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Article

Repeated Fish Removal to Restore Lakes: Case Study of Lake Væng, Denmark—Two Biomanipulations during 30 Years of Monitoring

Martin Søndergaard [1,2,*], Torben L. Lauridsen [1,2], Liselotte S. Johansson [1] and Erik Jeppesen [1,2]

1. Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark; tll@bios.au.dk (T.L.L.); lsj@bios.au.dk (L.S.J.); ej@bios.au.dk (E.J.)
2. Sino-Danish Centre for Education and Research, 8000 Aarhus, Denmark

* Correspondence: ms@bios.au.dk; Tel.: +45-871-5995

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Abstract: Biomanipulation by fish removal has been used in many shallow lakes as a method to improve lake water quality. Here, we present and analyse 30 years of chemical and biological data from the shallow and 16 ha large Lake Væng, Denmark, which has been biomanipulated twice with a 20-year interval by removing roach (*Rutilus rutilus*) and bream (*Abramis brama*). After both biomanipulations, Lake Væng shifted from a turbid, phytoplankton-dominated state to a clear, water macrophyte-dominated state. Chlorophyll *a* was reduced from 60–80 $\mu g \cdot L^{-1}$ to 10–30 $\mu g \cdot L^{-1}$ and the coverage of submerged macrophytes, dominated by *Elodea canadensis*, increased from <0.1% to 70%–80%. Mean summer total phosphorus was reduced from about 0.12 to 0.07 $mg \cdot L^{-1}$ and total nitrogen decreased from 1.0 to 0.4 $mg \cdot L^{-1}$. On a seasonal scale, phosphorus and chlorophyll concentrations changed from a summer maximum during turbid conditions to a winter maximum under clear conditions. The future of Lake Væng is uncertain and a relatively high phosphorus loading via the groundwater, and the accumulation of a mobile P pool in the sediment make it likely that the lake eventually will return to turbid conditions. Repeated fish removals might be a relevant management strategy to apply in shallow lakes with a relatively high external nutrient loading.

Keywords: biomanipulation; clear water; turbid water; chlorophyll *a*; nutrients; *Elodea*

1. Introduction

Biomanipulation by removing zooplanktivorous and benthivorous fish has been used as a method to restore and improve lake water quality for many years [1–5]. By decreasing the top-down control from fish on particularly large-sized zooplankton, the aim is to increase the filtration of zooplankton capacity on phytoplankton and thereby create clearer water. Removal of benthivorous fish species such as carp (*Cyprinus carpio*), gizzard shad (*Dorosoma cepedianum*) and bream (*Abramis brama*) may also improve lake water quality in shallow lakes by reducing the sediment resuspension and nutrient recycling caused by their feeding activity [6,7] and possibly also by reducing the amount of loose sediment, which otherwise would be more easily resuspended by wind [8].

Numerous examples of biomanipulation have been given in the literature during the past 30 years, including more general approaches integrating the results from a number of case studies [6,9–11]. Overall, clear effects of biomanipulation have often been recorded, among these a trophic cascade impacting most trophic levels; however, the effects have sometimes been weak [12–14]. In some biomanipulation projects, for example those undertaken in Danish lakes, a clear tendency to a return to the previous turbid conditions after 5 to 10 years has been observed [10]. The failure to establish a long-lasting clear water state and inability to create permanent effects have been ascribed to a number of factors. First, adequate reduction of the external nutrient loading is crucial to obtain permanent effects [2,3,15,16]. In shallow lakes, TP concentrations below 0.05 mg·L^{-1} have been suggested as a prerequisite of this [17]. Furthermore, a sufficient number of fish need to be removed, depending also on TP concentrations [18]. In shallow lakes, extensive cover of submerged macrophytes is important for stabilising and maintaining clear water conditions [19], and the overall biomanipulation success may depend on the internal nutrient loading and the interacting effects of nutrient concentrations, fish recolonization and macrophyte abundance [11,14,20].

Post-restoration data sets rarely cover more than a few years after restoration, which renders it difficult to draw any firm conclusions on how often long-term or permanent effects are achieved. Most lake restorations are conducted as a single intervention and repeated lake restorations via fish removal are rare. If a first biomanipulation is not successful in the long term, it has been suggested that a second biomanipulation demands less effort due to the slow recovery of some large benthivores, higher abundance of potential piscivores (e.g., perch, *Perca fluviatilis*) and reduced risk of high internal phosphorus loading [6].

In this paper, we describe the results obtained from two biomanipulation cases conducted with a 20-year interval in Lake Væng, Denmark. The lake has been monitored yearly since 1986 for a number of chemical and biological parameters. Our aim was to study the long-term effects of fish biomanipulation on different trophic levels and nutrient dynamics and to investigate the impacts of repeated fish removal as a lake restoration tool. Our hypothesis was that repeated fish removal would imitate the effects achieved by the first biomanipulation, but with less fishing effort.

2. Materials and Methods

2.1. Study Area

Lake Væng is a small and shallow lake situated in the central part of Jutland, Denmark (Figure 1, Table 1). The catchment is 9 km^2 and consists mainly of extensive agricultural areas and forests. The lake has a short hydraulic retention time (15–25 days) and hydraulic loading is dominated by groundwater, which comprises about 70% of the total hydraulic input. The lake sediment is soft and rich in organic matter.

From 1964, the lake received wastewater from a small village, but this was diverted in 1981, projected to reduce the external phosphorus loading from 4 to 1.5 g·P·m^{-2}·year^{-1} [2]. However, the lake remained eutrophic with a mean summer Secchi depth of 0.6–0.8 m (1981–1986), chlorophyll *a* concentrations of 76 μg·L^{-1} (1986) and dominance of cyanobacteria [20]. No known changes have occurred in the external nutrient loading since 1981. Lake Væng and topics related to its restoration have previously been described in various papers [6,21–24].

Table 1. Morphological and chemical characteristics of Lake Væng (mean values for the monitoring period).

Parameter	Value
Area (ha)	16
Mean depth (m)	1.2
Maximum depth (m)	1.9
Hydraulic retention time (days)	15–25
Hydraulic loading via groundwater (% of total water inlet)	70
Alkalinity (meq·L^{-1})	0.7–1.5

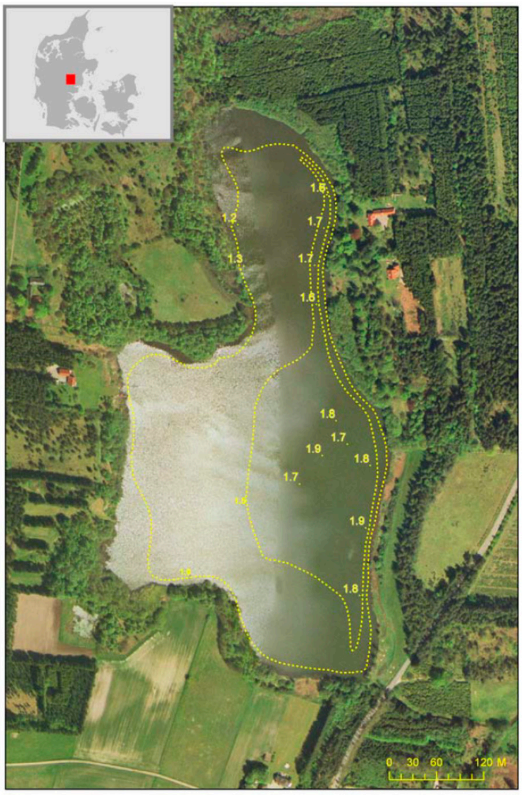

Figure 1. Bathymetry of Lake Væng with 1.0 and 1.5 m depth contours. Geographical location is shown in the upper left corner (56°02′ N, 9°39′ E).

2.2. Biomanipulation

Biomanipulation involving removal of zooplanktivorous and benthivorous fish species was conducted in Lake Væng using gill nets, fish traps, fish trawling and electrofishing. The first biomanipulation took place from autumn 1986 until summer 1988 and a total of 4 tons fish, corresponding to 201,000 individuals, were removed from the lake (Table 2). Of the fish removed, the zooplanktivorous roach (*Rutilus rutilus*) comprised 98% by number and 38% by weight, while bream comprised 1% by number and 62% by weight. Piscivorous pike (*Esox lucius*) and large perch caught during the fishing were released into the lake again. The removal was estimated to have reduced the total biomass of zooplanktivorous and benthivorous fish by approximately 50%–70% [21].

In the second biomanipulation from October 2007 to May 2009, less (2.8 tons) fish were removed and, again, roach and bream comprised the majority of the fish biomass removed, constituting, respectively, 32% and 55% (Table 2).

Table 2. Fish biomass (kg wet weight) removed during the first and the second biomanipulation in Lake Væng. "Others/mixed" comprise a few other species (ruffe (*Gymnocephalus cernua*) and ide (*Leuciscus idus*)) and unidentified fish species. No data are available on fish numbers in the second biomanipulation.

Species	1986–1988		2007–2009
	Number (% of Total)	Biomass (% of Total)	Biomass (% of Total)
Bream (*Abramis brama*)	2528 (1)	2457 (62)	1508 (55)
Perch (*Perca fluviatilis*)	0	0	65 (2)
Pike (*Esox lucius*)	0	0	24 (1)
Roach (*Rutilus rutilus*)	196,851 (98)	1505 (38)	882 (32)
Rudd (*Scardinius erythrophthalmus*)	708 (<1)	22 (<1)	28 (1)
Tench (*Tinca tinca*)	0	0	31 (1)
Others/mixed	846 (<1)	7 (<1)	212 (8)
Total	200,933	3991	2750

2.3. Sampling

Water samples for chemical analyses were taken with a tube sampler (diameter 7.5 cm) from the central part of the lake as an integrated sample from the surface to 10 cm above the sediment. From 1986 to 2015, sampling was conducted 1 to 4 times a month during summer (May–September) and in most years at least once a month during the rest of the year.

Submerged macrophytes were sampled every month during summer and usually at least once during winter (December–February) when macrophytes were present. Water depth, species composition, cover of individual species, total cover and plant height were recorded at 41–192 sampling positions (average = 89 positions, most at dense macrophyte cover), representing the whole lake area. Mean macrophyte cover (Cov, as percentage of the whole lake area, excluding filamentous algae) and mean plant volume inhabited (PVI, PVI = Cov $\times$ plant height/water depth) corresponding to the percentage of the water volume occupied by plants were calculated for each sampling date.

Fish stock composition and relative biomass were investigated with standardised methods every year from 1989 to 2011 and every second year from 2011 to 2015. The fish monitoring was conducted from 15 August to 15 September using eight to twelve 42 m long multiple mesh-sized gill nets with 14 different mesh sizes ranging from 6.25 mm to 75 mm [25,26]. The nets were set in different parts of the lake in late afternoon and retrieved after 18 hours. Except for unharmed large pike, fish caught during the monitoring were not released into the lake again. Quantitative measurements of the total fish stock and the individual species were expressed as catch per unit effort of weight (WPUE) or numbers (NPUE). It should be noted that bream is not easily caught in these survey nets and its relative abundance may have been underestimated.

2.4. Secchi Depth and Chemical Analyses

Secchi depth and chemical analyses included chlorophyll *a* (CHL), total phosphorus (TP), dissolved inorganic phosphorus (DIP), total nitrogen (TN) and dissolved inorganic nitrogen (DIN) calculated as the sum of the concentration of ammonium, nitrate and nitrite. All chemical analyses were performed using standard analytical procedures (see [27] for further details). Except for seasonal data or if otherwise mentioned, all chemical data presented are mean summer concentrations calculated as averages from 1 May to 30 September. In years with clear water, Secchi depth often reached the bottom of the lake and in these cases this was recorded as the Secchi depth.

2.5. Data Analyses

A summer winter ratio obtained by dividing mean summer concentrations (1 May–30 September) by mean concentrations the following winter (1 December–27 February) was calculated for TP (TPs_w), TN (TNs_w), DIP (DIPs_w) and CHL (CHLs_w). As DIN was close to or below the detection limit in most summers, a summer–winter ratio was not calculated for DIN. Statistical tests (*t*-test) were performed using SAS Proc *t*-test with Bonferroni correction to identify differences between the first and second biomanipulation and differences between years with high and low Cov.

Low Cov (Cov < 10%) and high Cov (Cov > 20%) were used to define turbid and clear water periods, respectively. A Cov threshold of ca. 20% has been shown to markedly influence phytoplankton biomass and the CHL:TP ratio in shallow Danish lakes [28].

3. Results

3.1. Fish

In the first years after the first biomanipulation, WPUE was <1.5 kg·net^{-1} and NPUE was <100 net^{-1} (Figure 2). From 1991, WPUE increased rapidly to 3–6 kg·net^{-1} and NPUE rose gradually during the next 10 years to 300–600 net^{-1}. After the second biomanipulation, WPUE declined to 2–4 kg·net^{-1} and NPUE to about 50 net^{-1}. During the whole period, roach and perch completely dominated the fish stock, and in most years they constituted more than 80% in both number and weight.

The relative importance of perch was highest in the first years after biomanipulation, particularly after the first intervention. For a few years after the first biomanipulation, pike constituted about 20% of WPUE, and in some years bream constituted 10%–20% of both WPUE and NPUE.

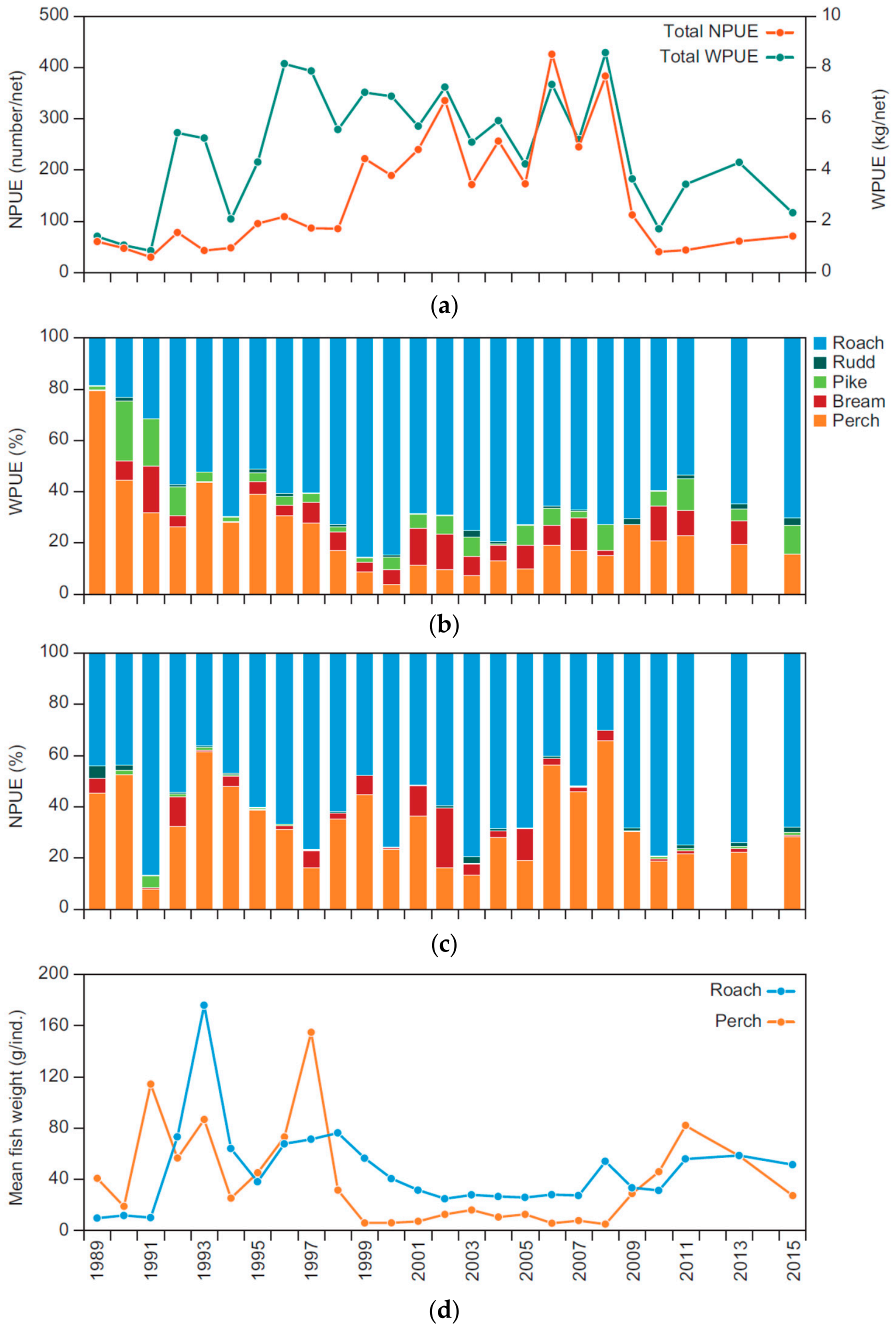

Figure 2. (**a**) Changes in relative fish biomass: WPUE (catch per unit effort of weight) and NPUE (catch per unit effort of weight of number); (**b**) relative fish biomass; (**c**) number composition; (**d**) mean weight of roach and perch during the monitoring period. The second biomanipulation was conducted from 2007 to 2009.

The individual weight of both perch and roach was highest in the first years after the two biomanipulations, albeit with large year-to-year fluctuations, particularly in the clear water periods (Figure 2). In the turbid period between the two biomanipulations (1999–2008), especially perch had a low individual weight, varying between 5.1 and 16.2 g·ind^{-1}, while its weight in the first clear water period (1989–1998) varied from 18.9 to 154.9 g·ind^{-1} and in the second clear water period (2009–2015) from 27.3 to 82.2 g·ind^{-1}. For the whole monitoring period, the mean weights of perch and roach did not differ significantly between periods with high and low Cov (Table 3).

Table 3. Mean (summer and/or winter with min-max shown in parentheses) Secchi depth, CHL, nutrient concentrations, Cov and fish data in years with low Cov (Cov < 10%: 1986–1988, 1993, 1997–2008, n = 16) and years with high Cov (Cov > 20%: 1989–1991, 1994–1995, 2009–2015, n = 12). No summer mean concentrations of NO_3 and NH_4 are given since these were often below the detection limit. The five last rows show summer–winter ratios of CHL (CHLs_w) and nutrients (TPs_w, DIPs_w, TNs_w and DINs_w). *t*-Test results for difference between low and high Cov ae given in the right column (significant differences are marked with bold). According to the Bonferroni correction for multiple comparisons, the results were considered significant for $p < 0.0025$, representing an overall alpha level of < 0.05.

Variable	Low Cov	High Cov	Difference Low-High Cov
Secchi, summer (m)	0.87 (0.61–1.46)	1.51 (1.24–1.78)	$\boldsymbol{p < 0.001}$
Cov, summer (%)	0.8 (0–4)	67.6 (22–86)	$\boldsymbol{p < 0.001}$
CHL, summer (μg·L^{-1})	62.1 (21.3–85.0)	16.9 (9.4–29.5)	$\boldsymbol{p < 0.001}$
CHL, winter (μg·L^{-1})	21.3 (11.6–40.3)	31.2 (5.1–253.5)	$p = 0.58$
TN, summer (mg·L^{-1})	0.97 (0.60–1.25)	0.40 (0.14–0.68)	$\boldsymbol{p < 0.001}$
TN, winter (mg·L^{-1})	0.97 (0.65–1.40)	1.01 (0.40–2.00)	$p = 0.69$
NO_3, winter	0.555 (0.407–0.655)	0.492 (0.185–0.715)	$p = 0.13$
NH_4, winter	0.083 (0–0.268)	0.102 (0–0.261)	$p = 0.52$
TP, summer (mg·L^{-1})	0.120 (0.082–0.151)	0.067 (0.034–0.169)	$\boldsymbol{p < 0.001}$
TP, winter (mg·L^{-1})	0.060 (0.40–0.73)	0.090 (0.040–0.283)	$p = 0.07$
DIP summer	0.049 (0.030–0.084)	0.036 (0.014–0.136)	$p = 0.17$
DIP winter	0.032 (0.020–0.039)	0.063 (0.023–0.120)	$\boldsymbol{p = 0.001}$
WPUE (kg)	6.3 (4.2–8.6)	2.5 (0.8–4.3)	$\boldsymbol{p < 0.001}$
NPUE	220 (43–426)	58 (30–112)	$\boldsymbol{p < 0.001}$
Perch_w (g)	28.1 (5–155)	48.6 (19–114)	$p = 0.22$
Roach_w (g)	51.4 (25–176)	36.6 (10–64)	$p = 0.32$
CHLs_w	3.47 (1.34–6.23)	1.46 (0.07–5.06)	$\boldsymbol{p = 0.0017}$
TPs_w	2.30 (1.43–3.03)	0.75 (0.21–1.68)	$\boldsymbol{p < 0.001}$
DIPs_w	1.61 (0.86–2.46)	0.49 (0.21–1.21)	$\boldsymbol{p < 0.001}$
TNs_w	1.02 (0.69–1.26)	0.36 (0.12–0.62)	$\boldsymbol{p < 0.001}$

3.2. Secchi Depth, CHL and Nutrient Concentrations

Summer means of Secchi depth, CHL and nutrient concentrations were highly variable during the 30 years of monitoring (Figure 3). After the first biomanipulation, Secchi depth increased from 0.6 m to 1.3–1.6 m from 1988 to 1996 but then declined to 0.6–0.8 m during the following 10 years. After the second biomanipulation (2010–2015), Secchi depth increased to above 1.5 m or the maximum depth of the lake. CHL varied correspondingly, with high concentrations (60–80 μg·L^{-1}) before the two biomanipulations and low concentrations (10–30 μg·L^{-1}) for a period after the two biomanipulations. CHL increased steadily from a minimum in 1991 (9.4 μg·L^{-1}) to a maximum before the second biomanipulation of 85 μg·L^{-1} in 2006. Secchi depth was significantly higher and CHL significantly lower in periods with high Cov than in periods with low Cov (Table 3).

Nitrogen and phosphorus concentrations also varied and were generally lowest in the clear water periods with high Cov following the two biomanipulations (TP: 0.03–0.17 mg·L^{-1}; TN: 0.1–0.7 mg·L^{-1}) and highest under turbid conditions with low Cov before the biomanipulations (TP: 0.08–0.15 mg·L^{-1}, TN: 0.6–1.3) (Table 3). Especially after the second biomanipulation from 2010 and onwards, TP and TN reached low levels (Figure 3).

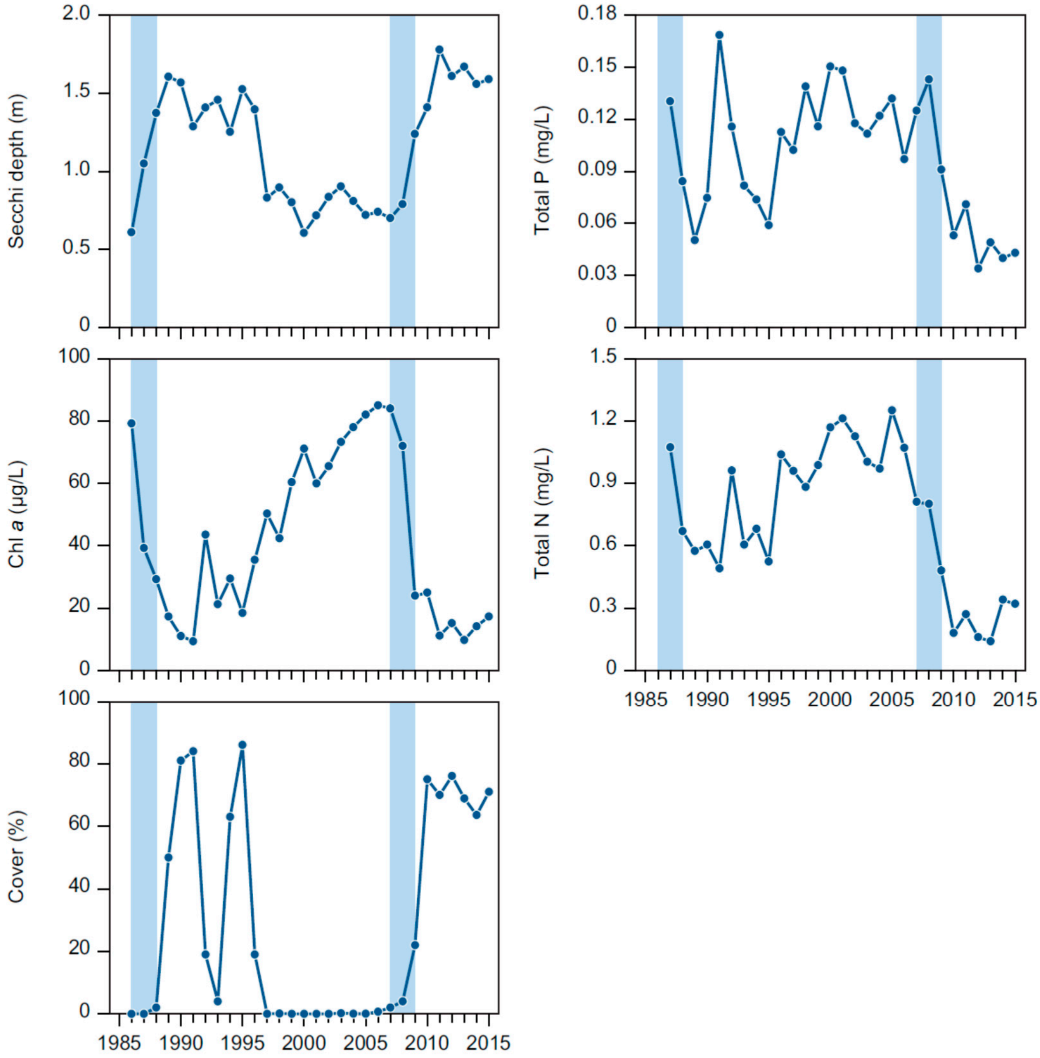

Figure 3. Mean summer Secchi depth, CHL, cover of submerged macrophytes and concentrations of nutrients (TN and TP) from 1986 to 2015. The two periods with fish removal (1986–1988 and 2007–2009) are indicated with blue columns.

On a seasonal scale over the past 10 years, CHL, phosphorus and nitrogen demonstrated marked seasonality, but the seasonal pattern changed after the biomanipulation (Figure 4). Before the second biomanipulation and before the reappearance of high macrophyte Cov (2006–2009), summer CHL and TP concentrations were significantly higher than during the following period when macrophyte Cov was high (2011–2015, Table 4). For example, mean summer TP reached 0.084–0.170 mg·L^{-1} at low Cov compared with 0.036–0.072 mg·L^{-1} at high Cov. In years with a summer peak in TP, this was partly due to increased DIP concentrations, but in years with winter TP peaks this was mainly due to increased DIP concentrations. As to TN, winter peaks were mainly caused by increased nitrate concentrations, ammonia being a contributory factor when submerged macrophytes reappeared. Only TP during summer was significantly different between years with high and low Cov. Before the macrophytes returned, CHL concentrations were usually highest during summer (up to 123–137 μg·L^{-1}), but after the return of macrophytes the concentrations were low in summer (mean summer CHL: 10–25 μg·L^{-1}) and comparatively high (up to 96 μg·L^{-1}) in winter or early spring (Figure 4).

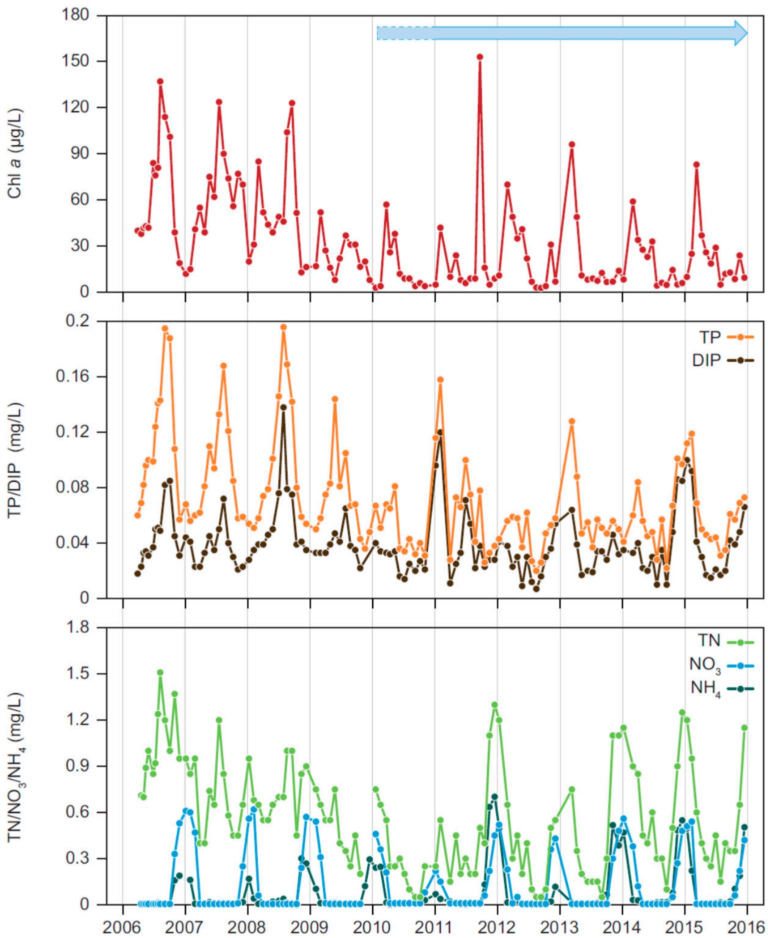

Figure 4. Seasonal variations in CHL, TP, DIP, TN, NO_3 and NH_4, during the past 10 years. The blue arrow denotes occurrence of high macrophyte cover.

Table 4. Mean CHL and nutrient concentrations during summer months (June, July and August) and winter/spring months (January, February and March) during a period with low Cov (2006–2009) and a period with high Cov (2011–2015) as shown in Figure 4. *t*-Test results for differences between years (summer or winter/spring months) with high and low Cov (significant differences are marked with bold). According to the Bonferroni correction for multiple comparisons, the results were considered significant for $p < 0.0045$, representing an overall alpha level of < 0.05. During summer, NO_3 was always below the detection limit (<0.01 mg·L^{-1}) and is not included.

Variable	2006–2009 (Low Cov) Mean (Min–Max)	2011–2015 (High Cov) Mean (Min–Max)	Difference (Low–High Cov)
CHL summer	70.7 (30.0–94.5)	11.5 (8.1–16.2)	$p = 0.028$
CHL winter	30.0 (30.8–45.3)	46.3 (18.9–96.1)	$p = 0.53$
TP summer	0.128 (0.084–0.170)	0.048 (0.036–0.072)	$\boldsymbol{p = 0.002}$
TP winter	0.059 (0.054–0.062)	0.086 (0.051–0.128)	$p = 0.15$
DIP summer	0.061 (0.047–0.098)	0.027 (0.016–0.049)	$p = 0.029$
DIP winter	0.029 (0.018–0.034)	0.057 (0.034–0.078)	$p = 0.047$
TN summer	0.79 (0.47–0.66)	0.27 (0.19–1.25)	$p = 0.045$
TN winter	0.77 (0.65–0.90)	0.75 (0.32–1.03)	$p = 0.84$
NO_3 winter	0.33 (0.25–0.42)	0.24 (0.01–0.47)	$p = 0.39$
NH_4 summer	0.012 (0.005–0.025)	0.009 (0.007–0.012)	$p = 0.58$
NH_4 winter	0.056 (0.008–0.104)	0.145 (0.013–0.251)	$p = 0.18$

The seasonality of nutrient concentrations dependency on clear or turbid conditions and/or the abundance of macrophytes is illustrated by the ratio between summer and winter concentrations of TP, DIP, TN and CHL relative to Cov (Figure 5). In years with low Cov (<10%), TP was 1.4–3.0 times (mean TPs_w = 2.30) higher during summer than during the following winter, but in years with high Cov (>20%) TP was in most years highest during winter (mean TPs_w = 0.75). The same pattern emerged for DIP (mean DIPs_w = 1.61 at low Cov and 0.49 at high Cov), TN (mean TNs_w = 1.02 at low Cov and 0.36 at high Cov) and CHL (mean CHLs_w = 3.47 at low Cov and 1.46 at high Cov). All three nutrient ratios and CHL differed significantly between high and low Cov (Table 3).

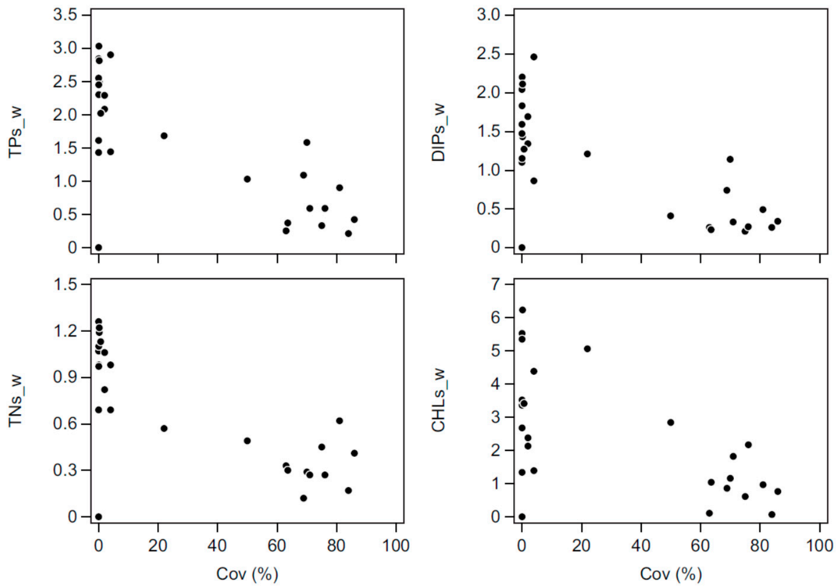

Figure 5. TPs_w, DIPs_w, TNs_w and CHLs_w relative to Cov from 1986 to 2015. No winter data available for 1992/1993 and 2002/2003.

A comparison of the various chemical variables following the first and second biomanipulation demonstrated no significant differences in nutrient concentrations under clear water conditions with Cov > 20% (Table 5). The only significant difference was found for summer TN, which was significantly lower after the second biomanipulation.

Table 5. Mean CHL and nutrient concentrations in years with Cov > 20% after the first (1989–1991, 1994–1995, $n = 5$) and the second biomanipulation (2009–2015, $n = 7$). *t*-Test results for differences between the first and second biomanipulation (significant differences are marked with bold). According to the Bonferroni correction for multiple comparisons, the results were considered significant for $p < 0.0056$, representing an overall alpha level of <0.05. No summer mean concentrations of DIN are given as NO_3 and NH_4 concentrations were often below the detection limits.

Variable	First Biomanipulation Mean (Min–Max)	Second Biomanipulation Mean (Min–Max)	Difference First—Second
CHL summer	17.3 (9.3–28.2)	18.8 (9.7–34.9)	$p = 0.76$
CHL winter	60.4 (5.0–265.5)	14.3 (3.8–29.3)	$p = 0.42$
TP summer	0.085 (0.050–0.168)	0.054 (0.034–0.093)	$p = 0.16$
TP winter	0.130 (0.039–0.242)	0.069 (0.049–0.104)	$p = 0.12$
DIP summer	0.049 (0.014–0.136)	0.027 (0.015–0.045)	$p = 0.39$
DIP winter	0.067 (0.023–0.136)	0.054 (0.032–0.086)	$p = 0.50$
TN summer	0.57 (0.47–0.66)	0.27 (0.14–0.44)	$\boldsymbol{p < 0.001}$
TN winter	1.35 (0.87–2.15)	0.92 (0.70–1.20)	$p = 0.07$
DIN winter	0.74 (0.60–0.90)	0.54 (0.39–0.68)	$p = 0.0084$

A box-plot representing the monthly average for the whole monitoring period (1986–2015) with either low or high Cov illustrates the seasonal differences in CHL and nutrients between turbid and clear periods (Figure 6). The results of a test of the differences between low and high Cov during the different months are shown in Table 6. At low Cov, maximum CHL generally occurred in August, and when Cov was high it usually peaked in March. CHL was significantly lower at high Cov than at low Cov from April to October. TP concentrations were markedly higher during summer, peaking in July–August, but only when Cov was low. From April to July and in September, TP was significantly lower at high Cov than at low Cov. Maximum TN tended to appear during summer when Cov was low and in winter when Cov was high. TN was significantly lower at high Cov than at low Cov from April to September. DIN was low during the whole summer season, from April to September at all Cov levels.

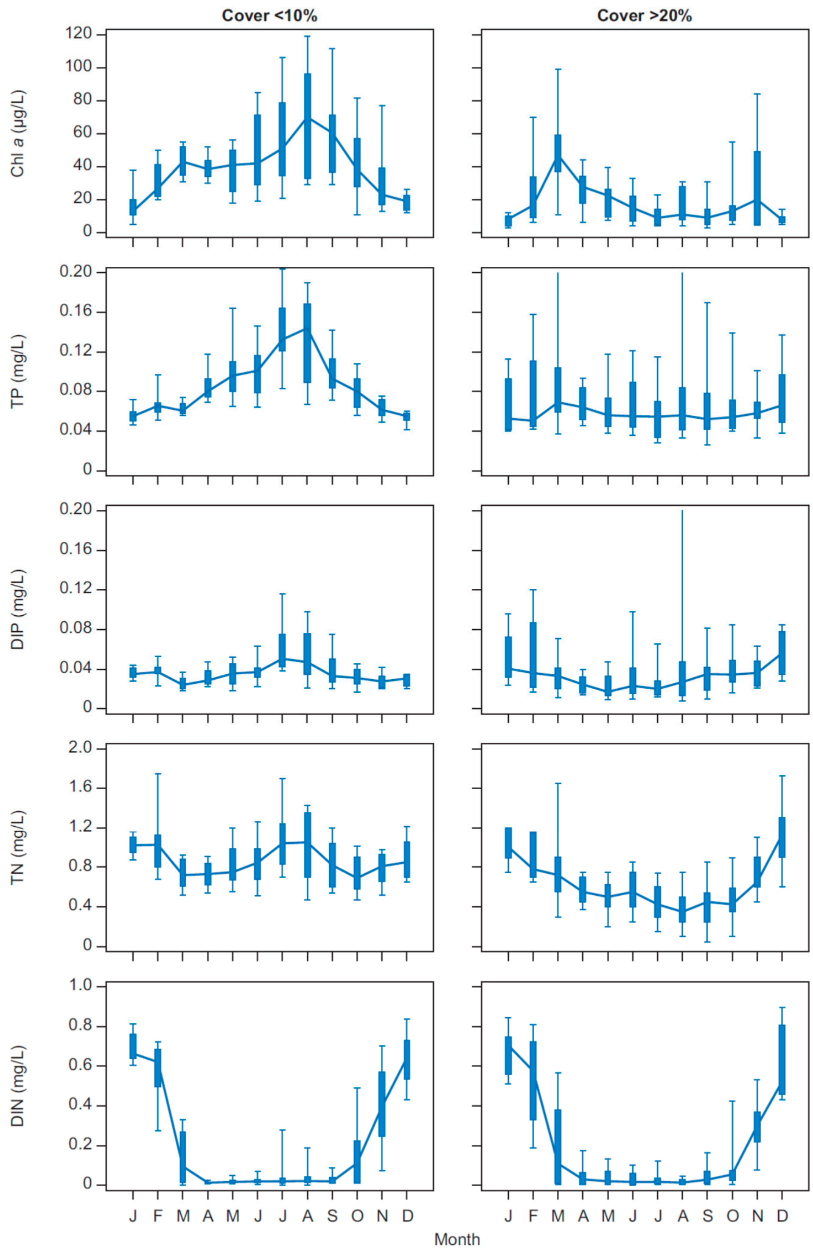

Figure 6. Box-plots showing monthly (January–December) seasonal CHL and nutrient concentrations during years with high Cov (Cov > 20%, 1989–1991, 1994–1995 and 2009–2015) and low Cov (Cov < 10%, 1986–1988, 1993 and 1997–2008). The box-plots show 10%, 25%, 75% and 90% fractiles. Median values are connected by a solid line.

Table 6. *t*-Test for monthly significant differences between low and high Cov for the variables and years shown in Figure 6: - ($p < 0.05$), -- ($p < 0.01$) and --- ($p < 0.001$) when the variable is lowest at high Cov and: + ($p < 0.05$) and ++ ($p < 0.01$) when the variable is highest at high Cov.

Variable, Month	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
CHL				---	--	---	---	---	---	---		-
TP				-	---	-	---		---			
DIP					-		---					++
TN				--	---	---	---	---	---			
DIN				+				-				

3.3. Submerged Macrophytes

Cov fluctuated widely during the investigation period, from <0.1% (no or very few macrophytes) to more than 80% (Figure 3). During the year of clear water after biomanipulation, Cov was relatively low, but maximum Cov occurred already 2–3 years after each biomanipulation. After the first biomanipulation, the macrophyte community collapsed in 1992–1993 and Cov decreased to <20%. After this, the macrophytes returned and reached high Cov again before they disappeared from 1997 and onwards until the second biomanipulation. After the second biomanipulation, Cov has remained high (64%–75% from 2010 to 2015).

The macrophyte community was dominated by *Elodea canadensis* throughout the periods with high Cov (Table 7). In some years, particularly after the first biomanipulation, *Potamogeton crispus* had a considerable cover, and in other years also the filamentous algae *Lemna trisulca*, *P. pusillus* and *P. obtusifolius* were richly represented. The macrophyte community exhibited higher richness (up to 10 taxa) after the second than after the first biomanipulation (up to four taxa).

The seasonality of the submerged macrophytes during the past 10 years shows a development from low to high Cov and PVI during a calendar year (Figure 7). In years with high density of macrophytes, they did not disappear during winter, but Cov, and in particular PVI, decreased to markedly lower levels (in most years from >30% to <10%).

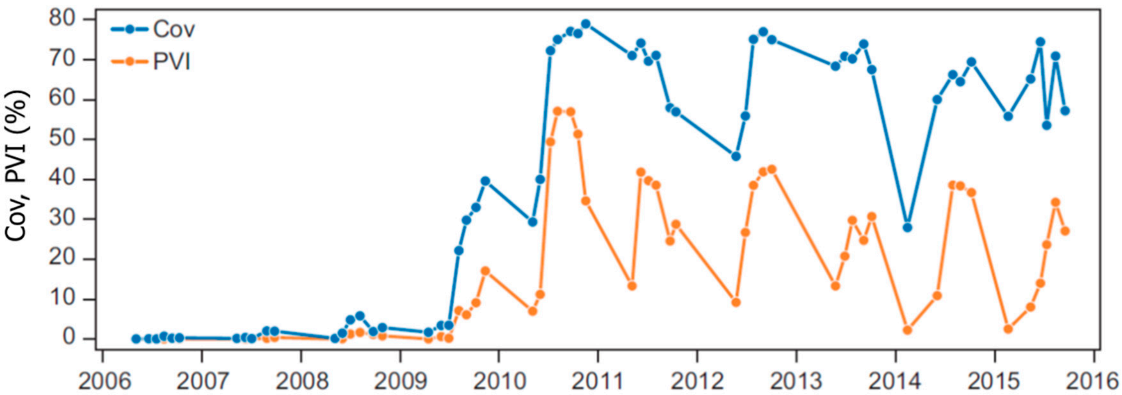

Figure 7. Seasonal Cov and PVI in Lake Væng during the past 10 years.

Table 7. Submerged macrophytes, filamentous algae and indication of the mean summer cover of each taxon from 1986 to 2015.

Species/Taxa	86	87	88	89	90	91	92	93	94	95	96	97	98	99	00	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
Elodea canadensis		-	-	■	■	■	■	=	■	■	■	-	-					-	-	-	-	-	=	■	■	■	■	■	■	■
Filamentous algae									=	-								-			-		-	=	-	■	=	=	-	=
Lemna trisulca															-									-	-	-	-	=	=	=
Nitella flexilis						-	-						-															-	-	-
Potamogeton berctoldii																								-				-	=	=
P. crispus	-	-	=	■	=	-	-	-	=	-						-		-	-		-	-	-			-		-		-
P. friesii											-												-				-	-		
P. obtusifolius																									-			-	-	=
P. pectinatus										-															-		-	-		-
P. pusillus																									-			-	=	=

Notes: Empty cell: absent; -: <1% cover; =: 1%–10% cover ; ■: >10% cover.

4. Discussion

We would like to emphasise three main findings from our long-term study of Lake Væng: (1) biomanipulation by fish removal can greatly improve lake water quality for a number of years and repeated fish removal can help maintain the positive effects; (2) the effects of the fish removal cascade all the way down to the level of nutrients, altering the nitrogen and phosphorus cycling markedly on both the yearly and seasonal scale; and (3) less fishing effort seems needed at the second biomanipulation to achieve similar effects as during the first manipulation.

The effects of both biomanipulation attempts in Lake Væng were comparable with those obtained in other shallow Danish lakes [10], and in many other parts of the world as well [9,11,29]. We are not able to ascertain whether the fish biomass was lower before the second than before the first biomanipulation in Lake Væng, but the chemical and biological effects of the second biomanipulation appear to be just as strong as after the first biomanipulation, even though 30% less fish was removed. This indicates that a follow-up fish removal requires less effort, in our case probably due to the presence of a relatively large population of small perch after the first manipulation. These small perch have the potential to reach an older, larger and piscivorous stage after fish removal and thereby contribute to controlling the roach population [6]. Perch mean weight increased after both biomanipulations, reflecting the potential of perch to reach the piscivorous stage when mass removal of planktivorous and benthivorous fish produces a dietary change in the remaining species towards increased utilisation of benthic resources [30].

The effects obtained from the first and second biomanipulation were remarkably similar: within 1–2 years after the fish removal, the lake had changed from a turbid to clear water state with a strongly reduced phytoplankton biomass, within 2–3 years the submerged macrophyte community (dominated by *Elodea* after both biomanipulations) had developed from almost absence to almost complete cover all over the lake from bottom to surface and, finally, the shifts between turbid and clear water changed the internal cycling of nutrients markedly and in the same manner. After the second biomanipulation, lower nitrogen concentrations and a more diverse macrophyte community have been observed, but overall the effects of the two biomanipulations have been very similar and they suggest a strong top-down impact of fish followed by similar cascading effects. Although nutrients and phytoplankton are not independent variables, many of these effects most likely reflect the reduced phytoplankton biomass created through by less nutrients and higher zooplankton grazing [6] and improved light conditions, enabling a shift from mainly pelagic to mainly benthic primary producers. The major decline in CHL after biomanipulation has previously been shown to produce relatively minor effects on gross production and respiration but a higher seasonal amplitude in net production [6], reflecting the shift in dominant primary producers and higher variability in the biomass of macrophytes. Not many case studies on repeated fish removals are available, but in Dutch Lake Zwemlust a second fish removal was observed to create similar effects as the first removal [31]. The absence of long-term effects was ascribed to the high nutrient loading, which only allows submerged macrophytes to dominate in the "window of opportunity" occurring in the first years after the fish removal.

Both biomanipulations had pronounced effects on the nutrient concentrations in Lake Væng, and TP and TN were reduced to about 50% of pre-biomanipulation levels with increasing water clarity. As no changes in external loading have occurred, the lower concentrations must be produced by changes in the internal nutrient dynamics. Particularly, the internal summer release of phosphorus, which is often seen in shallow eutrophic lakes [32–34], most likely decreased notably, which suggests a radical change in the way that the sediment acts as a sink or source of phosphorus depending on the biological structure. Similar effects have been observed in other biomanipulated lakes and in comparisons of inside and outside submerged macrophyte stands [10,35]. This may in part be attributed to improved light conditions at the sediment surface. This leads to higher benthic primary production [36,37], facilitating the uptake of phosphorus from the lake water and a subsequent reduction of the sediment phosphorus release due to the improved redox conditions. More details on the role of benthic algae in the phosphorus exchange between sediment and water are available in [38].

In a Dutch shallow lake ecosystem [39], phosphorus concentrations fluctuated strongly relative to variations in the growth of aquatic plants despite the fact that external phosphorus loading was stable. Years with low Cov had higher P concentrations than years with high Cov, the patterns thus being identical with those recorded in Lake Væng. In addition, lower sedimentation of phytoplankton may increase redox conditions in the sediment and reduced fish-induced resuspension may contribute to lower internal P loading. For nitrogen, decreased density of cyprinids (mainly roach and bream) was observed to lead to decreased N concentrations and increased N retention in four Danish lakes [40]. In Lake Væng, summer N-ret% increased from 22%–39% before to 60%–72% after the first biomanipulation, probably due to a decrease in organic N in the lake, reduced resuspension by fish and higher denitrification in the sediment [40].

The importance of biological structure for the internal cycling of nutrients is also seen by the shifts in the seasonal concentrations of phosphorus, reflecting the high fluctuations in the abundance of submerged macrophytes. A likely explanation for the peaks in high winter concentrations of TP and DIP in periods with high Cov could be phosphorus release from decomposing macrophytes and redox sensitive release of phosphorus accumulated in the sediment. A dense and senescent macrophyte biomass at the sediment surface can result in reduced redox conditions and a reduced P sorption capacity [41].

Highly dynamic and cyclical growth of submerged macrophytes and regime shifts between turbid and clear water conditions as those seen in Lake Væng have been recorded in several other lakes [39,42,43]. In Lake Væng, P retention has been high, particularly after the second biomanipulation as demonstrated by low P concentrations in the lake water. Consequently, a mobile pool of phosphorus has accumulated in the sediment, a pool that may eventually be released, particularly if the lake returns to turbid conditions. High macrophyte Cov may then in the long term increase the risk of a return to turbid conditions, a negative feedback that might be supported by the build-up of decaying plant material in the sediment [37]. It has been suggested that also the climate may contribute to the regime shifts through lowered macrophyte production, and in Lake Tåkern, Sweden, an eight-year cyclic periodicity of organic nitrogen has been observed to depend on the seasonal macrophyte production [44]. Nitrogen may also be important, particularly for creating favourable growth conditions for submerged macrophytes, and high N concentrations have negative impacts on macrophytes [45–47].

In Lake Væng, the seasonality in phytoplankton biomass changed markedly after the two biomanipulations, with a shift from maximum CHL concentrations during summer when the lake was turbid and without macrophytes to a maximum in winter when the lake was clear and rich in macrophytes. During summer in the presence of macrophytes, phytoplankton may be limited by grazing from zooplankton and other filtrators, by nutrients and the low light availability caused by the high macrophyte density. The significantly low DIN levels throughout summer under both clear and turbid conditions indicate that phytoplankton may be limited by nitrogen during the major part of their growing season, as also seen in other Danish shallow lakes and in other parts of the world [48,49]. During winter, increased nutrient availability, lower macrophyte abundance and less tall macrophytes may increase the phytoplankton biomass.

The numbers of grazing waterfowl, mute swan (*Cygnus olor*) and coot (*Fulica atra*) have fluctuated widely in Lake Væng from year to year concurrently with the abundance of submerged macrophytes [23]. Waterfowl may negatively impact the abundance of submerged macrophytes and thereby the stability of clear water conditions, but even though waterfowl were present in high numbers in some years the decline in macrophytes cannot likely be ascribed to waterfowl grazing. A study from UK concluded that the potential of waterfowl herbivory to shift a macrophyte-dominated state into a phytoplankton-dominated state is low due to the recovery of aquatic plants during the growing season when bird populations decline [50]. In winter 1991/1992 in Lake Væng, the number of coot and mute swan reached a maximum of, respectively, 800 and 300 individuals. During this winter, Cov decreased from 84% to 44% and PVI from 60% to 10%. Total macrophyte consumption by the waterfowl during this winter was estimated to 440 $kg \cdot DW \cdot ha^{-1}$ [23]. The P content of *Elodea* varies relatively widely, but assuming a mean content of 0.7% of the dry weight [51] this would correspond to a potential release (if all phosphorus in the plant tissue is released to the water) of 3 kg·P/ha or 0.3 mg·L^{-1} (with a mean depth of 1 m) during the whole winter. Thus, as the hydraulic retention in Lake Væng is only a few weeks, the increase caused by decomposing or ingested macrophytes can probably only explain a minor part of the increase seen in lake water nutrient concentrations.

The future environmental state of Lake Væng is uncertain and a number of unknown factors behind, for example, the fluctuating macrophyte abundance observed make predictions difficult. Factors in favour of clear water conditions are: (1) the macrophyte community appears more stable after the second biomanipulation with a more constant high abundance and presence of more species than after the first biomanipulation; (2) Nitrogen concentrations are lower now than after the first biomanipulation, augmenting the chances of submerged macrophytes maintaining their high abundance and thus stabilising clear water conditions [45,52]. Other factors could, however, indicate a return to turbid conditions: (i) the first biomanipulation did not create permanently clear water conditions; and (ii) the high nutrient concentrations in the groundwater may counteract the maintenance of clear water conditions [24]. The mean concentration of total phosphorus in the groundwater just below the lake bed is 0.162 mg·L^{-1} [24]. However, as TP concentrations vary considerably between the groundwater wells, it is difficult to establish a precise phosphorus loading of the lake. It has been estimated (GEUS, personal communication) that Lake Væng receives between 200 and 280 kg P yearly with the groundwater, corresponding to 1.3–1.8 g·P·m^{-2}·year^{-1} or a mean inlet TP concentration of 0.12 mg·L^{-1}. This is more than twice as high as the suggested maximum levels of 0.6–0.8 g·P·m^{-2}·year^{-1} [53] in order to ensure long-term success of biomanipulation in shallow lakes; (3) Paleolimnological investigations of the upper 0.5 m of the sediment involving calculation of diatom-inferred TP concentrations indicate that Lake Væng has been eutrophic for centuries, with TP concentrations fluctuating between 100 and 160 μg·L^{-1} [54]; (4) The dominance of *Elodea* may not favour stable clear water conditions as this submerged macrophyte species is known to show rapid changes in abundance. In a Dutch biomanipulation experiment, *Elodea* abundance showed high variability, ranging between high to sudden low abundances [55]; (5) The present high retention of phosphorus entails accumulation of a phosphorus pool in the surface of the sediment, and this might be released should the lake return to turbid conditions. Consequently, at the present nutrient levels, clear water conditions in Lake Væng are difficult to maintain, and regular repetition of biomanipulation therefore seems required.

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