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**Identifying nutrient and climate impacts on diatom dynamics in Loch Leven
from recent sediment records**

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Summary

1. Changes in nutrients and climate have occurred over approximately the same timescales in many European lake catchments. Here we attempt to interpret the sedimentary diatom record of a large shallow lake, Loch Leven, in relation to these pressures using information gained from analysis of long term datasets of water quality, climate and planktonic diatoms.
2. The core data indicate enrichment of Loch Leven starting initially in ~1800-1850, most likely attributed to nutrient inputs from agricultural practices in the catchment, with a more marked phase since ~1940-1950 caused by increased phosphorus inputs from sewage treatment works, land drainage and a woollen mill.
3. While the recent diatom plankton remains are dominated by taxa associated with nutrient-rich conditions, an increase in *Aulacoseira subarctica* relative to *Stephanodiscus* taxa since the mid-1980s suggests that reductions in external catchment sources of nutrients (since 1985) may have resulted in partial recovery. This observation accords well with the long-term monitoring series of water chemistry and phytoplankton.
4. On a decadal-centennial scale, the eutrophication signal in the sediment record outweighs any evidence of climate as a control on the diatom community. However, at an inter-annual scale, while the diatom data exhibit high variability, there are several changes in species composition in the recent fossil record of core LEVE12 that may be attributed to climatic controls. For example, high abundances of *A. subarctica* relative to unicellular centricales from 1969 to 1987 appear to be associated with a period of higher winter rainfall with consequences for increased flushing rates; and peaks in *Aulacoseira granulata*, *Aulacoseira granulata* var. *angustissima* and *A. ambigua* seem to coincide with years with warmer springs.
5. The study highlights the value of a palaeolimnological approach, particularly when coupled with long term datasets, for developing our understanding of environmental change at a range of temporal scales. It demonstrates that the diatom record in the sediment can be used effectively to track recovery from eutrophication,

but requires greater understanding of contemporary ecology to fully interpret climate impacts.

6. The study illustrates the complexity of ecosystem response to synchronous changes in nutrients and climate, and the difficulty of uncoupling the effects of these multiple, interacting pressures.

Introduction

Eutrophication, principally caused by phosphorus (P) and nitrogen (N) from agricultural sources, industrial waste and domestic sewage, remains one of the foremost environmental issues threatening the quality of surface waters (Smith, Joye & Howarth, 2006). Many lakes have been subject to nutrient loading over relatively long (centuries, decades) timescales (e.g. Bradshaw, Rasmussen & Odgaard, 2005) but over the last decade or so efforts to better manage and restore enriched systems have increased (e.g. Jeppesen *et al.*, 2007; Søndergaard *et al.*, 2007) and there are now numerous examples of lakes in recovery (Anderson, Jeppesen & Søndergaard, 2005). Current attempts to mitigate eutrophication could be frustrated, however, by the influences of a changing climate (Battarbee *et al.*, 2005; Jeppesen *et al.*, 2007, 2010). At present, the effects of climate change on lake ecosystems are poorly understood. It is often assumed, for example, that rising global temperatures will lead to deterioration in water quality because phytoplankton will become more abundant and warm-water associated cyanobacteria will begin to dominate lake ecosystems. Experimental studies on phytoplankton growth rates and seasonal community successions certainly appear to support this possible outcome (Reynolds, 1984; Moss *et al.*, 1997). However, other studies have shown that the reproductive and grazing rates of zooplankton are affected by changes in temperature (Hanazato & Yasuno, 1985). This suggests that an increase in water temperature could also significantly increase the grazing effect of the zooplankton community on the phytoplankton. If so, the net effect of an increase in water temperature could be lower, rather than higher, phytoplankton abundances. Changes in other meteorological parameters, such as wind speed, incident solar radiation and precipitation, may also have an influence. For example, increases or decreases in precipitation affect the nutrient supply from the catchment and flushing rate (Bailey-Watts *et al.*, 1990).

The potential confounding effect of climate change on remediation efforts is of particular concern to the implementation of the EU Water Framework Directive (WFD) (European Union, 2000). Under this Directive, all Member States must achieve good ecological status in lakes with a surface area of $>0.5 \text{ km}^2$ by 2015. If the definition of good ecological status is based, simply, on historical reference conditions that do not take the effects of climate change into account, Member States may be

faced with water quality targets that are impossible to achieve. In order to improve our understanding of how climate change will affect water quality in the future, we need a better knowledge of how this pressure has modified lake ecosystems in the past. This information is contained within existing historical and palaeolimnological records, but it is difficult to extract because many lakes (especially in populated lowland regions) that have been affected by climate change have also experienced changes in nutrient input over a similar timescale. The examination of long term limnological and climate records may provide a means of disentangling the effects of nutrient enrichment and climate change, and assessing interactions between the two.

This paper focuses on Loch Leven (Kinross, Scotland) and explores the potential of a palaeolimnological approach, in combination with long time series, to assess the impacts of nutrients and climate on a range of timescales from several centuries to seasons. A well documented eutrophication history and existence of comprehensive limnological (spanning the last forty years) and palaeolimnological datasets for the site provides a rare opportunity for examining ecological responses to enrichment and recovery. Further, the latter have occurred over a period when there has been a measurable impact of climate change on the loch. In particular, over the last 30 years, winter ice cover has become less frequent and less extensive, spring air temperatures have increased markedly and winter rainfall has significantly increased (Ferguson *et al.*, 2008; Carvalho *et al.*, in press). Studies considering limnological (e.g. Bailey-Watts *et al.*, 1990; Carvalho & Kirika, 2003) and palaeolimnological (e.g. Haworth, 1972; Bennion, Fluin & Simpson, 2004; Salgado *et al.*, 2010) data have been published previously, but the data have not been integrated to provide a more comprehensive picture of how lake ecosystem structure and functioning are impacted by, and recover from, nutrient pressures. Furthermore, to date such data have not been used to examine how climate and nutrients may interact. The use of sediment records can potentially provide a temporally integrated, longer-term view of ecological dynamics at the site than can be provided by the monitoring data alone, whilst the long term monitoring data series can be used to identify trends and relationships among variables recorded at a seasonal timescale which is less accessible to palaeolimnology (Battarbee *et al.*, 2005).

Loch Leven has been a particular focus for diatom based palaeoecological studies (Haworth, 1972; Bennion *et al.*, 2004). Diatoms (unicellular, siliceous algae: Bacillariophyceae) are one of the most widely used biological groups in palaeolimnological studies for tracking environmental change (e.g. Battarbee *et al.*, 2001). They are sensitive to changes in water quality and have been shown to be particularly good indicators of lake nutrient concentrations (Bennion, Juggins & Anderson, 1996; Hall & Smol, 2010). Here, we present diatom data analysed at a high resolution from a recent sediment core, in combination with existing diatom records from previous cores and long term datasets of phytoplankton, nutrient and climate variables, to track environmental change at a range of temporal scales.

Methods

Site details

Loch Leven is a large, shallow lake (lake area 13.3 km², mean depth 3.9 m, maximum depth 25.5 m), situated in lowland Scotland (56° 12' N, 3° 22' W; altitude 107 m) (Fig. 1). The loch has a well documented history of eutrophication and subsequent recovery following the implementation of a catchment management plan in the late 1980s and early 1990s. This resulted in a 60% reduction in the external load of phosphorus (P) to the loch over the period 1985 to 1995 (from ~20 t y⁻¹ to ~8 t y⁻¹), largely attributed to effluent control from a local woollen mill (D'Arcy, 1991) and improvements to local sewage treatment facilities (Bailey-Watts & Kirika, 1987, 1999). Diffuse P loads from agricultural sources contributed approximately 3.5 t TP y⁻¹ to the load reduction.

Long-term datasets

The lake has been monitored on a weekly to fortnightly basis since 1968 and significant changes in both climate variables and nutrient availability have been recorded over this period (Carvalho & Kirika, 2003; Ferguson *et al.*, 2008; Carvalho *et al.*, in press). Sampling for water chemistry and phytoplankton was carried out by boat from a mid-basin area, using a weighted polythene tube, providing an integrated water sample from the surface to a depth of around 25 cm from the sediment (usually around 3.5 m depth). Samples were stored and analysed using standard methods as described in Bailey-Watts & Kirika (1999). There are gaps in the phytoplankton record for the years 1983, 1984, 1986, 1987, 1991 when samples were not collected. Additionally, a horizontal phytoplankton net (53 µm mesh) tow sample was collected on each occasion to provide information on the dominant algae. As part of the present study, a subset of 34 of these samples, covering the period from 1996-2005, were analysed for diatom composition using standard procedures (Battarbee *et al.*, 2001) to provide information on the seasonality of the dominant species and to facilitate comparison with the sedimentary diatom records. Three to four phytoplankton samples, representing the main diatom growing seasons of each year, were chosen for analysis. Prior to diatom slide preparation, samples stored in formaldehyde were centrifuged (1500 rpm for 3 minutes) and rinsed in distilled water four times. Counts were carried out at x1000 magnification using a Leitz Ortholux II microscope. The main diatom floras used for taxonomic identification were Krammer & Lange-

Bertalot (1986, 1988, 1991a, b). At least 200 valves of centric diatoms and 100 valves of *Aulacoseira* spp. were counted for each sample.

As a result of the catchment management plan P concentrations in Loch Leven declined from an annual mean in excess of 100 μg total P (TP) L^{-1} in the early 1970s to 33 μg TP L^{-1} in 2008, which meets the water quality target of 40 μg TP L^{-1} annual mean for restoring the ecological health of the loch set by the Loch Leven Area Management Advisory Group (LLAMAG, 1993). The declining trend was non-linear, however, with a rapid decline in concentrations in the early 1970s thought to be largely due to the return of *Daphnia* to the lake in 1970 after an absence of 15-20 years (Gunn *et al.*, in press), a slight increase in the early 1990s caused by P recycling from the sediments (Carvalho *et al.*, in press), and another rapid decline in 2007 and 2008. Significant reductions in soluble reactive P (SRP) concentrations, a decline in May chlorophyll *a* concentrations and an increase in spring Secchi disc depth have also been recorded in the last two decades (Carvalho *et al.*, in press). Low SRP concentrations of $<10 \mu\text{g L}^{-1}$ are generally now present from February through to June (Carvalho *et al.*, in press). Indeed Fozzard *et al.* (1999) and May & Carvalho (2010) have reported signs of positive ecological change in the loch following the reduction of point sources of P, including lower algal abundances, increased water clarity, increases in macrophyte abundance in some years, and an increased diversity and abundance of invertebrates.

Daily air temperatures and rainfall measurements in the Loch Leven catchment have been recorded from 1968 to present at a meteorological station near the shore of the lake. The data have been used to calculate monthly averages for key climate variables for each calendar year. Additionally mean air temperature and total rainfall values have been calculated for spring, summer, autumn and winter periods to assess seasonal changes in climate. Data gaps were filled using estimations based on regression equations relating monthly weather data at Loch Leven with monthly records from the Royal Air Force base at Leuchars, 44 km north-east of Loch Leven (<http://www.metoffice.gov.uk/climate/uk/stationdata/>).

Analysis of trends in the climate data from 1968-2007 have revealed that air temperature displays a highly significant, increasing linear trend in spring and

significant, increasing trends in autumn and winter (Carvalho *et al.*, in press). Winter rainfall has exhibited a significant increasing trend over the monitoring period although all seasons showed high variability in rainfall. Notably the years 1990, 1995 and 2000 had particularly wet winters and 1976, 1996 and 2006 were particularly dry winters (Carvalho *et al.*, in press). Given that the most significant changes were seen in spring air temperatures and winter rainfall, these variables are used in the current study for assessing climate influence over observed shifts in the fossil diatom assemblages.

Sediment records

Three cores were collected from the loch: i) a short (33 cm) gravity core (LEVE6) was taken on 24-6-1997 from the North deep basin at a water depth of 23 m, ii) a 90 cm mini-Mackereth core (Mackereth, 1969) (LEVE11) was taken on 5-5-1999 at a water depth of 4 m near to Reed Bower Island, and iii) a 94 cm mini-Mackereth core (LEVE12) was taken on 1-6-2005 from the North deep basin at a water depth of 23 m (Fig. 1). The cores LEVE6 and LEVE11 were extruded in the laboratory at 1 cm intervals and core LEVE12 was sliced throughout at 0.25 cm contiguous intervals. Cores LEVE11 and LEVE12 were radiometrically dated using standard gamma assay procedures (Appleby *et al.*, 1986; Appleby, Richardson & Nolan, 1992) and chronologies were calculated based on ^{210}Pb using the constant rate of supply model (Appleby & Oldfield, 1978).

Selected sub-samples from each core (19 samples from LEVE6, 16 samples from LEVE11 and 61 samples from LEVE12) were prepared and analysed for diatoms using standard procedures (Battarbee *et al.*, 2001). At least 300 valves were counted in each sample using a Leitz research microscope at x1000 magnification and phase contrast. Principal floras used in identification were Krammer & Lange-Bertalot (1986, 1988, 1991a, b), as before. The small centric taxa *Stephanodiscus parvus* Stoermer & Hakansson and *Stephanodiscus minutulus* (Kütz.) Cleve & Moller were merged into *Stephanodiscus parvus/minutulus* owing to difficulties in splitting them consistently using light microscopy. All slides are archived at the Environmental Change Research Centre. Diatom data are expressed as percentage relative abundances for all cores. All diatom taxa shown in subsequent figures are listed in Appendix 1.

Summary groups were utilised to aid ecological interpretation of the diatom data and for comparison with the monitoring data. Thus, all diatoms were grouped by their habitat (planktonic vs. periphytic) and plankton species were summarised variously as *Aulacoseira* spp., unicellular centrales (genera *Cyclotella*, *Cyclostephanos* and *Stephanodiscus*) or ‘oligotrophic *Cyclotella*’ (*C. comensis* Grun. in Van Heurck, *C. aff. comensis*, *C. cyclopuncta* Håkansson & Carter, *C. ocellata* Pant., *C. kuetzingiana* var. *planetophora* Fricke in A. Schmidt, *C. rossii* Håkansson, *C. glomerata* Bachm. and *C. stelligera* (Cleve & Grun. in Cleve) Van Heurck). Species were also partitioned by their typical seasonal response in a temperate lake into i) those taxa frequently dominant in late winter-spring and autumn-early winter (January-May and October-January) when the water column is cooler and ill-illuminated (‘spring-autumn’ taxa), and ii) those often dominant in summer (May-September) when the water is warmer and subject to strong illumination through longer daylengths (‘summer’ taxa) (Appendix 1). Categorisation of each species was achieved by reference to published seasonality studies in which diatom taxonomic resolution was high (Kiss & Padisák, 1990; Köhler & Nixdorf, 1994; Gosselain, Descy & Everbecq, 1994; Reynolds & Irish, 2000; Sayer & Roberts, 2001) and importantly was also informed directly by the available phytoplankton records for Loch Leven (e.g. Bailey-Watts, 1988; Bailey-Watts *et al.*, 1990; and see Fig. 2). Given, the opportunistic nature of diatom algae (Reynolds, 1984) and the inherent temporal variability of environmental controls it is recognised that exact seasonal categorisation of species is impossible. Indeed where conflicting reports of diatom seasonality arose, or where no specific favoured season could be identified a species was divided between the two groups on a 50:50 basis.

Cluster analysis was performed on the full diatom dataset from LEVE12 to identify the major zones in the diatom profile using CONISS (Grimm, 1987), implemented by TILIA and TILIAGRAPH (Grimm, 1991). CONISS is a program for stratigraphically constrained cluster analysis by the method of incremental sum of squares. The high resolution of diatom counts for LEVE12 resulted in multiple samples (up to four) for some years. For comparison with the monitoring data and to remove noise, annual mean diatom composition data were generated by averaging the counts for all samples from a single year.

The diatom data from LEVE12 for the period 1969-2005 were compared with measured hydrochemical and climatological data using principal components analysis (PCA). PCA, an indirect ordination method, was used in preference to constrained techniques such as redundancy analysis (RDA) and canonical correspondence analysis (CCA) as our aim was to extract the main components of variation in the diatom record, not those components that were restricted to be linear combinations of the available environmental data. Previous trend analysis had identified that the most significant changes were in spring air temperatures and winter rainfall (Carvalho *et al.*, in press), consequently these two variables were selected for assessing climate influence over observed shifts in the fossil diatom assemblages, while annual mean TP provided the most complete dataset for assessing response to changes in nutrient concentrations.

Prior to analysis the diatom data were transformed using the Hellinger method of Legendre & Gallagher (2001) such that the resulting PCA represents Hellinger distance between samples rather than Euclidean distance. Measured hydrochemical and climatological data were fitted into the ordination space described by the first two principal components of the diatom data by projecting biplot vectors. This provides a linear representation of the individual variables within the ordination space and allows for easy comparison among the three selected variables. However, in unconstrained ordinations this linear representation may not be ideal as there is no reason to assume *a priori* that relationships between the variables and the ordination space will be linear. To address this issue, we used additive models to fit response surfaces for each variable to the two-component ordination space. A smooth 2-d function of the sample scores on PCA axes one and two was generated using thin-plate splines and used to model the individual measured hydrochemical and climatological variables in turn. The degree of smoothness in the thin-plate spline was estimated by generalised cross-validation. Ordinations and response surfaces were produced using the R statistical language (version 2.11.1-patched revision 52767; R Development Core Team, 2010) and function 'ordisurf' in the vegan package (version 1.17-4; Oksanen *et al.*, 2010).

We rejected partial constrained ordination as a means of measuring the effects of the three variables on diatom composition change for several reasons. Chief among these was that RDA and CCA fit axes that are linear functions of environmental variables

and we did not wish to make such a limiting assumption. Furthermore, partial ordination provides a single measure of the magnitude of effect for each environmental variable for the whole data set; here we are interested in developing a more finely grained assessment of the effects of the three environmental variables to investigate periods in the sediment core when species compositional shifts were related to the environment. The method of Simpson & Anderson (2009) would have provided a more direct assessment of the relative effects of the three variables plus identification of the timing of the effects. However, insufficient numbers of samples were available to justify the use of this more complex technique. Our indirect ordination followed by response surface modelling using an additive model is a sound compromise given our aims and the constraints of the available data.

Results

Seasonality of the diatom plankton

Analysis of the 34 horizontal phytoplankton net tow samples, covering the period 1996-2005, provided useful information on the seasonality of the key diatom taxa in Loch Leven (Fig. 2). *Stephanodiscus parvus* and *Aulacoseira subarctica* peaked in the winter to early spring period. *Cyclotella radiosa* and *Asterionella formosa* reached highest relative abundances in winter samples. In contrast *Diatoma elongatum*, *Aulacoseira granulata* and *Aulacoseira granulata* var. *angustissima* were most common in summer, and *Stephanodiscus hantzschii* forma *tenuis*, *Cyclostephanos invisitatus* and *Cyclotella pseudostelligera* bloomed in autumn.

Stephanodiscus hantzschii, *Cyclostephanos tholiformis* and *Aulacoseira ambigua* appeared in all seasons, but were generally most abundant in autumn also.

Fossil diatom records

Diatom preservation was good throughout all cores with totals of 196, 198 and 164 taxa for cores LEVE6 (deep water, high resolution), LEVE11 (shallow water, low resolution) and LEVE12 (deep water, high resolution), respectively. Based on the radiometric dating results, LEVE11 covered the longest time period. Sediment accumulation rates of 0.12-0.15 cm yr⁻¹ were estimated for this core for the period ~1930-1970, increasing gradually from this time to 0.33 cm yr⁻¹ by 1990 (Fig. 3), and

extrapolation of the accumulation rate for the 1930s suggests that the core dates back to ~1300 AD. However, owing to uncertainties surrounding the chronology, extrapolated dates must be viewed with caution and dates have not been assigned to the lower section. A more reliable chronology was established for the upper 12 cm of the core which is estimated to represent the period from 1934 ± 14 to 1999. Marked changes in diatom community composition were evident in LEVE11 (Fig. 4). Below approximately 20 cm (extrapolated date ~1850) assemblages were co-dominated by planktonic and periphytic taxa, the former being comprised of both 'spring-autumn' and 'summer' taxa. Common phytoplankters were *A. subarctica*, *C. radiosa*, *Tabellaria flocculosa* and 'oligotrophic *Cyclotella*'. Periphyton assemblages consisted largely of small, 'chain-forming' taxa from the *Staurosira*, *Staurosirella*, *Pseudostaurosira* complex (formerly *Fragilaria* and termed so hereafter) and of species in the genera *Achnanthes*, *Amphora*, *Cocconeis* and *Navicula* (not shown). Above 20-15 cm there was an increase in the planktonic component, in particular small unicellular centrals (notably *S. parvus/minutulus* and *S. hantzschii*), together with *A. formosa*, *D. elongatum*, *Fragilaria crotonensis* and *A. ambigua*. Conversely, *T. flocculosa* and oligotrophic *Cyclotella* spp. sharply declined. This upper section also sees a shift towards increased dominance of taxa in the 'spring-autumn' group and a marked reduction in small *Fragilaria* taxa along with many other epiphytic and benthic taxa. By contrast, the epipsammic species *Cymbellonitzschia diluviana* appeared for the first time and steadily increased.

Based on a correlation of diatom assemblage changes, the undated core LEVE6, most likely corresponds to the upper 10 cm of LEVE11 (i.e. post-1950). It therefore affords a higher resolution record of Loch Leven's recent past. Diatom shifts throughout LEVE6 were more subtle than in LEVE11 (Fig. 5). Nonetheless, above 25-30 cm there were some important compositional changes including reductions in unicellular centrals, in particular *S. parvus/minutulus*, *S. hantzschii* and *C. pseudostelligera*, a decrease in *A. ambigua*, and an increase in *A. subarctica* and periphytic taxa (although not in small *Fragilaria* taxa). To a large extent these changes were evident in LEVE11 above 6 cm (dated 1977 ± 5), although a definitive correlation of levels is difficult to make.

A yet higher resolution for the last 60 years is afforded by core LEVE12. Radiometric dating indicates that this core represents the period 1946 ± 14 to 2005 with rapid accumulation rates of $1-3 \text{ cm yr}^{-1}$ (Fig. 3). If our assumptions about the chronology of LEVE6 are correct then LEVE12 and LEVE6 represent a similar time period albeit with LEVE12 adding a further eight years to the top of the LEVE6 record (i.e. 1997-2005) which spans the recovery period in lake P concentrations. Changes in diatom composition (Fig. 6) and abundance (not shown) in LEVE12 were relatively subtle. As in LEVE6, planktonic taxa dominated the assemblages throughout the core (70-80%) and the community was comprised of a large number of species with no single taxon dominating. The most notable shifts in the core were a decline in *A. granulata* var. *angustissima* and *S. parvus/minutulus* relative to *A. subarctica* after ~1970. The increase in the latter relative to *S. parvus/minutulus* was also observed in LEVE6 at ~25 cm. Other notable similarities between LEVE12 and LEVE6 include the expansion of *C. diluviana* which occurs in LEVE12 at ~1955 and in LEVE6 at 30 cm, and the low relative abundance of *A. ambigua* from ~1987 to ~1997 in LEVE12 and in the upper 8 cm of LEVE6, suggesting that there is some consistency in the two deep water sediment records. The general decline in ‘summer’ diatoms in the 1980s-1990s is evident in LEVE12 in accordance with their observed decrease in the upper parts of LEVE6 and LEVE11. However, in recent years there has been marked inter-annual variability in the diatom assemblages of LEVE12 with pulses of *A. ambigua*, *A. granulata* and *A. granulata* var. *angustissima* in 1986-87, 1998-1999 and 2003-2004 resulting in peaks of ‘summer’ taxa in these years.

Ordination

The ordinations with response surfaces for the three main variables, annual mean TP, spring air temperature and winter rainfall (Fig. 7), show samples from 1969-1987 in LEVE12 (located on the left side of the plot) to be associated with the highest TP values and coolest spring air temperatures, with the exception of the 1970 sample which lies to the right of the diagram. The 1970 sample contained particularly high percentages of *A. subarctica* (40%) compared to neighbouring samples with abundances similar to those observed in samples from 2000-2003. The samples from 1988-1997 are located in the upper part of the plot associated with the highest winter

rainfall values and intermediate TP, and the most recent samples from 1998-2005 are positioned to the right of the diagram associated with the highest spring air temperatures and lower TP.

The percentage of variance in each variable explained by the response surface of the PCA axis scores is 74, 12.1 and 36 for TP, winter rainfall and spring air temperature, respectively. Only the fitted response surface for TP was statistically significant ($F = 2.569$, $p = 0.0339$, $df = 14.59$).

Discussion

The various time periods represented by the three cores from Loch Leven allow us to assess environmental change at a range of temporal scales. The following discussion firstly explores the evidence for change at the decadal-centennial scale, and then examines the observed shifts seen at an inter-annual scale in the sediment record, in combination with the monitoring data, with a particular focus on the role of climate and nutrients as potential drivers of change.

Decadal-centennial scale

At the decadal-centennial scale, the palaeolimnological data reveal three main phases in the recent ecological history of Loch Leven: i) a pre-enrichment phase (pre ~1850), ii) an enrichment phase (post ~1850), iii) a partial recovery phase (post ~1985).

Pre-enrichment phase (pre ~1850)

Core LEVE11 provides us with a long term perspective on the ecological history of Loch Leven. The core is estimated to extend back ~700 years based on extrapolation of sediment accumulation rates. The early assemblages of Loch Leven were comprised of a mixed planktonic and periphytic community with taxa found in circumneutral to slightly alkaline, oligotrophic to mesotrophic waters. The planktonic communities contained a mix of 'spring-autumn' and 'summer' taxa (e.g. Kiss & Padišák, 1990; Gosselain *et al.*, 1994; Reynolds & Irish, 2000). Several periphytic species associated with benthic substrates (submerged macrophytes, sediments, stones) were present, indicating favourable light conditions and an important component of benthic primary productivity. There was no major change in the diatom

assemblages until the ~20 cm level of LEVE11 (~1850), suggesting relatively stable conditions in the early part of the loch's history, although a higher resolution dataset for this period and analysis of variance is required to assess the degree of stability more fully.

The findings agree with an earlier study of the diatom history of Loch Leven's north basin (Haworth, 1972) where *Melosira italica* ssp. *subarctica* O. Mull. (now *A. subarctica*), *T. flocculosa* and oligotrophic *Cyclotella* taxa were observed along with small *Fragilaria* spp. and other periphytic taxa in the lower sections of a core. Furthermore, a recent study of plant macro-remains from a shallow water core collected offshore from St. Serfs Island suggests a submerged macrophyte community associated with oligotrophic to mesotrophic waters during this era including *Isoetes lacustris* L. and *Lobelia dortmanna* L. (Salgado *et al.*, 2010).

Enrichment phase (post ~1850)

The most marked change in the fossil diatom record occurred at around 20 cm in LEVE11 (~1850) with the appearance of planktonic taxa that had previously been in low abundance or had not been observed in the diatom record, namely *A. ambigua*, *S. parvus/minutulus*, *S. hantzschii*, and *C. pseudostelligera*. These taxa expanded from around 1940 to the core top (1999) in LEVE11. They are typically associated with nutrient-rich waters and occur in winter-spring and autumn suggesting a strengthening of the diatom bloom either side of the summer period. An increase in planktonic diatoms and turbidity in response to enrichment could have reduced the depth of light penetration thus reducing the habitat availability for benthic diatoms which decline during this period.

In accordance with our data, Haworth (1972) noted an appearance of *Melosira ambigua* (Grun. in Van Heurck) O. Mull. (now *A. ambigua*) and *S. hantzschii*, taxa indicative of enrichment, in the upper part of a core from the Northern basin of the loch. Although an accurate chronology was lacking for this core, it was concluded that there was evidence of early enrichment some time prior to 1816 AD followed by a subsequent more marked eutrophication phase. Furthermore, the post 1816 AD changes were thought to be associated with lowering of the lake level by 1.5 m in 1830 which resulted in an expansion of agriculture around the lake. The arrival of

Cymbellonitzschia diluviana in LEVE11 and its expansion in both LEVE12 and LEVE6 perhaps reflect the shallowing of the lake as the lowering process may have brought more sandy sediment from the littoral zone to the core site. *C. diluviana* is a small, low growing, epipsammic species which forms aggregates in the depressions of sand bars. It has the ability to stay firmly attached to sand grains, and can form resting stages that allow it to cope with wave action and changes in water level (Jewson, Lowry & Bowen, 2006). It is interesting to note, however, that despite the expansion of *C. diluviana* in our cores, there was no overall increase in periphyton, and indeed planktonic forms continued to dominate the assemblages. This suggests that the enrichment signal was sufficiently strong to over-ride the influence of lake level change on the diatom community. Nonetheless, a water level change of 1.5 m is not insubstantial and highlights the fact that there are multiple drivers of change and that Loch Leven's pre-disturbance baseline in terms of water depth and benthic habitat has shifted.

Our core data provide evidence of enrichment of the loch starting initially in ~1850 with a more marked phase since ~1940-1950. The former is most likely attributed to nutrients from expanding agricultural practices in the catchment, and the latter with P inputs from sewage treatment works, land drainage and P-rich discharges from the woollen mill, considered to be a substantial point source of P (Holden & Caines, 1974). The enrichment trend inferred from the diatom record is supported by the long term datasets which provide evidence of eutrophication, although the latter are limited in temporal extent as the monitoring programme did not start until 1968 and, therefore, post-dates the onset of enrichment. Nevertheless, available historical data record a decline in the extent of macrophyte beds since the late 1940s and cyanobacteria blooms as a regular feature on the loch since the 1960s (Fozzard *et al.*, 1999).

Recovery? (post ~1985)

An increase in *A. subarctica* relative to *Stephanodiscus* spp. in all cores from ~1970s to 1980s suggests recent biological recovery from eutrophication. The phytoplankton monitoring data confirm that unicellular centrics, principally *Stephanodiscus*, produced extensive spring blooms in the 1960s and early 1970s and that these have shown two step-wise declines, along with a trend towards increasing cell size with

larger species such as *Aulacoseira* spp. dominating over smaller unicellular centrics (Bailey-Watts, 1988; Bailey-Watts *et al.*, 1990; Carvalho pers. comm.). The first decline, in the 1970s, coincided with the re-colonisation of *Daphnia*. These large zooplankton feed particularly efficiently on smaller algae, and thereby increased grazing pressure on the small centric diatoms (Bailey-Watts *et al.*, 1990). The second decline, from the mid-1980s, however, is thought to be associated with nutrient reductions.

A considerable reduction in point sources of P from industrial and sewage effluents has been achieved since 1985 (May, Defew & Bennion, in press). However, agriculture in the Leven catchment remains a significant diffuse source of nutrients to the loch as much of the land is used for arable farming, and rural septic tanks also contribute to the P load. Nevertheless, Fozzard *et al.* (1999), May & Carvalho (2010), and Gunn *et al.* (in press) report signs of positive ecological change in the loch since the reduction of point sources of P, including lower algal abundances, increased water clarity, increased macrophyte colonisation depth, and an increased diversity and abundance of invertebrates. In line with these signs of improvement, a small increase in the periphytic taxa was observed in the cores from the mid-1980s. Whilst there appears to have been some recovery at the functional level, the sediment record suggests that those diatom taxa lost during enrichment, notably the oligotrophic *Cyclotella* spp., *T. flocculosa*, and the *Fragilaria* spp. have not yet returned. This may in part be due to the altered shoreline configuration and hence the shifting baseline which confounds attempts to return the flora to that seen in the past. It may also be that nutrient concentrations remain too high and indeed macrophyte abundance still remains low compared to pre-enrichment conditions.

The monitoring data show that chemical recovery of the loch has not followed a monotonic improving trend. The rapid decline in concentrations in the early 1970s pre-dates the restoration programme and is again thought to be largely due to the return of *Daphnia* in 1970 following their disappearance, attributed to dieldrin poisoning (Morgan, 1970; Gunn *et al.*, in press). The slight increase in measured annual mean TP in the early 1990s arose due to increased P recycling from the sediments which compensated for reductions in the external nutrient load (Spears *et al.*, 2007; Carvalho *et al.*, in press). Changes in climate may also have played a role in

driving observed recent changes in the loch and these are further discussed when examining finer scale variation in the diatom record (see below).

Inter-annual scale: variation in the diatom record over the last 40 years

The higher resolution diatom data for the recent period provided by LEVE6 and LEVE12 show that the diatom flora has been dominated by planktonic, nutrient-tolerant taxa for at least the last four decades. This is in accordance with plankton data which reveal a community of *Aulacoseira* taxa and unicellular centric diatoms, predominantly *S. parvus* and *S. hantzschii* with some *Cyclotella* and *Cyclostephanos* species (Bailey-Watts, 1988; Fig.2). A comparison of planktonic diatom composition in LEVE12 with that of phytoplankton records for the period 1996-2005 revealed a good match for the dominant taxa (Jeppesen *et al.*, 2010). Indeed, both records exhibit a decline in *C. radiosa* and *A. formosa*, and more recently in *A. subarctica*, *A. granulata* and *D. elongatum*, and a relative increase in *F. crotonensis*, *S. hantzschii* and *S. parvus/ minutulus*. This indicates that the core does reflect real compositional changes and suggests that the fossil assemblages are not just an artefact of variable dissolution or spatial variability induced by sediment mixing and bioturbation.

While there are consistencies in diatom changes recorded in all three cores (e.g. the *Stephanodiscus* decline in the upper part of the records), the assemblages exhibit high intra- and inter-annual variation which is also seen in the contemporary records. A detailed taxonomic analysis of diatom species in the spring maxima over 1968- 1985 revealed no obvious temporal pattern in the occurrence of any species, with no species dominating for more than two years (Bailey-Watts, 1988). Indeed, in this respect Bailey-Watts *et al.* (1990) noted that “The hallmark of Loch Leven phytoplankton dynamics, which has been studied since 1967, is in its extraordinary inter-annual variability”. This high variability in the phytoplankton community in the sediment record confounds attempts to determine clear trends reflective of improving water quality. While there is evidence of partial recent recovery since the 1980s in the diatom record when viewed at a decadal scale (see above), it is difficult to extract a clear recovery signal from the higher resolution study of LEVE12 where initial signs of improvement in the late 1980s (e.g. increase in *Aulacoseira* spp. relative to unicellular centric diatoms) are reversed in more recent years (2004-2005). This reversal of recovery was also evident in measured annual mean in-lake P concentrations which

show that water quality varies from year to year with values of $\sim 75 \mu\text{g TP L}^{-1}$ between 2003 and 2005 compared to $\sim 50 \mu\text{g TP L}^{-1}$ in 2000.

While errors associated with the core chronology require that a degree of caution is exercised when comparing core and plankton data directly, these datasets can be explored to assess overall trends. Although the diatom data in LEVE12 are noisy over the last 37 years, some structure in the data is evident with apparent diatom responses to nutrient and climate variables. The samples from 1969-1987 comprised relatively high percentages of taxa typically associated with highly productive lakes, such as *A. ambigua*, *S. parvus/minutulus*, *S. hantzschii*, and *C. pseudostelligera*. The woollen mill did not cease using P-based materials until 1988 and reduction of nutrient loads from the sewage treatment works did not occur until the 1990s. Hence 1969-1987 marks one of the most productive periods in the history of the lake and the high P concentrations are likely to have been a key control over diatom assemblages. Additionally the data show that spring air temperatures in 1969-1987 were on average 1°C lower than today and, therefore, temperature may also have played a role in influencing the diatom community. This highlights the problem of identifying the key controls on diatom dynamics and suggests that the measured variables are insufficient to capture the complexity of the multiple drivers.

I have deleted the sentence in the Discussion about percentage variance explained by each env variable but can we add anything in its place based on the significance of the fitted surface for TP compared with the climate variables?

The samples from 1988-1997 contained lower percentages of ‘summer’ taxa (*A. granulata*, *A. granulata* var. *angustissima* and *A. ambigua*) but higher amounts of ‘spring-autumn’ taxa (*A. subarctica*) than the 1969-1987 samples. Annual mean P concentrations remained relatively high in this period despite a reduction in external nutrient inputs, due to continued internal loading. However, winter rainfall values were at their highest during this ten year period suggesting that the observed subtle species shifts may have been driven by climatic factors. Loch Leven has a characteristically variable oceanic climate and a large catchment and hence rainfall exerts considerable influence on the loch. The amount of rainfall impacts on flushing rates which have been shown to affect the supply and dynamics of nutrients in Loch Leven, producing a highly variable water column and complex phytoplankton

sequences (Bailey-Watts *et al.*, 1990). A more rapid flushing rate in years of high winter rainfall may have favoured occurrence of *A. subarctica* over unicellular centric diatoms as the former has resting stages and can, therefore, in spring quickly accumulate numbers from the sediments while the latter, which do not have this facility may require a longer duration of low flushing rates to build up populations.

The samples from 1998-2005 saw a further increase in several of the 'spring-autumn' taxa (*A. subarctica*, *C. invisitatus*) but a decline in others typically associated with this season (*A. formosa*), and similarly a rise in the relative abundances of some of the 'summer' taxa (*A. ambigua* and *A. granulata* var. *angustissima*), with notably high percentages of these taxa in 1998-9 and 2003-4, but with decreases in others classed in the 'summer' group (*F. crotonensis*, *C. radiosa*). Ordination analysis suggests that these shifts may be associated with the decrease in annual mean P concentrations and/or the increase in spring air temperatures. Climate data from 1968 reveal an increasing trend in water temperatures, particularly during winter and spring (Carvalho & Kirika, 2003; Carvalho *et al.*, in press). Given that nutrient reduction and climate warming have occurred over the same timescale at Loch Leven it is difficult to disentangle the relative ecological influence of these drivers. The long term phytoplankton dataset for Loch Leven indicates that *Aulacoseira* abundance is associated with years when winter SRP and spring silica concentrations are low and winter temperatures are high and conversely high unicellular centric abundance is associated with years when winter SRP is high and winter rainfall is low (Carvalho, unpublished data). The 1996-2005 dataset indicates that *A. granulata*, *A. granulata* v. *angustissima* and *A. ambigua* generally bloom in late summer to early autumn and these taxa are known to tolerate warmer waters than *A. subarctica* (Lund, 1954) which tends to peak in late winter to early spring. Consequently, their higher abundances relative to *A. subarctica* may reflect changing climatic conditions, but in relation to different seasons and consequent effects on seasonal nutrient availability. Indeed 2003-4 saw spring temperatures of 8.4 and 8.2°C compared with an average of 7.6°C for 1998-2005. Reduced availability of silica for diatom growth may also be important as trend analysis reveals that soluble reactive silica concentrations for the first (1968-1977) and last (1998-2007) decade of monitoring have declined for all months of the year, leading to potentially limiting concentrations for diatom growth throughout the year (Reynolds, 2006). Diatoms have become the dominant algal group in the loch for

much of the year (Carvalho, unpublished data) and hence the reduction in silica may be due to greater diatom uptake. Most probably a complex interaction between nutrient and climatic factors contribute to the observed shifts in the sedimentary diatom assemblages and a greater understanding of contemporary diatom ecology, especially at the species level, at a seasonal scale is required before high-resolution palaeo-records can be adequately interpreted.

Concluding remarks

Our study highlights the value of a palaeolimnological approach for developing an understanding of environmental change at a range of temporal scales and for tracking recovery. However, the study underlines the difficulty of deciphering a climate signal in sites where recent eutrophication and climate change have occurred over the same period. At the decadal-centennial timescale eutrophication is clearly the dominant force driving ecological change in Loch Leven and any climate effects seem to be overpowered by the enrichment signal. Ideally remote sites with little anthropogenic influence are required for extracting climate signals but these rarely have long-term monitoring data to help validate the trends inferred from the sediment record.

At an inter-annual scale it was not possible to establish the relative importance of climate and nutrients in explaining the diatom assemblages and indeed interactions between these variables make separation of their effects difficult. A study of this kind would ideally be undertaken at a site with varved sediments but such highly resolved sequences are rarely found in shallow lakes. The high intra and inter-annual variability of the phytoplankton in Loch Leven relative to the signal also precludes detection of this climate signal at the fine scale. Indeed nutrient and climate variables explained only a small amount of the variation in the diatom assemblages and it is likely that small changes in local weather conditions, top-down control by fish and zooplankton, as well as chaotic and random factors have also played a role in controlling species composition. (Gav - Is it still ok to say this given that we have removed the results paragraph on percentage variance explained by nutrients and climate? How might we reword it?) Nevertheless the strong influence of flushing rate on temperature regimes, external nutrient supply and in-loch nutrient dynamics suggests that, at an inter-annual scale, changes in species composition in the recent

fossil record may be in part attributed to climatic factors. The study highlights the need for more autecological information on centric diatoms, particularly seasonality data, to better inform palaeoecological studies. The functional groups of Reynolds *et al.* (2002) would suggest that nutrients and light climate are likely to be key gradients for shifting between the unicellular and filamentous diatoms but information on the ecological preferences of individual diatom taxa in relation to temperature and flushing-rate is lacking.

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Appendix 1 List of diatom names with their authorities, and seasonal grouping for planktonic taxa (Sp-Au: spring-autumn taxa, Su: summer taxa, 50:50: divided between Sp-Au and Su groups on a 50:50 basis)

<i>Achnantheidium minutissimum</i> (Kutz.) Czarnecki	
<i>Amphora pediculus</i> (Kutz.) Grun.	
<i>Asterionella formosa</i> Hassall 1850	Sp-Au
<i>Aulacoseira ambigua</i> (Grun. in Van Heurck) Simonsen	Su
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	Su
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O. Mull.) Simonsen	Su
<i>Aulacoseira italica</i> var. <i>tenuissima</i> (Grun. in Van Heurck) Simonsen	Sp-Au
<i>Aulacoseira subarctica</i> (O.Mull.) Haworth	Sp-Au
<i>Cyclostephanos invisitatus</i> Theriot, Stoermer & Hakansson, comb. nov.	Sp-Au
<i>Cyclostephanos tholiformis</i> Stoermer, Hakansson & Theriot	50:50
<i>Cyclotella pseudostelligera</i> Hust.	50:50
<i>Cyclotella radiosa</i> (Grunow) Lemmerman	Su
<i>Cymbellonitzschia diluviana</i> Hust.	
<i>Diatoma elongatum</i> (Lyngb.) Ag.	Sp-Au
<i>Fragilaria crotonensis</i> Kitton	Su
<i>Fragilaria parasitica</i> (W. Sm.) Grun. in Van Heurck	
<i>Pseudostaurosira brevistriata</i> (Grun. in Van Heurck) Williams & Round	
<i>Staurosira construens</i> var. <i>binodis</i> (Ehrenberg) P.B.Hamilton	
<i>Staurosirella pinnata</i> (Ehrenb.) Williams & Round	
<i>Stephanodiscus hantzschii</i> Grun. in Cleve & Grun.	Sp-Au
<i>Stephanodiscus hantzschii</i> forma <i>tenuis</i> (Hustedt) Hakansson & Stoermer	Sp-Au
<i>Stephanodiscus parvus/ minutulus</i>	Sp-Au
<i>Synedra acus</i> var. <i>angustissima</i> (Grun. in Van Heurck) Van Heurck	
<i>Tabellaria flocculosa</i> (Roth) Kutz.	Su

Figure legends

Figure 1 Map of Loch Leven showing coring locations

Figure 2 Seasonality of diatom taxa in the phytoplankton net tow samples 1996-2005. Samples are ordered by date (y-axis), species data are in % relative abundance and mean monthly water temperature is expressed in °C

Figure 3 Depth-date profiles for Loch Leven cores LEVE12 (deep water) and LEVE11 (shallow water) based on the ^{210}Pb constant rate of supply model (Appleby & Oldfield, 1978)

Figure 4 Summary diatom diagram of LEVE11. Diatom data are expressed as % relative abundance. The point of major compositional change is shown by the horizontal line and the approximate time periods covered by LEVE6 and LEVE12 are shown for comparison

Figure 5 Summary diatom diagram of LEVE6. Diatom data are expressed as % relative abundance.

Figure 6 Summary diatom diagram of LEVE12. Diatom data are expressed as % relative abundance. The zones of compositional change are indicated by the horizontal lines (determined by CONISS). Measured environmental data (TP, Spring mean air temperature, Summer mean air temperature and Winter total rainfall) for 1969-2005 are shown.

Figure 7 Ordination of the 1969-2005 diatom data from LEVE12 with response surfaces for the three main environmental variables

Figure 1 Map of Loch Leven showing coring locations

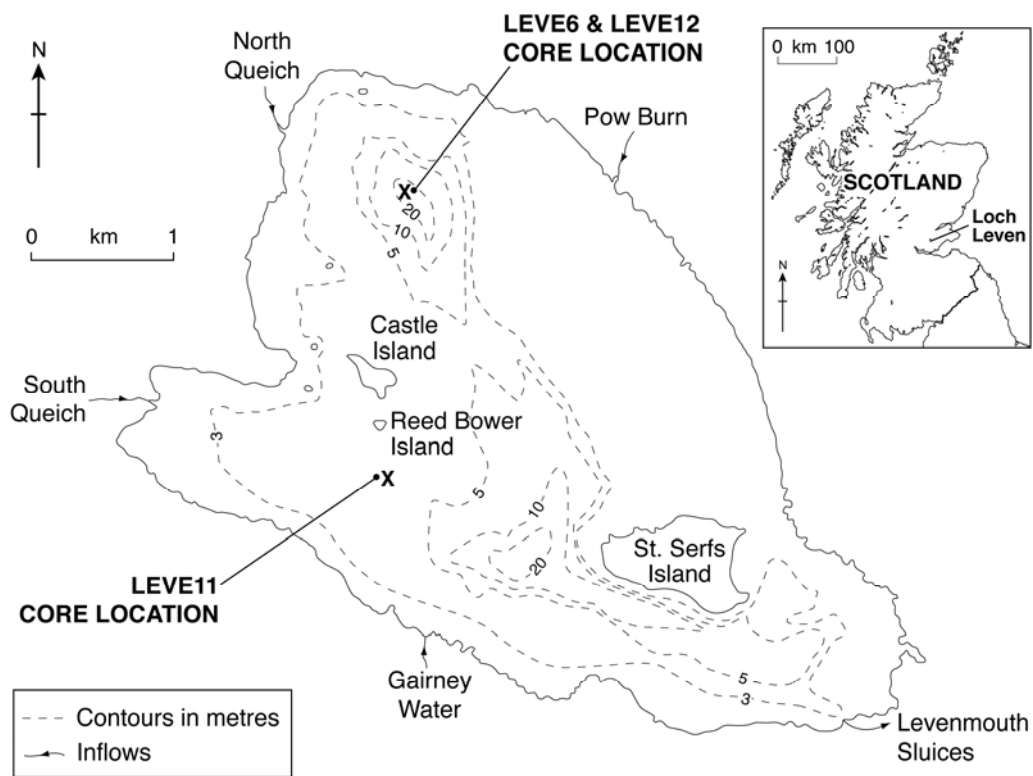


Figure 2 Seasonality of diatom taxa in phytoplankton net tow samples 1996-2005

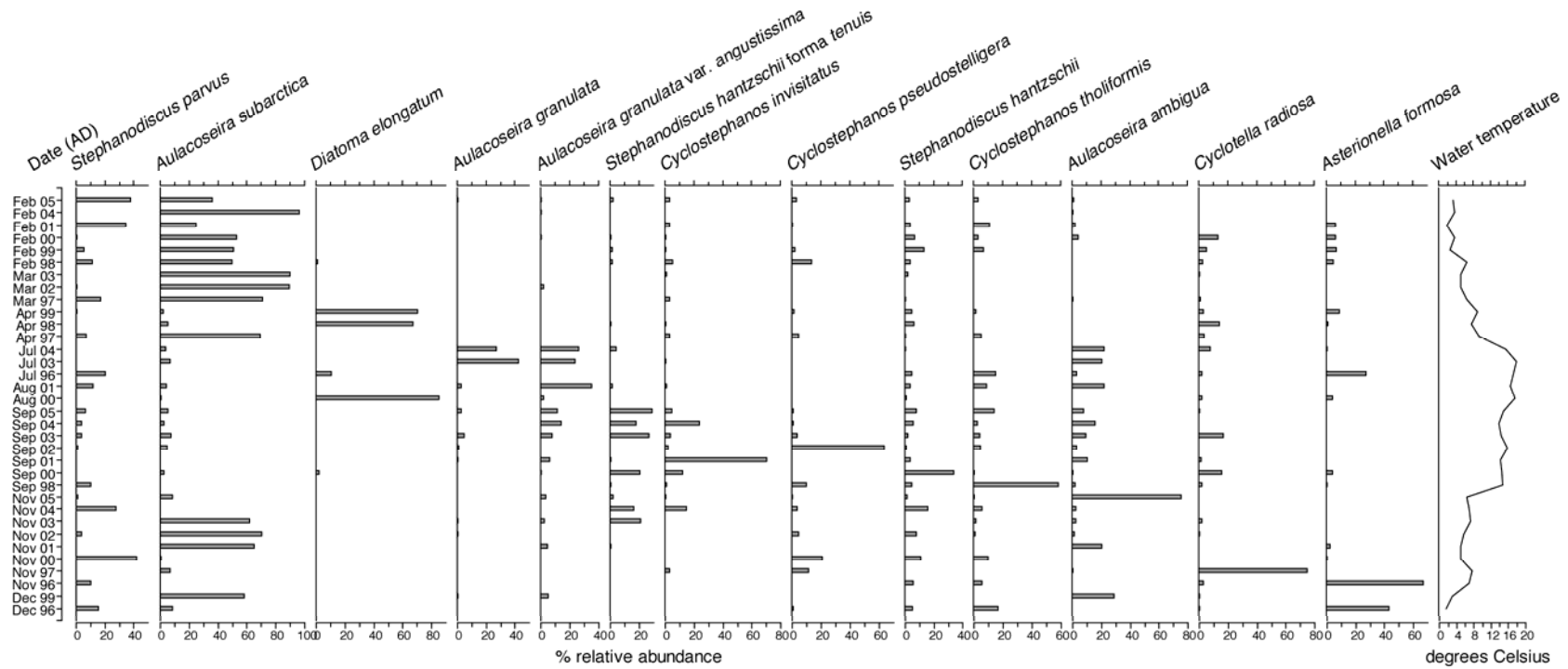


Figure 3 Depth-date profiles for Loch Leven cores LEVE12 (deep water) and LEVE11 (shallow water) based on the ^{210}Pb constant rate of supply model (Appleby & Oldfield, 1978)

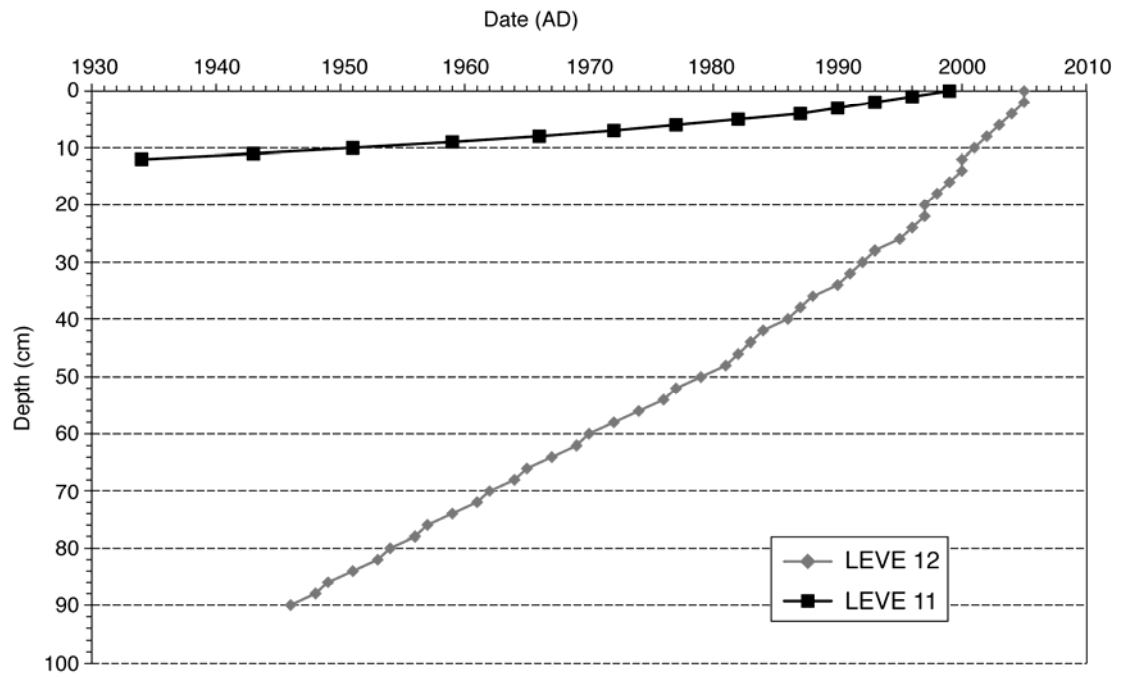


Figure 4 Summary diatom diagram of LEVE11. Diatom data are expressed as % relative abundance. The point of major compositional change is shown by the horizontal line and the approximate time periods covered by LEVE6 and LEVE12 are shown for comparison

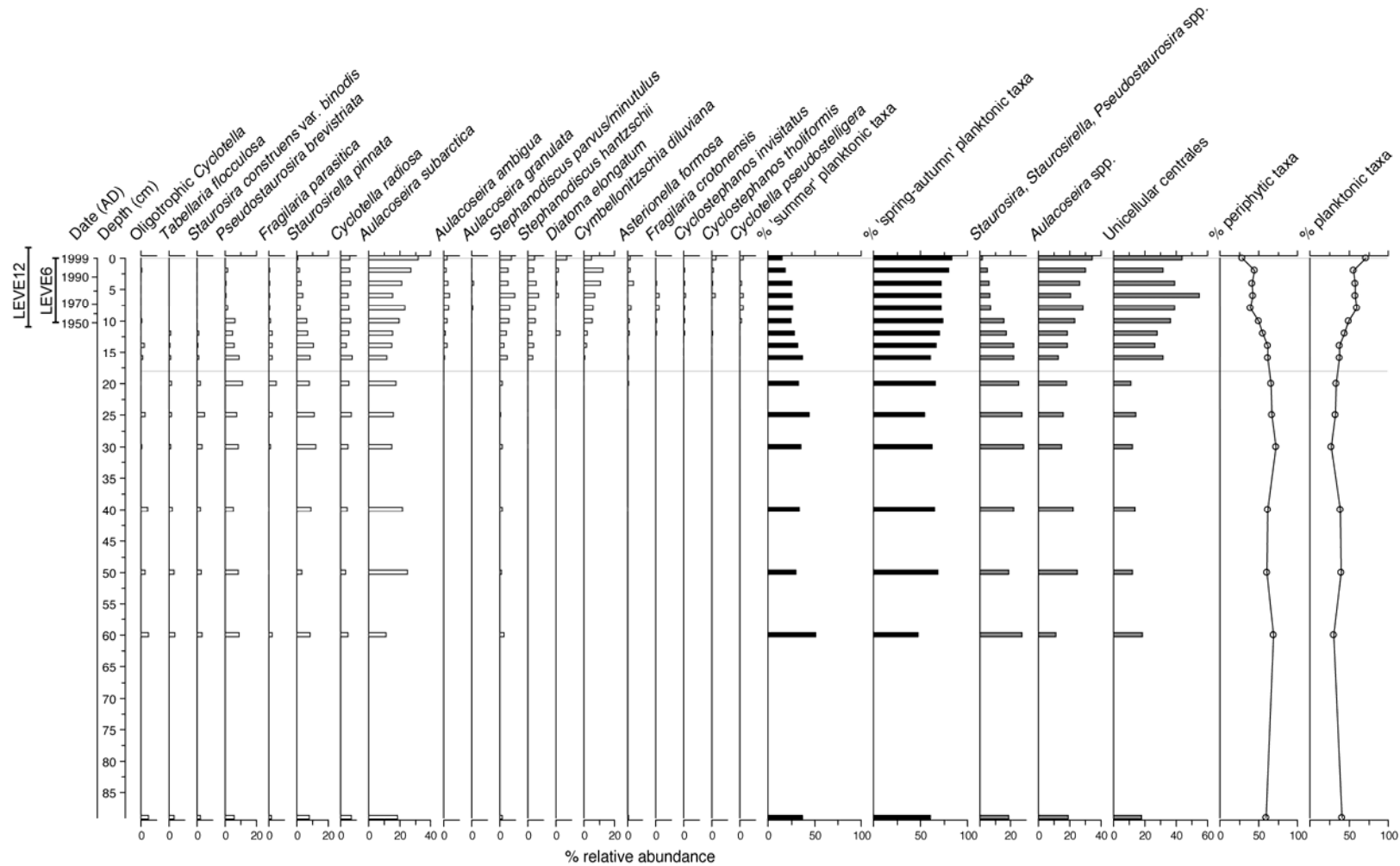


Figure 5 Summary diatom diagram of LEVE6. Diatom data are expressed as % relative abundance.

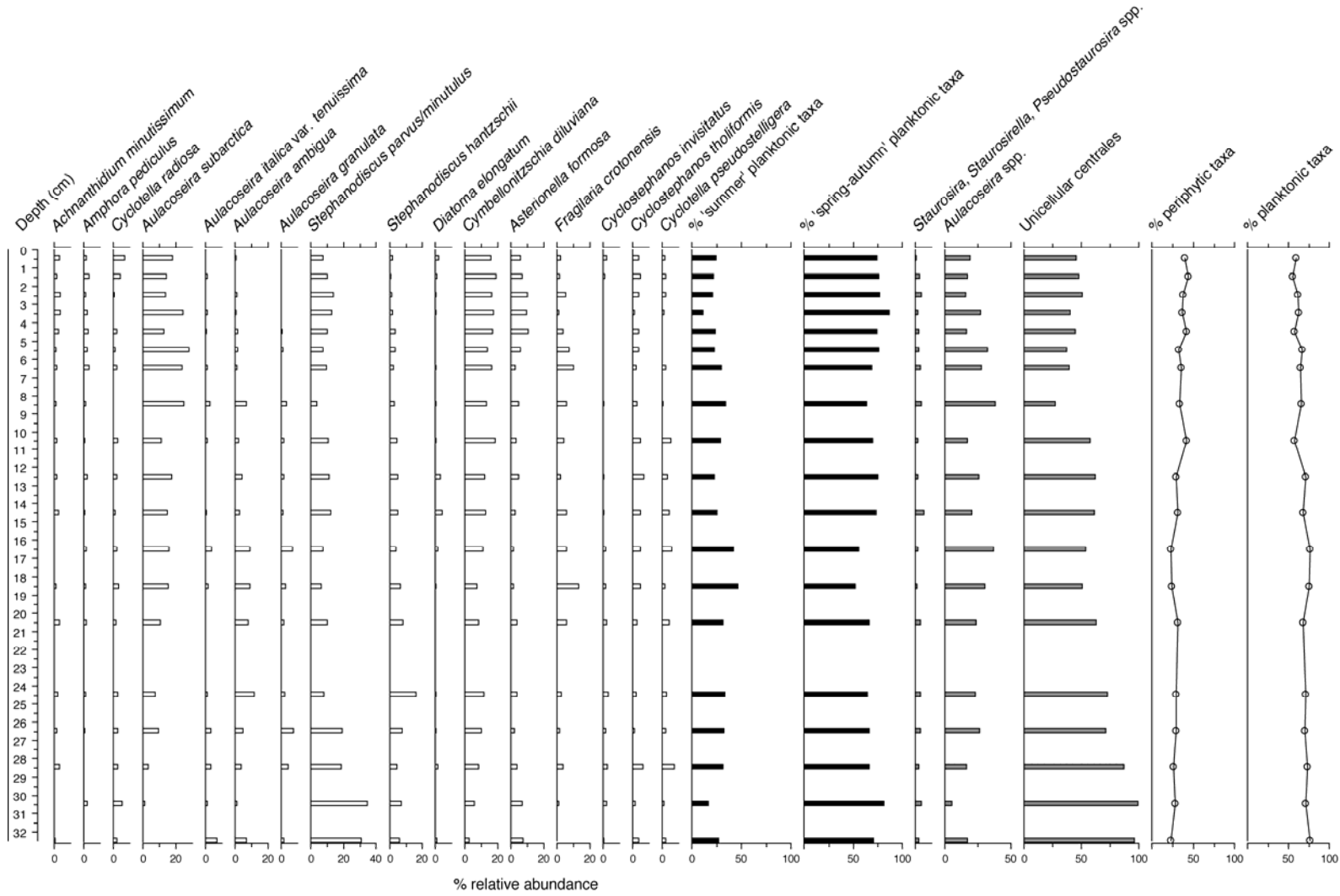


Figure 6 Summary diatom diagram of LEVE12. Diatom data are expressed as % relative abundance. The zones of compositional change are indicated by the horizontal lines (determined by CONISS). Measured environmental data (TP, Spring mean air temperature, Summer mean air temperature and Winter total rainfall) for 1969-2005 are shown.

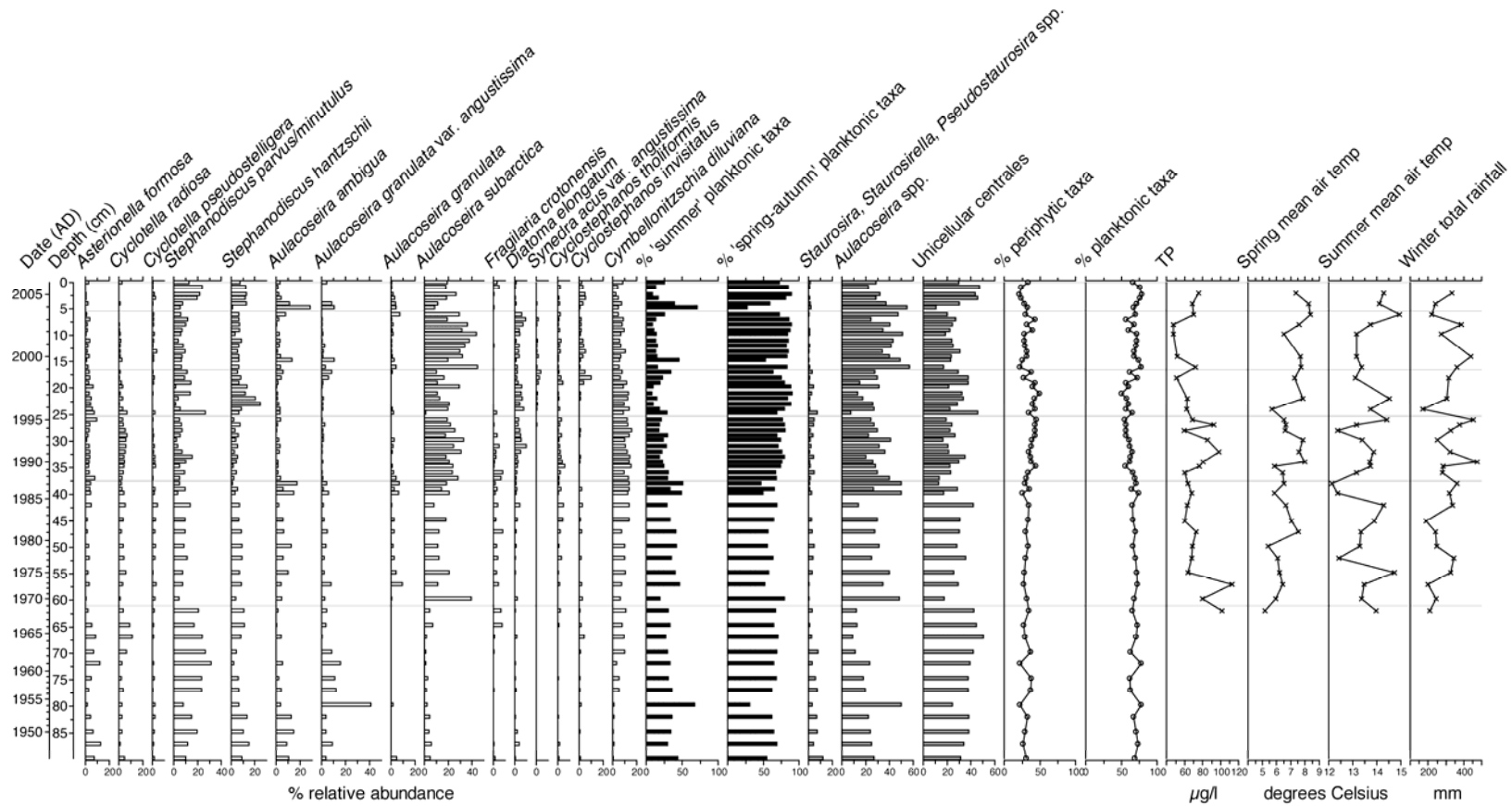


Figure 7 Ordination of the 1969-2005 diatom data from LEVE12 with response surfaces for the three main environmental variables

