

Article

Nutrients and Saltwater Exchange as Drivers of Environmental Change in a Danish Brackish Coastal Lake over the Past 100 Years

Jonathan P. Lewis ^{1,*}, David B. Ryves ¹, Martin Søndergaard ^{2,3}, Torben Linding Lauridsen ^{2,3}, Lisolette Sander Johansson ², Sh. Tserenpil ⁴, Peter Rasmussen ⁵ and Erik Jeppesen ^{2,3,6,7,*} 

¹ Geography and Environment, Loughborough University, Loughborough LE11 3TU, UK

² Department of Ecoscience, Aarhus University, Vejløvej 25, 8600 Silkeborg, Denmark

³ Sino-Danish Centre for Education and Research, Beijing 100049, China

⁴ Nuclear Research Center, National University of Mongolia, Ulaanbaatar 14200, Mongolia

⁵ Environmental Archaeology and Materials Science, National Museum of Denmark, Brede Værk, 1471 Lyngby, Denmark

⁶ Limnology Laboratory, Department of Biological Sciences and Centre for Ecosystem Research and Implementation, Middle East Technical University, 06800 Ankara, Turkey

⁷ Institute of Marine Sciences, Middle East Technical University, 33731 Mersin, Turkey

* Correspondence: j.p.lewis@lboro.ac.uk (J.P.L.); ej@ecos.au.dk (E.J.)

Abstract: Many northwest European lake systems are suffering from the effects of eutrophication due to continued loading and/or poor, ineffective management strategies. Coastal brackish lakes are particularly difficult to manage due to complex nitrogen, phosphorus, and salinity dynamics that may exert varying influence on lake biological communities, but long-term data on how these important and often biodiverse systems respond to change are rare. In this study, palaeolimnological data (including sedimentary parameters, diatoms, and plant macrofossils) and environmental monitoring data (for the last ~40 years) have been used to assess environmental change over the last 100 years in Kilen, a brackish lake in northwest Jutland, Denmark. Kilen has been regularly monitored for salinity (since 1972), TP (from 1975), TN (from 1976), and since 1989 for biological data (phytoplankton, zooplankton, and macrophytes), which allows a robust comparison of contemporary and paleolimnological data at high temporal resolution. The palaeolimnological data indicate that the lake has been nutrient rich for the last 100 years, with eutrophication peaking from the mid-1980s to the late 1990s. Reduced nutrient concentrations have occurred since the late 1990s, though this is not reflected in the sediment core diatom assemblage, highlighting that caution must be taken when using quantitative data from biological transfer functions in paleolimnology. Lake recovery over the last 20 years has been driven by a reduction in TN and TP loading from the catchment and shows improvements in the lake water clarity and, recently, in macrophyte cover. Reduced salinity after 2004 has also changed the composition of the dominant macrophyte community within the lake. The low N:P ratio indicates that in summer, the lake is predominately N-limited, likely explaining why previous management, mainly focusing on TP reduction measures, had a modest effect on the water quality of the lake. Despite a slight recovery, the lake is still nutrient-rich, and future management of this system must continue to reduce the nutrient loads of both TN and TP to ensure sustained recovery. This study provides an exceptional opportunity to validate the palaeolimnological record with monitoring data and demonstrates the power of using this combined approach in understanding environmental change in these key aquatic ecosystems.

Keywords: nutrient loading; total nitrogen; total phosphorus; salinity; eutrophication; diatoms; macrophytes; lake monitoring; transfer function



Citation: Lewis, J.P.; Ryves, D.B.; Søndergaard, M.; Lauridsen, T.L.; Johansson, L.S.; Tserenpil, S.; Rasmussen, P.; Jeppesen, E. Nutrients and Saltwater Exchange as Drivers of Environmental Change in a Danish Brackish Coastal Lake over the Past 100 Years. *Water* **2023**, *15*, 1116. <https://doi.org/10.3390/w15061116>

Academic Editor: Ryszard Gołdyn

Received: 31 January 2023

Revised: 7 March 2023

Accepted: 9 March 2023

Published: 14 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The importance of phosphorus in limnic systems is well understood and remains a key issue in lake management and ecosystem restoration [1–5]. However, coastal studies

have focused more on total nitrogen [6,7], as N is considered to be more often limiting in marine systems than P [8]. Whilst the relative roles of N and P in causing nutrient enrichment and eutrophication in aquatic systems have long been debated (e.g., [8]), it is now recognised that control of both P and N levels in the natural environment is often needed to prevent problems associated with nutrient enrichment in both shallow lakes and estuarine environments [9,10]. Nutrient enrichment and eutrophication has been a particularly prevalent problem in Danish lakes and coastal systems since the 1970s [11,12], resulting in the initiation of various monitoring programs and catchment scale strategies aimed at reducing fertiliser use and nutrient loading into these systems (e.g., [13,14]). These programs have had varying degrees of success across Denmark [15,16], but sustained anthropogenic pressure has meant that eutrophication remains an issue today and one that may increase again in the future [17]. Many Danish lakes and coastal systems are still eutrophic and, in some cases, are seeing previous trends in recovery being reversed [16,18].

In addition to nutrients, it is also critical to understand the relative influence of other potential confounding variables which might influence biological communities to formulate a holistic management strategy for the conservation of coastal and limnic systems [19–21]. Salinity changes in coastal systems (including coastal lakes) may also influence the ecological response to environmental change, directly through the physiological response of flora and fauna due to changing osmotic pressure [22] and indirectly by causing or enhancing stratification through density differences (e.g. [23]). Salinity stratification may occur both when inflows of higher salinity (marine) water sink below less dense fresh-brackish water above or if inflowing freshwater from the terrestrial catchment creates lower-density water lenses sitting above the brackish marine waters below. Such stratification can lead to anoxia in bottom waters and nutrient (especially P) release from underlying sediments, particularly as salinity stratification (unlike thermal stratification) may last substantially longer than the growing season and, potentially, for multi-annual periods [23]. Inputs from marine waters may also reinforce nutrient effects in brackish lakes by affecting trophic structure and interactions, which also has implications for water quality [24–27]. The complex interplay of nutrient loading (from terrestrial and/or marine sources), salinity dynamics, stratification, and nutrient release from the sediment can drive wholesale ecological changes within brackish lake and coastal systems [26,28,29]. This includes, but is not limited to, changing species composition across trophic levels, reduced biological diversity, increased phytoplankton abundance, subsequent reduced light penetration, loss of benthic flora and fauna, and anoxic conditions in deeper waters [10,26,30,31]. If conditions pass a critical threshold, a system may theoretically shift from a ‘stable’ state, making it increasingly difficult to reverse using conventional management methods [20,32], though recent data suggest that this is rare for shallow lake systems on longer timescales [33].

There are only a few studies that investigate long-term ecological changes in lakes affected by shifting salinities and simultaneous changes in anthropogenic pressures (e.g., nutrient loading, land use, climate warming) in coastal brackish lakes [26,27,34]. Kilen (in the Limfjord region of northern Jutland in Denmark) provides a rare opportunity to examine ecosystem dynamics in a coastal brackish lake, which is still narrowly connected to the Limfjord (and hence the North Sea) via a sluice-controlled channel, and subject to variable salinity and nutrient levels [35]. Here, we assess the ecological effects of changing environmental conditions (nutrient status, productivity, salinity) within the basin over the last ~100 years in response to human impact. The analysis is based on palaeolimnological multiproxy analyses, which include physical sedimentary analyses (sediment accumulation rate, organic, calcium carbonate, and minerogenic content), plant macrofossils and diatoms, from which quantitative inferences of total nitrogen and salinity have been generated using diatom-based transfer functions. We further include monitoring data from the 1970s (for TN, TP, salinity, and Secchi depth) and external nutrient loading and biological survey data for 1989–2019 [36].

Obtaining a well-preserved and comprehensive sediment record at a site with available long-term monitoring data is rare, particularly for coastal lakes. However, comparing

and combining these datasets is key for developing a much longer-term perspective of system dynamics and response to environmental, climatic, and anthropogenic influence than monitoring data can offer alone [37]. It is also important for the critical evaluation of palaeolimnological methods, such as biological transfer functions, which have come under scrutiny more recently [38,39]. Recent studies have shown that caution must be exercised when using such quantitative tools in modern paleolimnology (e.g., [38–40]) due to difficulties with establishing which variables can be reliably inferred. In particular, it can be challenging to isolate a clear signal for a particular variable when multiple (confounding) variables are frequently exerting influence on the target biological community [38,39]. Understanding the long-term and often complex biological response to changes in interacting variables, such as salinity, nutrients, and light, is vital, both to predict how coastal lake systems such as Kilen will respond to future climate and anthropogenic pressures affecting northwest Europe [41,42] and to inform strategies to underpin sustained management and conservation of these key aquatic ecosystems and environmental assets.

2. Materials and Methods

2.1. Study Area

Kilen (56°30′005″ N, 08°34′089″ E) is a shallow, brackish, eutrophic lake situated in the Limfjord region of northern Jutland, Denmark (Figure 1). The lake is ~5 km long and 1 km wide with a surface area of 319–334 ha and an average depth of 2.9 m (max depth of 6.5 m) [2,43]. Despite significant changes in its connection with the Limfjord due to human activity (as discussed below), the lake area has not changed substantially over the last ~150 years. Although shallow, in some years, Kilen has been periodically strongly stratified due to inflowing higher salinity water from the Limfjord, which underlies upper, less dense brackish water.

Prior to 1856/1857 (all dates common era; CE), Kilen was a marine fjord with relatively high salinity, being connected to the Limfjord system, which became open to the North Sea in the west following a storm in 1825 [44]. However, following the building of a road and rail embankment in 1856/1857, Kilen was isolated from the Limfjord, transitioning into a brackish lake system, which today is only connected to Struer Bay and the wider Limfjord system via a narrow sluice in the southeast corner (Figure 1). This sluice was built in 1984 and is nowadays maintained for regulating salinity, nutrient, and oxygen levels within the lake. Since its artificial creation as a lake (following its separation from the Limfjord), the lake has been subject to nutrient enrichment from surrounding industry and agriculture [35]. Kilen has been protected as a nature reserve since 1952, and today it is an important breeding and moulting ground for wetland birds, particularly the light-bellied brent goose (*Branta bernicla hrota*).

Catchment nutrient loading is dominated by diffuse sources from open land for both phosphorus and particularly for nitrogen (Table 1). Increasing nutrient concentrations (both P and N) from point sources were noted from the 1960s following the building of four fish farms and later directly recorded during a monitoring program in the 1970s [35]. Subsequent reduction of TP in the late 1970s is believed to have been related to improved water exchange with the Limfjord and reduced TP loading from these fish farms. An improved sluice installed in 1984 increased water exchange with the Limfjord and subsequently led to higher salinities, but the formation of occasionally anoxic saltwater pockets above the sediment led to greater sediment release of phosphorus [35]. Since then, sluice operation has varied, with periods when the lake has become fresher and at other times leading to an increase in salinity ([36]; and see below). Attempts have been made to reduce the nutrient input from both point sources, including from two trout rearing pond systems, and from diffuse sources, which together resulted in decreased inputs of both phosphorus and nitrogen from 1989 to 2005 (Table 1).

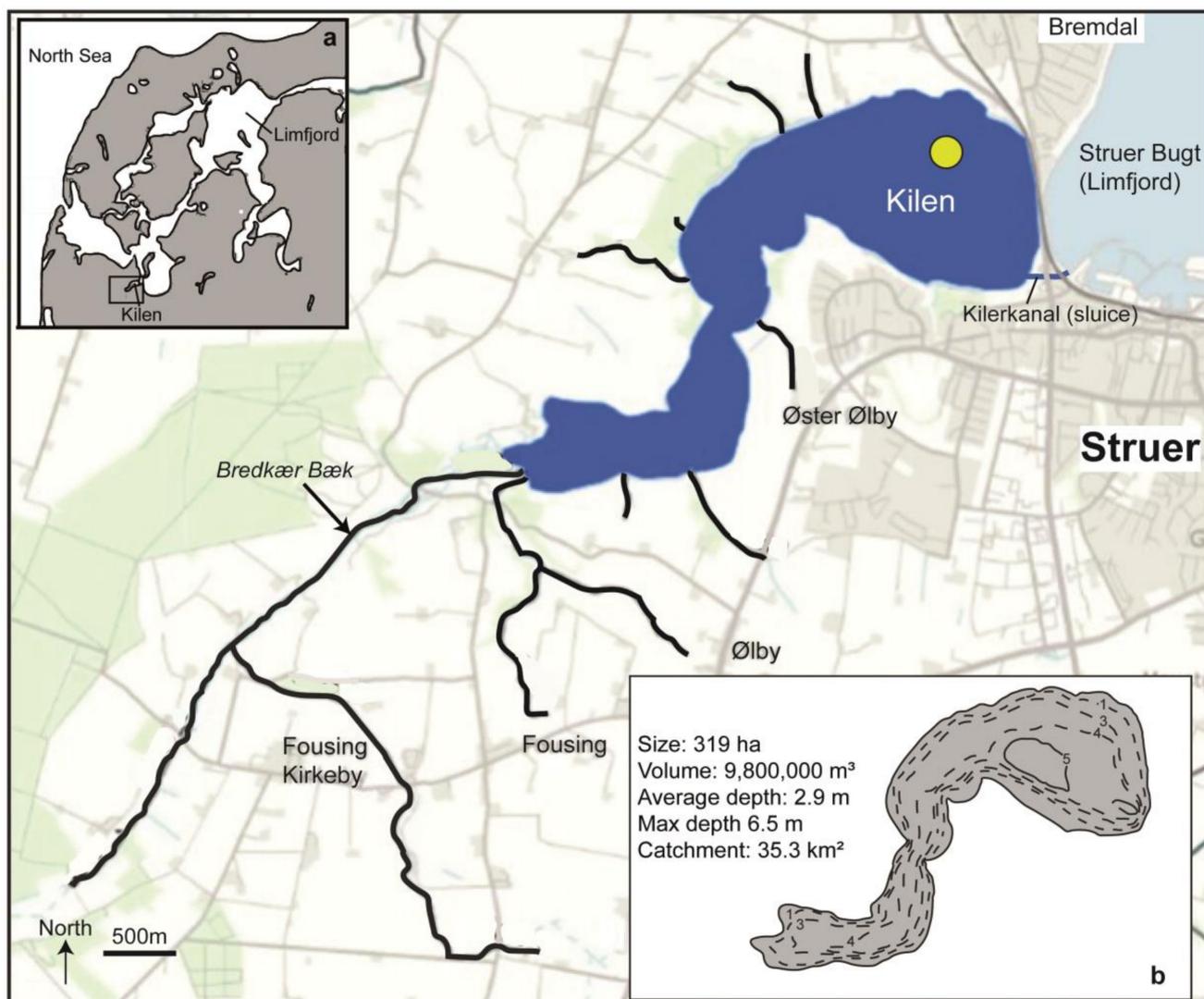


Figure 1. Map showing the study site Kilen, major fluvial inlets, modern sluice (the Kilerkanal), and coring location (yellow dot). (a) shows the location of Kilen in relation to northwest Jutland and the Limfjord. (b) shows simplified bathymetry of the lake and catchment characteristics.

Table 1. External loading from the different catchment sources to Lake Kilen during two periods with extensive data (1989–1993 and 1993–2001). Numbers in brackets represent the percentage contribution, with rounding (hence the sum may not always equal 100%) (modified from [45]).

	Period (Average)	Sewage and Rainwater	Fish Ponds	Scattered Villages	Open Land (Diffuse Sources)	Atmospheric Deposition	Total Input from Catchment
TP (kg y ⁻¹)	1989–1993	224 (8)	936 (34)	309 (11)	1204 (44)	50 (2)	2723 (100)
	1993–2001	52 (3)	344 (18)	70 (4)	1386 (73)	50 (2)	1902 (100)
TN (kg y ⁻¹)	1989–2003	621 (0.5)	7325 (6)	906 (0.8)	101,945 (87)	6680 (6)	117,477 (100)
	2001–2005	196 (0.2)	3202 (4)	302 (0.3)	74,240 (88)	6680 (8)	84,620 (100)

2.2. Sediment Sampling and Core Chronology

In April 2007, a HON-Kajak corer [46] was used to retrieve a sediment core at a depth of 3.9 m in the north-central part of the basin, collecting the uppermost 36 cm of sediment (3.90–4.26 m below present lake level) from the basin. Following retrieval, a preliminary

assessment was made of the core for lithological changes/hiatuses before it was extruded on-site into 0.5 cm sections.

For dating of the profile, a total of 23 freeze-dried samples (1 cm homogenised slices) were sent to the Gamma Dating Centre, University of Copenhagen, for analysis of ^{210}Pb , ^{137}Cs , and ^{226}Ra activity. The activity was measured via gamma spectrometry, carried out on a Canberra low germanium well-detector. ^{210}Pb was measured via its gamma peak at 46.5 keV, ^{226}Ra via the granddaughter ^{214}Pb (peaks at 295 and 352 keV), and ^{137}Cs via its peak at 661 keV. Dates were calculated using a modified constant rate of supply model [47], with linear extrapolation used for dates at the very bottom of the profile (outside the ^{210}Pb time scale).

2.3. Proxy Analyses

Loss-on-ignition (LOI) for organic matter, calcium carbonate (CaCO_3), and mineral content was performed at 1 cm resolution on aliquots of pre-dried sediment following standard methodology [48,49]. All samples from the HON-Kajak sediment core were wet sieved at 1 cm resolution in two size fractions (500 μm and 90 μm). From these fractions, all plant macrofossils and/or faunal remains were picked and identified where possible. Aliquots of sediment were subsampled at 0.5 cm resolution for diatom analysis. Diatom samples were prepared following standard methods [50,51] with diatoms mounted in Naphrax medium (refractive index = 1.73). A minimum of 300 diatom valves were counted per slide (excluding *Chaetoceros*, *Skeletonema*, and *Rhizosolenia* spp.; see below) using a DMRE Leica light microscope with phase contrast under oil immersion at $\times 1000$, and where possible, identified to species level. *Chaetoceros*, *Skeletonema*, and *Rhizosolenia* spp. could not be identified to the species level but were included in the diatom count for % calculations and other diatom metrics. Microspheres were added [52] for the calculation of diatom concentration and accumulation rates. The diatom stratigraphy was split into 3 significant zones in PsimPoll [53] using an optimal splitting method with information content and applying a broken stick model significance test (only diatom taxa > 1% relative abundance were included for zonation). Full methodological details are provided in [44,54,55].

2.4. Statistical Analyses and Diatom-Based Inference Model

To assess long-term nutrient changes, total nitrogen (TN) was inferred from the fossil diatom dataset with a WA-PLS-component 2 model based on the MOLTEN Western Baltic training set [56] using the program C2 v.1.7.7 ([57]). This modern-day training set incorporates 94 sites and spans a nitrogen gradient of 239–2890 μgL^{-1} TN and has a high predictive power under internal validation ($r^2 = 0.89$, RMSE = 0.09 \log_e units), though no independent test for spatial autocorrelation [40,58] has been performed. Due to recent scrutiny concerning using diatom-based transfer functions to infer nutrients (TN, TP) [38,59], we compared results with monitoring data over a 47-year period in addition to qualitative ecological interpretation to assess the performance of this model.

A diatom-salinity transfer function was also applied to the fossil diatom dataset, using a WA-PLS-component 1 model [60] based on a 210-site pan-Baltic modern training set adapted for salinity derived from the MOLTEN and DEFINE [56] datasets, as applied to earlier Holocene sequences in the Limfjord, including at Kilen [44,55,61]. Due to difficulties in taxonomic identification, *Chaetoceros*, *Skeletonema*, and *Rhizosolenia* spp. are not included in either of the diatom-inferred models.

2.5. Monitoring Data

Due to growing concern about widespread eutrophication in Danish lakes, Kilen was selected for environmental monitoring within the Danish Nationwide Monitoring Program [35,62]. Measurements began in the early 1970s (salinity from 1972; TP from 1975; TN from 1976), and since 1989, it has also included various biological data with varying

frequency (phytoplankton, zooplankton, macrophytes, and fish) [36]. For monitoring methods used in the present paper, we refer to [10].

3. Results

3.1. Age-Depth Model

Unsupported ^{210}Pb activity is detectable from background levels down to a depth of 30 cm. The concentration generally decreases exponentially with depth from its surface concentration of 241 Bq kg^{-1} down to $\sim 25 \text{ cm}$, after which irregularities occur in the profile (Figure 2a). Supported ^{210}Pb activity is detectable in all samples, remaining almost constant throughout. The ^{210}Pb age-depth model (Figure 2b) was validated by ^{137}Cs , detectable in all samples. ^{137}Cs levels also decrease with depth down until 1920 (as indicated by the ^{210}Pb stratigraphy). This predates its release into the atmosphere (~ 1945), suggesting a degree of sediment mixing or downward diffusion of Cs, and therefore Pb-based ages must be considered as minimum ages as mixing will result in the derivation of ‘younger’ ages. The maximum ^{137}Cs activity occurs at 8 cm, dated by ^{210}Pb as 1984, which can be considered broadly consistent with the fallout from the Chernobyl incident in 1986. Despite some clear signs of sediment mixing and irregularity in ^{210}Pb activity towards the bottom, the exponential decrease with depth in the upper section gives confidence in the results, and therefore, the dating is considered to be reasonably accurate.

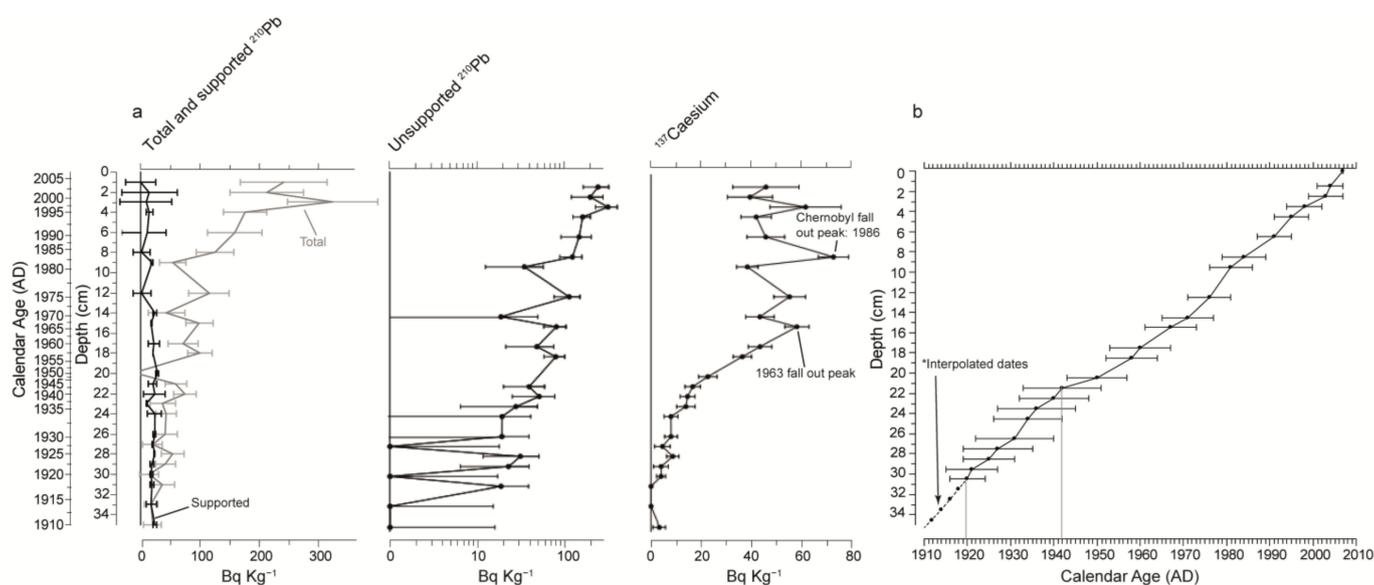


Figure 2. (a) Depth profiles of ^{210}Pb and ^{137}Cs for Kilen. (b) ^{210}Pb and ^{137}Cs based age–depth model for the Kilen Kajak core (collected in 2007). * The lowermost dates of the Kajak profile are extrapolated based on the average accumulation of 0.5 cm yr^{-1} from 1920–1942 (grey line), as the sedimentation rate changes little over this period. A potential hiatus means that this dating profile has not been matched with the ^{14}C dates available for the longer (Holocene) sedimentary sequence available for this site [44,54,55]. The sediment core was collected from a water depth of 390 cm.

3.2. Physical Sedimentary Parameters

The sediment accumulation rate (SAR, cm yr^{-1} , Figure 3) fluctuates markedly over the last 100 years, though the general trends indicate high but declining SAR in the lowermost part of the sequence, with the lowest values around 1964. After this date, a steady increase in SAR occurs up to the top of the sequence. Organic matter content (%OM) gradually increases throughout the profile (Figure 3) as the minerogenic percentage gradually declines. Calcium carbonate content (% CaCO_3) is generally low (average 7%) and relatively stable throughout the profile. When converted to accumulation rates (AR, $\text{mg cm}^{-2} \text{ yr}^{-1}$), all three components show the highest ARs in the earliest part of the record and follow a

similar pattern of general decline up until ~1960, with a short period of increased ARs from ~1960 to 1980, and further decline thereafter to the top of the sequence.

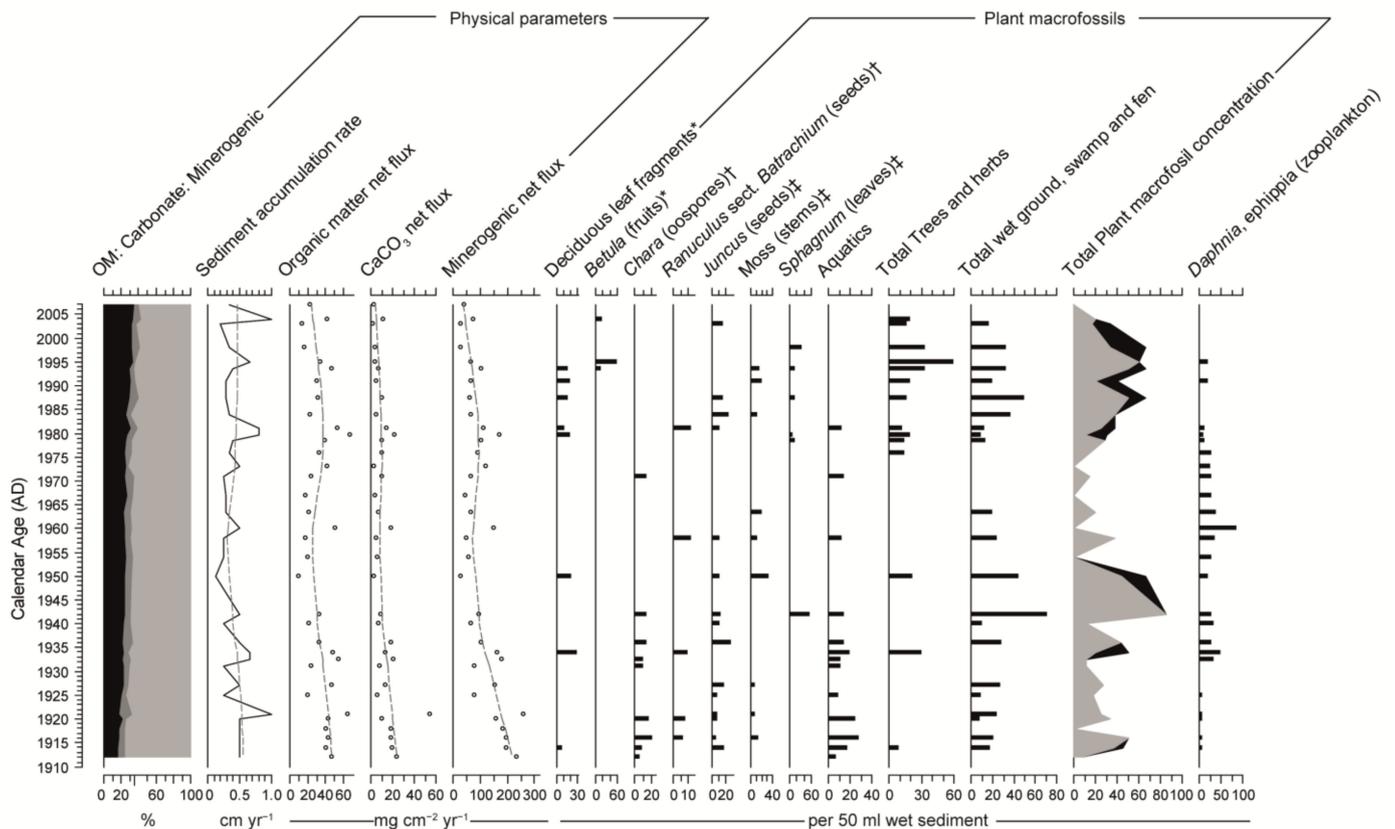


Figure 3. Physical sedimentary parameters and plant macrofossil concentrations (selected taxa) present in the Kilen Kajak sequence. Physical sedimentary parameters refer to sediment accumulation rate, loss-on-ignition (LOI) analyses for percentage organic matter, calcium carbonate (CaCO_3), and minerogenic content and flux (with 0.5 span Lowess smoothing) of these parameters based on the $^{210}\text{Pb}/^{137}\text{Cs}$ age–depth model. Plant macrofossil groupings: * = terrestrial taxa, † = aquatic taxa, ‡ = wet ground, swamp, and fen taxa.

3.3. Macrofossils

A variety of aquatic, wetland, and terrestrial plant macrofossils are present in the sediment sequence (Figure 3), with wet ground, swamp, and fen taxa generally present throughout (though briefly absent between 1964 and 1979), aquatic taxa common up until 1981 and terrestrial taxa sporadic in the lower sections, but frequent after 1976. The aquatic macrophyte *Chara* spp. (found as oospores) is frequent between 1910 and 1940, and seeds of the *Ranunculus* sect. *Batrachium* (water crowfoot) occur sporadically throughout the sequence. Of the wet ground/swamp/fen taxa, *Juncus* spp. seeds are frequent between 1910 and 1960 and again after 1980, whilst moss stems are regularly recorded throughout, and *Sphagnum* is frequent after 1979. Terrestrial taxa are mainly represented by deciduous leaf fragments, which occur sporadically throughout the sequence, though becoming more common between ~1980 and 1994, and *Betula* fruits, present in a few of the uppermost samples (after ~1994).

Faunal remains are generally scarce throughout the sequence (and devoid of ostracods, molluscs, and foraminifera, despite these groups being abundant in older sections of the Kilen sediment record [44]). The only notable faunal sub-fossils present are the ephippia of *Daphnia* spp. (cladoceran zooplankton), which exhibit a substantial increase in the early 1930s and remain frequent in the record up until ~1995 but are absent thereafter.

3.4. Diatoms

Figure 4 shows all diatom taxa found >3% in any sample, together with key diatom-based metrics. The diatom stratigraphy is divided into three statistically significant zones.

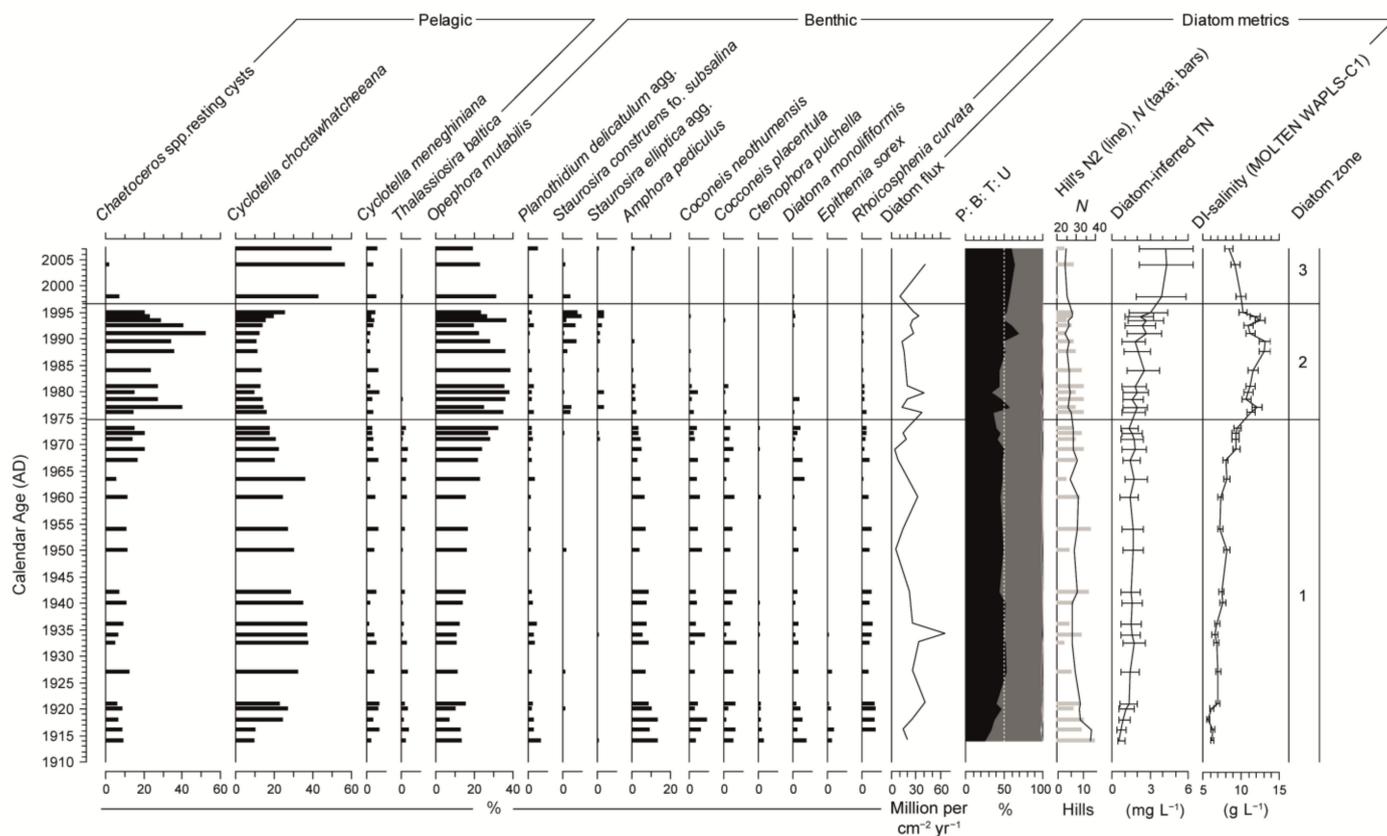


Figure 4. Kilen percentage diatom assemblage and associated metrics. Diatom-associated metrics include diatom flux based on the $^{210}\text{Pb}/^{137}\text{Cs}$ age–depth model; Pelagic: benthic: tychopelagic: undifferentiated (P:B:T:U) ratios; Hill’s N2 measure of diversity and number of taxa present (N), diatom-inferred total nitrogen (DI-TN) based on the MOLTEN Western Baltic dataset [56] and diatom-inferred salinity based on a MOLTEN- and DEFINE-derived salinity dataset [44,60].

KIL-D1 (1910–1974)—After an initial increase, *Cyclotella choctawatcheeana* is the most abundant pelagic species throughout this zone (10–38%) alongside ever-present and relatively consistent percentages of *Cyclotella meneghiniana* (2–7%) and *Thalassiosira baltica* (1–4%) and, up until 1967, *Chaetoceros* cysts (6–13%). *C. choctawatcheeana* percentages peak between 1918 and 1964 before gradually declining in the uppermost part of this zone (i.e., after 1964), concurrent with a rise in *Chaetoceros* cysts (14–21%). *Opephora mutabilis* (7–23%) and *Amphora pediculus* (6–14%; up until 1942 and 3–7% post-1942) are the most abundant benthic species, accompanied by less frequent occurrences of *Planorhithidium delicatulum*, *Cocconeis neothumensis*, *C. placentula*, *Diatoma moniliformis*, and *Rhoicosphenia abbreviata*. *Opephora mutabilis* increases in the upper section of this zone, particularly after 1960, and dominates the diatom benthos.

KIL-D2 (1974–1996)—*Chaetoceros* cysts dominate the pelagic diatom component, as *C. choctawatcheeana* (though still abundant) continues to decrease down to its lowest percentages since 1916 and *Thalassiosira baltica* becomes rare. *Opephora mutabilis* remains the most abundant benthic species, reaching its peak abundance in this zone (fluctuating between 20 and 38%, average ~30%). *Staurosira construens* fo. *subsalina* and *S. elliptica* agg. increase in abundance, whilst *Planorhithidium delicatulum* also remains present throughout this zone. However, several benthic species experience a decrease during this zone, becoming rare

after ca. 1980, most notably *A. pediculus*, *C. placentula*, *C. neothumensis*, *D. moniliformis*, and *R. abbreviata*.

KIL-D3 (~1996–2007)—*Chaetoceros* cysts experience a major decrease at the beginning of this zone and almost disappear from the record by the uppermost sample (i.e., 2007). *Cyclotella choctawatcheeana* increases dramatically, replacing *Chaetoceros* cysts as the main component of the diatom plankton and the most abundant overall. *Opephora mutabilis* (~19–31%) remains the main representative of the diatom benthos, with smaller amounts of *P. delicatulum* also present.

3.5. Diatom Metrics

Benthic diatom taxa dominate the assemblage at the bottom of the core profile, though this group declines up until 1927 as pelagic diatoms increase. Between 1927 and 1969, percentages of benthic and pelagic diatoms are relatively even and stable. After a brief decline in the proportion of pelagic diatoms (and concurrent increase in benthic diatoms) between 1976 and 1969, the pelagic diatom contribution gradually increases again throughout zones KIL-D2 and KIL-D3. Diatom diversity is generally highest in KIL-D1, despite some fluctuation, before gradually declining in the subsequent zones. Diatom accumulation rate (DAR) varies substantially over the sequence, following the pattern in sedimentation accumulation rate (SAR).

3.6. Monitoring Data

The nutrient input has been monitored in one of the two main inlets of the lake, accounting for about 48% of the total freshwater input to the lake [36]. Trend analysis revealed a significant ($p < 0.001$) linear decline in input from 1989 to 2020 in both loadings of TN and TP (Figure 5), amounting on average to 1.7% per year for TN and 1.6% for TP. This has occurred despite an increase in hydraulic loading of 0.7% per year. The main form of N entering the lake is as nitrate, while for phosphorus, it is a mixture of ortho-P and inorganic/organic-P, the latter usually coming in particulate form in Danish streams [63]. The discharge-weighted concentration shows a TN peak of 8–10 mg N l⁻¹ during 1993–1998, followed by a substantial decline until 2007 and a more moderate decline thereafter. A more irregular pattern is found for TP. The changes in TN in the lake in summer follow the concentration pattern of the inlet, thus showing a rapid lake response to a reduction in N loading, as in other shallow lakes [64]. The peak in N concentration was substantially lower than in the inlet, reflecting losses in the lake. The peak in TP occurred in the mid-1980s (about 0.4 mg L⁻¹) and fell below 0.1 mg L⁻¹ as an annual average in several of the monitored years. In accordance with the decline in lake nutrient concentrations, the phytoplankton biomass (chlorophyll-a) declined and the water clarity improved (as seen in changes in the Secchi depth). Judging from the Secchi depth (for which there are more data available than for chlorophyll-a), the biomass of phytoplankton was highest in the mid-1980s and declined sharply in the mid-1990s, coinciding with the decline in both TN and TP (Figure 6). Except for 2004, the TN:TP ratio (by mass) was below 10, indicating overall N limitation of phytoplankton growth in the lake in summer [9].

Following the increase in water clarity, the lake area covered by submerged macrophytes increased (~doubled) from below 4% in 2004–2010 to 8.3% in 2016 (Figure 7). During the monitoring period, a change occurred from the dominance of *Zannicellia palustris* and *Enteromorpha* sp. in 2004 to the dominance of *Potamogeton perfoliatus* and *Chara* sp. in 2016 (Table 2), associated with an overall reduction in salinity since the early-mid 1990s (Figure 6). This reduction in overall salinity likely also explains the disappearance of the two *Ruppia* species in the survey data after 2004.

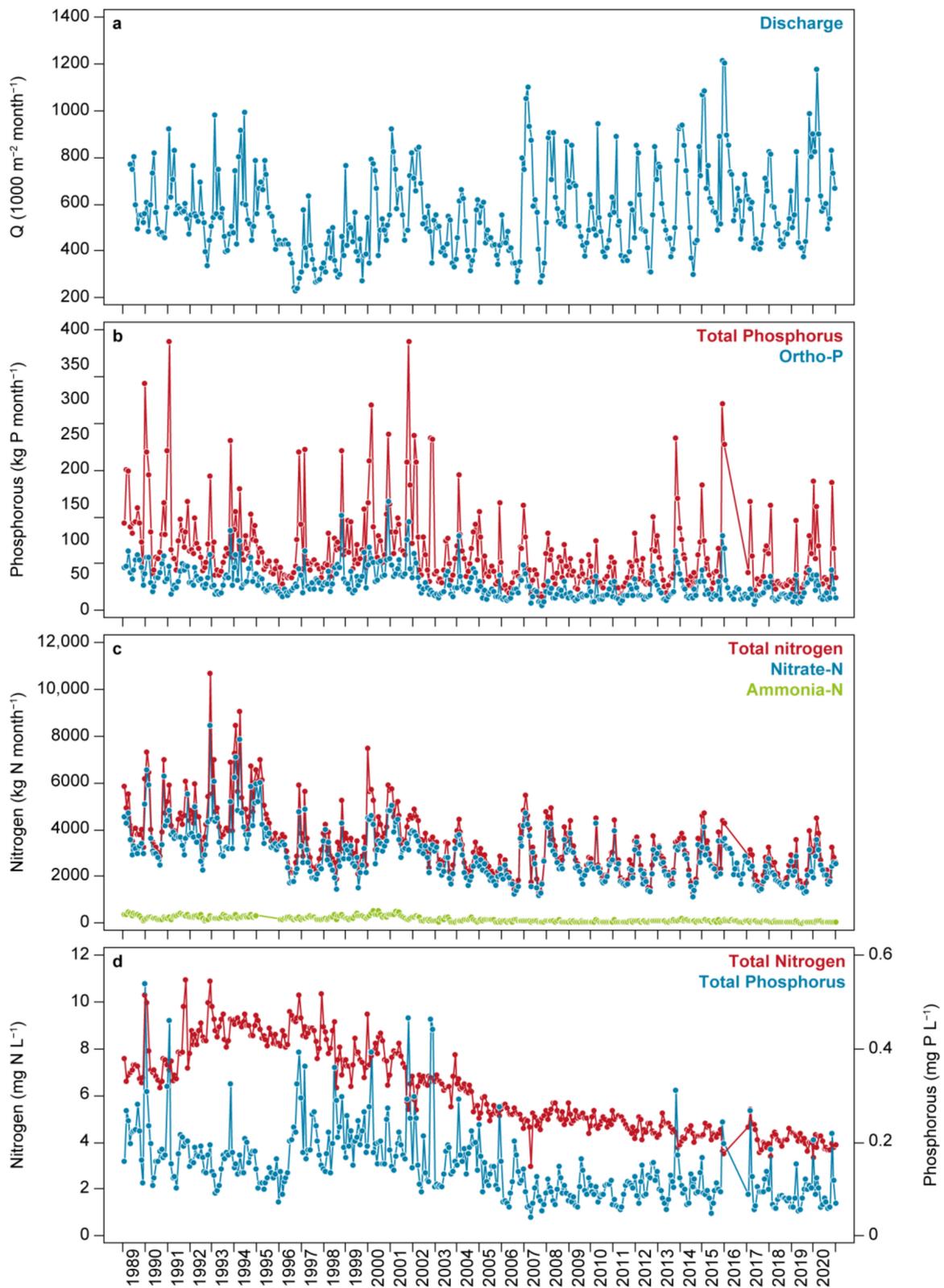


Figure 5. Monthly data for (a) monitored inlet (inflow) discharge; (b) loading of total phosphorus and ortho-phosphate-P; (c) loading of total nitrogen, nitrate-N, and ammonium-N; and (d) discharge weighted inlet concentration of total phosphorus and nitrogen in one of the two main inlets (Bredkær Bæk) to Lake Kilen from 1989 to 2019. This inlet accounts for about 48% of the total freshwater input to the lake.

Table 2. Presence of and most abundant (red) submerged macrophytes in the surveys conducted between 2004 and 2016.

Taxa	2004	2007	2010	2016
<i>Enteromorpha</i> sp.	X	X		
<i>Potamogeton crispus</i>	X		X	X
<i>Potamogeton pectinatus</i>	X	X	X	X
<i>Potamogeton perfoliatus</i>	X	X	X	X
<i>Potamogeton berchtoldii</i>		X		
<i>Ranunculus peltatus</i> ssp. <i>baudotii</i>	X			
<i>Ruppia cirrhosa</i>	X			
<i>Ruppia maritima</i>	X			
<i>Zannichellia palustris</i> var. <i>major</i>	X	X	X	X
<i>Zannichellia palustris</i> var. <i>repens</i>	X			
<i>Batrachium</i> sp.		X	X	
<i>Chara</i> sp.			X	X

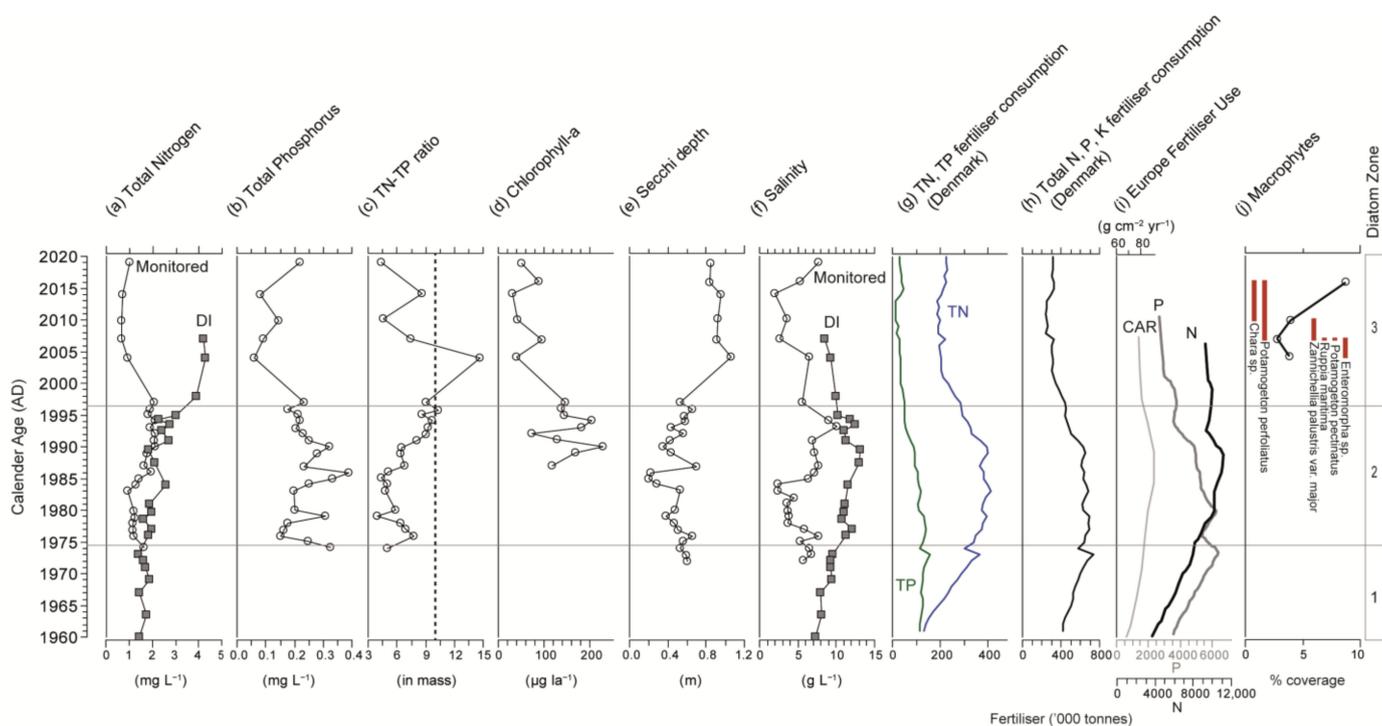


Figure 6. Summer means for Lake Kilen for the period 1976–2019 of (a) total nitrogen (TN); (b) total phosphorus (TP); (c) TN:TP ratio (as mass); (d) concentration of chlorophyll-a; (e) Secchi depth; (f) mean summer salinity in the surface waters. For TN (a) and salinity (f), diatom-inferred inferences (DI) are also plotted for comparison (also see Figure 4). (g) Total nitrogen and phosphorus fertilizer consumption in Denmark; (h) total NPK fertilizer consumption for Denmark (original source: www.ifastat.org/databases/plant-nutrition (accessed on 25 February 2023)); (i) Total European (EU-15) P and N fertilizer use and carbon accumulation rate (CAR) data for European lakes in the eutrophic-hypertrophic category (loess smooth; $n = 55$) after [65]; (j) Summary of macrophyte survey data for Kilen (based on data for dominant macrophytes in Table 2 and percentage macrophyte coverage in Figure 7).

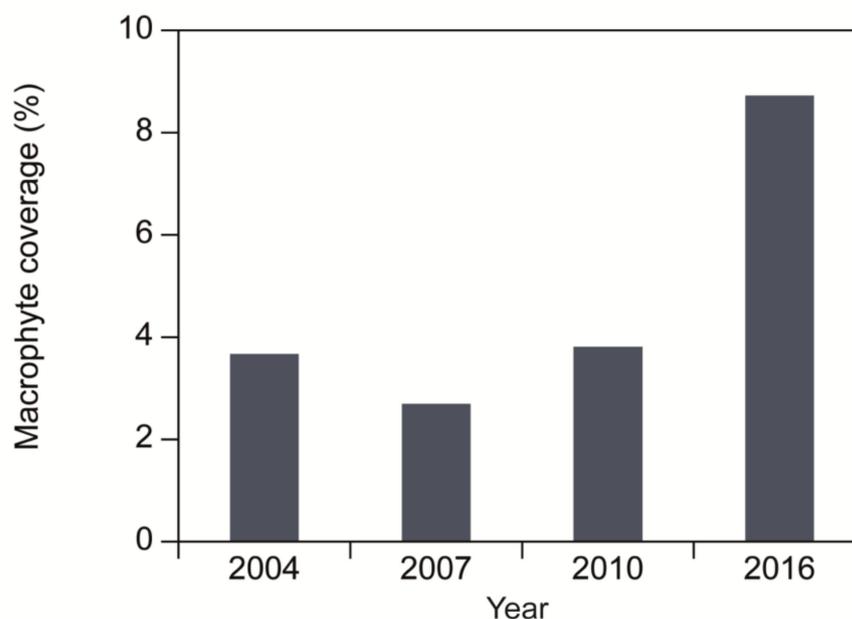


Figure 7. Plant coverage in percent of lake area (lake area = 319 ha).

3.7. Inferred Total Nitrogen and Salinity from Diatoms and Validation

Measured TN and salinity at Kilen over the last ~40 years allows the diatom-inferred model inferences from the fossil assemblages in the sediment core to be validated. In Figure 6, the DI-TN and DI-salinity model estimates are compared against measured TN concentrations and salinity for the period 1972–2019 [35,36,43]. Diatom-inferred TN values (Figure 4) exhibit a progression from lower values in KIL-D1 ($<1 \text{ mg L}^{-1}$ at the base of the sequence to $\sim 2 \text{ mg L}^{-1}$ at the top of KIL-D1), increasing through KIL-D2 to $\sim 3 \text{ mg L}^{-1}$ and with maximum values over the entire record inferred in the uppermost samples in KIL-D3 (up to 4.5 mg TN l^{-1}). The DI-TN and monitoring data generally show a similar pattern of TN change over the earlier part of this period up until around 1997, although DI-TN model inferences are slightly higher than the observed TN concentrations, probably due to the monitoring data being calculated as summer means rather than annual concentrations (as there are few winter data available). After 1997, there appears to be a divergence between the measured data and the DI-TN model inferences, though there is a lack of both monitoring data and sub-fossil diatom-based inferences available between 1997 and 2004. By 2004, monitoring data indicate a substantial drop in TN (along with decreased chlorophyll-a and improved water clarity), whilst the DI-TN model suggests increased nutrient values by 2004–2007 and intensified eutrophication within the lake, driven by very high *C. choctawhatcheana* percentages ($\sim 50\text{--}57\%$) in the uppermost samples (with the core collected in 2007). Monitoring continued after the core was collected in 2007 (Figure 6) and shows that TN concentrations began to decrease sometime after 1996, reaching about 0.75 mg L^{-1} by summer 2006, at which level it has remained.

In summary, the data suggest that the DI-TN model performs well between 1974 and 1995. However, due to the known problems with diatom-based TN inference models and particularly the influence of confounding variables [38,59], DI-TN inferences in the earlier part of the core must be treated with caution and used as a guide to nitrogen trends in support of ecological interpretation and other multiproxy data, rather than used as an indicator of absolute DI-TN values. This is clearly highlighted by the divergence in DI-TN and monitoring values from 1997.

The DI-salinity and measured salinity data show broadly similar patterns between $\sim 1970\text{--}1975$ and $1984\text{--}2007$. However, the model does not capture the pattern of declining salinity from $\sim 1975\text{--}1984$, with DI-salinity values rising over this period to as high as 12 g L^{-1} while measured values fall to $\sim 3 \text{ g L}^{-1}$ at a time when measured TN also rises.

The subsequent increase in salinity in the early 1990s is faithfully captured, however, with measured and inferred values both falling after this. Saline water input at depth and periodic salinity stratification likely explain why DI-salinity values are consistently higher than measured salinity values, which are taken from the lake surface (~0.5 m) during summer.

4. Discussion

4.1. Onset of Nutrient Enrichment: Early 20th Century

The Kilen palaeolimnological data suggest that eutrophication has been prevalent since the beginning of the century (Figure 4), consistent with other Danish lakes and estuaries and coastal sites (e.g., Roskilde Fjord, Mariager Fjord; [6,66]), and in other coastal sites across the Baltic Sea [67,68]. Even in the lowermost (earliest) samples of the Kilen core sequence, high relative abundance of diatoms associated with nutrient enrichment is present (i.e., *Cyclotella choctawhatcheana*, *C. meneghiniana*, *Thalassiosira baltica*, and *Amphora pediculus* [66,69–71]) and by ~1930, pelagic diatoms account for over half the diatom assemblage, a frequent indicator of a shift in ecosystem function under eutrophication [72,73]. Increasing eutrophication was accompanied by an increase in pelagic cladocerans (*Daphnia* spp.), possibly in response to increasing phytoplankton biomass available for grazing. A reduction in aquatic plant remains after ~1942 in the macrofossil record (Figure 3) likely reflects deteriorating light conditions within the lake due to an increase in phytoplankton biomass.

4.2. Increasing Impacts of Eutrophication: Mid to Late 20th Century

In the period between 1920 and 1964, there was minimal change in the diatom assemblage, suggesting that nutrient concentrations remained high but relatively stable. After ~1964, there was a change in the pelagic diatom community, from *C. choctawhatcheana* dominance to *Chaetoceros* spp. (present as resting cysts). This is possibly due to increased salinity, though it is difficult to be certain here what is driving this increase in *Chaetoceros* spp. as it is not possible to differentiate the *Chaetoceros* cysts to species level. However, *Chaetoceros* species are more typically found in coastal or marine environments, though some species of *Chaetoceros* are known to tolerate lower saline waters [74]. During this period, the DI-TN suggests nutrient levels continue to increase gradually in the basin, consistent with rising fertiliser use across Denmark and Europe (Figure 6; [65]), with eutrophic conditions becoming widespread across lakes and coastal areas in Denmark and the Baltic Sea [6,23,66,68,75,76]. However, whilst we can be confident that nutrient concentrations were high in Kilen at this time, we must treat the DI-TN reconstruction with some caution (discussed in more detail below).

The absence of *Chaetoceros* cysts from the DI-TN model means their relative increase is not influencing the DI-TN values despite their frequency between 1967 and 1995. Increasing DI-TN values after ~1976 are likely still being significantly driven by *Cyclotella choctawhatcheana*, which remains abundant, along with the reduction in several lower nutrient-affiliated benthic diatom taxa (discussed below). The classic symptoms of eutrophication are evident in the proxy records and monitoring data at Kilen: phytoplankton blooms, lower water clarity and light penetration, and declining relative abundance of benthic diatoms and macrophyte populations (Figures 3 and 4). *Opephora mutabilis* (increasing since 1964) dominates the benthic diatom community that remains, though it is possibly confined to the shallower parts of the basin due to low light penetration. *Opephora mutabilis* is not generally associated with nutrient-rich conditions and therefore might be responding to some other variable, such as suitable (sandy) substrate or changing salinity conditions [77].

The relative decline in benthic (brackish, epiphytic) diatom abundance and diversity begins after 1973, with a number of diatom taxa disappearing from the record by 1980–1981 (Figure 4). The environmental monitoring data for TP and TN (available since 1975; Figure 6) support nutrient-rich conditions, though TP actually declined in the late 1970s/early 1980s due to decreased P loading from fish farms [35]. The TN:TP ratio (below 10 almost

throughout) suggests that the overall system is N limited [9] in summer, and therefore the short-term decline in TP does not appear to reverse the nutrient signal in the diatom record (Figure 4) or lead to any improvement in water clarity (Figure 6). At this time, greater water exchange with the Limfjord is also documented [35], but increased salinity is not evident in measured salinity values until later (from 1985; Figure 6), despite being apparent in the DI-salinity reconstruction. This might be due to the creation of a halocline following saline water exchange (input), whilst measured values only reflect surface salinity (~0.2–0.5 m) where water samples were collected during this period [36]. Whilst no data are available for bottom water salinity before 1989, substantial differences between bottom and surface water salinity have been subsequently recorded in the lake in some years [36,78], suggesting plausible halocline formation, though the relationship between water exchange and salinity remains speculative due to the lack of comprehensive data on these aspects.

Alternatively, inferred DI-salinity values higher than those measured from 1973 to 1994 might be spurious due to problems modelling accurate values when multiple variables are exerting strong influences on biological communities (e.g., [38]; see discussion below). The increase in *Chaetoceros* cysts (a genus commonly found in brackish to marine systems) and *Ophephora mutabilis* in the late 1970s supports the observation of improved water exchange between the Limfjord and Kilen. High nutrient levels and increasing bottom water anoxia, perhaps intensified by strengthening stratification following saline inflows, and increased abundance of pelagic diatom taxa (shading out light since the mid-1970s) possibly drove a decline in benthic diatom taxa (and potentially a further loss of aquatic macrophytes, though no macrophyte data are available for this period and sub-fossil data is generally very sparse; Figure 3). In support of this argument, the Secchi depth data suggest poor and declining light penetration in the late 1970s (Figure 6).

After 1984, summer total nitrogen begins to increase, leading to peak TN conditions throughout the 1990s. Rising nutrient concentrations are reflected in the DI-TN reconstruction, despite the apparent decrease in the relative abundance of *C. choctawhatcheeana*. This decline is relative to *Chaetoceros* cysts (the latter not included in the DI-TN model due to problems differentiating cysts to species level), and the close agreement between the monitoring TN data and the DI-TN data suggest the model is performing relatively well at this time. Between 1984 and 1998, the monitoring and palaeolimnological data reflect typical eutrophication conditions, driven by high TN (rather than TP) concentrations in the lake and potentially increased bottom water anoxia. Sedimentary diatom assemblages are dominated by a pelagic, high nutrient-indicative taxa, with declining relative abundance of benthic diatoms and likely poor macrophyte coverage, all suggesting poor light penetration, supported by generally low Secchi depth (Figure 6), generally high chlorophyll-a concentrations. Installation of a new sluice system in 1984 improved water exchange with the Limfjord, resulting in high salinity ($>5 \text{ g L}^{-1}$ in surface waters) within Kilen until ~2005 (as reflected in the measured salinity data; Figure 6). Increased salinity also drove changes in the diatom record, with further increases in *Chaetoceros* resting cysts and the brackish benthic taxon *Staurosira construens* fo. *subsalina*.

4.3. Lake Recovery: Late 20th Century to the Present Day

After 1995, diatom assemblages suggest eutrophication intensified, a trend that continued up until 2007 (the uppermost sample of the sediment core and sub-fossil diatom record). Taking the sub-fossil record alone, this might appear to be a classic case of hyper-eutrophication, exhibiting increasingly eutrophic symptoms described above. However, this is not seen in the monitoring data, which point towards reduced nutrient concentration and a trend towards lake recovery (Figure 6). No monitoring data are available for the period between 1997 and 2004, but by 2004, summer nutrient concentrations had declined for both TP and TN, chlorophyll-a was lower, and water clarity had increased substantially (with Secchi depths ~0.5 m greater than pre-1997). The reasons for this contrast appear complex, perhaps explained by poorer diatom preservation in the upper layers of the sediment sequence and/or the response of diatom communities to confounding variables,

skewing the DI-TN reconstruction. The diatom assemblage in the uppermost sediment core samples is dominated by the spring-blooming pelagic taxon *Cyclotella choctawatcheeana*. Despite the improved water clarity suggested by the Secchi depth data, the relative abundance of benthic diatoms continues to decline, and diversity remains low. The decline in salinity after 1995 likely explains the decline in *Chaetoceros* cysts in the upper core sequence, possibly resulting from reduced efficiency of exchange with the Limfjord.

The trend towards lake recovery and greatly reduced TN and TP concentrations within the lake are driven by reduced loading of TN, nitrate, and ammonia since ~1996 and both TP and further TN reductions after 2004 (Figure 5), in keeping with the pattern across Denmark and Europe (Figure 6). This has led to changes in biological communities in Kilen, reduced overall chlorophyll-a concentrations, and improved water clarity within the basin (by ~0.5 m). A comprehensive macrophyte survey of 40 locations around the Kilen basin in 2004 found coverage of 15–24% in areas shallower than 1.5 m water depth, while no macrophytes were found below 3 m [36]. The substantial increase in macrophyte cover in recent years from <4% (of total lake area) between 2004 and 2010 to over 8% is indicative of improved water quality and clarity [18], while the change in the plant community from more saline-tolerant *Enteromorpha* and *Ruppia* species to species indicative of lower salinity such as *Potamogeton perfoliatus* and *Chara* spp. (Table 2) concur with the reduced salinity observed from 2004 to 2016 (Figure 6).

4.4. Drivers of Ecological Change in Brackish Systems: Nutrients and Salinity

The data presented in this study demonstrate the complexity of environmental dynamics and ecological response in this brackish lake system, which retains some degree of connection with the sea (in this case, the North Sea via the Limfjord). The nutrient loading of this lake is characterised by both point and diffuse source pollution (Table 1), with the lake being predominately TN-limited in summer for almost the entire period of monitoring (except for 2004, as indicated by the TN:TP ratio; Figure 6). Therefore, early attempts to manage this system by focusing on reducing TP loads from the catchment and the sediment via increasing water exchange with the sea (e.g., in 1986 via altering sluice dynamics through the Kilerkanal; [35]) did not have a strong effect on lake water quality. Whilst these measures were successful in reducing TP levels (and increasing salinity) after 1986, TN continued to rise (Figure 6), with N control only having an impact on in-lake TN concentrations in the early 2000s. For future management and sustained recovery of this system, greater focus is needed on N input reduction from the catchment. Although the monitoring data are scarce after 1997, and interpretations should therefore be made with caution, there are no clear indications that different sluice operating regimes leading to variable salinities in the 2000s had an important effect on the lake's trophic status.

4.5. Problems and Potential of Transfer Functions

When compared with monitoring data, the DI-TN model reconstructs TN well for a portion of the record (1974–1995), and may be reliable in the earlier sections of the record (given the agreement for observed and modelled TN in the mid- to late-1970s, and a convergence in observed and inferred salinity in the early 1970s), but shows a clear divergence after ~2000 (Figure 6). Nonetheless, it would be prudent to treat the longer-term reconstructions (i.e., pre-1974) with a degree of caution due to the role played by potential confounding variables. For example, a related coastal diatom TN model with many of the same MOLTEN sites included in this model used here [66] was shown to be problematic due to a composite variable (i.e., water depth) appearing to exert a dominant influence on the modern diatom training set (e.g., [38,66]), likely leading to erroneous TN reconstructions. Nevertheless, leaving absolute “quantitative” values aside, we remain confident that the sub-fossil diatom data and DI-TN model correctly infer that the lake was nutrient enriched in the early twentieth century.

Similar doubts can also be expressed with the DI-salinity model when compared against monitoring data, as whilst the model can perhaps pick up broad signals and larger

magnitude changes, it certainly struggles with shorter-term and/or lower magnitude salinity shifts (e.g., the drop in salinity between ~1974 and 1992). In the modern training set, salinity is the strongest variable [44], and the DI-salinity model has been tested for spatial autocorrelation [60], generating more confidence in its performance during times when salinity was likely the dominant driver (i.e., prior to 1856; [44,55]). However, this study shows that when other variables exert strong influences on the diatom community (e.g., nutrients, light, extent of macrophyte cover), then the performance of a diatom-based salinity model may be weakened.

Some of the issues for both the DI-TN and DI-salinity models may also be an artefact of the age–depth model used for the sediment core, as there are errors with any such models (affecting apparent timings between changes seen in the monitoring record and the sedimentary record). Similarly, the time-averaging and spatial integration that each 0.5 cm sediment slice represents will also have an impact, as a sample may represent more than one year (especially in older sections of the core), as well as integrating diatoms growing, and diatom valves reworked, from different habitats across the lake. In this study, verification by modern data was critical for clearly establishing the limitations of the diatom-based transfer functions employed, and are all too rarely possible or discussed, which makes Kilen an excellent site for examining this in detail.

5. Conclusions

The palaeolimnological and monitoring data indicate that the brackish lake Kilen has been nutrient rich for the last 100 years, with eutrophication peaking in the mid-1980s to late 1990s, and has also fluctuated in salinity over this time (from ~3 to >10 g L⁻¹). The lake has shown some recovery in trophic status since the late 1990s, predominately between ca. 1997 and 2004, driven by a reduction in TN and TP loading from both point and diffuse sources across the catchment, which has resulted in improvements in the lake water clarity and extent of macrophyte cover. However, this recovery was not fully captured by the palaeolimnological data or in the quantitative diatom-based nutrient (TN) model, highlighting potential limitations of transfer functions when multiple variables are exerting a strong influence on species communities. The low N:P ratio indicates that in summer, Kilen is predominately N-limited, likely explaining why previous management mainly focusing on TP reduction measures had a modest effect on the water quality of the lake. The lake is still eutrophic and does not fulfil the criteria of minimum Good Ecological Status according to the European Water Framework Directive. Therefore, the future management of this system must continue and include the reduction of nutrient loads of both TN and TP to ensure sustained recovery. The combination of multi-decadal monitoring data with multi-proxy palaeolimnological records offers a rare opportunity to validate these sedimentary archives critically, including the performance of diatom-inferred models. This approach highlights the value of high-quality palaeolimnological archives for tracking long-term variations in key environmental parameters as the context for understanding current and future coastal environmental change.

Author Contributions: Conceptualization and methodology, J.P.L., D.B.R., P.R. and E.J.; Investigation and formal analysis, J.P.L., P.R., E.J., L.S.J. and S.T.; resources, D.B.R., P.R., E.J. and T.L.L.; writing—original draft preparation, J.P.L., E.J. and D.B.R.; writing—review and editing, M.S., T.L.L., L.S.J., S.T. and P.R.; supervision, D.B.R. and P.R.; project administration, J.P.L., D.B.R., P.R. and E.J.; funding acquisition, D.B.R., P.R., E.J. and M.S. All authors have read and agreed to the published version of the manuscript.

Funding: Funding was provided from the Loughborough University Development Fund (Ph.D. funding for J.P.L.). The monitoring project was supported by Struer municipality, Aage V. Jensen Nature Foundation, project “Vestlige Vejler”. E.J. was also supported by the TÜBİTAK program BİDEB2232 (project 118C250). M.S. was supported by the Poul Due Jensen Foundation.

Data Availability Statement: All palaeo-data are available in the main text or can be supplied by contacting the corresponding author (J.L.). Monitoring data are available from Overfladevandsdatabasen (odaforalle.au.dk).

Acknowledgments: For palaeo-analyses, Beth Stavngaard, Ole Bennike, and Bent Odgaard are thanked for field and laboratory support. MOLTEN and DEFINE members are also thanked for transfer function production, discussion, and support. We thank Anne Mette Poulsen for their valuable editions. Two anonymous reviewers are thanked for their helpful comments.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Bradshaw, E.G.; Nielsen, A.B.; Anderson, N.J. Using diatoms to assess the impacts of prehistoric, pre-industrial and modern land-use on Danish lakes. *Reg. Environ. Chang.* **2006**, *6*, 17–24. [[CrossRef](#)]
- Jensen, J.P.; Pedersen, A.R.; Jeppesen, E.; Søndergaard, M. An empirical model describing the seasonal dynamics of phosphorus in 16 shallow eutrophic lakes after external loading reduction. *Limnol. Oceanogr.* **2006**, *51*, 791–800. [[CrossRef](#)]
- Schindler, D.W. Evolution of phosphorus limitation in lakes. *Science* **1977**, *195*, 260–262. [[CrossRef](#)] [[PubMed](#)]
- Carpenter, S.R.; Caraco, N.F.; Correll, D.L.; Howarth, R.W.; Sharpley, A.N.; Smith, V.H. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **1998**, *8*, 559–568. [[CrossRef](#)]
- Schindler, D.W. Recent advances in the understanding and management of eutrophication. *Limnology Oceanogr.* **2006**, *51*, 356–363. [[CrossRef](#)]
- Ellegaard, M.; Clarke, A.L.; Reuss, N.; Drew, S.; Weckström, K.; Juggins, S.; Anderson, N.J.; Conley, D.J. Multi-proxy evidence of long-term changes in ecosystem structure in a Danish marine estuary, linked to increased nutrient loading. *Estuar. Coast. Shelf Sci.* **2006**, *68*, 567–578. [[CrossRef](#)]
- Nixon, S.W. Coastal marine eutrophication: A definition, social causes and future concerns. *Ophelia* **1995**, *41*, 199–219. [[CrossRef](#)]
- Howarth, R.W.; Marino, R. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. *Limnol. Oceanogr.* **2006**, *51*, 364–376. [[CrossRef](#)]
- Paerl, H.W.; Scott, J.T.; McCarthy, M.J.; Newell, S.E.; Gardner, W.S.; Havens, K.E.; Hoffman, D.K.; Wilhelm, S.W.; Wurtsbaugh, W. It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environ. Sci. Technol.* **2016**, *50*, 10805–10813.
- Søndergaard, M.; Lauridsen, T.L.; Johnsson, L.S.; Jeppesen, E. Nitrogen or phosphorus limitation in lakes and its impact on phytoplankton biomass and submerged macrophyte covers. *Hydrobiologia* **2017**, *795*, 35–48. [[CrossRef](#)]
- Nørring, N.P.; Jørgensen, E. Eutrophication and agriculture in Denmark: 20 years of experience and prospects for the future. *Hydrobiologia* **2009**, *629*, 65–70. [[CrossRef](#)]
- Agency, D.E.P. *Environmental Impacts of Nutrient Emissions in Denmark*; No. 1.; Redegørelse fra Miljøstyrelsen: Odense, Denmark, 1991.
- Conley, D.J.; Markager, S.; Andersen, J.; Ellermann, T.; Svendsen, L.M. Coastal eutrophication and the Danish National Aquatic Monitoring and Assessment Program. *Estuaries* **2002**, *25*, 848–861. [[CrossRef](#)]
- Kronvang, B.; Andersen, H.E.; Borgesen, C.; Dalgaard, T.; Larsen, S.E.; Bøgestrand, J.; Blicher-Mathiasen, G. Effects of policy measures implemented in Denmark on nitrogen pollution of the aquatic environment. *Environ. Sci. Policy* **2008**, *11*, 144–152. [[CrossRef](#)]
- Søndergaard, M.; Jensen, J.P.; Jeppesen, E.; Møller, P.H. Seasonal dynamics in the concentrations and retention of phosphorus in shallow Danish lakes after reduced loading. *Aquat. Ecosyst. Health Manag.* **2002**, *5*, 19–29. [[CrossRef](#)]
- Søndergaard, M.; Jeppesen, E.; Lauridsen, T.L.; Skov, C.; Van Nes, E.H.; Roijackers, R.; Lammens, E.; Portielje, R. Lake restoration: Successes, failures and long-term effects. *J. Appl. Ecol.* **2007**, *44*, 1095–1105. [[CrossRef](#)]
- Trolle, D.; Nielsen, A.; Rolighed, J.; Thodsen, H.; Andersen, H.E.; Karlsson, I.; Refsgaard, J.; Olesen, J.; Bolding, K.; Kronvang, B.; et al. Projecting the future ecological state of lakes in Denmark in a 6 degree warming scenario. *Clim. Res.* **2015**, *64*, 55–72. [[CrossRef](#)]
- Søndergaard, M.; Davidson, T.A.; Lauridsen, T.L.; Johansson, L.S.; Jeppesen, E. Submerged macrophytes in Danish lakes: Impact of morphological and chemical factors on abundance and species richness. *Hydrobiologia* **2022**, *849*, 3789–3800. [[CrossRef](#)]
- Riemann, B.; Carstensen, J.; Dahl, K.; Fossing, H.; Hansen, J.W.; Jakobsen, H.H.; Josefson, S.B.; Krause-Jensen, D.; Markager, S.; Stæhr, P.A.; et al. Recovery of Danish coastal ecosystems after reductions in nutrient loading: A holistic ecosystem approach. *Estuaries Coasts* **2016**, *39*, 82–97. [[CrossRef](#)]
- Schindler, D.W. The dilemma of controlling cultural eutrophication of lakes. *Proc. R. Soc. B* **2012**, *279*, 4322–4333. [[CrossRef](#)]
- Roberts, L.R.; Sayer, C.D.; Hoare, D.; Tomlinson, M.; Holmes, J.A.; Horne, D.J.; Kelly, A. The role of monitoring, documentary and archival records for coastal shallow lake management. *Geo Geogr. Environ.* **2019**, *6*, e00083. [[CrossRef](#)]
- Williams, W.D.; Boulton, A.J.; Taaffe, R.G. Salinity as a determinant of salt lake fauna: A question of scale. *Hydrobiologia* **1990**, *197*, 257–266. [[CrossRef](#)]
- Conley, D.; Cartensen, J.; Ærtebjerg, G.; Christensen, P.B.; Dalsgaard, T.; Hansen, J.L.S.; Josefson, A.B. Long-term changes and impacts of hypoxia in Danish coastal waters. *Ecol. Appl.* **2007**, *17*, 165–184. [[CrossRef](#)]

24. Jeppesen, E.; Søndergaard, M.; Kanstrup, E.; Petersen, B.; Eriksen, R.B.; Hammershøj, M.; Mortensen, E.; Jensen, J.P.; Have, A. Does the impact of nutrients on the biological structure and function of brackish and freshwater lakes differ? *Hydrobiologia* **1994**, *275*, 15–30. [[CrossRef](#)]
25. Moss, B. Brackish and freshwater shallow lakes-different systems or variations on the same theme? *Hydrobiologia* **1994**, *275*, 1–14.
26. He, H.; Jeppesen, E.; Bruhn, D.; Yde, M.; Kjerulf Hansen, J.; Spanggaard, L.; Madsen, N.; Liu, W.; Søndergaard, M.; Lauridsen, T.L. Decadal changes in zooplankton biomass, composition and body mass in four temperate shallow brackish lakes subjected to various degrees of eutrophication. *Inland Waters* **2020**, *10*, 186–196. [[CrossRef](#)]
27. Christensen, I.; Pedersen, L.K.; Søndergaard, M.; Lauridsen, T.L.; Tserenpil, S.; Richardson, K.; Amorim, C.A.; Pacheco, J.P.; Jeppesen, E. Impact of zooplankton grazing on phytoplankton in north temperate coastal lakes: Changes along gradients in salinity and nutrients. *Hydrobiologia* **2023**, 1–18. [[CrossRef](#)]
28. Wu, H.; Liao, M.; Guo, J.; Zhang, Y.; Liu, Q.; Li, Y. Diatom assemblage responses to multiple environmental stressors in a deep brackish plateau lake, SW China. *Environ. Sci. Pollut. Res.* **2022**, *29*, 33117–33129. [[CrossRef](#)] [[PubMed](#)]
29. Mortensen, E.; Jeppesen, E.; Søndergaard, M.; Kamp Nielsen, L. (Eds.) *Nutrient Dynamics and Biological Structure in Shallow Freshwater and Brackish Lakes*; Springer: Dordrecht, The Netherlands, 1994; Volume 94, p. 507.
30. Alprol, A.E.; Ashour, M.; Mansour, A.T.; Alzahrani, O.M.; Mahmoud, S.F.; Gharib, S.M. Assessment of water quality and phytoplankton structure of eight Alexandria beaches, southeastern Mediterranean Sea, Egypt. *J. Mar. Sci. Eng.* **2021**, *9*, 1328. [[CrossRef](#)]
31. Smith, V.H.; Tilman, G.D.; Nekola, J.C. Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut.* **1999**, *100*, 179–196. [[CrossRef](#)]
32. Scheffer, M.; Carpenter, S.; Foley, J.A.; Folke, C.; Walker, B. Catastrophic shifts in ecosystems. *Nature* **2001**, *413*, 591–596. [[CrossRef](#)]
33. Davidson, T.A.; Sayer, C.D.; Jeppesen, E.; Søndergaard, M.; Lauridsen, T.L.; Johansson, L.S.; Baker, A.; Graeber, D. Bimodality and alternative equilibria do not help explain long-term patterns in shallow lake chlorophyll-a. *Nat. Commun.* **2023**, *14*, 398. [[CrossRef](#)] [[PubMed](#)]
34. Feike, M.; Heerkloss, R. Long-term stability of the seasonal succession of different zooplankton species in a brackish water lagoon (southern Baltic Sea). *Hydrobiologia* **2008**, *611*, 17–28. [[CrossRef](#)]
35. Ringkjøbing Amtskommune. *Kilen. Tilstand 1972–1986 samt Udviklingsmuligheder. Ringkjøbing Amtskommune, Teknik-og Miljøforvaltningen, Recipientafdelingen*; Ringkjøbing Amtskommune: Ringkøbing, Denmark, 1988; p. 26. (In Danish)
36. Jeppesen, E.; Johansson, L.S.; Bøgestrand, J.; Tserenpil, S. *Kilen. Report to Struer Kommune*; Ringkjøbing Amtskommune: Ringkøbing, Denmark, 2019; p. 33. (In Danish)
37. Mills, K.; Schillereff, D.; Saulnier-Talbot, E.; Gell, P.; Anderson, N.J.; Arnaud, F.; Dong, X.; Jones, M.; McGowan, S.; Massaferrero, J.; et al. Deciphering long-term records of natural variability and human impact as recorded in lake sediments: A palaeolimnological puzzle. *WIREs Water* **2017**, *4*, e1404. [[CrossRef](#)]
38. Juggins, S. Quantitative reconstructions in palaeolimnology: New paradigm or sick science? *Quat. Sci. Rev.* **2013**, *64*, 20–32. [[CrossRef](#)]
39. Anderson, N.J. Miniview: Diatoms, temperature and climatic change. *Eur. J. Phycol.* **2000**, *35*, 307–314. [[CrossRef](#)]
40. Telford, R.J.; Birks, H.J.B. The secret assumption of transfer functions: Problems with spatial autocorrelation in evaluating model performance. *Quat. Sci. Rev.* **2005**, *24*, 2173–2179. [[CrossRef](#)]
41. Jeppesen, E.; Canfield, D.E.; Bachmann, R.W.; Søndergaard, M.; Havens, K.E.; Johansson, L.S.; Lauridsen, T.L.; Tserenpil, S.; Rutter, R.P.; Warren, G.; et al. Toward predicting climate change effects on lakes: A comparison of 1656 shallow lakes from Florida and Denmark reveals substantial differences in nutrient dynamics, metabolism, trophic structure, and top-down control. *Inland Waters* **2020**, *10*, 197–211. [[CrossRef](#)]
42. IPCC. Summary for Policymakers. In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., et al., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2021; pp. 3–32.
43. Windolf, J.; Jeppesen, E.; Jensen, J.P.; Kristensen, P. Modelling of seasonal variation in nitrogen retention and in-lake concentration: A four-year mass balance study in 16 shallow Danish lakes. *Biogeochemistry* **1996**, *33*, 25–44. [[CrossRef](#)]
44. Lewis, J.P.; Ryves, D.B.; Rasmussen, P.; Knudsen, K.L.; Petersen, K.S.; Olsen, J.; Leng, M.J.; Kristensen, P.; McGowan, S.; Phillipsen, B. Environmental change in the Limfjord, Denmark (ca 7500–1500 cal yrs BP): A multiproxy study. *Quat. Sci. Rev.* **2013**, *78*, 126–140. [[CrossRef](#)]
45. Miljøstyrelsen. *Miljøtilstanden i Kilen 1989–2004*; Miljøstyrelsen: Odense, Denmark, 2004.
46. Renberg, I. The HON-Kajak sediment corer. *J. Paleolimnol.* **1991**, *6*, 167–170. [[CrossRef](#)]
47. Appleby, P.G. Chronostratigraphic techniques in recent sediments. In *Tracking Environmental Change Using Lake Sediments; Basin analysis, coring and chronological techniques*; Last, W.M., Smol, J.P., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2001; Volume 1, pp. 171–203.
48. Dean, W.E. Determination of carbonate and organic-matter in calcareous sediments and sedimentary-rocks by loss on ignition-comparison with other methods. *J. Sediment. Petrol.* **1974**, *44*, 242–248.
49. Heiri, O.; Lotter, A.F.; Lemcke, G. Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *J. Paleolimnol.* **2001**, *25*, 101–110. [[CrossRef](#)]

50. Renberg, I. A procedure for preparing large sets of diatom slides from sediment cores. *J. Paleolimnol.* **1990**, *4*, 87–90. [[CrossRef](#)]
51. Battarbee, R.W.; Carvalho, L.; Jones, V.J.; Flower, R.J.; Cameron, N.G.; Bennion, H.; Juggins, S. Diatoms. In *Tracking Environmental Change Using Lake Sediments; Terrestrial, Algal, and Siliceous Indicators*; Smol, J.P., Birks, H.J.B., Last, W.M., Smol, J.P., Eds.; Developments in Paleoenvironmental Research; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2001; Volume 3, pp. 155–202.
52. Battarbee, R.W.; Kneen, M.J. The use of electronically counted microspheres in absolute diatom analysis. *Limnol. Oceanogr.* **1982**, *27*, 184–188. [[CrossRef](#)]
53. Bennett, K.D. Psimpoll. 2003–2009. Available online: <http://www.chrono.qub.ac.uk/psimpoll/psimpoll.html> (accessed on 3 March 2023).
54. Lewis, J.P. *Holocene Environmental Change in Coastal Denmark: Interactions between Land, Sea and Society*; Loughborough University: Loughborough, UK, 2011.
55. Lewis, J.P.; Ryves, D.B.; Rasmussen, P.; Olsen, J.; van der Sluis, L.G.; Reimer, P.J.; Knudsen, K.-L.; McGowan, S.; Anderson, N.J.; Juggins, S. Marine resource abundance drove pre-agricultural population increase in Stone Age Scandinavia. *Nat. Commun.* **2020**, *11*, 2006. [[CrossRef](#)] [[PubMed](#)]
56. Andrén, E.; Clarke, A.L.; Telford, R.J.; Wesckström, K.; Vilbaste, S.; Aigars, J.; Conley, D.; Johnsen, T.; Juggins, S.; Korhola, A. Defining reference conditions for coastal areas in the Baltic Sea. *TemaNord* **2007**, *583*, 81.
57. Juggins, S. *C2 Data Analysis, Version 1.6.2*; Newcastle University: Newcastle, UK, 2014. Available online: <https://www.staff.ncl.ac.uk/stephen.juggins/software/C2Home.htm> (accessed on 2 January 2020).
58. Telford, R.J.; Birks, H.J.B. Evaluation of transfer functions in spatially structured environments. *Quat. Sci. Rev.* **2009**, *28*, 1309–1316. [[CrossRef](#)]
59. Juggins, S.; Birks, H.J.B. Chapter 14: Quantitative environmental reconstructions from biological data. In *Tracking Environmental Change in Lake Sediments; Data handling and numerical techniques*; Birks, H.J.B., Lotter, A.F., Juggins, S., Smol, J.P., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2012; Volume 5, pp. 431–494.
60. Warnock, J.; Andrén, E.; Juggins, S.; Lewis, J.P.; Ryves, D.B.; Weckström, K. A high-resolution diatom-based Middle and Late Holocene environmental history of the Little Belt region, Baltic Sea. *Boreas* **2020**, *49*, 1–16. [[CrossRef](#)]
61. Lewis, J.P.; Ryves, D.B.; Rasmussen, P.; Olsen, J.; Knudsen, K.-L.; Andersen, S.H.; Weckström, K.; Clarke, A.L.; Andrén, E.; Juggins, S. The shellfish enigma across the Mesolithic-Neolithic transition in southern Scandinavia. *Quat. Sci. Rev.* **2016**, *151*, 315–320. [[CrossRef](#)]
62. Kronvang, B.; Ærtebjerg, G.; Grant, R.; Kristensen, P.; Hovmand, M.; Kirkegaard, J. Nationwide monitoring of nutrients and their ecological effects: State of the Danish aquatic environment. *Ambio* **1993**, *22*, 176–187.
63. Kronvang, B.; Jeppesen, E.; Conley, D.J.; Søndergaard, M.; Larsen, S.E.; Ovesen, N.B.; Carstensen, J. Nutrient pressures and ecological responses to nutrient loading reductions in Danish streams, lakes and coastal waters. *J. Hydrol.* **2005**, *304*, 274–288. [[CrossRef](#)]
64. Jeppesen, E.; Søndergaard, M.; Jensen, J.P.; Havens, K.E.; Anneville, O.; Carvalho, L.; Coveney, M.F.; Deneke, R.; Dokulil, M.T.; Foy, B.; et al. Lake responses to reduced nutrient loading—An analysis of contemporary long-term data from 35 case studies. *Freshw. Biol.* **2005**, *50*, 1747–1771. [[CrossRef](#)]
65. Anderson, N.J.; Bennion, H.; Lotter, A.F. Lake eutrophication and its implications for organic carbon sequestration in Europe. *Glob. Chang. Biol.* **2014**, *20*, 2741–2751. [[CrossRef](#)]
66. Clarke, A.; Juggins, S.; Conley, D. A 150-yr reconstruction of the history of coastal eutrophication in Roskilde Fjord, Denmark. *Mar. Pollut. Bull.* **2003**, *46*, 1615–1629. [[CrossRef](#)]
67. Weckström, K.; Juggins, S.; Korhola, A. Quantifying background nutrient concentrations in coastal waters: A case study from an urban embayment of the Baltic Sea. *Ambio* **2004**, *33*, 324–327. [[CrossRef](#)]
68. Weckström, K. Assessing recent eutrophication in coastal waters of the Gulf of Finland (Baltic Sea) using subfossil diatoms. *J. Paleolimnol.* **2006**, *35*, 571–592. [[CrossRef](#)]
69. Håkansson, H. *Cyclotella striata* complex: Typification and new combinations. *Diatom Res.* **1996**, *11*, 241–260. [[CrossRef](#)]
70. Prasad, A.K.S.K.; Nienow, J.A.; Livingston, R.J. The Genus *Cyclotella* (Bacillariophyta) in Choctawhatchee Bay, Florida, with Special Reference to *C. striata* and *C. choctawhatcheeana* sp.-nov. *Phycologia* **1990**, *29*, 418–436. [[CrossRef](#)]
71. Ryves, D.B.; Clarke, A.L.; Appleby, P.G.; Amsinck, S.L.; Jeppesen, E.; Landkildehus, F.; Anderson, N.J. Reconstructing the salinity and environment of the Limfjord and Vejlerne Nature Reserve, Denmark, using a diatom model for brackish lakes and fjords. *Can. J. Fish. Aquat. Sci.* **2004**, *61*, 1988–2006. [[CrossRef](#)]
72. Cooper, S. Chesapeake Bay watershed historical land use: Impact on water quality and diatom communities. *Ecol. Appl.* **1995**, *5*, 702–723. [[CrossRef](#)]
73. Andrén, E. Changes in the composition of the diatom flora during the last century indicate increased eutrophication of the Oder estuary, south-western Baltic Sea. *Estuar. Coast. Shelf Sci.* **1999**, *48*, 665–676. [[CrossRef](#)]
74. Rines, J.E.B.; Hargraves, P.E. *The Chaetoceros Ehrenberg (Bacillariophyceae) Flora of Narragansett Bay, Rhode Island, U.S.A.*; J. Crammer: Berlin/Stuttgart, Germany, 1988; Volume 79, p. 196.
75. Conley, D.J.; Kaas, H.; Mohlenberg, F.; Rasmussen, B.; Windolf, J. Characteristics of Danish estuaries. *Estuaries* **2000**, *23*, 820–837. [[CrossRef](#)]

76. Carstensen, J.; Conley, D.J.; Andersen, J.H.; Ærtebjerg, G. Coastal eutrophication and trend reversal: A Danish case study. *Limnol. Oceanogr.* **2006**, *51*, 398–408. [[CrossRef](#)]
77. Sabbe, K.; Vyverman, W. Taxonomy, morphology and ecology of some widespread representatives of the diatom genus *Opephora*. *Eur. J. Phycol.* **1995**, *30*, 235–249. [[CrossRef](#)]
78. Fishcon APS. *Kilen-Miljø ved Ferskvand Eller Saltvand*; Fishcon APS: Wädenswil, Switzerland, 2004. (In Danish)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.