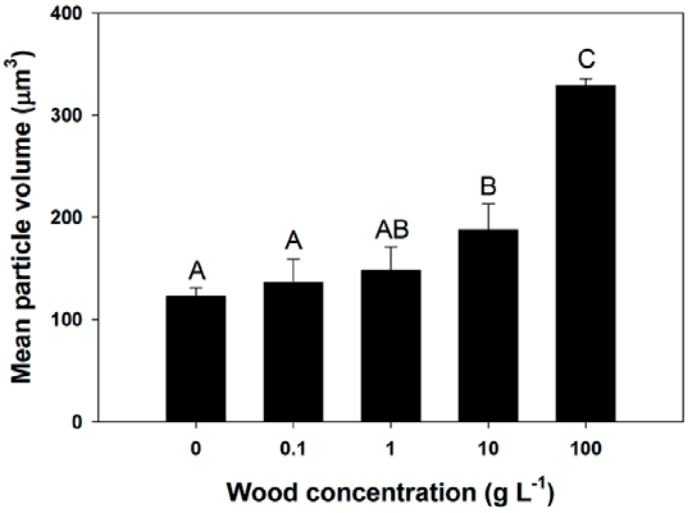


Fig. 3.C.3: Mean particle volume (µm³) of *S. obliquus* in filtrates of sawdust (wood, g L⁻¹) from wooden sheet pilings of pond Dongen. Different capitals indicate significantly different homogenous groups (Tukey test, *P* < 0.05). Error bars indicate 1 SD (n=4).

The filtrate of only the highest concentrations of sawdust (10 and 100 g L^{-1}) limited significantly the growth of the green alga *S. obliquus.* The growth limiting effect was not caused by discoloration of the medium, as shown by the absorption spectra (Fig. 3.C.4). Growth rates stayed positive and high, indicating an increase of phytoplankton. It is concluded that a strong limiting effect of the wooden sheet pilings on the algal growth in the compartments of pond Dongen is not plausible.

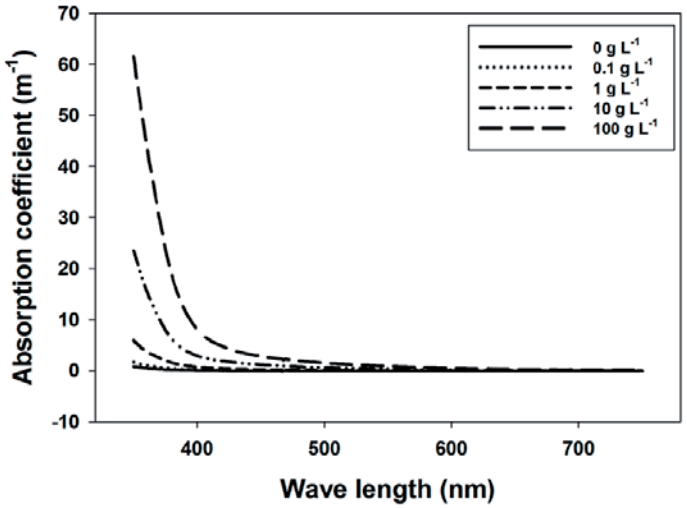


Fig. 3.C.4: Absorption (m^{-1}) of visible and photosynthetic active light (350 – 750 nm) in filtrated medium which had been incubated with sawdust from wooden sheet pilings of pond Dongen (g sawdust L^{-1}).

Appendix 3.D: Flocculation and sinking assay

Introduction

To examine the effect of PAC alone and in combination with LMB on the settling of the suspended particulate matter, an additional laboratory assay was conducted with water from the compartments 2, 3 and 4 in Eindhoven – collected on 31 July 2009. Nine samples of 100 mL of the well-mixed suspension were transferred to nine 125 mL glass tubes. Three tubes were treated with PAC (2.18 mg Al L^{-1}). To three other tubes, first LMB was added (mean 38 mg, SD±3 mg) by making a slurry with 5 mL water from the tube, which was then sprayed on top of the tube, followed by adding PAC (2.18 mg Al L^{-1}). The three remaining tubes remained untreated (controls). The tubes were placed for 2 h at 20° C where after the top 10 mL and the bottom 10 mL of each tube were pipetted off and collected separately in 30 mL vials. For each sample the chlorophyll-*a* concentration was measured with the PHYTO-PAM phytoplankton analyzer. As PAC is an acidic solution, pH was measured (WTW-pH320) in the tubes.

Data analysis

The chlorophyll-*a* concentrations in the test tubes used in the flocculation and sinking assay were analyzed by Mann-Whitney Rank Sum Test, *t*-test and one-way ANOVA to distinguish significant differences ($P < 0.05$).

Results

The initial mean total chlorophyll-*a* concentration was 119 µg L^{-1} (SD±19; n=3) of which 40% was comprised of cyanobacterial chlorophyll-*a* (48 ± 6 µg L^{-1}). A Mann-Whitney Rank Sum Test revealed that after two hours the mean total chlorophyll-*a* concentrations in the top and the bottom of the controls remained similar (T3 = 8.0; $P = 0.400$); top 113 ± 21 µg L^{-1} and bottom 130 ± 19 µg L^{-1} (Fig. 3.D.1). Cyanobacterial chlorophyll-*a* concentrations of the controls were also similar after two hours (t-test; $t_4 = 0.09$; $P = 0.930$) in the top (49 ± 4 µg L^{-1}) and bottom (48 ± 6 µg L^{-1}). Addition of PAC (2.18 mg Al L^{-1}) alone or in combination with LMB (380 mg L^{-1}) resulted in strong sedimentation of phytoplankton (Fig. 3.D.1).

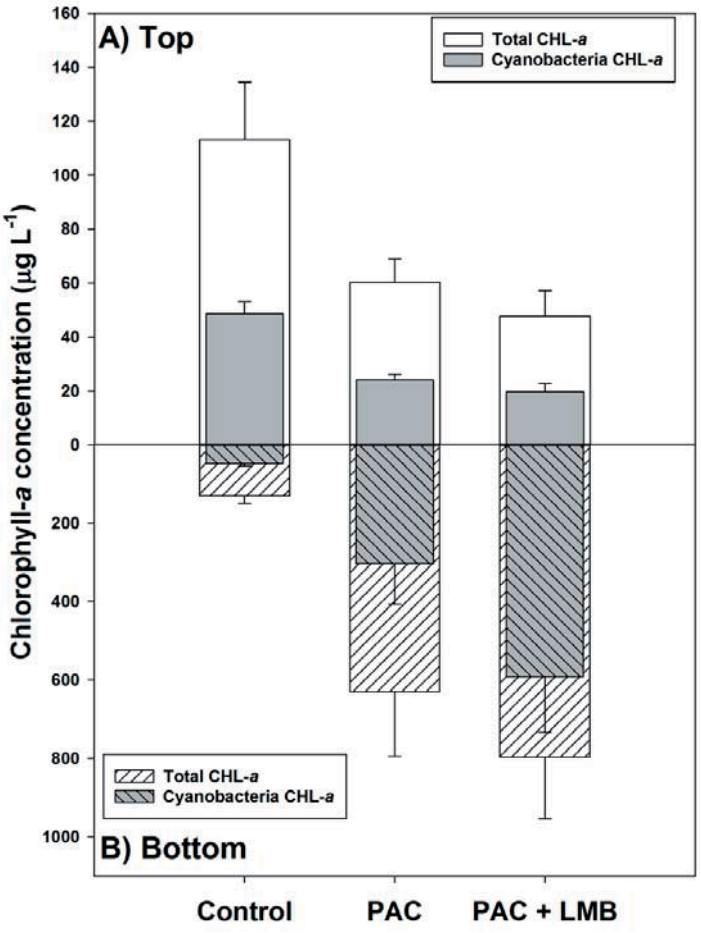


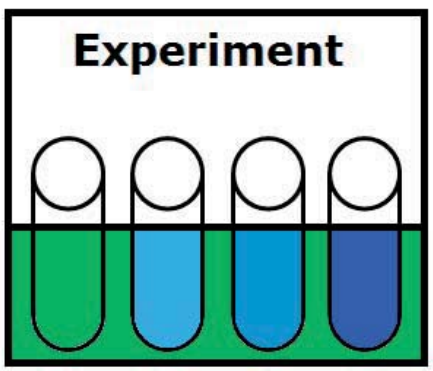
Fig. 3.D.1: Flocculation and sinking assay: total and cyanobacterial chlorophyll-*a* concentrations (µg L^{-1}) after two hours in the top and bottom of test tubes containing untreated mixed water from the compartments in pond Eindhoven (control) and treated with either PAC (2.18 mg Al L^{-1}) or PAC + LMB (2.18 mg Al L^{-1}, 380 mg LMB L^{-1}). Error bars indicate 1 SD (n = 3).

Total chlorophyll-*a* concentrations in the top of all of the tubes were significantly different (one-way ANOVA; $F_{2,8}$ = 17.5; *P* = 0.003) from each other, as were those in the bottom ($F_{2,8}$ = 20.7; *P* = 0.002). In both cases Tukey post-hoc comparison (P < 0.05) revealed that chlorophyll-*a* concentrations in the controls were significantly different from those in the PAC and LMB+PAC treatments. Similarly, cyanobacterial chlorophyll-*a* concentrations in the top were significantly ($F_{2,8}$ = 65.2; *P* < 0.001) higher in the controls than in the PAC or LMB+PAC treatments (Fig. 3.D.1). In the bottom, cyanobacterial chlorophyll-*a* concentrations in the controls were significantly lower than in the LMB+PAC treatment (H_2 = 7.2; *P* = 0.004). The pH in the controls (8.29 ± 0.04) was similar to the pH at the start of the experiment (8.23 ± 0.11; t-test, t_4 = 0.90; *P* = 0.417), but pH was significantly reduced ($F_{2,8}$ = 93.2; *P* < 0.001) in the PAC (7.74 ± 0.10) and LMB+PAC (7.55 ± 0.05) treatments. The LMB+PAC treatment resulted in a 58% reduction in total chlorophyll-*a* concentration in the top after two hours.

	P load (mg P m^{-2} d^{-1})
Separated sewer system	6.5
Deposition on lake	[illegible]
Water birds	0.2
Feeding of water birds	0.1
Litterfall	0.3
Bathing	0.1
Dog faeces and urine	0.1
Total	7.5

Diagnostics

Experiment



Lake restoration

CHAPTER 4

Restoration of urban Lake Kleine Melanen (The Netherlands): diagnostics, experiment and whole lake treatment

This chapter is based on:
The unfulfilled promise of urban Lake Kleine Melanen (The Netherlands): diagnostics, experiment and whole lake restoration.
Waajen, G., M. Lürling & R. van de Sande, *submitted.*

Abstract

Urban Lake Kleine Melanen (The Netherlands) regularly suffers from detrimental cyanobacterial blooms due to eutrophication. A restoration project was initiated to improve the water quality and fulfilment of ecosystem services, and to reduce the risk of winter fish kill. Diagnostics showed that the external P-load exceeded the critical P-loads by 44-121%. Discharges from the separated rainwater sewer system in the adjacent neighbourhood were the dominant P-source of the lake, causing 87% of the external P-load. Sediment P-release was high. While removal of sediment increased water depth and reduced the risk of winter fish kill, it did not reduce sediment P-release adequately. An enclosure experiment was carried out to test different sediment capping options and determine which to apply to the whole lake. Following dredging, sediment capping with sand and lanthanum modified bentonite were applied. Fish were removed and lake banks were reconstructed. The measures taken so far resulted in significant reductions of total-P, ortho-P and chlorophyll-*a* concentrations, yet cyanobacterial blooms still occur. Reduction of the P-load from the separated rainwater sewer system still has to be effectuated and the application of a low dose flocculant (polyaluminiumchloride) will finalize the restoration in near future. The study demonstrates that the reduction of the P-load from the rainwater sewer system is essential for further recovery, pointing out the importance of restoration based on diagnostics.

Introduction

Small lakes (< 10 ha) are common features in urban areas and they provide services for both human and ecological purposes (Bolund & Hunhammar, 1999; Hellström et al., 2000; Gledhill et al., 2008). These small urban lakes are strongly influenced by eutrophication (Brönmark & Hansson, 2002), causing high levels of phytoplankton biomass including cyanobacteria (Schindler et al., 2008). The cyanobacterial blooms, resulting in malodors, fish kills due to anoxia and high concentrations of potent cyanobacterial toxins, hamper the fulfilment of ecosystem services and threaten human and animal health (Paerl & Huisman, 2008). As the anthropogenic pressures on urban lakes are big and the demand for the proper fulfilment of their ecosystem services is high, water managers are in need of effective and robust restoration methods. The restoration of urban lakes, however is complex and necessitates tailor-made approaches (Thornton et al., 2013).

Phosphorus (P) and nitrogen (N) are both key nutrients in eutrophication of lakes (Conley et al., 2009). This led to the view that both P and N should be controlled to reduce cyanobacterial blooms (Conley et al., 2009; Paerl et al., 2014). Availability of all nutrients is required to create a bloom. For control however, according to Liebig's law of the minimum, only reduction of one nutrient is needed as the growth is controlled by the scarcest available nutrient. For the restoration of eutrophic lakes reduction of the external nutrient input is essential (Cooke et al., 2005) and P is the nutrient that can most easily limit cyanobacterial growth (Golterman, 1975; Carpenter, 2008; Schindler et al., 2008). Substantial N-reduction in lakes is virtually impossible due to high atmospheric deposition (Anderson & Downing, 2006) and N-fixation (Stal, 2015). Strong P-reduction will render N-reduction insignificant, which is supported by the outcome of whole-lake experiments targeting only P (e.g., Lürling & Van Oosterhout, 2013; Chapter 5). Insight into the P-sources and the critical P-load thresholds for transitions between turbid and clear water states (Scheffer et al., 1993) is essential for successful recovery of eutrophic urban lakes. A ratio 'actual P-load':'critical P-load' >1 indicates the necessity to reduce the external P-load in order to realize the clear water state (Janse et al., 2008) and mitigate cyanobacterial nuisance. In addition to the reduction of the external P-load, control of the internal P-load is often crucial for long-term positive effects (Gulati & Van Donk, 2002; Søndergaard et al., 2007) as internal P-loading can hamper lake recovery for many years (Søndergaard et al., 1999).

Lake Kleine Melanen is one of many eutrophic urban lakes in The Netherlands characterized by cyanobacterial blooms (Chapter 2). In 2009, a project was initiated to restore the habitat and water quality of the lake, to realize the clear water state (Scheffer et al., 1993; Scheffer & Van Nes, 2007) with opportunities for submerged macrophytes and to improve the ecosystem services. As winter fish kill due to hypoxia under ice (Fang & Stefan, 2000) was an issue in this lake, the water manager initially decided to increase the water depth with on average ~0.5 m through sediment removal. Increase of water depth reduces the risk of winter fish kill as it results in a lower oxygen depletion rate and higher oxygen content under ice cover (Mathias & Barica, 1980; Ellis & Stefan, 1989). Removal of

~0.5 m of the sediment to increase the water depth resulted in freshly exposed peat in a part of the lake, while in the rest of the lake a layer of soft sediment remained. As the lake's sediment had been negatively affected by mixed sewer overflows in the past, the remaining soft sediment provided a potential source of sediment P-release (Owens & Walling, 2002). The freshly exposed peat in the other part of the lake implied the risk of enhanced aerobic mineralization and mobilization of P (Geurts et al., 2010). To prevent P-mobilization from freshly exposed peat and to reduce P-release from the remaining soft sediment, sediment capping with clean sand was considered a promising measure (Pan et al., 2012), but the knowledge on the impacts of sand capping on P-release is scarce (Kim et al., 2007). Additionally, sand capping prevents soft sediment resuspension (Danielsson et al., 2007) and benefits the growth of submerged macrophytes by providing firm sediment (Istvánovics et al., 2008). Although sediment capping is used to block P-release, the use of a physical barrier alone will not eliminate the movement of dissolved P through the capping material. In Lake Kleine Melanen, groundwater seepage into the lake occurs during wet periods, implying the risk of P-intrusion through the physical barrier even though the diffusion of P through a sand layer is a slow process (Thoma et al., 1993; Himmelheber et al., 2008). The combination of physical capping with an active P-fixative increases the efficacy of blocking P-release from the sediment (Yuan et al., 2009; Pan et al., 2012) and was considered a promising measure for Lake Kleine Melanen.

An enclosure experiment was carried out (Fig. 4.1) to test different sediment capping options. We hypothesized that 1) sand capping will significantly reduce the total phosphorus (TP), ortho phosphate (o-P) and chlorophyll-*a* concentrations of the overlying water, and 2) the effectivity of sand capping is enhanced by the addition of a solid phase P-sorbent. As a part of the in-lake P is present in suspended particles and phytoplankton, which contributes to the internal P-recycling, removal of this P-stock promotes lake recovery (Van Oosterhout &

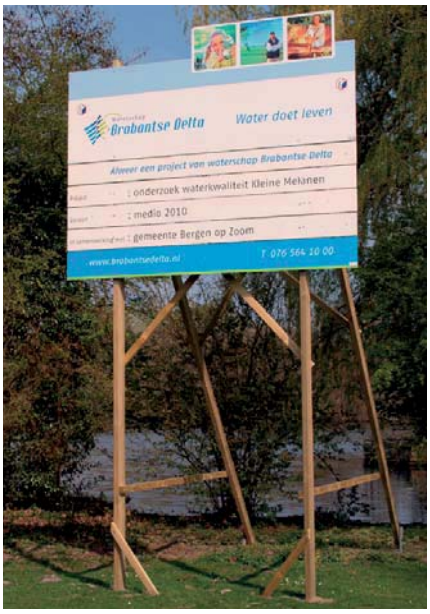


Fig. 4.1: Information sign indicating the enclosure experiment in Lake Kleine Melanen.

Lürling, 2011). A flocculant, if used together with ballast, effectively precipitates particulate P even from positively buoyant cyanobacteria and enhances o-P stripping from the water column (Lürling & Van Oosterhout, 2013). The efficacy of a flocculant was tested as part of the enclosure experiment and we hypothesized that the additional application of a low dose flocculant more strongly reduces the concentrations of TP, o-P and chlorophyll-*a*.

A high fish stock, dominated by carp (*Cyprinus carpio carpio*), is known to characterize eutrophic urban ponds (Chapter 2). As sediment resuspension by fish may keep the water turbid and prevent establishment of macrophytes (Cline et al., 1994; Meijer et al., 1999; Chumchal et al., 2005), biomanipulation on fish was included in the restoration planning. Food web control by flocculation of phytoplankton and biomanipulation on fish may switch a lake from the turbid phytoplankton dominated state to the clear water state (Fig. 4.2) and maintain the clear water state, provided the P-loading is low (Scheffer et al., 1993). A characteristic of the lake is the absence of marsh area, the zone with water depth of 0 – 1 m and habitat for helophytes and submerged macrophytes. The introduction of marsh area raises the critical P-load thresholds cP_{oligo} (indicating the switch from turbid to clear) and cP_{eutro} (indicating the switch from clear to turbid, Fig. 4.2), e.g., through the uptake of nutrients by the vegetation and providing a good habitat for predatory fish (Janse et al., 2008), and was included in the restoration planning.

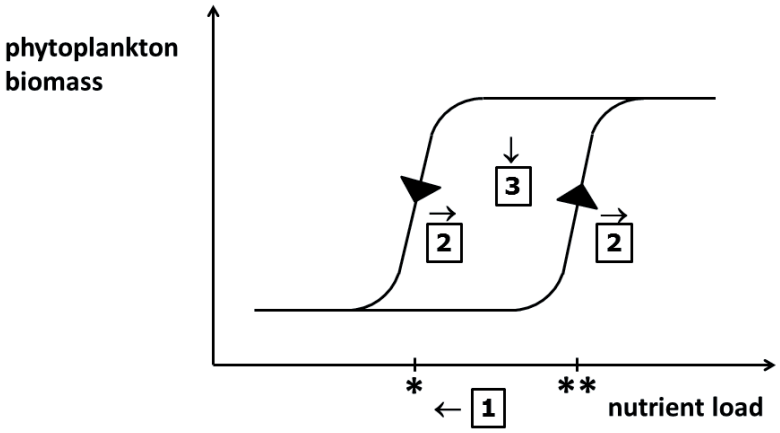


Fig.4.2: Nutrient load in relation to phytoplankton biomass showing a hysteresis curve. Triangular arrows indicate the switches from the clear water state to the phytoplankton dominated state (= cP_{eutro}) and vice versa (* = cP_{oligo}). Numbers indicate the elements of the ecosystem approach: 1 = reduction of nutrient load, 2 = increase of the carrying capacity of the clear water state for nutrients, 3 = food web control (modified from Hosper, 1989).**

This chapter presents the results of the diagnostics, the results of the enclosure experiment and describes the restoration efforts to date, including initial results. The restoration efforts combine biological, physico-chemical and reconstructional in-lake methods and catchment measures. Although the combination of biological and physico-chemical in-lake measures has been considered promising, the scientific evidence is poor (Jeppesen et al., 2012). To our knowledge, there are no comprehensive studies on urban lake restoration based on a P-budget and modelling derived critical P-load thresholds,

supplemented by in-lake and catchment measures and supported by experimental research. We hypothesized that the combination of the in-lake and catchment measures will reduce the risk of cyanobacterial blooms and will realize and maintain the clear water state with submerged macrophytes. The elaborated example of the restoration of Lake Kleine Melanen will support future rehabilitation of urban lakes and ponds and can contribute to their improvement.

Materials and methods

Baseline situation of Lake Kleine Melanen and restoration planning

Lake Kleine Melanen (The Netherlands) is a shallow lake, which was originally a marshy depression surrounded by heathland. During the 18th century peat was excavated, creating the present day lake. Presently the lake is situated in a residential area and it is surrounded by a small park (Fig. 4.3; Table 4.1).

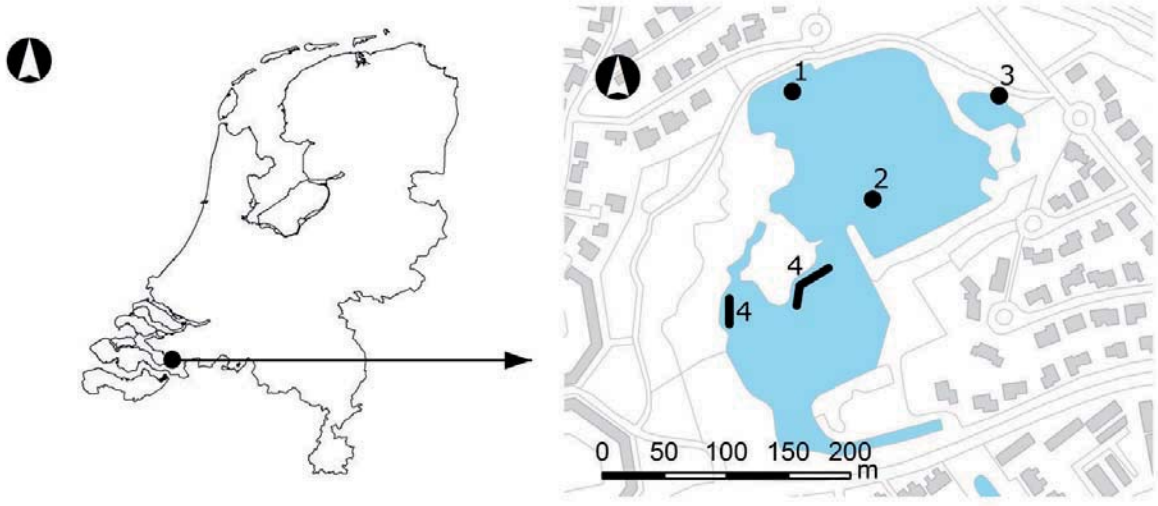


Fig. 4.3: Location of Lake Kleine Melanen in The Netherlands (left) and detailed map of the lake (right). 1 = sampling location 1993 - 2008, 2 = sampling location 2009 - 2014, 3 = location of outlet weir, 4 = location of the enclosure experiment.

Table 4.1: Characteristics of Lake Kleine Melanen before restoration (situation in 2009; TP and chlorophyll-*a* are the mean concentrations for the period 2008-2009).

longitude/latitude	N 51°30'44''/E 4°17'33''
Area (ha)	4
mean water depth (m)	1.0
sediment	>1 m mud on peat
cover submerged macrophytes (%)	0
fish biomass (kg ha^{-1})	605 (66% carp)
mean TP (mg L^{-1})	0.31
mean chlorophyll-*a* (µg L^{-1})	107

The banks are characterized by small-scale wooden bank protection and the lake has a layer of accumulated soft sediment (~1m thick) on a peat base. Until a decade ago, the lake was influenced by discharges of mixed sewage. Now, the lake receives rainwater collected from impervious surfaces in the residential area, entering the lake at four points via separated sewer overflows. An outflow-weir is situated in the NE corner of the lake. The water level of the lake is fairly constant throughout the year and the lake is used for recreational angling and the feeding of ducks and geese. The lake is artificially stocked with fish and it has a history of cyanobacterial blooms (*Microcystis* spp.*, Anabaena* spp.*, Aphanizomenon* spp.). In the winter of 2009/2010, a massive fish kill reduced the fish biomass. Submerged macrophytes are lacking, despite a few isolated specimens of *Ceratophyllum demersum*, *Lemna minor*, and *Nymphaea alba*.

The water balance and the P-loads were determined by modelling with a combination of measured and published values (Appendix 4.A). The PCLake Metamodel (Mooij et al., 2010; PBL, 2015) was used to determine the critical P-load thresholds cP_{oligo} and cP_{eutro} (Fig. 4.2). The external P-load was compared with the critical P-load thresholds, providing information of the necessary reduction of the P-load. This determined the extent of catchment measures needed to reduce the external P-load. Data of the soft sediment, the underlying peat and the pore water have been extracted from Waltjé et al. (2009). This includes density, dry matter and organic matter content and the concentrations of P, iron (Fe) and sulfur (S). The sediment P-release of the upper soft sediment layer and of the underlying peat were deduced from a known correlation between the P concentration of the pore water and the sediment P-release (Van der Wijngaart et al., 2012). The intended increase of the water depth by 0.5 m, the introduction of marsh area and the reduction of the discharges from the separated sewer system influenced cP_{oligo} and cP_{eutro} (Janse et al., 2008). For this reason, the designing of the final set of measures was done as an iterative process by repeatedly determining the effects of measures on cP_{oligo}, cP_{eutro} and the external P-load and redefining measures. As a part of the restoration planning, the fish stock was measured by a professional fishing company in September 2010 using a combination of electro fishing (5 kW) and trawl fishing, supplemented with information about the fish kill of the winter 2009/2010 (Kalkman, 2010c).

Enclosure experiment

An enclosure experiment was executed to determine which method of sediment capping works best and whether a flocculant has added value. Based on the water clearing properties and good flocculation polyaluminiumchloride (PAC) was used as flocculant (Delgado et al., 2003). A lanthanum modified bentonite clay (LMB, available as Phoslock®) was used as a solid phase P-sorbent as it has a strong binding capacity to o-P (Haghseresht et al., 2009), is relatively unaffected by changes in pH and redox state (Ross et al., 2008; Gibbs et al., 2011) and can reduce the sediment P-release even under conditions of groundwater seepage. When applied to the water surface, LMB strips additionally o-P from the water column

(Robb et al., 2003; Akhurst et al. 2004; Douglas et al., 2008; Ross et al., 2008; Egemose et al., 2010; Gibbs et al., 2011; Van Oosterhout & Lürling, 2013). The experimental period lasted from 8 March 2010 (day 0) to 3 June 2010 (day 87). The tested treatments were: sand capping (S; n=2), sand capping + LMB (SL; n=3), sand capping + LMB + PAC (SLP; n=3) and control (C; n=3). Eleven perspex cylinders (diameter 1.05 m, height 1.30 m) were positioned on two locations in Lake Kleine Melanen on 3 and 4 March 2010 (Fig. 4.3). The cylinders were pushed into the sediment leaving 20 cm above the water surface. They were open to the sediment and the air, covered with chicken wire to prevent waterfowl getting in and contained no fish. The water depth inside the cylinders was initially on average 84 cm. Commercially available desalinated clean sea sand from a local vendor was carefully added to the cylinders with a scoop on 4 March 2010 until a layer of 20 cm settled on top of the sediment. The thickness of the sand capping layer was tested with a perspex corer (Fig. 4.4).

Fig. 4.4: 20 cm of sand capping on top of the soft sediment in the enclosure experiment at Lake Kleine Melanen (4 March 2010).

On 8 March 2010 (day 0) LMB (253 g LMB enclosure^{-1}; 292 g LMB m^{-2}; 5% La; supplied by Phoslock Europe GmbH, Ottersberg, Germany) was mixed with lake water and added as a slurry to the water surface. 15 minutes after the addition of LMB, 25 mL PAC enclosure^{-1} (3.0 g Al enclosure^{-1}; 28.9 mL PAC m^{-2}, 3.5 g Al m^{-2}; Melfloc 39 polyaluminiumchloride, ρ = 1.37 kg L $^{-1}$, 8.9% Al, 22% Cl; supplied by Melspring International B.V., Arnhem, The Netherlands) was mixed into the PAC enclosures with a scoop. The LMB dose for the enclosure experiment was tailored to match the P-load and TP concentration of the lake water prior to the experiment (0.31 mg P L^{-1}, 3 December 2009). The LMB dose used (292 g LMB m^{-2}) is within the range used in 16 case study lakes (349 g LMB m^{-2} ± 189; Spears et al., 2013b). The PAC dose for optimal flocculation was experimentally determined 2 h before the application using glass jar tests.

Whole-lake measures

A number of measures was executed in Lake Kleine Melanen to date (Table 4.2, Fig. 4.5).

Table 4.2: Restoration measures and year of execution.

Measure	Year of execution
Fish removal	2010
Dredging	2010 - 2011
Pruning trees and shrubs	2011
Construction of marsh area	2011 - 2012
Sand capping and LMB	2012

Fig. 4.5: Aerial view of Lake Kleine Melanen after reconstruction. Photograph W. Cornelissen, 3 October 2013.

Fish were removed in September 2010 by electro fishing of the banks and trawl fishing of the open water, to prevent fish from being harmed during dredging and sand capping. Combined with the winter fish kill (February 2010), the total fish removal in 2010 was 75%, leaving behind a fish biomass of 152 kg ha^{-1} comprising all species originally present in Lake Kleine Melanen. As the fish kill of February 2010 affected mainly big carp (*Cyprinus carpio carpio*), the composition of the remaining fish stock differs from the original fish stock with a low biomass of remaining carp.

Dredging was done from November 2010 until May 2011. From the initial layer of soft sediment (mean thickness 0.93 m), in 3.5 ha of the lake the upper layer (mean thickness 0.64 m) was removed by a cutter dredger and a crane. In the remaining 0.5 ha of the lake the complete layer of soft sediment (mean thickness 1.30 m) was removed down to the peat substrate.

The trees and shrubs on the banks were pruned and some were felled in October and November 2011 to reduce the P-load by litter fall and provide more sun light for natural vegetation development on banks.

The marsh-area was introduced from December 2011 until February 2012 by removal of the wooden bank protection and construction of natural banks with faintly descending under water slopes (inclination 1 : 15), covering 20% of the lake area.

Sand capping and LMB were applied to the lake from August 2012 until October 2012. Clean coarse sand was used (D50>250 µm, ≤5% weight of particles < 65 µm), was mixed with lake-water and sprayed on the water surface (Fig. 4.6). Four successive sand layers each of 5 cm thick were deposited on top of the remaining soft sediment and peat. Although bioturbation enhances sediment P-release (Holdren & Armstrong, 1980), a sand layer of 20 cm prevents bioturbation by fish, as this is largely limited to the upper 10-15 cm of the sediment (Davis, 1974; Ten Winkel & Davids, 1985; Adámek & Maršálek, 2013). 16.57 tonnes of LMB were applied to the lake (414 g LMB m^{-2}), tailored to match the concentration of potentially bioavailable P in the upper 10 cm of the freshly exposed sediment after the dredging as has been determined by sequential P fractionation (Psenner et al., 1984; Hupfer et al., 2009; Institut Dr. Nowak, 2011). The LMB was mixed with the lowest 5 cm layer of sand, to improve positioning of the LMB on the lake's bottom and to reduce the risk of direct contact of the LMB with organic matter in the sediment as it is known that organic matter hampers the P-binding capacity of LMB (Geurts et al., 2011; Lürling et al., 2014b).

Fig. 4.6: Application of sand capping in Lake Kleine Melanen (7 September 2012).

The reduction of the major external P-source, the separated sewer overflows, will be addressed no sooner than 2017, because political considerations overruled the recommendations that were based on the diagnostics. After the reduction of the external P-load from the separated sewer overflows, flocculation and fish stock management to a new fish stock (~75 kg ha^{-1}) dominated by pike (*Esox lucius*) will finalize the restoration.

Sampling

Enclosure experiment

The enclosure experiment lasted from 8 March 2010 (day 0) until 3 June 2010 (day 87). Prior to the enclosure experiment, the lake was sampled on day -11. The enclosures and the lake were sampled on day -4 and 0-1-4-11-24-45-59-87 at a water depth of 30 cm. At day -4, the samples from the enclosures were taken 1.5 h after the addition of the sand, while at day 0 the samples were taken 1.5 h after the addition of the LMB and PAC. On every sampling date, the pH and oxygen (O_2) concentrations were determined in situ using a WTW Multi 350i meter (WTW, Weilheim, Germany). Chlorophyll-*a* concentrations were determined in the laboratory with a PHYTO-PAM phytoplankton-analyzer (Heinz Walz GmbH, Effeltrich, Germany), differentiated into cyanobacteria, green algae and diatoms and calibrated against the Dutch standard procedure (NNI, 2006b). Turbidity was measured using a Hach 2100P turbidity meter. TP and total nitrogen (TN) concentrations were analyzed in unfiltered samples and o-P in filtered samples (0.45 µm membrane filter, Polydisk), using a continuous flow analyzer (Skalar Analytical BV, Breda, The Netherlands) following the Dutch standard protocols according to NNI (2005a, 2005b, 2006a). Total lanthanum (TLa) analysis was conducted in unfiltered samples and filterable La (FLa) in filtered samples (0.45 µm membrane filter, Polydisk) according to NNI (2009) by inductively coupled plasma optical emission spectrometry ICP-OES in 2010 (detection limits 6-12 µg L^{-1}). The phytoplankton genus composition and the biovolumes were microscopically determined on days 56 and 87.

Whole lake monitoring

The lake was sampled during the daytime (between 8 and 14 h) at the water depth of 0.3 m in 1993, 1997, 2000, 2002-2005, 2008-2014 (for sampling locations see Fig. 4.3), mostly on a monthly basis. Secchi depth was determined using a 20 cm diameter Secchi disc. O_2 concentrations were determined in situ using a WTW Multi 350i meter (WTW, Weilheim, Germany). Concentrations of TP, o-P and chlorophyll-*a* (spectrophotometric method) were analyzed in the laboratory following the Dutch standard protocols (NNI, 2005a, 2005b, 2006b and predecessors). TLa analysis was conducted in unfiltered samples and FLa in filtered samples (0.45 µm membrane filter, Polydisk) according to NNI (2009), inductively coupled plasma optical emission spectrometry ICP-OES in 2010 (detection limits 6-12 µg L^{-1}) and inductively coupled mass spectrometry ICP-MS from 2012 until 2014 (detection limits 0.02-0.2 µg L^{-1}). The separated sewer overflows were sampled on 27 August 2010 and 11 November 2010 after rain storms on three locations. These samples were analyzed for the concentrations TP and o-P. On 30 July, 18 August and 31 August 2009 samples from the lake water were taken for microscopic determination of the dominating phytoplankton taxa.

Sediment core-samples were taken on 14, 15 and 16 September 2009 at six locations spread over the lake, using a Beeker core sampler (Eijkelkamp Agrisearch, Giesbeek, The Netherlands; Waltjé et al., 2009). From the cores, subsamples were taken from the top-layer of the soft sediment (0-10 cm) and from the top of the underlying peat (5-15 cm). The

pore water was anaerobically sampled from both layers using rhizons (0.1 µm; Eijkelkamp Agrisearch, Giesbeek, The Netherlands; Waltjé et al., 2009). The sediment samples were individually analyzed for concentrations of TP, S and Fe. Three soft sediment samples from the northern part of the lake were mixed, as well as three soft sediment samples from the southern part. From each mixed sample, the potential releasable P fraction was determined according to Golterman (1996). The same was done for the samples of underlying peat substrate. Pore water was analyzed for concentrations TP, S and Fe. Analyses were performed according the Dutch standard protocols (Waltjé et al., 2009).

To determine the amount of LMB to be used, the potentially releasable P fraction after the dredging was measured at eight locations spread over the lake, in samples taken with an Ekman-Birge sampler from the upper layers of the sediment (0-5 cm and 5-10 cm). Four samples of sediment from the northern part of the lake were mixed as well as four samples from the southern part. Each mixed sample was fractionated in TP and o-P (cf. Psenner et al., 1984; Hupfer et al., 2009; Institut Dr. Nowak, 2011).

Data analysis

The concentrations of chlorophyll-*a*, TP, o-P, TN, O_2, TLa and FLa, and the turbidity from day 0 until day 87 in the enclosure experiment were analyzed by non-parametric Kruskal-Wallis Test ($P < 0.05$) and post hoc analyses by pairwise comparisons using Dunn's (1964) procedure. A Bonferroni correction for six multiple comparisons was made in order to correct for Type I errors ($P < 0.05$). Parallel lines analysis of concentrations of o-P for individual enclosures from day 0 until day 59 was done in SigmaPlot 12.5 (Systat Software Inc.). Water quality variables of the lake were analyzed for the periods before (Jan 2008 – Aug 2010), during (Sep 2010 – Oct 2012) and after the whole lake measures (Nov 2012 – Oct 2014) by one-way repeated measures (rm)ANOVA followed by post hoc analysis with a Bonferroni adjustment ($P < 0.05$). TN data were natural log transformed to fulfil normality requirements. In case Mauchly's test indicated a violation of the assumption of sphericity, a Greenhouse-Geisser correction was used ($\varepsilon < 0.75$). A non-parametric Friedman test ($P < 0.05$) was used in case requirements for rmANOVA were violated. Analyses, except the parallel lines analysis, were done in SPSS 21 (IBM). When the concentration was below the detection limit, the value of half the detection limit was used in data analysis.

Results

Baseline situation of Lake Kleine Melanen and restoration planning

Over the period 2008-2009, the mean TP concentration was 0.31 mg L^{-1} and the mean chlorophyll-*a* concentration was 107 µg L^{-1} (Table 4.1). The water balance showed that 14% of the inflow originated from precipitation on open water and for 86% from precipitation on impervious surfaces collected in the separated sewer system (Appendix 4.A). The external P-load to the lake was 7.5 mg P m^{-2} d^{-1} (Table 4.3; Appendix 4.A), of which 87% originated from the separated sewer system. The remaining 13% of the external P-load consisted of

Table 4.3: External P-loads (mg P m^{-2} d^{-1}) of Lake Kleine Melanen before restoration.

	P-load (mg P m^{-2} d^{-1})
Separated sewer system	6.5
Deposition on lake	0.2
Water birds	0.2
Feeding of water birds	0.1
Litter fall	0.3
Bait angling	0.1
Dog faeces and urine	0.1
Total	7.5

several sources, with litter fall being the second largest (Table 4.3) supplying 4%. The critical P-load thresholds were 3.4 mg P m^{-2} d^{-1} (cP_{oligo}) and 5.2 mg P m^{-2} d^{-1} (cP_{eutro}) and were below the external P-load. The top layer of the peat had a higher amount of dry matter (25.1%) and organic matter (64.5%) than the top layer of the accumulated soft sediment (9.5% and 47.9%; Table 4.4A). The P, S and Fe concentrations of the top layer of the soft sediment were 7-18 times higher than the P, S and Fe concentrations of the top layer of the peat (Table 4.4A). P and Fe concentrations of the pore water in the top layer of the soft sediment were 7-38 times higher than the concentrations of P and Fe of the pore water in the top of the peat, while the S concentrations were in the same range (Table 4.4B). Based on visual observations during sampling, the structure of the soft sediment layer was homogeneous over depth (Waltjé et al., 2009). The P-release of the soft sediment, as deduced from a known correlation between the P concentration of the pore water and the sediment P-release (Van der Wijngaart et al., 2012), was 3.8 mg P $m^{-2}d^{-1}$.

Table 4.4: Sediment characteristics (density, % dry matter, % organic matter) and concentrations of P, Fe and S in sediment (A; g kg^{-1} DW) and pore water (B; mg L^{-1}). DW = dry weight. In parentheses ± SE (data modified from Waltjé et al., 2009).

A				g kg^{-1} DW		
	density (kg L^{-1})	dry matter (%)	organic matter (% of DW)	P	Fe	S
Soft sediment (upper 10 cm)	1.04	9.5 (2.5)	47.9 (3.3)	1.8 (0.3)	23.1 (3.6)	20.4 (3.2)
Peat (upper 10 cm)	1.33	25.1 (10.1)	64.5 (15.8)	0.16 (0.03)	1.5 (0.3)	3.9 (1.0)

B	mg L^{-1}		
	P	Fe	S
Soft sediment (upper 10 cm)	5.05 (0.35)	7.28 (0.76)	0.52 (0.01)
Peat (upper 10 cm)	0.87 (0.10)	0.19 (0.08)	0.73 (0.30)

The Fe:P (mol mol^{-1}) ratio of the pore water of the underlying peat was on average 0.1 indicating the risk of P-release from freshly exposed peat after sediment removal (Smolders et al., 2001; Geurts et al., 2010). The fish biomass before the fish kill in the winter 2009/2010 was 605 kg ha^{-1} comprising 67% carp, 12% roach, 11% pike, 4% perch (*Perca fluviatilis*) and 6% other species (Kalkman, 2010c). Cyanobacteria and Euglenaceae dominated summer phytoplankton (Table 4.5).

Table 4.5: Biovolume (mm^3 L^{-1}) of dominating phytoplankton taxa in Lake Kleine Melanen on 30 July, 18 and 31 August 2009.

	30 July	18 August	31 August
Anabaena ssp.	62.4	26.5	0.6
Microcystis ssp.	2.9	17.0	6.9
Trachelomonas ssp.	23.9	0.1	0.1
Woronichinia ssp.	0.2	14.8	12.8

Enclosure experiment

At day -4, the mean TP concentration in the enclosures was 0.39 mg L^{-1} (± 0.13 mg L^{-1}; n=11). During the course of the experiment (day 0 – 87), the treatments S, SL and SLP had an obvious effect on the TP concentration compared to the control enclosures C (Fig. 4.7). The effect was significant for the treatments SL and SLP (Table 4.6). The treatment SLP showed the significantly lowest TP concentration treatments SL and SLP (Table 4.6). The treatment

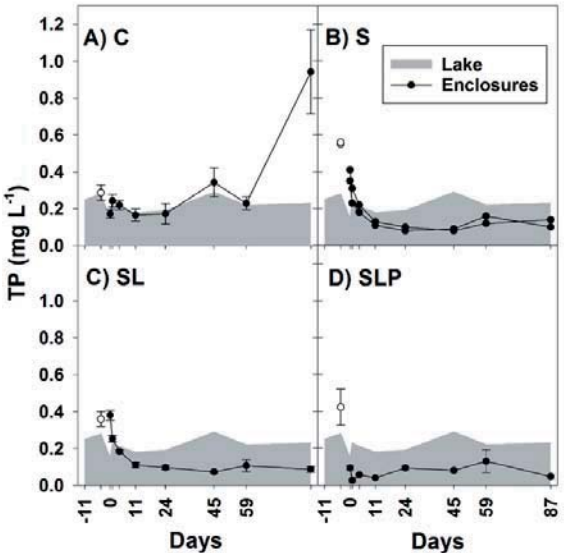


Fig. 4.7: Total phosphorus (TP) concentrations (mg L^{-1}) in enclosure experiment. Treatments: C (control, panel A), S (sand capping, panel B), SL (sand capping + LMB, panel C), SLP (sand capping + LMB + PAC, panel D). The grey areas indicate the TP concentration in the lake (days -11 – 87). Open symbols indicate the TP concentration on day -4, at 1.5 h after sand addition. Error bars indicate one SE (n = 3). In panel B the course of TP in each replicate is presented.

Table 4.6: Enclosure experiment: median concentrations of total phosphorus (TP, mg L^{-1}), ortho-phosphate (o-P, mg L^{-1}), total nitrogen (TN, mg L^{-1}), total and cyanobacterial chlorophyll-*a* (µg L^{-1}), total and filterable lanthanum (TLa, FLa, µg L^{-1}) and median turbidity (FTU) in control (C; n = 3) enclosures and in enclosures with sand capping (S; n = 2), sand capping + LMB (SL; n = 3) and sand capping + LMB + PAC (SLP; n = 3) during the 87 day experimental period. In parentheses ± 1SE except o-P. For o-P, the number of sampling events with a mean concentration < 0.01 mg P L^{-1} is given in parentheses (total number of sampling events = 8). The right column shows the results of non-parametric Kruskal-Wallis test. The capitals (A, B, C) indicate significantly different groups ($P < 0.05$).

	Control (C)	Sand (S)	Sand+LMB (SL)	Sand+LMB+PAC (SLP)	
TP (mg P L^{-1})	0.22^{A} (0.06)	0.14^{AB} (0.03)	0.12^{B} (0.02)	0.07^{C} (0.01)	$\chi^2(3) = 42.144$
					$P < 0.0005$
o-P (mg P L^{-1})	0.01 (3)	0.01 (4)	0.01 (6)	0.01 (7)	
TN (mg N L^{-1})	3.50^{A} (0.19)	1.95^{B} (0.20)	1.95^{B} (0.10)	1.75^{B} (0.11)	$\chi^2(3) = 48.006$
					$P < 0.0005$
Total chlorophyll-*a* (µg L^{-1})	121.8^{A} (19.4)	20.5^{BC} (14.4)	13.9^{B} (11.0)	6.6^{C} (1.7)	$\chi^2(3) = 31.462$
					$P < 0.0005$
Cyanobacterial chlorophyll-*a* (µg L^{-1})	10.0^{A} (3.0)	2.0^{AB} (2.2)	1.2^{AB} (1.6)	0.1^{B} (0.2)	$\chi^2(3) = 16.912$
					$P = 0.001$
Turbidity (FTU)	11.0^{A} (1.3)	14.5^{A} (11.3)	18.5^{A} (10.5)	2.6^{B} (0.5)	$\chi^2(3) = 22.399$
					$P < 0.0005$
TLa (µg L^{-1})	$< 12.0^{A}$ (0)	$< 12.0^{A}$ (0)	1327.5^{B} (276.8)	292.5^{B} (61.0)	$\chi^2(3) = 75.895$
					$P < 0.0005$
FLa (µg L^{-1})	$< 12.0^{A}$ (0)	$< 12.0^{A}$ (0)	67.3^{B} (14.0)	21.4^{B} (4.5)	$\chi^2(3) = 61.106$
					$P < 0.0005$

SLP showed the significantly lowest TP concentration (Table 4.6) The reduction was most obvious during the first 11 days of the experiment (Fig. 4.7). In the SLP treatment, the mean TP concentration over the entire 87 day experimental period was 0.07 mg P L^{-1}, compared to 0.31 mg P L^{-1} in the C enclosures and 0.21 mg P L^{-1} in the lake. The mean o-P concentration in the SL and SLP treatments was < 0.01 mg P L^{-1} which was lower than in the S treatment (0.021 mg P L^{-1}) and in the controls C (0.065 mg P L^{-1}) and in the lake (0.036 mg P L^{-1}). Parallel lines analyses showed that the o-P concentrations in the S enclosures had a similar increase over time (day 0 – 59) as in the C enclosures ($F_{1, 38} = 0.19$, $P = 0.67$), in contrast to the SL ($F_{1, 38} = 34.40$, $P < 0.0001$) and to the SLP enclosures ($F_{1, 38} = 30.51$, $P < 0.0001$) which reacted significantly different from the S enclosures. After day 59, the o-P concentrations in the C enclosures varied between 0.11 mg P L^{-1} and 0.65 mg P L^{-1} and increased more than in the S enclosures (0.06 – 0.07 mg P L^{-1}), while o-P concentrations in the SL and in the SLP enclosures remained at or below the detection limit of 0.01 mg P L^{-1} (Fig. 4.8). TN concentrations in S, SL and SLP enclosures had medians of 1.75-1.95 mg L^{-1} that were significantly lower than in the C enclosures (3.50 mg L^{-1}; Table 4.6). The TN concentration in the SLP treatment was lower than in the S and SL treatments, but this difference was not significant (Table 4.6).

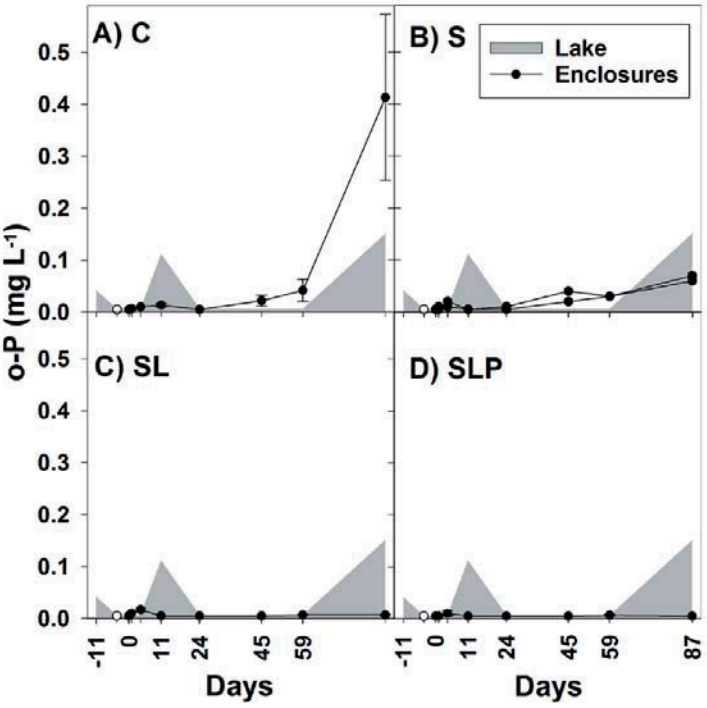


Fig. 4.8: Ortho-phosphate (o-P) concentrations (mg L^{-1}) in enclosure experiment. Treatments: C (control, panel A), S (sand capping, panel B), SL (sand capping + LMB, panel C), SLP (sand capping + LMB + PAC, panel D). The grey areas indicate the TP concentration in the lake (days -11 – 87). Open symbols indicate the o-P concentration on day -4, at 1.5 h after sand addition. Error bars indicate one SE (n = 3). In panel B the course of o-P in each replicate is presented.

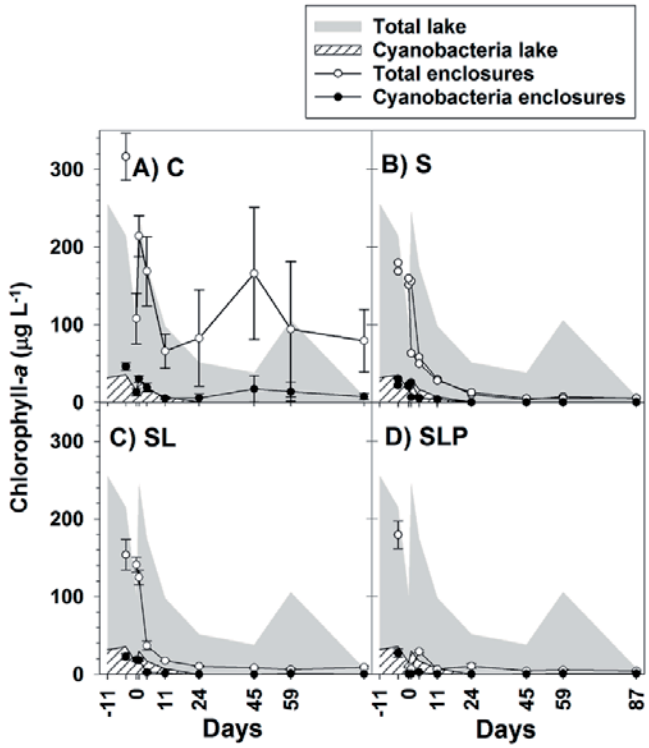


Fig. 4.9: Concentrations total chlorophyll-*a* (µg L^{-1}; open symbols) and cyanobacterial chlorophyll-*a* (µg L^{-1}; filled symbols) in enclosure experiment. Treatments: C (control, panel A), S (sand capping, panel B), SL (sand capping + LMB, panel C), SLP (sand capping + LMB + PAC, panel D). The grey areas indicate the total chlorophyll-*a* concentration and the hatched areas indicate the cyanobacterial chlorophyll-*a* concentration in the lake (days -11 – 87). Symbols at day -4 indicate the chlorophyll-*a* concentrations 1.5 h after sand addition. Error bars indicate one SE (n = 3). In panel B the course of total and cyanobacterial chlorophyll-*a* in each replicate is presented.

Total chlorophyll-*a* concentrations in the lake were high during the first 59 days of the experiment, varying between 38 and 246 µg L^{-1} (Fig. 4.9). Cyanobacterial chlorophyll-*a* concentrations in the lake during the experimental period reached a maximum of 30 µg L^{-1} on day 1 and then decreased to 0 µg L^{-1} on day 24. In the C enclosures the cyanobacterial chlorophyll-*a* concentration varied between 5 and 30 µg L^{-1} (Fig. 4.9A). The S, SL and SLP treatments showed significantly lower total chlorophyll-*a* concentrations than the C enclosures with the lowest concentration in the SLP treatment (Table 4.6). The SLP enclosures showed a significantly lower cyanobacterial chlorophyll-*a* concentration than the C enclosures, while the concentrations in the treatments S and SL were not significantly lower than in C (Fig. 4.9, Table 4.6). During the first three weeks of the experiment, the decrease in concentrations of total and cyanobacterial chlorophyll-*a* was more pronounced in the treatments SL and SLP than in S (Fig. 4.9).

Turbidity on day 0 was on average 120 FTU in the S enclosures and 137 FTU in the SL enclosures. In the SLP enclosures, the turbidity was 7 FTU on day 0, while the mean was 3 FTU during the entire experimental period. In the S and SL enclosures turbidity decreased below 10 FTU after 24 days. Turbidity in the SLP enclosures was significantly lower than in the S and SL treatments, which in turn did not differ significantly from the C enclosures (Table 4.6).

TLa and FLa concentrations in the lake, in the C enclosures and in the S enclosures were below the detection limit of 12 µg L^{-1} during the entire experimental period. On day 0, the mean concentrations were 4133 µg TLa L^{-1} and 47 µg FLa L^{-1} in the SL enclosures and 753 µg TLa L^{-1} and 42 µg FLa L^{-1} in the SLP enclosures. The initially increased TLa and FLa concentrations in the SL and SLP enclosures decreased over time, but started to rise again from day 45 on. On day 87, the mean TLa and FLa concentrations had increased to 790 µg TLa L^{-1} and 187 µg FLa L^{-1} in the SL enclosures and to 157 µg TLa L^{-1} and 40 µg FLa L^{-1} in the SLP enclosures. The concentrations TLa and FLa were lower in the SLP enclosures compared to the SL enclosures, but the differences were not significant (Table 4.6).

During the experimental period, the pH in the lake varied from 7.2 to 9.2 and in the C enclosures from 6.6 to 8.6. In the S enclosures, the pH varied from 8.0 to 9.0, in the SL enclosures from 7.2 to 8.6 and in the SLP enclosures from 7.1 to 8.2. Oxygen (O_2) concentrations varied in the lake from 9.0 mg L^{-1} (83% saturation) to 18.0 mg L^{-1} (138%), in the C enclosures from 5.7 mg L^{-1} (50%) to 24.5 mg L^{-1} (198%), in the S enclosures from 8.2 mg L^{-1} (72%) to 15.0 mg L^{-1} (133%), in the SL enclosures from 3.8 mg L^{-1} (33%) to 15.7 mg L^{-1} (117%), and in the SLP enclosures from 6.4 mg L^{-1} (56%) to 18.4 mg L^{-1} (142%).

Whole-lake measures

After the measures were taken, a reduction of the mean TP concentration from 0.30 ± 0.02 mg L^{-1} (Jan 2008 – Aug 2010) to 0.11 ± 0.01 mg L^{-1} (Nov 2012 – Oct 2014; Table 4.7) was observed. A rmANOVA revealed that the mean TP concentration after the measures were

Table 4.7: Whole lake measures: mean concentrations of total phosphorus (TP, mg L^{-1}), ortho-phosphate (o-P, mg L^{-1}), total nitrogen (TN, mg L^{-1}), chlorophyll-*a* (µg L^{-1}), total and filterable lanthanum (TLa, FLa, µg L^{-1}), oxygen (O_2, mg L^{-1}) and mean Secchi depth (m) in Lake Kleine Melanen before (Jan 2008 – Aug 2010), during (Sep 2010 – Oct 2012) and after (Nov 2012 – Oct 2014) measures were executed, based on monthly sampling except TLa and FLa. In parentheses ± 1SE. * significant difference before and after measures ($P < 0.05$).

	Before measures	During measures	After measures
TP (mg P L^{-1})*	0.30 (0.02)	0.30 (0.04)	0.11 (0.01)
o-P (mg P L^{-1})*	0.10 (0.02)	0.04 (0.01)	0.01 (0.00)
TN (mg N L^{-1})	2.30 (0.10)	2.79 (0.30)	1.95 (0.11)
Chlorophyll-***a*** (µg L^{-1})*	111 (20)	74 (11)	39 (5)
Secchi depth (m)	0.54 (0.03)	0.47 (0.06)	0.61 (0.05)
TLa (µg L^{-1})[1)]	< 0.2	434.8 (130.4)	38.1 (6.0)
FLa (µg L^{-1})[1)]	< 0.02	23.06 (5.57)	6.68 (1.15)
O_2 (mg L^{-1})	9.9 (0.8)	9.0 (0.5)	9.6 (0.4)

[1)] Measurements before LMB application on 15 August 2012 (n = 1), during LMB application (n=8) and after LMB application (n = 21)

implemented was significantly lower than before ($F_{1.2, 29.0} = 15.4$, $P < 0.0005$, post hoc with Bonferroni adjustment $P < 0.0005$). The mean o-P concentration showed a significant decline over time from 0.10 ± 0.02 mg L^{-1} (Jan 2008 – Aug 2010) to 0.01 ± 0.00 mg L^{-1} (Nov 2012 – Oct 2014; Table 4.7; Friedman's test $\chi^2(2) = 10.848$, $P = 0.004$; pairwise comparison with Bonferroni correction $P = 0.006$). The TN concentration reached a maximum of 9.1 mg L^{-1} during the dredging period in the beginning of 2011 and then gradually declined to a mean of 1.95 mg L^{-1} (Nov 2012 - Oct 2014; Table 4.7). Although mean TN concentrations were lowest after the measures were undertaken (Table 4.7), the decrease was not significant (rmANOVA $F_{2, 46} = 3.0$, $P = 0.06$). The mean chlorophyll-*a* concentrations declined significantly over time from 111 ± 20 µg L^{-1} (Jan 2008 – Aug 2010) to 74 ± 11 µg L^{-1} (Sep 2010 – Oct 2012) and 39 ± 5 µg L^{-1} (Nov 2012 – Oct 2014; Table 4.7; rmANOVA $F_{1.3, 30.2} = 4.7$; $P = 0.03$). The period after the measures were implemented showed a significantly lower chlorophyll-*a* concentration than before ($P = 0.005$; Table 4.7). Cyanobacterial blooms still appeared (maximum cyanobacterial chlorophyll-*a* concentration 32 µg L^{-1} on 24 July 2013 and 17 µg L^{-1} on 24 June 2014). During the implementation of measures (Sep 2010 – Oct 2012), the mean Secchi depth was 0.47 m and was lower than before (0.54 m) and after implementation (0.61 m), but the difference was not significant (Friedman's test $\chi^2(2) = 0.067$, $P = 0.967$; Table 4.7). The TLa concentration increased from < 0.2 µg L^{-1} just before the LMB application to a mean of 434.8 µg L^{-1} (SE ± 130.4 µg L^{-1}) during the LMB application and 38.1 µg L^{-1} (SE ± 6.0 µg L^{-1}) after the application (Table 4.7). The difference of the TLa concentration before and during the LMB application was significant (Friedman's test $\chi^2(2) = 16.0$, $P < 0.0005$; pairwise comparison with Bonferroni correction $P < 0.0005$), but no significance was revealed for the difference in concentrations before and after the measures were implemented ($P = 0.137$). The FLa concentration increased from < 0.02 µg L^{-1} just before the

LMB application to a maximum of 59.7 µg L^{-1} during the LMB application and then gradually decreased to a mean value of 3.27 µg L^{-1} (SE ± 0.65 µg L^{-1}) during 2014. The difference of the FLa concentration before and during the LMB application were significant (Friedman's test $\chi^2(2) = 9.8$, $P = 0.008$; pairwise comparison with Bonferroni correction $P = 0.008$), but no significance was revealed for the difference in concentrations before and after the measures were undertaken ($P = 0.073$). Before the measures were implemented (Jan 2008 – Aug 2010), the O_2 concentration fluctuated from 2.2 to 20.8 mg L^{-1} with a mean concentration of 9.9 mg L^{-1} (SE ± 0.8 mg L^{-1}; Table 4.7). During the implementation of measures (Sep 2010 – Oct 2012) the O_2 concentration fluctuated from 0.8 to 15.6 mg L^{-1} with a mean concentration of 9.0 mg L^{-1} (SE ± 0.5 mg L^{-1}; Table 4.7). After the measures were completed (Nov 2012 – Oct 2014) the O_2 concentration fluctuated from 5.4 to 14.4 mg L^{-1} with a mean concentration of 9.3 mg L^{-1} (SE ± 0.4 mg L^{-1}; Table 4.7). No significant difference was revealed in O_2 concentrations before and after the measures were undertaken (rmANOVA $F_{2, 48} = 0.135$; $P = 0.874$).

Discussion

Baseline situation of Lake Kleine Melanen and restoration planning

Diagnostics showed that the separated sewer system was the most important contributor to the external P-load (Table 4.3; Appendix 4.A). Although intent of the separated sewer system was to separate waste water from rainwater and to discharge only rainwater into the lake, the mean TP concentration of the discharges was more than thirteen times as high as in rainwater itself (Appendix 4.A). High TP concentrations in discharges from separated sewer systems have been shown in other studies (Boogaard & Lemmen, 2007; Reddy et al., 2014). Pollution of the collected rainwater is most likely the result of urban sources such as street dirt, dog and bird faeces, fertilizers and plant matter (Waschbusch et al., 2000; Deffontis et al., 2013). The other external P-sources contributed in total 13% to the external P-load (Table 4.3). The exceedance of cP_{eutro} was in line with the phytoplankton dominated state of the lake (Table 4.1). A prerequisite for realization and maintenance of the clear water state is the decrease of the external P-load below cP_{oligo}. A reduction of the P-load from the separated sewer system of 35-63% would reduce the total external P-load to the critical range where alternative stable states exist (Fig. 4.2). However, to restore the lake there is the need for a vigorous reduction of the average yearly external P-load to a level well below cP_{eutro}, due to natural variations of P-loads over time, heterogeneity in data that were used to calculate the P-loads (Appendix 4.A) and model errors up to 0.4 in PCLake results (Janse, 2005; Janse et al., 2008; Mooij et al., 2010).

After deepening of the lake, the sediment posed a risk for P-release from the remaining soft sediment and from freshly exposed peat (Smolders et al., 2001; Geurts et al., 2010). As sediment P-release can hamper lake recovery for many years (Søndergaard et al., 1999), the reduction of the in-lake P-source was incorporated in the restoration planning. Sediment capping was considered a promising measure to reduce sediment decomposition and P-release (Reible & Lampert, 2014), and the efficacy of this measure was tested in the

enclosure experiment.

The initial fish stock was dominated by carp, most of which were killed during winter 2009/2010. However, due to the appreciation of carp by anglers, recolonisation of the lake with carp was to be expected. Sediment resuspension by carp keeps the water turbid and prevents the establishment of macrophytes (Chumchal et al., 2005; Cline et al., 1994; Meijer et al., 1999). As submerged macrophytes play a key role in lake recovery and enhancing the clear water state (Scheffer et al., 1993), biomanipulation on fish was included in the restoration planning.

Enclosure experiment

The enclosure experiment showed significantly lower concentrations of TP and total chlorophyll-*a* in the SLP enclosures compared to the C, S and SL enclosures (Table 4.6), which is ascribed to P-fixation and precipitation by LMB and PAC (Lürling & Van Oosterhout, 2013). Turbidity in the SLP enclosures was significantly lower than in the S and SL enclosures (Table 4.6). Although sand capping and LMB (S, SL) initially increased turbidity, it decreased again during the first 24 days after application because of sedimentation of sand and clay particles (Hickey & Gibbs, 2009; Van Oosterhout & Lürling, 2011). In the SLP enclosures, turbidity was reduced instantly after application of the PAC, due to the strong and instant precipitating effect of PAC in combination with the LMB as ballast material (Lürling & Van Oosterhout, 2013). Compared to exclusively sand capping (S), lower o-P concentrations were not detected in the LMB applications (SL enclosures; Table 4.6). Another enclosure experiment likewise showed no o-P reduction after LMB addition (Lürling & Faassen, 2012). Interference with humic substances is a plausible explanation (Lürling et al., 2014b). In the presence of humic substances, complexation of La from LMB with humic substances prevents La to bind with phosphates (Lürling et al., 2014b). The organic matter content of the soft sediment (47.9%; Table 4.4A) and the DOC concentration of the lake water (mean DOC concentration in 2014 was 8.5 mg L^{-1}, SE ±0.8, n=6; unpublished data Water Authority Brabantse Delta) indicated the potential of complex-formation of La with humic substances (Lürling et al., 2014b). The SLP treatment however, showed more often o-P concentrations < 0.01 mg P L^{-1} (7 times out of 8) than in the S treatment (4 times out of 8). Both SL and SLP enclosures did not show the increase in o-P concentrations over time which was observed in the C and S enclosures (Fig. 4.8). This increase of o-P concentrations in the S enclosures contrasts with findings of Pan et al. (2012) who showed that sand capping reduced the sediment to water o-P flux. A capping layer of clean sand cannot prevent P diffusion from pore water to the overlying water (Pan et al., 2012) and the P transport through the capping layer can be enhanced by groundwater seepage and by sediment compression (Mohan et al., 2000). The most obvious reductions in concentrations of TP, o-P and chlorophyll-*a* were observed during the first 11 days of the experiment (Figs. 4.7, 4.8, 4.9). The SLP treatment showed an instant reduction of the concentrations of TP and chlorophyll-a, while in the treatments S and SL it took ~11 days to reach similar low levels (Figs. 4.7, 4.9). After this

initial period, the mean concentrations remained lowest in the SLP enclosures, although no significant difference between the treatments S, SL and SLP could be revealed for the concentrations of TP, cyanobacterial chlorophyll-*a* and total chlorophyll-*a* (days 11 – 87).

Application of LMB significantly raised TLa and FLa concentrations (Table 4.6), due to the La content of the LMB (Spears et al., 2013b). Although application of PAC lowered the median TLa and FLa concentrations in the SLP treatment, the difference with the SL treatment was not significant (Table 4.6). Due to product settling the TLa and FLa concentrations decrease over time (Spears et al., 2013b), as was observed in the SL and SLP enclosures during the first eleven days after the application, when the mean TLa concentration dropped from 4133 µg L^{-1} (day 0) to 127 µg L^{-1} (day 11) in the SL enclosures and from 753 µg L^{-1} (day 0) to 37 µg L^{-1} (day 11) in the SLP enclosures. Until day 11 the Dutch standard for TLa of 150 µg L^{-1} (http://www.rivm.nl/rvs/Normen/Eindresultaat?groep=normen&waarde=lanthanum%28freshwater%29&lijst=milieukwaliteit&veld=englishsubstancename_tagged, accessed 8 December 2014) was violated in the SL and SLP enclosures. The FLa concentration decreased in the same period from 47 µg L^{-1} to 16 µg L^{-1} in the SL enclosures, and from 42 µg L^{-1} to 11 µg L^{-1} in the SLP enclosures, both violating the Dutch standard for FLa of 10.1 µg L^{-1} (Sneller et al., 2000; http://www.rivm.nl/rvs/Normen/Eindresultaat?groep=normen&waarde=lanthanum%28freshwater%29&lijst=milieukwaliteit&veld=englishsubstancename_tagged, accessed 8 December 2014). Strikingly, from day 45 on an increase in TLa and FLa concentrations was observed in the SL as well in the SLP enclosures, violating the Dutch standards on day 87. The pattern of increases of FLa concentrations over time, after the initial decrease, is similar to results from an enclosure experiment in an urban pond in which LMB was applied in combination with the removal of soft sediment down to the hard sandy substrate. A plausible explanation is the uptake of La and excretion by zooplankton during diurnal migration (Engels et al., 2011). Spears et al. (2013b) postulate that after the initial decrease of the TLa concentration due to product settling, an increase of the TLa concentration can be ascribed to biologically and physically induced bed sediment disturbance processes, while the FLa concentration is regulated by physico-chemical conditions of the water. During sampling events, ebullition of gas bubbles from the sediment was observed in the S, SL and SLP enclosures, probably caused by the production of methane (Barros et al., 2014). The sediment disturbance caused by ebullition is likely to be important for the increase of the TLa concentrations shown in this study.

In conclusion the treatment SLP showed the strongest reduction of concentrations of TP and chlorophyll-*a*. Especially during the initial period (21 days) the reduction in concentrations and the clarifying effect were strongest in the SLP enclosures, which is important for whole lake application as it might open a window of opportunity for submerged macrophytes to get established when applied during springtime. Based on the results of the enclosure experiment, SLP was selected as the best treatment to scale up to the whole lake.

The increases of La concentration over time is a matter of concern as La has the potential of unintended ecotoxicological impacts (Lürling & Tolman, 2010; Spears et al.,

2013a; Van Oosterhout et al., 2014) and the violation of the water quality standards for TLa and FLa pose legal consequences. Although the knowledge on this aspect is limited (Copetti et al., 2016), the issue should be considered when preparing a treatment and the potential ecological consequences of the use of La should be weighed against the negative consequences of cyanobacterial blooms. SLP had the advantage over SL of a lesser increase of the TLa and FLa concentrations, calling for a short period between LMB and PAC applications.

pH values of 8.5 and above pose the risk of dissolution of sediment $Al(OH)_3$ to form toxic aluminate $Al(OH)_4^-$ (Reitzel et al., 2013a). pH in the lake showed a maximum of 9.2, probably driven by the photosynthesis of phytoplankton. The SLP treatment showed low chlorophyll-*a* concentrations (Table 4.6) with a maximum pH of 8.2. The mean concentration of filterable Al in the SLP enclosures got up to 82.7 µg L^{-1} and in the control enclosures to 54.0 µg L^{-1} (data not shown). The concentrations in the SLP enclosures remained below the indicative maximum permissible concentration for dissolved Al of 312 µg L^{-1} as used in legislation in The Netherlands (Van de Plassche, 2003), below the upper limit for dissolved Al of 100 µg L^{-1} in hard water lakes (Burrows & Hem, 1977) and below the value of 150 µg L^{-1} known to cause undesired effects (Reitzel et al., 2013a). Nevertheless, it shows that flocculation with PAC only should be applied when there is not a risk of pH > 8.5.

To minimize the risk of direct contact of La with organic matter in the lake's sediment and water, the water manager decided to apply the LMB mixed into the sand. This burial was not tested in the enclosure experiment, but was selected as a mitigating measure based on the results of this experiment.

Whole lake measures and synthesis

After the measures were taken (Nov 2012 – Oct 2014), significant reductions in the concentrations of TP, o-P and chlorophyll-*a* were observed (Table 4.7). However, the reduction of the chlorophyll-*a* concentration was limited and cyanobacterial blooms still occurred. Secchi depth did not differ significantly from the situation before the measures and hardly any submerged macrophytes appeared. Although the sand capping layer prevented resuspension of soft sediment (Danielsson et al., 2007) and benefited the growth of submerged macrophytes in providing a firm sediment (Istvánovics et al., 2008), the underwater light penetration was limited for the development of an extensive submerged vegetation. The maximum depth for submerged angiosperm colonization in Lake Kleine Melanen was limited to 1 m (Chambers & Kalff, 1985), implying the lake to be too turbid for extensive growth of submerged macrophytes. After the measures, the O_2 concentrations did not drop below 2 mg L^{-1}, a threshold indicative of fish kill (Fang & Stefan, 2000). The LMB application raised the FLa concentrations, violating the Dutch standard for ~9 months. This period is comparable to recovery periods for FLa concentrations found after LMB applications in 16 case study lakes (Spears et al., 2013b).

Despite improvements in water quality, restoration targets were not met. The major external P-source, discharges from the separated sewer system, has not yet been reduced. The order of the measures targeting the internal and external P-sources was not ideal. Although it is preferred to start with the reduction of the external P-sources (Søndergaard et al., 2007; Jeppesen et al., 2012), this was not feasible given the challenges associated with reducing the external P-load. Split responsibilities in water management of the lake fostered this situation. The water authority took care of the in-lake measures, while the municipality had the liability for the separated sewer system. The intended construction of a bypass around the lake will divert most of the sewerage to a different water body, less susceptible to cyanobacterial blooms. The bypass will reduce the P-input from the separated sewer system into the lake by 91% to 1.6 mg P m^{-2} d^{-1}. However, the bypass will also reduce the water inflow into the lake and increase the residence time. The combination of these measures results in adjusted critical P-loads cP'_{oligo} of 1.0 mg P m^{-2} d^{-1} and cP'_{eutro} of 3.5 mg P m^{-2} d^{-1}. Combined with the finalizing food web control, the realization of the bypass will provide proper prospects for the substantial increase of Secchi depth, the reduction of the cyanobacterial blooms and the expansion of submerged macrophytes.

The restoration planning provided a coherent set of measures. The measures support and supplement each other. Each measure by itself is not powerful enough to realize a sustainable clear water state. The in-lake measures alone did not clarify the water, due to the remaining external P-loading, ongoing turbidity and lack of macrophytes. Although the chlorophyll-*a* concentration was significantly reduced after measures were taken (Table 4.7), this cannot be solely ascribed to the measures as unknown factors might have influenced the results. This is illustrated by the course of the chlorophyll-*a* concentration in the nearby urban Lake Groote Melanen during the same period. This lake has similar features and a eutrophication problem as Lake Kleine Melanen, but no measures have been executed in Lake Groote Melanen. During the period Nov 2012 – Oct 2014 the mean chlorophyll-*a* concentration in Lake Groote Melanen was 48 µg L^{-1} compared to 118 µg L^{-1} in the period June 2009 – Aug 2010. TP concentrations, however, remained high (0.48 mg L^{-1} from Nov 2012 – Oct 2014; 0.39 mg L^{-1} from June 2009 – Aug 2010; unpublished data Water Authority Brabantse Delta). These findings underpin the necessity of diagnostics when preparing urban lake restorations. Due to high anthropogenic pressures upon Lake Kleine Melanen, the required measures recommended are comprehensive resulting in substantial costs (~€1,800,000). A side-effect of this study is the growing awareness of the authorities for the potential negative effects when planning separated sewer systems. A copy and paste approach of the measures for Lake Kleine Melanen to other situations is not recommended. Local pressures differ from site to site, demanding an approach tailored to the local situation (Thornton et al., 2013). Including the stakeholders, such as anglers, in restoration planning is essential for acceptance and support of diagnostics-results and prolonged efficacy of measures.

Conclusions

Exceedance of the critical P-load of Lake Kleine Melanen was largely due to discharges from the separated rainwater sewer system. Sediment removal did not eliminate the risk of sediment P-release. An enclosure experiment, testing different sediment capping options, showed that the best reductions of concentrations of TP and chlorophyll-*a* were realized by combining capping by sand and LMB with a low dose of the flocculant PAC. The most important in-lake measures – dredging, sand capping and LMB - did not realize the clear water state yet, although the concentrations of chlorophyll-*a* and TP were significantly reduced. As shown by diagnostics and underpinned by the results of the in-lake measures, the discharges from the separated sewer system have to be substantially reduced to realize the clear water state.

Supplementary information to Chapter 4

Appendix 4.A: Water balance and external P-loads of Lake Kleine Melanen

Introduction

The water balance and external P-loads of Lake Kleine Melanen are described.

Water balance

The water balance for Lake Kleine Melanen was based on the general equation (1) given by Nõges (2005):

$I + P – E – O \pm \Delta V = 0$ (1)

in which I = inflow, P = precipitation on open water, E = evaporation from open water, O = outflow, ΔV = change in storage.

Specific for Lake Kleine Melanen the equation (2) becomes:

$Pre + Pl + R – El \pm \Delta V = 0$ (2)

in which Pre = collected rainwater from impervious surfaces (31.8 hectares) in the residential area which flows into the lake, Pl = precipitation on the lake, R = rest (the total of seepage, runoff, infiltration and outflow over the weir; groundwater seepage and infiltration were low and depending on season, the total of seepage and infiltration on an annual basis was negligible), ΔV = change in storage.

Precipitation and evaporation data originated from the nearby meteorological stations Bergen op Zoom and Wilhelminadorpe. Pre was calculated from precipitation and evaporation resulting in drainage from a known impervious area of 31.8 ha. It was assumed that impervious surfaces had a maximum storage of 2 mm, filled by rain events and emptied by subsequent evaporation. The water level of the lake was measured on an hourly basis which data were used to calculate ΔV. The water balance was determined on a daily basis for the period 2000-2009 and averaged per month for a period of one year. The water balance components are given in Table 4.A.1. Precipitation was fairly constant throughout the year, with a minimum in April and a maximum in November. The collected rainwater from precipitation on impervious surfaces in the residential area was the major incoming water source, while outflow over the weir was the major outgoing component of the water balance.

Table 4.A.1: Mean water balance components (m^3 $month^{-1}$) for Lake Kleine Melanen, averaged over the period 2000-2009 (P_{re}= collected precipitation from impervious surfaces in residential area, P_l = precipitation on the lake, R = rest (predominantly outflow over the weir), E = evaporation, ΔV = change in storage.

month	P_{re}	P_l	R	E	ΔV
Jan	18,362	2,629	-20,530	214	249
Feb	20,140	3,005	-23,238	725	-819
Mar	14,746	2,475	-15,769	2,200	-749
Apr	7,977	1,593	-6,962	3,870	-1,262
May	16,062	2,764	-13,181	5,232	412
Jun	14,979	2,532	-12,750	5,958	-1,196
Jul	26,372	4,292	-24,549	5,503	612
Aug	23,838	3,905	-23,522	4,465	-244
Sep	16,572	2,783	-17,235	2,903	-783
Oct	19,470	3,072	-19,715	1.359	1,468
Nov	27,779	4,056	-29,406	454	1,975
Dec	24,881	3,498	-26,515	118	1,746
Total	231,177	36,603	-233,372	-32,999	1,409

External P-loads

The external P-loads were based on incoming loads by different sources: net precipitation from impervious surfaces, precipitation on the lake, water birds, feeding of birds, bait used by anglers, leaf litter from trees and shrubs surrounding the lake, and dog excrements that were left on the banks and transported to the lake by runoff. The external P-loads from these sources were based on measurements in this study and on references, as specified below.

• Precipitation from impervious surfaces. Immediately after heavy storm events on 27 August 2010 and 11 November 2010 samples were collected in this study from the separated rainwater sewer system at three different locations just before the rainwater was discharged into the lake. The locations are situated ~50 m from the shoreline, south and southeast of the lake. Samples were analyzed for total phosphorus (TP) and ortho-P (o-P) according the methods for surface water as applied at Lake Kleine Melanen. Runoff from impervious surfaces in the residential area at Lake Kleine Melanen contained a TP concentration of on average 0.41 mg P L^{-1} (SE ± 0.11) and o-P 0.18 mg P L^{-1} (SE ± 0.08); Table 4.A.2. Although results differ between rain storm events, the average results for TP in this study are similar to average results obtained from separated rainwater sewer systems collecting rainwater from impervious surfaces in residential areas in other parts of The Netherlands (Boogaard & Lemmen, 2007; mean TP = 0.42 mg P L^{-1}). The P-load caused by the separated rainwater sewer system was determined by multiplication of the mean TP concentration in the sewer system at Lake Kleine Melanen by the mean annual quantity of the discharged water (Table 4.A.1), resulting in a P-load on the lake of 6.5 mg P m^{-2} d^{-1}.

Table 4.A.2: Concentrations of total phosphorus (TP, mg L^{-1}) and ortho-P (o-P, mg L^{-1}) in the separated sewer system at Lake Kleine Melanen during two storm events. A, B, C: locations of discharge of the sewer system into the lake. 1, 2: storm events, 1 = 27 August 2010, 2 = 11 November 2010. ND = not detected.

	A		B		C	
	1	2	1	2	1	2
TP (mg L^{-1})	0.74	0.58	0.56	0.22	0.07	0.28
o-P (mg L^{-1})	0.49	0.16	0.06	ND	0.04	0.16

• Deposition on the lake. Monthly data for the o-P deposition were used, as given for the meteorological station Huijbergen at a distance of 10 km from Lake Kleine Melanen (Stolk, 2001). We assumed that o-P is 50% of TP in rainwater (Buijsman, 1989). Monthly precipitation on the lake was used as averaged over the period 2000-2009 (Table 4.A.1). TP concentration of rainwater was, corrected for the amount of monthly precipitation, on average 0.03 mg P L^{-1} (SE ± 0.01) and o-P 0.01 mg P L^{-1} (SE ± 0.01) (Stolk, 2001). The resulting P-load from precipitation on the lake is 0.2 mg P m^{-2} d^{-1}.

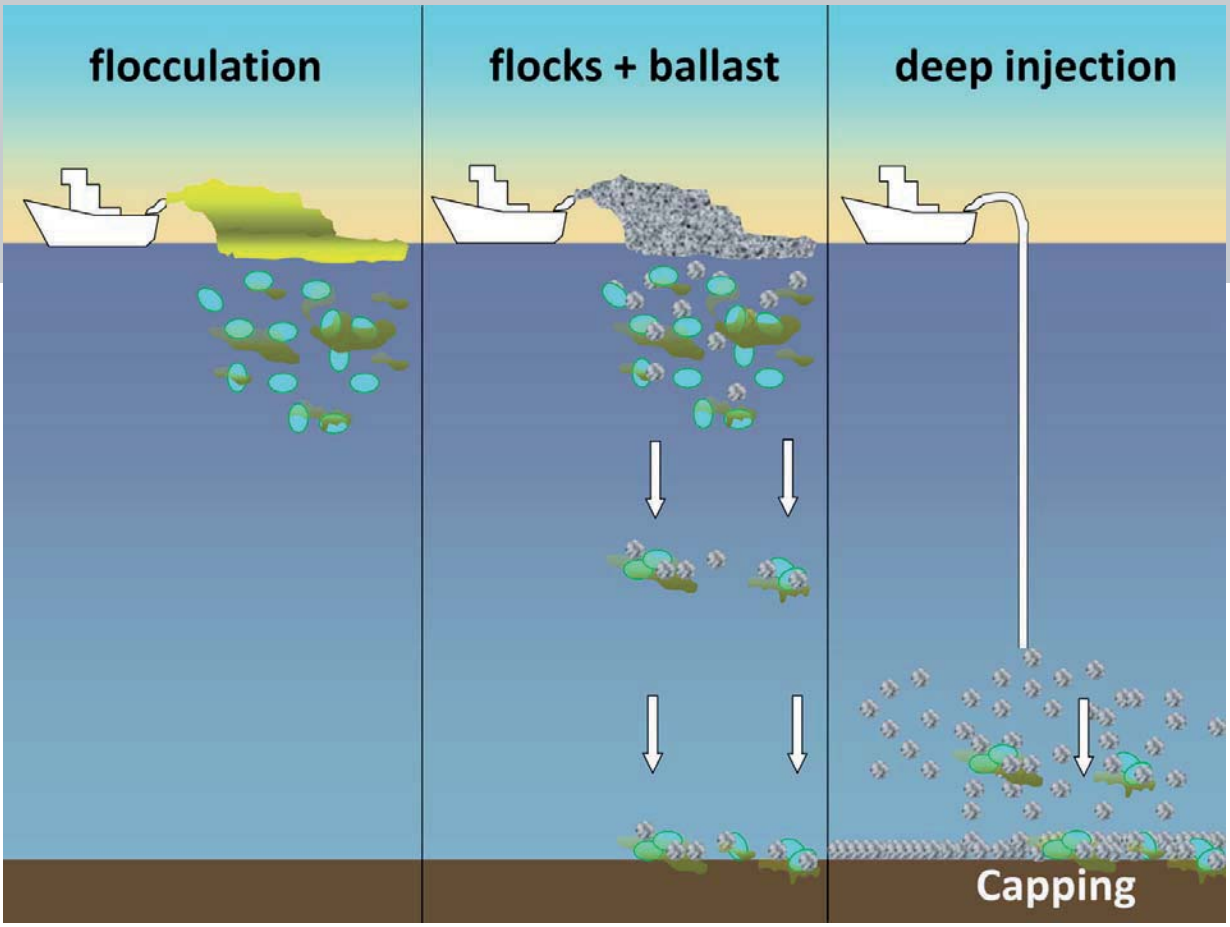
• Seepage of groundwater (including runoff) was a negligible small term of the water balance (<440 m^3 y^{-1}) and was based on seepage data from nearby areas (data not shown). As the volume is limited and the mean TP concentration of the phreatic groundwater in a nearby gauge was 0.06 mg P L^{-1} (*unpublished data*, 2003), this term is neglected as a P-source.

• Water birds. The amount of P-load from water birds was estimated as average from the food intake and dropping models (Hahn et al., 2007, 2008) and calculated with Waterbirds 1.1. Bird counts were done in this study during sampling events in 2009, 2010, and 2014. On average, the daily number of water birds on the lake were in Winter greylag goose (*Anser anser*) 10, Canada goose (*Branta canadensis*) 10, Eurasian coot (*Fulica atra*) 30, mallard (*Anas platyrhynchos*) 10, common gull (*Larus canus*) 15, cormorant (*Phalacrocorax carbo*) 1, and in Spring and Summer greylag goose 15 and Canada goose 10. This resulted in a P-load of 0.2 mg P m^{-2} d^{-1}.

• Feeding of water birds. During sampling events in this study, we observed that water birds, predominantly residential geese and mallards (*Anser anser*, *Branta canadensis* and *Anas platyrhynchos*) were excessively being fed on a regular basis with bread. According to Aalderink et al. (2009) we assumed that 50% of the added bread is eaten by the birds and that the maximum daily bread consumption per bird is 50 g. The part of the bread which was consumed was already included in the intake and dropping models. The part of the bread which was not consumed, provided an additional P-load. The P content of bread was based on Wolos (1992; 1.74 g P kg^{-1}), Aalderink et al. (2009; 4 g P kg^{-1}) and RIVM (2013; whole meal 2.00 g P kg^{-1}, white water based bread 0.84 g P kg^{-1}) with a mean of 2.15 g P kg^{-1}. The resulting P-load from the feeding of water birds was 0.1 mg P m^{-2} d^{-1}.

• Litter fall from trees and shrubs on open water contributed to the P-load. The area of branches over open water was determined from aerial photographs in this study. The amount of litter per unit of area was based on data from Liu et al. (2004) and set at 442 g DW m^{-1} y^{-1}, with an average P content of 0.22% (Dorney, 1985). The area of overhanging branches at Lake Kleine Melanen was 4346 m^2 resulting in a P-load of 0.3 mg P m^{-2} d^{-1}.
• The contribution of bait used by anglers depended on local conditions (Lewin et al., 2006). The local angling association had 25 members and organized 8 angling competitions on Lake Kleine Melanen a year (information provided by the angling association, 2011). Based on information from the angling association (available at www.derietvoorn.nl, assessed 18 November 2014) we assumed 15 participants each competition, of which 60% used bait at 1 kg per angling day (Wolos, 1992; Van Emmerik & Peters, 2009), resulting in 72 kg bait y^{-1} during competitions. Outside competitions we assumed 40.5 angling days per angler a year of which 53.4% used ground bait at 1 kg bait per angling day (Wolos, 1992), resulting in 541 kg bait y^{-1} outside competitions. The total amount of bait used in the lake was 613 kg y^{-1}. The average P content of bait was 2.27 g P kg^{-1}, resulting in a P-load of 0.1 mg P m^{-2} d^{-1} due to angling.
• Dog faeces and urine deposited on the banks were a P-source. The contribution of dog fouling to the P-load of the lake was based on a runoff of 25% of the P in excreta, an estimated dog population within a 3 hectare-range around the lake of 25, and an excretion of 240 g P dog^{-1} y^{-1} (Aalderink et al., 2009), resulting in a P-load of 0.1 mg P m^{-2} d^{-1}.

The total external P-loading was 7.5 mg P m^{-2} d^{-1}.


flocculation
flocks + ballast
deep injection
Capping

CHAPTER 5

Management of eutrophication in Lake De Kuil (The Netherlands) using combined flocculant - Lanthanum modified bentonite treatment

This chapter is based on:
Management of eutrophication in Lake De Kuil (The Netherlands) using combined flocculant - Lanthanum modified bentonite treatment.
Waajen, G., F. van Oosterhout, G. Douglas & M. Lürling, 2016.
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Abstract

Eutrophication of Lake De Kuil (The Netherlands, 6.7 ha, maximum depth 9 m) has frequently caused cyanobacterial blooms resulting in swimming bans or the issue of water quality warnings during summer. The eutrophication was mainly driven by sediment phosphorus (P)-release. The external P-loading was in the range of the critical loading for phytoplankton blooms. Hence, the reduction of the internal P-loading provided a promising way to reduce cyanobacterial blooms. To mitigate the cyanobacterial blooms, the combination of a low dose flocculant (iron(III)chloride; Flock) and a solid phase phosphate fixative (lanthanum modified bentonite; Lock) was applied in May 2009. This combined approach both removed cyanobacterial biomass from the water column and also intercepted P-released from the bottom sediments. Immediately after treatment, the Secchi depth increased from 1.5 m up to 5 m. Sediment P-release decreased from 5.2 mg P m^{-2} d^{-1} (2009) to 0.4 mg P m^{-2} d^{-1} (2010) but increased in later years. Mean summer concentrations of total P decreased from 0.05 mg L^{-1} (1992-2008) to 0.02 mg L^{-1} (2009-2014) and chlorophyll-*a* from 16 µg L^{-1} (1992-2008) to 6 µg L^{-1} (2009-2014). Mean summer Secchi depth increased from 2.31 m (1992-2008) to 3.12 m (2009-2014). The coverage of macrophytes tripled from 2009 to 2011. In the winter of 2010/2011 *Planktothrix rubescens* bloomed, but cyanobacterial biomass decreased during the summers after the Flock and Lock treatment in comparison to prior years. After the Flock & Lock the bathing water requirements have been fulfilled for six consecutive summers. As the sediment P-release has gradually increased in recent years, there is a risk of a reversion from the present mesotrophic state to a eutrophic state.

Introduction

In The Netherlands around 100 million tonnes of sand, gravel and clay is excavated from the Dutch subsurface annually, most of which is used immediately in construction (TNO, 2015). These excavations are not only located around the main river branches, but also in the sandy southern province North Brabant where at least 146 sand excavations are known. Such excavations fill with water and the resultant new lakes (≥ 6m water depth, ~10 ha mean area) are intensely used by citizens to whom they provide amenities including angling, boating, recreation and swimming. The implementation of the European Water Framework Directive (WFD; Council of the European Union, 2000) and the European Bathing Water Directive (BWD; Council of the European Union, 2006) has resulted in considerable attention to the maintenance of acceptable water quality. However, over decades many of the once oligotrophic waters have become turbid, phytoplankton dominated systems (Smith et al., 1999). The phytoplankton blooms, which are often cyanobacteria-dominated, are due to the fertilization with nutrients derived from local surface and groundwater inputs. A range of management interventions including flushing, mixing, the application of algaecides and biomanipulation are being used to mitigate the negative effects of eutrophication. Long-term restoration, however, can only be achieved if the total nutrient loading is sufficiently reduced (Søndergaard et al., 2007). Although several nutrients are relevant for phytoplankton growth, the emphasis in lake recovery is on phosphorus (P) reduction (Jeppesen et al., 2007b; Carpenter, 2008; Schindler et al., 2008), indicating still the present day validity of Golterman (1975) who stated: "*It is not important whether phosphate is currently the limiting factor or not, or even that it has ever been so; it is the only essential element that can easily be made to limit algal growth*." Phosphorus reduction implies the reduction of the external and the internal P-loading (Gulati and Van Donk, 2002, Søndergaard et al., 2007). In lakes created by excavations, the internal P-loading may often be the main driver for sustaining phytoplankton growth (Burger et al., 2007) and delays recovery for a substantial period after the reduction of the external P-loading (Jeppesen et al., 2005). Removal of sediments enriched in P is a common method to reduce the internal P-loading (Peterson, 1982), but it is not always effective (Geurts et al., 2010) and is expensive (Welch & Cooke, 2005) in terms of recovery, but also for dewatering and long-term disposal. This explains the need for alternative methods to control the sediment P-release (released as phosphate) and internal P-recycling in lakes. As a consequence, the interest in P-sorbing materials is growing (Hickey and Gibbs, 2009; Spears et al., 2013c).

A promising novel method has been applied in the former sand excavation site, Lake Rauwbraken (The Netherlands) that has experienced intensifying cyanobacterial blooms since 2000 onwards, before this official bathing site underwent restoration (Lürling & Van Oosterhout, 2013). A system analysis of the lake revealed that internal P-loading was about ten times higher than external P-input. In light of the high proportion of diffuse inputs, mostly through groundwater, a combined phytoplankton flocculation/precipitation and sediment capping treatment was developed. In short, the cyanobacteria biomass was

effectively removed from the water column using a low dose polyaluminiumchloride (PAC at < 1 mg Al L^{-1}) as flocculant with increased density provided by two tonnes of a lanthanum (La) modified bentonite (LMB). Thereafter, the settled matter and sediment were covered with an active barrier of the LMB designed to capture P-released from decaying settled matter, sediment and incoming groundwater (Lürling and Van Oosterhout, 2013). The LMB was developed by CSIRO Australia (Douglas, 2002) and has a strong binding capacity for P (Meis et al., 2012; Spears et al., 2013c) with the capacity to remove P from the water column during settling, and once settled being able to intercept P-released from the sediments (Robb et al., 2003; Akhurst et al. 2004; Ross et al., 2008; Egemose et al., 2010; Gibbs et al., 2011; Van Oosterhout & Lürling, 2013). The P-binding capacity of the LMB is not affected by anoxia (Douglas et al., 2004; Ross et al., 2008; Gibbs et al., 2011). The efficacy and strong P-binding properties of LMB made it a promising candidate for application in Lake De Kuil, where anoxic events near the sediment occurred annually. Apart from the single application of this 'Flock & Lock' method in Lake Rauwbraken, no knowledge exists of its efficacy in stratified lakes subject to seasonal anoxia.

The present study provides information on the efficacy of the Flock and Lock method in the stratified Lake De Kuil, where it was applied in a modified form. Whereas in Lake Rauwbraken the LMB was applied at the lake's surface (Lürling and Van Oosterhout 2013), in Lake De Kuil most of the LMB was applied through deep injection to improve positioning of the LMB on the lake's bottom and to prevent turbid waters due to surface application. Lake De Kuil is a recreational lake to which the WFD and BWD apply. The lake suffered from regular cyanobacterial blooms since the early '90s which led to violation of BWD standards resulting in swimming bans and issue warnings in most summers. A system analysis revealed no major water inflow, but only rain and groundwater and pointed towards periodic P mobilisation under anoxia in the deeper water and near the sediment supporting

Fig. 5.1: Positioning of a wind-driven mixing device (LAS International, Bismarck, ND, USA) in Lake De Kuil (4 March 1997).

the cyanobacterial blooms (Witteveen+Bos, 2006). A previous attempt to mitigate the nuisance caused by *Planktothrix rubescens* in Lake De Kuil was undertaken using artificial mixing from 1997 to 2005 (Ibelings et al., 1997; Fig. 5.1). Supported by the intermittent mixing regime cyanobacterial nuisance persisted with *Microcystis* sp. and *Anabaena planctonica* (Witteveen+Bos, 2006). This experience shifted the focus to the reduction of sediment P-release. As the results of the Flock & Lock treatment of Lake Rauwbraken were promising (Van Oosterhout & Lürling, 2011), this treatment was selected for Lake De Kuil.

However, doubts within the managing water authority towards the safe use of aluminium (Al) in a recreational lake meant that no permit for the use of PAC in a low dose as flocculant could be obtained. Hence, the combined use of iron(III)chloride ($FeCl_3$) as flocculant and LMB as ballast and solid phase P-sorbent were chosen. The lake was treated in May 2009. We report the results of the combined treatment of $FeCl_3$ as flocculant and LMB as active barrier system in a whole-lake application over the period 2009-2014, for which we hypothesized that the treatment would precipitate cyanobacteria rapidly out of the water column, inhibit internal P-loading, and minimize cyanobacterial biomass in the following years.

Material and methods

Lake De Kuil

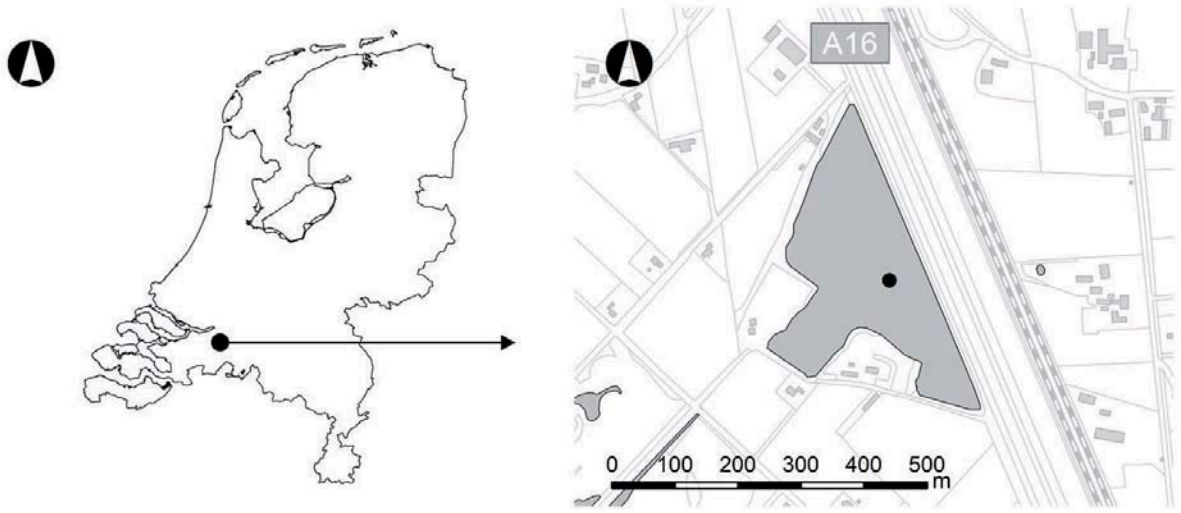


Fig. 5.2: Location of Lake De Kuil in The Netherlands (left panel) and schematic drawing of the lake (right panel) including the sampling station (●) and A16 motorway.

Lake De Kuil is located in the south western part of The Netherlands (N 51°37'22"; E 4°42'23"; Fig. 5.2). The lake was excavated in the 1950s to provide sand for the construction of the A16 motorway (Fig. 5.2). In 2000, one-third of the lake was filled with desalinated sea sand to allow for the widening of the motorway and the parallel high speed train track. Currently, the lake has a surface area of 6.7 ha, a maximum water depth of 9 m and an estimated

water volume of 268,000 m^3. Reflecting its morphometry, the lake has characteristics of both shallow and deep stratifying lakes. The banks have partly been enforced with rocks. Submerged and emergent vegetation is not abundant. Using combined navigation (GPS) and sonar equipment from a boat a clear pattern of an irregular lakebed form was documented. Deeper holes (8 - 9 m) and shallower parts (5 - 7 m) alternate and reflect sand excavation activities, where excavation was stopped once a clay base was reached. A shallow part of the lake is being used as an official bathing site, while the remaining part of the lake is in use for angling. From 1992 onwards annually reoccurring cyanobacterial blooms have been reported, starting with *Planktothrix rubescens* blooms in the first years in spring followed by summer blooms of *Anabaena* ssp., *Woronichinia naegeliana* and *Microcystis aeruginosa*. The cyanobacterial blooms resulted in swimming bans and the issue of warnings in most of the years. To reduce bloom occurrence, in 1997 two wind-driven vertical water-mixing devices were installed to induce destratification, however, because of their inefficiency the mixing was stopped in 2005. In 2008 the opening of the bathing season (May 1st) had to be postponed because of a cyanobacterial bloom, which prompted the authorities to investigate other management measures.

On average, the lake receives 61,750 m^3 water p.a. through direct precipitation and 14,128 m^3 p.a. through runoff from its catchment, while 50,170 m^3 p.a. is lost through evaporation and 25,708 m^3 p.a. leaves the lake through an outlet into a canal north of the lake (Supplementary information, Appendix 5.A). Rates of seepage and intrusion are similar and are of marginal importance to the water balance. While the lake outlet is equipped with a backflow stop, until the mid '90s water from the canal that was polluted with sewer overflow water, could enter the lake. Until 2003 the lake was influenced by sewage water from the building at the swimming site due to a leakage.

A high fish stock can influence water quality (Meijer et al., 1999). Before the start of the restoration project the fish stock determined in 2006 consisted of 50.2 kg fish ha^{-1} (Table 5.1). In winter time up to several hundreds of waterfowl reside on the lake.

The external P-loading of the lake was determined to be 0.27 mg P m^{-2} d^{-1} (Supplementary information, Appendix 5.A). The critical P-loading thresholds were determined with the PCLake Metamodel (Janse et al., 2008; Mooij et al., 2010; PBL, 2015) and ranged from 0.21 to 0.23 mg P m^{-2} d^{-1}. Based on Vollenweider (1976) the permissible and excessive P-loads were 0.3 and 0.6 mg P m^{-2} d^{-1}, in which the permissible P-load is the boundary between the oligotrophic and mesotrophic state (based on the lower limit of 10 µg P L^{-1}; Vollenweider, 1976) and the excessive P-load is the boundary between the mesotrophic and eutrophic state (based on the upper limit of 20 µg P L^{-1}; Vollenweider, 1976). Below the permissible load, the likelihood for phytoplankton blooms and anoxic conditions in the hypolimnion are considered small. Above this, the lake's ability to assimilate P without producing nuisance phytoplankton growth are poor (Rast and Thornton, 2005). In eutrophic conditions cyanobacterial blooms are likely to occur (Smith, 2003). General characteristics of the lake before the treatment are provided in Table 5.2.

Table 5.1: Fish stock of Lake De Kuil (fresh weight kg ha^{-1}) before (2006) and after the Flock & Lock treatment (2011, 2014). In parentheses n ha^{-1}.

	Fish stock kg ha^{-1} (n ha^{-1})		
Fish species	**19 June 2006**	**28 September 2011**	**12 August and 5 September 2014**
Perch (*Perca fluviatilis*)	13.2 (11,706.0)	12.7 (637.9)	10.7 (204.1)
Bream (*Abramis brama*)	11.8 (3.9)	6.2 (193.8)	8.5 (2.6)
Eel (*Anguilla anguilla*)	12.5 (65.5)	32.2 (87.3)	13.2 (30.8)
Carp (*Cyprinus carpio carpio*)	6.0 (1.3)	67.0 (11.6)	85.7 (12.8)
Roach (*Rutilus rutilus*)	0.8 (25.7)	2.1 (19.3)	
Pike (*Esox lucius*)	4.1 (2.6)	6.5 (6.4)	11.2 (7.7)
Tench (*Tinca tinca*)	1.8 (1.3)	4.7 (7.7)	1.0 (3.9)
Ruffe (*Gymnocephalus cernuus*)	<0.1 (1.3)	<0.1 (1.3)	
Spined loach (*Cobitis taenia*)	<0.1 (2.6)		<0.1 (5.1)
Rudd (*Scardinius erythrophthalmus*)		0.4 (2.6)	
Moderlieschen (*Leucaspius delineatus*)		<0.1 (1.3)	
Grass carp (*Ctenopharyngodon idella*)			6.6 (< 1.0)
Total	50.2 (11,810)	131.8 (970)	136.9 (268)

Table 5.2: Characteristics of Lake De Kuil.

Area (ha)	6.7
Mean water depth (m)	4
Maximum water depth (m)	9
Residence time (y)	3.7
LMB dose	13.65 tonnes superficially on 6.7 ha; 27.85 tonnes injected on 2.2 ha with water depth ≥ 6.4 m
$FeCl_3$ dose and area	4.38 tons (3 m^3) on 6.7 ha
pH (prior to treatment)	8.4

Chemicals and dosage

Dissolved $FeCl_3$ (ρ = 1.46 kg L^{-1}, 40% $FeCl_3$) was used as flocculant and lanthanum (La) modified bentonite (LMB; available as Phoslock®) was used as solid phase P-sorbent. As dissolved $FeCl_3$ is acidic, 200 kg calcium hydroxide (hydrated lime, $Ca(OH)_2$) in powdered form was used to prevent the pH declining below 6.5 as this has negative effects on both flocculation efficiency and on biota. The LMB dosage was based on the amount of total P (TP) in the water column prior to the application and on the amount of immediately and potentially releasable P in the top 5 cm of the sediment using a molar ratio of La:P of 1:1 as recommended by the manufacturer. The potentially releasable P in the sediment was the total of the fractions Fe/Mn bounded-P, reductive soluble organic-P and organic-P (microorganisms, humic, detritus) as derived in the

sequential P extraction (cf. Psenner et al., 1984). The dosage was 42 tonnes LMB (Supplementary information, Appendix 5.B). A jar test revealed that 2.2 mg Fe L^{-1} would suffice to effectively flocculate the particles in Lake De Kuil into large aggregates and to sink them with the addition of LMB as ballast. Based on the jar trials a total of 4.38 tonnes (3 m^3) $FeCl_3$ was added to the lake.

Lake treatment

Lake De Kuil was treated from 18 May to 22 May 2009, with the thermocline at ~5 m water depth. On 18 May 2009 3 m^3 of the $FeCl_3$ was diluted with lake water, buffered with calcium hydroxide ($Ca(OH)_2$) and sprayed on the lake surface from a barge. The subsequent day 13.65 tonnes LMB were mixed with lake water and sprayed through the manifold on the water surface to provide ballast for precipitation of the flocs (Fig. 5.3). The next two days (20/21 May 2009) 28.35 tonnes LMB were injected on 2.2 ha below the epilimnion at 5 m depth to the lake areas deeper than 6.4 m. The deep injection was applied to avoid rendering the lake turbid and to address the internally sediment P-release from the deepest part of the lake (Fig. 5.4; Appendix 5.B).

Fig. 5.3: Application of iron(III)chloride (18 May 2009, left panel) and lanthanum-modified bentonite (LMB, 19 May 2009, right panel) at Lake De Kuil.

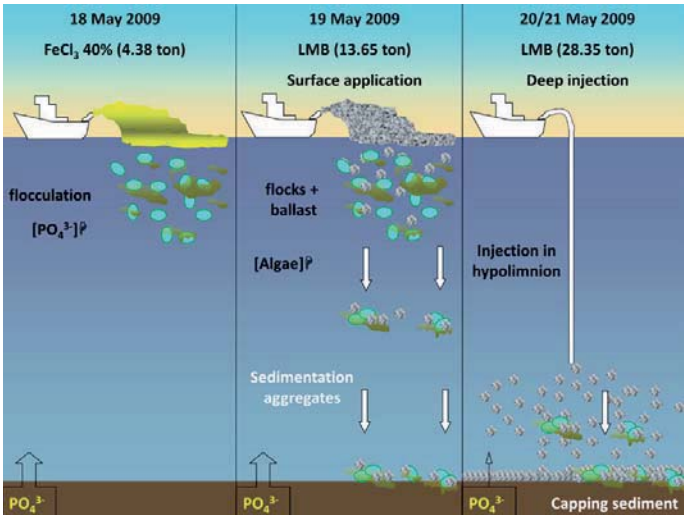


Fig. 5.4: Schematic drawing of the Flock & Lock application in Lake de Kuil in May 2009.

Sampling and water quality classification

Sampling occurred adjacent to a buoy that was placed near the centre of the lake (N 51°37'20", E 4°42'25"; Fig. 5.2) at one of the deepest points (8.7 m). Two litre water samples were taken at 1 m interval using a Uwitech water sampler. Samplings were done on 16 December 2008 (Fig. 5.5) and from 18 March 2009 biweekly until the application, when the lake was sampled daily. From June until December 2009 biweekly sampling was continued. Ice cover prohibited sampling until the beginning of March 2010 when the lake was sampled biweekly again until the end of 2010. Thereafter, the lake was sampled 16 times in 2011, 10 times in 2012, 9 times in 2013 and 10 times in 2014 at regular intervals. Measurements over sample dates and depth are presented as means. At the same location adjacent to the buoy, the Water Authority Brabantse Delta has sampled Lake De Kuil regularly since 1992 and samples were analyzed by the laboratory of the water authority according the standard protocol. These samples were taken at water depths of 0.3 – 0.5 m. These data were included in the long-term data set and compared with the results from similar water depths (0-1 m) obtained in the period 2009 – 2014.

Fig. 5.5: Two limnologists from Wageningen University on a sampling event at Lake De Kuil (16 December 2008).

Temperature (°C), pH, oxygen (O_2) concentration (mg L^{-1}) and O_2 saturation (%) were measured using a WTW- multi 350i meter. Water transparency was determined using a 30 cm black and white Secchi disc. The two litre water samples were transferred to the laboratory of Wageningen University and analyzed for cyanobacterial and total chlorophyll-*a* concentrations using a PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany). The PHYTO-PAM phytoplankton analyzer uses four different excitation wavelengths, allowing quantitative measurement of cyanobacteria, green algae, and diatoms (Heinz Walz GmbH, 2003; Lürling & Roessink, 2006) and calibrated against the Dutch standard procedure (NNI, 2006b). Turbidity was measured using a HACH 2100P turbidity meter. Unfiltered samples were analyzed for TP concentrations, while samples filtered through glass fibre filters (Whatman GF-C) were analyzed for ortho-P (o-P) concentrations using a Skalar SAN+ segmented flow

analyzer following the Dutch standard protocol (NNI, 1986). Filterable lanthanum (FLA, through Whatman GF-C) and total lanthanum (TLa) was measured by ICP-MS. The detection limits for FLa were 0.02 and for TLa 0.2 µg L^{-1}. In cases that the concentration was below the detection limit, the value of half the detection limit was used as concentration in data processing.

On 16 December 2008 five sediment cores were extracted from five locations distributed over the lake. From these five cores the thickness of the layer of soft sediment was determined visually, as well as the water depth at which they were taken and the organic matter concentration of the soft sediment. On 18 March 2009 eight cores were obtained from the deepest parts of the lake. Five cores were sliced into 2 cm thick slices. The five upper slices of each core were subjected to a sequential P extraction (cf. Psenner et al., 1984) to estimate the immediately and potentially releasable P in the top 5 cm of the sediment. The three remaining cores, along with six to eight sediment cores obtained at the sampling point on 16 February 2010, 15 March 2011, 20 March 2013 and 2 July 2014 were used for sediment P-release experiments. The overlying water from the cores for the sediment P-release experiments was gently siphoned off and replaced by anoxic demineralized water in the cores obtained in 2009 and in half of the cores obtained in 2010 to 2014, and oxygenated water in the remaining cores obtained in 2010 to 2014. The anoxic cores were closed with a rubber stopper while the others remained open and they were placed for 2 to 6 weeks in the dark at room temperature. Weekly, the o-P concentration in the overlying water was measured after filtration through a 0.45 µm membrane filter (Whatman NC45) using a Skalar SAN+ segmented flow analyzer following the Dutch standard protocol (NNI, 1986). Oxygen concentrations were measured and the water was replaced with new (an)oxic water. Sediment P-release was calculated from multiplying the measured P concentration with water column height, divided by incubation time.

In 2009, 2010, 2011 and 2015, submerged macrophyte coverage and species composition were determined by rake-sampling from a boat over 14 transects along the lake. After the Flock & Lock treatment the fish stock was measured again in September 2011 and August and September 2014 by professional fishing companies using combined electrofishing (5 kW) and trawl fishing. Zooplankton composition was determined from 4 May until 30 November 2009 in one liter samples that were poured over a 55 µm net and preserved with Lugol's solution prior to counting under an inverted microscope. Phytoplankton and filamentous macro algae taxa were identified by light microscope.

The trophic level of the lake was assessed according to the classification system of Forsberg & Ryding (1980) using mean summer TP and chlorophyll-*a* concentrations. The WFD operates with a system of five ecological classes to assess the ecological status of a water body (bad, poor, moderate, good and high status; Council of the European Union, 2000). We assessed the ecological status of Lake De Kuil and used TP, being the main environmental stressor for lake water quality, as key variable for lake water quality and chlorophyll-*a* as the response variable (Søndergaard et al., 2005). Boundaries for the ecological classes for macrophytes and fish were derived from Van der Molen et al. (2013).

Results

Water quality - short-term effects (1 week)

The water column mean chlorophyll-*a* concentration prior to the start of the application on 18 May 2009 was 13.0 ± 8.4 µg L^{-1} (median 16.5 µg L^{-1}; Fig. 5.6A). The highest chlorophyll-*a* concentration was observed in the upper 4 m of the water column (mean 19.9 ± 2.0 µg L^{-1}, of which 96% consisted of cyanobacterial chlorophyll-*a*). The mean chlorophyll-*a* concentration over the depth 5 - 8 m was 4.4 ± 2.1 µg L^{-1} (with 31% cyanobacterial chlorophyll-*a*). On 18 May a bloom of *Aphanizomenon flos-aquae* was developing with *A. flos-aquae* colonies visible to the naked eye present throughout the lake and scums accumulated in a part of the lake.

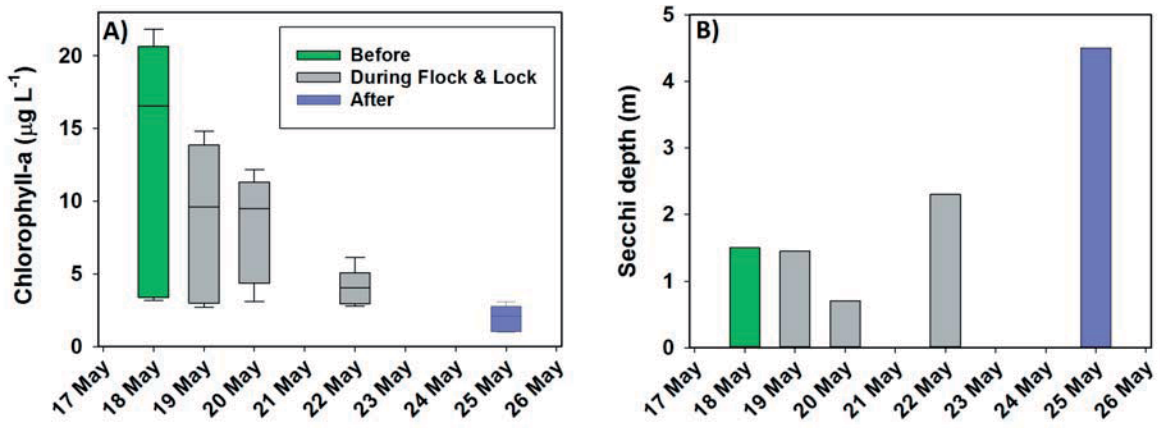


Fig. 5.6: Water column chlorophyll-*a* concentration (µg L^{-1}, panel A) and Secchi depth (m, panel B) before (18 May), during (19 – 22 May) and after (25 May) the application of Flock & Lock in 2009. Boxes in panel A indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles.

On 18 May 2009 the Secchi depth was 1.5 m and the water column mean turbidity was 6.6 ± 2.4 NTU (median 7.1 NTU). The addition of oxidized ferric (Fe^{3+}) to the lake produced ferric hydroxide flocs (Cooke et al., 2005). Immediately after the addition of the $FeCl_3$ the flocs were visible at the water surface. During the Flock & Lock treatment the Secchi depth decreased to 0.7 m. On 25 May 2009 the Secchi depth had increased to 4.5 m (Fig. 5.6B), while the mean turbidity had decreased to 2.9 ± 1.7 NTU (median 2.0 NTU) and the chlorophyll-*a* concentration to 1.9 ± 0.9 µg L^{-1} (median 2.1 µg L^{-1}; Fig. 5.6A). The Flock & Lock treatment effectively precipitated the scums and cyanobacteria colonies to the bottom sediment as confirmed via a visual inspection of the lake. The Secchi depth remained ~5 m during June 2009, while the mean chlorophyll-*a* concentration gradually increased to 14.9 ± 23.4 µg L^{1} on 29 June 2009.

The water column mean TP concentration on 18 May 2009 was 0.15 ± 0.19 mg P L^{-1} (median 0.04 mg P L^{-1}; Fig. 5.7A). The highest TP concentration (0.54 mg P L^{-1}) was measured near the sediment (water depth 8 m). During the application the TP concentrations dropped and on 25 May 2009 the mean was 0.03 ± 0.02 mg P L^{-1} (median 0.03 mg P L^{-1}; Fig. 5.7A). O-P concentrations followed a similar pattern (Fig. 5.7B) with a mean concentration on 18 May 2009 of 113 ± 147 µg P L^{-1} (median 26 µg P L^{-1}). The highest o-P concentration (409 µg P L^{-1})

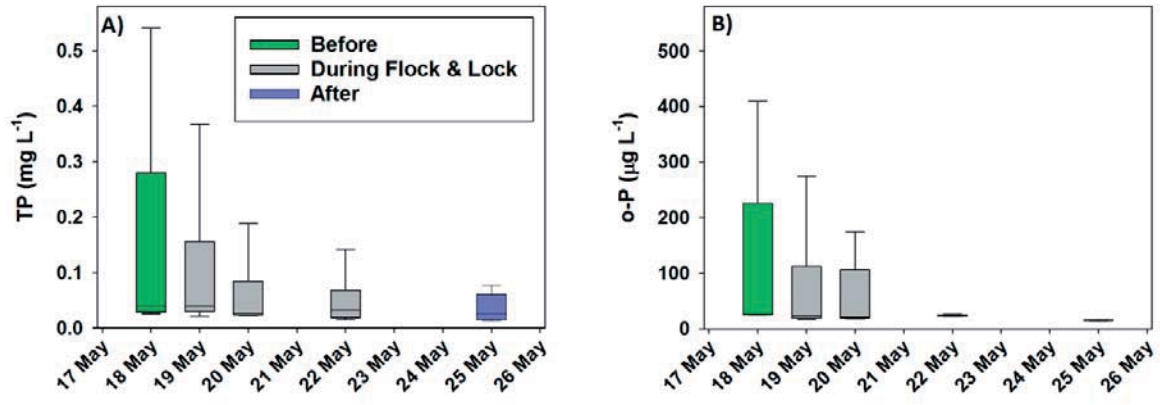


Fig. 5.7: Water column concentrations of total phosphorus (TP, mg L^{-1}, panel A) and ortho-phosphate (o-P, µg L^{-1}, panel B) before (18 May), during (19 – 22 May) and after (25 May) the application of Flock & Lock in 2009. Boxes indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles.

was measured near the sediment. During the Flock & Lock treatment the o-P concentrations dropped and on 25 May 2009 the mean was 15 ± 1 µg P L^{-1} (median 15 µg P L^{-1}).

Total La and filterable La reached maximum concentrations during the Flock & Lock application on 22 May 2009. The highest water column mean concentrations were 409 ± 353 µg TLa L^{-1} and 5.9 ± 6.4 µg FLa L^{-1} on 22 May 2009. The highest single concentrations were measured on 22 May 2009 in the hypolimnion, being 893 µg TLa L^{-1} at 8 m water depth and 15.9 µg FLa L^{-1} at 7 m water depth.

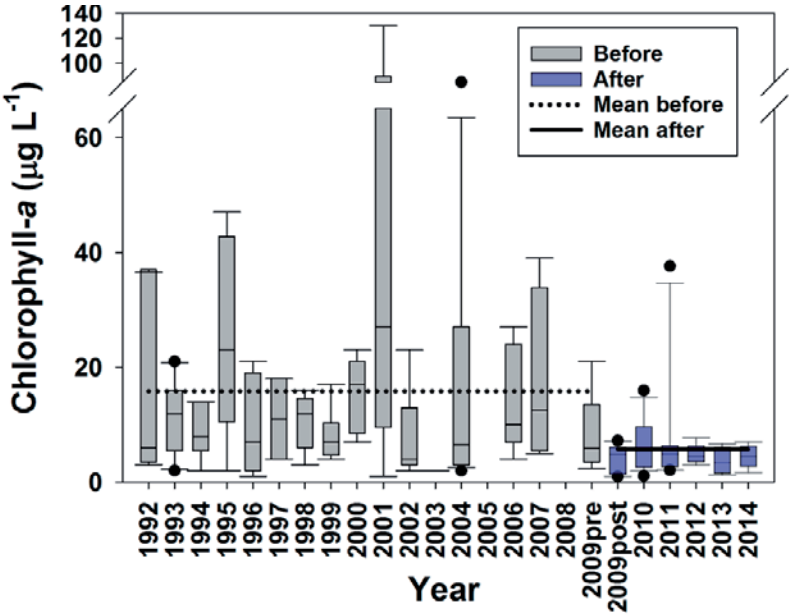


Fig. 5.8: Summer chlorophyll-*a* concentration (µg L^{-1}, April – September, at water depth 0 – 1 m) before (1992 – 2009pre, grey boxes) and after (2009post – 2014, blue boxes) the application of Flock & Lock. Boxes indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles. The dotted line indicates the mean chlorophyll-*a* concentration before Flock & Lock. The solid line indicates the mean after Flock & Lock.

Water quality - long-term effects (> 5 years)

Prior to the Flock & Lock treatment (1992 - 18 May 2009) the chlorophyll-*a* concentration during the summer (1 April – 30 September) varied from year to year (upper water layer 0 - 1m; Fig. 5.8) with a mean concentration of 15.8 µg L^{-1} and a maximum of 130 µg L^{-1} (9 August 2001). Cyanobacterial blooms hampered the recreational function of the lake in 1992, 1995, 1998, 2000 - 2004, 2006 and 2008. The blooms were dominated by *Planktothrix rubescens* in 1992 and 1995, and *Woronichinia naegeliana*, *Microcystis* ssp., *Anabaena* ssp. and *Aphanizomenon flos-aquae* in later years.

After the Flock & Lock treatment (25 May 2009 – 2014) the mean chlorophyll-*a* concentration during summer was 5.7 µg L^{-1}. The highest single chlorophyll-*a* concentration was 156.1 µg L^{-1} (24 August 2009 at water depth 7 m). At that moment the phytoplankton community was dominated by *Ceratium* sp. and picocyanobacteria with 46% of the chlorophyll-*a* consisting of cyanobacteria. After the autumn turnover of 2010, a persistent bloom of *P. rubescens* was observed from November 2010 until the end of April 2011 (Fig. 5.9A+B) with scums of ~50 m^2. During this bloom the highest single cyanobacterial chlorophyll-*a* concentration was 59.7 µg L^{-1} (15 March 2011 at the water surface outside of the scums). The disappearance of the bloom in the beginning of May 2011 produced malodor. Outside this *P. rubescens* bloom, the highest concentrations of chlorophyll-*a* and cyanobacterial chlorophyll-*a* were found each year during the months June - September in the hypolimnion (depth 5 – 8 m; Fig. 5.9). Occasionally short-term accumulations of *Microcystis* and *Aphanizomenon* with scums of less than 25 m^2 were observed.

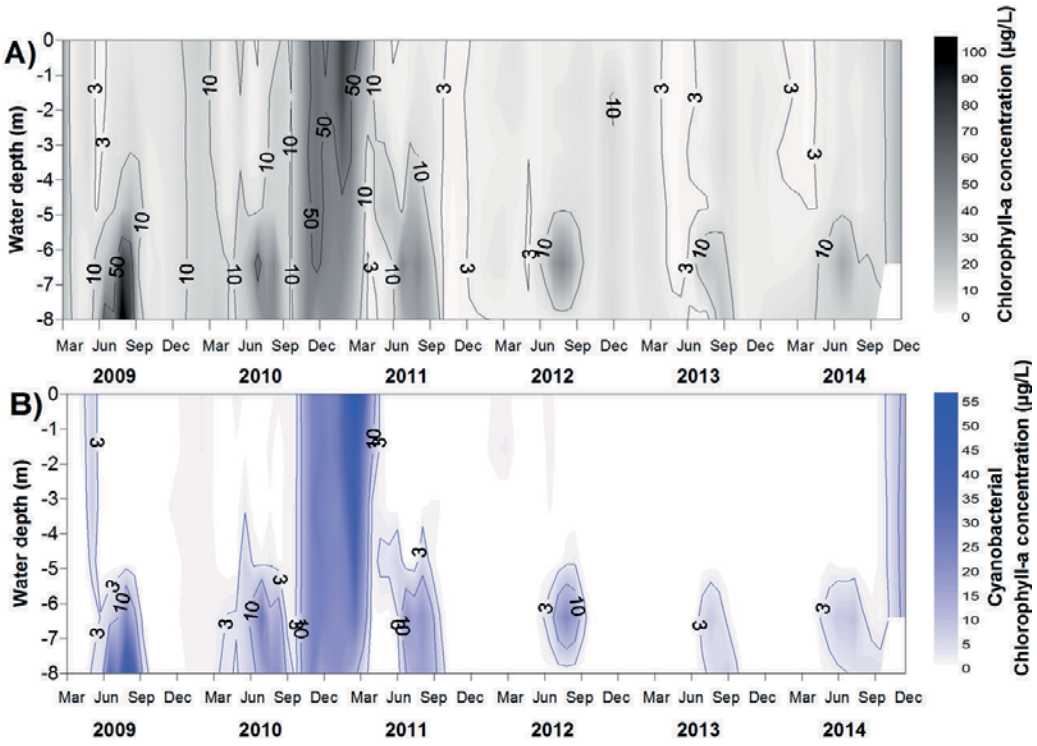


Fig. 5.9: Contour plots of chlorophyll-*a* concentrations (µg L^{-1}, panel A) and cyanobacterial chlorophyll-*a* (µg L^{-1}, panel B), March 2009 - December 2014. Flock & Lock treatment in May 2009.

The mean Secchi depth during summer before the Flock & Lock treatment (1992 – 2009) was 2.31 m (minimum 0.20 m on 24 May 1995; maximum 5.50 m on 26 May 2004). After the treatment (2009 – 2014) the mean Secchi depth was 3.12 m (Fig. 5.10) with a minimum Secchi depth of 0.42 m on 13 April 2011 during the *P. rubescens* bloom and a maximum of 5.25 m on 15 June 2009. In the period 2009 - 2014 the highest Secchi depths were observed during the first month after the treatment.

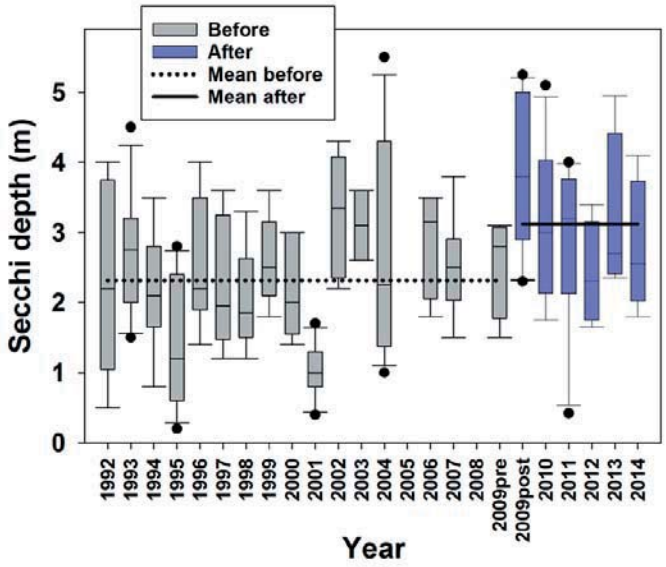


Fig. 5.10: Summer Secchi depth (m, April – September) before (1992 - 2009pre, grey boxes) and after (2009post – 2014, blue boxes) the application of Flock & Lock. Boxes indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles. The dotted line indicates the mean Secchi depth before Flock & Lock. The solid line indicates the mean after Flock & Lock.

The water column mean turbidity before the treatment (18 March – 18 May 2009) was 3.5 NTU. After the treatment (25 May 2009 – 2014) the mean turbidity was 4.6 NTU. The highest single turbidity after the treatment was observed on 13 April 2011 at the water surface (37.7 NTU).

The mean summer TP concentration at water depth 0 - 1 m decreased from 0.05 mg P L^{-1} (1992 – 2009 before Flock & Lock) to 0.02 mg P L^{-1} (2009 – 2014 after Flock & Lock; Fig. 5.11). The highest TP concentrations before the treatment were observed in the '90s and early '00s, with a maximum of 0.70 mg P L^{-1} on 18 September 2002. After the Flock & Lock treatment the highest TP concentration at water depth 0 - 1 m was 0.13 mg P L^{-1} on 26 May 2010. Over the whole water column, the highest TP concentrations were observed in the hypolimnion during the period June – September 2011 and in September 2012, with the maximum concentration of 0.61 mg P L^{-1} on 23 August 2011 at the water depth of 7 m. The o-P concentrations after the treatment reached the highest values in the hypolimnion during the period June - September 2011, with the maximum concentration of 442 µg P L^{-1} on 28 September 2011. The mean summer o-P concentration at water depth 0 – 1 m was 18 µg L^{-1} before the treatment (1992 -2009) and 6 µg L^{-1} after the treatment (2009 – 2014).

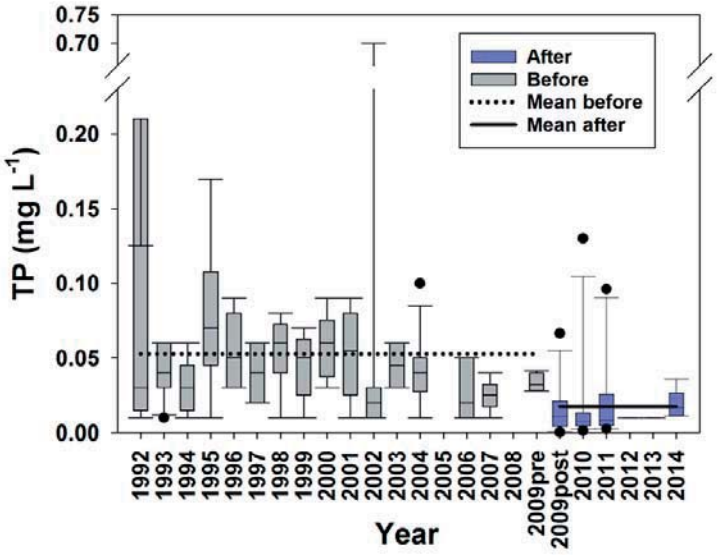


Fig. 5.11: Summer total phosphorus (TP) concentration (mg L^{-1}, April – September, at water depth 0 – 1 m) before (1992 – 2009pre, grey boxes) and after (2009post – 2014, blue boxes) the application of Flock & Lock. Boxes indicate 25th, 50th and 75th percentiles, whiskers indicate 10th and 90th percentiles. The dotted line indicates the mean TP concentration before Flock & Lock. The solid line indicates the mean after Flock & Lock.

Dissolved oxygen (DO) concentrations during the summers of 2009 and 2010 reached minima of 1.8 mg L^{-1} (25 May, 27 July, 12 August 2009) and 2.1 mg L^{-1} (29 September 2010) at 8 m. The following years more pronounced anoxia occurred with minima of 0.1 mg L^{-1} during longer periods in the summers of 2012, 2013 and 2014 at water depths of 6-8 m (Fig. 5.12). Before the Flock & Lock treatment the minimum DO concentration was 0.2 mg L^{-1} (13 September 2006 at the water surface).

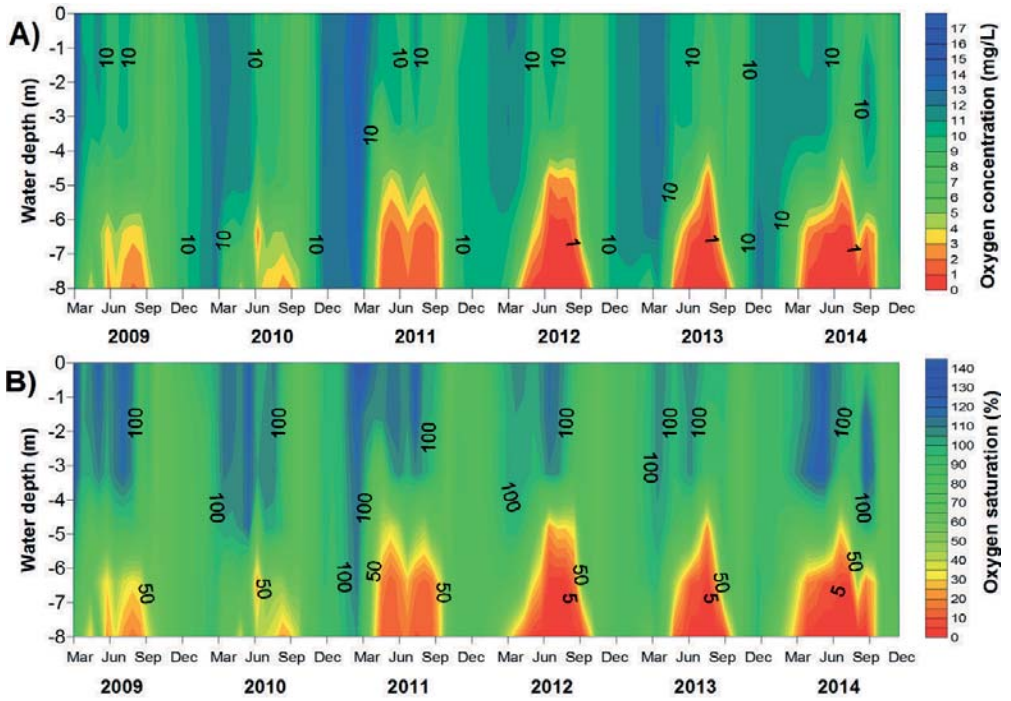


Fig. 5.12: Contour plots of dissolved oxygen concentration (mg L^{-1}, panel A; %, panel B), March 2009 – December 2014. Flock & Lock treatment in May 2009.

Before the Flock & Lock treatment the water column mean TLa concentrations varied from 0.2 ± 0.0 µg L^{-1} (18 May 2009) to 1.7 ± 1.1 µg l^{-1} (18 March 2009). The mean FLa concentrations varied from 0.02 ± 0.01 µg L^{-1} (21 April 2009) to 0.27 ± 0.07 µg L^{-1} (18 March 2009). The La concentrations during the application violated the Dutch water quality standards for TLa (150 µg L^{-1}) and FLa (10 µg L^{-1}; Sneller et al., 2000; https://rvs.rivm.nl/zoeksysteem/, accessed 19 March 2015). Violation of the standards persisted until 22 May 2009 for FLa and 2 June 2009 for TLa. After the application the TLa and FLa concentrations decreased and after 8 December 2010 (FLa) and 17 December 2013 (TLa) returned the pre-treatment concentrations respectively.

Sediment

Sandy sediment occurred in the more shallow parts of the lake (< 8 m depth) and darker sediment enriched in organic matter in the deepest parts (> 8m depth; Supplementary information, Appendix B, Table 5.B.1). The measured anoxic sediment P-release before the Flock & Lock treatment was 5.2 ± 2.6 mg P m^{-2} d^{-1} (Fig. 5.13). After the Flock & Lock treatment the sediment P-release had decreased to 0.4 ± 0.0 mg P m^{-2} d^{-1} (anoxic) in 2010. In the following years the sediment P-release (anoxic) increased to 6.3 ± 2.1 mg P m^{-2} d^{-1} in 2014. The measured sediment P-release under oxic conditions increased from 0.1 ± 0.0 mg P m^{-2} d^{-1} in 2010 to 4.8 ± 1.1 mg P m^{-2} d^{-1} in 2014 (Fig. 5.13).

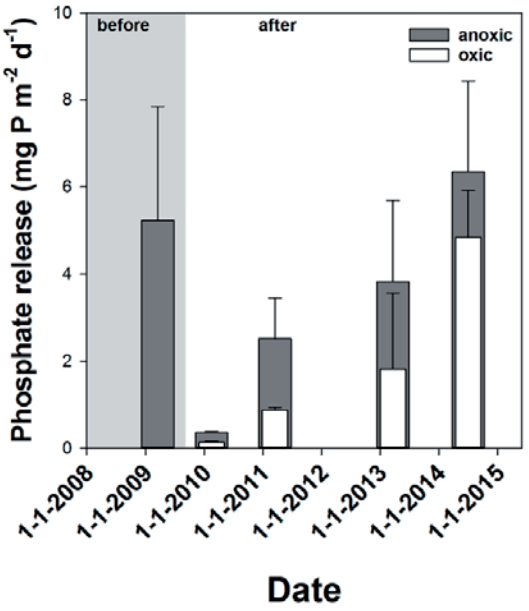


Fig. 5.13: Sediment phosphate release (mg P m^{-2} d^{-1}) before and after the Flock & Lock treatment in anoxic (2009-2014) and oxic (2010-2014) sediment cores.

Macrophytes

In July 2009 submerged and floating macrophytes (including filamentous macro algae) covered 1185 m^2 which increased to 2346 m^2 on 19 October 2009 at the end of the first growing season after Flock & Lock application (Table 5.3). In spring 2010 and 2011 the coverage was 1884 m^2 (9 June 2010) and 1825 m^2 (9 June 2011). At the end of the growing seasons 2010 and 2011, the coverage had increased to 6292 m^2 (19 October 2010) and 7589 m^2 (19 August 2011; Table 5.3). At the end of the growing season 2015, the coverage had decreased to 2191 m^2 (7 September 2015; Tabel 5.3). The maximum water depth at which macrophytes grew was 4-5 m in each year, except in 2015, when it was 3.5 m. During the period 2009-2011, the dominant species were *Elodea nuttallii* and *Chara vulgaris* (Table 5.3), while in 2015 filamentous macro algae dominated. The coverage of floating and submerged macro algae (i.a. *Spirogyra* ssp., *Enteromorpha* ssp.) increased from 439 m^2 (19 October 2009) to 1180 m^2 (19 October 2010) and 1249 m^2 (19 August 2011; Table 5.3). In 2015, the coverage of macro algae had decreased to 1006 m^2 (7 September 2015; Table 5.3).

Table 5.3: Coverage (m^2) of submerged and floating macrophytes and filamentous macro algae in Lake De Kuil after the Flock & Lock treatment (2009 – 2015).

	22 July 2009	19 October 2009	9 June 2010	22 July 2010	19 October 2010	9 June 2011	19 August 2011	7 September 2015
Chara vulgaris	8	569	733	854	380	282	3069	77
Filamentous macro algae	115	439	257	510	1180	919	1249	1006
Elodea nuttallii	196	972	417	2654	4479	274	2791	316
Potamogeton crispus	236	168	160	201	155	120	232	98
Potamogeton pectinatus	527	181	293	567	81	208	223	181
Other species	103	16	24	15	17	22	25	513
Total	1185	2346	1884	4802	6292	1825	7589	2191

Fish

The total fish biomass varied between 50.2 kg fresh weight ha^{-1} in 2006 and 136.9 kg fresh weight ha^{-1} in 2014 (Table 5.1). The fish density (n ha^{-1}) was the highest in 2006 (11,810 ha^{-1}) and had declined in 2011 (970 ha^{-1}) and further in 2014 (268 ha^{-1}; Table 5.1). The number of fish species varied from 9 in 2006, to 10 in 2011 and 8 in 2014 (Table 5.1). The most abundant species by number was perch (*Perca fluviatilis*), with n = 11,706 ha^{-1} in 2006, n = 637.9 ha^{-1} in 2011 and n = 204.1 ha^{-1} in 2014. Carp (*Cyprinus carpio carpio*) contributed the largest proportion of the fish biomass in 2011 and 2014.

Discussion

Diagnosis

The results of the study show that after the Flock & Lock treatment the water quality rapidly and substantially improved (Fig. 5.6, Fig. 5.7). System analysis indicated that the estimated internal P-loading (5.2 mg P m^{-2} d^{-1}) exceeded the external P-loading (0.3 mg P m^{-2} d^{-1}; Appendix 5.A) almost 20 times. The high internal P-loading was attributed to the legacy of former sewage discharges into Lake De Kuil that were ceased in the mid '90s and early '00s. Not dealing with this internal P-source might hamper recovery of the water quality for decades (Søndergaard et al., 1999). The external P-loading (0.27 mg P m^{-2} d^{-1}) was in the range of the critical loading (0.21-0.6 mg P m^{-2} d^{-1}) above which cyanobacterial blooms were likely to occur. This situation was promising for the reduction of the cyanobacterial biomass once the substantial reduction of the sediment P-release could be achieved.

Flock & Lock

Considering the irregular morphometry of the lake, removal of the P-rich sediment was viewed as problematic. In addition, costs of removal of 25.000 m^3 of sediment were estimated to be as high as € 1,200,000. Therefore, in situ immobilization of P was selected as the most promising management option. Given the early development and biomass of cyanobacteria which sometimes bloomed during winter (*P. rubescens*), a combined removal via flocculation of cyanobacteria from the water column and P-fixation was considered the most promising treatment. This Flock & Lock technique was applied first in 2008 in Lake Rauwbraken (The Netherlands), where it rapidly improved water quality (Lürling & Van Oosterhout, 2013). The Flock & Lock technique was applied to Lake De Kuil in May 2009. After the treatment the phytoplankton biomass decreased within a week from 13.0 µg chlorophyll-*a* L^{-1} on 18 May 2009 to 1.9 µg chlorophyll-*a* L^{-1} on 25 May 2009. Ballasted with LMB to counteract the positive buoyancy of the *Aphanizomenon* colonies during the bloom in May 2009, the ferric hydroxide flocs trapped the *Aphanizomenon* and sank to the bottom such that the majority of the *Aphanizomenon* bloom was effectively precipitated.

The combination of flocculant and ballast is viewed as an essential management technique as either of them alone is ineffective in removing a bloom of cyanobacteria from the water column (Lürling & Van Oosterhout, 2013). Due to an error, the flocculant was inadvertently applied before the LMB which caused the need to double the initially intended dose of ballast-LMB to be effective. Another effect of the incorrect order of application of flocculant and LMB was that the LMB did not absorb P from the water column to its full capacity, as most dissolved P (in addition to TP) had already been absorbed by the $FeCl_3$. In Lake Rauwbraken polyaluminiumchloride (PAC) was used as flocculant and in Lake De Kuil $FeCl_3$. This modification became necessary because a permit for the use of PAC in Lake De Kuil could not be obtained. Based on the known flocculating properties of $FeCl_3$ (Cooke et al., 1993) and its history in lake restoration (e.g., Peelen, 1969 in Cooke et al., 2005), $FeCl_3$ was chosen as the best alternative for PAC. Despite the poorer floc formation of $FeCl_3$ compared

to PAC (Delgado et al., 2003), after the Flock & Lock treatment Lake De Kuil clarified within a week with an increase of 3.0 m in Secchi depth (Fig. 5.6B), a reduction in mean turbidity of 2.9 NTU and a reduction in mean chlorophyll-*a* of 11.1 µg L^{-1} (Fig. 5.6A). This demonstrates that the combined treatment of $FeCl_3$ and LMB would precipitate cyanobacteria rapidly out of the water column. In Lake Rauwbraken the main portion of the LMB needed as P-fixative was applied at the lake's surface. As a result, the crystal clear waters of Lake Rauwbraken after the applied ballast-LMB plus PAC were made turbid again by additional LMB (Van Oosterhout & Lürling, 2011), after which it took weeks until this lake became clear enough to open a window of opportunity for submerged macrophytes to develop. Hence, in Lake De Kuil the remainder 68% of the LMB was injected into the hypolimnion, a procedure that prevented a turbidity increase in the epilimnion. The hypolimnetic injection of LMB addressed the areas of the lake where the sediment P-release was high due to anoxia and the accumulation of soft sediment. In the first year after the Flock & Lock treatment the sediment P-release was reduced by 93%, which is comparable to reduction rates in other Flock & Lock treatments that varied between 80% and 93% (Lürling & Van Oosterhout, 2013; Chapter 3).

Phosphorus and chlorophyll-a

The treatment targeted the sediment P-release and the concentrations of TP and chlorophyll-*a*. After the Flock & Lock treatment the trophic level of Lake De Kuil based on TP and chlorophyll-*a* (Forsberg & Ryding, 1980) shifted from a eutrophic to a mesotrophic state (Fig. 5.14). In parallel, the WFD classification for summer TP and chlorophyll-*a* concentrations shifted from a bad-poor-moderate status to a high status (Fig. 5.14). These shifts have been stable for six summers, which indicate the positive long-term effects of the treatment.

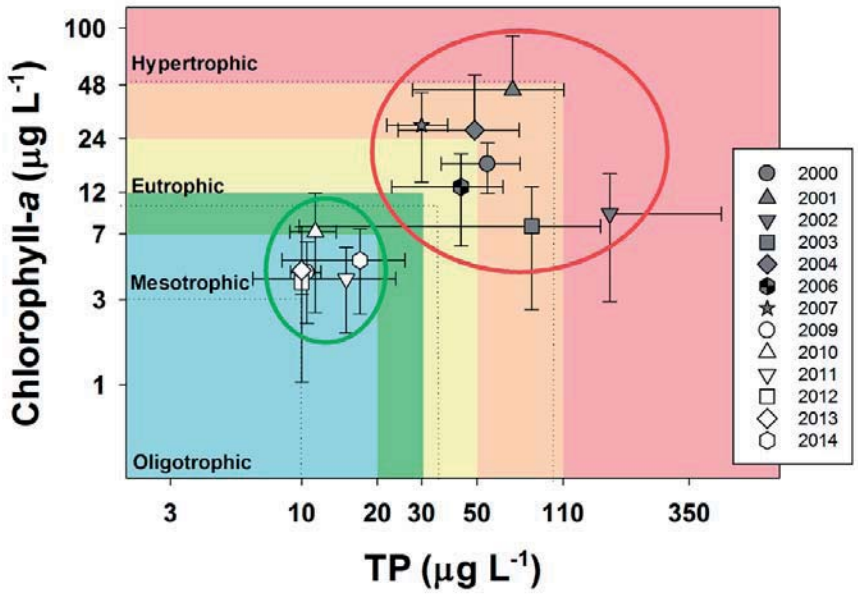


Fig. 5.14: Trophic state and Water Framework Directive (WFD) classification based on mean summer (April – September) concentrations of total phosphorus (TP, µg L^{-1}) and chlorophyll-*a* (µg L^{-1}). Colours indicate WFD status: blue = high, green = good, yellow = moderate, orange = poor and red = bad. Filled symbols: before Flock & Lock treatment (2000 – 2007); open symbols: after Flock & Lock treatment (2009 – 2014).

Nevertheless the long-term monitoring shows year to year fluctuations in the concentrations of TP and chlorophyll-*a* and in Secchi depth (Figs. 5.8, 5.10 and 5.11). Although no modifications other than the Flock & Lock treatment were imposed on the lake, unknown factors such as surface P inputs might have influenced the results. To illustrate this, the results over a multiyear period for Lake De Kuil are compared with Lake Zandwiel which is situated 3 km West of Lake De Kuil. Lake Zandwiel has similar features and eutrophic status to Lake De Kuil including a history of cyanobacterial blooms. No measures targeting the sediment P-release and the concentrations of TP and chlorophyll-*a* were undertaken in Lake Zandwiel. A reduction of summer TP and chlorophyll-*a* concentrations was not observed in Lake Zandwiel. The mean concentrations of TP in Lake Zandwiel were 0.16 ± 0.09 mg P L^{-1} (1995 – 2007) and 0.38 ± 0.15 mg P L^{-1} (2010 – 2013), and for chlorophyll-*a* 32.2 ± 29.2 µg L^{-1} (1995 – 2007) and 42.1 ± 61.1 µg L^{-1} (2010 – 2013; unpublished data Water Authority Brabantse Delta). This lack of long-term reductions in concentrations of TP and chlorophyll-*a* in Lake Zandwiel support the likelihood that the improvements in Lake De Kuil are the result of the Flock & Lock treatment as this was the only intervention.

Cyanobacterial blooms

Before the Flock & Lock treatment there were regular warnings by the authorities of poor swimming water quality and swimming bans due to cyanobacterial blooms. Since 2009 no swimming bans have occurred due to the reduction of cyanobacterial blooms. Nonetheless, after the Flock & Lock treatment each summer cyanobacteria developed in the hypolimnion (Fig. 5.9B), albeit at a reduced biomass. Occasionally elevated concentrations of *Microcystis* and *Aphanizomenon* were observed near the surface, often as local accumulations and most probably these cyanobacteria originated from overwintering cells on the sediment (Preston et al., 1980). Although the reduced P-release rate of Lake De Kuil in 2010 post Flock & Lock treatment was comparable to release rates in oligotrophic lakes (Nürnberg, 1994), in summer 2010 *P. rubescens* developed in the hypolimnion. During the autumn turnover of 2010, *P. rubescens* occurred throughout the water column and bloomed until spring 2011 (Fig. 5.9B). *P. rubescens* is known to proliferate in stratifying lakes after oligotrophication as a transitionary response to the lake's recovery provided TP concentrations are low (Jacquet et al., 2005). Based on the TP concentrations during the blooming period, the cyanobacteria could maximally have contained 11 kg P. The decaying biomass could have led to the higher sediment P-release. However, in sediment cores collected in February 2010, the anoxic P-release was 0.36 (± 0.03) mg P m^{-2} d^{-1}, which means that even if the entire lake bottom sediment during the entire year was releasing P at this rate, the 11 kg P would not have been reached. This indicates anoxic P-release from any P sorbed to any remaining Fe-flocs created during May 2009 could not have been the main source of P supporting the *P. rubescens* bloom in autumn/winter 2010.

Sediment P-release

The increased sediment P-release over time may reflect an underestimation of the LMB dose and hence a progressively decreasing binding capacity to address internal P-release. The dose was based on the potentially available P in the top 5 cm of the sediment and a supposed La content of the LMB of 5%. Analysis of the LMB that was used in Lake De Kuil showed a La concentration of 4.24 % by weight. Similarly, Gibbs et al. (2011) found a lower than specified La concentration of LMB of 4.54% and Reitzel et al. (2013a) 4.4%. Those lower concentrations reduce the P-binding capacity of the LMB. Potentially, 10% less P could have been fixed, which may have allowed more than 45 kg of additional P to be released into the water column.

Another possible factor leading to more sediment P-release is sediment resuspension induced by wind and biota (Kleeberg et al., 2013). Although the LMB will have initially settled upon the sediment as a thin capping layer (on average < 1 mm thick), resuspension would have mixed the LMB through the upper sediment. Indeed, Meis et al. (2012) showed that La from LMB was translocated to sediment depths of 8 cm within 28 days after an LMB application in a reservoir. The translocation of La was described to natural sediment disturbance processes as bioturbation (Meis et al., 2012). Likewise, chironomids have been shown to mix LMB deeper into the sediment (Reitzel et al., 2013c). Bioturbation is largely limited to sediment depths of 10-15 cm (Davis, 1974; Ten Winkel & Davids, 1985; Adámek & Maršálek, 2013), but Søndergaard et al. (1999) reported sediment P-release up to depths of 20 cm. The sequential P extraction from bottom sediment samples collected in our study revealed the presence of potentially bioavailable P at sediment depths of at least 10 cm (Supplementary information, Appendix 5B, Fig. 5.B.1). The underestimation of the sediment mixing depth is confirmed by Dithmer et al. (2016b), who found a relative homogenous La distribution over the upper 11 cm of the cores from Lake De Kuil analyzed. Whether present on top of the sediment in the initial capping layer or incorporated into the sediment, the LMB can be active in intercepting P released from the sediment. Mixing and a longer reaction time have been suggested to have a positive effect on P-binding by LMB (Van Oosterhout & Lürling, 2013) and long-term P-binding effects by LMB have been reported (Novak & Chambers, 2014).

LMB dose

Based on the recommended LMB:P ratio of 100:1 the dosed LMB would theoretically suffice to control the initial sediment P-release of 5.2 mg P m^{-2} d^{-1} for only three years. In addition, the recommended LMB:P ratio of 100:1 might have been too low to reduce the P concentrations to the desired level as a results of interaction of LMB with pH, major anions in the water (Reitzel et al., 2013b) and humic substances (Lürling et al., 2014b). This makes it likely that the amount of LMB that was used in Lake De Kuil was insufficient to absorb the P released over time. After the Flock & Lock treatment of Lake Rauwbraken the sediment P-release over the years is, in contrast to Lake De Kuil, stable at 15-20% of the P-release under anoxia compared to before (Lürling & Van Oosterhout, 2013). In Lake Rauwbraken the dosage of LMB was, equally to Lake De Kuil, based on the potentially releasable P in the top 5 cm of the

sediment (Lürling & Van Oosterhout, 2013). Differences, however, exist in the order of the application of the ballast-LMB and flocculant, as well as the type of flocculant itself (PAC and $FeCl_3$) that in case of Lake Rauwbraken is redox-insensitive that could have compensated for the lower amount of La in the LMB. Moreover, recent sediment core analyses revealed that the sediment mixing depth in Lake Rauwbraken, as indicated by the La profile, is 5 cm (Dithmer et al., 2016b). Hence, in Lake Rauwbraken the potentially released P pool seems to have been addressed adequately, whereas in lake De Kuil it may have been underestimated. Nonetheless, both lakes experienced a shift in trophic status lasting for at least six years. The flocculant-type, in addition to the communicating sediment depth of the P-absorbent are indicated as major factors influencing the observed increase in sediment P-release over time in Lake De Kuil. The increased internal loading, however, has yet to result in increased water turbidity and a proliferation of cyanobacterial blooms. It should be noted that the sediment P-release of 0.3 (± 5.1) mg P m^{-2} d^{-1} determined by Dithmer et al. (2016b) is lower than those determined in this study for cores taken in the same period (6.3 ± 2.1 mg P m^{-2} d^{-1}), which might reflect the spatial heterogeneity.

Secchi depth, macrophytes and fish

Despite year to year fluctuations in Secchi depth (Fig. 5.10), the long-term summer mean increased after the Flock & Lock treatment by 0.81 m to 3.12 m. However, maximum Secchi depths recorded for and after the Flock & Lock treatment were comparable: 5.50 m before the treatment (26 May 2004) and 5.25 m after the treatment (15 June 2009). Lake De Kuil experiences weak stratification during summer. Hence resuspension of sediment and mixing of material from the hypolimnion throughout the entire water column may occur. Albeit the long-term improvement of the Secchi depth in Lake De Kuil was limited, it coincided with the long-term decreases in mean summer concentrations of chlorophyll-*a* from 16 to 6 µg L^{-1}, of TP from 0.05 to 0.02 mg L^{-1} and of o-P concentration from 18 to 6 µg L^{-1}. Although these variables have been similar since the treatment (Figs. 5.8, 5.10 and 5.11), the increasing sediment P-release (Fig. 5.13) indicates the risk for future increase of the trophic status of the lake and will be a subject for further research.

The summer stratification in Lake De Kuil is generally weak due to the morphometry and shallow water depth. Lakes with intermediate mean water depths are shown to be the most susceptible for eutrophication and least manageable as they are too deep to be protected by macrophytes in the littoral zones and too shallow to mitigate sediment P-release through dilution in the hypolimnion (Genkai-Kato & Carpenter, 2005). However, following the Flock & Lock treatment a noticeable increase in macrophyte coverage was observed from few macrophytes to almost 12% area coverage two years after the treatment. Nevertheless, in 2015 the coverage had decreased to 3%, possibly indicating the future relapse to increased turbidity. The macrophytes, besides stabilising the sediment provided structure and promoted macrofauna (Chapter 6) and may also have had a beneficial influence on the fish stock. The fish stock increased from a low 50 kg ha^{-1} before to more

than 130 kg ha^{-1} after the treatment (Table 5.1). This increase was mostly due to adult carp reflecting the greater appreciation of fishermen (and consequently uncontrolled stocking) of the improved water quality. Adult carp is known to feed mainly on benthic invertebrates and is capable of resuspending sediments (Breukelaar et al., 1994; Scheffer, 2004). Anoxic conditions during summer prevent carp to enter the hypolimnion, which prevents carp to cause hypolimnetic resuspension. This, in addition with the carp biomass of 85.7 kg ha^{-1} (2014, Table 5.1) being below the threshold of 200 – 450 kg ha^{-1} where carp populations negatively influence macrophytes and other ecosystem elements (Weber & Brown, 2009), limits the reduction of the Secchi depth by carp. Pike biomass increased, which may also be directly related to improved water clarity and enhanced macrophyte cover.

The WFD classification for macrophytes based on maximum growth depth, species composition and abundance (Van der Molen et al., 2013) was high and did not change after the Flock & Lock treatment, although the vegetation coverage increased during the first three growing seasons and had decreased in 2015 (Table 5.3). Both *E. nuttallii* and *C. vulgaris*, characteristic of mesotrophic/eutrophic conditions (Søndergaard et al., 2010), became dominant over time and filamentous macro algae made up a substantial part of the aquatic vegetation (Table 5.3) reflecting mesotrophic to moderately eutrophic conditions (Simons, 1994). The fish community was according to the WFD classification defined as moderate before (2006) and after the treatment (2011, 2014) indicating a delayed response or no response at all of the fish community to the improved water quality. This indicates that the WFD classification systems for macrophytes and fish (Van der Molen et al., 2013) are poor indicators for eutrophication-impact relationships, although the systems are supposed to reflect the change in ecological status by the responses of the biota, rather than by changes in water quality variables (Birk et al., 2012).

Lanthanum

Before the Flock & Lock treatment the maximum concentration of FLa was 0.39 µg L^{-1} and of TLa 3.9 µg L^{-1} (data not shown). During the Flock & Lock treatment the concentrations of FLa and TLa increased to maxima of 15.9 µg FLa L^{-1} and 893 µg TLa L^{-1} and hence exceeded the Dutch standards for La up to 59% (FLA) and 495% (TLA), albeit only for a period of less than two weeks during and after the application. Despite the sharp decline in La concentrations following these initial maxima the concentrations remained elevated for one (FLa) to four years (TLa). These periods are longer than the 3-12 months recovery periods reported in a review of LMB applications in different lakes by Spears et al. (2013b), although sporadic increases of TLa concentrations after this period occurred. Sporadic increases of TLa concentrations, which were also observed in Lake De Kuil, are likely the result of bed disturbances (Spears et al., 2013b). After a LMB application, La can be found in organisms exposed to the LMB (Van Oosterhout et al., 2014). Although few ecological effects have been observed (Copetti et al., 2016), vigilant monitoring is required following each application. The Flock & Lock treatment included $FeCl_3$ addition. With respect to the low dose application

the mean total Fe and chloride concentrations before and after the treatment were in the same range, respectively 114 and 121 µg Fe L^{-1}, and 142 and 157 mg Cl^{-} L^{-1} (data not shown) and ecological impacts other than the result of eutrophication management were not to be expected. The experiment showed strong increases in macrophytes, higher fish stock, zooplankton developing normally after treatment (Supplementary information, Appendix 5.C), more macrofauna and less cyanobacteria, which illustrates improved water quality and limited, if any, ecotoxicity of the Flock & Lock treatment.

Tools for water management

The results of the treatment in Lake De Kuil support the hypothesis that the Flock & Lock treatment would precipitate cyanobacteria rapidly out of the water column and inhibit internal P-loading leading to a reduction in cyanobacterial biomass in the following years. Considering the significant costs of the Flock & Lock treatment in Lake De Kuil (~€ 140,000 in 2009) a solid preparation and planning exercise underpinning future Flock & Lock treatments should be undertaken. Estimation of the major P-sources as well the communicating sediment depth is particularly important. Targeting the whole potential labile sediment P in Lake De Kuil would have implied a doubling of the costs, which however is still considered more cost-effective than dredging particularly in the light of increased whole of life costs associated with dredge spoil treatment and disposal. This study demonstrates the usefulness of a whole of system analysis of P fluxes and the potential of the Flock & Lock treatment as powerful tools for water management and lake rehabilitation.

Conclusions

Based on the results of this study we conclude that:

- An approach targeting the internal P-loading of a lake is effective in managing the detrimental effects of eutrophication.
- A whole of system analysis of nutrient fluxes provides a sound basis for the assessment of the perspectives of P management.
- The application of the flocculant $FeCl_3$ combined with the solid phase P-fixative LMB (Flock & Lock) effectively precipitated a developing cyanobacterial bloom in Lake De Kuil and shifted the trophic state of the lake from eutrophic to mesotrophic, and maintained this state for at least six years by intercepting sediment P-release.
- After the Flock & Lock treatment of Lake De Kuil, due to the improvement in water quality, and in particular the substantial reduction in cyanobacterial biomass, no swimming bans had to be issued.
- Underestimation of the amount of potentially available P in the upper sediment layers is one probable reason for a gradual increase in sediment P-release over time. Hence the amount of LMB to be applied should be based not only on the potentially available P in the top 5 cm of the sediment, but potentially also to greater depths.

Supplementary information to Chapter 5

Appendix 5.A: Water balance and external P-loads

Introduction

The water system analysis for lake De Kuil consisted of the water balance and the P-loading budget for an average year during the period 2000-2010.

Water balance

Water balance calculations for Lake De Kuil were based on the general equation (1) given by Nõges (2005):

$I + P – E – O \pm \Delta V = 0$ (1)

in which I = Inflow (surface runoff, groundwater, surface water), P = Precipitation onto the water surface, E = Evaporation from the water surface, O = Outflow, ΔV = Change in storage during the period in question; all parameters in m^3 $year^{-1}$.

Water supply of Lake De Kuil is made up of precipitation onto open water and runoff from the adjacent area (2 ha). More specifically the water balance equation for Lake De Kuil is:

$P + SR – E - \Delta V – R = 0$ (2)

in which SR = Surface Runoff, R = Residual (resultant of outflow over a weir into a neighbouring canal, seepage and intrusion). As the water balance was based on a 10 year period, ΔV is set at 0. The main input data for the water balance consist of daily measurement of the precipitation at station Ginneken (9 km distance from Lake De Kuil; 2000-2010) and evaporation at a meteorological station at Gilze-Rijen Airport (16 km distance from Lake De Kuil; 2000-2010). Evaporation data are recalculated to open water evaporation using the relation between reference evapotranspiration according to Makkink and the open water evaporation according to Penman (Hooghart & Lablans, 1988). The adjacent area runs off to the lake and is mainly vegetated with grassland and covers 2 ha. We assumed that the surplus of precipitation (precipitation minus evaporation) runs off to the lake. The residual term consists mainly of outflow over a fixed weir into a nearby canal. During wet periods some seepage is to be expected and during dry periods some intrusion, assumed to sum to ~0 m^3 y^{-1}. The water balance for an average year during the period 2000-2010 for Lake De Kuil is given in Table 5.A.1.

Table 5.A.1: Water balance for Lake De Kuil (m^3 y^{-1}; average situation 2000-2010). Residual term is mainly the outflow over a weir.

IN	**Lake De Kuil**
Precipitation on open water	61,750
Runoff	14,128
OUT	
Evaporation from open water	50,170
Residual	25,708

External P-loads

The external P-loads are given for the lake in an average year. The P-loads are:

• Precipitation on open water. Data for the o-P concentration of rainwater were used from Stolk (2001; meteorological station Gilze-Rijen). It was assumed that the TP concentration in rainwater is two times the concentration of o-P (Buijsman, 1989), resulting in TP concentrations in rainwater of 0.031 mg L^{-1}. Dry deposition for TP was neglected. P-loads were determined on an annual basis by multiplication of the P concentration by the quantity of this source. Resulting P-load due to precipitation on open water is 0.08 mg P m^{-2} d^{-1}.

• Runoff from the adjacent area of grassland (2 ha) to the lake. Normally, the surplus of precipitation infiltrates and flows to the lake as shallow groundwater. Water samples of the shallow groundwater in a monitoring well at the south side of Lake De Kuil (at 16 m distance from the border of the lake) were taken four times during 2011 and analyzed for TP at the Deltawaterlab Laboratory (The Netherlands) following the standard protocol. The water level of the ground water in the monitoring well was around the water level of the lake and the water depth in the monitoring well was around 2.25 m. The results are given in Table 5.A.2. A ground water flow of 3300 m^3 y^{-1} and a mean TP concentration of 0.01 mg P L^{-1} (measurements below the detection limit are taken as half the detection limit) resulted in a P-load of 0.001 mg P m^{-2} d^{-1}. It was assumed that this figure did not differ strongly from previous years as the land use did not change.

Table 5.A.2: TP concentrations (mg P L^{-1}) of shallow ground water in a monitoring well near Lake De Kuil in 2011. < is below detection limit.

Date 2011	TP (mg P L^{-1})
8 March	< 0.01
11 May	0.02
7 September	0.02
10 November	< 0.01

• Water birds. The number and species of water birds for the years 2010 and 2011 were retrieved from the Dutch National Database Flora and Fauna, which is based on the site http://waarneming.nl (accessed on 1 February 2012). From these data, the mean abundance per species per season was determined (Table 5.A.3). The P-load caused by the birds is 0.10 mg P.m^{-2}.d^{-1}, which is the mean of the food intake and dropping models of Hahn et al. (2007, 2008) as calculated with Waterbirds 1.1 (available on http://www.nioo.knaw.nl/content/kwantitatieve-bepaling-van-de-aanvoer-van-voedingsstoffen-door-watervogels-zoetwaterhabitats). It was assumed that this figure did not differ strongly from previous years.

Table 5.A.3: Mean number of water birds per day on Lake De Kuil in 2010, per season. In parentheses is the number of observations. a = autumn, w = winter, sp = spring, su = summer.

	a/w (n=6)	sp (n=3)	su (n=5)
Mallard			
(*Anas platyrhynchos*)	68.5	1.3	2.2
Tufted duck[1)]			
(*Aythya fuligula*)	17.2	9.7	8.2
Grebe[2)]			
(*Podiceps cristatus*)	1.5	1.7	1.0
Eurasian coot			
(*Fulica atra*)	27.0	1.3	2.4
Black-headed gull			
(*Larus ridibundus*)	14.3		0.2
Greylag goose			
(*Anser anser*)		1.0	
Great cormorant			
(*Phalocrocorax carbo*)	0.2		
Lesser black-backed gull			
(*Larus fuscus*)			0.2

[1)] P-load comparable to mallard; [2)] P-load comparable to 0.5 great cormorant

• Litter fall. Litter falling from trees and shrubs caused a P-load into the lake. The P-load caused by litter falling on the ground is part of the P-load caused by runoff, but litter fall from branches directly over open water (area determined from aerial photographs) resulted in an additional P-load. The area of overhanging branches over Lake De Kuil in 2010 was 2100 m^2, while the mean P content of litter is 0.22 % (Dorney, 1985) and the average litter fall from deciduous trees is 442 g DW m^{-2} y^{-1} (Liu et al., 2004). This resulted in a P-load of 0.08 mg $P.m^{-2}.d^{-1}$.

• Bait used by anglers on Lake De Kuil caused a P-load. It was estimated that on average 1 angler km^{-1} shoreline is fished during 183 days y^{-1} (Van Emmerik & Peters, 2009), which is in line with direct observations during sampling events. The length of the shoreline of Lake De Kuil suitable for angling is 1020 m. 60% of the anglers used bait, 1 kg bait $angler^{-1}$ d^{-1} (Van Emmerik & Peters, 2009). The mean P content of ground-baits is 0.3% (Wolos et al., 1992). The resulting P-load caused by the use of bait is 0.01 mg P m^{-2} d^{-1}.

The summed amount of the external P-loads is 0.271 mg P m^{-2} d^{-1}.

Appendix 5.B: Determination of LMB dose for Lake De Kuil

Introduction

The required dose of LMB (Phoslock®) was based on 1) the immediately and potentially releasable and bio-available P in the top 5 cm of the sediment of the lake and 2) the amount of TP in the lake water. The amount of LMB that was needed was determined using a molar ratio of La:P = 1:1, as recommended by the supplier of the LMB (Phoslock Europe GmbH, Ottersberg, Germany). The supplier provided a proposal for the amount of LMB to be used in Lake De Kuil ('Proposal for Treatment of Zwemplas De Kuil Prinsenbeek' dated 10 May 2009). This amount of LMB was actually applied in the lake. Based on our own measurements, we provide the calculation of the LMB dose to be used in the lake and present it together with the calculation of the supplier.

Proposal supplier

The supplier of the LMB listed the amounts of P to be bound in Lake De Kuil and based the amount of LMB to be used on this listing (cf. Proposal for Treatment of Zwemplas De Kuil Prinsenbeek, 10 May 2009):

1. *P-load in the water column to be removed*: 20.3 kg P (based on measurements of the TP concentration on 18 March 2009, at water depths of 1 m and 8 m). Based on the suppliers assumption that 100 g of LMB removes 1 g of P, the removal of 20.3 kg P requires 2030 kg of LMB;
2. *Releasable P of the sediment to be removed*: 348 kg P (based on the total surface of the lake, the concentration of releasable P in the top 5 cm of the sediment and the expectation that the top 5 cm of the sediment is the layer to interact with the water column and be the potential source of releasable P). The removal of 348 kg P requires 34800 kg of LMB;
3. *Additional LMB* for the removal of not fully quantified external P inputs: 5000 kg LMB.

The total amount of LMB to be used is 41830 kg. Based on 1.05 ton LMB per pallet, the amount of LMB in the proposal was 42 tonnes (40 pallets). This amount was applied in May 2009.

Calculation LMB dose

Based on own measurements we calculated the LMB dose to be used:

1. *P-load in the water column to be removed*.

On 18 March 2009 we sampled the water column at each m of water depth in the middle of the lake. On 18 March 2009 the water temperature ranged from 7.9° C at the water surface to 5.8° C at water depths of 7 and 8 m. The water column mean TP concentration was 42.8 µg L^{-1}. Assuming the volume of the lake is 268,000 m^3, the TP-load of the water column to be removed is 11.5 kg P.

2. *Releasable P of the sediment to be removed*.

On 16 December 2008 the morphometry of the sediment was determined by an echosounder, which revealed an uneven under water sediment surface. The echosounder results showed that the deeper parts of the lake (8-9 m water depth) were covered with soft sediment, while the more shallow parts (5-7 m water depth) had a solid and more sandy bottom. On 16 December 2008 sediment cores were taken with a UWITECH sediment core sampler from 3 deep locations (7.8 – 8.9 m water depth) and 2 more shallow locations (6.1 – 6.4 m water depth). The samples were analyzed in duplicate for dry weight and for residue on ignition (NNI, 2007) to calculate the mean percentage organic and inorganic matter (Table 5.B.1). Hereto, from each sediment sample 2 x 20 mL were transferred into pre-weighed aluminium cups that were dried at 105°C, weighed and subsequently ashed at 550°C, after which they were weighed again and the percentage of (in)organic matter calculated according to the Dutch standard (NNI, 2007). The results underpin the echosounder results of accumulation of sediment rich in organic matter in the deepest parts of the lake and a mineral soil in more shallow parts (Table 5.B.1).

Table 5.B.1: Coordinates and water depth (m) of 5 locations were sediment samples were taken on 16 December 2008, the thickness of the sediment (cm) and the percentage (an)organic matter of the sediment.

Location	Coordinates	Water depth (m)	Sediment (cm)	Organic (%)	Inorganic (%)
1	N 51.62240; E 4.70697	7.8	8	1.5	98.5
2	N 51.62363; E 4.70599	8.9	21	9.8	90.2
3	N 51.62323; E 4.70553	6.4	7	5.0	95.0
4	N 51.62252; E 4.70676	8.7	20	11.3	88.7
5	N 51.62088; E 4.70834	6.1	1	1.6	98.4

Based on these findings, 5 additional sediment samples were taken on 18 March 2009 from the deep (> 8 m water depth) locations (Table 5.B.2). Due to temperature dependent variation in sediment P- release and seasonal variation in sedimentation of organic material (Søndergaard et al., 2003) the amount of P stored in the sediment is

Table 5.B.2: Coordinates and water depth (m) of 5 locations were sediment samples in were taken on 18 March 2009, the thickness of the sediment (cm) and the percentage (an)organic matter of the sediment.

Location	Coordinates	Water depth (m)	Sediment (cm)	Organic (%)	Anorganic (%)
I	N 51.62231; E 4.70695	8.5	14	3.3	96.7
II	N 51.62231; E 470695	8.5	14	2.7	97.3
III	N 51.62246; E 4.70688	8.7	18	3.1	96.9
IV	N 51.62312; E 4.70652	8.3	18	2.8	97.2
V	N 51.62378; E 4.70614	8.3	14	3.3	96.7

expected to be maximum in early spring. The top 10 cm of each core was divided into 2 cm thick slices and each slice was subjected to a sequential P extraction (cf. Psenner et al., 1984). In each step the soluble inorganic P and TP were measured using a Skalar SAN+ segmented flow analyzer. The potentially releasable P in the top 5 cm of the sediment was derived from this analysis as the sum of the immediately available P, the Fe/Mn bounded P (reductively labile P) and the organic-P (reductive soluble organic-P and the organic-P in microorganisms, humic and detritus) (Fig. 5.B.1). We used the data from the top 4 cm from cores I, II and IV and the top 6 cm from cores II and V to calculate the mean concentration releasable P as 0.3796 g releasable P kg^{-1} DW sediment in the top 5 cm. The specific weight of the sediment was measured as 1.086 kg DW L^{-1}. Based on our echosounder recording we estimated the deep part of the lake (≥ 6.4 m water depth), with sediment rich in organic matter to be 2.2 ha as was underpinned by visual inspection of randomly taken sediment cores, and we assumed that this is the part of the lake contributing to the sediment P-release. In the rest of the lake (< 6.4 m water depth) the bottom consisted of hard mineral material, which was underpinned by visual inspection of randomly taken sediment cores, and we assumed the sediment P-release from this area to be negligible.

We estimated the amount of releasable P in the top 5 cm of the sediment which had to be bound as 22,000 m^2 * 0.05 m * 0.3796 g releasable P kg^{-1} DW sediment * 1.086 kg DW L^{-1} = 453.5 kg P.

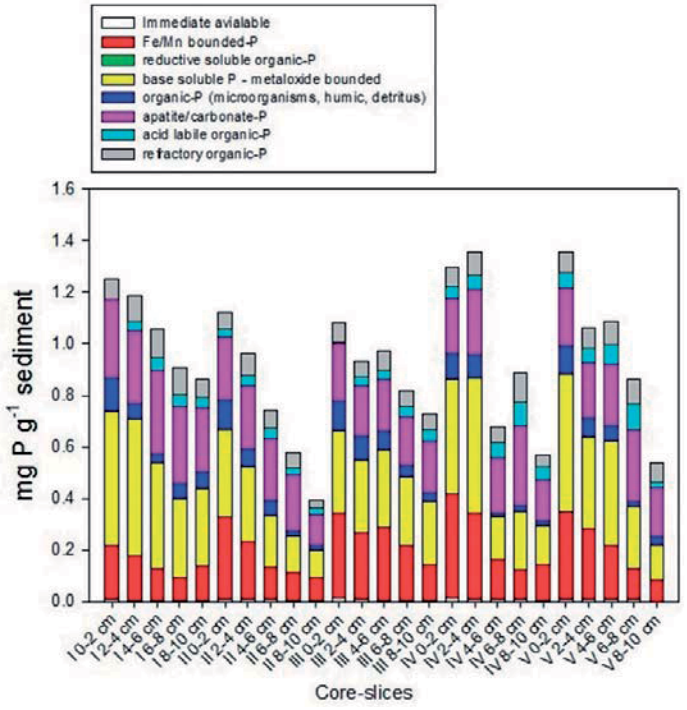


Fig. 5.B.1: Concentrations P in fractions of sediment samples, 0 - 10 cm depth divided into 2 cm thick slices, 18 march 2009.

3. Additional LMB for the removal of external inputs was not considered.

The elimination of the sediment P-release would bring the remaining P input in the region of the critical loading, which would be a promising situation for the realization of a state without recurring cyanobacteria blooms.

The total amount of P to be removed by LMB is 11.5 kg P in water column + 453.5 kg releasable P in top 5 cm of sediment = 465 kg P. We assumed that 1 mol La can potentially bind 1 mol P, giving a required dose of La of 138.9054/30.9738 resulting in 4.485 kg La which is required to bind 1 kg P. To bind 465 kg P we need 2086 kg La. The LMB supplier stated a 5% La in the LMB, which results in 41.7 tonnes LMB required.

Appendix 5.C: Zooplankton in Lake De Kuil in 2009

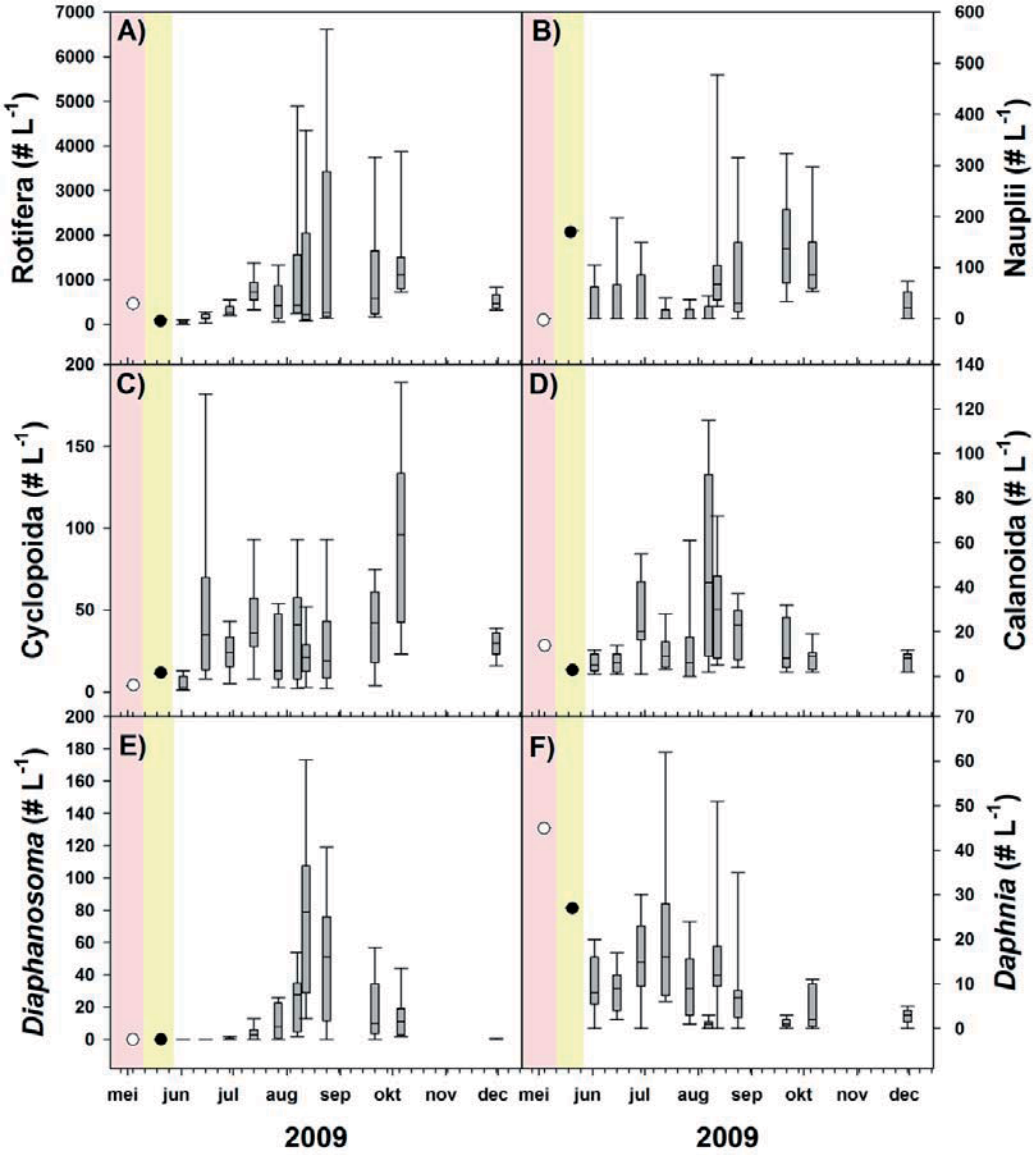


Fig. 5.C.1: Course of abundances (numbers per L) of major zooplankton groups (panel A rotifers; panels B-D copepods) and key species (Panel E, F water fleas) before the Flock & Lock application (white symbols, grey plane), during the application (black symbols, yellow plane) and in the remainder of 2009 (boxes). The boxes represent the data collection over the entire water column in 1 m intervals. The circles are from integrated samples.

Maarten Pauwels sampling macroinvertebrates at Lake De Kuil (22 June 2009)

CHAPTER 6

Effects on macroinvertebrate fauna of eutrophication management by combined flocculant – Lanthanum modified bentonite treatment in Lake De Kuil (The Netherlands)

This chapter is based on:
Effects on macroinvertebrate fauna of eutrophication management by combined flocculant – Lanthanum modified bentonite treatment in Lake De Kuil (The Netherlands). Waajen, G., M. Pauwels & M. Lürling, *submitted.*

Abstract

A low dose flocculant ($FeCl_3$), combined with lanthanum modified bentonite (LMB) as phosphate-binding agent, has been applied as eutrophication management method in Lake De Kuil (The Netherlands). After the treatment, the state of the lake shifted from hypertrophic to mesotrophic. Although macroinvertebrate fauna is important for lake ecosystems, the knowledge of its response to this lake restoration method is fragmented and scarce. Because insight in the macroinvertebrate fauna response is important to assess future applications, pre and post application macroinvertebrate assemblages were identified in Lake De Kuil. The research was accompanied by a microcosm experiment in which the effects of LMB, $FeCl_3$ and LMB + $FeCl_3$ were studied on macroinvertebrate communities. Results show the reduction of macroinvertebrate numbers and taxa during the first month following the application. The number of Gastropoda was strikingly reduced one month after the application. One year after the application, the macroinvertebrate numbers and taxa exceeded the pre-application situation and Gastropoda and Oligochaeta prospered. The short-term (one month) effects of the treatment are most likely due to the combination of physical impacts of the use of bentonite and chemical impacts of the use of $FeCl_3$, while long-term (one year) effects can be attributed to the shift in trophic state of the lake.

Introduction

Eutrophication is considered the primary water quality issue in the world facing many lakes and reservoirs (Smith & Schindler, 2009). Striking effects of eutrophication are the increases of turbidity and phytoplankton biomass, often accompanied by hazardous cyanobacterial blooms, the disappearance of submerged macrophytes and the decline of the biodiversity. Besides detrimental effects on the aquatic ecosystem itself, eutrophication negatively affects the societal ecosystem services of lakes and reservoirs (Smith, 2003; Steffensen, 2008).

Effective eutrophication management demands the reduction of both external and internal phosphorus (P) supplies (Jeppesen et al. 2007b; Schindler et al. 2008), whereby biological and chemical in-lake methods enhance lake recovery (Cooke et al., 2005; Pan et al., 2006b). The use of geo-engineering materials is of growing interest in eutrophication management, as these materials disrupt the negative feedback mechanisms that promote internal loading and provide opportunities for aquatic macrophytes to re-establish (Mackay et al., 2014). One of the materials which has been used in lake recovery is lanthanum modified bentonite (LMB; available as Phoslock®), which is highly efficient in P binding (Robb et al., 2003; Ross et al. 2008; Haghseresht et al., 2009; Van Oosterhout & Lürling, 2013). Currently about 200 water bodies across the globe have been treated with LMB (Copetti et al., 2016). Full scale treatments have shown to be effective in P removal, although water quality responses vary across treated lakes (Spears et al., 2016).

Due to suspended fine clay particles, LMB application may result in high turbidity for several weeks (Van Oosterhout & Lürling, 2011). To speed up the clarifying effect of LMB, even under conditions of a high biomass of positively buoyant cyanobacteria, the LMB application has been combined with a low dose flocculant. This technique, referred to as Flock & Lock, instantly changes the turbid phytoplankton dominated state into the clear water state while strengthening the P removal from the water column (Lürling & Van Oosterhout, 2013). In Lake De Kuil (The Netherlands), Flock & Lock combined LMB with the flocculant iron(III)chloride ($FeCl_3$). After the treatment, an instant and long-term shift from the initial hypertrophic/eutrophic state of the lake to a mesotrophic state was observed (Chapter 5). Lanthanum, which is the P-adsorbing ingredient in LMB, is known to promote plant growth (Xie et al. 2002; Hu et al. 2004; Babula et al. 2008; Jin et al. 2009), while LMB consolidates the sediment and improves macrophyte colonization (Egemose et al. 2010). After the Flock & Lock treatment in Lake De Kuil, the abundance of cyanobacteria decreased, submerged macrophyte coverage increased and the fish stock improved. The zooplankton developed normally (Chapter 5), although another Flock & Lock treatment in Lake Rauwbraken indicated a temporary disappearance of zooplankton (Van Oosterhout & Lürling, 2011). Despite the positive effects on water quality and ecological recovery of Lake De Kuil, awareness is needed for non-target side-effects of Flock & Lock treatments, or applications with solid phase P-fixatives such as LMB (Spears et al., 2013b).

The benthic macroinvertebrate community is essential for aquatic ecosystems and it plays an important role in the assessment of the ecological status of water bodies

(Council of the European Union, 2000). Despite concerns with respect to the environmental safety of LMB (Spears et al., 2013b; Van Oosterhout et al., 2014) and of $FeCl_3$ (Totaro et al., 1992) for macroinvertebrates, little is known of the effects of these materials, when applied in eutrophication management, on the aquatic macroinvertebrates (Copetti et al., 2016). Macroinvertebrates may experience exposure to the geo-engineering materials through ingestion and bioturbation (Lürling & Tolman, 2010; Reitzel et al., 2013c; Copetti et al., 2016). In assessing the effects on invertebrates due to the use of lanthanum, LMB and Flock & Lock (with polyaluminiumchloride – PAC – as flocculant), generally single species laboratory tests using zooplankton have been used, occasionally accompanied by field observations (Watson-Leung, 2009; Lűrling & Tolman, 2010; Van Oosterhout & Lűrling, 2011). Strikingly, studies concerning the effects of LMB on macroinvertebrate species (Clearwater, 2004; Watson-Leung, 2009) and on macroinvertebrate communities are very scarce (Meis, 2012; Bishop et al., 2014), while no information exists on the effects of the Flock & Lock technique on the macroinvertebrate community, despite the sediment is a main target of this technique.

Considering the potential of Flock & Lock as a eutrophication management tool, it is important to provide insight in the implications on the macroinvertebrate community. Based on observations during lake recovery from eutrophication (Köhler et al., 2005; Gunn et al., 2012), we hypothesized that the Flock & Lock treatment in Lake De Kuil would reduce the abundance of macroinvertebrates. As lake recovery increases the Chironomidae : Ologochatae ratio (Wetzel, 2001) and the number of taxa (Gunn et al., 2012), we also hypothesized similar changes to occur in Lake De Kuil. Therefore, we studied changes in macroinvertebrate assemblages from just before to over one year after the Flock and Lock treatment in Lake De Kuil. The research was accompanied by a microcosms experiment.

Materials and methods

Study site

Lake De Kuil is located in the southwest of The Netherlands (51°37'N, 4°42'E; Fig. 6.1) and is the result of sand excavations during the 1950's. Part of the lake is being used as an official bathing site. The surface area of the lake is 6.7 ha, the mean water depth is 4 m and the maximum depth is 9 m. During summer, the lake stratifies thermally. The lake has a history of cyanobacterial blooms (*Planktothrix rubescens*, *Anabaena* ssp., *Woronichinia naegeliana*, *Microcystis aeruginosa*), resulting in swimming bans and warnings. Precipitation and evaporation dominate the water balance, while eutrophication is mainly driven by sediment P-release. Detailed information on the water balance and the P-loading of the lake is given in Chapter 5.

From 18 to 22 May 2009 Flock and Lock was applied to Lake De Kuil. On 18 May 2009 4.38 tonnes of 40% $FeCl_3$ solution (3 m^3; on average 44.8 mL $FeCl_3$ m^{-2}), buffered with 200 kg powdered $Ca(OH)_2$, were sprayed to the lake's surface from a barge. On 19 May 2009 13.65 tonnes LMB were sprayed to the surface, mixed with lake water as a slurry. On 20 and

21 May 2009 28.35 tonnes LMB were injected into the hypolimnion (Chapter 5). In total 42 tonnes LMB (on average 627 g LMB m^{-2}) were applied.

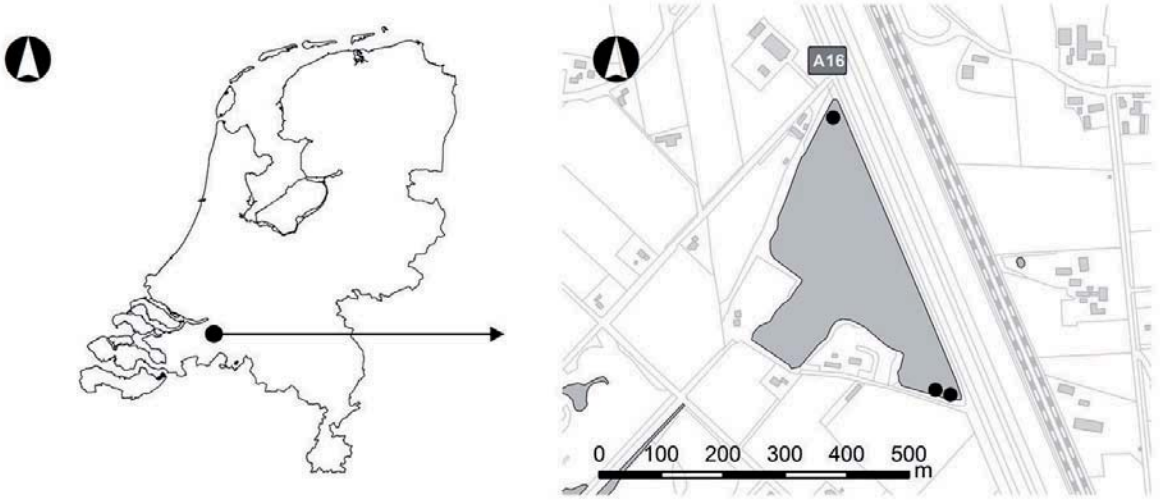


Fig. 6.1: Location of Lake De Kuil in The Netherlands (left panel) and schematic drawing of the lake (right panel) including the three sampling sites for macroinvertebrates (●) and A16 motorway.

Sampling

Three uniform sampling sites for macroinvertebrates were located in the north and south of the lake (Fig. 6.1). Macroinvertebrates were sampled from natural substrates and habitats. Vegetation, sediment and open water in the littoral zone (water depth 0.4 – 0.7 m) were sampled in proportion of occurrence using a hand net (h x w = 20 x 30 cm, mesh 0.5 mm). The total sample lengths at each site were 4 to 5 m. Net samples were collected prior and post Flock and Lock on 21 April 2009, 22 June 2009, 20 April 2010, 18 May 2010 and 22 June 2010.

Supplementary to the net samples, plastic substrate baskets (h x w x d = 20 x 20 x 20 cm, mesh 5 mm) were placed at the three sampling sites. The baskets were open to the top and were completely filled with sandy sediment (top 10 cm) from the littoral zone of the lake collected at site. The baskets were put in place prior and post Flock & Lock (Table 6.1), at water depth 0.4 – 0.7 m with the top of the baskets at the same level as the top of the surrounding sediment. Three substrate baskets were placed next to each other at each sampling site, incubated for 4 weeks and collected (Table 6.1). The macroinvertebrates from net samples and substrate baskets were sorted out in the laboratory under running tap water, using sieves with descending mesh width (minimum mesh 0.5 mm) and preserved in

Table 6.1: Placement dates and sampling dates of substrate baskets. Flock & Lock application 18 – 22 May 2009.

Placement	21 April 2009	25 May 2009	24 March 2010	20 April 2010	18 May 2010
Sampling	14 May 2009	22 June 2009	20 April 2010	18 May 2010	22 June 2010

70% ethanol. The total numbers of macroinvertebrates in the samples were counted. In 2009 the macroinvertebrates were microscopically divided into the taxonomic groups Gastropoda, Oligochaeta, Diptera, Malacostraca, Hirudinea, Trichoptera, Ephemeroptera, Arachnida, Coleoptera, Odonata, Turbellaria, Bivalvia and Heteroptera and counted, while in random parts of each basket sample and of two out of three net samples on each sampling date 35 – 211 specimen were microscopically identified as far as the species level until no more new taxa could be identified. In 2010 all macroinvertebrates were microscopically identified as far as the species level.

Microcosm experiment

Small artificial ecosystems (microcosms) were constructed in the laboratory in June 2009. Each microcosm consisted of a plastic container with a surface area of 35 x 22 cm. Each container was filled with 1 kg (wet weight, WW) of locally collected (not LMB-treated) pond sediment, which was sieved (mesh 1 mm) to remove organisms and debris, and 8 liters of tap water. After three days each container was populated with locally collected (not LMB-treated) 10 adult specimens *Gammarus pulex*, 5 adult specimens *Physa fontinalis*, 5 caddisflies (not identified to species level), 10 adult specimens *Asellus aquaticus*, 30 specimens *Daphnia magna* and 10 g (WW) *Elodea nuttallii*. Additionally 2 g (DW) of leaves of *Populus nigra* were added as a food source for *Asellus* and *Gammarus*.

Different treatments were tested and for each treatment the experiment was executed in fourfold. The treatments were: LMB (584 g m^{-2}), $FeCl_3$ (64.9 mL m^{-2}), LMB (584 g m^{-2}) + $FeCl_3$ (64.9 mL m^{-2}; Flock & Lock) and control (no treatment). The doses are in the range of the Flock & Lock treatment at Lake De Kuil (627 g LMB m^{-2}; 44.8 mL $FeCl_3$ m^{-2}). Simultaneously with the $FeCl_3$, 1.5 mL of 1M $Ca(OH)_2$ solution was added to establish a pH of 7.5. The macroinvertebrates and *Elodea* were added to the microcosms before the treatments were applied. Daphnia from a Wageningen University breed were added directly after the treatments. The other macroinvertebrates and *Elodea* were locally collected from locations that had not been LMB treated before. The experiment was executed at 21^{o}C with a day night regime of twelve hours each. The containers were aerated. To maintain water levels, occasionally tap water was added to the containers. After 4 weeks, macroinvertebrates were collected, identified and counted.

Data analysis

One-way ANOVA (original or log transformed data) and Welch test were used to determine differences between dates (field observations) and treatments (laboratory experiment), followed by Tukeys post hoc and Games-Howell test (SPSS 17.0, SPSS Inc.). Non-parametric Kruskal-Wallis test followed by pairwise Mann-Whitney U-tests were used in case normality requirements were not met. At $P < 0.05$ statistical significance was accepted. To express differences in the macroinvertebrate community diversity between sampling dates of corresponding samples, the Sørenson index for similarity was calculated as 2j/(a+b) where

j is the number of species found in both samples, a is the number of species in the sample from date A and b the number of species in the sample from date B (Magurran, 1988). For each comparison, results from the three locations for net sampling (one at the north and two at the south of the lake) and two baskets from the location at the north of the lake were used. Sørenson indices are given in comparisons of four periods:

a. before and after Flock & Lock in 2009 (samples taken on 21 April 2009 and 14 May 2009 versus 22 June 2009),
b. before Flock & Lock in 2009 compared to one year after Flock & Lock in the similar period in 2010 (samples taken on 21 April 2009 and 14 May 2009 versus 20 April 2010 and 18 May 2010),
c. one month after Flock & Lock in 2009 compared to one year after Flock & Lock in the similar period in 2010 (samples taken on 22 June 2009 versus 22 June 2010),
d. one year after Flock & Lock (samples taken on 20 April 2010 and 18 May 2010 versus 22 June 2010; this concerns similar seasonal periods as in comparison a).

Assessment of the ecological status of the macroinvertebrate community was done according to the classification system of the Water Framework Directive (WFD), based on results from net samples and baskets from the sampling location in the north of the lake. The classification system assesses the ecological status by means of an Ecological Quality Ratio (EQR) ranging from 0 to 1 and differentiating into five ecological classes (bad, poor, moderate, good and high status; Council of the European Union, 2000). The boundaries for the five ecological classes were derived from deep lakes in Van der Molen & Pot (2007). In the microcosm experiment, the survival rate is defined as the number of surviving individuals compared to the number of individuals at the start of the experiment.

Results

Macroinvertebrate density

The density of all macroinvertebrates (indicated as the number of all specimens m^{-2}) significantly declined to about one third, one month after Flock and Lock compared to before. The decline was observed both in net samples (from a mean of 485 specimens m^{-2} to 126 specimens m^{-2}) and in substrate baskets (from a mean of 2850 specimens m^{-2} to 1150 specimens m^{-2}; Table 6.2). One year after Flock & Lock, the total number of specimens (555-770 specimens m^{-2}) in the net samples was higher than before Flock & Lock, although the difference was not significant (Table 6.2). In the baskets, the total number of specimens one year after Flock & Lock (10416-12941 specimens m^{-2}) was significantly higher than before the treatment (Table 6.2). Different macroinvertebrate groups reacted differently over time during the period 14 May 2009 – 22 June 2010 (Figs. 6.2 and 6.3).

Table 6.2: Average density (total number of specimens – Σ n – per m^2) and total number (Σ) of taxa (± 1 SD) before and after Flock and Lock in net samples and in substrate baskets. The capitals (A, B, C) indicate significantly different groups ($P < 0.05$).

	Before application (April-May 2009)	One month after application (June 2009)	One year after application (April-May 2010)	One year after application (June 2010)
Net Σ n m^{-2}	485 (141)^A	126 (42)^B	770 (289)^A	555 (97)^A
Basket Σ n m^{-2}	2850 (222)^A	1150 (87)^B	10416 (5368)^C	12941 (7337)^C
Net Σ taxa	38.0	19.5	64.3 (10.0)	59.7 (5.9)
Basket Σ taxa	18.3 (1.5)^A	11.3 (3.2)^B	29.4 (8.6)^C	41.0 (7.6)^C

Dominant Gastropoda on 14 May 2009 and on the sampling dates in 2010 were *Bithynia tentaculata* and *Physa fontinalis*, in net samples and baskets. The numbers of Gastropoda showed significant differences per sampling date in net samples ($F_{4, 10} = 6.1$, $P = 0.009$) and in baskets ($\chi^2_4 = 10.8$, $P = 0.029$). The numbers on 22 June 2009 were significantly lower compared to 14 May 2009 and to the sampling dates in 2010. In 2010 the mean numbers were on a similar level as before Flock & Lock (Figs. 6.2 and 6.3).

Frequent Oligochaeta in net samples and baskets in 2010 were *Psammoryctides albicola*, *Lumbriculus variegatus*, *Stylaria lacustris*, *Lumbriculus variegatus* and *Limnodrilus hoffmeisteri*. The numbers of Oligochaeta showed significant differences per sampling date in net samples ($\chi^2_4 = 12.7$, $P = 0.013$; Fig. 6.2) and in baskets ($\chi^2_4 = 10.7$, $P = 0.030$; Fig. 6.3). In net samples, the numbers in 2010 were significantly higher than in 2009. In baskets, the numbers on 22 June 2009 were significantly lower than on the other sampling dates. Although the mean numbers of Oligochaeta in baskets in 2010 were higher than on 14 May 2009, the differences were not significant.

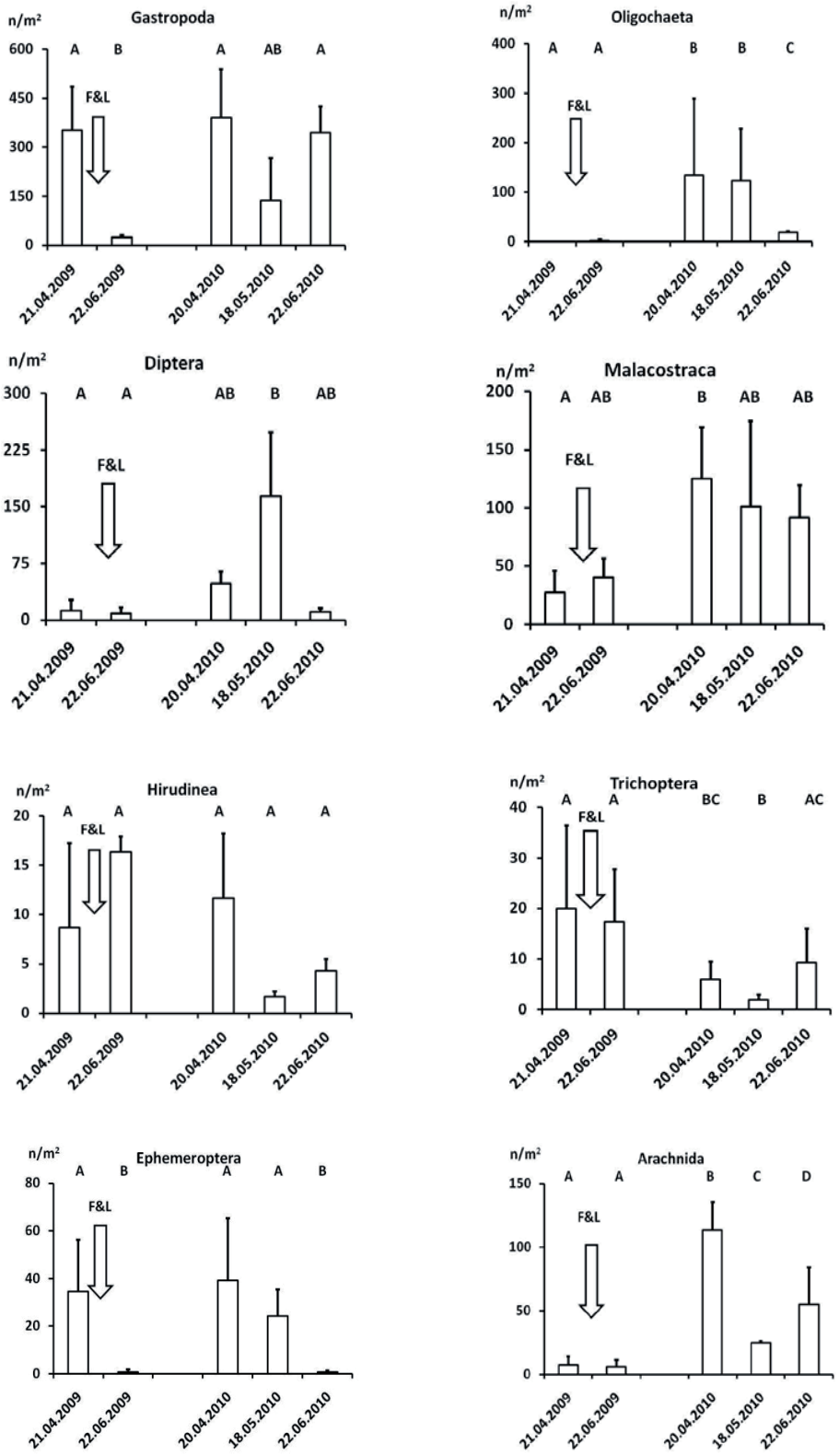


Fig. 6.2: Mean numbers of specimens per m² for macroinvertebrate groups in net samples. Sampling dates: 21 April 2009, 22 June 2009, 20 April 2010, 18 May 2010, 22 June 2010. The arrows indicate the Flock and Lock treatment. Error bars indicate 1 SD (n=3). Letters indicate significant relationships.

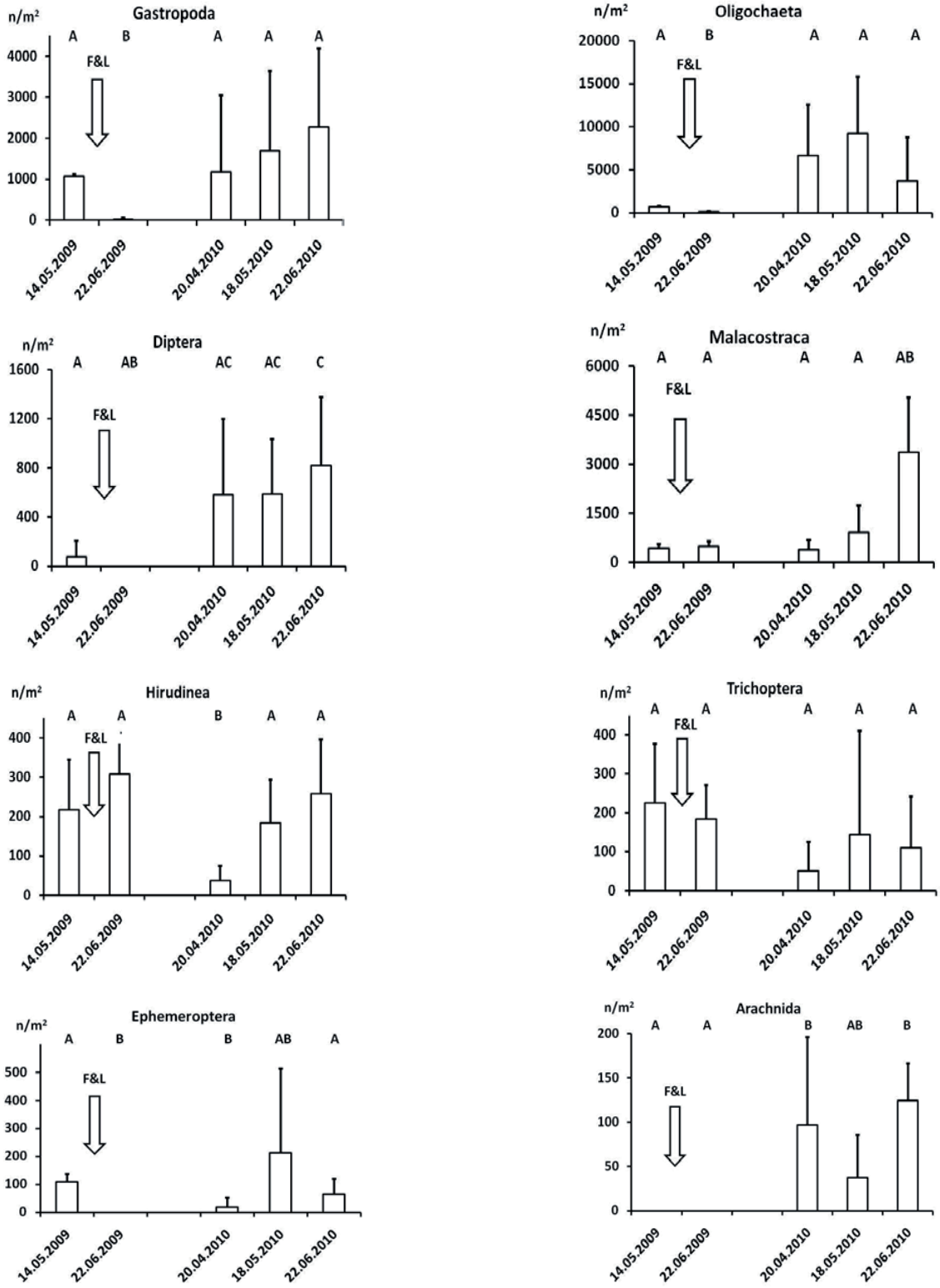


Fig. 6.3: Mean numbers of specimens per m² for macroinvertebrate groups in substrate baskets. Sampling dates: 14 May 2009, 22 June 2009, 20 April 2010, 18 May 2010, 22 June 2010. The arrows indicate the Flock and Lock treatment. Error bars indicate 1 SD (n=3 in 2009, n=6 in 2010). Letters indicate significant relationships.

Larvae of the Ceratopogonidae family dominated the Diptera, accompanied by high numbers of the Chironomidae *Endochironomus albipennis*, *Psectrocladius obvius* and *Glyptotendipes pallens* agg. The highest numbers of Diptera were found in the net samples and baskets in 2010 (Figs. 6.2 and 6.3). In the net samples, Diptera numbers on 18 May 2010 were significantly higher than in 2009 ($F_{4, 10}$ = 5.2, *P* = 0.016; Fig. 6.2). In the baskets, the numbers on 22 June 2010 were significantly higher than in 2009 (χ^2_4 = 10.7, *P* = 0.03; Fig. 6.3). Pre and post treatment numbers in 2009 did not differ significantly (Figs. 6.2 and 6.3).

The dominant species of the Malacostraca was *Asellus aquaticus* and the highest numbers were found on 2010 (Figs. 6.2 and 6.3). The mean number in the net samples taken on 20 April 2010 was significantly higher than on 21 April 2009 ($F_{4, 10}$ = 4.8, *P* = 0.02). Pre and post treatment numbers in 2009 did not differ significantly (Fig. 6.2, Fig. 6.3).

Frequent Hirudinea species were *Erpobdella octoculata*, *Glossiphonia complanata* and *Helobdella stagnalis*. No significant differences were shown in Hirudinea numbers pre and post Flock & Lock in 2009 in net samples and baskets (Figs. 6.2 and 6.3). On 20 April 2010, the mean number in baskets was significantly lower than on the other dates (χ^2_4 = 12.743, *P* = 0.013; Fig. 6.3). This difference was not shown in net samples (χ^2_4 = 8.3, *P* = 0.083; Fig. 6.2).

Frequent Trichoptera species were *Agraylea multipunctata* and *Mystacides longicornis.* The numbers of Trichoptera in the baskets showed no significant difference between the sampling dates (χ^2_4 = 5.1, *P* = 0.272). In net samples, the numbers on 20 April and 18 May 2010 were significantly lower than on other dates (χ^2_4 = 9.7, *P* = 0.046; Fig. 6.2). *Caenis horaria* and *Cloeon dipterum* were the dominant Ephemeroptera by numbers. The numbers of Ephemeroptera on 22 June 2009 were significantly lower than on 21 April 2009 in net samples (χ^2_4 = 10.6, *P* = 0.031; Fig. 6.2) as well as in baskets on 14 May 2009 (χ^2_4 = 10.0, *P* = 0.04; Fig. 6.3). In net samples, the pattern in 2010 was similar to 2009 with significantly lower numbers on 22 June compared to 20 April 2010 (Fig. 6.2). In baskets, the pattern in 2010 was opposite and on 22 June 2010 the number of Ephemeroptera was significantly higher than on 20 April 2010 (Fig. 6.3).

In 2009, the numbers of Arachnida in net samples were ~7 specimens m^{-2} (Fig. 6.2), while Arachnida were absent in baskets (Fig. 6.3). In 2010, frequent Arachnida species were *Hydrodroma descipiens*, *Hygrobates longipalpis* and *Piona pusilla. Argyroneta aquatica* was occasionally collected. Numbers of Arachnida were significantly higher in 2010 than in 2009 in net samples (χ^2_4 = 13.0, *P* = 0.011; Fig. 6.2) and in baskets (χ^2_4 = 13.7, *P* = 0.008; Fig. 6.3).

Coleoptera numbers were low, reaching up to 6 specimens m^{-2} in net samples and 42 specimens m^{-2} in baskets, both in 2010. Regular species were *Haliplus immaculatus* and *Haliplus lineolatus.* No significant differences were shown between the different dates in net samples (χ^2_4 = 2.1, *P* = 0.716). In baskets a significantly higher number was shown on 22 June 2010 compared to all other dates (χ^2_4 = 14.9, *P* = 0.005).

Odonata numbers were low, reaching up to 22 specimens m^{-2} in net samples on 20 April 2010 and 37 specimens m^{-2} in baskets on 20 April 2010. Most abundant species

was *Ischnura elegans.* In net samples, the numbers on 18 May and 22 June 2010 were significantly lower than on the other dates (χ^2_4 = 10.9, *P* = 0.027). In baskets, no significant difference was revealed in the numbers on different dates (χ^2_4 = 9.5, *P* = 0.051).

Turbellaria were absent in 2009 in net samples and baskets. In 2010, Turbellaria were present, reaching mean numbers up to 32 specimens m^{-2} in net samples on 20 April and 1154 specimens m^{-2} in baskets on 22 June. Regular taxa were *Dendrocoelum lacteum*, *Dugesia tigrina* and *Dugesia lugubris/polychroa.*

The Bivalvia were absent in 2009 in net samples and in baskets. In 2010, Bivalvia were present, reaching mean numbers up to 7 specimens m^{-2} on 18 May in net samples and 245 specimens m^{-2} in baskets on 22 June. Frequent taxa were *Dreissena polymorpha* and *Pisidium* ssp.

Heteroptera were absent in net samples taken in 2009 and reached numbers up to 3 specimens m^{-2} on 18 May and 22 June 2010. In the baskets they were present only on 20 April 2010 (mean number was 9 specimens m^{-2}). Taxa that were encountered were *Notonecta viridis* and *Micronecta* ssp.

Similarity of macroinvertebrate assemblages

Macroinvertebrate community composition changed over time in 2009 and 2010. The highest mean similarity was shown in samples taken one year after Flock & Lock (comparison d: samples taken on 20 April 2010 and 18 May 2010 versus 22 June 2010, Sørenson index = 0.54; Fig. 6.4). The lowest mean similarity was shown in samples taken one month after Flock & Lock compared to one year after Flock & Lock (comparison c: 22 June 2009 versus 22 June 2010 Sørenson index 0.21; Fig. 6.4). The differences between both comparisons c and d were significant ($F_{3, 8}$ = 13.6, *P* = 0.002). Differences between other periods (comparisons a and b) were intermediate (Fig. 6.4).

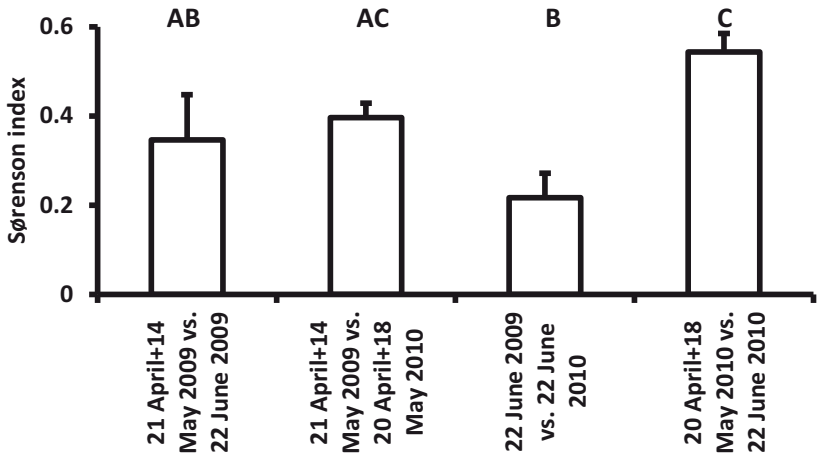


Fig. 6.4: Mean Sørensen index for macroinvertebrate samples of Lake De Kuil in a comparison of four periods. Error bars indicate 1 SD (n=3). Letters indicate significant relationships.

Ecological Quality Ratio (EQR)

The ecological status of the macroinvertebrate community was classified as moderate. The EQRs ranged from 0.46 to 0.52 (Fig. 6.5). No significant difference between sampling dates was shown ($F_{4, 10}$ = 0.45, *P* = 0.770).

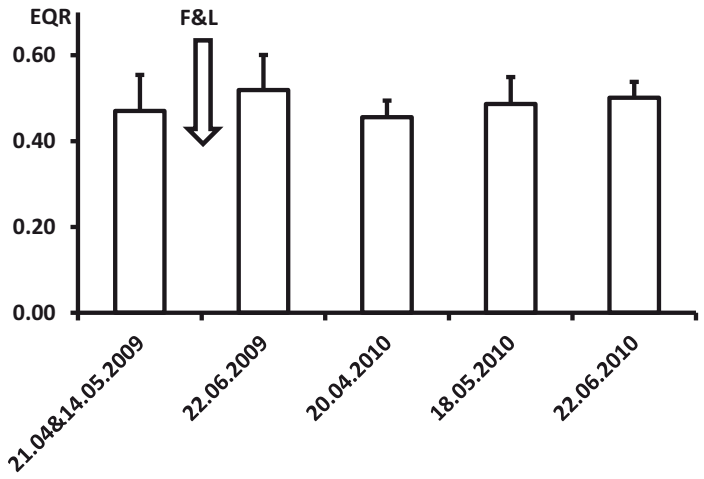


Fig. 6.5: Mean Ecological Quality Ratio (EQR) for the macroinvertebrate community of Lake De Kuil on sampling dates before and after the treatment. The arrow (F&L) indicates the Flock and Lock treatment. Error bars indicate 1 SD (n=3).

Microcosm experiment

The survival rates differed between the species and between the treatments (Fig. 6.6). The survival rates of *Asellus aquaticus* ranged from 40% in the exclusive LMB treatment to 133% in the control, but no significant difference between treatments was shown ($F_{3, 6.3}$ = 3.4, *P* = 0.091). The survival rates of *Gammarus pulex* were significantly lower in the LMB treatment (28%) and the LMB + $FeCl_3$ treatment (18%) than in the $FeCl_3$ treatment (73%) and in the control (68%; χ^2_3 = 12.3, *P* = 0.007). The Gastropod *Physa fontinalis* showed significant lower survival rates in the LMB treatment (85%) and the LMB + $FeCl_3$ (60%) compared to the control (150%), while the treatment $FeCl_3$ showed an intermediate survival rate of 110% (χ^2_3 = 10.7, *P* = 0.013). Survival rates over 100% indicate propagation during the experiment.

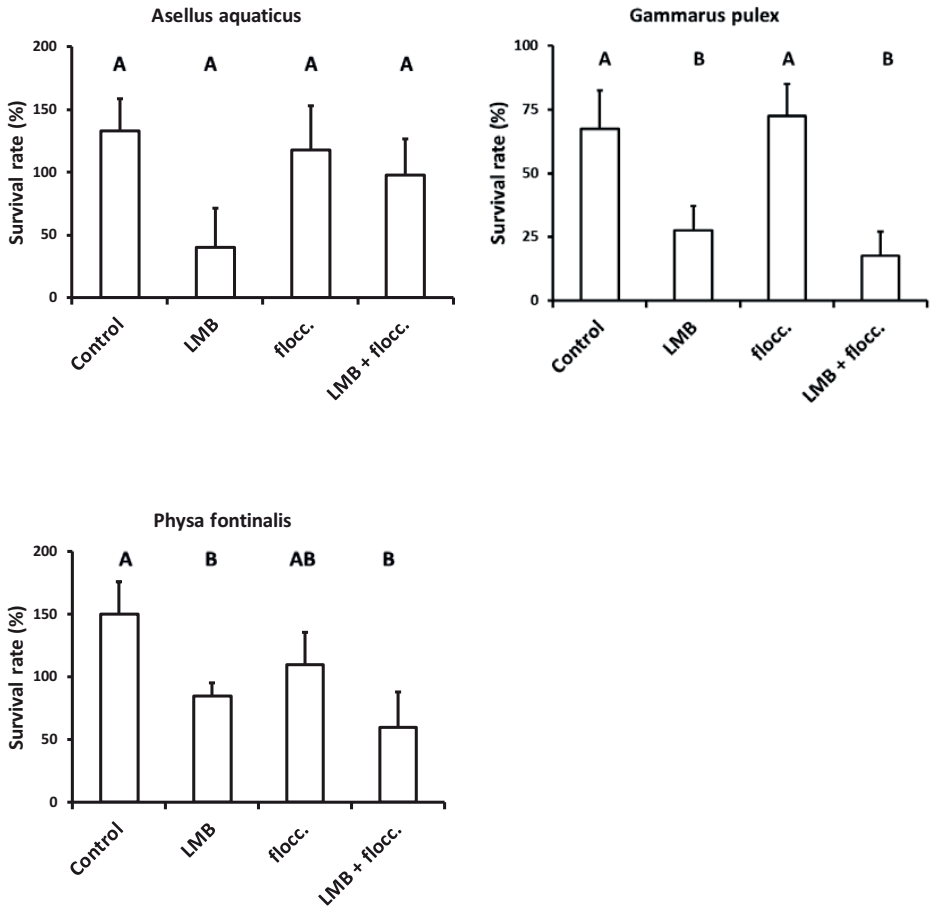


Fig. 6.6: Survival rates of Asellus aquaticus, Gammarus pulex and Physa fontinalis in the microcosm experiment. Treatments: LMB (lanthanum modified bentonite), flocculant (flocc. = $FeCl_3$), LMB + flocculant, and control. Error bars indicate 1 SD (n = 4). Letters indicate significant relationships.

Discussion

In Lake De Kuil, the Flock & Lock treatment combined the application of LMB with a low dose flocculant ($FeCl_3$) which effectively precipitated a developing *Aphanizomenon flos-aquae* bloom. The TP and chlorophyll-*a* concentrations shifted from eutrophic to mesotrophic, which state remained stable for at least six years, while cyanobacterial blooms have been reduced (Chapter 5). As the macroinvertebrate fauna contributes largely to the biodiversity of lakes (Van de Meutter et al., 2006) and is an essential element in the ecological assessment according to the WFD (Council of the European Union, 2000), insight in the implications of Flock & Lock on macroinvertebrates is important in the approval of Flock & Lock as an environmentally acceptable method for eutrophication management.

Short-term and long-term changes in macroinvertebrate fauna

In this study, two sampling methods were used (net sampling and substrate baskets). The baskets got quickly colonized and different taxonomic groups reached higher densities (specimens m^{-2}) in the baskets than could be deduced from the net samples. Despite these differences in densities due to the sampling methods, the results showed similar patterns over time within the macroinvertebrate groups. The macroinvertebrate community showed

a significant decline in number of taxa and number of specimens during the first month following the Flock & Lock treatment, both in net samples and baskets (Table 6.2). This observation was supported by the low similarity between the community one month after the treatment compared to the same season one year later (Sørenson index 0.21; Fig. 6.4). One year post Flock & Lock, the abundance of macroinvertebrates had increased and the number of taxa exceeded significantly the pre-treatment situation, both in net samples and baskets (Table 6.2). As most invertebrate taxa in lakes are associated with mesotrophic conditions (O'Toole et al., 2008), the shift of the eutrophic state to the mesotrophic state in Lake De Kuil is expected to improve conditions for a diverse macroinvertebrate community (Gong & Xie, 2001). Although declines in macroinvertebrates due to management interventions in lakes have been shown before (Van Kleef et al., 2006; Angeler & Goedkoop, 2010), following a LMB application no substantial community variations have been found (Bishop et al., 2014). Another study, however, showed that LMB application resulted in significant decreases of Chironomidae and Oligochaeta, while the abundance of the Gastropoda Bythiniidae did not vary substantially (Meis, 2012). As such, the responses of the macroinvertebrates to the LMB application in Meis' study (2012) contradicted other findings that pointed towards an increase of the Chironomidae to Oligochaeta ratio during lake recovery (Wetzel, 2001). Van Haaren & Soors (2013) indicated sediment type, organic enrichment of the sediment, local temperature regime and groundwater influences to be key factors determining the Oligochaeta fauna in parts of northwestern Europe, rather than the trophic state of the water body.

The taxonomic groups present in Lake De Kuil showed different short-term responses at the end of the first month following Flock & Lock. Particularly, the abundance of Gastropoda significantly declined (Figs. 6.2 and 6.3). A similar effect was not detected during the same season one year after the treatment. Other taxonomic groups showed a less obvious short-term response (e.g., Oligochaeta), or no response at all (e.g., Hirudinea; Figs. 6.2 and 6.3). One year after Flock & Lock, macroinvertebrates had increased in abundance and in the number of taxa (Table 6.2) and several groups (for instance Oligochaeta and Gastropoda) flourished (Fig. 6.2, Fig. 6.3). Although macroinvertebrate communities can be highly variable among years (Yee et al., 2000), the absence of adverse responses of the macroinvertebrate community one year after Flock & Lock is important when selecting management options for lakes. Nevertheless, the response of the Gastropoda abundance is manifest on the short-term (one month) following Flock & Lock. The short-term negative effect on Gastropoda numbers, as observed in the lake, is underpinned by the results of the microcosm experiment, in which *Physa fontinalis* was negatively affected by LMB , LMB + $FeCl_3$ and $FeCl_3$ (Fig. 6.6).

Physical and chemical impacts

Although Gastropoda are vulnerable to acidification, short-term effects of acidification during Flock & Lock are not to be expected (Servos & Mackie, 1986). As $FeCl_3$ is acidic, a

drop in pH below 6.5 was counteracted by the simultaneous addition of $Ca(OH)_2$. Within a few hours after the addition of $FeCl_3$, the pH in Lake De Kuil and in the microcosms rose well above 7.0, indicating an adverse effect of acidification by $FeCl_3$ not plausible. A negative effect of $Ca(OH)_2$ on Gastropoda is not to be expected. Yee et al. (2000) found no change in macroinvertebrates following lime treatments at a hundredfold concentration compared to our study. More likely however, are effects caused by the addition of bentonite and $FeCl_3$. The microcosm experiment indicated that the response to LMB exceeded the effect of $FeCl_3$. Treatments of algal and cyanobacterial blooms by clay addition and flocculation have been reported for marine and freshwater environments (Lewis et al., 2003; Guenther & Bozelli, 2004; Sengco & Anderson, 2004; Beaulieu et al., 2005; Pan, 2006; Verspagen et al., 2006; Hagström et al., 2010). Flocculation can affect macroinvertebrate communities by reducing phytoplankton biomass and food availability. Decrease of Secchi depth during the application and increase shortly after (Chapter 5) affects visual predation on macroinvertebrates and the clearance rate of filter-feeding organisms (Shumway et al. 2003; Archambault et al. 2004; Beaulieu et al. 2005; Hagstöm et al. 2010). Lewis et al. (2003) suggested the transfer of toxicity from toxin producing algae to underlying substrates containing benthos, caused by flocculation. Beaulieu et al. (2005) indicated physical and chemical changes of sediments due to flocculation by clay. These processes can affect deposit feeders, settlement of larvae and chemical fluxes across the sediment-water interface. Egemose et al. (2010) found that LMB increased sediment stability, which also may affect benthic invertebrates. These studies showed a plethora of effects due to flocculation by clays, depending on local conditions and the taxa involved. Özkundakci et al. (2011) indicated that the grain size of geo-engineering materials affects planktonic communities. In what way this might affect macroinvertebrates as well is unknown, but given the different life-strategies of many macroinvertebrates compared to zooplankton, the effect of grain size on macroinvertebrates is disputable. As a well-developed macroinvertebrate community depends on submersed macrovegetation, providing habitats and shelter (Wetzel, 2001), the observed increase of macrophytes following the Flock & Lock treatment in Lake De Kuil (Chapter 5) is beneficial. Van Oosterhout & Lürling (2011) hypothesized that the temporary disappearance of *Daphnia galeata* following Flock and Lock was due to the combination of physical effects caused by flocs, grazing inhibition, low food concentrations and the lack of predation refuge. For macroinvertebrates too, multiple causes are likely and the need exists for further assessment.

Aside from physical effects of flocculation, chemical effects on macroinvertebrates can be expected. Toxicity tests with LMB on macroinvertebrates indicated low adverse or no effects at all in the range of the LMB concentrations used in field applications (Clearwater, 2004; Watson-Leung, 2009; Copetti et al., 2016). Despite potential negative effects due to heavy metals in clays used for flocculation (Lewis et al., 2003), the environmental risks from metals leaching from LMB are expected to be absent (Lürling & Tolman, 2010). Effects of the use of LMB can be expected from the lanthanum itself. Standards for total and filterable

lanthanum have been violated in Lake De Kuil for less than two weeks after the Flock & Lock application (Chapter 5). Gibbs et al. (2011) showed a low but persistent leaching of lanthanum at the sediment surface after sole LMB application. Lürling & Tolman (2010) detected lanthanum in the filtrates from LMB suspension. The most striking adverse effect on macroinvertebrates in our study appeared on the short-term (one month) rather than on the long-term (one year), indicating a negative effect of a low and persistent leaching of lanthanum not to be important. Although lanthanum is known to be toxic to Daphnids (Barry & Meehan, 2000), no major detrimental effects on Daphnia are to be expected form LMB or its active component lanthanum when applied in eutrophication management (Lürling & Tolman, 2010). Although evidence is lacking, the results from studies on Daphnids and the observed recovery and prosper of the macroinvertebrate community after one year in Lake De Kuil and in other water bodies (Meis, 2012; Bishop et al., 2014) indicate that chemical effects of lanthanum are not likely to be the cause for observed effects.

Spraying of $FeCl_3$ solution is known to have detrimental effects on terrestrial snails and earthworms (Totaro et al., 1992). Addition of $FeCl_3$ in the aquatic system results in the formation of iron(III)phosphate (Cooke et al., 1993). Although iron(III)phosphate is naturally widespread, it is also known for its molluscicidal properties (Speiser & Kistler, 2002; Langan & Shaw, 2006). The advised dosage as a molluscicide of commercially available iron(III) phosphate ranges from 0.02 to 0.1 g Fe/m^2. As the dosed Fe in Lake De Kuil averaged 15.3 g Fe/m^2, iron(III)phosphate may have potentially affected Gastropoda. Iron(III)phosphate has been reported to have adverse impacts on other terrestrial macroinvertebrates as well (Langan & Shaw, 2006), indicating potential adverse effects on other components of the macroinvertebrate community of Lake De Kuil as well. As the findings in this study indicate the recovery of the macroinvertebrate community after one year, the adverse effects seem to be temporary.

Despite the observed changes in water quality (Chapter 5) and in abundance and taxa richness of macroinvertebrates following the Flock & Lock treatment, the macroinvertebrate community was classified as moderate during the entire course of study. Even though WFD classification systems intend to reflect ecological changes (Birk et al., 2012), this study showed that the WFD classification system for macroinvertebrates (Van der Molen & Pot, 2007) poorly indicated the lake recovery.

Conclusions

The effects of Flock & Lock in Lake De Kuil had a short-term (< one year) adverse impact on the macroinvertebrate community. The responses are likely to be caused by the combination of physical factors (decline of food availability, increase of visual predation, a transfer of toxicity from algae to benthonic habitats and a change in composition of the sediment) and effects of the formation of iron(III)phosphate. On the long-term (one year) an increase in macroinvertebrate density and taxa was observed, although the changes were not reflected by WFD classification results. A change in the macroinvertebrate community

can be of influence to higher-trophic-level predators, such as fish (Rodusky et al., 2008). Possible effects on macroinvertebrate communities, like risk of disappearance of rare species and effects to higher trophic levels, have to be considered when designing management schemes. Such effects have to be weighted up against detrimental effects of cyanobacterial blooms. A good practice of monitoring and evaluation of future treatments is essential for the progress in lake restoration.

Lanthanum modified bentonite at Lake De Kuil (18 May 2009)

CHAPTER 7

Bio-accumulation of lanthanum from lanthanum modified bentonite treatments

This chapter is based on:
Bio-accumulation of lanthanum from lanthanum modified bentonite treatments in lake restoration.
Waajen, G., F. van Oosterhout & M. Lürling, *submitted.*

Abstract

Lanthanum (La) modified bentonite (LMB) is one of the available mitigating agents used for the reduction of the phosphorus (P) recycling in eutrophic lakes. The potential toxicity of the La from LMB to aquatic organisms is a matter of concern. In this study the accumulation of La was investigated in the macrophyte *Elodea nuttallii*, in chironomid larvae and in several fish species during periods up to five years following in situ LMB applications. The application of LMB increased the La concentration of exposed plants and animals. During the first growing season following LMB application, the La content of *E. nuttallii* increased 78 to 127 fold. During the second growing season following LMB application, the La content decreased but was still raised compared to plants that had not been exposed to LMB. The La content of chironomids was doubled two years following LMB application, although the increase was not significant. Raised La concentrations in fish liver, bone, muscle and skin were observed two and five years subsequent to the LMB application. Liver tissues showed the highest increase, ranging from 6 to 20 fold increases two years following LMB application and from 6 to 13 fold increases after five years in pelagic and littoral fish. The La content of the liver from benthic *Anguilla anguilla* (eel) had increased 94 fold two years and 135 fold five years following LMB application. No acute and chronic effects of La accumulation were observed. We advocate the long-term study of effects of La accumulation following future LMB applications.

Introduction

Eutrophication of water bodies is considered a major water quality problem, often resulting in detrimental cyanobacterial blooms (Watson et al., 1997; Smith & Schindler, 2009). In urban regions, cyanobacterial blooms are widespread and the reduction of such blooms is of major concern to water managers (Chapter 2). A prerequisite for the long-term reduction of cyanobacterial nuisances is the reduction of nutrient loadings (Cooke et al., 2005; Jeppesen et al. 2012). Next to the reduction of external nutrient loadings, the reduction of the biologically available internal pool of nutrients is crucial, as this pool may hamper lake recovery for long periods (Søndergaard et al., 1999). In managing eutrophication, the focus is on the control of phosphorus (P) which is considered the key nutrient (Schindler et al., 2008; Chapter 1).

Lanthanum modified bentonite (LMB, commercially available as Phoslock®) has a high P-binding capacity. LMB, which has been developed by the Australian CSIRO (Douglas, 2002), can be an attractive means targeting the reduction of the sediment P-release and the inactivation of the water column associated P pool by the formation of poorly soluble lanthanum-phosphate (Lürling & Van Oosterhout, 2013; Chapters 3 and 5). Next to the P-binding capacity of LMB, it is important to take the ecotoxicological safety of its active ingredient lanthanum (La) into consideration. Uptake of dissolved La by aquatic organisms has been shown (Qiang et al., 1994; Yang et al., 1999) and the potential toxicity of La to aquatic organisms has been indicated a matter of concern (Barry & Meehan, 2000). To overcome the potential toxicity of La, LMB incorporates the La into a bentonite matrix (Haghseresht, 2006). This storage intends to make LMB ecotoxicologically compatible (Copetti et al., 2016). Being locked in the bentonite matrix, the La can either react with phosphate ions in the water or stay in the matrix (Ross et al., 2008). The storage of La in the bentonite matrix and the extreme low solubility of produced La-phosphate indicate no bioavailability of La from LMB and little toxicological risk has been suggested (Haghseresht, 2006). Nevertheless, La may leach from LMB following application (Lűrling & Tolman, 2010; Spears et al., 2013b) and increased insight in its potentially unintended treatment consequences is needed (Douglas et al., 2016). Uptake of La from LMB by animals has been shown in the laboratory (Van Oosterhout et al., 2014) and undergoing field application (Landman & Ling, 2006), however information on this topic is scarce. Next to the uptake of dissolved La, the ingestion of LMB has been suggested as a route for La to enter biota (Van Oosterhout et al., 2014). Bioavailability of La from LMB is a prerequisite for possible toxic effects. As to our knowledge, long-term information on bio-accumulation of La following LMB applications is lacking, we studied the La content of aquatic organisms from different trophic levels. This chapter presents the results of analyses of the La content of chironomid larvae, a selection of fish and the macrophyte *Elodea nuttallii* before and after field applications of LMB at Lake De Kuil (Chapter 5), pond Dongen and pond Eindhoven (Chapter 3), during periods up to five years following the application. We hypothesized that the presence of LMB in the water body does not increase the La concentration (μg g^{-1}) of exposed organisms.

Material and methods

Study sites, treatments and chemicals

Lake De Kuil (N 51° 37' 22"; E 4° 42' 23") has an area of 6.7 ha, an average water depth of 4 m and a maximum water depth of 9 m. Part of the lake is a bathing site, while the rest of the lake is used for angling. Regularly occurring cyanobacterial blooms resulted in swimming bans and warnings. On 18 May 2009, 4.38 tonnes of $FeCl_3$ solution (44.8 mL $FeCl_3$ m^{-2}; ρ = 1.46 kg L^{-1}, 40% $FeCl_3$) were applied to the lake as flocculant. From 19 until 21 May 2009, 13.65 tonnes LMB (Phoslock®, 5% La) were applied to the surface of the lake and 28.35 tonnes LMB were injected into the hypolimnion (on average 0.627 kg LMB m^{-2}). Characteristics of Lake De Kuil and of the treatment are provided in detail in Chapter 5.

In the shallow ponds Dongen (N 51° 37' 48''; E 4° 56' 28'') and Eindhoven (N 51° 48' 97''; E 5° 47' 66''), compartments of 210 – 400 m^2 each were constructed. In the compartments, from August 2009 until September 2011 treatments were tested managing the frequently occurring cyanobacterial blooms in the ponds:

1. dredging + biomanipulation (DB)
2. dredging + polyaluminiumchloride (PAC)+ biomanipulation (DPB)
3. LMB + biomanipulation (LB)
4. LMB + PAC + biomanipulation (LPB)
5. biomanipulation (B).

LMB (Phoslock®, 5% La) was applied in two compartments at pond Dongen on 4 September 2009 (0.75 kg LMB m^{-2}) and in two compartments at pond Eindhoven on 2 and 3 September 2009 (1.13 kg LMB m^{-2}), while at each pond one of the LMB-treated compartments additionally received the flocculant PAC (AquaPAC39, $Al_n(OH)_mCl_{3n-m}$; ρ = 1.37 kg L^{-1}, 8.9 % Al, 21.0% Cl), 16.7 mL PAC m^{-2} at pond Dongen and 16.3 mL PAC m^{-2} at pond Eindhoven. The other compartments did not receive LMB. The LMB and the flocculants $FeCl_3$ and PAC for the three sites were supplied and applied by Phoslock Europe GmbH (Ottersberg, Germany).

At the start of the experiment, macrophytes were lacking in the compartments. Biomanipulation included the introduction of *Elodea nuttallii* (Nuttall's waterweed, also known as western waterweed) and fish stock control. Each biomanipulated compartment received 1 kg of locally collected fresh *E. nuttallii* (on 28 April 2010 at Dongen and on 29 April 2010 at Eindhoven), from locations that had not been treated with LMB. Fish were removed from the compartments 1 – 5 at the start of the experimental period. These compartments were stocked with roach (Rutilus rutilus) and pike (Esox lucius) from locations that had not been treated with LMB, at pond Dongen in July and August 2010 and at pond Eindhoven on 28 September 2009. Characteristics of both ponds and of the treatments are provided in detail in Chapter 3.

Sampling and analysis

Lake De Kuil: chironomids and fish

Chironomid larvae were collected before (18 March 2009) and after the LMB treatment (30 June 2011) at 10 sites spread over Lake De Kuil with an Ekman-Birge sampler (top ~5 cm of the sediment). The chironomids were sorted out by sieving the sediment samples under running tap water (as described in Chapter 6) and the density (n m^{-2}) was determined. After collecting the chironomids, they were rinsed under running tap water to remove potentially attached LMB from the outside. The chironomids were stored at -18° C and then subsequently freeze-dried (-60° C), grinded and 12-16 h dried at 40° C. According to protocol C8-E6 (Wageningen University, Aquatic Ecology and Water Management Group; based on Van Griethuysen et al., 2004), approximately 20 mg of dried sample was destructed using successively 200 µl Ultrex HNO_3 (65%) and 100 µl H_2O_2 (30-35%) at 94° C. After complete evaporation, 2 mL of nano pure water was added and the samples were mixed (Vortex). Thereafter, 1 mL of each sample was diluted with 9 mL 0.1 M Ultrex HNO3. The destruates were analyzed for La (ICP-MS, Laboratory of the Department of Soil Siences, Wageningen University).

Fish from different species present in Lake De Kuil (eel, *Anguilla Anguilla*; perch, *Perca fluviatilis;* roach, *Rutilus rutilus;* bream, *Abramis brama;* pike, *Esox Lucius*; tench, *Tinca tinca;* Chapter 5) were caught before (9 April 2009) and after the LMB treatment (28 September 2011, Fig. 7.1; 28 August and 5 September 2014) using electrofishing (5 kW) and trawl fishing, by professional fishing companies (Visserijbedrijf P. Kalkman, Moordrecht, The Netherlands in 2009 and 2014; ATKB, Stellendam, The Netherlands in 2011). 1-6 specimens per species were collected at each sampling event. Roach was only caught in 2011. Length and fresh weight of the fish were measured (Table 7.1). As LMB particles or La may

Fig. 7.1: Measuring the length of eel during fishery at night time, Lake De Kuil 28 September 2011.

Table 7.1: Length (cm) and fresh weight (g) of the caught fish that were used for La analysis, caught before LMB application (9 April 2009) and after LMB application (28 September 2011, 28 August/5 September 2014) at Lake De Kuil.

	Before LMB		After LMB		After LMB	
	9 April 2009		28 September 2011		28 August and 5 September 2014	
Fish species	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Eel	66	420	68.5	612	71	555
	64	418	49	193	68	490
	67	526	81	1057	---	---
	64	388	42.2	120	---	---
	77	648	59	449	---	---
Perch	39	440	20	106	24	184
	16	42	18	83	24	178
	14	37	14.3	35	21	122
	15	33	16.6	64	23	158
	14	28	12.8	28	20	106
	---	---	---	---	23	162
Roach	---	---	19.5	89	---	---
	---	---	20	104	---	---
	---	---	28	291	---	---
	---	---	27	262	---	---
	---	---	20.8	102	---	---
Bream	62	2764	66	4649	66	4506
	---	---	62	3406	61	3188
	---	---	---	---	66	4900
	---	---	---	---	---	---
	---	---	---	---	---	---
Pike	76	2540	42.5	480	58	1180
	---	---	42	529	---	---
	---	---	46.5	723	---	---
	---	---	68.5	2127	---	---
	---	---	40.5	433	---	---
Tench	46	1480	50	2149	50	2540
	48	1734	55.5	3364	---	---
	53	2253	14	53	---	---
	42	1272	12.2	26	---	---
	47	1827	11.5	23	---	---

potentially get attached to the outside of fish, external tissue (a piece of the skin) and internal tissues (the liver, 4 cm of the spinal bone, 4-5 cm of the muscle from the tail) were dissected separately. The tissue samples were stored at -18° C and then destructed and analyzed for the La content, using the method described for chironomids.

Ponds Dongen and Eindhoven: macrophytes and fish

On 28 April 2010, before the stocking of macrophytes at ponds Dongen and Eindhoven, three samples were randomly taken from the stocking material of *E. nuttalli*. After the stockings, three samples of *E. nuttallii* were randomly taken with a garden rake from each compartment with macrophytes, at pond Dongen on 25 May 2010, 30 June 2010 and 23 August 2011 and at pond Eindhoven on 27 May 2010, 1 July 2010 and 24 August 2011. The fresh samples consisted of the complete plants including the roots and shoots. The samples were thoroughly rinsed with running tap water to remove possibly attached LMB, dead leaves, macro invertebrates and other impurities. The samples were centrifuged to remove adhering water and dried for 5 days at 50° C (Fig. 7.2). The dried samples were destructed and analyzed for La, using the method described for chironomids. The La concentrations of *E. nuttallii* from the LMB treated compartments were compared to the La concentrations of *E. nuttallii* from the non-treated compartments and stocking material.

Fig. 7.2: Processing macrophytes from pond Dongen and pond Eindhoven at the laboratory of Wageningen University. Left photograph by M. van Delft, 27 May 2010. Right photograph 30 August 2011.

As part of the biomanipulation treatment, the fish were removed from the compartments at the start of the experimental period and a new fish stock consisting of roach and pike was introduced. At the end of the experimental period, 5 specimens from the most abundant fish specie(s) were collected from each compartment at pond Dongen on 16 September 2011 and at pond Eindhoven on 6 September 2011. The fisheries were done by a professional fishing company (Visserijbedrijf P. Kalkman, Moordrecht, The Netherlands) using seines. From each fish, liver, bone, muscle and skin samples were taken. These tissue samples were stored at -18° C, freeze-dried and destructed and analyzed for the La content as described for chironomids.

Data analysis

The densities and La concentrations of chironomid larvae before and after the LMB application in Lake De Kuil violated normality and Mann-Whitney U tests were run for analyses. The La concentrations of *E. nuttallii* in the compartments of ponds Dongen and Eindhoven were analyzed by One Way ANOVAs, followed by post hoc Tukey tests. To fulfil the requirements for normality and homogeneity of variance, the data of the La concentrations of *E. nuttallii* were log transformed prior to running the ANOVAs. Data on fish were analyzed by One Way ANOVA. In case normality requirements were not met, fish data were analyzed running Kruskal-Wallis Wallis One Way Analysis of Variance on Ranks. Statistical analyses were performed in PASW Statistics 18 and SigmaPlot 12 (Systat Software Inc.).

Results

Lake De Kuil: chironomids and fish

On 18 March 2009, the density of chironomid larvae in Lake De Kuil was 48 m^{-2}, which was significantly lower (Mann-Whitney U; $P = 0.001$) than the density on 30 June 2011 (307 m^{-2}). The La concentration of the larvae before the LMB application (18 March 2009) was 6.6 µg g^{-1} DW and after the LMB application (30 June 2011) over twice as high (13.6 µg g^{-1} DW), although the difference was not significant (Mann-Whitney U; $P = 0.272$).

The length of the caught eels did not differ significantly between the three sampling

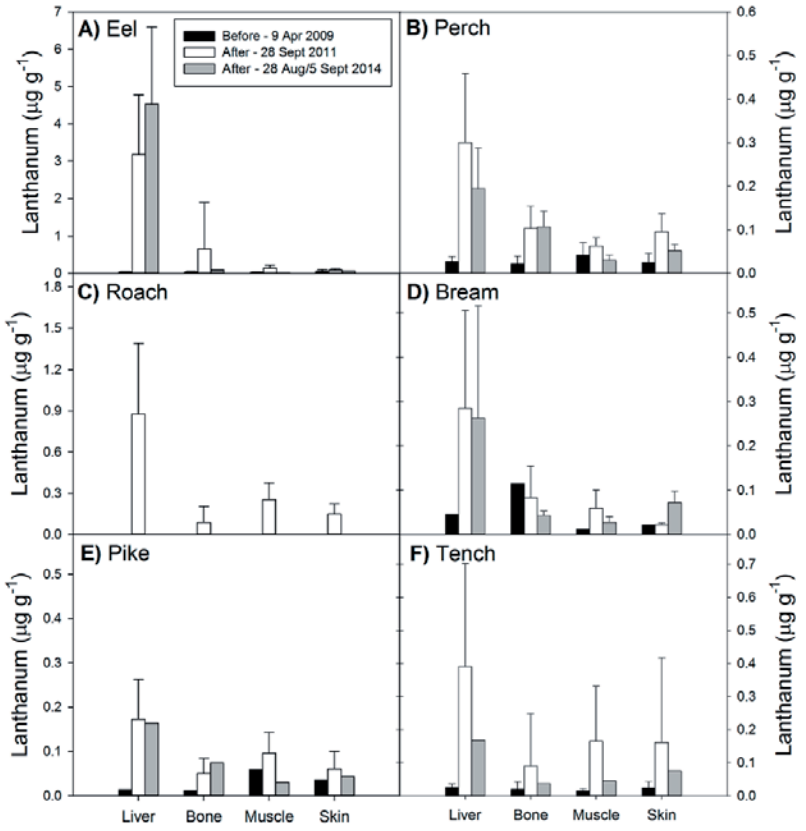


Fig. 7.3: Mean concentrations of La (µg g^{-1} DW) in liver, bone, muscle and skin of eel (A), perch (B), roach (C), bream (D), pike (E), and tench (F), before (9 April 2009) and after (28 September 2011 and 28 August/5 September 2014) the LMB treatment in Lake De Kuil. Error bars indicate 1 SD.

dates (Kruskal-Wallis One Way Analysis of Variance on Ranks: H_2 = 1.44; P = 0.539), neither did the weight of the eels (One way ANOVA $F_{2,11}$ = 0.02; P = 0.98). The length of the perch (H_2 = 6.56; P = 0.038) and their weight (H_2 = 6.53; P = 0.038) differed significantly, in which perch collected in 2014 were longer and heavier than perch collected in 2009 and 2011. The La concentrations in most tissues of the fish caught 2 and 5 years after the LMB treatment were raised compared to the situation before the treatment (Fig. 7.3).

Ponds Dongen and Eindhoven: macrophytes and fish

The mean La concentrations of *E. nuttallii*, which was harvested from the compartments not treated with LMB, varied from 0.35 – 7.03 µg g^{-1} at pond Dongen and from 0.14 – 13.53 µg g^{-1} at pond Eindhoven (Table 7.2). These concentrations were in the range of the mean La concentration of the stocking material (7.50 µg g^{-1}). *E. nuttalli* from the LMB treated compartments showed significantly higher La concentrations in comparison to the non-treated compartments in May 2010, in June/July 2010 and in August 2011, in both ponds (Table 7.2). The mean La concentration of *E. nuttallii* from the LMB treated compartments (Fig. 7.4) reached a maximum of 380.30 µg g^{-1} at pond Dongen and 871.33 µg g^{-1} at

Table 7.2: Mean La concentration of *Elodea nuttallii* (µg La g^{-1} DW) in compartments at pond Dongen and pond Eindhoven. Treatments: DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation, B = biomanipulation. In parentheses ± 1 SE (n = 3). Sampling date before stocking: 28 April 2010. Sampling dates after stocking at pond Dongen: 25 May 2010, 30 June 2010, 23 August 2011. Sampling dates after stocking at pond Eindhoven: 27 May 2010, 1 July 2010, 24 August 2011. The right column shows results of One way ANOVAs. The capitals (A, B, C, D) indicate significantly different groups (P < 0.05).

	treatment						
	La concentration (µg La g^{-1} DW)						ANOVA
	stocking material	DB	DPB	LB	LPB	B	
28 April 2010	7.50 (0.50)						
pond Dongen							
25 May 2010		3.50^{AB} (0.67)	7.03^{B} (0.85)	325.02^{C} (56.01)	380.30^{C} (103.54)	2.27^{A} (0.24)	$F_{4, 10}$ = 200.194 P < 0.001
30 June 2010		0.95^{A} (0.10)	5.49^{B} (1.01)	281.80^{C} (44.49)	341.07^{C} (100.89)	0.93^{A} (0.10)	$F_{4, 10}$ = 221.725 P < 0.001
23 August 2011		0.35^{A} (0.10)	0.31^{A} (0.07)	26.33^{B} (3.82)	8.55^{B} (3.53)	0.24^{A} (0.10)	$F_{4, 10}$ = 47.167 P < 0.001
pond Eindhoven							
27 May 2010		3.92^{A} (0.48)	2.67^{A} (0.23)	178.94^{B} (45.73)	442.41^{C} (52.82)	5.36^{A} (1.53)	$F_{4, 10}$ = 169.254 P < 0.001
1 July 2010		1.84^{A} (0.31)	1.96^{A} (0.96)	163.70^{C} (75.32)	871.33^{D} (93.23)	13.53^{B} (0.44)	$F_{4, 10}$ = 67.367 P < 0.001
24 August 2011		0.14^{A} (0.05)	0.68^{B} (0.27)	38.63^{C} (6.96)	53.54^{C} (15.42)	---	$F_{3, 8}$ = 102. 989 P < 0.001

Fig. 7.4: *Elodea nuttallii* in the compartment with treatment LB (LMB + biomanipulation) at pond Dongen during the second growing season (28 June 2011).

pond Eindhoven in 2010 (Table 7.2). In 2010, the raised La concentrations were the highest in treatment LPB in both ponds, although the difference with treatment LB was significant only for pond Eindhoven in (Table 7.2).

Despite the initial fish stock, consisting of roach and pike in compartments 1 – 5 at both ponds, a deviate fish stock was revealed in most of the compartments at the end of the experiment (Chapter 3). This hampered the comparison of the La concentrations of tissues from LMB-exposed and non-LMB exposed fish in individual species. For that reason, the mean La concentrations of liver, muscle, bone and skin tissues of all fish exposed to LMB (compartments LB and LPB) were compared to those of non-exposed fish (compartments with treatments DB, DPB and B). At pond Dongen, the mean La concentrations of liver (P = 0.001), bone (P = 0.017) and skin (P = 0.025) of the fish exposed to LMB were significantly higher (respectively 23.5 x higher, 1.8 x higher and 1.1 x higher) than those of fish not exposed to LMB (Fig. 7.5). When exposed to LMB, the mean La concentration of the muscle of the tail was 3.4 times higher than when not exposed to LMB, although this difference was not significant (P = 0.318).

At pond Eindhoven, the mean La concentrations of liver, muscle, bone and skin of the fish exposed to LMB were significantly higher ($P \leq 0.001$), than those of fish which had not been exposed to LMB (Fig. 7.6). The mean La concentrations of liver, muscle, bone and skin of the LMB exposed fish were respectively 17.2x, 2.5x, 2.9x and 3.2x higher than those of not exposed fish.

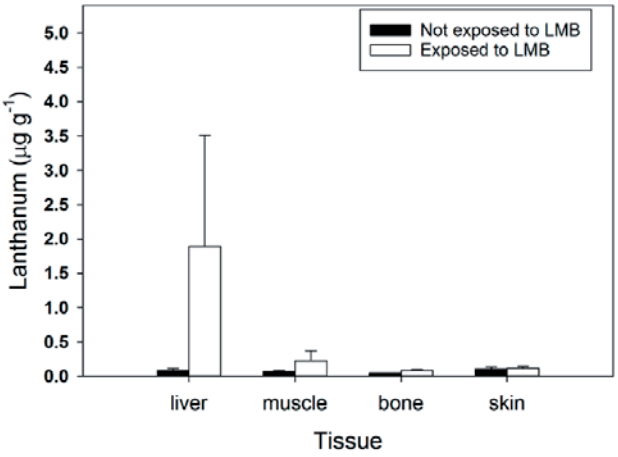


Fig. 7.5: Pond Dongen. Mean concentrations of La (µg g^{-1} DW) in liver, muscle, bone and skin of fish not exposed to LMB (treatments DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, B = biomanipulation) and of fish exposed to LMB (treatments LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation) at the end of the experimental period (16 September 2011). Error bars indicate 1 SD.

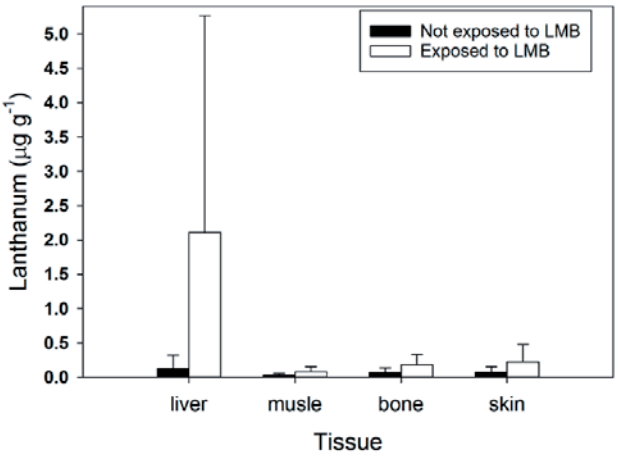


Fig. 7.6: Pond Eindhoven. Mean concentrations of La (µg g^{-1} DW) in liver, muscle, bone and skin of fish not exposed to LMB (treatments DB = dredging + biomanipulation, DPB = dredging + PAC + biomanipulation, B = biomanipulation) and of fish exposed to LMB (treatments LB = LMB + biomanipulation, LPB = LMB + PAC + biomanipulation) at the end of the experimental period (6 September 2011). Error bars indicate 1 SD.

Discussion

The results from this study show that La from LMB is taken up by fish and *E. nuttallii*. Hence, for these organisms we reject the hypothesis that the presence of LMB does not increase the La concentration of the exposed organisms. The study showed raised concentrations of La in macrophytes, chironomids and fish tissues, up to at least two years (macrophytes, chironomids) and five years (fish) following LMB application. Low baseline concentrations of La in invertebrates, macrophytes and fish that had not been exposed to dissolved La or LMB, have been shown before (Hao et al., 1996; Yang et al., 1999; Weltje et al., 2002b; Van Oosterhout et al., 2014), which was confirmed in this study. The baseline concentrations already indicate the bioavailability of La (Van Oosterhout et al., 2014).

Lanthanum in Elodea nuttallii and chironomids

The macrophyte *E. nuttallii* showed raised La concentrations following LMB application at pond Dongen and pond Eindhoven. In 2010, the increase of the La concentrations in *E. nuttallii* from the LMB treated compartments was 83 and 127 fold at pond Dongen and 78 and 90 fold at pond Eindhoven, compared to the non-treated compartments. In 2011, the La concentrations of *E. nuttallii* had decreased at both ponds, compared to 2010 (Table 7.2). Nevertheless, the La concentrations in plants from the LMB treated compartments in 2011 showed a 58 fold increase at Dongen and 112 fold increase at Eindhoven, compared to the non-treated compartments. Two years after the LMB application, the La concentration of the chironomids at Lake De Kuil was more than doubled, although the increase was not significant.

Lanthanum in fish tissues

In fish, the livers contained the highest La concentrations of the tissues that were tested (Figs. 7.3, 7.5 and 7.6). The La content of eel livers were the highest (Fig. 7.3). Compared with the baseline concentration of eel liver from Lake De Kuil of 0.034 µg La g^{-1}, the La concentration showed a 94 fold increase in 2011, two years after LMB application, and a 135 fold increase in 2014, five years after LMB application (Fig. 7.3). Perch, roach, bream, pike and tench from Lake De Kuil showed the highest La concentrations in 2011 (Fig. 7.3), except for roach that was only caught in 2011 (Table 7.1). Following LMB application, the increase of the La concentrations of livers of perch, bream, pike and tench ranged from a 6 fold in bream (with a baseline concentration of 0.046 µg La g^{-1}) to a 16 fold in tench (baseline 0.025 µg La g^{-1}) in 2011, and from a 6 fold in bream to a 13 fold in pike (baseline 0.013 µg La g^{-1}) in 2014 (Fig. 7.3). The results from Lake De Kuil are in line with the findings at pond Dongen and pond Eindhoven, where the La concentrations of liver in 2011 were highest, showing a ~20 fold increase in the LMB treated compartments, while increases in other tissues were more moderate (Figs. 7.5 and 7.6). In a laboratory experiment using dissolved La, Hao et al. (1996) showed high La uptake in internal organs of fish compared to bone and muscle. Differences in La uptake between fish species has been shown by Landman et al. (2007), following LMB application. Their study showed highest La concentrations in the liver and indicated a restricted period of less than twelve months with raised La concentrations following LMB application, suggesting depuration. The liver is the most important organ for the excretion of La (Bervoets et al., 2009) and several studies, including our study, showed the highest La concentrations in the liver, which may indicate excretory. Nevertheless, the findings from Lake De Kuil showed accumulation of La in liver tissues over a period of at least five years following LMB application (Fig. 7.3), while fish from ponds Dongen and Eindhoven showed raised La concentrations up to at least one year (Dongen; Fig. 7.5) and two years (Eindhoven; Fig. 7.6) after the treatment. Considering depuration, these findings indicate ongoing La uptake up to at least two to five years following LMB application. Although the

skin may potentially be subject to the external attachment of LMB particles or sorption of La from the water, it showed lower La concentrations than the liver (Fig. 7.3).

Impact of lanthanum from LMB

As the results clearly show in situ La uptake by fish following LMB application, the raised La concentrations of *E. nuttallii* and chironomid larvae also point towards uptake of La from LMB after in situ LMB application. Nevertheless, it may not be excluded that external attachment of LMB particles or external sorption of La contributed to the increase of La concentrations shown for chironomids and *E. nuttallii*. Accumulation of La by the invertebrate marbled crayfish (*Procambarus fallax* f. *virginalis*) has been shown in a 28 day laboratory experiment, resulting in raised La concentrations of the internal organs (ovaries, hepatopancreas, muscle) ranging from 0.8 to 306.3 μg g^{-1} DW (Van Oosterhout et al., 2014), while the animals had been exposed to 1000 mg LMB L^{-1}. This exposure was in the range of the LMB dosages of 157 mg LMB L^{-1} at Lake De Kuil, 753 mg LMB L^{-1} at pond Eindhoven and 1071 mg LMB L^{-1} at pond Dongen. Compared to these results, the La concentration of the chironomids at Lake De Kuil, two years after the LMB application, were low (13.6 μg g^{-1} DW). The La concentration of the sediment of Lake De Kuil prior to the LMB treatment (12 μg g^{-1} DW; Van Goethem, 2010) had increased over three orders of magnitude in 2014, ranging from 2 – 4 mg La g^{-1} DW (Dithmer et al., 2016b). This dramatic raise was not reflected by the increased La concentration of the chironomids. Due to the filter feeding habit of the chironomid larvae, they will be mainly influenced by the La concentration of the water, which was raised temporarily after the LMB treatment (Chapter 5). Next to the increased La concentration of the sediment after LMB application and the potential ingestion of La associated to particles, the release of filterable La (FLa) from LMB has been reported (Spears et al., 2013b; Chapter 5), indicating a potential La source for uptake by aquatic organisms. Uptake of dissolved La has been shown for duckweeds (*Spirodela polyrhyza*, Yang et al., 1999; *Lemna minor*, Weltje et al., 2002a), sago pondweed (*Potamogeton pectinatus*, Weltje et al., 2002b) and *E. nuttallii* (Zhang et al., 2015). In ponds Dongen and Eindhoven, the FLa concentrations in the water column in the LMB treated compartments was mostly well below 10.1 μg L^{-1}, the Dutch standard for FLa (Sneller et al., 2000), except during a short period after the application when macrophytes had not yet been introduced. At much higher dissolved La concentrations (5 – 20 mg La L^{-1}) in laboratory experiments, *E. nuttallii* is known to accumulate La in the shoots with most of the La bound to the cell walls (Zhang et al., 2015). In situ in ponds Dongen and Eindhoven, the La concentration of *E. nuttallii* in the LMB treated compartments reached a maximum during the first growing season (2010) and declined in the second growing season (2011; Table 7.2). Growth-induced dilution has been suggested as a mechanism lowering La concentrations in macrophytes over time (Weltje et al., 2002a).

The FLa concentration in Lake De Kuil peaked at 15.9 μg FLa L^{-1} during the LMB application (Chapter 5), while the maximum concentrations in pond Dongen (781 μg FLa L^{-1}) and pond Eindhoven (33.4 μg FLa L^{-1}) were measured two days after the LMB applications.

Spears et al. (2013b) described a range of peak FLa concentrations in 16 case study lakes ranging from 2 to 414 µg FLa L^{-1}, which is comparable to the order of magnitude in our data. The observed maxima exceeded the Dutch standard of 10.1 µg FLa L^{-1} for a few days at Lake De Kuil and pond Eindhoven, and up to five weeks at pond Dongen. No lethal toxic effects were observed during and following the LMB applications. The peak FLa concentrations at pond Dongen and Eindhoven were in the range of LC_{50} (50% effect concentration) causing mortality in *Daphnia carinata* and *Hyalella azteca* in laboratory tests within 2 – 7 days using soft water (Barry & Meehan, 2000; Borgmann et al. 2005). The increase of water hardness increases these LC_{50}s by one to two orders of magnitude (Barry & Meehan, 2000; Borgmann et al. 2005; Copetti et al., 2016) and, as the water bodies in our study were classified as hard water (e.g., the mean Ca concentration in Lake De Kuil in 2006 was 54 mg L^{-1} and in 2016 57 mg L^{-1}, the Ca concentration in pond Dongen on 23 December 2008 was 45 mg L^{-1}; the mean hardness in Lake De Kuil in 2016 was 213 mg $CaCO_3$ L^{-1}; data provided by water authority Brabantse Delta), lethal toxic effects were not likely.

Human exposure to La in LMB treated water bodies can be through the consumption of fish and the ingestion of water during recreational activities. La uptake by humans is confirmed safe up to six years, when administered in medication as phosphate binder (Hutchison et al., 2008; Zhai et al., 2015), with uptakes up to 1820 mg La day^{-1} (Fosrenol®, Shire Pharmaceuticals). Considering the application doses of 7.9 mg La L^{-1} at Lake De Kuil, 53.6 mg La L^{-1} at pond Dongen and 37,6 mg La L^{-1} at pond Eindhoven, the safe La uptake would equal a daily human consumption of 230 liters water from Lake De Kuil, 34 liters from pond Dongen and 48 liters from pond Eindhoven. Of all fish tissues, eel livers from Lake De Kuil had the highest La concentration (Figs. 7.3, 7.5 and 7.6), with a mean concentration of 4.5 µg La g^{-1} in 2014 (Fig. 7.3). This implies that the safe La uptake would equal a daily human consumption of 404 kg eel liver. It is considered highly unlikely that La from LMB poses a human health risk by the consumption of water or fish.

The shown improvements in water quality and biological community following the LMB applications were evident (Chapters 3, 4, 5 and 6). Nevertheless, this study showed La uptake in biota after LMB field-applications, which advocates for long-term research on this topic. For humans, the health risks from consumption of water or fish harvested from LMB treated lakes are considered negligible (Copetti et al., 2016).

Conclusions

La accumulation was shown in macrophytes, chironomids and fish following LMB applications. In fish, the highest concentrations were found in liver, indicating depuration of La. Bio-accumulation of La up to at least two and five years following LMB applications, indicates continuous uptake of La by fish. This La may originate from dissolved La, particles and through food web exchange. No ecotoxicological effects were observed up to five years following the in situ LMB applications, while human health risks are considered negligible.

Long-term monitoring following LMB applications is recommended to increase insight in the fate of La in biota from different trophic levels.

Removal of cyanobacterial scum from a city pond in Breda (The Netherlands; photograph Water Authority Brabantse Delta)

CHAPTER 8

Evaluation of several end-of-pipe measures proposed to control cyanobacteria

This chapter is based on:
Evaluation of several end-of-pipe measures proposed to control cyanobacteria.
Lürling, M., G. Waajen & L. de Senerpont Domis, 2016.
Aquatic Ecology 50: 499-519. DOI 10.1007/210452-015-9563-y

Abstract

While reduction of nutrient loading is a prerequisite for mitigation of harmful cyanobacterial blooms in nutrient enriched waters, in certain surface waters eutrophication control is not always feasible due to practical and economic constraints, or might be effective only in the long run. Yet, the urgent need to control cyanobacteria in water for drinking, irrigation, aquaculture, industry and recreation has spurred the development of a plethora of alternative methods that claim to be fast acting. Here, we provide a critical overview of several of these end-of-pipe measures: Effective microorganisms (EM®), golden algae (*Ochromonas*), plant/tree extracts, ultrasound and artificial mixing of non-stratifying waters. Most of the end-of-the-pipe measures claim to provide sustainable control of harmful cyanobacterial blooms, while at best only targeting symptom relief rather than eutrophication relief. Support for "effective" microorganisms, golden algae, plant extracts, ultrasound, and artificial mixing of non-stratifying waters to diminish eutrophication problems such that the resulting water quality meets societal and legislation demands is limited, and several proposed underlying mechanisms are doubtful. None of these curative measures seem the desired wide applicable solution to cyanobacterial nuisance; they should not be considered Columbus's egg. A critical evaluation of end-of-pipe measures is crucial for water authorities in their choice for mitigating measures.

Introduction

Excessive nutrient loading is the major cause of water blooms of cyanobacteria (blue-green algae), i.e., elevated densities throughout the water column (Smith et al., 1999; Conley et al., 2009). Water blooms may cause high turbidity and malodor of the water, while associated nocturnal oxygen deficiency can lead to fish kills (Smith et al., 1999; Paerl and Huisman, 2008). Such blooms may have significant detrimental environmental impacts by food web changes reducing biodiversity (Paerl et al., 2001; Paerl, 2008). Cyanobacterial blooms have caused drinking water shortages (Yang & Liu, 2010) and pose a serious health threat because cyanobacteria might produce potent toxins (Codd et al., 2005a; Dittmann & Wiegand, 2006). This is especially the case when in a stable water-column in lakes and ponds floating layers or surface scums develop that might be further concentrated on the leeside shore, resulting in a manifold concentration of the mostly intracellular contained toxins (Chorus et al., 2000).

Because of the paramount role of nutrient loading in the development of cyanobacteria blooms and scums, nutrient input reductions are the most obvious targets in controlling harmful cyanobacterial blooms (Paerl & Otten, 2013). The reduction of external nutrient loading is a prerequisite for improvement, but lake recovery can be delayed for decades due to internal phosphorus loading (Søndergaard et al., 1999). Consequently, hazardous cyanobacterial blooms may remain in these waters for many years. In other systems, such as open systems with intense agricultural influence, nutrient input reductions may not always be possible and cyanobacterial blooms will sustain. Such blooms are clashing with modern society's demand for good water quality (Steffensen, 2008) and are in conflict with the attainment of a good water quality needed to comply with both the EU Water Framework Directive (WFD; Council of the European Union, 2000) and the EU Bathing Water Directive (BWD; Council of the European Union, 2006). Despite water authorities displaying a great need for preventive measures leading to nutrient reduction and thus to the reduction of cyanobacterial blooms in the longterm, they also need short-term fast acting, curative treatments to mitigate cyanobacterial nuisance.

Curative methods should rapidly suppress the proliferation of cyanobacteria or destroy a massive bloom bringing immediate improvement of the water quality and should provide, at least in the growing season, access to the water for drinking, irrigation, aquaculture, industry and recreation (Jančula & Maršalek, 2011). Several of these curative measures, such as the application of cyanocides and algicides (Matthijs et al., 2016), and manipulations of the food web through macrophytes (Bakker & Hilt, 2016) and artificial mixers have been dealt with elsewhere (Visser et al., 2016). Nonetheless, many products remain of which quite a number often have been proposed to water authorities as end-all solutions in controlling cyanobacteria, especially following upon the typical heatwaves of 2003 and 2006 with numerous cyanobacterial bloom events in northwestern and central Europe. Here, we will critically review the claims and effectiveness of a selection of methods: i.e., "effective" microorganisms, golden algae, plant extracts, and ultrasound. The choice

for this selection is based on the scientific information available and the strong promotion or media attention the products received in The Netherlands over the last decade, where golden algae got quite some media attention, questions about "effective" micro-organisms made it into the National Parliament, while ultrasound, Barley straw and SolarBee® trials were conducted in situ. A critical review of these end-of-pipe measures might be helpful to water authorities in making a more balanced decision for effective treatments to control eutrophication and mitigate cyanobacterial nuisance.

Effective microorganisms (EM®)

The use of effective microorganisms (EM) has been advocated to be an end-all solution to a wide suite of water quality problems (e.g., URL1-5; Zakaria et al., 2010). A blend of EM can be kneaded into dried mud to form mud balls that can be thrown in water bodies. These "EM-mudballs" are based on a concept that was first developed by Higa (1998), who suggested that –through competitive exclusion- addition of EM-1® changes the microbial community towards dominance of beneficial species, while suppressing harmful bacteria. EM-cocktails are allegedly said to contain about 80 species of microorganisms, such as photosynthetic bacteria, lactic acid bacteria, actinomycetes, yeasts and fermenting fungi (Higa, 1998). However, this could not be confirmed by analysis of EM-1® samples that revealed the majority consisted of lactic acid bacteria (*Lactobacillus* and *Lactococcus* at 5 - 10 × 10^6 mL^{-1}) and yeasts (*Saccharomyces* and *Candida* at about 10^5 mL^{-1}), while other microorganisms were present in very low concentrations, or not present at all (Van Egeraat, 1998). Semi-quantitative PCR-DGGE could not confirm the stated richness of EM either (Van Vliet et al., 2006).

EM claims range from water purification, to sustainable end-all solution for water quality and sanitation problems. These claims can be found on various webpages and are based on anecdotal evidence rather than on scientifically confirmation with reproducible and consistent data (Higa & Parr, 1994). For example the statement "*Using EM-1® Microbial Inoculant on a regular basis will help to keep enough beneficial microbes in the system to keep nutrient levels low enough to prevent the growth of algae"* (URL6) is not supported by any scientific study.

There are only limited scientific studies on the use of EM to control nutrients or cyanobacteria in aquatic systems. A recent study showed that the combination of EM and submerged plants, *Hydrilla verticillata*, had good removal of total nitrogen (TN) and total phosphorus (TP) (Chen et al., 2013). Also humic substance removal by EM was studied and although the authors report good removal of humic substances by EM (Joo & Foldenyi, 2012), the lack of proper controls should be noted. In a 1200 m^2 pond in Poland fed by purified water from a sewage plant, several water quality variables were measured in a period before and after the addition of EM (Jóźwiakowski et al., 2009). Although the preliminary data of Jóźwiakowski et al. (2009) indicates a decrease in both TN and TP, the limited sampling regime (twice in winter, once in spring) does not allow for drawing definite conclusions

on the effectivity on the application of EM. Application of four mudballs and 2.5 L liquid material both containing EM to a 24 m^2 garden pond in Hungary led to elevated SRP levels and lower transparency indicating the inefficacy of the EM in controlling eutrophication (Padisák, 2014).

Controlled experiments in the laboratory found no growth inhibition for a laboratory strain of *Microcystis aeruginosa* and for *M. aeruginosa* from the field at a recommended dosage of 1 EM-mudball per square-metre (≈0.1–0.3 g L^{-1}) (Lürling et al., 2009; 2010). Suspensions of EM-mudballs up to 1 g L^{-1} were ineffective in reducing cyanobacterial growth. Cyanobacteria were inhibited only at very high EM-mudball concentrations (5–10 g L^{-1}), because of the very high amount of clay and high water turbidity (Lürling et al. 2009; 2010). Also a suspension of 'activated Effective Micro-organism' (EM-A) was not effective in reducing cyanobacteria growth and high concentrations of EM-A caused low pH of the water and nutrient enrichment (Lürling et al., 2009). High dosage of EM-mudballs caused water column oxygen depletion (Lürling et al., 2009; 2010), release of metals (Al, Cd, Cu, La and Pb) and release of phosphate (Lürling et al., 2009). Hence, such "effective microorganisms" formulations are not effective to prevent the growth of algae or in preventing cyanobacterial proliferation and/or terminating blooms. The Dutch authorities prohibited the application of EM-mudballs in Almere-Haven, because they contained heavy metals such as mercury as well as nutrients (Rijkswaterstaat, 2007).

Overall, the claim that EM will "*keep nutrient levels low enough to prevent the growth of algae*" in surface water is not supported by scientific evidence. In contrast, controlled experiments have clearly shown that cyanobacteria cannot be controlled with EM.

Golden algae (*Ochromonas*)

In August 2009 a press release based on a publication of a study with *Microcystis* and the chrysomonad *Ochromonas* (Van Donk et al., 2009) led to more than a dozen news items in the Dutch media with main emphasis on the green nature of this promising solution, and a comparison with use of natural enemies such as predatory mites in horticulture pest control was made (source: LexisNexis® *Academic*). Consequently, several water authorities considered the use of the shiny golden alga *Ochromonas* in controlling cyanobacteria.

It is already known for decades that chrysomonads (*Ochromonas danica, O. minuta*, and *Poterioochromonas malhamensis*) can feed on cyanobacteria (Daley et al., 1973; Cole & Wynne, 1974). They can ingest food items several times larger in diameter than their own which is around 10 µm (Zhang et al., 1996), seemed to modify or degrade microcystins (Ou et al., 2005) and are capable of growing on sole cyanobacterial food (Zhang & Watanabe, 2001). Chrysomonads, however, had no significant effect when *Microcystis* density was high (Zhang et al., 2009a). On the other hand, *Microcystis* can respond to the threat of being grazed by chrysomonads by building colonies (Burkert et al., 2001; Wang et al., 2010). Formation of large colonies in *Microcystis aeruginosa* was observed under

continuous grazing pressure by *Ochromonas* that allowed *Microcystis* population growth (Yang & Kong, 2012). Those colonies were protected from *Ochromonas* grazing, which was further corroborated in studies that showed low clearance rates on colonial *M. aeruginosa* compared to unicells (Yang et al., 2009c,d). Thus, a common bloom-forming organism like *Microcystis* had simply grown to sizes beyond the ingestion capacity of the chrysomonads. In eutrophic systems, the summer increase of phytoplankton contains a high proportion of inedible species (Sommer et al., 2012). There is no evidence that chrysomonads would be capable of grazing down those grazing-resistant phytoplankton, often comprised of large dinoflagellates and/or cyanobacteria (Sommer et al., 1986).

The chrysomonads themselves are on the menu of several larger metazoan grazers, mesozooplankton (Saunders et al., 1994). They can be toxic to mesozooplankton (Leeper & Porter, 1995; Boxhorn et al., 1998; Hiltunen et al., 2012), but it is assumed that at normal field abundances of these organisms toxic effects will be hardly detectable (Boenigk & Stadler, 2004). Grazing by chrysomonads on *Microcystis* can also reduce the toxicity of *Microcystis* to *Daphnia* (Zhang et al., 2009b). Therefore, chrysomonads are often controlled by mesozooplankton (Sommer et al., 2012).

While *Ochromonas* and *Poterioochromonas* might be widespread and common mixotrophs (Boenigk & Stadler, 2004; Van Donk et al., 2009), the incidence and intensity of cyanobacterial blooms has increased over the last decades (O'Neil et al., 2012), strongly suggesting that the chrysomonads fail to control cyanobacteria in the field. Mass culture and depositing chrysomonads in the field is advised against, because of the expected lack of effect and the addition of nutrients to the receiving water.

Plant/tree extracts

A whole array of plant extracts and chemicals exuded by macrophytes for controlling cyanobacteria is promoted. In a thorough review it was concluded that "*With many findings on new allelochemicals, it will become the most promising method to control algal bloom*" (Hu & Hong, 2008). Among the most studied is barley straw (extracts) that could reduce growth of unicellular and filamentous green algae (Gibson et al. 1990; Welch et al., 1990) and cyanobacteria such as *Microcystis aeruginosa* (Newman & Barrett, 1993). During straw decomposition under UV-supplemented visible light, hydrogen peroxide was produced that might have caused inhibition of *M. aeruginusa* (Iredale et al., 2012). This could explain the differences in sensitivity between eukaryotic algae and cyanobacteria as cyanobacteria in general are more sensitive to hydrogen peroxide than green algae and diatoms (Drábková et al., 2007). The active compounds, polyphenols (Pillinger et al., 1994; Ridge & Pillinger, 1996) have recently been identified as salcolin A and B (Xiao et al., 2014). Salcolin A caused after one day an increase in *M. aeruginosa* intracellular reactive oxygen species (Xiao et al., 2014). The findings have triggered speculations on the use of barley straw for controlling *M. aeruginosa* blooms (Shao et al., 2013) and more specific the use of salcolin in the "*future control of cyanobacterial harmful algae blooms*" (Xiao et al., 2014). However, a protective

role of microcystins against oxidative stress has been suggested too (e.g., Phelan & Downing, 2011), which finds further support in an experiment where a microcystin producing *M. aeruginosa* strain was better protected against oxidative stress caused by hydrogen peroxide than its microcystin-free mutant (Zilliges et al., 2011). Hence, increased oxidative stress caused by the decaying barley straw could potentially select for more toxic (microcystin producing) cyanobacteria.

Applying rotting barley straw (50 and 25 g m^{-3}) to shallow reservoirs inhibited phytoplankton and lowered cyanobacteria dominance (Everall & Lees, 1996; 1997). Repeated addition of barley straw (6 – 28 g m^{-3}) to a reservoir lowered phytoplankton and cyanobacteria cell numbers to one half or to one quarter (Barrett et al., 1999). A pond experiment with different barley straw dose (40 and 80 g m^{-2}) revealed inhibition of *M. aeruginosa*, *Anabaena* sp. and *Aphanizomenon* sp., no effect on *Nostoc* sp. and a stimulation of *Oscillatoria* sp. (Rajabi et al., 2010), while another pond experiment showed no difference between controls and barley treated ponds (Ferrier et al., 2005). The phytoplankton abundance in a loch treated with barley bales declined after 10 months compared to an untreated loch and remained low for subsequent 17 months (Harriman et al., 1997). In contrast, in another Scottish loch barley had no effect on phytoplankton and no evidence for such a delayed effect of straw bale placement was detected as April placement was followed by an August bloom (Kelly & Smith, 1996). Likewise, adding barley straw into a rice field did not reduce phytoplankton abundance (Spencer & Lembi, 2007) and in an enclosure study three different barley concentrations (5, 15 and 60 g m^{-3}) showed no effects on algae (Boylan & Morris, 2003). The latter authors mentioned a case of two ponds where a combination of barley straw and aerators reduced phytoplankton biomass (Boylan & Morris, 2003). Positive effects of barley straw on controlling *Microcystis* and cyanobacteria for some reservoirs in the UK were reported (Purcell et al., 2013), but these find no strong support in the underlying data.

In 1997 and 2000 barley straw was added to an urban pond in Roosendaal (The Netherlands) and in 2000 to a small lake near Waspik. For the latter, mean summer chlorophyll-*a* concentrations were 156 µg L^{-1} in 1999 prior to barley and 106 µg L^{-1} in 2000 after the barley placement (Water Authority Brabantse Delta). In the urban pond Parkvijver (Roosendaal, The Netherlands), mean summer chlorophyll-*a* concentrations were 167 µg L^{-1} in 1995 before barley placement, only 23 µg L^{-1} in 1997 during barley presence, 30 µg L^{-1} in 1998, 116 µg L^{-1} in 1999, while after a second barley placement in 2000 mean summer chlorophyll-*a* concentrations were 306 µg L^{-1}. In 1999, phytoplankton was dominated by chlorophytes reaching a maximum phytoplankton cell concentration of 3.4×10^5 cells mL^{-1} in August, while less than three months after the barley placement (beginning March 2000) cyanobacteria already flourished reaching densities of 0.77×10^5 cells mL^{-1} on May 29th 2000 (Fig. 8.1). End of July chlorophyll-*a*- and phytoplankton concentrations had dropped, but they increased again after a second application of barley straw in the beginning of August 2000 (Fig. 8.1).

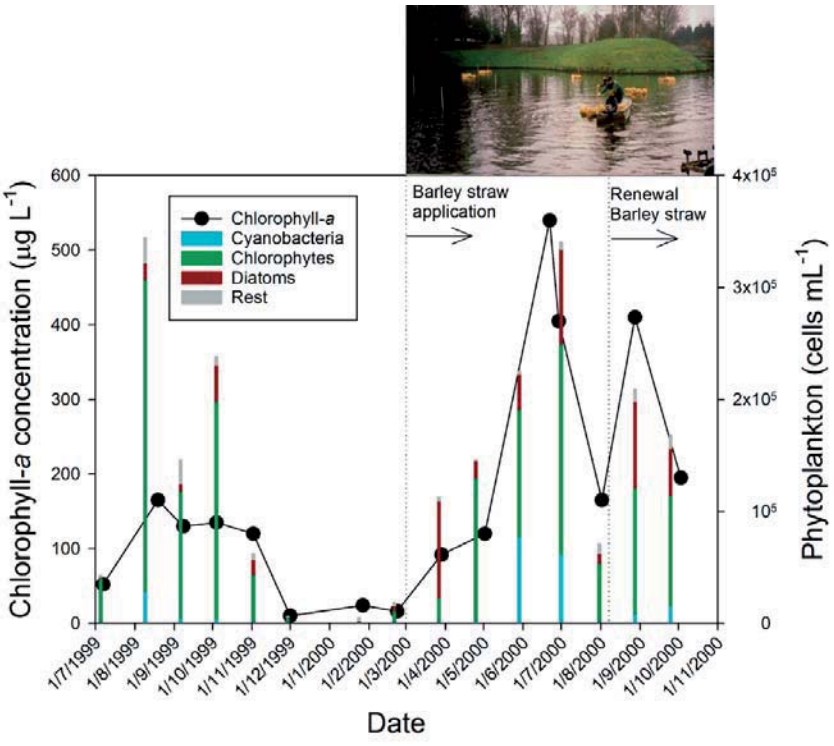


Fig. 8.1: Phytoplankton abundance (bars) for cyanobacteria (blue fill), chlorophytes (green fill) and diatoms (brown fill) as well as the course of the chlorophyll-*a* concentration (solid black line) over the period July 1999 – September 2000. The two vertical dashed lines represent the addition of barley straw. A photograph of the application is added. (Data from: AquaSense, 2000; Bijkerk, 2000).

The mixed results that emerge from field trials have also been obtained in more controlled laboratory assays. In general, *M. aeruginosa* appears among the most vulnerable organisms (Martin & Ridge, 1999; Ferrier et al., 2005). However, not all cyanobacteria seem susceptible to rotting barley straw; no effects were observed in *Pseudoanabaena* sp. (Brownlee et al., 2003) and growth stimulation has been observed in *Anabaena cylindrica*, *A. flos-aquae, Oscillatoria animalis* and *O. lutea* var. *contorta* (Martin and Ridge, 1999; Ferrier et al., 2005). Similarly, barley straw extract had variable effects on dinoflagellates; inhibition of some *Heterocapsa* species, no effect or stimulation of Gymnodiniales, and stimulation of Prorocentrales (Terlizzi et al., 2002). Commercially available barley straw extract had no effect on the ichthyotoxic *Prymnesium parvum* (Grover et al., 2007). Likewise, testing microbe-lift barley straw concentrated extract (1.5×10^{-4} to 15 ml L^{-1}) in our laboratory on *M. aeruginosa* (initial chlorophyll-*a* concentration 17 µg L^{-1}) revealed no growth inhibition (Fig. 8.2).

Decomposed barley straw extract inhibited *M. aeruginosa*, while fresh extract promoted growth (Ball et al., 2001). Microbial activity appears essential for barley straw to become inhibitory causing a lag period before straw becomes 'active'. Fine chopping of the straw can shorten this lag phase substantially (Iredeale et al., 2012).

As with effects on cyanobacteria, also varying effects on water quality variables have been reported. For example, while in an enclosure study barley straw (1.3 g L^{-1}) was effective in suppressing *Aphanizomenon flos-aquae*, dissolved oxygen levels dropped to near zero (Haggard et al., 2013). In contrast, others found higher oxygen levels in barley treated ponds

(Rajabi et al. 2010) or no effects in an enclosure study (Boylan & Morris, 2003). Moreover, the addition of barley straw to mesocosms increased phosphate levels fivefold to tenfold compared to the control (Haggard et al., 2013), whereas in another study no effects on nutrients were found (Boylan & Morris, 2003).

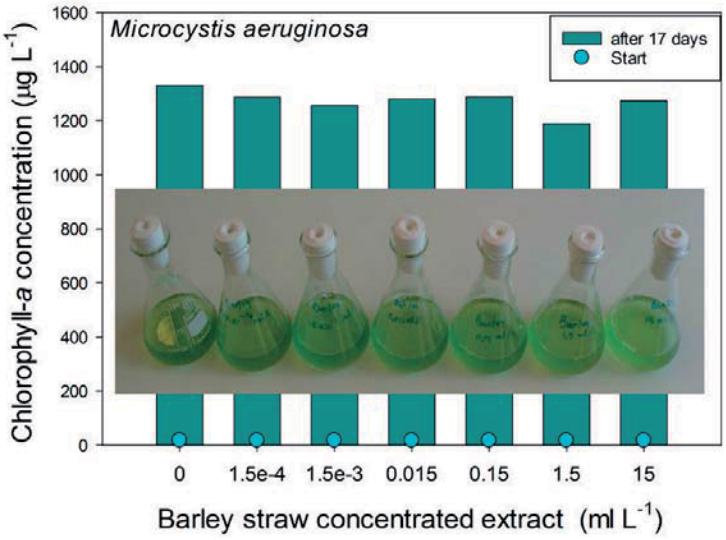


Fig. 8.2: Chlorophyll-*a* concentrations of *Microcystis aeruginosa* after 17 days growth in different concentrations of microbe-lift barley concentrated extract (0 – 15 mL L^{-1}). *M. aeruginosa* was grown in 100-mL Erlenmeyer flasks containing 50 mL of autoclaved WC-medium (Lürling & Beekman, 2006) that were placed in a Gallenkamp ORBI-SAFE Netwise Orbital Incubator at 20°C, in 60 rpm and in a 18:6 h light:dark rhythm that was programmed to gradually increase light intensity to a maximum of 130 µmol quanta m^{-2} s^{-1} and subsequently decreased again to darkness, which resulted in a daily average light intensity of ~ 57 µmol photons m^{-2} s^{-1}. A picture of the cultures after 17 days incubation is inserted (photograph by M. Lürling).

Although widely accepted that barley straw can be an "*effective control method*" (Purcell et al., 2013), an "*effective and environmentally-sound option for the control of cyanobacterial and microalgal blooms*" (Iredale et al., 2012) and "*very useful for controlling of M. aeruginosa based blooms*" (Shao et al., 2013), the lag phase, the different sensitivity of organisms, the contrasting field results, and the proposed underlying mechanism of oxidative stress imply that use of rotting barley straw should be met with care.

In addition to barley straw extract, many plant derived chemicals have anti-cyanobacterial activities. These compounds are mostly extracted from plant tissue and then tested on cyanobacteria, mainly *M. aeruginosa* (Table 8.1). Controlled experiments with extracts of *Fructus mume*, *Salvia miltiorrhiza* and *Moringa oleifera* as well as L-lysine and D-lysine gave no support that these plant extracts and amino-acid could be promising candidates for curative application in *M. aeruginosa* bloom control (Lürling & Van Oosterhout, 2014). From several studies, it is unclear if the effect is caused by the extract or by the solvent. Often rather high concentrations are needed, making applications expensive or virtually impossible (Shao et al., 2013). Moreover, concomitant input of nutrients could aggravate eutrophication (Lürling & Beekman, 2010; Shao et al., 2013), while high amounts of organic matter in combination with expected warming could lead to higher bloom toxicity (Ekvall et al., 2013).

Table 8.1: Overview of plants of which extracts (solvent given) have been tested on target cyanobacteria, including reported effects.

Plant	Target	Extract	Test	Effect	Reference
Solidago canadensis L.	*M. aeruginosa*	ethanol (No ethanol control)	Laboratory	At 0.3 – 0.5 g L^{-1} > 90% inhibition	Huang et al. (2013)
Pistia stratiotes	*M. aeruginosa*	methanol, ethyl acetate and n-hexane	Laboratory	20 - 500 mg L^{-1}; growth inhibition $EC_{50,7d}$ = 80-210 mg L^{-1}, recovery after 7 d	Wu et al. (2013)
Myriophyllum aquaticum	*M. aeruginosa*	water	Laboratory	Growth inhbition	Wu et al. (2008)
Chara australis Potamogeton crispus	*A. variabilis*	60% methanol Live plants, exudates	Laboratory	Clearing effect	Pakdel et al. (2013)
Barley straw 75% *Scirpus acutus* - 25% *Typha latifolia*	*Aphanizomenon flos-aquae*	water	Laboratory	1-5 g L^{-1}; growth inhibition 2.5 g L^{-1}; growth inhibition	Haggard et al. (2013)
Salvia miltiorrhiza	*M. aeruginosa*	ethyl acetate	Laboratory	1.25 – 20 mg L^{-1}; growth inhibition EC_{50} = 4.68 mg L^{-1}	Zhang et al. (2013)
Acacia mearnsii	*M. aeruginosa* Natural community	Not specified	Laboratory, Field mesocosms	3.623 mg L^{-1}; max 59% reduction 1-4 mg L^{-1}; strong growth reduction ~28% of cell density of control bloom	Zhou et al. (2012)
Ephedra equisetina	*M. aeruginosa* Natural community	Water	Laboratory, Ponds	50 -375 µg L^{-1}; decline CHL 1.25 mg L^{-1}; rapid decline in CHL, recovery after one week	Yan et al. (2012)
Myriophyllum verticillatum	*M. aeruginosa*	Tannic acid water	Laboratory	1 – 5 g L^{-1}; growth inhibition, but stimulation in presence of *Desmodesmus*	Chang et al. (2012)
Phragmites australis, Carex dispalata, Typha domingensis	*M. aeruginosa*	Water	Laboratory	unspecified dose; growth inhibition	Takeda et al. (2011)
Eichhornia crassipes	*Spirulina platensis, Nostoc piscinale*	methanol	Laboratory	20-250 mg L^{-1}; no effect	Shanab et al. (2010)
Purumus yedoensis, Acer buergerianum	*M. aeruginosa*	Water, casein/ acetone, Ethanol 50%	Laboratory	2.2 – 26 pg $cell^{-1}$ Decline in cell density	Shimada et al. (2010)

Table 8.1: Overview of plants of which extracts (solvent given) have been tested on target cyanobacteria, including reported effects.*(continued)*

Plant	Target	Extract	Test	Effect	Reference
Acacia mimosa	*M. aeruginosa*	Not specified	Laboratory	2-12 mg L^{-1}; growth inhibition, max ~50%, recovery occurs	Zhou et al. (2010)
Moringa oleifera	*M. aeruginosa*	Water	Laboratory	1.6 – 160 mg L^{-1}; growth inhibition > 20 mg L^{-1}, recovery observed	Lürling & Beekman (2010)
Stratiotes aloides	*Anabaena variabilis, Merismopedia tennuissima, Leptolyngbya boryana*	50% Methanol	Laboratory	0.1 – 4 g L^{-1} Growth inhibition *A. variabilis* No effect on *M. tennuissima* and *L. boryana*	Mohamed & Shehri (2010)
66 Chinese medicinal herbs	*M. aeruginosa*	Water boiled	Laboratory	16 extracts inhibited growth Minimum inhibitory concentration 0.39 – 25 g L^{-1}	Yang et al. (2009b)
Oryza sativa	*M. aeruginosa*	Methanol, ethyl acetate	Laboratory	0.01 – 10,000 µg l^{-1}; max 64% growth inhbition	Park et al. (2009)
Papaveraceae	*M. aeruginosa* *Synechococcus leopoliensis*	Water boiling	Laboratory	EC_{50}: 56 – 871 mg L^{-1} EC_{50}: 46 – 985 mg L^{-1}	Jančula et al. (2007)
Stratiotes aloides	*M. aeruginosa* *Anabaena* sp.	Water, methanol, acetone	Laboratory	0.5 – 3.75 g L^{-1}; inhibition > 1 g L^{-1}, stimulation at 0.75 g L^{-1}	Mulderij et al. (2007)
Myriophyllum verticillatum	*Anabaena* sp. *Limnothrix redekei*	50% methanol	Laboratory	0.5 – 2 mg ; clearing area found No effect on CHL	Hilt et al. (2006)
Elodea nuttallii *E. canadensis*	*Anabaena sp.* *An. variabilis* *Synechococcus elongatus* *Syn.* sp. *Syn. nidulans* *Pseudanabaena* cf. *catenata*	methanol		0.5 – 2 mg ; clearing area found 0.5 – 2 mg ; clearing area found 0 – 1 g L^{-1}; growth reduction 56-92%	Erhard & Gross (2006)
rice straw	*M. aeruginosa*	methanol	Laboratory	0.01 – 10 mg L^{-1}; growth reduction 98%	Park et al. (2006)
Stratiotes aloides	*M. aeruginosa*	Spent medium	Laboratory	Increased lag phase, slightly higher growth	Mulderij et al. (2005)

Plant extracts are often used as synonyms for allelochemicals, despite no proof on their exudation from living plants to the surrounding water exists. The effects of living plants on mitigation of cyanobacterial nuisance are reviewed by Bakker & Hilt (2016). Here we add a few words on allelochemicals, as they might contribute to stabilisation of clear-water states in shallow lakes (Hilt & Gross, 2008). Probably, the life time of active compounds is rather short. For example, in a study with spent medium of *Stratiotes aloides* in which two strains of *M. aeruginosa* were grown, a delayed lag phase was observed, but as exponential growth rates were equal or slightly higher than the controls (Mulderij et al., 2005), these exposed cyanobacteria apparently caught up rapidly pointing towards rapid decline of the active compounds. Hence, it remains to be seen if field applications of plant extracts or allelochemicals can produce a window of clear water long enough for submerged and presumably allelopatic active macrophytes to establish. Furthermore, a recent study showed while *M. aeruginosa* was inhibited by macrophyte allelochemicals when growing in pure culture, interacting with a green alga completely reversed inhibition into enhancement (Chang et al., 2012). This led the authors to conclude "*allelopathically-active macrophytes might thus support cyanobacteria rather than suppress them in situ*" (Chang et al., 2012).

Ultrasound

Ultrasound is sound of frequencies higher than those that can be detected by the human ear (Mason, 2007), i.e., approximately 20 kHz and higher. The frequency range ~20 – 200 kHz is considered low frequency ultrasound and used in industry and therapy, while frequencies up to 20 MHz are applied in medical diagnostics (Ahmadi et al., 2012). Ultrasound finds a large range of applications in medicine, science, industry, including various water treatments (Phull et al., 1997). The alleged potential of ultrasound controlling cyanobacteria in situ is based on several laboratory studies showing clear effects of ultrasound on cyanobacterial growth, the collapse of gas vesicles, cell wall disruption and disturbance of the photosynthetic activity (Wu et al., 2011; Rajasekhar et al., 2012a). The vast majority of these laboratory studies have used high power devices (e.g., 20-80 W, Hao et al., 2004a; 40-1200 W, Lee et al., 2001) that cause acoustic cavitation: a process in which compression and rarefaction create gas bubbles that may collapse (Neppiras, 1980). On collapse of the bubbles, several processes such as pressure gradients, shear forces, formation of radicals and hydrogen peroxide production may disrupt the cells (Joyce et al., 2003). However, such relatively high ultrasound intensities are difficult to apply in lakes and ponds as in larger volumes significantly less power is transmitted and consequently the impact on cyanobacteria will be far less (Rajasekhar et al., 2012a). This finds support in some field studies using higher power units - 10 units of 2 times 100 W (200 kHz) in a 365,000 m^3 reservoir (Lee et al., 2002) and one 630 W unit (22 kHz) in 9000 m^3 pond (Ahn et al., 2007). The pond study of Ahn et al. (2007) gave no support for strong cyanobacteria control by ultrasound, because both the control and treated pond were dominated by diatoms and green algae, the treated pond already at start had significantly lower chlorophyll-*a* concentration than the control,

while at the end of the experiment chlorophyll-*a* concentrations in both ponds were the same. Lee et al. (2002) reported that chlorophyll-*a* concentrations were lower in the two years of ultrasound treatment, which, however, finds no support in the data as chlorophyll-*a* concentrations (digitally extracted from figure 4 in Lee et al. 2002), yielded 81 (± 56) µg L^{-1} before and 74 (± 42) µg L^{-1} during ultrasound. The intensity in those studies would be around 5.5 x 10^{-9} W mL^{-1} (in Lee et al. 2002) and 7 x 10^{-8} W mL^{-1} (in Ahn et al., 2007). Such intensities are much lower than the intensities applied in laboratory studies (mean ± 1 sd: 0.115 ± 0.084 W mL^{-1}, n = 26, 20/40 kHz; Thomas et al., 1989; Francko et al., 1990; 1994; Al-Hamdani et al., 1998; Hao et al., 2004a,b; Zhang et al., 2006; Joyce et al., 2010; Rajasekhar et al., 2012b; Wu et al., 2012; Rodriguez-Molares et al., 2014).

Intensities of most, if not all, devices that are being sold for clearing lakes, ponds and aquaria are low. Some manufacturers even pointed out that for their commercial available transducers "*the occurrence of cavitation can be disregarded*" (URL7), or "*the method is not cavitation*" (URL8). The supposed mode of action "*is purely based on killing algae by bringing them in resonance*" (URL7). For cyanobacteria, the underlying assumption is that ultrasound will cause resonance and subsequent rupture or collapse of gas vesicles (Rajasekhar et al., 2012a). The resonance frequency (f_0) of gas bubbles can be estimated with the equation (Kotopoulis et al., 2009):

$$f_0 = \frac{1}{2\pi}\sqrt{\left(\frac{3\gamma}{R_0^2\rho}\left(p_0 + \frac{2\sigma}{R_0} + \frac{2\chi}{R_0}\right) - \left(\frac{2\sigma + 6\chi}{R_0^3\rho}\right)\right)} \qquad \text{(Eq. 1)}$$

in which γ is the polytropic exponent of the gas (1.39 for air), R_0 is the radius of the bubble (µm), ρ is the density of the surrounding liquid (1000 kg m^{-3}), p_0 is the ambient pressure (10^5 Pa), σ is the surface tension of the surrounding medium (Nm^{-1}) and χ is the membrane elasticity (Nm^{-1}). The surface tension and membrane elasticity were taken as in Kotopoulis et al. (2009); 0.072 and 0.044 Nm^{-1}, respectively. With this equation (Eq. 1), the resonance frequency can be calculated (Fig. 8.3). As a rule of thumb, the resonance frequency (in MHz)

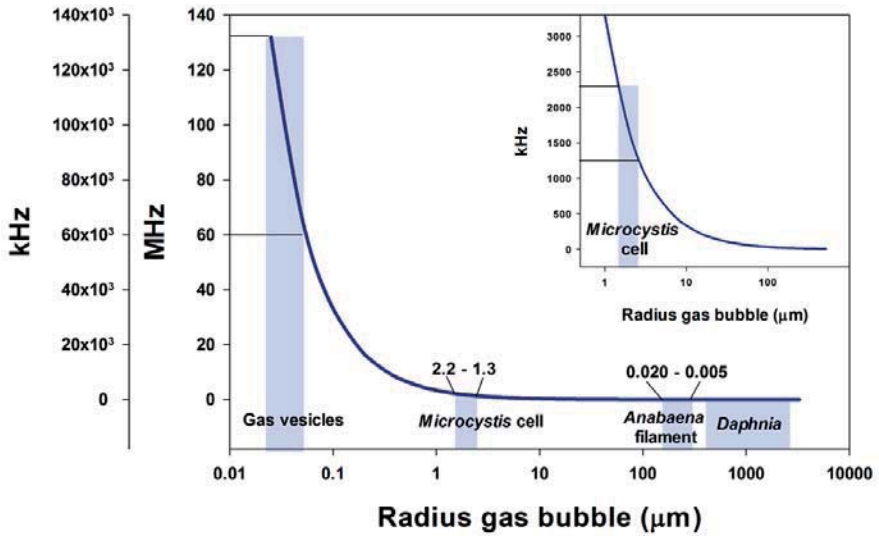


Fig. 8.3: Calculated resonance frequencies (using Eq. 1) of gas bubbles varying in radius (µm). Also included are the ranges for cyanobacteria gas vesicles, *Microcystis* cell, an *Anabaena* filament and *Daphnia*.

can also be approximated by 6.5/bubble diameter in µm (pers. comm. M. Postema, prof. in acoustics, University of Bergen, Norway).

From Eq. 1 follows that a sphere with a diameter of 5 µm would require 1.3 MHz, a 1 µm sphere 6.5 MHz, a 0.6 µm sphere 11 MHz, a 0.1 µm sphere 65 MHz and a 60 nm sphere 109 MHz to bring them into resonance. Here, the shape of the gas vesicles is assumed to be spherical, but in reality they have the form of a hollow cylindrical tube (Walsby & Hayes, 1989). Gas vesicles in *M. aeruginosa* have a diameter of 60-70 nm and maximum length of around 600 nm (Walsby, 1994; Dunton & Walsby, 2005), which means that it is highly unlikely that low frequency ultrasound (~20 – 200 kHz) from such commercial low power systems will provoke resonance of gas vesicles and subsequent collapse of gas vesicles in the cyanobacteria. However, acoustic cavitation may cause gas vesicle damage (Lee et al., 2001; Rodriguez-Molares et al., 2014), while in the acoustic field near the transducers a high acoustic pressure (power) will kill everything, not only cyanobacteria, but as mentioned before effective control of cyanobacteria in lakes and ponds by such devices is highly questionable.

Also some studies that had used higher frequency ultrasound (1700 kHz), contain some peculiarities, which make the suggested gas vesicles resonance doubtful; Tang et al. (2004) derived a resonance frequency of "*1.30 – 2.16 MHz because the R of the cyanobacteria vacuoles was in the range of 3–5* µm", while Hao et al. (2004b) mentioned that "*the gas vesicles are usually up to 1* µm *in length*". This seems a rather large exaggeration of gas vesicle sizes.

Absence of ultrasound induced gas vesicle rupture is supported by a controlled experiment with Flexidal AL-10 transducers (Lürling & Tolman, 2014a), in which there was not only no wipe out of *Cylindrospermopsis raciborskii*, but also no effect on buoyancy (Fig. 8.4). Similarly, *Anabaena* sp*., Microcystis aeruginosa* and the green alga *Scenedesmus*

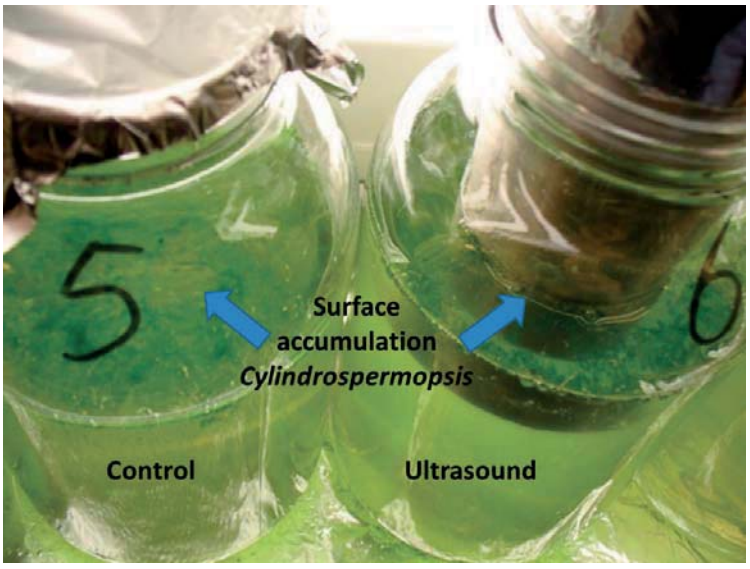


Fig. 8.4: Jars containing 800 mL *Cylindrospermopsis raciborskii* suspension after 10 days of exposure to ultrasound (6) where the surface accumulation is comparable to the control (5). Photograph by M. Lürling.

obliquus could not be controlled by these transducers that produced ultrasound at 20, 28 and 44 kHz with an acoustic power of 0.7 W (Lürling & Tolman, 2014a; Lürling et al., 2014a). The experiments unequivocally demonstrated that these devices were not able to clear even small volumes (800 mL – intensity of 8.5×10^{-4} W mL^{-1}) of cyanobacteria or chlorophytes thereby refuting the manufacturers claim that "*phytoplankton would be killed within one week*" (URL9). However, the zooplankton grazer *Daphnia magna* was killed within 15 minutes exposure (Lürling & Tolman, 2014a). In a follow up using Flexidal AL-05 transducers, ultrasound was not able to clear 85 L tanks over a 25 d experimental period (Lürling & Tolman, 2014b). Six tanks were inoculated with a mixture of green algae and cyanobacteria and stocked with some zooplankton grazers (*Daphnia*). While in controls the *Daphnia* strongly suppressed the phytoplankton creating clear water after 3 weeks, the ultrasound treatments turned into a green phytoplankton dominated soup (Fig. 8.5). This was caused by a strong detrimental effect of ultrasound on *Daphnia* as was demonstrated in additional experiments (Lürling & Tolman, 2014b).

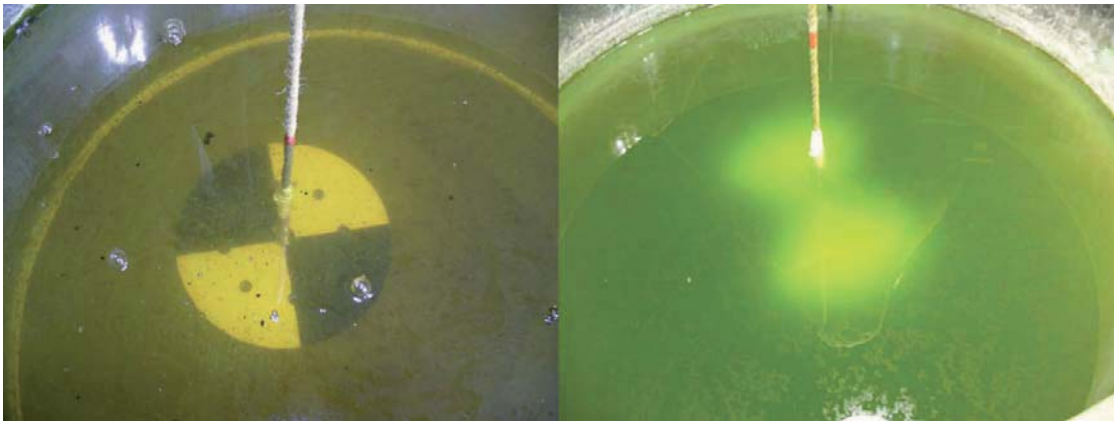


Fig. 8.5: Pictures after three weeks incubation of a non-exposed (Control, left panel) and ultrasound treated 85-L tank (right panel). Photographs by M. Lürling.

The findings that non-target organisms, such as *Daphnia*, can be killed by ultrasound from the commercial available transducers is in direct conflict with the claim that ultrasound is "*environmental friendly*" (Rajasekhar et al., 2012a). Actually, such claim and that ultrasound can be considered a "*green solution*" (Wu et al., 2011) find no support in the literature. None of the studies reviewed in Rajasekhar et al. (2012a) and Wu et al. (2011) included controlled experiments to examine the effect of ultrasound on non-target organisms such as *Daphnia*. High-power ultrasound is also used for disinfection of ballast water or raw water for drinking water preparation, where it may inactivate motile plankton (Hoyer & Clasen, 2002) or kill zooplankton, especially larger cladocerans (Holm et al., 2008). Cavitation might damage fish skin (Frenkel et al., 1999), while detrimental effects of ultrasound on macrophytes have been reported (Carstensen et al., 1990; Wu & Wu, 2006). Hence, high ultrasound intensities might come with danger for non-target organisms in the vicinity of the transducers.

The commercially available ultrasound devices had no water-clearing effect in relatively small volumes of 800 mL and 85 L (Lürling & Tolman, 2014a,b; Lürling et al., 2014a). Likewise, field trials with comparable devices that have been conducted in The Netherlands in 2007, yielded no evidence of an effect of ultrasound on cyanobacteria or phytoplankton (Govaert et al., 2007; Kardinaal et al., 2008). The study of Govaert et al. (2007) was conducted in two identical ponds of which one was treated with ultrasound produced by a Flexidal AL-50 transducer, while the other one served as control. During the four months of operation, chlorophyll-*a* concentrations in the control were around 64 (± 13) µg L^{-1} and in the ultrasound treatment around 69 (± 26) µg L^{-1} (data digitally extracted from Fig. 2 in Govaert et al., 2007). Moreover, no difference in phytoplankton composition was found (Govaert et al., 2007). Kardinaal et al. (2008) described two other field trials in The Netherlands; one in the southwest of The Netherlands in a harbour area near Tholen and the other one in a bay of recreational area De Gouden Ham near the river Maas. Surface scums and high *Microcystis* densities were observed on both sites despite the ultrasound treatment and the authors concluded that ultrasound was not effective in reducing cyanobacteria (Kardinaal et al., 2008).

In analogy to what has been reported for bacteria (Joyce et al., 2003; Mason, 2007) the effects of ultrasound on cyanobacteria can be grouped as:

- High-intensity (power) ultrasound in small volumes kills cyanobacteria. The devices are predominantly those that are meant for cleaning and sterilization.
- High-intensity (power) ultrasound in large volumes has no lethal effect on cyanobacteria. Filament breakage and colony declumping may occur.
- Low-intensity (power) ultrasound in small volumes does not kill cyanobacteria, but causes filament breakage and some growth reduction.
- Low-intensity ultrasound in large volumes has no effect on cyanobacteria.

The use of artificial mixing devices in non-stratifying waters

Whereas the use of artificial mixers in stratifying water has been dealt extensively (Visser et al., 2016), we would like to devote a few words to the use of artificial mixers in non-stratifying waters. In a review on the application of aeration in American reservoirs, Pastorok et al. (1980) showed that in only 50% of the cases artificial mixing was successful. An average depth > 40 m seems to be necessary to create the light conditions that bring the total phytoplankton biomass down (Klapper, 1991). In non-stratifying, mixed systems, the added advantage of being buoyant is limited to non-existent. In these shallow systems, mixing can be even counterproductive, by disrupting the sediment surface and enhancing phosphorus release (Blottiere et al., 2014). Indeed, Barbiero et al. showed that artificial mixing in a small, shallow impoundment close to New York (USA) not only did not result in the desired reduction of cyanobacterial dominance, but also led to an increased flux of phosphorus from the sediment, potentially even fuelling more biomass buildup (Barbiero et al., 1996a, b). Also in shallow lake Sheldon (z_{max} = 3.0 m), artificial mixing could not mitigate cyanobacterial

blooms (Oberholster et al., 2006). Although artificial mixers such as the Solarbee® have been claimed to mitigate water quality problems in shallower waters by taking away the competitive advantage that buoyant cyanobacteria have over other phytoplankton species that cannot control their buoyancy, the failure to reduce nuisance caused by phytoplankton in shallow systems tells another story (Hudnell et al., 2010; Barkoh et al., 2011; Bocchichio, 2012). In July 2013 a Solarbee® was installed in the shallow -average 2 m depth- Krabbeplas (The Netherlands) to control cyanobacteria nuisance. However, the already issued swimming ban lasted until beginning September 2013 as cyanobacteria remained flourishing in the lake. For instance, a water sample from August 28th 2013 measured with a PHYTO-PAM revealed 133 µg L^{-1} cyanobacterial chlorophyll-*a*. A warning for cyanobacteria in May 2014 was followed by several warnings and negative bathing advices from June 30th 2014 until September 2014. Hence, the Solarbee® was ineffective in controlling cyanobacteria nuisance in this lake.

In conclusion, we advise against artificial mixing of shallower waters, as sediment release of phosphorus may fuel cyanobacterial blooms rather than mitigate nuisance by cyanobacteria.

Conclusions and recommendations

Tackling nutrient inputs and internal loading is generally considered a prerequisite for water quality improvement and long-term reduction of cyanobacterial blooms (Cooke et al., 2005; Hilt et al., 2006), but the required preventive measures are not always feasible (Jančula & Maršálek, 2011) or may be insufficient to bring the water body in the desired state, which is often a clear water. In particular, in shallow lakes, a typical hysteresis can be observed (Scheffer et al., 1993) where increased nutrient loading first passes by rather unnoticed, stimulating submerged macrophytes, but once crossed a critical loading, the water body shifts into a phytoplankton dominated state (Fig. 8.6). This is often a cyanobacteria dominated state (Scheffer et al., 1997). Reducing the nutrient loading will express little effect on water clarity, unless reduction is very strong traversing a critical loading at much lower values than the one that caused a turbid state, where after the system returns to a clear water state (Fig. 8.6a). Between those transition boundaries the water body can have two alternative stable states, a turbid phytoplankton-dominated state and a clear water state with submerged macrophytes (Scheffer et al., 1993). Here end-of-pipe solutions might be of use when nutrient reduction alone has little impact on water clarity (Fig. 8.6b, arrow A) and a disturbance with a strong water clearing agent can bring the water back to a stable clear state (Scheffer et al., 1993).

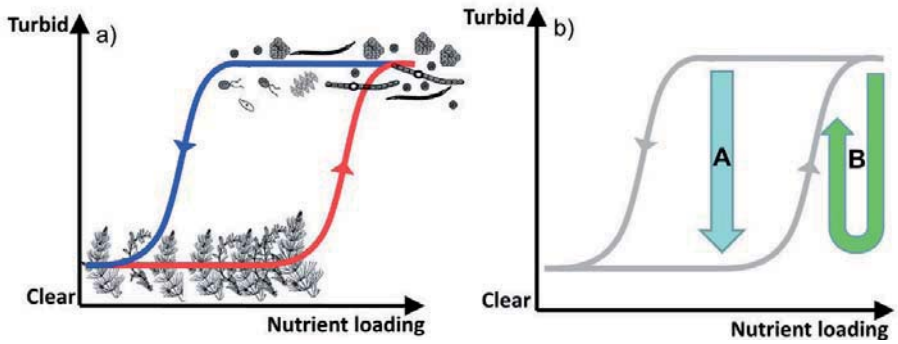


Fig. 8.6: a): Hysteresis in a typical shallow lake where increased nutrient loading leads to a phytoplankton-dominated state that can be brought back to a clear water state with submerged macrophytes by strong reduction in nutrient loading. The critical point of return is at lower loading than the transition to a turbid state (cf. Scheffer et al., 1993). b): Shock measures strongly reducing turbidity like end-of-pipe cyanobactericides will be able to bring a system in a clear water state when applied to a water that is below the critical nutrient loading for turbidity (arrow A). However, when applied above the critical loading the effects will be of short duration and the system will return to a turbid state (arrow B).

Hence, increased water transparency is one of the desired effects after fast reduction of the cyanobacterial biomass, but the curative products should also improve the water quality long enough to allow submerged macrophytes to establish. To determine if such a shock therapy might be feasible, a decent system analysis on nutrient loadings is crucial, because interceptions above the critical loading for the transition from clear to turbid (Fig. 8.6b, arrow B) will only be short-lived and the system will return to its turbid, cyanobacteria-dominated state.

To our opinion, the road to more evidence-based mitigation of cyanobacterial blooms, should always start with a system analysis of the specific water system. This implies a thorough investigation of its water and nutrient flows, the biological makeup of the system and the societal environment related to the functions of the specific water. There is broad consensus that nutrient enrichment leads to harmful cyanobacterial blooms (e.g., Paerl et al., 2011) and thus determining the nutrient inputs is a first logical step (Cooke et al., 2005). The nutrient flows separate in external load, water and non-water related, and internal load. In deep lakes acceptable loadings can be derived from empirical relationships (e.g., Vollenweider, 1976). The overall nutrient loading for shallow lakes can be evaluated by using the model PCLake (Janse et al., 2008) that will indicate the critical nutrient loads for clear to turbid water and vice versa for the specific water bodies. In relation to the biological makeup, it is crucial to determine whether a water body is packed or not with lake-bottom resuspending fish (carps, breams). If those fish are abundant in densities of hundreds of kg per hectare, the water will remain in a turbid state by resuspending sediments and preventing submerged macrophyte establishment (e.g., Cline et al., 1994; Roozen et al., 2007). In such cases, fish removal -maybe in combination with macrophyte introduction- should be considered an essential structural measure for rehabilitation. Besides efficiency and applicability, lake managers also need to consider safety, costs, and societal support of proposed measures. Consequently, the system analysis will lead to tailor-made solutions

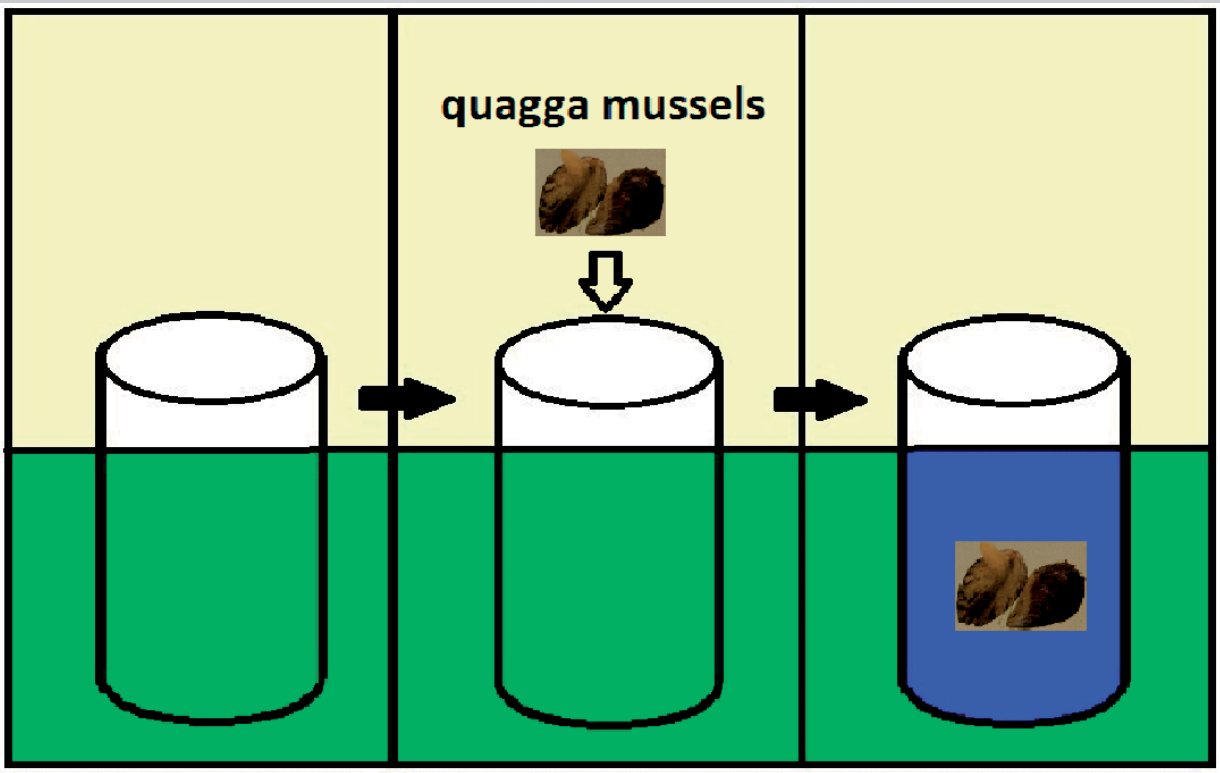
and most probably a set of measures rather than one single measure, but also leaving doing nothing as an option (Mackay et al., 2014).

The problem of cyanobacterial blooms and nuisance might be evident and may press water authorities to undertake immediate actions from obvious warnings to even trial and error activities with all kind of "miracle" techniques and products. In that context, numerous products have been proposed to Dutch water authorities as end-all solutions in controlling cyanobacteria. In this review we have critically evaluated the claims and effectiveness of "effective" micro-organisms, golden algae, plant extracts, ultrasound and artificial mixers in non-stratifying lakes. These were selected because of strong promotion or media attention the products/techniques received in The Netherlands over the last decade and of which information is available. There are many more or less comparable products/techniques that are tried by water authorities (e.g., "Dango-balls" URL10, "vortex-system" URL11, "oxatur" URL12, "oil screens" URL13) that have been left out of the evaluation, but of which efficacy is likewise controversial. In general, proposed underlying mechanisms are doubtful. The "effective" micro-organisms do not outcompete cyanobacteria, and there is no reason to expect they will improve water quality through diminished cyanobacteria/phytoplankton abundance. Golden algae cannot consume large cyanobacteria, and despite their omnipresence, they apparently fail to control blooms, but even if they did the resulting golden algae bloom would still keep water turbid. Plant extracts are needed in high quantities, might have short-lived effects, might have rather long lag phase and could come with negative side-effects such as exacerbated eutrophication, lower oxygen levels, or release of cyanotoxins, bringing too much uncertainties to warrant application. Commercially available ultrasound transducers emit frequencies that cannot collapse gas vesicles, and in contrast to the manufacturer's claim, they do not clear the water, but are harmful to other aquatic organisms, at least to *Daphnia*. The "positive" laboratory studies have all used high-power devices that are designed for cleaning and sterilization through disruption of cells, bacteria, spores or tissue. These devices cannot be used in situ, and even if they would, the much larger volumes would mean a strongly reduced power transmission and thus diminished impact on cyanobacteria. Hence, there is no music in fighting cyanobacteria with ultrasound. Finally, mixing can have positive effects in deeper, stratifying waters (see Visser et al., 2016), but mixing of shallower waters should be avoided as sediment release of phosphorus may fuel cyanobacterial blooms rather than mitigate nuisance by cyanobacteria. Therefore, none of the above seem the wide applicable solution to cyanobacterial nuisance; they should not be considered Columbus's egg.

Nonetheless, in addition to eutrophic lakes and ponds also in oligotrophic and mesotrophic lakes and ponds cyanobacteria might still be an important component of the plankton community (Lepistö et al., 2005; Carey et al., 2012). Even very low concentrations of cyanobacteria can become positively buoyant, accumulate at the water surface and be further concentrated on leeside shores. In such systems, reduction of nutrient loading is not the measure of first choice, while mechanical removal, sedimentation, or killing of the

accumulated material seems more feasible. The latter could be achieved with selected plant extracts (Table 8.1) that comes with abovementioned drawbacks, or hydrogen peroxide (Matthijs et al., 2012, 2016). Whether these will also be applicable when cyanobacteria scums have accumulated in harbours or in the vicinity of houses giving strong nuisance mainly through foul odors remains to be seen. Then probably skimming of cyanobacteria off the surface or sinking the intact cells with a coagulant and ballast (e.g., Li & Pan, 2013; Lürling & Van Oosterhout, 2013) might offer an alternative. There is a wealth of information on the latter technique, where natural soils and clays are modified with flocculants to effectively remove cyanobacteria from the water column (Pan et al., 2006a,b; 2011a,b; Zou et al., 2006; Noyma et al., 2016). An in situ experiment in Lake Taihu applying 25-31 mg L^{-1} (40-50 g m^{-2}) effectively cleared an isolated bay of cyanobacteria (Pan et al., 2011a). Inasmuch as in this technique entrapped cyanobacteria in flocs remain intact (Chow et al., 1999; Drikas et al., 2001), no release of toxins and nutrients during treatment occurs. Hence, the stripping of the water column from cyanobacteria might provide a promising alternative to the use of algaecides, such as copper sulfate or hydrogen peroxide, that may come with shortcomings such as toxins and nutrients release (Merel et al., 2013). In stratifying waters, cyanobacteria removal with flocculants and modified clay has yielded promising results too (Lürling & Van Oosterhout, 2013; Chapter 5). Nonetheless, more research on prevention of resuspension or liberation of viable cells from flocs in shallow waters is needed, where inclusion of calcium peroxide pellets (Noyma et al., 2016) or toxin-degrading bacteria (Li & Pan, 2015) may further improve performance.

Overall, water managers display a great need for effective treatments to control eutrophication and mitigate cyanobacterial nuisance. In order to solve the problems and to select the most promising set of measures, identification of the cause(s) is crucial. Based on the outcome of the essential system analysis, water managers may choose efficient, easy, safe and cheap measures from the tool box that ideally contains numerous preventive and curative measures. However, this critical review clearly showed that there is no strong support for several end-of-pipe measures, such as “effective” micro-organisms, golden algae, plant extracts, ultrasound and artificial mixers in non-stratifying systems to diminish eutrophication problems such that the resulting water quality meets societal and legislation demands. In that view, rapid interventions through flocking and sinking cyanobacteria with a coagulant and ballast seem more promising and further research should be devoted to these encouraging techniques.


quagga mussels

CHAPTER 9

Biomanipulation with quagga mussels (*Dreissena rostriformis bugensis*) to control harmful algal blooms in eutrophic urban ponds

This chapter is based on:
Biomanipulation with quagga mussels (*Dreissena rostriformis bugensis*) to control harmful algal blooms in eutrophic urban ponds.
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Abstract

Many urban ponds in The Netherlands and other countries suffer from eutrophication, resulting in harmful algal blooms which are often dominated by cyanobacteria. A sufficient reduction of nutrients, as prerequisite to mitigate cyanobacterial blooms in urban ponds, is not always feasible. Water managers are in need for applicable alternatives to mitigate these cyanobacterial blooms. The quagga mussel (*Dreissena rostriformis bugensis*) is a rapidly expanding bivalve species in many standing waters and rivers in The Netherlands. Because quagga mussels feed on algae, they could become a useful tool in controlling harmful algal blooms in urban ponds if provided with the appropriate substrate. We tested the hypothesis that quagga mussels can reduce phytoplankton biomass and induce a clear water state in a shallow hypertrophic urban pond. We executed an in situ enclosure experiment using eight enclosures (750 L) in an urban pond: four enclosures contained quagga mussels (0.3 g ww L^{-1}), and four control enclosures were set up without mussels. We used artificial substrate for the breeding of mussels and the transfer from the breeding location to the experimental site. In contrast to the controls, the enclosures with mussels showed significantly lower concentrations of total chlorophyll-*a* (2.4 µg L^{-1} in mussel enclosures versus 84.1 µg L^{-1} in controls), cyanobacterial chlorophyll-*a* (1.0 µg L^{-1} versus 7.3 µg L^{-1}) and total phosphorus (0.08 mg L^{-1} versus 0.17 mg L^{-1}), and had higher transparency (>0.80 m in mussel enclosures versus 0.57 m in controls) and higher soluble reactive phosphorus concentration (0.03 mg L^{-1} versus <0.01 mg L^{-1}). No effect of the mussels on microcystin concentrations was shown. The results show that quagga mussels are able to reduce the phytoplankton biomass in a hypertrophic urban pond, including cyanobacteria and induce a clear water state. We conclude that quagga mussels can be a promising tool in controlling algal blooms in urban ponds, in particular when a sufficient reduction of nutrients is not feasible. A preferred next step in the scaling up of the method is the determination of long-term effectiveness and side effects in a controlled application in an urban pond. Because the quagga mussel is an invasive alien species, new introductions should be considered carefully and water purification using quagga mussels is preferably applied in water systems where the species is already present.

Introduction

Eutrophication poses a threat to the water quality of many water bodies (Brönmark & Hansson, 2002; Smith & Schindler, 2009). In urban ponds and lakes, eutrophication often results in harmful algal blooms dominated by cyanobacteria (Downing et al., 2001; Oberholster et al., 2006; Schindler et al., 2008). Regionally this is a common phenomenon, for example in many urban ponds in The Netherlands (Chapter 2*)*. Cyanobacterial blooms in urban ponds cause a deterioration in water quality. As cyanobacteria are able to produce potent toxins, the blooms pose a risk to human and animal health and impair the values and usages of the water bodies involved (Falconer, 1999; Codd et al., 2005b; Steffensen, 2008). In mitigating the effects of cyanobacterial blooms, different strategies have been applied that intend to decrease the phytoplankton biomass and improve the underwater light climate (Gulati & Van Donk, 2002; Cooke et al., 2005; Søndergaard et al., 2008; Hickey & Gibbs, 2009; Jeppesen et al., 2012). The sufficient reduction in nutrient loading is essential in eutrophication control (Søndergaard et al., 2013). In urban ponds, this often appears to be arduous owing to the nature of the different nutrient sources and of the stakeholders involved (Gulati & Van Donk, 2002). Furthermore, an approach consisting solely of reducing nutrient loading is often not satisfactory, due to the resistance of shallow urban pond- and lake-ecosystems to shifting from an algae dominated turbid state to a macrophyte dominated clear state (Scheffer et al., 1993). In such cases, biomanipulation can be an additional management tool to accelerate the realization of a clear water state (Meijer, 2000). Even in nutrient rich ponds, biomanipulation can induce a clear water state, although the effectiveness may be short-termed (Peretyatko et al., 2009).

In its original concept, biomanipulation is defined as a series of manipulations of the biota of lakes and their habitats to reduce algal biomass and, in particular, cyanobacteria (Shapiro et al., 1975; Shapiro, 1990). In practice, biomanipulation is generally restricted to the removal of benthivorous and zooplanktivorous fish and to the introduction of piscivorous fish in order to stimulate the increase of filter feeding zooplankton, in particular *Daphnia* (Benndorf, 1995; Jeppesen et al., 2007a; Peretyatko et al., 2012). Grazing of zooplankton on phytoplankton may help to 'push' the system from a turbid to a clear state (Dionisio Pires, 2005). However, the grazing capability of zooplankton is limited, due to factors such as predation by invertebrates and juvenile fish, food-limitation and deleterious cyanobacteria (Benndorf et al., 2000; Scheffer, 2004; Gulati et al., 2008). Due to the great need of water managers to mitigate cyanobacterial blooms in urban ponds, attention has been given to the potential of using filter-feeding bivalves as additional or alternative phytoplankton grazers to improve water quality (Reeders & Bij de Vaate, 1990; McIvor, 2004; McLaughlan and Aldridge 2013).

Early enclosure experiments showed that the introduction of zebra mussels (*Dreissena polymorpha* Pallas) increased transparency in a temperate hypertrophic shallow lake, and it was suggested that this species could be used for biomanipulation purposes to improve lake water quality (Richter, 1986; Reeders & Bij de Vaate, 1990). *D. polymorpha*,

which is native in the Ponto-Caspian area, has spread to large parts of Europe and North America. It reached The Netherlands in 1826 (Kinzelbach, 1992 in Bij de Vaate et al., 2002). In some areas which have been invaded, *D. polymorpha* is regarded a nuisance, creating ecological and economic damage (Pimental et al., 2001; Sousa et al., 2009). Nevertheless, the use of *D. polymorpha* has been promoted as a potential useful organism to control cyanobacterial blooms, including its use in shallow Dutch lakes (Reeders & Bij de Vaate, 1990; Dionisio Pires, 2005). Nowadays, *D. polymorpha* is widespread in The Netherlands where it is not considered to be a nuisance but improves ecosystem function and structure (Dionisio Pires et al*.,* 2005). The related sympatric Ponto-Caspian quagga mussel (*Dreissena rostriformis bugensis* Andrusov, also referred to as *Dreissena bugensis*), has first been reported for The Netherlands in 2006 and has spread rapidly. This spread has resulted in a dominance shift from *D. polymorpha* to *D. bugensis* at many locations (Molloy et al., 2007; Matthews et al., 2013). *D. bugensis* is also a suspension feeder that filters water and extracts food consisting of phytoplankton and other food sources as detritus and bacteria from it (Baldwin et al., 2002; Tang et al., 2013). Until now, *D. bugensis* did not receive much interest as a potential tool for water management.

Due to the urgent need for effective management tools to control cyanobacterial blooms in urban ponds, Dutch water authorities have become increasingly interested in the use of dreissenids of regional origin as a tool to improve water quality. Despite the long-term and widespread nature of dreissenids in many water bodies in The Netherlands, they do not dominate in urban ponds. Considering the present dominance of *D. bugensis* over *D. polymorpha* in areas in The Netherlands, it is of great importance to provide a better insight in the potential of *D. bugensis to* mitigate cyanobacterial blooms. We hypothesized that the introduction of *D. bugensis* into a shallow hypertrophic urban pond would increase water transparency and reduce phytoplankton biomass including harmful cyanobacteria. To test this hypothesis, we executed an enclosure experiment in a semi-natural setting in a hypertrophic urban pond, and supported the field experiment with a laboratory assay to gain insight in the effect of feeding activity of *D. bugensis* on water quality.

Material and methods

Study site

The enclosure experiment was executed from August 20th 2012 (day 0) until September 3rd 2012 (day 14) in a pond in the city of Breda (The Netherlands, N 51° 36’ 48”/E 4° 47’ 30”). The shallow pond (mean water depth ~1 m) is part of a residential area and is affected by discharges from a separated sewer system, transporting mostly rainwater. The pond contains water throughout the year at a fairly constant level and is used for recreational angling, feeding of ducks and the extraction of irrigation water for allotments. Lawns with scattered groups of deciduous trees surround the pond. The banks of the pond are protected by wooden revetments. The experimental site is in full sunlight during most of the day. It has a sandy bottom, covered with a 20 cm layer of mud. Submerged macrophytes

and dreissenids are lacking and cyanobacterial blooms (*Microcystis* sp., *Anabaena* sp.) occur regularly during the summer. For the laboratory assay water from the neighbouring pond 'Linievijver' was used (The Netherlands, N 51° 36' 0"/E 4° 46' 58"), which regularly suffered from cyanobacterial blooms in the past.

Dreissenids

Dreissenid mussels for the enclosure experiment were grown on a plastic cage (30 x 45 x 64 cm) to provide an appropriate surface for larval settlement (Ackerman, 1994). The cage was made up of a three dimensional braid of 3 mm wide plastic strips, providing open spaces of 1.5 – 2.5 cm. An empty cage was incubated at a water depth of ~3 m in spring 2011, on the bottom of a reservoir (N 51° 58' 26.5" / E 5° 14' 13.4") with an open connection to the river Rhine, north of the city of Culemborg. The sediment of the reservoir had a large population of *D. bugensis*. The cage provided a hard substrate for mussel larvae to attach to and was completely colonised by dreissenids at the start of the experiment.

Enclosure experiment

On August 17th 2012 (day -3) eight enclosures were created using transparent perspex cylinders that were positioned at 1 m intervals in a straight line (Fig. 9.1), at a mean water depth of 0.86 m (SE 0.01 m). The cylinders had a height of 1.30 m and a diameter of 1.05 m. They were pushed into the sediment to a depth of 0.2 m and were open to the sediment and the air. Approximately 750 L of pond water was enclosed in each cylinder. On top they were covered with chicken wire to prevent waterfowl getting in. The mussel cage was retrieved from the breeding reservoir on day 0 and transported to the experimental site within two h. The cage was divided into four equal parts (30 x 45 x 16 cm). A similar, empty cage was divided into equally sized parts. On day 0, four enclosures were each stocked with one of the cage-parts colonised with mussels, at a water depth of 15 – 45 cm. The mean wet weight

Fig. 9.1: Experimental site. The enclosures are covered with chicken wire to prevent waterfowl getting in.

(ww) of the mussel flesh in the enclosures was 0.292 g L^{-1} (SE 0.020). Four enclosures served as controls and they received a cage part that had no mussels. The position of controls and mussel treated enclosures was randomised.

From each cage-part the ww of mussels was determined at day 0 and at day 14. At the end of the experiment, randomly ~50% of the fresh mussel-biomass was scrubbed from the cage-parts with a hand brush, and ~100 specimens were randomly collected from each cage-part. In total 392 specimens were identified. The distinction between *D. bugensis* and *D. polymorpha* was based on shell-morphological features (Bij de Vaate & Jansen, 2007). Besides identification of each specimen the shell lengths were measured. From the scrubbed mussels from each enclosure, 10 random samples were taken. Each sample consisted of several hundred mussels. Because the animals were too small to determine the weights of the flesh, we determined the weights of the total animals (flesh + shells). The samples were dried in an oven at 105° C for 34 h before dry weight (dw) was measured. The samples were then ashed at 550° C for 3 h and weighed again to determine residue on ignition. To determine the ratio (flesh weight)/(total weight), we collected a second similar crate from the same breeding location on April 23rd 2013. This second crate had been incubated for one year and carried larger and easier to handle animals. From this second crate, we determined species composition and shell lengths of 309 randomly collected mussels, and the weights of 78 randomly collected individuals of *D. bugensis.* The ratio (flesh weight)/(total weight) of these mussels was applied to the results of the enclosure experiment, as the data from the second crate revealed no allometric relation of shell lengths (range 2.4 – 6.5 mm) to the ratio (flesh weight)/(total weight) (Pearson $r(9) = -0.064$, $P = 0.851$).

Field sampling and analyses

During the experimental period August 20th (day 0) until September 3rd (day 14) 2012 the enclosures and the pond were sampled at days 0, 1, 2, 4, 7, 10, 14. Additionally the pond was sampled prior to the setup of the enclosures on August 16th (day -4). On each sampling day, from each enclosure and the pond 10 L water was collected using a sampling tube (whole water column integrated samples) and mixed in a 10 L plastic bucket. After mixing, 2-5 sampling bottles with a total content of 3 L were filled with water from the bucket for further analysis. The sampling bottles were transported in a cooled, dark container before being analyzed. The water that was removed from the enclosures by sampling was gradually replaced by seepage through the sediment and no major differences in water levels inside and outside the enclosures were observed during the course of the experiment.

On each sampling date, the Secchi depth was determined using a Secchi disk; pH and oxygen (O_2) concentrations of the water were measured using a WTW Multi 350i meter (WTW, Weilheim, Germany). Chlorophyll-*a* concentrations (four algae groups: 'green' algae – Chlorophyta and Euglenophyta -, 'brown' algae – Bacillariophyta, Chrysophyta and Dinophyta -, 'blue-green' algae – Cyanobacteria -, 'red' algae – Cryptophyta -) were measured using a FluoroProbe (Moldaenke bbe, Schwentinental, Germany; Catherine *et al.*, 2012) 4

days before the start of the experiment and on days 0, 2, 4, 7, 10 and 14. Total chlorophyll-*a* refers to the sum of these four groups. To determine the relation between the results of the FluoroProbe measurements and the regularly used spectrophotometric analysis, the chlorophyll-*a* concentration of each enclosure was analyzed according to NNI (2011) 4 d before the start of the experiment and on days 0, 7 and 14. Phytoplankton composition and biovolume were microscopically determined on days 0 and 14. Analyses of soluble reactive phosphorus (SRP), total phosphorus (TP), total nitrogen (TN), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), suspended solids, residue on ignition and microcystins (MCs) were quantified 4 d before the start of the experiment and on days 0, 7 and 14. TP and TN concentrations were analyzed in unfiltered samples using a continuous flow analyzer (Skalar Analytical BV, Breda, The Netherlands) following the Dutch standard protocols according to NNI (2005a, 2005b, 2006a). SRP, NO_3^-, NO_2^- and NH_4^+ concentrations were analyzed in filtered samples (0.45 µm membrane filter, Polydisk) by a continuous flow analyzer (Skalar) using NNI protocols (NNI, 1997, 2005b, 2006a). Suspended solids were collected on a 7 µm paper filter (Whatman). Dry-weights of the solids were determined after 16 h drying at 105°C and residue on ignition was subsequently determined after 1.5 h heating at 550°C according to NNI (2005c, 2010). Samples for MC analyses were stored at -20° C until further processing. Concentrations of eight MC variants (dm-7-MC-RR, MC-RR, MC-YR, dm-7-MC-LR, MC-LR, MC-LY, MC-LW and MC-LF) were analyzed using LC-MS/MS as described in Lürling & Faassen (2013). On days 0, 7 and 14 zooplankton was sampled with the sampling tube (whole water column integrated samples). On each date, 25 L from each enclosure was filtered over a plankton net (55 µm), and conserved with Lugol's solution until microscopic analysis of biovolume in each sample.

Assay

The grazing activity of *D. bugensis* on a green alga (*Scenedesmus obliquus* SAG 276/3A), a cyanobacterium (*Microcystis aeruginosa* NIVA CYA 140) and on a mixture of both was examined in a short laboratory assay. *Microcystis aeruginosa* NIVA CYA 140 is a single celled strain and a known toxic microcystin producer (Dionisio Pires *et al.*, 2004b). Mussels for the assay were collected on the same breeding location as for the enclosure experiment. The mussels were raked from the sediment on July 16th 2012 using a handnet (mesh 0.5 mm) at a water depth of ~1.5 m, and were transported to the laboratory within two h. About 150 mussels (shell lengths 13-20 mm) were identified as *D. bugensis* and acclimated at 19.0° C in a container with 10 L unfiltered aerated pond water, originating from the Linievijver pond, under natural light conditions. The mussels were daily fed with the green alga *S. obliquus,* while 50% of the container water was refreshed every 4-5 days with unfiltered pond water from the Linievijver pond.

Scenedesmus obliquus and *M. aeruginosa* were grown in WC-medium (Guillard & Lorenzen, 1972) as a batch monoculture in 0.5 L Erlenmeyer flasks at 21° C (± 2° C), with a 16 : 8 light : dark regime on a rotating shaking table (80 rpm) during three weeks prior

to the assay. To minimize the possible influence of the WC-medium on the grazing activity of the mussels, we centrifuged the phytoplankton cultures *S. obliquus* for 20 min (5,180 rcf, Heraeus Varifuge GL) and *M. aeruginosa* for 10 min (3,000 rcf, MSE Harrier 18/80). Sedimented phytoplankton was resuspended in filtered (0.45 µm, Whatman NC 45) pond water and centrifuged again before used as food for mussels.

24 h before the start of the grazing experiment, the mussels were rinsed under running demineralised water and six mussels were placed in each of 9 glass jars containing 700 mL of filtered pond water (pond Linievijver) to which food (*S. obliquus*) was added. Jars were placed in a dark incubator at 19° C on a rotating shaking table set at 80 rpm to keep food in suspension. From experiments with *D. polymorpha* it is known that dreissenids are not disturbed by shaking (Dionisio Pires et al., 2004a). Two h before the start of the assay, the mussels were rinsed again and provided with fresh filtered pond water collected from the Linievijver pond. Just before the assay, the mussels were rinsed again. Then the assay was performed with six mussels held in a container filled with 700 mL filtered pond water taken from the Linievijver pond. The containers were placed on a rotating shaking table set at 80 rpm in a dark incubator at a temperature of 19° C. Depending on the treatment, the mussels were fed solely on *S. obliquus* (SAG 276/3A at 263 µg chlorophyll-*a* L^{-1}), *M. aeruginosa* (NIVA CYA 140 at 103 µg chlorophyll-*a* L^{-1}) or a mixture of both (*S. obliquus* at 90 µg chlorophyll-*a* L^{-1}, *M. aeruginosa* at 40 µg chlorophyll-*a* L^{-1}). Each treatment was in triplicate. As a control, the same algal cultures (singular species and mixture) were added in triplicate to containers with filtered pond water which contained six sets of empty *D. bugensis* shells. The treatments and the controls were run simultaneously during two h. Every 20 min a 2 mL sample was taken from each container for measurement of green algal and cyanobacterial chlorophyll-*a* using a PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany). After two h the mussels were taken out of the containers. To get an indication whether the phytoplankton was actually ingested or incorporated in (pseudo)faeces after it had been filtered by the mussels, the containers were thoroughly shaken after mussel removal to suspend particles. Immediately after being shaken, the containers were sampled again and chlorophyll-*a* concentrations were determined. After the grazing experiment, all mussels were rinsed, dried and ashed for determination of the clearance rate (CR) as described below. The weights of the animals were determined as dw and ash-free dry-weight (afdw) of the flesh.

Data analyses

The clearance rate is a measure of the grazing activity of the mussels. It refers to the volume of water from which the mussels have removed all of the food per unit of time (Bunt *et al.*, 1993) and is calculated according to the equation (Coughlan, 1969):

$$\text{CR} = \frac{V}{nt} * \left(Ln\ \frac{C0}{Ct} - Ln \frac{C'0}{C't} \right),$$

in which CR is the clearance rate (L g dw^{-1} h^{-1}, with dw as dry weight of the flesh); V the volume of the container (L); n the dw of the mussel flesh (g); t the time (h); C0 the chlorophyll-*a* concentration (µg L^{-1}) in the enclosures with mussels at t = 0 and Ct at t = t; C'0 the chlorophyll-*a* concentration in the controls at t = 0 and C't at t = t.

The dependent samples T-test was used to analyze whether the ww's on day 0 differed from day 14 and to analyze whether the chlorophyll-*a* concentrations with and without dissolved (pseudo)faeces differed. To analyze whether the separate water quality variables of the controls and of the mussel enclosures showed significant differences, we used the independent samples T-test for equality of means. In case requirements for normality or equality of variances could not be fulfilled, we used the Mann-Whitney U-Test for equality of medians, with treatment as the grouping variable. The total and cyanobacterial chlorophyll-*a* concentrations (FluoroProbe) were analyzed using repeated measures ANOVAs. Total chlorophyll-*a* concentrations were log-transformed to fulfil variance homogeneity requirements. Cyanobacterial chlorophyll-*a* concentrations from day 0 were left out as they did not meet the requirement of equality of error variances. We used Greenhouse-Geisser estimates as the assumptions of homogeneity of co-variances were violated ($\varepsilon < 0.75$). As the data of the CRs of the assay showed no homogeneity of variances, we used the Welch test. The analyses were performed in SPSS Statistics 21 (IBM). At $P < 0.05$ we accepted statistical significance.

Results

Enclosure experiment

The dreissenids used in the enclosure experiment in 2012 consisted of 99.74% of *D. bugensis* and 0.26% of *D. polymorpha*. The characteristics of *D. bugensis* used in the enclosure experiment as well as *D. bugensis* collected in 2013 for the determination of weight-ratios, are given in Table 9.1. The dreissenids collected in 2013 consisted of 97.09% of *D. bugensis*

Table 9.1: Characteristics of *D. bugensis* on day 14 of the enclosure experiment in 2012 (A) and as collected in 2013 for determination of ratios (B). ww = wet weight, dw = dry weight, afdw = ash free dry weight. All weights as flesh. In parentheses ± 1SE.

	(A) 2012	(B) 2013
Mean shell length (mm)	2.26 (0.04; n=392)	5.80 (0.52; n=78)
Minimum shell length (mm)	0.9	1.6
Maximum shell length (mm)	7.0	23.9
Mean ww flesh (g L^{-1})	0.292 (0.020)	
Mean dw flesh (g L^{-1})	0.019 (0.002)	
Mean afdw flesh (g L^{-1})	0.018 (0.002)	
Ratio (flesh ww)/(total ww)		0.32
Ratio (flesh dw)/(total dw)		0.06
Ratio (flesh afdw)/(total afdw)		0.59

and 2.91% of *D. polymorpha*. Considering the abundance of *D. bugensis* in the dreissenid-composition that was used, we refer to this species when presenting and discussing the results of the enclosure experiment.

There is a strong positive correlation between total chlorophyll-*a* concentrations as measured with the FluoroProbe and spectrophotometric chlorophyll-*a* (NNI, 2011) , r_s = 0.768, $P < 0.0005$. In the rest of this section we refer to chlorophyll-*a* as measured with the FluoroProbe.

During the experiment, *D. bugensis* decreased the concentrations of total chlorophyll-*a* by 97% (from 81.0 to 2.4 µg L^{-1}; $F_{1.888,\ 5.664}$ = 73.378, $P < 0.0005$) and of cyanobacterial chlorophyll-*a* by 72% (from 3.6 to 1.0 µg L^{-1}; $F_{1.255,\ 3.766}$ = 20.120, P = 0.012; Fig. 9.2, Table 9.2). On day 0, the phytoplankton community was dominated by the Chlorophyta

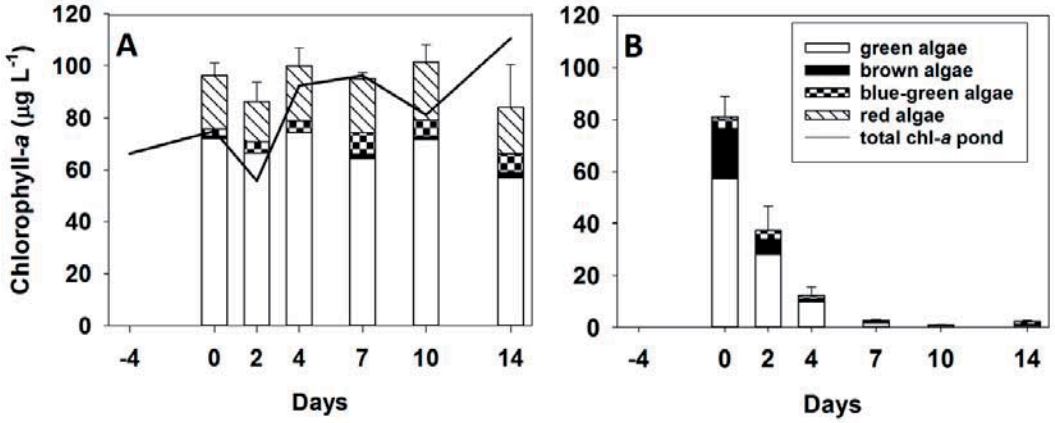

Fig. 9.2: Chlorophyll-*a* concentrations (µg L^{-1}) in the enclosure experiment. Panel A: mean of algae groups in control enclosures (bars) and total chlorophyll-*a* in the pond (line). Panel B: mean of algae groups in enclosures with *D. bugensis*. Green algae = Chlorophyta and Euglenophyta, brown algae = Bacillariophyta, Chrysophyta and Dinophyta, blue-green algae = Cyanophyta, red algae = Cryptophyta. Error bars indicate 1 SE of the total chlorophyll-*a* concentration.

Table 9.2: Mean concentrations of water quality variables in control enclosures and in *D. bugensis* enclosures. In parentheses ± 1SE. Significant differences between control enclosures and *D. bugensis* enclosures on the same date are indicated in bold ($P < 0.05$).

	day 0		**day 14**	
	controls	***D. bugensis***	**controls**	***D. bugensis***
total chlorophyll-*a* (µg L^{-1})	96.4 (4.7)	81.0 (8.0)	**84.1** (16.5)	**2.4** (0.3)
cyanobacterial chlorophyll-*a* (µg L^{-1})	2.4 (0.6)	3.6 (0.1)	**7.3** (1.6)	**1.0** (0.1)
TP (mg P L^{-1})	**0.19** (0.01)	**0.23** (0.00)	**0.17** (0.03)	**0.08** (0.01)
SRP (mg P L^{-1})	<0.01 (0.00)	<0.01 (0.00)	**<0.01** (0.00)	**0.03** (0.01)
TN (mg N L^{-1})	**1.98** (0.05)	**2.25** (0.09)	1.58 (0.23)	1.38 (0.48)
NO_3^- (mg N L^{-1})	<0.10 (0.00)	<0.10 (0.00)	**<0.10** (0.00)	**0.18** (0.03)
pH	8.1 (0.2)	8.2 (0.0)	**8.7** (0.1)	**7.9** (0.1)
suspended solids (mg L^{-1})	20 (0)	20 (0)	**16** (2)	**<5** (0)
O_2 (mg L^{-1})	**12.3** (0.5)	**10.0 (**0.4)	13.4 (1.2)	10.6 (0.7)
temperature (°C)	**24.6** (0.2)	**23.6** (0.1)	19.5 (0.4)	18.9 (0.6)

Volvocales indet. and *Actinastrum hantzschii*, the Cryptophyta *Cryptomonas* sp. and the Baccilariophyta *Fragilariophyceae* indet. (Table 9.3). On day 14, the dominating taxon in the controls was *Actinastrum hantzschii*, and in the mussel enclosures it were *Volvocales* indet. (Table 9.3).

Table 9.3: Mean biovolume ($mm^3 L^{-1}$) of dominating phytoplankton taxa on day 0 and day 14. In parentheses ± 1 SE.

	day 0		day 14	
	Controls ($mm^3 L^{-1}$)	***D. bugensis* ($mm^3 L^{-1}$)**	**Controls ($mm^3 L^{-1}$)**	***D. bugensis* ($mm^3 L^{-1}$)**
Volvocales indet.	4.320 (1.261)	3.582 (2.664)	1.574 (0.841)	0.693 (0.161)
Actinastrum hantzschii	3.258 (1.649)	2.585 (0.204)	14.091 (9.599)	0.008 (0.008)
Cryptomonas sp.	2.671 (1.675)	1.387 (0.334)	4.245 (1.931)	0.202 (0.062)
Coscinodiscophyceae indet.	2.240 (0.944)	2.478 (0.535)	6.640 (4.228)	0.017 (0.011)
Fragilariophyceae indet.	2.301 (1.902)	2.277 (1.029)	0.926 (0.756)	0.024 (0.024)
Synura sp.	0.830 (0.723)	2.073 (0.725)	0 (0)	0 (0)
Closterium sp.	1.750 (1.038)	0.204 (0.138)	1.034 (0.644)	0.015 (0.016)
Dinophyceae indet.	1.000 (1.000)	1.667 (0.639)	1.333 (1.334)	0.039 (0.039)
Trachelomonas sp.	1.587 (0.691)	0.869 (0.486)	0.781 (0.474)	0.016 (0.019)
Tetraedriella regularis	1.331 (1.332)	0 (0)	0 (0)	0 (0)
Aphanocapsa sp.	0.147 (0.077)	0.151 (0.060)	0.065 (0.038)	0.001 (0.002)
Aphanizomenon sp.	0.249 (0.139)	0.034 (0.020)	1.128 (0.698)	0 (0)
Aulocoseira sp.	0.251 (0.108)	0.532 (0.324)	3.805 (2.988)	0.041 (0.041)
Microcystis sp.	0.073 (0.041)	0.012 (0.012)	0.281 (0.172)	0.006 (0.006)
Scenedesmus sp.	0.113 (0.041)	0.109 (0.077)	0.930 (0.807)	0.007 (0.005)

After the introduction of *D. bugensis*, the Secchi depth increased from 0.48 m (day 0) to bottom view (0.82 m) on day 4 (Figs. 9.3 and 9.4). The pond remained turbid with a Secchi depth between 0.30 and 0.40 m. The Secchi depth of the controls ranged from 0.39 m (day 1) to 0.56 m (day 14).

Table 9.2 shows the mean concentrations of water quality variables at the start (day 0) and at the end of the experiment (day 14). Although TP concentrations differed significantly between controls (TP = 0.19 mg P L^{-1}) and *D. bugensis* enclosures (TP = 0.23 mg P L^{-1}) at day 0 ($t(6) = -4.39$, $P = 0.005$), both means were lower than the concentration in the pond (day 0, TP = 0.35 mg P L^{-1}). On day 14 the TP concentration in the *D. bugensis* enclosures (TP = 0.08 mg P L^{-1}) had dropped significantly compared to the controls (TP = 0.17 mg P L^{-1}; $U = 2.0$, $P = 0.04$). SRP concentrations showed the opposite pattern, at day 14 the concentration in the *D. bugensis* enclosures (SRP = 0.03 mg P L^{-1}) was significantly higher compared to the controls

(SRP < 0.01 mg P L^{-1}; U = 0.0, *P* = 0.01). At day 14, TN concentrations did not differ significantly between *D. bugensis* enclosures (TN = 1.38 mg N L^{-1}) and controls (TN = 1.58 mg N L^{-1}; U = 5.5, *P* = 0.465), while NO_3^- concentrations in the *D. bugensis* enclosures (NO_3^- = 0.18 mg N L^{-1}) were significantly higher than in the controls (NO_3^- < 0.10 mg N L^{-1}; U = 2.0, *P* = 0.04). The controls showed a significantly higher pH at day 14 (pH = 8.7) than the *D. bugensis* enclosures (pH = 7.9; t(6) = 9.31, *P* < 0.001). A similar pattern was observed for the suspended solids concentration, with higher concentrations in the controls (suspended solids = 16 mg L^{-1}) than in the *D. bugensis* enclosures (suspended solids < 5 mg L^{-1}; U = 0.0, *P* = 0.01). NO_2^- and NH_4^+ concentrations were mostly below detection limit (< 0.01 mg N L^{-1}).

Fig. 9.3: Control enclosure with an empty crate (left panel) and enclosure with quagga mussels on a crate (right panel) on day 14.

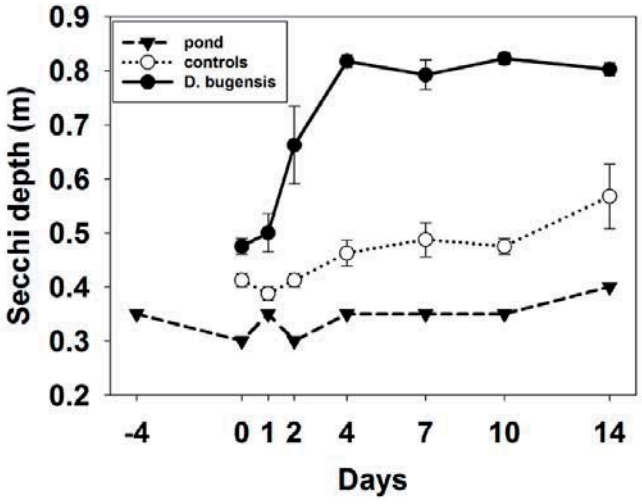

Fig. 9.4: Secchi depth (m) of the pond, of the means of the control enclosures and of the enclosures with *D. bugensis*. Error bars indicate 1 SE.

Concentrations of eight microcystin (MC) variants in the pond and in the enclosures were often below the detection limit (Table 9.4). Total MC concentrations were accordingly low (< 1 µg L^{-1}), with the highest concentrations found in the pond on day 7 (MC-LR = 0.37 µg L^{-1} and MC-RR = 0.36 µg L^{-1}). The variant MC-LR was detected in the pond on each of the dates, while in the enclosures only MC-LR was detected on days 0 and 7. There is no

obvious difference between control and *D. bugensis* enclosures as MCs were in general not detectable (Table 9.4).

Table 9.4: Maximum concentrations (µg L^{-1}) of eight microcystin variants in the pond and in any of the control enclosures and the enclosures with *D. bugensis* on days -4, 0, 7 and 14 (. = not detectable).

	day	Pond (µg L^{-1})	Control (µg L^{-1})	*D. bugensis* (µg L^{-1})
dmMC-LR	-4	.		
	0	.	.	.
	7	0.02	.	.
	14	.	.	.
dmMC-RR	-4	.		
	0	.	.	.
	7	0.14	.	.
	14	.	.	.
MC-LF	-4	.		
	0	.	.	.
	7	.	.	.
	14	.	.	.
MC-LR	-4	0.05		
	0	0.05	0.01	0.01
	7	0.37	0.04	0.02
	14	0.21	.	.
MC-LW	-4	.		
	0	.	.	.
	7	.	.	.
	14	.	.	.
MC-LY	-4	.		
	0	.	.	.
	7	.	.	.
	14	.	.	.
MC-RR	-4	.		
	0	.	.	.
	7	0.36	.	.
	14	0.17	.	.
MC-YR	-4	.		
	0	.	.	.
	7	0.05	.	.
	14	0.03	.	.

The mean CR reached a maximum of 1.35 L g^{-1} dw *D. bugensis* h^{-1} for the period from day 2 to day 4 and then declined to -0.55 L g^{-1} dw *D. bugensis* h^{-1} for the period from day 10 to day 14 (Fig. 9.5). All four enclosures showed a similar pattern. During the course of the experiment, the mean ww of the mussels increased from 0.279 g L^{-1} (SE 0.026) on day 0 to 0.292 g L^{-1} (SE 0.020) on day 14. Although an increase of ww can be expected and is in accordance to results in other studies (Baldwin et al., 2002), in our study the increase is not significant (t(3) = -1.186, *P* = 0.321).

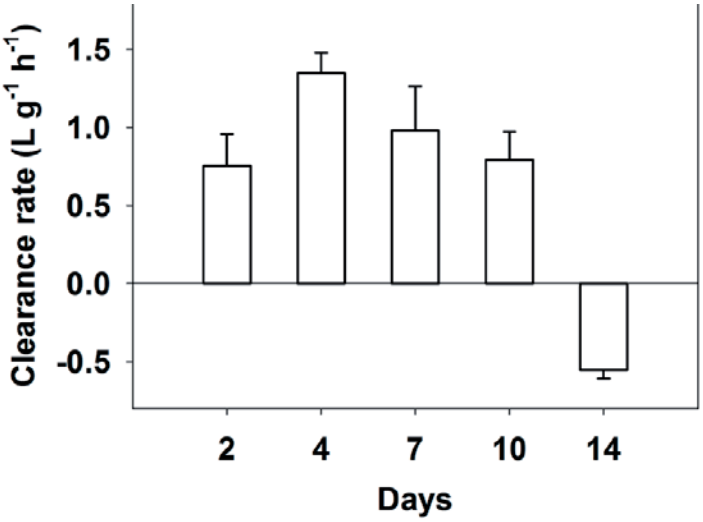

Fig. 9.5: Mean clearance rates (L g^{-1} dw h^{-1}) of *D. bugensis* in the enclosure experiment. Error bars indicate 1 SE.

During the course of the experiment, the biovolume of zooplankton declined in all enclosures. The decline in the enclosures with *D. bugensis* was 90%, and 60% in the controls at day 14 (Fig. 9.6). At day 14 the biovolumes of Copepoda (0.92 $mm^3 L^{-1}$) and Cladocera (0.41 $mm^3 L^{-1}$) were significantly lower in the mussel enclosures than in the controls (Copepoda 2.73 $mm^3 L^{-1}$, t(6) = 4.522, *P* = 0.004; Cladocera 2.97 $mm^3 L^{-1}$; t(6) = 9.430, *P* < 0.001). All major taxa showed a decline on day 14 in the mussel enclosures compared to the controls, except for copepod nauplii (Table 9.5).

Table 9.5: Mean biovolume ($mm^3 L^{-1}$) of dominating zooplankton taxa on day 14. In parentheses ± 1SE.

	Controls ($mm^3 L^{-1}$)	***D. bugensis* ($mm^3 L^{-1}$)**
Leptodora kindtii	0.849 (0.201)	0.041 (0.029)
Diaphanosoma brachyurum	1.970 (0.350)	0.320 (0.077)
Thermocyclops oithonoides	1.935 (0.420)	0.187 (0.048)
Nauplii	0.280 (0.026)	0.466 (0.048)
Acanthocyclops robustus	0.092 (0.031)	0.063 (0.034)

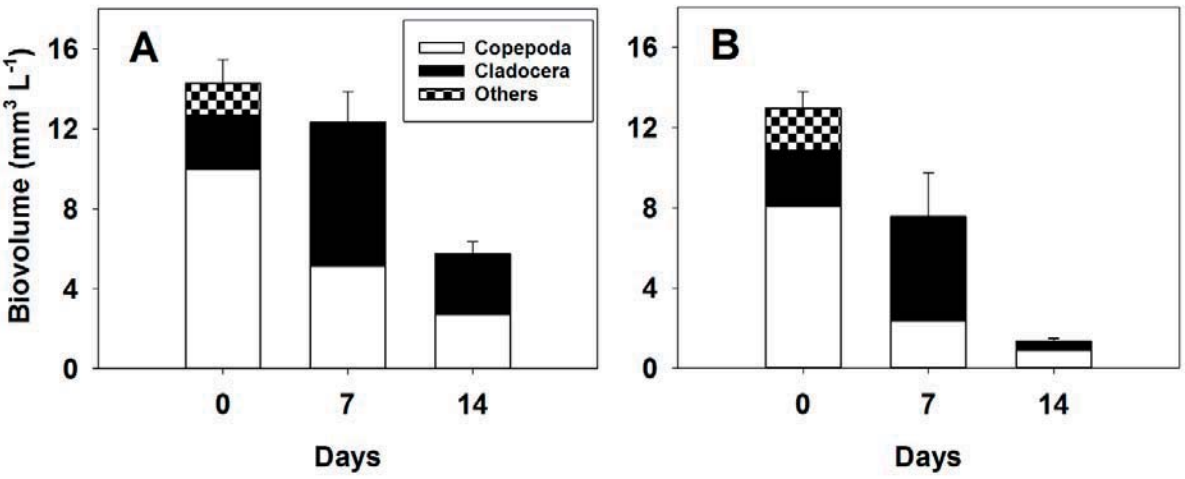

Fig. 9.6: Biovolume (mm^3 L^{-1}) of zooplankton groups in enclosure experiment. Panel A: mean biovolume of zooplankton groups in control enclosures. Panel B: mean biovolume of zooplankton groups in enclosures with *D. bugensis*. Error bars indicate 1 SE of total biovolume (n=4).

Assay

The effect of the feeding activity of *D. bugensis* on *S. obliquus* and *M. aeruginosa* and on a mixture of both was tested in an assay. The mean total chlorophyll-*a* concentrations dropped from 104 (SE 7) to 28 (SE 10) µg L^{-1} using *M. aeruginosa*, from 263 (SE 18) to 119 (SE 18) µg L^{-1} using *S. obliquus*, and from 40 (SE 2) to 11 (SE 1) µg L^{-1} and from 90 (SE 1) to 63 (SE 5) µg L^{-1} using a mixture (*M. aeruginosa* and *S. obliquus* respectively). Although the mean CRs ranged from 2.66 (mixture; SE 0.18) to 3.19 (*S. obliquus*; SE 0.44) and 6.18 L g^{-1} dw *D. bugensis* h^{-1} (*M. aeruginosa*; SE 1.75), they did not differ significantly between the three treatments ($F_{2, 3.0787}$ = 2.080, P = 0.268).

At the end of the assay, the containers were shaken to dissolve particles from (pseudo)faeces. Containers with only *S. obliquus* and containers with the mixture showed a significant increase in mean chlorophyll-*a* concentrations after being shaken, from 119.4 to 327.0 µg L^{-1} (t(2) = -9.858, P = 0.010) and from 74.2 to 180.0 µg L^{-1} (t(2) = -13.226, P = 0.006) respectively. In the containers with only *M. aeruginosa*, the mean chlorophyll-*a* concentration increased after the shaking from 28.0 to 72.1 µg L^{-1}, but the increase was not significant (t(2) = -2.858, P = 0.104).

Discussion

Effect on water quality and phytoplankton

The crates provided a good breeding substrate for *D. bugensis* and the mussels survived the transfer from the breeding location to the experimental site. The introduction of *D. bugensis* reduced the total chlorophyll-*a* concentrations by 97% and the cyanobacterial chlorophyll-*a* by 72% during a 14-day enclosure experiment (Fig. 9.2). The Secchi depth in the mussel enclosures increased from 0.48 m to > 0.82 m within 4 days (Fig. 9.4). The results of the enclosure experiment support the hypothesis that the introduction of *D. bugensis* into a shallow hypertrophic urban pond increases the transparency of the water and reduces

phytoplankton biomass. The mean total chlorophyll-*a* concentration reached a minimum of 1.0 µg L^{-1} on day 10 compared to the initial concentration of 81 µg L^{-1} (Fig. 9.2). The grazing effect of the mussels stretched over the whole enclosure depth, despite the stable water column (Ackerman et al., 2001; Zhang et al., 2011). The effect of *D. bugensis* on chlorophyll-*a* concentrations in the enclosure experiment was in line with the results from the assay and results from experiments with *D. polymorpha* in a eutrophic lake (Richter, 1986). In the experiment with *D. polymorpha* (Richter, 1986), transparency increased from 30 cm to >120 cm in 11 days after the introduction of mussels in enclosures in a shallow eutrophic lake. Although *D. bugensis* and *D. polymorpha* differ in environmental requirements, CR, and breeding and habitat preferences (Diggins, 2001; Baldwin et al., 2002; Orlova et al., 2005), the effect of the filtering activities is in outline similar as they reduce phytoplankton biomass and increase water clarity (Mida et al., 2010; Vanderploeg et al., 2010). In the laboratory clearance rate experiment we observed a decline of the green alga *S. obliquus* and of a toxic strain of the cyanobacterium *M. aeruginosa* in single food species treatments, as well as in mixed cultures. The removal of *M. aeruginosa* in the mixed cultures (-73% chlorophyll-*a* within two h) is similar to the removal in the sole *M. aeruginosa* cultures (-73% chlorophyll-*a* within two h). The mean CRs of the sole cultures did not differ significantly from the mean CRs of the mixture. Striking effects of the introduction of *D. bugensis* in the enclosures in the urban pond were the decline of the seston, the TP concentration and pH, while the concentrations of SRP and NO_3^- increased. These observations correspond with observations in natural lakes after invasions with *D. bugensis* and *D. polymorpha*, where dreissenid filtration activities decreased chlorophyll-*a* and TP concentrations (Mida et al., 2010; Cha et al., 2013) when algal cells and other particles were removed. Although *D. polymorpha* is known for the direct excretion of NH_4^+ (Gardner et al., 1995), our data show no evidence for such excretion by *D. bugensis*. At days 0 and 14, all NH_4^+ concentrations in the treated enclosures and in the controls were below the detection limit (< 0.10 mg NH_4^+-N L^{-1}). On the other hand, we observed that the NO_3^- concentrations increased significantly in the treated enclosures (Table 9.2). Mida et al. (2010) mentioned an increase of NO_3^- after the invasion by *D. bugensis* in a natural lake. The increase is most likely caused by the decrease of the NO_3^- utilization by the diminished phytoplankton biomass (Mida et al., 2010), as well as oxidation of NH_4^+ to NO_3^- by bacteria. Conroy et al. (2005) conclude that nitrification influences the phytoplankton assemblage, as NH_4^+ is the preferred form of nitrogen for cyanobacteria-taxa such as *Anabaena* and *Microcystis* (Dortch, 1990) which often dominate blooms in urban ponds (Chapter 2). As such, nitrification can contribute to a reduction of the growth of cyanobacteria (Conroy et al., 2005). Increase of the SRP concentrations are caused by dreissenid excretions and less uptake by phytoplankton as the result of grazing loss (Zhang et al., 2011). This internal P-loading can enhance the growth of phytoplankton and, stimulated by the increased water clarity, of macrophytes and benthic algae (Higgins et al., 2008). This is supported by our observation of the expansion of filamentous green algae on the mussel cages (Fig. 9.3, right panel) and on the inside walls of the mussel enclosures.

D. polymorpha is known to promote cyanobacterial blooms in North America, due to the selective rejection of toxic colonial *Microcystis* in pseudofaeces (Vanderploeg et al., 2001; Knoll et al., 2008). In Dutch lakes however, *Microcystis* blooms did not occur after the introduction of *D. polymorpha*. It has been hypothesized that this was due to a minimal grazing effect of *D. polymorpha* in Dutch lakes (Vanderploeg et al., 2001). Knoll et al. (2008) showed a decline of the potentially toxic cyanobacterium *Anabaena* in lakes with *D. polymorpha*. Dionisio Pires et al. (2005) showed that *D. polymorpha* was able to remove colonial and filamentous cyanobacteria from the water, whether they were toxic or not. Similar to *D. polymorpha*, *D. bugensis* is known as a selective filter feeder, being able to graze on *Anabaena* (Tang et al., 2013). Due to the apparent contradiction in the outcome of these and other studies (e.g., Baker et al., 1998), the effect of the introduction of *D. bugensis* on the cyanobacteria concentration in a shallow urban pond cannot be predicted unambiguous on forehand. Our enclosure experiment showed that the cyanobacteria concentration in the mussel enclosures was significantly lower than in the controls, indicating a grazing effect of *D. bugensis* on cyanobacteria. Despite the absence of fish in the enclosures, naturally present zooplankton failed to reduce the algal biomass in the control enclosures (Fig. 9.2A). The assay showed that toxic *M. aeruginosa* and *S. obliquus* were grazed in single and in mixed cultures. As the grazing activity of the mussels depends not only on food quality but also on particle concentration, the initial differences in algal biomass in the assay might have biased the CR of in particular the single *S. obliquus* treatment (highest algal biomass) downward as mussels may have experienced a higher food level (Baldwin et al., 2002). Chlorophyll-*a* from *M. aeruginosa* and *S. obliquus* was found in egested material in the assay. This indicates that both species can be excluded from ingestion and rejected as pseudofaeces (Baker et al., 1998). As pseudofaeces can contain viable algal cells (Baker et al., 1998), the pseudofaeces may become a potential source for algal regrowth after remobilization into the water column. However, we did not observe a net effect of this in the enclosure experiment, as concentrations of all algae groups decreased in the presence of *D. bugensis*. In less sheltered, natural situations in urban ponds, resupension of viable algal cells in pseudofaeces by wave action and bioturbation might become a source of algal regrowth. We did not observe an increase of cyanobacterial toxins in the enclosure experiment, which is in accordance with the low concentration of cyanobacteria in the mussel enclosures. Although MC concentrations in the pond were low, the measured concentrations in the mussel enclosures were lower but not significantly different from the controls.

The mean CR in well mixed cultures in the assay was 2.66 L g^{-1} dw h^{-1}. In the presence of a mean of 15.18 g dw *D. bugensis* per enclosure (mean content 750 L) this implies that from an enclosure theoretically all the food particles will be removed in 18.5 h. Due to refiltration of water previously cleared of algae (e.g., by mussels in the centre of the crate), inhomogeneity of the suspension (Yu and Culver, 1999), and the variation of environmental conditions, the actual removal of algal biomass in the enclosures is slower, which is represented in a lower CR in the enclosures (Fig. 9.5). Algal concentrations in the

treated enclosures decreased asymptotically (Fig. 9.2B). After an initial increase of CR, we observed a decrease and hypothesized that the cause was a depletion of food which reduced filtering activity (Horgan & Mills, 1997). At the end of the experiment we observed a negative CR, due to the combined effect of the increase of chlorophyll-*a* concentration in the mussel enclosures and the decrease in the controls due to fluctuations.

Copepoda and Cladocera biomass decreased under the influence of *D. bugensis*. During the course of the enclosure experiment, the biovolumes of the larger grazers *Daphnia* and *Bosmina* were low in the pond as well in the controls and in the treated enclosures, showing that the observed increased transparency is due to the introduction of *D. bugensis*, rather than to the grazing of zooplankton. The reduction of zooplankton in the mussel enclosures, is probably the combined effect of competition for algae and the consumption of small zooplankton by *D. bugensis* (Thorp & Casper, 2002; Kissman et al., 2010).

Tool for water management

Based on the rapid decrease of chlorophyll-*a*, including cyanobacteria and the increase of transparency related to the introduction of *D. bugensis*, it is obvious that biomanipulation with this species has potential as a tool for water management. Despite small differences in Secchi depths between controls and mussel enclosures at the start of the experiment, the clarifying effect of *D. bugensis* is evident. Treated enclosures reached bottom view. Measures that create clear water will open a window for submerged macrophytes to establish and support the 'push' of the pond into a clear water state. However, as *D. bugensis* is also known as a detrimental invasive species, appropriate care should be considered. Uncontrolled spreading of the species in areas where it is absent, should be prevented. Factors to be considered are the intensity and consequences of the algal blooms, the regional and national legislation with regard to algal blooms and to the introduction of species, the current presence of the species in the region, the isolation and natural values of the water body involved, and the feasibility for mitigating eutrophication effects by other means. In controlling algal blooms, the best approach is the structural reduction of nutrient loads in combination with fish stock control and the reconstruction of bathymetry and hydrology. In reality however, this structural approach is not always feasible in urban ponds and there is an urgent need for alternatives. As *D. bugensis* is widely spread in most of the major basins and catchment areas of The Netherlands, the controlled use of the species as a tool for water management, seems obvious. Despite the spread of *D. bugensis* in The Netherlands, many urban ponds do not offer the appropriate conditions for such an extensive growth of the mussel population that clarifying effects can be expected. As a wait and see approach in solving the water quality problems of urban ponds is not an option for water managers, active support of the expansion of *D. bugensis* by its introduction and provision of artificial substrate might offer a way to reduce the harmful algal blooms. Our research showed that the controlled use of *D. bugensis* can create enough transparency for macrophytes to develop. The artificial substrate that we used supported the breeding of the mussels and

the transplantation to the urban pond. On the other hand, literature is not unambiguous with respect to negative effects of dreissenid introductions, and our results contradict the increase of cyanobacteria and MCs that were shown in earlier studies (e.g., Vanderploeg et al., 2001). To make progress in this field, we favor the execution of controlled small scale field experiments with special attention to the state of external nutrient loading, the regeneration of nutrients in the water body, the establishment and growth of macrophytes, filamentous algae, and benthic and pelagic cyanobacteria over several seasons and to the duration of effects. Such an approach is preferred over an extended enclosure experiment as it excludes confounding enclosure-effects such as the growth of filamentous green algae on the inside walls of the enclosures and includes the effects of wind, fish and other (known or unknown) influencing factors. Due to the nature and eutrophic state of many urban ponds, experiments could well be conducted in this water type.

Conclusions

The crates that were used provided a suitable substrate for the regional breeding of *D. bugensis* and for the transfer from the breeding site to the application site in an urban pond. The enclosure experiment showed that the introduction of *D. bugensis* in a hypertrophic urban pond reduced phytoplankton including cyanobacteria and induced a clear water state. This clarity can support conditions for submerged macrophytes to establish, which is essential for the perpetuation of the clear water state. TP concentrations decreased, while SRP and NO_3^- concentrations increased. In urban ponds, the introduction of *D. bugensis* can become a promising tool in controlling harmful algal blooms, in particular in situations where the sufficient reduction of nutrients is not feasible. The next step in the scaling up of the method can be a controlled whole-pond application to determine long-term effects and side-effects. As *D. bugensis* is an invasive alien species, introductions should be considered with care.

CHAPTER 10

Summarizing discussion and conclusions

Introduction

Eutrophication is globally considered the primary water quality issue for most of the freshwater ecosystems, often resulting in harmful algal blooms dominated by cyanobacteria (Smith & Schindler, 2009). In urbanized areas, small eutrophic freshwater lakes and ponds are vulnerable for cyanobacterial blooms. This counteracts the societal need for safe and aesthetically acceptable urban waters and for their use (Birch & McCaskie, 1999; Steffensen, 2008). The demand for the proper fulfilment of ecosystem services of urban lakes and ponds, calls for the reduction of the cyanobacterial nuisances. As the anthropogenic pressures upon urban waters are high, water managers experience during daily work that meeting the societal demands is tough. Difficulties in mitigating the cyanobacterial nuisance in urban lakes are enhanced by the complicated nature of freshwater ecosystems and the poor scientific proof for effective approaches (Mackay et al., 2014). Despite the importance of urban lakes for human society, there has been little scientific attention for these small water bodies (Céréghino et al., 2008; Downing, 2010; Hassall, 2014). To make progress in scientifically based rehabilitation of urban lakes and ponds, studies were undertaken, targeting the long-term reduction of cyanobacterial nuisance.

This thesis aims to improve insight into the scope and scale of the cyanobacterial nuisances in small freshwater lakes and ponds in the urbanized southern part of The Netherlands (Chapters 1 and 2). The source-oriented rehabilitation approach targeting the clear water state, which is generally considered the best way to realize long-term positive effects (Scheffer, 2004; Cooke et al., 2005; Jeppesen et al., 2007a; Jančula & Maršalek, 2011; Jeppesen et al., 2012; Taranu et al., 2015), is tested in several urban lakes suffering from cyanobacterial blooms (Chapters 3, 4 and 5). Diagnostics revealed the causes of the cyanobacterial blooms and guided towards the appropriate management approach for each site, which was supported by controlled laboratory studies and mesocosm field studies. Management interventions included the reduction of the external nutrient input from the catchment, the reduction of the internal nutrient input (through dredging, and passive and reactive sediment capping), reconstruction and biomanipulation. Efficacy of rehabilitation efforts in whole lake applications were monitored and evaluated. Side-effects of the application of LMB and flocculant on macroinvertebrate communities and on the bio-accumulation of La were studied (Chapters 6 and 7). Integrating results at various scales (Fig. 10.1) provided insight in efficacy of source-oriented treatments and safety issues. The source-oriented approach, however, is not always feasible due to societal, financial and technical restrictions. Then, curative methods have to be considered as alternatives. Several widely proposed curative methods have been evaluated for their efficacy (Chapter 8). A promising curative method is the application of quagga mussels, which was tested in a mesocosm experiment (Chapter 9). The findings of these studies are discussed, and a road map for the reduction of cyanobacterial nuisance in urban waters is given.

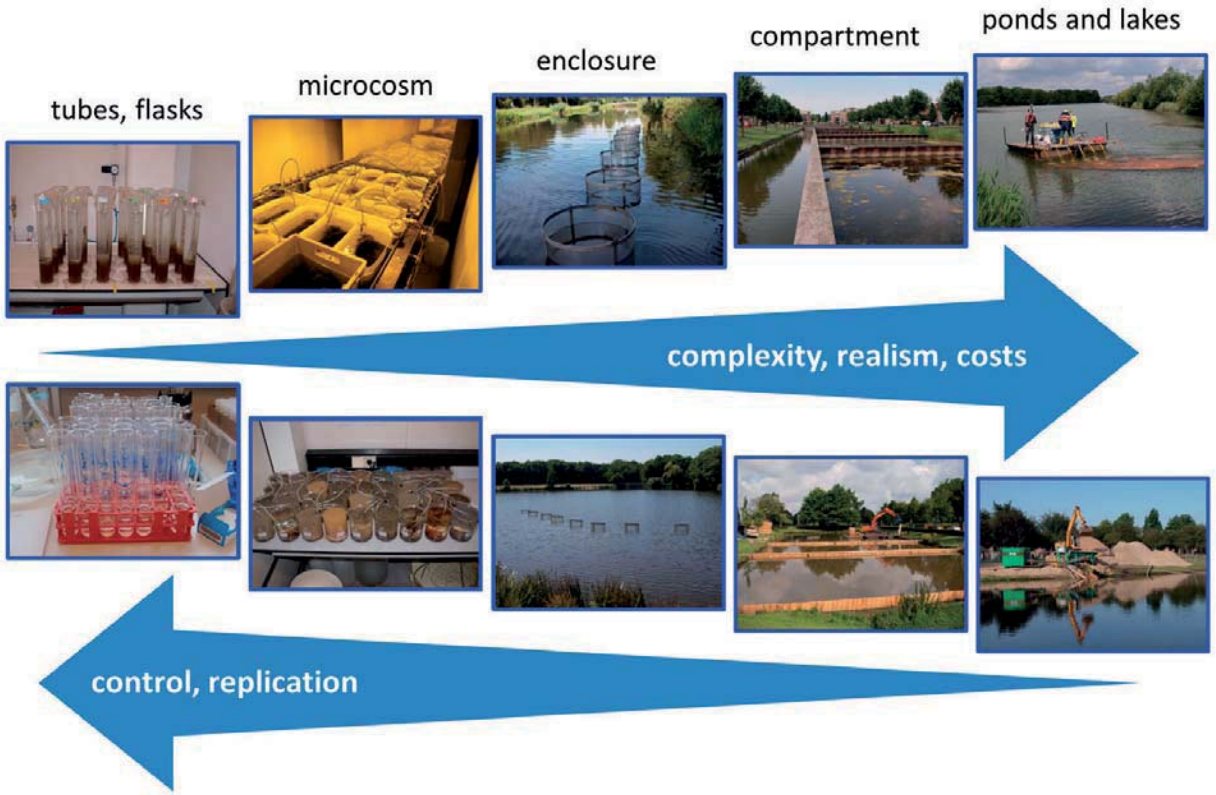

Fig. 10.1: Research at various scales from controlled and replicated laboratorial experiments to whole lake treatments yielding a powerful balance between statistical power and reality (modified from Lürling et al., 2016). The photographs relate to studies described in this thesis (the chapter number is given in parentheses). From left to right, upper line: testing sediment from pond Eindhoven (3), microcosms with macroinvertebrates (6; photograph by M. Pauwels), enclosure experiment with quagga mussels (9), compartments in pond Eindhoven (3), Flock & Lock treatment in Lake De Kuil (5). From left to right, lower line: laboratory assay testing LMB and flocculant (3), microcosms with macroinvertebrates (6), enclosure experiment testing flocculation in Waalwijk (10), compartments in pond Dongen (3), sediment capping in Lake Kleine Melanen (4).

Cyanobacterial nuisances, diagnostics and curative methods

Although shallow, stagnant small lakes and ponds are common features in urban areas in the south of The Netherlands, they do not receive as much attention from water managers as large water bodies, which are considered more important for the WFD (Council of the European Union, 2000; Boix et al., 2012). As urban lakes are year-round accessible and are situated in densely populated areas, they provide the most important public contact with lakes (Birch & McCaskie, 1999). Nevertheless, water quality monitoring in urban lakes is not common, resulting in poor information on the water quality state of these waters. Sightings of cyanobacterial blooms, indicative for nutrient enrichments, are reported by the public at dozens of locations each year, most of which are situated in urban areas (Chapter 2). The same situation is likely for other regions as well (Stoaianov et al., 2000; Ibelings et al., 2012; Faassen & Lürling, 2013). While external and internal nutrient loadings fuel the cyanobacterial blooms, wind induced mixing conditions may favor different cyanobacteria to proliferate (Condie & Webster, 2001; Reynolds et al., 2002; Padisák et al., 2009). High fish biomasses in urban lakes, often dominated by carp, enhance cyanobacteria by sediment

resuspension, causing turbidity of the water, and by reducing large herbivorous zooplankton (Meijer et al., 1990; Scheffer et al., 1993; Reynolds et al., 2002). The high concentrations of cyanobacterial toxins that were detected in urban lakes, exceeded over 3,300 times the standard for safe bathing water (Ibelings et al., 2012) and threaten citizens' health if ingested (Chapter 2).

The findings underpin the importance of eutrophication management for the reduction of cyanobacterial blooms in urban lakes. The reduction of nutrient loading is generally accepted as the major prerequisite for the sustainable long-term recovery of lakes and for the reduction of cyanobacterial blooms (Paerl & Otten, 2013; Mackay et al., 2014; Chapter 1). This thesis focuses on the reduction of biologically available P and considers P to be the key nutrient for eutrophication management (OECD, 1982; Schindler et al., 2008; Chapters 3, 4 and 5), as the reduction of N to concentrations limiting the phytoplankton growth is often not possible (Douglas et al., 2016). Diagnostics of the causes of the cyanobacterial blooms at a specific site is essential for the guidance towards effective management approaches. Diagnostics, also referred to as a water system analysis, includes the main water flows and P-loads and the composition of the biological community. The pressures upon water bodies are site-specific, dictating diagnostics to be site-specific. The water bodies involved in this thesis differed greatly in external and internal P-loads, as well in their critical P-loads (Table 10.1). Unexpectedly, discharges of rainwater from a separated sewer system, intended to separate clean rainwater from waste water, appeared to be the most important P source at several sites (Table 10.1; Chapters 3 and 4). The P pollution of the collected rainwater is most likely caused by street dirt, dog and bird droppings, plant matter, fertilizers and other urban sources (Waschbusch et al., 2000; Deffontis et al., 2013). Information on the P-loadings, supplemented with site-specific information of the fish and macrophyte communities, pointed towards appropriate management approaches. Accuracy of diagnostics, however, has its limitations, due to uncertainty ranges in model derived critical P-loads (Janse et al., 2010) and in the determination of the actual P-loads (Chapters 3, 4 and 5) for example caused by year-to-year fluctuations. Future research is needed to further increase the accuracy. Nevertheless, the diagnostics presented in this thesis revealed the major P-sources at each site, showed the required reductions in P-loadings and guided towards additional remediation. This converged into coherent packages of mutually reinforcing measures, in which each of the measures by itself would not suffice to induce the clear water state and reduce the cyanobacterial blooms. Not taking the outcome of diagnostics into full account, results in a questionable approach, as was disappointingly shown for pond Eindhoven (Chapter 3) and Lake Kleine Melanen (Chapter 4). Conversely, an approach tailored to the results of the diagnostics, targeting the external and internal P-loads and the food web, offers good options for successful management of cyanobacterial nuisances for several years, as was demonstrated for pond Dongen (Chapter 3), Lake De Kuil (Chapter 5) and Lake Groote Melanen (Fig. 10.2). In designing the site-specific management approach, ecological processes based the engineering and combined natural

Table 10.1: Phosphorus (P) loads and critical P-loads (mg P m^{-2} d^{-1}) at pond Dongen, pond Eindhoven, Lake Kleine Melanen and Lake De Kuil before rehabilitation. In bold the major external P source for each site.

	P-load (mg P m^{-2} d^{-1})			
	Pond Dongen	**Pond Eindhoven**	**Lake Kleine Melanen**	**Lake De Kuil**
External P sources				
Precipitation	0.07	0.11	0.20	0.08
Runoff (including dog faeces and urine)	**1.12**	4.69	0.10	0.001
Water birds	0.14	0.03	0.20	**0.10**
Feeding water birds	0.52	-	0.10	-
Feeding fish (bait)	0.42	0.44	0.10	0.01
Leaf litter	0.04	-	0.30	0.08
Rainwater discharge (separated sewer system)	-	**17.16**	**6.50**	-
Pumped groundwater	0.40	-	-	-
Total external P-load	2.71	22.43	7.50	0.27
Sediment P-release	5.42	1.66	3.80	5.20
Critical P-loads				
Turbid to clear	0.60	1.00	3.40	0.30*
Clear to turbid	1.50	3.10	5.20	0.60*

* Based on Vollenweider (1976), Chapter 5, permissible and excessive P-load

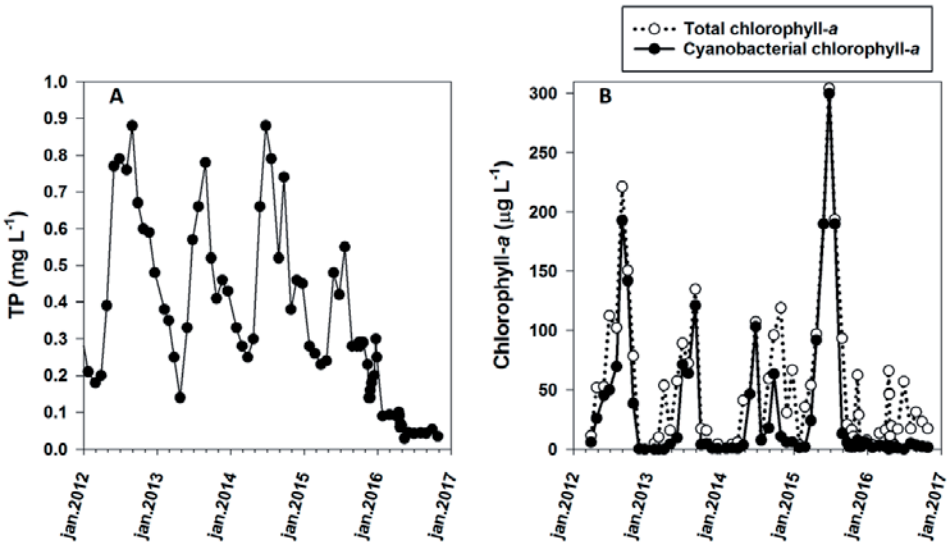

Fig. 10.2: Total phosphorus (TP) concentrations (mg P L^{-1}, panel A) and total and cyanobacterial chlorophyll-*a* concentrations (µg L^{-1}, panel B) in Lake Groote Melanen (Bergen op Zoom, The Netherlands), 2012-2017 (unpublished data Water Authority Brabantse delta). Restoration measures were based on diagnostics and focused on the reduction of external and internal P-loadings and on food web control. Implementation period of the major measures: sediment dredging April – July 2015, reconstruction of banks and reduction of external P-loading October – November 2015, sediment capping with sand and LMB November 2015 – January 2016, Flock & Lock April 2016, removal of bream and carp June 2016.

and anthropogenic demands (eco-engineering). Despite the efforts made by water managers, the anthropogenic pressures upon urban waters are not always fully manageable, as was demonstrated for uncontrolled fish stocking in pond Dongen (Chapter 3). The diagnostics-based approach targets the limitation of cyanobacterial growth by nutrient limitation, but longevity of the measures is unknown and may exceed 2 years (pond Dongen, Chapter 3) and 6 years (Lake De Kuil, Chapter 5). Future research, including long-term monitoring, is needed to increase insights in longevity of the approach for urban waters. When a limited external P-load reduction, remaining sediment P-release and limited development of macrophyte communities restricts the longevity, recurrent management actions rather than a one-time effort are likely needed for prolonged efficacy (Søndergaard et al., 2007). Contribution of stakeholders, such as local residents, anglers and the city authorities is crucial for commitment and an effective approach (Chapters 2, 3 and 4).

Curative methods in general do not provide long-term solutions, as they address the symptoms rather than the underlying causes (Sterner et al., 2006; Chislock et al., 2013). Without a comprehensive nutrient control strategy, the curative methods likely need (frequently) repeated application. They may be appropriate in situations where the source-oriented approach is not feasible, and they may contribute to the efficacy of source-oriented methods once nutrient reduction undershoots the upper critical loading threshold (Fig. 8.6b). Emphasis on such quick fixes, however, may distract the attention of politicians and policymakers from the structural long-term source-oriented approach (Sterner et al., 2006). Nevertheless, curative methods receive attention and a variety of methods has been developed. Curative methods often claim to meet demands for quick and simple elimination of nuisances (Chapter 8). The public awareness for such methods is often high and media attention has been witnessed (Fig. 10.3). While curative methods are at times promising,

Fig. 10.3: TV shot at the experimental site with quagga mussels (Chapter 9). Photograph by N. van Bruggen, 20 August 2012.

it has been shown that the profound claims of several approaches to end cyanobacterial nuisance could not be realized and that the longevity of positive effects, if any, is limited (Chapter 8). Nevertheless, further development of effective curative methods is essential as it is not always feasible to realize the suffice reduction in nutrient loads and the source-oriented approach may exceed the societal desired time scale for recovery. In this perspective, the use of flocculants to strip the water column from algae (Fig. 3.D.1) is promising. The additional use of ballast material (e.g., clay or LMB) enhances the sedimentation of flocs, and even a bloom of positively buoyant cyanobacteria can be instantly removed, clarifying the water (Lürling & Van Oosterhout, 2013; Chapter 5). The advantage of such a "Flock & Sink" method over the use of algaecides is the reduction of the risk for serious cell damage and subsequent release of cyanotoxins and nutrients into the surrounding water (Jančula & Maršálek, 2011; Noyma et al., 2016). Toxins from sedimented cells can be metabolized at the sediment and nutrients can be bound by the ballast material, in case it has P adsorption capacity, or become available for the growth of submerged macrophytes (Pan et al., 2011a;

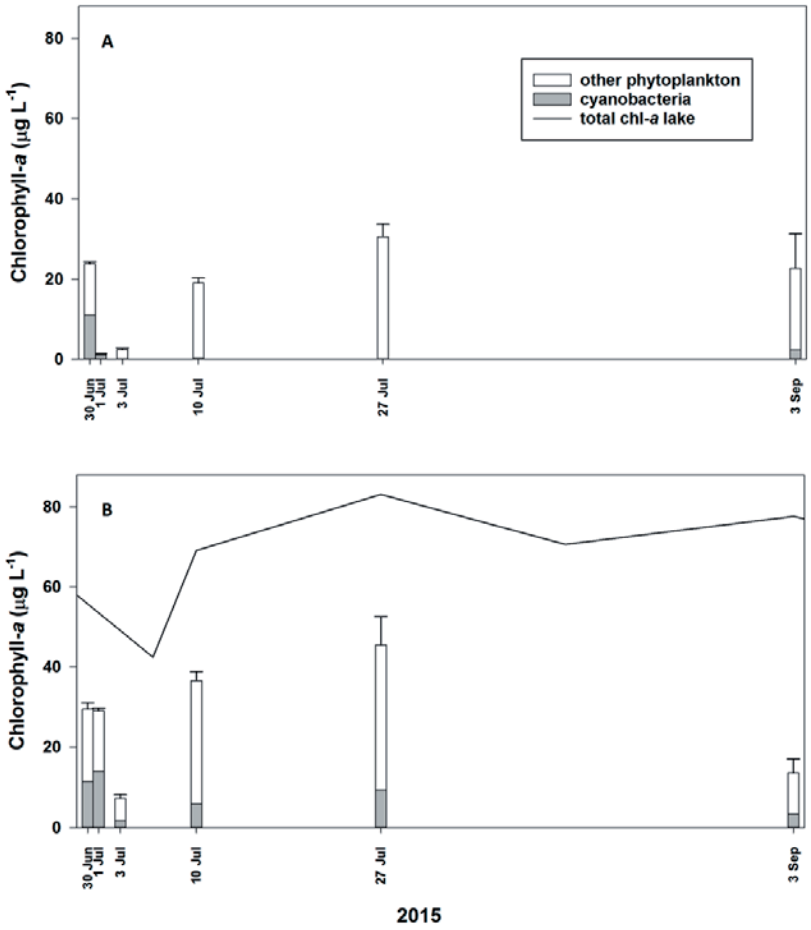

Fig. 10.4: Chlorophyll-*a* concentrations (µg L^{-1}) in a flocculation experiment with eight enclosures (700 L each) in a small shallow eutrophic lake in Waalwijk (The Netherlands; unpublished data Water Authority Brabantse Delta). The experiment lasted from 30 June 2015 until 3 September 2015. On 30 June 2015 the flocculant PAC was added to four enclosures (86 mL PAC in each enclosure; PAC ρ = 1.37 kg L^{-1}, 8.9% Al; panel A), while four other enclosures acted as control (panel B). Bars: mean chlorophyll-*a* concentrations in the enclosures, grey = cyanobacteria, white = other phytoplankton. Error bars indicate 1 SE of the total chlorophyll-*a* concentration (n = 4). The solid line represents the total chlorophyll-*a* concentration in the lake.

Li & Pan, 2015; Noyma et al., 2016). The effect of sole flocculation may be short-lived and the system may return to its phytoplankton dominated turbid state within weeks to months, indicating a high nutrient loading (Fig. 10.4).

Another promising method implies filter feeding bivalves (Reeders & Bij de Vaate, 1990; McIvor, 2004; McLaughlan & Aldridge, 2013). The first steps on the road to a field application were set in the enclosure experiment that is described in Chapter 9. A follow up to this experiment has already been given in the pilot project 'Linievijver', initiated by the Water Authority Brabantse Delta. In the 1.1 hectare shallow urban lake Linievijver (Breda, The Netherlands), an artificial reef consisting of 1600 crates overgrown with quagga mussels has been constructed in 2013-2014 (Fig. 1.4). Prior to 2014, the isolated eutrophic lake suffered from cyanobacterial blooms in most summers. While no other interventions have been made to the lake, the water quality improved and no cyanobacterial blooms have been reported since the completion of the reef (Fig. 10.5). In the neighbouring eutrophic urban lake Emerput, without management interventions, cyanobacterial blooms remained an issue and concentrations of cyanobacterial chlorophyll-*a* rose up to 89 µg L^{-1} (28 July 2015; unpublished data Water Authority Brabantse Delta).

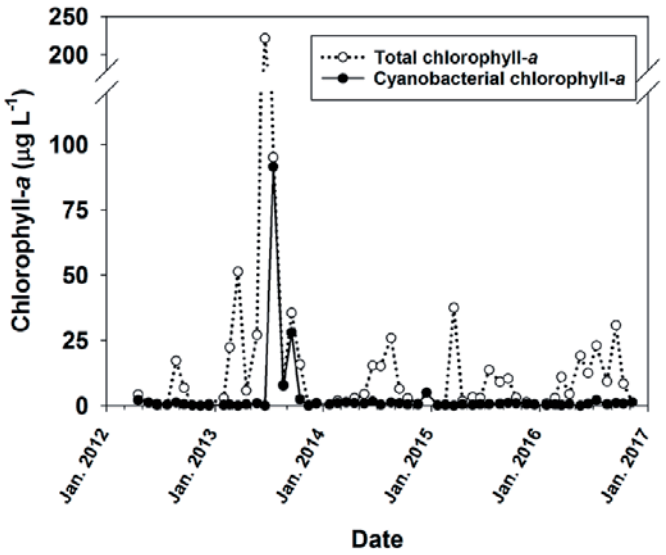

Fig. 10.5: Total and cyanobacterial chlorophyll-*a* concentrations (µg L^{-1}) in urban lake Linievijver (Breda, The Netherlands), April 2012 – September 2016 (unpublished data Water Authority Brabantse Delta). An artificial reef with quagga mussels was completed in March 2014.

Implications for water management

Based on the findings provided in this thesis, a flow chart for the reduction of cyanobacterial nuisance in eutrophic urban lakes is given (Fig. 10.6). The guideline provides the major steps for the reduction of cyanobacterial nuisance. Long-lasting solutions, targeting the P reduction and clear water state, are preferred (Chapters 3, 4 and 5). Curative measures are considered as short-term alternatives or second best solutions. Nevertheless, they are demanded for, if effective.

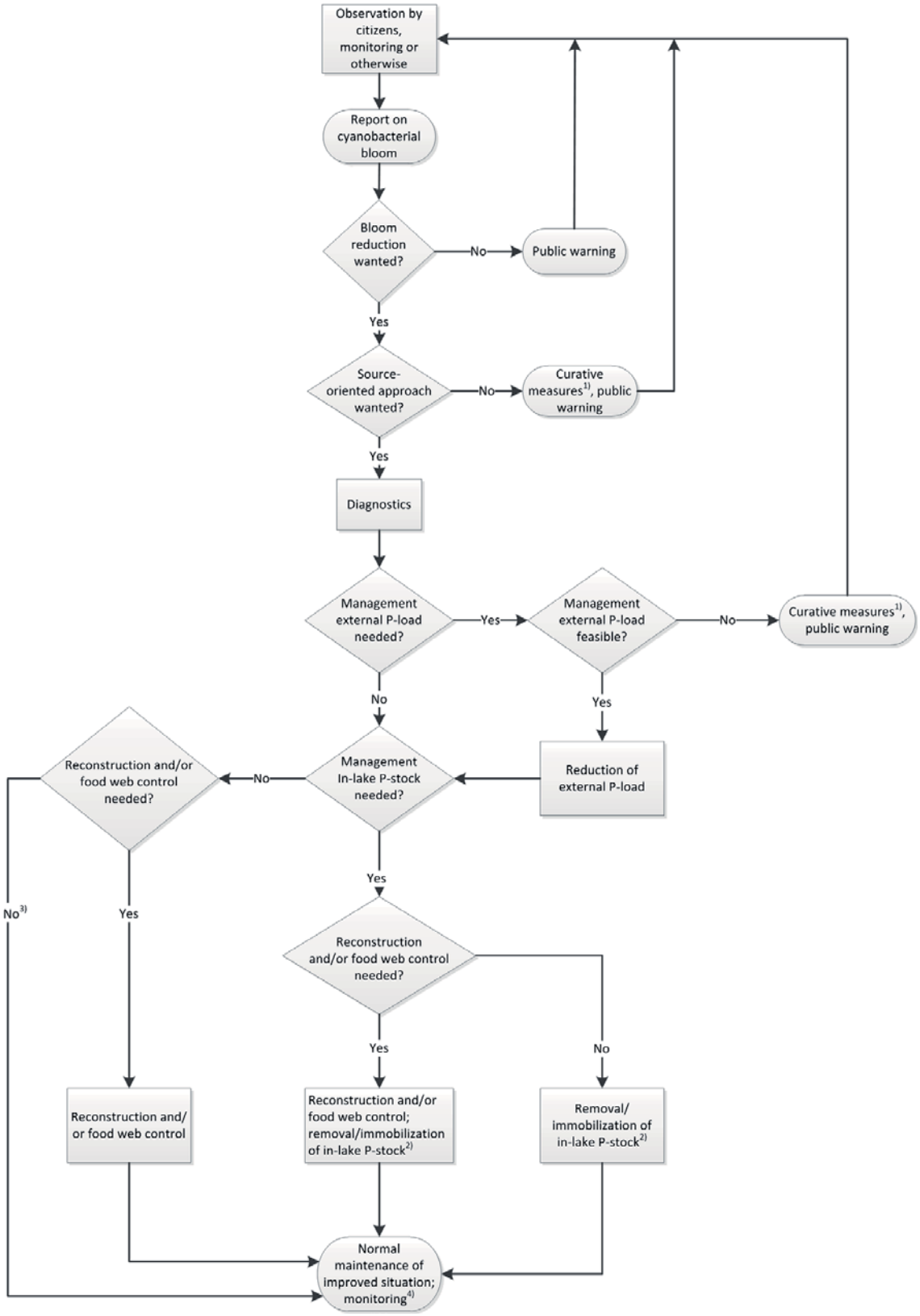

Fig. 10.6: Flow chart for the reduction of cyanobacterial nuisance in urban freshwater lakes and ponds.
1) E.g., scum removal, Flock & Sink, bivalves, hydrogen peroxide.
2) E.g., dredging, sediment capping with sand and P-fixative, Flock & Lock.
3) Optionally need for curative measures, e.g., in oligotrophic and mesotrophic lakes.
4) Normal maintenance consists of regular water management. Monitoring of water quality variables provides information on efficacy of rehabilitation and potential adjustments of water management.

Once diagnostics reveals the actual P-loads, the critical P-loads and the composition of the biological community, designing an appropriate approach is the next step towards the management of cyanobacterial blooms. Reductions of the external P-loads from the catchment to the range of the critical P-loads, or ideally below this range (Fig. 1.5), require catchment-wide actions. Due to high anthropogenic pressures on Dutch urban lakes, considerable efforts may be involved in this and the support of stakeholders, such as the municipality, is essential. The four case study lakes showed substantial differences in external loads and the dominant P-sources (Table 10.1). In order to reduce external P-loads, each site demanded a specific approach. In situations where no reduction of the external P-load to (at least) the intermediate state was feasible, the source-oriented approach was not recommended and transition to a long-lasting clear water state was not likely. In these cases, curative methods can be considered, if any relief is pursued. Despite considerable efforts that were made in the case study lakes, reduction of the external P-loads to the range of the critical P-loads was the best to be achieved, necessitating additional measures for the transition from the turbid state to the clear state. As the pool of legacy P in the sediment would delay the response of the lake to reduced external P-loads (Søndergaard et al., 1999), the internal P-sources were addressed. Additionally, food web control to reduce the effects of biological resistance to transition (Søndergaard et al., 2007) and reconstruction measures to increase the critical P-loads (Janse et al., 2008) were applied. When reductions of the external P-loads to (at least) the intermediate state seemed feasible, the design followed an iterative process in which the total of measures was refined in sequential steps. Various external P-sources were selected for reduction to undershoot the critical P-load (Chapters 3 and 4). Once the feasible reductions of the external P-loads were clarified, the hydrological implications of these reductions on the critical P-loads were derived. The same accounts for the consequences of alterations in water depth and for the construction of marsh area. This thesis compared the effectiveness of methods to control sediment P-release: dredging (Chapters 3 and 4), passive sediment capping with sand (Chapter 4) and reactive sediment capping with the P-fixative LMB (Chapters 3, 4 and 5).

Carp often dominates the high fish biomass of urban lakes (Chapters 2, 3 and 4). A high biomass of carp prevents submerged macrophytes to establish, thereby perpetuating the turbid state and high concentrations of chlorophyll-*a* (Breukelaar et al., 1994; Zambrano & Hinojosa, 1999; Chumchal et al., 2005). A low biomass of carp and accompanying benthivorous and zooplanktivorous fish as bream, is considered a prerequisite for the clarification of shallow eutrophic urban lakes. However, if societal demands impede the significant reduction of high carp and bream biomass, realization of the clear state is likely to be obstructed or at best delayed, as was shown in the control compartments at ponds Dongen and Eindhoven (Chapter 3). As the case study lakes pond Dongen, pond Eindhoven and Lake Kleine Melanen were excessively stocked with carp, management of sediment P-release deemed ineffective without reduction of the fish stock. Therefore, biomanipulation on fish and introduction of macrophytes were included in the treatments. Results showed that

biomanipulation alone had only a temporary positive effect on water quality (pond Dongen, Chapter 3; De Backer et al., 2012) or showed no improvement at all (pond Eindhoven, Chapter 3). Combining biomanipulation with measures reducing sediment P-release showed to be effective for water quality improvement, reducing the concentrations of TP and chlorophyll-*a*. The use of LMB showed to be a cost-effective alternative to dredging in reducing TP and phytoplankton biomass and improving water transparency. Attention should be given to the mixing depth of the top sediment layer, which affects the necessary LMB dose. The decline of the TP concentrations in the LMB treated lakes was most likely attributed to the binding capacity of the LMB (Chapters 3 and 5). An adjusted approach, however, was needed in Lake Kleine Melanen (Chapter 4) where high concentrations of organic matter were likely to hamper the in situ immobilization of P by LMB (Lürling et al., 2014b) and dredging did not reduce sediment P-release substantially due to characteristics of the freshly exposed sediment. The enclosure experiment in Lake Kleine Melanen showed the combination of passive sediment capping and a reactive LMB barrier to be effective in reducing the concentrations of TP and chlorophyll-*a* (Chapter 4).

Geo-engineering materials, such as P-fixatives and flocculants, can be powerful tools in eutrophication management (e.g., Chapter 5). Considering the great demand for (cost-) effective eutrophication management and the diversity of surface waters, there is a need for further development of geo-engineering materials. For the future development and testing of these compounds, international collaboration is preferred and an independent research centre could strongly support innovations, boosting the development and validation of effective, safe and cheap materials (Lürling et al., 2016).

A low dose flocculant (PAC and $FeCl_3$) flocculated and precipitated the phytoplankton, even when positively buoyant cyanobacteria were abundant (Chapters 3, 4 and 5). This instantly opened a window of opportunity for submerged macrophytes to get established, as was shown in the enclosure experiment at Lake Kleine Melanen (Chapter 4) and in the whole lake application at Lake De Kuil (Chapter 5). The additional advantage of PAC (Lopata & Gawronska, 2008) and $FeCl_3$ to bind SRP (Boers et al., 1992) was not evident however (Chapters 3, 4 and 5). As such, combining LMB with a flocculant is advisable only early in the growing season or in waters with perennial blooms. Based on the better floc formation of PAC (Delgado et al., 2003) and the redox-sensitivity of iron (Smolders et al., 2006), PAC is preferred over $FeCl_3$. Nevertheless, the risk of sediment resuspension should be taken into account when preparing treatments with PAC, as this may enhance Al dissolution (Reitzel et al., 2013a).

Addition of naturally occurring elements such as La, Al and Fe, thereby exceeding the background concentrations, gives rise to concern about the environmental safety, as each of these elements can be toxic (Das et al., 1988; Smolders & Roelofs, 1996; Gensemer & Playle, 1999). In the low dose applications of Al and Fe as flocculant, the issue can be addressed in the legal permit requirements and be tested prior to the application. Neither of these elements gave rise to an acute or a long-term toxicity issue in the case study lakes

(Chapters 3 and 5). After the application of LMB, the La content of fish and macrophytes was significantly raised, indicating bio-accumulation (Chapter 7). Bioavailability of La from LMB has been shown for invertebrates (Van Oosterhout et al., 2014) and is a matter of concern. No toxic effects on fish and macrophytes were observed in the compartments and whole lake application up to five years following LMB application, and human health risks are considered negligible. Nevertheless, long-term information on ecotoxicological side-effects of LMB applications is still predominantly lacking (Copetti et al., 2016), justifying the need for future long-term monitoring.

Summarizing conclusions

The studies that are described in this thesis, demonstrate that cyanobacterial blooms in urban ponds and small lakes are widespread. Such blooms hamper the fulfilment of the ecosystem services of these waters for citizens, and the need for improvement of the water quality is high. Claims of curative measures to provide fast control of cyanobacterial blooms often appear to be false. While the rehabilitation of eutrophic urban ponds and lakes has been neglected for a long time, studies embodied in this thesis show that improvement of the water quality targeting the clear water state and of the long-term reduction of the concentrations of cyanobacteria are feasible. As each site has its unique characteristics and pressures, site-specific diagnostics are essential to address the cause(s) of the cyanobacterial blooms. The results of diagnostics point towards the effective management option, mostly comprising of a coherent set of interventions, each of which alone is not powerful enough to realize the target. A copy and paste approach of site-specific measures to other locations is not recommended. In reducing sediment P-release, LMB can be an attractive alternative for the removal of sediment, given the right conditions, while the additional use of a low dose flocculant is beneficial early in the growing season or in waters,

Fig. 10.7: Panel on the bank of an urban pond (Breda, The Netherlands), informing the public and anglers about their impact (e.g., from feeding birds and fish) on cyanobacterial nuisance.

with perennial blooms. Sand capping may be an additional tool. Rehabilitation, however cannot be seen as a once and for all time operation given the high anthropogenic pressures upon city waters. Depending on the remaining pressures, management interventions can be required to maintain a good water quality and prevent relapse to the turbid state. This implies the efforts of all stakeholders (Fig. 10.7). Nevertheless, doing nothing can be an alternative option for the water manager, when considering the technical prospects, costs and support from stakeholders for improvement. Good monitoring practices of long-term trends in water quality and of the biological community are key elements for sustainable water management, warranting the fulfilment of ecosystem services (Fig. 10.8). Regular adjustments of negative trends, if any, are by far more efficient and cost-effective than occasional bottom-up restorations from a relapsed turbid state.

Fig. 10.8: Clarity at Lake De Kuil (22 May 2009, after the Flock & Lock treatment; Chapter 5).

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Summary

Small lakes and ponds are common features in urban areas and they contribute to the quality of citizens' life. A poor water quality, however, can easily give rise to nuisance. A major cause for a poor water quality is a high concentration of plant-nourishing nutrients, eutrophication. In (semi-)standing waters, eutrophication often results in a high biomass of blue-green algae (cyanobacteria), turbid water and the disappearance of submerged aquatic plants. The cyanobacterial blooms can be accompanied with fish kills due to anoxia, the development of unpleasant surface scums and malodors. As cyanobacteria can produce potent toxins, they impose a serious risk for citizens' health, pets and wildlife. The cyanobacterial blooms hamper the anthropogenic use of the water and can have negative economic impacts. Water managers experience that the reduction of cyanobacterial nuisance is arduous. As long-term positive effects of management interventions are not often achieved, there is need for effective approaches.

The objective of this study was to investigate the efficacy of promising methods to reduce cyanobacterial nuisance in city waters, targeting the clear water state and promoting the growth of aquatic plants. Various methods were tested, in the laboratory, in small and large compartments and were eventually applied in whole ponds and lakes. It is widely accepted that the reduction of nutrient inputs is essential for long-term positive effects. This study focused on the reduction of the input and the availability of the key-nutrient phosphorus. It was shown that cyanobacterial nuisance was wide spread in urban ponds and lakes in the Dutch province of North Brabant. The phosphorus inputs of four urban lakes in this province were addressed. The study lakes differed greatly in the phosphorus sources and loads, depending on site-specific characteristics. These differences affected the selection of measures. It was shown that in-lake measures were effective in realizing the long-term abatement of the cyanobacterial nuisance, provided the external phosphorus input was limited. If the external phosphorus input could not be limited sufficiently, in-lake measures did not result in the long-term reduction of cyanobacterial nuisance.

To reduce the bioavailable phosphorus stock in the lake with in-lake measures, sediment capping with a phosphorus-binding agent (lanthanum modified bentonite, LMB) can be effective and cheaper than sediment removal by dredging. The additional use of a flocculant may have added value and suppressed cyanobacterial blooms quickly and effectively. Aquatic plants and macroinvertebrates responded positively to the achieved improvement of the water quality. Accumulation of lanthanum was shown in aquatic plants and fish, following LMB exposure. No toxic effects of lanthanum from LMB were observed. Depending on site-specific characteristics, dredging or LMB did not suffice to limit the available phosphorus stock in the lake. For this situation, the additional capping of the sediment with sand was tested and subsequently applied in a lake. Management of the fish biomass and lake reconstruction can support rehabilitation. The results of this study underpin the importance of a site-specific diagnosis (water system analysis). The diagnosis clarifies the underlying causes of cyanobacterial nuisances and is essential for a site-specific

tailored set of measures. This study showed that a site-specific set of measures reduced cyanobacterial nuisance effectively for a long term.

As eutrophication control is not always feasible or might be effective only in the long run, curative measures are needed for symptom relief. Several curative end-of-pipe measures that are often suggested were evaluated: effective microorganisms (EM®), golden algae, plant extracts, ultrasound and artificial mixing of non-stratifying waters. No strong support for the efficacy of these measures could be shown. Next to the above mentioned application of flocculant, the use of freshwater quagga mussels is promising. The efficacy of the mussels was experimentally tested and it was shown that the introduction of mussels in a hypertrophic urban pond reduced the phytoplankton biomass, including cyanobacteria, and induced a clear water state. The quagga mussel is an invasive alien species and new introductions should be considered carefully.

Based on the results from this study, the thesis provides a road map for water managers for the reduction of cyanobacterial nuisances in urban ponds and lakes.

Samenvatting

In stedelijke gebieden komen veel vijvers, meertjes en andere waterpartijen voor. Deze stadswateren dragen bij aan een goede en fijne leefomgeving, maar een slechte waterkwaliteit kan tot overlast leiden. Een belangrijke oorzaak van een slechte waterkwaliteit is een hoge concentratie plantenvoedingsstoffen, eutrofiëring. In stilstaand en traag stromend water leidt eutrofiëring vaak tot sterke blauwalgengroei (blauwalgenbloei). Het water wordt dan troebel en ondergedoken waterplanten verdwijnen. Er kunnen vieze en stinkende drijflagen aan het wateroppervlak ontstaan en er is kans op vissterfte. Doordat blauwalgen sterke gifstoffen kunnen maken, leidt blauwalgenbloei tot ernstige gezondheidsrisico's voor mens en (huis)dieren, zoals honden die in het water spelen. Blauwalgenbloei legt beperkingen op aan het gebruik van het oppervlaktewater en kan negatieve economische gevolgen hebben. In de praktijk vormt het verminderen van blauwalgenoverlast in stadswateren een lastig en hardnekkig probleem. Waterbeheerders hebben behoefte aan effectieve methoden om blauwalgenoverlast aan te pakken.

Deze studie had als doel te onderzoeken welke kansrijke aanpak effectief blauwalgenoverlast bestrijdt en de waterkwaliteit langdurig verbetert, met helder water en goede groeimogelijkheden voor waterplanten. Verschillende methoden zijn hiervoor getest, in het laboratorium, in kleine en grote compartimenten en daarna toegepast op hele vijvers en meertjes. Het beperken van de toevoer van voedingsstoffen wordt in het algemeen gezien als de beste aanpak voor langdurig positieve effecten. Dit onderzoek richtte zich op het beperken van de belangrijke voedingsstof fosfor. In het onderzoek werd aangetoond dat blauwalgenoverlast in stadswateren in de provincie Noord-Brabant wijdverbreid voorkomt. Voor vier van deze Noord-Brabantse stadswateren werd de aanvoer van fosfor gekwantificeerd. De fosforbelasting van deze wateren verschilde onderling sterk, qua omvang en herkomst. De verschillen hadden belangrijke gevolgen voor de te treffen verbetermaatregelen. De maatregelen vormden een samenhangend pakket, specifiek afgestemd op de plaatselijke omstandigheden. Bij voldoende beperking van de van buitenaf komende toevoer van fosfor, konden maatregelen in de waterpartij zelf de blauwalgenoverlast langdurig terugdringen. Echter, bij onvoldoende beperking van de van buitenaf komende fosfortoevoer lukte het niet om met maatregelen in de waterpartij blauwalgenoverlast langdurig terug te dringen. Het was dan dweilen met de kraan open.

Om de voor algengroei beschikbare fosforvoorraad in de waterpartij aan te pakken, kan het gebruik van een fosforbindend middel (met lanthaan verrijkt bentoniet, LMB) effectief zijn en is goedkoper dan het verwijderen van bagger van de waterbodem. Het aanvullend gebruik van een vlokmiddel bleek in bepaalde gevallen meerwaarde te kunnen hebben en blauwalgenbloei werd daarmee snel en effectief de kop ingedrukt. Waterplanten en macrofauna reageerden positief op de gerealiseerde waterkwaliteitsverbetering. Ophoping van lanthaan uit LMB werd vastgesteld bij waterplanten en vissen. Toxische effecten door

lanthaan werden niet waargenomen. Afhankelijk van de bodemopbouw en de hydrologische situatie, volstond baggeren of LMB niet altijd om de voor algengroei beschikbare hoeveelheid fosfor voldoende te beperken. Als aanvullende maatregel werd het bezanden van de waterbodem getest en vervolgens toegepast in een meertje. Visstandbeheer en het opnieuw inrichten van de waterpartij versterkten de positieve effecten van fosforreductie.

De resultaten van dit onderzoek onderstrepen het belang van een locatie specifieke diagnose (watersysteemanalyse). Deze analyse maakt de oorzaken van de blauwalgenoverlast inzichtelijk. Dit inzicht is essentieel voor het opstellen van een op maat gesneden maatregelenpakket. Met dit onderzoek is aangetoond dat met een locatie specifiek maatregelenpakket blauwalgenoverlast in stadswateren effectief en langdurig bestreden kan worden.

Het voldoende terugdringen van de fosforbelasting van het oppervlaktewater is in de praktijk niet overal mogelijk, of pas effectief op lange termijn. Symptoombestrijding kan in dergelijke situaties bijdragen aan het tijdelijk beperken van blauwalgenoverlast. Een aantal vaak genoemde symptoombestrijdingsmaatregelen werd geëvalueerd: effectieve micro-organismen (EM®), goudalgen, plantenextracten, ultrageluid en menging van ondiepe wateren. Er bleken geen duidelijke aanwijzingen te zijn voor de effectiviteit van deze maatregelen. Naast het al genoemde vlokmiddel, dat voor tijdelijke verlichting kan zorgen, is het gebruik van quagga mosselen kansrijk om blauwalgenoverlast te beperken. Aangetoond werd dat de mosselen in een zeer voedselrijke situatie de hoeveelheid algen en blauwalgen beperkten en het water helder maakten. De quagga mossel wordt beschouwd als een invasieve exotische soort. Het is belangrijk om een afweging te maken van voor- en nadelen, risico's en alternatieven, voordat de soort wordt gebruikt.

Op basis van de studieresultaten is een routekaart voor het bestrijden van blauwalgenoverlast in stadswateren opgesteld voor waterbeheerders.

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About the author

Guido Waajen (1958) was born in Maasniel, in the Province of Limburg (The Netherlands). He graduated for his MSc in Biology at the Wageningen University in 1982. In 1982 and 1983 he worked as a volunteer researcher for the State Forestry. In 1983 and 1984 he did his civil service as a conscientious objector for the Province of Noord-Brabant. From 1984 until 1987 he had temporary positions at the Province of Noord-Brabant, the Province of Limburg and the Province of Gelderland, the Wageningen University, and the Oranjewoud Consultancy in Heerenveen. From 1988 until 1990 he worked as a hydrobiologist at the Water Authority of Schieland in Rotterdam. In October 1990 he started to work for the Water Authority Brabantse Delta in Breda, first as head of the small Department of Surface Water Quality, and later as head of the Department of Water Systems and as head of the Research Department. The common theme in his work was aquatic ecology and the improvement of surface water quality. During his work for the Water Authority Brabantse Delta, he gradually got more and more involved in management tasks, thereby slowly loosing the ecological work. In 2007 he decided to initiate a career shift and to dedicate himself fully to aquatic ecology, with a special interest for measure-effect relationships, while he remained working for the Water Authority Brabantse Delta. During the first half year of 2009, he combined his work at the water authority with the position of interim managing director for the Deltawaterlab, the joint laboratory for the water authorities Brabantse Delta, Hollandse Delta and Delfland. In 2009 he was one of the initiators of the Water Framework Directive Innovation project 'Reduction of Cyanobacterial Nuisance', together with Miquel Lürling from the Wageningen University. This project generated the oppertunity of conducting this PhD, which Guido was authorized to combine with his regular job at the water authority. The combination of the regular work and the applied ecological researches, offered the unique opportunity to adjust the scientific research to the needs of water manager, to provide a tailor-fit scientific basis to the real-world problems from his regular work, and to implement the finding of the studies instantly into the regular work of the water authority. Currently, Guido works as a biologist at the Water Authority Brabantse Delta and is involved in projects targeting the reduction of cyanobacterial nuisances in city waters and the improvement of the ecological status of the water bodies designated by the European Water Framework Directive. Since 2001 he chairs the Dutch national Platform on Lake Restoration (PEHM). Guido is married, has two daughters and lives in Breda (The Netherlands).

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Paranymphs: Annemiek Waajen and Irene Waajen

Cover photograph by Guido Waajen: detail of the sculpture 'Blauwe Algen' at Oisterwijk Sculptuur (17 May – 3 June 2012, Oisterwijk, The Netherlands). 'Blauwe Algen' (Cyanobacteria) was created by the artist Monique Bastiaans (Valencia, Spain) and was inspired on a story on Algas Azules by the writer Eduardo Galeano (Uruguay; Bocas del tiempo, 2004; Siglo XXI de España Editores, S.A.):

"Antes del antes, en los tiempos de la infancia del mundo, cuando no había colores ni sonidos, ellas, las algas azules, ya existían. Echando oxígeno, dieron color a la mar y al cielo. Y un buen día, un día que duró millones de años, a muchas algas azules se les dio por convertirse en algas verdes. Y las algas verdes fueron generando, muy poquito a poco, líquenes, hongos, musgos, medusas y todos los colores y los sonidos que después vinieron, vinimos, a alborotar la mar y la tierra. Pero otras algas azules prefirieron seguir siendo como eran. Así siguen estando. Desde el remoto mundo que fue, ellas miran el mundo que es. No se sabe qué opinan."

"Vroeger nog dan vroeger, in de kindertijd van de wereld, toen er nog geen kleuren of geluiden waren, bestonden zij al, de blauwe algen. Door het produceren van zuurstof gaven ze kleur aan de zee en de lucht. En op een goeie dag, een dag die miljoenen jaren duurde, kregen veel blauwe algen het in hun hoofd om te veranderen in groene algen. En de groene algen genereerden heel langzaam, beetje bij beetje, korstmossen, schimmels, mossen, kwallen en alle kleuren en geluiden die hierna verschenen, onbeduidend, om het land en de zee te verstoren. Maar andere blauwe algen verkozen te blijven zoals ze waren. Zo zijn ze nog steeds. Vanuit de verre wereld die er was, kijken ze naar de wereld die nu is. Niemand weet hun mening erover."

Eduardo Galeano
(Dutch translation by Monique Bastiaans)

The use of the cover photograph of the sculpture was kindly permitted by the artist.
The quotation of Algal Azules (Eduardo Galeano) was kindly permitted by the publisher.
All photographs in this thesis by Guido Waajen, unless otherwise stated.

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Propositions

1. Control of noxious algal blooms in urban ponds and lakes makes sense.
   (this thesis)

2. Effective rehabilitation of urban ponds and lakes is based on diagnostics.
   (this thesis)

3. The political attitude towards the eurocrisis and dieselgate leave no doubt about the approach of the impending Water Framework Directive crisis.

4. Monitoring and evaluating the effects of interventions is crucial for proper management.

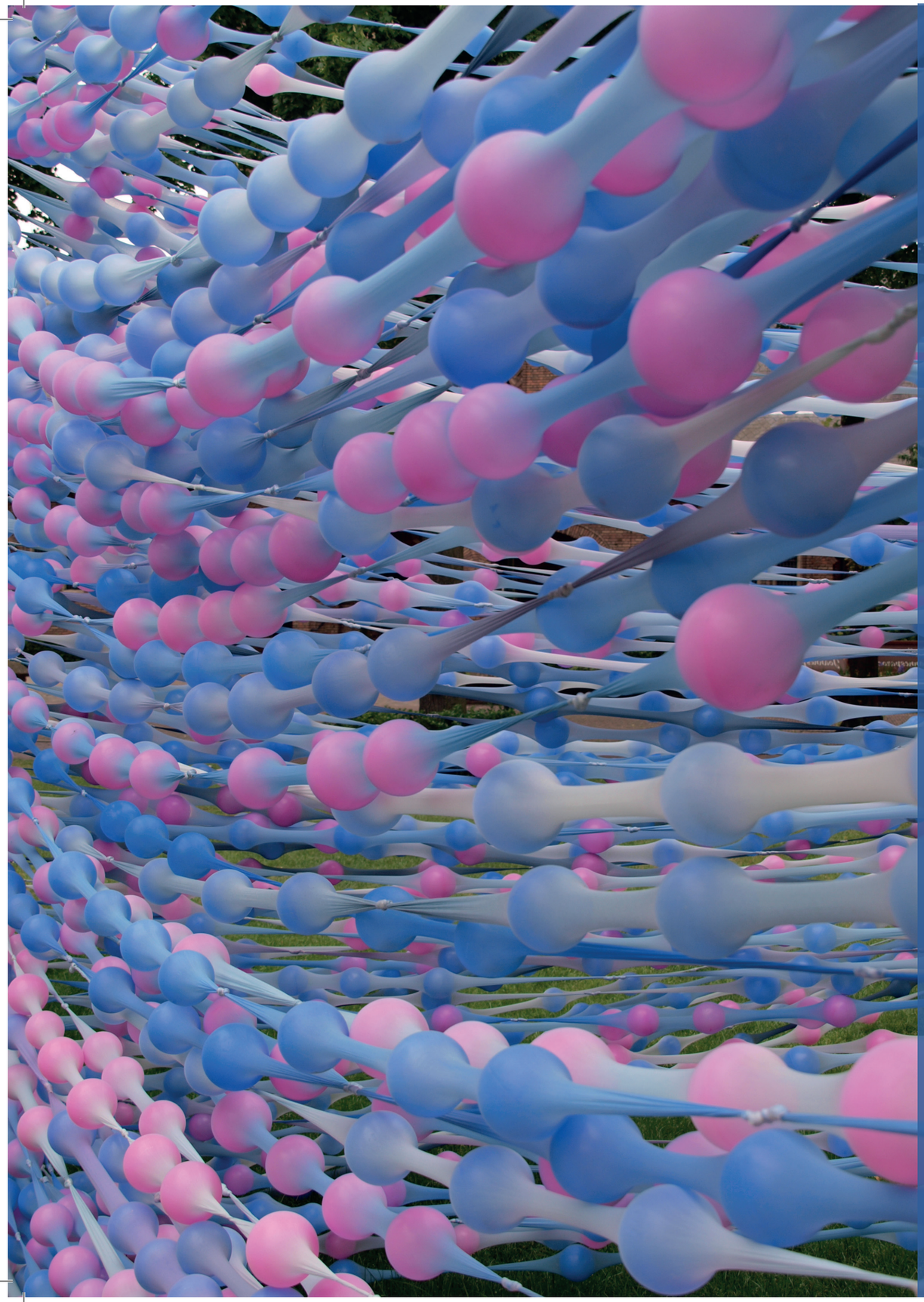
5. Without a lively network, limnologists dehydrate.

6. Supporting a PhD candidate to combine his or her research with a regular job, reflects good staff management.

Propositions belonging to the thesis, entitled

'Eco-engineering for clarity. Clearing blue-green ponds and lakes in an urbanized area'.

Guido W.A.M. Waajen
Wageningen, 3 May 2017



Eco-engineering for clarity Clearing blue-green ponds and lakes in an urbanized area

Guido Waajen

Eco-engineering for clarity

Clearing blue-green ponds and lakes
in an urbanized area

Guido Waajen