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DEPARTMENT OF BOTANY

BOT4000W

HONOURS IN BOTANY

MUDDY MEMORIES: ENIVRONMENTAL CHANGE AT HLUHLUWE-IMFOLOZI GAME RESERVE, KWA-ZULU NATAL, SOUTH AFRICA: EVIDENCE FROM DIATOMS

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This project aims to show how changes in diatom community structure over time provide evidence to interpret historical climate changes in the Hluhluwe area.

1) Abstract

Diatoms are microscopic algae found in almost all aquatic environments. They are habitat specific and have silica frustules which preserve well in sediments. They have been used extensively to provide a multitude of palaeoecological data relating to pH, nutrient loads and water levels. Here, the diatoms in a lake sediment core from Hluhluwe-Imfolozi, South Africa, are analysed to show fluctuations in littoral to benthic taxa, and brackish to freshwater taxa as indicators of lake level changes over time. Changes in lake level act as a proxy for warm/dry or cold/wet periods over time. During the last 240 years lake levels have remained consistently low but nevertheless indicate four alternating periods of cold/wet and warm/dry climate. From c. A.D. 1770 (the last few decades of the Little Ice Age) to the c. A.D. 1840 the climate was cool and wet, but nonetheless gradually warmed up and became drier. From c. A.D. 1840 through to c. A.D. 1920 a there was a gradual increase in temperature and corresponding decrease in rainfall. Then, from c. A.D. 1920 till c. A.D. 1980 there was another colder, wetter period. Finally, very recently, from around c. A.D. 1980 till the present day, Hluhluwe-Imfolozi has experienced warmer, drier conditions once. Pollution-tolerant diatoms increase during the last 100 years and indicate eutrophication of the lake, a consequence of anthropogenic activity in the region. The diatom sequence from Phindiswene provides a high resolution climatic proxy for the critical period covering the latter stages of the Little Ice Age (LIA), post LIA warming, and recent increasing anthropogenic impacts.

Key Words

Palaeoecology, diatoms, Lake sediment core, South Africa, climate variation.

Contents

2) Introduction

Diatoms are microscopic algae found in almost all aquatic environments where they often dominate microalgal assemblages (Stoermer and Smol, 1999). They are characterized by high species diversity and niche specificity, and thus their community composition is typically governed by physico-chemical and biological variables present in their habitat (Fritz *et al.*, 1999). They are abundant where water is at least occasionally present and, moreover, are ecologically sensitive (Stoermer and Smol, 1999). They are well preserved in sediments as their 'skeletons' (frustules) consist of two valves made of resistant opaline silica (Wolfe, 2003). In addition to this, the cell walls of the valves have taxonomically diagnostic shapes, sizes and sculpturing (Stoermer and Smol, 1999) making their identification easy. They are also often cosmopolitan species, meaning many of the same species are often found all over the world (Taylor *et al.* 2007). The stratigraphic sequence of diatoms in lacustrine sediments can offer a valuable source of evidence for the reconstruction of high temporal resolution environmental change (Anderson, 1995). These characteristics have enabled diatoms to be used as indicators of past climates and make them useful for gaining insight into environmental variables (such as pH, conductivity and cation/anion concentration and composition) and climate changes (Wolin and Duthie, 1999). Diatom analysis is thus being increasingly used to determine factors responsible for observed environmental changes.

Diatom communities respond rapidly to limnological changes that are directly or indirectly influenced by climate. Davis (1985) and Hughen *et al.* (2000) showed that diatom community structure from a core taken on Baffin Island traced climatic changes very precisely during, and immediately after, the Little Ice Age. Previous studies by Ekblom and Stabell (2008) and Gillson and Ekblom (2009) have looked at changes in the ratio of benthic to planktonic diatom taxa as indicators of lake water levels over time. An increase in the proportion of benthic species is indicative of low lake levels and hence higher temperatures and/or lower rainfall (Ekblom and Stabell 2008). A relative increase in the proportion of planktonic species is indicative of high lake levels and hence lower temperatures and/or higher rainfall (Ekblom and Stabell 2008). Other studies have looked at changes in the ratio of littoral/boundary to benthic diatom taxa as an indicator of lake level change (Sarmaja-Korjonen & Hyvarinen 2002). In the same study, fluctuations from freshwater to brackish water states, as indicated by the diatom taxa, correlated well with lake level fluctuations. In particular, brackish water states were associated with low lake levels, whilst freshwater states were more typically associated with higher lake levels (Sarmaja-Korjonen & Hyvarinen 2002).

The use of palaeoecological data is becoming increasingly important as a tool for reserve management (Saunders *et al.* 2008) and these kind of analyses can be applied in a broader context towards the summer rainfall region of South Africa. This project aims to investigate fluctuations in lake levels at Phindiswene, a site near the Hluhluwe-Imfolozi Game Reserve in Kwa-Zulu Natal. Fluctuations in lake levels will be used as a proxy for tracking palaeoclimate changes over time. High lake levels are indicative of cool/wet periods whilst low lake levels are indicative of warm/dry periods. In particular, this project will investigate changes in the proportion of benthic, planktonic, aerophylic and littoral taxa as an indication of fluctuating lake levels and as a proxy for cool/wet or warm/dry periods over time. It will also investigate the ratio of brackish to freshwater species as a potential corroboration of the aforementioned lake level indicators.

This project forms part of a broader project which includes analyzing fossil phytoliths to calculate *Broad-leaved tree cover density* (D/P), *Humidity - Aridity index* (Iph%), climatic index and water stress index. These indices will be used to estimate climatic conditions at the study site and corroborate climate fluctuations indicated by the diatoms. The broader project also includes using stable carbon and nitrogen isotopes as indicators of tree to grass ratio fluctuations over time. Lastly, pollen and charcoal grains in the cores will also be investigated in an attempt to reveal changes in tree density and fire frequency over time. The combination of these studies will provide a strong palaeoclimatic and palaeoecological record for the Hluhluwe-Imfolozi Game Reserve and will develop a chronology of changes in fire, herbivory and tree density over time and space.

3) Methods

Field Work and Study Site

Two sediment cores were collected from Phindiswene, a site near Memorial Gate in Hluhluwe, Kwazulu-Natal (figure 1) in 2009 using a Russian corer (Aaby and Digerfeldt 1986). One of the cores was taken for pollen, charcoal and carbon and nitrogen stable isotope analysis (core named PhindA). The other was taken for diatom and phytolith analysis (core named PhindB). The cores were then taken back to UCT and subsampled every four centimetres so that they could be processed in the laboratory and provide high resolution data.

KwaZulu-Natal has a humid, subtropical climate due to onshore easterly atmospheric flow and the warm Agulhas current that contributes to convective rainfall, high soil moisture and a dense vegetation cover (Walker 1990). Mean annual rainfall in Kwa-Zulu Natal varies from 760 mm in the northern interior to 1250 mm along the coast and in the mountains (Neumann *et al.* 2010).

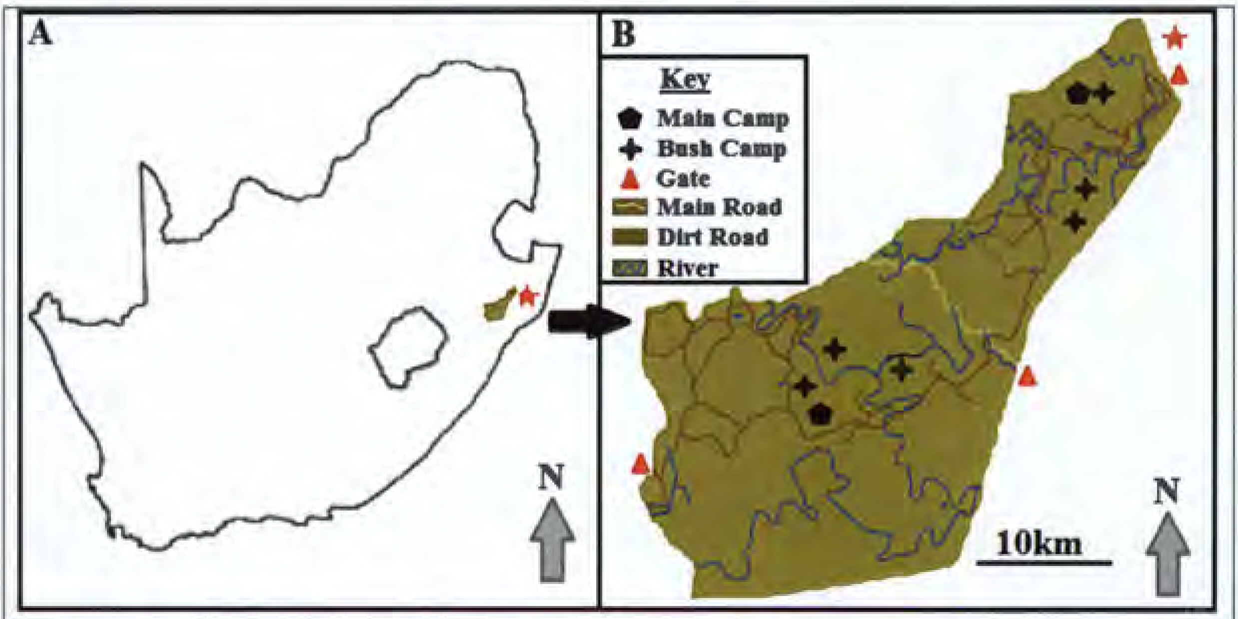

Figure 1: The site location (indicated by the red star) within the context of South Africa (A) and in relation to the Hluhluwe-Imfolozi Game Reserve (B). The lake is located near Memorial Gate in the north East of the reserve and has coordinates.

Diatom Extraction

The core was sub-sampled every four centimetres, starting at 4cm from the surface and going to a depth of 1.4 metres below the surface. The diatoms were extracted from the sediment from each of these sub-samples using the following chemical steps:

1. The sediments were initially treated with hot 10% HCl to remove all carbonates from the sample. This was achieved by placing a small quantity of sediment into a beaker, mixing it with 10% HCl and heating gently for 15 minutes while swirling the contents. This was repeated until all carbonates were dissolved (Fritz, 1991).
2. Following this, the residue was diluted with distilled water and allowed to settle overnight, with excess supernatant liquid removed through pipetting the following morning.
3. The sample was then washed in 20ml of 30% H_2O_2 and heated gently in a water bath until all organic matter was removed. This was repeated several times until complete removal of organic matter had taken place.
4. Coarse organic matter (e.g. roots) was removed from the residue using a 0.3mm sieve.
5. The resultant residue was centrifuged and washed with distilled water at least three times.
6. The subsequent sample contained clays and finer mineral matter. These particles were removed by swirling the residue in a beaker. Swirling and then waiting for 30 seconds enabled the diatoms to sediment out and the resulting clays were discarded as suspended matter in the excess water.
7. The prepared sample was then checked by preparing wet mounts to ensure an adequate removal of diluent material and a proper separation of frustules into single valves.
8. Following these steps, 3 drops of the diatom solution was pipetted onto a clean cover slip (Battarbee, 1986) and diluted with a few drops of deionised water (Barnett, 1997). The solution was then left to settle (Battarbee, 1986).
9. The water in the solution was left to evaporate on a hot plate at a low temperature (~40°C).
10. After all the water had evaporated, the coverslip was mounted onto the microscope slide using a resin of high refractive index (Battarbee, 1986); for this purpose Pleurax (R.I. = 1.73) was utilised. The slides were then left to dry in the fume cupboard.
11. On each slide 350 diatoms were then identified and counted.

Identification of species was achieved using taxa descriptions and imagery from Kelly *et al.* (2005) and Taylor *et al.* (2007) and with the use of a plate put together during the identification process (refer to appendices 1, 2, 3, 4 and 5).

Scanning Electron Microscope

Photographs of some of the specimens were taken using the scanning electron microscope (SEM). Wet, unmounted sample was dried out on SEM stubs. The stubs were then placed in an argon gas vacuum and coated in gold to enhance the image quality when under the microscope. Once this was done the stubs were placed in the SEM and those diatoms that were seen were photographed.

Statistical Analysis

Two AMS dates were calibrated for each of the cores using the calibration curve for the southern hemisphere (McCormac *et al.* 2004).

Diatom community structure was analysed and displayed using a computer programme, *Tilia* (Grimm 1997). Graphs were produced on *Tilia* showing the ratio of life forms (benthic, aerophylic etc.) and showing water type (brackish, fresh, salt etc.). Life form and water type classifications are summarised in appendices 6 and 7. Additional graphs showing mean values in each of the climate 'zones' were also created along with a graph to show fluctuations in eutrophic species over time.

4) Results

Radiocarbon chronology

The calibrated median age at 100cm depth (PhindB100) was A.D. 1685. The 2r range for this date was A.D. 1490–1660 (95.4%) and A.D. 1510–1650 (68.2%), (Figure 2A). The uncalibrated date for PhindB100 was 325 ± 30 BP (lab number: Poz-36534). The second AMS date, taken at 142cm depth, revealed a very young age and was most likely contaminated by modern day dust deposits (T. Goslar *pers. comm.* 2010). As a result of this, this date has not been included in this report.

The surface layer of sediment was assumed to represent the present day with the date of 325 (±30) BP being established for the sample at 100cm. These two dates were plotted on a graph and the line equation (y=3.5x) was used to calculated the dates of the other samples (figure 2B). In each case the error can also be assumed to be 30 years either side of the prediction.

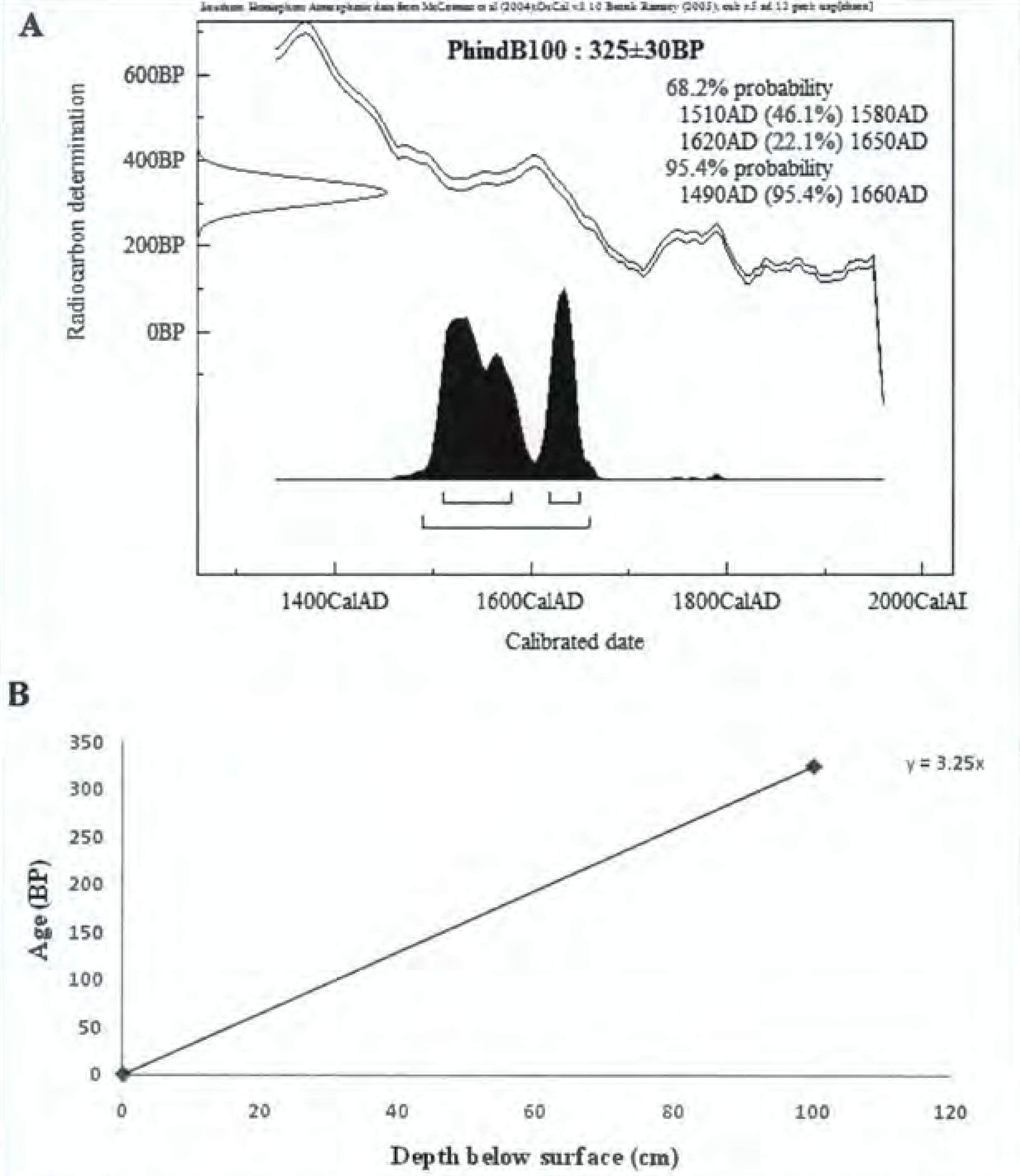

Figure 2: Calibrated radiocarbon date for a sample of the lake sediment core taken at 100cm (A) and the relationship between age and depth at different points along the core (B). Note the line equation for (B) is included (y=3.25x). (A) courtesy of T. Goslar and the Poznan Radiocarbon Laboratory (2010).

Diatoms: Benthic to Littoral/Boundary Ratio

Two unexpected findings must be noted from the onset. First, between the depths of 72cm and 76cm there was a sharp change in lithology of the core. In particular, the top 72cm of the core consisted of very organic and low clay sediments. Below this, there was a change and the sediments became much more clay rich. The clays in the sediment at this point were abrasive and destroyed the diatom frustules, leaving behind only the occasional, unidentifiable, fragment. Because of poor preservation which could bias results, these sediments at the lower depths were thus left out of the analysis.

Secondly, whilst diatoms were incredibly abundant for the top 72 cm of the core, there were no planktonic species recorded in any of the samples. This meant analysing the ratio of planktonic to benthic taxa was not possible. In light of this, the focus shifted to looking at the ratio of littoral (boundary) to benthic taxa. Boundary or littoral taxa indicate an intermediate phase between aerophylic taxa (which occur in wet mud and low to empty lake levels) and benthic taxa (which indicate comparatively higher lake levels (albeit still low)) (Sarmaja-Korjonen & Hyvarinen 2002). Similarly, the ratio of brackish water species to freshwater species was also analysed. An increase in the ratio of brackish species over time can indicate lowering lake levels as nutrients and salts become more concentrated in the lake so favouring brackish water species (Sarmaja-Korjonen & Hyvarinen 2002).

The analysis of boundary to benthic taxa gave rise to five zones or periods of lake level fluctuation by cluster analysis (figure 3). In contrast, the analysis of brackish to freshwater taxa gave rise to just four zones (figure 4). The first three zones from each analysis show similar trends to one another. Specifically, Zone 1 corroborates the trends seen in zone 1B and vice verse, zone 2 corroborates the trends seen in zone 2B and vice verse, and zone 3 corroborates the trends seen in zone 3B and vice verse. In figure 3, the fifth zone is shown as a distinct zone, separate from zone 4, because cluster analysis revealed zone 5 to be an outlier. Nonetheless, the fifth and fourth zones of figure 3 still correspond well with zone 4B of figure 4.

The oldest zone of figure 3, zone 5 (c. A.D. 1770-1800, Z5, 72-64cm), shows an initial period of increase in boundary species. In particular, boundary species such as *Rhopalodia gibberula* (appendix 1) increase substantially. Towards the end of this period, however, there is an equally dramatic reversal as *Rhopalodia gibberula* numbers decrease and consequently boundary species individuals become less abundant (figures 3 and 5A).

Zone 4 (c. A.D. 1800-1850, Z4, 64-48cm) represents a fairly long, stable period with a steady increase in the ratio of boundary species (figure 3). In particular, *Rhopalodia gibberula* increases steadily. When averaged for the whole zone, the number of boundary species out numbers the abundance of benthic species individuals within this zone (figure 5A). Notably, the aerophylic species, *Pinnularia borealis*, is also present in this zone.

Zone 3 (c. A.D. 1850-1930, Z3, 48-24cm) represents a very turbulent period with sharp fluctuations between boundary dominated and benthic dominated diatom communities (figure 3). Nonetheless, when averaged, the zone shows a slight dominance of benthic species (figure 5A).

Zone 2 (c. A.D. 1930-1980, Z2, 24-8cm) represents a stable period of benthic dominated diatom taxa. Benthic species abundance, when averaged, is far higher than boundary species in this zone (figure 5A).

Zone 1 (c. A.D. 1980-present day, Z1, 8-0cm) shows a steady increase in boundary species and is classified as its own zone for this reason (figures 3 and 5a). Both zone 1 and zone 2 exhibit another intriguing pattern, an increase in the abundance of eutrophic species (figures 3 and 6).

Diatoms: Brackish to Freshwater Ratio.

Zone 4B (c. A.D. 1770-1840, Z4B, 72-52cm) shows a general decrease in brackish diatom species over time following an initially high concentration of brackish species at the 72cm mark (figure 4). This pattern is particularly evident with *Nitzschia palea*, which steadily decreases from the beginning of the zone towards the end of it (figure 4). Similarly, the two saline species, *Diploneis subovalis* and *Nitzschia clausii*, are particularly common at the beginning of the zone but drop off towards the end of it. The initial presence of brackish species correlates well with the averaged data in figure 5B, which suggests a relatively high concentration of brackish species, and with zones 5 and 4 from figure 3.

Zone 3B (c. A.D. 1840-1920, Z3B, 52-28cm) shows a fairly long, stable period with consistently low concentrations in brackish and saline diatom species over time (figure 4 and 5B). Fluctuations in some key freshwater species are fairly dramatic but are invariably compensated for with equal, but opposite, fluctuations in other freshwater species. In particular, *Rhopalodia gibberula and Navicula microcari* appear to share an inverse relationship with *Nitzschia linearis* which is most obvious during this period.

Zone 2B (c. A.D. 1920-1980, Z2B, 28-8cm) shows a sharp increase in the abundance of brackish species that lasts for the entire period (figure 4 and 5B). In particular, *Nitzschia palea* and *Caloneis bacillum* (appendix 4F) show large increases in their respective concentrations during this period. The second half of this period is also represented with a large increase in the abundance of widespread species such as *Denticula subtilis* and *Achnanthidium exiguum* (appendix 4D)

Zone 1B (c. A.D. 1980-present day, Z1B, 8-0cm) shows a sharp increase in the abundance of freshwater species (figure 4 and 5B). In particular, *Rhopalodia gibberula*, increases substantially. The sharp increase of freshwater species relative to benthic species means this zone is classified on its own and corroborates the same classification when looking at zone 1 (figure 3).

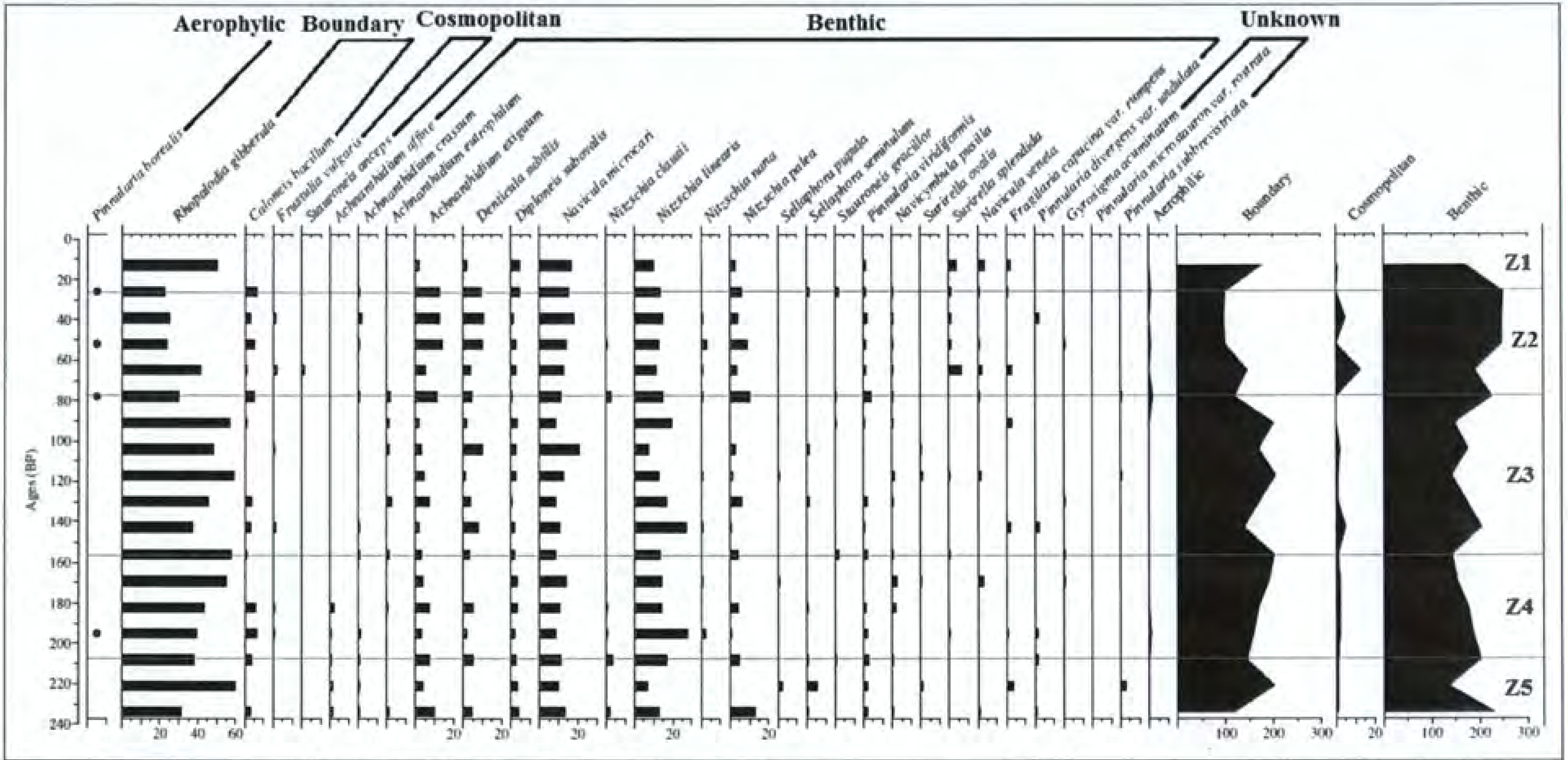

Figure 3: The diatom assemblage from Hluhluwe-Imfolozi, showing the most common aerophylic, boundary, cosmopolitan and benthic diatoms.

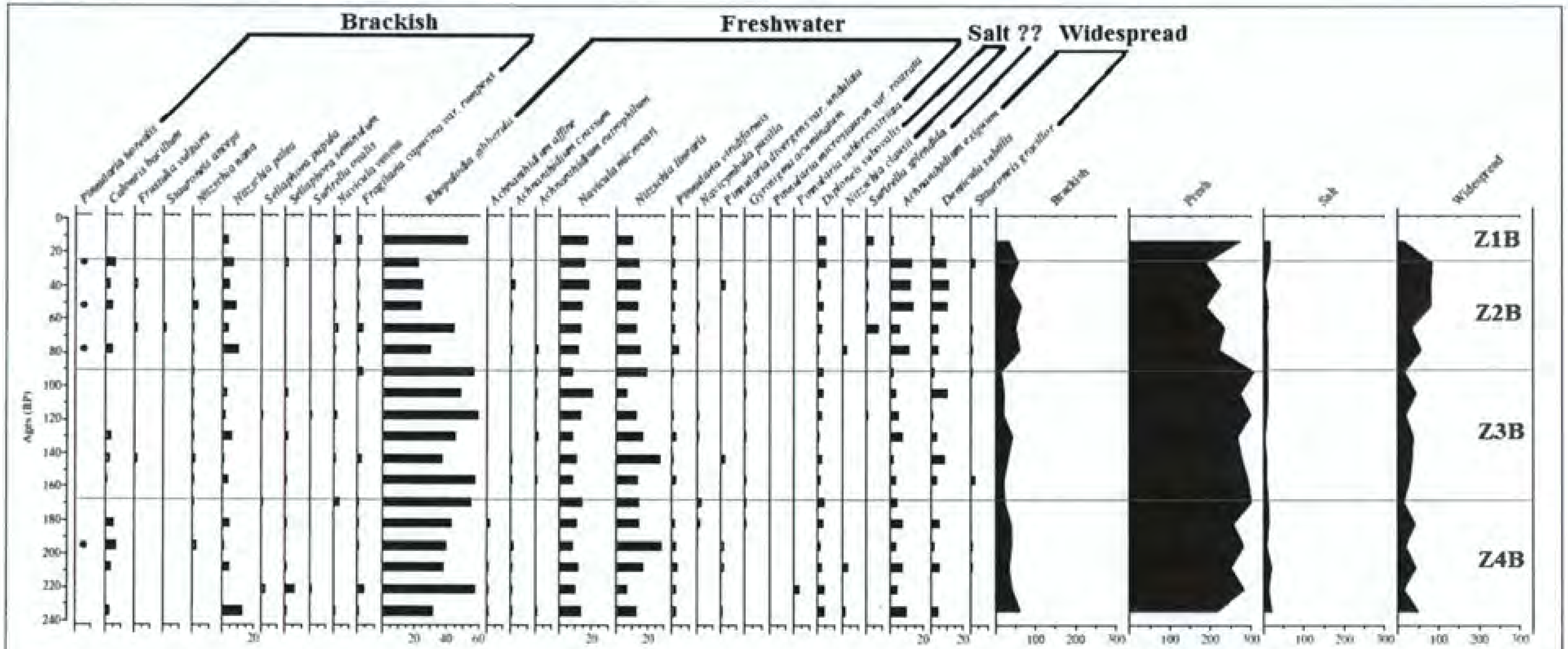

Figure 4: The diatom assemblage from Hluhluwe-Imfolozi, showing the most common brackish water, freshwater, salt water, and widespread diatoms. The favoured water type of *Surirella splendida*, indicated by the two question marks (??) is not described in the literature.

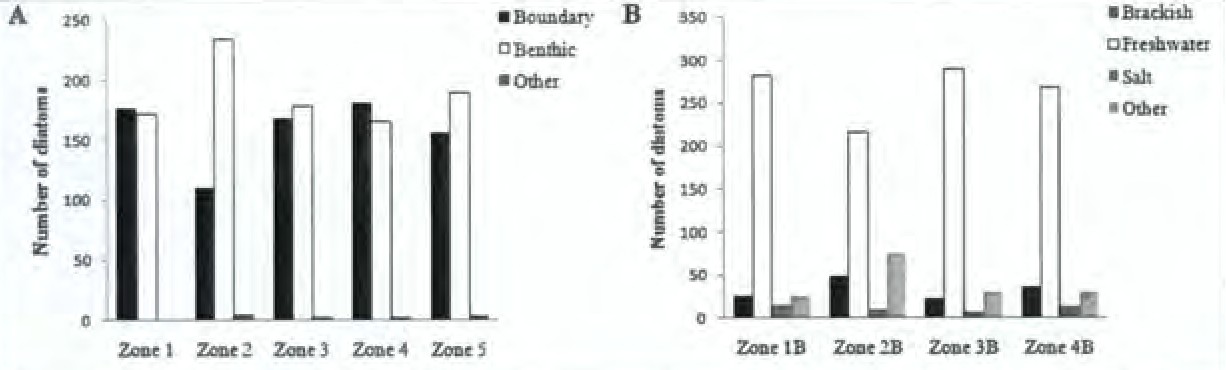

Figure 5: Mean number of diatoms taken from each sample within the zones for (A) benthic or boundary species and (B) brackish or freshwater species.

Eutrophic species

A sharp increase in the number of eutrophic species was also noted for zones 1B and 2B when compared to the other zones (figure 6).

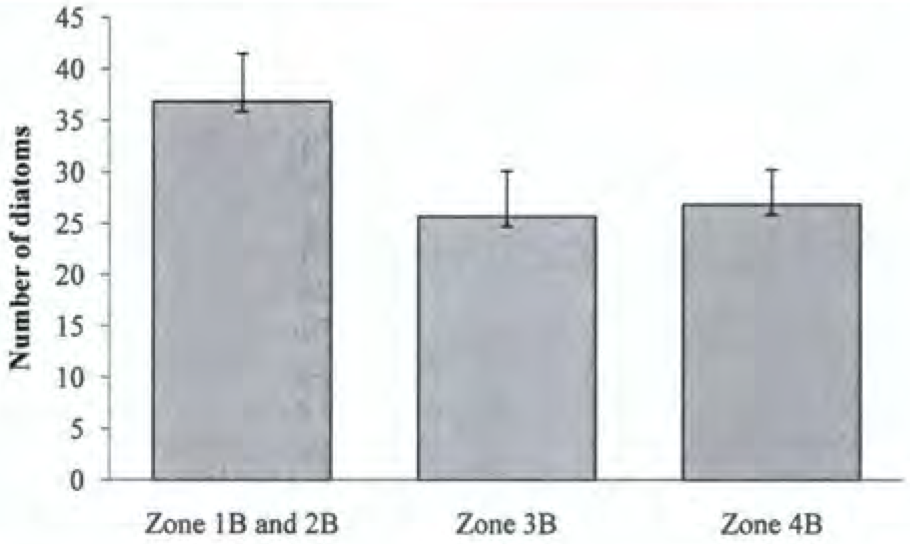

Figure 6: The number of diatoms that are eutrophic for each zone. Zone 1B and 2B have been included together as Zone 1B consists of only one sample. Standard error is indicated with the error bars.

Saline influences?

Three hypersaline species were found within the samples of the core. These were *Diploneis subovalis* (appendix 4A), *Nitzschia clausii* and a possible centric diatom which was tentatively classified in the *Chaetoceros* genus as a *Chaetoceros*-type (appendix 5). Owing to its poor preservation, uncertain identification and exclusively marine ecology, the centric diatom count was not included the results section. *Diploneis subovalis* is widespread along the core and occurs in fairly consistent, even numbers in all the samples. *Nitzschia clausii*, however, occurs only in zones 2B and 4B.

5) Discussion

Aerophylic, boundary and benthic ratios and Brackish to freshwater ratios as indicators of climate.

The rapid increase in boundary species, particularly *Rhopalodia gibberula*, at the start of the zone 5 (c. A.D. 1770 - 1800, figures 3 and 5A), combined with the relatively high ratio of brackish species in zone 4B (c. A.D. 1770 - 1840, figure 4 and 5B) suggests that there is initially a fairly rapid drop in water levels suggesting a corresponding trend towards a drier, warmer climate. *Rhopalodia gibberula* can tolerate fairly high water temperatures (Taylor *et al.* 2007), so the increase in its abundance supports the idea that conditions were warmer and drier at that time.

An increase in brackish species is indicative of lowering water levels and consequential increasing salt and pollutant concentrations (Sarmaja-Korjonen & Hyvarinen 2002). This is exemplified by the appearance of a saline species, *Nitzschia clausii*, at the time (figure 4). Boundary species can handle either extreme and their response to increasing water levels is delayed when compared to the response of brackish species which have to handle the change in water chemistry that is a consequence of increasingly wetter periods. Zone 5 of figure 3 and zone 4B. of figure 4 suggest a brief, small trend towards increasing lake water levels as one approaches the end of the respective zones. This increase in lake water levels corresponds well with a single tree ring record, from the Karkloof area, which showed a brief period of increased rainfall (Neumann *et al.* 2008).

Zone 4 (c. A.D. 1800-1850, 64-48cm, figures 3 and 5A) shows a period of steady decline in lake levels. The Little Ice Age (LIA; c. A.D. 1400 – 1800) preceded this period (Neumann *et al.* 2008; Gillson and Ekblom 2009; Neumann *et al.* 2010) and has corroborated the cooler but drier climate suggestions for Zones 5 and 4B respectively. The corresponding warming that followed the LIA is thus well represented by the increase in boundary species seen in zone 3. The increase in boundary species abundance suggests a shift towards a warm, dry period immediately after the LIA (Neumann *et al.* 2010). At the very least, this period is indicative of a period of drought, causing lake water levels to lower and enabling aerophylic species, such as *Pinnularia borealis*, to establish. The presence of an aerophylic species suggests that the lake water levels may have been critically low (Kelly *et al.* 2004). This also corresponds well with zone 4B. It is likely that this warming is a product of post LIA warming and the start of anthropogenic warming. *Gyrosigma acuminatum, Sellaphora seminulum* and *Nitzschia palea* are also present in fairly high concentrations at this time. The presence of these eutrophic species, at a stage when lake levels were low, is indicative of a concentration of pollutants and salts induced by evaporative water loss (Sarmaja-Korjonen & Hyvarinen 2002), rather than by anthropogenic influences on the catchment area. These species continue on in reasonably high numbers in zones 3 and 3B.

Zone 3 (c. A.D. 1850-1930, 48-24cm, figures 3 and 5a) represents a fluctuating period of short periods of drought followed by short periods of relief in which rainfall events replenished the lake. Zone 3B (c. A.D. 1840-1920, 52-28cm, figures 4 and 5b) supports this with similarly fluctuating periods of drought and rainfall indicated by fluctuations in brackish to freshwater species ratios. Neumann *et al.* (2008) found similar trends with fluctuations in Chenopodiaceae pollen grains (indicative of warm, dry, saline habitats) and Ericaceae pollen grains (indicative of colder, wetter habitats) during a similar time period.

Zone 2 (c. A.D. 1930-1980, 24-8cm, figures 3 and 5A) shows a period of particularly low numbers of boundary individuals with *Rhopalodia gibberula* being particularly low at this time. This suggests a period of high water levels relative to the water levels for the other zones over the last 240 years (figure 3). The indication, therefore, is that this was a colder wetter period. However, *Caloneis bacillum*, another boundary species, is very common during this period when compared to its abundance in other zones. Furthermore, Zone 2B (c. A.D. 1920-1980, 28-8cm, figures 4 and 5B) shows a high proportion of brackish individuals suggesting that lake levels should be low during this period, contradicting the high lake levels indicated by the low representation of *Rhopalodia gibberula* in zone 2.

However, an increase in widespread species (species that can tolerate brackish or freshwater), like *Achnanthidium exiguum* and *Denticulus subtilis,* suggest that conditions in the lake might have been able to support a variety of ecological niches at the time. This is exemplified by the appearance of *Pinnularia borealis* in these sediments; an aerophylic species associated with drying. The abundance of these aerophylic diatoms is, nonetheless, extremely low, suggesting localised drying of peripheral pools rather than widespread drying of the main basin.

There is a marked increase in the densities of taxa which are able to withstand very high to critically high levels of pollution in zones 2 and 2B and in zones 1 and 1B (both c. A.D. 1980-present day, 8-0cm, figures 3, 4 and 6). In particular, taxa such as *Navicula veneta*, *Nitzschia palea*, and *Sellaphora seminulum*, all of which are indicators of eutrophication (Kelly *et al.* 2004; Taylor *et al.* 2007), show substantial increases in abundance in these zones (figure 6). This indicates eutrophication of the lake or a flux of pollutants into the lake system. The increase in pollution-tolerant species corresponds with a decrease in the ratio of littoral taxa and therefore an increase in lake levels. Sarmaja-Korjonen & Hyvarinen (2002) suggest that a decrease in the ratio of littoral taxa is associated with increasing lake levels, decreased salinity and a dilution of lake pollution. This then poses a puzzling scenario where eutrophic species increase in what, in theory, should be more oligotrophic waters. The implication is that this increase in eutrophic taxa might be the product of anthropogenic influences on the catchment area as a whole. It seems likely that during this wetter period, runoff into the lake system might have brought with it an increase in pollutants caused by human action in the area. Previous studies in the area have had similar findings and suggest that recent human impact has resulted in a significant increase in the delivery of topsoil-derived material to the floodplain wetlands of the Mkuze River (Plater *et al.* 2003) and to Lake Eteza (Neumann *et al.* 2010); both of which are in northern Kwa-Zulu-Natal. The resulting pollutants in the system have caused a change in diatom community structure (Plater *et al.* 2003).

Zone 1 and Zone 1B represent very clear trends towards lower lake levels. Grenfell *et al.* (2009) allude to the role of humans, and more specifically, agriculture, as a possible cause for the drying up of rivers and lakes in the area. It is likely that along with climate, anthropogenic influences have a significant bearing on the results from these two zones, though this interpretation must be treated cautiously because of the short duration of these zones. It is possible that records from local weather stations might be able to validate the findings for these zones.

The indication from the zones is that climate has fluctuated from cold and wet to warm and dry, then back to cold and wet, and finally back to warm and dry over the last 240 years.

Saline Diatoms

There is no clear pattern or distribution with the saline species. *Diploneis subovalis*, for example, is present in all but one of the samples (at 24cm, figure 3 and 4) and is fairly consistent in number in all the samples. Kelly *et al.* (2004) describe species of the *Diploneis* genus as being generally marine species favouring high concentration salt waters. However, Taylor *et al.* (2007) describe *Diploneis subovalis* as a species which tolerates high salt concentrations in freshwater, suggesting it is not necessarily limited to sea water. The presence of what appears to be *Chaetoceros-type* centric diatom fragments (not included in the results) in two of the samples suggests that the *Chaetoceros* genus is not necessarily exclusively marine as described by Taylor *et al.* (2007) The almost permanent presence of saline species, such as *Diploneis subovalis*, corroborates the idea that the lake has had consistently low water levels (which have fluctuated between low, very low and dry) over the last 240 years.

6) Conclusions

The diatom sequence from Phindiswene provides a high resolution climatic proxy for the critical period covering the latter stages of the Little Ice Age (LIA), post LIA warming, and recent increasing anthropogenic impacts.

The lake has had consistently low water levels during, at least, the last 240 years. The main conclusions are summarised as follows.

1. The absence of any planktonic diatom species, along with the persistent occurrence of saline species, such as *Diploneis subovalis*, suggest that over the last 240 years the lake has had consistently low water levels that have probably fluctuated between low, very low and dry states.
2. The consistent occurrence of saline species corroborates the consistently low water level trend over the past 240 years.
3. Four (possibly five) 'climate zones' occurred over the last 240 years with fluctuations from cold and wet to warm and dry back to cold and wet and finally back to warm and dry.
4. Recent anthropogenic influences have caused an increase of eutrophic species in the lake.

Originally it was hoped that a comparison of planktonic to benthic species could be done as an indication of fluctuating lake levels over time. However, no planktonic species were recorded from any of the samples.

7) Acknowledgements

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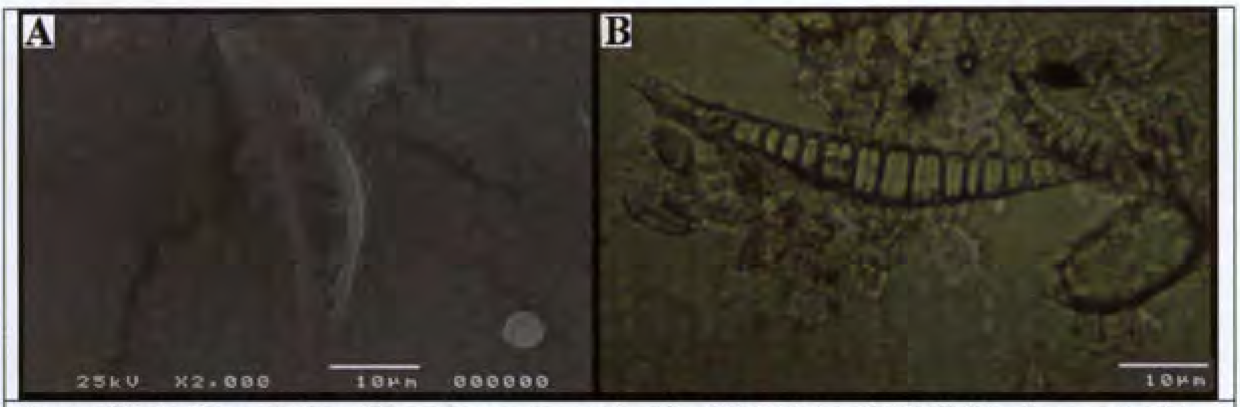
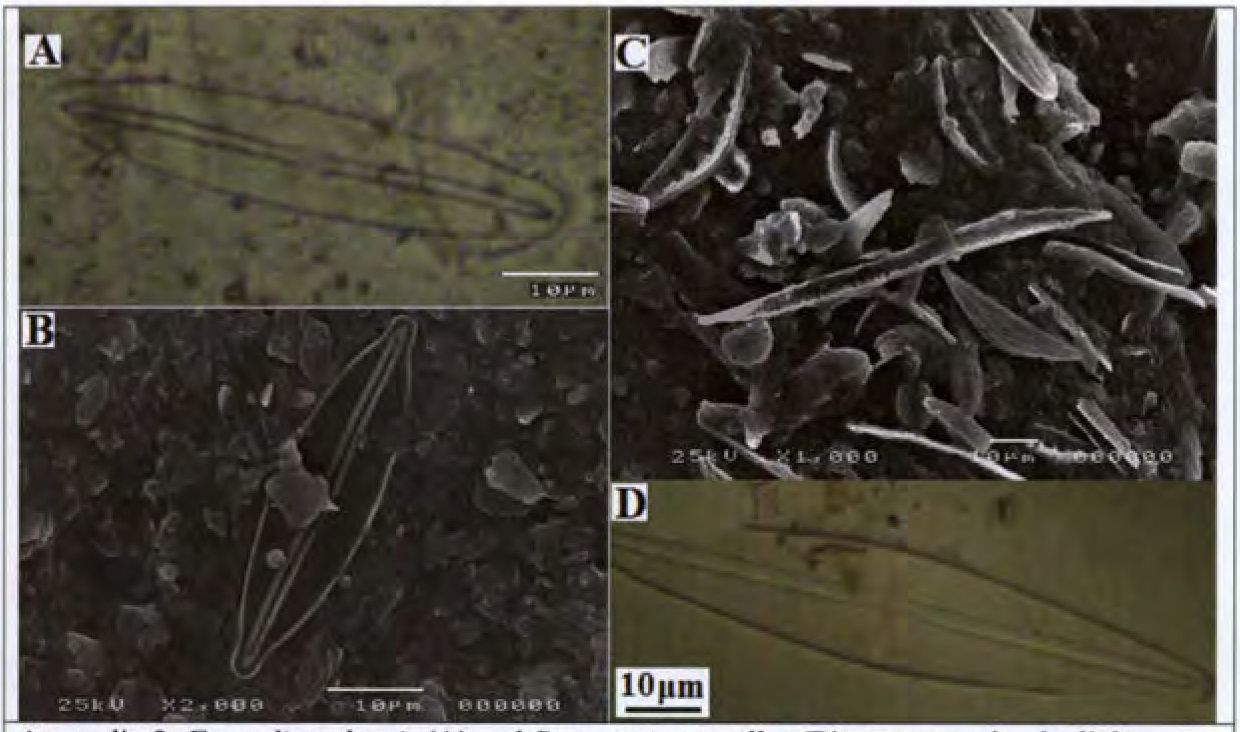
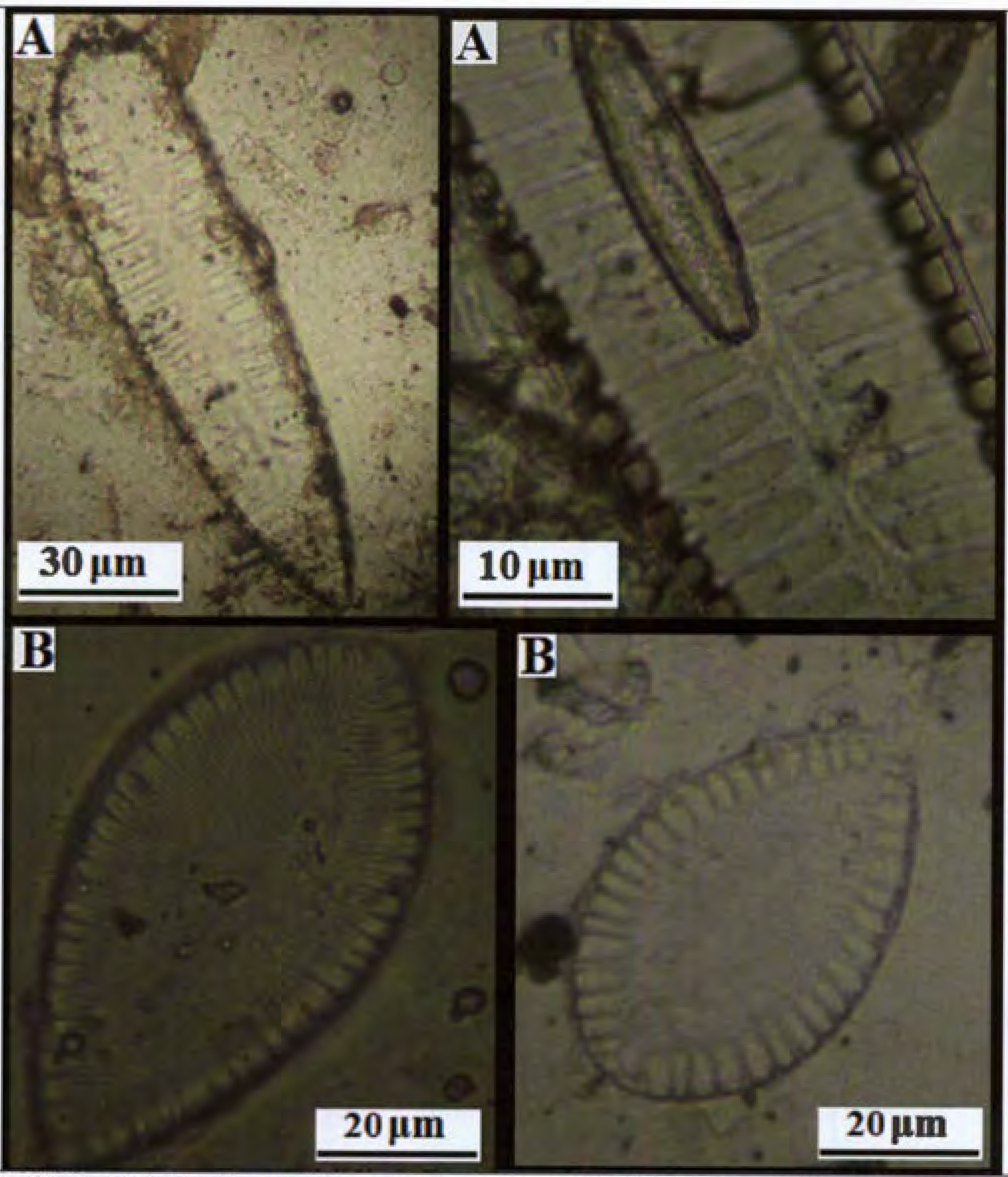
9) Appendices



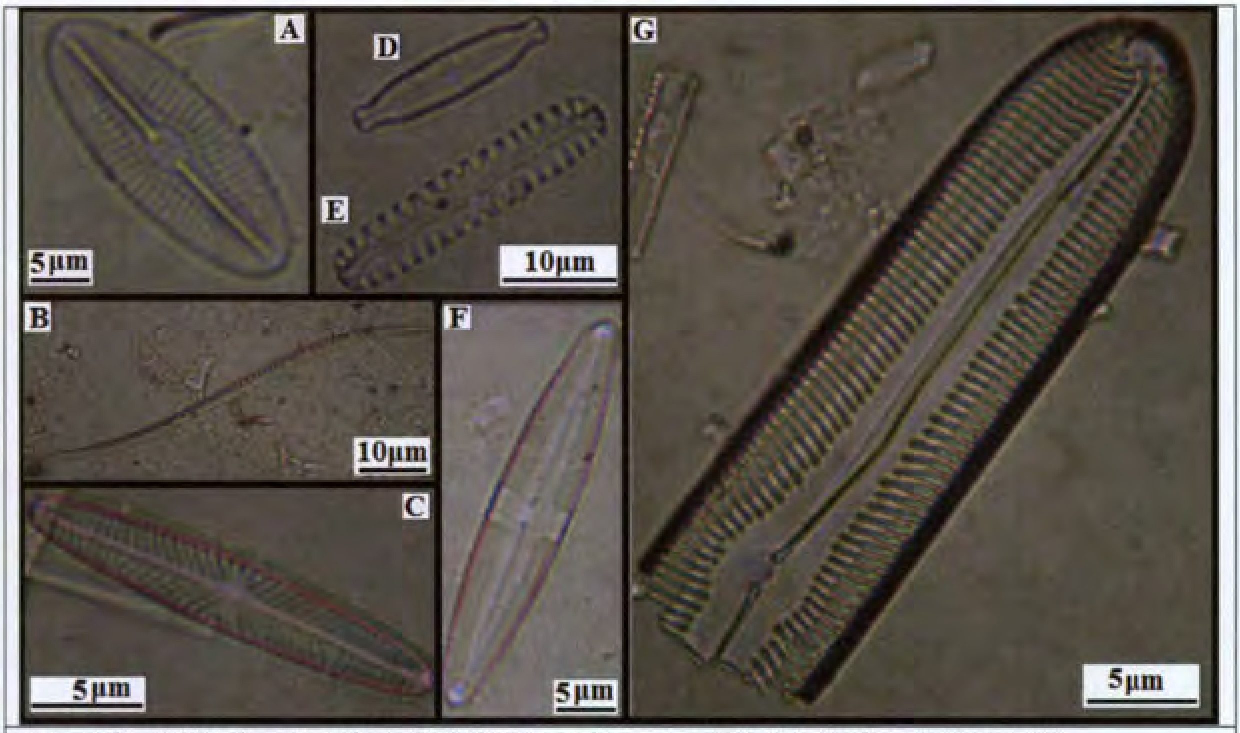
Appendix 1: *Rhopalodia gibberula* as seen under the (A) SEM and (B) light microscope (at 1000X magnification). Photos: (A) D. Ohland and (B) N. Fordyce (2010).



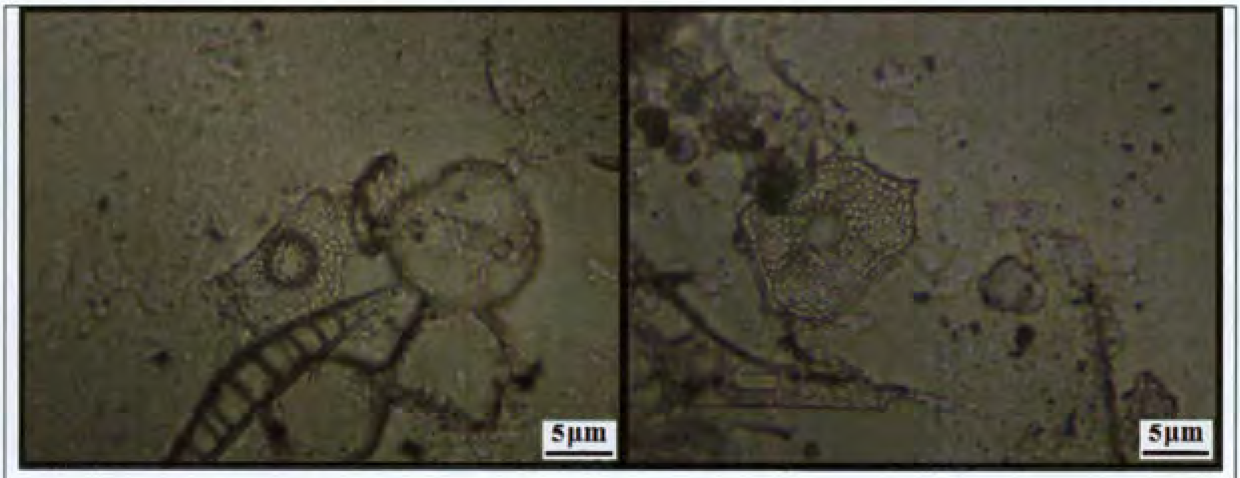
Appendix 2: *Frustulia vulgaris* (A) and *Stauroneis gracillor* (D) as seen under the light microscope (Photos: N. Fordyce, 2010). *Frustulia sp.* (B) nd *Nitzschia linearis* (C) as seen under the SEM (Photos; D. Ohland, 2010).



Appendix 3: *Surirella splendid* (A) and *Surirella ovalis* (B) as seen under the light microscope. Photos: N. Fordyce (2010).



Appendix 4: *Diploneis subovalis* (A), *Nitzschia nana* (B), *Navicula microcari* (C), *Achnanthidium exiguum* (D), *Pinnularia borealis* (E), *Caloneis bacillum* (F) and a fragment of *Pinnularia viridiformis* (G) as seen under the light microscope at 1000X magnification. Photos: N. Fordyce (2010).



Appendix 5: Possible centric diatom fragments (possibly *Chaetoceros*) indicating some marine influence on the lake system. Photos: N. Fordyce (2010).

Appendix 6: Diatom species list, life form and reference for the life form description.

Species	**Life form**	**Reference**
Pinnularia borealis	Aerophylic	Kelly *et al.*, 2004
Caloneis bacillum	Boundary	Kelly *et al.*, 2004
Rhopalodia gibberula	Boundary	Yacobaccio & Morales, 2005
Frustulia vulgaris	Cosmopolitan	Kelly *et al.*, 2004
Stauroneis anceps	Cosmopolitan	Kelly *et al.*, 2004
Achnanthidium affine	Benthic	Taylor *et al.*, 2007
Achnanthidium crassum	Benthic	Taylor *et al.*, 2007
Achnanthidium eutrophilum	Benthic	Taylor *et al.*, 2007
Achnanthidium exiguum	Benthic	Taylor *et al.*, 2007
Denticula subtilis	Benthic	Taylor *et al.*, 2007
Diploneis subovalis	Benthic	Taylor *et al.*, 2007
Navicula microcari	Benthic	Taylor *et al.*, 2007
Nitzschia clausii	Benthic	Kelly *et al.*, 2004
Nitzschia linearis	Benthic	Kelly *et al.*, 2004
Nitzschia nana	Benthic	Taylor *et al.*, 2007
Nitzschia palea	Benthic	Kelly *et al.*, 2004
Sellaphora pupula	Benthic	Kelly *et al.*, 2004
Sellaphora seminulum	Benthic	Kelly *et al.*, 2004
Stauroneis gracillor	Benthic	Taylor *et al.*, 2007
Pinnularia viridiformis	Benthic	Nguetsop *et al.*, 2004
Navicymbula pusilla	Benthic	Saros and Fritz, 2000
Surirella ovalis	Benthic	Kelly *et al.*, 2004
Surirella splendida	Benthic	Akar & Sahin. 2006
Navicula veneta	Benthic	De Fabricius *et al.* 2003
Fragilaria capucina var. rumpens	Benthic	Taylor *et al.*, 2007
Pinnularia divergens var. undulata	Benthic	Ziller & Economou-Amilli, 1998
Gyrosigma acuminatum	Unknown	
Pinnularia microstauron var. rostrata	Unknown	
Pinnularia subbrevistriata	Unknown	

Appendix 7: Diatom species list, water type and reference for the water type description

Species	**Water Type**	**Reference**
Caloneis bacillum	brackish	Kelly *et al.*, 2004
Fragilaria capucina var. rumpens	brackish	Taylor *et al.*, 2007
Frustulia vulgaris	brackish	Kelly *et al.*, 2004
Navicula veneta	brackish	De Fabricius *et al.* 2003
Nitzschia nana	brackish	Taylor *et al.*, 2007
Nitzschia palea	brackish	Kelly *et al.*, 2004
Pinnularia borealis	brackish	Kelly *et al.*, 2004
Sellaphora pupula	brackish	Kelly *et al.*, 2004
Sellaphora seminulum	brackish	Kelly *et al.*, 2004
Stauroneis anceps	brackish	Kelly *et al.*, 2004
Surirella ovalis	brackish	Kelly *et al.*, 2004
Achnanthidium affine	fresh	Taylor *et al.*, 2007
Achnanthidium crassum	fresh	Taylor *et al.*, 2007
Achnanthidium eutrophilum	fresh	Taylor *et al.*, 2007
Gyrosigma acuminatum	fresh	Sarmaja-Korjonen & Hyvarinen, 2002
Navicula microcari	fresh	Taylor *et al.*, 2007
Navicymbula pusilla	fresh	Saros and Fritz, 2000
Nitzschia linearis	fresh	Kelly *et al.*, 2004
Pinnularia divergens var. undulata	fresh	Ziller & Economou-Amilli, 1998
Pinnularia microstauron var. rostrata	fresh	Kelly *et al.*, 2004
Pinnularia subbrevistriata	fresh	Kelly *et al.*, 2004
Pinnularia viridiformis	fresh	Nguetsop *et al.*, 2004
Rhopalodia gibberula	fresh	Yacobaccio and Morales, 2005
Chaetoceros sp.	salt	Taylor *et al.*, 2007
Diploneis subovalis	salt	Kelly *et al.*, 2004
Nitzschia clausii	salt	Kelly *et al.*, 2004
Surirella splendida	Unknown	
Achnanthidium exiguum	widespread	Taylor *et al.*, 2007
Denticula subtilis	widespread	Taylor *et al.*, 2007
Stauroneis gracillor	widespread	Taylor *et al.*, 2007