Biomanipulation as a Restoration Tool to Combat Eutrophication: Recent Advances and Future Challenges


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Abstract

Eutrophication resulting from high nutrient loading has been the paramount environmental problem for lakes world-wide for the past four decades. Efforts are being made in many parts of the world to reduce external nutrient loading via improved wastewater treatment or diversion of nutrient-rich inflows. However, even after a reduction of the external phosphorus loading, the effects obtained may be unsatisfactory. This may reflect an insufficient reduction in the external nutrient loading to effectively limit phytoplankton growth. However, the lack of success may also be due to chemical or biological within-lake inertia preventing or delaying improvements. To overcome the resilience and thereby reinforce recovery, a number of physico-chemical and biological restoration methods have been developed.

In this chapter, we describe recent developments of biological restoration methods related to eutrophication, their short-term and long-term effects, and discuss the possibility of using combined physico-chemical and biological methods to improve the long-term stability of restoration and to reduce restoration costs. As comprehensive reviews of the effect of fish manipulation in cold temperate lakes are numerous, for these waterbodies, we highlight recent results, including effects on biodiversity and metabolism, and present new approaches of biomanipulation. Our particular focus is, however, directed at biomanipulation in warm lakes and on combined treatments which are far less well described in the literature.

1. INTRODUCTION

As the demand for food and other ecosystem goods and services increases in line with the growing global human population and improved welfare, the pressure on all natural marine, terrestrial and freshwater alike,
has intensified dramatically and is expected to reach unprecedented levels by the end of this century (Millennium Ecosystem Assessment, 2005), with consequences over both ecological and evolutionary timescales (Hagen et al., 2012; Moya-Larano et al., 2012). Freshwaters, in particular, face a multitude of anthropogenic stressors (e.g. Hladyz et al., 2011; Layer et al., 2010, 2011), which have led to the development of numerous bio-assessment, biomonitoring and restoration schemes to mitigate or, even reverse, these human impacts, that have had varying degrees of success (Feld et al., 2011; Friberg et al., 2011). Although the effects of climate change have recently become an area of increasing research activity in aquatic ecology (Ledger et al., 2012; Meerhoff et al., 2012; Mintenback et al., 2012; Mollmann and Dieckmann, 2012; O’Gorman et al., 2012; Peck et al., 2012), eutrophication as a result of high nutrient loading has been the paramount environmental problem for lakes world-wide for the past four decades, and it will continue to be a major issue for the foreseeable future (Carpenter et al., 1999). This has led to turbid water, often toxic cyanobacteria blooms, biodiversity loss, and changes in biological community structure and ecosystem functioning (Jeppesen et al., 2000). During the past decades, measures have been taken in many parts of the world to reduce external nutrient loading via improved wastewater treatment or diversion of nutrient-rich inflows. Nutrient loading is, however, still increasing, particularly in developing countries and, thus, the most serious problems may not yet have been manifested on a global scale (Le et al., 2010).

Reducing the external nutrient loading is the key measure to restore eutrophied lakes and should have the highest priority (Cooke et al., 2005). A multi-faceted approach is often needed to attain sufficiently low external nutrient loading to improve the ecological status of lakes, particularly in more densely populated areas. Such approaches include phosphorus (P) stripping and occasionally nitrogen (N) removal at sewage works, sewage diversion, greater use of phosphate-free detergents, higher demands for animal fertiliser storage capacity and strict fertilisation plans and green cover of agricultural fields in winter. In addition, nutrient retention and N loss in lake catchments can be enhanced by re-establishing wetlands, stabilising river banks to reduce erosion, re-establishing a natural riparian zone and by allowing flooding of riverine areas (Jeppesen et al., 2011).

Notwithstanding significant reductions in nutrient loading, chemical or biological within-lake resilience can delay the establishment of clear water even when the external nutrient loading is sufficiently low to limit
phytoplankton growth. Chemical resilience is largely due to P release from the sediment pool accumulated during high loading (Søndergaard et al., 2003). Depending on the loading history and release mechanisms, this internal P loading typically persists for 10–15 years after the loading reduction until new equilibrium conditions are established (Jeppesen et al., 2005). Biological resilience can emerge through (i) resilience in the fish community and (ii) delayed return of submerged macrophytes. Zooplanktivorous and bottom-feeding fish species often dominate in nutrient-rich turbid lakes and may prevent a shift to a clear water state after nutrient loading reduction as they disturb the sediment when foraging and by preying on the zooplankton. Submerged macrophytes are important for creating and maintaining stable clear water conditions, particularly in temperate and cold areas (Jeppesen et al., 1998; Moss, 1990; Scheffer et al., 1993; Vanderstukken et al., 2011), but they typically disappear or decline significantly in abundance when nutrient loading is high. Their recovery following nutrient loading reduction may be delayed (Lauridsen et al., 2003) due to high turbidity (caused by fish and resuspension of loose sediment), insufficient seeds or turion banks in the sediment and grazing by waterfowl (Barko and Smart, 1986; Søndergaard et al., 1998).

Several physico-chemical and biological restoration methods related to eutrophication have been developed to overcome this ecological inertia and thereby reinforce recovery (for an extensive overview, see Cooke et al., 2005; Moss et al., 1996). In this chapter, we discuss the recent development of biological restoration methods (Table 1, Diagram 1), their effects and the prospects for using combined physico-chemical and biological methods to improve the long-term stability of restorations with a reduction in cost. As the effect of fish manipulation in cold temperate lakes has been comprehensively reviewed (Benndorf, 1995; Cooke et al., 2005; Hansson et al., 1998; Jeppesen and Sammalkorpi, 2002; Lathrop, 2007; Mehner et al., 2004; Meijer et al., 1999; Moss et al., 1996; Perrow et al., 1997; Sierp et al., 2009; Søndergaard et al., 2008), we focus on more recent results, including the effects on biodiversity and lake metabolism, and discuss new approaches to biomanipulation for cold temperate lakes. The main emphasis is given to biomanipulation in warm and warming lakes and new methods, including combined chemical and biological restoration. We also include a number of case studies, mostly from temperate Danish lakes, as a set of specific examples of more general phenomena. A brief description of these lakes and restoration methods used is given in Table 2 ordered as they appear in the text.
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<tr>
<th>Method</th>
<th>Processes involved</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Removal of zooplankton-eating fish to control phytoplankton</td>
<td>Zooplankton- and/or benthic invertebrate-eating fish are removed to increase the number of large zooplankton and their grazing on phytoplankton</td>
<td>McQueen (1998); Søndergaard et al. (2008)</td>
</tr>
<tr>
<td>Removal of benthic omnivorous fish to control phytoplankton</td>
<td>Benthic filter-feeding omnivorous fish (typically warm water herbivores–detritivores, for instance, common carp, tilapia, Prochylodus sp.), which naturally reach high carrying capacity, are removed to decrease sediment bioturbation and internal nutrient recycling</td>
<td>Starling et al. (2002); Lazzaro et al. (2003); Lazzaro and Starling (2005)</td>
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<tr>
<td>Stocking of predatory fish to control phytoplankton</td>
<td>Fish-eating fish are added to decrease the number of zooplankton–eating fish and improve zooplankton numbers and phytoplankton grazing</td>
<td>Benndorf (1995); Skov and Nilsson (2007)</td>
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<tr>
<td>Stocking of pelagic herbivorous fish to control phytoplankton</td>
<td>Pelagic filter-feeding herbivorous fish (e.g. bighead carp, silver carp) are stocked to remove phytoplankton, notably cyanobacteria</td>
<td>Arcifa et al. (1986); Starling (1993); Starling et al. (1998)</td>
</tr>
<tr>
<td>Macrophyte transplantation and protection</td>
<td>Submerged macrophytes are established and protected from plant-eating birds or fish to maintain high macrophyte coverage</td>
<td>Lauridsen et al. (2003); Qiu et al. (2001)</td>
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<tr>
<td>Stocking with herbivorous fish to control macrophytes</td>
<td>Plant–eating fish (grass carp) are added to reduce excessive growth of submerged macrophytes</td>
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<td>Introduction of mussels</td>
<td>Mussels are introduced to increase filtration of the water and create clearer water</td>
<td>Roy et al. (2010); Gulati et al. (2008)</td>
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Diagram 1 Conceptual model on the interactions between nutrients and the main components of a lake trophic web as affected by classic biomanipulation to enhance trophic cascade effects and other restoration measures. The nature of the interactions, whether positive or negative, bottom-up (via nutrients), top-down (consumption) or others (e.g. mechanical, chemical), is highlighted. The simultaneous application of both biomanipulation and a treatment to reduce internal nutrient loading may generate synergistic effects.

2. FISH MANIPULATION

In this chapter, we give an overview of fish manipulation methods and their effects on the short- and long-term ecology of temperate lakes where they have been used extensively. We then discuss their applicability to warm lakes and illustrate this with two case studies.

2.1. Fish manipulation in temperate lakes

This section focuses on fish removal, piscivorous fish stocking and effects of fish manipulations and discusses the use of repeated fish manipulation. We also discuss how the sediment record may help elucidating the effects of fish manipulation.
2.1.1 Removing zooplanktivorous and benthivorous fish

The most frequently used biomanipulation method (Table 1) is removal of plankti–benthivorous fish. This method has been extensively used over the past 20 years in northern temperate lakes in Europe (Søndergaard et al., 2007). It has been most successful in small shallow lakes but has had variable long-term effectiveness. Removal of a high proportion of the planktivorous

<table>
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<tr>
<th>Lake, country</th>
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<th>Mean depth (m)</th>
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<tr>
<td>Lake Christina, USA</td>
<td>46°05’N, 95°44’W</td>
<td>16</td>
<td>4.3</td>
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<td>Lake Vesijärvi, Finland</td>
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<td>Lake Væng, Denmark</td>
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<td>1.2</td>
<td>Repeated fish removal</td>
<td>Water chemistry and biota</td>
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<tr>
<td>Huizhou West Lake, China</td>
<td>23°06’N, 114°23’E</td>
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<td>1.0</td>
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</tr>
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<td>Lake Kollelev, Denmark</td>
<td>55°47’N, 12°27’E</td>
<td>0.05</td>
<td>1.8</td>
<td>Fish removal with and without sediment capping</td>
<td>Water chemistry and biota</td>
</tr>
<tr>
<td>Lake Fure, Denmark</td>
<td>55°48’N, 12°24’E</td>
<td>9.32</td>
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<td>Lake Engelsholm, Denmark</td>
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<tr>
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<td>Colonisation by zebra mussels</td>
<td>Water chemistry and biota</td>
</tr>
</tbody>
</table>

2.1.1 Removing zooplanktivorous and benthivorous fish

The most frequently used biomanipulation method (Table 1) is removal of plankti–benthivorous fish. This method has been extensively used over the past 20 years in northern temperate lakes in Europe (Søndergaard et al., 2007). It has been most successful in small shallow lakes but has had variable long-term effectiveness. Removal of a high proportion of the planktivorous
and benthivorous fish stock during a 1- to 2-year period has been recommended to avoid re-growth of the original stock and to stimulate the growth of young specimens of fishes that potentially become piscivores when they reach a sufficient size (Hansson et al., 1998; Jeppesen and Sammalkorpi, 2002). A simple, feasible strategy of fish removal is to catch non-moving fish with active gear and active moving fish with passive gear using information on the seasonal behaviour of fish, such as spawning or foraging migration and shoaling of the target species. In addition, ice fishing during winter, when fish may aggregate near the bottom (Ventelä et al., 2007), and removal of fish seeking winter refuge in adjacent streams (Brönmark et al., 2010) have also been used (Annadotter et al., 1999). A drastic method is to apply a piscicide (typically rotenone) treatment to part or the entire lake and its tributary streams (Eilers et al., 2011; Hanson and Butler, 1994). Rotenone affects all fish species, as well as invertebrates, and thus requires ethical considerations, and legal permissions may be difficult to obtain. Nonetheless, rotenone treatment has been used to regulate fish communities for sport fishing and to eliminate invasive species (McClay, 2000) as well as native but aggressive species that are assumed to pose a danger to fishermen (e.g. piranha, Serrasalmus spp., see Lazzaro et al., 2003).

An efficient reduction of the zooplanktivorous fish biomass generally achieves dramatic, short-term cascading effects in eutrophic lakes. This takes the form of a shift to dominance by large zooplankton, reduced phytoplankton biomass (and much less noxious cyanobacteria), and improved transparency (Hansson et al., 1998, Meijer et al., 1999), an increase in benthic feeding and herbivorous waterfowl (Allen et al., 2007) and a higher proportion of piscivorous fish (e.g. perch, Perca fluviatilis L., and pike, Esox Lucius L.), in part promoted by the development of submerged macrophytes. Strong cascading effects of fish removal have also been found in ponds, also leading to a decrease in cyanobacteria (Peretyatko et al., 2012).

In some temperate lakes, however, a reduction in cyanobacterial biomass and improved water clarity have been achieved after biomanipulation without a trophic cascade, which has been attributed to reduced P release from the sediment, not least due to less fish foraging at the bottom following the biomanipulation (Horppila et al., 1998). Biomanipulation only achieves the desired results when fish removal is sufficiently extensive (Fig. 1, Meijer et al., 1994; Jeppesen and Sammalkorpi, 2002; Søndergaard et al., 2008), and Jeppesen and Sammalkorpi (2002) estimated the annual amount of fish removal required to significantly improve water quality in shallow temperate lakes based on biomanipulation experiments as: Catch-required = 6.9 TP^{0.52} with catch in kg ha^{-1} and TP in µg L^{-1}. 
This target catch per year is higher than the estimates of fish biomass based on TP concentrations for deep stratified lakes (Hanson and Leggett, 1982), indicating that lower catches are likely needed to create a significant increase in water clarity in such lake types (Jeppesen and Sammalkorpi, 2002).

### 2.1.2 Stocking of piscivorous fish

An alternative or supplementary method to fish removal is stocking of potential piscivores (Benndorf, 1995; Berg et al., 1997; Drenner and Hambright, 1999; Ha et al., 2012), which may enhance water clarity through a trophic cascade (Carpenter and Kitchell, 1993) and/or a behavioural cascade (Romare and Hansson, 2003). By preying on zooplanktivorous and benthivorous fish, piscivores diminish prey fish abundance, resulting in lower fish-induced resuspension, lower predation on large zooplankton and lower translocation of nutrients from sediments to water via feeding and excretion. However, stocking of pelagic foraging piscivores, such as zander (*Sander lucioperca* L.), may also affect water clarity more indirectly through a behavioural cascade. Prey fish may be forced to take refuge in the littoral zone, which would not only release predation on pelagic zooplankton (Braband and Faafeng, 1994; Romare and Hansson, 2003).
but also decrease nutrient translocation from the littoral zone to open water (Braband et al., 1990; Kairesalo et al., 1999).

The basic tools are stocking with nursery or pond-raised fingerlings, often accompanied by catch and mesh size limits for fishing (Jeppesen and Sammalkorpi, 2002; Mehner et al., 2004). The most often used piscivores in northern temperate lakes are pike, zander, walleye (Stizostedion vitreum (Mitchill)), various trout species and largemouth bass (Micropterus salmoides (Lacepede)) (Drenner and Hambright, 1999).

The effects of introducing piscivorous fish have varied considerably but have often been poor compared with the effects of fish removal (Drenner and Hambright, 1999). Pike has been used in numerous biomanipulation experiments, often with high stocking densities (1000–4000 fish ha\(^{-1}\)) of fry in spring, aiming at almost instantaneous removal of 0\(^{+}\) planktivores (Berget al., 1997; Prejs et al., 1994). The seasonal timing of the 0\(^{+}\) pike stocking might influence the efficiency of pike controlling the 0\(^{+}\) cyprinids (Skov, 2002), and the strongest effect is expected if the stocking coincides with the appearance of the newly hatched larvae of the dominant prey species (Prejs et al., 1994; Skov, 2002). While strong cascading effects of stocking large numbers of pike have been observed in Polish Lake Wirbel (Prejs et al., 1994) and Danish Lake Lyng (Berg et al., 1997; Søndergaard et al., 1997), the effects have been weak in many other lakes (Skov, 2002). Pike stocking may be most successful in systems where they have gone extinct and other strong pelagic predator species, such as perch or pikeperch, are present, as was the case in Lake Lyng (Berg et al., 1997), thereby creating a high predation risk on prey fish in both the pelagic and the littoral zone. Pike stocking has also been used to control exotic bullfrogs (Lithobates catesbeianus (Shaw)) in Australian ponds, which also host mostly small and planktivorous fish (e.g. pumpkinseed, gibbosus L., and topmouth gudgeon, Pseudorasbora parva Temminck and Schlegel) (Louette, 2012).

Zander in Europe and walleye in the USA have also been popular species to stock for the control of planktivores (Benndorf, 1995; Lathrop et al., 2002). As an example, Potthoff et al. (2008) stocked walleye in a series of shallow lakes in Minnesota (USA) to control fathead minnows (Pimephales promelas Rafinesque) and shift the lakes to a clear water state. Six lakes were stocked with walleye fry (12,000 individuals or 0.05 kg ha\(^{-1}\)), six with walleye age-1 and older (21–130 individuals and 6–8 kg ha\(^{-1}\)), while six lakes served as controls. Stocking of fry decreased fathead minnow and increased cladoceran abundance. Phytoplankton chlorophyll \(a\) (Chl \(a\)) also decreased at the fry-treated sites. By contrast, stocking of age-1 and older walleye induced few changes at the stocking densities tested. The success of
walleye fry appeared to be partly attributable to their rapid diet shift to piscivory and their ability to consume and suppress all life stages of planktivores (fry, juveniles and adults). For zander, stocking with fingerlings (up to 200 fish ha\(^{-1}\)) in summer has been recommended. However, later age-0 juveniles are a better alternative (up to 20 fish ha\(^{-1}\)), but since they are stocked in late autumn, a minimum fish length of 15 cm is recommended to prevent high overwinter mortality (Mehner et al., 2002). Most responses to walleye and zander fry additions have been short-lived, however, which may be in part attributed to gape limitation of these two species, leaving bigger planktivores or benthivores (e.g. bream, Abramis brama (L.), common carp, Cyprinus carpio L.) with size refuges (Lammens, 1999). Repeated stocking has therefore been used and recommended (Benndorf, 1995). Moreover, repeated stocking of piscivores in combination with size and bag limits for recreational fisheries had a strong positive effect on zooplankton body size and water clarity in a number of reservoirs in Germany (Scharf, 2007). Other piscivores used with at least short-term effects are brown trout (Salmo trutta L.) (e.g. Scharf, 2007) and rainbow trout (Oncorhynchus mykiss Walbaum) (Ha et al., 2012); however, these species may be non-native to the location they are introduced to, entailing undesirable conservation and biodiversity consequences.

An alternative or a supplement to stocking is to adjust the size structure of the population of piscivores. For example, Sharma and Borgström (2008) removed large pike in Lake Årungen, Norway, aiming to achieve a higher contribution of small pike and large perch in the fish community. There was a consequent decline in the abundance of small perch and roach (Rutilus rutilus L.), as well as a habitat shift of small specimens towards the littoral, releasing the predation pressure on the pelagic large-bodied zooplankton. However, long-term consequences remain unclear.

### 2.1.3 Longevity of the fish manipulation effects

There are only a few long-term studies (>10 years) of the effects of fish manipulation and the results are ambiguous. However, in most studies where strong short-term effects were seen, a gradual return to the turbid state and higher abundance of zooplanktivorous fish have occurred after 5–10 years. In particular, the trophic cascade effects in the pelagic seem to weaken in the long term due to return of zooplanktivorous fish; in contrast, the impact on benthic fish apparently lasts longer and may even be permanent. The most comprehensive comparative study of long-term responses so far is from 27 Danish Lakes (Fig. 2, Søndergaard et al., 2008).
this study, abundance of benthivores, such as bream, was demonstrated to remain lower than before the restoration, creating significantly reduced levels of suspended matter and improved water clarity in the long term. However, the grazing effect, as indicated by the zooplankton:phytoplankton biomass ratio, returned to pre-manipulation levels and cyanobacteria returned and were dominant, indicating almost complete nullification of the strong trophic cascade in the pelagic observed 10–15 years after the fish manipulation, likely reflecting a return of planktivorous roach. A similar response with long-term effects on bream and mainly short-term effects on roach has been observed in biomanipulated Cockshoot Broad, UK (Hoare et al., 2008).

Nonetheless, some examples exist of lakes with persistent effects, especially on water quality (see, for instance, examples below). A necessary prerequisite for obtaining a clear water state in the long term is an adequate external nutrient loading reduction, which corresponds to annual mean TP concentrations $<0.05$ mg P l$^{-1}$ for shallow temperate lakes (Jeppesen et al., 2000) and $<0.02$ mg P l$^{-1}$ for deep temperate lakes (Sas, 1989). However, internal loading may remain high for decades after loading reduction (Jeppesen et al., 1991; Søndergaard et al., 2003). A drawback of the fish manipulation is that lakes retain more P in the clear water state after manipulation, leaving more sediment P available for internal loading if the system returns to the turbid state than if fish manipulation had not been conducted (Søndergaard et al., 2007).

2.1.4 Repeated measures—fish manipulation in Lake Væng, Denmark

Repeated fish removal may be a way to maintain the clear-water state if lakes are deemed likely to shift back to the turbid state. We anticipate that the repeated manipulation effort does not have to be as comprehensive as the first for several reasons: (a) as shown for temperate Danish lakes, some of the large benthivorous fish show slow recovery (Søndergaard et al., 2008), probably due to food limitation and in some lakes also extensive growth of macrophytes; (b) small potential piscivores, such as perch, are

Figure 2 The effects of removal of more than 200 kg fish ha$^{-1}$ relative to the levels less than 3 years before intervention on Secchi depth (A), suspended solids (B), chlorophyll $a$ (C) in 27 Danish shallow lakes (summer means). Also shown are biomass of total zooplankton (D), *Daphnia* (F) and other cladocerans (G) the zooplankton:chlorophyll $a$ biomass ratio (H), phytoplankton biomass (E) and the relative biomass share of cyanobacteria (I) (number of lakes = 10). Light boxes show significant differences from the pre-removal situation. The boxes show 10% and 90% fractiles (outer lines) and 25% and 75% fractiles (boxes) (modified from Søndergaard et al., 2008).
typically more abundant after the first biomanipulation and tend to shift to piscivory more quickly; (c) presence of submerged macrophytes, seeds or turions may facilitate faster development of plants compared to the first manipulation and (d) the mobile pool of P in the sediment has likely declined (buried deeper or released via the outlet).

The shallow Lake Væng in Denmark (Table 2) is an example of repeated biomanipulation. The lake catchment area is 9 km² and consists of agricultural and forested land and a gravel pit. The water residence time is 15–25 days. Following sewage diversion in 1981 the lake remained in a turbid state (Søndergaard et al., 1990). In an attempt to restore the lake, approximately four tonnes of benthivorous and zooplanktivorous fish (mainly bream and roach) were removed during the years 1986–1988 by use of gill, fyke and pound nets as well as electrofishing.

Following fish removal, phytoplankton Chl a and water turbidity decreased substantially (Fig. 3). Two years after the biomanipulation Secchi depth improved, submerged macrophytes recolonised, first *Potamogeton crispus* L. and then *Elodea canadensis* Rich., with the latter completely covering the lake within 1–2 years (Lauridsen et al., 1994). However, from 1998 macrophytes disappeared or occurred only in low densities, coinciding with an increase in the abundance of roach (Fig. 4), and small perch became dominant. Immediately after the fish removal, seasonal TP in the lake changed considerably (Fig. 3). When submerged macrophytes were abundant, Chl a and TP were also low. Later, when macrophytes disappeared and the water turned turbid, TP reached high summer concentrations again.

In an attempt to shift the lake back to the clear state, 2.7 tonnes (68% of the first biomanipulation effort) of fish consisting of bream and roach were removed during 2007–2009. Catch per unit effort in gillnets (CPUE) decreased from ca. 5–7 kg net⁻¹ before the fish removal to 2–3 kg net⁻¹ afterwards (Fig. 4). Following the second biomanipulation, mean summer Chl a was reduced, Secchi depth increased, TP and TN both declined markedly and coverage of submerged macrophytes increased substantially (Fig. 3). Macrophyte recovery thus followed the pattern observed after the first fish removal in the 1980s, with fast re-growth after improved water clarity. The macrophyte community became completely dominated by *E. canadensis* a few years after the first manipulation. Biomasses of roach and bream have been low since 2009 compared to the years before fish manipulation (Fig. 4).

Although the longevity of the new restoration is obviously unknown, the effort needed to bring the lake back to the clear state was clearly much
less extensive than that for the first biomanipulation, which is in line with our expectations.

### 2.1.5 Effects of fish manipulation judged from paleoecological studies

The limited number of suitable case studies of biomanipulation makes it difficult to judge the long-term effects. However, considerable information is stored in the sediment, and this ecological archive could be extremely valuable for understanding past ecological changes. Figure 3 presents a comparison of chlorophyll $a$ and macrophyte coverage (A), various phosphorus (B) and nitrogen (C) specimens in Lake Væng, Denmark. Fish removal was undertaken during 1986–1988 and 2007–2009, respectively.

**Figure 3** Changes in chlorophyll $a$ and macrophyte coverage (A), various phosphorus (B) and nitrogen (C) specimens in Lake Væng, Denmark. Fish removal was undertaken during 1986–1988 and 2007–2009, respectively.

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**Biomanipulation as a Restoration Tool to Combat Eutrophication**
valuable as most biomanipulation experiments were conducted in the 1980s and 1990s, that is, a sufficiently long time ago to enable detection of changes in the sediment provided that mixing is not too severe.

Among the few existing studies, the shallow Lake Christina in the USA (Table 2) (Hanson and Butler, 1994) has been biomanipulated three times since 1987, each time with only short-term (5–10 years), but dramatic effects (shifts to clear water with abundant macrophytes). The long-term changes in the lake were evident from changes in diatoms and cladocerans in the sediment record (Hobbs et al., 2012). However, the periodic shifts between clear and turbid states generated by biomanipulation are not as clearly reflected by the sediments as by the contemporary data, which may reflect sediment disturbance due to the large size and shallowness of the lake.

One of the best proxy indicators of changes in fish abundance in the sediment record is the abundance and size of *Daphnia* taxa (Jeppesen et al., 2001, 2002), in particular, relative to the abundance of smaller pelagic taxa such as *Bosmina* (Davidson et al., 2010), due to size selective predation by fish (Brooks and Dodson, 1965). An example of biomanipulation and its effects accurately recorded in lake sediment comes from Nykänen et al. (2010) who studied changes in subfossil cladocerans in annually layered sediment in the Enonselkä basin of Lake Vesijärvi, Finland (Table 2). Measures to restore the Enonselkä basin included reduction of the external nutrient loading and mass removal of planktivorous and benthivorous fish during 1993–1996 (Kairesalo et al., 1999). Water clarity increased and the lake changed from

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**Figure 4** Fish weight per unit effort (WPUE) in Lake Væng, Denmark. Biomanipulation was undertaken during 1986–1988 and 2007–2009, respectively (highlighted).
a eutrophic to a mesotrophic state, but some signs of increased turbidity were observed after 5–10 years of recovery. After the removal of fish, the mean size of *Daphnia* ephippia and *Eubosmina crassicornis* ephippia and carapaces, and the ephippia ratio of *Daphnia*/(*Daphnia* + *E. crassicornis*), increased significantly, all indicating reduced fish predation (Nyka¨nen et al., 2010) (Fig. 5). Expanding littoral vegetation along with improved water clarity owing to

### Figure 5  Sediment record of subfossil cladocerans in Lake Väsajärvi, Finland, before and after fish removal conducted to restore the lake (modified from Nykänen, 2010). (A, left) The ratio *Daphnia*/(*Daphnia* + *E. crassicornis*) (based on ephippia), the proportion of the cornuta form to the straight antennule form of *Bosmina longirostris*, and the proportion of rotifer resting eggs to cladoceran remains as percentages, all proxies for fish predation. (A, right) Mean size of *Daphnia* ephippia, *E. crassicornis* ephippia and carapaces and *B. longirostris* carapaces (ephippia E, carapaces C). *Daphnia* ephippia in varve 2001 were damaged and therefore not measured. (B) Planktonic/littoral (P/L) ratio, total concentration of planktonic and littoral remains per unit organic matter (OM), concentrations of mud-, plant- and rock-/sand-associated littoral species, as well as Shannon’s diversity index (H) for the entire subfossil community.
higher contribution of littoral species was clearly detected. Moreover, the species diversity index for the entire subfossil community increased.

Effects of fish stocking and removal have also been demonstrated in several studies by analysing remains of cladocerans, chironomids and/or pigments (Buchaca et al., 2011; Leavitt et al., 1989; Sayer et al., 2012; Verschuren and Marnell, 1997), in several cases showing strong effects of the trophic cascade and the resulting change in the pelagic zooplankton community and increased or decreased algal crop. The accuracy with which the sediment can record these events is dependent on a number of factors, including lake size, depth and fetch, which will dictate the degree of temporal smoothing of the sediment record, the size of the disturbance and perhaps the nutrient status of the sites. However, a clear-cut response to a change in the fish predation pressure in terms of abundance and size of *Daphnia* ephippia, not to mention the rapid evolutionary response, was recorded from the sediment record of a small lake, Oud Heverlee Pond, Belgium (Cousyn et al., 2001). Furthermore, a number of more naturally occurring fish kill events and their ecological consequences have been tracked via the sediment record (Amsinck et al., 2005; Carl Sayer et al., unpublished data). Carl Sayer et al. (unpublished data) found a sharp shift in the sediment record reflecting a step change in ecology. Thus, the sediment record holds great potential to assess the longevity of success of biomanipulation and whether the shift back to more turbid conditions is rapid or gradual, and it may perhaps be used to detect early warning signals as well. In agreement with contemporary observations (e.g. Søndergaard et al., 2007), the limited number of studies using the lake sediment record points to short-term, decadal scales effects of extensive changes in fish biomass on lake ecology.

Some further perspectives on the ecological condition of biomanipulated systems without contemporary data may be provided by the sediment record. Plant macrofossils can record the shifts in the dominant components of the macrophyte flora and provide some indication of the abundance of certain taxa, such as charophytes (Davidson et al., 2005). Other indicators may reveal trophic shifts through change in the relative abundance of benthic and pelagic taxa (Davidson et al., 2010; Vadeboncoeur et al., 2003).

### 2.2. Fish manipulation in warm lakes

In warm locations (Mediterranean, subtropical and tropical lakes), where omnivory among fishes is common (González-Bergonzoni et al., 2012), stocking of pelagic filter-feeding fish has, at times, been used as a
biomanipulation tool. Typically, various carp and tilapia species are introduced (Arcifa et al., 1986; Jones and Poplawski, 1998; Starling, 1993) to reduce noxious filamentous cyanobacteria, rather than total algae biomass, through direct feeding. This practice seems inefficient when fish reach a high carrying capacity. In such circumstances, nutrient recycling by fish may outperform their filtration of phytoplankton, resulting in phytoplankton enhancement with a frequent shift from edible to inedible forms (Attayde and Hansson, 2001; Figueredo and Giani, 2005). Examples are limited, but several characteristics suggest that biomanipulation is a more complex task in warm lakes than in cold and temperate areas.

In this section, we briefly describe how fish assemblages in warm lakes differ from those in northern temperate lakes and discuss the challenge that this presents to biomanipulation, followed by two case studies from warm lakes in China and Brazil.

2.2.1 Fish assemblages in warm lakes

It is debatable whether the fish manipulation approach used in northern temperate lakes can be used with success in warm lakes (Jeppesen et al., 2005; Lazzaro, 1997) due to several key differences in fish community structure between warm (i.e. tropical, subtropical and Mediterranean) and cold or temperate fish assemblages, as partly highlighted in recent reviews (i.e. Jeppesen et al., 2010; Meerhoff et al., 2012). The occurrence of similar patterns in the structure of fish assemblages in different geographic locations suggests that they are generally more related to climate than to biogeographic history (Meerhoff et al., 2012).

Firstly, fish species richness is often higher in warm lakes, with the exception of lakes with high hydrological stress such as some tropical closed-basin African lakes (Léveque, 1997). A global meta-analysis has shown increasing fish species richness, in particular, of omnivores, in shallow lakes towards lower latitudes (González-Bergonzoni et al., 2012). A consistent pattern emerges at latitudinal continental scales in shallow and deep lakes in Europe (Brucet et al., submitted for publication) and North America (Griffiths, 1997; Mandrak, 1995) as well as in cross-comparison studies (Meerhoff et al., 2007a; Teixeira-de Mello et al., 2009). The same pattern of higher species richness in warmer locations was found in altitudinal studies worldwide (Amarasinghe and Welcomme, 2002). Recent investigations suggest that littoral and benthic production promote fish diversity in lakes (Vander Zanden et al., 2011), which might contribute to explain the higher diversity in the typically plant-associated
fish assemblages in warm lakes (Conrow et al., 1990; Delariva et al., 1994; Meerhoff et al., 2003, 2007a; Teixeira-de Mello et al., 2009).

Secondly, the fish assemblages in many warm lakes, independent of trophic state, are often more functionally diverse (Moss, 2010) and dominated by omnivorous species (Lazzaro, 1997; Teixeira-de Mello et al., 2009). In addition, top-down control by piscivorous fish is apparently weaker in warm lakes than in temperate lakes, as suggested by a study on a series of subtropical lakes (Gelós et al., 2010). However, in shallow lakes of the Pantanal floodplains, it seems that drastic changes in the relative abundance within fish species assemblage (subtropical area) may promote turbid or clear water states (Mormul et al., 2012).

Thirdly, biomass and density of fish assemblages seem to increase with increasing ambient temperature, as found in a series of shallow lakes of varying nutrient concentrations along a climate gradient in Europe (Gyllström et al., 2005), and supported by cross-comparison studies conducted in both freshwater (Meerhoff et al., 2007a,b; Teixeira-de Mello et al., 2009) and brackish shallow lakes (Brucet et al., 2010).

Fourthly, in many warm lakes, fish reproduction occurs throughout the year (Fernando, 1994; Paugy and Lévêque, 1999), and an increase in the number of reproductive events with decreasing latitude (i.e. increasing temperature) has been reported for some species (Goyenola et al., 2011). In temperate systems, in contrast, most freshwater fish species have seasonal reproduction, with peaks in spring or summer (Wootton, 1984). Small, short-lived species with generally opportunistic strategies are more abundant at low latitudes, whereas large, longer-lived species predominate at high latitudes, as found in a very exhaustive research on North American fishes (Mims et al., 2010).

Finally, fish assemblages in warm lakes are typically characterised by smaller individuals than in temperate and cold lakes (reviewed in Meerhoff et al., 2012), as detected in several studies in freshwaters, both along latitudinal gradients (North America, Griffiths, 1997; South America, Lacerot, 2010; Europe, Brucet et al., submitted) and in cross-comparison studies (Brucet et al., 2010; Teixeira-de Mello et al., 2009).

As small fishes are more zooplanktivorous and have a much higher energy demand per unit of biomass than large fish (Kalff, 2002), the dominance of small fishes in such high abundances leads to a higher predation pressure on large zooplankton than in similar colder lakes, where the effect of juvenile fish is typically strongest in mid-late summer (Jeppesen et al., 1998). Moreover, the diurnal refuge potential of aquatic plants for large zooplankton against visual-feeder fishes, which is often seen in temperate shallow lakes (Burks et al., 2002; Timms and Moss, 1984), is almost absent in warm lakes (Iglesias et al., 2007; Meerhoff et al., 2006, 2007b; Tavşanoğlu et al., 2012).
Supporting the hypothesis of higher fish predation as a key factor in shaping the zooplankton size structure in warm lakes (Fernando, 2002; Jeppesen et al., 2007; Meerhoff et al., 2007a,b), occurrence of large Daphnia spp. in subtropical lakes, has been associated with the absence of fish (Iglesias et al., 2011). The control of phytoplankton by large zooplankton, typically observed in temperate lakes, is rarely found in warm lakes, as indicated by the low zooplankton:phytoplankton biomass ratio found in several latitudinal gradient studies (reviewed in Meerhoff et al., 2012), and as explained by theoretical models analysing fish reproduction in warm systems (Van Leeuwen et al., 2007). This predation pressure on zooplankton may represent a further limitation of the usefulness of the classic biomanipulation in tropical and subtropical lakes.

2.2.2 Fish manipulation examples in warm lakes: Huizhou Westlake, China

Huizhou West Lake is a shallow lake in tropical China (Table 2, Fig. 6). The lake has a total surface area of about 1.6 km². The lake is divided into several basins connected through waterways. Water temperature in the lake ranges from 12 to 35 °C over the season and the hydraulic retention time is ca.
3 months. The lake was dominated by submerged macrophytes before the 1960s. Following the initiation of fish aquaculture in the lake during the 1970s and increased waste water input, the lake became eutrophic and submerged macrophytes have been absent since the 1980s (Li, 2009).

In spite of restoration efforts, including effluent diversion and sediment removal (Li, 2009), the lake has remained eutrophic and turbid (Fig. 7). Furthermore, fish stockings have occasionally been conducted, the fish community being dominated by omnivorous and benthivorous species including Nile tilapia (*Oreochromis niloticus* L.), common carp and Cruscian carp (*Carassius auratus* L.).

In order to improve water quality, a large-scale biomanipulation experiment was conducted in a 1-ha basin (restoration area, Fig. 6) of the lake at the end of 2004. The biomanipulation included fish removal, followed by submerged macrophyte transplantation and stocking of piscivorous fish. After isolating the area from the rest of the lake, the water level was lowered to ca. 60 cm and ca. 200 kg ha$^{-1}$ of fish, including tilapia, common carp, Crucian carp, mud carp (*Cirrhina molitorella* Cuvier et Valenciennes), silver

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**Figure 7** Comparison of total nitrogen (A), chlorophyll $a$ (B), total phosphorus (C) and total suspended solids (D) in the restored and unrestored area in Huizhou West Lake, China. The highlighted period in grey shows pre-restoration levels.
carp (*Hypophthalmichthys molitrix* (Valenciennes)) and bighead carp (*Aristichthys nobilis* (Richardson)) were removed over a 2-month period. Submerged macrophytes, including *Hydrilla verticillata* Royle, *Vallisneria natans* L. and *Myriophyllum spicatum* L., were planted and coverage reached 60% in May–June, 2005, and > 80% in summer 2006. Some piscivorous fish were stocked after macrophyte transplantation, including snakehead (*Channa argus* Cantor) and mandarin fish (*Siniperca chuatsi* Basilewsky).

Annual mean TN was reduced to less than 1.0 mg L\(^{-1}\) and TP to less than 0.050 mg L\(^{-1}\). Fish removal likely reduced sediment resuspension, while submerged macrophytes protected the sediment from resuspension, and macrophytes and benthic algae may have reduced the nutrient release from the sediments (*Zhang et al.*, 2012).

Annual mean concentration of Chl \(a\) also dropped to less than 10 \(\mu g\) L\(^{-1}\) in the restored area, while total suspended solids were \(\ll 5\) mg L\(^{-1}\), respectively, reflecting a decrease in both inorganic suspended solids and phytoplankton in the restored part. Densities of planktonic crustaceans, both cladocerans and copepods, were extremely low in both the restored and unrestored areas, suggesting that zooplankton grazing played a minor role in controlling phytoplankton in the restored area. So, the biomanipulation in this tropical shallow lake did not increase the number of zooplankton grazers as seen in north temperate lakes (*Søndergaard et al.*, 2008), likely reflecting high predation by the abundant small fish including fish fry. Many fishes can spawn several times a year in this climate region, as is the case for species such as tilapia and Crucian carp which are abundant in the lake (Z. Liu, unpublished data).

The restoration study of Huizhou West Lake demonstrated that fish removal and transplantation of submerged macrophytes can restore the clear water state in a tropical eutrophic lake via enhanced bottom-up control and reduced sediment resuspension, even without increasing zooplankton grazing on phytoplankton. The experimental design does not allow us to disentangle the relative strength of these two mechanisms nor can we evaluate the long-term impacts of the restoration methods yet.

### 2.2.3 Fish manipulation examples in warm lakes: Lake Paranoá, Brazil

Lake Paranoá is a tropical urban reservoir in Brasilia (Table 2, Fig. 8). During 1970–1990, the release of partially treated domestic wastewater led to deterioration of the lake water quality, particularly in two basins (Bananal and Riacho Fundo basins, Secchi depth < 0.5 m, and Chl \(a\) > 40 \(\mu g\) L\(^{-1}\), Fig. 9).
Eutrophication intensified until early 1990, with recurrent cyanobacteria blooms of the colonial *Microcystis aeruginosa*, and a permanent bloom of the filamentous cyanobacteria *Cylindrospermopsis raciborskii*, associated with high TP (0.03–0.08 mg L\(^{-1}\)) and TN (1.8–2.5 mg L\(^{-1}\)) concentrations (Branco and Senna, 1996; Mattos et al., 1992). The Brasilia Water and Sewage Corporation (CAESB) treated the surface blooms with periodical applications of copper sulphate.

Omnivorous Nile tilapia, Congo tilapia (*Tilapia rendalli* (Boulenger)) and common carp dominated the fish assemblage (average >500–790 kg ha\(^{-1}\) in the eutrophic areas) during the 1970–1990s (Starling, 1998). The highest fish biomasses occurred in both basins (Lazzaro et al., 1998; Lebourges-Dhaussy et al., 1999a,b) despite a 300-tonnes fish removal following two major episodes of fish kill in 1997. These cyanobacteria bloom-induced fish kills were regarded as an “accidental biomanipulation”, since water quality during the period following the massive removal of dead fish improved noticeably (Starling et al., 2002).

A 10-year research programme was initiated in 1987 including fish stock assessments and laboratory and *in situ* mesocosm experiments to quantify the role of different key species of filter-feeding omnivorous fish (Nile tilapia,
common carp and silver carp) with the aim to develop effective measures to restore the lake. The focus was on fish–cyanobacteria interactions and whether a large-scale fish manipulation could contribute to significantly improve the water quality (Lazzaro and Starling, 2005). A major result was that omnivorous Nile tilapia, owing to its benthivorous habits, was a major P recycler contributing to N limitation, which is favourable to cyanobacteria growth. In contrast, omnivorous silver carp, as an obligate filter feeder, effectively suppressed cyanobacterial biomass without detrimental reduction of the zooplankton grazing pressure because of the typically small size of the zooplankton herbivores. For more details, see Starling and Lazzaro (1997) and Lazzaro and Starling (2005).

The restoration of Lake Paranoá involved several steps. Starting in 1993, the external nutrient loading was drastically reduced (90% of N and P removal from raw sewage, resulting in a 70% reduction in P external loading) but did not lead to a drastic improvement in water quality. Thus, at the end of the 1998 dry season, the reservoir was flushed by opening the dam gates. As a result, the Chl \(a\) concentration dropped from 90 \(\mu g \text{L}^{-1}\) in 1992 to 9 \(\mu g \text{L}^{-1}\) in 1999, Secchi depth transparency reached 2.5 m and green algal species progressively replaced \(C. \text{raciborskii}\) (Burnett et al., 2001) (Fig. 9). From 1999 and onwards, less dramatic yearly flushing has been performed.

Since 1998, the biomanipulation has involved removal of omnivorous tilapias and common carp from the Bananal and Riacho Fundo branches by professional cast-net fishery. Yet, the average yield (8 tonnes per month in 1999–2001) has been declining since then. The contribution to lake internal P by the removed fish can be estimated in terms of P release rates to reach the same magnitude as the external P load to this area.

Analysing temporal series, Angelini et al. (2008) assessed that the activation of the water treatment plants (1993) had no effect on the Chl \(a\) and TP concentrations, whereas both significantly declined after proper operation of these plants (1996), and water transparency increased notably after the 1998 flushing event and following biomanipulation.

Currently, the lake shows oligo-mesotrophic characteristics (Batista, 2011), with higher phytoplankton richness, lower phytoplankton primary productivity, reduced fisheries yield and increased fish biodiversity in the captures, including a strong reduction of tilapia and re-occurrence of the native cichlid \(Geophagus\) sp. The best outcome of this restoration programme is that Lake Paranoá is currently being certified for its appropriate water quality to become a major drinking water supply source for the Brasilia Federal District.
The restoration has thus been successful, although it remains difficult to disentangle the effect of the different measures (flushing and biomanipulation).

### 3. MACROPHYTE ESTABLISHMENT AND PROTECTION

Submerged macrophytes play a fundamental structuring role in shallow lakes (Jeppesen et al., 1998; Moss et al., 1996), and their re-establishment and protection are thus of key importance for long-term recovery. Therefore, exclosures to protect macrophytes against herbivory have been used as an alternative or supplementary restoration tool to fish manipulation (Table 3). Such exclosures allow the macrophytes to grow in a grazer-free environment from where they may expand colonisation across the entire lake.

There are many examples where submerged macrophytes do not readily return to former levels (with respect to both abundance and species composition) despite reduced external nutrient loading and favourable light conditions (Bakker et al., 2012; Hilt et al., 2006; Sand-Jensen et al., 2008). This may be due to lack of sufficient propagules and low spreading potential or limited connection with other aquatic systems acting as source (Strand and Weisner, 2001). However, herbivory by fish (Prejs, 1984; Körner and Dugdale, 2003; Hutorowicz and Dziedzic, 2008), crayfish (Rodriguez et al., 2004) and waterfowl (Lauridsen et al., 1993; Marklund et al., 2002; Søndergaard et al., 1996) may also hamper their recovery (Lodge et al., 1998), although it may also promote higher macrophyte diversity under certain conditions (Hidding et al., 2010; Sandsten and Klaassen, 2008).

Here, we first describe macrophyte manipulation in temperate lakes where many experiments have been performed; then, we highlight the few available case studies from warm lakes, followed by a brief discussion about the potential and need for measures in warm lakes. Finally, we discuss the future perspectives of macrophyte manipulation.

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**Figure 9** Changes in yearly means of key environmental variables as an average for the lake (left panel) and the most eutrophic Riacho Fundo Branch (right panel) from 1992 to 2011. The three key events that may be responsible for the oligotrophication process of Lake Paranoá are shown: (A) activation of two water-treatment plants (Jan 1993), (B) proper operation of these plants (Jan 1996) and (C) major flushing event (sudden release of water in Nov. 1998). To reduce cyanobacterial blooms, the algaecide copper sulphate was applied in various amounts until 2006 (lower right panel).
<table>
<thead>
<tr>
<th>Natural or man-made plant establishment</th>
<th>Methods and effects</th>
<th>Temperate/ warm, T/W</th>
<th>Lake name and country</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>- Fish manipulation</td>
<td>T</td>
<td>L. Zwemlust, The Netherlands</td>
<td>van Donk and Otte (1996)</td>
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<td></td>
<td><em>E. canadensis</em></td>
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<td>- Fish manipulation</td>
<td>T</td>
<td>L. Væng, Denmark</td>
<td>Lauridsen <em>et al.</em> (1993)</td>
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<td><em>E. canadensis</em></td>
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<tr>
<td></td>
<td>- Fish kill</td>
<td>T</td>
<td>L. Arreskov, Denmark</td>
<td>Sandby and Hansen (2007)</td>
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<tr>
<td></td>
<td>Establishment of a diverse submersed flora</td>
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<tr>
<td></td>
<td>- Fish manipulation, establishment of submerged macrophytes</td>
<td>T</td>
<td>L. Ringsjön, Sweden</td>
<td>Strand (1999)</td>
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<td></td>
<td>- Protection of submerged macrophytes</td>
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<tr>
<td>No significant effect due to abundant natural vegetation</td>
<td>T L. Björkesåkrašjön, Sweden</td>
<td></td>
<td>Marklund <em>et al.</em> (2002)</td>
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Table 3 Overview of natural and man-made macrophyte re-establishment following restoration attempts in shallow lakes—cont’d

<table>
<thead>
<tr>
<th>Natural or man-made plant establishment</th>
<th>Methods and effects</th>
<th>Temperate/warm, T/W</th>
<th>Lake name and country</th>
<th>References</th>
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<tr>
<td>Man-made</td>
<td>- Protection of submerged macrophytes (birds or fish)</td>
<td>T</td>
<td></td>
<td>Hanlon et al. (2000); Lauridsen et al. (1996); Shireman and Maceina (1981)</td>
</tr>
<tr>
<td></td>
<td>- Transplantation and protection of submerged macrophytes from bird and fish grazing</td>
<td>T</td>
<td>L. Engelholm, Denmark</td>
<td>Lauridsen et al. (2003)</td>
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<tr>
<td></td>
<td>- Introduction of shoots, seeds and seed pods of <em>Vallisneria americana</em> and protection against grazing</td>
<td>T</td>
<td>Freshwater tidal area, USA</td>
<td>Moore et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>- Removal of herbivores, transplantation and protection of plants (large scale)</td>
<td>W</td>
<td>Wuli Bay, China</td>
<td>Chen et al. (2009), Ye et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>- Removal of herbivorous fish and transplantation of macrophytes (large scale)</td>
<td>W</td>
<td>L. Qinhu, China</td>
<td>Z. Liu (unpublished data)</td>
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<tr>
<td></td>
<td>- Transplantation in combination with stocking of piscivorous fish (large scale)</td>
<td>W</td>
<td>L. Huizhou, West Lake, China</td>
<td>Z. Liu (unpublished data)</td>
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<td></td>
<td>- Establishment of artificial plant beds</td>
<td>T</td>
<td>Lake Stigsholm, Lake Væng</td>
<td>Schou et al. (2009) and Boll et al. (2011)</td>
</tr>
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</table>

The table includes only studies where full-scale manipulations or natural fish community changes have occurred, plus small/larger scale experiments in which the aim was to establish submerged macrophytes by protecting areas from bird and/or fish grazing.
3.1. Macrophyte manipulation in north temperate lakes

Studies in UK (Chaichana et al., 2011; Irfanullah and Moss, 2004), Denmark (Lauridsen et al., 2003; Søndergaard et al., 1996) and Germany (Hilt et al., 2006) have shown higher survival and number of plants and longer total shoot length in enclosures that prevent bird access. A large-scale experiment conducted in Danish Lake Engelsholm, where three submerged macrophyte species were planted in three 250 m² exclosures (protected from bird grazing, Lauridsen et al., 2003), demonstrated an up to fourfold increase in plant coverage in two growth seasons. Furthermore, the experiment revealed that submerged macrophytes were capable of colonising non-transplanted but protected areas. No macrophytes were observed outside the protected areas, illustrating the high grazing pressure, despite relatively few herbivorous birds in the lake. The importance of macrophyte establishment outside the maximum diving depth of herbivorous birds was a key factor for the macrophyte re-establishment in the lake.

Transplantation and protection of submerged macrophytes are a time- and resource demanding restoration process, particularly in large systems. Many eutrophic submerged macrophyte species have a very large growth potential and are able to completely overgrow shallow lakes within a few seasons (e.g. Lauridsen et al., 1993; Rørslett et al., 1986). Elodea species can develop from absence to complete cover within 1–2 years (Meijer et al., 1994; Søndergaard et al., 1998). It therefore remains an open question under which circumstances it would actually be necessary to transplant and protect macrophytes from grazing. In a recent review, Bakker et al. (2012) concluded that after abiotic conditions for plant macrophyte growth are reached it is imperative to study the role of propagule availability and herbivory more closely in order to define what exactly can be restored and what cannot. There may be unfavourable conditions that can explain why submerged vegetation does not return naturally if clear water conditions have been established, as suggested by Marklund et al. (2002) and Lauridsen et al. (2003). Once these uncertainties have been clarified, transplantation would mainly be relevant where propagule availability and spreading/colonisation potential are both low. However, protection from herbivory would be expected to be most important in shallow lakes which often need to recover from very low (or zero) macrophyte abundance. Waterfowls are also important, and in a recent meta-analysis on their impacts, Wood et al. (2012) found a positive linear relationship between waterfowl abundance and the reduction in plant standing crop when the waterfowl density was expressed as kg ha⁻¹, but not when expressed as individuals per hectare, emphasising the importance of species body mass.
If the restoration goal includes the re-introduction of species typically dominating in nutrient poor lakes, or species which may have become regionally extinct or have allelopathic effects to enhance clear water conditions, species-specific macrophyte transplantations may be relevant. The need to reintroduce species to areas where they have become extinct has been a subject of recent discussion, and a whole new research field on this topic is emerging (Seddon et al., 2007). In Danish Lake Fure, for instance, the number of macrophyte species almost returned to pre-enrichment levels after an external loading reduction, but short angiosperms, mosses and characeans disappeared along with all other vegetation in deeper waters (5–8 m) and have only partly recovered recently (Sand-Jensen et al., 2008). Instead, tall angiosperms became dominant and only 4 out of 10 characean species originally present reappeared. The review by Bakker et al. (2012) showed that the macrophyte community in none of six European lakes studied had recovered completely, but all lakes showed a different community composition with fewer species compared with the situation 20–100 years ago. It is important to emphasise that a lack of recovery of plants may also reflect excessive N loading after P loading reduction, as several studies indicate that plant recovery and species richness are sensitive to high N (Barker et al., 2008; James et al., 2005; Jeppesen et al., 2005; Moss et al., 2012).

An alternative way forward to restore the macrophytes is to spread seeds or whole shoots. The restoration success of *V. americana* was studied in different types of transplantations in Virginia, USA, by Moore et al. (2010), who found that whole shoot transplants resulted in the most rapid cover of the bottom, whereas direct dispersal of individual seeds or intact seed pods was effective, but slower. They also concluded that protection of the plants from herbivory was critical to restoration success, and for large-scale restorations, they suggested that large founder colonies may be needed to withstand grazing pressures for expansion outside herbivore exclosures. The success of transplantation also depends on suitable substrate conditions, with sufficient organic content and limited toxic substances (Bornette and Puijalon, 2011; Smolders and Roelofs, 1996; Van der Welle et al., 2007).

If plants do not respond immediately either naturally or to the above transplantations methods, then artificial plants may initially help to create and stabilise a clear water state by serving as a daytime refuge for zooplankton against predation by fish. Schou et al. (2009) found that a ca. 3–5% lake area covered by artificial plants doubled zooplankton densities in shallow Lake
Stigsholm, Denmark. Moreover, these plants could serve as a substrate for plant-associated macroinvertebrates. Boll et al. (2012) found plant-associated macroinvertebrate densities of 342,000 and 133,000 ind. m$^{-2}$ of lake bottom at high- and low artificial plant density (80% and 40% PVI, plant volume inhabited), respectively, which was substantially higher than the densities of benthic macroinvertebrates (3500 ind. m$^{-2}$) in sediment without plants. As submerged vegetation may serve as profitable feeding grounds for small perch, it is likely that artificial plant beds can enhance recruitment to the piscivorous stage, as when submerged vegetation is expanding (Hargeby et al., 2005). The optimal plant density for efficient foraging by small, non-piscivorous perch has yet to be determined.

3.2. Macrophyte manipulation in warm lakes

While in temperate systems the introduction and development of aquatic plants are considered key steps in restoration (Moss et al., 1996), in the tropics and subtropics, many aquatic plants, particularly exotics, are often considered a nuisance and subject to eradication measures. Plant beds in nutrient-enriched lakes may become so abundant that they impede transportation, hydroelectricity generation and fisheries and reduce the recreational value for anglers (Bini et al., 1999; Cilliers et al., 1996; Moreira et al., 1999). Moreover, excessive growth of several species, like M. spicatum, or the free-floating Pistia stratiotes L. and Eichhornia crassipes (Mart) Solms (the latter two native to South America) in many lakes and reservoirs in the USA, South America, Asia and Africa, or the North American E. canadensis in Europe, may alter lake ecosystems and constitute a serious threat to the native flora and fauna. Biomanipulation by stocking of herbivorous grass carp (Ctenopharyngodon idella Valenciennes) or the introduction of specialist phytophagous insects, such as weevils (Euhrychiopsis lecontei) and water veneer moth (Acentria ephemera), have been used to control some of these plants (Cooke et al., 2005).

Manipulation of dense mats of free-floating plants has been conducted in an attempt to remove suspended solids and nutrients from artificial systems (e.g. from sewage or industrial runoff, Dellarossa et al., 2001), as well as to complement other restoration measures (e.g. Lake Rodó, Uruguay, Rodriguez-Gallego et al., 2004). The need for rapid transport of the typically huge volume of removed biomass (to avoid nutrients from returning to the system) might impose logistic problems for this method.

In contrast to temperate lakes, few comprehensive full-scale studies on the use of macrophytes for restoration are available for subtropical lakes.
The experimental results demonstrate the usefulness of plant refuges as a restoration tool in small shallow lakes (Chen et al., 2009; Ye et al., 2011). In the studies by Ye et al. (2011) and Chen et al. (2009), herbivorous fish were excluded from a 5-ha shallow area followed by transplantation of submerged macrophytes into the same area. In one area, there was an increase in submerged macrophyte coverage, whereas in another area, a decrease was observed due to overgrowth by floating-leaved macrophytes (Ye et al., 2011), with transplanted submerged plants having limited success (Chen et al., 2009). Consequently, it was concluded that transplantation of submerged macrophytes may not necessarily be successful in itself in this particular case, but in combination with emergent and floating-leaved macrophytes, they had a positive impact on water quality. Also in Lake Qinhu, China, a relatively large-scale successful transplantation of macrophytes following removal of herbivorous fish has been performed (Z. Liu, unpublished data). In Lake Qinhu, macrophytes have expanded and have a positive effect on the system, contributing to maintaining a newly established clear water state.

For regulated lakes, alteration in water level may help promote the submerged macrophytes (Cooke et al., 2005), as it may improve the light environment for macrophyte growth (Blindow, 1992; Nõges and Nõges, 1999; Coops et al., 2003; Bekliog˘lu et al., 2006) depending on lake morphology (Bekliog˘lu et al., 2006). Accordingly, mesocosm studies in a Turkish shallow lake showed that macrophytes could resist higher nutrient loading due to a high, evaporation-triggered, water level reduction during summer, overriding the deleterious effect of periphyton- and phytoplankton-induced turbidity (Bucak et al., 2012; Özkan et al., 2010).

### 3.3. Macrophyte manipulation: Future perspectives

The use of macrophyte establishment and protection as a lake restoration method will remain an important measure in the future, but there are many unsolved issues: under which circumstances are these measures needed? can the methods be optimised? and how can long term effects be assured? A possible conflict between recreational users and nature conservation remains as promotion of indigenous submerged macrophytes is an important measure to restore shallow lakes but may, if plants become very abundant, cause nuisance to boating and swimming and obstruct the water flow (van Nes et al., 2002). In many densely populated and agricultural countries, nutrient levels in lakes will remain at such high levels that both situations may occur. The predicted increase in eutrophication in the future can boost both
turbidity-tolerant submerged and free-floating plants, since they are not affected by the underwater light regime. The choice of the best plant species to promote will thus be of key importance.

4. ENHANCEMENT OF ALTERNATIVE HERBIVORES: STOCKING MUSSELS

Stocking of aquatic mussels may potentially help induce clear water in lakes after nutrient loading reduction (Gulati et al., 2008), although full-scale stocking experiments have not, to our knowledge, been conducted yet. Here, we overview the potential role that naturally occurring and introduced mussels may play in lake ecosystems and discuss how they might restore eutrophied lakes.

Mussels can have an important impact on shallow lake ecosystems via several mechanisms, including suspension feeding (e.g. Strayer et al., 1999), deposit feeding (Vaughn et al., 2004), grazing (Hill et al., 1992; Karatayev et al., 2009a,b), predation (e.g. Howells et al., 2006), bio-deposition (Stewart et al., 1998), bioturbation (e.g. Karatayev et al., 2007) and shell production (e.g. Gutierrez et al., 2003).

Large unionid mussels, such as *Anodonta*, *Unio* and *Hyridella* spp., are sometimes abundant in well-mixed macrophyte-dominated systems and can filter the entire water volume in a few days (Ogilvie and Mitchell, 1995; Strayer et al., 1994;Welker and Walz, 1998). Ogilvie and Mitchell (1995) observed that the *Hyridella menziesii* population of Lake Tuakitoto (New Zealand) filtered a volume of water equal to that of the lake (826,000 m³) once every 32 hours. Unionid mussels are also capable of filtering and ingesting cyanobacteria (Bontes et al., 2007; Dionisio Pires et al., 2007). However, these mussels often disappear from turbid lakes, probably due to predation of their larvae by fish (Gulati et al., 2008). The re-introduction of these species may therefore potentially be a useful tool for the restoration of the clear water state, but so far, this has received little attention. If a lake is in a turbid state and fish are abundant, however, re-introduction of mussels without removing fish is, in fact, unlikely to have any effect, unless the introduction involves large quantities of adult mussels that can escape fish predation.

The zebra mussel (*Dreissena polymorpha*), which is native to the lakes of southeast Russia, could be another candidate. It has become an invasive species in many countries (e.g. it was discovered in North America around 1986, Herbert et al., 1989) and affects sediment chemistry, grain size and
organic matter content via bioturbation. It also increases light penetration into the water column due to filter feeding, induces changes in near bed flows and shear stress due to the presence of shells and provides colonisable substrate and refuges by their shells.

Zebra mussels may, through filter feeding, markedly reduce phytoplankton while increasing water clarity (Caraco et al., 1997; Karatayev et al., 1997; Reinders et al., 1993) and thus often trigger a shift in primary production from the phytoplankton-dominated towards a macrophyte-dominated community (MacIsaac, 1996; Phelps, 1994). Ricciardi et al. (1998), however, demonstrated competition between zebra mussels and North American endemic bivalve species (Unionoida), increasing the risk of extinction of the latter, and the former are a nuisance for recreational boating and commercial shipping as well as for raw water using industries, potable water treatment plants and electric power stations (see Ludyanskiy et al., 1993; Roberts, 1990; Sousa et al., 2009).

Changes in the composition of phytoplankton and zooplankton communities following zebra mussel establishment have been observed in many systems (Bastviken et al., 1998; Ten Winkel and Davids, 1982). For zooplankton, Pace et al. (1998) showed that zebra mussels caused a strong size-dependent decline in microzooplankton in the Hudson River. How zebra mussels affect the potential toxic cyanobacteria is debatable. Invasion of North American lakes by zebra mussels has been argued to cause the widely observed increase, in particular, of species of toxigenic cyanobacteria, *Microcystis* (Knoll et al., 2008; Raikow et al., 2004; Sarnelle et al., 2010; Vanderploeg et al., 2001). In contrast, a decline of *Microcystis* has been observed in other studies (Baker et al., 1998; Dionisio Pires et al., 2004, 2005; Smith et al., 1998). Dionisio Pires et al. (2005) suggested that the mussels may be used as a biofilter for the removal of harmful cyanobacterial blooms in shallow (Dutch) lakes where the mussels are already present and not a nuisance.

Cascading effects on water clarity, macrophyte growth and the nutrient levels have been observed in many lakes. For example, studies in Oneida Lake in North America showed a clear improvement of light climate and macrophyte vegetation extending to deeper areas in the presence of zebra mussels (Idrisi et al., 2001; Mayer et al., 2002) and macrophyte diversity increased (Zhu et al., 2006). There is therefore no doubt that stocking of zebra mussels has promoted a clear water state in several eutrophic lakes. However, it is an invasive species to most countries, and serious effects on other components of the local food webs have been described. It is therefore important
to test native species for their potential in lake restoration and the considerable diversity of bivalves in warm regions represents an interesting opportunity.

5. COMBINED TREATMENTS

The high probability of a return to the turbid state some years after biomanipulation calls for supplementary methods. One such possibility is to combine biomanipulation with chemical treatment of the water to precipitate phosphorus and to reduce internal phosphorus loading and thus phytoplankton growth. The resulting higher water clarity may potentially reinforce recovery by altering the top-down control of zooplankton by fish through a trophic and/or a behavioural cascade. An alternative is to combine biomanipulation with oxygenation of the hypolimnion, which may not only result in reduced internal loading but also affect the fish community and eventually the entire pelagic ecosystem. If oxygen appears in the hypolimnion, the conditions for macroinvertebrates improve. In addition, piscivorous fish species may be favoured. Perch, in particular, may obtain better foraging possibilities in the benthos, thereby allowing them to pass from consuming zooplankton through a macroinvertebrate eating stage before becoming piscivores (pass the “macroinvertebrate bottleneck”, Persson et al., 1991), facilitating a higher control of planktivorous fish. A behavioural cascade is also expected if water clarity increases in the epilimnion, as it enhances grazing and thus reduces sedimentation and thereby the need for oxygenation in the hypolimnion. The hypothesis is therefore that a combined treatment may be more effective than individual application of methods, due to synergistic effects (Diagram 1), and may reduce the costs of restoration and perhaps the need for subsequent intervention.

Here, we describe dual techniques, including biomanipulation combined with chemical treatment and with hypolimnion oxygenation, respectively, and give two case studies from Danish lakes. We further highlight the need for controlled experiments within this field.

5.1. Biomanipulation combined with chemical treatment

Chemical restoration aims at supplying new sorption sites for phosphate onto the surface sediment (sediment capping) (e.g. Cooke et al., 2005; Koschel et al., 2006). Phosphate adsorbs readily to calcite (CaCO$_3$), hydroxides of oxidised iron (Fe$^{3+}$) and aluminium (Al$^{3+}$). Aluminium addition has been used for restoration in some 150 lakes in the USA and Europe,
and the reported longevity of positive effects varies from a few to 10–15 years, seemingly depending on dosage and on how well external P loading is controlled. Lanthanum (La) and the La-modified benthonite can also be used (Meis et al., 2011). The commercial La product Phoslock™ was introduced in the late 1990s and treatment has been reported for ~10 lakes or basins, but a long-term positive effect has yet to be documented.

The precipitation of P and a reduction in internal loading followed by chemical restoration result in higher water clarity, which, in turn, may reinforce recovery by altering the top-down control of zooplankton by fish through a trophic and behavioural cascade. Following treatment with aluminium in deep Danish Lake Nordborg, a change in the fish community structure, abundance and habitat selection occurred within a year or less (Lund et al., 2010). The proportion of perch increased in overnight gill net catches, at the expense of roach. Similarly, growth and average size of all key species increased. The habitat distribution of perch and roach changed from a high proportion in the upper pelagic zone towards increasing proportions in the deeper pelagic and profundal zones, even though the abundance of large-bodied zooplankton increased and the abundance of benthic macroinvertebrates decreased (E. Jeppesen et al., unpublished results). These results indicate that enhanced risk of predation from piscivorous perch was more important for the habitat distribution change of the fish than food availability, resulting in less predation on zooplankton, higher grazing on phytoplankton and thus higher water clarity than expected from the aluminium treatment. They also demonstrated that chemical restoration may rapidly and strongly affect the trophic structure and dynamics of the system.

It is therefore likely that a combination of aluminium treatment or other chemical restoration methods with biomanipulation may have stronger and perhaps also more long-lasting effects than when the various methods are applied individually and that synergistic effects may even reduce the cost of restoration as a less extensive chemical and less fish manipulation effort will be needed. However, evidence of this is so far still limited.

5.1.1 Dual treatment: chemical treatment and fish manipulation in Lake Kollelev, Denmark

Shallow Lake Kollelev (Table 2) is an example of combined chemical and biological treatment. The lake is divided into three basins connected by channels. Until 1998, the lake received waste water or storm water with overflow, but after waste water diversion, the lake remained hypertrophic. Different in-lake measures were applied in the period 1999–2005 to
improve water clarity (Fig. 10, Appendix). Iron addition to two of the basins in 1998 had little effect. Biomanipulation including cyprinid removal and perch stocking in all basins in 1999 was also ineffective, while aluminium treatment of basins 1 and 2 in 2003 immediately resulted in lowered lake water TP. Meanwhile, no improvements in Secchi depth were observed in any of the basins. A new biomanipulation in all three basins resulted in an immediate and strong improvement in water clarity in the aluminium-treated basins, but a much less pronounced and only gradual improvement of the Secchi depth in the untreated basin, coinciding with a gradual decrease in TP.

This case study indicates that only the combined treatment with aluminium (bottom-up control) and biomanipulation (top-down control) ensured a rapid improvement in water clarity. However, as this experiment is not replicated, we cannot exclude that other factors may be involved, and
controlled follow-up experiments are needed before any firm conclusions can be drawn.

5.2. Biomanipulation combined with hypolimnion oxidation

Another possibility restricted to (summer-) stratified lakes is to combine biomanipulation and addition of oxidisers to an otherwise anoxic hypolimnion. Oxygenation (addition of pure oxygen) has been used worldwide (for recent reviews, see Cooke et al., 2005; Liboriussen et al., 2009) in summer-stratified lakes. It improves the redox sensitive sorption of phosphate to iron in the sediment and thereby reduces the internal P loading. Alternatively, electron acceptors such as nitrate may be used as alternative oxidisers. When using nitrate, a liquid solution of nitrate is added by stirring it into the upper sediment layer or by injecting it into the water just above the sediment (Ripl, 1976; Søndergaard et al., 2000). The advantage of using nitrate is that it will penetrate the sediment to a greater depth than oxygen, although the oxygen concentration in the hypolimnion will be less affected.

5.2.1 Dual treatment: oxygenation and fish removal in Lake Fure, Denmark

Lake Fure is a deep lake (Table 2) with a hydraulic retention time of 10.6 years. The annual external P loading to the lake peaked in 1969 (ca. 37 tonnes in 1969) followed by a major decline due to improved sewage treatment and sewage diversion. Since an intensive monitoring programme was established in 1989, both the total annual P and N loading as well as annual/summer mean TP, TN and Chl a have decreased in the lake (Appendix, Fig. 11). To reinforce recovery, a combined biomanipulation–oxygenation restoration initiative was initiated in 2003. Biomanipulation was conducted from March 2003 to June 2006. In total, 213 tonnes of coarse fish (226 kg ha$^{-1}$) were removed from the lake and adjacent waters using different types of pound nets and dragnets. Of the total catch, 173 tonnes were target species, particularly roach and small bream. There was a clear change in the composition of the fish community towards a much higher percentage in both abundance and biomass of piscivorous fish, primarily perch (Frederiksborg County, 2006, Table 4). Oxygenation of the hypolimnion (method described in Appendix) has been conducted during stratification from June to November since 2003 (Ramboll, 2012).

The combined restoration led to major changes in key physico-chemical variables. The hypolimnion became oxic during the entire stratification period, and concentrations of ammonia, ortho-phosphate and, with those,
Figure 11 Time series of Secchi depth (A), chlorophyll a (C), total phosphorus (D), orthophosphate (E), total nitrogen (F), nitrate (G) and ammonia (H) in the surface water and hypolimnion (data include the stratification period only) in Lake Fure from 1989 to 2011. Also shown are saturation (I) and temperature in the surface water (B) and near the sediment at a mid-lake deep station. Oxygenation started in 1993 and fish removal occurred between 2003 and 2006.
also TN and TP decreased markedly (Fig. 11; Liboriussen et al., 2009) and immediately, while Chl \(a\) decreased, though with periodic peaks in concentrations after 2006, and vice versa for Secchi depth.

Unfortunately, only few fish surveys are available in the years following the fish manipulation, making it difficult to judge the effect of this measure. No clear effect on CPUE was found, but apparently the individual biomass of fish increased, being much higher in 2006 than in any of the surveys prior to 2003 (Table 4), but the total CPUE in terms of weight was also high. A fish survey in 2006 revealed several species, including large piscivores in the hypolimnion below 15 m, after oxidation.

Zooplankton individual biomass and the proportion of *Daphnia* among cladocerans are valuable indicators of fish predation pressure in lakes as fish select for large species (Brooks and Dodson, 1965; Jeppesen et al., 2011). Summer means of these ratios followed largely the changes in fish CPUE, being low in the early 1990s when the smelt population and total CPUE of fish by weight were high, peaked in 2003–2004 when the lake was biomanipulated, but decreased again in the following years. The changes in zooplankton were overall mirrored in the phytoplankton Chl \(a\), which was lower in years where large-bodied zooplankton were dominant (Fig. 12), emphasising the role of top-down control in the system. The immediate response to oxygenation of nutrient concentrations and the more slow response of zooplankton size and Chl \(a\) following three years of fish manipulation suggest a combined effect of the two restoration measures as hypothesised. However, like for Lake Kollelev, the study does not allow firm disentangling of the effects of the treatments or evaluation of potential synergistic effects.

<table>
<thead>
<tr>
<th>Year</th>
<th>CPUE (no.)</th>
<th>CPUE (g)</th>
<th>Mean body mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>155 (15%)</td>
<td>7086 (10%)</td>
<td>45.7</td>
</tr>
<tr>
<td>1996</td>
<td>62 (39%)</td>
<td>3669 (35%)</td>
<td>59.1</td>
</tr>
<tr>
<td>1999</td>
<td>87 (38%)</td>
<td>5031 (30%)</td>
<td>57.9</td>
</tr>
<tr>
<td>2002</td>
<td>77 (51%)</td>
<td>4293 (49%)</td>
<td>55.7</td>
</tr>
<tr>
<td>2003</td>
<td>96 (44%)</td>
<td>5296 (62%)</td>
<td>54.1</td>
</tr>
<tr>
<td>2006</td>
<td>86 (38%)</td>
<td>7372 (26%)</td>
<td>85.7</td>
</tr>
</tbody>
</table>

Shown are total Catch Per Unit Effort of fish in gillnets in terms of numbers (no) and weight (g WW), percentage contribution of perch (in parenthesis) and average body mass (g WW) of fish in surveys in late summer in the main basin of the lake during 1991–2006.
6. EFFECT OF BIOMANIPULATION ON BIODIVERSITY

Shifts in trophic structure may trigger complex changes in biodiversity. In theory, a more balanced fish community after fish manipulation and less top-down control by planktivorous fish should cascade to higher diversity at the lower tropic level (zooplankton and phytoplankton) unless key-stone species (such as large *Daphnia*) completely dominate the zooplankton and benthic invertebrates. Moreover, provided that submerged macrophytes re-colonise and become more abundant after biomanipulation, habitat complexity in the lake littoral will increase, which would lead to more diverse microhabitats and an increase in biodiversity. A comprehensive study of 96 European lakes covering organisms from fish to bacterioplankton showed that taxon richness was best associated with abundance of submerged macrophytes (Declerck et al., 2005).

Here, we present analyses of changes in richness of phytoplankton and zooplankton due to biomanipulation by analysing data from before and after fish manipulation in Lake Engelsholm and zebra mussel introduction in Lake Faarup (Denmark). Main results are given below, while a more thorough analysis including statistics can be found in the Appendix. We expected that fish removal would lead to enhanced biodiversity of the plankton following a shift to a more diverse fish assemblage and reduction in nutrients and that mass development of zebra mussels would, in contrast, lead to a reduction due to selective removal of some taxa of both zooplankton and phytoplankton despite an expected reduction in the nutrient level.

Figure 12 Changes in summer mean (May 1–Oct 1) individual biomass of cladocerans and *Daphnia* (A) and summer mean chlorophyll *a* and individual biomass of cladocerans (B) in Lake Fure from 1989 to 2011.
6.1. Effects of fish manipulation on biodiversity: Lake Engelsholm, Denmark

Shallow Lake Engelsholm (Table 2) was biomanipulated in 1992–1993 to reinforce its recovery after nutrient loading reduction (Søndergaard et al., 2007). Nineteen tonnes of cyprinids were removed and the estimated biomass of cyprinids subsequently decreased from 675 to 150–300 kg ha\(^{-1}\) (Møller, 1998). Biomanipulation led to a substantial reduction of Chl a, TP and TN as well as an increase in Secchi depth (Fig. 13). Change point analysis (Hinkley, 1970) identified a shift \((p < 0.01)\) in phytoplankton biomass trends in July 1993, immediately after the fish biomanipulation (Fig. 14). There was a 10-fold decrease in both median and minimum phytoplankton biomass (10th percentile) following the shift (Table 5). Time series analysis identified no trend in phytoplankton biomass before biomanipulation and a negative trend afterwards (Fig. 14). Mean within-year variation in phytoplankton biomass almost doubled after the shift. Both median phytoplankton richness and evenness increased after the shift (Table 5), whereas within-year variation increased for richness, but decreased for evenness, indicating a year-round higher evenness of phytoplankton. Following biomanipulation, phytoplankton composition shifted from year-round dominance of cyanobacteria to, first, stronger seasonal succession among chlorophytes, cyanobacteria and diatoms, followed by higher dominance of the remaining groups (Fig. 14).

There were also marked changes in zooplankton in the lake. Median zooplankton biomass as well as its mean within-year variation decreased after biomanipulation (Table 5). There was no trend in the zooplankton biomass before and a negative trend after biomanipulation. Zooplankton richness and its within-year variation increased slightly with a positive trend after the shift. The fraction of rotifers in zooplankton biomass increased, while cladocerans decreased following biomanipulation (Fig. 14).

6.2. Effects of mussel invasion on biodiversity: Lake Faarup, Denmark

Lake Faarup is a stratified lake (Table 2) with a water residence time around 0.5–0.7 year. The majority of the nutrient loading of both N and P is diffuse (large part via groundwater input) from the cultivated catchment. Zebra mussels were observed for the first time in 1993 and veliger larvae in the plankton from 1998 and onwards (Andersen, 2009). The density in 2000 was recorded 1300 m\(^{-2}\). Since 1995, a major decrease has occurred
in summer mean Chl $a$, TP and TN. Accordingly, Secchi depth has increased (Fig. 15). As the external loading of TN and TP has not changed during the study period, the drastic changes can most likely be attributed to the colonisation and a gradual increase in zebra mussel densities.

Compared to fish-manipulated Lake Engelsholm, the changes in the Lake Faarup planktonic communities were more gradual (Fig. 16). Although zebra mussel was first observed in 1993, the change point analysis identified a shift
Lake Engelsholm

Phyto. biovolume (log (mg L\(^{-1}\) + 1))

Phyto. genera richness

Phyto. genera evenness

Zoop. DW (µg L\(^{-1}\))

Zoop. biomass composition

Zoop. genera richness

Zoop. genera evenness

Phyto. biovolume Zoop. biomass

<table>
<thead>
<tr>
<th>Phyto. biovolume</th>
<th>Zoop. biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Others</td>
<td>Rotifers</td>
</tr>
<tr>
<td>Diatoms</td>
<td>Copepods</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Cladocerans</td>
</tr>
<tr>
<td>Chlorophytes</td>
<td></td>
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Biomanipulation as a Restoration Tool to Combat Eutrophication
(\(p < 0.01\)) in phytoplankton biomass trends in August 1997 (Fig. 15), coinciding with the observation of high densities of zebra mussel larvae in plankton. Median phytoplankton biomass decreased sixfold, and both phytoplankton genera richness and evenness decreased as well by 40% (Table 5). Both phytoplankton biomass and diversity measures showed a decreasing trend after the shift. Also mean within-year variation in phytoplankton biomass and diversity measures increased considerably after the shift. The change in phytoplankton community composition between these periods was mostly reflected by a decrease in cyanobacteria dominance and a gradual increase in heterogeneity accompanied by an increase in abundance of diatoms and the remaining groups, with little change in chlorophytes in the latter period.

Median zooplankton biomass in Lake Faarup decreased by 50% after 1997, while its mean within-year variation increased (Fig. 16). Both zooplankton genera richness and evenness were higher before 1997 and within-year variation in both zooplankton genera richness and evenness increased after 1997. Considering community composition, cladoceran dominance gradually decreased, whereas the fraction of rotifers of the zooplankton biomass rose after the shift (Fig. 16).

Judged from the results obtained from Lake Engelsholm and Lake Faarup, changes in top-down control via fish removal enhanced the diversity of phytoplankton and zooplankton (but also led to a reduction in cladoceran biomass, contrary to expectations), while mass development of zebra mussel reduced the diversity of both planktonic communities as predicted and enhanced within year variation. The latter is supported by other studies (Caraco et al., 1997; Pace et al., 1998; Ten Winkel and Davids, 1982), showing a differential strong impact of zebra mussels on specific phytoplankton and zooplankton taxa and size classes. However, more studies are clearly needed to draw any firm conclusions about the generality of our findings concerning the effect of biomanipulation on biodiversity.

**Figure 14** Time series for phytoplankton biomass (log-transformed, A), phytoplankton biomass composition across four main groups (chlorophytes, cyanobacteria, diatoms and others, B), phytoplankton genera richness (C) and evenness (D), as well as zooplankton biomass (E), zooplankton biomass composition across three main groups (cladocerans, copepods and rotifers, F) zooplankton genera richness (G) and evenness (H) in Lake Engelsholm, Denmark. Grey and white backgrounds indicate the shift in the phytoplankton biovolume trend identified by change point analysis. Blue lines in scatter plots denote yearly running means excluding winter samples. Lines in scatter plots and polygons in composition plots were left unconnected if two samples were more than 30 days apart.
7. EFFECT OF BIOMANIPULATION ON LAKE METABOLISM

The effect of biomanipulation on ecosystem metabolism is debatable and not well studied. It is expected that phytoplankton production is reduced due to the enhanced grazer control of phytoplankton. However, this may be compensated by an increase in production by benthic algae, submerged macrophytes and epiphyton due to an increase in water clarity. Studies of lakes with contrasting nutrient levels have shown only slightly higher system production in nutrient-rich phytoplankton-dominated systems than in oligo-mesotrophic lakes dominated by benthic production.
(Vadeboncoeur et al., 2003; Liboriussen and Jeppesen, 2003) or macrophytes and associated epiphyton (Vadeboncoeur et al., 2003). A modelling study by Genkai-Kato et al. (2012) indicates that the phosphorus loading threshold for a shift in dominance from benthic to pelagic production decreases with increasing mean depth, and also that the system production decreases with increasing mean depth (mean depth range 1.6–3.6 m), particularly at high nutrient loading where phytoplankton dominates. So, the effect of fish manipulation on ecosystem metabolisms and thus oxygen

![Figure 15](image-url)
state of the lake will depend on the degree of compensatory benthic production following a decline in Chl $a$, being highest in shallow lakes. In shallow lakes with high biomass of benthic fish, fish manipulation may further enhance production as light limitation, triggered by fish-induced high concentration of suspended matter, is diminished.

Here, we provide three examples of the effects of biomanipulation on the metabolism in lakes, from fish-manipulated Lake Væng and Lake Engelsholm, and from Lake Faarup, subjected to invasion by zebra mussels. The oxygen metabolism in Lake Væng has been followed 4 years during and after the second biomanipulation event (see Section 2.1.4) using high-frequency (every 15 min) sampling of oxygen, temperature and Chl $a$ (methods in Appendix). As predicted, the major decline in Chl $a$ had comparatively minor effects on gross production and respiration (Fig. 17A). Net production (March 1–Nov 15) ranged between 0.49 and 0.52 mg O$_2$ L$^{-1}$ d$^{-1}$ in 2007 and 2008 became negative in 2009 ($-$0.65 mg O$_2$ L$^{-1}$ d$^{-1}$) and increased to 0.23 mg O$_2$ L$^{-1}$ d$^{-1}$ in 2010, coinciding with extensive growth of *Elodea* (Fig. 17 B). A major decline in net production was found in autumn 2010 following a drastic decline in the macrophytes, so the amplitude in net production tended to vary more over the seasons after restoration (Figs. 3 and 17B).

As in Lake Væng, drastic reductions in Chl $a$ in Lake Engelsholm and Lake Faarup have not resulted in changes (regressed against year, $p>0.5$, Fig. 18) in oxygen saturation (averages for Mar 1–Dec 1), and the saturation ranged between 115% and 128% in Lake Engelsholm and between 112% and 161% in Lake Faarup after the respective shifts in trophic structure. Given the strong reduction in planktonic biomass, the high saturation values suggest a shift from pelagic to benthic production, as also evidenced by modelling of primary production in Lake Engelsholm (Genkai-Kato et al., 2012). Potentially, the oxygen supersaturation may also reflect production of submerged macrophytes together with their attached periphyton. Plant colonisation of Lake Engelsholm gradually occurred, but plant coverage remained low (maximum in 2010: 12.5% coverage, but only 2.5% of the lake volume inhabited by plants (PVI)). Likewise, plant coverage in Lake Faarup was below 7.4% and PVI $<0.5%$. Macrophytes can therefore only marginally have contributed to

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**Figure 16** Time series for phytoplankton biomass (log-transformed, A), phytoplankton biomass composition across four main groups (chlorophytes, cyanobacteria, diatoms and others, B), phytoplankton genera richness (C) and evenness (D), as well as zooplankton biomass (E), zooplankton biomass composition across three main groups (cladocerans, copepods and rotifers, F), zooplankton genera richness (G) and evenness (H) in Lake Faarup, Denmark (see legend of Fig. 14 for further details).
Figure 17  Seasonal variation in (A) system gross production (GPP) and respiration (R) and (B) net production and chlorophyll a in Lake Væng, Denmark, during March 1–November 15 before (2007–2008) and after (2009–2010) the second biomanipulation event. Based on high-frequency measurements of oxygen and temperature (four times per hour, monthly means shown).

Figure 18  Time series for oxygen and temperature in biomanipulated Lake Engelsholm and Lake Faarup, colonised by zebra mussels.
the lake metabolism in these two lakes, and the high summer production may therefore be attributed to an increase in production by benthic algae.

Judged from these three examples, all from relatively shallow lakes, oxygen metabolism is overall marginally or only shortly affected by biomannipulation of fish or mass development of zebra mussels, though seasonality might change (higher net production in summer and lower in winter) if macrophytes become abundant. A different scenario is likely to prevail in deep lakes where loss of pelagic production may not be fully compensated by an increase in benthic/macrophyte production leading to lower net production, at least in the short term, where respiration of previously settled organic matter (from the time when production was high) may be important.

8. FUTURE CHALLENGES FOR BIOLOGICAL LAKE RESTORATION

We are currently facing an increasing pressure on aquatic systems, which is expected to worsen considerably in the future. One of the challenges is the global development resulting in a substantially higher human population. This will entail a much higher agricultural production, a stronger need for fertilisation and, consequently, potentially higher eutrophication of waterbodies (Howden et al., 2007). In addition, climate warming will overall lead to higher eutrophication or worsening of eutrophication symptoms of lakes (Jeppesen et al., 2009, 2010; Moss et al., 2011, but see Nøges and Nøges, 1999) due to higher external and/or internal loading of nutrients and a shift in trophic structure and dynamics in lakes (Jeppesen et al., 2009, 2010, 2012; Meerhoff et al., 2007a,b, 2012).

It is also to be expected that warming and enhanced precipitation in Central and Northern Europe, as well as in other parts of the globe, induced by climate change, will enhance the allochthonous carbon inflow to lakes and reduce carbon burial, leading to higher in-lake dissolved organic concentrations and often a higher humic content (Sobek et al., 2007; Tranvik et al., 2009), which may affect the trophic dynamics in lakes (Karlsson et al., 2009). Moreover, higher winter survival of young fish due to reduced ice cover (Jackson et al., 2007) may lead to enhanced fish predation pressure on the zooplankton (Balayla et al., 2010; Ruuhijärvi et al., 2010) and, consequently, to lower grazing on phytoplankton. Accordingly, it will be more difficult to maintain or obtain a high ecological quality of lakes to fulfill the criteria of minimum good ecological status stipulated by, for example, the European Water Framework Directive, without substantial control of the nutrient input to the lakes. This enhances the demand for additional within-lake restoration measures to improve the ecological state of lakes.
Here, we discuss further the biological and dual restoration for use in temperate and warm lakes and provide recommendations. A conceptual scheme on the main in-lake processes and potential success of the different restoration methods for lakes is given in Diagram 2 and will form the basis for the discussion.

Diagram 2  Main feedback loops responsible for the existence of different states in lakes (turbid or clear water) and the potential effects of different restoration measures under different climates. The relative strength of the connections (including the expected success of restoration measures) is indicated by the width of the arrows. Different responses, evidenced by empirical investigations, due to climatic differences are highlighted by arrows with colour codes for warm (red or light grey) and cold (blue or dark grey) lakes. The potential climate effects on other in-lake processes remain a topic for further research. The qualitative effect of each route in the diagram can be determined by multiplying the signs along the connections. Based on the schemes for alternative states of shallow lakes proposed by Scheffer et al. (1993) and modified by Meerhoff and Jeppesen (2009).

9. DISCUSSION OF METHODS AND RECOMMENDATIONS

Here, we discuss further the biological and dual restoration for use in temperate and warm lakes and provide recommendations. A conceptual scheme on the main in-lake processes and potential success of the different restoration methods for lakes is given in Diagram 2 and will form the basis for the discussion.
9.1. Temperate lakes

While there are examples of positive effects of removing a large proportion of fish, the lakes often return partly or fully to the state before manipulation after 10–15 years. This lack of long-term success may well have contributed to the reduction in the number of biomanipulation projects initiated during the recent decade. In Denmark, for example, biomanipulation activities peaked in 1995–1996 (Søndergaard et al., 2007). With the future challenges of climate effects exacerbating eutrophication, time has therefore come to re-think the theory and methodology. Repeated measures combined with detection of early warning on relapse offer one way forward. The earlier the adjustments are made, the less effort is required. Such measures may be combined with protection of crucial size classes of piscivores from angling or commercial fishing, and stocking of native piscivores.

A promising alternative or supplement is the dual treatment combining biomanipulation with sediment capping or oxygenation of the bottom water (the latter in stratified lakes only). While oxygenation is costly and needs to be continued for a very long time period (Liboriussen et al., 2009), sediment capping is comparatively cheap in the long term. However, also here adjustment may be needed as relapses have been observed after 5–10 years in most of the restoration case studies. We argue that combined and repeated (based on early warning signals) sediment capping and biomanipulation treatment likely are a way forward. With dual treatment, it is, however, impossible to elucidate the specific role of the different treatments or determine whether synergistic effects occur. Controlled experiments are therefore needed, where single and dual treatments are run at the same time, and with untreated controls. Such experiments are easily done for sediment capping and biomanipulation, while it is more difficult, though not impossible at large scales, to undertake combined hypolimnion oxygenation–biomanipulation experiments. Experiments of this kind cannot stand alone, however, as scale also matters (Schindler, 1998). Therefore, a greater range of full-scale dual treatment experiments are required before any firm conclusions can be drawn, and the optimal sets of methods adapted to lake type and nutrient level can be assessed. Ideally, such experiments should be based on combined single and dual treatment in the same lake.

There are still many unresolved issues regarding the use of macrophyte establishment and protection as a lake restoration method, and research gaps to address. However, based on previous experiences, it is shown that macrophytes may re-establish if a macrophyte source is available (via seed banks
or connectivity to other waterbodies), and the plants are tolerant to eutrophic conditions, since such plants are typically fast growing and able to overgrow a potential grazing pressure from herbivores. If a potential macrophyte source is unavailable, however, natural re-establishment may be prevented. In such cases, introduction of macrophytes by simple transplantations may be a solution, although large-scale or full-scale experiments are still lacking. In most cases, where transplantations have been successful, macrophytes have been protected from herbivores. Artificial plants or other structured items are an alternative to be considered if plant establishment is delayed, at least in small lakes, in order to maintain a clear water state after, for instance, fish removal until the plants appear. However, more research is needed within this field to find the optimal cost effective solution.

9.2. Warm lakes

So far, few studies have investigated the applicability of biomanipulation theory, based on the trophic cascade (i.e. with the ultimate aim to enhance zooplankton grazing on phytoplankton) to tropical and subtropical freshwater lakes (e.g. Scasso et al., 2001). Due to the characteristics of the typical fish assemblage in warm lakes described above, the “classical biomanipulation” model (i.e. introduction of piscivores and/or removal of planktivores) has serious limitations. It is therefore likely that a removal-induced reduction of the biomass of omnivorous or planktivorous fishes will be quickly compensated by the adjustment of the remaining population, and the impact of fish manipulation will therefore be of only very brief duration (Jeppesen et al., 2005, 2009), as found in subtropical and tropical lakes after massive fish kills (Iglesias et al., 2011; Nagdali and Gupta, 2002). However, removal of fish can also promote a reduction in nutrient loading in warm lakes. In subtropical Lake Apopka (Florida, USA), it was estimated that, in the short term, most of the P demand of phytoplankton is met through recycling of P, which greatly exceeds external P loading. Depending on population biomass, phosphorus excretion by a resident fish population was similar in magnitude to the P release by diffusive flux from the sediments (Schaus et al., 2010), an indication that a reduction in the fish stock might enhance nutrient control of phytoplankton.

Widespread omnivory potentially allows the fish stock in warm locations to attain a higher carrying capacity than obligate zooplanktivores or strictly pelagic fishes, which augments the potential control of the large zooplankton. Many of the fish species show partial niche overlap, which expectedly
increases predator control of prey items (Aguiaro and Caramaschi, 1998; Lazzaro, 1997). Other factors could also affect the success of biomanipulation in warm lakes but are poorly elucidated. There is scarce knowledge of the trophic role of many fish species, given the much larger number of species typically present in warm areas (González-Bergonzoni et al., 2012). This implies a potentially large number of fish species to be controlled, but also a window of opportunity to test several species or alternative trophic groups in a biomanipulation programme. For instance, periphyton-feeding fishes could potentially facilitate the establishment of submerged macrophytes via removal of their epiphyton competitors; however, we have found no experimental test of this.

Most of the existing experimental studies in warm areas have examined food web interactions in mesocosms in eutrophic lakes and reservoirs, with the aim to control cyanobacterial blooms via enhanced grazing by omnivorous filter-feeding fish (Arcifa et al., 1986; Jones and Poplawski, 1998; Northcote et al., 1990; Starling, 1993). Caution must be taken in the choice of the manipulative species, however, due to the side effects associated with the release of competition within the phytoplankton community (for the preferential consumption of certain phytoplankton taxa) and the increased rate of nutrient recirculation, such as that associated with the use of tilapia (Menezes et al., 2010). For instance, as a result of the consumption of both phytoplankton and zooplankton, silver carp (ideally non reproductive) could be used for biomanipulation only when the primary aim is to reduce nuisance blooms of cyanobacteria that cannot be effectively controlled by large herbivorous zooplankton, such as in tropical nutrient-enriched lakes (Radke and Kahl, 2002).

For most fish species, management practices are still under discussion in the scientific literature. Incorrect timing for the harvest of silver carp from the system could result in the release of grazing on phytoplankton and the boosting of blooms (Xiao et al., 2010). Similarly, overstocking of silver and bighead carps could enhance the predation pressure on zooplankton rather than on phytoplankton (Ke et al., 2008). Besides, the few existing whole-lake biomanipulation examples in the subtropics (e.g. Scasso et al., 2001) have often removed fish biomass at levels lower than those typically successful in temperate lakes, thus limiting the extent of their conclusions regarding the effort needed and long-term effects.

The absence of a native piscivorous fish culture in many tropical countries precludes the application of biomanipulation. Generally, aquaculture has so far focused on a few species (i.e. tilapia, common carp and grass carp),
mostly for protein production that typically has negative effects on water quality and biodiversity. It is remarkable that in many countries, state institutions are responsible for the introduction and dispersal of fish species with—in many cases—known undesirable effects on water quality and biodiversity (Agostinho et al., 2004). A change in the management paradigm seems crucial for successful restoration and biomanipulation of warm lakes.

Other biomanipulation alternatives, such as those described here, have to our knowledge not yet been seriously applied in warm lakes, perhaps in part due to the high cost (e.g. of some of the chemical restoration methods). The typical scarcity of large zooplankton as phytoplankton grazers highlights the relevance of focusing on other grazers. As in temperate zones, in warmer areas, there are several native and exotic filter-feeding bivalves that could control phytoplankton biomass. There are several case studies on the effects of the zebra mussel in the temperate zone, while in South America, studies on the effects of the food web of the invasive Corbicula fluminea and Limnoperna fortunei, and a few on native species, such as Diplodon spp, are still scarce.

The management of aquatic vegetation represents another potential alternative to restoration in warm areas, given the large diversity of species and of functional groups and their potential effects on water clarity. Despite the fact that submerged plants do not seem to offer similar refuge for large zooplankton in warm lakes as in temperate lakes due to high fish aggregation among the plants (Iglesias et al., 2007; Meerhoff et al., 2003, 2006, 2007b), submerged plants may improve water clarity by competing for nutrients with phytoplankton (Kosten et al., 2009) and by releasing allelopathic substances (Vanderstukken et al., 2011). We thus urge for more research on these promising topics of high relevance under the scenario of increasing eutrophication, particularly in developing countries with warm climates.

The few study cases available raise some interesting research topics and questions for the future, that is, (a) shifting a warm eutrophic lake towards an alternative oligotrophic state seems possible if the manipulation pressure is strong and encompasses both external nutrient loading and internal recycling; but (b) the recuperation process of warm lakes may be faster than in the temperate zones due to lower accumulation of organic matter and nutrients (higher metabolism); (c) the resilience associated with the internal nutrient load might therefore be lower in warm systems, whereas the resilience associated with fish might be higher than in the temperate zone and (d) the success of flushing indicates that repeated measures might be needed in order to maintain the effectiveness of the biomanipulation (as also highlighted for temperate lakes in the previous sections). The effort required,
the frequency of application, the success of dual treatments, and the duration of effects of any methods remain open topics of high interest for research.

10. CONCLUSIONS

While short-term effects of biomanipulation have often been successful, provided that they have been sufficiently strong, the long-term perspectives have for various reasons been less positive. Repeated measures may maintain the initial positive effect of biomanipulation, but more research is needed to optimise the frequency and extent of such events. A promising alternative is combining biological and physico-chemical techniques to make the restoration more robust and perhaps less expensive due to synergistic effects of the treatments. We encourage controlled experiments and large-scale field studies and modelling to elucidate the effects of such dual methods, as the scientific evidence is so far poor. More research is particularly needed to determine the potential of biomanipulation or dual methods in warm lakes. It is important to emphasise, though, that the key measure to restore eutrophied lakes is the reduction in external nutrient loading. In-lake restoration only serves the purpose of reinforcing recovery, treating symptoms or improving/maintaining a high environmental quality temporarily until the external loading can be significantly reduced.

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APPENDIX. OVERVIEW OF DATA, METHODS AND SPECIFIC ANALYSIS FOR HITHERTO UNPUBLISHED STUDIES

Section 2.1.4: Biweekly to monthly samples during all seasons using standard methods

Sections 4, 5 and 6.1/6.2: Data are derived from the National Monitoring Programme on Lakes in Denmark with typically sampling 19 times per year,
most intensively in summer (Jeppesen et al., 2000; Kronvang et al., 1993; Sondergaard et al., 2003).

Section 5.1.1: Iron and aluminium salts form hydroxides through acid producing hydrolysis steps when dissolved in water. It is the neutral hydroxides that precipitate phosphorus. Most often the salts are spread uniformly in the surface water from a boat. The product is normally concentrated to $\sim 25\%$ in hydrochloric or sulfuric acid.

The shallow Lake Kollelev (Table 2) is divided into three basins connected by channels. Basin 2 is upstream and basin 3 is downstream the main basin 1. Until 1942, the lake received untreated or mechanically treated wastewater and has until 1998 received storm water with overflow of waste water. After 1998, the annual average inlet P concentration was $<0.1$ mg P L$^{-1}$, but the lake remained hypertrophic. Therefore, different in-lake measures were applied in the period 1999–2005 to improve water clarity (Fig. 10): (1) In 1998, 24 g m$^{-2}$ iron was added to basins 2 and 3 in order to bind P in the sediment. Although lake water P dropped slightly in basin 2, iron rapidly became reduced in the sediment and high P levels returned. (2) In 1999, cyprinid fish (2500 kg in total) were removed in all three basins and perch (300 kg) were added, but with no improvements in Secchi depth or lower TP (Fig. 10). (3) In spring 2003, basins 1 and 2 were treated with aluminium (54 and 32 g Al m$^{-2}$) which immediately resulted in lowered lake water TP, an effect that has lasted in the following years. A similar drop in lake water TP was not observed in basin 3, although gradual improvement has been observed until 2011. This is likely a result of the downstream position and a water retention time of $\sim 4$ months in basin 3 (compared to retention times of $\sim 2$ years in basins 1 and 2). Meanwhile, no improvements in Secchi depth were observed in any of the basins in 2003 or 2004. (4) In 2004–2005, a new biomanipulation (removal of 2200 kg cyprinid fish) was conducted in all three basins. In basins 2 and 3, there was an immediate and strong improvement in water clarity lasting until 2011. In basin 3, a much less pronounced and gradual improvement of the Secchi depth has occurred coinciding with a decrease in TP.

Section 5.2.1: Lake Fure: Oxygen is supplied from an oxygen tank at the shore through distribution pipes to 100-m long perforated diffusers placed in star formation (each consisting of between 10 and 21 diffusers) at the three deepest places in the lake. The oxygen added amounted to 50 and 500 tonnes per year. Linear regression showed that both the total annual P and N loading have decreased significantly with time ($r^2 = 0.70$, $F_{1,19} = 45.72$, $p < 0.001$ and $r^2 = 0.54$, $F_{1,19} = 22.10$, $p < 0.002$,
log-transformed data) and so have annual mean concentrations of TP ($r^2 = 0.67$, $F_{1,20} = 40.54$, $p < 0.001$), TN ($r^2 = 0.67$, $F_{1,20} = 40.58$, $p < 0.001$) and Chl a ($r^2 = 0.49$, $F_{1,20} = 19.58$, $p < 0.001$) as well as summer concentrations ($r^2 = 0.66$, $F_{1,20} = 39.48$, $p < 0.001$; $r^2 = 0.37$, $F_{1,20} = 11.52$, $p < 0.003$; $r^2 = 0.50$, $F_{1,20} = 20.81$, $p < 0.001$, respectively, for TP, TN and Chl a).

Section 6: Data are derived from the National Monitoring Programme on Lakes in Denmark (Jeppesen et al., 2000; Kronvang et al., 1993; Søndergaard et al., 2003). Data were linearly interpolated for Julian days, while winter samples (Nov 15–March 15) were excluded from the analyses due to uneven winter sampling intensity across years, while the figures include all data. Taxonomic identifications were aggregated to genus level, whenever possible. We performed time series analysis for monotonic trends based on Kendall rank correlation (Mann, 1945) before and after the shift. Significant Kendall trends were denoted with a star sign if robust for serial autocorrelation after a block bootstrap test. Within-year variations in plankton biomass and diversity measures were calculated as coefficients of variation per year averaged across years within a period. All the analyses were performed by R (R Development Core Team, 2011) using the Kendall package (McLeod, 2011) for monotonic trend analyses in time series; the changepoint package (Killick and Eckley, 2012) for changepoint analyses and the vegan package (Oksanen et al., 2012) for Shannon evenness calculation.

Section 6.1: There was a 10-fold decrease in both median and minimum phytoplankton biomass (10th percentile) following the shift (Table 5). Time series analysis identified no trend ($p = 0.411$) in phytoplankton biomass before biomanipulation and a negative trend ($\tau = -0.10$, $p = 0.018$; afterwards Fig. 14). Mean within-year variation in phytoplankton biomass almost doubled after the shift. Both median phytoplankton richness and evenness (Shannon diversity, Magurran, 1988) increased after the shift (Table 5), whereas within-year variation increased for richness, but decreased for evenness, indicating a year-round higher evenness of phytoplankton. Both richness and evenness exhibited a negative trend before biomanipulation ($\tau^* = -0.49$, $p < 0.001$ for richness and $\tau = -0.22$, $p = 0.006$ for evenness), which was replaced by a positive trend ($\tau^* = 0.39$, $p < 0.001$ for richness and $\tau = 0.12$, $p = 0.006$ for evenness). Following biomanipulation, phytoplankton composition shifted from year-round dominance of cyanobacteria to, first, stronger seasonal succession among chlorophytes, cyanobacteria and diatoms, followed by higher dominance of the remaining groups (Fig. 14).

There were also marked changes in zooplankton in the lake. Median zooplankton biomass as well as its mean within-year variation decreased after
biomanipulation (Table 5). There was no trend in the zooplankton biomass before ($p = 0.563$) and a negative trend after biomanipulation ($\tau^* = -0.17$, $p < 0.001$). Zooplankton richness and its within-year variation increased slightly with no trend ($p = 0.499$) before and a positive trend ($\tau = 0.10$, $p = 0.031$) after the shift. Zooplankton evenness decreased slightly, but its within-year variation increased before the shift, while the negative trend ($\tau = -0.20$, $p = 0.012$) was replaced by no trend ($p = 0.27$) following the shift. The fraction of rotifers in zooplankton biomass increased, while cladocerans decreased following biomanipulation (Fig. 14).

**Section 6.2:** The density of zebra mussels was recorded in 2000 to 1300 m$^{-2}$. Since 1995 a major decrease has occurred in log-transformed values of summer mean Chl $a$ ($r^2 = 0.52$, $F_{1,11} = 11.95$, $p < 0.005$), mean TP ($r^2 = 0.32$, $F_{1,11} = 5.29$, $p < 0.04$), mean TN ($r^2 = 0.56$, $F_{1,11} = 13.75$, $p < 0.004$) and annual mean Chl $a$ ($r^2 = 0.77$, $F_{1,9} = 31.37$, $p < 0.001$), but not in annual mean TP and TN ($p > 0.05$). Accordingly, Secchi depth has increased (Fig. 15). As the external loading of TN and TP has not changed during the study period ($p > 0.21$ for both TN and TP), the drastic changes can most likely be attributed to the colonisation and a gradual increase in zebra mussel densities.

Median phytoplankton biomass decreased 6-fold, and both phytoplankton genera richness and evenness decreased as well by 40% following the shift (Table 5). Before the shift, there were no significant trends in phytoplankton biomass and evenness ($\tau = -11$, $p = 0.067$ and $\tau = -10$, $p = 0.074$, respectively), although ($\tau = -12$, $p = 0.035$) phytoplankton richness declined. However, both phytoplankton biomass and diversity measures showed a decreasing trend after the shift ($\tau = -0.17$, $p = 0.011$; $\tau^* = -0.27$, $p < 0.001$ and $\tau = -0.16$, $p = 0.018$, respectively).

Mean within-year variation in phytoplankton biomass and diversity measures increased considerably after the shift. The change in phytoplankton community composition between these periods was mostly reflected by a decrease in cyanobacteria dominance and a gradual increase in heterogeneity accompanied by an increase in abundance of diatoms and the remaining groups, with little change in chlorophytes in the latter period. Median zooplankton biomass in Lake Faarup decreased by 50% after 1997, with no trend ($p = 0.104$) before and a negative trend ($\tau^* = -0.28$, $p < 0.001$) after the shift, while its mean within-year variation increased (Fig. 16). Both zooplankton genera richness and evenness were higher before 1997 with a positive trend ($\tau^* = 0.20$, $p < 0.001$ for richness; $\tau = 0.12$, $p < 0.045$ for evenness). However, zooplankton genera richness exhibited no trend ($p = 0.223$) and
evenness had an increasing trend ($\tau = 0.14$, $p = 0.038$) after 1997 (Fig. 16). Within-year variation in both zooplankton genera richness and evenness increased after 1997.

**Section 7:** Gross primary production (GPP) and respiration (R) were estimated from the formula given by Erlandsen and Thyssen (1983) and Kelly et al. (1983):

$$\left[ \frac{d \text{DO}}{d t} \right] = \left[ K_2 1.0241(T^{-20})(\text{DO}_{\text{sat}} - \text{DO}_t) \right] - \left[ \rho_{20} 1.07(T^{-20}) \right]$$

$$+ \left[ \pi_{20} 1.035(T^{-20}) \frac{I_t}{\eta + I_t} \right]$$

[Oxygen change] = [Air water exchange]
- [Temperature-dependent respiration]
+ [light and temperature-dependent production]

where

* $T$: water temperature OC
* $\text{DO}_{\text{sat}}$: dissolved oxygen saturation concentration mg l$^{-1}$
* $\text{DO}$: dissolved oxygen concentration mg l$^{-1}$
* $I_t$: light intensity Einst m$^{-2}$ 30 min$^{-1}$
* $K_2$: is the air–water transport coefficient (cm h$^{-1}$) at 20 °C, depending on wind speed. Several empirical relations between the transport coefficient and wind speed have been established:

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<th>$k$</th>
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<tr>
<td>a</td>
<td>$2.07 + 0.215 , U , 1.7$</td>
<td>Cole and Caraco (1998)</td>
</tr>
<tr>
<td>b</td>
<td>$0.72 , U$</td>
<td>Crusius and Wanninkhof (2003)</td>
</tr>
<tr>
<td>c</td>
<td>$0.168 + 0.228 , U , 2.2$</td>
<td>Crusius and Wanninkhof (2003)</td>
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</table>

where $U$ is the average wind speed (m s$^{-1}$) recorded at a standard height of 10 m. We used an average value for the transport coefficient derived from the three empirical relations.

$\rho_{20}$, $\pi_{20}$, and $\eta$ are parameters estimated (using the secant non-linear method, PROC NLIN, in the statistical software SAS 9.3). We used this method instead of the simple Odum method commonly used in recent lake metabolism research, as Eq. (1) takes into account that respiration is temperature dependent and that temperature can vary substantially on a diel basis, not least in the shallow lakes encompassed by this study. Moreover, simultaneous calibration of the parameters $\rho_{20}$, $\pi_{20}$ and $\eta$ (respiration and photosynthesis parameters) has been shown to be
a robust method by stream researchers (Mahlon et al., 1983). Net ecosystem production was derived as the difference between GPP and R as a daily accumulated value (mg O₂ day⁻¹) and DO was given as the dissolved oxygen percentage (DO%) relative to saturation levels, calculated as a function of T (Lin, 2001):

\[
\text{DO}_{\text{sat}} = 14.652 - 0.41022T + 0.0079910T^2 - 0.000077774T^3 \quad [2]
\]

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