Quantitative Reconstruction of Eutrophication Histories in Central Mexican Lakes

Emma Louise Hill, BSc. (Hons)

October 2006

Thesis submitted to the University of Nottingham for the degree of

Doctor of Philosophy

October 2006

Abstract

The primary aim of this study was to develop a diatom based nutrient transfer function for central México, and apply it to a sediment core from Laguna Zirahuén, Michoacán México, (19° 26' N, 101° 44' W) reconstruct change in nutrient availability over the last ca. 200 years. The principle techniques employed were analysis of diatoms, stable isotopes of carbon and nitrogen and lipid biomarkers. These were supplemented by magnetic susceptibility and isotopic measurements of modern aquatic and terrestrial organic matter. In order to interpret the diatom record and quantitatively reconstruction the change in the diatom flora a calibration data set, from 30 lakes, was collected during both the wet and the dry seasons.

Detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) were used to explore species-environment relationships and to establish which environmental variables made independent and significant contributions to species variance. Results showed that electrical conductivity and chlorophyll-a caused a significant and independent proportion of variance in the species data (12.54%, p = 0.001), this was supported by variance partitioning and the ratio of CCA axis one to CCA axis two. Weighted averaging regression and calibration was then used to create an inference model to reconstruct changes in Chlorophyll-a. The strength of the model was assessed using the squared coefficient of determination (r^2 = 0.83) and root mean square of errors (RMSE = 0.32). Application of cross validation resulted in a substantial drop in r^2 and increase in RMSEP, this is most likely associated with the small and heterogeneous nature of the data set.

Numerical reconstruction of the change in nutrient status was complicated by a high number of non-planktonic species and a lack of modern analogues. In the upper 3 cm the trajectory of change inferred by the reconstruction was, however, in line with other existing contemporary nutrient data from the lake. There is a marked decrease in productivity between 24 and 9 cm, shown by declining concentrations of $\delta^{13}C$, lipid biomarkers, $\delta^{13}C$ and diatom inferred Chl-a and corresponding increase C/N. This may correspond to the decline in the copper smelting industry in the basin the in the late 19th early 20th century. A rise in productivity is noted over the top 3 cm of the core, possibly the last 15-20 years. The majority of proxies showed a marked response to the deposition of the Paricutín tephra.

Despite the fact that electrical conductivity is the primary variable in controlling diatom species variation, it is evident that human impact on lake basins though increased levels of nutrients in having an impact on the ecology of the lake. In the case of Laguna Zirahuén its sensitivity and the current trajectory of change may be an important consideration in its future management.

Acknowledgements

This thesis was undertaken with the support of Natural Environment Research Council (NERC) grant (NER/S/A/2002/10358) at the University of Edinburgh, Institute of Geography and then the School of Geography, University of Nottingham.

I'm indebted to my principle supervisor Prof. Sarah Metcalfe without whose support, advice, encouragement, help with field work in México (particularly for getting me another core from Zirahuén) and the odd reality check this would have been a whole lot harder. Drs Steve Juggins and Greg Cowie were also really helpful with suggesting/providing reading on statistics and lipids, and their help on these matters was invaluable.

I'd like to thank Prof. Melanie Leng and Dr Tim Heaton at the NERC Isotope Geochemistry Laboratory at BGS, Keyworth for undertaking carbon and nitrogen analyses for me and for their advice and helpful and insightful comments on my results. I'm also grateful to Dr Nicola Cayzer at the Grant Institute of Earth Sciences, University of Edinburgh and David Clift at the School of Chemical, Environmental and Mining Engineering, University of Nottingham for their help with SEM work. Graham Tulloch at the BGS, Edinburgh provided access to x-raying facilities. A big thank you has to go to Steve Mowbray (Grant Institute) without whose help over most things GC-MS and GC-FID related was greatly appreciated. Thanks also to Ben Aston who has allowed me to use some of his modern isotope data from the Zirahuén basin.

I am grateful to a number of people in México who made my time out there all the more enjoyable. In particular a big thank you goes to the Lopez family, Isabel Israde and family, Virginia Segura and family and Miroslave Macek all of whom provided generous hospitality at some point during my time in México. Dr Javier Alcocer allowed me to use the laboratory facilities at FEZ Iztacala, UNAM, México City and allowed me to accompany his group on field trips to the Oriental and the Nevado de Toluca. Thanks to Gloria Vilaclara for her help and advice on lakes, nutrients and algae in México and to Margarita Caballero and Dr Maria Aurora Armienta for organising and processing some of my samples for K and Na analysis at the Instituto Geofisica, UNAM, México City. A number of people at INIRENA, Universidad Michoacána San Nicolas de Hidalgo, Morelia helped me in numerous ways. In particular I'd like to thank Dr Arturo Chacon-Torres for allowing me access to laboratory facilities, and for providing field vehicles and field equipment as well as providing useful insights into the lakes of Michoacán. A big thank you also goes to Dr Fernando Bernal-Brooks for sharing his extensive knowledge of lakes in Michoacán with me and for coming out in the field with me. Thanks also goes to Dana, Cubo,

Julio, Yola, Alé, Mabe, Heriverto, Judith, Laura, Luis, Alfonso, Rosario, Jorge and Eli in Morelia or México City for their help in the field of in the lab.

In the UK thank you's also go to Anthony Newton, for helping me out in a number of ways including providing diagrams, field kit, general chat and cups of tea when I was back up at KB doing lipid work, advice and proof reading a couple of chapters, to Julie Mitchell (Edinburgh), Teresa Needham, Ian Conway and Graham Morris (Nottingham) for help in the lab or with kit. Thanks to Suzanne McGowan for proof reading a chapter and for general chat about ordination and geochemistry. I'd also like to thank George Wolff for talking to me about lipids, John Anderson and Dave Ryves for helpful comments on transfer functions and Georgina Endfield and Sarah O'Hara for providing literature on central México. A big thank you has got to go to all the people I've shared offices with over the last few years and for putting up with my swimming/running/bike kit being left in the office, for occasionally taking over their desks and for talking to myself rather loudly: Kerry-Anne, Lindsay, Colin and Krista at Edinburgh and Gemma, Zoe, Briony, Azman and Mulemwa and occasionally Dave at Nottingham. These people and others, in both Edinburgh and Nottingham; Sarah Mc, Clem, Kirsty, Becky, Lou, Jo, Lorena, Hattie, Laura, Ben, Steve, Innes, Louise, Sam, Clare, Andy C, Ed and Sarah G who either had to live with me (therefore providing great chat and red wine) or just dragged me to the pub, out climbing or came running with me were a great help in terms of keeping me sane and offering great chats over a pint. Special thanks also go to Anna, Jo, Ella, Emma and India for coming to visit, or letting me descend, for making me go out and listening to me rant over various issues.

A massive THANK YOU also has to go to my parents Sue and John without who's constant support (emotional, practical, financial and culinary) and encouragement I wouldn't have made it this far. So this is dedicated to them, with love.

Contents

Chapter 3. Nutrients

Chapter 4. Environmental Biogeochemistry

Chapter 5. Methodology

Chapter 6. Modern Limnology

Chapter 7. Species-Environment Relationships: The Central Mexican calibration set and Development of a Transfer Function for Nutrient status

Chapter 8. The Palaeolimnology of Laguna Zirahuén

Chapter 9. Conclusions

Appendices

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

List of Tables

Chapter 1

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

List of Plates

Chapter 1

Chapter 2

Chapter 6

Chapter 8

Table of Appendices

Appendix 1

Appendix 2

Appendix 3

Appendix 4

Glossary and abbreviations

Glossary

Lakes

Allochthonous:	Sediment or organic matter transported to the site of deposition
Autochthonous:	Sediment or organic matter deposited *in-situ*
Eutrophic:	Nutrient rich waters of high productivity
Eutrophication	The process of nutrient enrichment
Mesotrophic:	Waters of intermediate nutrient levels
Oligotrophic:	Nutrient poor water of high clarity and low primary productivity
Epilimnion:	The warm, upper, circulating, layer of water in a thermally stratified lake
Metalimnion:	Transition between the epilimnion and hypolimnion, where the thermocline is greatest.
Hypolimnion:	Non circulating lower layer of cold water
Lentic:	Freshwater ecosystem where there is no continuous flow i.e. lakes and ponds
Lotic:	Freshwater ecosystem where there is continuous flow i.e. rivers

Diatom Ecology

Epiphytic:	Living attached to vegetation
Facultative Planktonic:	Usually associated with periphytic habitats but often found in plankton
Periphytic:	Living attached to a substrate or submerged object
Planktonic:	Free floating, suspended in water column
Centrales:	Centric Diatoms, no raphe.
Pennales:	Elongated species, distinguished between by raphe type and presence or absence
Araphidinaea:	Penate diatoms with no raphe, e.g. *Synedra* species
A:C ratio:	Index of trophic status based on the number of frustules of planktonic araphid to planktonic centric species

General

Ejido:	Communal land holdings
Haciendas:	Mexican colonial farming estates
Mesoamerica:	Area of land ranging from N central México to Central American Lowlands (Incorporating Central/Southern México, Guatemala, Belize El Salvador and western Honduras.

Non-Standard Abbreviations

CCA:	Canonical Correspondence Analysis
CNA:	Comisión Nacional del Agua (National Water Commision – México)
DCA:	Detrended Correspondence Analysis
EC:	Electrical Conductivity
ENSO:	El Niño Southern Oscillation
GC-FID	Gas Chromatogram-Flame Ionisation detector
GC-MS	Gas Chromatogram-Mass Spectrometer
HBI	Highly Branched Isoprenoid
ITCZ:	Inter Tropical Convergence Zone
MGVF:	Michoacan-Guanajuato Volcanic Zone
NAFTA:	North American Free Trade Agreement
OECD:	Organization for Economic Cooperation and Development
PCA:	Principle Components Analysis
RMSE:	Root Mean Square of Errors
RMSEP:	Root Mean Square of Error of Prediction
SEM:	Scanning Electron Microscope
SMN:	Servicio Meteorológico Nacional (Mexican Meteorological Service
SRP:	Soluble Reactive Phosphorus
TIC	Total Ion Chromatogram
TMVB:	Trans Mexican Volcanic Belt
TP:	Total phosphorous
UNEP:	United Nations Environment Programme
WA:	Weighted averaging

Chapter 1. Introduction: Research Context, Background and Rationale

1.1. Research Aims

In the absence of long term monitoring data information accumulated in the sediment record of lakes through diatoms, stable isotopes, mineral magnetism and organic biomarkers can be used to provide evidence of past changes in ecosystem functioning and past levels of human impact (Smol 1992). Diatoms are good indicators of environmental change due to their sensitivity to factors such as pH, conductivity and nutrients (Anderson *et al.*, 1990; Battarbee 1984; Gasse *et al.*, 1995), good preservation in the sediment and the ability to identify their remains to species level (Bennion, 1995). Quantitative models for inferring changes in nutrient status, to determine anthropogenic impact have been successfully developed for temperate regions of Europe and North America (e.g. Bennion 1994; Hall & Smol 1992) and for Australia and New Zealand (Tibby, 2004; Reid, 2005) but do not yet exist for the northern tropical Americas, as there has been a lack of systematically collected nutrient and corresponding diatom data. The aim of this study is, therefore, to develop a diatom based nutrient transfer function for central México. The quantitative relationship established between diatoms and nutrients will then be used to reconstruct changes in nutrient concentration over time in Laguna Zirahuén, in the highlands of central México, and inferences will be supported by other proxy data. Carbon and nitrogen isotopes have been successfully used to reconstruct change in aquatic productivity (e.g. Brenner, 1999; Schelske & Hodell 1991). In addition isotopes have been used to corroborate diatom inferred changes in nutrient status (Ekdahl *et al.*, 2004; Rosenmeier *et al.*, 2004). More recently lipid biomarkers have also been used to infer shifts in organic matter production and infer changes in aquatic productivity (e.g. Hanisch *et al.*, 2003; Prartono & Wolfe, 1998). This study aims to use these additional proxies to attempt to ascertain a bigger picture of the nature and rate of nutrient increases in Laguna Zirahuén.

To achieve these aims the following research questions will be addressed:

1) Does diatom species variation have a significant relationship with nutrient variables such as TP?
2) Can a transfer function, with high precision and low errors, be successfully created from this relationship, thus enabling quantitative reconstruction of change in nutrient status?
3) Do other proxy data, in the form of stable isotopes and organic lipid biomarkers, corroborate diatom inferred changes in nutrient status?
4) Can a multi-proxy palaeoenvironmental record of change improve our understanding of human/environment interactions?
5) Can this information be used to inform environmental management of lake ecosystems in México?

The thesis provides a reconstruction of changes in nutrients over approximately 200 years, which correspond to the late colonial period and the period of independence from Spain after 1810. The results of this thesis therefore have the potential to form a basis for management of unique, fragile ecosystems in central México through the identification of trajectories of change and identification of causes of change. The rest of this chapter provides some background on the geology, climate and cultural history of México, providing the context for central México and presents the rationale behind the research. An outline of the rest of the thesis is also provided.

1.2. Rationale

The lakes of central México are, generally, closed systems. They are, therefore, sensitive to changes in climate, through fluctuations in lake level due to variation in the precipitation-evaporation balance, and have shown associated changes in diatom species composition. Consequently their sediments provide a means of tracking climatic change. Palaeolimnological work on Mexican lakes, specifically Lago de Pátzcuaro and the lakes of the Vallé de México, had been carried out since 1944, when Deevey used pollen stratigraphy to track climatic changes. This was followed in 1956 by further study of Pátzcuaro by Hutchinson *et al.* (1956), however these studies lacked firm chronological constraints. Around this time limnological monitoring was also carried out, by De Buen, on the lakes of Michoacán, which focused on basic measurements of, among others, pH, temperature, water depth and observations of aquatic plants, zooplankton and phytoplankton (e.g. De Buen 1941a; 1943; 1944a). After the mid 1950s there was a reduction in palaeolimnological investigation until renewed efforts in the 1970s and early 1980s, with a focus on climatic change (Bradbury, 1971; Watts & Bradbury 1982; Metcalfe 1985). This was later followed by more work on palaeoenvironmental evidence of anthropogenic activity (e.g. Metcalfe *et al.* 1989; Metcalfe *et al.*, 1991; O'Hara *et al.*, 1993; Street-Perrott *et al.*, 1989) as it had become apparent that environmental change in central México over the late Holocene could not solely be ascribed to climatic changes. In order to elucidate the relationship between climate, environment and society Davies (2000) undertook a study of two lakes in central México, complemented by a modern calibration set to determine the relationship between diatom species variation and electrical conductivity and alkalinity, as proxies for climatic change. It was, however, not possible to make a reliable numerical down core reconstruction of these hydrochemical variables in Laguna Zirahuén and Laguna Juanacatlán. Both lakes reflected a diatom stratigraphy driven by anthropogenic activity, one major impact of which was probably an increase in nutrient availability. Limnological and palaeolimnological studies have shown that a number of the lakes in central México have become nutrient rich, some since pre-Hispanic times (Bernal-Brook & MacCrimmon, 2000b; Chacón-Torres, 1993a, Davies *et al.*, 2004; Metcalfe, 1995; Metcalfe & O'Hara, 1992). Many of these studies have been focused on only a few lakes in central México namely, Chapala, Pátzcuaro, Zirahuén, Cuitzeo and the lakes of the Vallé de México. There is, therefore, a lack of systematically collected nutrient and species data, this has prevented quantitative reconstruction of these changes. This is the first study to collect a dataset consisting of contemporary water chemistry, including nutrients, and corresponding diatom flora to establish a relationship between humans and their environment. Such a data set will provide information on lake ecosystem response to anthropogenic nutrient input, in the absence of a long term and extensive monitoring network.

1.3. Geology

México can be divided in to eight regions based on geology (Figure 1.1); the Sierra Madre Occidental (west), Sierra Madre Oriental (east), Sierra Madre del Sur (south), the Central High Plateau, coastal plains of the Gulf of México, the Trans Mexican Volcanic Belt, Isthmus of Tehuantepec, the Yucatan peninsula, and the mountains of north western Central America (Maldonado-Koerdell, 1964). During the early Tertiary Period tectonic uplift and volcanic activity led to the formation of high plateau areas and the formation of the Sierra Madre Occidental. After a period of reduced volcanic activity in the mid Pliocene, during which landscape was shaped by intense erosion, activity was renewed in the late Pliocene resulting in the deposition of silicate rich rhyolitic lava flows (West, 1964). Tectonic uplift, beginning in the Tertiary, resulted in the formation of the Central Plateau, which rises from the USA border in the North to reach its maximum elevation in Central México, at 19°N. The Central Plateau can be divided into the Mesa Central and the Trans Mexican Volcanic Belt (TMVB), and the Mesa del Norte (see Figure 1.1). The Plateau has an average height of 1500 masl and is bounded to the east by the Sierra Madre Oriental, with peaks reaching up to ca. 6000 masl, to the west by the Sierra Madre Occidental and to the south by the Sierra Madre del Sur. The geology of the central México and the TMVB is discussed in more detail in Chapter 2.

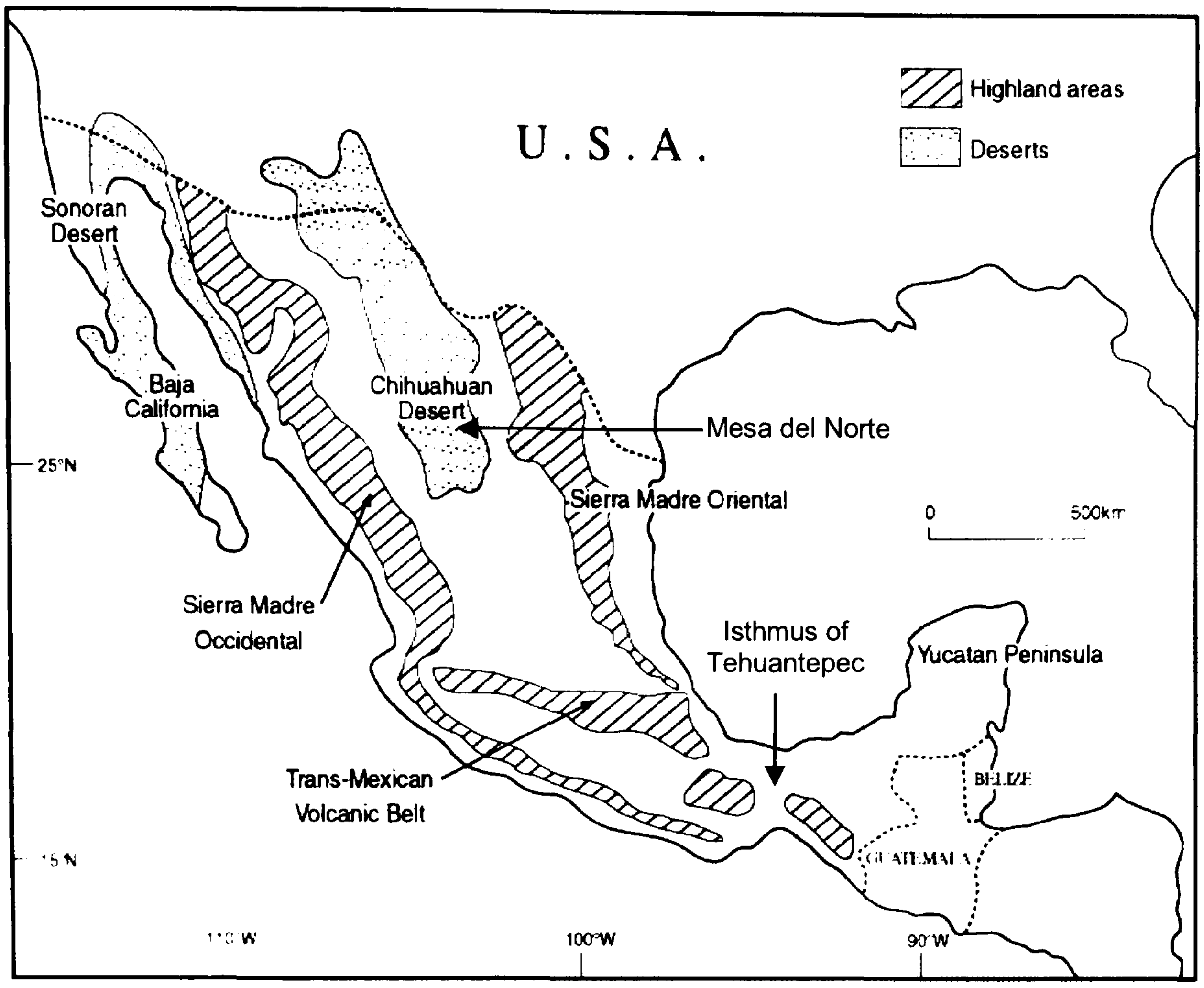


Figure 1.1. Geology of México.

1.4. Climate

México covers a wide latitudinal range from 16° to 32°N and longitudinal range from 82° to 115° W, and in addition has a wide altitudinal range, from the costal lowlands up to nearly 6000 masl in the Sierra Madre Oriental mountain range. These features therefore have an impact on the prevailing climatic regime, with altitude modifying the effects of latitude.

A dominant feature of the climate of México is the seasonal change in circulation patterns, termed the Mexican Monsoon (Douglas *et al.* 1993), which is driven by the north-south migration of the Inter Tropical convergence Zone (ITCZ) (see Figure 1.2). In the northern hemisphere summer the northerly migration of the ITZC results in the northerly movement of the sub-tropical high pressure and allowing deep easterly flows (the Trade Winds) that bring warm moist tropical air off the Gulf of México flowing south east to northwest across the Mexican Plateau (Mosiño-Alemán & García 1974) consequently bringing higher levels of precipitation across the country. In winter the ITCZ moves south over the equator and this seasonal reversal in atmospheric circulation patterns results in 80% of precipitation falling during the wet reason between June and September. The seasonal variation in precipitation between the wet and dry season is shown in Figure 1.3.

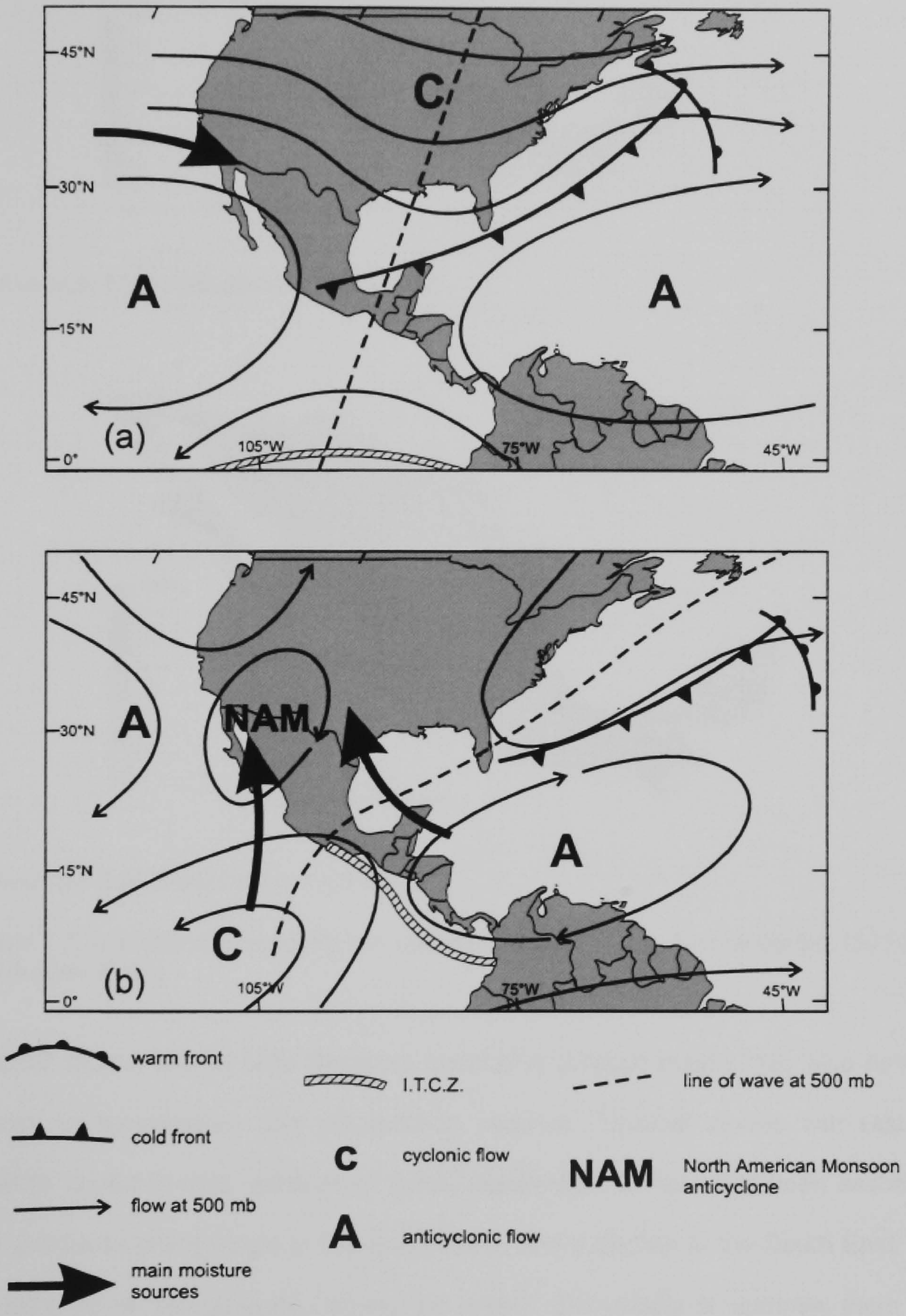
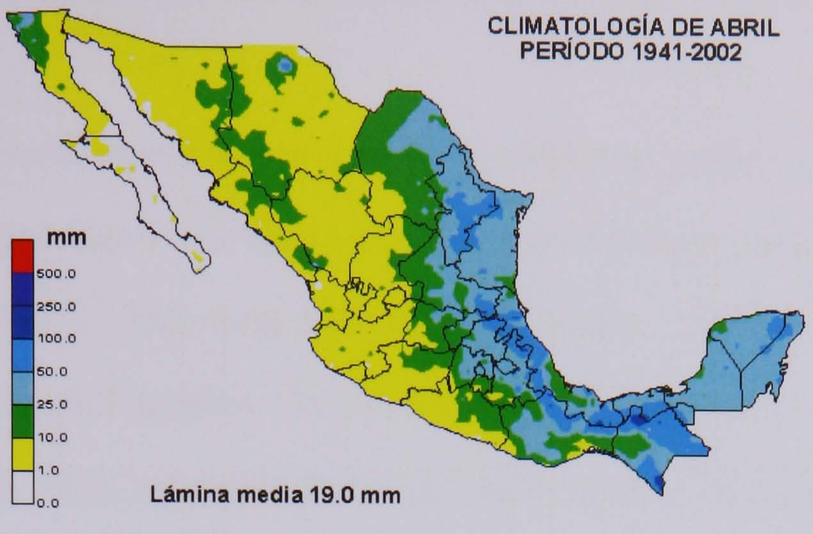
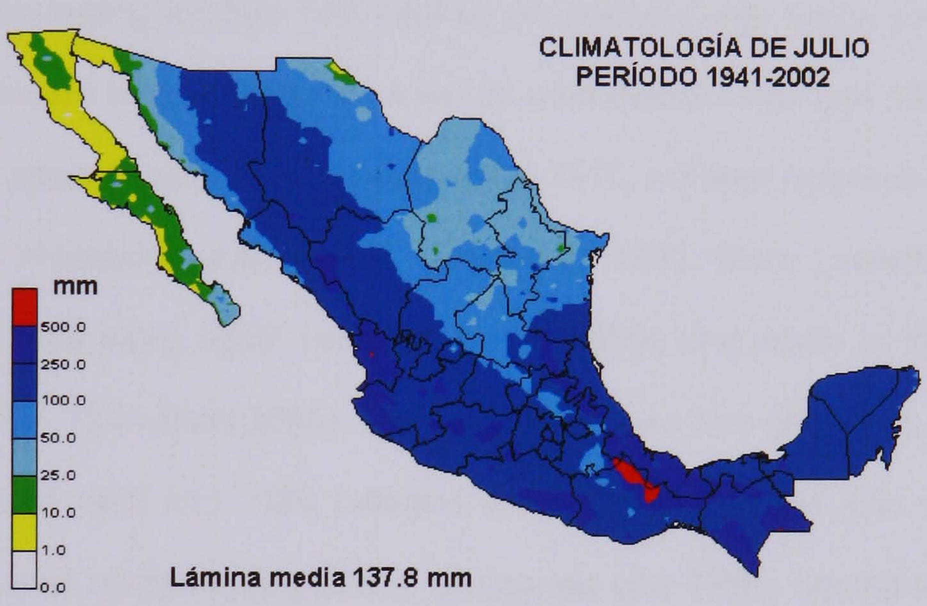


Figure 1.2 Major features of the atmospheric circulation over México in a) winter and b) summer, showing major sources of moisture (Metcalfe *et al.*, 2000 with permission)



a) Average April precipitation 1941-2002



b) Average July precipitation 1941-2002

Figure 1.3. Variations in precipitation between April and July, for the period 1941-2002 (CNA-SMN 2005)

Tropical storms and El Niño Southern Oscillation (ENSO) events may also have an impact on temperature and precipitation regimes. Tropical storms can result in extreme rainfall events, particularly during September. El Niño has been associated with increased precipitation in the North West, and a decline in the South East, over the Isthmus of Tehuantepec, during the winter. Conversely in summer there is a reduction in precipitation over most of México and Central America, due to a southerly shift of the ITCZ (Magaña *et al.* 2003). Reverse conditions are true of La Niña, with normal to above average levels of precipitation in the summer and a reduction in precipitation in the North West and an increase in the South East. Therefore, particularly in the North West ENSO events can have serious socio-economic and environmental impacts. The impacts are less well understood in central México.

The instrumental record of precipitation is short and broken, with only a few records being collected from a few sites within the Vallé de México since the late 19th century (Metcalfe, 1987). Two long uninterrupted series exist for central México, from Guadalajara and Tacubaya (México City), which date back to the beginning of the 20th century. More extensive recording began across the country from the 1920-30s onwards (Jauregui, 1997). Gaps in the data do exist however, with, for example, a cessation in data collection during the 1980s, due to economic crisis. Instrumental data show that during the late 19th century precipitation was below average with two periods of drought experienced, the driest found between 1892 and 1896. A period of drought was again experienced between 1900-1910, but was recorded within a period of generally increasing precipitation (Metcalfe, 1987). More recently (Figure 1.4) extreme droughts were again recorded in the 1950s and again in the early 1980s (Jauregui, 1997, CNA-SMN 2005). The late 1990s were also drier, with particularly dry winters noted in 1996 and 1999, between which 1997 and 1998 also saw lower than average summer rainfall (CNA, 2005). During the late 1950s rainfall levels began to increase again, with the highest levels ever recorded between 1966 and 1977 (Metcalfe, 1987). Assessment of annual rainfall patterns in 1982 (dry) and 1984 (wet) in Mexico city have shown that rainfall increased during the early part of the year, probably associated with the impact of incursions of northerly air, the *nortes*, while the summer monsoon rainfall was considerably lower than the wet conditions recorded in 1984 (Metcalfe, 1987). Such fluctuations have been linked to changes in atmospheric circulation patterns and shifts in the ITCZ, with periods of higher precipitation linked to an increase and periods of drought to a decrease in cyclonic activity. For a more in-depth review of climatic variation in México see Metcalfe *et al.*, 2000.

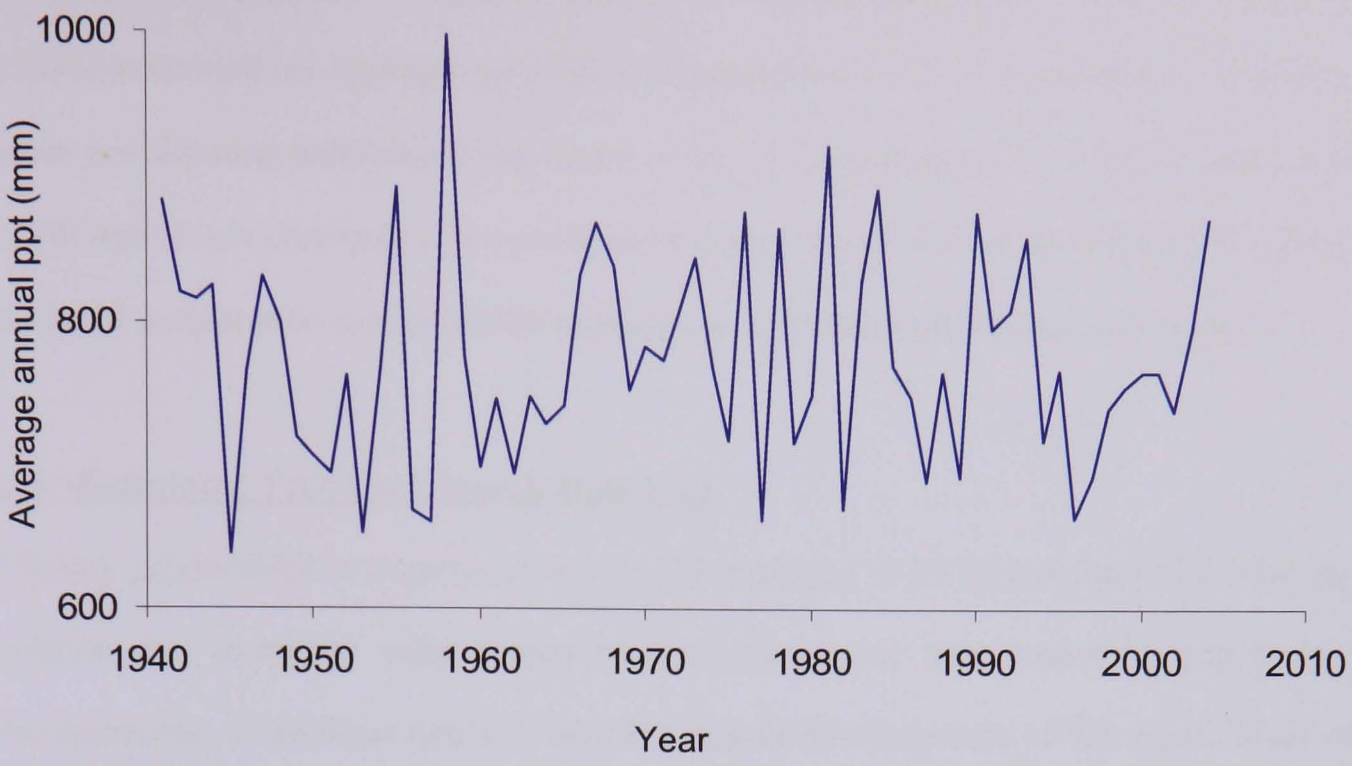


Figure 1.4 Average annual precipitation for México 1941-2004 (CNA-SMN 2005)

México also experiences a wide thermal regime, due to its wide latitudinal and altitudinal range. This results in the formation of three main thermal regimes: *tierra caliente* (< 1500 masl), *tierra templada* (1500-3000 masl) and *tierra fría* (> 3000 masl), of which the latter two are important in the area covered by this study (see Chapters 5 & 6). Highest temperatures are reached along coastal and northern desert areas with lowest at the tops of the highest, glaciated, peaks. In the northern states of Coahuila, Sonora and Chihuahua summer temperatures may exceed 35°C, but drop to between 5° and 0°C during the winter dry season months. In contrast México City in the central highlands records temperatures between 27°C and 5°C. Temperatures fluctuate less markedly in the lowland costal states such as Chiapas. Variations are also seen in the timing of the warmest month, with March noted as receiving the highest temperatures in Chiapas, where as in the north of México June, July and August record the highest temperatures (Rzedowski, 1986).

Intergovernmental Panel on Climate Change (IPCC) scenarios have predicted relatively small temperature changes in México due to global warming. Predicted changes in precipitation are less well constrained but it is expected that subtropical areas will experience a decrease in precipitation (Houghton *et al.*, 2001). For Central America predictions range from no change to a 10% decrease in mean precipitation.

Using general circulation models (GCMs) for a doubling in CO_2 Liverman & O'Brien (1991) estimated an average rise in temperature between 2.38 and 5.44°C for Mexico, while precipitation predictions are, again, more variable ranging from a 23% decline to a 3% rise. Such changes in temperature and precipitation have the potential to affect levels of evaporation and available moisture and consequently water availability.

1.5. Hydrology, Tropical Lakes & Nutrients.

Primary productivity in tropical lakes has the potential to be higher than in temperate regions due to higher solar irradiance, therefore lower light limitation, and higher temperatures, in addition nutrient recycling due to the dynamics of the mixed layer of lakes, tends to be more efficient (Lewis, 2000). Consequently topical lakes have potential for higher natural levels of productivity and a higher sensitivity to eutrophication, resulting in more severe symptoms, which may persist longer. Phosphorus has generally been recognised as playing a vital role in the process of eutrophication, with sources of P being both anthropogenic and natural in origin. Consequently management, and quantitative assessment, of eutrophication has focused upon phosphorus. In addition, P is generally easier to regulate through relatively simple management techniques, which has been achieved in temperate regions with a good degree of success. Phosphorus is generally considered to be the limiting nutrient in lakes, however evidence indicates that nitrogen limitation is more common in tropical lakes than P limitation (Talling & Lemoella, 1998). Evidence for this includes a more widespread occurrence of nitrogen fixing cyanobacteria in un-polluted waters and also from bioassay studies. Nitrogen, rather than P, limitation thought to occur for a number of reasons, which will be discussed in Chapter 3, but includes higher natural P availability. Tropical lakes are therefore, considered to benefit from P, but also from nitrogen removal (Lewis, 2000). This information is important in trying to quantify change in nutrient status in Mexican lakes, relative to the similar work that has gone on in the temperate regions, which have focussed on total phosphorus.

1.6. Archaeology

The ancient cultures of México form part of the area referred to as Mesoamerica (Krichhoff, 1949) which extended from central México into the southern Central American states of Guatemala, Belize, Honduras and El Salvador. The cultural history Mesoamerica can be subdivided into periods (see Table 1.1) the Archaic (< 2500 BC), Formative or Pre-Classic (2500 BC – 300 AD), Classic (300 – 900 AD) and Post-Classic (900-1521 AD).

Agricultural productivity aided the development of early cultural groups in México, such as the Olmec. The Pre-Classic was dominated by the Olmecs, who occupied the Gulf coast states of southern Veracruz and Tabasco. The origins of the Maya in the Yucatan peninsula also dates back to the Preclassic, with the earliest sites dated around 1000-300 BC (Brenner *et al.*, 2001). By the late Pre-Classic agriculture had intensified, in line with population growth, settlements grew in size and became increasingly politically and hierarchically ordered and towards the end of the period monumental architecture had developed. This increasing urbanisation and agricultural intensification marked the end of the Pre-Classic and the beginning of the Classic around AD 150 – 200.

Table 1.1 Pre-Hispanic cultural history of México

Date (AD/BC)	**Years BP (approx)**	**Period**	**Major cultures**	**Significant developments**
AD 1521	479	Hispanic		Spanish Conquest
AD 1200-1521	800-479	Post Classic	Aztec, Purépecha, Mixtec	Aztec triple alliance formed
AD 900-1200	1100-800	Early Postclassic	Toltec state	Aztecs arrive in Basin of Mexico
AD650-900	1350-1100	Epiclassic	Maya	Competing regional capitals Peak of Maya society & subsequent collapse
AD150-650	1850-1350	Classic	Teotihuacán Maya	Destruction of Teoithuacan Peak of Teotihuacan civilizations.
150 AD-1800 BC	1850-3800	Pre Classic	Teotihuacan Chupícuaro Olmec	Start of building of Pyramid of the Sun at Teotihuacán
> 1800 BC	> 3800	Archaic		Origins of village life Early agriculture

The timing of the Classic period differs for different regions, being ascribed to between AD 250 and 900, based on the activity of the Classic Maya in the Yucatan, Belize and Guatemala. In contrast the beginning of the Classic in Central México is based on the start of the construction of Teotihuacán (Figure 1.5), to the north east of México City in the second century AD. The end of the Classic is marked by the peak in supremacy of Teotihuacán and its subsequent collapse around AD 650. The Classic and the Post-Classic are separated by the Epiclassic, between AD 650 and 900. This was a period when a number of individual, decentralised, city states developed new political and trade alliances and new cultural systems. During the Epiclassic, the Mayan Empire reached its peak, prior to going through a major collapse between AD 800 and 900. In addition to this, societies which had been formed after the fall of Teotihuacán also went into decline.

The early Postclassic (AD 900-1200) was dominated by the Toltecs, who settled at Tula north of the Vallé of México. The most well known society of the Post Classic was the Aztecs, whose capital was located at Tenochtitlan in the Vallé of México. The Aztecs were contemporary with the Purépecha to the west and the Tlaxcalans and Mixtecs to the south. The arrival of the Spanish in the 16th century had a profound impact on the indigenous population of México. Population estimates for the Vallé de México are variable, ranging from 1.16 to 2.96 million (Whitmore 1991) suffering an estimated loss of ~65% between 1519, when Cortez entered the Basin, and 1620. Based on computer simulation, Whitmore (1991) estimated a population of 1.59 million on the eve of conquest falling to ~180,000 by 1607. The archaeological and post colonial history of central México, will be discussed in more detail in Chapter 2.

Figure 1.5. Archaeological sites mentioned in the text, cultural groups labelled in red (from Davies, 2000 used with permission)

1.7. Agriculture

Maize was the basis of settled life in México, and throughout Mesoamerica in the pre Colombian period (Coe & Koontz, 2002). The origins of sedentary society were based around the domestication of maize and other cultigens, such as squash. The origin of maize has however, been regularly contested. Archaeological and more recent molecular biological studies have indicated that the domestic, cultivated *Zea mays* was developed from the wild form *Zea mexicana* (*teosinte*), dating back to the Preclassic. The date for this is however disputed; López & López (2002) cite 4,500 years BP, while Coe & Koontz (2002) cite 3,800 years BP for transition. Small maize cobs found by Manglesdorf *et al.* (1967) in cave sediments in the Tehuacan valley, Puebla, east of México City, were initially assigned a date of 7,600 years BP, based on dating of associated materials, however, recent dating of the cobs themselves yielded a date of 5,500 years BP (Coe & Koontz 2002). Marine and lacustrine palynological studies reviewed by Brown (1985), from the Isthmus of Tehuantepec and west into Central México, indicated the widespread adoption of maize cultivation from *ca.* 4,500-3,500 years BP. González-Quintero (1986) found evidence of the adoption of maize cultivation in the Vallé de México from ca. 5000 years BP, which increased to a peak around 2,600 years BP. Maize, while important, does not appear to be the first and is not the only cultigen in Mesoamerica. Recently, evidence has been found for the domestication of squash (*Cucurbita pepo*), dated to *ca.* 8,000 BC (Smith, 2003). Other long lived staples include chilli and beans.

The arrival of the Spanish, as indicated above resulted in a massive decline in the populations of indigenous peoples in México, but also changed land use and tenure. Butzer (1988) indicated that Colonial agriculture encompassed the key characteristics of Spanish and indigenous agriculture to form a new, more successful system. *Relaciones*, Spanish ethnographic documents, indicate that by 1580 exotic crops had been rapidly integrated into agricultural practice, but were still only of secondary importance in the indigenous diet (Sanders, 1991). Several authors have postulated that as the indigenous population declined, there was a simultaneous increase in the size of herds of grazing animals due to an increase in available land (Melville, 1994; Sanders, 1991). The Spanish, and thus their agricultural techniques such as the introduction of grazing animals and plough technology, were not evenly spread across México, and depending on region, techniques were integrated with varying degrees of success (Butzer, 1988). Maize was retained as the primary cultigen, but in certain areas animals were more resilient than plants, and were thus maintained with more success (Butzer, 1991). Although pastoralism was a key feature of Spanish agriculture in the New World, cultivation was also important, but this required a large labour force, which, in the aftermath of indigenous population decline, was not readily available.

In the fight for independence (1810) from Spain, *haciendas*, large farming estates, had either been destroyed or abandoned and as a result agricultural production was at a low. A number of policy decisions were made to try to increase production. Article 27 of the new Constitution brought in modest agrarian reform in 1915. Its aim was to modernize the communal land system (*ejido* system), by allowing rental or sale of these lands, and to encourage foreign investment (Markiewicz, 1993). Further changes were made to agricultural policy from 1930 onwards to aid increased production of staple crops to ensure domestic food security. The focus however, tended to be on irrigated land for the growth of cash crops (Appendidi & Liverman, 1994). By the 1950s, policy favoured private investment and led to a decline in *ejido* land, and thus basic food crops. In the 1940s, under Avila-Camacho, technological development of agriculture and further expansion of irrigation was encouraged to increase production of subsistence crops such as maize and wheat, where between 10 and 25% of cultivated land in each region had to be dedicated to the production of these crops. Emphasis was also placed on production of crops for industrial use, and campaigns were initiated to encourage fertilizer use (Appendidi & Liverman, 1994; Markiewicz, 1993). Such incentives and technologies were beneficial to those who could afford them and did lead to an increase in production, particularly on irrigated land. As a result there was a growth in the divide between the rich, primarily farming irrigated land and the poor, farming the rain-fed crop land. Fertilizer use did, however, increase amongst the farming population as a whole during the 1970s, which Appendidi & Liverman (1994) attribute to a decline in soil fertility as a result of constant cropping and an increase in soil erosion.

1.8. Water Resources

Freshwater is imperative not only for drinking water supply, but also in terms of the economic resources it provides, through recreation and irrigation. Globally, research has increasingly shown a rapid decline in water quality as well as quantity. (Naiman, 1995). Based on figures in the latest IPCC report "Climate Change 2001" (McCarthy *et al.* 2001) approximately 1.7 billion people, globally live in countries that are water-stressed, a number which is projected to rise by 2025, associated with the rising global temperatures and changes in precipitation linked to global warming, coupled with population growth. Population growth and resultant demand for increased agricultural productivity, growth in tourism and industrial development place a high demand upon water resources, exacerbated by lack of recharge and by pollution, such as eutrophication. While the impacts of global climatic change are less well understood for México, relative to other regions, there are indications that in México and Central America by ca. 2025, 70% of the population in this region will live in areas of low water supply (McCarthy *et al.* 2001). In 1995 the UNEP estimated that ~27% of

Latin America did not have access to clean water. This is made more problematic by the lack of maintenance of the existing water infrastructure systems and discharge of domestic, agricultural and industrial effluent into aquifers and standing water bodies. This leads to increased levels of nutrients and other inorganic and organic pollutants such as polychlorinated biphenyles (PCBs), which can be detrimental to human health. Other potential impacts on water quality and quantity include acid deposition, toxic heavy metal contamination, introduction of exotic species, uncontrolled water abstraction, changes in thermal regimes and erosion (Naiman *et al.* 1995; Wetzel, 1992). Freshwater demand is particularly high in arid and semiarid regions such as northern and highland México where in addition to the problems cited above natural water scarcity exacerbates demand.

In México, in 2002 average annual availability was reported at 4,841 m^3 per capita (Aldama, 2002). By 2005, reported in the latest Comissíon Nacional del Agua report (Estadisticas del Agua, 2005) average water availability had declined to 4505 m^3/person/yr. Average annual rainfall in México is around 740mm, showing a strong increasing gradient north west to south east (see Figure 1.4), 72% of which is lost through evaporation and transpiration (García-Caderon & Lanza-Espino 2002). Water availability is also affected by seasonality and altitude, with 90% of river discharge occurring between May and October, corresponding to the rainy season, and only 5% of water resources located above 2000 masl (Alcocer *et al* 2000). México has numerous lakes, a large number of which are small, between 0.01 and 0.1 Km^2, (Alcocer *et al.* 2000; García-Caderon & Lanza-Espino, 2002) and which are concentrated in small areas of the country, chiefly central México and the Yucatán peninsula. Highest natural levels of water availability correspond to the southern and eastern states of Chiapas, Tabasco, Veracruz and northern Oaxaca (CNA 2005). Northern and central México constitute 77% of the population but only has access to 32% of the country's water, 1835 m^3/person/yr, while the southern states have an availability of 13,290 m^3/person/yr. The majority of water in México is used for agricultural purposes (76%), the majority of which is from surface water sources. In contrast domestic water is derived primarily from groundwater stores. Abstraction of groundwater can significantly affect water table levels and has been seen to lead to a reduction in lake level. For example lakes in the Vallé de Santiago, southern Guanajuato, were originally around 50m deep (See Plate 1.1), declining to 35m by 1985 and then around 10m in the last 10 years later and are now only a few centimetres deep (Alcocer, 2002). Nationally, in 2000, approximately 72,000 million m^3 of water was withdrawn from both surface and subsurface resources. The population of México is expected to rise to around 120 million (from ca. 106m) by 2020, and is therefore expected to place increased pressure on already stretched resources. Consequently there is an expected decrease in natural water availability over this time, to a national average availability of 3808 m^3/person/yr, with the greatest declines are predicted in the northern states along the Rio Bravo (US border), Baja California and the Valle de México. Water quantity is not the only problem México faces; increasing population, waste water, lack of treatment facilities and salinization are degrading water quality, which exacerbates the problem of access to clean, fresh water. Surface water monitoring by the Comisión Nacional del Agua (CNA) and their most recent study (Estadisticas del Agua en México 2005) found the 5.3% were extremely contaminated. Subterranean water resources are also exploited in México, associated with high populations and low levels of surface water availability, particularly across central México and the Mesa del Norte. In northern México, particularly western Sonora, a number of these aquifers have been so highly exploited they are now subject to saline intrusions.

1.8.1. Water Management strategy

Resources in some areas, particularly arid/semi-arid regions are being used quicker than their renewal times: 1-100 years for lakes and up to 300 years for groundwater (Wetzel 1992). Since the Revolution in 1910, water use for irrigation, industry and urbanisation has been supported by the Federal Government, which controlled exploitation, management and development of water resources and has been inherently tied to land and agricultural policy. The first major legislation was implemented in 1926 in which water was made a national resource (Whiteford &

Bernal, 1996). The framework for Mexican water policy lies in the creation of the National Irrigation Commission and Irrigation Law of 1926. Water development and management were integrated in 1946 within the Secretariat of Water Resources (Sectretaríat de Recursors Hidráulicos), the Ley Federal de Aguas was implemented in 1972 and in 1975 the first National Water Plan was developed. In 1976, due to reorganisation of the Federal Government, management of water and agriculture was integrated, although management of urban water supply and sewage systems was controlled by the Secretariat of Human Settlements and Public Works, but was later decentralised (Aldama, 2002). Federal funding for water however, declined between 1972 and 1992 (Whiteford & Bernal, 1996). Consequently co-ordination and implementation of water policy became difficult, causing conflict, particularly between rural and urban users and at the same time scarcity was accentuated by contamination from pesticides, erosion and, sewage and industrial effluent (Aldama, 2002; Whiteford & Bernal, 1996). At present only around 14 % of industrial and municipal wastewater is treated. Constitutional amendments in 1982 allowed the establishment of new institutions and aimed to improve environmental protection regulation. In 1989 the Comisión Nacional del Agua (CNA) was created with federal authority in control of water management (Aldama, 2002). The current aims of the CNA include efficient use of water for agriculture, improved and increased quality and coverage of drinking water, sewage and sanitation facilities, sustainable management of basins and aquifers, promotion of social inclusion and to reduce flood and drought risk (CNA, 2003). These aims are to be achieved though several national strategies such as consolidated administration of water and improved regulation to achieve increased sustainability, efficiency and productivity. In 1992 the Ley de Aguas Nacionales was enacted and aimed to achieve integrated water management, sustainable development and public participation, coupled with a polluter pays system and to achieve efficiency and self sufficiency. The implementation of this led to a slight improvement in the water quality of some surface water bodies through monitoring and technological improvements. On the whole, however, surface water quality has however continued to decline and is coupled with increased demand, growing disparities between rural and urban users and between states and regions (Aldama, 2002).

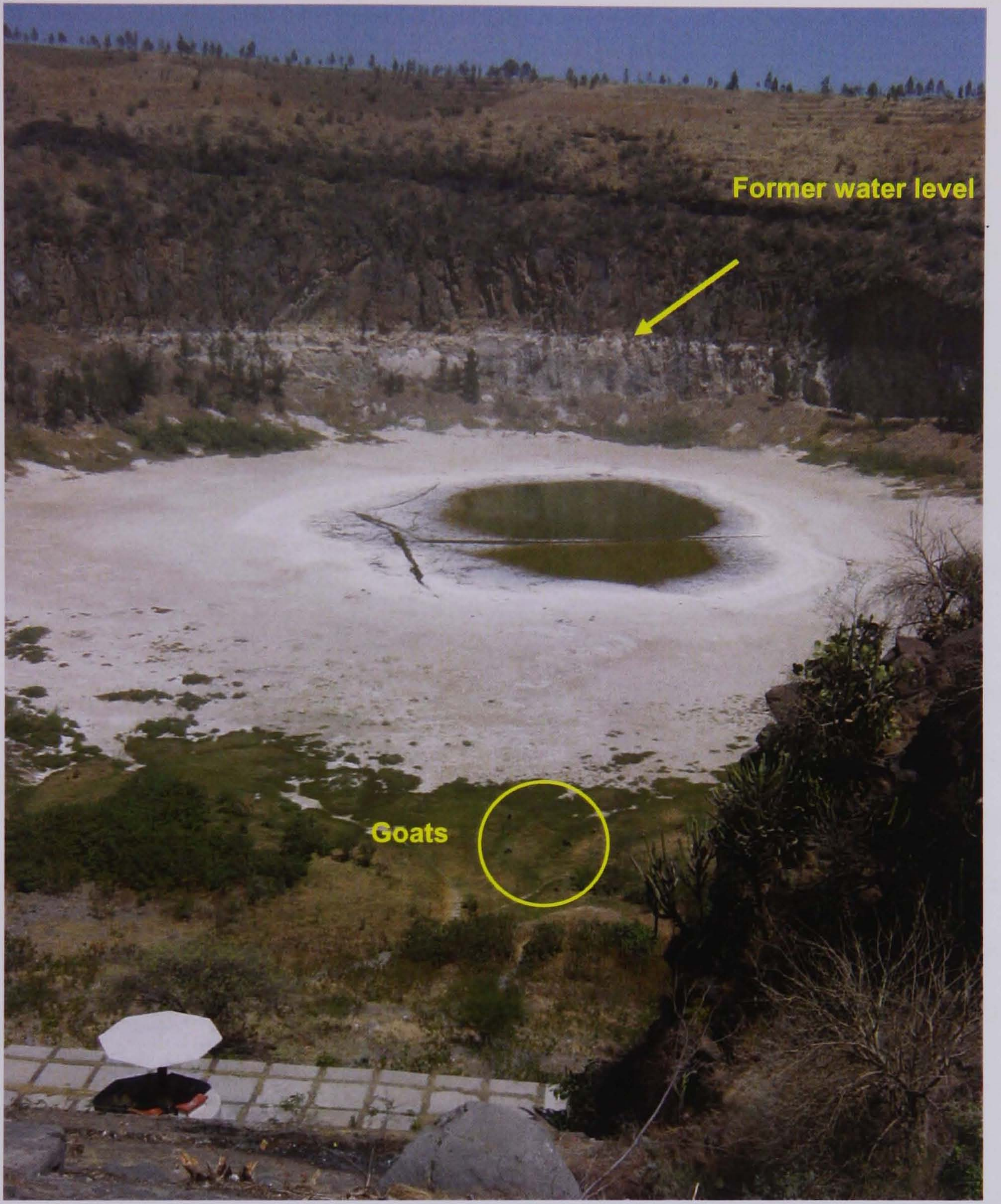


Plate 1.1. La Alberca, Valle de Santiago March 2003 showing original and current water depth (note goats – for scale)

1.9. Approach and Outline

The thesis is arranged into nine chapters. Chapter one has presented a general outline of climatic variability and human development in México as a whole, and introduced issues related to eutrophication such as human activity, agriculture and water resources policy, which provides a more general background for the thesis.

The relationship between humans, climate and environment in central México is complex, but must be understood for an accurate interpretation of palaeoenvironmental records in the study area. Chapter 2 provides a more specific review outlining the climate, environment and human history of central México, building on the information outlined in this chapter. It discusses previous palaeolimnological research and the future implications for water resources and society in central México. Chapter 3 reviews in detail the role played by nutrients in biological productivity and the implications of increase nutrient availability through human activity, i.e. eutrophication. It describes existing evidence, both limnological and palaeolimnological, for eutrophication in central Mexican lakes. Chapter 4 provides a review of geochemical methodologies, isotope and lipid analysis, for tracking changes in trophic states and outlines reasons for changes in these proxies.

The research approach is discussed in detail in Chapter 5. The methodology chapter describes, site selection, in terms of coring and modern sampling, and methods of modern and fossil analyses of chemical, biological and physical properties. Diatom analysis and the use of multivariate statistics to relate diatom species variation to environmental changes were the main techniques employed in this study. Consequently the main focus of this chapter is on the methodologies involved in the collection of the modern data set and its analysis.

The results of modern water chemistry and diatom sampling and the relationship between water chemistry variables is discussed Chapter 6. The results of the modern sampling are compared with other available chemical data. This chapter also discusses complexity in the data set, in terms of relationships between variables, such a phosphorus, productivity and water clarity. This is developed further in Chapter 7, which discusses, in detail, the outcomes of multivariate statistical analysis of relationships between diatoms and their environment, though gradient analysis and the development of a diatom based nutrient transfer function. Chapter 7 also reviews the development of quantitative methods of inferring changes in nutrient status over time. The environmental history of Laguna Zirahuén is presented in Chapter 8, in terms of previous research on the Zirahuén basin, and through the outcomes of the sedimentary analyses of the diatom stratigraphy, isotope and lipid analyses. This chapter also discusses the application of the transfer function and what the data as a whole show in terms of changes in nutrient status over time. This will be compared to former palaeolimnological work on the Zirahuén basin, namely Davies (2000) and other limnological work, such as Bernal-Brooks & MacCrimmon (2000b) to aid validation of the transfer function. Chapter 9 draws together the two main strands of the thesis, the modern diatom-environment relationships, in terms of nutrient availability and the palaeolimnological evidence of change in nutrient status in Laguna Zirahuén.

Chapter 2. Environment & Human Impact in Central México: Background to the Study Area

2.1. Introduction

The environment of central Mexico has been shaped by volcanic and tectonic activity (section 1.3), which resulted in the formation of numerous hydrologically closed lake basins and fertile volcanic soils across the central México (TMVB). Consequently central México includes a wide variety of climates and natural environments, which has favoured massive social and urban development over a prolonged period of time (Section 1.6 & 1.7). The majority of México's population is, and has in the past, been concentrated in central México along the TMVB, associated with the availability of water and its suitability for agriculture. Environmental impact has therefore, been prolonged and at times intense. Distinguishing between climatically induced and human induced environmental and limnological changes has consequently become difficult. As evidence presented below indicates, part of the problem has been associated with high levels of nutrient input into many of the lakes. In order to fully comprehend past, present and future trends in the nutrient status of Mexican lakes it is necessary to have a background of the physical and human conditions under which these changes have evolved. Discussed below are the issues of climate, environment and human activity which affect the lakes basins of central México.

2.2. Geology

The TMVB (Figure 1.1), along which the lakes in this study are located, extends from the Pacific to the Gulf coast between 19° and 21° N, it includes several strato-volcanoes peaks including: Popocátepetl, Iztaccihuatl, Pico de Orizaba, Nevado de Toluca and the Volcán de Colima all of which reach over 4000 masl (Maldonado-Koerdell 1964). The formation of the TMVB is associated with faulting along east-west trending lines of weakness, in particular the Clarion Fracture Zone, on the Pacific floor (West 1964a) perpendicular to the motion of the Cocos-North American plate boundary. In addition the division of the San Andreas Fault off the Nayarit coast led to the formation of a graben-like fault, stretching across Jalisco and Michoacán, resulting in the formation of Lago de Chapala (Maldonado-Koerdell 1964) (see Figure 1.1 & 5.1)

The TMVB, which forms the southern edge of the Central Plateau, linking the Sierra Madre Oriental and Occidental, can be subdivided into 5 volcanic zones (Demant, 1981; 1992).

1) Tepic-Chapala Graben – forms the most westerly region of the TMVB and trends NW-SE. This area is predominantly andesitic and dacitic and is older than the majority of the TMVB, with the exception of the Sierra Madre Oriental. There are 4 principle volcanoes in the area, including Ceboruco and San Juan.
2) Colima Graben – occupies the area south of Guadalajara and trends north-south. It is connected to the Tepic-Chapala Graben by a series of tectonic depressions occupied by large Playas such as Sayula or lakes such as Zapotlan and Atotonilco. At the southern end of the graben are two large strato-volcanoes the Nevdao de Colima and the Volcán de Colima, the latter is México's most active volcano.
3) Michoacán – The Michoacán-Guanajuato Volcanic Field (MGVF) of Northern Michoacán and southern Guanajuato occupies the majority of this zone. It is characterized by ~1000 monogenetic volcanic cinder cones, small shield volcanoes and lava domes and flows, which are Quaternary in age formed by short lived events and which are rarely active after formation (Hasenaka & Carmichael 1987). The area between these cinder cones consists of lava, volcanic ash and mud flows or olivine basalt, andesite and rhyolitic flows (Hasenaka & Carmichael, 1987; West, 1964a). The Michoacán region is perhaps most well known for the eruption of Paricutín between 1943 and 1952.
4) Toluca-Puebla Region – stretches from the Queretaro-Taxco fault system to include the Toluca Basin (or Upper Lerma Basin), Basin of México and the Puebla Basin. These basins are all high upland areas located between

2,200 and 2,600 masl. This zone is characterised by a large number of high strato-volcanoes. The Nevado de Toluca lies in the Toluca basin, Popocatépetl and Iztaccihuatal in the Sierra Nevada around the Basin of México and La Malinche in the middle of the Puebla Basin.

5) Oriental Basin – is located at the eastern end of the TMVB in the western foothills of the Sierra Madre oriental in a north-south trending graben, with the eastern limit marked by the Cofre de Perote and Pico de Orizaba, the highest peak in México (5,750 masl). The area is composed of basalts and rhyotlites. There are a series of maar lakes, such as Lagunas Alchichica and Atexcac in the north of the Oriental Basin.

Although the surface configuration of Central México was similar to the present during the Quaternary, it has been under constant modification by further volcanic and tectonic activity and subsequent erosion which formed a number of closed basins. Areas of internal drainage include several basins within the TMVB, such as the Upper Lerma Basin or the Oriental Basin, where interruption of normal exterior drainage, by tectonic or volcanic activity, led to the formation of closed basins containing numerous small lakes. The formation of lava flows and cinder cones aided the formation of closed lake basins through lava damming of streams or the formation of lakes within the cinder cones themselves (West 1964a). Based on the volcanic origin of parent materials, soils in central México are andesitic or basaltic and are consequently highly fertile and phosphate rich and are highly susceptible to erosion. When associated with the deposition of rhyolitic flows during the Tertiary, which have subsequently been exposed by folding and faulting, soils are also silicate rich. Such an environment provides fertile soils, lacustrine basins and a source of material, such as obsidian, that can be used to make tools, thereby creating an environment suitable for the evolution of sedentary life.

2.3. Climate, Water & Vegetation

Land in central México falls into the altitudinal zones *tierra templada* and *tierra fria.* Atmospheric circulatory patterns and the effects of altitude, as discussed in Chapter 1, result in warm wet summers and cool dry winters. The TMVB receives an average of 1000 mm per year, ranging between 400 mm per year in the north and 1500 mm per year in the south. Figures 1.4 and 1.5 showed the current rainfall pattern (1941-2002) for Mexico. This highlights the moisture gradient across central México, due to seasonality and topography, running north-west (Northern Jalisco and Southern Guanajuato) to south-east (Southern Estado de México & Morelos). The majority of rainfall is received during the rainy season between June and September; however, timing of maximum rainfall varies between states. Based rainfall data for the period 1941-1996 maximum precipitation is recorded in July in Michoacán, Estado de México, México (Distrito Federal) and Jalisco; in August in Nayarit and in September in Puebla and Colima (CNA SMN, 2005). Temperatures also vary accordingly, usually peaking prior to the onset of the rainy season in April and May, or in the first month of the rainy season. Temperatures also show a general increase southwards, but are modified by the effects of altitude. For example, in Michoacán Morelia (1900 m asl), has recorded average temperatures for the period 1951-1980, 14.6 to 20°C (CNA-SMN, 2005), while near by Pátzcuaro (2035 m asl) shows lower average temperatures (1971-1986) between 7 and 14°C (Chacón-Torres & Muzquiz-Iribe, 1991). Variations in levels of precipitation and temperature, which have an impact on evaporation, will affect lake levels and, therefore, the chemical characteristics of the lakes in central Mexico.

Little research has been conducted into the impact of ENSO events on the climate of central Mexico, seasonality and its subsequent impacts on the chemical, physical and biological characteristics of lake basins. Mosiño & Morales (1988) found high variability in the impact of El Niño events on precipitation, with strong events causing a reduction in precipitation and moderate strength events associated with abundant rainfall in central México. In contrast Jauregui (1995) found that for certain periods El

Niño has been significantly correlated with drought conditions. Consequently it is apparent that the effects of El Niño on central México are not yet well understood. Caballero *et al.*, (2003) note that reduced precipitation in Central México is related to ENSO events, associated with the deflection of the ITCZ southwards and a reduction in tropical storm activity. However, their palaeolimnological research on Laguna Alchichica in the Oriental Basin, eastern Central México however, showed no evidence of a response to ENSO. It would appear that the impact of ENSO events in central México is small, relative to its impacts elsewhere in México.

2.3.1. Water

As outlined in Chapter 1 the majority of surface waters in México can be classified as excellent, in terms of biological oxygen demand. However, water bodies which were classified as contaminated or very contaminated were found to be concentrated in central México, primarily in the Valle de México. In the Valle de México 72% of surface water resources are either contaminated or very contaminated, in the Balsas basin 28.2% are excellent, while the same amount are classified as contaminated, in contrast the majority of resources in the Lerma-Santiago basin are acceptable-excellent, with 19.2% classified as contaminated (CNA, 2005). In the Balsas and Lerma-Santiago-Pacifico basins the majority of water for agricultural, industrial and domestic use comes from these surface water sources. Despite the apparent cleanliness of water resources in the Balsas and Lerma-Santaigo basin the efficiency of chlorination in Jalisco and Michoacán is below the national average, with the latter only reaching 75% efficiency, while in contrast Estado de México and the Distrito Federal and Puebla are above the national average. In these areas, as with the majority of the country water availability is predicted to decline by 2025. The Balsas basin is expected to fall from 2703 to 2403 m^3/person/yr, the Lerma-Santiago from 1820 to 1583 m^3/person/yr and the Valle de México from 188-162 m^3/person/yr (CNA 2005). Central México accounts for 76% of the total population and 51% of the total agricultural land, but only has access to 12% of the country's available water supply and 5% of freshwater bodies (Alcocer, 2002; Liverman, 1992). In addition to poor or declining water quality in central México other problems, which may exacerbate this, are also becoming apparent. Liverman & O'Brien (1991) estimated a decline in moisture availability of 5-15% between 1991-2016. This is coincident with a rise in demand for water resources, particularly in the Basin of México, which resulted in a decline in water availability by 11 to 23% which has the potential to place further stress on water resources, agricultural production, human health and nutrition. The area covered by this study corresponds to the hydrological administrative regions of the Lerma-Santiago-Pacifico drainage basin (Jalisco, Colima and southern Guanajuato), the Balsas Basin (Michoacán, Estado de Mexico and Morelos) Valle de México (Estado de México and Distrito Federal) and partly to the region of the Golfo Centro (Puebla).

The focus of Mexican water policy has been on water availability for irrigation and drinking water which are predominantly derived from groundwater and reservoirs (Liverman & O'Brien 1991). Declining water availability and quality from such sources may place long term focus on natural lake basins, basins which have suffered prolonged and recently intensified impact through population growth and agricultural policy. Future trends in population growth and in climate change predict increasing stress on water resources and as a result on agricultural production and human health. This is particularly important as population collapse, when agricultural practices have appeared to have been carried out in an unsustainable manner combined with adverse, dry, climatic condition have been noted in México's history, such as the collapse of Teotihuacán and of the Maya in the Yucatan (Brenner *et al.*, 2001). This highlights the potential vulnerability of a society where agriculture is still highly important, where water resources are declining in both quality and quantity. An understanding of the impacts human activity, through agricultural policy, land use and population growth, on lake basins in the past and at present, is necessary to understand future rates and trajectories of change. Such an understanding allows appropriate management plans to be developed and applied to ensure the sustainability of these systems. In addition the ability to separate the effects of human activity from those of climate in the sedimentary record may allow a better interpretation of the climate signal and thus interpretation of patterns and cycles of change and potential responses to the effects of global warming.

2.3.2. Vegetation

Highland areas of México are dominated by pine/oak forest and cloud forest. The latter is restricted to small areas along the eastern upper slopes of the Sierra Madre Oriental. In contrast pine/oak forests are dominant along the Sierra Madre Oriental and Occidental and along the TMVB.

Vegetation composition in Central México reflects the sub-tropical climate, with cooler temperatures and more defined winter conditions at higher altitudes. *Pinus* (pine) forests dominate from ~2300 up to ~4000 masl, with the dominant species often changing with increasing altitude, *Quercus* (oak) is usually abundant between 2350/2500 and 3100 masl and *Abies* (fir) are also important between 2700/2800 and 3500 masl (Lozano-García & Ortega-Guerrero 1994). The tree line is located at ~4000m with *Juniperus* (junpier), grasses, shrubs and herbs between this level and ~4300m, above which the environment is tundra like with incomplete ground cover, which is herb dominated (Wagner, 1964). Palynological studies throughout central México have highlighted that *Pinus* and *Quercus* have been dominant over the last ~35Ka (Lozano-Garcia & Ortega-Guerreo 1994; Straka & Ohngemach 1989), but with fluctuations in the amount of non-arboreal pollen (NAP), such as *Chenopodiaceae-Amarantaceae* (Cheno-Ams) over this time period. The appearance of *Zea mays* (cultivated maize) in palynological records is accompanied by a decline in arboreal pollen (AP) and an increase in NAP, indicating clearance for agriculture and settlement.

2.4 Human activity in Central México

Section 1.6 briefly discussed the cultural history of México (see Table 1.1 and Figure 1.6). The following section discusses in more detail the history of central México, the cultural groups and their relationship with, and impact on, the environment. This section also outlines the impact of the Spanish Conquest on the indigenous populations of central México and the environment.

2.4.1 The Pre-Hispanic periods

During the Preclassic the dominant cultures in central México were the Chupícuaro and Cuicilco. Chupícuaro culture was centred in Guanajuato, north west of the Basin of México while the Cuicilco were in the south of the Basin of México. The late Preclassic is characterised by the appearance of temple pyramids, most notably the Cuicilco pyramid, which is now surrounded and partially covered by lava. The Classic period is characterised by the development of complex societies, the most dominant of which was Teotihuacán, located north east of the Basin of México. It occupied a large area, approximately 300 Km^2, one half of which was probably suitable for farming, with the city itself occupying 12 Km^2. A number of springs produced a plentiful supply of water, used for farming and there is evidence to show that this may have been used for irrigation (Coe & Koontz, 2002). The influence of the city state extended from Central México south to Oaxaca, east towards the Gulf coast and south east towards the Isthmus of Tehuantepec and the Maya lowlands.

The fall of Teotihuacán marked the end of the Classic and transition into the Epiclassic and then the Postclassic (see Table 1.1). At this time migration increased and central México was repeatedly invaded from the north by nomadic Chichimec tribes, thought to be driven south by adverse climatic conditions, by the prospect of fertile soils or driven south by invasions from further north (Coe & Koontz 2002; Metcalfe, 1985; Prescott, 1843). The Toltecs were the dominant society in Central México during the early Post-Classic who settled at Tula, in the present state of Hidalgo. Tula was a locally important centre which was small in comparison to Teotihuacán, but did have trade links across Mesoamerica. They did however, suffered a similar fate to Teotihuacán, and the city was abandoned in the 12th century.

From conflict between city states after the fall of the Toltec state, arose the Aztec Empire, also thought to be related to the southward movement of Chichimec tribes. A number of Aztec traits are thought to be related to the Teotihuacán and the Toltec's. A number of Aztec gods, such as the rain god Tlaloc, were modified from Teotihuacán deities. Similarities are noted in the construction of the Aztec capital Tenochtitlan with both Tula and Teotihuacán. The Aztecs established themselves in the Basin of México and founded their capital Tenochtitlan on an island in Lake Texcoco, from ~AD 1325 onwards. The Aztec diet was heavily dependent upon maize and other agricultural product such as beans and squash. Consequently a number of deities were dedicated to maize and to rain, with offerings, including human sacrifice, being made. With population growth came agricultural intensification, resulting in a shift from rain-fed and slash and burn cultivation to more high yield methods, involving terracing, raised fields (*Chinampas*) and irrigation. Consequently the environment became a cultivated landscape, with little natural vegetation as these methods allowed previously unsuitable land to be brought under cultivation. Intensification was also encouraged due to increasing social stratification and the resultant need to make tributes and pay taxes, in addition to meeting basic subsistence needs (Smith, 2003).

The Aztec empire extended over much of central México across to the Gulf and Pacific coasts and down to the western part of the Isthmus of Tehuantepec. They attempted to expand their empire, but never gained full control of Central México, being prevented from doing so by the Purépecha to the west and also by the Tlaxcalans to the east and the Mixtecs in the south east (Coe & Koontz, 2002; Smith, 2003). In attempts to expand their empire the Aztecs were brought in to conflict several times with the Purépecha which, in the 1470s, resulted in Aztec defeat and the loss of ca. 20,000 Aztec soldiers (Smith, 2003), consequently the Purépecha were considered to be equal in power to the Aztecs.

Based on the *Relación de Michoacán*, the earliest ethnographic document for Michoacán produced by the Spanish ca. 1541, the Purépecha were also descendent from the in-migration of Chichimec tribes to Michoacán (Coe & Koontz 2002; Michelet 1996). Initially they settled on the shore of Laguna Zacapu in the early Post classic. The capital was then moved to the town of Pátzcuaro in AD 1325. Pátzcuaro was later succeeded as the Purépecha capital by Ihuátzio (Plate 2.1) and then Tzintzuntzan (Plate 2.2) on the northeast shore of Lago de Pátzcuaro, where it remained until the arrival of the Spanish in AD 1522. They controlled all of modern day Michoacán, parts of Colima, Jalisco and Guanajuato (see Figure 2.1). Consequently, they controlled a number of other lake basins, including Zapotlan to the west, Cuitzeo and Yuriría to the north and Zirahuén to the south of Pátzcuaro. The former two were religious, trading, administrative and defensive settlements while Zirahuén was used by the Purépecha notability for recreational and religious purposes. Purépecha religion was different from a number of Mesoamerican cultures, for example there was no analogous rain god to Tlaloc. There were however alternative deities: Kwerawáperi who controlled rain and drought, and their primary god was Kurikaweri, the sun god and god of war, to whom huge offerings of fire wood were made, necessitating extensive deforestation (Coe & Koontz 2003; Street-Perrott *et al.,* 1989). Despite having no specific rain god water was central to the Purépecha belief system, with myths arising over its origins, sources and disappearance (Acevedo *et al.,* 1982). The Purépecha are also noted for their metallurgy, which was unique to this culture in Central Mexico. Copper was most widely used to create tools for agriculture and arrow heads (Beltrán, 1986)

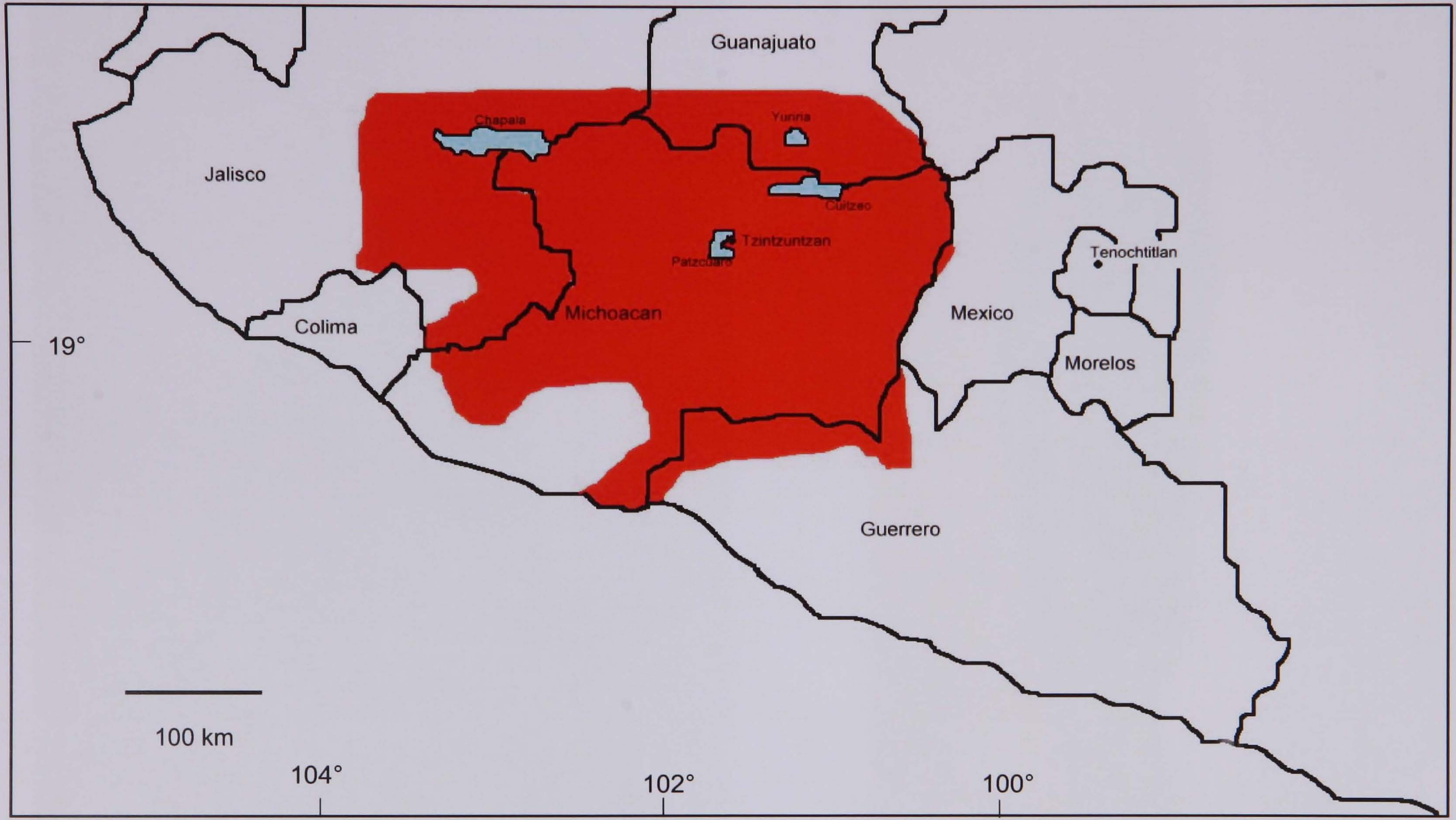


Figure 2.1 Purépecha Empire

Plate 2.1. Ihuátzio

Plate 2.2. Tzintzuntzan

Pollard & Gorenstein (1980) estimate the population in the Pátzcuaro basin at the time of contact to be between 60,000 and 100,000 (between 25,000 and 35,000 at Tzintzuntzan) with 8 religious centres and more than 79 other settlements. The majority of the population was thought to live either on the shore of Lago de Pátzcuaro or on the lower slopes of the basin. As with the majority of Meosamerican cultures their diet was based on maize, beans and fish. Around 95% of available agricultural land was cultivated and irrigation and terrace agriculture were used to intensify production. It has been suggested that this exceeded the basin's capacity to produce enough food, necessitating the import of maize from outside (Pollard & Gorenstein, 1980). Borah & Cook (1963), in contrast, estimated the population of Purépecha in Michoacán to be around 1.3 million on the eve of Conquest., while other estimates place the population of Michoacán around 750,000 just prior to conquest (Beltrán, 1986)

2.4.2. Colonial period and Independence

The arrival of the Europeans in México in the 1500s resulted in unprecedented socio-economic and demographic changes associated with the introduction of exotic flora, fauna, new technologies and diseases. The extent of impact is, however, often contested (Whitmore, 1991). In the 16th century the Spanish encountered a large population that was highly developed, but in the wake of contact numbers declined rapidly. The rapid decline in the indigenous population has been attributed to a series of epidemics, such as small pox, to which the indigenous population had no acquired immunity. Population decline has also been associated with war, famine and a decline in fertility (Whitmore, 1991).

After the Conquest, the Spanish began to exploit México's mineral wealth, to aid the development of New Spain. Raising livestock, sheep and cattle was also important, with less emphasis placed on cultivation due to its large labour force requirement (Suchliki, 2001). During the period in which the indigenous population declined, the Spanish population was still small, consequently population density was low and pressure on the environment was reduced. There is, however, a degree of debate over the impact that the introduction of Spanish agricultural techniques had on the environment (c.f. Butzer, 1988; 1993; Melville, 1994). Concepts such as the "Pristine Myth" have advocated that pre-Hispanic society was in harmony with the environment using it in a sustainable, non-intrusive manner and that European activity had an immediate and adverse impact on the environment through the introduction of new farming techniques (Butzer, 1993; Denevan, 1992). Such prolonged human and related agricultural activity as there has been in central México, would have had a profound impact on the environment, prior to the Conquest. This has led to the question of 'natural vegetation' and in palaeoenvironmental terms, makes separation of climatic and anthropogenic signals problematic (see section 2.5). The Spanish did introduce plough technology, grazing animals such as cattle, sheep and goats and new exotic cultigens such as citrus, wheat, barley and sugar cane. While the extent of the impact of these introductions on the environment is debated, it can be noted that they did modify human-environment interactions. Re-intensification of cultivation was related to the recovery of the indigenous population and growth of the Spanish population from the 1700s (Butzer, 1991), which gave rise to increased pressure on environmental resources. In addition to a renewed period of land degradation, population growth also resulted in conflict over access to land and resources. Spanish administrative policies, acquisition of land during the period of indigenous depopulation and expansion of *haciendas* meant that the indigenous population had lost their means of economic independence as they had little access to land (Endfield & O'Hara, 1999). Consequently disputes over land and resources and civil unrest escalated and resulted in more marginal land being brought under cultivation and further lack of access to fertile land. This was accentuated by the prevailing climatic conditions and disputes over water resources also increased during this time (Endfield & O'Hara, 1997). Like the Aztecs and the Purépecha, the Spanish also made offerings, to the Virgin de Los Remedios, for more favourable climatic conditions. During particularly dry periods the statue of the Virgin was moved from its more permanent position outside México city to the Metropolitan cathedral.

2.5 Palaeoenvironmental Records in Central Mexico

As the lakes in Central México are, on the whole, hydrologically closed they are climatically sensitive. Their sediments therefore provide a useful record of climatic changes in this area. Due to the lack of long term instrumental records and to an extent, historical documents and instrumental records recording climatic changes, the palaeo record is most useful for tracking long term climatic changes and their impact on the environment. A number of palaeoclimatic records exist for lakes in Central México. More recent studies carried out in central México include Arnaud *et al.* (1997), Bradbury (1971; 1989; 2000), Caballero (1995; 1998), Davies *et al.* (2002; 2004; 2005), Lozano-Garcia & Ortega Guerrero (1998), Metcalfe (1985; 1988) and Watts & Bradbury (1982) on lakes in the Basin of México and the state of Michoacán. The increase in number and spatial distribution of studies, in addition to the diversity of methods used and improved dating control, have improved understanding of climatic and corresponding environmental change in the area and variations in human activity.

2.5.1. Palaeoclimatic records of Environmental Change

Initial palaeoclimatic records date back to the work of Deevey (1944) and Hutchinson *et al.* (1957) both of which focused on Lago de Pátzcuaro, the former using pollen analysis and the latter a combination of diatom and pollen evidence. Deevey concluded that there had been relatively little change in the pollen sequence over the time-period covered by the (undated) core, but that there had been small scale changes. Based on the palynological evidence Deevey interpreted the early assemblage as being indicative of moist conditions (documented by arboreal pollen) that were replaced by drier conditions (documented by non-arboreal pollen). The later study, on the same core, (Hutchinson *et al.*, 1956) of pollen and diatom sequences at Pátzcuaro, showed a general progression from moist to dry conditions, punctuated by wetter and drier periods. Correspondingly, the diatom record shows a progressive shift from cool deep water to shallow warm conditions with a high ionic content. The lack of an absolute chronology, however, limits the value of these early studies.

Dated palynological and diatom evidence was later used by Watts & Bradbury (1982) to reconstruct change in the Pátzcuaro Basin over the last ~44 Ka, updated by Bradbury (2000) to cover the last 48 Ka. Results showed levels of *Pinus, Quercus,* and *Alnus* to be high throughout the sequence but with varying levels of non arboreal pollen. An abundance of *Juniperus, Artemisia*, and *Ambrosia* were found in the early part of the sequence, 33-11 Ka, interpreted as indicating drier conditions than at present. There was a rapid decline in *Alnus* around 5 Ka, and a notable increase in non arboreal pollen and the appearance of *Zea mays* in the late Holocene. Such changes over the last 5 Ka are linked to anthropogenically induced environmental change, although it is noted that increased aridity may have contributed to the decline in *Alnus*. Diatoms at the base of the core, *Pinnularia maior*, *Cocconeis placentula, Navicula mutica* and *Hantzschia amphioxys* are interpreted as representing marsh-margin environment. Between 47 and 10 Ka the diatom assemblage was characterised by planktonic flora such as *Stephanodicus oregonicus, Cyclotella stelligera* and *Aulacoseira ambigua* indicating deeper conditions. In the Holocene the assemblage was dominated by attached and motile diatoms, *Fragilaria* species and *Aulacoseira ambigua*, indicating a shallower but fresh water environment. Late Holocene sediments indicate a more eutrophic environment with a diatom assemblage dominated by *Stephanodiscus* species and *Aulacoseira granulata*.

Palaeoclimatic records in the state of Michoacán also exist for Laguna Zirahuen, Laguna Zacapu and La Piscina de Yuríria. A core taken from Laguna Zacapu covers ~4 Ka of environmental change (Metcalfe 1995). Lake level is interpreted from the diatom stratigraphy as low between ~3800 and 2400 ^{14}C y BP with deeper periods noted between 2900-2800 ^{14}C y BP and at ~2200 ^{14}C y BP. After which time lake level was thought to decline rapidly. After AD 960 data implied a shift to fresher more alkaline conditions. Increases in *Stephanodiscus* species, χ_{lf}, Fe, Al and P in the late Post-Classic and early Hispanic periods are interpreted as an increase in catchment disturbance resulting in soil erosion and cultural eutrophication, and thus complicate a climatic interpretation of the record (Metcalfe 1995; Metcalfe *et al.,* 1989). Diatom records from La Piscina de Yuríria (Metcalfe & Hales, 1990) indicate that the lake has been alkaline and eutrophic for a prolonged period. Based on the diatom stratigraphy and detrital sedimentary indicators the period around 4100 ^{14}C y BP gave rise to a shallow, alkaline eutrophic lake. The presence of *Amphora coffeaformis* and *Chaetocerous muelleri* is interpreted as a shift to more saline conditions ~3320 ^{14}C y BP, after which time conditions become more dilute until ca. 2840 ^{14}C y BP. The upper core indicates drier conditions especially ~1000y BP, with increasing evaporative concentration and a shift to a Cl^- dominated system. Data from Zirahuén (Davies *et al.*, 2004; 2005) show an abrupt increase in the abundance of the planktonic species *Fragilaria crotonensis* and *Cyclotella ocellata*, in the last 20 years, which in correspondence with the existing limnological data (Bernal-Brooks & MacCrimmon 2000b) was interpreted as a shift to higher levels of nutrient availability.

Historical records have been used to document changes in lake level and vegetation changes over the recent past to supplement the sedimentary record when the sediment record become complex (c.f. Davies *et al.,* 2005; Endfield & O'Hara 1999; Metcalfe, 1987; O'Hara, 1993, O'Hara & Metcalfe, 1995; 1997). As with changes in lake level, historical documents from the colonial period can also be used to interpret changes in vegetation. They are however often subject to a degree of bias and in addition are temporally restricted. As a result they do not account for character of changes in the Pre-Hispanic period, only comment on what the environment was like upon the arrival of the Spanish, and as such do not describe Central México's natural vegetation, but a landscape that has been profoundly altered by anthropogenic activity.

As noted above, in a number of lakes palaeolimnological studies have encountered problems in interpreting the sedimentary record in climatic terms alone, due to the prolonged human activity in the area. It is evident from the interpretation of the sediment record in central México that anthropogenic activity has played a role in determining environmental changes. Shifts to increases in the abundances of the eutrophic indicator species *Stephanodiscus*, in Lago de Pátzcuaro and Laguna Zacapu, in association with changes in other sediment parameters such as magnetic susceptibility and phosphorus content and human modification of the water balance, point to a signal driven by human activity. Consequently such changes confound the sole climatic interpretation of the record, in the late Holocene. Echoing Bradbury (1971) Markgraf (1989) highlights this point stating that *'after 6 Ka palaeoenvironmental changes in Central America can no longer be ascribed to climate change alone'*. Human impact on the environment, as recorded by lake sediments is discussed in more detail below in section 2.5.2.

2.5.2. Palaeoenvironmental Evidence of Human Activity

Palaeolimnological evidence indicates that the pre-Hispanic landscape had been affected by dramatic and persistent anthropogenic impact, which provides evidence against the "Pristine Myth". There is, however, still debate over the extent and nature of the impact of Colonial agriculture. The following discusses the palaeoenvironmental evidence for changes anthropogenic activity over the recent past.

Brown (1984; 1985) notes that the pollen record has been strongly influenced by human activity and that change in vegetation cannot necessarily be ascribed to climatic change. This observation is not one which has been limited to palynology. It has been note by several studies, using diatoms and other proxy data, that erosion, water extraction and eutrophication have all played a role in obscuring the climate signal (Davies 2000; Metcalfe & O'Hara 1992; Metcalfe *et al.,* 1989, 1991, 1994). Studies show two phases when influx of detrital material was high in La Piscina de Yuríria, Zacapu and Pátzcuaro during the Prehispanic period. The first in the Preclassic/classic, around 2,500-1,200 yrs BP in Pátzcuaro (O'Hara *et al* 1993), associated with the late Preclassic, and around 3500-3000 yrs BP in Zacapu and La Piscina de Yuríria (Metcalfe *et al.* 1989). The second phase occurred during the Post-Classic around 1100 yrs BP. In the Pátzcuaro basin this second event was more intense and wide spread than the first and occurred around 850 years BP. This was associated with the arrival of the Purépecha, subsequent deforestation and increased agriculture (O'Hara *et al.,* 1993). Sediment flux data do not point towards accelerated erosion due to the introduction of plough and draft animals by the Spanish after Conquest, and if anything implies a decline in levels of erosion, related to a decline in the indigenous population and subsequent forest regeneration (O'Hara *et al.,* 1993). Similar landscape recovery is also noted at La Piscina de Yuriria (Metcalfe *et al.,* 1994). In contrast Fisher *et al.* (2003), using sedimentological, geo-archaeological and archaeological evidence from the south west of Lago de Pátzcuaro, suggest that it is settlement, rather than agriculture that is responsible for erosion and landscape degradation. They identify two erosive phases (1885-1230 yrs BP and 485-45 yrs BP) which they associate with periods of low population density. A period of landscape stability, thought to be dependent upon terrace maintenance, is identified as occurring between these two erosive phases, cited as a period of high population density, population growth and consequently urbanization. Human impact on lake basins is not restricted to soil erosion and changing vegetation communities, human activity has also been seen to have had a rising impact on water quality. Palaeoenvironmental evidence and monitoring records (Bernal-Brooks, 1988; Chacón-Torres *et al.* 1989; Metcalfe *et al.,* 1989) have indicated increasing and rapid eutrophication of lakes in Central México. In addition to the influx of eroded material, numerous studies on the lakes of Michoacán indicate increasing levels of nutrient flux, which has had a profound impact on the diatom flora.

The Zirahuén basin was expected to be relatively pristine prior to Conquest, as it had only been used by the Purépecha nobility in the Post-Classic, as a result the basin was perceived to be less exploited. Although previously uncultivated land was brought into use by the Spanish, it is likely that cultivation also occurred on already degraded tracts of land (Endfield & O'Hara 1999). Palaeolimnological evidence supports this, showing that in the pre-Hispanic period there was significant catchment disturbance and after Conquest this declined associated with a reduction in the indigenous population (Davies *et al.*, 2004). Archival records suggest that by the mid 18th Century several *Haciendas* had been established in the basin and record a degree of deforestation and the introduction of livestock into the area. This period of renewed catchment disturbance is also recorded in the sediment record through higher rates of sediment accumulation. More recently a shift in the diatom assemblage has indicated a shift to more nutrient rich conditions, with the appearance of *Cyclotella ocellata* and *Fragilaria crotonensis* over the last 15 years, associated with increased settlement and tourism in the basin. This has also been noted in the limnological monitoring data (Bernal-Brooks & MacCrimmon, 2000b; Chacon-Toreds & Rosas-Monge, 1998). This is discussed in more detail in Chapter 8.

2.6 Summary

As has been seen from Chapters 1 and 2, the geological formation of central México has affected climate, soil type and as a result vegetation providing a suitable location for human activity. Evidence has shown that several of the lakes in central México have become eutrophic with palaeolimnological evidence showing that diatoms appear to respond, primarily to changes in nutrient loadings, although this has yet to be quantified. The following chapter provides more detail on key nutrients and nutrient cycling in lakes, in order to provide further background on the processes integral to this study

Chapter 3. Nutrients in Lakes

3.1. Introduction

Nutrients are essential for respiration and production of proteins and, when present in abundance, lead increased plant productivity. They are naturally available in water bodies where they are available from the atmosphere, catchment soils, and vegetation (terrestrial and aquatic). Rates of primary productivity are affected by the availability of resources such as nutrients, and light, with different classes, genera and species having different physiological requirements. Consequently availability of resources, whether from natural or anthropogenic sources, has an effect on phytoplankton community composition and inter-species competition develops. Alteration of nutrient cycles and consequently biological cycles has implications for biodiversity and system function and as a result on social issues. Population growth in México (as elsewhere) has led to increased stress on catchments, competition and demand for freshwater and has consequently exacerbated the need for adequate management. This is reliant upon a sound understanding of nutrient sources, sinks, cycling and how changes manifest themselves, now and in the past. The following chapter provides background on nutrient cycling in lakes, the eutrophication process, and its impacts, the palaeolimnological approach to modelling eutrophication and documents changes in nutrient status in Mexican lakes.

3.2. Role of nutrients in productivity

Primary producers such as phytoplankton require macronutrients such as carbon (C), oxygen (O), hydrogen (H), nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), chlorine (Cl) and in addition they need micronutrients such as iron (Fe), copper (Cu), silica (Si), molybdate (Mo), and cobalt (Co). When these nutrients are available in abundance, they are generally considered to increase productivity. Correspondingly, if one or more of these nutrients is in short supply it can limit productivity. Different classes and genera of phytoplankton are seen to have different nutrient requirements, thus variations in the relative availability of nutrients can bring about shifts in community composition. The development of initial studies by Pearsall (1930; 1932) on water chemistry-phytoplankton interactions established the primary roles of N, P and in the case of diatoms Si, in driving primary productivity, phytoplankton abundance, and community composition (Reynolds 1984). The following discusses forms, sources and cycling of P, N and Si in lakes and competition for these resources.

3.2.1. Phosphorus & Nitrogen

Phosphorus is an essential element in the conversion of carbon dioxide (CO_2) and water (H_2O) to form glucose ($C_6H_{12}O_6$) during photosynthesis. This requires phosphorus as adenosine diphosphate (ADP) and adenosine triphosphate (ATP). Phosphorus is also a constituent of nucleic acids and phospholipids, consequently it is essential to plant growth and development. Natural sources of P come from allochthonous and autochthonous sources, such as terrestrial vegetation, from erosion or from aquatic flora and fauna. In freshwater P is present in organic and inorganic phases with the majority found in the former, either in the biota or adsorbed to particulate organic and inorganic material. The most significant form of inorganic P is orthophosphate (PO_4^{3-}). The most commonly measured forms of P are total phosphorus (TP) and soluble reactive phosphorus (SRP). TP constitutes organic and inorganic P in particulate and dissolved form. Particulate P can either be minerogenic, P in the biota or P adsorbed to dead organic matter. Dissolved P (SRP) can be present either as orthophosphate, polyphosphates (commonly derived from chemical detergents) or as organic colloids and represents the proportion of P that is immediately available to the biota (Reynolds, 1984).

Phytoplankton also require nitrogen, which is available in organic and inorganic, dissolved and particulate forms, for the synthesis of amino acids and proteins. Inorganic N is highly soluble and is present as nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and dissolved molecular N. Together NO_3^-, NO_2^- and NH_4^+ constitute combined total inorganic nitrogen (TIN). Nitrogen is also available for uptake by phytoplankton in organic form, derived from the biota, which represents approximately 50% of the total soluble nitrogen (Wetzel, 2001). As NO_3^- and NO_2^- need to be reduced prior to utilisation, ammonium is used preferentially as it is most easily assimilated by phytoplankton, however NH_4^+ availability in the epilimnion is usually low (Reynolds, 1984).

Figure 3.1 shows the movement and cycling of P within the epilimnion of lakes Outflow, dilution and sedimentation of organic and minerogenic material, are the major factors involved in controlling P concentration within a lake. In well flushed lakes, those with short residence times, outflow and dilution are the main mechanisms for P loss, giving rise to short P residence times. In closed lake basins or those with long hydraulic residence times, sedimentation accounts for the majority of P loss (Marsden, 1989). P residence times are, however, affected by the thermal structure of a lake with slower rates of dilution in stratified lakes and by P release from the sediment, usually when the hypolimnion becomes anoxic. Phosphorus may also be lost via co-precipitation with calcium carbonate ($CaCO_3$), through uptake by zooplankton or bacteria, chemical transformation or isolation in the hypolimnion. Loss to the hypolimnion and to zooplankton however, can be temporary. Nutrients taken up by zooplankton may be re-released through excretion and sedimentation upon death. In this form, P is usually taken up rapidly by phytoplankton (Wetzel, 2001). Forms of P trapped in the hypolimnion may be made biologically available again as they are cycled through the whole water column during overturn. Within the water column, particulate forms of P may become soluble and thus biologically available, depending on particle size and the length of time spent in the water column. P may also become adsorbed or released from organic, colloidal or minerogenic material thus lost or recycled respectively.

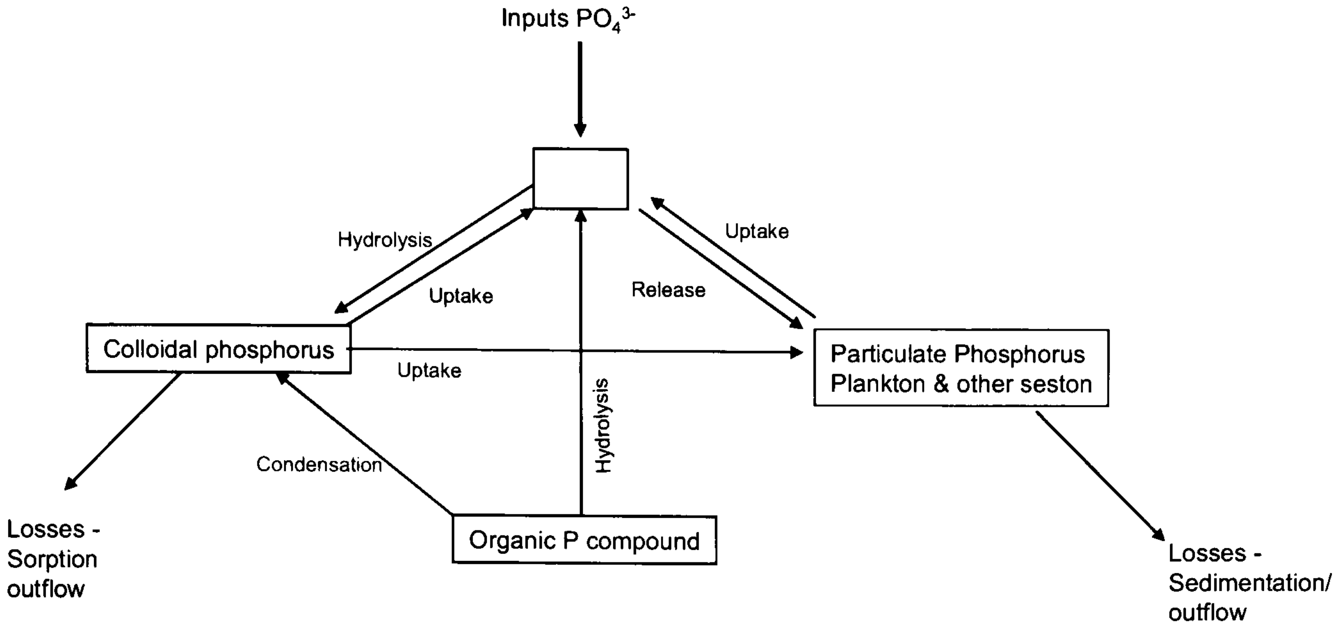


Figure 3.1 Phosphorus cycling in the epilimnion (from Wetzel 2001)

The Nitrogen cycle in lakes is displayed in Figure 3.2. Nitrogen can be derived by nitrogen fixation from the atmosphere, in the water column and in the sediments. The atmospheric reservoir of N is large, but N_2 is not highly soluble in water. It can however, can be derived from the atmosphere through N fixation by heterocystous cyanobacteria. Dissolved organic nitrogen (DON) is released by phytoplankton, fish and zooplankton and is primarily made up of amino acids and is rapidly recycled by bacteria and phytoplankton (Wetzel, 2001). The release and subsequent uptake of DON is only one pathway of N cycling in aquatic systems. Ammonium can be generated by the dissimilation of NO_3 or as a product of organic matter break down. NH_4^+ may be converted in the nitrification process through oxidation to NO_2^- in the presence of the nitrifying bacteria *Nitrosomonas*. It may also become deprotonised at high pH values to form NH_3 gas. Alternatively NH_4^+ may be lost from the system to the sediment when it becomes sorbed to particulate material. The process of nitrification also converts NO_2^- to NO_3^- in the presence of *Nitrobacter*, thus increasing the availability of NO_3^- but *Nitrobacter* cannot tolerate low temperatures or high pH conditions consequently in such conditions NO_2^- can accumulate. NO_3 may be assimilated by bacteria, resulting in the reduction of NO_3^- back to NH_4^+ or in the presence of denitrifying bacteria it may be reduced to NO_2^- and subsequently to N_2 gas. Consequently N may be lost from an aquatic system by sedimentation, outflow or through the evolution of N_2 and NH_3 gases which are released into the atmosphere.

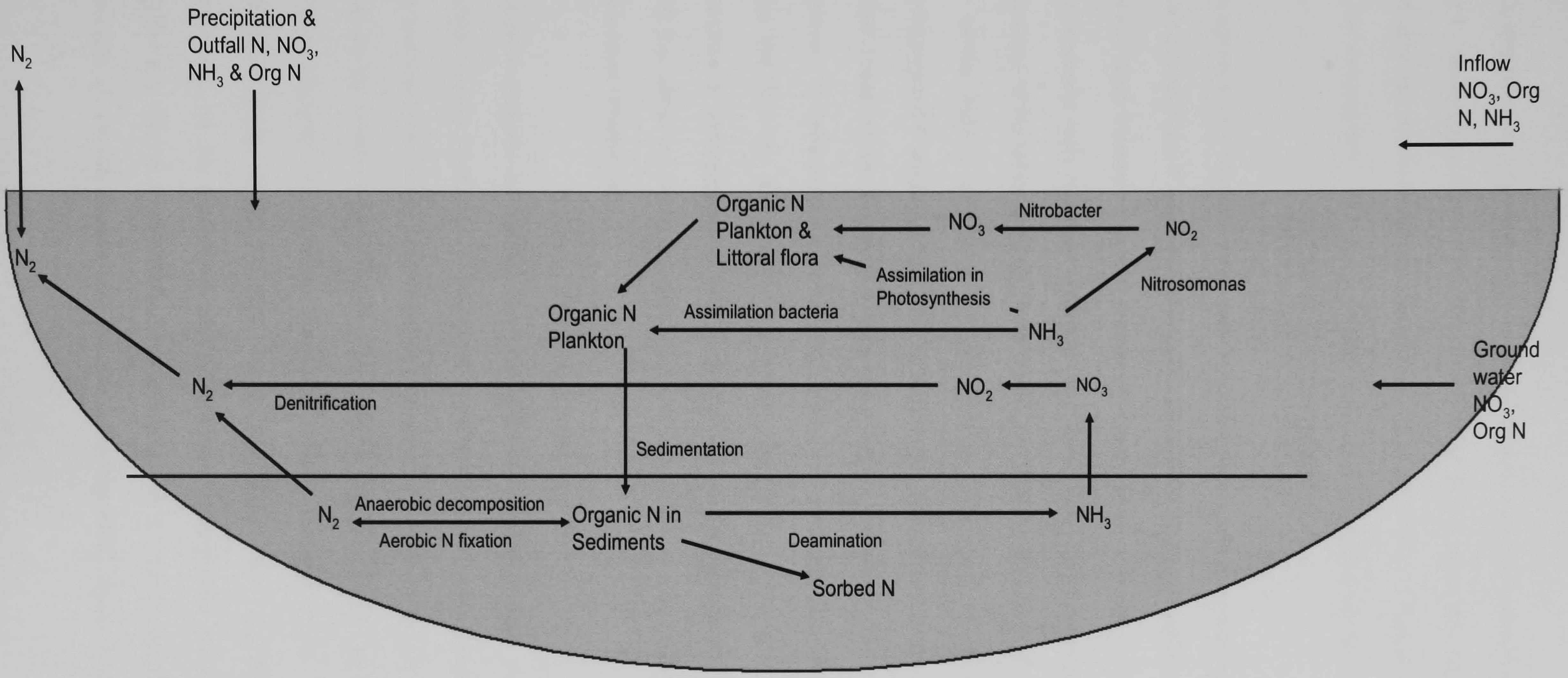


Figure 3.2. The Generalised Nitrogen Cycle for freshwater systems (Modified from Wetzel 2001)

NO_2 and NH_4 are usually present in low concentrations in the range 0-0.1 mg l^{-1} and 0.15 mg l^{-1} respectively. Reynolds (1984) notes that in the anaerobic hypolimnion of small eutrophic lakes concentrations of ammonia can reach 1 mg l^{-1}. Concentrations of NO_3 are higher ranging from 0-10 mg l^{-1} in unpolluted freshwaters (Wetzel, 2001).

Phosphorus and nitrogen both have anthropogenic sources. Unlike nitrogen there is no direct atmospheric P source, but lakes may receive P from atmospheric fallout of particles derived from industry, fire, or particles of soil or biota. The fallout of P rich material is consequently high in urban, industrial and agricultural areas (Wetzel, 2001). With the exception of the fallout from agricultural sources, these are generally unlikely as the lakes of central México are generally distant form urban and industrial centres. Other anthropogenic P sources include domestic sewage, storm water drainage and agricultural runoff, these will be discussed in more detail below. Inorganic forms of N are also highly abundant in sewage and in runoff from agricultural and deforested land and, in places, from wet and dry atmospheric deposition. The relative proportions of each of these sources, i.e. atmospheric vs. run-off, are spatially and temporally variable depending on land use within the basin, meteorological conditions, extent and location of industry, and agriculture (Wetzel, 2001).

Nutrients may also be released from the sediment, depending upon the conditions at the sediment-water interface (i.e. aerobic or anaerobic) and the concentration of P already in the sediment. The ability of the sediment to retain P is reduced as the concentration of P rises so the sediment becomes saturated and its sorptive capacity is reduced and thus a smaller proportion of P within the water column is drawn down into the sediment (Marsden, 1989). Strong reducing, anoxic, conditions may result from high levels of productivity, or be associated with progressive hypolimnetic de-oxygenation of the hypolimnion during stratification. The generation of a reducing environment leads to reduction of Fe (III) oxides and Mn (IV) releasing PO_4^{3-} which is adsorbed to these minerals (Marsden, 1989; Wetzel, 2001). Phosphorus, which has become soluble can then migrate upwards through the sediment and may subsequently be released into the overlying water in anaerobic conditions, if dissolved oxygen concentration is less than 2 mg l^{-1} (Marsden, 1989). If the upper layer of sediment remains aerobic, where the interstitial water has an oxygen concentration of >2 g m^{-3}, it provides a barrier to the diffusion of P across the sediment-water interface (Marsden, 1989) through the oxidation of Fe, to which P is then re-adsorbed. If the interstitial water becomes fully deoxygenated, then Fe becomes reduced and P can rapidly diffuse into the hypolimnion. This process usually occurs in deep lakes where stratification occurs allowing progressive deoxygenation of the hypolimnion. Due to a thick oxidized layer, P is generally retained within the sediment in aerobic conditions, in well mixed or shallow lakes. Massive levels of P release from the sediment have been associated with periods of anoxia due to stratification and subsequent redistribution through the water column during overturn in Shagawa Lake, Minnesota (Larsen *et al.*, 1979) and in Scottish lakes (Gibson *et al.*, 1996). High external P loading can lead to a reduction in the sediments' capacity to adsorb any additional P as the sediment becomes saturated (Anderson, 1995; Marsden, 1989). Consequently phosphorus remains in the water column where it may become biologically available. NH_3 may be re released from the sediments into the water column through ammonia volatilization when ammonium concentrations and the pH of the hypolimnion are high. In addition hypolimnetic anoxia, loss of the oxidised microzone, reduces the sediments capacity to adsorb and retain NH_4^+ resulting in release from the sediment. However, if light can penetrate to the sediments and support a benthic community they can assimilate NH_4-N from the interstitial waters and prevent its release from the sediment.

There are also mechanisms for P release from aerobic sediments. Re-suspension of the sediment due to wind induced turbulence has been seen to bring nutrients back into the water column in shallow lakes (de Anda *et al.*, 2001; Kristensen *et al.*, 1992; Reynolds, 1992), but only when wind speed exceeds a critical velocity (Carper & Bachman, 1984) and may therefore be an important procees in lakes such as Chapala, Atotonilco and Zapotlan. In shallow tropical lakes temperature has also been seen to have an effect on layer stability and consequently on turbulence (Lewis, 2000) and therefore recycling of nutrients back into the epilimnion. P exchange across the sediment water interface can also be affected by NO_3^- concentration and pH. Warmer temperatures and higher NO_3^- availability are thought to stimulate biological activity which can lower the redox potential of the surface sediment allowing the reduction of Fe and release into solution of P (Jensen & Andersen 1992). Desorption of P from its Fe complex, due to substitution for OH-, has been found to occur above pH 8 (Marsden, 1989). Through mass balance experiments on cores from four shallow, eutrophic Danish lakes Jensen and Anderson (1992) found that the release of SRP was primarily controlled by changes in temperature. Higher temperatures reduce the depth of the oxidized layer and in winter high NO_3^- concentrations led to a suppression of P release by increasing the sorptive capacity of the sediment. Addition of NO_3^-, when concentrations of inorganic N were low in the late summer, led to P release though increase mineralization. In one lake increased pH, associated with a phytoplankton bloom, led to de-sorption of P and thus increased SRP release into the water column. Jensen & Anderson (1992) also note that other factors, such as mineralization of easily degradable organic matter, play a role in rates of P release as mineralization occurs more rapidly under aerobic conditions (Marsden, 1989). Increased P release, from hypertrophic sediments at higher temperatures has also been noted by Søndergaard (1989) from Lake Søbygaard, Denmark.

3.2.2. Silica

In freshwaters, silica is used by chrystophytes and diatoms to strengthen their cell walls, it therefore constitutes a macro- rather than a micronutrient. The amount of silica required by each of diatom is, however variable, due to differences in size and frustule ornamentation, with and between species (Bailey-Watts, 1976a). Si is derived from catchment soils or the biota itself (Reynolds, 1984). Consequently, its availability is geographically variable, with the highest concentration being noted in basins of volcanic origin, such as central Mexico. There are two main forms of SiO2, dissolved silicic acid and particulate silica, the latter is derived either from the biota or sorbed to minerogenic material (Wetzel, 2001). High seasonality is noted in Si availability, which is associated with uptake and utilisation by diatoms. Seasonal declines in the diatom population may also return dissolved Si to the water column. In Loch Leven, Scotland, dissolution was found not to be important, due to the shallow depth of the lake (Bailey-Watts, 1976b), this may also be applicable to the lakes of Central México. The decline in the diatom population may also lead to loss of silica to the sediments through sinking (Bailey-Watts, 1976b). Variability in Si concentration has been seen to have an impact on not only the diatom community composition, but on phytoplankton community composition as a whole (Kilham et al., 1986; Tilman et al., 1986; van Donk & Kilham, 1990). Silica may be lost through sedimentation and through uptake by the biota. Increased ability of diatoms to remove silica from the water column has been seen to be related to abundance of P, i.e. in more eutrophic conditions (Bailey-Watts, 1976a).

3.2.3. Competition for nutrients and resources

A resource has been defined as any substance, or factor, which can lead to increased growth rates as its availability is increased and is consumed by organisms (Tilman, 1982). Algal growth rates are determined by access to light and nutrient resources, demands for these resources results in interspecies competition. Tilman (1982) defines competition as depression of a competitors reproductive rate because of shared consumption and thus depletion of shared resources. That is, two or more species may depend upon the same resource for maximal growth, but the species with the greatest ability to assimilate the resource will dominate. Assuming steady state conditions, the dominant algal group can be predicted on the basis of known resource requirements for the genera and the supply of the resource. The ability to predict the outcome of resource competition is complicated as competitive ability for one resource is often associated with reduced competitive ability for another (Tilman et al., 1986). Prediction also becomes more complex when a species competes with another for more than one resource. Numerous bioassay tests have been carried out to assess the dominance of certain diatom species (Interlandi et al., 1999; Kilham et al., 1986; van Donk & Kilham, 1990) and of chlorophytes, cyanophytes and diatoms (Tilman et al., 1986) at varying resource ratios: N:P, Si:P and light:P. In situ enrichment experiments (e.g. Henry et al., 1985) have also been carried out, which help to determine which resources drive changes in productivity.

The strong correlation between algal growth and P concentration has lead to the assumption that the majority of lakes are P limited, shown through the process of eutrophication, experimental whole lake enrichments and bioassay studies. Such an assertion is supported by evidence that shows a decrease in P loading results in a corresponding decline in algal growth (Schelske & Stoermer, 1972). A correlation between N and algal growth has also been noted, particularly in tropical localities (Lewis, 2000; Talling & Lemoalle, 1998). In tropical and temperate eutrophic lakes, nitrogen is often considered the main nutrient limiting phytoplankton growth (James et al., 2003, Reynolds, 1984), based on its low abundance relative to P, which is usually more readily available. In the tropics where temperatures are higher than at temperature latitudes chemical weathering takes place faster, and consequently the natural supply of P, especially in volcanic areas, is greater (Lewis, 2000). Talling & Lemoalle (1998) do however present evidence of P limitation in lakes in East Africa, although in lakes which are at high elevations, and are therefore cooler. It has also been suggested that the potential for N loss to denitrification is greater in tropical lakes due to higher temperatures in the sediments (Lewis, 2000). Increases in P loading result in shifts to lower N:P and Si:P ratios and corresponding shifts in the dominant algae, species which are good competitors for the limited resource, i.e. N or Si. Diatoms, based on their Si requirements, are dominant at high Si:P ratios, and have been found in bioassay studies to be dominant at high N:P ratios (Tilman et al., 1986). In contrast, cyanophytes are abundant at low N:P ratios, based on their ability to fix atmospheric nitrogen.

In variable resource ratio and temperature experiments, Tilman *et al.* (1982) found the ability to utilise resources in cyanophytes and diatoms to be temperature dependent. In a bioassay study of phytoplankton from Lake Superior they found that cyanophytes had maximum competitive ability at high temperatures (above 20°C), while diatoms were more competitive at temperatures lower than 15°C. Such nutrient temperature interactions have also been noted between diatom species (van Donk & Kilham, 1990). At high temperatures they found *Asterionella formosa* was dominant at high Si:P, while *Stephanodiscus hantzschii* dominated at low Si:P. At lower temperatures *Fragilaria crotonensis* was dominant at all but the lowest Si:P ratios, where *A. formosa* dominated. In general their work found that the minimum cell requirement increased with decreasing temperature, however *S. hantzschii* showed an increased P requirement with increasing temperature. Tilman *et al.* (1986) and Interlandi *et al.* (1999) both show the competitive ability of *Synedra* species for P, with species dominating at high Si:P and high N:P ratios. The latter, however, show no correlation between the abundance of *Synedra* and Si:P, associated with the high ambient Si concentration in the Greater Yellowstone ecosystem. Similar results are found in tropical lakes (Kilham *et al.*, 1986). In their study of lakes in the east African Rift Valley, Kilham *et al.* (1986) also found intra-specific competition between *Synedra* species, *S. ulna* was found to have higher P requirements than *Synedra acus*. Similarly *Aulacoseira granulata* was found to grow best in Si rich conditions and had higher P requirements than *Aulacoseira ambigua*, which was also intolerant of low light conditions. Thus, in these lakes, *A. ambigua* was found to be widely distributed in oligo-mesotrophic lakes and *A. granulata* in eutrophic lakes. *Stephanodiscus species* were also found to have high P but low Si requirements and were found to grow well in poor light environments. Consequently the dominance of certain *Stephanodiscus* species in eutrophic conditions is often associated with its ability to persist in low light and low Si conditions, but only at high P concentrations.

Prediction of species succession in response to changes in nutrient/resource availability can be complicated by several factors: grazing, sinking and alleopathy (Tilman et al., 1986) and by factors that alter substrate utilisation, such as temperature or salinity (van Donk & Kilham, 1990; Saros & Fritz, 2000; 2002). Nutrients and temperature are seen to interact, both in observed seasonal succession in lakes and in bioassay studies. Moss et al. (2003) showed that increases in temperature alone were insufficient to cause major shifts in community composition, but were associated with an interaction between nutrients and/or grazing and temperature. This is supported by the work of Tilman et al. (1986) and van Donk & Kilham (1990) discussed above. Saros & Fritz (2000; 2002) found that variations in salinity and anion balance affect diatom species nutrient requirements, uptake ability and competitive ability. Under high salinity conditions Chaetocerous and Cyclotella were found to have superior competitive ability for N, while Anomoeoneis species were always found to have poor competitive ability for N.

Such studies have implications for interpreting shifts in phytoplankton community composition and competition in Mexican lakes where Si is not limiting, but conductivity can be high and temperatures warm. In contrast to temperate lakes, tropical lakes, including many Mexican lakes, are often considered nitrogen limited (Hernández-Avilés et al., 2001; Lind et al., 1992). There is also evidence, however for N/P co-limitation, at least seasonally, in some lakes (Bernal-Brooks & MacCrimmon, 2000; Bernal-Brooks et al., 2002; Hernández-Avilés et al., 2001). Limitation of algal growth by nutrients and resources other than P has implications for management, in such cases a reduction in external P loading may not have the required effect on algal abundance. In situ experiments in Lake Jacaretinga in Amazonia, Brazil (Henry et al. 1985), showed no statistically significant increase in productivity when P or P with NO^{3-} were added. The addition of NO^{3-} alone did significantly stimulate growth, and after several days was found to be almost fully assimilated, with a concomitant decline in productivity.

3.3. Eutrophication

The Organisation for Economic Co-operation and Development (OECD) defines eutrophication as 'the nutrient enrichment of waters which results in the stimulation of an array of symptomatic changes among which are increased production of algae and macrophytes and deterioration of water quality...found to be undesirable and interfere with water uses' (OECD, 1982).

Lentic systems, water bodies where there is no continuous flow, may be classified as oligotrophic (nutrient poor); eutrophic (nutrient rich) or an intermediary, termed mesotrophic. The term eutrophication is usually applied to describe a succession from an oligotrophic to a eutrophic system and concomitant increased primary productivity. Where this process occurs as a natural succession, it is termed ontogeny (Moss, 1998; Wetzel, 2001), where is it usually associated with increase organic carbon, rather than N or P (Whiteside, 1983). Where human activity has accelerated the process the term cultural eutrophication is used. These terms to describe the nutrient status of lakes were introduced from the study of nutrients in peatland soils by Weber in 1907, and were subsequently applied to the study of freshwaters by Naumann in 1919. They were later redefined by Thienemann based on hypolimnetic anoxia and benthic fauna (O'Sullivan, 1995). There are also extremes at either end of the scale: ultra oligotrophic or hypertrophic. These trophic states were later quantified, in the late 1970s, by the OECD, using TP, Chl-a and Secchi depth, for the purposes of management. From the OECD definition, it is evident that the problems eutrophication instigates can be numerous. They will be discussed in more detail below.

3.3.1. Eutrophication and the OECD

The elevation of nutrient concentration in lakes has been recognised as being accelerated by pollution associated with anthropogenic activity, and as a process that had detrimental side effects (Hasler, 1947). Consequently, it is important to define causes, effects and possible solutions in order to establish adequate management proposals. Vollenweider's work (1968) commissioned by the OECD, aimed to synthesise existing data on eutrophication and conduct further study into the phytoplankton nutrient requirements, relationships between nutrients and productivity and establish critical loads for N and P, and nutrient sources. The report also highlighted the role of competition for nutrients between phytoplankton and the role of other limiting factors such as light (discussed above) on system response to nutrient loading. Another key finding of Vollenweider's work was that limnological monitoring, up to that time (1968) was insufficient for making broad generalisations and for producing guidelines for management due to its lack of spatial and temporal resolution (OECD 1982). The results of the study resulted in the establishment of the Steering Group on Eutrophication in 1971 whose goal was to develop and co-ordinate the evaluation of eutrophication control. Their main objectives were to gain a better, quantitative, understanding of the relationship between nutrient loading and system response, to determine whether N or P was the primary factor driving eutrophication and to elucidate the role played by other factors, such as basin morphometry, on trophic response. The OECD cooperative programme on eutrophication undertook more detailed monitoring studies, primarily conducted on lakes in the temperate regions of Northwest Europe and North America. Results were considered to yield adequate enough results to establish general statistical behaviour. T he study included little of no monitoring data from arctic, high alpine, saline, closed and sub tropical lake basins. Quantitative relationships, derived from regression equations, were established between trophic indicators and the trophic status of lakes, biomass and P or N concentration, and trophic indicators and loading. Such data offered a methodology for water quality assessment and allowed stringent limits to be place around each trophic state, which was necessary to provide environmental managers with a sound basis for assessment.

Two systems were developed to assess water quality; the open boundary and the closed (fixed) boundary systems, which provided limits for trophic state based on minimum and mean TP and chlorophyll-a (Chl-a) concentration and Secchi depth. The fixed boundary system (see Table 3.1) was more arbitrary but more easily applicable, with clearly defined limits for each variable. It is this system which has been used to classify sites in this study. Consequently the assignment of a site to a certain trophic state needs to be based on all variables, not just one or two. The open boundary system has a greater degree of flexibility, as it can account for outlying sites as the value for each variable is based on the group mean and standard deviation. Research therefore allowed, to an extent, prediction of trophic state or nutrient concentration based on levels of primary productivity or water residence times. As their study was, however, limited to temperate regions, its applicability globally is somewhat limited.

Table 3.1 OECD limit values for fixed boundary trophic classification system, with TP and Chl-a in μg l^{-1} and secchi depth in metres (OECD, 1982)

Trophic category	TP	Mean Chl-a	Max Chl-a	Mean Secchi Depth (m)	Min. Secchi Depth (m)
Ultra-oligotrophic	<4.0	< 1.0	< 2.5	> 12.0	> 6.0
Oligotrophic	<10.0	< 2.5	< 8.0	> 6.0	> 3.0
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypertrophic	> 100	> 25	> 75	< 1.5	< 0.7

3.3.2 Phosphorus Productivity Relationships

As noted above, in general, increases in TP lead to increases in productivity and consequently photosynthesis resulting in the formation of Chlorophyll-a. Increased TP therefore leads to increased Chl-a, which results in a decrease in transparency, measured by secchi depth. Consequently, as noted above, these three variables have been used by the OECD to assigned trophic status to a lake. The OECD results showed a positive log linear relationship between TP and Chl-a and a negative correlation between increasing Chl-a/TP and declining secchi depth. A strong positive relationship has been found between TP and Chl-a in lakes in Southern Ontario, Canada, (r2 = 0.93) (Dillion & Rigler, 1974) and also in Japanese lakes (r2 = 0.975) (Sakamoto, 1966). The results of the former were based on lakes which were olio- and mesotrophic (primarily the former) and the authors note that the relationship would have been improved if the range was extended. The relationship between TP and algal productivity and consequently secchi depth is, however, complex. Despite this, the strength of the relationship between Chl-a and TP has been used to justify a reduction in P-loading as a solution to eutrophication (Smith & Shapiro, 1981). This model was applied to a national study of lakes in the USA (Lorenzen, 1979), where results showed a reduction in TP would only result in a decline in productivity in a small number of lakes. Smith & Shapiro (1981) do, however, note that this prediction is a factor of the model used, rather than the actual relationship between TP and productivity. They highlight that factors such as seasonality, bloom time, variability in zooplankton type and abundance, temperature and light availability have a strong control over algal productivity, and this must be accounted for when sampling. Other factors such as nitrogen limitation and turbidity affect the relationship between TP and Chl-a and, therefore, the ability to predict levels of productivity. The role played by turbidity in affecting the relationship between TP and algal growth (and consequently secchi depth) is particularly apparent in some Mexican lakes, which will be discussed in more detail in Chapter 6.

Based on a USEPA data set of 493 lakes and 548 lakes from published data McCauley et al. (1989) found a sigmoid (non-linear) relationship between TP and Chl-a. This was associated with the effect of N on the TP/Chl-a relationship. The effect of N on algal productivity, when TP concentrations are high, is great as it becomes the limiting nutrient.

Algae will therefore respond rapidly to increased N, rather than P, which is already present in abundance. They therefore concluded that in hypertrophic systems, a reduction in TP would only have a negligible effect on productivity. Prairie et al. (1989) also highlight the role of N in algal productivity and stress the implications of applying a TP/Chl-a model to lakes which are N-limited.

3.3.3. Natural vs. anthropogenic eutrophication

Some lakes have been referred to as naturally eutrophic, i.e. prior to human impact P concentrations and levels of productivity were high. Moss et al. (1994), classify the north-west Midland Meres of the UK, as naturally eutrophic, based upon 19th century records of blooms of cyanobacteria and because the lakes have a high P concentration, but no obvious source. Through palaeolimnological investigation Lake Hollingsworth, Florida, USA has also been described as naturally eutrophic (Brenner et al., 1995). Although reconstruction only dates back to ~1843, this was prior to European settlement in the area, and is therefore associated with a time of little human activity, but diatom inferred phosphorus levels indicate eutrophic concentrations. Such a high TP loading, in both Florida and the NW Midland Meres, was associated with base rich underlying geology.

The idea of naturally eutrophic lakes in populated lowland areas has been contested by Anderson (1995), who acknowledges that while naturally nutrient rich lakes do exist, they are limited to lakes in relatively pristine catchments on P rich bedrock (c.f. Murphy et al., 1983). Such conditions apply to neither the NW Midland Meres nor Florida. The 19th century reference to the presence of blue-green algae on the NW Midland Meres was most likely related to early farming and associated activities, such as the use of animal waste as manure. Such long term human impact is cited as resulting in a saturation of the soil and sediments ability to retain P. The palaeolimnological record from Lake Hollingsworth indicates a shift from oligo- mesotrophic species to eutrophic diatom species, despite the nutrient rich catchment. It is Anderson's (1995) conclusion that lowland lakes cannot be naturally eutrophic, but that they have merely been eutrophic for a long time. It is only with a coupling between limnology and palaeolimnology that this can be properly ascertained.

3.3.4. Causes, consequences, solutions

3.3.4.1. Causes

The factors involved in eutrophication have been recognised since the early work of Naumann (1919) and Thienemann (1918). These studies highlighted the link between the catchment and the lake itself, and in addition trophic state could be constrained by nutrient supply, basin morphology, hydrodynamics and factors such as climate and human activity (Bernhardt, 1992). External causes can roughly be divided into those that are 'point' source, or 'diffuse' source. Point sources include industrial, domestic and municipal effluent, in contrast diffuse sources are associated with agriculture and atmospheric loading and are, therefore, more difficult to isolate and thus manage. The vast majority of increased P flux to lakes has been within the last 60 years and is primarily associated with lack of sewage treatment (or treatments which do not remove P from the effluent) and the use of phosphate based detergents. Agricultural runoff, from animal husbandry and fertilisers also contribute to eutrophication. Phosphates in fertilisers are, however, often insoluble and thus do not become biologically available before they are lost to the sediment (Reynolds, 1992). Soil erosion makes a major contribution to TP, associated with weathering of P rich minerals such as apatite (Reynolds, 1992), a proportion of which will become biologically available. This proportion is dependent on grain size, as smaller grains remain in suspension longer and thus are more likely to become soluble. Data collected in the United States indicated that the highest proportion of P loading was derived from urban sources, 1.0 g $m^{-2} yr^{-1}$. In contrast loading derived from agriculture amounted to 0.5g m^{-2} yr^{-1} (Lee *et al.*, 1978 cited in Mason, 1996). Studies, both palaeolimnological and limnological, have highlighted more specific point sources of eutrophication and their impacts. For example effluent from creameries in Northern Ireland (Anderson, 1989), high population density and associated sewage at Lake Victoria, East Africa (Hecky, 1993) and a combination of lawn fertilisation, storm-water runoff and phosphate mining around Lake Hollingsworth, Florida (Brenner, 1995), have been caused shifts from eutrophic to hypertrophic conditions. Fluctuations in lake level have also been found to cause shifts in nutrient concentration in several lakes. Loss of depth in Lago de Cuitzeo in Mexico has been associated with increased nutrient concentrations (Alverado Diaz *et al.*, 1985). Rippey *et al.*, (1997) found reduced flushing rates (i.e. increased residence time), linked to dry climatic conditions, caused a rise in (diatom inferred) TP concentration in White Lough, Northern Ireland through increased P release from surface sediment. Reduced depth in shallow lakes has allowed increased mixing and sediment re-suspension (Alverado Diaz *et al.*, 1985) and subsequent P release (see section 3.2.1). Increased P residence time in the water increase the likelihood of P becoming biologically available (Rippey *et al.*, 1997). Increased lake level, during high levels of precipitation has also been seen to cause an increase in TP concentration in Lago de Chapala (de Anda *et al.,* 2001), where inundation of nutrient rich agricultural land led to increased fluxes of P and N in run-off to the lake. As discussed in section 3.2.1, P can be released from the sediments in anaerobic conditions, and occasionally in aerobic conditions.

Although, in the majority of cases, eutrophication is associated with external P inputs, internal P loading has also played a role in either maintaining P concentration after remediation, or in exacerbating external P loading. Lake sediments contain a higher concentration of P than the overlying water, present in numerous forms, some of which are more soluble than others. Peak concentrations occur in the upper centimetres (Wetzel, 2001) and, as discussed in section 3.2.1, this P can be released from the sediments under anaerobic conditions, and occasionally in aerobic conditions.

3.3.4.2. Consequences

The effects of eutrophication on a water body are numerous and often linked. The most well known impacts of nutrient enrichment are prolific algal growth, resulting in high Chl-a concentrations and consequently a decline in water clarity. Associated with increased photosynthetic activity are increased pH due to increase CO_2 production through respiration and as phytoplankton and macrophytes die, and are decomposed, a decline in O_2. Such conditions have a profound impact upon the biological community, in terms of community composition and population size. Changes in the dominant species in a class, or in the dominance of a particular class, are common. Such changes are based upon species' competitive abilities for resources such as light, P, N and SiO_2, which have been discussed in detail above. Eutrophic lakes are characterised by low species diversity, but high population densities usually restricted to the epilimnion (Ryding and Rast, 1989). Green algae are usually found in abundance in water of low nutrient content, while cyanophytes dominate in eutrophic waters. Diatom species shifts are noted between trophic states. Generally species such as *Cyclotella stelligera* has been found in nutrient poor to slightly enriched waters (Gasse, 1986; Zeeb *et al.*, 1994). Genera with a better competitive ability for P such as *Fragilaria* and *Stephanodiscus* are found in eutrophic conditions. Changes in zooplankton are also noted. Although, in general, they increase in parallel with the increase in phytoplankton, certain species of zooplankton are not capable of assimilating cyanobacteria. They are, therefore, low in abundance or absent from the community in systems where cyanobacteria dominate in highly eutrophic conditions. This can affect phytoplankton abundance though changes in predation.

Shallow lakes have been found to occupy two steady states, over a range of nutrient concentrations, either dominated by aquatic vegetation (in clean water conditions) or by high algal productivity in nutrient rich, turbid conditions (Irvine *et al.*, 1989). In clear water conditions macrophytes have the ability to out-compete phytoplankton for nutrients and in addition can provide a refuge for zooplankton, bringing about a decline in phytoplankton through grazing and shading. Eutrophication is associated with the loss of rooted macrophytes and an increase in abundance of floating, non-rooted macrophytes, such as water hyacinth and a shift towards phytoplankton dominance, due to the increased turbidity of the water. A number of the eutrophic/hypertrophic lakes are dominated by water hyacinth while those of lower nutrient concentrations by rooted macrophytes. Not enough data exist yet to determine there has been a shift form one state to the other associated with increased nutrient availability. Changes in O_2 availability and pH are brought about by the death and growth of aquatic plants, such factors in conjunction with changes in light availability and plankton species composition bring about a change in fish species composition.

Factors such as these have implications for human use of water sources. Those that are most pressing are usually, drinking water quality and aesthetic value. Although in México drinking water is not sourced from lakes. Increased phytoplankton numbers make filtration difficult, consequently the process becomes inefficient, in terms of time and cost. Small celled phytoplankton may pass through filters and remain in the water, where they may be degraded through the action of fungi and bacteria. Consequently water can develop an undesirable taste, odour and colour (Mason, 1996). In addition, the abundance of cyanobacteria can release toxins, *Microcystis*, *Anabaena* and *Aphanizomenon* have been found to release potent toxins which have been associated with liver damage and paralysis in animals. In Rutland Water, East Midlands, UK blooms of *Mycrocystis* were associated with sheep death (Mason, 1996), while in Lake Alexandrina, Adelaide, Australia blooms of *Nodularia spumigena* were also associated with livestock death (Lawton & Codd, 1994). Such loss of grazing animals may have consequences for long term economic stability, particularly in developing countries. Toxins released by cyanobacteria have yet to be found to have a long-term detrimental impact on human health, but have been noted to cause minor illness. Increased nitrates are also associated with eutrophication, and have been linked to 'blue baby' syndrome, which resulted in the implementation of legislation to control nitrate levels in agricultural runoff in the UK and the EU under the Nitrate Directive. In addition, nitrate in water has been found to have a carcinogenic effect on the stomach and digestive tract in livestock (Miravish, 1985 in Soares, 2000). Diatoms have also been found to produce unpleasant tasting water, although they can be filtered out more easily than cyanobacteria in the treatment process (Moss, 1998). Due to the revenue derived from tourism in many parts of the world associated with lakes, such as Zirahuén, eutrophication is a major problem. Algal and macrophyte blooms present drinking water, aesthetic, navigation and fish stock problems.

3.3.4.3. Solutions

Ideally, restoration of a water body would involve its return to natural conditions (OECD, 1982), allowing for natural soil fertility, geology and climate, which can be established through palaeolimnological studies. Total restoration is however is not always possible. Restoration schemes are, therefore, often focused on achievement of a desired status which will make the water body fit for purpose, rather than returning it to its pre-disturbance state. Restoration requires an assessment of surrounding land use, catchment characteristics, proximity of urban areas and atmospheric conditions and that baseline conditions are established.

Effective restoration of lake ecosystems to pre-disturbance, or desired conditions, requires prior knowledge of natural nutrient status. In many cases long term monitoring data do not extend back far enough to provide that knowledge. In addition, monitoring records often do not provide full insight into the natural variability of the system in question. Consequently, palaeolimnological records have been seen to provide an alternative (Anderson, 1998; Bennion *et al.*, 1996). Such records not only offer an indication of baseline condition, but can also indicate rate and trajectory of system change. Such information can then be used to infer causes of change and define accurate restoration targets.

In general, it is considered that a decrease in external P supply will result in a corresponding, proportional, decline in lake P concentration (Marsden, 1989) and consequently algae, although the role played by internal cycling must also be considered. Management of eutrophication can be divided into two main classes: treatment of the causes or treatment of the symptoms. The former provides a long-term solution, but often at greater cost, while the latter can provide more immediate results, which can be particularly useful if the aim is restoration of the aesthetic value of a lake. Treatment of the causes of eutrophication can tackle both diffuse and point sources, although as the latter are more readily identifiable, they are often the main target of remediation. Point sources of eutrophication can be reduced through elimination of P by chemical precipitation using Al or Fe salts, either in inflowing tributaries or in treatment plants. The installation of tertiary sewage treatment plants in Lake Mälaren, Sweden was seen to bring about a 90% decline in P supply from sewage effluent (Bradshaw & Anderson, 2001). Waste water can be diverted away from the water body in question either to a treatment plant or further down stream away form sensitive areas, thus transferring the problem elsewhere. Diversion of effluent from a creamery on Lough Augher, Northern Ireland lead to a decline in lake TP, inferred from diatoms, from 140 μgl^{-1} to ~80 μgl^{-1} (Anderson *et al.* 1990). Diffuse sources of pollution can be tackled through control of basin land use, or urban and agricultural runoff. These include control of domestic fertiliser use, prevention of storm water drainage and sewage overflow during flooding, and minimisation of soil erosion through alteration of tillage and crop routines. Controlled use of agricultural fertilisers and the use of buffer zones to reduce runoff and trap sediment have also been used to control influx of nutrients to lake basins.

Direct treatment of the symptoms of eutrophication provides an alternative when the treatment of the causes may be socially or economically un-viable (Ryding and Rast, 1989). There are numerous within lake treatments which can alter biological, chemical or physical characteristics of the lake. Biological include removal of algal scums, or through biomanipulation which is a top-down approach through the selective introduction, encouragement or removal of species to control the phytoplankton population (Moss, 1992). Commonly biomanipulation involves increasing the population of herbivorous zooplankton by reducing the population of planktivorous fish, thereby increasing the grazing pressure on the phytoplankton. Such manipulation of the food web was seen to be successful in Round Lake, Minnesota where addition of rotenone led to a reduction in the population of planktivorous fish (Shapiro & Wright, 1984). The result of this was a decline in chlorophyll-a, an increase in the population of the zooplankton *Daphnia* and an increase in littoral macrophyte cover. Physical methods for either removing or isolating P include aeration of the hypolimnion through mixing or its removal altogether, or covering or removal of P rich surface sediments. This prevents P release from the sediment. Flow regimes and flushing rate can be altered to prevent P accumulation, aid loss by outflow or dilute the water body. Lake response to remediation is varied and depends upon numerous factors, and consequently is to a certain extent, site specific. Factors such as duration of enrichment, catchment land use and political control over the basin can affect effective implementation of remediation measures and lake response to them.

3.4. Diatoms & Nutrients

As photosynthetic organisms, diatoms are reliant upon phosphorus and light availability, but, due to their structural requirements, they are also reliant upon silica availability (SiO_2). As described above different species have different requirements and different competitive abilities for these resources, (see section 3.2.3). When Si is not limiting, as in most of the lakes in Central Mexico, it is P availability that has been shown to drive diatom community composition and size (c.f. Battarbee *et al.*, 2001). As has already been established, P is an essential element in causing eutrophication and tracking changes in P is essential to effective management and restoration. The instability of P in sediments means that to track past changes in P loading using the sediment record, an alternative has to be found to simply measuring sedimentary P concentration. This instability of P in the sediments, the role of P in driving diatom productivity and species variation led to the use of diatoms as indicators of changes in nutrient status. The use of diatoms for inferring trophic status and problems associated with this are discussed below.

3.4.1. Diatoms as indicators of trophic state

Initial attempts to track changes in nutrient loading were semi-quantitative. Nygaard (1949) attempted to assess trophic state through a ratio of Centrales, centric species, to Pennales, elongated species, (C:P ratio) on the basis that Centrales were indicative of eutrophic waters, while Pennales indicated lower nutrient availability. Stockner & Benson 1967 proposed the Araphidineae to Centrales ratio (A:C) based on an observed increase in araphid taxa and corresponding decline in centric taxa at the top of sediment cores in Lake Washington, USA (thought to be a response to human impact). The A:C ratio was seen to work on the Experimental Lakes Area, (Ontario, Canada), lakes in the English Lake District (UK), and on Lake Washington (USA). It was, however, found to be inapplicable to lakes which had been meso-eutrophic for all or part of their histories, or to shallow lakes (Stockner, 1971; Brugam, 1979). This was associated with the dominance of these lakes by centric species with superior competitive ability under low light conditions. Other studies have been more semi-quantitative, relying upon published autoecological information (e.g. Anderson *et al.*, 1990).

More recently, studies have become quantitative. Agbeti & Dickman (1989) assigned a trophic indicator status based on published autoecological information and then performed multiple linear regression on species groups and TP. The resultant diatom trophic index was found to be significantly correlated with TP, however, the model was found inappropriate for lakes dominated by a few species. In addition, not all species were found to have a positive correlation with TP, associated with roles played by other environmental variables. The methods of Agbeti & Dickman (1989) and of Brenner *et al.*, (1993) used classical regression, where a linear relationship between species and the environment was assumed. Subsequent research has shown that species tend to have a Gaussian distribution response to environmental variables, with an optima and a tolerance range. Consequently multivariate statistical methods were developed, initially applied to the study of pH (Birks *et al.*, 1990) but later applied to eutrophication (Hall & Smol, 1992).

3.4.2. Quantitative Reconstruction of Trophic State

Agbeti (1992) assessed the role of diatom inferred trophic indices against weighted average (WA) regression/calibration methods, and found that WA methods were more accurate. The WA method (discussed in more detail in Chapter 7) has now been used with some success, primarily in temperate regions; North America (Hall & Smol, 1992: Christie & Smol, 1993; Fritz *et al.*, 1993) and North West Europe (Anderson & Rippey, 1994; Bennion, 1994; Bennion *et al.*, 1996). The predictive ability of WA methods is, however, hindered if the length of environmental gradient is short. Bennion *et al.*, (1996) merged 6 regional training sets in order to increase predictive ability, by increasing the range of TP concentrations, and species found, and created a transfer function applicable to north-west Europe. Models have also been developed for more sub-tropical regions such as south-east Australia (Tibby, 2004), New Zealand (Reid, 2005), Florida (Brenner *et al.*, 1993) and California (Bloom *et al.*, 2003). As yet no such models have been developed for Central America. Most models have some degree of accuracy, with a high correlation between observed and inferred TP, but Bennion *et al.* (1996) found a large discrepancy between diatom inferred and historically measured TP.

3.4.3. Complications

The inclusion of benthic and epiphytic species, particularly *Fragilaria* species, into quantitative models has been seen to introduce a degree of error. The periphytic species tend to respond to factors other than epilimnetic TP concentrations and derive some of their nutrients either from the sediment or from plants to which they are attached (Wetzel

2001). In addition, light, pH and temperature conditions differ. On this basis, some studies have tried removing the periphytic species from models (Bradshaw *et al.*, 2002, Philibert & Prairie, 2002). Results, however, are only marginally better for the plankton only model, relative to the plankton plus periphyton model. In shallow lakes the number of *Fragilaria* species is often high, thus masking changes shown by other non periphytic taxa. In addition, the ubiquitous nature of *Fragilaria* species under varying TP concentrations has been problematic for several studies (Bennion, 1995; Bennion *et al.*, 1996; Sayer, 2001). To overcome the problem posed by such species, Sayer (2001) recommends increasing the count of non-*Fragilaria* species, although this can be time consuming (Bennion 1995). An alternative is to remove *Fragilaria* species from the analysis, but has been unsuccessful in the UK due to the paucity of the data left behind (Bennion et al., 2001)

As noted in section 3.2.3, diatom nutrient requirements and ability to take up nutrients has been seen to be affected by salinity and anion composition of a water body (Saros & Fritz 2000; 2002). Frustule silicon concentration was found to decrease with increasing salinity and in *Cyclotella meneghiniana* amino acid production was seen to increase, consequently the N requirement of this species was believed to increase. Salinity can affect competitive ability between taxa for nutrients, in algal bioassay tests *Cymbella* species were found to be poor competitors for N at high salinity, relative to *Chaetocerous* species. Consequently salinity may have a strong impact on the diatom species assemblage through an impact on nutrient requirements and competitive ability for nutrients and therefore transfer function results.

3.5. Changes in Nutrient status in Mexican Lakes

The understanding of interactions between nutrients and diatom assemblages is limited in tropical systems, and while some temperate processes can be applied to tropical lakes, others cannot, such as length of growing season, light availability and the assumption of P-limitation (Lewis, 2000). Limnological data do exist for a few lakes in central México but are spatially and temporally limited. Palaeolimnological evidence for changes in nutrient status do exist, but no numerical reconstruction exists. Quantification is important as both limnological and palaeolimnological evidence show a rapid shift towards eutrophication.

3.5.1 Palaeolimnological evidence of nutrient enrichment

Palaeolimnological evidence for eutrophication comes from five basins: Lago de Pátzcuaro (Metcalfe *et al.*, 1989), Laguna Zacapu (Metcalfe, 1986), La Piscina de Yuríria (Metcalfe & O'Hara, 1992), the Upper Lerma Basin (Metcalfe *et al.*, 1991) and Laguna Zirahuén (Davies, 2000; Davies *et al.*, 2004). These studies have all had a long term palaeoclimatic or palaeoenvironmental focus, and have looked at changes over at least the last 500 years. They infer increased nutrient availability over varying time scales. Studies of Lago de Pátzcuaro have indicated a rise in "eutrophic" diatom species over the last 900 years, associated with agriculture and deforestation in the Post-Classic and the Hispanic periods. Increased nutrient availability was inferred from a rise in *Stephanodiscus subtilis* during the Post-Classic and Hispanic periods at Laguna Zacapu (Metcalfe, 1986). Diatom and sedimentological evidence from La Piscina de Yuríria indicates that the lake has been high in nutrients over the last 4000 years BP, but has become more enriched in the last 500 years (Metcalfe & Hales, 1990; Metcalfe & O'Hara, 1992). The presence of *Nitzschia amphibia, Nitzschia palea, Cyclotella meneghiniana* and *Aulacoseira ambigua* were interpreted as indicating turbid eutrophic conditions. This was thought to be associated with land clearance and soil erosion between 4500 and 900 year BP. This period is followed by a decline in nutrient concentration, although the abundance of *Nitzschia palea* in the surface sediment was interpreted to infer renewed nutrient flux (Metcalfe, 1991). Recent studies of Zirahuén have indicated a rise in nutrient input into the lake in the last 20 years. An increase in *Fragilaria crotonensis* and an abrupt shift from *Cyclotella stelligera* to *Cyclotella ocellata* ~15 years ago is attributed to recent increases in nutrient loading (Davies *et al.*, 2004). Such rapid change may imply high sensitivity to minor changes and an approach to a threshold type change in system functioning to give more unfavourable conditions. This will be discussed in more detail in Chapter 8, with reference to palaeolimnological proxy data.

The palaeolimnological studies indicate that some lakes have naturally high base levels of nutrients. The diatom record at La Piscina de Yuríria, for example indicates high nutrient levels over the last 4000 years BP, i.e. prior to intensified human impact (Metcalfe & Hales, 1990). The apparent sensitivity of lakes such as Laguna Zirahuén to changes in nutrient concentration further highlights the need for limnological and palaeolimnological research into nutrient sources, sinks and cycling. Information on past changes may inform upon future changes; the potential for new steady states, biological and chemical shifts and consequently future human-environment interactions. This is particularly important as the lakes of the TMVB are hydrologically closed and are more sensitive to small changes in nutrient loading.

3.5.2. Contemporary evidence for eutrophication

The majority of lakes with palaeolimnological studies also have instrumental records, although the time scales covered by these is limited. The bulk of studies have focused upon Lago de Pátzcuaro although data do exist for other lakes. Measurements taken between 1983-1990 indicated that Lago de Pátzcuaro, with an average TP concentration of 64.4 μgl^{-1}, was eutrophic, a high proportion of which was biologically available (Chacón-Torres, 1993). However, the lake is not homogeneous and differences in TP concentration occur between the north and south (Chacón-Torres, 1992) and open water and littoral areas (Alcocer & Bernal-Brooks, 2002). Detailed remote sensing studies (Chacón-Torres, 1992; 1993) have indicated that the northern basin was eutrophic to hypertrophic, central areas of the basin were mesotrophic and the south was oligo-mesotrophic conditions. This south to north gradient is also manifested in chlorophyll-a concentrations, peaking at 146 μgl^{-1} in the north, and is related to wind circulation patterns. Alcocer & Bernal-Brooks (2002) also note spatial differences within Lago de Pátzcuaro, although in contrast to

Chacón-Torres (1992; 1993) with peak TP, SRP and Chl a concentrations found in the littoral areas of the south east basin. Particularly high Chl-a concentrations were noted around the main towns, due to municipal sewage effluent flux to the lake. In 1993 an estimated 94,368 tonnes of P entered the lake derived from untreated sewage from ~20 towns and villages and from rural sources, although the latter was estimated to contribute to only 24.6% of the total (Chacón-Torres 1993). The impact of nutrient enrichment in Lago de Pátzcuaro is accentuated by the endoreic nature of the basin and consequently long residence times. The lake is important commercially, socially, economically, and historically and the threat posed to it by nutrient enrichment is high, particularly in addition to other environmental problems suffered. Of particular importance is the sustainability of the fishing industry, especially the endemic pescado blanco (white fish), *Christoma estor* (Chacón-Torres, 1993). Current solutions are focused on treating the symptoms and include dredging, removal of water hyacinth blooms from the southern part of the basin.

Lago de Chapala is the largest lake in Mexico and has been referred to as the most important (de Anda *et al.*, 2001). Studies on phosphate budgets in Lago de Chapala indicate that the majority of phosphates are derived from the Río Lerma (~50%), and from internal P loading (de Anda *et al.*, 2001). Monitoring data taken between 1974 and 1998 indicate that the lowest PO_4 levels were found in the period 1974-80, since which time concentration has risen, with an average for this time period of 300 $\mu g\ l^{-1}$. This rise in P concentration corresponds to the installation of 14 primary sewage treatment plants, which do not remove PO_4^{2-}, to meet the demand of a growing population. de Anda *et al.* (2001) and Lind *et al.* (1992) have noted net accumulation of phosphates at the east end of the lake associated with peak inflow from the Río Lerma and water circulation. High turbidity has been noted by both studies and has been linked to enhancement of PO_4 release from the sediment. P peaks in PO_4 concentration have also been noted during flood events due to increased run-off.

Lago de Cuitzeo, Mexico's second largest lake, has been classified as hypertrophic, primarily due to inflow of the Río Grande de Morelia (and thus effluent from the city of Morelia), agricultural runoff and loss of lake depth, and consequent increased concentration of nutrients (Alverado-Diaz *et al.*, 1985). In addition, catchment soils, vertisols and lithosols, have high inherent fertility. Between 1979-81 156 t P yr^{-1} were estimated to have drained into the Río Grande de Morelia and subsequently into Cuitzeo. This resulted in a P concentration of 310 µg l^{-1}. Such high P concentration resulted in large algal blooms, but with a reduction in number of species. A large increase in the population of the cyanobacteria *Oscillatoria* was noted in 1981. The growth in abundance of this species was related to the loss of lake depth and to a rise in nutrients, factors which both contributed to reduced transparency. In such conditions *Osciallatoria* has been shown to thrive.

Laguna Zirahuén is also of high economical, social and historical importance in Michoacán. The lake provides a unique resource in terms of recreation and fishing. Based on epilimnetic measurements it has been classified as oligo-mesotrophic, with a range of total phosphate concentrations for the lake between 3 and 20 µgl^{-1} (Bernal-Brooks & MacCrimmon, 2000). However, as mentioned earlier the sediment record has indicated a recent abrupt shift in trophic status. Potential sources of phosphates come from 32 settlements in the catchment, all without sewage treatment facilities, although some do use septic tanks, and from agriculture which accounts for 60% of land use in the basin (Bernal-Brooks, 1998). Despite these sources, Bernal-Brooks & MacCrimmon (2000) classify Zirahuén as insensitive to nutrient enrichment. This apparent insensitivity to potentially high nutrient loading has been ascribed to several factors: N and P co-limitation, dilution of nutrient rich inflow, differences in inflow water and lake water density, associated with temperature differences (Bernal-Brooks & MacCrimmon, 2000). In addition Zirahuén is a deep (~40m), warm monomictic lake, but despite stratification the hypolimnion has in the past been prevented from becoming anoxic due to density currents

(Bernal-Brooks, 1998), but has recently shown O_2 depletion at depth during the summer. Consequently, in the recent past internal P loading has not been considered a major source of P. Despite the apparent insensitivity to external P sources, and lack of internal P loading there is evidence that the lake has shown shifts to a higher trophic state, evidence which is corroborated by the palaeolimnological record. Between 1987 and 1996, a 45% increase in TP and associated 3.0 m decline in Secchi depth (due to Chl-a increase) was noted. The lake, however, still lacks the classic signs of eutrophication and can still be classified as oligo-mesotrophic (Bernal-Brooks 1998).

3.6. Conclusion

The majority of lakes studied in central Mexico are either eutrophic or show signs of nutrient enrichment. The major sources of nutrients in the area appear to be untreated sewage and the use of phosphate based detergents. The role played by run-off from agricultural land also appears to be important. The effects of high levels of nutrients can be seen clearly in lakes such as Pátzcuaro and Chapala, and have potential to occur in other lakes. There may be potential for thresholds to be reached and abrupt changes in ecosystem function at a new steady state. With low adaptive capacity, social and economic growth may no longer be sustainable under a new steady state. Nutrient enrichment in several lakes has a long history and may have increased ecosystem sensitivity to change. In addition, the complication of resource competition by co-limitation and by salinity and the potential for P release from the sediments may require consideration for management purposes.

There is a need for a long-term perspective in the management of eutrophication and the apparent sensitivity and rate at which systems seem to be changing, highlights this need. Comprehensive monitoring records for lakes in central México are, however, limited. In temperate regions a lack of long term monitoring data has been overcome by the creation and application of diatom based TP transfer functions (Anderson 1995; Bennion, 1994;

Bennion *et al.* 1996; Hall & Smol 1992, Tibby 2004). In Mexico the lack of instrumental records has hindered the creation of quantitative models for inferring changes in nutrient status. It is hoped, however, that the creation of a new calibration data set for central México, which includes nutrient data, will result in a suitable model for reconstruction of eutrophication histories. The results will be discussed in Chapters 6 and 7.

Chapter 4. Environmental Biogeochemistry

4.1. Introduction

Lake basins act as sinks for allochthonous and autochthonous organic matter and thus provide a record of local, and often regional, responses to environmental change. The biota are affected by, *inter alia*, changes in climate which bring about changes in molecular and isotopic composition of organisms, which on death, may be preserved in the palaeolimnological record. Organic matter transferred to the sediment comprises lipids, carbohydrates and proteins which act as a record of natural and anthropogenic influences within the catchment (Meyers, 2003). However, material is susceptible to degradation during sinking and after burial, although some compounds are more susceptible than others. Despite this, valuable palaeoenvironmental information is still retained. Proxies derived from organisms include total organic carbon, carbon and nitrogen stable isotopes and biological marker molecules, such as fatty acids, aliphatic hydrocarbons and sterols. Such compounds record changes in organic matter source, abundance and diagenesis. There are two main sources of organic matter: vascular, such as shrubs, trees and aquatic macrophytes, and non vascular plants, such as algae. The former are rich in cellulose while the latter are protein rich and contain little or no cellulose.

Analysis of sedimentary biomarkers, stable isotope analysis and elemental analyses will provide additional information on aquatic productivity and inputs of organic matter into the lake ecosystems in central México. While diatoms are an integral part of the project, they constitute only one part of the lake ecosystem and its sediment record. Thus an assessment of other species of algae and plants over time is essential to the understanding of the ecosystem response to environmental change over time. Discussed below are the background and application of studies into chemical and biogeochemical analyses of lake sediments.

4.2. Carbon and Nitrogen

Total organic carbon (TOC) can be used as a proxy for the amount of organic matter present in lacustrine sediments and accounts for ~50% of all organic matter, known via loss on ignition (LOI) at 550 °C. The abundance of TOC is influenced by the initial production of biomass and by subsequent degradation (Meyers, 2003). As such it represents an integration of OM from different origins and is a product of differential preservation and delivery rates as well as depositional processes. Rates of preservation are seen to vary within lakes, for example in Lake Victoria, East Africa, TOC is seen to decline with increasing water depth (c.f. Talbot & Lærdal, 2000). For these reasons interpretation of spatial and temporal changes in TOC can be complicated. Terrestrial and aquatic sources of organic matter can be distinguished using the ratio of carbon to nitrogen (C/N) on the basis that terrestrial plants contain high amounts of cellulose, and low amounts of protein and are thus C rich and N poor. Terrestrial plants are usually considered to have C/N greater than 20 while aquatic plants are in the range 4-10 (Meyers, 1994), thus variations in the ratio can be used to track shifts in OM source. Variations in OM sources have been measured by Meyers & Teranes (2001) and showed mixed phytoplankton samples from Pyramid Lake, Nevada, *Sphagnum* and red oak leaves to have C/N ratios of 6, 9 and 29 respectively. Consequently down core changes in the C/N ratio have been used to track historical changes in the dominant OM source and can be used to infer shifts in trophic state, i.e. increased productivity. Brenner *et al.* (1999) used C/N ratios to aid assessment of changes in trophic state and establish baseline conditions of four hypertrophic Florida lakes. Using molar C/N ratios, in conjunction with $\delta^{15}N$ and $\delta^{13}C$, they showed an overall shift to lower C/N ratios in three of the four, but with a degree of variability down core. This shift to lower C/N was either progressive (Lake Hollingsworth) or abrupt (Lakes Clear and Griffin). The abrupt shift, from 14 to 10 and from 8.5 to 7.5 in Lakes Griffin and Clear respectively has occurred from the beginning of the 20th century and is consistent with a shift from macrophyte to phytoplankton dominated systems, due to increased nutrient availability with increased human activity.

Deforestation, associated with European settlement ~200 years BP, in the catchment of Lake Pleasant, Massachusetts, has been associated with a corresponding rapid increase in C/N from 16.1 to 25.2, due to increased delivery of catchment material, this peak was followed by a return to pre-disturbance levels (Kaushal & Binford 1999). In a small crater lake in México a peak in C/N, corresponding to a *Pinus* maximum, has been linked to elevated carbon flux associated with increased *Pinus* (Metcalfe *et al.*, 1989). Flannery *et al.* (1982) found C/N to be inversely correlated with OM, TP, TN and Chl-a concentrations implying that with increasing nutrient supply and subsequent eutrophication, dominance by phytoplankton led to a decline in the C/N ratio. Of the lakes studied, those with the highest trophic state had the lowest C/N ratios with phytoplankton assemblages dominated by cyanobacteria and consequently the C/N ratio is a reflection of the N-rich autochthonous biomass rather than anthropogenic allochthonous inputs. Meyers *et al.* (1984) and Flannery *et al.* (1982) note that C/N profiles should be interpreted with caution as the C/N of terrestrial material falls while that of algae increases over time due to preferential early diagenesis of N. The magnitude of change and often down core position of change is considered indicative of anthropogenic impact, particularly in those basins that are highly disturbed. In addition, as with many sedimentary proxies, one core may not necessarily be representative of whole basin conditions.

4.3. Stable Isotopes

Changes in palaeoproductivity can be tracked using stable carbon and nitrogen isotopic ratios: $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ($\delta^{13}C$ and $\delta^{15}N$ respectively). Stable isotope geochemistry allows an assessment of OM source, abundance, productivity rate and changes in nutrient availability. The $\delta^{13}C$ has been widely used to track changes in productivity, while the use of $\delta^{15}N$ (initially used in marine sediments), is more recent in lacustrine settings.

4.3.1. Nitrogen

Shifts in $\delta^{15}N$ can be attributed to variation in the availability of the two stable isotopes of N, changes which may be brought about by either natural or anthropogenic effects, referred to as fractionation. Fractionation can be physical, brought about by processes such as freezing or evaporation, or can be chemical occurring in biochemical processes such as denitrification and inorganic reactions, (e.g. ammonia volatilization) such processes usually have a characteristic signal (Talbot, 2001). Consequently, sources of N have distinct signatures (see Table 4.1), but the biochemical cycling within lakes and prior to input of N is complex, relative to C (see below), which makes interpretation of the signal more difficult (Talbot, 2001; Leng *et al.*, 2005).

Table 4.1 Typical $\delta^{15}N$ values for OM in lacustrine environments (compiled from Leng *et al.*, 2005; Meyers & Lallier-Vergès, 1999; Talbot, 2001)

Source	**$\delta^{15}N$ (‰)**
Land plants	+2 to +10
Aquatic macrophytes	-10 to >0
Plankton	+2 to +14
Lacustrine sediment	-2 to +20
Atmospheric N_2	~0
Precipitation	-5 to +5
Soil OM	~ 0 to +8
Sewage (animal/human)	+8 to +18
Fertilizers	-4 to +4
Ground water (unpolluted)	0 to +10

Algae generally use dissolve inorganic N (DIN), usually as NO_3^-, as their primary N source, while land plants utilize N_2 either directly from the atmosphere or via N-fixing soil bacteria. It is this difference that allows OM sources to be tracked using $\delta^{15}N$. Typical dissolved NO_3^- values are in the range +7 to +10‰ while N_2 values are ~0‰ (Meyers & Teranes, 2001). Values differ, however, in OM derived from terrestrial and aquatic plants. Algae tend to discriminate against ^{15}N and preferentially use ^{14}N due to its lower bond strength (Talbot, 2001), resulting in lower $\delta^{15}N$ values of the sedimenting OM. With increasing productivity the lighter isotopes are used up and consequently phytoplankton begin to discriminate less against the heavier isotope. Therefore the sedimenting OM becomes ^{15}N enriched and results in an increase in $\delta^{15}N$.

The nitrogen isotope signal is complicated by several factors: water column denitrification in anoxic conditions, shifts in the phytoplankton community composition, anthropogenic inputs of sewage, nutrient limitation and lake pH. Studies of Lake Erie, North America, have shown that seasonal anoxia resulted in conversion of NO_3^- to N_2 (denitrification) leaving the remaining NO_3^- enriched in ^{15}N, a signal which has been transmitted down stream to Lake Ontario (Hodell & Schelske, 1998). This has also been noted in Lake Baldeggersee, Switzerland (Teranes & Bernasconi, 2000). Shifts from diatom or chlorophyte (green algae) to cyanophyte (blue-green algae) dominated communities can bring about changes in $\delta^{15}N$. A shift to a system dominated by N-fixing cyanobacteria, using atmospheric N_2 as their primary N source, results in a decline in $\delta^{15}N$ of the sedimenting OM and has been recorded in Lake Baldeggersee (Teranes & Bernasconi, 2000), Clear Lake, Florida (Brenner *et al.* 1999) and Lake Bosumtwi, Ghana (Talbot & Johannessen, 1992). Gu *et al.*, (1996) and Brenner *et al.* (1999), however, note that primary productivity as a whole, rather than species composition is important in controlling $\delta^{15}N$ and that factors other than N fixation need to be considered, particularly in lakes of lower nutrient concentrations. Human and animal wastes are enriched in ^{15}N and give rise to $\delta^{15}N$ in the range +8 to +18‰ (Heaton, 1986). An up-core increase in the signal at Lake Baldeggersee has been interpreted as progressive enrichment of the DIN pool in ^{15}N due to agricultural and urban runoff (Teranes & Bernasconi, 2000). The process of ammonia volatilization, loss of NH_3 by degassing, is pH dependent. At high pH values, between 9.1-9.6, such as those in Lake Bosumtwi, ammonium is present as ammonia, which is subsequently easily lost. This process gives rise to strong fractionation leading to enrichment of the remaining DIN in ^{15}N and has caused shifts in $\delta^{15}N$ by up to 34‰ (Talbolt & Johannessen, 1992). Whether a lake is P or N limited can also present problems in interpreting variation in the $\delta^{15}N$ as P-limited systems phytoplankton abundance is driven by P not N availability. In such cases productivity had little impact on the DIN pool and consequently $\delta^{15}N$. Anoxia, either natural or as a result of eutrophication, can also alter the δ^{15} signal, though the inhibition of nitrification. This consequently leads to the build up of ammonia or denitrification, which can increase $\delta^{15}N$ of the remaining DIN. This may be complicated by isotopic fractionation due to ammonia assimilation which leads to a decrease in the $\delta^{15}N$ (Leng *et al.*, in press).

Many studies have focused on variation of $\delta^{15}N$ in sedimenting organic matter (Bernasconi *et al.*, 1997; Hodell & Schelske, 1998) while others have used it to track changes in primary productivity and eutrophication (Gu *et al.*, 1996; Neumann *et al.*, 2002; Teranes & Bernasconi, 2000) or environmental changes (Hassan *et al.*, 1997: Talbot & Johannessen, 1992). Such studies have highlighted the difficulties in interpreting the $\delta^{15}N$ signal. Hodell & Schelske (1998) and Bernasconi *et al.* (1997) used sediment traps, in varying water depths, to track seasonal and interannual change in OM flux and composition and also to explore the influence of diagenesis. Hodell & Schelske (1998) show the seasonal pattern in the $\delta^{15}N$ signal to be the opposite of the $\delta^{13}C$ signal. i.e. peaks in $\delta^{15}N$ correspond to declines in $\delta^{13}C$. Values are high in the winter-spring period during mixing and during the stratified period, decline rapidly and remaining low during the summer. This seasonal decrease is associated with phytoplankton blooms, but may also be related to recycling of ^{14}N by picophytoplankton, which are a major constituent of the summer bloom. A decrease of 6‰ is noted during the stratified period and has been associated with a change in OM source, dominated by isotopically light phytodetritus in the stratified period and by isotopically heavy detrital OM in the mixing period. Slight differences between the shallow and deep traps are noted, average values are +7.8 and +8.6‰, respectively, but the shallow traps does not consistently display lower $\delta^{15}N$ values than the deep trap. A similar increase with depth and seasonality has also been noted in Lake Lugano, Switzerland from 7.22 to 7.95‰ between the upper and lower traps and showing a seasonal variation from +4 to +16‰ (Bernasconi *et al.*, 1997). Sediment collected during the summer is characterized by isotopically light material, and the winter by heavier OM. Two storm events are recorded during the sampling period and show a decrease in $\delta^{15}N$, associated with elevated inputs of allochthonous material. Winter samples were found to be the most positive, at a time when nitrate concentrations were greatest, implying that other factors are involved in controlling the $\delta^{15}N$. Suggestions for this include alteration of the OM by degradation and size fractionation, however, when considered in conjunction with other data this is considered to be improbable. They suggest that the rise in $\delta^{15}N$ was associated with effluent from a local water treatment plant; but they note that the lack of research in this area hinders further interpretation.

The natural variation in the $\delta^{15}N$ signal can be masked by the impact of an anthropogenic signal, through stimulation of eutrophication and/or inputs of sewage or accelerated erosion. The use of $\delta^{15}N$ to aid interpretation of nutrient histories can therefore be profitable. Lake Arendsee, Germany, was initially oligotrophic and since the beginning of the 20th century has suffered progressive eutrophication, associated with increased agricultural activity and sewage input. Eutrophication led to blooms of cyanobacteria, but following remediation measures blooms have not been seen since 1995 (Neumann *et al.*, 2002). From ~1934 $\delta^{15}N$ shows an overall decline from +5.4 to 5.6‰ to a low of +0.3‰ in mid 1995, after this time there is an increase in values to +2.5 to +3.6‰ in the uppermost sediments. There is, however, a slight excursion to higher values (+6.4‰) between 1971 and 1974. This period of elevated $\delta^{15}N$ corresponds to one of elevated nutrient loading and aquatic productivity resulting in the production of OM with a heavier isotopic value. Low $\delta^{15}N$ values have been associated with blooms of cyanobacteria, due to their ability to fix atmospheric N_2 which has an isotopically light $\delta^{15}N$ value. Lake Baldeggersee has also undergone eutrophication and corresponding productivity increases in the last 100 years with large blooms of cyanobacteria noted between 1990 and 1992 (Teranes & Bernasconi, 2000). In contrast to Neumann *et al.* (2002), they show a progressive increase in $\delta^{15}N$ up-core from +6 to +7‰ in ca. 1880 to +10 to +13‰ at the core top. In addition sediment trap data show $\delta^{15}N$ between +11.9 and +12.4‰. Such values are greater than typical values for lacustrine sediments, commonly in the range +1-5‰ (Meyers, 1997), and correspond to a rise in external nutrient loading and resultant hypolimnetic anoxia. Increased nutrient supply is attributed to increased flux of effluent from agricultural and municipal sources, which have isotopically heavy $\delta^{15}N$ values (+10 to +25‰) and in addition, anoxia has been shown to increase denitrification rates and thus nitrate concentration leading to production of OM that is isotopically heavy. As the lake has been subject to remediation measures, such as artificial aeration, anoxia and thus denitrification have declined. Teranes & Bernasconi (2000) therefore infer that up core changes in $\delta^{15}N$ are predominantly controlled by the influx of isotopically heavy anthropogenic waste. Rosenmeier *et al.* (2004) also track changes in eutrophication through the use of $\delta^{15}N$ in Lake Petén Itzá, Guatemala. They show a steady increase from +1.9‰ to +2.7‰. This was attributed to inputs of soil and sewage due to recent urbanisation and growth in intensive and commercial agriculture, but they note that the magnitude of change did not correspond to such inputs, i.e. < +8‰. This is thought to be due to the role of cyanobacteria, which results in $\delta^{15}N$ values similar to atmospheric N ca. 0‰, which would offset the effect of sewage and soil inputs.

4.3.2. Carbon

Variations in the $\delta^{13}C$ of organic matter arise from variations in source associated with differential use of the carbon isotopes by different plants. Photosynthetic plants are noted as following one of two pathways, either the Calvin-Benson cycle (C_3 plants such as trees and shrubs) or the Hatch-Slack cycle (C_4 plants such as tropical grasses) with isotopic composition being strongly dependant upon the pathway followed. C_3 plants are enriched in ^{12}C, relative to C_4 plants, yielding $\delta^{13}C$ values around -26‰, while C_4 plants are generally found to be ^{13}C depleted, with $\delta^{13}C$ values around -13‰. Phytoplankton (classified as C_3 plants) use dissolved inorganic carbon (DIC) and preferentially use ^{12}C, resulting in $\delta^{13}C$ values that are isotopically light, relative to their carbon source.

Consequently a rise in productivity leads to depletion of the ^{12}C in the DIC reservoir and subsequently $\delta^{13}C$ of newly sedimenting OM rises as the heavier isotope (^{13}C) is utilized instead. C_3 algae have been found to have $\delta^{13}C$ values similar to C_3 land plants (Meyers, 1994) and the two are thus difficult to distinguish based on $\delta^{13}C$ alone. They can however be distinguished between on the basis of their C/N values (see Figure 4.1). The similarity between C_3 terrestrial plants and algae arises as C_3 land plants use atmospheric CO_2 and C_3 algae use dissolved CO_2 which is in isotopic equilibrium with the atmosphere (Meyers, 2003). This dissolved CO_2 is, however, limited and once depleted, algae use HCO_3^- which is isotopically heavy, relative to atmospheric CO_2 resulting in a heavier isotopic signal, which can approximate that of C_4 plants. The contribution of allochthonous and autochthonous material to lacustrine OM can therefore yield a wide range of $\delta^{13}C$ values, between -8 and -35%. Aquatic plants have been found to yield more positive $\delta^{13}C$ values than C_3 plants, but still follow the C_3 photosynthetic pathway (O'Leary, 1981). Submerged macrophytes, like algae, use dissolved CO_2 and HCO^- and can yield $\delta^{13}C$ values between -10‰ and -50‰, but more commonly between -12 and -30‰ (Boutton, 1991; Leng & Marshall, 2004). Such a wide range depends upon whether these aquatic plants are using CO_2 from the atmosphere or the DIC. For example submerged macrophytes in Lake Okahumpka, Florida, were found to have values ca. -8‰ while in Lake Apopka emergent macrophytes, using CO_2 from the atmosphere, were found to have $\delta^{13}C$ values of -26‰ (Brenner *et al.*, 1999).

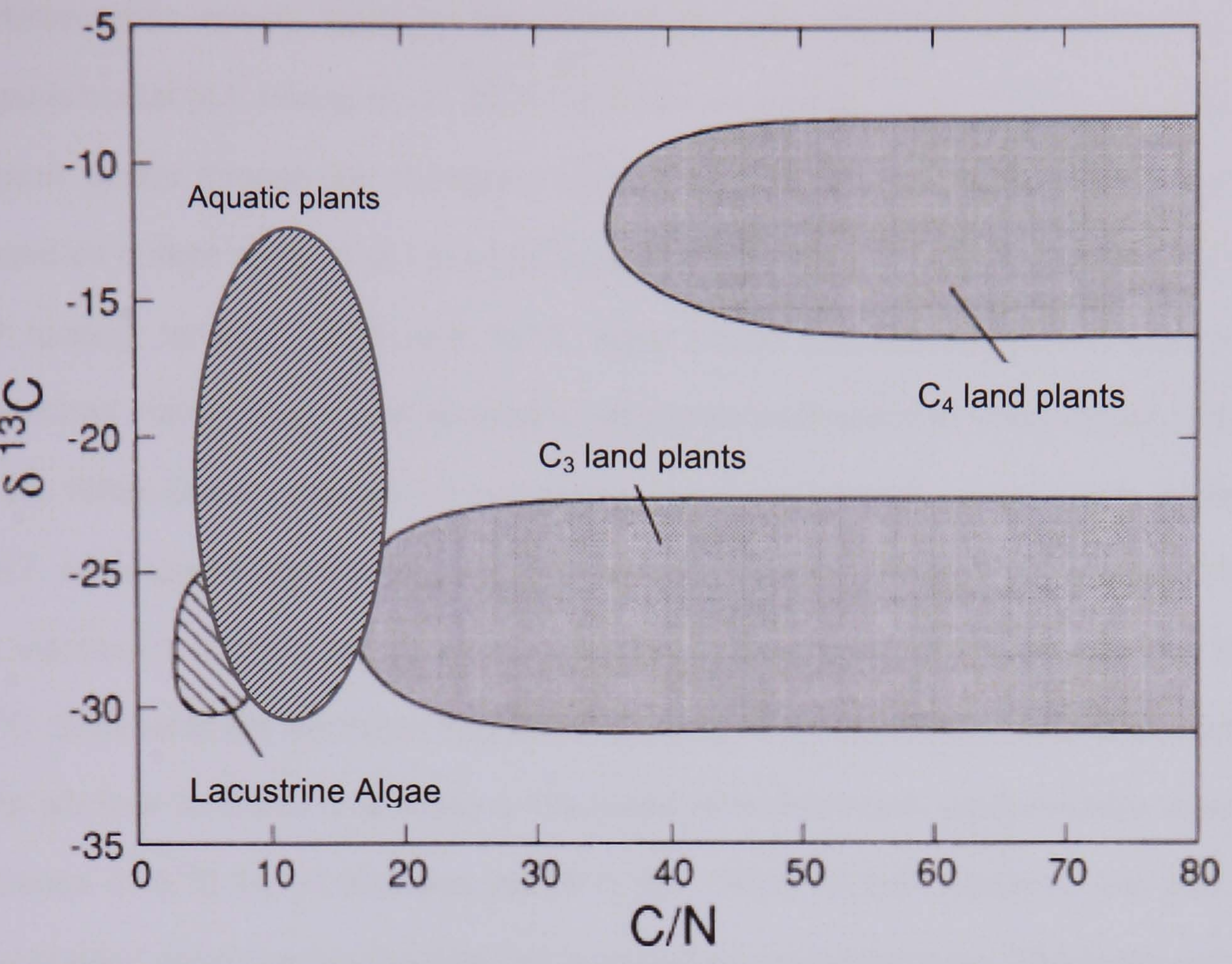


Figure 4.1. Generalised $\delta^{13}C$ and C/N values as major sources of plant organic matter to lake sediments (modified from Meyers, 1994; Meyers & Lallier-Verges, 1999)

The stable carbon isotope signature has been used to aid palaeoclimatic reconstruction (Huang *et al.,* 2001; Jasper & Gagosian, 1989; Krishnamurthy *et al.*, 1986), variations in productivity (Bernasconi, 1997; Hodell & Schelske, 1998) and tracking changes in eutrophication (Neumann *et al.*, 2002; Teranes *et al.*, 1999). This is based on the different isotopic signature produced by the different photosynthetic pathways (see Figure 4.1). Examination of Mexican lacustrine sediment on a NW to SE climatic gradient (Huang *et al.*, 2001) indicates that climatic factors such as precipitation and temperature drives the relative abundances of C_3 and C_4 plants. Based on $\delta^{13}C$ of the C_{29} *n*-alkane for lakes Babícora, in northern México, and Quexil, in Guatemala, opposing trends are noted in the abundance of C_3 vs. C_4 plants and consequently in the $\delta^{13}C$, with increased abundance of C_3 corresponding to a decline in $\delta^{13}C$ during the LGM in Northern México and dominance by C_4, and more positive $\delta^{13}C$ values in southern México. Low $\delta^{13}C$ values in cool periods have also been noted in Indian lacustrine sediments (Krishnamurthy *et al.*, 1986).

Anthropogenic activity, such as forest clearance, can affect the $\delta^{13}C$ of allochthonous organic matter (c.f. Huang *et al.*, 2001), and can also influence the $\delta^{13}C$ of autochthonous organic matter through the changes in aquatic productivity and community composition. Based on diatom analysis of Lake Baldeggersee sediments (Lotter, 1998) indicated peak TP loading between 1956 and 1974, since which time remediation measures and watershed management have resulted in progressive water quality improvement (Teranes *et al.*, 1999). Diatom inferred TP indicated a rise in concentration from ~1909, peaking in 1977, correspondingly OM production increased, observed through changes in C/N and an increase in $CaCO_3$ accumulation, changes in productivity are also recorded by the $\delta^{13}C$. Sediments are laminated and the $\delta^{13}C$ signal of the seasonal varves is distinct, with light summer laminations showing a 1‰ lower value than dark autumn/winter layers. An increase in $\delta^{13}C$ by ~1.0‰, was noted in the 1940s, in both seasons, but with more pronounced enrichment in the dark late summer/autumn/winter layer. The preferential use of ^{12}C by elevated algal productivity in response to increased TP loading, resulted in ^{13}C enrichment of the DIC reservoir, while anoxic conditions preserved the ^{12}C enriched signal of OM in the sediment. This was subsequently recorded as a progressive enrichment of the sedimentary $\delta^{13}C$, in both seasons.

Neumann *et al.*, (2002) in a study of Lake Arendsee, Germany, showed an increase in $\delta^{13}C$ of organic carbon, from -24.2‰ to -21.2‰ between 1935 and 1985, thereafter a decline to -22‰. Very heavy values (-17‰) were noted, associated with the deposition of a marl layer in 1995 and recent sediments show a return to isotopically light (-24‰) values. Fluctuations in $\delta^{13}C$ of inorganic carbon are different with peaks noted in the early 1970s up to +11‰ decreasing thereafter and increasing to around 3.8‰ in the most recent sediment. Neumann *et al.* (2002) associate changes in $\delta^{13}C_{TOC}$ with changes in productivity, while changes in $\delta^{13}C_{TIC}$ are related to a combination of factors: photosynthetic activity, rates of burial and diagenesis and by the signature of the allochthonous material. Eutrophication is expected to result in an increase in $\delta^{13}C_{TOC}$

through CO_2 limitation associated with enhanced productivity as CO_2 limitation has been noted to lower isotopic fractionation, leading to an overall shift to lighter $\delta^{13}C_{TOC}$ values through forced ^{13}C uptake. Such CO_2 limitation has been noted in hydrologically closed small lake basins with long residence times such as Arendsee and therefore potentially Mexican lakes. Coincident with increasing $\delta^{13}C_{Toc}$ is a decrease in $\delta^{13}C_{TIC}$, interpreted as increased biologically induced authigenic calcite formation, again associated with CO_2 limitation which subsequently led to a shift to lighter $\delta^{13}C_{TIC}$ values. The implementation of remediation measures to reduce eutrophication led to a decline in $\delta^{13}C_{TOC}$ and a corresponding increase in $\delta^{13}C_{TIC}$ as reduced productivity occurred in a system which was no longer CO_2 limited. Excursions to higher $\delta^{13}C$ values associated with increased productivity in response to nutrient loading, through P-rich soil flux, have also been noted in Lake Ontario (Hodell & Schelske, 1998). Here increased algal productivity led to removal of ^{12}C and thus isotopically light OM and consequently ^{13}C enrichment of TIC, which became isotopically heavy. Lake sediment based studies of Lake Ontario show a shift to lighter $\delta^{13}C$ values to a peak around -24.5‰ in response to nutrient loading. The implementation of remediation measures saw a drop in $\delta^{13}C$ in recent sediment in response to reduced productivity (Meyers, 2003). Generally, increase in primary productivity, associated with increased nutrient availability, through eutrophication, is expected to result in increased $\delta^{13}C$. Rosenmeier *et al.* (2004) however record a marked decrease in $\delta^{13}C$ from -22.5% to -25.2%, indicating an alternative source of ^{13}C depleted material. They suggest sewage inputs as a possible source of the relatively light $\delta^{13}C$ signal.

4.4. Sedimentary biomarkers

Lacustrine sediments contain a range of organic chemical residues, or lipids, which can identify the origin of the material contributing to sediment accumulation, as well as degradation processes. Early culture experiments by Cranwell (1976) showed potential for the use of sedimentary lipids in productivity assessment. Accumulation, preservation and conversion of organic compounds can vary between lake basins, based on variation in physical, chemical and biological characteristics and is also variable between compounds. Lipids can be defined as substances that are insoluble in water, but are extractable in organic solvents such as Hexane, Dichloromethane (DCM) or Methanol (MeOH) and include aliphatic hydrocarbons, sterols, alcohols and fatty acids (Killops & Killops, 2005). Free and bound lipids may be extracted from the sediment, but this study will only focus on the former. Free lipids can be subdivided into neutral and acid compounds. The former includes alcohols, *n*-alkanes and sterols, while the latter includes fatty acids. Methodologies for lipid extraction, fractionation, quantification and identification are given in more detail in Chapter 5. Compounds preserved in the sediment are referred to as biological marker compounds, or biomarkers, and are characteristic of certain sources (Table 4.2). They are, however, rarely species specific and are often only representative of a class or phylum. Two major sources are readily identifiable: allochthonous and autochthonous organic matter, based on their biochemical composition, where they either lack or contain cellulose as with C/N ratios. Although compounds are susceptible to degradation, such as the loss of functional groups (side chains, hydroxyl groups, double bonds), the carbon spine (normal, linear hydrocarbon chain Section 4.5.1) and stable derivatives of their precursor compounds are often recognizable and therefore can easily be attributed to source (Meyers, 2003). Processes such as saturation of double bonds, removal of a hydroxyl group may alter the molecule, however the carbon 'spine' tends to remain the same and these are used to infer source (Meyers & Ishiwatari 1993). This project, however is concerned with time scales, of $<10^3$ years, so it is likely that diagenesis will be less advanced.

Table 4.2. Sources of major lipid biomarkers

Organic Matter Source	Lipid Biomarkers
Algae	nC_{17}-C_{19}, Cholesterol,
Diatoms	Brassicasterol, highly branched isoprenoids
Aquatic macrophytes	nC_{21}-C_{25}
Terrestrial Plants	nC_{29}-C_{31}, campesterol, β-sitosterol, stigmasterol
Trees & Shrubs	nC_{29}
Grasses	nC_{31}

4.4.1. Aliphatic hydrocarbons

Simple hydrocarbons, alkanes (C_nH_{2n+2}); straight chain, branched or cyclic, have been most extensively used in studies of organic matter delivery to lakes (Ficken *et al.*, 2000; Filley *et al.*, 2000), diagenesis (Meyers & Ishiwatari, 1993) and as a proxy for eutrophication (Hollender *et al.*, 1992; Silliman & Schelske, 2003). Alkanes are saturated (i.e. they have carbon-carbon single bonds) and are consequently relatively stable, with a low susceptibility to degradation. *n*-Alkanes are straight, un-branched carbon chains and are usually found in lacustrine sediments in the range of *n*-C_{15} to *n*-C_{35}. It has generally been considered that *n*-alkanes with chain lengths >20 C atoms are indicative of vascular plants, conversely algae are represented by *n*-alkane chain lengths <20 C atoms. Terrestrial plants are dominated by C_{27}, C_{29} and C_{30} *n*-alkanes which are associated with epicuticular waxy leaf coatings, while aquatic macrophytes are dominated by C_{21}, C_{23} and C_{25} (Ficken *et al.*, 2000). Algae and bacteria are dominated by the C_{17} *n*-alkane. Consequently it is possible to determine the relative contributions of allochthonous and autochthonous organic matter based on the relative abundances of the aforementioned *n*-alkanes. Due to their low susceptibility to diagenesis relative to other compounds, however, they may be over represented (Meyers, 1994). From C_4H_{10} alkanes may be branched alkanes, forming structural isomers that have the same chemical formula but have different physical and chemical properties due to their differing structures. These are also common in lacustrine settings, cyanobacteria for example produce 7- or 8-methylheptadecane (Me_nC_{17}) (Filley *et al.,* 2000).

4.4.2. Sterols

Terpenoids are synthesized from C_5 isoprene units and can be classified on the basis of the number of the constituent isoprene units (Killops & Killops, 2005). They generally contain an oxygen functional group and form cyclic units, but may also be acyclic, such as squalene (see Appendix 4). Triterpenoids contain six isoprenoid units and are thought to be primarily derived from the precursor acyclic unit squalene ($C_{30}H_{50}$), which is ubiquitous in organic matter. Steroids are one major class of tirterpenoids, are pentacyclic (5 rings) and are formed by the enzymatic oxidation of squalene and subsequent cyclisation which forms cycloartenol and lanosterol precursors to many plant steroids and act as rigidifiers to plant cell membranes. Steroids which contain an alcohol group are commonly referred to as sterols. Common sterols in plants include cholesterol (Cholest-5-en 3β-ol), Dinosterol, and β-sitosterol (24-ethylcholest-5-en 3β-ol). Like hydrocarbons certain sterols are indicative of certain sources (Table 4.2); diatoms are usually found to contain a high amount of brassicasterol (24-methylcholesta-5,22E-dien-3β-ol) (Killops & Killops, 2005) while Nishimura & Koyama (1977) found cholesterol to be dominant in algae from Lake Suwa, Japan, and β-sitosterol was found to be dominant in vascular plants from the catchment. β-sitosterol has however been found to be the dominant sterol in cyanobacteria from saline lakes in Antarctica (Matsumoto *et al.*, 1982). Characteristics such as the number of double bonds, position of methyl groups and configuration of the branched side chain on the C_{24} molecule allow distinction between algal β-sitoterol and higher plant β-sitosterol (Killops & Killops, 2005). This does however require a more advanced method of analysis, such as NMR, beyond the scope of this study to determine the exact derivation.

4.5. Tracking changes in nutrient status using biomarkers

The distinction between OM source and abundance of biomarkers have been used to track changes in productivity and trophic status by many investigators. Gaskell & Eglinton (1976) reported high concentrations of cholesterol, campesterol and β-sitosterol in the surface sediments of Rostherne Mere and declining concentrations of cholesterol down core, which were interpreted at a record of increase algal productivity associated with eutrophication. A subsequent study of the same lake by Prartono & Wolfe (1998) showed a progressive shift in organic matter to an autochthonous source, inferred by a progressive increase in abundance of 7-MeC$_{17}$ (from cyanobacteria), unsaturated and branched fatty acid isoprenoids and C$_{27}$, C$_{28}$ and C$_{29}$ sterols. This dominance of cyanobacteria in recent sediments at Rostherne is supported by pigment analysis by Livingstone (1984). Prartono & Wolfe (1998) identify a clear difference between upper and lower sediment in biomarkers, attributable to an increased abundance of algal, bacterial and cyanobacterial inputs. This increase in autochthonous input of OM is also recorded by a shift to more negative δ^{13}C values. A similar progressive shift towards an algal dominated system associated with a 7-fold increase in nutrient flux is noted in Lake Apopka, Florida by Silliman & Schelske (2003). The ratio between long hydrocarbon chains and short hydrocarbon chains (LHC/SHC) was used to highlight the low input of terrigenous material. They note however, while the LHC/SHC is low indicating dominance of surface sediments by phytoplankton, there is a low concentration of short chain hydrocarbons. No *n*-C$_{15}$ and little *n*-C$_{17}$ and *n*-C$_{19}$ were found in the sediment, associated with physical factors such as turbulence and subsequent resuspension. Filley *et al.* (2001) show Mud Lake, Florida to have progressed from land, to marsh to shallow eutrophic lake over the last 8,500 years BP, a transition which is matched by changes in the abundance and types of biomarkers preserved within the sediment. The branched alkanes 7- and 8-methylheptadecane were only found in the upper 15-20 cm, but were the second most abundant compound. In this zone *n*-C$_{17}$, the most abundant compound, also increased rapidly in abundance while longer chain aliphatic hydrocarbons increase slightly prior to declining in the uppermost sediments. Phytol and the C$_{16}$-C$_{17}$ *n*-alcohol increased in concentration from ~25cm depth while the C$_{22}$-C$_{25}$ *n*-alcohol increases substantially from 50cm. Such changes were used to infer shifting sources of organic matter input into the lake.

The application of lipid biomarkers to the study of tropical lakes has been more limited. Research has been conducted on Lagoa de Cima, Brazil (Gomes & Azevedo, 2003), located in an agricultural catchment dominated by cultivation of sugar cane. Concentrations of individual aliphatic hydrocarbons and total *n*-alkanes and chain length were seen to vary between the lake, its two main inflows and its outflow. The lake and its inflows were dominated by hydrocarbons in the range C_{23}-C_{26}, while the outflow was dominated by C_{12}-C_{22} indicating differing sources of OM. This is also noted in the C/N ratio, where values drop to below 4 at the outflow, indicating the role of phytoplankton, in contrast the lake sediments have a C/N value of ~10, resulting from vascular and non-vascular OM sources. This mixed input is also supported by the bimodal distribution of the *n*-alkanes. The Lagoa de Cima lake sediments show a large peak at C_{29} and a second smaller peak at C_{17}, thus indicating an input by terrestrial plants organic matter and by algae. In contrast the Ururaí outflow shows peaks at C_{20} and C_{29} which Gomes & Azevedo (2003) interpret as mixed microbial, algal and terrestrial plant input. Extractable sterols were dominated by cholesterol, β-sitosterol, sigmasterol and campesterol, with cholesterol dominating the sterol contribution to the Ururaí sediments while the others offer only a minor contribution (Azevedo, 2003). Such an increase was interpreted as an increase in input of OM from plankton and bacterial sources. In contrast the dominant sterols in the lake and the inflow sediments are interpreted as indicative of higher plants. Thus in combination, these two studies indicate a mixed allochthonous/autochthonous input through the inflows, but that sedimenting organic matter in the lake is dominated by autochthonous OM production, which is subsequently recorded in the outflow sediments, but was not due to the impact of the surrounding human activity.

4.6. Mineral Magnetism

The magnetic susceptibility (χ) of material is a measure of its magnetizability and provides an indication of the amount of iron bearing minerals in the material. Different forms of iron have different origins and therefore different magnetic susceptibilities (Dearing, 1999). Magnetic behaviour can be subdivided into 5 groups: ferromagnetism, very highly magnetic material such as pure iron; ferrimagnetism, highly magnetic including natural minerals such as magnetitie and magheamite, canted antiferromagnetic and paramagnetic minerals, which have low magnetic susceptibilities and includes minerals such as haematite and diamagnetic material, such as quartz, calcium carbonate and organic matter give a very weak or even negative magnetic signal. Consequently the magnetic signal exhibited by materials in the environment is a combination of these. Basic measurements of magnetic susceptibility include low frequency susceptibility, which provides information on the total concentration of ferrimagnetic material.

Such minerals are present in lake sediments derived from, or beyond the catchment, from soil erosion, or volcanic eruption, atmospheric fallout and subject to chemical and biological diagenetic change *in situ.* Evidence indicates, however, that the majority of magnetic minerals are derived from allochthonous sources, i.e. the catchment (Thompson & Oldfield, 1986). Sediments also contain a proportion of weakly magnetic (diagenetic) material in the form of organic matter or calcium carbonate (Dearing, 1999). The magnetic signal preserved in lakes sediments has been used extensively to track changes in soil erosion and sediment sources (Dearing, 1990; Dearing *et al.*, 1990; Dearing *et al.*, 2001). Mineral magnetism can also be used to track productivity driven diagenesis (Oldfield, 1999) and in tephra studies (Walden *et al.*, 1991). In central México mineral magnetism has been used to track erosion and environmental in the Lago de Pátzcuaro Basin (O'Hara, 1993) and in Lake Chalco (Lozano-Garcia & Ortega-Guerrero, 1998). In Mexican lake sediments highest concentrations of magnetic minerals are found in tephra layers, where in Lake Chalco χ values reached 200 x 10^{-6} m^{-3} kg^{-1}, in contrast to lacustrine sediments which had values of between 0.2 and 20 (Lozano-Garcia & Ortega-Guerrero, 1998). O'Hara *et al.,* (1993) found similar results, with low χ corresponding to sediments rich in organic matter and calcium carbonate, i.e. materials classified as diamagnetic. In contrast clay units rich in ions such as Si, Al and Fe are generally low in organic matter and have higher χ values and are interpreted as erosion events. Magnetic susceptibility has also been used to track detrital inputs, of climatic or anthropogenic origin in La Piscina de Yuriría, and Laguna Zacapu (Metcalfe *et al.*, 1989).

4.7. Conclusions

Separation of the organic and elemental fractions of lacustrine sediment provides additional information which can provide support for the fossil biological record. Changes in the C/N ratio have been found useful in distinguishing between terrigenous, aquatic and algal sources. The C/N ratio has also been seen to be subject to diagenetic alteration and consequently requires consideration in conjunction with other proxies such as the $\delta^{13}C$. It is apparent that the $\delta^{15}N$ signal is complicated by numerous factors, both natural and anthropogenic. In terms of tracking changes in eutrophication the signal is complicated by the addition of human and animal waste, but also by the presence of cyanobacteria. As Teranes & Bernasconi (2000) note, however, the magnitude of shift caused by the influx of sewage is greater than can normally be expected due to natural variability. Cyanobacterial blooms are usually associated with eutrophication, however as Gu *et al.* (1996) point out not all productive lakes are dominated by cyanobacteria, consequently shifts in $\delta^{15}N$ to lighter values would have to be attributed to other factors. When interpreting stratigraphic changes in $\delta^{15}N$ therefore it is important to consider all potential N sources within the catchment, physical, chemical and biological conditions of the lake and to consider other proxy data. Shifts in the $\delta^{13}C$ signal to heavier values are usually associated with increased productivity, and can therefore be used to track natural annual and seasonal variability as well change due to anthropogenic activity. Variations in the carbon isotopic signature of lacustrine sedimentary organic matter can be attributed to factors such as temperature driven variance in fractionation, CO_2 limitation, pH and phytoplankton community composition. Values of $\delta^{13}C$ for lacustrine algae and C_3 land plants are however very similar and therefore separation of the signal requires an additional proxy, such as C/N (Meyers, 1994).

Organic matter is primarily by altered biochemical oxidation within the water column, and through resuspension and bioturbation even after sedimentation may still be subject to degradation. Once fully buried, however, diagenetic processes are slowed but some compounds are however, more susceptible than others. Sedimentary biomarkers such as *n*-alkanes and sterols are also subject to diagenesis, but are more stable than pigments and can therefore provide alternative and additional information on biological, physical and chemical characteristics of a lake and its catchment. Meyers & Ishiwatari (1993) note however that short chain hydrocarbons have a higher susceptibility to degradation than long chains, which has potential to hinder interpretation of the signal. The magnetic susceptibility signal has also been found to be subject to diagenesis, in response to eutrophication (Anderson & Rippey 1988), however determining this is beyond the scope of this project.

There are a number of complications involved in interpreting the chemical signature preserved in lacustrine sediment, however when a number of these approaches are used in conjunction, together with information on basin characteristics and additional proxy data, such as diatoms, it may be possible to overcome some of these difficulties and provide a reliable inference of the impact of changing nutrient availability on lake basins.

Chapter 5. Methodology

5.1 Introduction

To achieve the aims of this study an approach of modern diatom and water chemistry sampling and sediment coring was used. In the temperate regions sampling for diatom transfer functions is generally undertaken on a monthly or quarterly basis as this encompasses the full range of seasonality, i.e. summer, autumn, winter and spring. In this study a calibration set of the modern diatom flora and contemporary water chemistry was collected over two field seasons, wet and dry season, as this encompasses the full range of seasonality observed in Mexican lakes. Fieldwork was under taken between March and June 2003 (dry season) and July to September 2004, (wet season). The author acknowledges that one of the problems inherent to nutrient transfer functions is the seasonality in the availability of nutrients, which though increased sampling may be reduced, but the sampling regime applied to this study was the logistical maximum. Not enough is known about tropical lakes to determine seasonality more precisely, although ideally samples would have been taken on at least a monthly basis to cover this. Sediment cores were taken during the wet season.

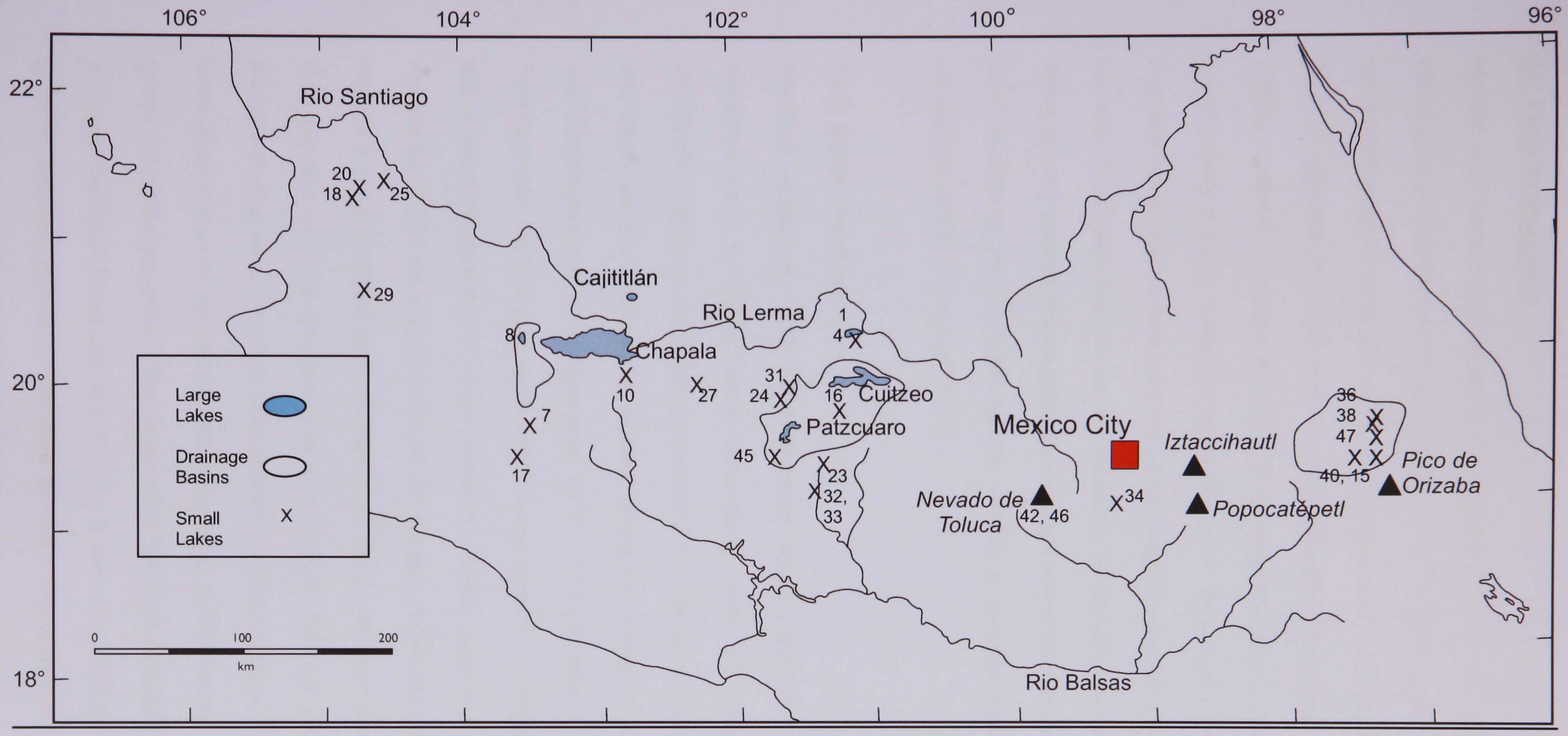
To create a numerical reconstruction of this change in nutrient status a wide nutrient gradient was required to capture as wide a range of species diversity as possible, thereby providing modern analogues for the fossil species. To track diatom species variation in response to nutrient variables, other environmental variables needed to be kept as constant as possible. Consequently only lakes on the TMVB were sampled, thereby variables driven by factors such as climate (temperature, EC and ionic composition) and geology (pH and ionic composition) remained similar. Thirty lakes were sampled across the Trans-Mexican Volcanic Belt (see Figure 5.1, Table 5.1) from the states of Puebla to Nayarit, between 97-105°W and 19-22°N. Thirty is generally considered to be the sample minimum required for a calibration data set, but to sample more would have required sampling lakes off the TMVB, this would have introduced further complexity through different geological and climatic conditions. Even though the data set would therefore be small it was decided to only sample lakes from the TMVB. These lakes were selected for sampling on the basis that they represent a variety of levels of human impact, providing as wide an environmental gradient as possible. Results will be broadly compared with modern results of Metcalfe (1985) and Davies (2000), however, as these studies lacked nutrient data, the nutrient concentrations from this study will be compared with available published data.

Modern sample sites are numbered and discussed based on their TP concentration, and are thus clustered into trophic categories, based on the OECD fixed boundary classification system (see Chapter 3), site codes can be seen in Table 5.1 below.

Table 5.1. Sample codes for calibration dataset.

Site	Code	State	Origin
Lago de Yuríria	1	Guanajuato	volcanic
Lago de Yuríria (vegetation)	2		
Lago de Yuríria (littoral)	3		
La Piscina de Yuríria	4	Guanajuato	Volcanic crater lake
La Piscina de Yuríria (vegetation)	5		
La Piscina de Yuríria (littoral)	6		
Zapotlan	7	Jalisco	Volcanic
Atototonilco	8	Jalisco	Volcanic
Atotonilco (vegetation)	9		
Los Negritos	10	Michoacán	Volcanic crater lake
Chapala	11	Jalisco	Volcanic
Cajititlán	12	Jalisco	
Cajititlan (vegetation)	13		
Cuitzeo	14	Michoacán	Volcanic
Tecuitlapa	15	Puebla	Volcanic crater lake
La Alberca (Teremendo)	16	Michoacán	Volcanic crater lake
Laguna la Maria	17	Colima	Volcanic
San Pedro	18	Nayarit	Volcanic
San Pedro (vegetation)	19		
Tepetiltic	20	Nayarit	Volcanic crater lake
Pátzcuaro Centre	21	Michoacán	Vvolcanic
Pátzcuaro centre (vegetation)	22		
San Gregorio	23	Michoacán	Volcanic
Zacapu	24	Michoacán	Volcanic
Santa Maria del Oro	25	Nayarit	Volcanic crater lake
Santa Maria del Oro (vegetation)	26		
Cámecuaro	27	Michoacán	Volcanic
Cámecuaro (vegetation)	28		
Juanacatlán	29	Jalisco	Volcanic
Juanacatlán (littoral)	30		
La Hoya de los Espinos	31	Michoacán	Volcanic crater lake
La Laguna	32	Michoacán	Volcanic
La Alberca (Tacambaro)	33	Michoacán	Volcanic crater lake
Zempoala	34	Morelos	Volcanic/glacial (?)
Zempoala (vegetation	35		
Alchichica	36	Puebla	Volcanic crater lake
Alchichica (vegetation)	37		
Quechulac	38	Puebla	Volcanic crater lake
Quechulac (vegetation)	39		
Atexcac	40	Puebla	Volcanic crater lake
Atexcac (vegetation)	41		
Lago del Sol	42	Estado de México	Volcanic crater lake
Lago del Sol (vegetation)	43		
Lago del Sol (littoral)	44		
Laguna Zirahuén	45	Michoacán	Volcanic
Lago de la Luna	46	Estado de México	Volcanic crater lake
La Preciosa	47	Puebla	Volcanic crater lake
La Preciosa (vegetation)	48		

Figure 5.1. Central México with sites



5.2. Field Methodology

Modern algal samples and epliminetic samples/measurements were taken from either the deepest part of the lake or, where this was not feasible, away from any sources of contamination and interference such as sewage outlets, vegetation or boat dockyards. Such an approach has been used and deemed suitable for shallow lakes by Bennion (1995). It should be noted that with increasing limnological research a degree of heterogeneity is being noticed in larger lakes (c.f. Alcocer and Bernal-Brooks, 2002), thus one epilimnetic sample may not necessarily be representative of the whole lake, but was all that was feasible on this occasion. All diatom samples were taken once, during the dry season (March 2003) and surface sediment samples were taken at the same location as the epilimnetic sample. In shallow well mixed lakes one sample was considered sufficient (Bennion, 1994).

5.2.1. Water chemistry

Electrical conductivity (EC), total dissolved solids (TDS) and temperature, were measured using a Mettler Toledo conductivity meter; pH using a Mettler Toledo MP120 pH meter; and depth using an ecosounder. Secchi depth measurements were also taken using a weighted secchi disk, taking the average of the depth where the disk disappeared and then reappeared. Up to 1.5 l of epilimnetic water was collected from each site, and analyses were carried out as soon as possible after collection. The author acknowledges that for certain variables, nitrogen species in particular, analyses need to be carried out in short order. In some cases, particularly lakes in more remote regions (i.e. Juanacatlan) up to 10 days could elapse between sampling and analysis. Until samples could be analysed they were kept as cold and dark as possible. The majority of sites were sampled once, however based on size, Cuitzeo was sampled twice (east and west) and Pátzcuaro three times (north, central and south), to take account of published spatial differences within the lakes. Despite the fact that Chapala is larger than both Pátzcuaro and Cuitzeo it was only sampled at one point, as it became logistically difficult to take more samples. The diatom preservation in samples from the west of Cuitzeo, and north and south of Pátzcuaro yielded poor results, therefore neither the diatom assemblage nor the water chemistry will be discussed in more detail. Alkalinity, silicates (SiO2), chlorides (Cl-) and phosphates (PO4) were also measure in the field using Hach kits, samples were all filtered prior to analysis using Whatman ashless filters and were allowed to come to room temperature. Analyses were performed according to test kit instructions. In addition to these analyses a note was also taken of altitude, latitude, longitude and of any human activity, vegetation, land use.

5.2.2. Modern Algal Sampling

Surface sediment samples were taken from all lakes and epilithic and epiphytic samples were taken where possible (see Table 5.1.). Surface sediment samples were the integral part of the modern data set as they constitute a spatially and temporally integrated sample (Bennion, 1994; Battarbee et al., 2001), including taxa derived from all habitats: littoral, planktonic and epiphytic. Therefore only the surface sediment samples are included and discussed in the statistical analysis (Section 6.7 and Chapter 7). The results of the analysis of the epilithic and epiphytic samples are presented in Section 6.6, but are not discussed further as they were not included in the statistical analyses. Surface sediment samples were taken using an Ekman dredge, from the deepest part of the lake, where possible, from which the upper ca. 0-1 cm was extracted and stored in polythene bags and kept cold and dark. It is acknowledged that in some cases, primarily the deepest lakes, that the top 0.5-1 cm is more likely to be representative of a period of up to and exceeding 10 years. For example, Davies (2000) showed a core from the deepest part of Laguna Zirahuén to have a sediment accumulation rate of 1 cm in 10 years while in the shallower lakes the top centimetre may be more indicative of the last few years. This cannot be accurately assessed as studies of sediment accumulation rates in Mexican lakes are limited. A further limitation to the methodology was the use of an Ekman dredge, as they are considered to be rather destructive (N.J. Anderson, pers. Comm., 2004) and does not ensure that the surface sediment is undisturbed. The author acknowledges this limitation, but it was the only feasible option at the time, a future alternative would be the use of a Kjack core which would ensure only the top 0.5 cm were extracted.

5.2.3. Sediment Coring

Laguna Zirahuén was chosen for the long term focus of the study for a number of reasons. This lake, although in the Purépecha heartland was not densely settled until around 300 years ago (Endfield, 1997), consequently it is almost unique in Mexican terms in being clear and relatively unpolluted. Furthermore Zirahuén has recently shown an abrupt shift to higher nutrient availability in both the limnological monitoring (Bernal-Brooks & MacCrimmon 2000b) and in palaeolimnological research (Davies 2000, Davies et al., 2004; 2005). Such data would have therefore provided a means of validating the predictive ability of the transfer function, to support any inferences made and to tentatively attribute a cause to changes in the diatom inferred productivity. A second set of cores were taken from Laguna Zacapu which lacked limnological monitoring (Metcalfe 1985; 1988), but which had extensive palaeolimnological data to test former inferences and again the correspondence between these and the diatom inferred nutrient inference. In addition this would allow the impact of human activity on lentic ecosystems to be assessed between 2 very different systems, affected by anthropogenic activity in different ways, but from the same geographic and climatic region.

Although several cores had already been taken from Laguna Zirahuén, and indeed one from Agua Verde itself, it was decided to take new material for the purposes of this study due to the lack of material from former studies and to ensure consistency between proxies. Two cores were to be taken from each lake as lipid geochemistry required between 2 and 5 grams dry weight of sediment to yield a good signal. This could still retain enough material for other analyses and to allow part of the core to be archived. Two sediment cores were therefore taken from Laguna Zacapu and three from Laguna Zirahuén during the wet season using a mini-Kullenberg corer with 1m, transparent PVC tubes, with 75 mm external diameter, attached to the coring head.

Cores were packed with oasis, to absorb water and minimize movement during transport and they were then sealed. Cores were kept as cold and dark as possible, until they could be section and frozen or kept in cold store at 4 °C.

At Laguna Zacapu cores were taken on the western side of the lake near Angostoura, in approximately 9m of water (ZAC1/04 and ZAC2/04). When these sediment cores arrived in the UK it was apparent that the sediments had been very watery, and that in transport the water had leaked from the core tube to no longer providing the sediment with support. The remaining material had therefore moved about in the core tube substantially. These cores could not, therefore, be used, as it could not be ensured that the stratigraphy had remained intact. They will, therefore, not be discussed further. The cores taken from Laguna Zirahuén were from the Agua Verde sub basin on the south west side of the lake. The first two cores (AV1/04 and AV2/04) were taken in approximately 12m of water in September 2004. Problems were also encountered with these two cores, when they were X-rayed and subsequently extruded it became apparent that the Paricutín tephra layer was at a 45 degree angle, implying either than the cores had been taken on a slope or that there had been movement of the upper, most recent part of the core in transport. In addition when diatom slides were made up using the upper most material no *Cyclotella ocellata* were identified, and as this core was taken in a similar depth of water to that of Davies (2000) it was assumed that the top of the core had either been lost or moved. As a result the third core was taken by S. Metcalfe in November 2004 (AV3/04) in 8m of water, using the same methodology.

5.3 Laboratory Methodology

5.3.1 Water chemistry

The majority of water chemistry analyses of nutrients and major ions, were carried out in Mexico at the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), (Morelia, Michoácan), in the Laboratorio de Limnología, FEZ Iztacala, (UNAM, Mexico

City) or at the Instituo de Geofisíca, (UNAM, Mexico City). Analyses undertaken were therefore performed using their standard protocols. The analysis of sodium (Na) and potassium (K) for all lakes sampled during the wet season were analysed on using ion chromatography (Metrohm 792 Basic IC) at the University of Nottingham. Dry season samples from Lago de la Luna, Lago del Sol and Laguna Zempoala were carried out at the University of Edinburgh, and analysis of total phosphorus (TP) for these lakes was carried out by Alex Kirika at the Centre for Ecology and Hydrology, Edinburgh. While it is acknowledged that carrying out the same analysis in several different laboratories is not ideal, it was the only logistical option at the time. All wet and dry season data ere presented in Appendix 1, average annual data are presented in Chapter 6 and discussed as average data though-out the rest of the thesis.

5.3.1.1 Ionic composition

Prior to analysis, all samples were allowed to reach room temperature and were, usually, filtered. Total water hardness was determined by titration with 0.01M EDTA. 25 ml samples were filtered and diluted to 50 ml using distilled water 1ml of standard buffer solution and 0.1 g endochrome black indicator were then added and titrated to an end point. Calcium (Ca) was determined using 25 ml of sample were used, diluted to 50 ml with distilled water, unless total alkalinity was greater than 250 mg l^{-1}, in which case 50 ml of sample was used. 1ml of sodium hydroxide was added followed by indicator and titrated to and endpoint using 0.01M EDTA. Magnesium (Mg) was subsequently calculated from hardness and Ca. Firstly results for total hardness and Ca were converted from mgl^{-1} to milli-equivalents per litre (meq l^{-1}) in the following ways.

$$\textbf{Hardness (meq } \mathbf{l^{-1}}\textbf{) = Hardness mg } \mathbf{l^{-1}} \textbf{ x 0.08199} \qquad \textbf{(Equation 1)}$$

$$\textbf{Ca meq}\mathbf{l^{-1}} \textbf{ = Ca mg}\mathbf{^{-1}} \textbf{ x 0.0499} \qquad \textbf{(Equation 2)}$$

Thus the concentration of Mg, in mgl^{-1}, is calculated from the results of equations 1 and 2 in the following way.

$$\mathbf{Mg\ (mg\ l^{-1}) = 0.12 \times (Hardness\ meql^{-1} - Ca\ meq\ l^{-1})} \qquad \textbf{(Equation 3)}$$

Filtered 50 ml samples were used for analysis of sulphates (SO_4). 5 ml of buffer solution and 0.1g of barium chloride solid were added to samples, which were then allowed to stand for 5 minutes to allow precipitation. Turbidity was then measured on a Milton Roy spectrophotometer set on 420 nm. Analyses of Mg^{2+}, Ca^{2+} and SO_4^{2-} for samples taken from Lago del Sol, Lago de la Luna and Laguna Zempoala were analysed at FEZ Iztacala using Hach test kits.

Potassium (K) and sodium (Na) analyses on acid treated samples, were analysed using flame spectrophotometry. Samples analyzed in Edinburgh were calibrated using standard K and Na solutions at 0.5, 1, 5 and 10 ppm. All data for major ions was converted from mg l^{-1} to meq l^{-1} for comparison with Metcalfe (1985) and Davies (2000) according to the conversion factors given in Hem (1970).

5.3.1.2 Nutrient analyses

Nitrate (NO_3), nitrite (NO_2) and ammonium (NH_4) were measured using a Perkin Elmer spectrophotometer, these were subsequently converted in to nitrate-nitrogen, nitrite-nitrogen and ammonium-nitrogen using standard equations. A filtered 50 ml aliquot sample was used for analysis of $NO_{3.}$ 1 ml of hydrochloric acid was added to each sample to adjust the sample pH. Concentration of nitrate nitrogen was measured on the spectrophotometer using UV light at 270 nm. NO_2 and NH_4 were both calculated colourimetrically. Two millilitres of colour reactive was added to a 50 ml filtered aliquot sample for NO_2-N. A pink colour was allowed to develop for 10 minutes before measurement. A 25 ml filtered sample was used for NH_4-N, to which 3 drops of mineral stabilizer, three drops of polyvinyl dispersing agent and one drop of Nesslers reagent were added and left for a pink colour to develop for one minute. Each sample was read three times and an average of these results taken.

Total phosphorus (TP) and soluble reactive phosphorus (SRP) are analyzed using the molybdate/ascorbic acid method, and all glass wear was acid washed prior to analysis. To analyses TP an unfiltered 50ml was used for digestion, to release the phosphorus bound into the suspended sediment, using 0.5 g of potassium persulphate solid or 8.0 ml of 5% potassium persulphate solution. Conical flask tops were tightly covered using aluminium foil. Samples were digested in an autoclave for 30 minutes. After digestion, samples were allowed to cool to room temperature then 10.0 ml of a reactive mixture of aqueous ammonium molybdate, sulphuric acid, ascorbic acid and potassium antimonial (see Lind 1985 for full details), was added to the digested sample. For SRP analysis, samples were filtered and 8.0ml of reactive mixture, plus 2 ml of deionized water, was added. On addition of the reactive mixture, both TP and SRP samples were allowed to stand for 10 minutes to allow colour development and were then measured using spectrophotometry at 880nm. Results are given in $\mu g\ l^{-1}$, based on a pre-installed calibration curve using a standard phosphate solution. As can be seen from data in Appendix One a number of sites yielded SRP values greater than TP, for logistical reasons it was not possible to re run these samples. As a result SRP analyses were not carried out on the wet season samples and were left out of the final dataset. It is acknowledged that SRP represents what is biologically available and therefore its inclusion in a calibration data set makes more sense than TP.

5.3.1.3 Chlorophyll-a

Chlorophyll-a (Chl-a) was performed at FEZ Iztacala, following the methodology of Arar and Collins (1997) outlined below. All equipment was washed using distilled water then 100% acetone. Up to 100 ml of sample were passed through a 45 mm glass-fibre filter paper (GF/F). Samples were dried and stored in paper towel and then in silica gel below 10°C, until required. Chl-a was extracted from the paper using 5ml of 90% acetone. The filter paper was cut into smaller pieces to make extraction easier, placed in a 50 ml glass tube. A Teflon plunger attached to a stable drill was used to disintegrate the filter paper. Care was taken not to over heat the solution, as this would aid degradation of the pigment (Arar and Collins, 1997). Once the filter paper had been reduced to a suitable point, the solution was transferred to a 10ml plastic centrifuge tube covered in black tape and another 5ml of 90% acetone was added. Samples were left for 20 hours at 4°C. After extraction, samples were centrifuged for 12 minutes at 1500 RPM. The extract was then decanted into a clean test tube. An aliquot of sample, between 0.05 and 2 ml, was diluted to 8 ml using 90% acetone and the volume of Chl-a was read, three times, using fluorimetry. A blank of 8 ml 90% acetone was read first for calibration. Extract volume, the volume of sample filtered the reading of each dilution, the dilution factor and the blank reading need to be recorded (for full details see Arar and Collins, 1997). Full results can be seen in Appendix 1.

The volume of Chl-α in the extract can be calculated in the following way

$$\mathbf{C_{eu} = Rb \times F_2} \qquad \textbf{(Equation 4)}$$

Where C_{eu} is the volume of Chl-α in the extract, Rb is the average fluorescence response and F_2 is the value of the blank.

Volume of Chl-α is then calculated thus;

$$\mathbf{C_{su} = \frac{C_{eu} \times \text{extract volume} \times DF}{\text{Sample volume}}} \qquad \textbf{(Equation 5)}$$

Where C_{su} is the volume of Chl-α in the sample, C_{eu} is the volume of Chl-a in the extract (as calculated above) and DF is the dilution factor, the volume by which the extract has been diluted to 8ml.

5.4. Diatom Preparation

Aquatic vegetation samples were prepared at FES Iztacala and stored in 20 ml glass vials for ease of transport back to the UK. Approximately 1g of vegetation was placed in 30-100 ml of hydrogen peroxide (H_2O_2) and then boiled. More H_2O_2 was added until all vegetation had dissolved. Samples were then transferred to centrifuge tubes and centrifuged at 1500 rpm for 4 minutes. After centrifuging the supernatant was decanted and replaced with distilled water and the process was repeated three times to ensure removal of all H_2O_2. Samples were then transferred to 15 ml glass vials for transport.

Surface sediment samples were prepared following the method of Battarbee (1986). Samples were air dried for 48 hours and a 0.5 g aliquot of this used for preparation. Air dried samples were placed in 250 ml conical flasks and ~10 ml of 30%, by volume, hydrochloric acid (HCl) was added to remove any carbonate present in the sediment. Conical flasks were placed on a hot plate to catalyse the reaction. Once samples had begun to effervesce they were removed from the heat. Any reaction to the HCl on initial addition, or on heating, was duly noted. Between 50 and 100 ml of H_2O_2, was added to each flask. Samples were subsequently placed back on the hot plate to catalyze the reaction between the organic matter in the sediment and the H_2O_2. Any material stuck together in large clumps was gently broken down using a clean stirring rod. Any reactions which were particularly vigorous were removed from the heat until the reaction calmed down. Once the reaction had ceased samples were left on the hotplate to reduce before more H_2O_2 was added. H_2O_2 was added until any reaction to new addition was minimal. The residual fluid in the flasks was allowed to reduce to approximately 30 ml. The addition of H_2O_2 only in some cases proved inadequate to reduce the amount of organic matter. To such samples approximately 10-20 ml of concentrated nitric acid was added and heated. Samples were again left to reduce to ~30 ml and allowed to cool before being transferred to a 50 ml centrifuge tube. Samples were centrifuged between 3 and 4 times to get rid of any residual acid and peroxide, at 2000 rpm for 10 minutes. After each time the supernatant was decanted off leaving the pellet in the bottom of the tube, tubes were then refilled using deionised water and re-centrifuged. After the final time the samples were diluted to 30 ml, using deionised water. A 1 ml aliquot of this was diluted further with 9 ml of deionised water. 19 mm round cover slips and slides were cleaned using petroleum ether and labelled 0.5 ml aliquots of the 1:9 dilutions using a Finipipette, were placed onto a cover slip and allowed to evaporate over night. Cover slips were mounted on to the slides using Naphrax® mounting agent. Any air bubbles were usually dispersed as the Naphrax® cooled, or were dispersed by gently putting pressure on the cover slip.

Samples from the third Zirahuén core (AV3/04) were prepared at a resolution of 1 cm for the top 10 cm of the core then every 2 cm between 12 and 40 cm, and every 4 cm thereafter (see Table 5.2.). The method of Renberg (1990), for preparing large batches of samples, was used. Samples were frozen, freeze dried and ~0.2 g aliquot was placed in a boiling tube, 1ml 10% HCl was added to remove any carbonates followed by ~1ml of 30% H_2O_2. Samples were covered and placed in a water bath at 80° for 1 hour, after this time another 1ml of H_2O_2 was added and sampled were left in the water bath for a further 2 hours. Samples were removed from the heat and left to settle for 3 days. Supernantent was then removed and the samples were washed with de-ionised water and left to settle again. This was repeated twice more to remove any traces of acid and peroxide. Samples were diluted at 0.25:9.75ml and were then mounted as above.

5.5 Microscopy

Slides were viewed under x1000 bright field or phase contrast using immersion oil on an Olympus BX 50 microscope (modern sediment samples) or Zeiss Axioscope 2 plus (core AV3/04 and vegetation samples). On some slides the concentration of diatoms and sediment made it difficult to count. These samples were diluted further until it was possible to see the majority of valves clearly. It has been shown that for a count of 100-200 valves the increase in the number of new taxa is high, whereas the increase in new taxa is minimal above 500 valves. Battarbee (1986) deems a count of between 400-600 valves adequate for analysis. Thus, in this study, a minimum of 400 valves were counted for each site. In some cases the diatom preservation was poor and thus a count of over 300 valves was made. Where it became apparent that one species was strongly dominant the count was increased to ~900 valves. A transect of each coverslip was made including everything within the field of view. A note was also made of any sponge spicules, chrystophyte cysts and mallomonas scales encountered as such species provide additional ecological information. Digital images were recorded of species using a JVC colour video camera and the Matrox Intellicam program. Images were stored as Tagged Image Format (TIF) files in ImageAxis. For larger species several images were taken and subsequently merged using Adobe Photoshop.

For Scanning Electron Microscope (SEM) work modern and core samples were diluted to 0.25ml in 10ml and placed on 12mm glass cover slips and attached to SEM stubs using carbon coated adhesive. All equipment was washed in ethanol. SEM stubs and cover slips were then sputter coated with gold. Modern diatom SEM images were taken on a Philips XL30-CP at the University of Edinburgh, School of GeoSciences, Grant Institute of Earth Sciences SEM facility and fossil images were taken on a FEI Quanta 600 at the University of Nottingham, School of Chemical, Environmental and Mining Engineering. A range of magnifications were used between 2000 x up to 33000 x, with a typical gun current of 20kv.

5.5.1 Diatom Identification

Diatom species were identified using the standard flora of Germain (1981), Krammer and Lange-Bertalot (1986; 2004a; 2004b; 2004c) and Patrick and Reimer (1966; 1979). Based on the similarity of Mexican flora to that of East African two flora published by Gasse (1980; 1986) on East African diatoms were also used. Identification of problem taxa such as *Stephanodiscus* and *Cyclostephanos* was aided by the use of a number of publications by Håkansson and others, in particular Håkansson (1986), Håkansson (1989) and Håkansson and Kling (1989). Identification under light microscope proved problematic for some species, problem samples and species were therefore studied under SEM (see above)

5.6. Statistical Analyses Of Species And Environmental Data – The Central Mexican Calibration Data Set

5.6.1. Introduction

To assess relationships between variation in the diatom species assemblages and environmental variables a number of statistical analyses were undertaken, namely hierarchical clustering (TWINSPAN), gradient analysis (CANOCO) and weighted averaging regression and calibration. The logic behind and processes involved in these analyses are discussed below and in detail in Chapters 6 (TWINSPAN) and 7 (Gradient analysis and weighted average regression and calibration).

5.6.2 Data analysis and normalization

The central Mexican calibration set consists of 30 surface sediment samples from 30 sites. For the purposes of ordination, 135 taxa present with a relative abundance of ≥ 1% have been included in this study. This approach has also been adopted by Tibby (2004) and retains ecological information, while reducing the impact of rare species. Taxonomic data sets are usually heterogeneous, noisy and often contain many zero values, noise can be reduced by transformation. The relative percentage abundance of taxon, over 1%, in this data set have been square root transformed to stabilized the variance (Bennion, 1994; Brooks, Bennion and Birks, 2001; Jones and Juggins 1995).

Seventeen environmental variables (annual averages) were included in the analyses: water depth at sample site, surface water temperature, pH, EC, $HCO_3^- + CO_3^{2-}$, Cl^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, $K^+ + Na^{2+}$, TP, NO_3-N, NO_2-N, NH_4-N, SiO_2, Chl-a and Secchi depth. The distribution of environmental variables was assessed SPSS for windows (ver. 11.0) to determine the degree to which data were skewed, relationships between variables and clustering of variables. If it was necessary to normalize the distribution, square root or $\log_{10}$ transformations were performed. Secchi depth, depth and SiO_2 were square root transformed and EC, TP, TN and Chl-a were log transformed. Some sites registered zero values for certain environmental variables (see Tables 6.1, 6.2 and 6.4). As zero values are not registered by CANOCO, half of the lowest number in that data stream was used as an alternative. Sites from the Nevado de Toluca and La Cuenca Oriental, excluding Alchichica, lack TP data for the wet season, their values are therefore based on dry season data only.

5.6.3. Cluster Analysis

Classification of large taxonomic data sets through cluster analysis, aids interpretation and can provide additional or supportive information for gradient analyses. It highlights taxonomic similarity and dissimilarity between sites, by identifying groups of similar individuals (Manly, 1986). In this study, a hierarchical polythetic divisive classification has been used, using the program TWINSPAN (Hill, 1979).

The aim of cluster analysis is to partition data into groups; TWINSPAN groups similar sites and species into clusters and arranges them into a hierarchy. TWINSPAN assumes that some species are representative of certain sites and consequently have a preference for one side of the dichotomy or the other (Van Tongeren, 1995), these differential species are however qualitative. Consequently for each species, pseudospecies are assigned based on presence or abundance at pseudospecies cut-levels, making the data quantitative. Cut-levels are chosen by the analyst to reflect the relative abundance of species in the data set and are thus subjective. The more abundant a species is, the more pseudospecies it will have. In this study pseudospecies cut levels were chosen to account for the high abundance of certain species at certain sites, and a high number of species in very low abundance and were set at 5%, 10%, 25%, 50% and 75% with a maximum of 6 levels of division, with a minimum of 5 species at each. Only the surface sediment samples were used and only species present in an abundance of greater than 1% at one or more sites were included in the analysis.

Initially all species and site data are grouped in one large cluster and are subsequently divided into smaller clusters based on species similarity between sites, to an end point where the data are still meaningful (Gauch and Whittakar, 1981). To create a dichotomy sample data are ordinated to achieve maximum dispersion between sites along correspondence analysis (CA) axis 1, the axis is then split in two at its centroid to form two new clusters. The groups formed are either termed negative (*0) or positive (*1). Second and subsequent divisions of clusters are performed using species abundance on the positive or negative side of the first dichotomy (i.e. *00 or *01 for group *0 or *10 and *11 for group *1). Sample data are therefore refined using species data, which when integrated are used to create a site-by-species table and provide an indication of taxonomic similarity and dissimilarity between sites.

5.6.4. Ordination/Gradient analysis

Ordination (gradient analysis) was performed using the programme CANOCO for windows (v 4.5, ter Braak) and CANODRAW for windows (v 4.0 Šmilauer). Correspondence analyses are sensitive to rare species as they exert undue influence over the model, such species can be given a low weight by CANOCO so that their influence is minimized and therefore all ordinations were run with rare species downweighting.

A principle components analysis (PCA) was initially run to determine relationships between variables. The environmental variables were entered into the analysis as species data, no variables or sites were excluded. Biological data were assessed following the methodology of Lotter *et al.* (1997). Detrended correspondence analysis (DCA) was used to determine whether linear or unimodal numerical techniques were appropriate for further analyses and to identify any outlying species or sites which exert undue influence on the data set. DCA was performed using the whole data set

(30 sites and 135 species), with detrending by segments, non-linear rescaling and downweighting of rare taxa. It was determined that gradient lengths were greater than 2SD, implying a unimodal species-response model, and thus that unimodal methods of gradient analysis were appropriate. Canonical correspondence analysis (CCA) was then used to establish which environmental variable(s) made a significant contribution to species variation.

Initially the statistical significance and explanatory power (marginal effects) of each individual variable was tested through a series of constrained CCAs, with each variable entered as the sole constraining variable. The percentage of variance in the taxonomic data set explained by each variable was calculated and its statistical significance assessed using an unrestricted Monte Carlo permutation test with 999 unrestricted permutations. Variables which were significant ($p < 0.05$) in explaining variation in the data set were then used in a CCA, performed without forward selection, to determine if any multi-collinearity existed between variables, any variables with variance inflation factors (VIFs) greater than 20 were removed sequentially until all fell below 20. CCA was then re-run with forward selection to determine which variables made an independent and significant contribution to diatom species variation. Statistical significance of the variables was tested using Monte Carlo permutation tests (999 unrestricted permutations). Partial CCAs and partially constrained CCAs were then run on the significant variables to assess a) the unique and independent contribution of each variable, b) the conditional variance between the variables and c) any unexplained variance (Borcard *et al.*, 1992).

Canonical correspondence analysis was also used to explore the relationship between changes in the diatom stratigraphy relative to changes in the other proxy data (Chapter 8, Figure 8.26). Simple CCA was performed with the fossil species data square root transformed and rare species downweighted, using CANOCO (ter Braak & Smilauer (1997-2004). Core samples (resolution from diatom stratigraphy) were used with an environmental data set consisting of isotopic changes, mineral magnetic analysis, C/N ratio, TOC, LOI and %N.

5.6.5. Weighted average regression and calibration

Weighted average (WA) regression and calibration for the construction of a TP transfer function was performed using C2 version 1.4 (Juggins, 2004). WA and WA partial least squares (WAPLS) were both run initially without cross validation, and then with jack-knife and bootstrap cross validation, the latter with 500 bootstrap cycles. Each analysis was initially run with all samples included, to detect any outliers, any identified outliers were then deleted from the model. Core AV3/04 was used as supplementary data to perform a reconstruction, with species >1% at one level included, where a reconstruction was performed on log TP.

5.7. Palaeoenvironmental analyses

5.7.1 Introduction

Prior to analysis core AV3/04 was x-rayed at the British Geological Society in Edinburgh, to detect any microscopic tephra layers. The core was then cut longitudinally, half of which was sectioned and immediately frozen for later biogeochemical analyses and the other half was stratigraphically described in terms of texture and colour, using a Munsel colour chart. Sampling resolution varied depending upon the proxy, due to time and cost constraints. A break down of this can be seen in Table 5.2.

5.7.2. Water and Organic matter content

The organic matter content, analysed every centimetre of the sediment from Agua Verde, Laguna Zirahuén (AV3/04) was assessed though LOI. Samples were weighed, dried overnight at 110°C and reweighed to calculate the water content of the sediment. These samples were then placed in a furnace over night at 550 °C to remove any organic matter. The organic matter content the difference between the weight of the sample before and after heating at 550°C.

Table 5.2 Matrix of depth and palaeoenvironmental analyses

Y = anaylsis carried out, X = analysis carried out but sample lost/contaminated

Depth	LOI	C_{org}	X_{lf}	Diatoms	Lipids	$\delta^{13}C$	$\delta^{15}N$	C/N
0	Y	Y	Y	Y		Y		Y
1	Y	Y	Y	Y	Y	Y	Y	Y
2	Y	Y	Y	Y	Y	Y		Y
3	Y	Y	Y	Y		Y	Y	Y
4	Y	Y	Y	Y	Y	Y		Y
5	Y	Y	Y	Y		Y	Y	Y
6	Y	Y	Y	Y	Y	Y		Y
7	Y	Y	Y	Y		Y	Y	Y
8	Y	Y	Y	Y	Y	Y		Y
9	Y	Y	Y	Y		Y	Y	Y
10	Y	Y	Y	Y	Y	Y		Y
11	Y	Y	Y			Y	Y	Y
12	Y	Y	Y	Y	Y	Y		Y
13	Y	Y	Y			Y	Y	Y
14	Y	Y	Y	Y	Y	Y		Y
15	Y	Y	Y			Y	Y	Y
16	Y	Y	Y	Y	Y	Y		Y
17	Y	Y	Y			Y	Y	Y
18	Y	Y	Y	Y	Y	Y		Y
19	Y	Y	Y			Y	Y	Y
20	Y	Y	Y	Y	Y	Y		Y
21	Y	Y	Y			Y	Y	Y
22	Y	Y	Y	Y		Y	Y	Y
23	Y	Y	Y			Y		Y
24	Y	Y	Y	Y	X	Y		Y
25	Y	Y	X			Y	Y	Y
26	Y	X	Y	Y		Y	Y	X
27	Y	Y	Y			Y		Y
28	Y	Y	Y	Y	Y	Y		Y
29	Y	Y	Y			Y		Y
30	Y	Y	Y	Y		Y	Y	Y
31	Y	Y	Y			Y	Y	Y
32	Y	Y	Y	Y	Y	Y		Y
33	Y	Y	Y			Y		Y
34	Y	Y	Y	Y		Y	Y	Y
35	Y	Y	Y			Y	Y	Y
36	Y	Y	Y	Y	Y	Y		Y
37	Y	Y	Y			Y		Y
38	Y	Y	Y	Y		Y	Y	Y
39	Y	Y	Y			Y	Y	Y
40	Y	Y	Y	Y	X	Y		Y
41	Y	Y	Y			Y		Y
42	Y	Y	Y			Y		Y
43	Y	Y	Y			Y		Y
44	Y	Y	Y	Y	Y	Y		Y
45	Y	Y	Y			Y		Y
46	Y	Y	Y			Y	Y	Y
47	Y	Y	Y			Y		Y
48	Y	Y	Y	Y	X	Y	Y	Y
49	Y	Y	Y			Y		Y
50	Y	Y	Y			Y	Y	Y
51	Y	Y	Y			Y	Y	Y
52	Y	Y	Y	Y	X	Y		Y
53	Y	Y	Y			Y		Y
54	Y	Y	Y			Y	Y	Y
55	Y	Y	Y			Y	Y	Y
56	Y	Y	Y	Y	Y	Y		Y
57	Y	Y	Y			Y	Y	Y
58	Y	Y	Y			Y		Y
59	Y	X	Y			Y	Y	Y
60	Y	Y	Y	Y		Y		Y
61	Y	Y	Y			Y		Y
62	Y	Y	Y			Y		Y
63		Y	Y					
64				Y				

5.7.3. Magnetic susceptibility

All samples from core AV3/04, were air dried to measure mass specific susceptibility, every centimetre (see Table 5.2). Low frequency susceptibility (χ_{lf}) was measured on a Bartington MS2B dual frequency sensor, high frequency (χ_{hf}) and thus frequency dependent susceptibility ($\chi_{fd\%}$) were not measured as previous studies of Agua Verde have shown that χ_{hf} has yielded negative results (Davies *et al.*, 2004). Magnetic susceptibility measurements of the air were taken in between each sample to determine the degree of equipment drift for the duration of the analysis. χ_{lf} was then determined by the following equations

$$\chi_{lf} = (\kappa-(A+C)/2)/(10/M)$$

Where K is the reading given by the MS2B for the sediment, A is the air susceptibility prior to measuring sediment, C is air susceptibility after measuring sediment and M is the air dried mass of the sediment used for analysis (Bartington 2004).

5.7.4. Carbon Isotopes, C/N and TOC

All samples for stable carbon isotope, total organic carbon and C/N analysis, (at 1 cm resolution, see Table 5.2) were air dried and then placed in 5% HCl overnight to remove any carbonates present in the sediment. Samples were then filtered and washed three times with 300 ml of deionized water to remove any residual acid and any chlorides formed. Sediment was removed from the filter paper and transferred to a watch glass and placed in an oven at 40°C overnight. Samples were then homogenized using a porcelain mortar and pestle and transferred to 14 ml glass sample vials for transport. An aliquot, between 5 and 10 µg of sediment, was placed in a tin capsule which was firmly sealed for subsequent TOC, C/N and $\delta^{13}C$ analysis at the NERC Isotope Geochemistry Laboratory at BGS, Keyworth, using the following procedure. Stable carbon isotope analysis, and related %N, %C, and C/N analyses, were carried out by combustion in a Carlo Erba NA1500 (series 1) on-line to a VG

TripleTrap and Optima dual-inlet mass spectrometer. Samples were placed in the carousel of the Carlo Erba and were sequentially dropped into a continuous flow of helium carrier gas, in a 1020°C furnace. A pulse of oxygen gas causes oxidation of the tin, ensuring full combustion of the sample. Product gases typically include CO_2, N_2, H_2O, SO_2, and possible traces of nitrogen oxides. CO_2 and N_2 remaining in the He stream are separated through a GC column, which allows N_2 to elute first by restricting the flow of CO_2, and pass through a detector which measures the gas concentrations by thermal conductivity to yield %C and %N of the sample. In each run two samples of a laboratory standard (broccoli) were analysed after each ten unknown samples. The $\delta^{13}C$ values of these laboratory standard's versus VPDB has been accurately determined by comparison with international calibration and reference materials (NBS). This allowed the $^{13}C/^{12}C$ ratios of the unknown samples to be converted to $\delta^{13}C$ values versus VPDB, results are presented per mil (‰) deviations relative to VPDB. Replicate analysis of well-mixed samples has indicated a precision of $\pm$<0.1‰ (1 SD). Atomic C/N ratios, determined from %C and %N, were determined by reference to an Acetanilide standard, replicate analysis of well-mixed samples indicated a precision of $\pm$<0.1.

5.7.5 Nitrogen Isotopes

Core samples for N-isotope analysis were frozen as soon as the sediment was extruded and were then freeze dried and homogenised. Samples were analysed every 2-4 cm (see Table 5.2). Aliquots (ca, 9 mg) of sample were placed in tin capsules and were tightly sealed. $^{15}N/^{14}N$ analysis was performed on a ThermoFinnigan 'Flash EA', Conflo III interface, comprising of an elemental analyser linked under continuous flow to a Delta+XL mass spectrometer. Samples were loaded on to a Carlo Erba carousel and were sequentially dropped into a continuous flow of helium gas. They then underwent oxidative combustion at 900 °C to release N_2 and CO_2 gas for $\delta^{15}N$ and C/N analysis. Isotope ratios were calculated as $\delta^{15}N$ versus atmospheric N_2 by comparison with a broccoli (freeze dried and homogenised) standard, which are run after every 10 unknown samples. Values of $\delta^{15}N$ for the samples are reported in per mil (‰) versus AIR and are calculated using the $\delta^{15}N$ values of the standards versus AIR derived by measurement against IAEA-N-1 and IAEA-N-2 (assuming their $\delta^{15}N$ vs AIR values = +0.4 and +20.3‰, respectively). Analytical precision of plant materials is typically <0.3‰ (1 S.D.).

5.7.6. Organic Lipid Geochemistry

Analysis of sedimentary biomarkers, such as sterols, alkanes and fatty acids, can be used to identify sources of OM (Meyers, 2003), such analysis was performed on core sediments (AV3/04) from Laguna Zirahuén in the Institute of Earth Sciences, School of GeoSciences, University of Edinburgh. Prior to extraction the core was stored at 4° C, the core was then sectioned at a 2 to 4 cm resolution (Table 5.2), samples were frozen, freeze dried and homogenized. Glass wear, glass wool, silica and alumnia were placed in a muffle furnace over night at 500 °C to remove any residual organic matter and were then rinsed three times using deionized water, methanol (MeOH) and dichloromethane (DCM). This method modified from that of Prartono and Wolff (1998) and Hanisch *et al.*, (2003) and from Wolff (Pers Comm., 2005). Unfortunately samples taken from 24, 40, 48 and 52 cm were lost due to spillage or contamination, which makes the resolution at the bottom of the core quite coarse.

5.7.6.1 Solvents and standards

The following procedure provides the extractable lipids: neutrals (*n*-alkanes, sterols and alcohols) and fatty acids only. Bound lipids were not analyzed in this study. Solutions of hexa-methylbenzene (HMB), a fatty acid mix (even carbon chains lengths of C_{14} to C_{24}) and an internal standard mix, consisting of a fatty acid (5β-cholanic acid), a sterol (β-sitosterol), an *n*-alkanes (hexatriacontane; C_{36}), an *n*-alcohol (octadecanol) and o-terphenyl, were made up in iso-octane to make solutions of ca. 100 mg l^{-1}. In addition, a 1000 mg l^{-1} solution of a DRO *n*-alkane mix (even carbon chain lengths of C_{12} to C_{28}) was used, diluted in iso-octane to 100 mg l^{-1}. For calibration, a 300 µl of solution was made up from equal volumes of HMB, the internal standard mix and the fatty acid mix or the *n*-alkane mix. Other solvents required were 6% KOH/MeOH, DCM extracted water, *n*-hexane, ultra clean (DCM extracted) HCl (6N), clean silica gel, 20% ethylacetate (ethylethanoate) in *n*-hexane (20%), sodium sulphate ($NaSO_4$) and bis-(trimethylsilyl)-trifluoroacetanide (BSTFA) for derivitisation. Ultra clean, DCM extracted silica and alumnia were also required.

5.7.6.2. Extraction

The core was prepared and run in two halves in order to reduce the potential for contamination, 0-18 cm and 20-56 cm. An aliquot of freeze dried sample, between 2 and 5 g was placed in a Teflon centrifuge tube to which a spike of 50 µl of internal standard mix added and then compounds were extracted using 15 ml of 1:1 MeOH:DCM, vortex mixed and placed in an ultrasonic bath for 15 minutes and then centrifuged at 3000 rpm for 15 minutes. The supernantant was transferred to a clean pear shaped flask and the process was repeated a further two times, i.e. three extractions. As in this study, a number of other studies have used the ultrasonic extraction process with success (e.g. Gomes & Azevedo 2003; Hanisch *et al.*, 2003). An alternative, but more time consuming, technique is Soxhlet extraction (Fisher *et al.*, 2003; Jeng & Huh, 2004; Prartono & Wolff 1998), which as is perhaps more effective, which may remove more of the lipids from the sediment. As yet there does not appear to be any evidence to show whether one technique is more appropriate than the other. Furthermore access to Soxhlet extraction was not possible during this study.

Pear shaped flasks (now containing ~45 ml of extracted material) were placed on a rotary evaporator in a water bath set at 30°C, to evaporate the solvent, until the supernatant was reduced to between 1 and 2 ml. The extract was transferred to a glass scintillation vial, which had been rinsed in DCM. To dissociate the fatty acid fraction (i.e. from COOH to COO^- and H^+), the extract was hydrolysed with 2ml of 6% KOH/MeOH, the reaction was catalysed by placing the samples in a heating block at 60°C for 1 hour. The neutral fraction was extracted with *n*-hexane (4x1 ml), samples were agitated then allowed to settle and the supernatant removed to a clean scintillation vial and the solvent was reduced to dryness under N_2 gas to remove residual hexane. The fatty acids were then recovered through acidification with 70 µl of clean (DCM extracted) 6N hydrochloric acid (HCl). The fatty acids were then extracted using hexane (4x1ml) in the same way as the neutral fraction. The acid fraction was then cleaned up over a silica column, in a Pasteur pipette, with 20% ethylethanoate in hexane as the eluent and the residue dried down under N_2 gas. It had been noted that the saponification step using KOH/MeOH to dissociate the acid fraction and then re-acidifying after the neutral fraction has been removed with HCl, has often not work as the acids may remain in the resultant emulsion (George Wolff pers. comm., 2005). Indeed the recovery of the acid fraction in this study was poor and therefore will not be discussed further. An alternative, to retain the acid fraction, is to use the total lipid extract, although this may overload the GC column detector, but should yield the fatty acid compounds. The methodology used by Fisher *et al.* (2003), which used a similar method of fractionation, using KOH in methanol, but left for 24 hours to ensure full dissociation, followed by use of a methylating agent (BF_3-MeOH), yielded good recovery of fatty acids and may represent a better alternative for future analyses.

The neutral fraction was run on the GC-FID in two sections, the upper section (0-18cm) was made up with 1ml of HMB and 1ml of iso-octane, 50 µl of this solution was removed and derivitised with BTSFA in the same way as before. These samples were then run on the GC-FID, and the remaining sample dried down under N_2 gas and frozen for preservation. The lower section of the core (20-56 cm) was run separately and samples were brought into solution in HMB and iso-octane, with HMB diluted from 144 mg l^{-1} to 96 mg l^{-1} (2:1 ratio with iso-octane). And were then derivitised with an equal volume of BSTFA, as before. Due to problems with calibration of the neutral fraction, upper core samples were run for a second time at a later date (April, 2005). They were brought into solution from frozen with an equal volume (250 µl each) of iso-octane and HMB (50% dilution of original 144 mg l^{-1} solution), and were then derivitised with BTSFA. The core was split into two parts to reduce risk of contamination, and then run on the GC-FID, and as noted above the upper samples were run twice. Replicates from bottom were, however, not re-run when the top was run on the GC-FID for the second time. Ideally, given more time all samples would be run in one batch to assure standardisation of GC conditions or bottom samples run with the top, therefore allowing for an assessment of discrepancies.

Initial GC-MS analysis indicated that the yield of simple *n*-alkanes was poor, these compounds often co-elute with other compounds and are therefore obscured, but they are also usually only present in low concentrations (Wolff pers. comm. 2005). An additional step was therefore used to extract the *n*-alkanes, from the neutral extract. Two to three drops of DCM was used to bring the extract back into solution, from frozen. Alumina (63-230 mesh, neutral grade Merck, soxhlet extracted and placed in a muffle furnace overnight to remove moisture and cooled to room temperature) was added to the sample so that all the extract covered the alumina. Samples were then left to air dry, so all the moisture evaporated. A small Pasteur pipette was blocked with clean glass wool and filled with 3cm of alumina, which was then covered by clean $NaSO_4$ powder The sample, now adhered to the alumina, was transferred to the column (one for each sample). To recover the n-alkanes 2ml of hexane was eluted through the column. The n-alkane eluent was dried, under N_2, and then brought back up in a 1:1 ratio of iso-octane and HMB, 100 µl of this solution was then analysed on the GC-MS to identify peaks and retention times. The remaining neutral fraction was recovered using a DCM/MeOH mix in ratio of 1:1, 2ml of which was eluted through the column, this was then dried down under N_2 gas and frozen.

5.7.6.3 Analysis

Samples were analysed using a gas chromatography-flame ionization detector (GC-FID) and then subsequently on a GC-mass spectrometer (GC-MS). GC-FID analysis was carried out on a Hewlett Packard 5890-A with a split/splitless (on-column) injector fitted with a silica column (30m x 0.32mm) with helium as the carrier gas. The oven temperature was set at an initial temperature of 75 °C and programmed to rise to 320 °C after 1.5 mins at a rate of 6 °C/min, the oven temperature was then held at 320 °C for 18 min. Data were recovered using Chrom Perfect Spirit version 4.4.2.2 (Justice laboratory software, 1998). The GC-MS was carried out on a Hewlett Packard 5890-A with a split/splitless (on-column injector) which was programmed with the same conditions as the GC-FID. The GC-FID was used to quantify compounds using an internal calibration in Chrom Perfect (1998), while the GC-MS was used to identify peaks. Identifications were made by comparison of relative retention times and mass spectra of the analytes and the internal standards, and by identification of analyte total ion chromatograms (TICs) when compared with the library software (Wiley Library 275) and published results.

Five internal standards were used in this study to aid quantification of the different compound classes, as it cannot be guaranteed that they will behave in the same way (Greg Cowie pers. comm 2004), Hexamethylbenzene (HMB) was used as the quantification standard, added immediately prior to injection onto the GC column. Some problems with these standards were identified during the quantification step. As this methodology was partially based on one used for extraction of lipids in a marine setting internal standards were included that in the marine realm are either naturally not present or present in negligible amount. In lacustrine settings however β-sitosterol is recovered, often in high concentrations (Gladu *et al.*, 1991). As a result this compound could not be used to quantify sterol behaviour. The use of HMB as a quantification standard, in lacustrine settings is unusual and has been noted as being unsuitable due to its volatility (G. Wolff pers. comm., 2005). Consequently unknown compounds in this study had to be quantified using the ratio of original amount to recovered amount of other internal standards (in this case o-terphenyl). There are inherent problems with this as if recovery is poor it can lead to large errors and therefore does not give an indication any loss. It is recommended that for further analysis alternative internal standard compounds are used, such as 2,21-dimethyldocosane or 5α-chloestane which are known not to occur naturally and have been used elsewhere (Fisher *et al.*, 2003; Muri *et al.*, 2004 respectively) and that an alternative, more stable, recognised compound is used for quantification, such as 5α-cholestane (Fisher *et al.*, 2003).

Chapter 6: Modern Limnology

6.1 Introduction

The aim of this chapter is to present the water chemistry data and corresponding diatom flora, which constitute the calibration set of 30 lakes. These data will be used to make linkages between changes in the epilimnion and diatom species composition to establish which variable(s) are significant in driving variation. Data presented here are the mean of the wet and dry season data, for separate wet and dry season data see Appendix 1. Sites are discussed and numbered based on trophic status classification, using the OECD fixed-boundary system, discussed in Chapter 3. Site codes can be seen in Table 5.1.

6.2 Previous Limnological Research

Limnological investigation in central México has mainly focused on the three largest and thus most important lakes: Chapala, Cuitzeo and Pátzcuaro. Initial studies date back to 1940, when de Buen, based at the Estación Limnológica at Pátzcuaro, undertook a detailed study of Lago de Pátzcuaro's depth and temperature profiles, zooplankton and phytoplankton populations. This was succeeded by more in-depth work into all aspects of limnology of Pátzcuaro and Zirahuén (de Buen, 1941; 1943; 1944a; 1944b) but has not been systematic since that time. Numerous studies have been carried out over the last 20 years on Lago de Pátzcuaro (Alcocer & Bernal-Brooks, 2002; Chacón-Torres, 1993a; Chacón-Torres *et al.*, 1991) and Laguna Zirahuén (Chacón-Torres & Muzquiz-Iribe, 1991; Bernal-Brooks, 2000a; 2000b) focusing on aspects such as nutrient availability and water quality, water balance and phytoplankton. Limnological data for Lago de Chapala date back to Chávez (1973) who focused on climate, hydrology, flora and fauna. Recent work on Chapala has concentrated more on annual phytoplankton variability, nutrients and nutrient limitation (Lind *et al.*, 1992a; Lind *et al.*, 1992b; Lind & Dávalos-Lind, 2002). Other lakes in central México have also been the foci of more recent paleaolimnological and limnological research, such as Alchichica (Alcocer & Lugo, 2003) and the Lago del Sol and Lago de la Luna on the Nevado de Toluca (Loffler, 1972; Banderas & Gónzalez, 2002; Alcocer *et al., in press*).

Other projects, which ultimately had a longer term palaeoenvironmental or palaeoclimatic goals, have also undertaken studies of modern water chemistry in order to provide a basis for understanding changes in the palaeo-record, through the provision of modern analogues. These have helped to widen the available information on lakes in Central México (Metcalfe 1985; Caballero, 1995; Davies 2002), but have lacked comprehensive nutrient data. Despite these studies, data on Mexican lakes are still limited. Limnological conditions of several lakes are monitored, both with measurements made in the field and in the laboratory: Alchichica, in the state of Puebla, is measured on a monthly basis by researchers at FEZ Iztacala, UNAM and Pátzcuaro and Zirahuén, in Michoacán, are also monitored on a regular basis by UMSNH, Morelia. There is, however, a lack of data for the majority of lakes in the states of Jalisco, Colima and Nayarit. The available data do provide a good long-term background for comparison with the following water chemistry data.

6.3 Contemporary Limnological conditions

6.3.1 Sample Sites & Physical Characteristics

All lakes sampled (see Figure 5.1, Table 5.1) lie within the Trans Mexican Volcanic Belt (TMVB); between 21 and 19° N and 105 and 97°W and are all located between altitudes of 700 and 4,206 m asl. (Table 6.1). Due to their tectonic and volcanic origin the majority of lakes are closed. Surface area and volume have not been measured during the course of this study, but were noted to vary considerably. Depth ranged between 0.2 and 53 m (see Table 6.1). Samples were taken from near the edge of La Piscina de Yuriría and Cuitzeo, thus the given depth is for the sample site rather than the maximum of the lake. The crater lakes tend to be the deepest, with the exception of Tecuitlapa, (3 m) and La Piscina de Yuriría, both of which are subject to human modification of water balance through groundwater extraction and spring diversion (see Plate 6.1). The remainder of the lakes sampled were formed by tectonic uplift or lava damming (see Plate 6.2) and are, with the exception of Zirahuén, shallow (between 1-22 m). Increased pressure on water resources, through population growth and anthropogenic modification of basins in central México, has had an effect on lake depth through groundwater abstraction and possibly sediment influx. Laguna Zirahuén now has a measured depth of 40 m (Bernal-Brooks & MacCrimmon, 2000a), a decline from 1942 when de Buen (1943) recorded the depth as 46 m. This decline has been associated with a combination of sedimentation through catchment erosion and climatic changes (Davies *et al.*, 2004). Similarly a decline in the maximum depth of Lago de Pátzcuaro has also been noted, declining from 14.4 m (de Buen, 1944) to 9.4 m (Alcocer & Bernal-Brooks, 2002). Such factors must be borne in mind when considering depth and volume variation between lakes, as variations may not necessarily be ascribed to climate alone.

As a result of altitude, temperature is modified in relation to that of other tropical lakes, consequently there is a strong relationship (r^2 = 0.79) between the two variables (Figure 6.1). Averaged surface water temperatures range between 10.5 and 30.1°C (see Table 6.1). The lakes of the Nevado de Toluca were the coolest with temperatures of 10.55°C, Lago de la Luna (46) and 11°C in Lago del Sol (42) respectively, associated with their high altitude, 4,200 m.a.s.l. Highest temperatures were found in Sta. Maria del Oro (25), Atotonilco (8), Los Negritos (10) and La Laguna (32), due to their lower altitudes.

Table 6.1 Field data (annual mean; wet and dry)

Site	Site Number	Longitude (°W)	Latitude (°N)	Altitude (m.a.s.l.)	Depth (m)	Surface Temperature (°C)	pH	EC (µS cm^{-1})	Secchi depth (m)
Lago de Yuríria	1	101 10 54	20 15 30	1740	1.8	19.1	8.39	657	0.19
La Piscina de Yuríria	4	101 07 14	20 12 09	1730	0.2	20.85	9.56	2908	0.085
Zapotlan	7	103 28 05	19 46 00	1570	3.6	23.45	8.66	793	0.265
Atotonilco	8	103 40 27	20 23 33	1367	1	28.9	8.9	3286.5	0.02
Los Negritos	10	102 36 52	20 03 34	1566	7.8	28.55	7.49	3932	0.705
Chapala	11	103 19 03	20 17 14	1551	1.4	24.85	8.37	966.17	0.23
Cajititlán	12	103 18 40	20 42 05	1519	1.8	25.85	8.44	919.3	0.17
Cuitzeo	14	100 51 18	19 56 22	1551	0.5	28.1	9.3	2651	0.045
Tecuitlapa	15	097 32 34	21 19 15	2360	2.5	22.05	8.61	1402	0.185
La Alberca (Teremendo)	16	101 14 59	19 41 21	1892	9.9	19.05	8.54	381	0.3
Laguna La Maria	17	103 42 10	19 27 15	1240	21	24.85	8.34	620	1.08
San Pedro	18	104 43 22	21 11 50	1265	7.3	25.45	8.84	329.15	0.39
Tepetiltic	20	104 41 00	21 19 15	1410	3	23.95	8.05	147.58	1.89
Pátzcuaro (c)	21	101 37 00	19 32 43	2044	5.1	22.35	9.56	1017	0.335
San Gregorio	23	101 38 42	19 33 45	3100	1.8	18.4	6.94	21.05	0.44
Zacapu	24	101 45 04	19 40 10	1980	3.6	20.75	8.5	146.05	0.97
Sta Ma. Del Oro	25	104 33 51	21 21 53	700	53	30.15	8.77	1440	5.585
Cámecuaro	27	102 12 33	19 54 10	1551	2.15	21.55	7.36	189.94	2.15
Juanacatlán	29	104 44 00	20 37 17	1995	22	20.5	7.41	125.02	2.615
Hoya de los Espinos	31	101 46 03	19 54 09	1980	40.1	22.8	8.53	1029.5	5.15
La Laguna	32	101 28 28	19 12 05	1487	5.8	28.45	8.31	108.65	0.525
La Alberca (Tacambaro)	33	101 27 43	19 12 43	1452	35	27.1	8.34	166.4	0.865
Zempoala	34	099 18 52	19 02 53	2808	4.75	18.65	7.5	79.15	2.065
Alchichica	36	097 23 49	19 24 41	2325	64	20.13	8.92	13210	5.505
Quechulac	38	097 20 56	19 22 23	2344	40	20.38	8.63	725.5	5.905
Atexcac	40	097 27 14	19 19 55	2359	39	19.78	8.43	11676	4.875
Lago del Sol	42	099 45 10	19 06 11	4200	11	11	6.92	59.17	6.3
Laguna Zirahuén	45	101 45 13	19 25 52	2075	40	22.15	8.41	114.05	4.43
Lago de la Luna	46	099 45 08	19 06 26	4206	8	10.55	5.20	16.95	7.85
La Preciosa	47	097 22 56	19 22 27	2337	45	20.13	8.49	2004.83	3.41

Plate 6.1 La Piscina de Yuríria, spring water being pumped back into the lake

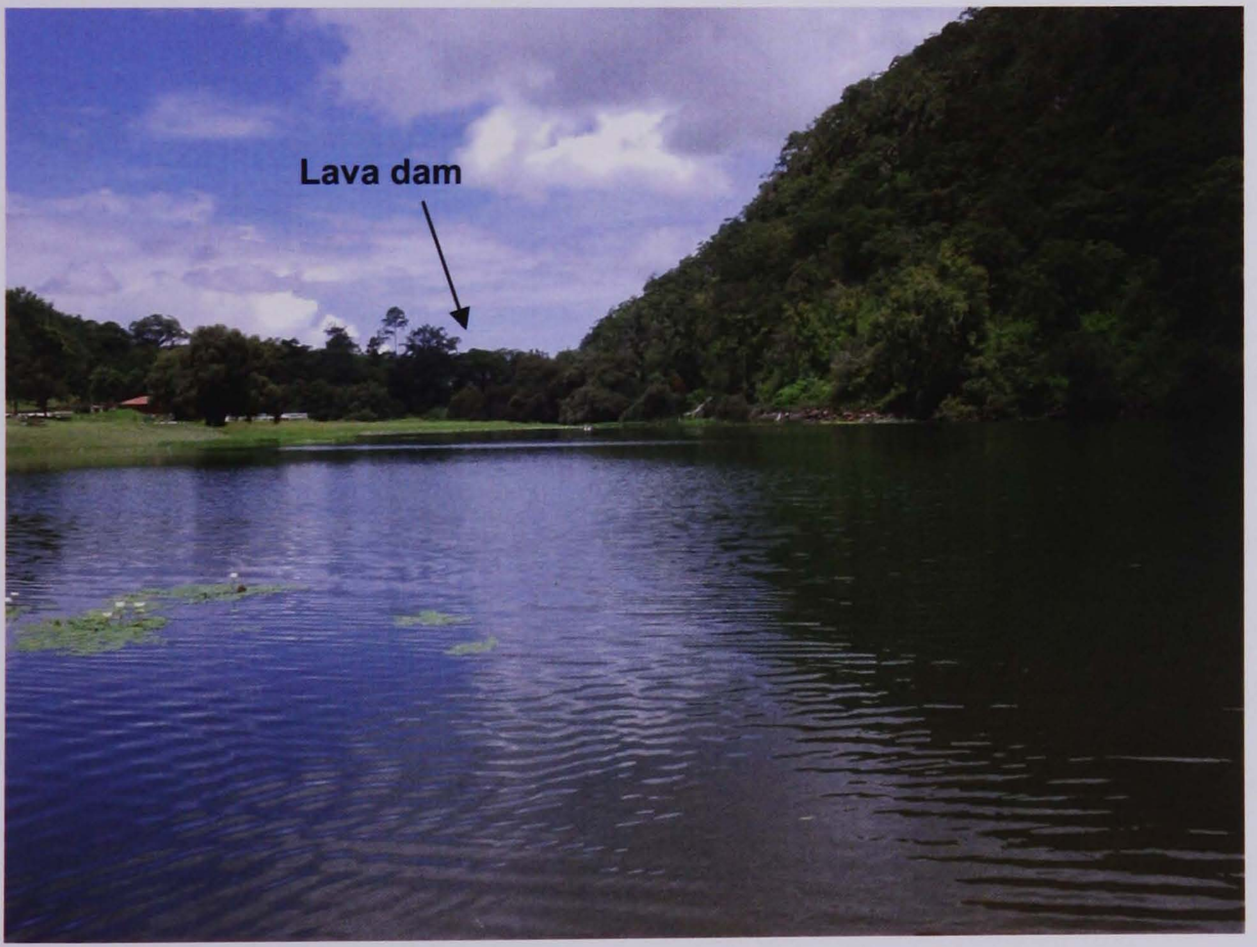


Plate 6.2 Lava dam resulting in the formation of Laguna Juanacatlán

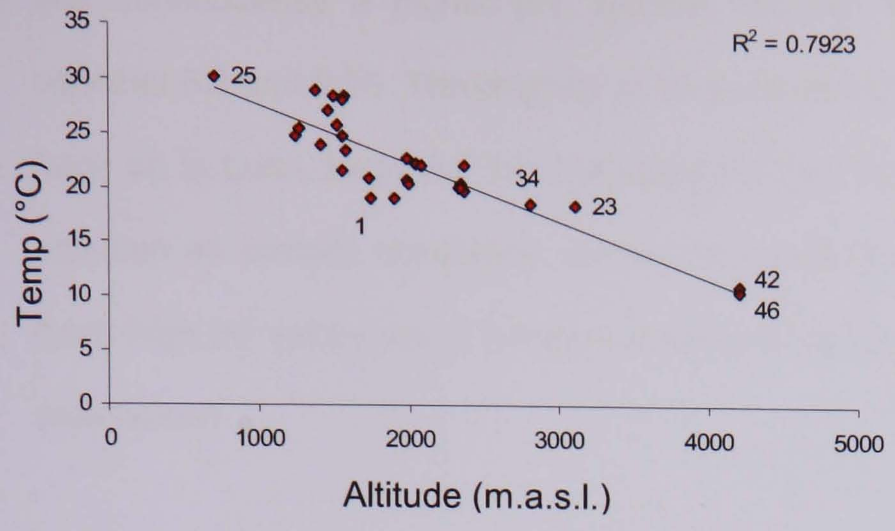


Figure 6.1 Altitude vs. temperature

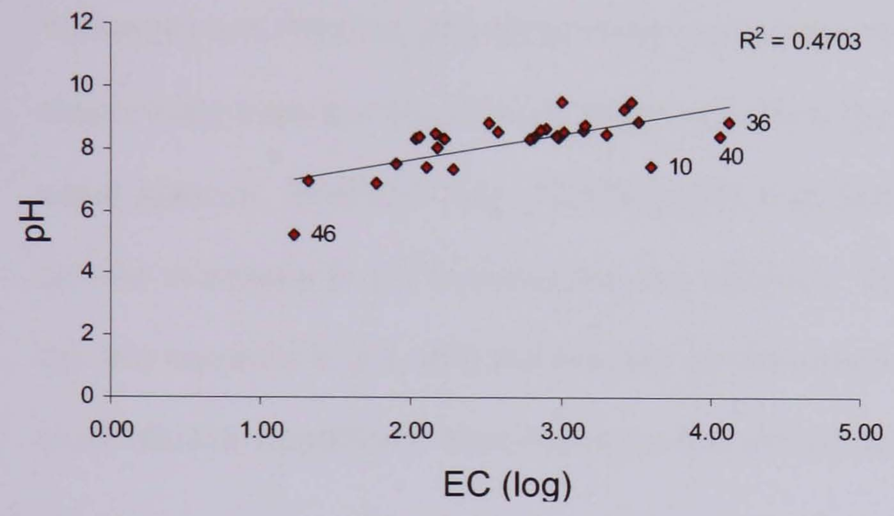


Figure 6.2. Electrical conductivity (log transformed from µS cm^{-1}) vs. pH

6.3.2 Salinity & pH

Electrical Conductivity (EC) is a measure of the ionic concentration of water, primarily the major cations and anions (discussed in greater detail below). Conductivity increases as a function of increasing ionic concentration. The range in EC (see Table 6.1), is between 16.95 µS cm^{-1} for Lago de la Luna (46) and 13,210 µS cm^{-1} in Alchichica (36), with an average of 1704 µS cm^{-1}. Alchichica and Atexcac (40) both display EC values an order of magnitude greater than other lakes in the data set. Only 4 sites have an EC value below 100 µs cm^{-1}: San Gregorío (23), Lago de la Luna (46), Lago del Sol (42) and Zempoala (34).

The relationship between pH and EC (Figure 6.2) is positive with an r^2 value of 0.47. In general, lakes with a high pH have a high EC, associated with higher ionic concentration and thus higher concentrations of HCO_3 and CO_3, which drive alkalinity, and consequently a higher pH. Central Mexican lakes have pH values ranging between 5.2 and 9.56. The majority of lakes have a pH above 7, with the exception of Lago de la Luna, Lago del Sol and Lagunilla San Gregorío, which are at the highest altitudes so climatic conditions, surrounding soils and vegetation act to reduce pH. Such high pH values are a function of dominance by carbonate and bicarbonate ions (see below)

Through dilution during the rainy season, a decline in EC is expected between the dry and the wet season. This occurs with a few exceptions, Tecuitlapa (15), La Piscina de Yuriría (4) and Atexcac (40) show large increases in EC, 2776, 1003 and 950 µS cm^{-1} respectively from the dry season, which may be a factor of human modification of the water balance. Zirahuén (45), Tepetiltic (20) and San Pedro de Lagunillas (18) show smaller increases in EC between the two seasons. Variations are also noted between the two seasons in pH, with the majority of sites showing an increase in pH, a shift to more alkaline conditions, from the dry to the wet season, most likely due to dilution.

6.3.3 Cation & Anion Composition

The ionic composition of water is dominated by 8 principle solutes: bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), chloride (Cl^-) and sulphate (SO_4^{2-}) anions and potassium (K^+), sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations (Eugster & Jones, 1979). Other ions such as nitrogen species, phosphate and silicate ions are also important, but to a lesser degree. The relative concentrations of such ions are determined by the composition of underlying bedrock, precipitation and subsequent modification by ionic fractionation processes such as evaporation and though anthropogenic activity such as groundwater abstraction. Ionic composition can, therefore, vary between regions and basins, within the same geological region

Annual average alkalinity ranges between 0.1 meq l^{-1}, in Lago de La Luna (46) to 26.8 meq l^{-1} in Atexcac (40), see Table 6.2. There is a positive correlation, r^2 = 0.66, between alkalinity, log transformed, and pH (Figure 6.3), related to the increase in HCO_3^- and CO_3^{2-} with increasing pH.

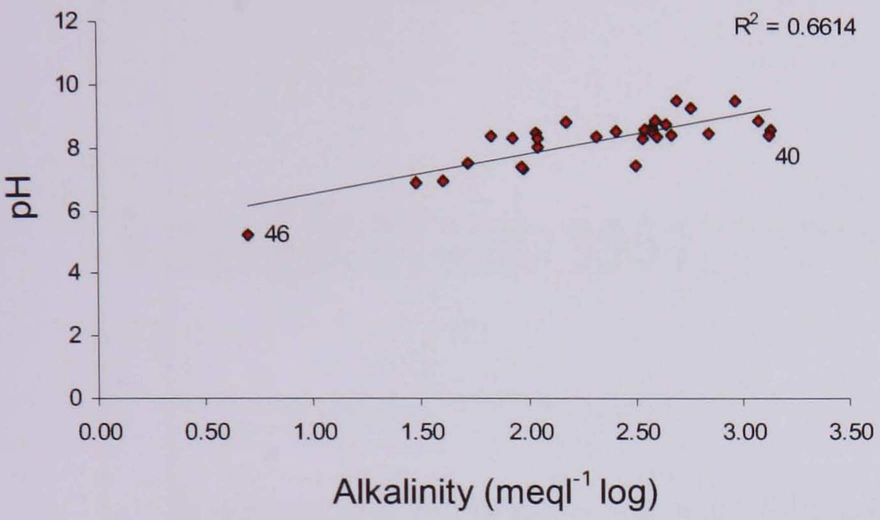


Figure 6.3. Alkalinity (log transformed from meq l^{-1}) vs pH

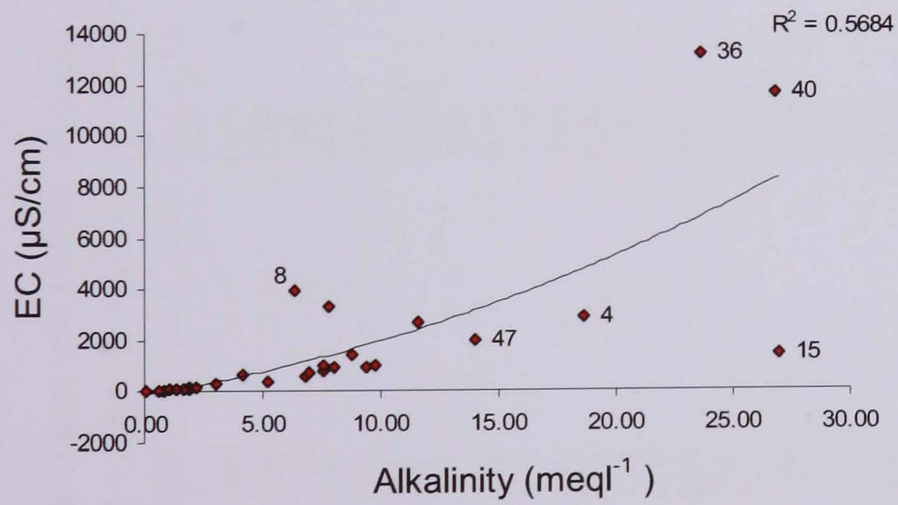


Figure 6.4 Electrical conductivity vs. alkalinity

Table 6.2 Ionic data all in meq l^{-1}(annual mean; wet and dry season)

Site	Site Codes	$HCO_3^- + CO_3^{2-}$ (Alk)	Cl^-	SO_4^{2-}	K^+	Na^+	Ca^{2+}	Mg^{2+}
Lago de Yuríria	1	4.2	2.28	0.08	0.62	5.19	1.62	2.5
La Piscina de Yuríria	4	18.6	11.44	1.26	1.59	94.85	2.72	2.39
Zapotlan	7	7.6	3.12	0.17	0.73	5.17	1.4	0.74
Atotonilco	8	7.8	5.26	0.83	0.81	219.05	1.36	3.27
Los Negritos	10	6.4	28.83	0.75	1.85	280.28	1.04	1
Chapala	11	8	3.54	0.21	0.79	7.68	2.08	2.06
Cajititlán	12	9.4	3.91	1.3	0.97	10.95	2.24	2.04
Cuitzeo	14	11.6	15.39	0.51	1.23	47.88	4	2
Tecuitlapa	15	27	6.6	0.85	1.01	12.90	2.02	1.76
La Alberca (Ter)	16	5.2	0.74	0.09	0.38	1.98	1.26	1.28
Laguna La Maria	17	6.8	1.27	0	0.08	2.48	1.6	2.71
San Pedro Lagunillas	18	3	1.34	0.04	0.69	1.7	0.98	0.58
Tepetiltic	20	2.2	0.58	0.06	0.22	0.29	3.23	2.07
Lago de Pátzcuaro	21	9.8	1.98	0.06	0.97	9.04	1.12	1.68
Laguna San Gregorio	23	0.8	0.5	0.09	0.05	0.11	0.39	0.68
Laguna Zacapu	24	2.18	0.59	0.09	0.54	0.54	0.62	1.24
Sta Maria del Oro	25	8.8	13.22	0.04	0.47	15.10	1.62	5.24
Camecuaro	27	1.9	0.66	0.15	0.07	0.83	0.56	1.46
Juanacatlán	29	1.88	0.46	0.10	0.05	0.4	0.81	0.75
Hoya de los Espinos	31	7.6	10.41	0.03	0.57	6.54	1.96	4.13
La Laguna	32	1.7	2.04	0.47	0.15	1.35	0.42	1.24
La Alberca (Tac)	33	2.2	0.66	0.04	0.09	0.33	0.76	1.4
Laguna Zempoala	34	1.05	0.36	0	0.04	0.36	0.55	1
Alchichica	36	23.6	117.69	1.14	4.42	934.70	11.08	32.34
Quechulac	38	7	4.06	0.25	0.18	4.29	2.10	4.59
Atexcac	40	26.8	132.81	1.4	2.24	530.77	14.57	30.59
Lago del Sol	42	0.6	0.28	0.04	0.29	0.06	0.36	0.99
Laguna Zirahuén	45	1.35	0.42	0.09	0.3	0.32	0.68	0.78
Lago de la Luna	46	0.1	0.2	0.02	0.02	1.02	0.61	0.43
La Preciosa	47	14	10.29	1.09	0.41	12.23	1.31	15.56

There is also a strong positive relationship between increasing EC and increasing alkalinity (Figure 6.4), as increasing conductivity implies an increase in concentration of the major ions, such as those responsible for the increase in alkalinity. Increased evaporative concentration can cause a shift from CO_3^{2-}-HCO_3^- domination to Cl^- domination (Eugster & Jones 1979). Alchichica (36) and Atexcac (40) both show high salinities and high alkalinities, however, EC in these lakes is associated with Na^+ and Cl^- ions, thus CO_3^{2-} and HCO_3^- concentration and pH are not as high as might be expected, and the relationship between the two variables is not linear.

Anion composition is displayed in Figure 6.5a, and indicates that the majority of lakes display a high proportion of carbonate and bicarbonate anions, over 40%; a low concentration of SO_4^{2-}, less than 12%, and a variable proportion of Cl^-, between 12.2 and 80.1%. La Laguna (32) shows highest proportion of SO_4^{2-} representing 11% of the dominant anions. The highest SO_4^{2-} concentrations are found in Atexcac (40) 1.4 meq l^{-1}, Alchichica (36) 1.14 meq l^{-1} and La Piscina de Yuríria (4) 1.26 meq l^{-1}. With the exception of Alchichica, Los Negritos (10) and Atexcac, Cuitzeo (14), Sta. Maria del Oro (25) and La Hoya de los Espinos (31) all sites show less than a 60% concentration of Cl^-. The aforementioned lakes all show a higher proportion of Cl^- than CO_3^{2-}/HCO_3^-. Santa Maria del Oro, Hoya de los Espinos, La Preciosa (47), Cuitzeo and Lago de la Luna (46) also display high proportions of Cl^- relative to other sites, (over 20%), but are still CO_3^{2-}/HCO_3^- dominated.

The majority of lakes sampled are dominated by the divalent cations, Mg^{2+} and Ca^{2+}. Particularly high levels of Ca^{2+} are noted in Juanacatlán (29) and in Tepitiltic (20). The latter, whilst showing dominance by Ca^{2+} also has a relatively high proportion of Mg^{2+} (35.6%), and shows little by Na^+ + K^+, (8.7%). In contrast Juanacatlán shows a greater proportion of Na^+ + K^+ (22.2%) and Mg^{2+} (37.3%). Zapotlan (7), La Piscina de Yuríria (4), Alchichica (36), Tecuitlapa (15) and Cuitzeo (14) all show over 70% dominance by Na^+ + K^+. Na^+ is. At all sites Na^+ is present in greater abundance than K^+.

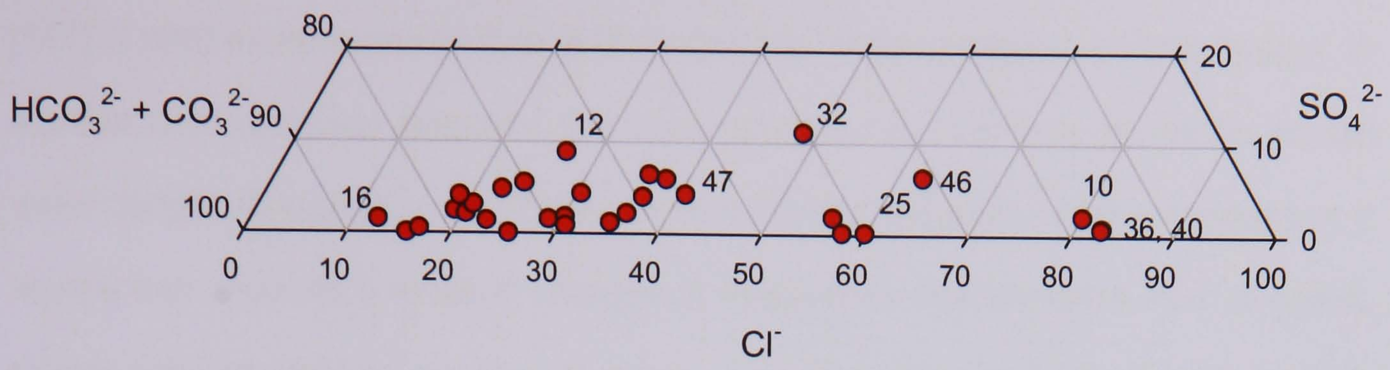


Figure 6.5. a) Anion composition (from meq/l)

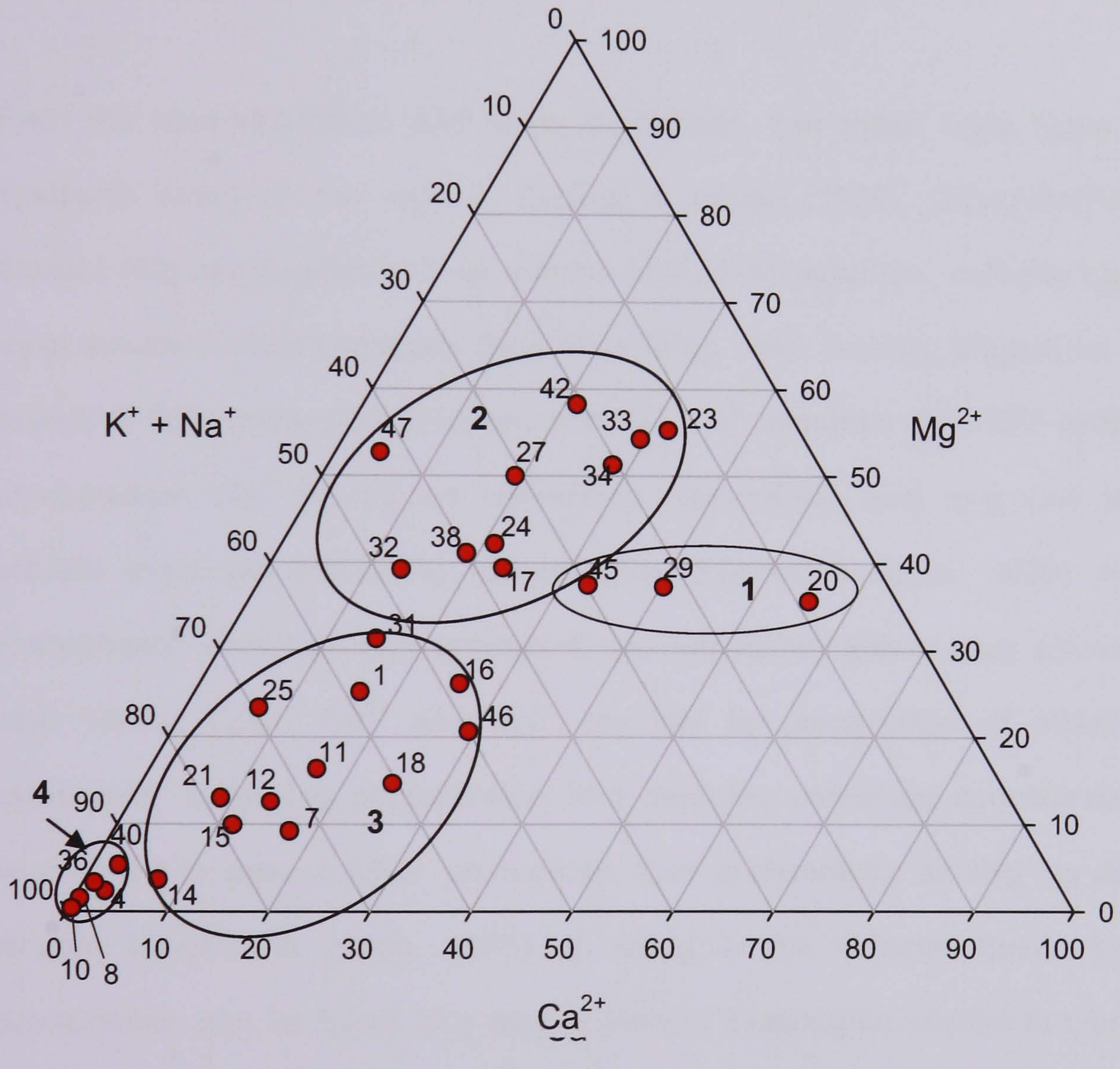


Figure 6.5 b) Cation Composition (from meql^{-1})

As the majority of basins sampled are closed water is lost through evaporation and seepage. Inflow is received through direct precipitation and runoff, primarily during the wet season, small springs or streams and ground water flow. Water balance is mainly driven by variations in seasonal precipitation and evaporation (i.e solute dilution and concentration) and groundwater abstraction. Consequently basins are often susceptible to a negative water balance and thus increasing evaporative concentration. In 1991, for example, Lago de Pátzcuaro average annual evaporation (1477.9 mm) exceed precipitation (979.2 mm) resulting in a negative water budget. In addition to precipitation seepage, has been estimated to contribute 8% to the annual water budget (Chacón-Torres 1993a). Brine evolution, between dominant cations and anions, can occur as a result of changes in evaporative concentration, loss of gases, mixing and temperature change. The first of these is the most effective (Eugster & Hardie, 1978), stimulating processes such as mineral precipitation, ion exchange and sorption and thus the preferential removal or return to solution of certain solutes.

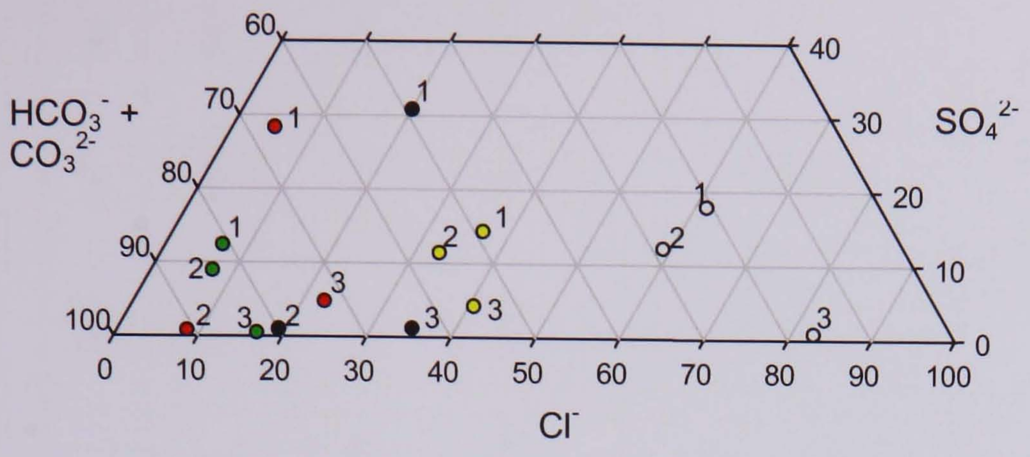
From the observed cation and anion distribution, four major brine types may be assigned, based on the work of Eugster & Hardie (1978). Only Alchichica (36), Atexcac (40) Los Negritos (10) fall into the sodium-chloride type, pathway III_{B2}. These lakes are low in total carbonate (less than 20%), while showing proportions of Cl^- in excess of 80% of the sum of anions and Na^+ + K^+ represent over 90% of the cation concentration. Na^+ and Cl^- are considered conservative ions, and thus remain in solution even with increasing concentration (Eugster & Jones, 1979). Increased concentration stimulates the fractionation and subsequent loss of other solutes. In this case HCO_3^-, CO_3^{2-}, Ca^{2+} and Mg^{2+} are lost by precipitation of alkaline earth carbonates. Increasing concentration and declining carbonate concentration have been noted to have a linear relationship, thus preferentially leaving Na and Cl in solution (Eugster & Jones, 1979). In lakes in the Oriental Basin, increasing concentration can be linked to a greater level of evaporation relative to precipitation due to the orographic rain-shadow effect caused by the Sierra Madre Oriental, atmospheric dust loading and ground water abstraction. The Ca^{2+}-Mg^{2+}-CO_3^{2-}-HCO_3^- group consists of Juanacatlán (29), Zirahuén (45) and Tepetiltic (20). These lakes display low alkalinities and circum-neutral pH values and follow Eugster and Hardie's (1978) pathway I. They are thus dilute and close to the composition of inflow waters and have experienced little brine evolution. The majority of lakes fall into one of the two following brine types, either the Mg^{2+}-CO_3^{2-}-HCO_3^- type (pathway III_A) or the Na^+-CO_3^{2-}-HCO_3^- (pathway III_B) and thus show progressive loss of alkaline earths.

6.3.4 Comparison on Ionic Composition Data

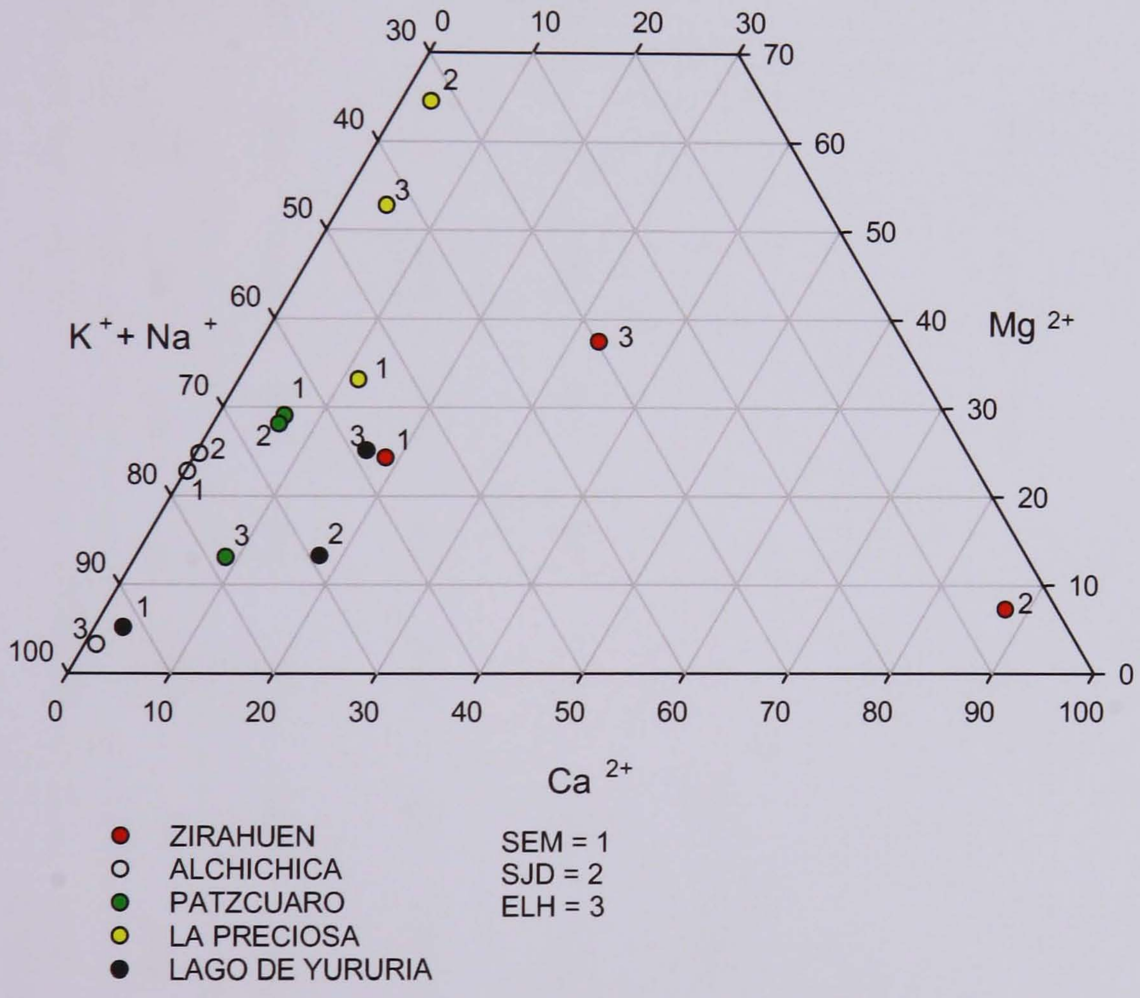
Cation and anion data for eight sites in this study (ELH) will be compared with that of Metcalfe (SEM), where samples were taken in 1981/82 and Davies (SJD); taken in 1997/98 (Figure 6.6). Such comparisons can be used to show actual, rather than diatom inferred, change over time.

Between 1982 and 1998 the anion composition of Alchichica shows an increase in HCO_3^- + CO_3^{2-} and corresponding decline in SO_4^{2-}, with little Cl^- change. In contrast the average data for this study shows Alchichica (36) to have experienced a large increase Cl^-, shifting from ~58% to 82% dominance, rise in HCO_3^- + CO_3^{2-} and a further SO_4^{2-} decline, with an overall SO_4^{2-} decline by almost 20%. Correspondingly between 1981/2, 1998 and 2003/4, in line with the increase in Cl^-, there has been a concomitant rise in K^+ + Na^+ increasing from ~75% dominance to 95%, a fall in Mg^{2+} and with little change in Ca^{2+}. La Preciosa (47) shows a similar shift over time, a decline and then increase in Cl^-, with a progressive increase in K^+ + Na^+ and a decline in both SO_4^{2-} and Mg^{2+}. Pátzcuaro (21) shows a massive decline in SO_4^{2-} content between 1981/2 and the two most recent studies. In 1982 SO_4^{2-} represented just over 30% of the anion composition, but in later studies, it accounted for between 1 and 2% correspondingly HCO_3^- + CO_3^{2-} increased from 50% to between 64 and 80%, with the greatest proportion being recorded in 1998. Very little difference was noted between 1981/2 and 1998 in the cation composition of Pátzcuaro. Between 1998 and 2003/4 however, an increase in K^+ + Na^+ and corresponding decline in Mg^{2+} has been noted. Such a difference may be associated with difference in sampling location, although Pátzcuaro is shallow, and has been considered to be relatively homogenous, studies have shown it to be quite heterogeneous (Alcocer & Bernal-Brooks, 2002). A consistent rise in the proportion of Ca^{2+} and Mg^{2+}, and corresponding decline in K^+ + Na^+, has been noted at Lago de Yuríria (1). The proportion of Cl^- at this site has increased between 1981 and 2004, with a major increase noted between 1998 and 2004, the proportion of HCO_3 + CO_3 varies between studies. Cation composition at Zirahuén (45) shows little change over time, with approximately a 10% decline in Ca^{2+} and 10% increase in K^+ + Na^+. Anion composition is more variable, showing little change in the contribution of HCO_3^- + CO_3^{2-} but a decline in SO_4^{2-}, by ~15%, and a corresponding rise in Cl^- of around 10%.

Results may also be compared with Chacón-Torres & Rosas-Monge (1998), Bernal-Brooks & MacCrimmon (2000b) for Zirahuén; Chacón-Torres (1993) for Pátzcuaro and Vilaclara *et al.* (1993) for Alchichica (see Table 6.3). Bernal-Brooks & MacCrimmon (2000b), with data covering 1995-1996, show low concentrations of Ca and Mg^{2+} but are within the range of Metcalfe (1985), Davies (2000) and this study. Alkalinity concentration shows a slight rise between Davies, Bernal-Brook and MacCrimmon and this study. In contrast Chacón-Torres & Rosas-Monge, using data averaged over 1989-1994, show much lower cation concentrations than other studies, however, alkalinity is higher. Chacón-Torres (1993) shows K^+ + Na^+ to be the dominant cations in Lago de Pátzcuaro, in line with Metcalfe (1985), Davies (2000) and this study, but with overall lower concentrations of all cations. In addition alkalinity is recorded at a lower concentration than Davies (2000) and this study, but higher than Metcalfe (1985). An increase in Cl^- concentration and corresponding fall in SO_4^{2-} is noted between 1985 and 2004.



a) anion composition



b) Cation comparison

Figure 6.6 comparative ionic data (ELH 2003/4, SJD 1998, SEM 1982)

Table 6.3 Comparative ionic data (all in meq l^{-1})

Study	**Zirahuén**					**Pátzcuaro**					**Alchichica**					
	HCO_3^- + CO_3^{2-}	**Cl^-**	**Ca^{2+}**	**Mg^{2+}**	**K^+ + Na^+**	**HCO_3^- + CO_3^{2-}**	**Cl^-**	**Ca^{2+}**	**Mg^{2+}**	**K^+ + Na^+**	**HCO_3^- + CO_3^{2-}**	**Cl^-**	**SO_4^{2-}**	**Ca^{2+}**	**Mg^{2+}**	**K^+ + Na^+**
SEM (1982)	0.8 (HCO_3^- only)	0.06	0.47	0.43	0.25	6.35	0.58	0.66	2.23	5.89	28.75	85.1	24.9	0.25	33.7	114
SJD (1998-1999)	1.1	0.1	0.44	0.49	0.34	10.48	0.91	0.75	3.31	7.71	41.14	84.63	17.71	0.51	36.13	109.2235
ELH (2003-2004)	1.35	0.42	0.68	0.78	0.62	9.8	1.98	1.12	1.68	10.1	23.6	117.69	1.14	11.08	32.34	939.13
Chacón & Rosas (1998)	1.4	-	0.088	0.39	0.147											
Bernal & MacCrimmon (2000b)	1.1	-	0.4	0.7	-											
Chacón (1993)						7.8		0.68	1.81	3.21						
Vilaclara et al. (1993)											18.54	85.24	21.36	0.6	3.22	92.76

6.4 Nutrients

The results of the nutrient analysis are fully reported in Appendix 1, the average annual results are presented in Tables 6.4 & 6.5.

6.4.1 Phosphorus concentration

Lakes in central México are likely to be naturally nutrient rich, although not necessarily eutrophic, given that the surrounding soils are primarily phosphate rich andesites. In addition, the majority of lakes sampled are either in areas of high population density or have catchments with a high percentage of agriculture. They are, therefore, susceptible to receiving nutrient rich runoff derived from untreated sewage, fertilizer or animal waste. The overall range of annual mean TP concentration was 0.8-1782 $\mu g\ l^{-1}$ (see Table 6.4) from Laguna Zirahuén and Atotonilco respectively (see Figure 5.1). Based on the OECD fixed boundary system (Table 3.1) a break down of sites, based on TP concentration and nutrient status indicates that the majority of lakes sampled

Table 6.4 Nutrient data, annual mean (from wet and dry season measurements. SRP measurements are wet season only.

Site	Site Codes	TP (μgl^{-1})	SRP (μgl^{-1})	NO_3-N (mgl^{-1})	NO_2-N (mgl^{-1})	NH_4-N (mgl^{-1})	SiO_2 (mgl^{-1})	Chl-a (μgl^{-1})
Lago de Yuríria	1	584.91	5.384	0.0303	0.00588	0.01961	70	75.38
La Piscina de Yuríria	4	653.24	120.09	0.1827	0.01652	0.05795	100	127.35
Zapotlan	7	285.10	125.49	0.0271	0.00493	0.00777	21.5	34.71
Atotonilco	8	1782.65	2860.27	0	0.09169	0.14474	70	25.01
Los Negritos	10	523.15	695.04	0.0102	0.08161	0.00743	240	11.24
Chapala	11	475.057	570.76	0.0648	0.00532	0.01042	18.5	8.34
Cajititlán	12	1040.35	1398.13	0.1087	0.00858	0.02191	64	32.81
Cuitzeo	14	927.37	101.8	0.1729	0.03788	0.05364	100	81.10
Tecuitlapa	15	453.912	378.87	0	0.0128	0.02545	87	60.86
La Alberca (Ter)	16	66.63	7.95	0.0153	0.00204	0.00687	150	34.56
Laguna La Maria	17	60.5	42.32	0.0103	0.0004	0.00595	100	15.69
San Pedro Lagunillas	18	47.29	18.02	0.0099	0.00014	0.01697	25.5	43.65
Tepetiltic	20	82.06	84.52	0.0279	0	0.00441	35.25	12.24
Lago de Pátzcuaro	21	71.53	59.19	0.0379	0.00361	0.00499	26	9.76
Laguna San Gregorio	23	40.08	7.77	0.0631	0.00234	0.00709	4.5	79.95
Laguna Zacapu	24	43.44	0	0.0037	0.00037	0.00176	110	11.56
Sta Maria del Oro	25	98.52	65.08	0.0068	0.00006	0.00601	90	23.43
Camecuaro	27	27.78	30.48	0	0.00119	0.00220	127.5	0.45
Juanacatlán	29	34.21	0	0.008	0	0.00172	36.5	4.57
Hoya de los Espinos	31	25.19	0	0.0014	0.00041	0.00138	95	0.84
La Laguna	32	30.49	18.41	0.0063	0.00146	0.00531	37.5	20.11
La Alberca (Tac)	33	10.32	12.65	0.0046	0.00037	0.00345	110	69.38
Laguna Zempoala	34	16.71	13.62	0	0.49115	0.00107	18.5	2.53
Alchichica	36	9.04	6.66	0.0029	0.00008	0.02582	61	1.78
Quechulac	38	8.9695	113.9	0	0	0.00204	66.5	5.9
Atexcac	40	8.1571	153.44	0	0.00052	0.03358	140	0.92
Lago del Sol	42	12.063	6.9	0	0.70697	0.0	3.5	0.87
Laguna Zirahuén	45	0.8	0	0	0	0.00514	1	1.74
Lago de la Luna	46	3.734	2.4	0	0.81197	0	3	0.54
La Preciosa	47	11.447	121.73	0	0	0.00758	111.5	0.79

were eutrophic or hypertrophic (see Table 6.5). The majority of sites show an increase in TP between the dry and the wet season, most likely associated with increased run-off from naturally nutrient rich, fertilized soils and drain overflow. Atotonilco exhibits the greatest rise in TP concentration, from 665.5 to 2899.8 μg l^{-1}, while Santa Maria del Oro changes from a mesotrophic state in the dry season (27.9 μg l^{-1}), to a hypertrophic state in the wet season (169.43 μg l^{-1}). This rise in TP is coincident with a large bloom of cyanobacteria (see Plate 6.4), which has been noted by local people to occur every 2-3 years. Only Hoya de los Espinos, La Alberca (Teremendo) and Juanacatlán show declines in TP concentration presumably due to dilution. The catchments of these basins are well vegetated and there are few P sources within them, thus increased rainfall will not necessarily result in increased P in-wash, but would dilute the concentration already present in the lakes.

Table 6.5 Trophic classification of calibration set lakes based on OECD classification, using TP only

Ultra oligotrophic	**Oligotrophic**	**Mesotrophic**	**Eutrophic**	**Hypertrophic**
Zirahuén	Alchichica	Camecuaro	La Alberca (Ter)	Lago de Yuriría
Lago de la Luna	Quechulac	Juanacatlán	Laguna la Maria	La Piscina de Yuriría
La Preciosa	Atexcac	Hoya de los Espinos	San Pedro	Zapotlan
	Lago del Sol	La Laguna	Tepetiltic	Atotonilco
		La Alberca (Tac)	Pátzcuaro	Los Negritos
		Zempoala	San Gregorio	Chapala
			Zacapu	Cajititlán
			Sta Maria del Oro	Cuitzeo
				Tecuitlapa

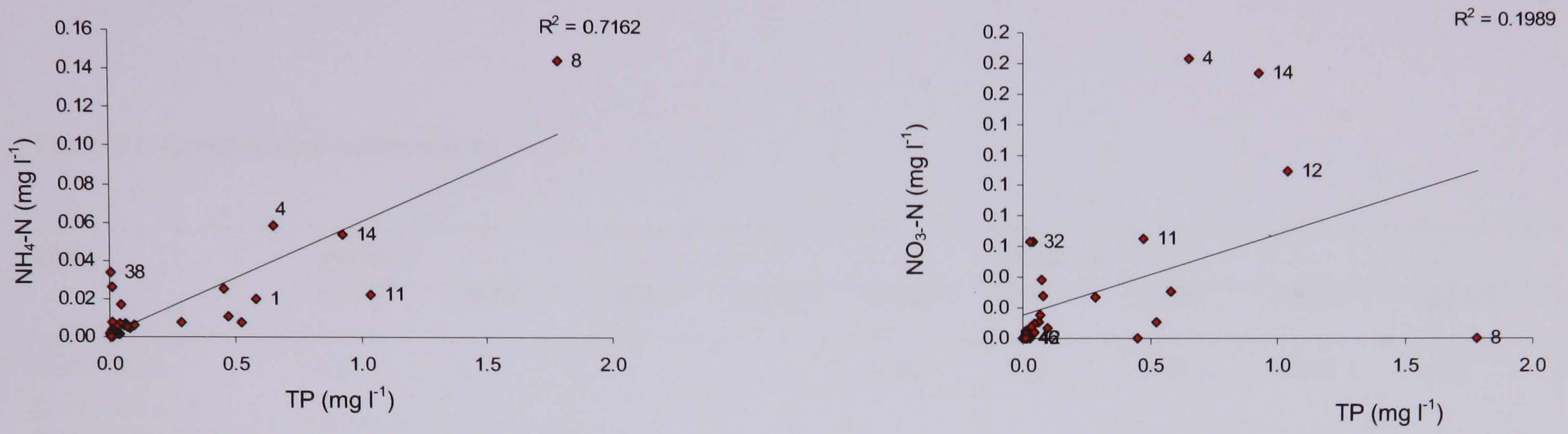
Three epilimnetic samples were taken from Lago de Pátzcuaro during the dry season, based on previous reports of differences in nutrient concentrations within the basin, these samples showed that the north had the greatest TP concentration of 58.5 μg l^{-1}, the south had a lower concentration of 43.55 μg p l^{-1} and the central sample site a concentration of 44.85 μg P l^{-1}. Analyses of remote sensing data and of primary productivity and phosphorus concentration by Chacón-Torres (1992; 1993) indicated that the northern basin, near the towns of Quiroga and Tzintzuntzan was eutrophic-hypertrophic and the southern basin was mesotrophic, despite the higher population in that area. The data presented here support such an assertion, as TP is higher in the north than in the south, however, unlike Chacón-Torres' data both the north and the south in this study could be classified as eutrophic.

Some lakes display TP concentrations which do not seem to relate to activity in the catchment. Zirahuén has a high population density, with a total of 32 settlements and ~60% of the catchment is cultivated, but there is no visual response of the epilimnetic water to this as TP was measured as between 0 and 1.59 µg l^{-1}. The measurements, however, appear to be erroneous, particularly as Zirahuén is situated in an area of P rich bedrock (Davies *et al.,* 2004). In contrast, Juanacatlán is in an upland area with, until recently little/no human activity in the basin, but can be classified as mesotrophic (TP = 34.21 µg l^{-1}), despite no obvious sources of phosphorus. La Hoya de los Espinos has a TP concentration (40.3 µgl^{-1}) that classes it as eutrophic, but the catchment contains no settlement and no agriculture, it is solely used for recreation, and such a this loading may be natural.

6.4.2 Nitrogen concentration

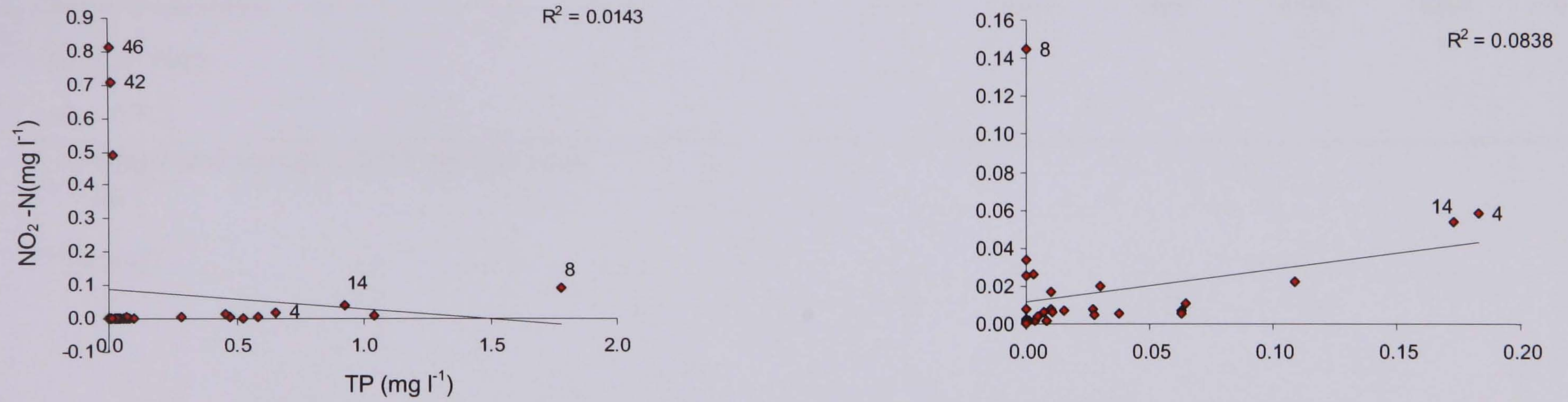
The majority of lakes show the following dominance of NO_3-N > NH_4-N > NO_2-N. Only Atotonilco (8), Tecuitlapa (15), San Pedro (18), Camecuaro (27), Zempoala (34), Alchichica (36), Quechulac (38), Atexcac (40), Zirahuén (45) and La Preciosa (47) show greater concentrations of NH_4-N than NO_3-N (Table 6.4). Several sites have NO_3-N concentrations which were below detection, such as Atotonilco, but in general lakes classified as oligotrophic have low NO_3-N concentrations. La Piscina de Yuriría (4), 0.18 mg $^{-1}$, and Cuitzeo (14), 0.17 mg l^{-1} have the highest concentrations. Hypertrophic lakes, in general have the highest concentrations of NO_3-N and NH_4-N, although Alchichica and Atexcac also exhibit higher concentrations than other lakes low in TP, although NO_3-N was below detection in the latter (Figure 6.7a and b).

Higher concentrations of NO_3-N are also noted in Atotonilco (0.09 mg l^{-1}) and Cuitzeo (0.04 mg l^{-1}). Increases in NO_3-N are, to an extent, matched by increases in NH_4-N see Figure 6.7d.



a) TP vs. NH_4

b) TP vs. NO_3



c) TP vs. NO_2

d) Relationship between NO_3-N and NH_4-N

Figure 6.7 Relationship between TP and Nitrogen compounds showing line of best fit.

Table 6.6 Comparative nutrient data

Study	**Zirahuén**					**Pátzcuaro**					**Chapala**		**Lago del Sol**
	TP **	SRP**	NO_3-N***	NO_2-N***	NH_4-N***	TP**	SRP**	NO_3-N***	NO_2-N***	NH_4-N***	TP**	NO_3-N***	TP**
ELH (2003/4)	0.8	0	0	0	0.002	71.5	59.9	0.038	0.004	0.005	475.1	0.06	6.6
Bernal-Brooks & MacCrimmon (2000b)	8-20	-	-	-	-	-	-	-	-	-	-	-	-
Chacón-Torres & Rosas-Monge (1998)	8.69	5.39	0.003	0.002	0.22	-	-	-	-	-	-	-	-
Chacón-Torres (1993)	-	-	-	-	-	64.4	39.2	0.003	0.006	0.037	-	-	-
Lind et al. (1992)	-	-	-	-	-	-	-	-	-	-	439.6*	0.149*	-
Löffler (1972)	-	-	-	-	-	-	-	-	-	-	-	-	2.8

* Average concentration for 5 sample sites

** = $\mu g\ l^{-1}$

*** = $mg\ l^{-1}$

6.4.3 Comparison of new nutrient data with previous results

General comparisons can be made between this study and published data for four lakes. Due to the potential seasonal variability in nutrient concentrations and unknown differences in sampling timing, location and methodology these comparisons are made tentatively. Table 6.6 shows a comparison of the new nutrient data and previously published nutrient data for Zirahuén, Pátzcuaro, Chapala and Lago del Sol. Total Phosphorus levels in Laguna Zirahuén have been previously measured as 8.7 µg l^{-1} (Chacón-Torres & Rosas-Monge, 1998) and 3-20 µg l^{-1}, (Bernal-Brooks and MacCrimmon, 2000b). Despite apparent low nutrient concentrations limnological measurements have noted a shift from oligo/mesotrophic conditions towards eutrophy between 1987 and 1996, based on shifts in TP, TN and Secchi depth (Bernal-Brooks, 1998). NO_3-N and NO_2-N concentrations were below detection in this study, however NH_4-N was measured at 0.002 mg l^{-1}, this contrasts with Chacón-Torres & Rosas-Monge (1998) who found higher concentrations of all N species. At Pátzcuaro Alcocer & Bernal-Brooks (2000) measured TP between 1998 and 1999 to range between 60 and 81 µg l^{-1} compared to 71 µg l^{-1} supporting a continued shift to higher nutrient concentration. Lind *et al.*, (1992) worked extensively on Lago de Chapala and reported TP and nitrogen species concentrations which are only marginally lower than recorded here, inferring a maintained level of impact. The TP concentration in Lago del Sol measured in 1972 (Löffler 1972) is ca. six times lower than that measured 31 years later in this study. Such a large increase in TP may be due to a higher level of use, in terms of grazing and recreational use, now. More recent research (Alcocer *et al.*, in press) indicates that Lago del Sol has TP concentrations of 6 µg l^{-1}. This corroborates that TP has increased since Löffler's measurements in 1972, as Alcocer *et al.* (in press) and this study show values in the range of 6-12.1µg l^{-1} for Lago del Sol. Data also exist for Lago de Cuitzeo which has in the past been seen to have high levels of TP (0.31 mg l^{-1}) and of NO_3 (22 mg $^{-1}$) associated with domestic and agricultural run-off, particularly from drainage from Morelia through the Río Grande de Morelia (Alverado Diaz *et al.*, 1985). Data for Cuitzeo in this study were taken from the more dilute eastern basin, which may account for much lower NO_3 concentrations, TP has however, risen over the intervening time period.

6.4.4 Silicate concentration

Silicate concentrations range between 0 and 240 mg l^{-1} (Table 6.3) with an average of 70.79 mg l^{-1}. The highest silicate concentrations were found in Los Negritos, Atexcac and La Alberca (Teremendo) and the lowest in Zirahuén, the Nevado de Toluca (42, 46) and San Gregorio (23). With a few exceptions, namely the lakes of the Nevado de Toluca, crater lakes display the highest concentrations of SiO_2. The average Si content of these lakes is greater than the global average of 13 mg l^{-1} (Wetzel 2001), although lakes of volcanic origin tend to show higher concentrations, due to the silicate rich bedrock and also because silica dissolution is pH sensitive and increases above 7 (Telford *et al.*, 2004). SiO_2 concentrations compare well with SiO_2 concentrations found in African lakes, which are similar in origin to the central Mexican lakes. Conditions are, therefore, favourable for diatom production.

The majority of sites showed a decline in SiO_2 between the dry and the wet season, particularly Los Negritos and Camecuaro, by 160 and 145 mg l^{-1}, respectively, while three sites showed no shift in concentration. Particularly large increase in SiO_2 concentration were noted in the crater lakes of the Oriental Basin, possibly related to the underlying bedrock, or concentration associated with climatic factors.

6.5 Transparency and phytoplankton productivity

The complex relationship between nutrient concentration (TP) and algal growth (using Chl-a as a proxy), and the subsequent relationship with secchi depth transparency has been discussed in detail in Chapter 3. These three variables have been used in conjunction to assign trophic state (OECD, 1982). Many central Mexican lakes however, are shallow, well mixed and consequently highly turbid with a high suspended sediment load (see Plate 6.3). Phytoplankton growth, stimulated by increase nutrient availability, can therefore be restricted by light limitation due to suspended sediment, resulting in a less clear cut relationship between these 3 variables.

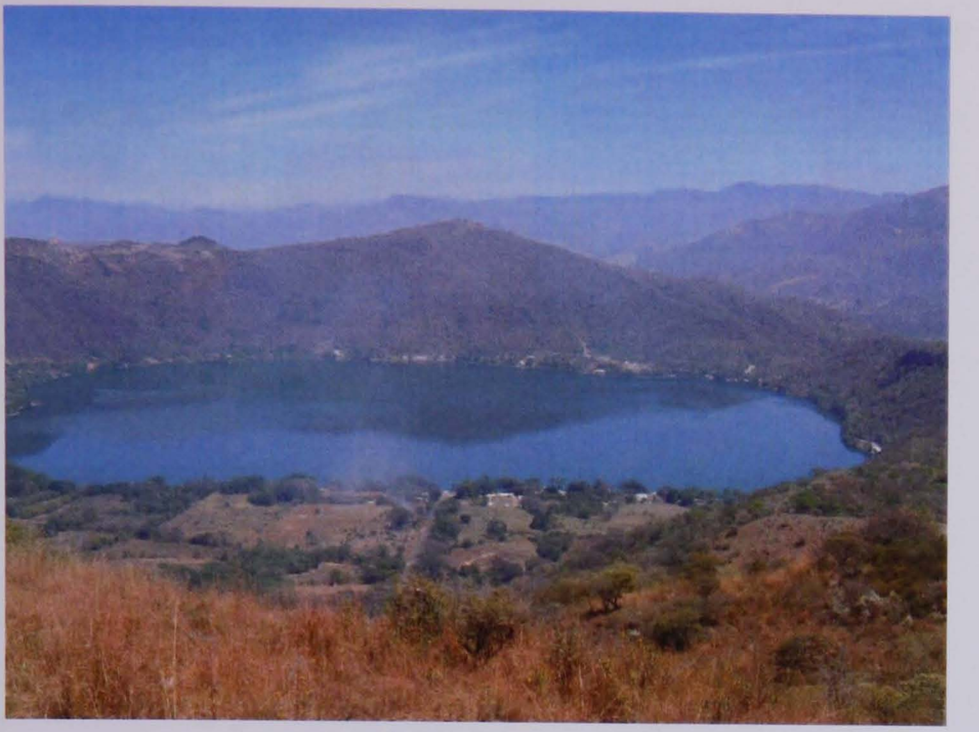
Annual mean secchi depth transparencies range between 0.02m and 7.85 m at Atotonilco (8) and Lago de la Luna (46) respectively (Table 6.1). In general low secchi depth transparencies are generally found in the lakes of higher nutrient status, i.e. those with higher Chl-a concentrations. At Camecuaro (27) and Tepetiltic (20) secchi depth transparency is equal to depth. 15 lakes show an increase in transparency between the dry season and the wet season, despite a rise in Chl-a concentration. La Alberca (Teremendo) (16) (Plate 6.3) shows a rise in secchi depth by 0.24 m due to an apparent reduction in algal productivity. The greatest increase in secchi depth, of 1.97m, is at Alchichica (36). This may be due to inputs of biologically unavailable P. 14 sites show a decline in transparency, the largest noted at Sta. Maria del Oro (25) of 6.57m, associated with an intense algal bloom (see Plate 6.4).

a) March 2003

b) July 2004

Plate 6.3. La Alberca (Teremendo) highlighting the differences in chlorophyll-a concentration between a) 2003 dry season and b) 2004 wet season

a) March 2003

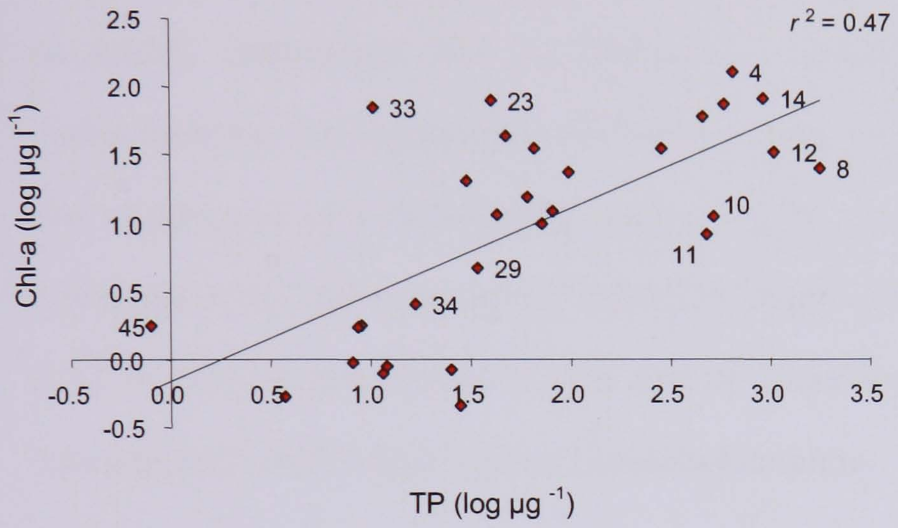


b) July 2004

Plate 6.4) a) Sta Maria del Oro March 2003 relative to b) algal bloom at Sta Maria del Oro, July 2004

Chl-a concentrations are in the range 0.45 µg l^{-1} (Cámecuaro; 27) to 127.35 µg l^{-1} (La Piscina de Yuríria; 4). Chl-a concentrations, in general correspond to their TP concentrations, and OECD trophic state, as expected i.e. high in hypertrophic lakes and low in oligotrophic lakes. There are, however, exceptions to this, the hypertrophic Atotonilco (8) and Cajititlán (12) have Chl-a concentrations of 25.01 µg l^{-1} and 32.81 µg l^{-1} respectively. These Chl-a concentrations, when considered independently, could classify these lakes as eutrophic, rather than hypertrophic. In contrast La Laguna (32) and La Alberca (Tacambaro) (33) have higher Chl-a concentrations than expected, relative to their mesotrophic TP concentrations. A rise in Chl-a concentration may be expected during the wet season, due to in-wash of nutrients from the catchment, which occurs at all but four sites.

a) TP vs. Chl-a



b) Chl-a concentration and Secchi depth relationship.

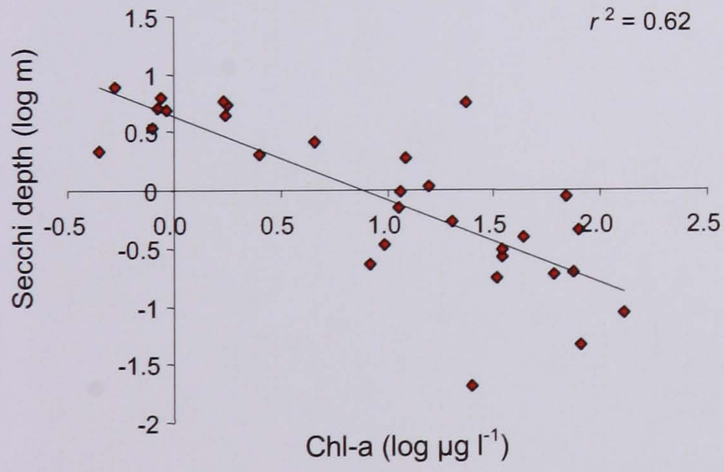


Figure 6.8. Relationships between TP, Chl-a and Secchi depth.

A rise in TP is expected to yield a rise in algal productivity and thus in Chl-a concentration and there is a positive relationship ($r^2 = 0.47$), between TP and Chl-a, both log transformed (Figure 6.8a). The majority of the lakes that can be classified as hypertrophic or eutrophic do show correspondingly high (>25 µg l^{-1}) Chl-a concentrations. For example La Piscina de Yuríria (4) has a high TP (653.24µg l^{-1}) and high Chl-a concentration (127.35 µg l^{-1}).

The relationship between log transformed Chl-a and secchi depth (Figure 6.9b) is relatively strong, but compared to OECD findings (1982) is not as strong as expected. Chl-a has however, been found to be a good indicator of phytoplankton productivity, even in the most turbid lakes (Lind *et al.*, 1992). In the central Mexican data set there is a negative correlation between the two variables indicating that an increase in Chl-a is usually associated with a decline in transparency. Despite the low Chl-a concentrations, transparency at Atotonilco is still low, at 0.02 and 0.17m, supporting the assignment of a hypertrophic status, based on OECD guidelines. This can be attributed to the high suspended sediment content within the water column (see Plate 6.5). In contrast San Gregorio (23) can be classified as eutrophic but has a Chl-a concentration (43.65 µg l^{-1}) in the hypertrophic range.

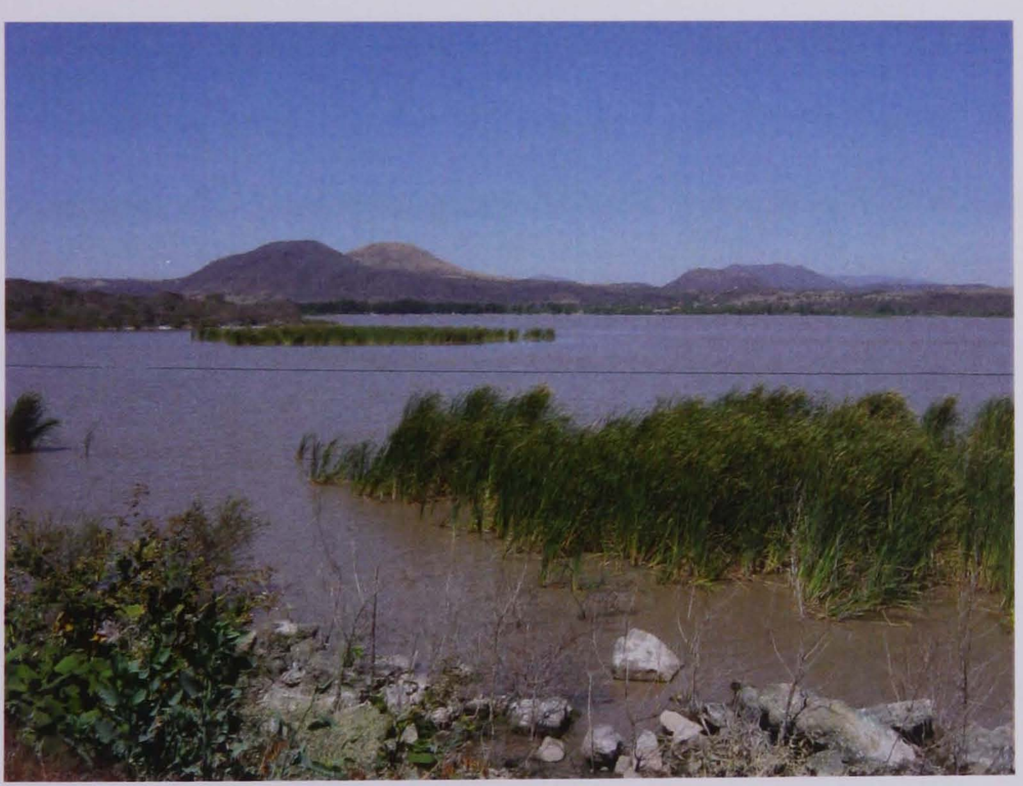


Plate 6.5 High suspended sediment load in Lago de Atotonilco, March 2003

These data may be compared with other studies from Chapala, Pátzcuaro and Zirahuén. Chl-a concentrations in Chapala, measured by Lind & Dávalos-Lind (2002) vary between 19 µg l^{-1} in the east of the basin and 7 µg l^{-1} in the west. The sample in this study was taken from the western basin (11.24 µg l^{-1}) and is within that range.

Chl-a concentrations have been found to be 3.8 μg l^{-1} (Chacón-Torres & Rosas-Monge, 1998) in Laguna Zirahuén and 59.8 μg l^{-1} for Pátzcuaro (Chacón-Torres, 1993), both of which are substantially higher than this study. However the methodology for analysis of Chl-a used in this study differs from that used by Chacón-Torres, which may account for a proportion of the difference.

As noted above, the relationship between trophic status and algal productivity is not clear cut, with some hypertrophic sites showing low Chl-a concentrations and low secchi depth transparencies and some mesotrophic sites showing high Chl-a and low secchi depths. Many of the lakes in the data set are shallow and highly turbid leading to sediment resuspension. It is this factor which reduces secchi depth and inhibits Chl-a production through light limitation of algae, despite the high availability of TP.

6.6 Modern Flora

Variation in resource requirements and competitive ability leads to a shift in species composition along a nutrient gradient, (see Section 3.2.3). Lakes with high TP concentrations are usually considered to be dominated by diatom genera such as *Fragilaria*, and planktonic genera *Stephanodiscus* and *Aluacoseira granulata* (Mason, 1996), which have high P requirements and are competitive in low light conditions. In contrast oligotrophic lakes tend to be dominated by species such as *Cyclotella stelligera*, with low P requirements, *Aulacoseira ambigua* and *A. distans* and *Asterionella spp* (Haworth, 1976; Kilham *et al.*, 1986). Species counts and LM and SEM diatom images are presented in Appendix 2. A total of 219 species, from 36 genera, were identified. 135 species, from 28 genera, will be discussed further as they constitute more than 1% of the assemblages. The sites are discussed based on their trophic state (see Table 6.5), classified based on TP values from the OECD fixed boundary system. These assemblages can be seen in Figure 6.9a to d.

6.6.1 Hypertrophic and eutrophic lakes

In Lago de Yuríria *Aulacoseira granulata, A. granulata* var. *angustissima* and *Cyclotella meneghiniana* dominated the assemblage (26.6%, 22.6% and 14.3%, respectively). There are few epiphytic species, despite the abundant growth of water hyacinth in the lake (see Plate 6.6). In total epiphytic or attached species constitute only 2.5% of the surface sediment assemblage. The epilithic sample taken from Lago de Yuríria was dominated by *Gomphonema truncatum* and the epiphytic sample by *Nitzschia inconspicua* and *Navicula radiosa* var. *tenella*. These species are not noted in the surface sediment sample.

Plate 6.6 Water Hyacinth growth on Lago de Yuríria

While classified as hypereutrophic (TP = 584 ugl^{-1}), La Piscina de Yuriria is highly saline (EC = 2908 µS cm^{-1}), alkaline (18.6 meq l^{-1}) and Na-CO_3-HCO_3 dominated. The surface sediment assemblage was dominated by Craticula halophila (56.1%) (Appendix 2, Plate 41) and *Chaetocerous muelleri* (5.3%) some of which were present as resting spores (see Appendix 2, Plate 36). Also present were *Anomoeoneis sphaerophora* f. *costata* (3.7%), *Denticula thermilis* (4.9%), *Nitzschia communis* (5.1%), *Nitzschia ovalis* (3.3%) and *Rhopalodia gibberula* (3.5%). This assemblage differs considerably from that of Metcalfe (1985) and of Davies (2000), the former showing dominance by *Navicula elkab* (22%) and the latter dominance by *A. spaerophora*, *A. spaerophora* f. *costata* and *Navicula elkab*. Such a difference may be accounted for by a shift in the water chemistry of this basin, a fall in pH from 11 in (1985) to 10.8 (1998) to 9.46 (2003/4) and EC from 26,000 to 8130 to 1907 μScm^{-1}, possibly associated with anthropogenic alteration of the water balance through redirection of groundwater back into the lake as observed in Plate 6.3. Although it should be noted that the taxonomic separation of *N. elkab* and *C. halophila* is difficult. An epiphytic sample was taken from La Piscina de Yuríria, however it yielded poor diatom preservation, fewer than 300 valves found. An epilithic sample was also taken and was found to be dominated by *N. radiosa var. tenella* (39.6%) and by *Nitzschia liebetruthii* (19.1%)

Zapotlan was dominated by the planktonic *Cyclotella meneghiniana*, a species found to be abundant in East Africa in lakes with a pH between 7.1-8.2 and with an average alkalinity of 20 meq l^{-1} (Gasse, 1986). Schoeman (1973, cited in Gasse, 1986) also noted that this species is abundant in lakes with a high nitrogen concentration and while this lake does have a high N content, it is by no means the highest. Other important species include *Aulacoseira granulata*, *A. granulata* var. *angustissima*, *Fragilaria (=Pseudostaurosira) brevistriata*, *F. pinnata* var. *trigona*, and *Cyclotella pseudostelligera*. All other species constitute less than 8% each. There are few 'eutrophic' indicator species present in this lake (i.e. *N. palea* and *Stephanodiscus* species) despite its high TP concentration (285.1 $\mu g\ l^{-1}$). There is little ecological information for *F. pinnata* var. *trigona* but, based on the work of Gasse it has been found in Na-HCO_3 waters. Such an assertion would seem to fit the observed cation and anion composition.

Atotonilco (TP = 1782.65 $\mu g\ l^{-1}$) was dominanted by *Nitzschia amphibia*, which constituted 60.2% of the assemblage, with other species representing less than 7% each. *Gomphonema parvulum* was the second most abundant species (6.8%). However as this sample was taken from the close to the lake shore, where there was extensive reed growth, the abundance of this species may be over represented relative to a sample taken from the lake centre. *Stephanodiscus astraea* var. *intermedia* and *S. asteroides* var. *intermedia* were identified, but in low abundance (1.7 and 2.7% respectively). The diatom assemblage in this lake appears to be more representative of the prevailing ionic composition, pH and salinity of the water, than its nutrient content. The abundance of *N. amphibia* may be a result of the high NO_2-N (0.09 mg $^{-1}$) and NH_4-N (0.144 mg l^{-1}) content, but its dominance of the surface sediment assemblage may be related to the high abundance of this species (28.6%) in nearby vegetation. The epiphytic sample was dominated by *Amphora veneta* (36.3%) which was only noted in low abundance in the surface sediment sample (3.7%).

Los Negritos has one of the most diverse diatom assemblages. 30 of the 45 species identified represent less than 1% each. *Nitzschia perminuta* and *Cyclotella menghiniana* are most abundant, representing 11.7 and 12.4% respectively. Despite being hypertrophic (TP = 376.29$\mu g l^{-1}$) the assemblage seems more indicative of ionic composition, salinity and pH.

Lago de Chapala was dominated by *Stephanodiscus* species and by *Fragilaria construens* var. *construens* (17.4%). *Stephanodiscus asteroides var. intermedia* was the most abundant (18.9%) with *S. astraea var. minuta* (12.9) and *S. minutus* (12.3%). All *Stephanodiscus* species were highly silicified and many were broken around the perimeter, thus their spines were not in evidence, making identification difficult. One *Stephanodiscus* species (Plate 174) remained unidentified, but only represented 2.5% of the assemblage. This was similar to an unidentified species in Davies (2000). It was characterised by variation in striae length, with very short striae between each of the longer striae around the edge of the valve. In addition, in the centre, of the valve are a number of punctae. In a shallow, turbid lake such as Chapala, dominance by *Stephanodiscus* species is not unexpected as such species, when P is not limiting, are superior competitors for P under low light conditions (Kilham *et al.*, 1986). The depth of the lake (maximum 6.90m) would account for the abundance of small facultative planktonic *Fragilaria* species.

Cajititlán was dominated by *Aulacoseira granulata* (35.3%) and *A. granulata var. angustissima* (19.7%). In East African lakes species in this genera are found to have high Si requirements and will be dominant if P is not limiting (Kilham *et al.*, 1986). The dominance of *A. granulata* despite low SiO_2 may be indicative of the competitive ability of this species in low light conditions (Kilham *et al.*, 1986). An unidentified *Stephanodiscus* species is the third most abundant species (13.6%) (plates 164-167). In contrast to the surface sediment sample, the epiphyton sample was dominated by *Nitzschia amphibia* (30.7%) and *N. palea* (20.8%). The latter was not represented in the surface sediment sample while the former is only present in low abundance (2.2%). Also present in the epiphytic and surface sediment samples, but in low abundance were *Aulacoseira granulata var. angustissima* (9.11 and 19.72%), *Cyclotella glomerata* (5.0 and 4.1%) and *Gomphonema parvulum* (4.4 and 1.6% respectively).

The diatom sample from the eastern side of Lago de Cuitzeo, was dominated by *Fragilaria brevistriata, Fragilaria (=Staurosirella) pinnata* and *Fragilaria (=Pseudostaurosira) construens var. venta. Fragilaria construens f. subsalina, Cocconeis placentula var. lineata* and *F. construens f. construens*. The sample was taken from a depth of 0.5m which may account for the dominance of these facultative planktonic species.

The surface sediment sample from Tecuitlapa was dominated by the epiphytic species *Achnanthes minutissima* (28.9%) and the planktonic species *Cyclotella meneghiniana* (22.3%). *Nitzschia amphibia* was also abundant (16.0%). Floating, emergent and submerged aquatic vegetation within the lake is limited, however, algal growth resulting in a high Chl-a concentration (17.53 $\mu g\ l^{-1}$) gives the lake a distinctive colour, and may act for as a substrate for the growth, in abundance, of *A. minutissima*.

6.6.2 Eutrophic Lakes

The surface sediment assemblage of La Alberca (Teremendo) (TP = 72.8 µg l^{-1}) is dominated by *Gomphonema parvulum*, which constitutes 65.8%. Other species in this assemblage constitute less than 5% each, and include *Synedra ulna*, *Nitzschia* species and *Fragilaria construens* f. *construens*. Dominance by *G. parvulum* is not unsurprising given the degree of reed growth around the perimeter of the lake and green algae growth within the lake, both of which could act as a substrate for this species. Based on personal observation nutrient sources include sewage runoff, from a small number of dwellings within the crater and animal waste. The latter is cited as a major control factor on the abundance of *G. parvulum* by Patrick and Reimer (1966), and although their work is based on the United States, similar ecological conditions for this species seem to prevail here.

No one species showed clear dominance in Laguna La Maria, however, six species are more abundant than others. *Aulacoseira granulata* var. *angustissima*, *Cyclotella glomerata*, *C. pseudostelligera*, *Fragilaria construens* var. *construens, F. construens* var. *venta* and *Nitzschia amphibia* are all present at between 10% and 15%. La Maria can be classified as eutrophic and Mg-CO_3-HCO_3 dominated. The presence of *C. glomerata* and in particular *C. pseudostelligera* would support its TP assigned trophic state.

The surface sediment diatom assemblage from Lagunillas San Pedro consisted, almost entirely, of *Achnanthes minutissima* (93.3%). Other species included *Aulacoseira granulata* and varieties, *Cyclotella meneghiniana, Fragilaria pinnata* and *Stephanodiscus medius*, however only *A. granulata* constitutes more than 1% of the assemblage. The dominance of *A. minutissima* is most likely related to the abundant reed growth around the lake. The epiphytic sample taken from the reed beds is similarly dominated by *A. minutissima* (94.8%), confirming the role of habitat in controlling the surface sediment assemblage. Small percentages (<2%) of *Synedra* *tenera, Aulacoseira granulata, Cocconeis placentula, Gomphonema parvulum* and *Nitzschia palea* are also present in the epiphytic sample.

Aulacoseira ambigua was the dominant species in Tepetiltic. It has been found in African lakes to have optimal development in small, shallow lakes and low alkalinities (Richardson *et al* 1978) and has been found in association with *Eunotia* species at low alkalinities, as at this site (Gasse, 1986). *A. minutissima* is the second most abundant species, associated with the high growth of submerged aquatic vegetation and weakly alkaline conditions.

The diatom assemblage from the central basin of Lago de Pátzcuaro was dominated by *Aulacoseira granulata* and *Aulacosira ambigua*. The modern sample taken by Davies (2000) was also dominated by *A. granulata*. Of secondary importance in this sample was *Cocconeis placentula* var. *lineata*, which was also of importance in Davies' sample. The corresponding water chemistry for this sample would classify it as mildly eutrophic and Na-Mg-CO_3-HCO_3 dominated. A number of eutrophic indicator species were identified, but none in any great abundance. *Stephanodiscus astraea* var. *intermedia* and *S. astraea var. minuta*, represent 4.2% collectively, *S asteroides* var. *intermedia* was also identified, but represented less than 1%. Previous studies (Metcalfe *et al.*, 1989) have shown an increase in *Stephanodiscus* species since the Post-classic (the last 900 years) at Pátzcuaro, often at high percentages in the core material, in contrast with the modern sample. Preservation in the surface sediments has however been noted as poor over the last 20 years associated with high turbidity. Fragments of *Stephanodiscus niagarae* were also found, but as this species is considered extinct in Mexico (Vilaclara, pers. comm., 2003) these fragments are most likely as a result of in-wash from older diatomites in the catchment. The epiphytic sample was dominated by *Gomphonema clavatum* (19.%), *G. parvulum* (9.2%), *Navicula radiosa* var. *tenella* (11.9%), *Nitzschia palea* (25%) and *Synedra goulardii* (15.1%). These species were not present in the surface sediment assemblage, species in the genera *Nitzschia* and *Synedra* are more susceptible to breakage in a turbid environment such as Pátzcuaro, which may account for their absence from the surface sediment. A variation of *S. goulardii* was present, but only at a relative abundance of 1.4%. These valves are similar to the nominate variety but they are more strongly attenuated around the central area (Plate 178). Such a variation has also been noted by Metcalfe (1985) in Lago de Pátzcuaro.

San Gregorio is at an altitude of 3,100 m.a.s.l, and consequently has a cooler surface water temperature (18.1°C) and is also circum-neutral (pH = 6.98), mesotrophic (TP = 25.35µgl^{-1}) and Mg-CO_3-HCO_3 dominated. As a result there were a greater number of species with a preference for slightly acidic conditions, such as *Anomoeoneis brachysira, Eunotia bilunaris* and *E. naegelii*, than at other sites. These species are, however, only present in low percentages. *Achnanthes minutissima* and *A. minutissima* var. *microcephela* dominate the assemblage (40 & 10.6% respectively). Species considered indicative of oligotrophic conditions in temperate lakes, such as *Cyclotella stelligera*, *Cymbella amphicephela and C. descripta* are also present. Their presence here may indicate that these species are capable of tolerating more nutrient rich conditions than their European/North American equivalents. It can be noted that some individuals in the *A. minutissima (microcephela)* group have irregular apices (Plate 10). Such a characteristic may be as a result of environmental stress causing deformation during reproduction (David Mann, pers. comm., 2003). This assemblage is quite different from that of Davies (2000) with a reduction in *Eunotia* species and the increased abundance of *Achnanthes minutissima*.

The diatom flora of Laguna Zacapu has been the subject of numerous publications by Metcalfe (1985; 1986; 1988; 1995). This work has indicated that the lake, while never oligotrophic, has recently become more eutrophic, as indicated by a rapid increase in small *Stephanodiscus* species, particularly after ~1190 AD. The modern sample taken in 2003 during the dry season was dominated by *Stephanodiscus subtilis* indicating the persistence of high nutrient loadings. Other species in the assemblage, including *Nitzschia palea, Cyclotella pseudostelligera* and *Cyclotella glomerata*, are also indicative of high nutrient availability. Two *Synedra* species, *S. ulna* and *S. acus* are also present in high percentages, which according to Richardson *et al.* (1978), are indicative of high silica availability, Zacapu has one of the highest Si concentrations (110 mg l^{-1}) in the data set. In addition the appearance of spindle shaped *Synedra* species, such as *Synedra acus* have been found to be indicative of nutrient enrichment (Zeeb *et al.*, 1994). The dominance of the assemblage by eutrophic species would seem to indicate that diatom variance in the lake is driven by anthropogenic impact on the basin.

The majority of species present in Santa Maria del Oro are widely tolerant of ionic composition, temperature and pH. Species present in abundance at this site included *Aulacoseira granulata* and *Nitzschia amphibia.* Dominance by planktonic species is not unexpected for a lake of such depth (53m), as light limitation would inhibit dominance by benthic species. Present above 1% but constituting less than 5% were *Achnanthes minutissima* var. *minutissima, Aulacoseira monospina, Cocconeis placentula* var. *euglypta, Fragilaria brevistriata, Navicula lanceolata, Nitzschia inconspicua* and *Rhopalodia brebissonii. Aulacoseira monospina* is differentiated from *A. granulata* on the basis of the presence of one very long spine and a triangular shaped gap in punctae on the valve face. This species was deemed a new species by Gasse (1980) but there is little ecological information for it. It was also noted by Metcalfe (1985; 1995) in the Zacapu basin. There was also an abundance of sponge spicules in the modern sample, all of which are greater than 100 µm in length. An epiphytic sample from the lake was dominated by two species *R. brebissoni* (61.4%), and *Fragilaria fasciculata.* The former is present in the surface sediment, but its abundance is low (2.8%), while *F. fasciculata* was not found in the surface sediment and is unique to this site.

6.6.3. Mesotrophic lakes

Lago de Camecuaro was dominated by *Fragilaria pinnata, Achnanthes acares* and *Nitzschia amphibia*, which represent 18.0%, 12.5% and 10.9% respectively. There are a few species in high abundance which indicate high nutrient availability, such as *Gomphonema parvulum* (4%) and *Nitzschia palea* (1.8%). The presence of *Achnanthes lanceolata* ssp. *dubia*, even in low percentages, seems to corroborate the trophic state, based on the published data (Patrick & Reimer, 1966). The abundance of *Fragilaria* species may be accounted for by the shallow depth of this lake. Such an assertion would be supported by the presence of *Amphora pediculus*, shown to prefer shallow water conditions in East African lakes (Gasse, 1986). The extensive growth of algae on the surface sediment accounts for the high abundance of epiphytic species. Species are, overall, indicative of well aerated waters. A *Terpinsoe* species also appears but only represents 1%; this is an upper estuarine species and would seem to indicate contamination, possibly though the introduction of terrapins, which are present in the lake.

Laguna Juanacatlán was dominated by *Fragilaria crotonensis* and *Fragilaria construens* var. *construens, F. construens* var. *venta* and *Fragilaria pinnata*. Given the wide tolerance ranges of these species, the small *Fragilaria* species in particular, it is difficult to infer the role played by nutrients in determining species composition, a problem which has been noted by Bennion (1994) and Sayer (2001). The majority of other species in the assemblage constitute between 0.17 and 3.3%, of which *Fragilaria brevistriata* and *Achnanthes lanceolata* ssp. *frequentissima* were the most abundant. Littoral and epiphytic samples were also taken at this site, although both yielded poor diatom counts (<300 valves). The former was dominated by *F. crotonensis, Nitzschia amphibia* and *Achnanthes minutissima* var. *saprophila.* The epiphytic sample was dominated by *Cocconeis placentula* (42.6%) and *Gomphonema angustatum* (12.1%), neither of which were found in the surface sediment. The lack of epiphytic species in the surface sediment sample can be accounted for by the limited growth of emergent and submerged vegetation.

La Hoya de los Espinos is a deep (40.1m), saline, crater lake with no obvious signs of intensive human impact. However mean annual epilimnetic TP loading (25.19 $\mu g l^{-1}$)

classifies it as mesotrophic. This is supported by the modern diatom assemblage, which was dominated by *Nitzschia palea* and *Cyclotella meneghiniana*. Such an assemblage would seem to indicate the strong role played by nutrients in controlling the diatom variance, but also high silica availability (Metcalfe, 1986). In addition to diatoms there is also an abundance of sponge spicules preserved in the sediment indicating low turbidity, as expected in a deep, sheltered lake.

La Laguna and La Alberca, near the town of Tacambaro, are very close to one another, and thus experience similar climatic and geological conditions. While both basins are closed, La Alberca is a crater lake and La Laguna appears to be lava dammed. They also experience different levels of human impact; the water level in La Alberca is artificially controlled through a sluice gate. Both lakes are dominated by *Achnanthes minutissima*, 65.6 and 83% respectively. La Laguna shows more species diversity with secondary dominance by *Cyclotella stelligera*, 11.5% of the assemblage. La Alberca shows a higher number of *Synedra* species than La Laguna, which may be accounted for by the high silica availability in that lake.

Zempoala is shallow, (4.75m) mesotrophic (TP = 16.7μgl^{-1}), Mg-CO_3 dominated and has a medium salinity and alkalinity, with a neutral pH. The lake is dominated by *Fragilaria crotonensis*, which constitutes 58.3% of the assemblage. Secondary to this species is *Aulacoseira granulata* var. *angustissima*, which also has a preference for shallow lakes. Based on the data for East African lakes, the latter has a preference for lakes which are alkaline and moderately saline conditions (Gasse, 1986), however, it was also reported in lakes with lower alkalinities, such as Zempoala. The results found in this study are in contrast to the results presented by Caballero (1995) who found *Achnanthes lanceolata* to be dominant. Habitat would seem to control species variation within this lake. Although only a small proportion (1.2%) of the surface sediment assemblage was *Achnanthes minutissima,* the epiphytic sample was dominated by the species (53.8%). This sample also contains a high percentage of

Asterionella formosa (15.8%), this mesotrophic-eutrophic indictor species is present also present in the surface sediment but in low abundance (3%).

6.6.4 Oligotrophic Lakes

Lago de la Luna is acidic and ultra oligotrophic and has a surface temperature of 8.5°C. The species in greatest abundance is an *Encyonema* species (Plate 69-72) as yet unidentified, but referred to as *Cymbella perpusilla* by Caballero (1995), but *Cymbella affine* by Vilaclara (unpub. data). However in secondary abundance, (24%) is *Achnanthes subatomoidies*, which has been reported in oligotrophic circum-neutral waters of low salinity in alpine lakes (Krammer & Lange-Bertalot, 1991). It appears that in Mexico this species represents similar conditions. Despite being of the same origin and of similar geology, Lago del Sol has quite a different water chemistry, it is oligotrophic and circum-neutral, with a pH of 6.9. Accordingly the diatom assemblage is different from that of Lago de la Luna. Lago del Sol has a much more diverse species assemblage made up of 30 species. Lago del Sol was dominated by *Aulacoseira italica* (16%), *Navicula vitabunda* (12.7%), *Cymbella sp 1* (12.14%) (Plate 69-72) and *Cavinula* (=*Navicula*) *pseudoscutiformis* (10.62%). Epiphytic and epilithic samples also contained a high proportion of *C. pseudoscutiformis* and of *Achnanthes minutissima,* although the latter is only present in an abundance of 8.6% in the surface sediment. The different water chemistries of the two lakes is reflected by an increase in species in Lago del Sol which are indicative of slightly more saline conditions, but waters which are still quite cool. It is noted that *Stenopterobia delicatissima* is present in greater abundance in Luna, despite its cited preference (Krammer & Lange-Bertalot 1991) for medium salinity conditions. The appearance of *N. vitabunda* in Lago del Sol would support the higher epilimnetic TP concentration relative to Lago de la Luna.

Zirahuén was dominated by the *Cyclotella ocellata* and by *Fragilaria crotonensis,* in line with the assemblage observed by Davies (2000). Other species represent less than 3% of the total but include *Fragilaria construens* var. *venter, Cyclotella schumannii, Aulacoseira ambigua, Achnanthes minutissima* and *Cyclotella stelligera.*

Published water chemistry data for the lake indicate that it is olig- mesotrophic, which is supported by the abundance of *Fragilaria* species. In East African lakes *C. ocellata* has been found in lakes which are highly alkaline, saline and Na-HCO_3. This is in contrast to the conditions observed at Zirahuén, which is weakly alkaline, fresh and Mg-CO_3 dominated. *C. stelligera* is rarely found, in the UK, even in nutrient poor lakes due to its preference for very low nutrient concentrations (Bennion, pers. comm. 2003). This is, however, in contrast to the findings of Stoermer et al. (1985) who found *C. stelligera* to increase rapidly in abundance in response to nutrient enrichment. Although an exact count on the number was not made, *Mallomonas* scales were abundant in this sample, which may be the species *Mallomonas acaroidies*.

Despite being subject to the similar modes of formation and the same broad climatic conditions the lakes of the Cuenca Oriental are highly variable in terms of their water chemistries and consequently diatom assemblages. Alchichica is dominated by *Cyclotella choctawhatcheeana* (70.3%) (Plate 43 & 43) and *Cyclotella quillensis* (23.3%) (Plate 51), all other species constitute less than 2% of the assemblage, but include *Amphora pediculus* and *Nitzschia liebetruthii*. Species such as *C. quillensis* have been found in the North American Great Plains to be present only in lakes with salinities greater than 3 g l^{-1} (Fritz *et al.*, 1999). Atexcac was also dominated by *C. choctawhatcheeana*, which constitutes 94.2% of the assemblage; only 2 other species are present over 1%; *Denticula elgans* (1.4%) and *Nitzschia perminuta* (2.4%). *C. quillensis* appears to be indicative of oligotrophic conditions and of water with medium to high salinity (Krammer & Lange-Bertalot 1991). Carvalho *et al.* (1995) found *C. choctawhatcheeana* to be characteristic of Na/Mg-SO_4 dominated waters, however other published material shows it to be characteristic of Cl dominated waters (Carvalho *et al.*, 1995 and refs therein). Generally this species is indicative of saline, brackish waters, although it cannot be accurately used to infer anion composition. In addition salinity optima for *C. choctawhatcheeana* appear to vary depending on the anion dominance of the system (Carvalho *et al.*, 1995). Lake depth and the limited growth of aquatic vegetation in the Oriental, due to salinity and rapid shelving of the lake bed, accounts for the dominance of planktonic species. The epiphytic samples are dominated by *Nitzschia liebetruthii* and *Cocconeis placentula* in Alchichica while Atexcac is dominated by *Achanthes minutissima* and an *Amphora* sp.

La Preciosa and Quechulac are fresher than Alchichia and Atexcac and have different ionic compositions and thus diatom flora. Quechulac was dominated by the epiphytic species *Cocconeis neodiminuta, Achnanthes lanceolata* ssp. *frequentissima* and by *Fragilaria brevistriata*. The epiphytic sample from Quechulac contrasts with the epiphytic species found in the surface sediment sample. The epiphytic sample was dominated by 4 species, *Achnanthes minutissima, Cocconeis placentula, Fragilaria capucina* var. *radians* and *Gomphonema olivaceum* var. *minutissima*, which were not identified in the surface sediments. In comparison La Preciosa was dominated by *Nitzschia palea* (32.8%) and *Cyclotella meneghiniana* (21.3%). There was also an bundance of *Fragilaria* species, and of epiphytic species, such as *Cymbella* and *Amphora*. *Mastogloia smithii* and its variety, *M. smithii var. lacustris*, which are not found in any other sites, were identified in La Preciosa. Gasse (1986) cites this species are rare in East African lakes but it appears to have a preference for chloride or carbonate dominated waters, with medium to high pH and alkalinity with a wide salinity tolerance. The nominate variety is associated with brackish water in the littoral zone (Krammer & Lange Bertalot, 1986) whereas *M. smithii* var. *lacustris* is associated with waters of a lower conductivity. The key difference between the nominate form and its variety is habitat with the latter only found in the littoral zone (Hustedt, 1959). Breen (2002) also identified these two species in Belize, her work showed a distinct taxonomic variation due to habitat, but within the *M. smithii* var. *lacustris*. In addition her work showed a much lower EC optima and tolerance for this species (610 µS cm^{-1}) compared to this study (2004 µS cm^{-1}). La Preciosa's epiphytic sample was dominated by *Achnanthes minutissima* (33%), *Gomphonema olivaceum* (10.36%) and *Epithemia adnata* (9.5%). La Preciosa and Quechulac can be classified as ultra-oligotrophic and oligotrophic respectively, and based on their diatom assemblages ionic composition and salinity appear to be greater determinants in taxonomic variation. *Nitzschia palea*, which is considered to be a eutrophic indicator species when occurring in large numbers (Schoeman, 1978) and *C. meneghiniana* and *Fragilaria* species also indicate elevated nutrient availability. Such an assemblage in La Preciosa is in contrast with its ultra-oligotrophic nutrient status.

In conclusion, the results of the modern surface sediment diatom analysis shows that there is a degree of progression in species variance across the trophic gradient. Hypertrophic and eutrophic sites are the only sites to contain *Stephanodiscus* species, commonly considered to be indicative of eutrophication (Bennion, 1994). There are a number of other species, such as *Gomphonema parvulum, Fragilaria crotonensis* and *Nitzschia palea* that are also considered to be indicative of nutrient rich conditions, but were present in this calibration set across the nutrient gradient. This can be assessed in more detail using Two-Way-Indicator-Species-Analysis (TWINSPAN), which is discussed in the following section.

Figure 6.9a Hypertrophic lakes

Figure 6.9b Eutrophic lakes

Figure 6.9c Mesotrophic lakes

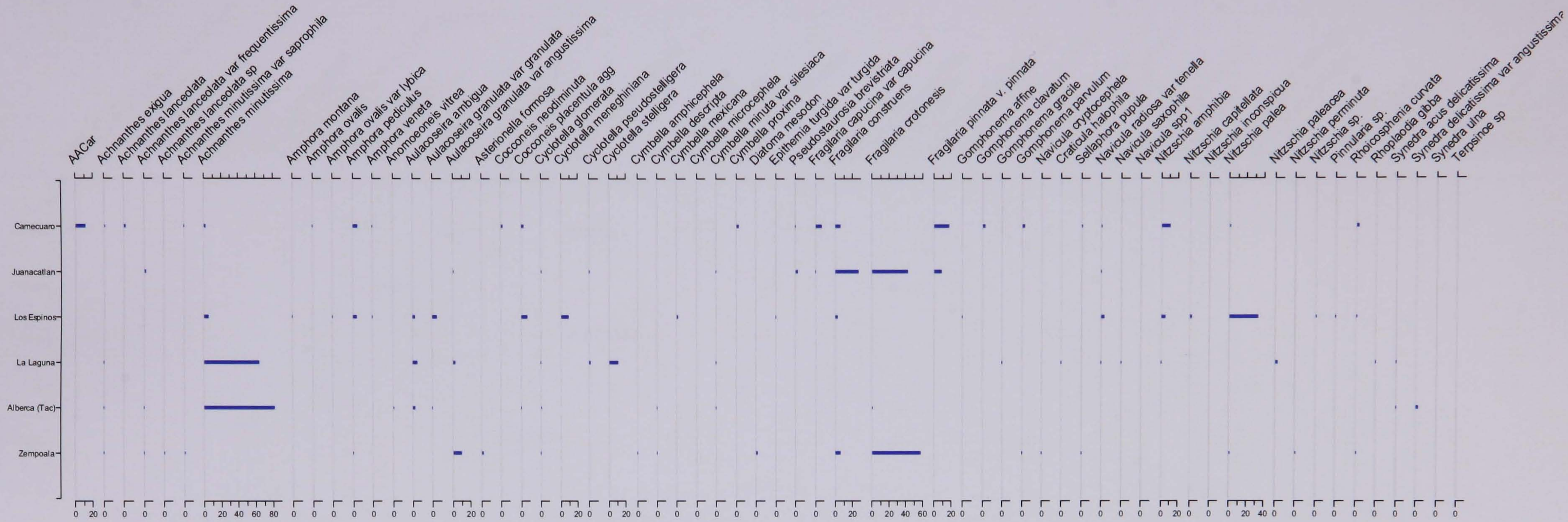
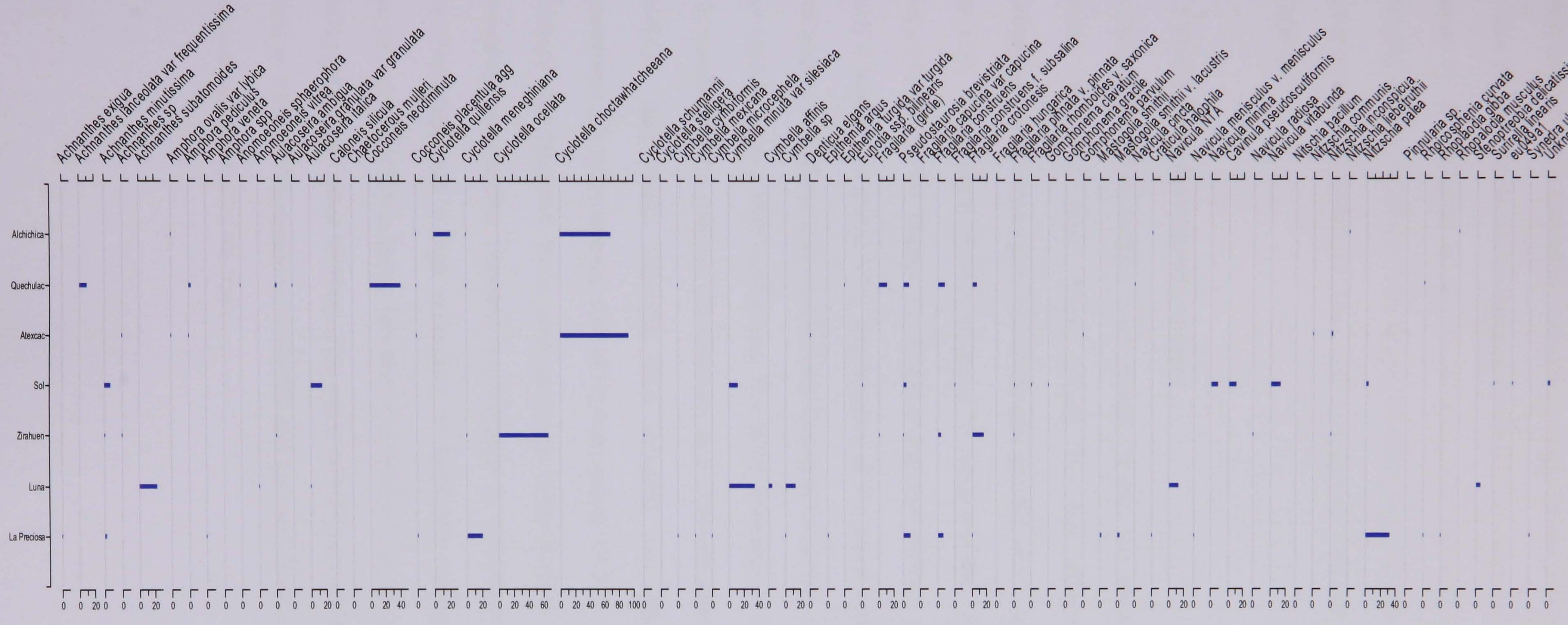


Figure 6.9d Oligotrophic lakes



6.7 TWINSPAN Classification of Modern Diatom Samples & Species

Classification of large taxonomic data sets through cluster analysis, aids interpretation and can provide additional or supportive information for multivariate analyses (see Chapter 7). It highlights taxonomic similarity and dissimilarity between sites, by identifying groups of similar individuals (Manly, 1986). In this study, a hierarchical polythetic divisive classification was used (see Section 5.6.3) the results are presented in Table 6.7.

6.7.1 TWINSPAN classification of sites

The first TWINSPAN division (into groups *0 and *1) separated Alchichica and Atexcac from all other sites, based on the abundance of *Cyclotella choctawhatcheeana* and *Cyclotella quillensis,* species which are not present at any other sites. The second division, of group *0, separates the lakes of the Nevado de Toluca into group *00. Group *01 is split at the third division so that La Piscina de Yuríria is isolated, in group *011. The isolation of this site is most likely related to the dominance of *Chaetocerous muelleri* and *Craticula halophila,* which are not found at other sites in such a high abundance. The fourth division (i.e into groups *0100 and *0101) separates La Alberca (Teremendo) and Zirahuén from the remaining sites. Tecuitlapa, San Pedro, Tepetiltic, San Gregorio, La Laguna and La Albeca (Tacambaro) are subsequently split off to from group *01011. With the exception of Tecuitlapa and Tepetiltic, these sites have high proportions of the epiphytic species *Achnanthes minutissima*. Tepetiltic is dominated by *Aulacoseira ambigua* (43.62%), while Tecuitlapa has a high proportion of *Cyclotella meneghiniana* (22.3%) and *Nitzchia amphibia* (16%). These two sites do, however, contain higher abundances of *A. minutissima* than other samples. This group was divided further with Tecuitlapa split off first at TWINSPAN division 6, Tepetiltic and La Laguna split off at level 7. Laguna Zacapu is also separated off, from

Table 6.7. TWINSPAN classification of samples.

***0**	Lago de Yuríria, La Piscina de Yuríria, Zapotlan, Atotonilco, Los Negritos, Chapala, Cajititlán, Cuitzeo, Tecuitlapa, La Alberca (Ter), La Maria, San Pedro, Tepetiltic, Patzcuaro, San Gregorio, Zacapu, Sta Ma. del Oro, Camecuaro, Juanacatlan, La Hoya de los Espinos, La Laguna, La Alberca (Tac), Zempoala, Quechulac, Lago del Sol, Zirahuen, Lago de la Luna, La Preciosa
***1**	Alchichica, Atexcac
***00**	Luna Sol
***01**	Lago de Yuríria, Zapotlan, Atotonilco, Los Negritos, Chapala, Cajititlán, Cuitzeo, Tecuitlapa, La Alberca (Ter), La Maria, San Pedro, Tepetiltic, Patzcuaro, San Gregorio, Zacapu, Sta Ma. del Oro, Camecuaro, Juanacatlan, La Hoya de los Espinos, La Laguna, La Alberca (Tac), Zempoala, Quechulac, Zirahuen, La Preciosa
***010**	Lago de Yuríria, Zapotlan, Atotonilco, Los Negritos, Chapala, Cajititlán, Cuitzeo, Tecuitlapa, LA Alberca (Ter), La Maria, San Pedro, Tepetiltic, Patzcuaro, San Gregorio, Zacapu, Sta Ma. del Oro, Camecuaro, Juanacatlan, Espinos, La Laguna, La Alberca (Tac), Zempoala, Quechulac, Zirahuen, La Preciosa
***011**	P Yuriria
***0100**	Alberca (Ter) Zirahuen
***0101**	Lago de Yuríria, Zapotlan, Atotonilco, Los Negritos, Chapala, Cajititlán, Cuitzeo, Tecuitlapa, La Maria, San Pedro, Tepetiltic, Patzcuaro, San Gregorio, Zacapu, Sta Ma. del Oro, Camecuaro, Juanacatlan, Espinos, La Laguna, La Alberca (Tac), Zempoala, Quechulac, Preciosa
***01010**	Lago de Yuríria, Zapotlan, Atotonilco, Los Negritos, Chapala, Cajititlán, Cuitzeo, La Maria, Patzcuaro, Sta Ma. del Oro, Camecuaro, Juanacatlan, Espinos, Zempoala, Quechulac, Zirahuen, Preciosa
***01011**	Teucitlapa, San Pedro Tepetiltic, Laguna, Alberca (Tac)
***010100**	Zacapu
***010101**	Lago de Yuríria, Zapotlan, Atotonilco, Los Negritos, Chapala, Cajititlán, Cuitzeo, La Maria, Patzcuaro, SMO, Camecuaro, Juanacatlan, Espinos, Zempoala, Quechulac, Preciosa
***010110**	Tecuitlapa
***010111**	San Pedro Tepetiltic San Gregorio Laguna Alberca (Tac)
***0101010**	L Yuriria Zapotlan Atotonilco, Chapala, Cajititlan, Cuitzeo, Maria Patz, SMO, Camecuaro, Juanacatlan Zempoala
***0101011**	Negritos Espinos Quechulac Preciosa
***01010100**	Atot Chap Cuitz Maria Cam Juan Zemp
***1010101**	L Yur Zapo Cajit Patz SMO

group *01010, at level 6, due to the presence, in abundance, of *Stephanodiscus subtilis*. Group *010101

Lago de Yuríria, Zapotlan, Atotonilco, Negritos, Chapala, Cajititlán, Cuitzeo, Pátzcuaro, Zacapu, Sta. Maria del Oro, Camecuaro, Juanacatlan, La Hoya de los Espinos, Zempoala, Quechulac, Zirahuen and La Preciosa make up group *010101. Los Negritos, La Hoya de los Espinos, Quechulac and La Preciosa are subsequently isolated to form group *0101011. Group *0101010 is broken down to cluster Atotonilco, Chapala, Cuitzeo, La Maria, Camecuaro, Juanacatlán and Zempoala together (group *01010100). These sites cover a range of trophic conditions and as such no one species is dominant at all of them, but they do have an abundance of *Fragilaria* sp. and *Nitzschia amphibia* in common. Group *01010101 is made up of the remaining sites (Lago de Yuríria, Zapotlan, Cajititlán, Pátzcuaro and Santa Maria del Oro) which display relatively high nutrient concentrations and all have a high proportion of *Aulacoseira granulata* and its variety *angustissima*. The TWINSPAN classification of sites does therefore seem to reflect a conductivity gradient, rather than a nutrient gradient as was initially expected.

6.8 Conclusions

One of the primary aims of the thesis was to develop a calibration set for central México, which in contrast to former studies, included nutrient data. This chapter has provided the results of that study in terms of environmental variables and corresponding diatom flora. The main conclusions that can be drawn from this are as follows:

- Many of the lakes sampled were saline, with most showing a decline in salinity between the dry season and the wet season, due to dilution. Lakes are primarily either the Mg^{2+}-CO_3^{2-}-HCO_3^- or Na^+-CO_3^{2-}-HCO_3^- dominated, with the exception of 3 Na^--Cl^- highly concentrated lakes: Los Negritos, Alchichica and Atexcac) and 3 Ca^{2+}-HCO_3^--CO_3^- dilute lakes: La Hoya de los Espinos, Juanacatlán and Zirahuén. The majority of lakes also have pH values greater

8, based on the annual mean, the high altitude lakes of San Gregorio and the lakes of the Nevado de Toluca show more acidic conditions.

- Where full ionic data exist from other studies comparisons were made and for example Zirahuén shows a slight shift towards higher K^+ + Na^+ concentrations and decline in SO_4^{2-} and an increase between 1981 and 2004. Major shifts in the dominant ions are noted at Pátzcuaro and Alchichica. Such shifts in ionic composition and salinity are not unexpected given the increase in levels of precipitation over this time period.
- Lakes in the central México calibration dataset show a TP gradient of 0-1782.65µg l^{-1} and the majority can be classified as hypertrophic or eutrophic with TP concentrations greater than 40µg l^{-1}.
- Many lakes show a rise in nutrient concentration between the dry season and the wet season, associated with increased influx of nutrients from the catchment due to increased run-off. Data were compared with other studies for, among others, Pátzcuaro, Zirahuén and Chapala, where data collected in this study for Zirahuén shows a considerably lower TP concentration than in previous studies (Bernal-Brooks and MacCrimmon 2000), while concentrations at Pátzcuaro, Chapala have increased relative to previous studies.
- With higher concentrations of phosphorus higher algal productivity was expected, and leading to a higher concentration of the photosynthetic pigment Chl-a and lower secchi depth transparency. Based on the data collected here there is a positive relationship between log transformed TP and Chl-a concentration and correspondingly a negative relationship between Chl-a and secchi depth with, in general, hypertrophic sites showing high Chl-a concentrations and low secchi depth and correspondingly nutrient poor sites such as Alchichica have low Chl-a concentrations and high secchi depths. Some hypertrophic lakes such as Atotonilco and Chapala have low Secchi depth transparencies, which may be interpreted as high algal production, however, they have lower than expected Chl-a concentrations. This may be

associated with light limitation of algal production by a high suspended sediment concentration.

- Many of the species identified were ubiquitous across the trophic gradient, however, *A. granulata C. meneghiniana* and *N. amphibia* were present in greatest abundances in hyper- and eutrophic sites. *Stephanodiscus* species are limited to Chapala (11), Cajititlán (12) and Zacapu (24). Oligotrophic sites had lower species diversity but greater abundance of epiphytic species. *Fragilaria* species were found to be abundant at the majority of sites, which may be associated with the shallow depth of many lakes as these species thrive in shallow water environments (Bennion, 1994; Sayer, 2001).
- TWINSPAN classification does not split sites clearly into trophic categories, and seems to reflect the conductivity gradient. Group *0101011 included the hypertrophic site Los Negritos (10) and the oligotrophic sites La Preciosa (47) and Quechulac (38). In addition Atotonilco (8), which has the highest TP concentration, was grouped with the mesotrophic sites Juanacatlán (29) and Zempoala. With the exception of San Gregorio (23), Zacapu (24) and Tepetiltic (20) however, all sites are saline, with EC values greater than 300 µS cm^{-1}.
- Lakes of higher nutrient status were, in general, not dominated by typical eutrophic indicator species, probably associated with the role played by salinity in driving diatom variation. The role played by habitat in driving diatom variation is also apparent. Sites in TWINSPAN group *01011 cover a range of TP concentrations but are dominated by epiphytic species, *A minutissima* in particular, hence their similarity.

The relationship between diatom flora and their environment can be explored further using unconstrained and constrained ordination and weighted averaging regression and calibration. Despite the apparent role of EC in driving species variance it is clear that there is a role played by nutrient availability. Ordination (or gradient analysis) can establish which environmental variables are significant in driving changes in the diatom flora and WA can establish the accuracy with which an environmental variable can be reconstructed. These issues are presented and discussed in Chapter 7.

Chapter 7. Species-Environment Relationships: The Central Mexican calibration set and Development of a Transfer Function for Nutrient status

7.1. Introduction

The construction of a successful diatom based transfer function is dependent upon establishing that a particular environmental variable, in this case TP, explains a significant and independent proportion of variance in the diatom assemblage. To establish the nature of the relationship between diatom species variation (response variables) and environmental variables (predictor variables) a modern calibration data set is required (Smol, 2002). This consists of surface sediment samples, and physical and chemical lake characteristics. The modern relationship between the predictor and response variable can be explored through unconstrained and constrained ordination, using the programme CANOCO (ter Braak, 1987). Based these relationships inferences can made, using weighted averaging (WA) regression and calibration, to establish relationships between fossil data and environmental changes. This chapter discusses some of the background associated with the creation of diatom based nutrient transfer functions and the creation of a nutrient transfer function for central México.

7.2 Quantitative Reconstruction of Palaeoenvironmental Change

Species abundance and variance is controlled by physical, chemical and biological factors imposed by the environment, either as a result of natural or anthropogenic forcing (Round *et al.*, 1990). Consequently species variation can be related to present day environmental conditions and, assuming that over time species responses to environmental variables have not changed, these relationships can be used to infer environmental changes from fossil species assemblages. Such data can then provide information on baseline or natural conditions and provide an indication of causes of system change.

Quantitative environmental reconstruction is based on 5 assumptions (Imbrie & Webb 1981):

1) That species are systematically related to the environment in which they live
2) The environmental variable of interest is (linearly) related to an ecologically important determinant in the ecosystem under study
3) Mathematical models used to relate species variation to environmental variables are adequate
4) Other environmental variables have a negligible influence on taxonomic variation
5) The taxa in the training set are well represented in core sediments and that species response to the environmental variable in question has not changed over the time span of the assessment i.e. there has been no change in species optima and tolerance for the environmental variable

Diatoms have long been recognised as indicators of pH and the impact of acid rain on lacustrine systems in the late 1960s and early 1970s brought about the need to track and quantify changes in pH and identify the causes of acidification to aid lake restoration. This required the construction of quantitative methods of palaeoenvironmental reconstruction. Initial methods such as Index B (Renberg & Hellberg, 1982) inferred pH from Hustedt's (1939) pH preference groups and did not make use of contemporary data on the distribution of individual taxa (ter Braak & van Dam, 1989). A number of numerical procedures have been developed since to quantitatively relate contemporary species distribution and abundance to environmental variables and thereby make quantitative inferences on the fossil data based on contemporary relationships. More recent attempts to make quantitative palaeoenvironmental reconstructions, in particular changes in pH (Davis & Anderson, 1985), involved the use of multiple linear regression but still assumed a linear relationship between species variation and environmental variables (ter Braak & van Dam 1989).

Species responses to environmental variables are, however, usually unimodal and can be represented as a Gaussian response curve: species are most abundant around an optimum value of the environmental variables and cannot survive when values are too high or too low, i.e. they have a tolerance range (Birks *et al.*, 1990). Consequently, statistical methods of inferring environmental variables have since been developed based on this principle. ter Braak and van Dam (1989) compared maximum likelihood (ML) and weighted averaging (WA) with multiple regression as methods for reconstructing pH. While the former was found to be slightly better than WA, with a lower prediction error, it was computer intensive and more sensitive to outliers in noisy biological data sets. Consequently WA has become the main method used in palaeolimnology as it combines simplicity with good performance and is applicable to a number of environmental variables (Birks, 1995). To determine whether an environmental variable is suitable for use in environmental reconstruction, its role in driving species change must be established. To create a suitable transfer function an environmental variable must explain a significant and independent proportion of taxonomic variance. This can be established from the relationships between the modern surface sediment samples and the contemporary water chemistry data, using gradient analysis.

7.2.1 Ordination/Gradient Analysis

Ordination, or gradient analysis, is a multivariate technique which relates response variables, such as taxonomic data, to measured predictor variables (environmental variables) or hypothetical (latent) gradients (Lepš & Šmilauer, 2003) and is therefore suitable for assessment of ecological data. Gradient analysis arranges sites along axes in two-dimensional space based on species data, resulting in the ordering of sites, whereby sites with similar species compositions are close together and conversely, sites with vastly different species compositions are at opposite ends of the axes. There are two forms of gradient analysis: indirect gradient analysis (unconstrained ordination), where gradients are inferred from species composition, and direct gradient analysis (constrained ordination), where environmental gradients are known and related to species composition.

7.2.1.1 Indirect gradient analysis

Detrended correspondence analysis (DCA) is an indirect gradient analysis technique for unimodal species data, and as predictor variables (environmental variables) are not included in the analysis, the ordination is unconstrained. It is used to counteract the arch effect, where the positions of samples on the second ordination axis are strongly dependent upon their positions on the first ordination axis. DCA is also used to combat compression of data at the extreme ends of the gradient due edge effects that effect correspondence analysis (Hill & Gauch, 1980). DCA maximises the amount of spread along ordination axes in the species data, independent of environmental data. Species spread along the axes can be used to *infer* environmental gradients as DCA only uses species and site data. This has a certain degree of benefit over direct gradient analysis, as direct gradient analysis includes environmental data, which may have a limited gradient and a higher degree of uncertainty (ter Braak, 1995). DCA can be used to indicate whether taxa have responded to the theoretical gradients in a linear or unimodal way, and consequently whether unimodal (gradients > 2 SD) or linear methods (gradients < 2 SD) of constrained ordination are more appropriate (Birks, 1995). More recently, the use of unimodal methods has been suggested only if gradients are > 4 SD (Lepš & Šmilauer, 2003). DCA can also be used to identify any outliers, which lie ≥2 SD from the mean. DCA provides species and sample scores derived by weighted averaging (WA), where species scores are calculated by WA regression from initial site scores. Subsequently site scores are calculated by WA calibration from the species scores. This unconstrained ordination provides information on species variation and should be coupled with a constrained ordination technique such as canonical correspondence analysis (CCA) direct gradient analysis.

7.2.1.2 Direct Gradient Analysis

Constrained ordination, such as CCA, is used to explore, simplify and express underlying patterns and relationships between the species assemblage composition and measured environmental variables (Charles & Smol, 1991). CCA, in a similar manner to DCA, maximizes niche separation and presents artificial gradients, based on the measured environmental gradients (Dixit *et al.*, 1999). Thus sites and species are ordered along the environmental gradients, providing an indication of species assemblage changes in response to these gradients. Biological variability is explained as a function of the environmental variables, i.e. the ordination axes are constrained by the environmental variables. Similar sites and species are therefore grouped together with, for example, species at sites with high TP concentrations located at one end of the gradient and sites dominated by species that show a preference for low TP concentrations at the opposite end of the gradient (e.g. Charles & Smol, 1991). CCA can, therefore, be used to establish which environmental variables explain large and significant proportions of taxonomic variation, and consequently which are suitable for reconstruction.

7.2.1.3 Variance partitioning

Variance partitioning is performed using a series of CCAs and partial CCAs and is used to establish the independence and strength of each of the main environmental gradients. This process establishes a) the unique contribution of each environmental gradient, b) interactions between pairs of gradients and c) any unexplained variance in the taxonomic data set (Borcard *et al.,* 1992; Gasse *et al.*, 1995). Partial CCAs and variance partitioning have been used in numerous studies to build a case for the construction of various transfer functions (c.f. Gasse *et al.*, 1995; Hall *et al.*, 1999; Jones & Juggins, 1995).

7.2.2 Weighted averaging regression and calibration

Weighted averaging (WA) is a two-step process; modern diatom responses to contemporary environmental variables are modelled first (regression). This involves a modern calibration set comprised of surface sediment diatom samples (the response variables) and the environmental variable of interest, across as wide a gradient as possible (the predictor variable). The modelled response from the modern data can then be used to infer past conditions from the fossil data. WA is based on the assumption that a species with a particular optimum, such as TP, will be most abundant in lakes with TP values at or close to this optimum (ter Braak & van Dam 1989). To estimate the optimum of a particular species, an average is taken of all the values at all the sites at which that species occurs, weighted by its abundance. This is referred to as a regression problem. Once defined, the optimum can be used to infer TP of a lake based on its diatom assemblage. In addition, species can be weighted based on their tolerance rages, $WA_{(tol)}$, for example *Fragilaria* species have been shown to have a broad TP tolerance (Bennion, 1994; 1995; Sayer, 2001) and are, therefore, poor indicators of TP. In this case they can be downweighted so they do not exert any undue influence on the analysis. It should be considered, however, that as calibration sets are often small, like in this study, they may not provide an accurate estimate of species tolerance and, in addition, the inclusion of all species found at all sites has often been found to improve WA estimates (Birks, 1995).

The process of WA regression and calibration involves averages being taken twice, which results in shrinking of the environmental variable towards the mean of the calibration set (Charles and Smol, 1991). The WA method provides an option for correcting the shrinkage of inferred values through either classical deshrinking, where inferred values are regressed on measured values, or the opposite, inverse deshrinking, with measured regressed on inferred values. Fitted values are then taken as the estimate of the environmental variable (ter Braak & Juggins, 1993). Past research has shown that classical deshrinking moves inferred values further away from the mean than inverse deshrinking and is therefore better for inferring values that lie at extreme ends of the gradient. In contrast, inverse deshrinking minimizes the root mean square of errors, one of the factors used to assess the models predictive capacity.

The predictive abilities of WA can be assessed by the coefficient of determination (r^2) and by root mean square of error of prediction (RMSE) (Bennion, 1994). The r^2 provides an indication of the strength of the relationship between the measured and inferred values and is, ideally, as close to 1 as possible. Apparent RMSE (observed – inferred) has been found to provide the most useful criteria for assessing predictive ability as it provides an indication of variation expected when applying the transfer function to new assemblage data (Charles & Smol, 1991). When based solely on the training set data, RMSE often underestimates, while r^2 usually over estimates (Birks, 1995). More reliable estimates can be derived using one of two cross-validation techniques: jack-knife or bootstrap. The former involves removing one sample from the calibration set and deriving inference equations for the remaining samples and applying the model to the left out sample. This process is repeated for each sample and the error is calculated using the estimates of the samples when left out of the model. Bootstrapping is a computer intensive re-sampling procedure which aims to mimic sampling variation in the actual calibration set and as it uses the full training set size, unlike the jack-knife procedure, it is less prone to bias. For each of the bootstrap cycles (between 500 and 1000 typically used) a subset of calibration samples is selected, at random, with replacement from the original calibration set to form a bootstrap calibration set the same size as the original calibration set (Birks *et al.*, 1990). Consequently some samples may be selected more than once and others are not selected and the latter are used to form a bootstrap test set. WA regression and calibration are carried out in each cycle using the bootstrap training set and this is used to infer water chemistry values for the test set, those from the original set, not included in the bootstrap set.

Improvements have been made to simple WA using partial least squares regression (PLS) to give weighted averaging partial least squares (WAPLS) analysis. This is an extension of WA which uses the residual correlation in the diatom data to improve the predictive power of the WA regression coefficients (ter Braak & Juggins, 1993). In simulations, WAPLS has been shown to give up to 70% reduction in RMSEP in data sets with little "noise" however in noisy data sets the reduction is smaller, and as it considers the role played by other variables it is therefore thought to be more accurate. The model was then applied to the Danish and the combined northwest Europe TP calibration data sets and was shown to provide more accurate predictions than the simple WA (Bennion *et al.*, 1996; Bradshaw *et al.*, 2002).

7.3 Species-environment relationships in Central Mexico

7.3.1 Indirect Gradient analysis

A PCA was run on the environmental data to determine the number and relative strengths of gradients present in the data set (see Figure 7.1). PCA indicated a number of strong environmental gradients namely cations, anions, transparency, pH, TP, SiO_2 and conductivity. The results echo those found in Chapter 6, with a strong relationship found between $K^+ + Na^+$ and conductivity, the relationship between anions and cations and between increasing Chl-a and declining secchi depth. This can been observed in the PCA diagram by a small angle between the vectors

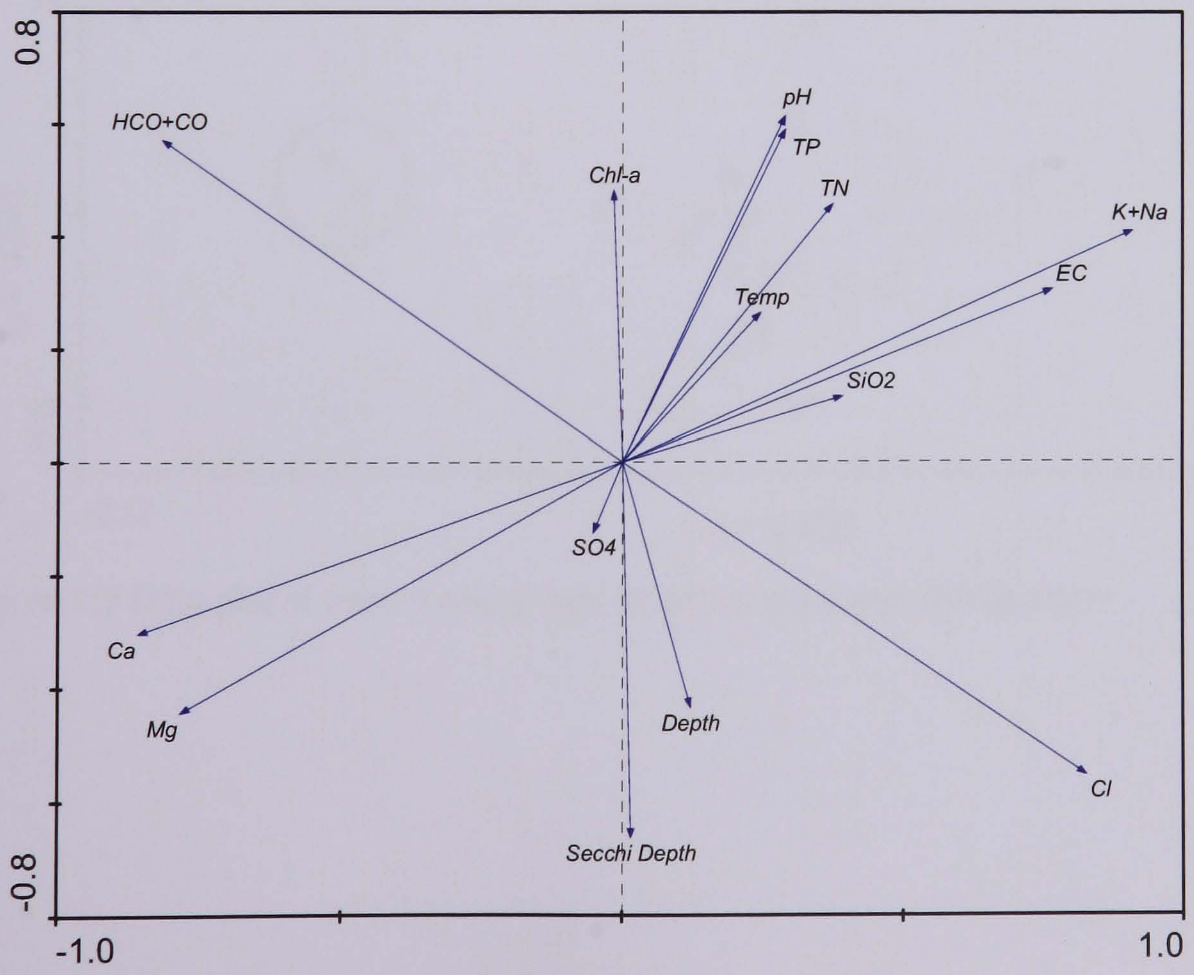


Figure 7.1. PCA of environmental variables

DCA of the diatom surface sediment species data, 30 sites and 135 species, detected 3 clear outliers, sites 36, 40 and 46 (Figure 7.2. see Table 5.1 for site codes). Alchichica and Atexcac (36 and 40 respectively) are both dominated by *Cyclotella choctawhatcheeana* (70.3% and 94.2% respectively), a species which is unique to these two sites. Lago de La Luna is dominated (34.5%) by *Cymbella minuta* var. *silesiaca*, which is present in other sites, but not at such high abundances. DCA was subsequently run again with these outliers removed (see Figure 7.3), as they exert undue influence on the ordination. In this second DCA, eigenvalues for axes 1 and 2 are 0.47 and 0.3 respectively and account for 15.8% of the species variance, with a total inertia of 4.82. Such a low explained variance is expected for noisy datasets containing many taxa and many zero values. Gradient lengths were 3.94 and 3.11 on axes 1 and 2 respectively indicating that unimodal methods are appropriate for constrained ordination. Based on the results presented above, unconstrained ordination axes are > 2 SD, species show a unimodal response to environmental variables and thus CCA was used for further analyses.

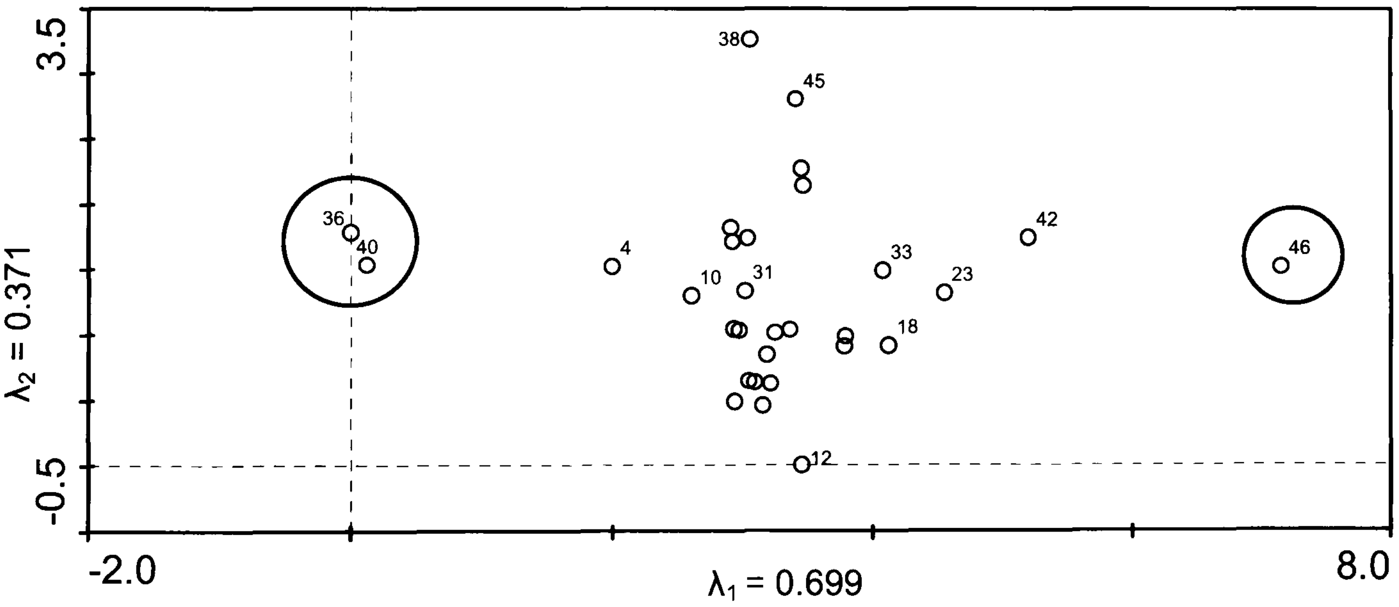
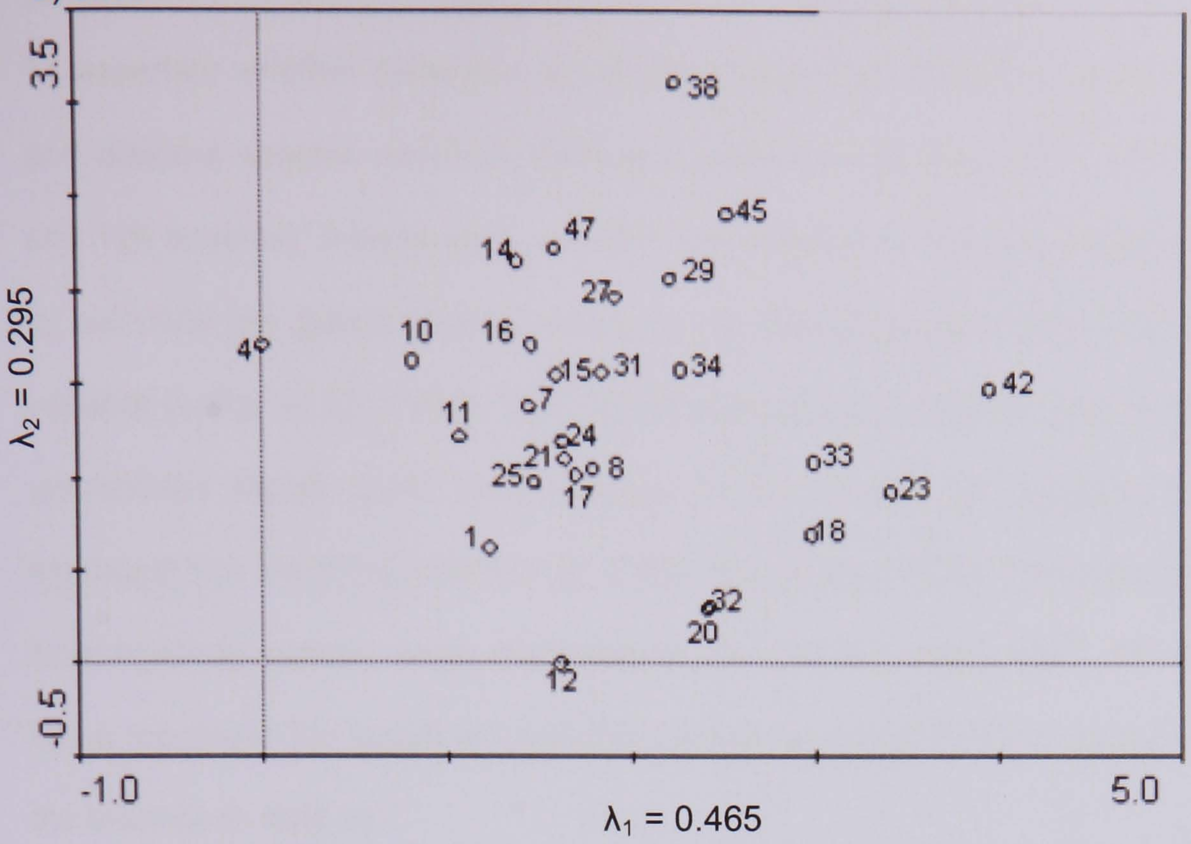


Figure 7.2 DCA plot of axes 1 and 2 with 30 active sites and 135 species

a) sites



b) species

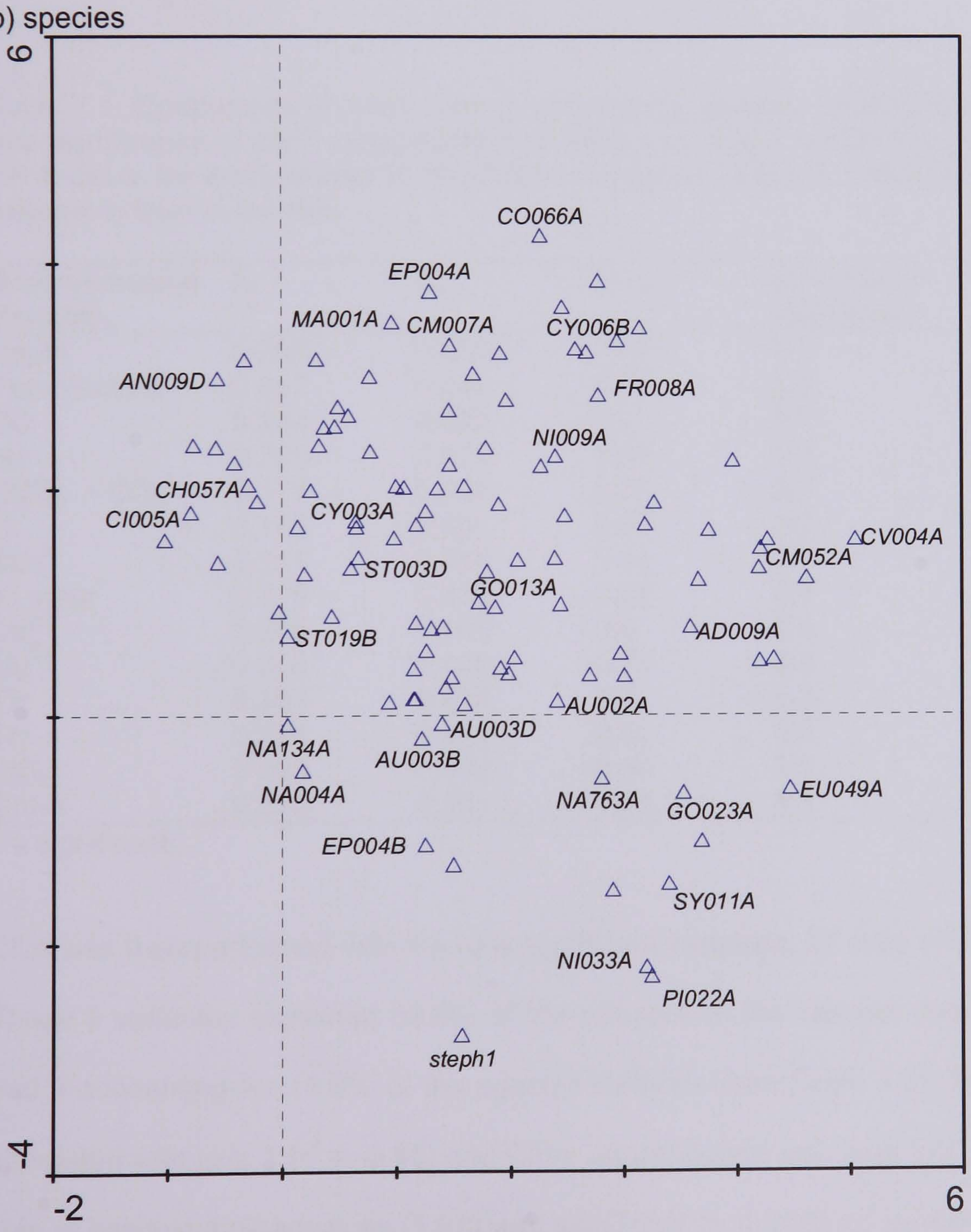


Figure 7.3 DCA plot of axes 1 and 2 with outliers removed, 27 active sites (Alchichica, Atexcac and Lago de la Luna made passive) and 129 active species

7.3.2. Direct Gradient Analysis

To ascertain whether there is a significant relationship between nutrient concentration and diatoms species variation, CCA was performed on the above data set (27 sites and 129 species). Initially each variable was entered as the sole constraining variable to calculate the percentage variance in the biological data accounted for by each variable (Lotter *et al.*, 1997). The significance of each variable was tested using 999 unrestricted Monte Carlo permutations. Of the original 14 variables (secchi depth excluded) five were insignificant ($p > 0.05$): depth, Ca and the three anions (Table 7.1). Consequently surface water temperature, EC, pH, $K^+ + Na^+$, Mg^{2+}, TP, TN, SiO_2 and Chl-a represent the significant variables which best explain the majority of variance in the taxonomic data set.

Table 7.1. Eigenvalues of axes 1 and 2, percentage variance caused by each variable and significance of each variable when entered as the sole constraining variable. EC, TP & Chl-a are emboldened to emphasise explained variance and λ_1/λ_2 as these are referred to later in the text.

Environmental Variable	λ_1	λ_2	λ_1/λ_2	% variance explained	p-value
Depth	0.203	0.459	0.44	4.2	0.243
Temperature	0.257	0.446	0.58	5.3	0.035*
EC	**0.344**	**0.432**	**0.8**	**7.1**	**0.001***
pH	0.255	0.432	0.59	5.3	0.02*
$HCO_3^- + CO_3^{2-}$	0.177	0.459	0.39	3.7	0.536
Cl^-	0.185	0.45	0.41	3.8	0.436
SO_4^{2-}	0.141	0.458	0.31	2.9	0.895
$K^+ + Na^+$	0.315	0.435	0.72	6.5	0.001*
Ca^{2+}	0.268	0.443	0.6	5.9	0.051
Mg^{2+}	0.308	0.432	0.73	6.4	0.001*
TP	**0.306**	**0.438**	**0.7**	**6.3**	**0.001***
TN	0.291	0.438	0.5	6.0	0.001*
SiO_2	0.285	0.432	0.66	5.9	0.002*
Chl-a	**0.273**	**0.45**	**0.61**	**5.7**	**0.008***

* = significant

CCA was then performed with the nine significant variables, 27 sites and 129 species. These 9 variables explained 39.6% of the variance in the species data, with axes 1 and 2 accounting for 13.8% of the species variance (see Table 7.2), EC was highly correlated with axis 1 ($r^2 = -0.85$) and Chl-a concentration with axis 2 ($r^2 = -0.71$). The sum of canonical eigenvalues (1.91) was significant ($p < 0.05$) as assessed using 999 unrestricted Monte Carlo permutations. Species environment correlations were high for axis one and two (0.954 and 0.937 respectively) and accounted for 34.8% of the variance in the diatom-environment relationship. This indicates a strong relationship between the 9 measured environmental variables and the 129 taxa (see Table 7.3). Although there was a high correlation between EC and Mg and K + Na, variance inflation factors (VIFs) are all below 20, indicating little multi-collinearity between variables (c.f. Appendix 3).

Table 7.2. Summary statistics for CCA with 9 environmental variables, 27 sites and 134 species

CCA Axes	**1**	**2**	**3**	**4**	
Eigenvalue	0.382*	0.284	0.253	0.237	
Species environment correlations	0.954	0.937	0.917	0.891	
Cumulative percentage variance					
- of species data	7.9	13.8	19.1	24	
- of species-environment relationship	20	34.8	48.1	60.5	
Total Inertia					4.821
Sum of canonical eigenvalues					1.911**

* p = 0.052
** p = 0.006

Table7.3. Weighted correlation matrix 9 significant environmental variables

	Temp	EC	pH	K^+Na^+	Mg^{2+}	TP	TN	SiO_2	Chl-a
Temp	1.000								
EC	0.520	1.000							
pH	0.391	0.64	1.000						
K^++ Na^+	0.466	0.872	0.625	1.000					
Mg^{2+}	-0.53	-0.80	-0.65	-0.88	1.000				
TP	0.444	0.626	0.353	0.689	-0.78	1.000			
TN	0.479	0.447	0.504	0.544	-0.65	0.82	1.000		
SiO_2	0.366	0.567	0.153	0.386	-0.28	0.295	0.053	1.000	
Chl-a	0.36	0.222	0.411	0.35	-0.5	0.653	0.785	0.075	1.000

CCA was then re-run with forward selection to determine which variables made independent and significant contributions to explaining the variation in the taxonomic data set. This showed that only EC and Chl-a were significant, ($p < 0.05$, and allowing for Bonforonni correction). Table 7.4 gives the summary statistics for the CCA using only the two significant variables. Together they account for 12.6% of the variance in the species data, where axis 1 and 2 have eigenvalues of 0.349 and 0.256 respectively and have high species-environment correlations. These eigenvalues are only slightly lower than when 9 variables were used, but species-environment correlations are higher, indicating these two variables drive the majority of variance in the taxonomic data set.

Table 7.4. Summary statistics for results of CCA with forward selection with 27 sites, 129 species and two environmental variables.

CCA axes	1	2	3	4	
Eigenvalues	0.349*	0.256	0.432	0.396	
Species-environment correlations	0.938	0.911	0	0	
Cumulative percentage variance					
- of taxonomic data	7.2	12.6	21.5	29.7	
- of species-environment relationship	57.6	100	0	0	
Total Inertia					4.821
Sum of canonical eigenvalues					0.605**

*p=0.001
**p=0.001

Partial CCAs were then carried out to estimate a) the unique variance explained by each variable and b) the conditional variance between the pairs of variables and c) remaining unexplained variance. Results (Fig 7.4) indicate that Chl-a accounts for 43.1% of the variance in the species-environment relation and 5.41% of the variance in the species data. As can be seen from Figure 7.4 CHl-a is significant, but EC makes a greater contribution to the variance in the species data. There is little conditional variance between the two variables, 2% of the species environment relationship and 0.25 in the species data. This is also highlighted by the large angle between the arrows, seen in the in the CCA biplot (Figure 7.5).

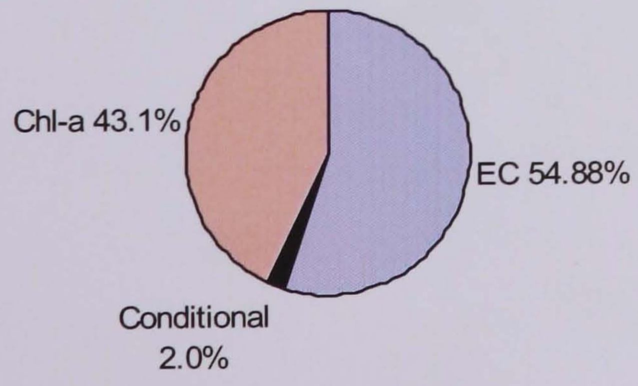
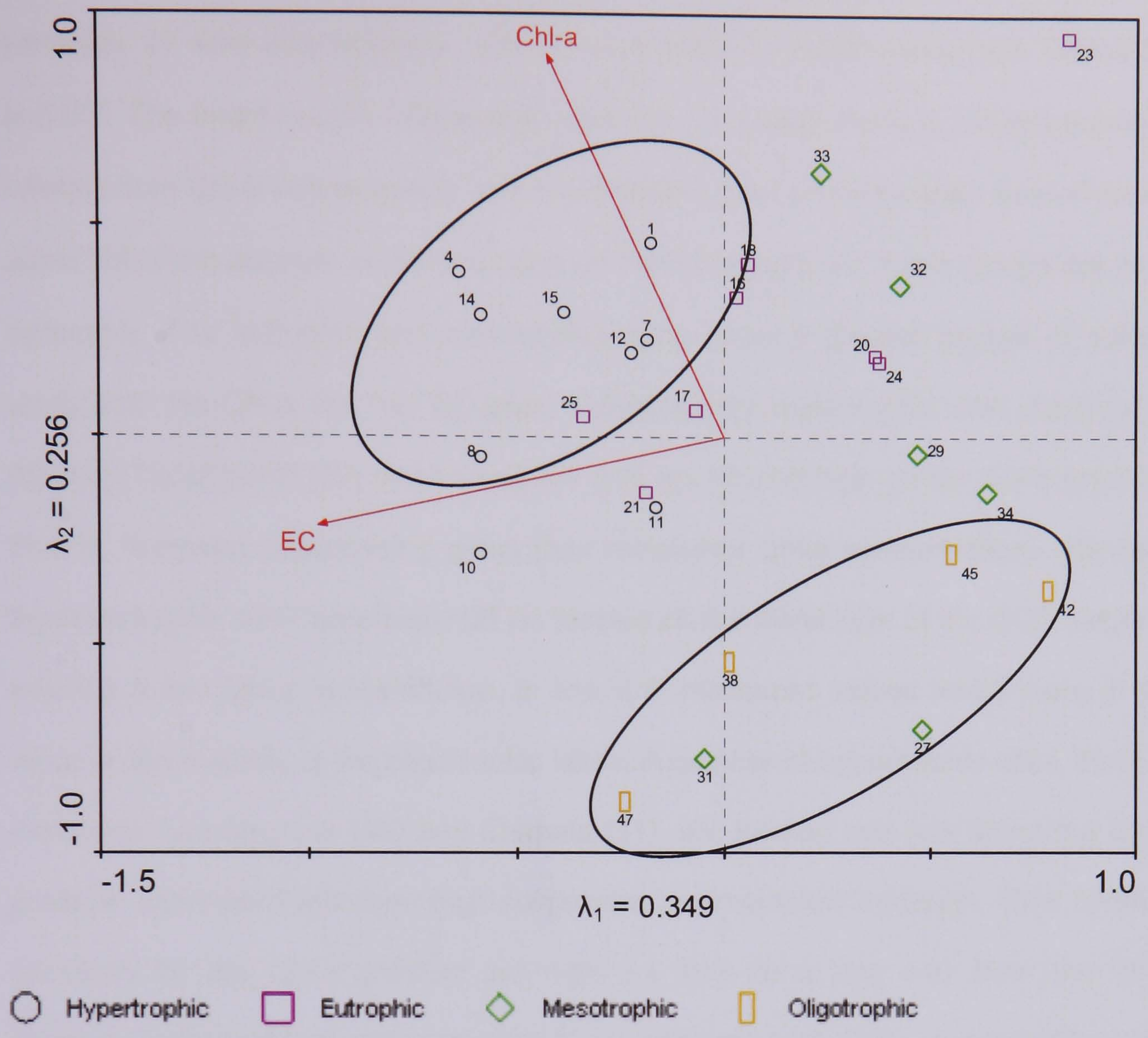


Figure 7.4 Results of variance partitioning (of species-environment relationship)

a) Samples, classified by trophic state (for codes see Table 5.1)



b) Species, only common species shown (N2 > 3)

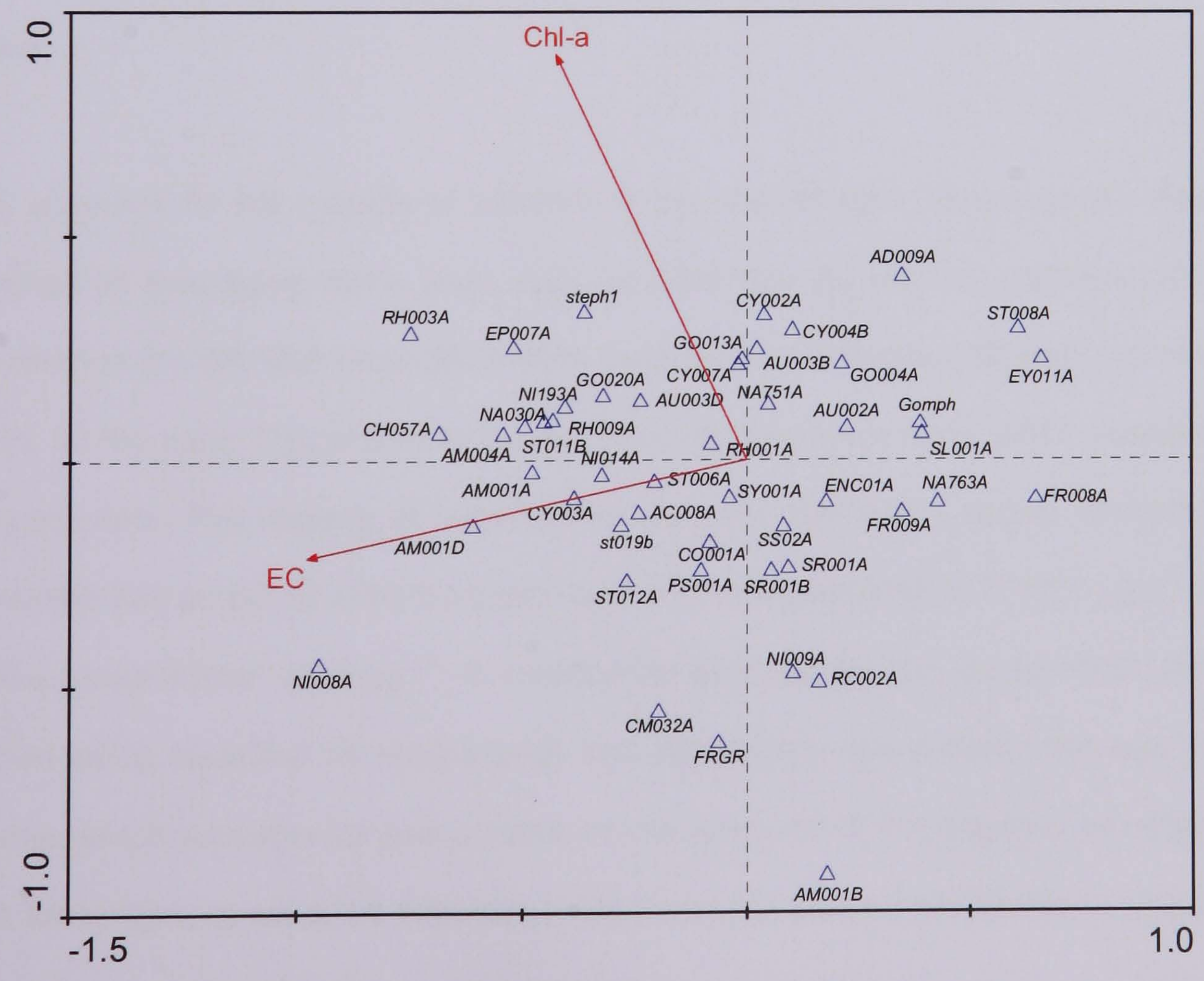


Figure 7.5. CCA biplot of 27 sites, two environmental variable and 129 species

Figure 7.5 presents results of ordination in a CCA biplot, based on two environmental variables, 27 sites and 129 taxa, with sites classified by trophic state (see Tables 3.1 and 6.7. The biplot roughly shows that sites are separated along a nutrient gradient, inferred from Chl-a concentration, with hypertrophic sites predominantly located on the upper left of the diagram and oligotrophic on the lower right, i.e. these groups are most distinctive. The eutrophic and mesotrophic sites show a greater degree of spread along both the Chl-a and the EC axes. Consequently mesotrophic sites such as La Alberca (Tacambaro) (33) and La Laguna (32) are located high on the Chl-a gradient. This is, however, unsurprising given their measured Chl-a concentrations. Similarly the mesotrophic site Camecuaro (27) is located on the lower right of the CCA diagram, inferring a low Chl-a concentration, in line with measured values which were in the range of the majority of the oligotrophic lakes. A number of hypertrophic sites, such as Atotonilco, Los Negritos (10) and Chapala (11), are located mid way along the Chl-a gradient, associated with their high suspended sediment concentration. Their residual distances for the Chl-a gradient are high, i.e they lie a long way from the Chl-a gradient, indicating that although they have relatively high Chl-a concentrations the strength of the relationship between nutrient concentration and species variation is weak.

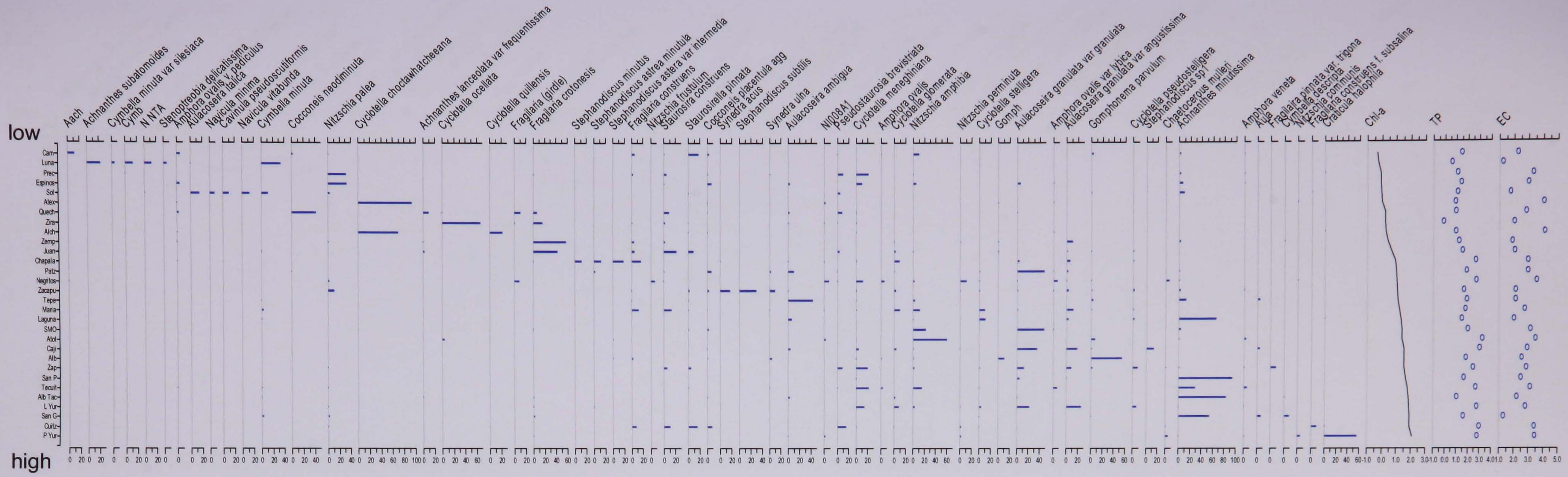
EC accounts for the majority of variance in the species data, consequently sites are aligned so that highly saline sites, such as Atotonilco (8) and Los Negritos (10), are located on the left, with more dilute sites, such as San Gregorio (23) and Lago del Sol (42), on the right. This accounts for the mixed distribution of sites, when classified by trophic state. The majority of hypertrophic sites are also highly saline; Atotonilco for example has an EC of 3286.5 µS cm^{-1} and a TP concentration of 1782.7 µg l^{-1} and a Chl-a concentration of 25 µg l^{-1}. In contrast Santa Maria del Oro and La Preciosa (47), whilst being classified as mesotrophic and oligotrophic respectively, are both highly saline, which accounts for their position on the lower left of the diagram, as oppose to the lower right, as would be expected if Chl-a was the primary explanatory variable.

Species spread is strongly associated with axis 1, indicating the primary role played by EC. If nutrients were the primary cause of variation in the diatom data set, then it would be expected that eutrophic indicator species, such as *Stephanodiscus* spp, *Gomphonema parvulum* (GO013A), *Cyclotella glomerata* (CY007A) and *Nitzschia palea* would be located on the upper left of the CCA biplot, relating to a high Chl-a concentration, but are located in the centre of the diagram. This is a function of sites dominated by these species, *Stephanodiscus* spp in particular, exhibiting low Chl-a, but high TP concentrations, associated with high turbidity and suspended sediment loading. *Nitzschia palea*, which is generally considered to be indicative of polluted water (Germain, 1981), has a high abundance in La Preciosa (47) and La Hoya de los Espinos (31), which exhibit low nutrient and Chl-a concentrations, consequently this species is located low on the Chl-a axis. This species has been noted to have a preference for alkaline conditions, but with a wide tolerance for conductivity (Gasse, 1986) which may account for its location, as these sites which have relatively high alkalinities and conductivities. Other hyper- or eutrophic, but saline, sites such as Los Negritos (10) and Piscina de Yurira (4) are dominated by species with a preference for high salinity conditions, such as *Chaetocerous muelleri* (CH057A), *Nitzschia perminuta* (NI193A) and *Rhopalodia gibberula* (RH003A) and are located in the mid to far left, indicating the role played by EC in driving diatom variation at these sites.

7.3.3 Weighted Averaging Models for Reconstruction of Nutrient Status

The results of CCA indicate that the strongest gradient driving taxonomic variation is EC, but that Chl-a is strongly correlated with axis 2 and thus represents a significant and secondary gradient in driving diatom variation. Chl-a can be used as a proxy for nutrient concentration as it combines the effect of both phosphorus and nitrogen limitation (Jones & Juggins, 1995). Chl-a has been used in several studies to infer changes in nutrient status for this reason (Reid, 2005; Reavie & Smol, 2001).Consequently as the existence of N limitation and N-P co-limitation has been detected in Laguna Zirahuén (Bernal-Brooks & MacCrimmon 2002b) the use of Chl-a rather than TP may be more appropriate. When each variable is entered as the sole constraining variable, the ratio of canonical axes 1 and 2 can be used as an indication of the strength of a variable in driving diatom variation (Bennion, 1994). In the current study λ_1/λ_2 for Chl-a = 0.61 (see Table 7.1), and while TP was slightly higher (0.7) this variable was insignificant ($P > 0.05$) in the forward selection process. In this study the λ_1/λ_2 for the variable of interest was greater than in some former studies, e.g. Bennion (1994) used TP with $\lambda_1/\lambda_2 = 0.5$, although she found TP to be the most significant variable in driving diatom variation. Transfer functions have also been created when the variable in question has been a secondary gradient. Siver (1999) created a successful transfer function for TN as even though it was correlated with axis 2 (with pH as the primary variable), furthermore it yielded a significant and high λ_1/λ_2 value of 0.49.

When sites are arranged along a Chl-a gradient and a TP gradient, in ascending order, and species are arranged by their WA score, an overall shift in dominant species is noted (see Figure 7.6). As established in Chapter 6 this highlights that certain species are only present at extreme ends of the gradient, while other species such as *A. minutissima*, *Cyclotella meneghiniana* and *F. construens* which are more ubiquitos. *Achnanthes minutissima* is present in greater abundance at higher Chl-a concentrations (see Figure 7.7). This species is indicative of well aerated, oxygen rich waters (Gasse, 1986). Photosynthesis results in the production of oxygen, as well as Chl-a and it may be possible that it at a certain level of photosynthesis, prior to oxygen depletion that conditions are conducive for the abundant growth of this species. It can also be seen from Figure 7.6b and Figure 7.7b that the response of *A. minutissima* to TP indicates a preference for mid range TP concentrations, but seems to be able to persist when there are low light conditions. This arrangement of sites and species further highlights the role of EC in species variation as sites with high nutrient concentrations usually are sites with high EC values, and as a result the species distribution mirrors this.


low
high
Aach
Achnanthes subatomoides
Cymbella minuta var silesiaca
Cymb
N NTA
Stenoptreobia delicatissima
Amphora ovalis v. pediculus
Aulacoseira italica
Navicula minima
Cavinula pseudoscutiformis
Navicula vitabunda
Cymbella minuta
Cocconeis neodiminuta
Nitzschia palea
Cyclotella choctawhatcheeana
Achnanthes lanceolata var frequentissima
Cyclotella ocellata
Cyclotella quillensis
Fragilaria (girdle)
Fragilaria crotonesis
Stephanodiscus minutus
Stephanodiscus astrea minutula
Stephanodiscus astera var intermedia
Fragilaria construens
Nitzschia frustulum
Staurosira construens
Staurosirella pinnata
Cocconeis placentula agg
Synedra acus
Stephanodiscus subtilis
Synedra ulna
Aulacoseira ambigua
NI008A1
Pseudostaurosia brevistriata
Cyclotella meneghiniana
Amphora ovalis
Cyclotella glomerata
Nitzschia amphibia
Nitzschia perminuta
Cyclotella stelligera
Gomph
Aulacoseira granulata var granulata
Amphora ovalis var lybica
Aulacoseira granulata var angustissima
Gomphonema parvulum
Cyclotella pseudostelligera
Stephanodiscus sp1
Chaetocerous mulleri
Achnanthes minutissima
Amphora veneta
Aula
Fragilaira pinnata var. trigona
Cymbella descripta
Nitzschia communis
Fragilaria construens f. subsalina
Craticula halophila
Chl-a
TP
EC
Cam
Luna
Prec
Espinos
Sol
Alex
Quech
Zira
Alch
Zemp
Juan
Chapala
Patz
Negritos
Zacapu
Tepe
Maria
Laguna
SMO
Atol
Caji
Alb
Zap
San P
Tecuit
Alb Tac
L Yur
San G
Quitz
P Yur
0 20 40 60 80 100
-1.0 0.0 1.0 2.0 3.0
0.0 1.0 2.0 3.0 4.0
1.0 2.0 3.0 4.0 5.0

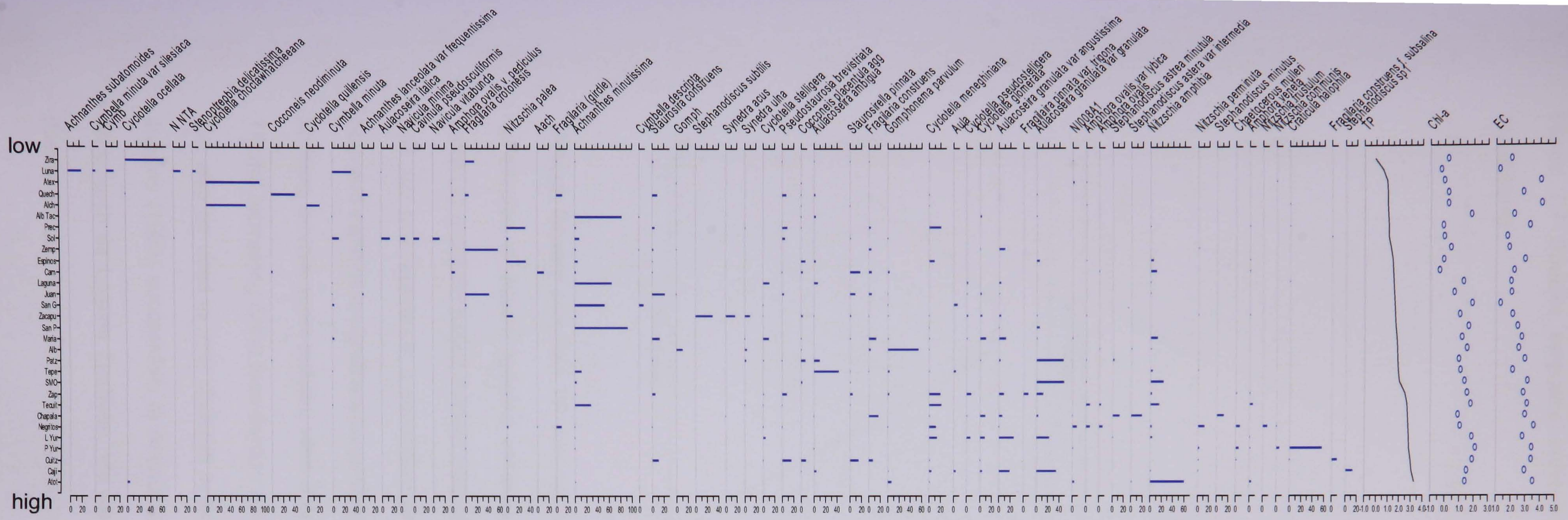
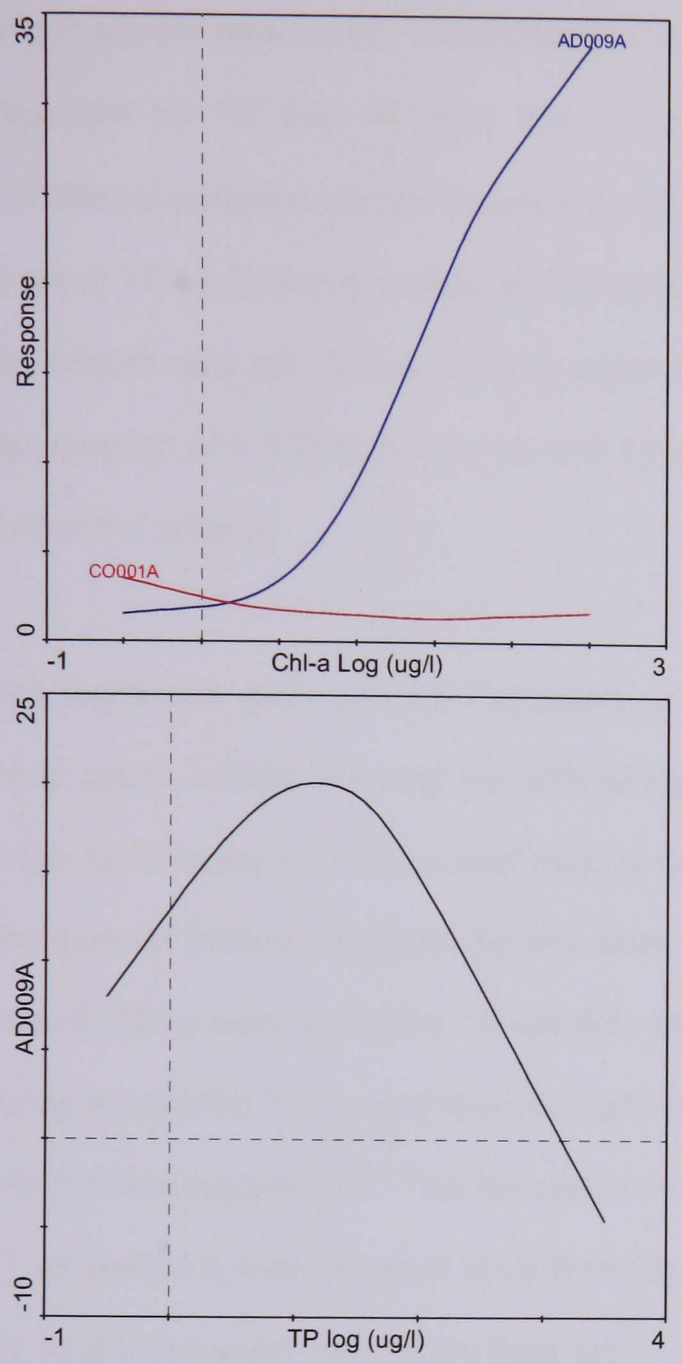

low
high
Achnanthes subatomoides
Cymbella minuta var silesiaca
Cymb
Cyclotella ocellata
N NTA
Stenopterobia delicatissima
Cyclotella choctawhatcheeana
Cocconeis neodiminuta
Cyclotella quillensis
Cymbella minuta
Achnanthes lanceolata var frequentissima
Aulacoseira italica
Navicula minima
Cavinula pseudoscutiformis
Navicula vitabunda
Amphora ovalis v. pediculus
Fragilaria crotonesis
Nitzschia palea
Aach
Fragilaria (girdle)
Achnanthes minutissima
Cymbella descripta
Staurosira construens
Gomph
Stephanodiscus subtilis
Synedra acus
Synedra ulna
Cyclotella stelligera
Pseudostaurosira brevistriata
Cocconeis placentula agg
Aulacoseira ambigua
Staurosirella pinnata
Fragilaria construens
Gomphonema parvulum
Cyclotella meneghiniana
Aula
Cyclotella pseudostelligera
Cyclotella glomerata
Aulacoseira granulata var angustissima
Fragilaria pinnata var. trigona
Aulacoseira granulata var granulata
NI008A1
Amphora ovalis var lybica
Amphora ovalis
Stephanodiscus astrea minutula
Stephanodiscus astera var intermedia
Nitzschia amphibia
Nitzschia perminuta
Stephanodiscus minutus
Chaetocerous muelleri
Amphora veneta
Nitzschia frustulum
Nitzschia communis
Craticula halophila
Fragilaria construens f. subsalina
Stephanodiscus sp1
TP
Chl-a
EC
Zira
Luna
Alex
Quech
Aich
Alb Tac
Prec
Sol
Zemp
Espinos
Cam
Laguna
Juan
San G
Zacapu
San P
Maria
Alb
Patz
Tepe
SMO
Zap
Tecuit
Chapala
Negritos
L Yur
P Yur
Cuitz
Caji
Atot

Figure 7.7 Species response curve for (a) Chl-a and (b) TP for *Achnanthes minutissima*



Through forward selection TP was not found to make a significant and independent contribution to diatom species variance, but Chl-a was, the latter will be used to construct a diatom based nutrient transfer function. Although, Chl-a is correlated with axis two and represents a secondary gradient, this variable does have a high λ_1/λ_2 ratio and explains a significant proportion of the variance in the species data (5.4%). In addition, despite some outlying sites, there is a strong relationship between increasing TP and increasing Chl-a (see Section 6.9 and Figure 6.9), which may imply that Chl-a can also be used to infer changes in trophic status, as has been done by Jones & Juggins (1993) successfully in Antarctica, and in New Zealand (Reid, 2005). Some sites, such as Laguna Zirahuén, are also known to be N limited, or N-P co-limited (Bernal-Brooks & MacCrimmon, 2000), consequently a TP transfer function would be less applicable to these sites. Diatoms species do not, however, respond directly to Chl-a, but to the underlying changes in TP and TN concentration, changes in which affect aquatic productivity and therefore Chl-a concentration. Variance partitioning was repeated for TP and EC and this indicated that there was a greater degree of conditional variance shared between these two variables, relative to Chl-a and EC. As a result TP explained a smaller amount of unique and significant explained variance in the diatom data set. These features indicate the suitability of Chl-a, rather than TP, for the creation of a diatom based transfer function for inferring changes in nutrient status in Central México.

WA regression and calibration equations were derived from a final calibration dataset of 27 lakes. Initially WA was run with all sites to determine if there were any outliers. Lago de la Luna, Alchichica and Piscina de Yuriría were removed as they did not fit the general pattern displayed by the other sites, due to their very high, or low mean annual Chl-a concentrations. In addition these sites also had unusual diatom floras, being dominated by species that were generally not present at other sites or present in much lower abundances. This dataset covers a wide Chl-a gradient of 0.79-127.35 µg l^{-1}, as well as a wide range of other limnological conditions (see Table 7.5 and Chapter 6). Inferences were based on a data set 27 sites (Alchichica, La Piscina de Yuriría and Lago de la Luna were removed) and 132 common species which were square root transformed and present in an abundance of at least 1%, in one or more samples.

Table 7.5. Selected limnological characteristics for 27 lakes in calibration data set.

Variable	**Mean**	**Max**	**Min**
Depth (m)	12.5	53	0.2
Temperature (°c)	22.92	31.5	10.55
Electrical conductivity (µS cm^{-1})	971.07	3286.5	16.95
pH	8.33	9.6	6.9
Alkalinity (mg l^{-1})	330.8	1350	30
TP (µg l^{-1})	264.39	1782.65	0
Chl-a (µg l^{-1})	29.27	127.35	0.79
Secchi Depth (m)	1.71	5.9	0.02

Results (Table 7.6 & Figure 7.8) show that a WA with classical deshrinking gave the best results, with a low apparent RMSE (0.32), with a low maximum bias (0.23) and a high correlation between observed and inferred values (r^2 = 0.83). WA with tolerance downweighting (WA$_{(tol)}$), which takes species tolerance into account by downweighting each species by its variance for Chl-a, did not improve the results significantly as they yielded higher RMSE(P), higher maximum bias and lower r^2. Figure 7.8 shows plots of observed vs. diatom inferred (log) Chl-a concentration and observed vs. residual Chl-a for WA and WA$_{(jack)}$, with inverse and classical deshrinking. This indicates that there is a close relationship between observed and modelled species response to nutrients, when no cross validation was used. Simple WA resulted in an underestimation of Chl-a at the higher end of the gradient and overestimation at the lower end of the gradient, as seen through the trend in the residuals. The application of jack-knife cross validation (with classical deshrinking) resulted in a large increase in the bias (to 1.36), seen through the trend in the residuals, to increase over and under estimation at the low and high ends of the gradient respectively. This also led to a poor relationship between the observed and modelled species response to Chl-a (r^2 = 0.09) and high errors (RMSPE = 0.7). RMSE and RMSEP are based on logged Chl-a in µg l^{-1}.

Table 7.6. Comparison of apparent Root Mean Square of Errors (RMSE) of prediction, maximum bias and correlation coefficients between observed and inferred log Chl-a (µg l^{-1}) values for WA, WA(tol), WAPLS and WAPLS components 1-3, with inverse and classical deshrinking with no cross validation and with jack-knife cross validation. Models in bold are those which will be discussed further

Calibration procedure	**Deshrinking type**	**Cross validation**	**Apparent RMSE/RMSEP (Chl-a log µg l^{-1})**	**Correlation observed vs inferred (r^2)**	**Maximum Bias**
WA	Inverse	N/A	0.29	0.83	0.35
WA	**Classical**	**N/A**	**0.32**	**0.83**	**0.23**
WA (tol)	Inverse	N/A	0.3	0.82	0.48
WA (tol)	Classical	N/A	0.33	0.82	0.29
WA	Inverse	Jack knife	0.69	0.08	1.4
WA	**Classical**	**Jack knife**	**0.7**	**0.09**	**1.4**
WA (tol)	Inverse	Jack knife	0.93	0.01	1.6
WA (tol)	Classical	Jack knife	1.0	0.01	1.8
WAPLS	Component 1	N/A	0.29	0.83	0.32
WAPLS	Component 2	N/A	0.16	0.95	0.19
WAPLS	Component 3	N/A	0.08	0.99	0.11
WAPLS	Component 1	Jack knife	0.67	0.12	1.34
WAPLS	Component 2	Jack knife	0.66	0.15	1.23
WAPLS	Component 3	Jack knife	0.56	0.14	1.19

In this data set cross validation by jack-knifing (leave one out), resulted in a large fall in the correlation between the observed and inferred values, a rise in RMSEP and a greater spread of the residuals, consequently higher maximum bias. This is because the data set is small and heterogeneous resulting in of a number of species only being present in abundance at one site (see Figure 7.9). As a result the measured gradient and inferred range for these species, such as *Stephanodiscus subtilis*, only present at Laguna Zacapu, is limited (see Siver, 1999). Consequently with only one data point optima and tolerances cannot be fully measured and therefore accurately estimated for these species. Problems also arise as the full gradient of some species has not been measured. This is illustrated above though the response of *Achnanthes minutissima* to Chl-a (Figure 7.7), which does not show a standard Gaussian response curve. WAPLS, which is a more complex model, did not offer a substantial improvement over WA.

Figure 7.8a.

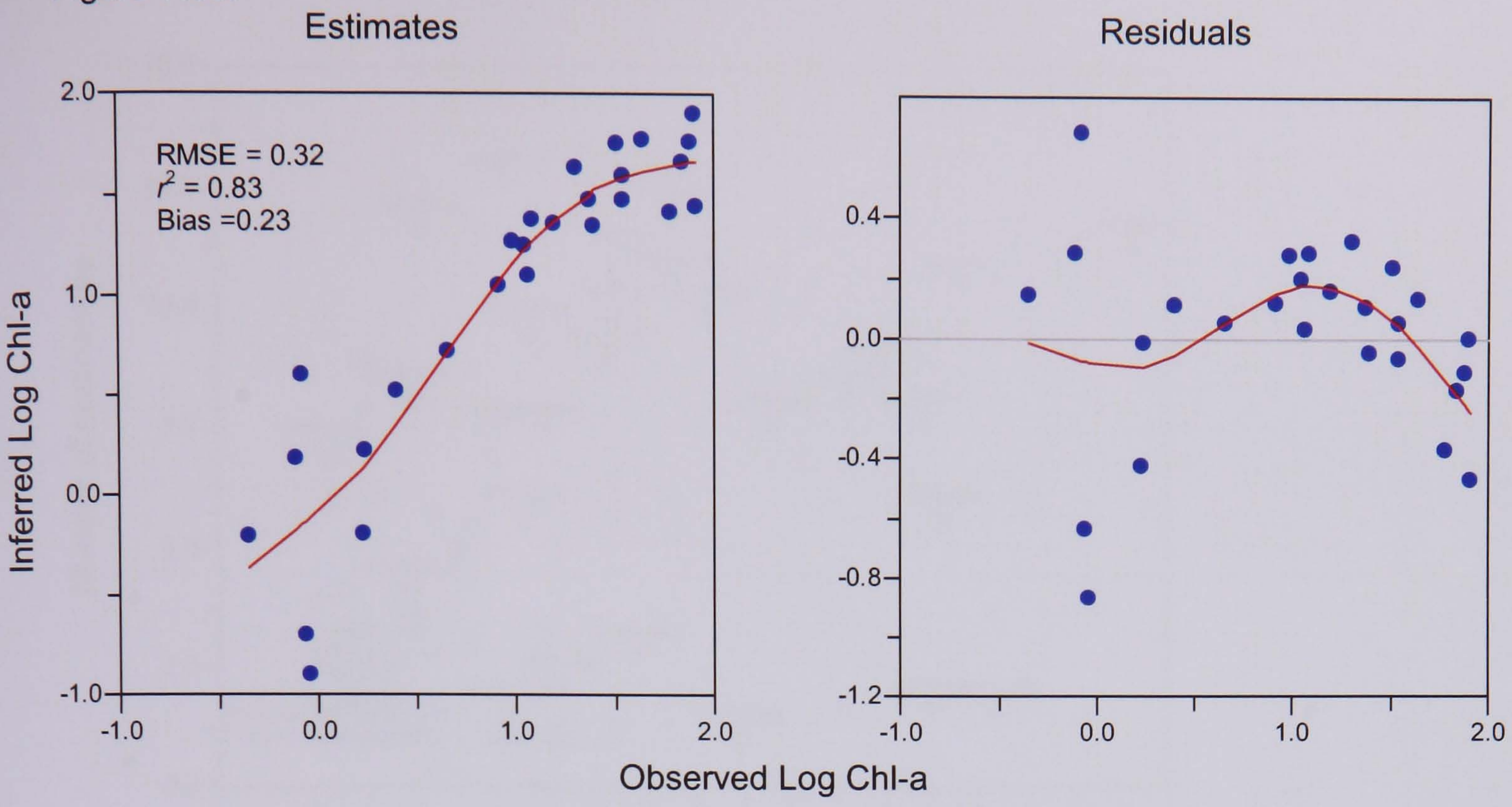


Figure 7.8b

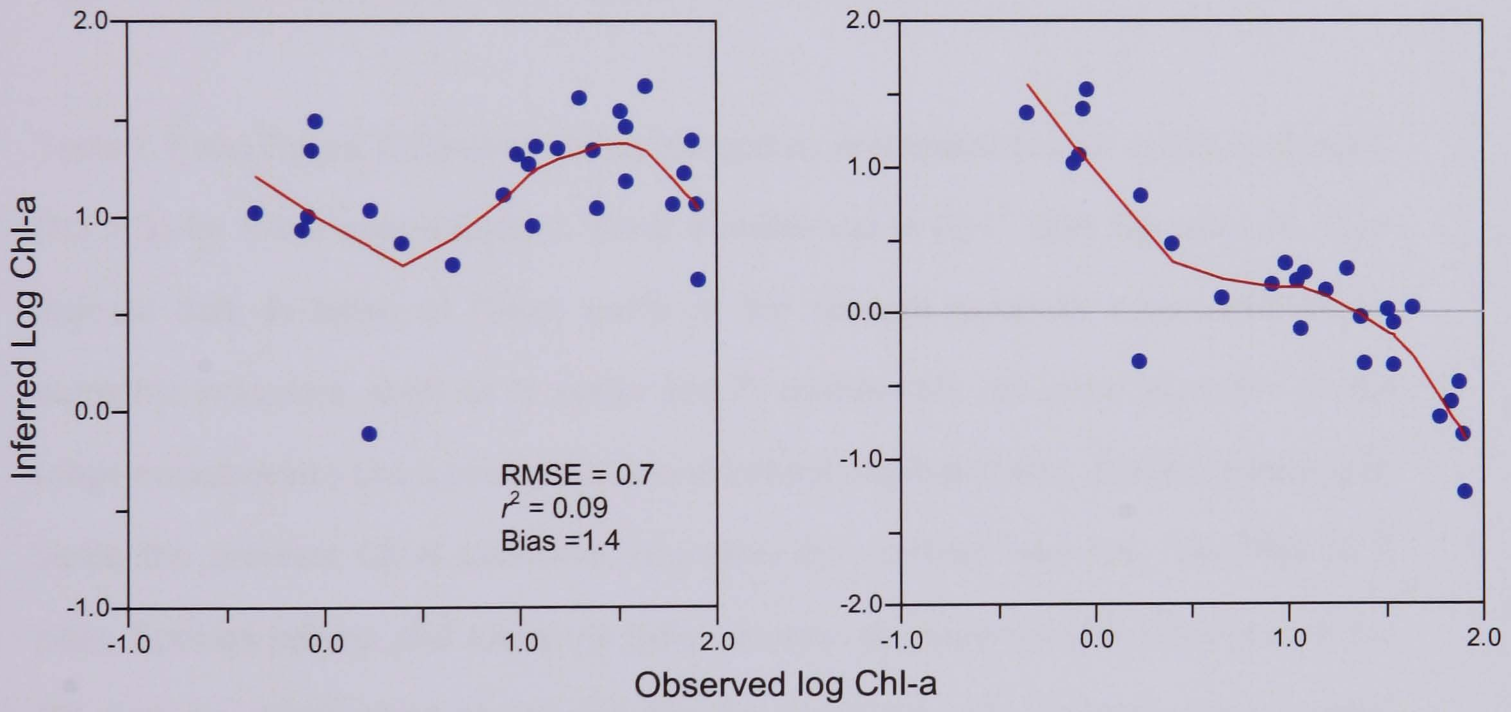


Figure 7.8. Results of WA model for Chl-a (log from μg l^{-1}) with a) classical deshrinking, without cross validation and b) classical deshrinking and jack-knife cross validation

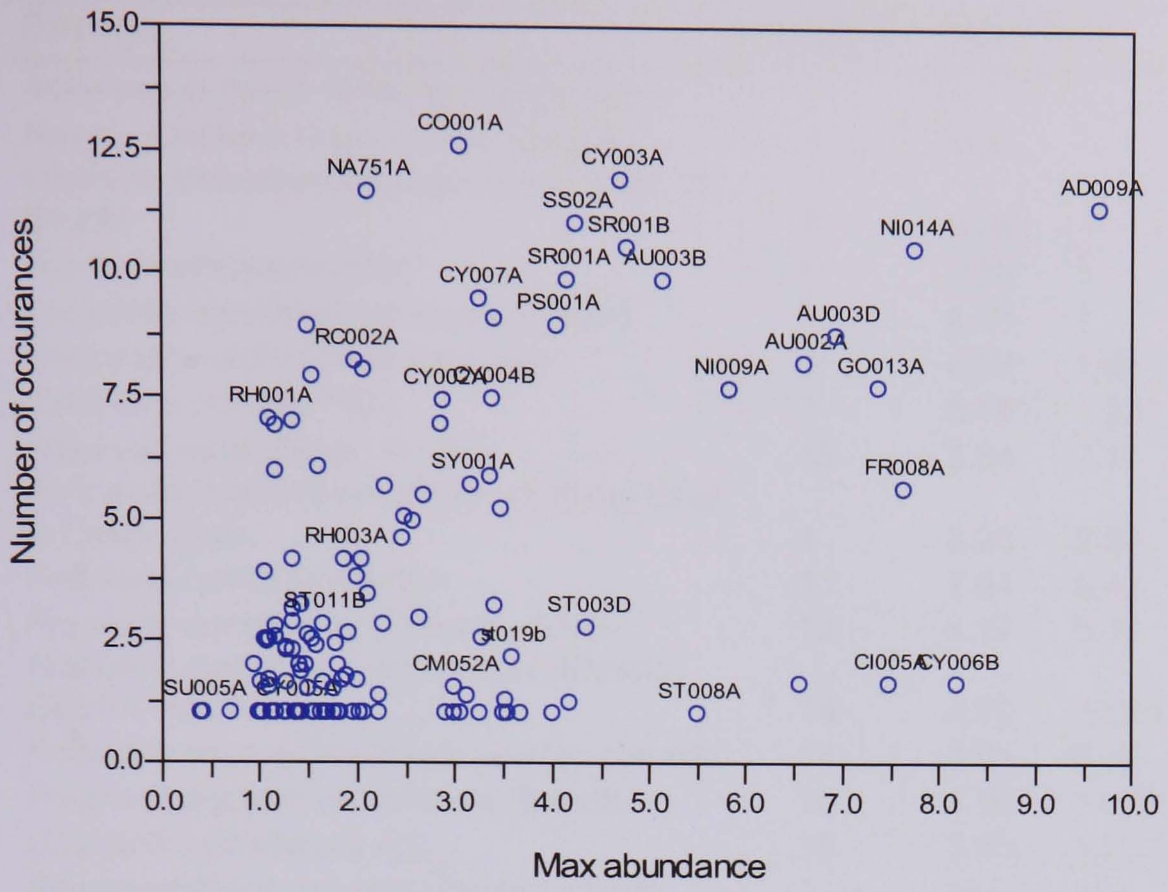


Figure 7.9 Species abundance vs. maximum abundance.

Table 7.7 and Figure 7.8 below, shows the optima and tolerances for common species (N2 > 3) for Chl-a concentrations (back transformed to µg l^{-1} from log values). They indicate that, in terms of Chl-a, some of the species generally considered to be eutrophic indicators, such as *N. palea* and *F. crotonensis*, are more abundant at low (oligo-mesotrophic) Chl-a concentrations in central Mexican lakes. *Cymbella descripta* yields the greatest Chl-a tolerance, however, this species was only identified at 3 sites. Species optima and tolerance data can also be generated for this data set for TP, and the distributions of species can be compared with those of other nutrient transfer function (see Table 7.8).

Table 7.7 Chlorophyll-a optima and tolerances (µg l^{-1}) for common species (Max >3) in the calibration data set

Species	**Count**	**Max**	**N^2**	**Optima**	**Tolerance**
Aulacoseira italica (Ehrenb.) Simonsen	1	4	1	0.87	4.42
Navicula minima Grun. in Van Heurck	1	3.01	1	0.87	4.42
Cavinula pseudoscutiformis (Hust.) Mann & Stickle	1	3.26	1	0.87	4.42
Navicula vitabunda Hust.	1	3.56	1	0.87	4.42
Cyclotella choctawhatcheeana Prasad	1	9.71	1	0.92	4.42
Cocconeis neodiminuta Krammer	3	6.57	1.64	1.49	3.09
Cyclotella ocellata Pant.	3	8.19	1.62	2.86	5.37
Nitzschia palea (Kutz) W. Sm	13	5.84	7.14	3.17	6.92
Achnanthes lanceolata (Breb. ex Kutz) Grun in Cleve Grun	4	3.28	2.58	3.43	4.22
Fragilaria crotonesis Kitton	11	7.64	5.42	4.51	3.92
Fragilaria construens (Ehrenb.) Grun.	13	4.17	9.88	6.13	5.07
Fragilaria construens var. *venter* (Ehrenb) Grun in Van Heurck	14	4.79	10.54	6.29	5.38
Fragilaria brevistriata (Grun.) in Van Heurck	14	4.05	8.95	7.38	6.93
Fragilaria pinnata var. *pinnata* Ehrenb.	16	4.25	11.04	7.58	6.78
Cocconeis placentula agg	18	3.06	13.12	7.85	6.40
Stephanodiscus minutus (Ehrenb.) Grun.	1	3.5	1	8.34	4.42
Cymbella minuta var. *minuta* Hilse ex Rabenh.	9	3.48	5.21	9.39	7.48
Synedra ulna (Nitzsch.) Ehrenb.	8	3.17	5.7	9.82	4.37
Aulacoseira ambigua (Grun. in Van Heurck) Simonsen	13	6.6	7.82	9.85	3.77
Stephanodiscus astraea minutula (Kutz.) Grun.	3	3.59	2.16	10.97	2.46
Synedra acus Kutz.	2	4.18	1.22	11.19	1.26
Stephanodiscus subtilis Van Goor	1	5.51	1	11.56	4.42
Stephanodiscus astraea var. *intermedia* Fricke	4	4.35	2.8	13.02	2.09
Cyclotella meneghiniana Kutz.	18	4.72	11.36	13.09	5.74
Nitzschia amphibia Grun.	18	7.76	10.55	14.57	4.71
Nitzschia perminuta (Grun) M Perag	12	3.41	8.08	17.74	2.79
Aulacoseira granulata var. *angustissima* Müller	13	5.15	9.45	17.85	2.94
Cyclotella glomerata Bachm.	13	3.26	9.49	18.18	2.69
Gomphonema parvulum (Kutz.) Kutz.	16	7.38	7.71	18.46	4.28
Achnanthes minutissima Kutz.	21	9.67	11.19	18.91	5.06
Cyclotella stelligera (Cleve & Grun in Cleve) Van Heurck	12	3.38	7.48	18.98	2.97
Aulacoseira granulata var. *granulata* (Ehrenb.) Simonsen	15	6.95	8.8	19.02	3.05
Stephanodiscus sp1	1	3.69	1	32.82	4.42
Fragilaria construens f. subsalina (Hust.)	2	3.13	1.3	39.76	24.72
Cymbella descripta (Hust.) Krammer & Lange-Bertalot	3	3	1.56	52.48	6.29

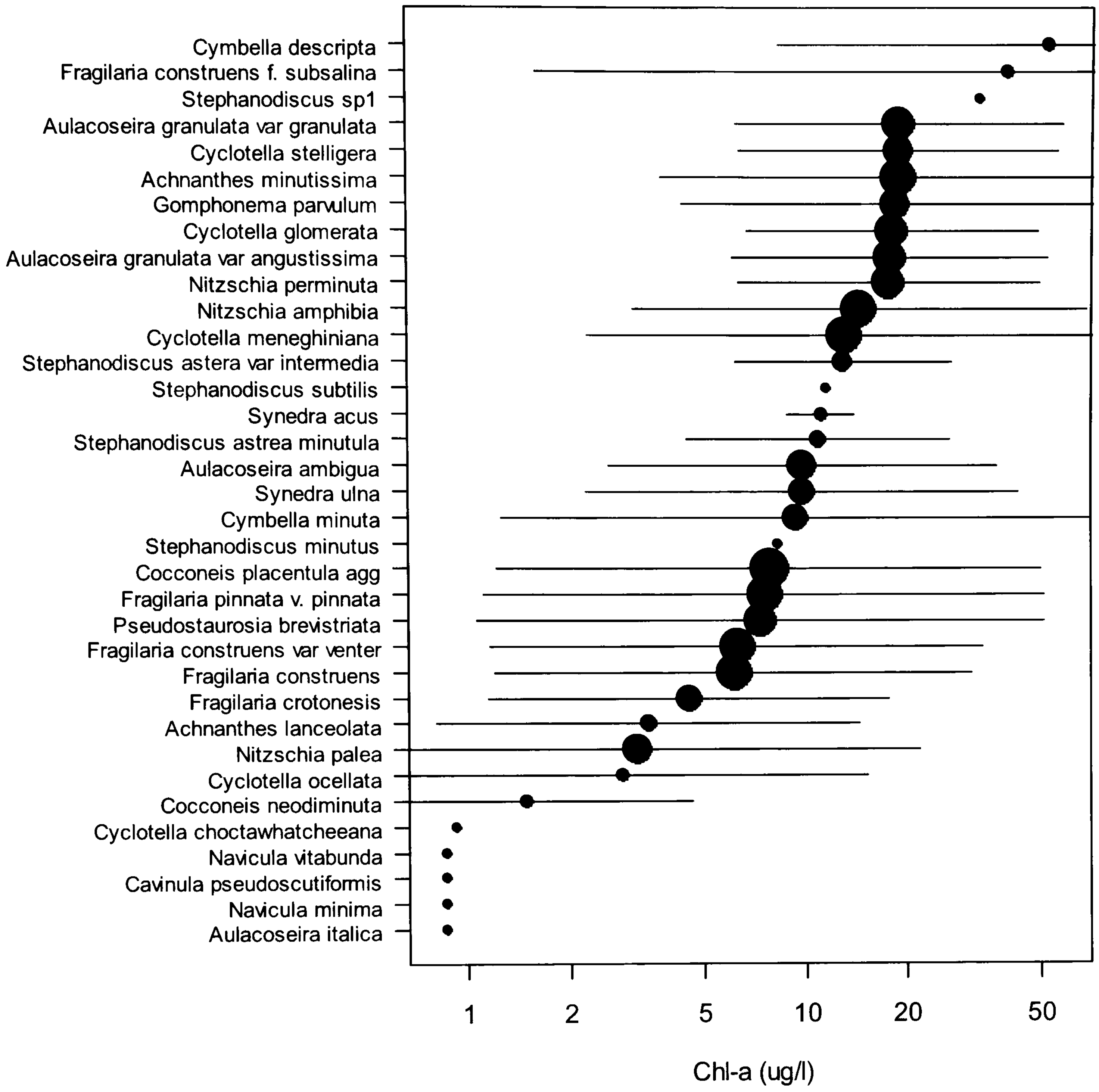


Figure 7.10. Species optima and tolerances for common species in calibration set

Table 7.8 WA TP optima for selected species, compared with WA TP optima ($\mu g\ l^{-1}$) from NE England (Bennion, 1994), British Colombia (Hall & Smol, 1992), SE Australia (Tibby, 2004) and NE United States (Dixit *et al.*, 1999)

Name	**Central México**	**S-E UK**	**British Colombia**	**S-E Australia**	**N-E USA**
Achnanthes exigua (Grun.)	110.7		12.7		13
A. minutissima (Kütz)	41.2	66.1	9	25.33	13
Amphora ovalis (Kütz)	250.6				22
A. ovalis v. pediculus (Kütz) Cleve	24.7	114.3	10.4	22.52	
A. ovalis v. lybica (Erh.) Cleve	226.0		11.2	177.8	
A. veneta (Kütz)	299.8			112.7	
Aulacoseira ambigua (Müller)	47.7	95.7	16.9	33.9	14
A. granulata v. angustissima (Müller)	155.7	126.5	23.3	52.6	
A. granulata v. granulata (Ehr.) Ralfs	155.0			49	29
Cocconeis placentula (Ehr.)	75.5	89.9	13.0	53.5	28.0
Craticula halophila (Grun. ex Heurck) Mann	142.4	151		37.4	
Cyclotella meneghiniana (Kütz)	160.7	408.3	9	66.8	66
C. pseudostelligera (Hust.)	132.0	158.1		26.5	
C. stelligera (Cleve & Grun. in Cleve) Van Heurck	72.1		9.7	16.2	7
Cymbella microcephela (Grun. in Van Heurck)	63.4	28.7	11.6		11
C. minuta v. minuta (Hilse ex Rabenh.)	45.1		10.2		13
Fragilaria brevistriata (Grun. in Van Heurck)	61.2	94.8	10.1		13
F. construens (Ehrenb.) Grun.	64.1	97.9	16.6		13
F. construens v. venter (Ehrenb. Grun. in Van Heurck)	45.5	71.1	12	29.8	8
F. crotonesis (Kitton)	14.9		13.9	12.3	14
F. pinnata v. pinnata (Ehr.)	82.3	93.8	15	17.8	14
Gomphonema gracile (Ehr.)	164.6				18
G. parvulum (Kütz) Kütz	137.2	138.4	10.2	47.9	
Navicula radiosa var tenella (Breb. ex Kutz.) Grun. ex Van Heurck	106.8	101.6			11
Nitzschia amphibia (Grun.)	208.0	164.1		67.2	19
N. palea (Kütz) W. Sm	32.9	129.1		69.4	18
N. perminuta (Grun. in Van Heurck) M. Perag.	226.3				12
Rhoplaodia gibba (Her.) O Müll.	98.8		11		
Sellaphora pupula (Kutz.) Mereschkowsky	65.6		12.1	46.6	13
Synedra ulna (Nitzsch) Ehrenb.	66.9	155.6		38.3	15

Table 7.8 shows that species TP optima for central México are, in general, higher than estimates for temperate North America, often by an order of magnitude, but lower, often by up to 100 $\mu g\ l^{-1}$ compared to the lakes of south east England. The latter relationship is not consistent, with species such as *Nitzschia amphibia* showing higher optimum in the central Mexican data set compared to the south east England data set (Bennion, 1994). The gradients sampled by these studies were, however, shorter; 25-

646 µg l^{-1} for SE England, 5-45 µg l^{-1} for British Colombia and 6.8-451 µg l^{-1} for SE Australia . The values for México and England are generally with in the same trophic state classification, while those of North America tend to be in the oligo-mesotrophic range compared to meso-hypertrophic range exhibited by the same species in Mexican lakes. Consequently there is a lack of agreement of species preferences for nutrient availability between tropical and temperate regions.

WA transfer function results can be compared with other nutrient based transfer functions such as those for SE England (Bennion, 1994), Australia (Tibby, 2004), NW Europe (Bennion *et al.*, 1995), Antarctica (Jones & Juggins, 1995) and New Zealand (Reid, 2005) for TP and Chl-a (Table 7.9) to assess the relative strength of this transfer function. Hall & Smol (1992) only produced a simple WA model with no cross validation, and when applied to a sediment core from Kamloops Lake inferred TP was found to agree strongly with monitoring data, indicating the high predictive ability of the transfer function. Their correlation between observed and inferred values was lower than in this study, but more importantly associated errors and bias were lower. The models listed above all produce better results when cross validated than this study. In the majority of cases the modelled variable was found to be the primary variable driving diatom variation, based on CCA results and, in addition dataset were more homogenous. Siver (1999) created a model for TN, despite being a secondary variable, while Reid (2005) created a model for TP from New Zealand lakes where it was considerably weaker than other measured variables, and had been found to be insignificant in a CCA, with Chl-a performing the best. Despite this, these two studies still yielded lower errors on the variables than in the current study. It should be noted however that these studies were based on generally larger and more homogenous data sets, and differ considerably from this study.

Table 7.9 Comparisons for WA/WAPLS diatom inferred phosphorus, nitrogen and chlorophyll models with this study

Geographic area & Reference	**Calibration set size**	**Nutrient Variable**	**Variable range (µg l^{-1})**	**r^2 (apparent)**	**RMSE (apparent) (log µg l^{-1})**	**RMSEP (boot/jack) log µg l^{-1}**
British Columbia (original) (Hall & Smol 1992)	37	TP	5-28	0.73	0.25	n/a
SE England (Bennion 1994)	31	TP	25-646	0.79	0.161	0.28 $_{\text{(boot)}}$
NW Europe (Combined) WAPLS (Bennion *et al.* 1996)	164	TP	5-1190	0.91	0.22	0.21$_{\text{(jack)}}$
Australia WAPLS (Tibby 2004)	33	TP	6.8-451	0.94	0.112	0.233$_{\text{(jack)}}$
Ontario (Reavie & Smol 2001)	59	TP	4-54	0.637	7 (mg l^{-1})	10 $_{\text{(boot)}}$
New Zealand (Reid 2005)	48	TP	1.85-171.25	0.5$_{\text{(jack)}}$	n/a	0.24$_{\text{(jack)}}$
Antarctica (Jones & Juggins 1993)	61	Chl-a	0.05-10.06	0.74	0.32	0.38$_{\text{(jack)}}$
New Zealand (Reid 2005)	49	Chl-a	0.38-26.07	0.63$_{\text{(jack)}}$	n/a	0.21 $_{\text{(jack)}}$
Ontario (Reavie & Smol 2001)	59	Chl-a	0.6-8.4 (mg l^{-1})	0.516	0.168	0.297$_{\text{(boot)}}$
Connecticut (Siver 1999)	50	TN	119-714	0.47		93 µg l^{-1} $_{\text{(boot)}}$
Central Mexico (this study 2003/4)	27	Chl-a	0.79-127.35			

7.4. Conclusions

A strong and significant relationship between an environmental variable, such as Chl-a or TP, and variation in the species assemblage is required in order to create a successful inference model. A number of studies have shown strong relationships between diatom species assemblages and lake water chemistry, for example in terms of pH, TP or EC. The nature of the species-environment relationship in central México does not always reflect expected results, such as the identification of eutrophic indicator species in few sites, despite the high number of nutrient rich lakes . As discussed in Chapter 6 and in Section 7.6 above, earlier studies have shown that lakes in central México have been eutrophic for prolonged periods of time, or in the case of Laguna Zirahuén have recently started to show shifts towards eutrophication.

This study used the data presented in Chapter 6 to assess the relationship between environment and diatom species variance. The main conclusions are as follows:

- Detrended correspondence analysis found 3 sites to be outlying, these were excluded from further ordinations
- In a constrained ordination five of the measured variables in the calibration set were found to be insignificant ($p > 0.05$). CCA, with forward section, found only two variables made a significant and independent contribution to diatom variance: EC and Chl-a. EC represented the primary gradient in driving diatom variation, but Chl-a did also make an independent and significant, but secondary, contribution to the species variation, shown by variance partitioning and a high λ_1/λ_2 (0.61). It was therefore suitable for use to infer changes in nutrient status, as a proxy for the combined influence of TP and TN.
- The relationship between Chl-a and TP is complex, as outlined in Chapters 3 and 6, particularly in Mexican lakes due to their turbidity and high suspended sediment content. Results indicated it was more suited to the construction of a transfer function than TP, based on variance partitioning, which showed a greater degree of shared variance between EC and TP.
- Simple WA with classical deshrinking, with no cross validation, yielded the best results, with the lowest bias in the residuals, showing a lower tendency to over and under estimate at the low and high ends of the Chl-a gradient respectively and had a high correlation between the observed and inferred Chl-a values.
- To obtain a more reliable inference jack-knife cross validation of the data needs was applied. As a number of the species are present at only one site, however, this resulted in large errors, and would therefore lead to an inaccurate reconstruction.

The data indicate that even though multivariate statistics show that Chl-a is a secondary gradient but it is one which is significant and independent, furthermore other studies have used secondary variables to create successful transfer functions (e.g. Siver 1999). Consequently evidence for the application of a Chl-a transfer function to provide a numerical reconstruction of nutrient change is appropriate. This will be discussed in Chapter 8, in conjunction with other supporting evidence.

Chapter 8. Palaeolimnology of Laguna Zirahuén

8.1. Introduction

This chapter provides more background on the Zirahuén Basin, in terms of its physical setting, geology and climate and explores the history of human activity in the basin, from the Post Classic period onwards, to provide a context for the palaeolimnological results. The results of the palaeolimnological investigation are then presented. The stratigraphic, mineral magnetic and chronological framework are discussed initially. The results of the diatom analysis are then discussed in terms of stratigraphic change and then numerical reconstruction, in relation to the results presented in Chapter 7. The results of the organic geochemistry; stable isotopes and lipid biomarker analysis, are then presented. The results from all these analyses are then synthesised to present a sequence of changes in nutrient availability over the last ca. 200 years in the Zirahuén catchment.

8.2. The Zirahuén Basin: Physical Environment

Laguna Zirahuén is located 2075 m above sea level in the south of the Trans-Mexican Volcanic Belt (TMVB), at 19° 26' N and 101° 44' W (see Figure 8.1 & 5.1). It is a hydrologically closed (endorheic) basin with an area of 260.81 km^2 of which the lake occupies 4% (10.48 km^2) (Figure 8.2). Laguna Zirahuén has been the subject of a number of limnological and palaeolimnological studies in recent years (Bernal Brooks, 1998; Bernal Brooks & MacCrimmon, 2002a, 2002b; Chacón Torres & Rosas Monge, 1998: Davies *et al.*, 2004; De Buen, 1943). Consequently such studies provide good background information on long term environmental changes (up to 1000 years) in the catchment, as well as short term changes in water chemistry (e.g. alkalinity and total phosphorus) and physical parameters such as fluctuations in water depth, which can provide a means of validation of quantitative inferences from diatom inferred nutrient change.

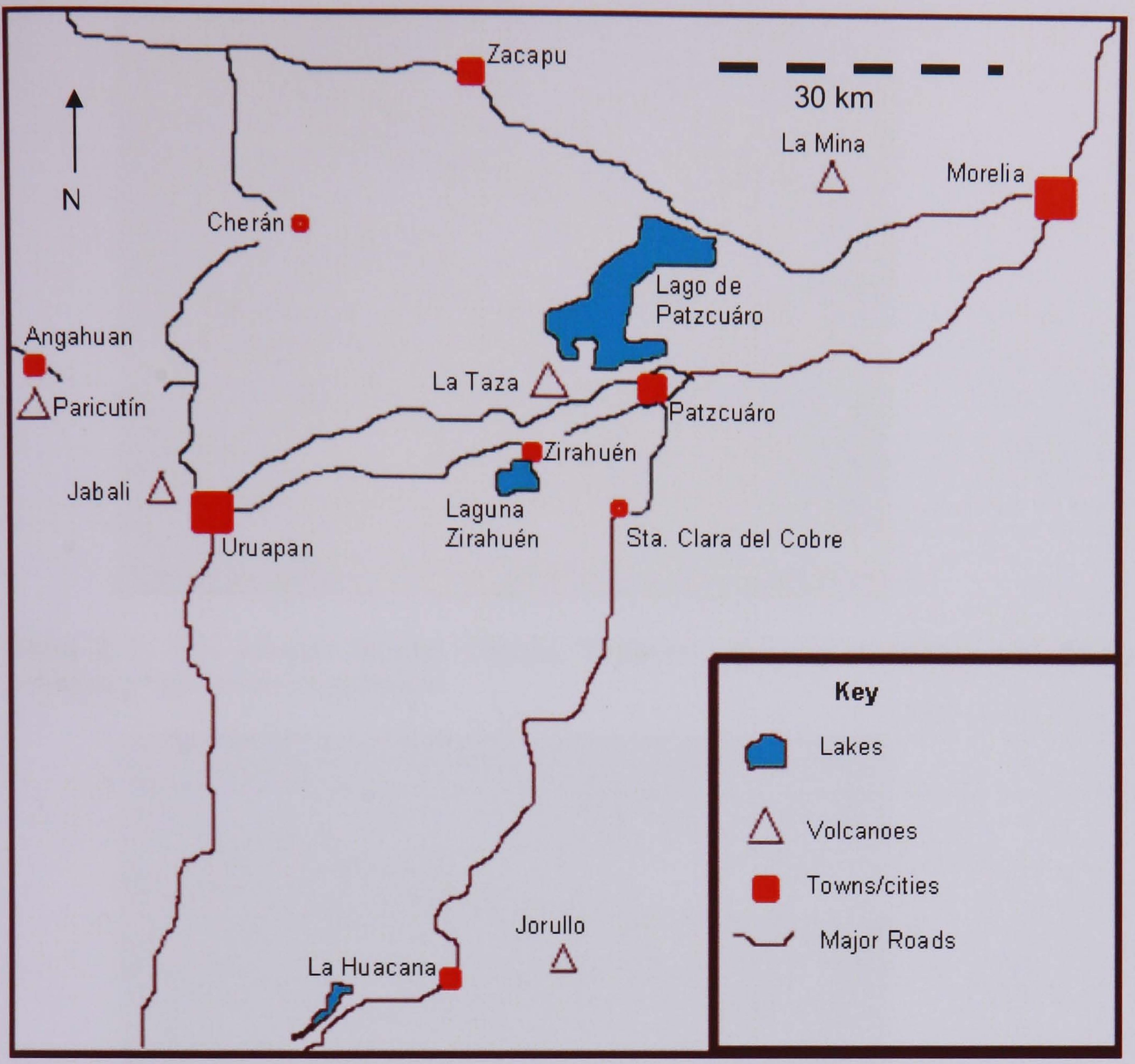


Figure 8.1. Location of Laguna Zirahuén in relation to other sites mentioned in the text.

8.2.1 Geology and Soils

The Zirahuén basin has an average watershed height of 2,500 m asl, with a maximum elevation of 3,260 m asl and is dominated by basaltic and andesitic bedrock of Tertiary and Quaternary age. Soils are derived from the volcanic bedrock geology and are primarily nutrient and iron oxide rich Andosols which occupy approximately 75% of the basin. They have a very distinct red colour and are highly susceptible to erosion (see Plates 8.1 and 8.2). At higher altitudes soils include lithosols, luvisols and feozems (Chacón-Torres & Rosas-Monge, 1998) and at lower altitudes, around the lake are alluvial deposits.

Plate 8.1. Soil erosion around Laguna Zirahuén, showing distinctive red colour indicating high levels of oxidation.

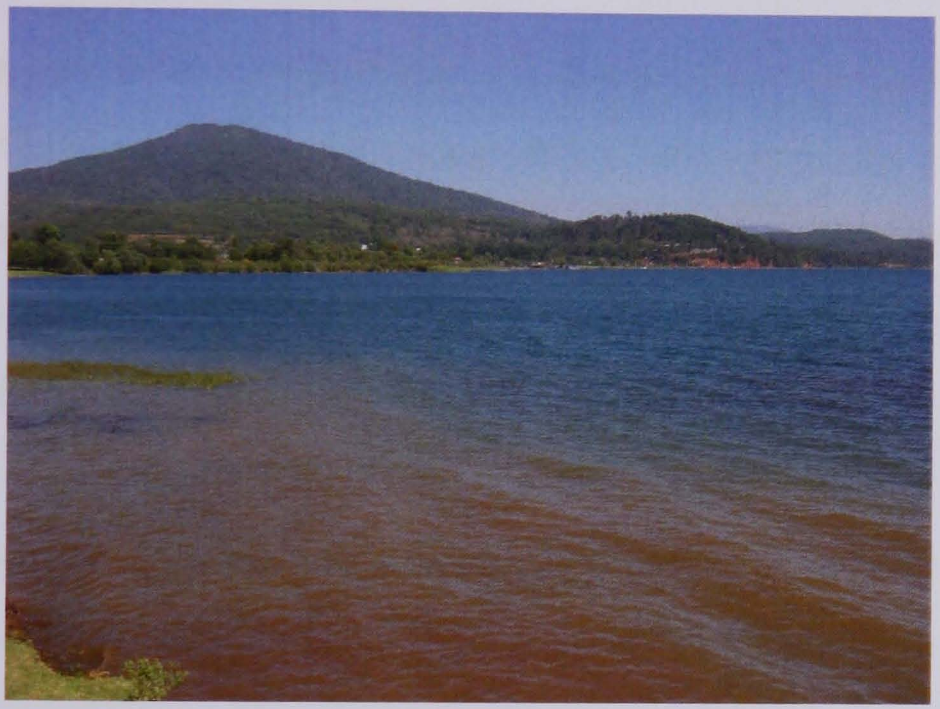


Plate 8.2. Soil erosion into Laguna Zirahuén

Two of the youngest monogenetic cinder cones in the MGVF are in close proximity to Laguna Zirahuén, Volcán Jorullo (AD 1759-1774) to the south and Volcán Paricutín (AD 1943-1952) to the west (Figure 8.1). Historical data indicate that the volcanic activity of Jorullo peaked in AD 1764 with lava flows reaching their maximum extent by AD 1766. Tephra was deposited in Queretaro 200km away, on the shore of Lago de Pátzcuaro, and there were reports of clouds blocking out the sun in Morelia (then Valladolid) and in the town Pátzcuaro (Newton *et al.*, 2004). The eruption of Paricutín, which began on February 20th 1943, when a fissure appeared in a farmer's field, has been one of the most intensively studied volcanic events of the 20th century (Davies *et* *al.*, 2004; Newton *et al.*, 2004). The eruption was documented at its most vigorous between March and June 1943 when ash was said to have reached México City, 320 Km east. The majority of volcanic ash was thought to have been produced within the first 2 years of the eruption (Newton *et al.*, 2004). The tephra produced by these two eruptions, Paricutín and Jorullo, are geochemically very similar, but there are slight differences in the SiO_2 concentration that allow them to be distinguished (Davies *et al.*, 2004). Where they are present, they provide useful isochrone markers for dating and estimation of accumulation rates in lake sediments.

Zirahuén lies within the Lerma-Chapala-Santiago system of internal drainage, classified as México's hydrological region 12 (Lerma-Santiago) (CNA, 2003), which can be subdivided into 5 basins where the Zirahuén basin occupies south west portion of the Yuríria-Cuitzeo-Pátzcuaro basin (INEGI, 1985). The basin is located within the Mexican Guanajuato Volcanic Field (MGVF) and was formed by the damming of the Río La Palma stream by lava flows during the Pleistocene (Chacón-Torres & Muzquiz-Iribe, 1991). Initially it was believed that Zirahuén, Pátzcuaro and Cuitzeo formed from a north flowing tributary of the Río Lerma and that the lakes had been isolated by successive compartmentalisation by volcanic activity. The stepwise change in altitude (Zirahuén at 2,075 m asl through to Cuitzeo at 1,820 m asl) and similarity between endemic fauna, particularly the pescado banco (*Christoma estor*) in each lake, were presented as evidence for this (De Buen 1943). Alternatively Bernal-Brooks (1998) infers that the Río La Palma was more likely a tributary of the Río Balsas which flowed south rather than north. Although there is some dispute over the exact nature of the formation of Zirahuén, it is clear that the basin was formed as a result of volcanic activity, resulting in the damming of the La Palma stream and that the area was highly volcanically active during the late Tertiary and Quaternary periods.

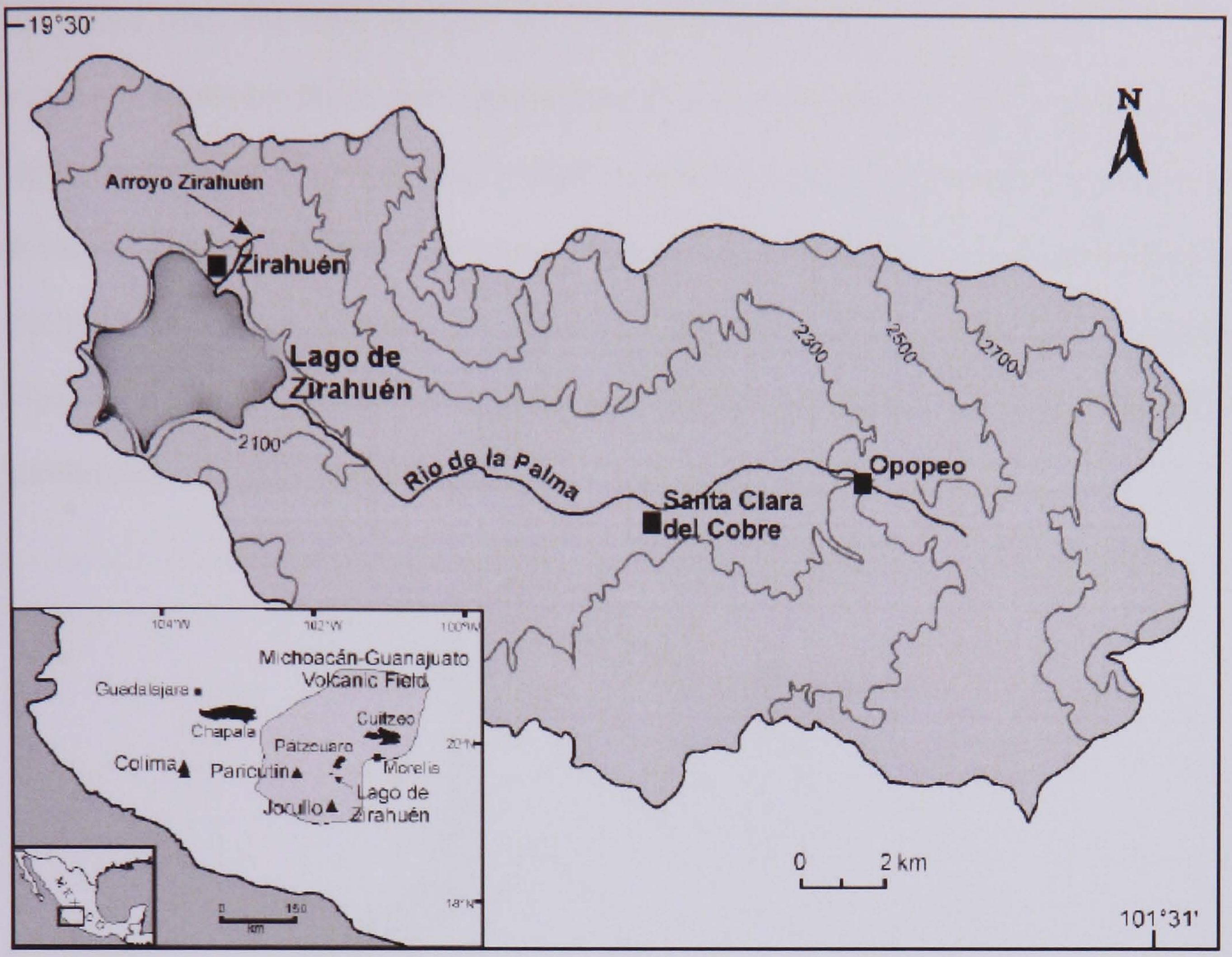


Figure 8.2. The Laguna Zirahuén basin indicating major settlements and inflows (from Davies 2002 – with permission).

The Río La Palma (or El Silencio) is the only permanent inflow into Zirahuén and originates to the east from springs near to Opopeo (Figure 8.2). Zirahuén is, however, also fed by a number of intermittent streams, present during the rainy season, such as the Arroyo Zirahuén to the north which flows though the town of Zirahuén and by unchannelised surface run-off. As the lake is closed, the primary mechanism of water loss is seepage. At present the lake has a maximum depth of about 40m located to the west in front of the village of Copandaro (Figure 8.3), compared to 46 m in 1942 (De Buen, 1943). This loss of depth, since 1942 (see Section 6.3) has been attributed to increased sedimentation due to high levels of soil erosion within the catchment combined with climatic change and hence increased evaporation (Bernal-Brooks & MacCrimmon, 2000a). Recent palaeolimnological evidence has shown that despite high levels of catchment disturbance there has been no significant increase in sedimentation rates over this time (Davies *et al.*, 2004). The sub basin Agua Verde, located on the south west side of the lake has a maximum depth of 12m and is separated from the main body of the lake by a rock barrier 4m beneath the water surface. Despite its depth and stratification Zirahuén formerly did not become anoxic regularly, although Bernal-Brooks (1988) shows that it has at times has come close to doing so, between 1986-87. Aerobic conditions were maintained by density currents due to the differences between the lake water and that of its in flow Río La Palma (see Figure 8.2). The lake does now show oxygen depletion at depth. Figure 8.3 shows the bathymetry, showing core location.

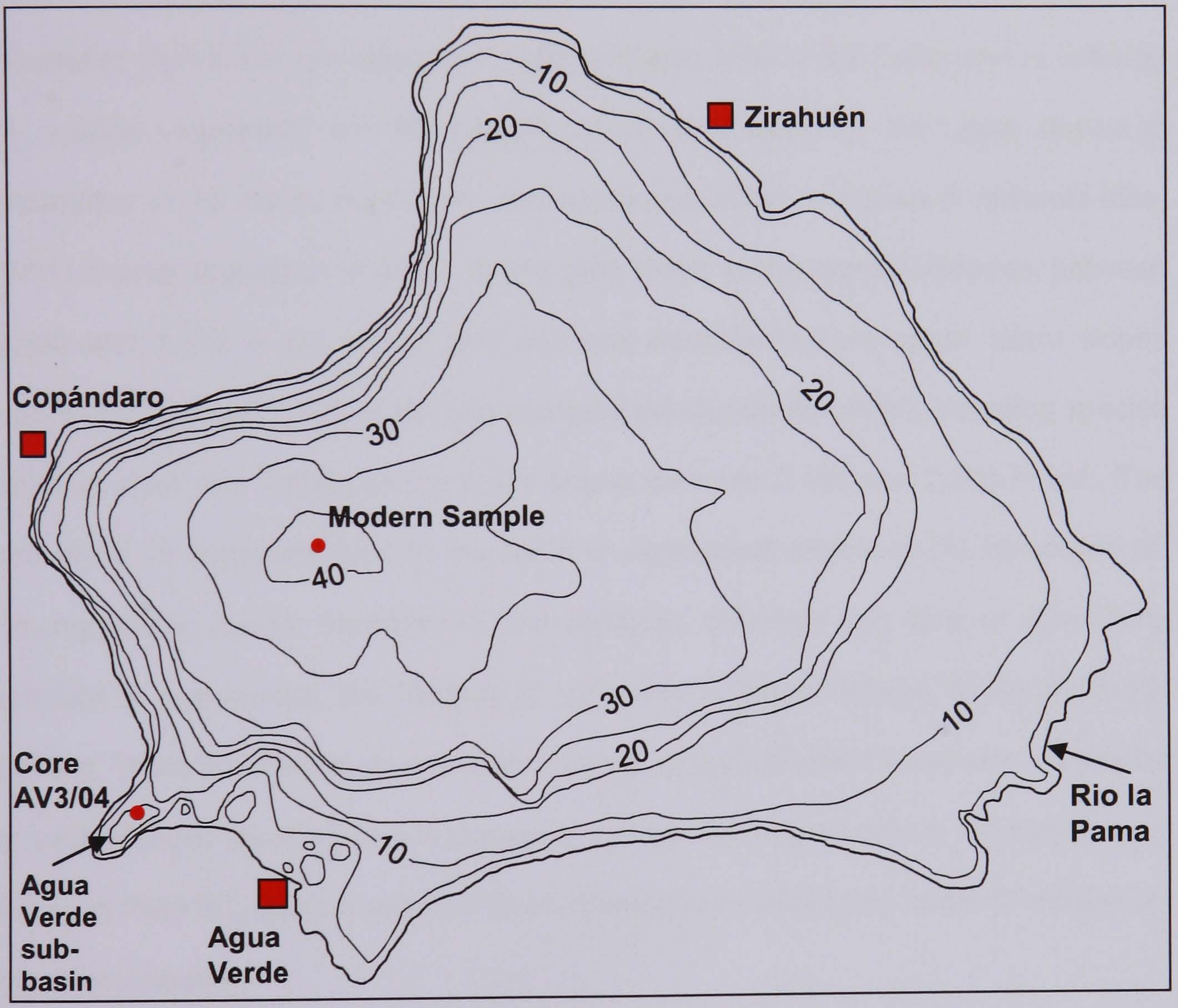


Figure 8.3. Bathymetry of Laguna Zirahuén (Chacón-Torres, Unpub data).

8.3. Climate and Vegetation

The Zirahuén Basin lies within the *Tierra fría* zone of the Michoacán Highlands (see Section 1.4), with topography exerting a strong control over annual precipitation and temperature regimes and consequently over vegetation. Currently the meteorological station closest to Laguna Zirahuén is located at Villa Escalante (Santa Clara del

Cobre) and has collected precipitation data and rainfall data for 52 years between 1947 and 1999 and recorded an average of 1006.3 mm and an annual average temperature of 16.2 °C. There has also been station at Zirahuén since ca. 1944, but records have been inconsistent and ceased around 1989 (Davies, 2000). Precipitation reaches a maximum during the rainy season between July and August. The highest average temperatures (~27-28 °C) have been recorded in April and May, just prior to the onset of the rainy season. During these months the minimum air temperature can fall as low as 1.6 °C, but the lowest overall temperatures are generally recorded between December and February. This impacts on the thermal regime of the lake, as discussed above and on vegetation. Approximately 40% of the catchment is covered by "natural vegetation" and the majority of forest is found on the upper slopes of mountains in the north, north-east and south-east (Chacon-Torres & Muzquiz-Iribe, 1991). Forest vegetation includes fir and pine forest at the highest altitudes, between 2,500 and 3,300 m asl, mixed pine and oak woodland on the upper sierra slopes between 2,300 and 2,800 m asl and montane deciduous woodland, including species such as alder and willow, on the lower slopes between 2,100 and 2,300 m asl,. The remainder of vegetated land in the basin is agricultural dedicated the cultivation of, among others, maize, blackberries and avocado, although only 20% of agricultural produce is commercial, the majority is subsistence (Bernal-Brooks & MacCrimmon, 2000b). There are distinct seasonal differences, associated with the seasonal variation in precipitation, resulting in a variance in the amount of vegetation cover, as seen below in Plate 8.3. Such a variation has implications for increased levels of erosion, as discussed above.

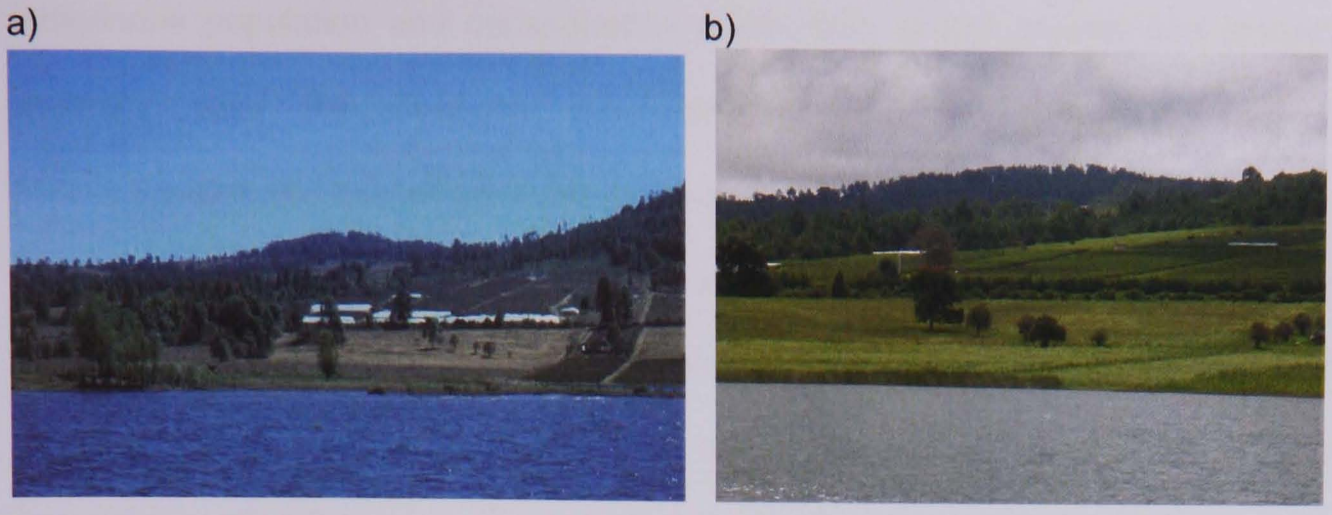


Plate 8.3 Differences between a) dry season (May 1998) and b) wet (September 2004,) in Zirahuén basin (photos taken by SE Metcalfe)

8.4. Human Activity

As outlined in Chapter 2 human activity in Michoacán has been prolonged and intense. Human impact on the environment pre-dates the Conquest, as shown by the sediment record (e.g. O'Hara *et al.*, 1993; Metcalfe *et al.*, 1994) thus conflicting with "The Pristine Myth" (Denevan, 1992). Sediment records from Lago de Pátzcuaro, the centre of the Purépecha Empire, indicated that there were several phases of pre-Hispanic erosion, the first associated with the appearance of cultivated maize ca. 3,600 yrs BP, a second episode was noted between 2,500 and 1,200 yrs BP and a final, pre-Hispanic episode around 850 yrs BP associated with the arrival of the Purépecha in the Pátzcuaro Basin (O'Hara *et al.*, 1994). The Zirahuén basin was however, subject to less intensive activity.

Published palaeoenvironmental records from Zirahuén are on a much shorter time scale, at present only covering the last 1000 years, but also show a significant period of erosion between ca 1000 and 800 yrs BP, associated with the Purépecha and again at ~150 yrs BP (Davies *et al.*, 2004). Historical records indicate that, in the pre-Hispanic period population density in the Zirahuén basin was low, as it was primarily used as a religious and recreational retreat by the Purépecha nobility (see Section 2.4.1), although there is no archaeological evidence to support this (Endfield, 1997). As a result it was settled and exploited to a lesser extent than lakes such as Pátzcuaro (Endfield & O'Hara, 1999). The arrival of the Spanish resulted in a fall in the indigenous population and consequently a reduction in soil erosion and landscape recovery. After the Conquest palaeoenvironmental reconstructions from both Pátzcuaro and Zirahuén show a decline in soil erosion most likely associated with the recovery of vegetation cover and slope stabilisation. In addition archival evidence supports reduced human activity, with the Zirahuén basin being largely forested (Davies, 2000) and indicates that the area remained under Purépecha control immediately after Conquest (Endfield & O'Hara, 1999; Relación de Michoacán, 1541) and that exploitation of the basin by the Spanish was minimal until the early 17th century. Population records for this time also indicate that the Zirahuén basin was sparsely settled in the early Colonial period, with only 270 in Zirahuén in 1619 and 130 in habitants in Santa Clara in 1620 (Carrillo, 1996).

The indigenous population increased in line with an increase in the Spanish population from the 17th century onwards. As a result, competition for and pressure on resources, such as land, wood and water, increased. From the mid to late 18th (ca. AD 1750) century onwards the sediment record shows increased soil erosion and a shift to dominance by planktonic diatoms species (Davies *et al.*, 2004) due to renewed catchment disturbance. This was thought to be associated with the establishment of a number of large Spanish farming estates (*haciendas*) by 1789. Several *haciendas* were established in the vicinity of the lake and near the town of Santa Clara del Cobre (Figure 8.1 & 8.2) in the east of the basin, which were associated with the cultivation of maize, wheat and for grazing (Endfield & O'Hara, 1999). In 1822 Lejarza (1974) reported 14 ranches and six *haciendas* in the Santa Clara area. This increased level of human activity led to a high degree of deforestation with the uplands in the south of the basin being described as "barren and stripped of vegetation" (Endfield & O'Hara, 1999). Consequently this and access to forest resources, became a source of contention between the indigenous peoples of the area and the *haciendas* who were perceived by the former to be the cause of land degradation and deforestation.

The Purépecha were renowned for their metallurgic skills, working with gold and silver for the creation of ornaments and with copper for practical instruments such as axes. The village of La Huacana, among others, were thought to be the primary copper refining centres in the Pre-Hispanic period (West, 1948). After the Conquest the Spanish took over the indigenous mines and introduced European copper smelting techniques, but retained the Tarascan metallurgists. Between 1607 and 1614 the industry was moved to Santa Clara (West, 1948). Although copper was imported from deposits ca. 100 km away, pine forests in the basin provided the charcoal required for the smelting process. This increased demand for forest resources from the early 1600s exploiting the dense pine forest surrounding Santa Clara. Eight royal refineries had been established in the Zirahuén basin by 1789. This has been noted in the sediment record, with increased levels of copper and lead, around the time of the deposition of the Jorullo tephra in AD 1759-1764 (Davies *et al.*, 2004). The industry provided employment and the population grew from the early 1600s onwards. The copper industry had however virtually disappeared by the early 20th century, with only a few coppersmiths remaining, who now produce copperware for tourists. Populations continued to rise throughout the colonial period, by 1822 Zirahuén had a population of 2,813, while Santa Clara del Cobre had a population of 4,073 (Lejarza, 1974).

The most recent available census data, taken in 1995, indicated that Santa Clara remained the largest town in the basin, with Opopeo as the second largest, with populations over 10,000. Both of these settlements lie on the main inflow to the lake, the Río la Palma. Zirahuén had a population of around 2000, in addition to which there are a number of smaller settlements. Recently, the area has begun to be marketed as a tourist resort. As a result there has been increase development in the basin, with a number of hotels and cabanas built and there is a proposal for a golf course on the south west side of the lake, above the Agua Verde sub basin. This has potential to influence the water balance of the area and act as an additional source of pollution from increased use of fertilizers. Increases in commercial farming may also result in increased levels of fertilizer use. Access to the basin has been improved with an exit from the main Morelia-Uruapan highway being built. Currently 60% of the catchment is given over to agriculture, which has recently included the development of more commercial agriculture with the establishment of a blackberry farm on the south side of the lake.

8.5. Hydrology, Nutrients and Biology of Laguna Zirahuén

Thermal stratification of the lake occurs between April and December, with over turn between January and March. In January epilimnetic temperatures have been recorded around 15°C with little noticeable difference with increasing depth. As atmospheric temperatures increase, the difference between the epilimnetic and hypolimnetic temperature also increases. Chacón-Torres & Rosas-Monge (1998) report a maximum surface water temperature of 23°C and 17°C in the hypolimnion, resulting in the stratification of the water column. Stratification begins to break down towards the end of the rainy season as the temperature difference declines.

As mentioned above approximately 60% of the catchment is used agriculture. There are 32 settlements (approximately 24,156 inhabitants) from which organic waste and phosphorus rich detergents are input directly into the lake (Bernal-Brooks *et al.*, 2002). Chacón-Torres & Rosas-Monge (1998) estimated that urban sources contribute to 22.9% of the total phosphorus loading, although many have septic tanks for waste treatment, while diffuse agricultural sources represent a greater proportion (58.54%). The remainder comes from rural village sources, which do not have sewage treatment facilities. Consequently this input of organic and nutrient rich material has the potential to change this oligo-mesotrophic system to one of higher nutrient status, and associated problems. Indeed, shifts from an oligotrophic state to a more nutrient rich state have been noted in the recent past (Bernal-Brooks & MacCrimmon, 2000b).

Despite this, limnological monitoring, including that of this study, shows that Zirahuén can still be predominantly classified as oligotrophic (Chacón-Torres & Rosas-Monge 1998), with low TP concentrations, between 3 and 20 µg l^{-1}, and correspondingly low

Chl-a concentrations, between 0 and 4 μg l^{-1}. Higher levels of primary productivity are noted in Agua Verde, as its sheltered, shallow conditions with organic matter inputs are conducive to the accumulation of nutrients. The period between 1987 and 1996, when anoxic conditions were almost reached in the hypolimnion, corresponds to a period when concentrations of total phosphorus and total inorganic nitrogen increased by 36% and 45% and transparency declined by 50% (Bernal-Brooks & MacCrimmon, 2000b). Bernal-Brooks & MacCrimmon (2000b) propose four hypotheses as to why Zirahuén has not responded with a high level of productivity to the potentially high levels of nutrients available. First, that although P loading in the inflow is high the difference in temperature immediately carries nutrients down into the hypolimnion where they become isolated; secondly that the concentration of P in the Rio La Palma may be diluted 10-fold when it enters the lake; thirdly that co-limitation by both N and P, rather than P alone or finally, that P precipitation from the epilimnetic zone into sediment close to the shore line. A combination of the effect of density currents and N-P co-limitation was cited as the most likely cause. Indeed, data have shown Zirahuén to be predominantly N-limited with periods of co-limitation. This N-P co-limitation has also been supported by algal bioassays on samples from the epilimnion, hypolimnion and the La Palma stream, based on 4 samples taken throughout the year (Bernal-Brooks *et al.* 2002). Results show that phytoplankton do not respond to the sole addition of nitrogen, but that the addition of phosphorus alone yields a large growth rate, particularly in the La Palma stream and in the hypolimnion. It is the co-addition of both N and P which was found to yield the maximum phytoplankton growth rates, thus providing further evidence for N-P co-limitation. This supported bioassay work by Hernández-Avilés *et al.* (2001) on 30 central Mexican lakes, which showed 80% of lakes in central México, including Laguna Zirahuén, had greater simulated growth in response to the addition of N and P simultaneously. Bernal-Brooks & MacCrimmon (2000b) showed that although P concentration in the La Palma stream is high, it may be present in a form which is not biologically available, which may explain Zirahuén's apparent insensitivity to nutrient loading.

Studies of the aquatic biology of Laguna Zirahuén are limited in terms of number and in terms of accounting for variations species abundance with depth, productivity and succession (Chacón-Torres & Muzquiz-Iribe, 1991). Early studies by De Buen (1943) showed that Chlorophytes (green algae) were the most abundant accounting for 80-94% of the total phytoplankton, and that the genus *Staurastrum* was dominant. De Buen also notes that *Anabaena* (a cyanobacteria) was found, but only in Agua Verde. Mendoza-Gonzáles *et al.* (1985) undertook a more detailed study of seasonal shifts in phytoplankton species and abundance, their work showed that species diversity was greatest during the spring and declined throughout the rest of the year and that chlorophytes were dominant. Cyanophytes (blue-green N-fixing algae), dominated by *Microcystis* and *Chroococcus*, first appear during the summer and increased in abundance into the autumn. This succession is linked to changes in the thermal regime, stratification, turn-over and consequently nutrient cycling and availability. The appearance of cyanophytes in the summer through to the winter is associated with stratification, as they prefer stable water column conditions. The high species diversity in the spring is linked to turn-over when nutrients are cycled throughout the water column.

The impacts of human activity, in terms of soil erosion and resultant higher nutrient loading, are already beginning to manifest themselves in the sediment record, despite apparent epilimnetic insensitivity. Davies *et al.* (2004) show an abrupt shift in diatom flora to a system dominated by *Cyclotella ocellata* and *Fragilaria crotonensis* in the last 15 years (see Section 6.4.3), interpreted as a shift to higher nutrient availability. Increased abundance of *C. ocellata*, in response to higher nutrient availability has been noted in Lakes Albano and Nemi, Italy, but was in association with other small centric species indicative of high nutrient availability such as *Stephanodiscus minutulus* (Ryves *et al.*, 1996). Van Dam *et al.* (1994) also found *C. ocellata* to be indicative of meso-eutrophic systems in a detailed study of lakes in the Netherlands. This contrasts with the findings of Stoermer *et al.* (1987) where *C. ocellata* was found to be most abundant in the areas of Lake Erie, North America, which were the most oligotrophic. This was also found in Lake Ontario, where in nutrient enrichment experiments *C. ocellata* was only identified in the least disturbed regions (Stoermer *et al.*, 1985).

In phytoplankton samples the increased abundance of *C. ocellata* in Zirahuén was also noted in the phytoplankton composition by Tevera & Martíez-Almeida (2005). Chacón-Torres & Muzquiz-Iribe (1991) found *Aulacoseira* species to be the dominant diatom in the phytoplankton of Laguna Zirahuén around 1989, which was supported by the identification of *Aulacoseira distans* var. *alpigena* was found to constitute 40% of a modern sample taken from the lake by Metcalfe in 1982 (Metcalfe, 1985). *Fragilaria crotonensis* is also known to increase in response to increase nutrient availability. Stoermer *et al.* (1985) showed a positive relationship between increases in *Fragilaria crotonensis* and increased nitrate availability in the North American Great lakes (see also Section 6.6.3). This corresponds to the findings of Interlandi *et al.* (2003) who showed *F. crotonensis* to be a poor N competitor. This species has also been found to be dominant in response to nutrient additions in Kootenay Lake, British Colombia. It should be noted however that these systems are considerably different from Mexican systems.

8.6. Interpretation of Sediment Record

A short (<1m) mini Kullenberg core (AV3/04) was taken in approximately 8m of water, from the Rincon de Agua Verde sub-basin on the south west side of the lake for palaeoenvironmental analyses (see Chapter 5). Former studies have shown that although Agua Verde is separated from the main body of the lake by a rock barrier approximately 4m beneath the water surface, sedimentation within the sub-basin was representative of catchment wide change (Davies, 2000; Davies *et al.*, 2004). Furthermore Davies *et al.* (2004) showed that a short core from the sub-basin provided a high resolution record of the last ~ 300 years of environmental change in the Zirahuén basin, which corresponded well to other cores taken from different areas of the lake, indicating its suitability for coring.

Past limnological and palaeolimnological studies in conjunction with historical archival records can be used to aid interpretation of sediment proxies. Allochthonous and autochthonous changes in organic matter, water chemistry, trophic state and catchment disturbance can all be recorded by the sediment record through changes in the diatom stratigraphy, sediment geochemistry and physical properties of the sediment such as organic matter content and magnetic susceptibility. As diatoms are highly sensitive to change in light, nutrient availability and to change in other water chemistry variables they, provide an important record of shifts in the algal community in response to environmental changes, such as changes in trophic state (Bennion, 1994, Battarbee *et al.*, 2001). Quantification of this change, through WA regression and calibration equations, as discussed in Chapter 7, can provide a more reliable inference on past environmental change than semi quantitative reconstruction on the basis of publish ecological tolerances (Bennion, 1994; Hall & Smol, 1992; Tibby, 2004). This is particularly important as ecological preferences of species have been seen to vary between regions (see Section 8.5).

Sedimentary organic matter provides another important record of change in within lakes and their catchments, through proxies such as stable isotopes and lipid biomarkers (Chapter 4). Such data can be potentially be used to corroborate the diatom based reconstruction of nutrient change in Laguna Zirahuén, and have been successfully used in conjunction with the diatom record to track change in eutrophication elsewhere (see Chapter 4 for details). They provide an additional record of autochthonous production, as diatoms are not the only part of the system, and also present a more detailed picture of autochthonous vs. allochthonous organic matter inputs into the lake.

The diatom stratigraphy was constrained into four zones using TWINSPAN. When applied to the organic and physical data these TWINSPAN zones did not correlate with observed changes. Organic zones one, two and three were therefore applied on the basis of observed changes in the stratigraphy of $\delta^{13}C$, C/N and $\delta^{15}N$, but were also applied to aid interpretation of LOI, magnetic susceptibility, lipids and organic carbon and nitrogen. The overlap can be observed more clearly in Table 8.1.

Table 8.1. Comparison of proxy data zones applied to core AV3/04

	Depth (cm)	**Diatoms**	**Organic & inorganic proxies**
	0	Diatom Zone AV3/04 4	Organic Zone 3
	1		
	2		
	3		
	4		
	5		
	6		
	7	Diatom Zone AV3/04 3	
	8		
Paricutín Tephra	9		Organic Zone 2
	10		
	11		
	12		
	13		
	14		
	15		
	16		
	17		
	18		
	19		
	20		
	21	Diatom Zone AV3/04 2d	
	22		
	23		
	24		
	25	Diatom Zone AV3/04 2c	
	26		
	27		
	28		
	29		
	30		
	31	Diatom Zone AV3/04 2b	
	32		
	33		
	34		
	35	Diatom Zone AV3/04 2a	
	36		Organic Zone 1
	37		
	38		
	39		
	40		
	41		
	42		
	43		
	44		
	45	Diatom Zone AV3/04 1	
	46		
	47		
	48		
	49		
	50		
	51		
	52		
	53		
	54		
	55		
	56		
	57		
	58		
	59		
	60		
	61		
	62		
	63		
	64		

8.7. Stratigraphy and Physical Properties

8.7.1. Stratigraphy and tephrochronology

Only one tephra layer was identified in core AV3/04, which was black in colour, fine grained and present at a depth of 9cm, although it was quite diffuse. It was identified on both visual inspection and X-radiographs. Based on its similar depth, texture and colour to the upper tephra layer in core AV/98 from the same area of the lake (Davies, 2002) it is assumed that the tephra layer in this core (AV3/04) is derived from Paricutín, dated to 1943. In the core taken by Davies from the Agua Verde sub-basin in 12m of water in 1998 (AV/98), two tephra layers were identified, that of Paricutín and the second, at a depth of 43 cm, is identified as Jorullo (AD 1759-1764). Historical archives confirm that there had been no volcanic activity between these dates. Despite no chemical analysis of the tephra layer in this study it can therefore be assumed, with reasonable certainty that the tephra identified in this study was from Paricutín. This tephra layer therefore acts as a marker placing AD 1943 at a depth of 9 cm. The tephra in AV3/04 acts as a chronological marker for the upper sediment indicating a deposition rate of ca. 0.15cm per year, assuming a relatively constant sedimentation rate this is in line with Davies (2000) who showed a sediment accumulation rate of 0.14 based on 210-lead dating. The sediment below the tephra is younger than 1764, based on the absence of the Jorullo tephra, although it cannot be constrained further.

The core stratigraphy was, in terms of structure quite monotonous and consisted of organic, clay rich, sediments ranging in colour from dark greyish brown (10YR 4/2) to dark olive brown (2.5Y 3/3) and dark olive grey (5Y 3/2); the results are presented in Figure 8.4. The basal unit of the core, from 64 to 62 cm, is a very dark grey/brown, clay. The unit above this, 62-52 cm is also high in clay, but is dark olive brown in colour, this unit also contains two large, rounded clasts one at the top of the unit, 52-53.5cm, the other which is larger at a depth of 57.2-59.2cm. These were rounded, light yellow/brown in colour with a degree of red-brown oxidation on the outside. Above this is a deep dark olive grey, clay unit between 51 and 29 cm. Within this, at a depth of 37 cm, there is a diffuse ca. 2 cm accumulation of black gritty sediment, possibly organic matter. The fourth unit 29-27 cm is similar in colour and texture, to that of the basal unit but appears, on visual inspection, to contain a higher proportion of black, gritty material; similar to that found at 37 cm. Above this is a unit of very dark grey sediment which is predominantly clay. Furthermore there were sections within layer which contain a slightly higher proportion of silt. A number of small, angular stones were also present through out this unit, with the most notable at depths of 18, 20 and 26 cm and several smaller more friable stones are also present in this layer. As mentioned above a black, silty fine grained material is present at 9 cm and is presumed to be the Paricutín tephra of 1943. The upper unit of the core is made up of dark brown clay which contains no clasts. This contrasts to the Agua Verde (AV/98) core of Davies *et al.* (2004) who found that the sediment above the Paricutín tephra layer was oxidised reddish silt, from the in-wash of catchment sediments.

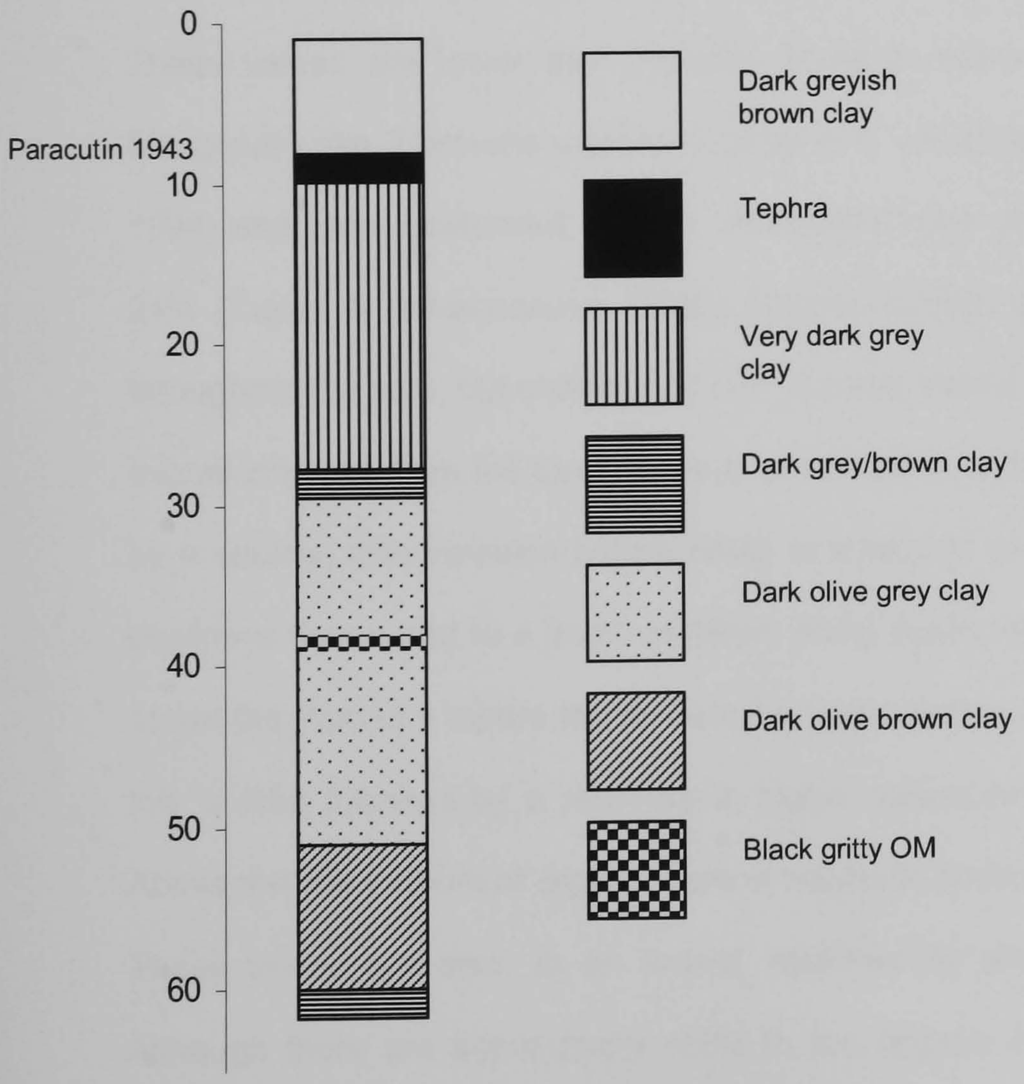


Figure 8.4 Stratigraphy of core AV3/04

Organic matter content (%OM), measured through Loss-on-Ignition (LOI), shown in Figure 8.5, was generally high but fluctuated throughout the core. Average %OM was 17.6% showing a range from 9.4 to 21.9%. Particularly low values are noticed at the base of the core (13.7%) and at depths of 47 cm (9.4%), 45 cm (12%), 31 cm (14.3%) and 9 cm (14.3%). Peak %OM values are noted at 57 cm (21.3), 36 cm (21.9), 32 cm (21.7%) and 20 cm (21.9%). Organic matter content fluctuated showing abrupt changes below 31 cm. At the top of the core %OM remains more constant between 9 and 31 cm with excursions to higher values at 24, 21 and 11 cm. Above the Paricutín tephra (which is coincident with a low in %OM), larger fluctuations are again recorded.

Organic matter typically contains 50% carbon (Meyers & Teranes 2001), consequently LOI values are expected to be, roughly twice the organic carbon content. Figure 8.5., however, indicates that the organic carbon content in core AV3/04 is substantially lower than the LOI values, with an average of 3.8 % and a range of 3.5 to 4.2%. These values are lower than typically found in many tropical lakes, for example Mangrove Lake, Bermuda exhibits % organic C values between 18 and 40% (Meyers 1994) and Lake Bostumtwi, Ghana, sediments have yielded values between 3 and 24% (Talbot & Johannessen 1992). Organic carbon content shows little variation throughout the core, but changes do mirror those shown in the LOI profile. There is an overall increase from the base of the core to a peak at 31 cm of 4.0%, this is followed by a decline, little variation before rising to a second peak of 4.1% at 20cm. A large decline is then noted to a depth of 18cm, rising again thereafter to peak at 4.2%. Just above the Paricutín tephra the amount if organic carbon falls to its lowest value (3.5%) this is then followed by a recovery to higher values to a depth of 2 cm (ca. 1984). Above this the amount of organic carbon begins to decline towards the top of the core. These trends are also, to an extent, matched by shifts in the organic N profile. Although there are some major shifts in the organic N profile there is little overall variation, with a range of 0.32‰ to 0.43‰ and an average of 0.36‰. Highest values are noted in the surface sediment, with generally low values noted between 18cm and the deposition of the Paricutín tephra.

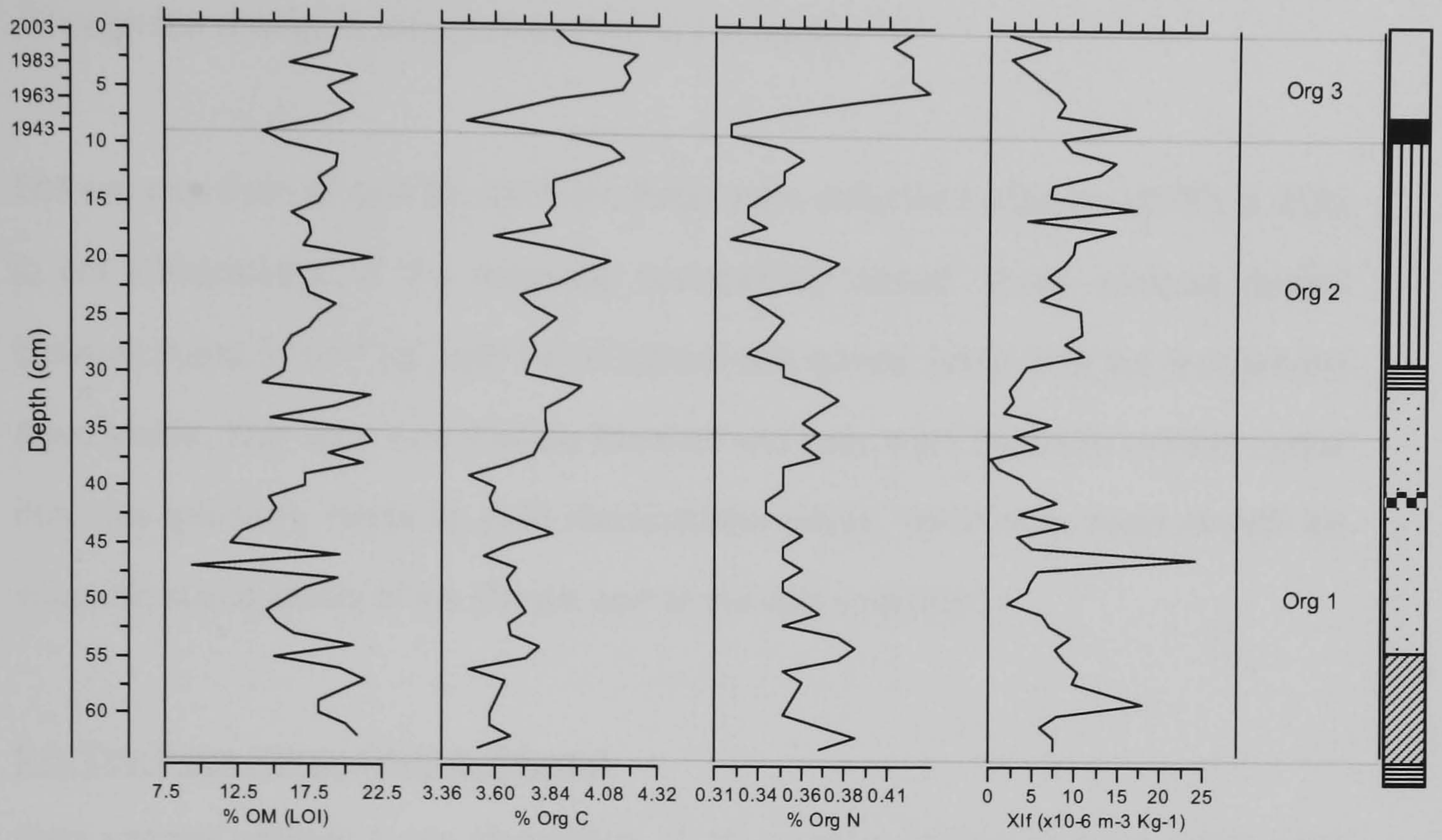


Figure 8.5 Loss on Ignition (% organic matter) and organic carbon and nitrogen and magnetic susceptibility profiles for AV3/04 (showing stratigraphy, see Figure 8.4 for description)

8.7.2. Magnetic susceptibility

Figure 8.5 shows the highly variable results of the mass specific low frequency magnetic susceptibility (χ_{lf}) analysis. Results vary between a low of 0.2 x 10^{-6} m^{-3} kg^{-1} and a peak of 24.2 x 10^{-6} m^{-3} kg^{-1} logged at depth of 37 and 46 cm respectively, with average values of 7.5 x 10^{-6} m^{-3} kg^{-1}. Six major peaks are identified at depths of 59cm (18.1 x 10^{-6} m^{-3} kg^{-1}), 46 cm (24.2 x 10^{-6} m^{-3} kg^{-1}), 17cm (14.6 x 10^{-6} m^{-3} kg^{-1}), 15 cm (17.0 x 10^{-6} m^{-3} kg^{-1}), 11cm (14.5 x 10^{-6} m^{-3} kg^{-1}) and 8 cm (16.9 x10^{-6} m^{-3} kg^{-1}), these point are coincident with small to large rock fragments found in the sediment, as identified by X-radiograph images. Low χ_{lf} values are noted at depths of 50 cm (2.1 x 10^{-6} m^{-3} kg^{-1}), 37 cm (0.3 x 10^{-6} m^{-3} kg^{-1}) and 33 cm (1.8 x 10^{-6} m^{-3} kg^{-1}). Values of χ_{lf} fluctuate markedly throughout the core, however, a phase of lower values was noted between 45-29 cm after which values increase again to a peak at 15cm remaining high until 8cm. Above the 8cm however there is a decline in χ_{lf} towards the surface to 2.2 x 10^{-6} m^{-3} kg^{-1} at the top of the core, there is however an excursion from 2.3 to 6.8

$x\ 10^{-6}\ m^{-3}\ kg^{-1}$ at a depth of 1 cm. These values are slightly higher than those recorded at Agua Verde by Davies *et al.* (2004) who, above the level of the Jorullo tephra, show a peak of 8 x $10^{-6}\ m^{-3}\ kg^{-1}$. They note a slight decline in χ_{lf} above the Paricutín tephra, although the change is less marked than in this study.

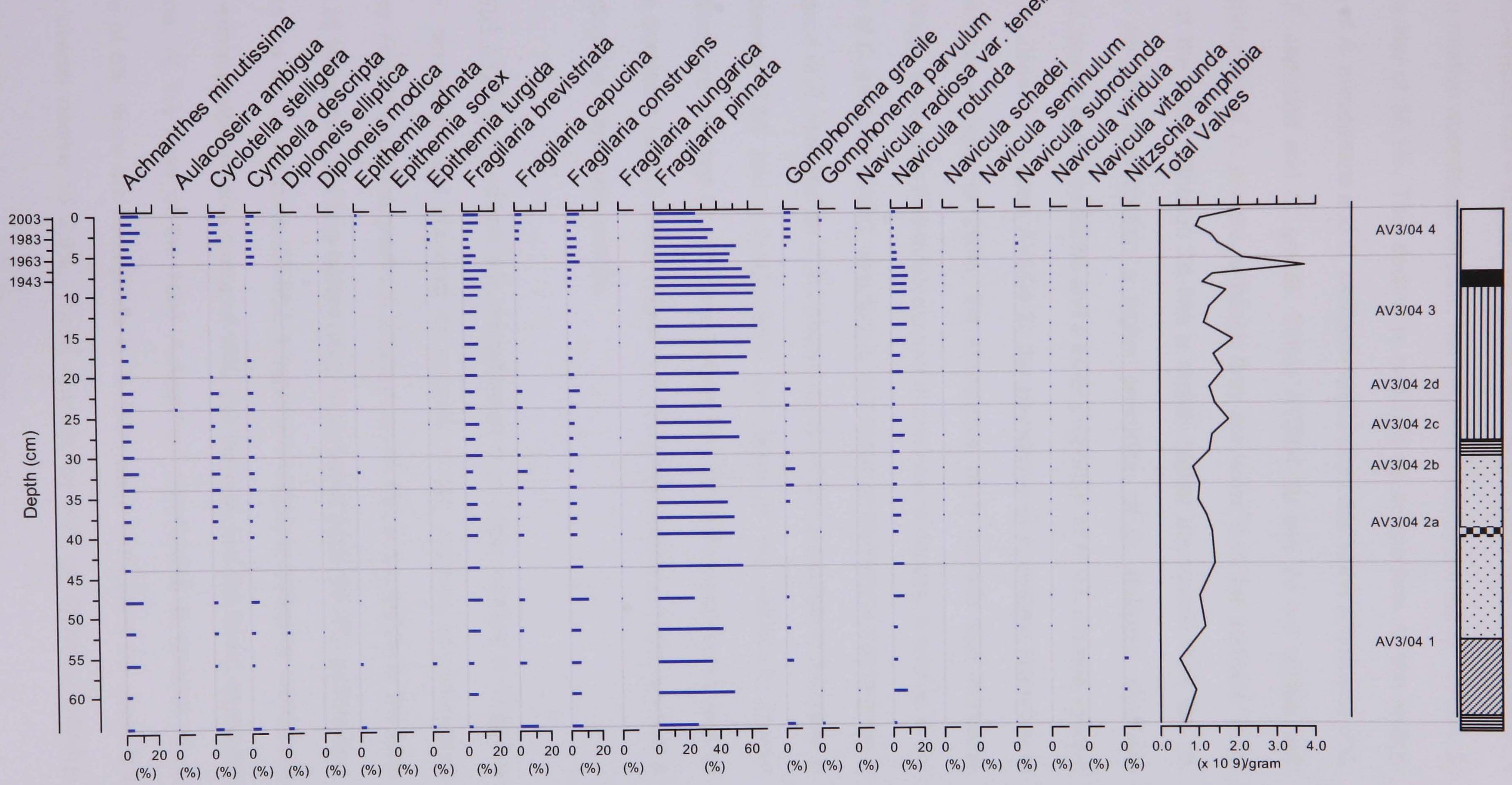
Soil samples from around the Zirahuén Basin were collected by Davies (2000) in order to aid interpretation of the magnetic susceptibility record. These samples ranged between 8 and 14 $\mu m^3\ kg^{-1}$ with the exception of a sample taken from the area around Agua Verde. This area was densely forested and soils were therefore organic matter rich, this generally tends to yield diamagnetic values, which may have diluted the magnetic susceptibility of the sample and of the lake sediment.

8.8. The Agua Verde Diatom Record

Only species present in an abundance of 1% or more at one or more levels were included for clarity in Figure 8.6 and Figure 8.7, which presents diatom habitat and as noted in Chapter 5 the zones were defined on the basis of TWINSPAN analysis. The core is described from the base upwards. Diatom preservation was good throughout and total diatom concentration ranged between 0.5 and 3.7 x 10^9 valves per gram dry sediment. Overall facultative planktonic species dominate and planktonic species are the least common. The majority of species were found to be indicative of freshwater conditions. Only one species remained unidentified, but is similar to *Navicula rotunda*, but has a higher straie count (See Appendix 2, Plates 128-131 for LM and SEM images)

Zone AV3/04 1 (64-48 cm) - The base of the core is dominated by *Fragilaria pinnata* (SS002A) which varies from between 24 and 50%. Other abundant species include *Fragilaria brevistriata* (PS001A), *F. capucina* (FR009A), *F. construens* (SR001A) and *Achnanthes minutissima* (AD009A). The latter shows an increase towards the top of the zone, but with a sight decline at 52cm, *F. brevistriata* also increases to the top of the zone. In contrast *F. capucina* shows a decline from 12 to 4.4%, and is not present in the sample from 60 cm. *Gomphonema gracile* (GO004A) and *Cyclotella stelligera* (CY004A) also decline in abundance towards the top of the zone. The sample from 60 cm depth is markedly different from the others in this zone, and is consequently isolated by TWINSPAN. This sample is characterized by a higher proportion of *F. pinnata* and *Navicula* c.f. *rotunda* (NA090A) compared to the rest of the zone, while species such as *F. capucina*, *C. stelligera*, *Cymbella descripta* (CM052A) and *G. gracile* were not identified in the sample. The total number of valves in this zone is between 0.51 and 1.17×10^9 per gram of dry sediment, which is the lowest overall concentration for the core. While facultative planktonic species dominate in this zone they are present in the lowest concentration for the whole core and epiphytic species are present in their highest proportions, relative to the rest of the core.

Figure 8.6. Diatom stratigraphy for core AV3/04, showing species present with and abundance of >1% at one or more levels



Zone AV3/04 2 (44-22 cm) – Based on the TWINSPAN classification of samples zone 2 can be sub-divided into 4 sub-zones. At the base of the zone (44cm) *F. pinnata* is present in a relative abundance of 56%, but declines towards the top of AV3/04 2a (32cm) to a low of 35.1%. This decline is matched by an increase in the relative abundance of *A. minutissima* and *C. stelligera* with a slight rise in the abundance of *C. descripta, F. capucina* and *G. gracile*. Zones AV3/04 2b and 2d are similar, with similar abundances of *F. pinnata*, although they are lower than the samples in the remainder of the zone, although 2d has a slightly higher abundance than the 2b. These two samples also contain a higher proportion of *C. stelligera, Cymbella descripta, G. gracile* and *F. capucina* and a lower proportion of *N.* c.f. *rotunda*, relative to the rest of Zone 2. In Zone AV3/04 2c the abundance of *F. pinnata* increases to peak at 54% at a depth of 28cm, the abundance of *N rotunda* also increases correspondingly, while both *F. brevistriata* and *F. construens* decline. A decline in the abundance of *C. stelligera* and *C. descripta* is also noted, while there is little change in the abundance of *A. minutissima*. Total diatom concentration increases in this zone to values between 0.85 and 1.7×10^9 valves per gram of dry sediment. Lowest concentrations are reached in sub zone AV3/04 2b. Facultative planktonic species remain the dominant habitat group (Figure 8.8) but they decline in numbers with a corresponding rise in epiphytic species.

Zone AV3/04 3 (20-7cm) – Zone 3 is characterised by a large increase in *Fragilaria pinnata, F. brevistriata* and *Navicula* c.f. *rotunda* at the expense of *Achnanthes minutissima*. *Fragilaria pinnata* peaks in abundance (66.4%) in the middle of the zone at a depth of 16cm, then declines before rising to a second peak (65.9%) at 9cm then declining to the top of the zone (56.6%). *Fragilaria brevistriata* increases throughout the zone, with a maximum abundance of 16%, (for the core and the zone), at the top of the zone. At the base of the zone *Achnanthes minutissima* is present in an abundance of 6%; there are fluctuations in its abundance throughout the zone, but shows an overall decline to 2.9%. These species, as well as *Diploneis elliptica,* *Fragilaria construens* and *Navicula subrotundata,* show a rise in abundance between 10 and 9cm, which corresponds to the deposition of the Paricutín tephra, which may indicate an increase in competitive ability due to additional silica. *Navicula* c.f. *rotunda* shows a similar pattern of shifts in abundance to *F. pinnata*, with an increase from 8.1% to 12.1% at 12cm, followed by a slight decline, to 10.3%, at 9cm depth, and then a subsequent rise at 8cm, to 11.5%, and further decline to the top of the zone. Valve concentration peaks at 7cm and ranges between 1.1 and 3.7 x 10^9 valves per gram of dry sediment. The proportion of the total species assemblage made up by facultative planktonic species is greatest in this zone, between 70 and 81%. The concentration of these facultative planktonic species peaks at 9cm, prior to the peak in total valve concentration. Periphytic species also reach their maximum concentration for the core in this zone. The concentration of planktonic species in this zone is particularly low.

Zone AV3/04 4 (6-0cm) – Zone 4 represents, approximately, the last 40 years of sediment deposition and environmental change within the basin and shows some rather abrupt changes in the diatom assemblage. *Fragilaria pinnata* remains the dominant species with a maximum abundance of 53.5% reached at 4cm. Above this there is a decline it its abundance to 35.4%, then to a minimum of 27.5% in the surface sediment. *Fragilaria brevistriata* is generally lower in abundance than in Zone 2, with abundance remaining relatively constant throughout Zone 1, with the exception of the two upper most samples where abundance rises from 6.6% at 3 cm to 10.4 and 10.2% at 1 cm and 0 cm respectively. The abundance of *Fragilaria construens* also remains relatively constant throughout the zone, with the exception of the sample from 2 cm depth where it declines from 6.9 to 3.7%. From 6 cm depth onwards abundances of *A. minutissima* and *Cymbella descripta* increase markedly from 2.8% and 1.1% at the top of Zone 3 to 9.9 and 5.8% respectively. Both fluctuate slightly, decreasing at 4cm and again at 2cm, but *A. minutissima* shows an overall increase to 11.9%, while the abundance of *C. descripta* remains relatively constant. *Cyclotella stelligera*, *Fragilaria capucina* and *Gomphonema gracile* show an abrupt increase in abundance at 3cm. *Epithemia turgida*, *Epithemia adnanta* and *Gomphonema parvulum* show a similar response at the same depth, although the change is less marked. *Navicula* c.f. *rotunda* decreases in abundance from the base of the zone to a low of 1.7% at 1cm, but increases, to 3.5%, in the surface sediment. *Aulacoseira ambigua*, present throughout the core in very low abundances, peaks in abundance at 4cm (2.8%) then experiences a decline to 0.7% at 3cm before recovering to between 1.9 and 1.3%. *Navicula seminulum*, which was again identified throughout the core, is also present in the greatest abundance in this zone.

Total valve concentration declines considerably in Zone 4, ranging between 0.9 to 2.0 x 10^9 valves per gram of dry sediment reaching a minimum at 3cm prior to increasing rapidly thereafter. In this zone the relative proportions of epiphytic and planktonic species increase in importance (Figure 8.8) although facultative planktonic species still remain the dominant habitat group. The proportion of epiphytic species ranges between 18 and 33% in this zone, with a maximum reached in the surface sediment.

As seen in Figure 8.6 and 8.7 the diatom stratigraphy was dominated by epiphytic species and benthic (facultative planktonic) *Fragilaria* species. This indicates light penetration throughout the depth of the water column (Sayer, 2001), to the surface sediment persisting though the length of the core. This would have allowed the development of benthic species and of submerged macrophytes which would have provided a substrate for epiphytic species. As mentioned above the core was taken in 8m of water, but due to the observed degree of lake level rise in the last couple of years it is suspected that this would have been around 6m deep more recently. Consequently the core is more indicative of a littoral, rather than open water community which would account for the low proportion of planktonic species, relative to the photographic evidence (see Plate 8.4). Valve concentration is generally low, relative to the Agua Verde Core studied by Davies (2002) who recorded concentrations between ~2 and 15 x 10^9 valves per gram of dry sediment. In this study maximum valve concentration only reaches 3 x 10^9 valves per gram of dry sediment. The lower concentration recorded in this study could reflect dilution of the diatom signal by inputs of catchment material and the correspondence of periods of increased χ_{lf} correspond to periods of lower valve concentrations tends to confirm this. There is a slight deviation from this at the top of the core both χ_{lf} and valve concentration decrease, after ca. 1963.

Plate 8.4. Laguna Zirahuén in 1998, illustrating a low lake level (taken by S.E. Metcalfe)

Two periods of increased planktonic abundance are noted, the first in Zone 2 and the second in Zone 4, since 1943. These periods correspond to periods of slightly lower or declining abundances, of facultative planktonic species and may therefore reflect increased water depth. Wet conditions were recorded in the late 1950s, with the highest ever recorded levels of precipitation recorded in México City between 1966 and 1970 (O'Hara & Metcalfe 1995). High levels of precipitation have also been recorded in the Zirahuén Basin at Villa Escalante since 1943 with peaks noted around 1946, 1952 and 1958-59 (Davies, 2000). Data do, however, show that Michoacán and Zirahuén were quite dry during 1980s with decreasing precipitation from the late 1970s until the early 1990s (Antaramian & Muzquiz, 1997; CNA, 2005).

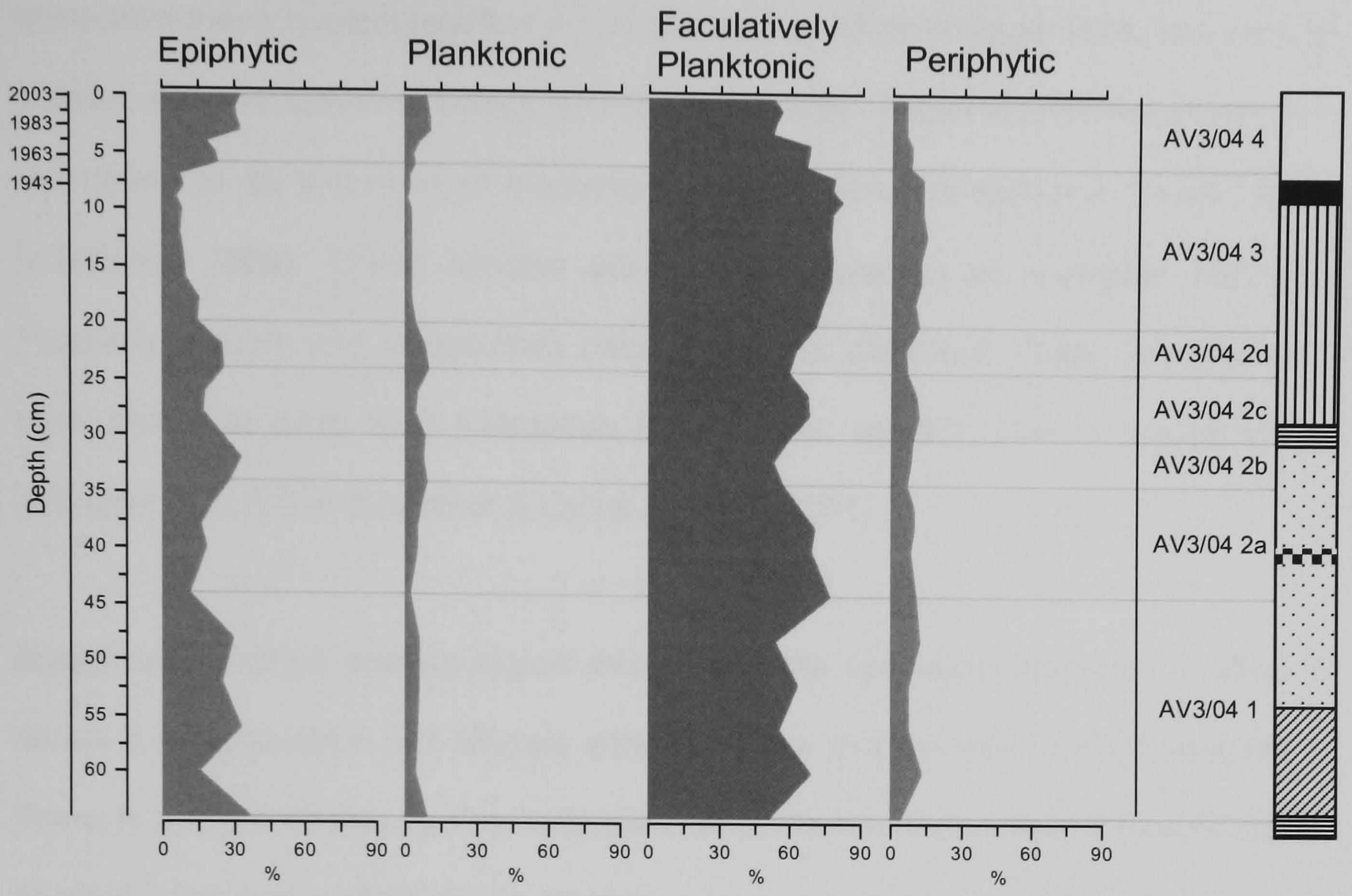


Figure 8.7 Diatom habitat

8.8.1 Diatom Inferred Changes in Nutrient Availability Based on Auto-ecological Information

The diatom assemblage of Zone AV3/04 1 is generally indicative of high nutrient availability. The presence of species such as *Navicula viridula* and *Gomphonema parvulum* in greater abundances here could be interpreted as a response to higher levels of pollution at these levels. In East African lakes *N. viridula* has been found to be indicative of lakes polluted by cattle waste (Gasse, 1986) and was found by Jörgensen (1948) to be indicative of eutrophic conditions. In the Zirahuén basin, however, agriculture is dominated by cultivation, rather than grazing, consequently the input of animal waste is minimal. *Nitzschia amphibia* is present throughout the core but attains its greatest abundances in this zone with particularly high abundance in the lowest 2 samples (60 and 64 cm). In the Florida Everglades *N. amphibia* has been found to be an indicator of eutrophication, increasing in abundance in response to nutrient enrichment (McCormick & Stevenson 1998) and has also been found to be indicative of higher nutrient availability in more temperate regions (Van Dam *et al.*, 1994). *Epithemia adnata, E. sorex* and *E. turgida* had high abundances in Zone 1, corresponding to high abundances of *Gomphonema gracile.* Zalat & Vildary (2005) interpreted these species together in complex with in lakes in Egypt, to be indicative of shallow, alkaline systems, subject to influx of sewage. These *Epithemia* species are also found to be indicative of mesotrophic to eutrophic conditions in Florida lakes (Whitmore, 1989). These species are, however, present in negligible amounts. *Fragilaria* species and *Achnanthes minutissima* are dominant. These species have been shown to have wide tolerances for nutrients, but the latter is though to be indicative of pollution (Krammer & Lange-Bertalot, 1991)

Based on published auto-ecological information, the species composition in Zone 2 shows a mixture which can tolerate either very low to moderate nutrient availability. There is a slight decline in *Fragilaria* species corresponding to rise in abundance of epiphytic taxa and a slight rise in planktonic taxa. As mentioned in Chapters 3 and 7 *Fragilaria* species are known to have wide nutrient tolerance, but *A. minutissima* is also reported as having a wide tolerance for nutrient availability, having been found in oligotrophic to hypertrophic conditions (Van Dam *et al.* 1994). In East Africa *G. gracile* also shows a preference for neutral to weakly alkaline conditions and was found to exhibit a preference for low nutrient conditions. In contrast *G. gracile* has been identified in lakes in The Netherlands as showing a preference for eutrophic conditions (Van Dam *et al.*, 1994) while in lakes in Florida Whitmore associated this species a wide range of conditions from oligotrophic to eutrophic. Van Dam *et al.* (1994) found *C. descripta* to be indicative of oligotrophic conditions in The Netherlands. In the modern calibration set this species was only identified in three lakes, all of which, based on their TP concentrations, were mesotrophic. Such a limited range does however limit the applicability of such an assumption. Due to the high levels of Chl-a found in two of these lakes, La Alberca (Tacambaro) and San Gregorio the Chl-a optima is high, 52.5 $\mu g\ l^{-1}$. *Cyclotella stelligera* was also seen to increase in abundance in Zone 2. In the UK this species is only found in post glacial sediments where nutrient availability has been very low, oligotrophic. In the USA, however, *C. stelligera* was found to increase in abundance rapidly in response to nutrient enrichment and was found to the most tolerant of *Cyclotella* species to eutrophication (Stoermer *et al.*, 1985). In contrast, Hall & Smol (1992) report this species as having a TP optimum of 9.7 $\mu g\ l^{-1}$, which is in the mesotrophic to oligotrophic range, which are similar to the findings of Bennion (1994) (see Section 6.6.4). The increase in *C. stelligera* and rise in overall abundance of planktonic species would seem to indicate deeper water conditions. This may indicate maintained, or a slight elevation in nutrient availability.

The small *Fragilaria* species have been found to tolerate a wide range of nutrient conditions from oligotrophic to hypertrophic (Sayer, 2001; Van Dam *et al.*, 1994). This interpretation varies slightly as Whitmore (1989) shows more constrained tolerance limits in these species in Florida lakes, between mesotrophic and eutrophic conditions. This contrasts with studies of temperate lakes as Bennion (1994) found the small *Fragilaria* species to increase in abundance at less concentrated mean annual TP values. Van Dam *et al.*, (1994) found *F. capucina* and *F. construens* to be indicative of mesotrophic conditions, although they also found *F. construens* in eutrophic waters. The abundance of the small *Fragilaria* species also indicative of shallow, weakly alkaline, circum-neutral conditions (Bradbury, 1989; Davies *et al.*, 2004). Stoermer (1978) also found *F. capucina* to be indicative of more eutrophic habitats in the North American Great Lakes. In the UK Sayer (2001) found *F. brevistriata* and *F. pinnata* to be bottom dwelling, living on stable sediment or on the bases of emergent macrophytes, indicating that to increase in abundance they would need increased water column clarity. The increase of these species in Zone 3 may therefore indicate increased water clarity through declining nutrient availability and possibly lower inputs of catchment material, as χ_{lf} is low, which would also account for the decline in *F capucina* and *F. construens*, which have higher nutrient tolerances.

Zone 4 is similar to Zone 1 with a greater abundance of *Gomphonema parvulum* relative to the rest of the core, and as discussed above could be indicative of renewed increase in pollution. In Zone 4, since 1943, there has been an increase in the epiphytic taxa *Epithemia adnata, E. sorex, E. turgida* and *Gomphonema gracile,* which as noted above in complex have been identified as being indicative of pollution. *Gomphonema parvulum* is highly tolerant of pollution and high nutrient loadings (Patrick & Reimer, 1961) and in the UK was found to have a TP optimum of 138.4 μg l^{-1}, indicative of eutrophic-hypertrophic conditions (Bennion, 1994). This species, while present throughout the core, achieves its highest abundances at the Zone AV3/04 4. *Aulacoseira ambigua* has been reported, in temperate regions, as being indicative of oligotrophic to mesotrophic conditions (Hall & Smol 1992; Ramstack *et al.*, 2004). In contrast Brugham (1979) showed *A. ambigua* to be found predominantly, in moderately eutrophic water, he also noted that in Linsley Pond, USA, this species shows no constant pattern of response to trophic state. An increase in *A. ambigua* and corresponding decline in *Fragilaria* species was noted in Lake George during the mid to late Holocene, and was interpreted as a, natural, increase in nutrient availability (Haworth, 1972). Proportions of *Fragilaria brevistriata, F. capucina* and *F. construens* all increase towards the top of Zone 4. This may reflect higher nutrient availability at the top of the core, as *F capucina* and *F. construens* are generally considered to have nutrient tolerances in the meso- to eutrophic category (Van Dam *et al.*, 1994). A decline in *Fragilaria pinnata* was noted in Zone 4, which as the nutrient tolerance for this species in central México indicates oligo-mesotrophic conditions, would corroborate an increase in nutrient availability and declining water clarity.

The diatoms stratigraphy of AV3/04 differs considerably from that of Davies (2000), core AV/98, also taken from the Agua Verde sub-basin but in 12 m of water. The primary difference is in the lower species diversity and the notable absence of *Cyclotella ocellata* and *Fragilaria crotonensis* in this study. Other species present in abundance in the core AV/98, but present in low abundances in this study, including *Nitzschia amphibia* and *Aulacoseira distans*. *Cyclotella stelligera* and *Diploneis elliptica* are also present in much greater abundances in the AV/98 core. *Fragilaria* species are present and abundant in AV/98 but not to the same extent as AV3/04, *Achnanthes minutissima* and *Cymbella descripta*, are only present in AV/98 at low abundance. Davies *et al.* (2004) identify *Gomphonema gracile, Navicula seminulum* and *Epithemia* species at only a few levels, while in this core they are found throughout. Such differences may be accounted for by the difference in core location, as the core in this study was taken from a more littoral location than AV/98. Further more the lack of *C. ocellata* and *F. crotonensis* may be associated with the loss of the top of core AV3/04, although without more precise dating it is difficult to determine this.

8.9 Diatom inferred change in nutrient status

As can be seen above, published auto ecological information for diatom species can vary by region, it is therefore preferable to have region specific estimations of species response to environmental change. Multivariate statistical analysis of environmental and species data (Chapter 7) showed that Chl-a, as a proxy for overall nutrient concentration, made a significant and independent contribution to diatom species variation. As a result, weighted average regression and calibration equations were derived for Chl-a. Results showed that simple WA with classical deshrinking provided the best model (see Section 7.3.3) and has been used to reconstruct Chl-a concentration quantitatively, and thus infer changes in nutrient status, in Laguna Zirahuén. Only species present in an abundance of greater than 1% at one or more samples were used in the analysis. The fossil data set therefore contained 24 species. The summary diagram, common species only, is shown above (Figure 8.6) and the diatom inferred Chl-a reconstruction below (Figure 8.8). The model has also been applied to Davies (2000) core; AV/98 (Figure 8.9).

The simple WA model shows a back transformed Chl-a range of 7.5 to 15.9 µg l^{-1} and a mean of 13.6 µg l^{-1} (WA) at the base of the core. After a slight decline at the base of the core the model infers a general rise to 30cm, except for a slight decrease between 48cm and 36cm. Above 30cm there is a progressive decline until 1943, when the Paricutín tephra was deposited, where Chl-a concentration is inferred as the lowest for the whole core at 7.5 µg l^{-1}. With the exception of a slight decrease between around mid 1960s and late 1970s (6-4cm), Chl-a concentration increase, and remain high, to a secondary peak of between 13.9 and 15 µg Chl-a l^{-1}, for the whole core, at a depth of 2 cm (ca. 1990). In around 1997 there is a drop off in Chl-a concentration, to around 10.9 µg l^{-1} (WA). The concentration of Chl-a increases to a maximum in the surface, most recent, sediment to reach 15.9 µg l^{-1}. Of the common fossil species identified only 63% had modern analogues.

The diatom stratigraphy seems to indicate similar levels of nutrient availability in Zones 1 and 4. Diatom inferred Chl-a (WA with classical deshrinking and no cross validation) for Zone AV3/04 1 shows lower Chl-a concentrations at the base of the core and higher levels at the top of the core. Diatom inferred Chl-a generally increases up the zone to reach a maximum of 15.8 µg l^{-1} with an average of 13.5 µg l^{-1}. Zone 1 shows lower inferred Chl-a concentrations and ranges between 13.7-9.8 µg l^{-1} with an average of 11.8 µg l^{-1}. In diatom Zone AV3/04 2 the available auto-ecological information seems to imply a low to moderate levels of nutrient availability. In contrast the diatom inferred Chl-a for Zone AV3/04 2 indicates a meso-eutrophic system with inferred Chl-a concentrations in the range of 10.75 to 14.6 µg l^{-1} with an average of 12.9 µg l^{-1}. In Zone AV3/04 3 the rise in *Fragilaria* species could be interpreted as higher water clarity through reduced nutrient availability. Furthermore the diatom inferred Chl-a shows a rapid decline in Chl-a, supporting lower levels of productivity, within this zone from 12.3 µg l^{-1} at the base (20cm) to 7.5 µg l^{-1} at 9cm, around 1943.

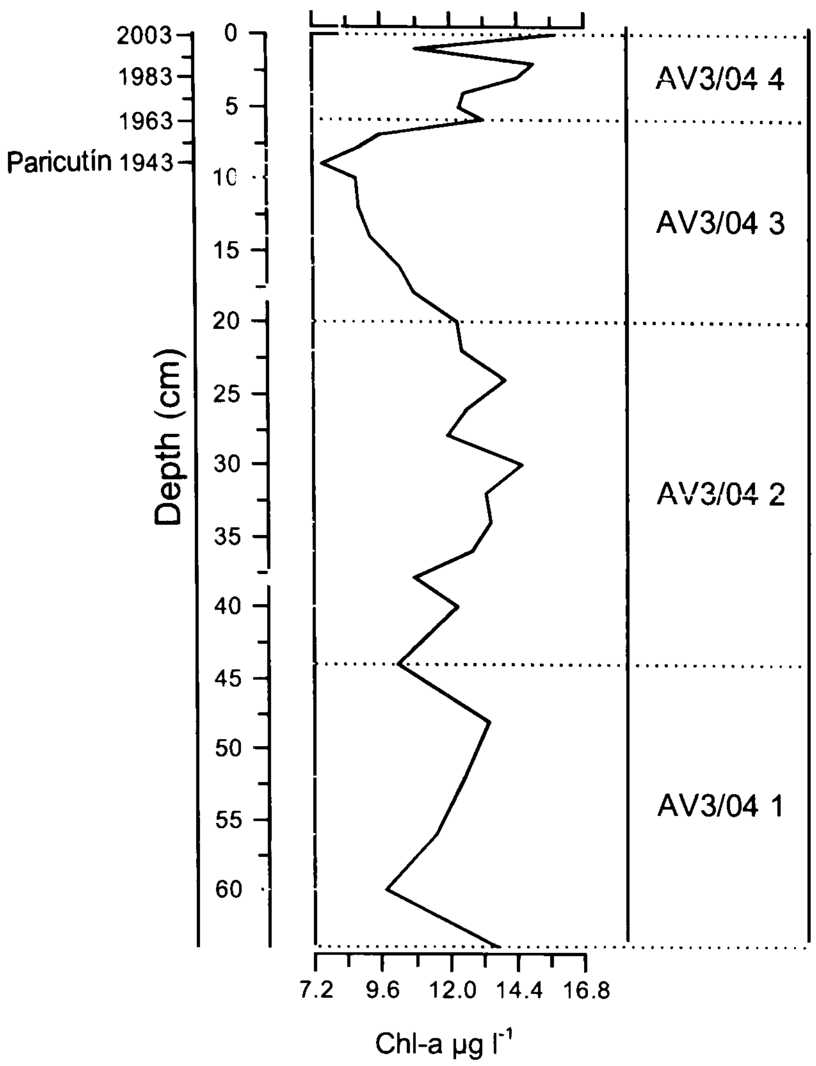


Figure 8.8. Results of simple WA with classical deshrinking for core AV3/04

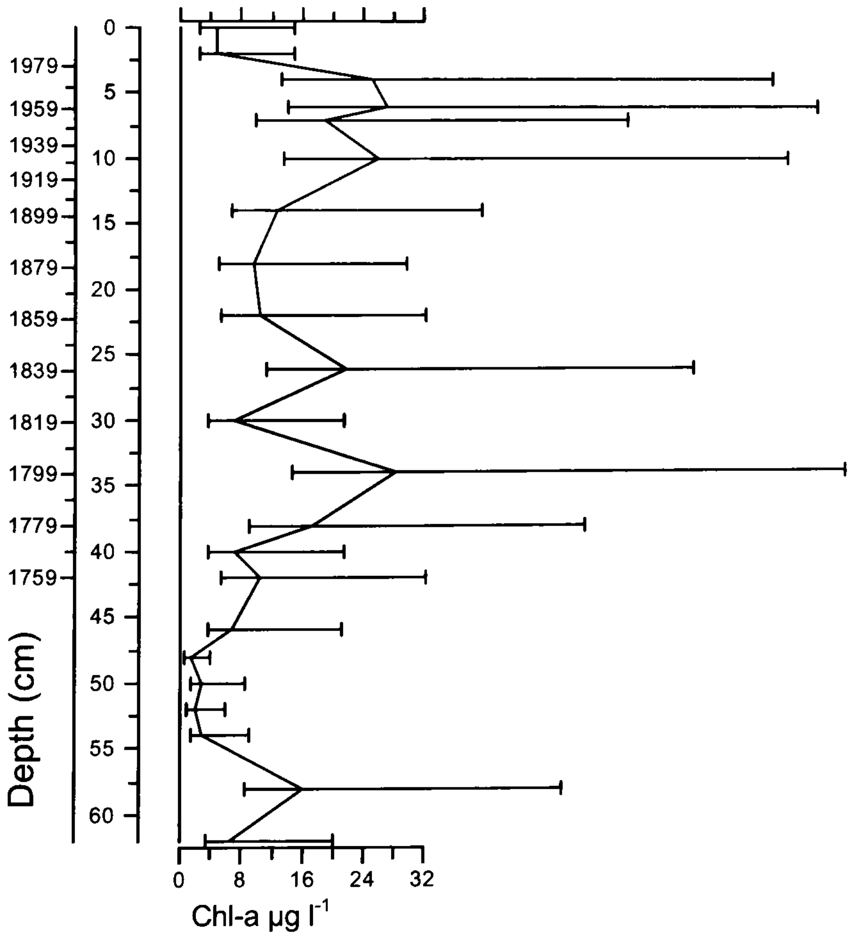


Figure 8.9. Results of application of simple WA with classical deshrinking to core AV/98.

The fossil material from this study varies substantially compared to the earlier study of the same area by Davies (2000), discussed in more detail above. As a result the application of the transfer function discussed above to her core (AV/98) resulted in a very different reconstruction of nutrient availability (Figure 8.9). The range of Chl-a concentrations inferred from AV/98 are much wider than in this study, between 1.3-28.4 µg l^{-}. The most abrupt change in the diatom flora in AV/98 occurs in the last 15 years when *Cyclotella ocellata* and *Fragilaria crotonensis* rapidly increase in abundance, previously interpreted as a shift to higher nutrient availability. Based on the modern calibration set collected for this study these two species were assigned optima of 2.9 and 4.5 µg l^{-1} respectively (see Table 7.7), which are in the oligo-mesotrophic range. As a result, diatom inferred Chl-a implies decreased nutrient availability over this period. In AV/98 maximum Chl-a concentrations are inferred for around the early 1800s, compared to this study. Minimum inferred concentrations are also at very different times, found at 46cm in AV/98, compared to 9 cm, corresponding to the deposition of the Paricutín tephra in this study. In the upper sediments inferred Chl-a in core AV/98 shows a large decrease in concentration during the 1970s from 25.3 to 4.9 µg l^{-1}. This corresponds to a period of inferred increasing Chl-a for this study. Of the fossil species identified in AV/98, 83‰ were not present in the modern material, consequently had no modern analogues, such as *Eunotia alpina, Eunotia minor* and *Navicula concentrica*. Some of the species identified in core AV/98 were identified in this study, but were present in very low abundances so were not included in the modern calibration set. This does indicates a severe no-analogue problem.

Changes in nutrient concentration, and the fit of the fossil to the modern samples, can also be tracked through plotting core sample and species in ordination space. Following the method of Bigler *et al.*, (2002), the 'goodness of fit' of the fossil samples to the modern calibration data set can be assessed by passively fitting fossil samples in CCA ordination space, defined by the modern samples, with Chl-a and EC as the sole constraining variables (Figure 8.10). As can be noted from Figure 8.10 the core samples differ from the modern surface sediment assemblage, with core samples being located further to the left of the modern Zirahuén sample in the CCA biplot. It should be noted that the modern was collected from the deepest part of Laguna Zirahuén and therefore had a very different diatom assemblage compared to the core. Furthermore available data indicate that Chl-a concentrations are higher in Agua Verde than they are in the main lake basin. These two factors may therefore contribute to the different locations on the CCA biplot.

In general terms, the shifts in Chl-a concentration inferred by WA correspond well to that inferred by plotting fossil samples in ordination space, with periods of low Chl-a, such as 60cm, 44cm, 38cm, 28cm and most prominently 9-10cm, plotting low on the Chl-a axis on the CCA biplot. Samples in Zone AV3/04 4 are located to infer higher Chl-a concentrations, on the upper right of the biplot, and thus greater nutrient availability. As the fossil samples are fitted into the CCA passively, i.e. after the modern samples, they do not influence the definition of the ordination axes (ter Braak & Šmilauer 2002) and thus must lie within the constrained ordination axes determined by the calibration set. A more reliable reflection of the relationship between modern and fossil material can be gained by plotting the modern and fossil samples together, actively in a DCA (Figure 8.11). This further highlights the lack of modern analogues in the fossil data set, in that the fossil samples lie over one standard deviation away from the main body of the data set.

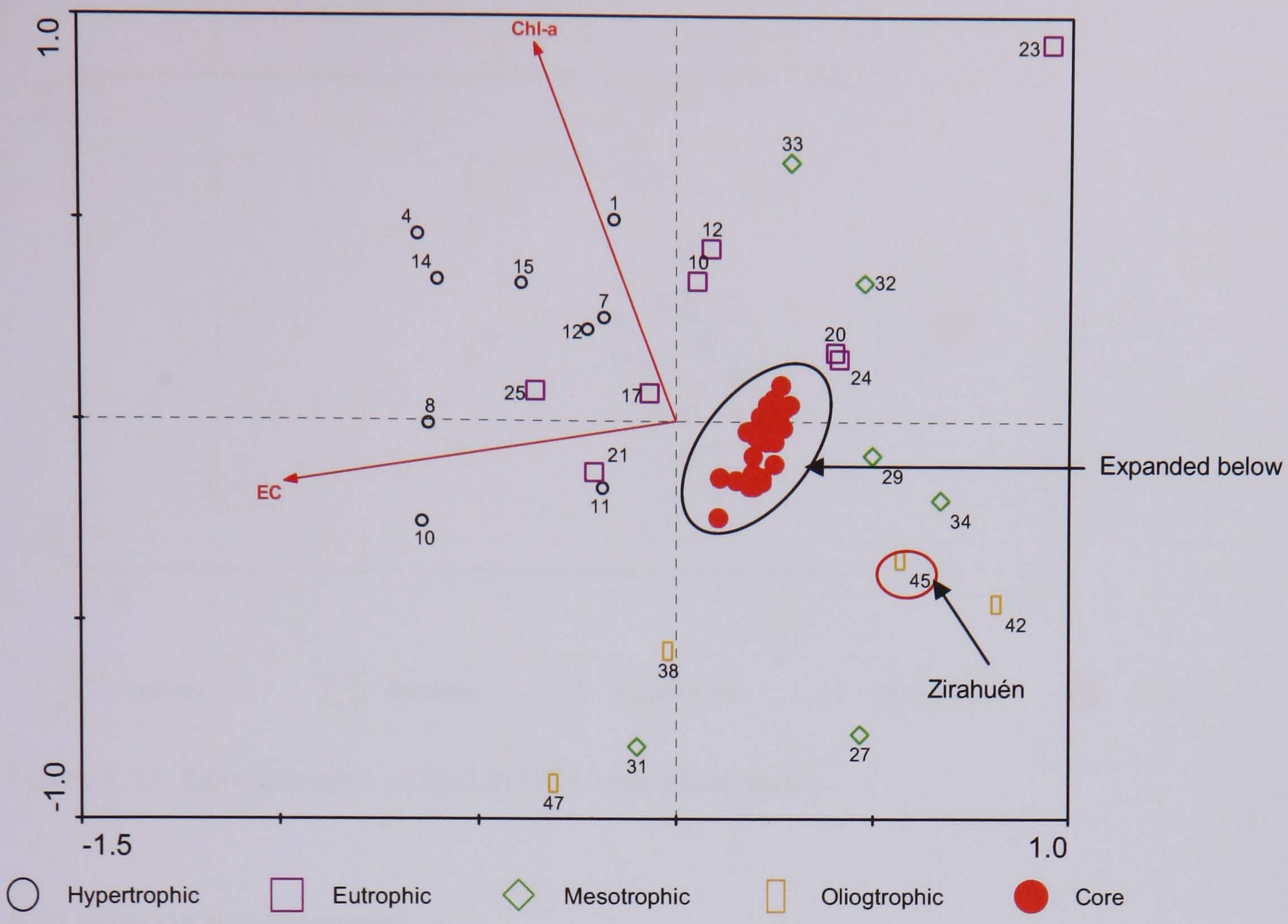


Figure 8.10. a) CCA biplot of modern samples with core samples plotted passively in ordination space

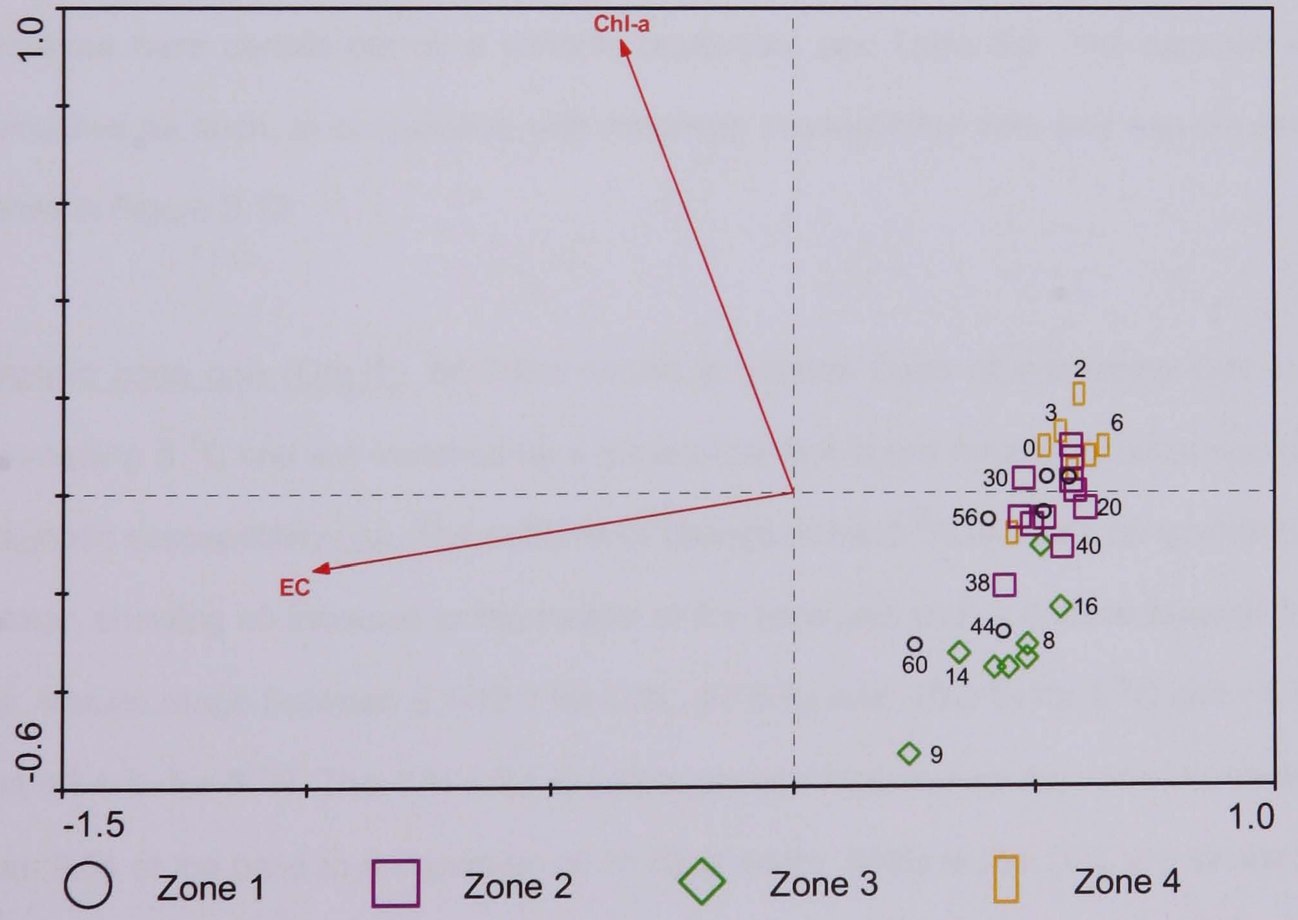


Figure 8.10. b) CCA biplot of core samples only

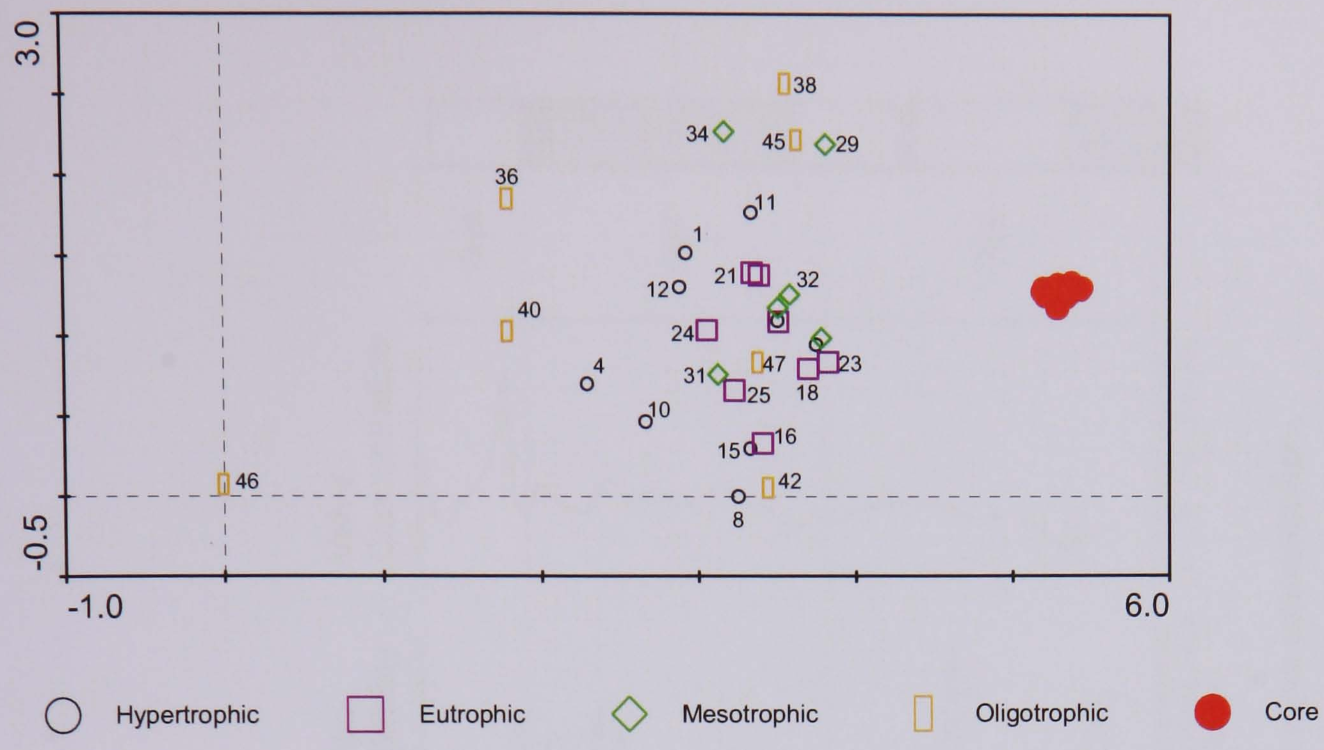


Figure 8.11. Core samples plotted in DCA ordination space

8.10. Isotope geochemistry

The organic data can be split into three zones, different to the diatom zones, on the basis of general corresponding trends in the different proxies (see Table 8.1). Analyses were carried out on a variable resolution, see Table 5.2. The data will be discussed as such, in conjunction with magnetic susceptibility data and can be seen below in Figure 8.12

Organic zone one (Org 1), 64-36cm shows a general trend of increasing C/N and decreasing δ^{13}C and are matched by a general decline in low frequency mass specific magnetic susceptibility (χ_{lf}). The patterns of change in the δ^{15}N and loss on ignition are similar, showing an increase to the middle of the zone and then a decline towards the top. Values range between 9.1-12.1 for C/N, -23.5 ‰ and -20.5 ‰ for δ^{13}C and +1.45 and +3.4 ‰ for δ^{15}N. The C/N ratio shows a general increase up the zone increasing from 9.54 at the base to a maximum of 10.72 at 44cm. Shifts in the TOC are similar to those of C/N, despite a poor relationship between the two variables (see Figure 8.13). Increase in the C/N is, generally, matched by shifts to more negative values in the δ^{13}C, while lower C/N are matched by less negative δ^{13}C values. From the base of Org 1 to between 52 and 48cm, δ^{15}N increases from +2.05 ‰ to peak between +2.9‰

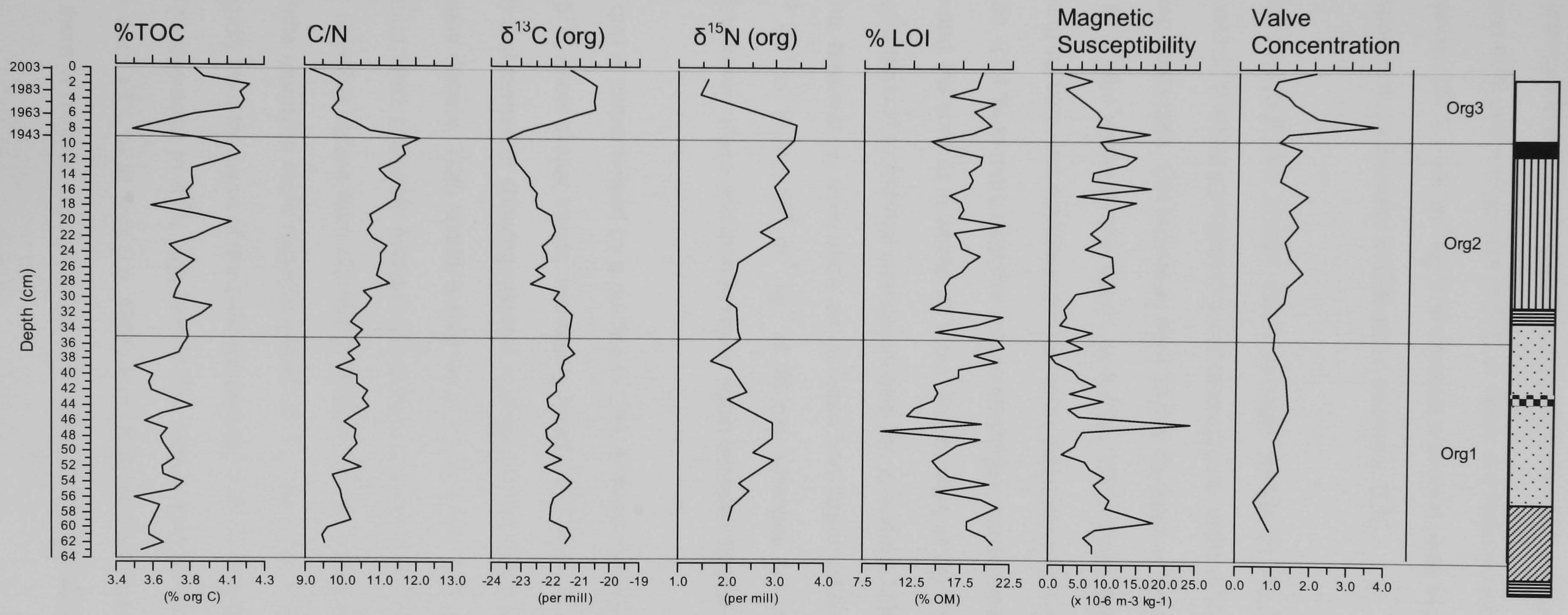


Figure 8.12. % TOC, C/N ratio, $\delta^{13}C$, $\delta^{15}N$ profiles, compared with magnetic susceptibility, LOI and diatom valve concentration

and +2.94 ‰. Above 48 cm $\delta^{15}N$ values decline towards the top of the zone to reach a minimum of +1.65 ‰ at 39 cm. As discussed in Section 8.7.3 magnetic susceptibility can be used to infer changes in detrital allochthonous inputs from the catchment. Low values of C/N, TOC and $\delta^{15}N$ and a slight rise in $\delta^{13}C$ at 46cm are matched by a large excursion to high χ_{lf} values (24.2 x 10^{-6} m^{-3} kg^{-1}). In contrast a second peak in χ_{lf} lower down at 59 cm corresponds to an increase in C/N and a decline in $\delta^{13}C$.

Organic zone 2 (Org 2) (35-9 cm) shows much stronger trends in the proxies measured. Both C/N and $\delta^{15}N$ show a progressive increase up core, while $\delta^{13}C$ shows the reverse, decreasing down core. C/N increases from 10.6 at the base of the zone to 12.1 at 10 cm. The $\delta^{15}N$ also peaks at this depth, at 3.4 ‰. TOC shows the same general trend increasing up core, but exhibits more variability than either C/N or $\delta^{15}N$. The $\delta^{13}C$ declines from -21.4 ‰ to more negative values reaching a maximum low of -23.5 ‰ for the zone and the core as a whole, at 9 cm, the location of the Paricutín tephra. These trends correspond to minimal changes in both the χ_{lf} and the LOI. From the bottom of the zone, however, χ_{lf} does show an increase from lower values at 33 cm of 1.8 x 10^{-6} m^{-3} kg^{-1} to 11.6 x 10^{-6} m^{-3} kg^{-1} at 28 cm. Changes are minimal thereafter, until a shift to lower values and then increase again between 16 and 15 cm.

Organic Zone 3 (9-0 cm) is characterised by a decline in C/N, $\delta^{15}N$ and χ_{lf} while $\delta^{13}C$ increases. TOC and $\delta^{13}C$ show similar trends, increasing rapidly from 9 cm to a peak at 5cm (ca. AD 1970) and thereafter showing minimal change to 2 cm depth (ca. AD 1990) whereupon values decline. TOC shows a shift from 3.5 % to 4.2 % in around 1970 and 4.3% in around 1990, thereafter falling to 3.9 at the top of the core. The $\delta^{13}C$ shows a shift to less negative values from -22.9‰ in 1943 to 20.5‰ between 1970 and 1990 and then shifts rapidly to more negative values of -21.3‰ to the top of the core. C/N declines rapidly from the base of the zone decreasing from 12.1 to 9.7 at 6 cm (ca. AD 1964). The decrease in $\delta^{15}N$ lags behind the shift in C/N by ca 12-13 years. Values decline from +3.4 ‰ at 7cm (ca. 1957) to +1.5 ‰ at 3 cm (ca 1984). From 1964 to ~1991 there is minimal change in the C/N, but in the upper sediments

(2-0cm) it declines further, in a similar manner to the $\delta^{13}C$ and TOC, from 10.0 to 9.1. Shifts in the upper part of the core are matched by a decline in the χ_{lf} from 16 x 10^{-6} m^{-3} kg^{-1} to 2.2 x 10^{-6} m^{-3} kg^{-1} at the top of the core, indicating a reduction in detrital input from the catchment. Both maximum and minimum values and the largest shifts of TOC, C/N, $\delta^{13}C$ and $\delta^{15}N$ are reached in within this zone, or at the base of the zone. These peaks are however often offset, with lowest TOC occurring 1cm above the peak in C/N and the lowest $\delta^{13}C$. In contrast $\delta^{15}N$ peaks at 7cm, although this may be a function of the difference in sampling resolution.

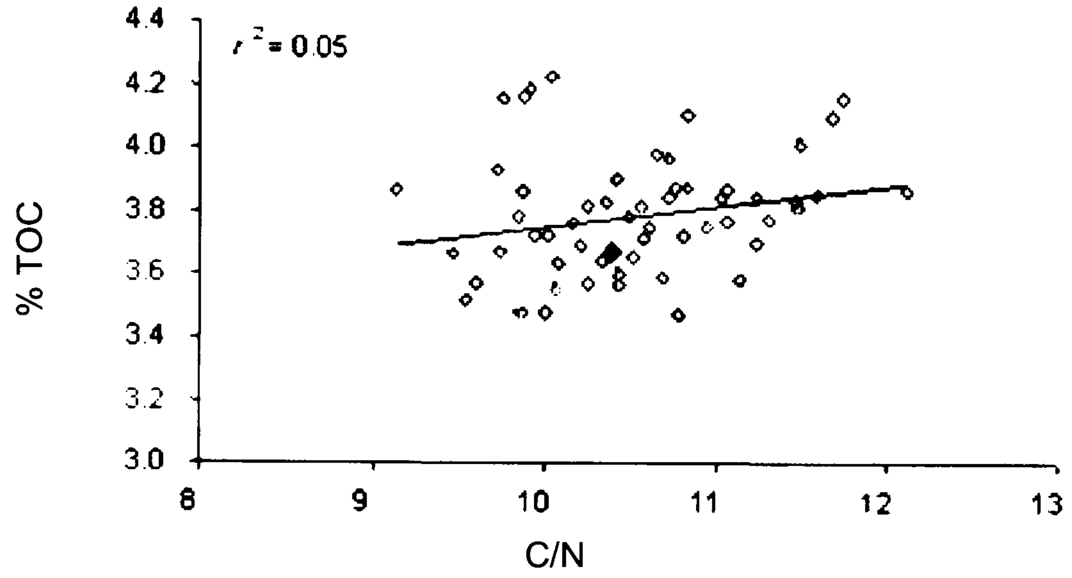


Figure 8.13. Relationship between C/N and TOC for Core AV3/04

As noted in Chapter 4 (Section 4.3.2) algae and C_3 plants have similar $\delta^{13}C$ values but variable C/N, consequently both metrics are used (to distinguish between them see Figure 4.1). The data from core AV3/04 was plotted in the same way (Figure 8.14). The reported values for this core (AV3/04) lie outside the standard ranges reported for C_3, C_4 plants and algae (Meyers & Teranes 2001). The values recorded in this study (as seen in Figure 8.14 below) are less negative than C_3, but more negative than C_4, ranging between -20.5‰ and -23.5‰ with an average of -21.9‰. In addition the C/N values recorded in this study are much lower (9.1 to 12.1) than standard C/N values reported for C_3 and C_4 plants, which are generally >20. C/N values are, however, in the range of a mixed (algal/terrigenous/aquatics) or algal signal, i.e. >10. Algae are generally thought to have $\delta^{13}C$ values between -25‰ and -30‰, lower than bulk values measured in core AV3/04. During periods of higher productivity algae can begin to use HCO_3^- rather than dissolved CO_2 which can lead to elevated $\delta^{13}C$ values up to -9‰ (Meyers & Teranes, 2001). In a study of C/N and $\delta^{13}C$ of plant matter Hornibrook *et al.* (2000) found a number of aquatic macrophytes to yield C/N values between 13 and 19 with a corresponding range of $\delta^{13}C$ -18.4‰ to -27.7‰. C/N values and δ^{13} may therefore be in the range of aquatic macrophytes or reflect a mixed signal of terrestrial and aquatic vascular and non-vascular plants.

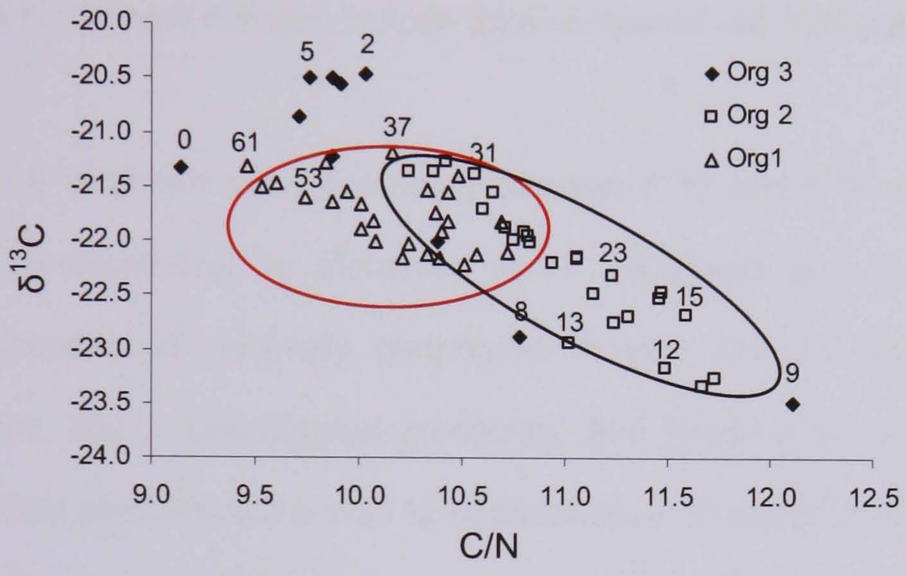


Figure 8.14 C/N vs. $\delta^{13}C$ data of fossil data from core AV3/04

Figure 8.14 also highlights the similarity between the upper and lower parts of the core in terms of organic matter composition and therefore organic matter source. When considered in conjunction with the Org C and χ_{lf} signals, this could be indicative a more algal signal in both zones Org 1 and Org 3, compared with Org 2. It may also indicate that similar processes have controlled the input and post-depositional processes in these zones. This is also seen in the relationship between $\delta^{15}N$ and C/N (Figure 8.15). Organic zone 1 however, generally has higher C/N and $\delta^{15}N$ values and lower $\delta^{13}C$ values than Zone 3. There are exceptions to this, with samples from 8 and 9 cm (in Zone 3) showing higher C/N ratios, than the rest of the zone, which are more indicative of aquatic or terrigenous input. These samples correspond to the deposition of the Paricutín tephra or immediately afterwards. The $\delta^{13}C$ of samples 8 cm and 9 cm are around 2 ‰ lighter (more negative) than the majority of the rest of the samples in the zone.

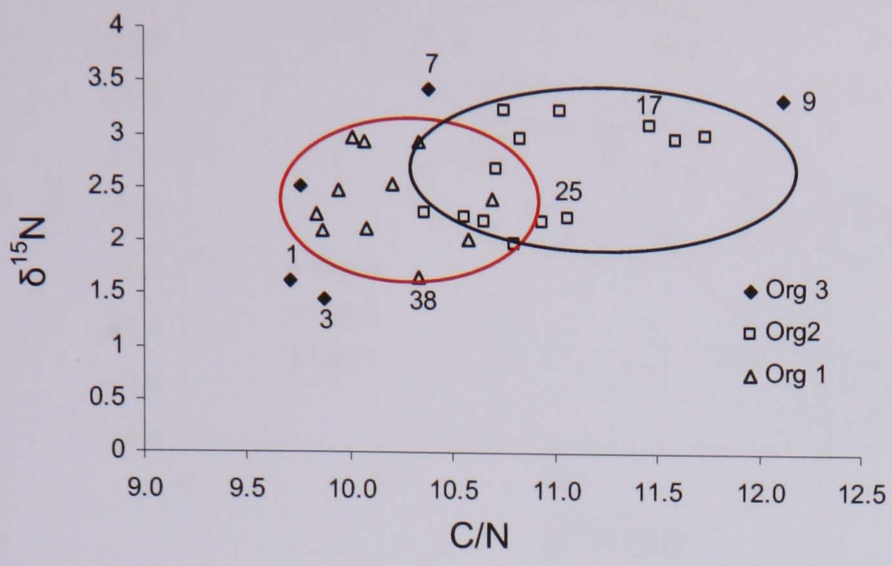


Figure 8.15 Fossil nitrogen isotope data compared with C/N data.

Figure 8.16 shows the relationship between $\delta^{13}C$ and $\delta^{15}N$ and shows a decline in $\delta^{13}C$ corresponding to increased $\delta^{15}N$. Increased primary productivity, due to eutrophication is generally considered to give rise to increased $\delta^{13}C$ and $\delta^{15}N$ (Meyers, 2003). Limnological monitoring and palaeolimnological investigations over the recent past indicate a shift to higher nutrient availability, which would be expected to lead to higher $\delta^{13}C$ values in the upper sediments, as the ^{12}C is locked into the organic matter accumulated in the sediment, which is observed here. It would also be expected to lead to higher $\delta^{15}N$ values, but decreasing values are seen here. Eutrophication is usually associated with a shift to nitrogen limiting conditions, due to higher P availability, which leads to an increase in the proportion of N-fixing cyanobacteria (Mason, 1996). As cyanobacteria derive their N from the atmosphere, primary production when dominated by cyanobacteria would be expected to yield $\delta^{15}N$ values close to 0 ‰. Consequently declining $\delta^{15}N$ is often a sign of eutrophication (Talbot, 2001), resulting in the negative relationship between the two variables.

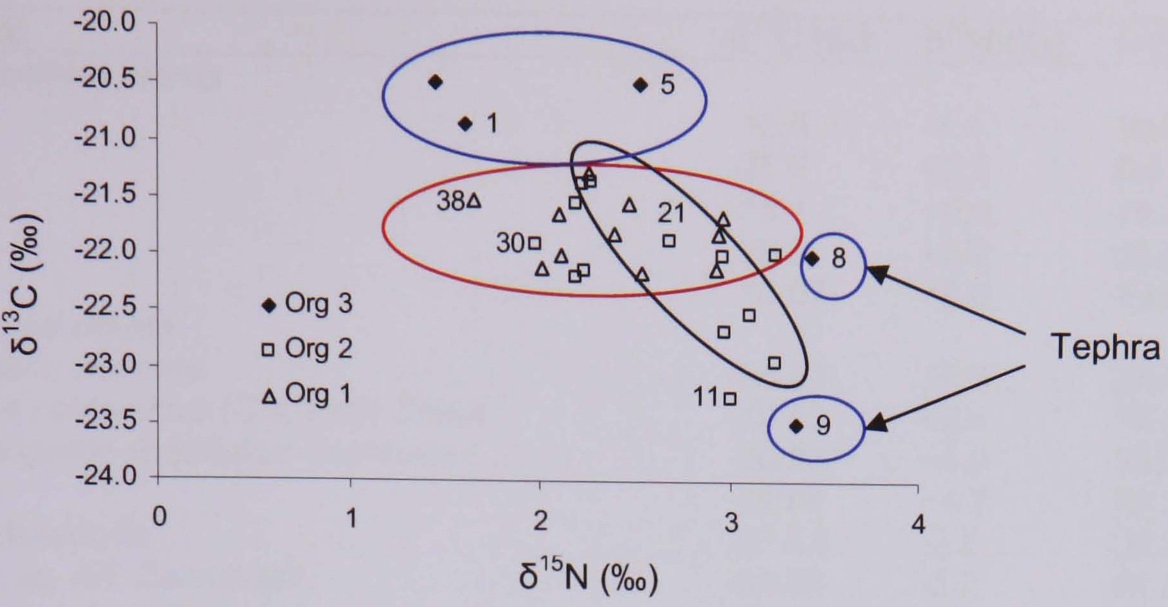


Figure 8.16. $\delta^{15}N$ vs. $\delta^{13}C$ for organic matter samples from Core AV3/04, samples are separated by organic zone, as discussed above

As noted in Chapter 4 the $\delta^{15}N$ signal is more complex than the $\delta^{13}C$ making interpretation more difficult. The $\delta^{15}N$ can exhibit a range of up to 100‰, but generally lacustrine sediments lie between -5 and +20‰ (Talbot, 2001). Certain sources do have distinctive signatures (see Table 4.1). Based on Talbot's review of $\delta^{15}N$ in lakes phytoplankton have $\delta^{15}N$ values which lie between +2 and +14‰, land plants between +2 and +10‰ and aquatic macrophytes are isotopically light with values between 0‰ and -10‰. The latter contrasts with Jones *et al.* (2004) who reported $\delta^{15}N$ values for aquatic macrophytes in lakes the UK between -3.1‰ and 7.3‰. In this study $\delta^{15}N$ ranges between +1.5‰ and +3.4‰ and consequently could be interpreted as exhibiting a mixed signal. N-fixing cyanobacteria and the impact of anthropogenic inputs can act to modify $\delta^{15}N$, but (as discussed in section 4.3.1) it can also be modified by internal processes such as ammonia volatilization or denitrification. Therefore interpretation of the $\delta^{15}N$ signal needs to be undertaken in conjunction with the other available proxy data to ascertain organic matter sources and will be discussed in more detail below.

Table 8.2. Results of modern plant material isotope analysis

Sample	δ^{13}C (‰)	δ^{15}N (‰)	C/N
Unidentified plants			
Zn1	-13.5	-7.4	10.8
Zn2	-25.9	+2.6	9.6
Zn3	-15.1	+0.9	19.4
Zn4	-26.5	+2.2	45.6
Zn5	-10.07	+6.0	19.0
Identified plants			
Baccharis Conferta	-25.93	+0.6	23.4
Scirpus californicus (C.A Mey) Steua	-10.08	+2.4	13.3
Potamogeton amplifolius Tuckheim	-20.23	+5.0	13.2
Grass	-28.86	+4.2	16.3
Pinus leiophylla	-27.62	-2.5	26.3
Agave sp. Aff. Salmiana?	-24.66	-2.3	59.2
Cupressus lusitanica	-28.61	-1.8	15.1
Baccharis salicifolia (Ruiz and Paron) pers.	-31.44	+3.1	27.6
Montanoa?	-32.32	+11.3	21.5
Soils			
Meadow by lake	-15.2	+1.9	5.6
Dry Scrub	-21.4	+3.3	12.7
Pine Forest	-24.5	+2.6	6.6
Oak Forest	-26.3	+0.8	17.6
Shoreline	-25.7	+3.2	7.8

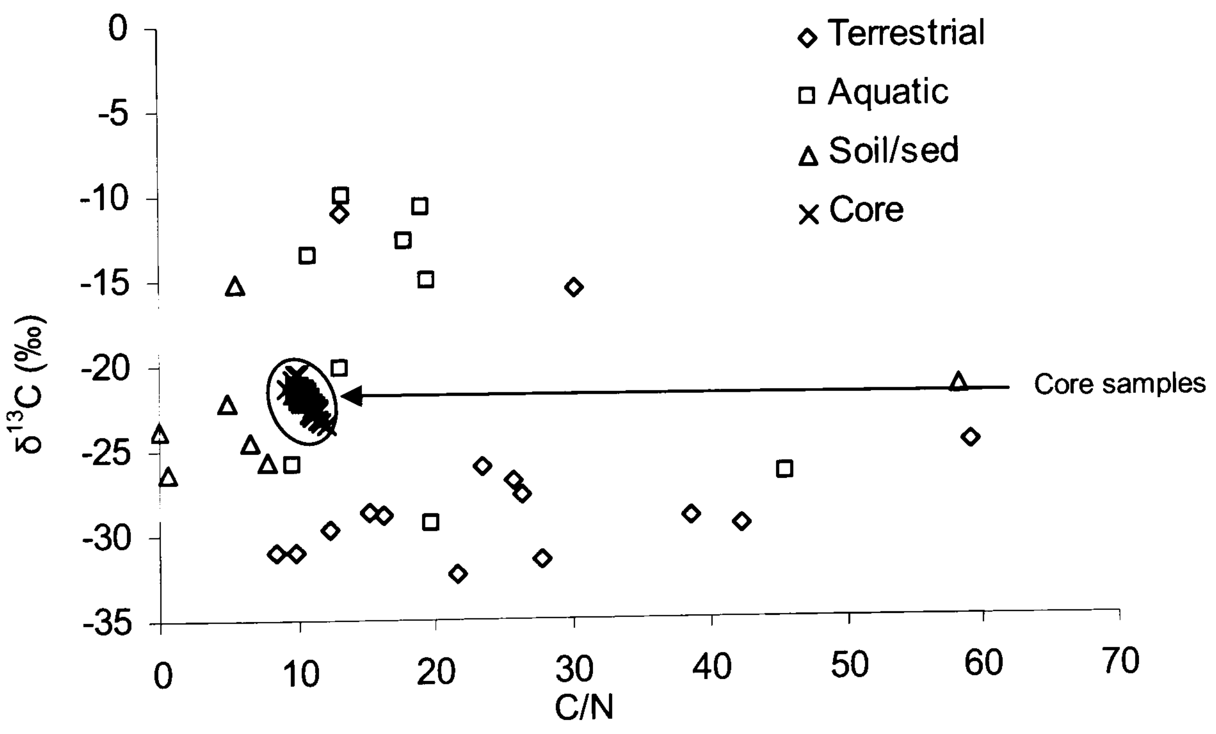


Figure 8.17 Core and modern samples for δ^{13}C and C/N

The results of isotope analyses from the core sediments can be compared with modern isotope results from analyses of soil and terrestrial and aquatic vegetation (collected by Ben Aston, used with permission) in the Zirahuén catchment, which can help to identify sources (Figure 8.17; 8.18, Table 8.2). Soils from around the Zirahuén catchment yield δ^{13}C values between -15.2 and -26.3‰ with the lowest (most negative) values taken from samples within oak woodland and the highest from meadow soil samples. C/N values range between 5 and 17.6. Nitrogen isotopes values ranged between +0.8 and +4.6‰, with the highest values recorded in soils beneath pine woodland. Terrestrial vegetation samples were taken from a wide range of sites. Values for $\delta^{13}C$ range between -10.96 and -32‰ and C/N range between 8.4 and 59.2, i.e. reflective of both C_3 and C_4 plants, and between -1.8 and +11.3‰ for $\delta^{15}N$. Maximum values of $\delta^{13}C$ were found in grass samples, i.e. C_4 plants, but these samples yielded low C/N values of 13.2. The lowest, most negative, $\delta^{13}C$ values were found in samples of the shrub *Baccharis salicifolia*, which yielded C/N values of 21.5 and the highest $\delta^{15}N$ value. The highest terrestrial C/N values were found in *Cupressus lusitanica* (Cedar). Nine aquatic vegetation samples were taken, five of which were not identified. These aquatic samples range between -10.08 and -26.5‰ for $\delta^{13}C$, 9.6-45.6 for C/N and between -7.4 to +6‰ for $\delta^{15}N$. The most negative values of both $\delta^{13}C$ and $\delta^{15}N$ were found in one of the unidentified samples collected in this study and from and emergent plant; *Scirpus californicus* (Reed)

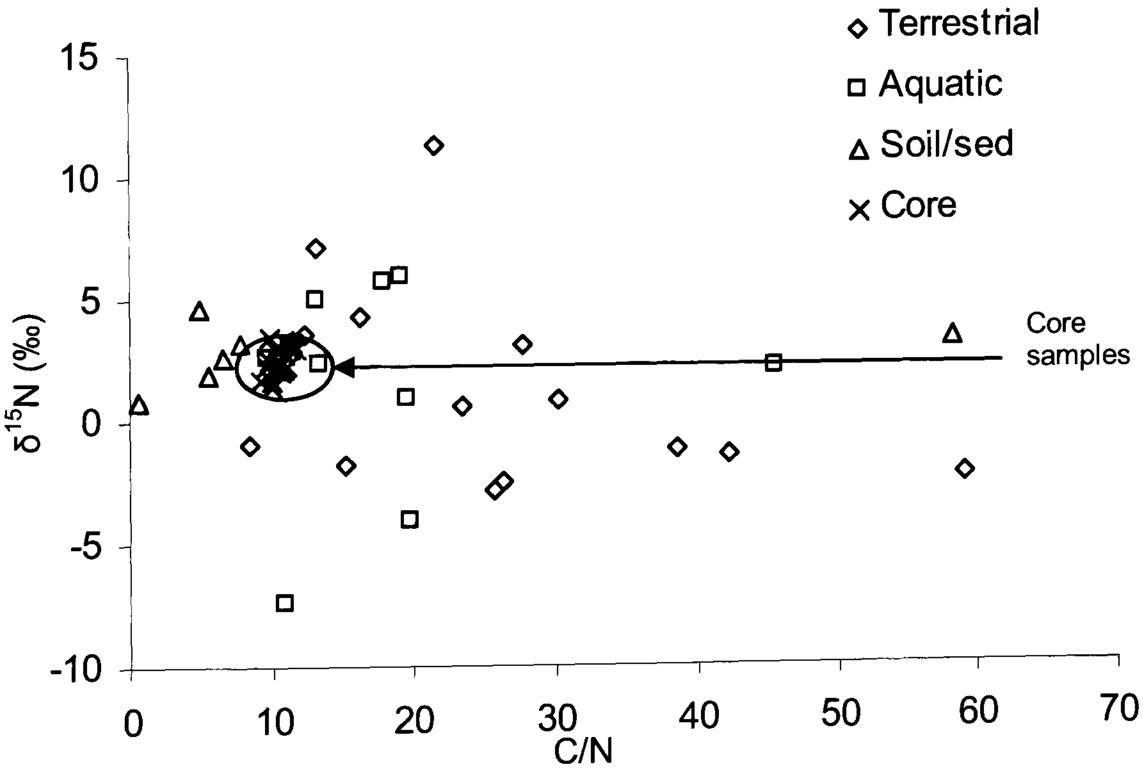


Figure 8.18. C/N vs.$\delta^{15}N$ for core and modern samples

The core sediment samples, which should represent a combination of these sources, sit towards the middle of these data on the $\delta^{13}C$ axis, but are low on the C/N axis. Comparison between the C/N modern data and the core data would seem to indicate sedimentary organic matter of a more aquatic origin. It is only an assumption that the modern plants represent the organic matter in the core and they are aquatic plants that have yielded C/N values of more than 10 while some terrestrial plants have values of less than 20. For example *Montanoa* (woody shrub) and *Solanum nigricans* (shrub found in oak woodland) yielded C/N ratios of 8.4 and 12.2 respectively, when, in general terrestrial plants are considered to have C/N values >20 (Meyers, 1994). Stable nitrogen isotopes in soil organic matter has been recorded between 0 and +8‰ (see Table. 4.1), and values recorded in the Zirahuén basin are therefore within this range. Aquatic macrophytes have been recorded with nitrogen isotope values which are more negative, between -10 and > 0% (Talbot, 2001), which contrasts with the values reported by Peterson & Fry (1987) who report values between -4 and +4‰, and samples therefore lie within this latter range. Generally, terrestrial plants and soil have high C/N values and low $\delta^{13}C$ values (relative to aquatic material); this is corroborated by the modern data. These may be associated with either higher or lower $\delta^{15}N$, due to the wide global range of $\delta^{15}N$ (Leng *et al.* 2005).

8.11 Lipid Geochemistry

Analyses of lipid biomarkers were carried out on 16 samples from core AV3/04. Only sterols, phytol, squalene, and n-alkanes were positively identified, through GC-MS analysis. Four compounds with total ion chromatograms resembling those of highly branched isoprenoids were also identified, but there were no published or library matches for these. Complications with the methodology used (see Section 5.7.6.2) arose during quantification; therefore the results are discussed here in terms of patterns of change rather than in terms of actual measured changes.

Figures 8.19 and 8.20 show the gas chromatograms for samples from the top and bottom of the core (0 and 56 cm). The more complex compounds such as sterols and isoprenoids elute towards the end of the run, as do the longer chain compounds, as seen by the sequence of sterols (C_{27}-C_{29}). The chromatograms are very similar, except for the area underneath the chromatogram, and the "hump" rising significantly above the baseline between 30 and 50 minutes. This area is commonly referred to as an unresolved complex mixture (UCM) of compounds, which cannot be separated on the GC (Meyers, 2003). This UCM is usually indicative of petroleum hydrocarbons (Bourbonniere & Meyers, 1996). In the Rochester Basin of Lake Ontario, N. America, Bourbonniere & Meyers (1996) showed an increase in the area of the UCM up core, with a notable increase since 1900, from which they inferred that this was associated with European arrival in the basin and subsequent petroleum hydrocarbon accumulation. In core AV3/04 the opposite seems to be the case, with increased "hump" size down core. As the UCM is a function of oil biodegradation, the increased "hump" size could be related to increased degradation with time, as it seems unlikely that more petroleum would have entered the lake in the 1800s compared to the last 60 years.

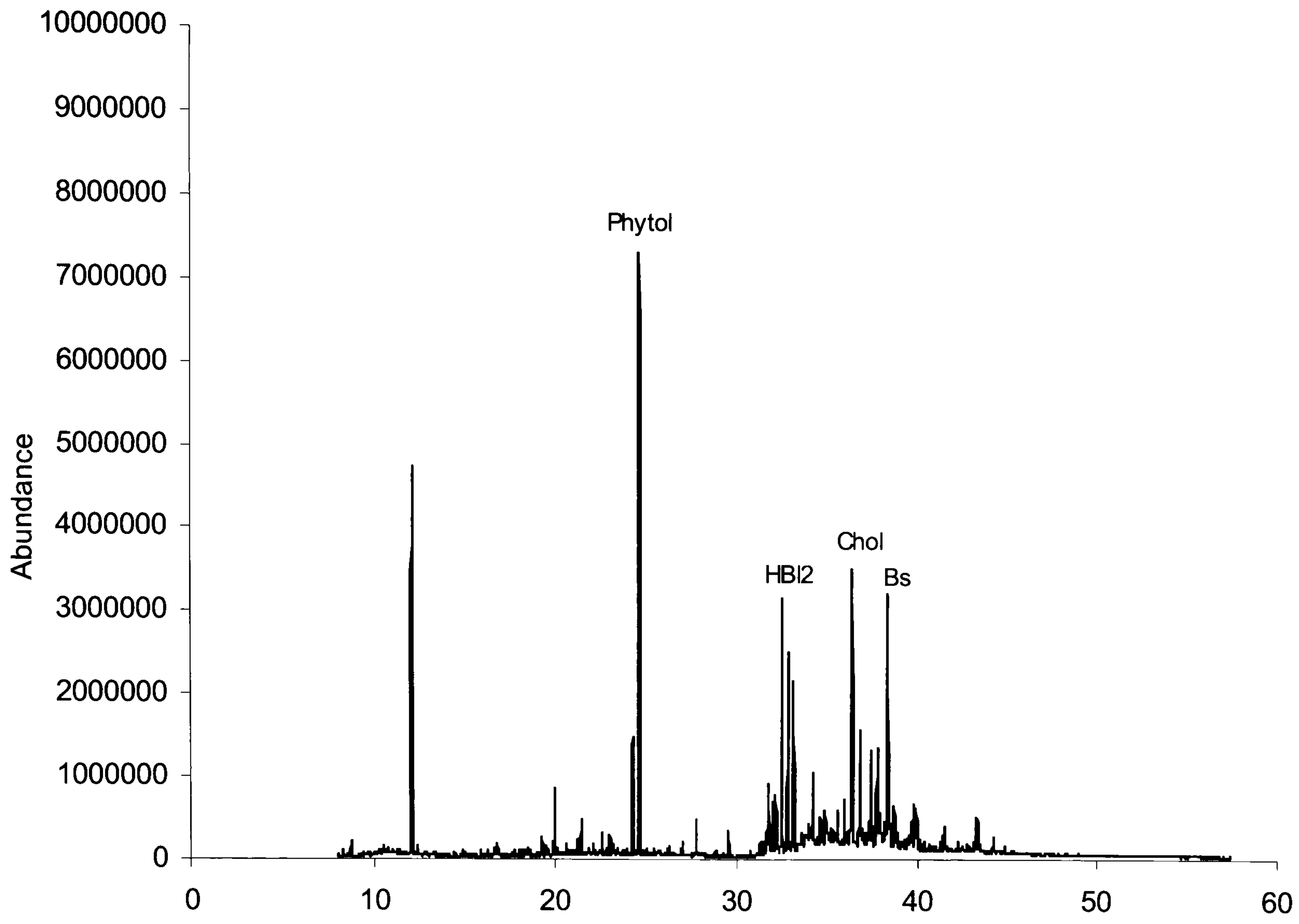


Figure 8.19a Gas chromatogram for 0cm in core AV3/04 (HBI = Highly branched isoprenoid, Chol = cholesterol, Bs = β sitosterol)

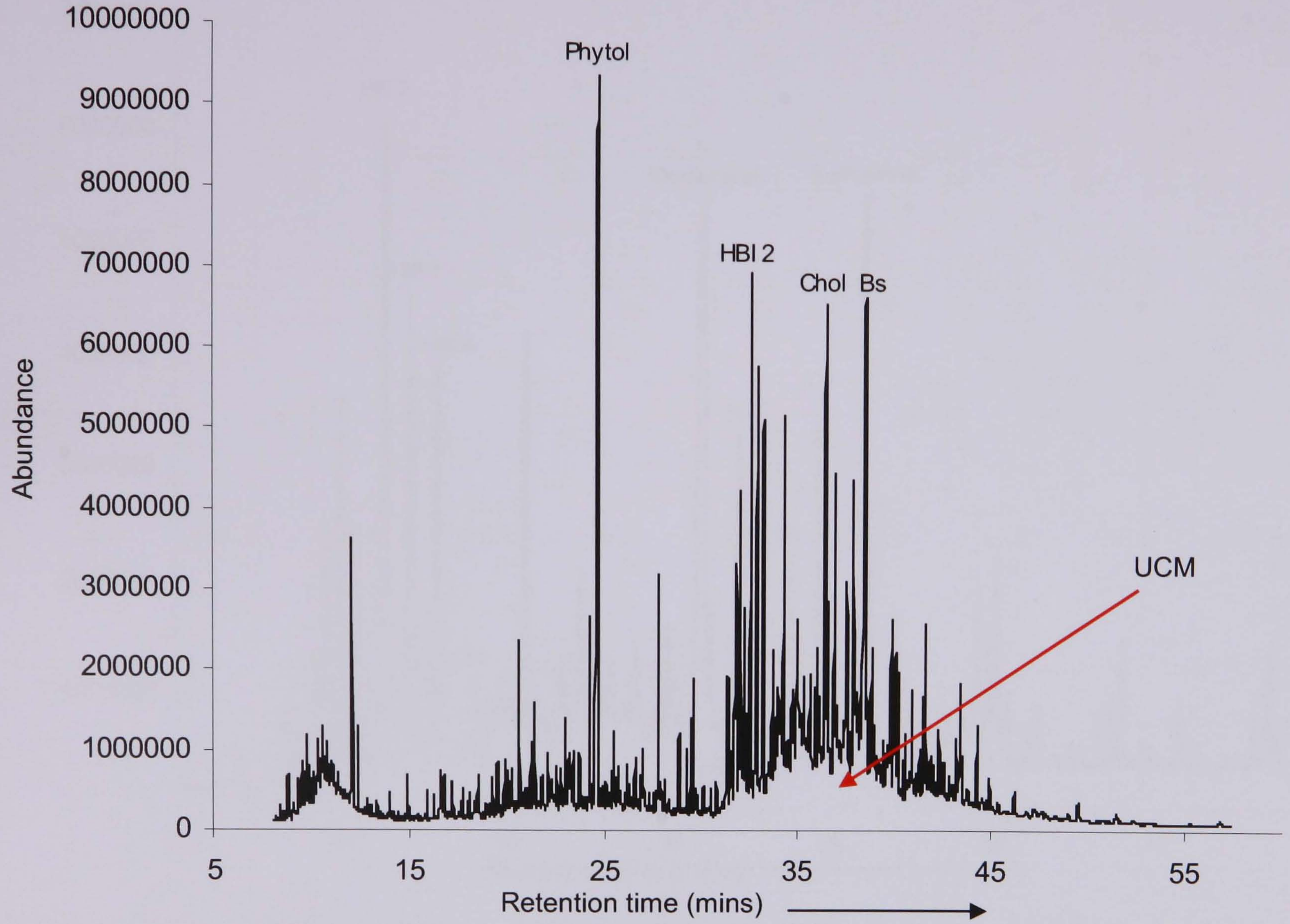


Figure 8.19b Gas Chromatogram for sample 32cm (HBI = Highly branched isoprenoid, Chol = cholesterol, Bs = β sitosterol)

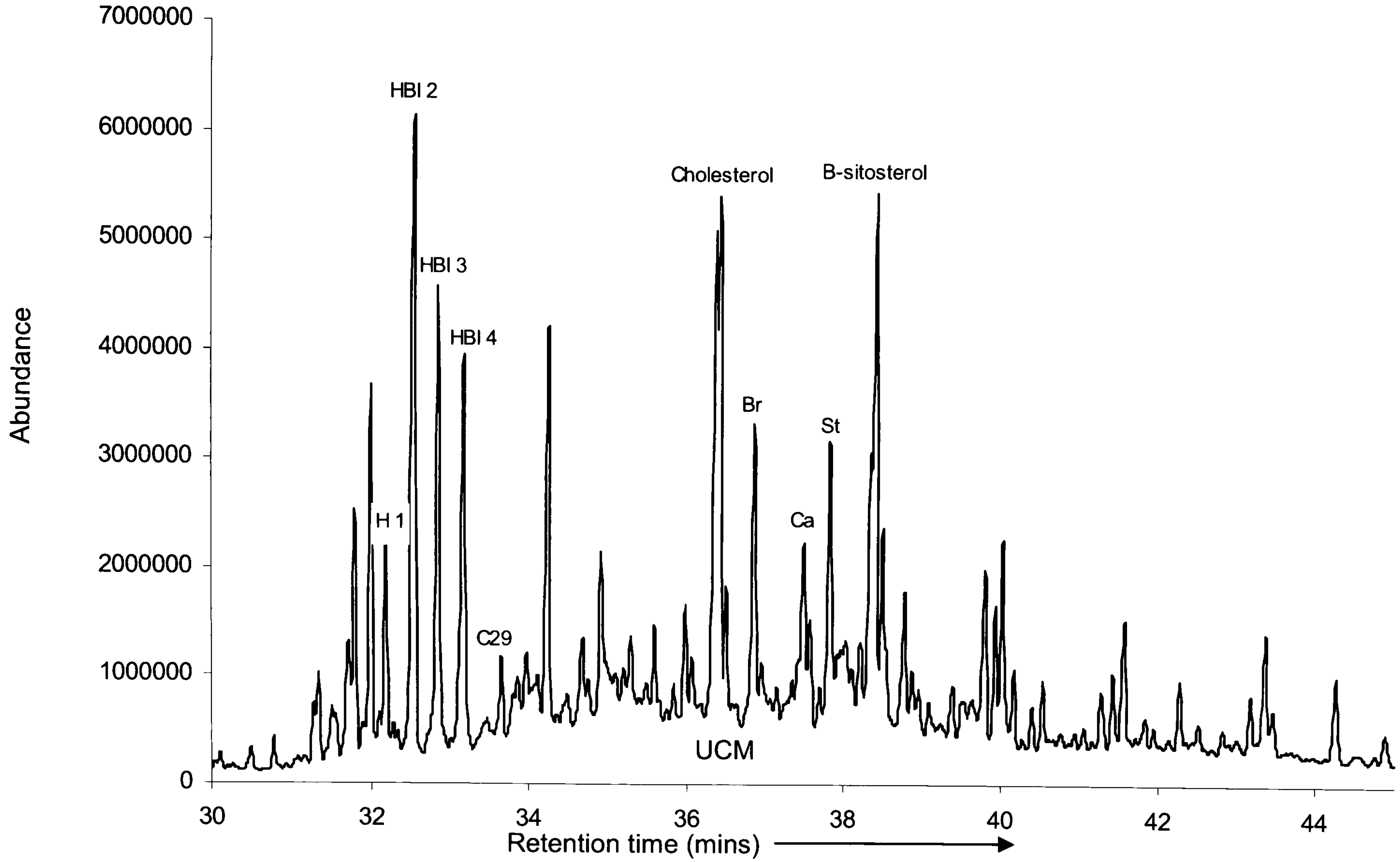


Figure 8.20. Fragmentogram for 30-45 min for sample 56 cm showing sterols and isoprenoids (Br = Brassicasterol, Ca = Campesterol, St = Stigmasterol)

8.11.1. *n*-alkanes

As noted in Chapter 4 short chain *n*-alkanes (C_{15}-C_{19}) are typical of algae; mid chain, C_{21}, C_{23} and C_{25} are indicative of aquatic plant and long chain *n*-alkanes are derived from terrestrial plant matter (see Table 4.2). The C_{17} *n*-alkane is however more widely considered to be indicative of algae (Jaffé *et al.*, 1996), with many studies showing a unimodal distribution of *n*-alkanes, which maximises at the C_{17} homologue. In this study only C_{23} and C_{25} were not positively identified, the results are displayed below in Figure 8.21.

At the base of the core, sometime after 1759, the n-alkanes are dominated by C_{17} and $C_{27,}$ there is a brief switch over at 44 cm when C_{17} becomes dominant, but up to 20 cm C_{27} is the dominant *n*-alkane. All compounds identified increase at 32 cm, with the largest increase experienced in the concentrations of the long chained n-alkanes C_{27}, C_{29} and C_{31}. This shift to more terrestrial material is associated with a decrease in $\delta^{13}C$ and an increase in C/N. After this there is a decline in the concentration of all compounds to 20 cm. At this point both C_{27} and C_{17} decline further, while the remaining identified compounds, increase rapidly to peak at 14 cm, with the exception of C_{23} which increases steadily towards the surface. In the top half of the core C_{17} shows the lowest concentration of all the identified *n*-alkanes, while C_{29} dominates, with the exception of the upper 4 cm where C_{19} has the highest concentration.

Above 10 cm, all compounds show a decline, seemingly in response to the deposition of the Paricutín tephra. The *n*-alkanes C_{19}, C_{23}, C_{29} and C_{31} show a pronounced decrease after 1943 and only begin to increase between 1949 and 1956 (8-6 cm). A similar trend is seen in the C_{17} and C_{27} concentrations decrease and subsequent recoveries are less marked. In the upper 4 cm, approximately the last 27 years, the majority of the *n*-alkanes decrease in concentration, the trend exhibited by C_{29} is particularly marked. C_{19} and C_{27}, however, continue to show an upward trajectory, although at a much lower rate. With increased levels of productivity at the top of the core, based on diatom inferred Chl-a and a decline in C/N and $\delta^{15}N$, dominance by the shorter chain *n*-alkanes would be expected. These trends are only matched by a shift to higher concentrations of C_{19}.

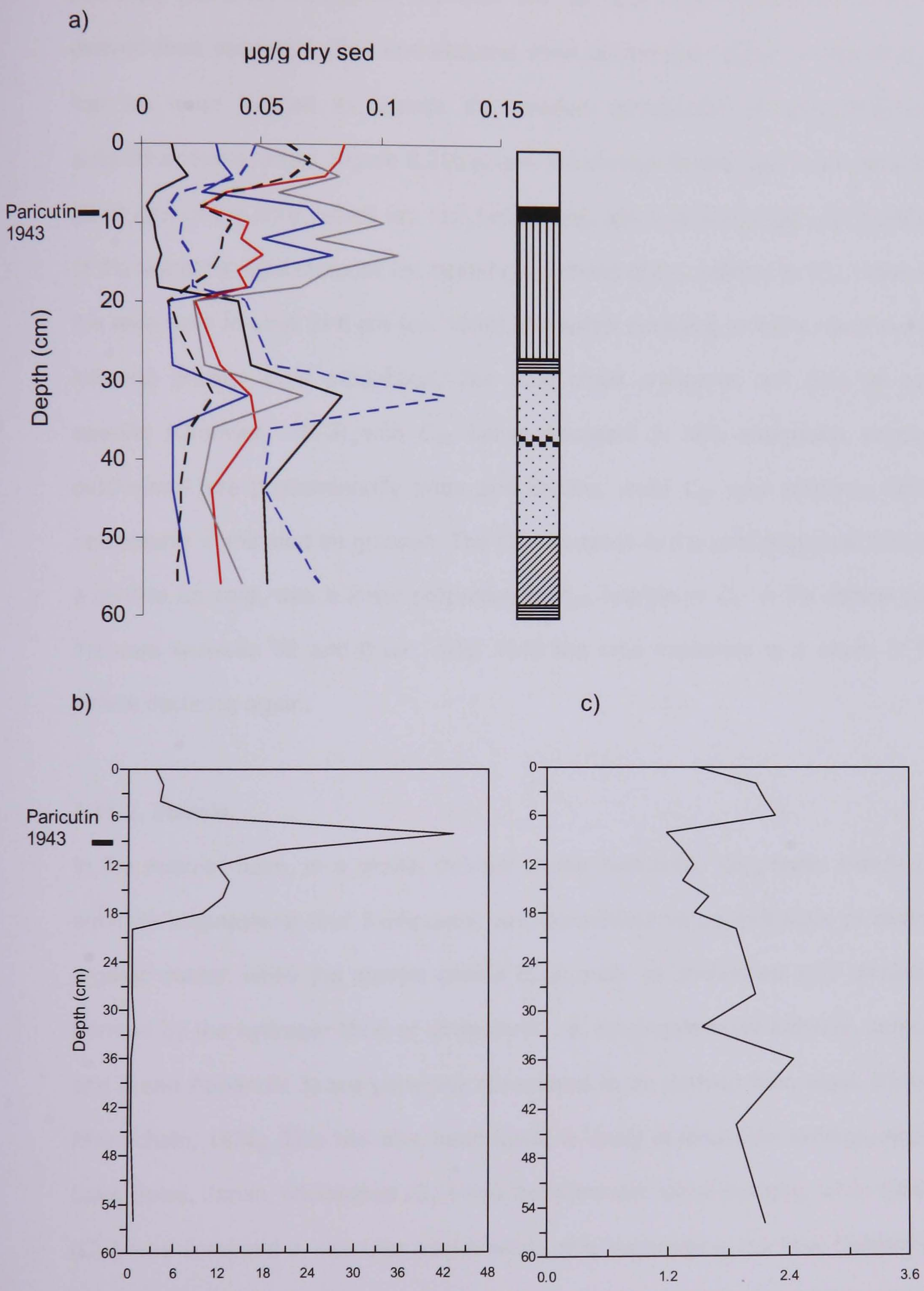


Figure 8.21 a) Stratigraphy of changes in n-alkanes in core AV3/04 (C_{17} = Black, C_{19} = Red, C_{23} = dashed, C_{27} = blue dashed, C_{29} = Grey, C_{31} = Blue) b) Ratio of C_{29}/C_{17} c) Ratio of C_{29}/C_{31}

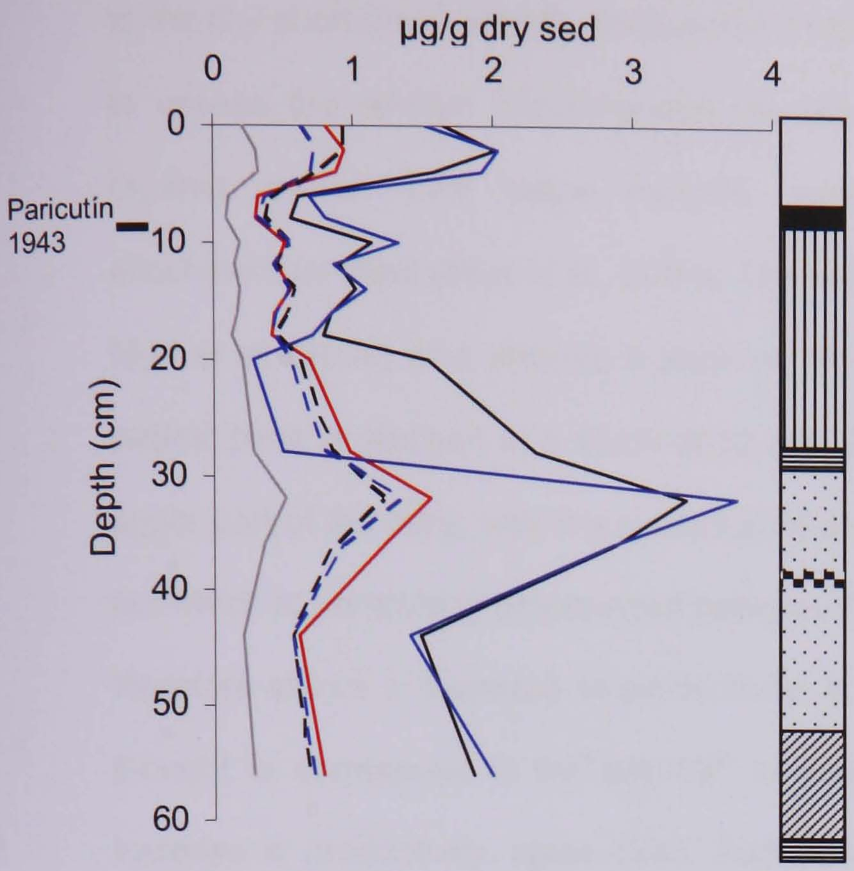
In the absence of positive identification of all *n*-alkanes it was not possible to create a carbon preference index, which would have allowed an assessment of the relative dominance of allochthonous over autochthonous inputs. As C_{29} and C_{17} were positively identified throughout the core and as long chain *n*-alkanes are primarily derived from terrestrial plant material and short chains from algae, a ratio of C_{29}/C_{17} can be used instead to assess the relative contribution of allochthonous vs. autochthonous sources. Figure 8.21b shows the change in this ratio down core. In the lower part of the core values are low, below one, which is consistent with dominance of the signal by algal material, i.e. higher proportions of C_{17}, relative to C_{29}. From 18cm the ratio rises to peak at 8 cm (ca. 1949), thereafter declining to the surface sediment inferring greater algal production. The long chain *n*-alkanes can also be source specific (Cranwell, 1973) with C_{29} being dominant in lake sediments where the catchments are predominantly trees and shrubs, while C_{31} was primarily found in catchments dominated by grasses. The C_{29}/C_{31} ratios in the core (Figure 8.21c) show a decline up core, with a lower proportion of C_{29}, relative to C_{31} in the central part of the core between 32 and 8 cm. After 1943 the ratio increases to a depth of 6 cm before declining again.

8.11.2. Sterols

In the marine realm, in a similar manner to the *n*-alkanes, long chain sterols (C_{29}), such as stigmasterol and β-sitosterol, are considered to be indicative of terrestrial organic matter, while the shorter chains (C_{27}) such as cholesterol and cholestanol, (formed by the hydrogenation of cholesterol, i.e. no double bond between carbons 5 and 6 see Appendix 3) are generally considered to be derived from algae (Huang & Meinschein, 1976). This has also been found to apply to lacustrine settings, where in Lake Suwa, Japan, cholesterol (C_{27}) was the dominant sterol in algae, while sitosterol (C_{29}) was dominant in vascular plants within and surrounding the lake (Nishimura & Koyama, 1977). Long chain sterols have also been identified in algae (Volkman *et al.*, 1986a). Brassicasterol (C_{28}) has been found to be indicative of diatoms, and is often referred to as diatom sterol (Volkman *et al.*, 1986), but has also been found in higher plants (Nichols *et al.*, 1990). Campesterol (C_{28}), stigmasterol (C_{29}) and sitosterol (C_{29}) are commonly considered to be derived from terrestrial plant sources (Huang & Meinschein, 1976; Volkman, 1986; see Table 4.2). In the East China sea campesterol was found to be indicative of dinoflagellates and diatoms (Jeng & Huh, 2004), in addition stigmasterol has been found to be the dominant sterol in *Amphora coffaeaformis*, while sitosterol is the dominant sterol in *Navicula pelliculosa* (Gladu *et al.*, 1991). Although these species of diatom were not identified in the core material from Laguna Zirahuén, this does indicate that long chain sterols cannot necessarily be interpreted as solely being derived from terrestrial sources. Therefore in terms of interpreting trends in sterols this section will focus on general trends rather than on individual sterols. The results are displayed below in Figure 8.22.

Cholesterol and β-sitosterol are the most abundant sterols throughout the majority of the core, while cholestanol is present in the lowest concentrations (Figure 8.22a), this is evident from the chromatography through peak area and peak height. At the bottom of the core the trends exhibited by β-sitosterol and cholesterol are very similar, with a similar trend shown between stigmasterol, campesterol and brassicasterol, but at lower concentrations. In a similar pattern to the *n*-alkanes, the sterols peak at a depth of 32 cm. Above this depth there is a divergence in the β-sitosterol and cholesterol profiles, with β-sitosterol showing a rapid and abrupt decrease in concentration to reach lower concentrations below those displayed by campesterol, brassicasterol and stigmasterol. Cholesterol also decreases in concentration between 32 and 18 cm but the magnitude of change is less marked. From 18cm the trends in both compounds are well matched. Since 1943 all sterols show an increase in concentration, with a particularly rapid increase between 6 and 2 cm (ca. 1964-1991). In the upper, most recent, sediment, with the exception of campesterol, all sterols decline in concentration.

a)



b)

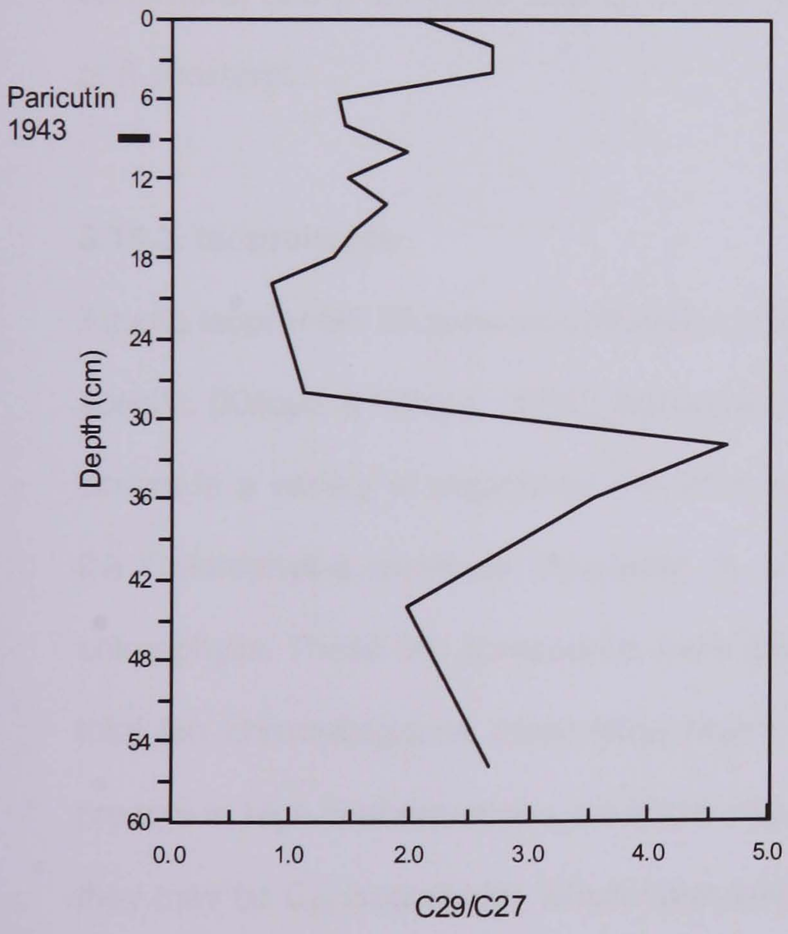


Figure 8.22 a) Stratigraphic changes in sterols (cholesterol = black, cholestanol = grey, brassicasterol = red, campesterol = dashed, stigmasterol = blue dashed, B-sitosterol = blue) b) Ratio of long (C_{29} – β-sitosterol + sitgmasterol) to short chain (C_{27} – cholesterol + cholestanol) sterols.

Figure 8.22b shows the ratio of the long chain C_{29} sterols, β-sitosterol + stigmasterol to the C_{27} short chain sterols, cholesterol + cholestanol, which this can again be used to assess the relative allochthonous vs. autochthonous contributions to lacustrine organic matter. Low ratios indicate autochthonous input and higher ratios allochthonous input (Muri *et al.*, 2004). The ratio of C_{29}/C_{27} is generally low, relative to Muri *et al.* (2004) who showed a peak of 10, and ranges between 0.9 and 4.7. The largest peak is reached at a depth of 32 cm. Values are generally low throughout the upper part of the core, with the exception of a slight shift to higher values from 20 cm, but which is particularly pronounced between 6 and 2 cm (ca. 1964-1992). The record therefore shows a decrease in productivity, in the central part of the record, roughly thought to correspond to the late 19th to early 20th century. This is followed by an increase in productivity, since 1943. Furthermore this is in line with the inferenc4es made based on the change in the other proxy data. The correspondence between cholesterol and β-sitosterol seems, in this case, to indicate a more ubiquitous source of β-sitosterol.

8.11.3. Isoprenoids

Acyclic isoprenoid alkanes and alkenes occur widely in organisms, but can be source specific (Killops & Killops, 2005). Squalene is a C_{30} polyunsaturated isoprenoid which occurs in a variety of organisms. Phytol is an acyclic diterpenoid which forms part of the Chlorophyll-a molecule (Appendix 3), but also forms part of a number of other chlorophylls. These two compounds were positively identified, while 4 compounds with total ion chromatograms resembling highly branched isoprenoids (HBIs) were also present in high concentrations. As these HBIs elute prior to squalene, but after phytol, they may be C_{25} isoprenoids which have been identified having their major sources in marine diatom species (Gomes & Azevedo, 2003), however they are not as widespread in lacustrine environments as marine environments (Belt *et al.*, 2001). The results can be seen below in Figure 8.23.

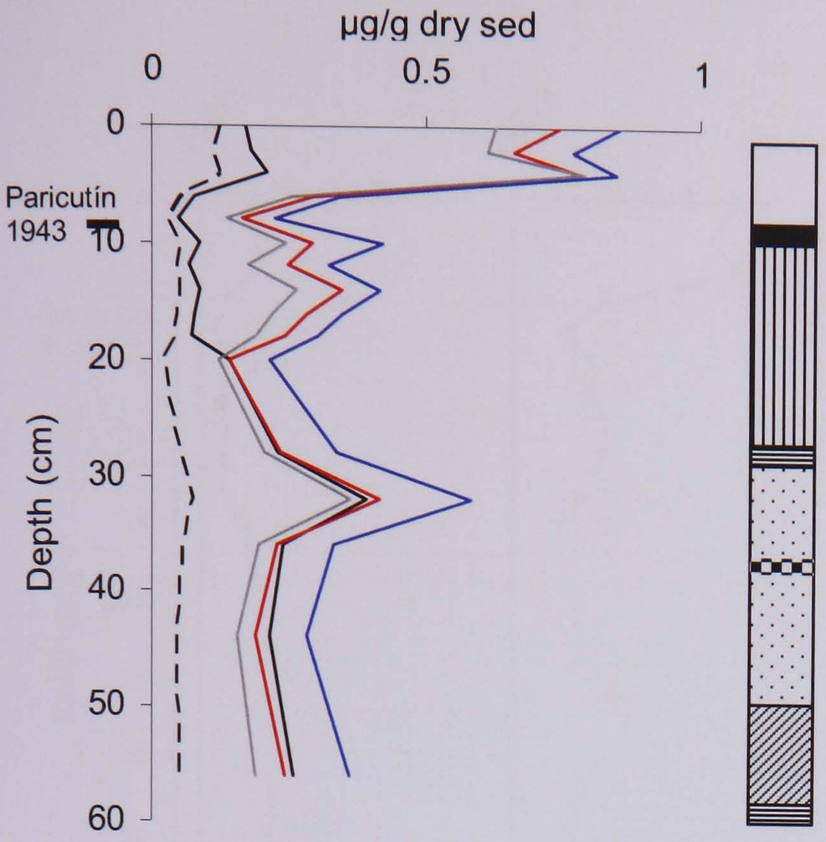


Figure 8.23 Highly Branched Isoprenoids (HBI 1 = black, HBI 2 = blue, HBI 3 = red, HBI 4 = Grey, Squalene = dashed)

Highly branched isoprenoid 2 is the dominant compound throughout the sequence, while the concentration of squalene is generally low. At the base of the core (56-20 cm) the unidentified HBIs and squalene show the same trend, exhibiting a peak at 32 cm. This is followed by a decrease to 20 cm above which HBIs 2, 3 and 4 show an overall increase towards the surface, with a particularly rapid rise in concentration noted from 8-4 cm (ca. 1949-1977). From 20 cm HBI 1 diverges slightly from the other analytes with a further decline in concentration to 18 cm. To a depth of 6 cm (ca 1964), there is little change in the concentration of HBI 1, after which the pattern of change is similar to the other HBIs, but it never recovers similar concentrations. The presence of HBIs, as they have been reported as being synthesized by diatoms in marine sediments and may be expected to show a similar response to valve concentration. The relationship between the isoprenoids and diatom valve concentration at the bottom of the core (Diatom zones 1 and 2), however, shows a negative correlation (Figure 8.24). In diatom zone 3 there is a greater degree of correlation between change in the concentration of HBI 3 and valve concentration, although in the upper most zone the trends diverge again, with the exception of the upper 2cm, representing the last 12 years.

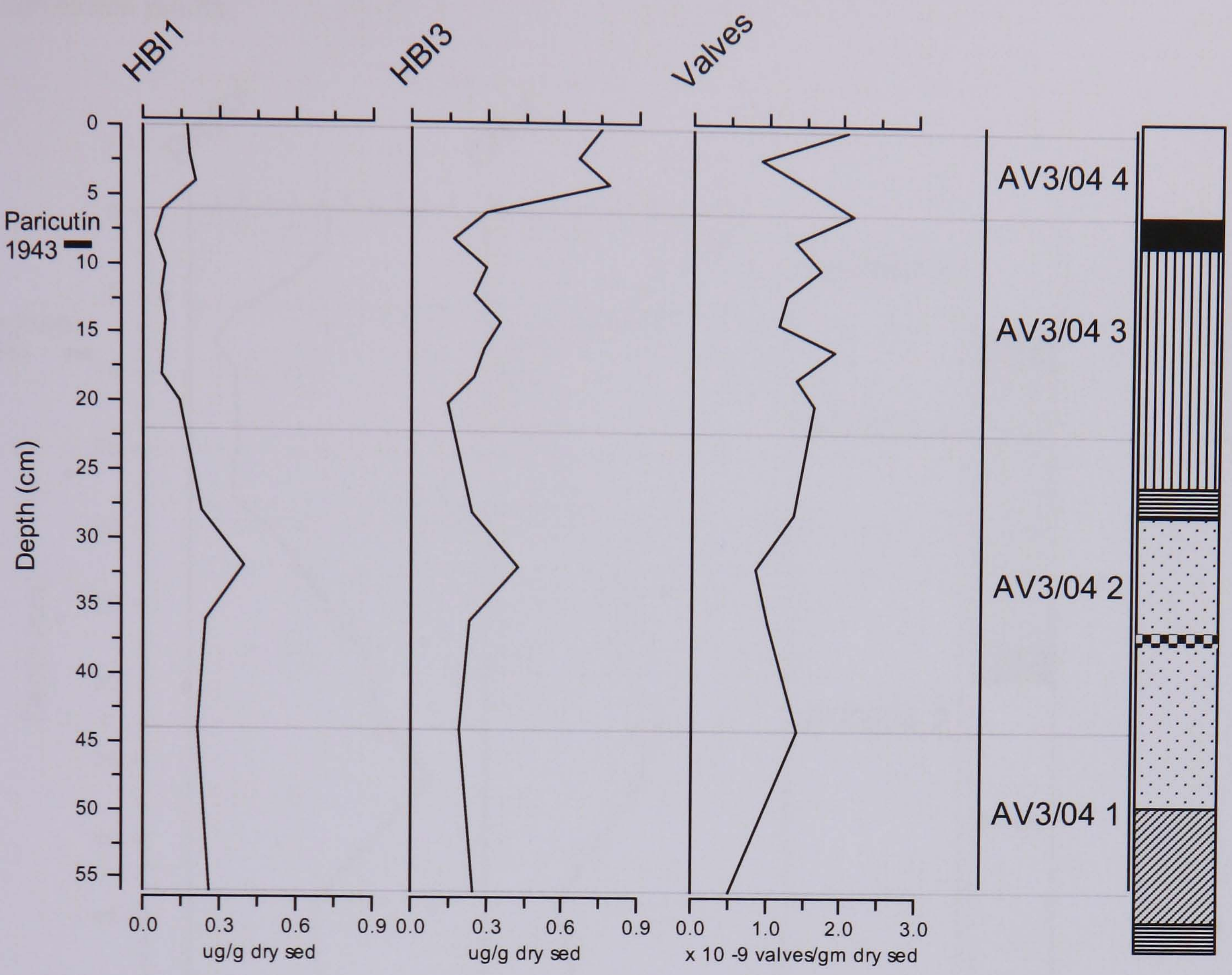


Figure 8.24 Comparison between HBIs and diatom valve concentration.

As phytol is a chlorophyll-a derivative a relationship between phytol and diatom inferred Chl-a may be expected. As seen in Figure 8.25 there is a degree of similarity in the two proxies. Both phytol and diatom inferred Chl-a increase from the bottom to the middle of Zone AV3/04-2. From 36cm phytol concentration begins to decline, prior to diatom inferred Chl-a (DI-Chla), which declines between 32 and 28 cm. After 28 cm Chl-a shows minimal change to the top of the zone. In zone AV3/04 3 diatom inferred Chl-a decreases from 12.6 to 8.8 µg l^{-1} at 8 cm (1943), while there is minimal change in phytol concentration until 10 cm where it seems to show a decline, possibly associated with the deposition of the Paricutín tephra. Both proxies show an increase from 1943 through zone AV3/04 4 to the top of the core. Phytol therefore seems to provide an independent proxy for algal productivity and the trajectory of change shown by this proxy adds further support to a period of decreasing, then increasing productivity, and therefore nutrient availability. It should be noted, that phytol will also be representative of other sources of Chl-a, such as aquatic macrophytes and terrestrial plants.

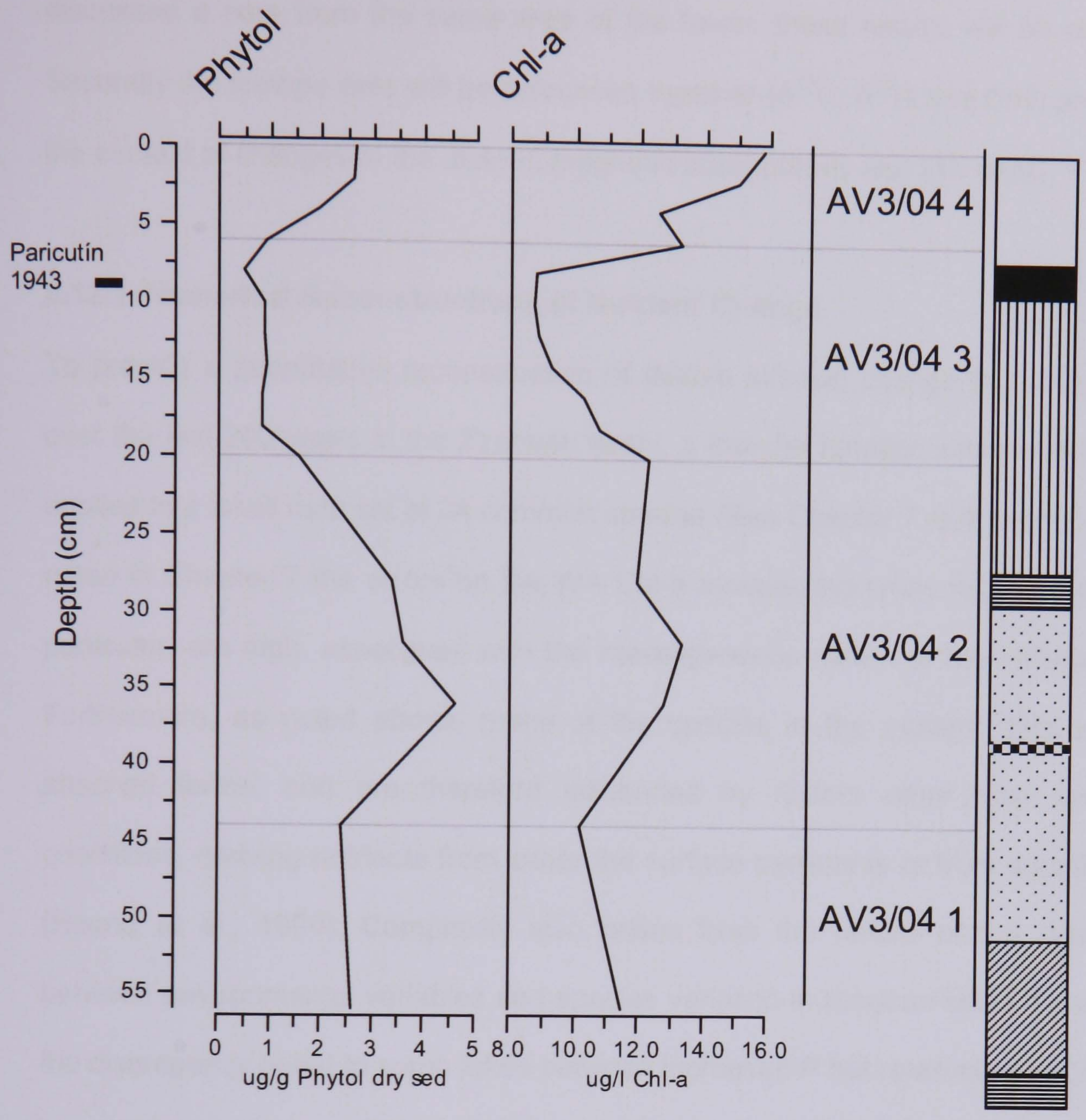


Figure 8.25 Comparison between phytol and diatom inferred Chl-a (simple WA)

8.12. Synthesis of Sedimentary Proxy Data

The sedimentary records of environmental change in Laguna Zirahuén collected in this study are presented, individually, above. This section discusses the implications of those data independently and then draws together the data to draw some overall conclusions about what the data, as a whole, indicate in terms of changes in nutrient status in Laguna Zirahuén over the last ca. 200 years. As diatoms in a core from Agua Verde and central Mexican lakes as a whole have been discussed elsewhere in relation to alkalinity, pH and conductivity (Davies *et al.*, 2002; Davies *et al.*, 2004) the discussion here will focus upon the implication of the species assemblage in terms of nutrient status. The diatom stratigraphy and the implications of the diatom based chlorophyll-a (Chl-a) reconstruction are discussed first. In addition, as Davies (2000) discussed a core from the same area of the basin, these results will be compared. Secondly the isotope data will be discussed together ($\delta^{13}C$, $\delta^{15}N$ and C/N) and then in the context of changes in the diatom, magnetic susceptibility and LOI data.

8.12.1. Numerical Reconstructions of Nutrient Change

To provide a quantitative reconstruction of diatom inferred change in nutrient status over the last 200 years in the Zirahuén basin, a transfer function was developed and applied to a fossil data set of 24 common species (See Chapter 7 and Section 8.9). As noted in Chapter 7 the errors on the WA Chl-a models, the cross validated model in particular, are high, associated with the heterogeneous nature of the calibration set. Furthermore, as noted above, many of the species in the modern data set were attached forms, and are therefore influenced by factors other than epilimnetic conditions, deriving nutrients from either the surface sediments or from aquatic plants (Round *et al.*, 1990). Complexity also arises from the nature of the relationship between environmental variables and species variation in Mexican lakes, for example the discrepancy noted in some lakes between increased P but relatively low Chl-a and the presence of N limitation of N/P co-limitation. Consequently the diatom inferred change in Chl-a and therefore nutrient availability, should be interpreted with caution.

Contemporary measurements of Chl-a are much lower than the inferred in the upper sediments by the models presented above. In this study mean annual Chl-a, for the main part of the lake, was recorded as 1.7 $\mu g\ l^{-1}$. Chacon-Torres & Rosas-Monge (1998) recorded a higher mean concentration of 3.8 $\mu g\ l^{-1}$ between 1989 and 1994, but note that the Agua Verde sub basin has yielded Chl-a concentrations of around 5.2 $\mu g\ l^{-1}$ as it is sheltered and therefore allows a greater level of primary productivity. Such values are lower than inferred values for the equivalent time periods, around 15 $\mu g\ l^{-1}$, which may be related to the lack of modern analogues. In core AV3/04, 37% of the most common species (> 1% abundance) were not found in the modern calibration data set. In addition some of the species identified in the fossil data set were present in low abundances or at few sites in the modern training set. For example *Cymbella descripta* was only identified in three of the modern samples, San Gregorio, La Alberca (Tacambaro) and Zempoala, and was only present at one site in an abundance greater than 1% (San Gregorio). Consequently problems arise over the assignment of accurate optima and tolerances for such species. Problems with accurate inferences also arise from the dominance of the fossil data set by *Fragilaria* species, which are known to have wide nutrient tolerance ranges (Bennion, 1994; Sayer, 2001). The core was taken in a location where the whole water column and the surface sediments were in the photic zone, allowing the proliferation of benthic species. Consequently it was not been possible to create a robust quantitative diatom based reconstruction of nutrient changes in Laguna Zirahuén over the last ~200 years, as they tend to not work as well with benthic species, which derive their nutrients from multiple sources. Both the CCA and WA reconstructions indicate a rise in primary productivity in the last 60 years, with a steeper increase noted in the last ca. 30 years. This offers support to the results of Davies *et al.* (2004), and limnological monitoring (Bernal-Brooks & MacCrimmon, 2000b) which, as discussed, above suggest a shift to higher nutrient availability over approximately the last 20 years.

The application of the transfer function to Davies' (2000) sediment core indicated a very different change in nutrient availability over the length of the core compared to this study. The abrupt increase in the abundance of *C. ocellata* and the appearance of *F. crotonensis* apparently correspond to a decline in diatom inferred Chl-a and then a stabilisation of concentration. The lower inferred Chl-a is associated with the low species optima and tolerances assigned to *C. ocellata* and *F. crotonensis* based on the calibration set, which are only derived from a few sites, and are therefore not necessarily accurate. This contrasts with the inference made by Davies (2000) based on published auto-ecological evidence and with contemporary measurements of changes in primary productivity over the last ca. 20 years (Bernal-Brooks & MacCrimmon 2000b). The inferred Chl-a concentrations from Davies core do, however, predict concentrations which are more in line with contemporary measured values. The general trajectory of change in diatom inferred nutrient status from this study appears to match other published data, it has not been possible to create a reliable numerical reconstruction of that change.

8.12.2. Statistical Analysis of Palaeoenvironmental Data

The results of a CCA with fossil diatoms as species data and other proxies as environmental data (Figure 8.26) indicate a strong negative relationship between $\delta^{13}C$ and C/N (r^2 = -0.98). Strong positive relationships are noted between the C_{17} with cholesterol, β-sitosterol and phytol (r^2 = 0.8-0.9). As seen from the biplot, increased C_{17} is related to decreasing χ_{lf}, C/N and long chain n-alkanes. Long chain *n*-alkanes are derived from similar sources, i.e. terrestrial plants, therefore an increase in C_{31} may be expected to be matched by a similar increase in C_{29}, the results show a strong positive relationship between the two, with a high correlation coefficient (r^2 = 0.97). As noted in Section 8.11.2 (Figure 8.23) the trends in cholesterol and β-sitosterol were very similar (r^2 = 0.92, which is contrary to the assertion that the latter is derived from higher plants, and provides an indication that β-sitosterol may have a more ubiquitous origin, in this case, possibly algal. Together the other proxy data do however, show indicate similar changes to the diatom flora.

The results indicate that diatom Zone 3 (AV3/04 3) is characterised by higher inputs of catchment material (increasing magnetic susceptibility); higher C/N values, indicating a mixed terrestrial/aquatic signal and increasing long chain *n*-alkanes, indicative of terrestrial plant matter input. This zone also sits low on the Chl-a axis indicating a period of lower primary productivity. Furthermore the lower, more negative, values of $\delta^{13}C$, and a lower concentration of phytol, sterols and the short chain *n*-alkanes, indicate further a relative decline in aquatic productivity. The results show three clear outliers, the samples from 2, 4 and 6 cm, associated with their high concentrations of the C_{19} *n*-alkane. The other samples are generally well spread across both axes. The CCA biplot also highlights the similarity between the central area of the bottom zone, 56-48 cm, (AV3/04 1) with the top of the core (AV3/04 4), inferring higher nutrient availability at these times. The plot also indicates that the sample from 9 cm, corresponding to the deposition of the Paricutín tephra, is the point at which inferred algal productivity is lowest.

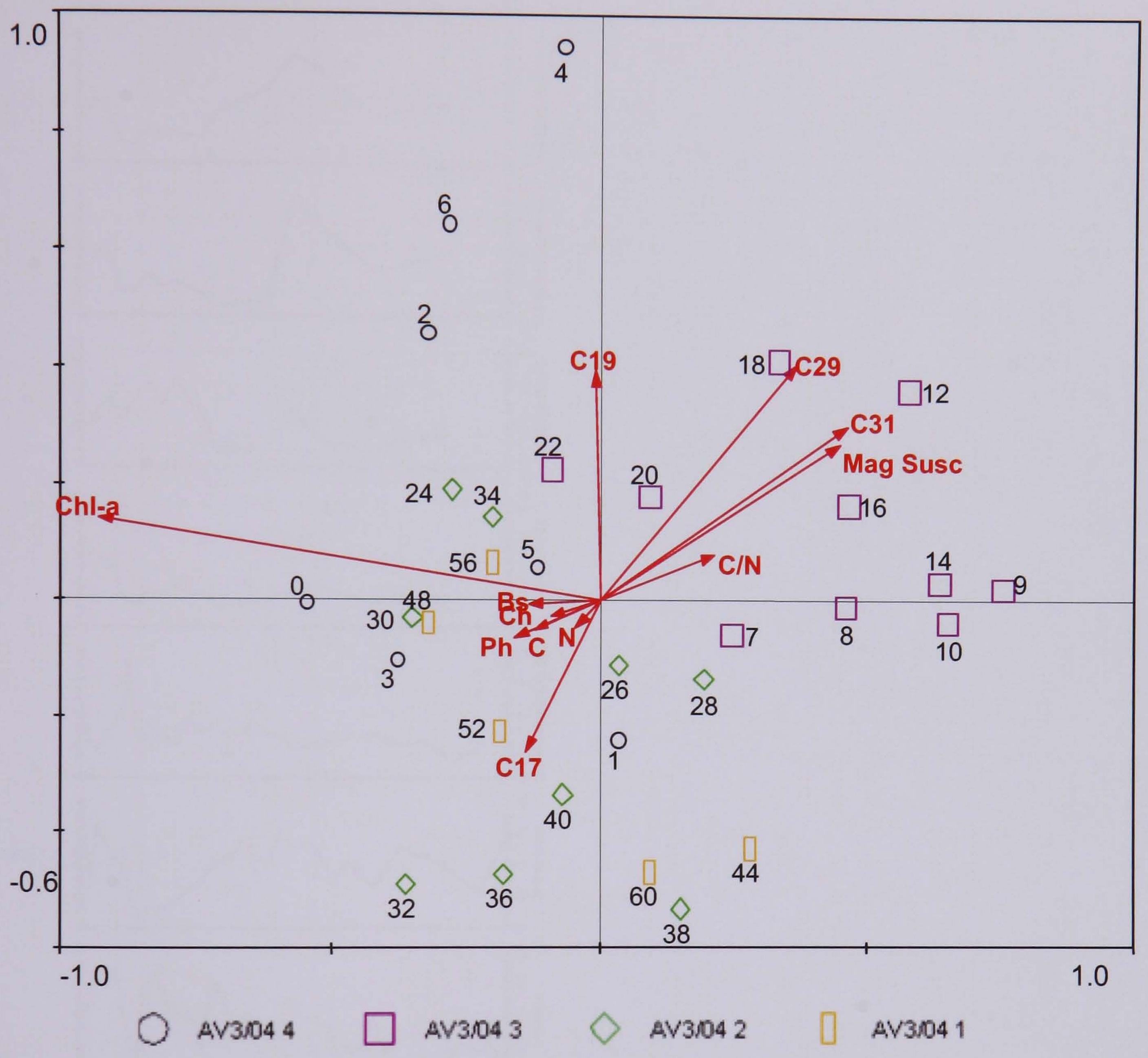
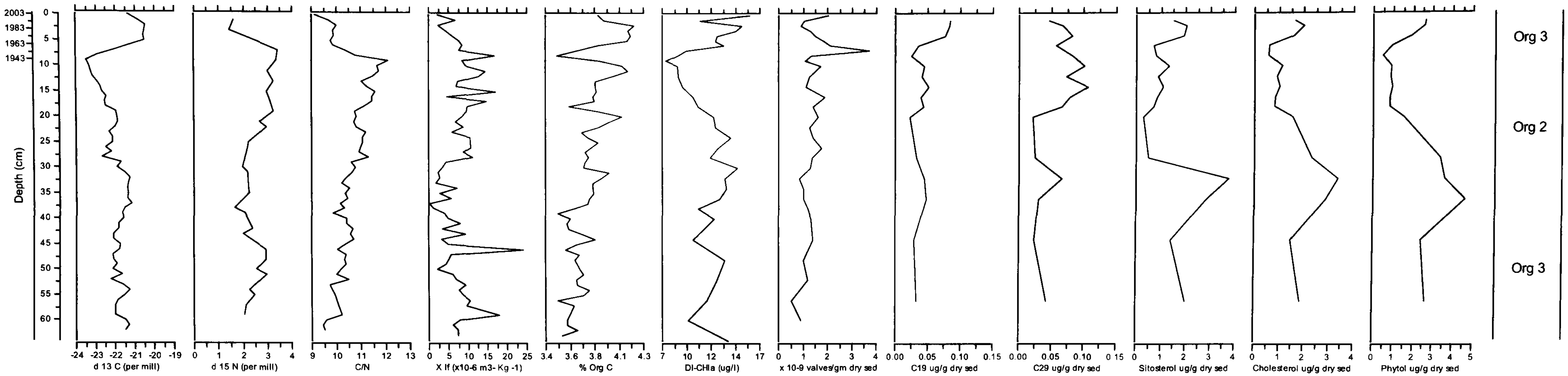


Figure 8.26. CCA plot of fossil species and environmental data (Ph = phytol, Bs = β-sitosterol, Ch = Cholesterol, C = δ^{13}C, N = δ^{15})

Figure 8.27 Composite stratigraphic plot of main proxies



8.12.3. Sequence of Nutrient Changes

Figure 8.27 shows some of the main proxy data obtained during this study, constrained by organic zones, for ease of comparison. Shifts in isotopic composition, lipid biomarkers and diatom assemblages together can be used together to interpret changes in nutrient input into Laguna Zirahuén. The results are summarised and presented diagrammatically in Figure 8.28

Depth	Diatoms	Sedimentary evidence	Environmental Interpretation	Human Activity
0-2cm	Increased plankton epiphytes, (*C. stelligera* & *G. gracile*), decreased *F. pinnata*	Low χ_{lf} decreasing $\delta^{13}C$	Low catchment input. Increased algal productivity & possibly cyanobacterial signal due N limitation due to higher P.	Increased population and intensive commercial agriculture with growing tourist industry
7-2 cm	High *A. minutissima* & *F. construens*	Decreasing χ_{lf} Minimal change in $\delta^{13}C$ & C/N		
9-7 cm (Diatom Zone 3)	Highest abundance of *F brevistriata* and *F. pinnata* Increased *F. construens*	High χ_{lf} Increasing $\delta^{13}C$ Decreasing $\delta^{15}N$ & C/N	Recovery after deposition of tephra, and increasing aquatic productivity.	Increasing population
20-7cm (Diatom Zone 3)	Increasing *F. pinnata*, high *F brevistriata*	Increased C/N, $\delta^{15}N$, long chain *n*-alkanes & χ_{lf} with decreasing $\delta^{13}C$ phytol, short chain *n*-alkanes & sterols	Higher terrigenous inputs. Decreasing nutrients and lower primary productivity	Decline of smelting industry (decreased population?)
44-20 cm (Diatom Zone 2)	*A. minutissima, C descripta* Increased facultative planktonic species to mid zone followed by brief decline then recovery	χ_{lf}, $\delta^{15}N$ decline to mid zone then increase. Minimal change in C/N Increase then decrease in $\delta^{13}C$	Slightly higher levels of Chl-a, higher nutrient availability	Copper smelting at Santa Clara del Cobre? Development of haciendas?
64-44 cm (Diatom Zone 1)	*A. minutissima*, *F. brevistriata*, *F construens*, *F pinnata*	Low C/N, increasing slightly Increased $\delta^{15}N$ Decreasing χ_{lf} Minimal $\delta^{13}C$ change	Initially higher levels of productivity, but declining mid-zone Lower levels of terrigenous input	

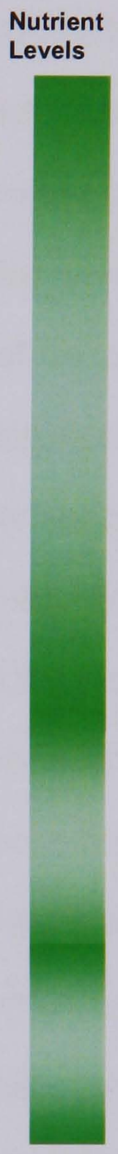


Figure 8.28 Sequence of change in nutrient availability at Laguna Zirahuén

Together proxy data seem to indicate minimal change in availability of nutrients at the base of the core, in Diatom Zones AV3/04 1, corresponding to lower Organic Zone 1 (64-35 cm). Within this zone (AV3/04 1) the decline in χ_{lf}, is interpreted as reduction in detrital allochthonous input from the catchment, but this corresponds to a slight increase in the C/N ratio between 64 and 44 cm which would indicate an increase in the contribution of vascular plants to the organic matter. This period is matched by a decline in the long chain *n*-alkanes, but a small increase in the concentration of the

C_{23} *n*-alkane (see also Table 4.2). This could imply an increase in the contribution of aquatic macrophytes (Ficken *et al.*, 2000), rather than terrestrial plants, to the organic matter. Furthermore this is supported by the proportion of epiphytic diatom species. Despite the decline in "traditional" algal biomarkers DI-Chla increases from 60 cm to 44 cm.

In Diatom Zone AV3/04 2a, upper part of Organic Zone 1 (44-35 cm), DI-Chla, $\delta^{13}C$ and phytol show a slight increase, and together are interpreted as a slight increase in aquatic productivity, and therefore increase in nutrient availability. Increased algal abundance is supported by an increase in abundance of the sterols and an increase in the short chain *n*-alkanes (see Figure 8.27). The approximate corresponding time period covered by this part of the core is post 1764, based on the absence of the of the Jorullo tephra, and may therefore correspond to the impact of the development of the copper smelting industry in the basin. There is also a rise in the concentration of the long chain *n*-alkanes and $\delta^{15}N$ values are more in the range of aquatic macrophytes. The signal between 44 cm and 35 cm may therefore represent a more mixed signal, but with an increased abundance of aquatic macrophytes, which would also account for the rise in the number of epiphytic diatom species. Further more this zone (AV3/04 2a) roughly corresponds to the zone in core AV/98, taken by Davies *et al.* (2004), that also shows an increase in epiphytic species. This is also supported by the continued rise in the concentration of C_{23}. In addition a number of prolonged droughts have been recorded in the historical documents, occurring during the 1700s continuing until the 1820s. These droughts were particularly severe during the mid to late 1700s (Endfield & O'Hara, 1997, O'Hara & Metcalfe, 1995; 1997). This has been noted by Davies *et al.* (2004) who interpret changes in the diatom stratigraphy from the mid to late 1700s as an expansion of marsh area consequently promoting an increase in epiphytic diatom species abundance though lake level lowering associated with a drier climate. Expansion of the marsh area may also have acted as a buffer zone, trapping any terrigenous input. The decline in terrigenous organic matter may also be related to a reduction in precipitation and therefore a reduction in run-off.

Diatom Zones AV3/04 2b-3, corresponding to Organic Zone 2 (between 35 and 9cm) are thought to correspond to the late 19th early 20th Centuries, up to ca. 1943. These zones show quite marked changes relative to the zones below. Throughout AV3/042b – AV3/04 3 (Organic Zone 2) there is a shift in the diatom inferred Chl-a towards lower productivity and therefore nutrient availability and higher $\delta^{15}N$, magnetic susceptibility and C/N, with lower, more negative, $\delta^{13}C$ indicate an organic matter signal more indicative of reduced aquatic productivity. The trends established in the other proxy data are maintained to 9 cm (1943) with a continued decrease in $\delta^{13}C$ and increases in C/N and $\delta^{15}N$. The increasing trend in the magnetic susceptibility signal corresponds to a period where the sediment contained a number of small stones, inferring catchment disturbance and allochthonous input. This is probably coincident during a time of increased catchment disturbance beginning in the 18th century, which has been maintained to the present day (Davies, 2000; Endfield & O'Hara, 1997). The C_{27} and the C_{17} show a progressive decrease up the core to 9 cm, while above 20 cm the other *n*-alkanes show an increase towards the surface sediment. The decline in the C_{27} *n*-alkane may be related to a reduction in organic matter contributed by trees, from which this compound is thought to be derived (Brincat *et al.*, 2000). Pollen analysis has been conducted on a core from Laguna Zirahuén (Ellis, 1998) and shows a decline in tree pollen towards the top and a corresponding rise in grass pollen. Unfortunately the core location and chronology are unspecified and consequently cannot be easily matched with this study. There may also have been a decrease in productivity associated with decreased human activity in the basin, after the decline of the copper smelting industry in the late 19th, early 20th century. This could be interpreted though decreased short chain *n*-alkanes and sterols concentrations, but also through the decrease in the $\delta^{13}C$ and diatom inferred Chl-a. This is contradicted by the increase in the $\delta^{15}N$, which is usually indicative of an increase in primary productivity or inputs from sewage and soils (Rosenmeier *et al.*, 2004). The magnitude of change is not really great enough to support such an assertion, as soils typically yield $\delta^{15}N$ values around +3 to +12‰, while sewage is in the range +10 to +20‰ (Heaton, 1986; Talbot, 2001). The shift in the $\delta^{15}N$ could therefore, be associated with a change in source, such as increased abundance of grasses and shrubs, from which the C_{31} *n*-alkane is thought to be derived (Cranwell, 1973; Meyers *et al.*, 1998).

An increase in the long chain *n*-alkanes from 20 cm, lower AV3/04 3 and mid Org 2, corresponds to a continued rise in the C/N ratio, which could indicate a mixture of vascular and non-vascular plants, and therefore a rise in allochthonous organic matter input, or a relative decline in algal productivity. Increase terrigenous input may be expected to result in a decrease in the concentration of diatom valves though dilution, however, there is minimal change in the concentration over this zone. Furthermore increased terrigenous material could be expected to reduce water clarity, however, *Fragilaria pinnata* increases in relative abundance over this time and this species has been noted as requiring clear water conditions in order to develop (Sayer, 2001). The C/N ratio is still not strictly terrigenous, as terrigenous material usually yields values greater than 20. This change may therefore reflect input from aquatic which would also provide a substrate for *F. pinnata* (Sayer, 2001). The change in the C/N ratio is matched by a decline in $\delta^{13}C$ to lighter, more negative values. These are, however, not in the range of terrestrial C_3 plants, particularly when assessed in relation to the corresponding C/N data. This would further support the increase in abundance of aquatic plants. The downturn in $\delta^{13}C$ could also be related to sewage input as anthropogenic waste (Rosenmeier *et al.*, 2004). Raw sewage has been noted to have $\delta^{13}C$ values between -26.2 and -22.0‰ (Burnett & Schaeffer, 1980). This period of increase relates to the early to mid 20th century, when population levels would have been lower. In addition the Rio la Palma flows into the opposite side of the basin to Agua Verde, and with dilution effects, the impact of sewage at this time may have been lower.

The upper 9 cm (Org 3 and upper AV3/04 3 and all of AV3/04 4), approximately the last 60 years, show the most marked changes in the organic data and shifts in the diatom assemblage. The proxy data also appear to infer a response notable response to the deposition of the Paricutín tephra. After 1943, for approximately 6-12 years, from 9 cm to 7cm, there is an abrupt increase in diatom valve concentration, which appears to be greater than the natural variation, from 1.09 to 3.72 x 10^9 valves per gram dry sediment. This corresponds to a shift to greater inferred primary productivity through the $\delta^{13}C$, C/N, diatom inferred Chl-a and the C_{19} *n*-alkane. After around 1949 (above 8 cm) diatom valve concentrations do return to their pre tephra levels. Other proxies indicating increase aquatic productivity continue to increase until around 1961. Lacustrine changes in response to the deposition of volcanic tephra such as diatom valve concentration and diatom assemblage have been noted elsewhere (Telford *et al.*, 2004, Barker *et al.*, 2000). Generally deposition of Si rich tephra would be expected to yield an increase in diatom abundance due to increased competitive ability (Kilham *et al.*, 1986), however in Laguna Zirahuén diatom response to tephra deposition has been negligible (Telford *et al.*, 2004). It is thought that this may be due to high levels of background silica availability due to the erosive nature of the catchment or the high anthropogenic nutrient loading which may mute the impact of the tephra on the diatoms (Davies *et al.*, 2004). The apparent sensitivity of species to tephra deposition identified in this core may therefore indicate lower Si availability in this area.

The C/N ratio shifts to a more algal dominated system decreasing from 12.1 to 9.1 in the upper 9cm (Zones Org 3, upper AV3/04 3 and AV3/04 4) which corresponds to a rise in the $\delta^{13}C$. This would indicate a more aquatic organic matter dominated system, with lower inputs of catchment material. Higher aquatic productivity would be expected to lead to an increase in both $\delta^{15}N$ and $\delta^{13}C$ as phytoplankton preferentially remove the lighter ^{12}C and ^{14}N from surface water DOC and DIN reservoirs during photosynthesis, leading to a progressive enrichment of the sinking OM with the heavier isotopes over time (Meyers & Tereanes, 2001). Increased aquatic primary productivity and lower terrestrial inputs could be expected to lead to an increase in the total diatom valve concentration and although there is an overall increase, it is not of the magnitude of the other proxy data. Increased primary productivity is supported by an increase in the concentration of C_{19}, phytol and the sterols. The concentration of

C_{17} also increases over this time period, although the rise is not as great as might be expected, particularly as this is the primary *n*-alkane in aquatic algae (Cranwell *et al.*, 1988). There is evidence, in marine settings, to show that increase C_{19} over C_{17} may be indicative of a bacterial rather than an algal signal (Jeng & Huh, 2004), although it is unclear whether this would transfer to lacustrine settings. In the upper 2cm the concentrations of all sterols decline, if considered in conjunction with the trend in the long chain C_{29} and C_{31} this may indicate a shift in source and that the C_{29} and C_{28} sterols are actually derived from terrestrial plants.

The decrease in $\delta^{15}N$ over the last ~60 years may be associated with an increase in the fixation of atmospheric nitrogen by cyanobacteria which yields values close to that of atmospheric N, 0 ‰ (Rosenmeier *et al.* 2004). Zirahuén is dominated by green algae (Chacon-Torres & Muzquiz Iribe, 1991; Tavera & Martínez-Almeida, 2005), but cyanobacteria, primarily *Mycrosystis* and *Snowella* are identified between May and July. *Snowella* cf. *lacustris* was identified in the lake throughout the year, but reached its maximum abundances in the summer when it accounted for up to 63% of the biomass. Quantitative studies of phytoplankton in Laguna Zirahuén are, however, limited so it is difficult to determine accurately if there has been substantial change in the relative abundance of cyanobacteria. The increase in $\delta^{15}N$ prior to the level of the Paricutín tephra (3.5 ‰ between 9 and 8 cm), corresponding to higher levels of χ_{lf}, may be related to inputs of soil nitrate which yields values between +3 ‰ and +12 ‰ (Rosenmeier *et al.*, 2004) or with soil organic matter which tends to have values between 0 ‰ and +8 ‰ or the inputs of fertilizer associated with soil (-4 to +4 ‰) (Talbot, 2001). The latter may be of particular importance due to the increased level of commercial agriculture in the catchment. Following an abrupt increase (3 ‰ in 4cm) the $\delta^{13}C$ showed a stabilisation and then a decrease to the top of the zone. The initial increase in $\delta^{13}C$ may be associated with increase productivity, as this period (ca. 1943-1967) corresponds to a peak in diatom valve concentration 9 cm (ca. 1955), possibly responding to the Paricutín tephra. It also corresponds to sharp rise in the diatom inferred Chl-a concentration, which would support increased primary productivity as a cause of the rise in $\delta^{13}C$. Furthermore C/N continues to decrease over this period, indicating higher aquatic productivity. It may therefore be possible that the shifts in the stable carbon and nitrogen isotopes due to increase aquatic productivity have been offset by factors such as atmospheric N fixation, and pulses of terrigenous material.

It is clear that the aquatic biota of Laguna Zirahuén, although not primarily driven by changes in nutrient availability are responding to variations in nutrients. The proxies used in this study seem to correspond well to one another and the changes in the diatom flora (Figure 8.27). Although the changes in the diatom flora are not as pronounced in the most recent part of the record as those noted by Davies (2000), the results do seem to show a rapid and progressive degradation of water quality over the last 40 years. Furthermore, the proxy data indicate that increased nutrient availability is affecting the system as a whole, and does not solely impact on the diatom community. Results imply a shift to a higher nutrient status since 1943, most likely in relation to anthropogenic activity. Despite this the lake does not seem to show any obvious visible signs of disturbance. The future growth in the use of the basin as a tourist destination, the construction of a golf course in addition to further population and agricultural expansion has potential to degrade the lake further. To prevent further deterioration a number of strategies could be implemented, such as the treatment of domestic waste (Anderson & Rippey, 1994) but should focus not only on removal of phosphorus but also on nitrogen. In addition to domestic sewage, as the majority of the catchment is agricultural, there is a potential increase in fertiliser use particularly with the construction of a golf course, a management strategy could also focus on diffuse pollution. Although this is more difficult, measures to reduce erosion, such as reforestation, and therefore accumulation of phosphorus and nitrates in the soil have been successfully adopted in the USA to reduce agricultural run-off and nutrient flux to water bodies (Sharpley *et al.*, 1992). It may therefore be appropriate to implement an integrated soil and water conservation policy, which could also tackle abstraction of water for use by the golf course and commercial agriculture.

8.13 Conclusions

The main conclusions which can be drawn from the palaeolimnological record from Agua Verde and from the application of a Chl-a transfer function to reconstruct changes in nutrient status over time are as follows:

- The lower part of the core (64 cm to 35 cm, Organic Zone 1), thought to correspond to the late 18th or early 19th century, is characterised by minimal change in the organic proxies, stable isotopes and lipid biomarkers, or in the diatom flora and therefore nutrient availability. The proxies indicative of algal organic matter, short chain *n*-alkanes, cholesterol and phytol do show a slight increase, which correspond to a period of increasing diatom inferred Chl-a. This probably corresponds to the initial impact of the copper smelting industry.
- The central part of the core (35 cm to 9 cm, Organic Zone 2) most likely the late 19th early 20th century, is characterised by a progressive decline in productivity, decreased DI-Chla, phytol and $\delta^{13}C$ and increased allochthonous inputs, seen through increased C/N and long chain *n*-alkanes. Although both C_{29} and C_{31} increase over this time period the relative change in these (shown though the ratio of C_{29}/C_{30}) could indicate a dominance of the lacustrine organic matter by grasses rather than trees and shrubs. It is thought that this period relates to the beginning of the decline in the copper smelting industry, and possible de-population resulting in a reduction in nutrient flux to the lake, but prior to landscape recovery after deforestation.
- Since 1943 (the upper 9 cm, Organic Zone 3) the most marked changes have been noted in the proxy data, with abrupt shifts in the stable isotopes, C/N, DI-Chla, phytol, the short chain *n*-alkanes and cholesterol and β-sitosterol. This implies a relative shift in organic matter source to one dominated by autochthonous inputs. This is probably related to continued population and agricultural growth and a resultant increase in nutrient input and availability which would result in increased aquatic productivity.

The chlorophyll-a reconstruction is not as accurate as hoped, with a large discrepancy between DI-Chla and observed Chl-a. This is most likely due to dominance of fossil material by species with wide nutrient tolerances such as *Fragilaria pinnata* and a lack of modern analogues, as well as the inherent areas in the model, associated wth the heterogeneous nature of the modern data set. The trajectory of change at the top of the cores is, however, consistent with other limnological and palaeolimnological evidence of increased nutrient availability over the last 20 years. Furthermore, as show in Figure 8.27 there is good correspondence between variables to offer support to the DI-Chla. In particular there is a strong correspondence between the pattern of change in both DI-Chla and phytol, a derivative of chlorophyll-a. Phytol concentration therefore offers further support to the inference made on the upward trajectory of increased nutrient status from DI-Chla in the recent past, despite the inaccuracy of the DI-Chla concentrations. This is the first study to use lipids in Mexican lakes, and to combine their use with diatoms as well as stable isotopes. This highlights that the use of lipids for tracking changes in eutrophication is a suitable technique that warrants further investigation. Furthermore, this study indicates the response of diatoms and algal productivity as a whole to tephra deposition. The former was noted to be more pronounced than in other studies of the area.

Chapter 9. Discussion and Conclusion

9.1. Introduction

Results of this study, presented in Chapters 6, 7 and 8, show that although diatom variance was not primarily related to nutrient availability it can be used effectively to show how Laguna Zirahuén has received an increase in nutrient supply over the last 60 years, an inference supported by other proxy data. In this final chapter the results are discussed in relation to the research questions outlined in Chapter 1, it explores the achievement of the thesis aims, the implications of the results and the potential for future development of the results through further research.

9.2 Discussion of Results

Does diatom species variation have a significant relationship with nutrient variables, such as TP?

Based on the results of former studies it was felt that nutrients played a stronger role in driving diatom variance, but they lacked the nutrient data to test this hypothesis. This study is, therefore, the first study from the northern tropical Americas to collect a full seasonal water chemistry data set that included nutrients. Furthermore this study represents the first study since the early 1980s to sample 30 lakes within the same 18 month period. In addition this study includes seven sites which have not been sampled before. The data collected during the course of this study showed that a high proportion of the lakes in central México (56%) could be classified as eutrophic or hypertrophic, but also spanned the full trophic gradient, i.e. there were lakes with TP values in the oligotrophic range. Initially it therefore seemed reasonable to infer that diatom species variance may have been driven by nutrient availability. The results also showed that other environmental variables in the calibration set also covered a wide gradient, such as electrical conductivity and the dominant ions. Furthermore the results of the modern study showed that lakes which had the same geological origin and similar climatic conditions, such as those in the Oriental basin, or those in Michoacán, namely Zacapu, Pátzcuaro, Zirahuén and Cuitzeo, could have remarkably different water chemistries and therefore diatom flora. A number of diatom species were only identified at one site. In addition some of the typical eutrophic indicator species, based on published auto-ecological information, such as *Nitzschia palea* and *Gomphonema parvulum* were not found in the most nutrient rich sites. Furthermore *Stephanodiscus* species, usually considered to be highly indicative of eutrophication were only identified at 3 sites. Consequently the statistical analysis (TWINSPAN and gradient analysis) of the environmental and species data showed that, even with the inclusion of nutrient data, diatom species variance was primarily due to changes in electrical conductivity. Although results did not show TP, or even TN to be significant in driving diatom variation, Chl-a was found to make an independent and significant, but secondary, contribution to species variance and had a high λ_1/λ_2 value. In addition Chl-a has been used with some success in other studies as it accounts for variations in all nutrients. It was therefore deemed suitable to be forward to build a diatom based nutrient transfer function.

Can a transfer function, with high precision and low errors, be successfully developed from this relationship?

Initial statistical analyses indicated that Chl-a could be used to build a transfer function, despite not being the primary variable in driving diatom species variance. The results showed that simple WA with classical deshrinking provided the best model, with reasonable results (r^2 = 0.83, RMSE = 0.32 log µg l^{-1}). There was still a degree of bias though, with over estimation at the low end of the gradient and under estimation at the high end of the gradient, although this is inherent in WA models. When jack-knife cross validation was applied the errors and bias increased further and the relationship between the observed and inferred values decreased substantially (r^2 = 0.09, RMSEP = 0.7 log µg l^{-1}). This was due to the very heterogeneous nature of the data set, with a number of species present at only one site. In addition species optima and tolerances could not be accurately assigned, due to the low number of occurrences of a number of species. Unfortunately the transfer function was not as robust as was hoped to be make accurate inferences on the levels of change in productivity.

When applied to sediment core AV3/04 the simple WA model, (i.e. without cross validation) showed the trajectory of change in DI-Chla was consistent with existing limnological data, but estimations of Chl-a concentration were higher than observed concentrations. This was probably due to lack of modern analogues between the core and the modern data, of the common fossil species identified, 37% were not found in the modern flora. It may also have been related to the intra-annual variability of Chl-a and the use of an annual average based on two samples to construct the calibration set. Patterns of nutrient change can also be modelled by plotting the fossil data passively in ordination space determined by the modern data. This provides further support for the WA model, in terms of trajectory of change, showing a recent rise in the level of primary productivity in Laguna Zirahuén. This also highlighted the lack of modern analogues for the fossil data. The multivariate statistical analysis of the modern data and its application to the fossil diatom data provided a useful indication of the trajectory changes occurring over the last 200 year at Laguna Zirahuén, but could not be used to provide a quantitative estimate of that change.

Do other proxy data, in the form of stable isotopes and organic lipid biomarkers, corroborate diatom inferred changes in nutrient status?

This is the first study, to the author's knowledge, that has applied lipid analysis to lacustrine systems in the subtropics, and the first to combine the use of diatoms, lipid and isotopes to assess eutrophication. Geochemical fossil proxy data used in this study provided substantiating evidence on changes in diatom inferred change in nutrient availability in the Zirahuén basin over the last 200 years. The data corroborate the diatom based inference of decrease nutrient availability in the late 19th early 20th Century followed by a progressive rise after 1943. The high degree of similarity between the pattern of change in the concentration of the Chlorophyll-a derivative phytol and DI-Chla provides an independent method for confirmation of DI-Chla changes at Laguna Zirahuén. In addition a rise in the concentration of lipid biomarkers indicative of increased algal abundance, supported by decreased C/N and increased $\delta^{13}C$, was noted towards the top of the core offering further support to diatom based inferences. Cholesterol and β-sitosterol show very similar trends, implying a similar origin, rather than being solely derived from aquatic or terrestrial sources respectively, therefore offering support to the findings of Galdu *et al.*, (1991). This study tentatively identified four highly branched isoprenoids (HBIs) which have, in marine systems, been identified as having diatoms as a primary source. Unfortunately the results of this study showed that there was not a strong correspondence between diatom valve concentration and the concentration of HBIs. Despite this there is currently little data on HBIs and their sources in lacustrine systems, particularly those in tropical regions. This study therefore represents, to the author's knowledge, an initial exploration of such compounds. The overall geochemical results, despite the limitations of the lipids methodology supported diatom based inferences as well as limnological investigation of increase nutrient availability over approximately the last 20 years. Furthermore the results of the lipids analysis were corroborated by stable isotope analyses. Stable carbon isotopes were also in good agreement with other longer term carbon isotope data from Zirahuén, which implies no major sources changes in organic matter delivery to the lake. It was hoped that lipid analysis would be able to help interpret change in the complex $\delta^{15}N$ signal, and the available data do help infer that the $\delta^{15}N$ pattern of change is related to shifts in autochthonous vs. allochthonous change.

Can a multi-proxy record of change improve our understanding of human/environment interactions?

Laguna Zirahuén had been considered to have sustained a relatively low level of anthropogenic disturbance, relative to other lakes along the TMVB. Nevertheless the system appears to have shown quite marked responses to human activity since 1759. This study has provided the first detailed, 200 year record of nutrient and productivity changes in a central Mexican lake, which has shown a more holistic approach, to track whole system response, rather than solely diatom response, to perturbation through the use of lipids, isotopes and diatoms. The results have shown that not only have diatoms had a pronounced reaction to what appear to be anthropogenic perturbations, but through the use of geochemical proxies, algal productivity as a whole has also been impacted to a similar degree. The data have also shown that the biology of the lake had a marked response to the deposition of the Paricutín tephra. Diatom valve concentration shows return to pre-tephra concentrations shortly after 1943, but from this point productivity shows a continued rise to the present day. As such this may imply that primary productivity in Laguna Zirahuén is driven by other classes of algae rather than diatoms. The use of lipids has also enabled tentative inferences to be made on the nature of the allochthonous inputs of organic matter, and therefore catchment conditions. Together the fossil proxy data all corroborate a marked shift in levels of productivity, and therefore nutrient inputs, over the most recent period covered by the core, last 20 years. This would offer support to Davies (2000) assertion of a non-linear system response to these perturbations leading to the creation of new steady states, with threshold like response

Can this information be used to inform environmental management of lake ecosystems in central México?

The ability to assess past ecosystem change in response to anthropogenic perturbation, particularly where monitoring data are spatially and temporally limited, provides a framework for assessment of potential system response to further perturbation, can detect any underlying system complexity and provides a base line for management, as discussed above. The data provided by this study give an indication that there is an upward trend towards higher levels of primary productivity at Laguna Zirahuén, which correlates with increased human activity around the basin, since 1943, but this has increase further over approximately the last 20 years. This could provide an early warning signal and could be taken forward to set realistic targets for management purposes, particularly in a system which is considered to be "apparently insensitive to nutrient loading". In addition the unique environment surrounding Zirahuén is becoming more developed though commercial agriculture, population growth and tourism, and may be subject to higher levels of nutrient loading which could be detrimental, in social, economic and environmental terms. The data do not extend far enough back to provide baseline conditions, prior to human impact, but do provide enough information to show that human activity has had a pronounced impact on primary productivity over the recent passed. Due to the future demand upon water resources in central México and declining quality and quantity of subterranean resources and of reservoirs there may be a possible focus on lakes, lakes which are still not really featured in current environmental/water policy. This study has shown that lake water quality is declining and that many of lakes across central México are already in a degraded state either due to nutrient inputs of through loss of depth. This study has also provided more information on nutrient-productivity relationships in tropical lakes and has provided the largest data set for the whole of central México in the last 20 years. The modern data collected in this study provided some interesting insights into seasonal changes in nutrients in across a much wide range of lakes than studies before it. Analysis of the modern data showed that lakes show a seasonal pattern of change, with the majority showing increased nutrient availability and therefore increased productivity in the wet season. Three lake did not conform to this and therefore indicated that the seasonality of tropical lakes still requires investigation. An increase in electrical conductivity between the dry season and the wet season is also noted at the majority of lakes.

9.3. Prospects for Future Work

This study set out to construct a diatom based nutrient transfer function for lakes in central México and although a robust transfer function was not created, as outlined above, some significant findings did emerge from the research that would be worth developing.

For several lakes there have been four studies conducted since 1982, including this one. In this study comparisons between the ionic compositions of five lakes in 1982 (Metcalfe, 1985), 1998 (Davies, 2000) and this study (2003/4) were made. Further work could be conducted on other lakes and for other variables to assess their change over the recent past. For example Chapala, Cuitzeo, La Piscina de Yuriría and Quechulac could also have been compared and changes in pH, and electrical conductivity could also be assessed. Furthermore diatom data also exist for these sites and more in-depth assessment of the corresponding diatom changes may also prove useful, particularly in the light of some of the pronounced changes at San Gregorio and La Piscina de Yuriría. Such data, across a wide geographical area may be able to add further information to existing knowledge on the impact of climatic changes across central México and on resultant chemical and corresponding biological changes. This may be able to aid predictions of the future impact of climate change and the impact that this may have on society. In addition it may be possible to access long term monitoring data on nutrient concentrations for Lago de Pátzcuaro and possibly Laguna Zirahuén dating back to the 1940s from the Limnological Station at Pátzcuaro, such data could provide further validation of the inferences made in this study. This may also help pinpoint the causes of nutrient loading through a higher resolution picture of change over time. A more regular programme of nutrient sampling to include Chl-a, TP, SRP, nitrogen species and total dissolved solid may be worthwhile, as they are known, in temperate regions to be highly variable throughout the year. Less is known about seasonality in tropical lakes. This may improve our understanding of which systems are P, N or N/P limited and may also improve our understanding of the relationship between Chl-a and nutrient availability. More detailed information such as this could contribute further to the understanding of nutrient cycling in tropical lakes, this may help aid interpretation of the $\delta^{15}N$ signal.

The low predictive ability of the transfer function could possibly be improved through the exclusion of periphytic taxa from the fossil and modern data set as such species draw nutrients from other sources and do not solely reflect epilimnetic changes in nutrient availability. Studies have, however, shown that in temperate lakes, the inclusion of all diatom species (benthic, epiphytic and planktonic) in a numerical model to reconstruct changes in nutrients yield statistically stronger models than those which only include planktonic taxa (Bradshaw *et al.*, 2002; Philibert & Prairie, 2002). The work of Philibert & Prairie (2002) on lakes in Quebec, Canada, shows that even in deep lakes where planktonic species dominate, the benthic model and the full model gave better errors and a higher correlation between measured and inferred TP than the plankton only model. Furthermore the exclusion of species with wide tolerance ranges, such as *Fragilaria* species, due to the paucity of the data left behind, only leads to a marginal improvement (Bennion *et al.,* 2001). Other possibilities include habitat specific sampling where models are created based on modern planktonic, epiphytic and benthic communities and then applied to relevant sections of the core (Sayer, 2001). These methods have not yet been tested in the tropics and may therefore warrant further investigation. One final possibility to potentially aid improvement of the transfer function could be to include the East African dataset, as these lakes are geologically and climatically similar to those in central México. Many of the East African lakes are large and deep, and not as highly impacted and this may introduce further complexities into the dataset.

As diatoms are not primarily driven by nutrients in Mexican systems it is probably worth focussing on other elements to track changes in eutrophication. This initial investigation of the use of lipids clearly shows potential, although not without its problems, which may easily be rectified to slight methodological changes. To facilitate interpretation of the record it may be worth using algal cultures to attempt to ascertain which classes or genera produce which compounds. Furthermore lipid analysis could be complemented by pigment analyses which are more taxonomically specific than lipids. Basic pigment analysis, of chlorophylls only, has been carried out on one lake in central México (Alchichica), but showed a decline in pigment concentration with depth, interpreted as degradation (Margarita Caballero pers. comm., 2003), but has been used with great success in a number of other studies, although primarily in temperate regions (Hall *et al.*, 1997; 1999).

9.4. Conclusions

This study has provided the first in-depth, multi-proxy study of changes in nutrient availability in Laguna Zirahuén over approximately the last 200 years and across the lakes of central México. The principle conclusions are as follows:

- The study sampled 30 lakes across central México, seven of which had not been sampled on a seasonal basis before and included measurements of phosphorus and nitrogen as well as Chlorophyll-a, which had not previously been measured
- Research showed seasonal change within the majority of lakes, in terms of nutrient availability and primary productivity. This is the first study to show such change across such a wide area. It does require further investigation, on a monthly basis to determine the exact nature of the seasonal changes in nutrient availability
- When ionic data were compared to existing data (1982 and 1998) in five lakes a number showed a shift towards greater ionic concentration. Major changes were noted in the ionic composition of Zirahuén, Pátzcuaro and Alchichica, with a shift to more saline conditions. This is worth further investigation; expansion of the number of lakes compared, the inclusion of data from Caballero (1995) and the inclusion of change in pH and EC over that time
- Monitoring by the Commission Nacional del Agua indicated that the majority of surface waters in central México were not degraded. This study showed that out of a data set of 30 lakes 57% could be classified as eutrophic or hypertrophic. Only the deep or isolated lakes could be classified as oligotrophic
- Despite the inclusion of nutrients both cluster analysis and gradient analysis showed that diatom species variance was primarily driven by electrical conductivity, although it was also closely tied to nutrient availability, with Chlorophyll-a as the secondary, but significant, variable
- Differences were also noted in the diatom flora of certain sites between three studies (1982, 1998 and this study). In particular the flora at San Gregorio and

La Piscina de Yuriría varied considerably relative to the studies that had come before. There was a notable rise in the abundance of *Achnanthes minutissima* and a decline in *Eunotia* species at the former and a reduction in *Navicula elkab* and increase in *Navicula (=Craticula) halophila* at La Piscina de Yuriría.

- A number of differences were noted between published ecological information on nutrient tolerances for certain species, namely *Cyclotella stelligera*, and the inferred nutrient status based on this study. This highlights further, possible slight taxonomic variations as well as the need to develop regional data sets, rather than making inferences on species preferences derived from other regions and simply applying them elsewhere
- Creation of a diatom based transfer function to infer Chl-a was unsuccessful, as Chl-a was not the primary variable and the heterogeneous nature of the data set
- The application of the transfer function to the fossil diatom stratigraphy, showed a trajectory of change in aquatic productivity that tentatively matches existing limnological data, i.e. an increase in the last 20 years. Furthermore, during a period known to have had a reduction in population and deforestation, there is a corresponding period of decreased diatom inferred Chl-a (DI-Chla)
- Diatom based inferences of changes in aquatic productivity are well matched by other proxy data. In particular there is a strong correspondence between DI-Chla and phytol, which is a chlorophyll derivative
- Lipid analysis is still in its infancy with respect to its application to lacustrine systems, and even more so in its use in tropical systems. This study therefore indicates the potential of this technique in the assessment of trophic status in lakes where diatoms are not primarily driven by nutrients
- A number of methodological issues arose from this study. They include the inclusion of epiphytic and benthic species in transfer function, the type of extraction use in lipid analysis and sampling regime

- From the study a number of issues also arose that could be taken forward. They include a more in depth comparative analysis of existing modern sampling to assess changes over time and further lipid analyses, including potential cultivation of algae to perhaps determine more specifically where the compounds are derived from

References

Abbott, M.B., Wolfe, B.B., Aravena, R. & Wolfe, A.P., Seltzer, G.O., (2000) Holocene hydrological reconstructions from stable isotopes and palaeolimnology: Cordillera, Bolivia. Quaternary Science Reviews, 19, 1801-1820.

Acevedo, C.R., Aguilar, A.A., Carrillo, M.R., L.E., D., M.E., E., Guerrero, J.J., Lozano, L.M., Ramirez, A., Rodriguez, A.R., Sanchez, O., Serna, M.G., Serna, P.M., Solis, V.M., Valdez, A. & Velasco, H., (1982) Mitos de la meseta Tarasca: Una analisis estructural. UNAM, Ciudad de México.

Adams, M.S. & Prentki, R.T., (1986) Sedimentary pigments as an index of trophic status of Lake Mead. Hydrobiologia, 143, 71-77.

Agbeti, M. & Dickman, M., (1989) Use of lake fossil diatom assemblages to determine historical changes in trophic state. Canadian Journal of Fisheries and Aquatic Sciences, 46, 1013-1021.

Agbeti, M.D. (1992) Relationship between diatom assemblages and trophic variables: a comparison between old and new methods. Canadian Journal of Fisheries and Aquatic Sciences, 49, 1171-1175.

Ahlgren, G. (1988) Phosphorus as a growth-regulating factor relative to other environmental factors in cultured algae. Hydrobiologia, 170, 191-210.

Alcocer, J. (2002) Surface water - major challenges in México. LakeLine, 22, 28-31.

Alcocer, J., Bernal-Brooks, F.W., (2002) Spatial and temporal heterogeneity of physical and chemical variables for an endoheric, shallow water body: Lake Patzcuáro, México. Archive fur Hydrobiologie, 155(2), 239-253.

Alcocer, J., Escobar, E. & Lugo, A. (2000) Water use (and abuse) and its effects on the crater-lakes of Valle de Santiago, México. Lakes and Reservoirs: Research and Management, 5, 145-149.

Alcocer, J., Oseguera, L.A., Escobar, E., Peralta, L. & Lugo, A. (2004) Phytoplankton Biomass and water chemistry in two high mountain, tropical lakes in central México, México City. Arctic, Antarctic, and Alpine Research: Vol. 36, 342–346.

Alcocer, J. & Sarma, S.S.S., (2002) Advances in Mexican Limnology: basic and applied aspects. Kluwer Academic Publishers, Dordrecht/Boston/London.

Aldama, A.A. (2002) Water resources in México. Lake Line, 22(4), 12-19.

Altabet, M.A., Francois, R., (1994) Sedimentary nitrogen isotopic ratio as a recorder for surface ocean nitrate utilization. Global Biogeochemcial Cycles, 8, 103-116.

Alverado-Diaz, J., Zubieta-Rojas, T., Ortega-Murillo, R., Chacon-Torres, A., Espinoza-Gomez, R., (1985) Hipertrofication en un Lago Tropical Somero (Lago de Cuitzeo, Michoácan, México). Boletin del Departamento de Biologia, Universidad Michoácan, 1, 1-22.

De Anda, J., Shear, H., Maniak, U. & Reidel, G. (2001) Phosphates in Lake Chapala, México. Lakes and Reservoirs: Research & Management 6, 313-321

Anderson, N.J. (1989) A whole-basin diatom accumulation rate for a small eutrophic lake in Northern Ireland and its palaeoecological implications. Journal of Ecology, 77, 926-946.

Anderson, N.J. (1990) Inferring diatom palaeoproduction and lake trophic status from fossil diatom assemblages. In: 11th International Diatom Symposium, 11 (Ed. by C.A.O. Sciences), pp. 539-547. California Academy of Sciences.

Anderson, N.J. (1990) Variability of the sediment diatom assemblages in an upland, wind-stressed lake (Loch Fleet, Galloway, S.W. Scotland). Journal of Paleolimnology, 4, 43-59.

Anderson, N.J. (1990) Variability of diatom concentrations and accumulation rates in sediments of a small lake basin. Limnology and Oceanography, 35, 497-508.

Anderson, N.J. (1994) Comparative planktonic diatom biomass responses to lake and catchment disturbance. Journal of Plankton Research, 16, 133-150.

Anderson, N.J. (1994) Diatom based phosphorus transfer functions - errors and validation. In: Ecology and Palaeoecology of Lake Eutrophication (Ed. by S.T. Patrick, N.J. Anderson), pp. 39-40. GEUS (Geological survey of Denmark and Greenland), Salten Skov, Silkeborg, Denmark.

Anderson, N.J. (1995) Temporal scale, phytoplankton ecology and palaeolimnology. Freshwater Biology, 34, 367-378.

Anderson, N.J. (1995a) Naturally eutrophic lakes: reality, myth or myopia? Trends in ecology and evolution, 10, 137-138.

Anderson, N.J. (1995b) Using the past to predict the future: lake sediments and the modelling of limnological disturbance. Ecological modelling, 78, 149-172.

Anderson, N.J. (1997) Historical changes in epilimnetic phosphorus concentrations in six rural lakes in Northern Ireland. Freshwater Biology, 38, 427-440.

Anderson, N.J. (1997) Reconstructing historical phosphorus concentrations in rural lakes using diatom models. In: H. Tunney, O.T. Carton, P.C. Brookes, A.E. Johnston (Eds.), Phosphorus loss from soil to water (Ed. by H. Tunney, O.T. Carton, P.C. Brookes, A.E. Johnston), pp. 95-117. CAB International, New York.

Anderson, N.J. (1998) Variability of diatom-inferred phosphorus profiles in a small lake basin and its implications for histories of lake eutrophication. Journal of Paleolimnology, 20, 47-55.

Anderson, N.J. (2000) Diatoms, temperature and climatic change. European Journal of Phycology, 35, 307-314.

Anderson, N.J., Rippey, B., & Stevenson, A.C. (1990). Change to a diatom assemblage in a eutrophic lake following point-source nutrient re-direction - a paleolimnological approach. Freshwater Biology, 23, 205-217.

Anderson, N.J. & Battarbee, R.W., (1992) Aquatic community persistence and variability: a palaeolimnological perspective. In: 34th Symposium of the British Ecological Society (Ed. by P.S. Giller, A.G. Hildrew, D.G. Raffaelli), pp. 233-259. Blackwell Sceintific Publications, Cork.

Anderson, N.J. & Odgaard, B.V., (1994) Recent palaeolimnology of three shallow Danish lakes. Hydrobiologia, 275/276, 411-422.

Anderson, N.J., Renberg, I. & Segerstrom, U. (1995) Diatom production responses to the development of early agriculture in a boreal forest lake-catchment (Kassjon, northern Sweden). Journal of Ecology, 83, 809-822.

Anderson, N.J. & Rippey, B. (1988) Diagenesis of magnetic minerals in the recent sediments of a eutrophic lake. Limnology and Oceanography, 33, 1476-1492.

Anderson, N.J., Rippey, B., (1994) Monitoring lake recovery from point source eutrophication: the use of diatom-inferred total phosphorus and sediment chemistry. Freshwater Biology, 32, 625-639.

Anderson, N.J., Rippey, B. & Gibson, C.E. (1993) A comparison of sedimentary and diatom-inferred phosphorus profiles: implications for defining pre-disturbance nutrient conditions. Hydrobiologia, 253, 357-366.

Anderson, N.J., Rippey, B. & Stevenson, A.C. (1990) Change to diatom assemblage in a eutrophic lake following point source nutrient redirection: a palaeolimnological approach. Freshwater Biology, 23, 205-217.

Aneona, I., Batalla, M.A., Caballero, E., Hoffman, C.C., Llamas, R., MArtin del Campo, R., Ochotereia, I., Rioja, E., Roca., J., Samano, A., Vega, C. & Villagran, F. (1940) Prospecto biologico del Lago de Patzcuáro. Anales del Instituto de Biologia, UNAM, 11, 415-450.

Appendini, K. & Liverman, D.M. (1994) Agricultural policy, climate change and food security in México. Food Policy, 19, 149-164.

Arar, E.J. & Collins, G.B. (1997) In Vitro determination of chlorophyll-a and pheophytin a in marine and freshwater algae by flourescence, pp. 22. National exposure research laboratory, Office of research and development, USEPA, Cincinnati.

Arnauld, C., Metcalfe, S.E. & Petrequin, P. (1997) Holocene climatic change on the Zacapu Lake Basin, Michoácan: Synthesis of results. Quaternary International, 43/44, 173-179.

Arnauld, C., Fauvet-Berthelot, M.-F. & Michelet, D. (1990) Les Tarasques du Michoácan. Les Dossiers d'Archeologie, 145, 16-21.

Arts, M.T., Robarts, R.D. & Evans, M.S. (1997) Seasonal changes in particulate and dissolved lipids in a eutrophic prairie lake. Freshwater Biology, 38, 525-537.

Austin, M.P. (2002) Spatial prediction of species distribution: and interface between ecological theory and statistical modelling. Ecological Modelling, 157, 101-118.

Avila Garcia, P. (1996) Escasez de Agua en una region indigena: el caso de la Meseta Purepecha. El Colegio de Michoácan, Zamora, Michoácan, México.

Bailey-Watts, A.E. (1976a) Planktonic diatoms and some diatom-silica relations in a shallow eutrophic Scottish loch. Freshwater Biology, 6, 69-80.

Bailey-Watts, A.E. (1976b) Planktonic diatoms and silica in Loch Leven, Kinross, Scotland: a one month silica budget. Freshwater Biology, 6, 203-213.

Banderas-Tarabay, A. & Gonzalez-Villela, R. (2002) Limnologia de El Sol, un lago alpino tropical. In: G. De La Lanza, J.-L. Garcia (Eds.), Lagos y Presas de México (Ed. by G. De La Lanza, J.-L. Garcia), pp. 63-79. AGT Editor, México DF.

Barker, P., Telford, R.J., Merdaci, O., Williamson, D., Taieb, M., Vincens, A. & Gibert, E. (2000) The sensitivity of a Tanzanian carter lake to catastrophic tephra input and four millenia of climate change. The Holocene, 10, 303-310.

Barnes, M.A. & Barnes, W.C. (1978) Organic Compounds in Lake Sediments. In: A. Lerman (Ed.), Lakes: Chemistry, Geology, Physics (Ed. by A. Lerman), pp. 127-152. Springer Verlag, New York.

Bartington Instruments (2004). Operating manual for the MS2 magnetic susceptibility system. Bartington Instruments, Oxford.

Bartlein, P.J., Webb III, T. & Fleri, E. (1984) Holocene climatic change in the Northern Midwest: Pollen-derived estimates. Quaternary Research, 22, 361-374.

Battarbee, R.W. (1984) Diatom analysis and the acidification of lakes. Philosophical Transactions of the Royal Society of London, Series B, 305, 451-477.

Battarbee, R.W. (1986) Diatom Analysis. In: B.E. Bergund (Ed.), Handbook of Holocene Palaeoecology & Palaeohydrology, pp. 527-570. John Wiley & Sons Ltd, Chichester.

Battarbee, R.W. (1999) The importance of palaeolimnology to lake restoration. Hydrobiologia, 395/396, 149-159.

Battarbee, R.W., Carvalho, L., Jones, V.J., Flower, R.J., Cameron, N.G., Bennion, H. & Juggins, S. (2001) Diatoms. In: J.P. Smol, H.J.B. Birks, W.M. Last (Eds.), Tracking Environmental Change Using Lake Sediments: Terrestrial, Algal and Siliceous

Indicators, 3, Developments in paleoenvironmental research, pp. 155-202. Kluwer Academic Press, Dordrecht, The Netherlands.

Belt, S.T., Allard, W.G., Masse, G., Robert, J.-M., & Rowland, S.J. (2000), Highly branched isoprenoids (HBIs): Identification of the most common and abundant sedimentary isomers: Geochimica et Cosmochimica Acta. 64, 3839-3851.

Belt, S.T., Allard, W.G., Johns, L., Konig, W.A., Masse, G., Robert, J.-M. & Rowland, S.J. (2001) Variable stereochemistry in highly branched isoprenoids from diatoms. Chirality, 13, 415-419.

Belt, S.T., Masse, G., Allard, W.G., Robert, J.-M. & Rowland, S.J. (2001), C25 highly branched isoprenoid alkene of the planktonic diatom Pleurosigma genus: Organic Geochemistry 32, 1271-1275.

Bengtsson, L. (1975) Phosphrous release from a highly eutrophic lake sediment. Verhandlungen Internationale Vereinigung für Limnologie, 19, 1107-1116.

Bengtsson, L. & Persson, T. (1978) Sediment changes in a lake used for sewage reception. Polskie Archiwum Hydrobiologii, 25, 17-33.

Bengtsson, L. & Enell, M. (1986) Chemical Analysis. In: B.E. Bergund (Ed.), Handbook of Holocene Palaeoecology & Palaeohydrology, pp. 423-451. John Wiley & sons Ltd, Chichester.

Bennion, H. (1994) A diatom-phosphorus transfer function for shallow, eutrophic ponds in southeast England. Hydrobiologia, 275, 391-340.

Bennion, H. (1995) Surface sediment diatom assemblages in shallow, artifical, enriched ponds, and implications for reconstructing trophic status. Diatom Research, 10, 1-19.

Bennion, H., Wunsam, S. & Schmidt, R. (1995) The validation of diatom-phosphorus transfer functions: an example from Mondsee, Austria. Freshwater Biology, 34, 271-283.

Bennion, H., Juggins, S. & Anderson, N.J. (1996) Predicting epilimnetic phosphorus concentrations using an improved diatom based transfer function and its application to lake eutrophication management. Environmental Science and Technology, 30, 2004-2007.

Bennion, H., Monteith, D. & Appleby, P. (2000) Temporal and geographic variation in lake trophic status in the English Lake District; evidence from (sub)fossil diatoms and aquatic macrophytes. Freshwater Biology, 45, 394-412.

Bennion, H. & Smith, M.A. (2000) Variability in the water chemistry of shallow ponds in southeast England, with special reference to the seasonality of nutrients and implications for modelling trophic status. Hydrobiologia, 436, 145-156.

Bennion, H., Appleby, P.G. & Phillips, G.L. (2001) Reconstructing nutrient histories in the Norfolk broads, UK: implications for the role of diatoms total phosphorus transfer functions in shallow lake management. Journal of Paleolimnology, 26, 181-204.

Bernal-Brooks, F. (1998) The lakes of Michoácan (México): a brief history and alternative point of view. Freshwater Forum, 20-34.

Bernal-Brooks, F. (2002) Classic studies of lakes and reservoirs across México. LakeLine, 22, 24-27.

Bernal-Brooks, F.W., Davalos Lind, L. & Lind, O.T. (2002) Assessing trophic state of an endoheric tropical lake: the algal growth potential and limiting nutrients. Archive fur Hydrobiologie, 153, 323-338.

Bernal-Brooks, F.W., & MacCrimmon, H.R. (2000a) Lake Zirahuen (México): an assessment of the morphometry change based on evidence of water level fluctuations and sediment inputs. In: M. Munawar, Lawrence, S.G., Munawar, I.F. & Malley, D.F. (Ed.), Aquatic Ecosystems of México: Status and Scope, Ecovision World Monograph Series. pp. 61-76. Backhuys Publishers, Leiden, The Netherlands.

Bernal-Brooks, F.W. & MacCrimmon, H.R. (2000b) Lake Zirahuen (México): A pristine natural reservoir visually insensitive to expected cultural eutrophication. In: M. Munawar, Lawrence, S.G., Munawar, I.F. & Malley, D.F. (Ed.), Aquatic ecosystems in México: Status and scope, Ecovision World Monograph Series. pp. 77-88. Backhuys, Leiden, Netherlands.

Bernasconi, S.M., Barbieri, A. & Simona, M. (1997) Carbon and nitrogen isotope variations in sedimenting organic matter in Lake Lugano. Limnology and Oceanography, 42, 1755-1765.

Bigler, C., Grahn, E., Larocque, I., Jeziorski, A. & Hall, R.I. (2003) Holocene environmental change at Lake Njua (999 m a.s.l.), northern Sweden: a comparison with four small nearby lakes along an altitude gradient. Journal of Paleolimnology, 29, 13-29.

Bigler, C., Larocque, I., Peglar, S.M., Birks, H.J.B. & Hall, R.I. (2002) Quantitative multiproxy assessment of long-term patterns of Holocene environmental change from a small lake in Northern Sweden. The Holocene, 12, 481-496.

Binford, M.W., Brenner, M., Whitmore, T.J., Higuera-Gundy, A., Deevey, E.S. & Leyden, B. (1987) Ecosystems, palaeoecology and human disturbance in subtropical and tropical America. Quaternary Science Reviews, 6, 115-128.

Birks, H.J.B. (1994) Can diatom based quantitaitve environmental reconstruction methods be improved. In: Ecology and palaeoecology of Lake Eutrophication (Ed. by S.T. Patrick & N.J. Anderson), pp. 41. GEUS (Geological Survey of Denmark and Greenland), Salten Skov, Silkeborg, Denmark.

Birks, H.J.B. (1995) Quantitative palaeoenvironmental reconstructions. In: D. Maddy, J. Brew (Eds.), Statistical Modelling of Quaternatry Science Data, Technical Guide No 5, pp. 161-251. Quaternary Research Association, Cambridge.

Birks, H.J.B. (1996) Contributions of Quaternary palaeoecology to nature conservation. Journal of Vegetation Science, 7, 89-98.

Birks, H.J.B. (1998) Numerical tools in palaeolimnology - Progress, potentialities and problems. Journal of Paleolimnology, 20, 307-332.

Birks, H.J.B., Line, J.M., Juggins, S., Stevenson, A.C.& ter Braak, C.J.F. (1990) Diatoms and pH reconstruction. Philosophical Transactions of the Royal Society of London, Series **B**, 327, 263-278.

Bloom, A.M., Moser, K.A., Porinchu, D.F., MacDonald, G.M., (2003) Diatom-inference models for surface-water temperature and salinity developed from a 57 lake calibration set from the Sierra Nevada, California, USA. Journal of Paleolimnology, 29, 235-255.

Borah, W.W. & Cook, S.F. (1963) The aboriginal population of central Mexico on the eve of the Spanish conquest. University of California Press, Berkeley

Borcard, D., Legendre, P. & Drapeau, P. (1992) Partialling out the spatial component of ecological variation. Ecology, 73, 1045-1055.

Bourbonniere, R.A. & Meyers, P.A. (1996) Anthropogenic influences on hydrocarbon contents of sediments deposited in eastern Lake Ontario since 1800. Environmental Geology, 28, 22-28.

Boutton, T.W. (1991) Stable carbon isotope rations of natural materials: II. Atmospheric, terrestrial, marine and freshwater environments, (in Coleman, D.C. & Fry, B. eds), Carbon Isotope Techniques: New York, Academic Press Inc., p. 173-185.

Bradbury, J.P. (1971) Paleolimnology of Lake Texcoco, México. Evidence from diatoms. Limnology and Oceanography, 16, 180-200.

Bradbury, J.P. (1978) A paleolimnological comparison of Burntside and Shagawa Lakes, northeastern Minnesota. In: Ecological Research Series, 51 pp, Corvallis, Oregon.

Bradbury, J.P. (1982) Holocene chronostratigraphy of México and Central America. Striae, 16, 46-48.

Bradbury, J.P. (1989) Late Quaternary lacustrine paleoenvironments in the Cuenca de México. Quaternary Science Reviews, 8, 75-100.

Bradbury, J.P. (2000) Limnologic history of Lago de Patzcuáro, Michoácan, México for the past 48,000 years: impacts of climate and man. Palaeogeography, Palaeoclimatoloy, Palaeoecology, 163, 69-95.

Bradshaw, E.G. & Anderson, N.J. (2001) Validation of a diatom-phosphorus calibration set for Sweden. Freshwater Biology, 46, 1035-1048.

Bradshaw, E.G., Anderson, N.J., Jensen, J.P. & Jeppesen, E. (2002). Phosphorus dynamics in Danish lakes and the implications for diatom ecology and palaeoecology. Freshwater Biology, 47, 1963-1975.

Bratton, J.F., Colman, S.M. & Seal II, R.R. (2003) Eutrophication and carbon sources in Chesapeake Bay over the last 2700 yr: Human impacts in context. Geochimica et Cosmochimica Acta, 67, 3385-3402.

Brehm, V. (1942) Plancton del lago de Patzcuáro. Revista de la Sociedad Mexicana de Historia Natural, 3, 81-85.

Brenner, M. (1983) Palaeolimnology of the Petén Lake District, Guatemala II: Mayan population density and sediment and nutrient loading of Lake Quexil. Hydrobiologia, 103, 205-210.

Brenner, M. & Binford, M.W. (1988) Relationships between concentrations of sedimentary variables and trophic state in Florida lakes. Canadian Journal of Fisheries and Aquatic Sciences, 45, 294-300.

Brenner, M., Hodell, D.A., Curtis, J.H., Rosenmeier, M.F., Binford, M.W., Abbott, M.B., (2001) Abrupt Climatic Change and pre-Colombian Cultural Collapse. In: V. Markgraf (Ed.), Interhemispheric Climate Linkages (Ed. by V. Markgraf), pp. 87-103. Academic Press.

Brenner, M., Whitmore, T.J., Curtis, J.H., Hodell, D.A. & Schelske, C.L. (1999) Stable isotope (d13C and d15N) signatures of sedimented organic matter as indicators of historic trophic state. Journal of Paleolimnology, 22, 205-221.

Brenner, M., Whitmore, T.J., Curtis, J.H. & Schelske, C.L. (1995) Historical ecology of a hypereutrophic Florida lake. Lake and Reservoir Management, 11, 255-271.

Brenner, M., Whitmore, T.J., Flannery, M.S. & Binford, M.W. (1993) Paleolimnological methods for defining target conditions in lake restoration: Florida case studies. Lake and Reservoir Management, 7, 209-217.

Brooks, S.J., Bennion, H. & Birks, H.J.B. (2001) Tracing lake trophic history with a chironomid-total phosphorus inference model. Freshwater Biology, 46, 513-533.

Brown, R.B. (1984) The paleoecology of the northern frontier of Mesoamerica. In: Department of Anthropology. University of Arizona, Tucson.

Brown, R.B. (1985) A summary of late-Quaternary pollen records from México west of the Isthmus of Tehuantepec. In: V.M. Bryant, R.G. Holloway (Eds.), Pollen records of Late Quaternary North American Sediments pp. 71-93. American Association of Stratigraphic Palynologists, Dallas.

Brugam, R.B. (1979) A re-evaluation of the Araphidineae/Centrales index as an indicator of trophic status. Freshwater Biology, 9, 451-460.

Brugam, R.B. (1983) The relationship between fossil diatom assemblages and limnological conditions. Hydrobiologia, 98, 223-235.

Brugam, R.B., Grimm, E.C. & Eyster-Smith, N.M. (1988) Holocene Environmental Change in Lily Lake, Minnesota Inferred from Fossil Diatom and Pollen Assemblages. Quaternary Research, 30, 53-66.

Brugam, R.B. & Patterson, C. (1983) The A/C (Araphidineae/Centrales) ratio in high and low alkalinity lakes in eastern Minnesota. Freshwater Biology, 13, 47-55.

Brylinsky, M. &Mann, K.H. (1973) An analysis of factors governing productivity in lakes and reservoirs. Limnology and Oceanography, 18, 1-14.

Burt, T. & Haycock, N. (1991) Farming and nitrate pollution. Geography, 60-63.

Butzer, K.W. (1988) Cattle and Sheep from Old to New Spain: Historical Antecedents. Annals of the Association of American Geographers 78, 29-56

Butzer, K.W. (1991). Spanish colonization of the New World: Cultural Continuity and Change in México. Erdkunde, 45, 205-219

Butzer, K.W. (1993) No Eden in the New World. Nature, 362, 15-17.

Butzer, K.W. & Butzer, E.K. (1997) The 'natural' vegetation of the Mexican Bajio; archival documentation of a 16th-century savanna environment. Quaternary International, 43/44, 161-172.

Byrne, R., & Horn, S. (1989) Prehistoric agriculture and forest clearance in the Sierra de los Tuxtlas, Veracruz, México. Palynology, 13, 181-193.

Caballero, M. & Ortega-Guerrero, B. (1998) Lake levels since about 40,000 years ago at Lake Chalco, near México City. Quaternary Research, 50, 69-79.

Caballero, M., Vilaclara, G., Rodriguez, A. & Juarez, D. (2003) Short-term climatic change in lake sediments from Lake Alchichica, Oriental, México. Geofisica Internacional, 42, 529-537.

Caballero, M., Lozano-Garcia, S., Ortega-Guerrero, B. & Urrutia-Fucugauchi, J. (1995) Historia ambiental del sistema lacustre del sureste de la Cuenca de México. In: E.S.

Otto (Ed.) Segundo Seminario Internacional de Investigadores de Xochimilco, pp. 12-22, México.

Caballero, M. Resultados preliminares del análisis de diatomeas en sedimentos del Lago Chalco, México, pp. 9. UNAM, México.

Caballero, M.E. (1995) Late Quaternary palaeolimnology of Lake Chalco, the Basin of México: new evidence for palaeoenvironmental and palaeoclimatic change in central México during the last 45,000 years. Unpub. PhD Thesis, Department of Geography, pp. 276 + plates. University of Hull, Hull.

Caraco, N.F. (1995) The influence of human populations on P transfers to aquatic systems: a regional scale study using large rivers. In: H. Tiessen (Ed.), Phosphorus in the global environment. John Wiley & Sons.

Caraco, N.F., Cole, J.J. & Likens, G.E. (1989) Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. Nature, 341, 316-318.

Cardoso, J.N., Gaskell, S.J., Quirk, M.M.& Eglington, G. (1983) Hydrocarbon and fatty acid distributions in Rostherne Lake sediment (England). Chemical Geology, 38, 107-128.

Carlson, R.E. (1977) Trophic state index for lakes. Limnology and Oceanography, 22, 361-369.

Carney, H.J. (1982) Algal dynamics and the trophic interactions in the recent history of Frains lake Michigan. Ecology, 63, 1814-1826.

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N. & Smith, V.H. (1998) Non-point pollution of surface waters with phosphorus and nitrogen. Ecological Applications, 8, 559-568.

Carper, G.L., Bachmann, R.W., (1984) Wind re-suspension of sediments in a Prairie lake. Canadian Journal of Fisheries and Aquatic Sciences, 41, 1763-1767.

Carrillo Cázares, A. (1996). Partidos y Padrones del Obispado de Michoacán 1680-1685. El Colegio de Michoacán, Morelia, Michoacán, México, 561pp

Carvalho, L. (1994) Top-down control of phytoplankton in a shallow hypertrophic lake: Little Mere (England). Hydrobiologia, 275/276, 53-63.

Carvalho, L.R., Cox, E.J., Fritz, S.C., Juggins, S., Sims, P.A., Gasse, F. & Battarbee, R.W. (1995) Standardizing the taxonomy of saline lake Cyclotella spp. Diatom Research, 10, 229-240.

Chacon-Torres, A. (1993a) Lake Patzcuáro, México: Watershed and water quality deterioration in a tropical high-altitude Latin American lake. Lake and Reservoir Management, 8, 37-47.

Chacon-Torres, A. (1993b) Lake Patzcuáro, México: effects of turbidity in a tropical high altitude lake. Tropical Freshwater Biology, 3, 251-272.

Chacon-Torres, A. (1989) A limnological study of Lake Patzcuáro, México, with a consideration of the applicability of remote sensing techniques. Unpub. PhD Thesis Institute of Aquaculture, University of Stirling. 340 pp.

Chacon-Torres, A., Ross, L.G. & Beveridge, M.C.M. (1989) Lake Patzcuáro, México: results of a new morphometric study and its implications for productivity assessments. Hydrobiologia, 184, 125-132.

Chacon-Torres, A. & Muzquiz Iribe, E. (1991) El Lago Zirahuen, Michoácan, México reconocimiento ambiental de una Cuenca Michoácan. Universidad San Nicolás de Hidalgo, Morelia, 30pp

Chacon-Torres, A., Mungia, R.P. & Muzquiz Iribe, E., (1991) Síntesis limnología del Lago de Patzcuáro, Michoácan, México. Universidad San Nicolás de Hidalgo, Morelia, 48 pp

Chacon-Torres, C., Ross, L.G., Beveridge, M.C.M. & Watson, A.I. (1992) The application of SPOT multispectral imagery for the assessment of water quality in Lake Patzcuáro, México. International Journal of Remote Sensing, 13, 587-603.

Chacon-Torres, A., Muzquiz-Iribe, E., (1997) Climatic trends, water balance and Lake Patzcuáro, a tropical high altitude lake. Quaternary International, 43/44, 43-51.

Chacon-Torres, A. & Rosas-Monge, C. (1998) Water quality characteristics of a high altitude oligotrophic Mexican lake. Aquatic ecosystem health and management, 1, 237-243.

Chacon-Torres, A. & Rosas-Monge, C. (2002) Water education and training to meet the challenges in México. Lake Line, 22, 32-34.

Chalie, F. & Gasse, F. (2002) Late Glacial-Holocene diatom record of water chemistry and lake level change from the tropical East African Rift Lake Abiyata (Ethiopia). Palaeogeography, Palaeoclimatology, Palaeoecology, 187, 259-283.

Chavez, E.A. (1973) Datos Hidrobiologicos del Lago de Chapala, Jalisco. Revista de la Sociedad Mexicana de Historia Natural, 34, 125-146.

Christie, C.E., & Smol, J.P. (1993) diatom assemblages as indicators of lake trophic status in southeastern Ontario lakes. Journal of Phycology, 29, 575-586.

Clement, R.M. & Horn, S.P. (2001) Pre-Columbian land-use history in Costa Rica: a 3000-year record of forest clearance, agriculture and fires from Laguna Zoncho. The Holocene, 11, 419-426.

Coe, M.D. (1984) The Maya. Thames & Hudson Ltd., London. 190 pp

Coe, M.D. & Koontz, R. (2002) México: from the Olmecs to the Aztecs. Thames & Hudson, London. 248 pp

Coe, M.D. & Flannery, K.V. (1981) Microenvironments and Mesoamerican prehistory. In: J.A. Graham (Ed.), Ancient Mesoamerica; selected readings (Ed. by J.A. Graham), pp. 25-34. Peek Publishing, Palo Alto.

Comisión Nacional del Agua (2005) Estadísticas del Agua en México 2005. Comisión Nacional de Agua, México City, 105 pp

Conley, D.J., Kilham, S.S. & Theriot, E. (1989) Differences in silica content between marine and freshwater diatoms. Limnology and Oceanography, 34, 205-213.

Conserva, M.E. & Byrne, R. (2002) Late Holocene vegetation change in the Sierra Madre Oriental of central México. Quaternary Research, 58, 122-129.

Correll, D.L. (1998) The role of phosphorus in the eutrophication of receiving waters: a review. Journal of Environmental Quality, 27, 261-266.

Cranwell, P.A. (1973) Chain-length distribution of n-alkanes from lake sediments in relation to post-glacial environmental change. Freshwater Biology, 3, 259-265.

Cranwell, P.A. (1976) Decomposition of aquatic biota and sediment formation: lipid components of two blue-green algal species and of detritus resulting from microbial attack. Freshwater Biology, 6, 481-488

Cranwell, P.A., Creighton, M.E. & Jaworski, G.H.M. (1988) Lipids of fours species of freshwater chrystophytes. Phytochemistry, 27, 1053-1059.

Cremer, H. & Wagner, B. (2003) The diatoms flora in the ultra-oliotrophic Lake El'gygytgyn, Chukotka. Polar Biology, 26, 105-114.

Cross, S.L., Baker, P.A., Seltzer, G.O., Fritz, S.C. & Dunbar, R.B. (2001) late Quaternary climate and hydrology of tropical South America inferred from an isotopic and chemical model of Lake Titicaca, Bolivia & Peru. Quaternary Research, 56, 1-9.

Cullen, P. & Forsberg, C. (1988) Experiences with reducing point sources of phosphrous to lakes. Hydrobiologia, 170, 321-336.

Curtis, J.H., Brenner, M., Hodell, D., Balser, R.A., Islebe, G.A.. & Hooghiemstra, H. (1998) A multi proxy study of Holocene environmental change in the Maya lowlands of Peten, Guatemala. Journal of Paleolimnology, 19, 139-159.

da Costa, G.M., de Grave, E., Bowen, .H., de Bakker, P.M.A. & Vandeberghe, R.E. (1995) Variable-temperature Mossbauer spectroscopy of nano-sized maghemite and Al-substituted maghemites. Clays and Clay Minerals, 43, 562-568.

Davies, N. (1982) The Ancient Kingdoms of México. Penguin, London. 272 pp

Davis, R.B. & Anderson D.S. (1985) Methods of pH calibration of sedimentary diatom remains for reconstructing history of pH in lakes. Hydrobiologia, 120, 69-87

Davies, S.J. (2000) Environmental change in the west central Mexican highlands over the last 1,000 years: evidence form lake sediments. Unpub. PhD thesis, University of Edinburgh p 253 + plates

Davies, S.J., Metcalfe, S.E., Caballero, M.E. & Juggins, S. (2002) Developing diatom based transfer functions for central Mexican lakes. Hydrobiologia, 467, 199-213.

Davies S.J., Metcalfe S.E., MacKenzie A.B., Newton A.J., Endfield G.H. & Farmer J.G. (2004) Environmental changes in the Zirahuén Basin, Michoacán, Mexico, during the last 1000 years. Journal of Paleolimnology, 31, 77-89.

Davies, S.J., Metcalfe, S.E., Bernal-Brooks, F., Chacón-Torres, A., Farmer, J.G., MacKenzie, A.B. & Newton, A.J. (2005) Lake sediment record sensitivity of two hyrdologically closed upland lakes in México to human impact. Ambio 34, 470-475

de A. Azevedo, D. (2003) A preliminary investigation of the polar lipids in the recent tropical sediments from Aquatic environments at Campos dos Goytacazes, Brazil. Journal of the Brazilian Chemcial Society, 14, 97-106.

de Buen, F. (1941) El Lago de Patzcuáro recientes estudios limnologicos. Revista Geografica, 20-45.

de Buen, F. (1941) La Vida en las agua dulces. Revista Universidad Michoácana, 25-26, 209-231.

de Buen, F. (1943) Los lagos Michoácanos: Characteres generales el lago de Zirahuén. Revista de la Sociedad Mexicana de Historia Natural, 4, 211-232.

de Buen, F. (1944) Limnoiologia de Patzcuáro. Anales del Instituto Biología, 15, 261-263.

de Buen, F., (1944) Los lagos Michoácanos. II Patzcuáro. Revista de la Sociedad Mexicana de Historia Natural, 5, 99-125.

de Cserna, Z., Alvarez, R., (1995) Quaternary drainage development in central México and the threat of an Environmental disaster: a geological appraisal. Environmental & Engineering Geoscience, 1, 29-34.

de Gomes, A. & Azevedo, D., (2003) Aliphatic and aromatic hydrocarbons in tropical recent sediments of Campos dos Goytacazes, RJ, Brazil. Journal of the Brazilian Chemcial Society, 14, 1-11.

Dearing, J.A. (1991) Lake sediment records of erosional processes. Hydrobiologia 214, 99-106.

Dearing, J.A., Hu, Y., Doody, P., James, P.A. & Brauer, A. (2001) Preliminary reconstruction of sediment-source linkages for the past 6000 years at the Petit Lac d-

Annecy, France based on mineral magnetic data. Journal of Paleolimnology, 25, 245-258.

Deevey, E.S. (1944) Pollen analysis and Mexican archaeology: an attempt to apply the method. American Antiquity, 10, 135-149.

Deevey, E.S., Binford, M.W., Brenner, M. & Whitmore, T.J. (1986) Sedimentary records of accelerated nutrient loading in Florida Lakes. Hydrobiologia, 143, 49-53.

Demant, A. (1981) L'Axe Néo-volcanique Transmexicain Etude Volcanologique et Pétrographique Signification Géodynamique. Université de Droit, D'Économie et des Sciences D'Aix-Marseille pp. 259

Demant, A. (1992) Marco Geologico Regional de la Laguna de Zacapu, Michoaca, México. In: D. Michelet (Ed.), El Proyecto Michoácan 1983-1987: Medio ambiente e Introduccion a los trabajos arqueologicos, 4, pp. 55-70. Centre D'Etudes Mexicaines et Centramericanes México, México.

Denevan, W.M. (1992) The pristine myth: the landscape of the Americas in 1492. Annals of the Association of American Geographers, 82, 369-385.

Dillon, P.J. & Rigler, F.H. (1974) The phosphorus-chlorophyll relationship in lakes. Limnology and Oceanography, 19, 767-773.

Dixit, A.S., Hall, R.I., Leavitt, P.R., Quinlan, R. & Smol, J.P., (2000) Effects of sequential depositional basins on lake responses to urban and agricultural pollution: a palaeoecological analysis of the Qu'Appelle Valley, Sasketchewan, Canada. Freshwater Biology, 43, 319-337.

Douglas, M.W., Maddox, R.A. & Howard, K. (1993) The Mexican Monsoon. Journal of Climate, 6, 1665-1677.

Downing, J.A., McClain, M., Twilley, R., Melack, J.M., Elser, J., Rabalais, N.N., Lewis Jnr, W.M., Turner, R.E., Corredor, J., Soto, D., Yanez-Arancibia, A., Kopaska, J.A. & Howarth, R.W. (1999) The impact of accelerating land-use change on the N-cycle of tropical aquatic ecosystems: Current conditions and projected changes. Biogeochemistry, 46, 109-148.

Eadie, B.J., Chambers, R.L., Gardner, W.S. & Bell, G.E. (1984) Sediment trap studies in Lack Michigan: Resuspension and chemical fluxes in the southern basin. Journal of Great Lakes Research, 10, 307-321

Ekdahl, E.J., Teranes, J.L., Guilderson, T.P., Turton, C.L., McAndrews, J.H., Wittkop, C.A. & Stoermer, E.F. (2004) Prehistorical record of cultural eutrophication from Crawford Lake, Canada. Geology, 32, 745-748.

Ellis, C.C. (1998) A preliminary investigation of the environmental history of Zirahuén lake basin situated in the highlands of central México. Unpub. undergraduate dissertation. School of Geography, University of Edinburgh. 78p

Elser, J.J., Marzolf, E.R. & Goldman, C.R. (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Canadian Journal of Fisheries and Aquatic Sciences, 47, 1468-1477.

Endfield, G.H., & O'Hara, S.L. (1997) Conflicts over water in 'The Little Drought Age' in central México. Environment and History, 3, 255-272.

Endfield, G.H., O'Hara, S.L. (1999) Degradation, drought and dissent: an environmental history of colonial Michoácan, west central México. Annals of the Association of American Geographers, 89, 402-419.

Endfield, G.H., O'Hara, S.L. & Metcalfe, S.E. (1998) Palaeoenvironmental reconstructions in the central Mexican highlands: a re-appraisal of the traditional theory. In: R.A. Nicholson, T.P. O'Connor (Eds.), People as an agent of environmental change, Symposia of the Association for Environmental Archaeoogy No. 16. Oxbow books.

Englehart, P.J., Douglas, A.V. (2001) The role of eastern north Pacific tropical storms is the rainfall climatology of western México. International Journal of Climatology, 21, 1357-1370.

Engstrom, D.R., Wright, H.E. (1984) Chemical stratigraphy of lake sediments as a record of environmental change. In: E.Y. Haworth, J.W.G. Lund (Eds.), Lake Sediments and Environmental History, pp. 11-67. University of Minnesota Press, Minneapolis.

Eugster H.P. & Hardie L.A. (1978) Saline Lakes. In: A. Lerman (Ed) Lakes: Chemistry, Geology, Physics, p237-294. Springer-Verlag, New York.

Eugster, H.P. & Jones, B.F. (1979) Behaviour of major solutes during closed-basin brine evolution. Amercian Journal of Science, 279, 609-631.

Evans, S.T. (1992) The productivity of Maguey terrace agriculture in central México during the Aztec period. In: T.W. Killion (Ed.), in Gardens of Prehistory: The archaeology of settlement agriculture in Greater Mesoamerica, pp. 92-113. University of Texas Press, Tuscaloosa.

Ficken, K.J., Li, B., Swain, D.L. & Eglington, G. (2000) A n-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. Organic Geochemistry, 31, 745-749.

Ficken, K.J., Wooller, M.J., Swain, D.L., Street-Perrott, F.A., & Eglington, G. (2002) Reconstruction of a subalpine grass doiminated ecosystem, Lake Rutundu, Mount Kenya: a novel multi-proxy approach: Palaeogeography, Palaeoclimatology, Palaeoecology 177, 137-149.

Filley, T.R., Freeman, K.H., Bianchi, T.S., Baskaran, M., Colarusso, L.A. & Hatcher, P.G. (2001) An isotopic biogeochemical assessment of shifts in organic matter input to Holocene sediments from Mud Lake, Florida. Organic Geochemistry, 32, 1153-1167.

Findlay, D.L., Kling, H.J., Ronicke, H. & Findlay, W.J. (1998) A palaeolimnological study of eutrophied Lake Arendsee (Germany). Journal of Paleolimnology, 19, 41-54.

Fisher, C.T., Pollard, H.P., Israde, I., Garduno, V. & Banerjee, S.K. (2003) A reexamination of human-induced environmental change within the Lake Patzcuáro Basin, Michoácan, México. Proceedings of the National Academy of Sciences of the United States of America, 100, 4957-4962.

Fisher, E., Oldfield, F., Wake, R., Boyle, J.F., Appleby, P.G. & Wolfe, G.A. (2003) Molecular marker records of land use change. Organic Geochemistry, 34, 105-119.

Flannery, M.S. Snodgrass, R.D. & Whitmore, T.J. (1982) Deepwater sediments and trophic conditions in Florida lakes. Hydrobiologia, 92, 597-602.

Foy, R.H., Smith, R.V., Jordan, C. & Lennox, S.D. (1995) Upward trend in soluble phosphorus loadings to Lough Neagh despite phosphorus reduction at sewage treatment works. Water Research, 29, 1051-1063.

Foy, R.H. & Withers, P.J.A. (1995) The contribution of agricultural phosphorus to eutrophication. The fertilizer society, conference proceedings, 27th April 1995.

Fritz, S.C., Juggins, S., Battarbee, R.W. & Engstrom, D.R. (1991) Reconstruction of past changes in salinity and climate using a diatom-based transfer function. Nature, 352, 706-708.

Fritz, S.C., Kingston., J.C. & Engstrom, D.R. (1993) Quantitative trophic reconstruction from sedimentary diatom assemblages: a cautionary tale. Freshwater Biology, 30, 1-23.

Fritz, S.C., Metcalfe, S.E. & Dean, W. (2001) Holocene climate patterns in the Americas inferred from paleolimnological records. In: V. Markgraf (Ed.), Interhemispheric Climate Patterns, pp. 241-263. Academic Press, San Diego.

Garcia-Rodriguez, F., Mazzeo, N., Sprechmann, P., Metzeltin, D., Sosa, F., Treutler, H.C., Renom, M., Scharf, B. & Gaucher, C. (2002) Paleolimnological assessment of human impacts in Lake Blanca, SE Uruguay. Journal of Paleolimnology, 28, 457-468.

Garcia-Rodriguez, J. & Tavera, R. (2002) Phytoplankton composition and biomass in a shallow monomictic tropical lake. Hydrobiologia, 467, 91-98.

Gaskell, S.J. & Eglington, G. (1976) Sterols of a contemporary lacustrine sediment Geochimica et Cosmochimica Acta, 40, 1221-1228.

Gasse, F. (1980) Les Diatomées lacustres plio-pléistocenes du Gadeb (Éthiopie), Paris. Revue Algologique. 249 pp

Gasse, F. (1986) East African Diatoms taxonomy. Ecological distribution. Cramer, Berlin. pp 201

Gasse, F. (1987) Diatoms for reconstructing palaeoenvironments and palaeohydrology in tropical semi-arid zones: examples of some lakes from Niger since 12,000 BP. Hydrobiologia, 154, 127-163.

Gasse, F., Barker, P., Gell, P.A., Fritz, S.C. & Chalie, F. (1997) Diatom-inferred salinity in palaeolakes: an indirect tracer of climatic change. Quaternary Science Reviews, 16, 547-563.

Gasse, F., Juggins, S. & Khelifa, L.B. (1995) Diatom based transfer functions for inferring past hydrochemical characteristics for African lakes. Palaeogeography, Palaeoclimatology, Palaeoecology, 117, 31-54.

Gasse, F., Talling, J.F. & Kilham, P. (1983) Diatoms assemblages in East Africa: classification, distribution and ecology. Review. Hydrobiolgia Tropical, 16, 3-34.

Gasse, F. & Tekaia, F. (1983) Transfer functions for estimating palaeoecological conditions (pH) from East African diatoms. Hydrobiologia, 103, 85-90.

Gauch, H.G. (1982) Multivariate analysis in community ecology. Cambridge University Press, Cambridge .

Gburek, W.J. & Sharpley, A.N. (1998) hydrological controls on phosphorus loss from upland agricultural watersheds. Journal of Environmental Quality, 27, 267-277.

Gell, P.A., Tibby, J., Fluin, J., Leahy, P., Reid, M., Adamson, K., Bulpin, S., MacGregor, A., Wallbrink, P., Hancock, G. & Walsh, B. (2005) Accessing limnological change and variability using fossil diatom assemblages, south-east Australia. River Research and Appications, 21, 257-269.

Gelpi, E., Schneider, H., Mann, J. & Oro, J. (1970) Hydrocarbons of geochemical significance on microscopic algae. Phytochemistry, 9, 603-612.

Germain, H. (1981). Flore des diatomées eaux douces et saumâtres. Société Nouvelle des Editions Boubée, Paris, pp 444

Gibson, C.E., Foy, R.H. & Bailey-Watts, A.E. (1996) An analysis of the total phosphorus cycle in some termperate lakes: The response to enrichment. Freshwater Biology 35, 525-532.

Gladu, P.K., Patterson, G.M., Wikfors, G.H., Chitwood, D.J., and Lusby, W.R., (1991), Sterols of some diatoms: Phytochemistry, 30, 2301-2303.

Gleick, P.H. (Ed) (1993). Water in Crisis: A Guide to the World's Fresh Water Resources. Oxford University Press, Oxford, 473 pp

Godinez, J.L., Ortega, M.M., Garduno, G., Olivia, M.G., Vilaclara, G., (2001) Traditional Knowledge of Mexican continental algae. Journal of Ethnobiology, 21, 57-88..

Goericke, R., Montoya, J.P., Fry, B., (1994) Physiology of isotopic fractions in algae and cyanobacteria. In: Lajtha, Mickener (Eds.), Stable isotopes in ecology and environmental science, pp. 187-221. Blackwells Science Publishing, London.

Graham, J.A., (1981) Ancient Mesoamerica: selected readings. Peek Publishing, Palo Alto.

Grossi, V., Beker, B., Geenevasen, J.A.J., Schouten, S., Raphel, D., Fontaine, M.-F., and Sinninghe Damste, J.S., 2004, C25 highly branched isoprenoid alkenes from the marine benthic diatom Pleurosigma strigosum: Phytochemistry, 65, 3049-3055.

Gu, B., Schelske, C., Brenner, M., (1996) Relationship between sediment and plankton isotope rations (D13C and D15N) and primary productivity in Florida lakes. Canadian Journal of Fisheries and Aquatic Sciences, 53, 875-883.

Guasch, H., Paulsson, M.& Sabater, S. (2002) Effect of Copper on algal communities from oligotrophic calcareous streams. Journal of Phycology, 38, 241-248.

Guneralp, B. & Barlas, Y. (2003) Dynamic modelling of a shallow freshwater lake for ecological and economic sustainability. Ecological modelling, 167, 115-138.

Haberyan, K.A. (1990) The misrepresentation of the panlktonic diatom assemblage in traps and sediments: southern Lake Malawi. Journal of Paleolimnology, 3, 35-44.

Håkansson, H. 1986 A taxonomic reappraisal of some Stephanodiscus species (Bacillariophyta) British Phycological Journal 21, 25-37

Håkansson, H., (1989) A light and electron microscopical investigation of the type species Cyclotella (Bacillariophyceae) and related forms, using original material. Diatom Research, 4, 255-267.

Håkansson, H. & Kling, H. (1989) A light and electron microscope study of previously described and new Stephanodiscus species (Bacillariophyceae) from central and northern Canadian lakes, with ecological notes on the species. Diatom Research, 4, 269-288.

Hall, R.I., Leavitt, P.R., Quinlan, R., Dixit, A.S. & Smol, J.P. (1999) Effects of agriculture, urbanization and cliamte on water quality in the northern Great Plains. Limnology and Oceanography, 44, 739-756.

Hall, R.I., Leavitt, P.R., Smol, J.P. & Zirnhelts, N. (1997) Comparison of diatoms, fossil pigments and historical records as measures of lake eutrophication. Freshwater Biology, 38, 401-4117.

Hall, R.I. & Smol, J.P. (1992) A weighted averaging regression and calibration model for inferring total phosphorus concentration from diatoms in British Columbia (Canada) lakes. Freshwater Biology, 27, 417-434.

Hall, R.I. & Smol, J.P. (1996) Paleolimnological assessment of long term water quality changes in south-central Ontario lakes affected by cottage development and acidification. Canadian Journal of Fisheries and Aquatic Sciences, 53, 1-17.

Hall, R.I. & Smol, J.P. (1999) Diatoms as indicators of lake eutrophication. In: E.F. Stoermer & J.P. Smol (Eds.) The diatoms: applications for the environmental and earth sciences, pp. 128-168. Cambridge University Press, Cambridge.

Hanisch, S., Ariztegui, D. & Puttmann, W. (2003) The biomarker record of Lake Albano, central Italy-implications for Holocene aquatic ecosystem response to environmental change. Organic Geochemistry, 34, 1223-1235.

Hasenaka, T. & Carmichael, I.S.E. (1987) The Cinder cones of Michoácan-Guanajuato Central México: petrology and chemistry. Journal of Petrology, 28, 241-296.

Hasler, A.D. (1947) Eutrophication of lakes by domestic drainage. Ecology, 28, 383-395.

Hassan, K.M., Swinehart, J.B. & Spalding, R.F. (1997) Evidence for Holocene environmental change from C/N ratios, and d13C and d15N values in Swan Lake sediments, western Sand Hills, Nebraska. Journal of Paleolimnology, 18, 121-130.

Hausmann, S. & Lotter, A.F. (2001) Morphological variation within the diatom taxon Cyclotella comensis and its importance fro quantitative temperature reconstruction. Freshwater Biology, 46, 1323-1333.

Havens, K., Aumen, N.G., James, R.T. & Smith, V. (1996) Rapid ecological changes in a large subtropical lake undergoing cultural eutrophication. Ambio, 25, 150-155.

Havens, K.E., East, T.L., Hwang, S.J., Rodusky, A.J., Sharfstein, B. & Steinman, A.D. (1999) Algal responses to experimental nutrient addition in the littoral community of a subtropical lake. Freshwater Biology, 42, 329-344.

Havens, K.E., East, T.L., Rodusky, A.J. & Sharfstein, B. (1999) Littoral periphyton responses to nitrogen and phosphorus: an experimental study in a subtropical lake. Aquatic Botany, 63, 267-290.

Havens, K.E., Kukushima, T., Xie, P., Iwakuma, T., James, R.T., Takamura, N., Hanazato, T. & Yamamoto, T. (2001) Nutrient dynamics and the eutrophication of shallow lakes Kasumigaura (Japan), Donghu (PR China), and Okeechobee (USA). Environmental Pollution, 111, 263-272.

Havens, K.E., Phlips, E.J., Cichra, M.F. & Li, B.L. (1998) Light availability as a possible regulator of cyanobacteria species composition in a shallow subtropical lake. Freshwater Biology, 39, 547-556.

Haworth, E.Y. (1976) Two Late glacial (late Devensian) diatom assemblage profiles from Northern Scotland. New Phytologist 77, 227-256

Haworth, E.Y. (1977) The sediments of Lake George (Uganda) V: the diatom assemblages in relation to the ecological history. Archive fur Hydrobiologie, 80, 200-215.

Haworth, E.Y. (1984) Stratigraphic changes in algal remains (diatoms and chrysophytes) in the recent lake sediments of Blenham Tarn, English Lake District. In: E.Y.L. Haworth, J.W.G. (Ed.), Lake sediments and environmental history, pp. 165-190. University of Minnesota press, Minneapolis.

Heaton, T.H.E. (1986). Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chemical Geology 59, 87-102

Hecky, R.E. (1993) The Eutrophication of Lake Victoria. Verhandlungen Internationale Vereinigung für Limnologie 25, 39-48.

Hecky, R.E., Campbell, P. & Hendzel, L.L. (1993) The stoichiometry of carbon, nitrogen and phosphorus in particulate matter of lakes and oceans. Limnology and Oceanography, 38, 709-724.

Heine, K. (1987) Anthropogenic sedimentological changes during the Holocene in México and Central America. Striae, 26, 51-63.

Hem, J.D. (1970) Study and interpretation of the chemical characteristics of natural water (2nd Ed). Geological Survey Water Supply Paper 1473, United States Government Printing Office, Washington: 363 pp

Henry, R., Hino, K., Tundisi, J.G. & Ribeiro, J.S.B. (1985) Responses of phytoplankton in Lake Jacaretinga to enrichment with nitrogen and phosphorus in concentrations similar to those of the River Solimoes (Amazonia, Brazil). Archive fur Hydrobiologie, 103, 453-477.

Hernadez-Aviles, J.S., Bernal-Brooks, F., Velarde, G., Ortiz, D., Lind, O.T., Davalos-Lind, L., (2001) The algal growth potential and algal growth-limiting nutrients for 30 of México's lakes and reservoirs. Verhandlungen Internationale Vereinigung für Limnologie 27, 3583-3588.

Hill, M.O. (1979) TWINSPAN: a FORTRAN program for arranging multivariate data in an ordered two way table by classification of the individuals and attributes. Cornell University, Ithica, New York.

Hill, M.O., Bunce, R.G.H. & Shaw, M.W. (1975) Indicator species analysis, a distinctive polythetic method of classification and its application to a survey of native pinewoods in Scotland. Journal of Ecology, 63, 597-613.

Hill, M.O. & Gauch, H.G. (1980) Detrended correspondence analysis: an improved ordination technique. Vegetatio, 42, 47-58.

Hird, S.J. & Rowland, S.J. (1994) An investigation of the sources and seasonal variations of highly branched isoprenoid hydrocarbons in intertidal sediments of the Tamar Estuary, UK. Marine Environmental Research, 40, 423-437.

Hodell, D.A., Schelske, C., (1998) Production, sedimentation and isotopic composition of organic matter in Lake Ontario. Limnology and Oceanography, 43, 200-214.

Hoefs, M.J.L., Sinninghe Damste, J.S., & De Leeuw, J.W. (1995) A novel C25 highly branched isoprenoid polyene in Recent Indian Ocean sediments. Organic Geochemistry, 23, 263-267.

Holdren, G.C. & Armstrong, D.E. (1980) Factors affecting phosphorus release form intact lake sediment cores. Environmental Science and Technology, 14, 79-87.

Hollander, D.J., McKenzie, J.A. & Lo ten Haven, H. (1992) A 200-year sedimentary record of progressive eutrophication in Lake Greifen (Switerland): Implications for the origin of organic-carbon-rich sediments. Geology, 20, 825-828.

Holtan, H., Kamp-Neilsen, L. & Stuanes, A.O. (1988) Phosphorus in soil, water and sediment: an overview. Hydrobiologia, 170, 19-34.

Huang, W.-Y. & Meinschain, W.G. (1976), Sterols as indicators of organic materials in sediments. Geochimica et Cosmochimica Acta, 40, 323-330.

Huang, Y., Street-Perrott, F.A., Metcalfe, S.E., Brenner, M., Moreland. M. & Freeman, K.H. (2001) Climate change as the dominant control on glacial-interglacial variations in C3 and C4 plant abundance. Science, 293, 1647-1651.

Hubble, D.S. & Harper, D.M., (2002) Nutrient control of phytoplankton in Lake Naivasha, Kenya. Hydrobiologia, 488, 99-105.

Huisman, J., van Oostveen, P. & Weissing, F.J., (1999) Critical depth and critical turbulence: two different mechanisms for the devlopment of phytoplankton blooms. Limnology and Oceanography, 44, 1781-1787.

Hutchinson, G.E., Patrick, R. & Deevey, E.S. (1956) Sediments of Lake Patzcuáro, Michoácan, México. Bulletin of the Geological Society of America, 67, 1491-1504.

Hutchinson, G.E. (1973) Eutrophication: The scinetific background of a contemporary practical problem. American Scientist, 61, 269-279.

Imbrie, J. & Kipp, N.G. (1971) A new macropaleontological methodfor quantitative paleoclimatology: application to a late Pleistocene Caribbean Core: In Turekian (Ed)

The late Cenozoic glacial ages. Yale University Press, New Haven. Connecticut. pp 71-181

Interlandi, S.J., Kilham, S.S. & Theriot, E.C. (1999) Responses of phytoplankton to varied resource availability in large lakes in the Greater Yellowstone Ecosystem. Limnology and Oceanography, 44, 668-682.

Interlandi, S.J., Kilham, S.S. & Theriot, E.C. (2003) Diatom-chemistry relationships in Yellowstone Lake (Wyoming) sediments: Implications for climatic and aquatic process research. Limnology and Oceanography, 48, 79-92.

Irvine, K., Moss, B. & Balls, H. (1989) The loss of submerged plants with eutrophication II. Relationship between fish and zooplankton in a set of experimental ponds, and conclusions. Freshwater Biology , 89-107

Jaffe, R. & Hausmann, K.B. (1995) Origin and early diagenesis of arborinone/isoarborinol in sediments of a highly productive freshwater lake. Organic Geochemistry, 22, 231-235.

James, C., Fisher, J. & Moss, B. (2003) Nitrogen driven lakes: The Shropshire and Cheshire meres? Archive fur Hydrobiologie, 158, 249-266.

Jasper, J.P. & Gagosian, R.B. (1989) Glacial-interglacial climatically forced d13C variations in sedimenraty organic matter. Nature, 342, 60-62.

Jauregui, E. (1997) Climate changes in México during the historical and instrumental periods. Quaternary International, 43/44, 7-17.

Jauregui, E. (2003) Climatology of land-falling hurricanes and tropical storms in México. Atmosfera, 16, 193-204.

Jauregui, E. & Klaus, D. (1976) Some aspects of climate fluctuations in México in relation to drought. Geofisica Internacional, 16, 45-61.

Jeng, W.-L. & Huh, C.-A. (2001), Comparative study of sterols in shelf and slope sediments off north eastern Taiwan. Applied Geochemistry, 16, 95-108

Jeng, W.-L. & Huh, C.-A. (2004), Lipids in suspended matter and sediments form the East China Sea Shelf. Organic Geochemistry, 35, 647-660.

Jensen, H.S. & Andersen, F.O. (1992) Importance of temperature, nitrate and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. Limnology and Oceanography, 373, 577-589.

Jinglu, W., Gagan, M.K., Xuezhong, J., Weilan, X. & Sumin, W. (2004) Sedimentary geochemical evidence for recent eutrophication of Lake Chenghai, Yunnan, China. Journal of Paleolimnology, 32, 85-94.

Jones, J.R. & Hoyer, M.V. (1982) Sportfish harvest predicted by summer chlorophyll-a concentrations in Midwestern lakes and reservoirs. Transactions of the American Fisheries Society, 111, 176-179.

Jones, R.I., King, L., Dent, M.M., Maberly, S.C. & Gibson, C.E. (2004) Nitrogen stable isotope ratios in surface sediments, epilithion and macrophytes from upland lakes with differing nutrient status. Freshwater Biology, 49, 382-391.

Jones, V.J. & Juggins, S. (1995) The construction of a diatom-based chlorophyll-a transfer function and its application at three lakes on Signy Island (maritime Antarctic) subject to differing degrees of nutrient enrichment. Freshwater Biology, 34, 433-445.

Jordan, P. & Rippey, B. (2003) Lake sedimentary evidence of phosphorus, iron and manganese mobilisation from intensively fertilized soils. Water Research, 37, 1426-1432.

Jordan, P., Rippey, B. & Anderson, N.J. (2002) The 20th century whole-basin trophic history of an inter-drumlin lake in an agricultural catchment. Science of the Total Environment, 297, 161-173.

Joyce, A.A. & Mueller, R.G. (1992) The social impact of anthropogenic landscape modification in the Rio Verde drainage basin, Oaxaca, México. Geoarchaeology, 7, 503-526.

Kallqvist, T. & Berge, D. (1990) Biological availability of phosphorus in agricultural run-off compared to other phosphorus sources. Verhandlungen Internationale Vereinigung für Limnologie, 24, 214-217.

Kauppila, T., Moisio, T. & Salonen, V-P. (2002) A diatom based inferrence model for autumn epilimnentic total phosphorus concentration and its application to a presently eutrophic boreal lake. Journal of Paleolimnology, 27, 261-273.

Kauppila, T., Knuuttila, S.T., Sandman, K.O., Eskonen, K., Luokkanen, S. & Lieuhu, A. (1990) Role of landuse in the occurance of blue green algal blooms. Verhandlungen Internationale Vereinigung für Limnologie 24, 671-676.

Kaushal, S., Binford, M.W. (1999) Relationship between C:N ratios of lake sediments, organic matter sources, and historical deforestation in Lake Pleasant, Massachusetts, USA. Journal of Paleolimnology, 22, 439-442.

Kenney, W.F., Waters, M.N., Schelske, C.L. & Brenner, M. (2002) Sediment records of phosphorus-driven shifts to phytoplankton dominance in shallow Florida lakes. Journal of Paleolimnology, 27, 367-377.

Kilham, P., Kilham, S.S. & Hecky, R.E. (1986) Hypothesized resource relationships among African planktonic diatoms. Limnology and Oceanography, 31, 1169-1181.

Kilham, S.S., Theriot, E.C. & Fritz, S.C. (1996) Linking planktonic diatoms and climate change in the large lakes of the Yellowstone ecosystem using resource theory. Limnology and Oceanography, 41, 1052-1062.

Killops, S. & Killops, V. (2005) Introduction to Organic Geochemistry (2nd Ed). Blackwell Publishing, Oxford 393 pp

King, L., Barker, P. & Jones, R.I. (2000) Epilithic algal communities and their relationship to environmental variables in the lakes of the English Lake District. Freshwater Biology, 45, 425-442.

Kirchoff, P. (1981) Mesoamerica: its geographical limits, ethnic composition and cultural characteristics. In: J.A. Graham (Ed.), Ancient Mesoamerica; selected readings, pp. 1-10. Peek publications, Palo Alto.

Koster, D., Racca, J.M.J. & Pienitz, R., (2004) Diatoms-based inference models and reconstructions revisited: methods and transformations. Journal of Paleolimnology, 32, 233-246.

Krammer K. and Lange-Bertalot H. (1986). Sßusswasserflora von Mitteleuropa Bacillariophyceae. 1. Teil: Naviculaceae, vol. 2/1, Gustav Fischer, Stuttgart, 876 pp.

Krammer K. and Lange-Bertalot H. (1988). Sßusswasserflora von Mitteleuropa. Bacillariophyceae. 2. Teil: Epithemiaceae, Bacillariaceae, Surirellaceae, vol. 2/2. Gustav Fischer, Stuttgart, 596 pp.

Krammer K. and Lange-Bertalot H. (1991). Sßusswasserflora von Mitteleuropa. Bacillariophyceae. 3. Teil: Centrales; Fragilariaceae, Eunotiaceae, vol. 2/3, Gustav Fischer, Stuttgart, 576 pp.

Krammer K. and Lange-Bertalot H. (1991). Sßusswasserflora von Mitteleuropa. Bacillariophyceae. 4. Teil: Achnanthaceae, vol. 2/4, Gustav Fischer, Stuttgart, 437 pp.

Krishnamurthy, R.V., Bhattacharya, S.K. & Kusumgar, S. (1986) Palaeoclimatic changes deduced from 13C/12C and C/N ratios of Karewa lake sediments, India. Nature, 323, 150-152.

Kristensen, P., Sondergaard, M. & Jeppesen, E. (1992) Re-suspension in a shallow eutrophic lake. Hydrobiologia, 228, 101-109.

Lambert, J.M., Meacock, S.E., Barrs, J. & Smartt, P.F.M. (1973) Axor and monit: two new polythetic-divisive stratergies for heirarchical classifictation. Taxon, 22, 172-176.

Larsen, D.P., van Sickle, J., Malueg, K.W. & Smith, P.D. (1979) The effect of wastewater phosphorus removal on Shagwa Lake, Minnesota: Phosphorus supplies, lake phosphorus and chlorophyll-a. Water Research, 13, 1259-1272.

Lawton, L.A. & Codd, G.A. (1991). Cyanobacterial (blue-green algal) toxins and their significance in the UK and European waters. Journal of IWEM, 5, 460-465

Leavitt, P.R. (1993) A review of factors that regulate carotenoid and chlorophyll deposition and fossil pigment abundance. Journal of Paleolimnology, 9, 109-127.

Leavitt, P.R. & Hodgson, D.A. (2001) Sedimentary pigments. In: J.P. Smol, H.J.B. Birks & W.M. Last (Eds.), Tracking Environmental Change Using Lake Sediments Volume 3: terrestrial, algal and siliceous indicators, 3, pp. 295-325. Kluwer Academic Press, Dordrecht, The Netherlands.

Lee, G.F., Jones, R.A. & Rast, W. (1978) Eutrophication of water bodies: insights for an age old problem. Environmental Science and Technology 12 900-908

Lee, G.F., Jones, R.A. & Rast, W. (1979) Availability of phosphorus to phytoplankton and its implications for phosphorus management strategies. In: Phosphrous management strategies for lakes, pp. 259-308. Ann Arbor Science Publishers Inc, Cornell University.

Legesse, D., Gasse, F., Radakovitch, O., Vallet-Coulomb, C., Bonnefille, R., Verschuren, D., Gibert, E. & Barker, P. (2002) Environmental changes in a tropical lake (Lake Abiyata, Ethiopia) during recent centuries. Palaeogeography, Palaeoclimatology, Palaeoecology, 187, 233-258.

Lehermann, M.F., Bernasconi, S.M., Barbieri, A. & McKenzie, J.A. (2002) Preservation of organic matter and alteration of its carbon and nitrogen composition during simulated and in situ early diagenesis. Geochimica et Cosmochimica Acta, 66, 3573-3584.

De Lejarza, J.J.M. (1974). Analisis Estadístico de la Provincia de Michoacán en 1822. Coleccion "Estudios Michoacanos" IV. Fimax Puplicistas, Morelia, Michoacán, México, 321 pp.

Leng, M J and Marshall, J D. (2004) Palaeoclimate interpretation of stable isotope data from lake sediments. Quaternary Science Reviews, 23, 811-831.

Leng, M.J., Lamb, A.L., Heaton, T.H.E., Marshall, J.D., Wolfe, B.B., Jones, M.D., Holmes, J.A. & Arrowsmith, C. (2005) Isotopes in Lake Sediments, in Leng, M.J., ed., Isotopes in Palaeoenvironmental Research, p. 147-184.Dordrecht, The Netherlands, Springer

Lepš, J. & Šmilauer, P. (2003) Multivariate analysis of ecological data using CANOCO: Cambridge, Cambridge University press, 269 pp

Lewis, W.M. (2000) Basis for the protection and management of tropical lakes. Lakes & Reservois: Research and Management. 5 35-48

Limon, J.G., Lind, O.T., Vodopich, D.S., Doyle, R., Trotter, B.G., (1989) Long- and short-term variations in the physical and chemical limnology of a large, shallow, turbid tropical lake (Lake Chapala, México). Archive fur Hydrobiologie Supplement, 83, 57-81.

Lind, O.T. (1985) Handbook of common methods in limnology. Kendal/Hunt Publishing Co., Dubuque, Iowa, USA, 199 pp.

Lind, O.T., Doyle, R., Vodopinch, D.S., Trotter, B.T., Glass, J., Davalos-Lind, L., Limon, J.G., (1992a) Lago Chapala: factores que controlan la produccion de fitoplancton. Ingenieria Hidraulica en México, 7(2/3), 17-29.

Lind, O.T., Doyle, R., Vodopich, D.S., Trotter, B.G., Limon, G. & Davalos-Lind, (1992b) Clay turbidity: regulation of phytoplankton production in a large, nutrient-rich tropical lake. Limnology and Oceanography, 37(3), 549-565.

Lind, O.T. & Davalos Lind, L.O. (2002) Interaction of water quality with water quantity: the lake Chapala example. Hydrobiologia, 467, 159-167.

Line, J.M., Birks, H.J.B., (1990) WACALIB version 2.1 - a computer program to reconstruct environmental variables from fossil assemblages by weighted averaging. Journal of Paleolimnology, 3, 170-173.

Liverman, D.M., (1990) Drought impacts in México: Climate, agriculture, technology and land-use in Sonora and Puebla. Annals of the Association of American Geographers, 80(1), 49-72.

Liverman, D.M., (1992) Global warming and Mexican agriculture: some preliminary results. In: Reilley J.M., M. Anderson (Eds.), Economic issues in global climatic change: Agriculture, forests and natural resources, pp. 332-352. Westview, Boulder.

Liverman, D.M. & O'Brien, K.L. (1991) Global warming and climatic change in México. Global Environmental Change, 351-364.

Livingstone, D. (1984) Preservation of algal remains in recent lake sediments. In: E.Y. Haworth, J.W.G. Lund (Eds.), Lake Sediments and Environmental History, pp. 191-202. University of Minnesota Press, Minneapolis.

Loffler, H. (1972) Contribution to the limnology of high altitude mountain lakes in Central America. International Review ges Hydrobiologia, 57, 397-408.

Long, A., Benz, B.F., Donahue, D.J., Jull, A.J.T. & Toolin, L.J. (1989) First direct AMS date on early maize from the Tehuacan, México. Radiocarbon, 31, 1035-1040.

Lopez, M. (2002) Pre-Coloumbian technology: water management in México. LakeLine, 22, 20-23.

Lopez-Sarrelangue, D.E. (1969) Ribeas del Lago de Patzcuáro. Arls de México, 120.

Lorenzen, C.J. (1979) Ultraviolet radiation and phytoplankton photosynthesis. Limnology and oceanography 24, 1117-1120

Lotter, A.F. (1998) The recent eutrophication of Baldeggersee (Switzerland) as assessed by fossil diatom assemblages. The Holocene, 8, 395-405.

Lotter, A.F. (2001) The palaeolimnology of Soppensee (central Switzerland), as evidenced by diatom, pollen and fossil pigment analysis. Journal of Paleolimnology, 25, 65-79.

Lotter, A.F., Birks, H.J.B., Hofmann, W. & Marchetto, A. (1997). Modern diatom, cladocera, chironomid and chrysophyte cyst assembages as quantitative indicators for the reconstruction of past environmental conditions in the Alps I: Climate. Journal of Paleolimnology, 18, 395-420.

Lotter, A.F., Birks., H.J.B., Hofmann, W. & Marchetto, A. (1998) Modern diatom, cladocera, chironomid and chrysophyte cyst assembages as quantitative indicators for the reconstruction of past environmental conditions in the Alps II: Nutrients. Journal of Paleolimnology, 19, 443-463.

Lozano-Garcia, M. & Ortega-Guerrero, B. (1994) Palynological and mineral magnetic susceptibility records of Lake Chalco, central México. Palaeogeography, Palaeoclimatoloy, Palaeoecology, 109, 177-191.

Lozano-Garcia, M. & Ortega-Guerrero, B. (1998) Late Quaternary environmental changes of the central part of the Basin of México; correlation between Texcoco and Chlaco basins. Review of Palaeobotany and Palynology, 99, 77-93.

Lynn, S.G., Kilham, S.S., Kreeger, D.A. & Interlandi, S.J. (2000) Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom Stephanodiscus minutulus (Bracillariophyceae). Journal of Phycology, 36, 510-522.

MacNeish, R.S. (1981) Ancient Mesoamerican civilization. In: J.A. Graham (Ed.), Ancient Mesoamerica; selected readings (Ed. by J.A. Graham), pp. 65-76. Peek Publishing, Palo Alto.

Magaña, V.O., Vazquez, J.L., Perez, J.L. & Perez, J.B. (2003) Impact of El Niño on precipitation in México. Geofisica Internacional, 42, 313-330.

Makulla, A. & Sommer, U. (1993) Relationships between resource ratios and phytoplankton species composition during spring in five north German lakes. Limnology and Oceanography, 38, 846-856.

Manzanilla, L. (2002) The impact of climatic change on past civilizations. A revisionist agenda for further investigation. Quaternary International, 43/44, 153-159.

Maldonado-Koerdell, M. (1964) Geohistory & Paleogeography of Middle America. In Wauchope, R. Handbook of Middle American Indians. University of Texas Press, Austin pp 3-32

Markiewicz, D. (1993) The Mexican Revolution and the limits of agrarian reform, 1915-1946. Lynne Rienner Publishers, Boulder, USA 215 pp

Markgraf, V. (1989) Palaeoclimates in Central and South America since 18,000 yr BP based on pollen and lake-level records. Quaternary Science Reviews 8, 24.

Marsden, M.W. (1989) Lake restoration by reducing external phosphorus loading: the influence of sediment phosphrous release. Freshwater Biology, 21, 139-162.

Mason, C.F. (1996) Biology of Freshwater pollution. Longman, Harlow pp 356

Mazumder, A. & Havens, K.E. (1998) Nutrient-chlorophyll-Secchi relationships under contrasting grazer communities of temperate versus subtropical lakes. Canadian Journal of Fisheries and Aquatic Sciences, 55, 1652-1662.

McAuliffe, J.R., Sundt, P.C., Valiente-Banuet, A., Casas, A. & Viveros, J.L. (2001) Pre-Colombian soil erosion, persistent ecological changes, and collapse of a subsistence agricultural economy in the semi-arid Tehuacan Valley, México's 'Cradle of Maize'. Journal of Arid Environments, 47, 47-75.

McCauley, E., Downing, J.A. & Watson, S. (1989) Sigmoid relationships between nutrients and chlorophyll among lakes. Canadian Journal of Fisheries and Aquatic Sciences 46, 1171-1175

McCormick, P.V. &Stevenson, R.J. (1998) Periphyton as a tool for ecological assessment and management in the Florida Everglades: Journal of Phycology, 34, 726-733.

Mechling, J.A. & Kilham, S.S. (1982) Temperature effects on silicon limited growth of the Lake Michigan diatom Stephanodiscus minutus (Bracillariophyceae). Journal of Phycology, 18, 199-205.

Melack, J.M. (1995) Transport and transformations of P, fluvial and lacustrine ecosystems. In: H. Tiessen (Ed.), Phosphrous in the Global environment pp. 244-254. John Wiley and Sons Ltd.

Mellville, E.G.K. (1990) Environmental and social change in the Valle de Mezquital, México 1521-1600. Comparative Studies in Society and Environment, 32, 24-53.

Mendoza-Gonzáles, A.C., Huerta-Múzquiz, L. & Flores-Granados, C. (1985) Estudio floristic de fitoplancton del Lago Zirahuen, Michoácan, México. Phytologia, 59, 1-8.

Mestre, E. (2002) Lake management: a matter of water governance. LakeLine, 22, 35-40.

Metcalfe, S.E., (1985) Late Quaternary Environments of Central México: A Diatom Record. D. Phil. Thesis, University of Oxford, School of Geography, pp. 565 + plates.

Metcalfe, S.E. (1986) Diatoms in a core from Laguna Zacapu, Michaocan, México. In: Proceeding of the 9th Symposium on Living and Fossil Diatoms (Ed. by F. Round), pp. 251-264, Bristol.

Metcalfe, S.E. (1987) Historical data and climatic change in México - a review. The Geographical Journal, 153, 211-222.

Metcalfe, S.E. (1988) Modern diatom assemblages in central México: the role of water chemistry and other environmental factors as indicated by TWINSPAN and DECORANA. Freshwater Biology, 19, 217-233.

Metcalfe, S.E. (1995) Holocene environmental change in the Zacapu Basin, México: a diatom based record. The Holocene, 5, 196-208.

Metcalfe, S.E. (1997) Palaeolimnological records of climatic change in México - frustrating past, promising future? Quaternary International, 43/44, 111-116.

Metcalfe, S.E., Street-Perrott, F.A., Brown, R.B., Hales, P.E., Perott, R.A. & Steininger, F.M. (1989) Late Holocene human impact on lake basins in Central México. Geoarchaeology, 4, 119-141.

Metcalfe, S.E & Hales, P. (1990) Holocene diatoms in a Mexican crater lake - La Piscina de Yuriria. In: 11th International Diatom Symposium, pp. 501-515.

Metcalfe, S.E., Street-Perrott, F.A., Perrott, R.A. & Harkness, D.D. (1991) Palaeolimnology of the Upper Lerma Basin, Central México: a record of climatic change and anthropogenic disturbance since 11,600 yr BP. Journal of Paleolimnology, 5, 197-218.

Metcalfe, S.E. & O'Hara, S.L. (1992) Sensibilidad de lagos mexicanos a alteraciones en el medio ambiente; ejemplos del Eje Neovolvanico. Ingenieria Hidraulica en México, 7, 107-121.

Metcalfe, S.E., Street-Perrott, F.A., O'Hara, S.L., Hales, P.E. & Perrott, R.A. (1994) The palaeolimnological record of environmental change: examples from the arid frontier of Mesoamerica. In: A.C. Millington & K. Pye (Eds.), Environmental change in drylands: Biogeographical and geomorphological perspectives pp. 131-145. John Wiley and Sons Ltd.

Metcalfe, S.E., O'Hara S.L., Caballero, M. & Davies, S.J., (2000) Records of Late Pleistocene-Holocene climatic change in México - a review. Quaternary Science Reviews, 19, 699-721.

Meyers, P.A. (1994) Preservation of elemental and isotopic source identification of sedimentary organic matter. Chemical Geology, 114, 289-302.

Meyers, P.A. (1997) Organic geochemical proxies of paleooceanographic, paleolimnologic and palaeoclimatic processes. Organic Geochemistry, 27, 213-250.

Meyers, P.A., (2003) Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentide Great Lakes. Organic Geochemistry, 34, 261-289.

Meyers, P.A., Tenzer, G.E., Lebo, M.E. & Reuter, J.E. (1998) Sedimentary record of sources and accumulation of organic matter in Pyramid Lake, Nevada, over the past 1,000 years. Limnology and Oceanography, 43, 160-169.

Meyers, P.A. & Kawka, O.E. (1984) Geolipid, pollen and diatom stratigraphy in post glacial lacustrine sediments. Organic Geochemistry, 6, 727-732.

Meyers, P.A. & Ishiwatari, R. (1993). Lacustrine organic geochemistry - an overview of indicators of organic-matter sources and diagenesis in lake-sediments. Organic Geochemistry 20, 867-900

Meyers, P.A. & Lallier-Verges, E. (1999) Lacustrine sedimentary organic matter records of Late Quaternary paleoclimates. Journal of Paleolimnology, 21, 345-372.

Meyers, P.A. & Teranes, J.L. (2001) Sedimentary Organic Matter. In: W.M. Last, J.P. Smol (Eds.) Tracking environmental change using lake sediments. Volume 2: Physical and geochemical methods, 2 pp. 239-269. Kulwer Academic Press, Dordrecht, The Netherlands.

Michelet, D. (1996) El origen del Reino Tarasco protohistorico. Arqueologia Mexicana, 9, 24-27.

Miettinen, J., Gronlund, E., Simola, H. & Huttunen, P. (2002) Palaeolimnology of Lake Pieni-Kuuppalanampi (Kurkijoki, Karelian Republic, Russia): isolation history, lake ecosystem develpoment and long-term agricultural impact. Journal of Paleolimnology, 27, 29-44.

Mosiño-Alemán, P.A. & García, E. (1974) The climate of México. In: Bryson, R.A. & Hare, F.K. (Eds.), World climate survey: Climates of North America, 2, World survey of climatology pp. 345-405. Elsevier.

Moss, B. (1992) Uses, abuses and management of lakes and rivers. Hydrobiologia, 243/244, 31-45.

Moss, B., Bekliogul, M., Carvalho, L., Kilinc, S., McGowan, S. & Stephen, D. (1997) Vertically-challenged limnology; contrasts between deep and shallow lakes. Hydrobiologia, 342/343, 257-267.

Moss, B., McGowan, S. & Carvalho, L. (1994) Determination of phytoplankton crops by top-down and bottom-up mechanisms in a group of English lakes, the West Midland meres. Limnology and Oceanography, 39, 1020-1029.

Moss, B., Mckee, D., Atkinson, D., Collings, S.E., Eaton, J.W., Gill, A.B., Harvey, I., Hatton, K., Heyes, T. & Wilson, D. (2003) How important is climate? Effects of warming, nutrient addition and fish on phytoplankton in shallow lake microcosms. Journal of Applied Ecology, 40, 782-792.

Muller, B., Lotter, A.F., Sturm, M. & Ammann, A. (1998) Influence of catchment quality and altitude on the water and sediment composition of 68 small lakes in Central Europe. Aquatic Sciences, 60, 316-337.

Mullins, C.E. (1977) Magnetic susceptibility of the soil and its significance in soil science - a review. Journal of Soil Science, 28, 223-246.

Muri, G., Wakenham, S.G., Pease, T.K. & Faganeli, J. (2004) Evalutaion of lipid biomarkers as indicators of changes in organic matter delivery to sediments from Lake Planina, a remote mountain lake in NW Slovenia. Organic Geochemistry, 35, 1083-1093.

Murphy, T.P., Hall, K.J. & Yesaki, I. (1983) Coprecipitation of phosphate with cacite in a naturally eutrophic lake. Limnology and Oceanography, 28, 58-69.

Naiman, R.J., Magnusson, J.J., McKnight, D.M. & Stanford, J.A. (1995). The Freshwater Imperative: A research agenda. Island Press 256 pp

Neumann, T., Stobauer, A., Walpersdof, E., Stuben, D. & Kunzendorf, H. (2002) Stable Isotopes in recent sediments of Lake Arendsee, NE Germany: response to eutrophication and remediation measures. Palaeogeography, Palaeoclimatology, Palaeoecology, 178, 75-90.

Newton, A.J. & Metcalfe, S.E. (1999) Tephrachronology of the Toluca Basin, central México. Quaternary Science Reviews, 18, 1039-1059.

Niederberger, C. (1981) Early sedentary economy in the Basin of México. In: J.A. Graham (Ed.), Ancient Mesoamerica; selected readings (Ed. by J.A. Graham), pp. 77-100. Peek Publishing, Palo Alto.

Nurnberg, G.K. (1987) A comparison of internal phosphorus loads in lakes with anoxic hypolimnia: laboratory incubation versus in situ hypolimnetic phosphorus accumulation. Limnology and Oceanography, 32, 1160-1164.

Nygaard, G. (1949). Hydrobiological studies on some Danish ponds and lakes. Part II. The quotient hypothesis and some new or little known phytoplankton organisms. Kongle. Danske. Vidensk. Selskab Biol. Skrift. 7, 293 pp

O'Hara, S.L. (1993) Historical evidence of fluctuations in the level of Lake Patzcuáro, Michoácan, México over the last 600 years. The Geographical Journal, 159, 51-62.

O'Hara, S.L., Street-Perrott, F.A.. & Burt, T.P. (1993) Accelerated soil erosion around a Mexican highland lake caused by prehispanic agriculture. Nature, 362, 48-51.

O'Hara, S.L., Metcalfe, S.E. & Street Perrott, F.A. (1994) On the arid margin: the relationship between climate, humans and the environment. A review of evidence from the highlands of central México. Chemosphere, 29, 965-981.

O'Hara, S.L. & Metcalfe, S.E. (1995) Reconstructing the climate of México from historical records. The Holocene, 5, 485-490.

O'Hara, S.L. & Metcalfe, S.E. (1997) The climate of México since the Aztec period. Quaternary International, 43/44, 25-31.

Olsson, S., Regnell, J., Persson, A. & Sandgren, P. (1997) Sediment-chemistry response to land-use change and pollutant loading in a hypertrophic lake, southern Sweden. Journal of Paleolimnology, 17, 257-294.

O'Neill, P. (1998) Environmental Chemistry. Blackie Academic & Professional, London.

Organisation for Economic Co-operation and Development (1982) Eutrophication of waters: Monitoring assessment and control. OECD, Paris, 154 pp

Ortega-Guerrero, B., Thompson, R. & Urrutia-Fucugauchi, J. (2000) Megnetic properties of lake sediments from Lake Chalco, central México, and their palaeoenvironmental implications. Journal of Quaternary Science, 15, 127-140.

O'Sullivan, P.E. (1995) Eutrophication. International Journal of Environmental Studies, 47, 173-195.

Palacios-Fest, M., Carreno, A.L., Ortega-Ramirez, J. & Alvarado-Valdez, G. (2002) A palaeoenvironmental reconstruction of laguna Babicora, Chihuahua, México based on ostrocod palaeoecology and trace element shell chemistry. Journal of Paleolimnology, 27, 185-206.

Palerm, A. (1981) The agricultural basis of urban civilization in Mesoamerica. In: J.A. Graham (Ed.), Ancient mesoamerica; selected readings, pp. 101-116. Peek Publishing, Palo Alto.

Palmer, M.W. (1993) Putting things in even better order: the advantages of canonical correspondance analysis. Ecology, 74, 2215-2230.

Patrick, R. & Reimer, C.W. (1966) The Diatoms of the United States. Exclusive of Alaska and Hawaii, Vol. 1. The Academy of Natural Sciences of Philadelphia, Philadelphia, 688 pp

Patrick, R. & Reimer, C.W. (1975) The Diatoms of the United States. Exclusive of Alaska and Hawaii. Vol 2 part 1. The Academy of Natural Sciences of Philadephia, Phiadelphia, 213 pp

Pearson, F.J. & Coplen, T.B. (1978) Stable Isotope studies of Lakes. In: A. Lerman (Ed.), Lakes: Chemistry, Geology, Physics, pp. 375-333. Springer Verlag, New York.

Pei, H., & Wang, H., (2003) Eutrophication research of West Lake, Hangzhou, China: modeling under uncertainty. Water Research, 37, 416-428.

Peterson, B.J. & Fry, B. (1987) Stable isotopes in ecosystem studies. Annual review of ecology and systematics, 18, 293-320.

Philibert, A. & Prairie, Y.T. (2002) Is the introduction of benthic species necessary for open-water chemical reconstruction in diatom-based transfer functions? Canadian Journal of Fisheries and Aquatic Sciences, 59, 938-951.

Pionke, H.B., Gburek, W.J., Sharpley, A.N. & Schnabel, R.R. (1996) Flow and nutrient export patterns for an agricultural hill-land watershed. Water Resources Research, 32, 1795-1804.

Pollard, H.P. (1993) Tariacuri's Legacy: The Prehispanic Tarascan State. University of Oaklahoma Press. 266 pp

Pollard, H.P. & Gorenstein, S. (1980) Agrarian potential, population and the Tarascan State. Science, 209, 274-277.

Prairie, Y.T., Duarte, C.M., & Klaff, J. (1989) Unifying nutrient chlorophyll relationships in lakes. Canadia Journal of Fisheries and Aquatic Sciences. 46, 1176-1182

Prartono, T. & Wolfe, G.A. (1998) Organic geochemistry of lacustrine sediments: a record of the changing trophic status of Rostherne Mere. Organic Geochemistry, 28, 729-747.

Racca, J.M.J., Philibert, A., Racca, R. & Prairie, Y.T. (2001) A comparison between diatom-based pH inference models using artificial neural networks (ANN), weighted averaging (WA) and weighted averaging partial least squares (WA-PLS) regressions. Journal of Paleolimnology, 26, 411-422.

Racca, J.M.J. & Prairie, Y.T. (2004) Apparent and real bias in numerical transfer functions in palaeolimnology. Journal of Paleolimnology, 31, 117-124.

Ramstack, J.M., Fritz, S.C., Engstrom, D.R. & Heiskary, S.A. (2003) The application of a diatom based transfer function to evaluate regional water-quality trends in Minnesota since 1970. Journal of Paleolimnology, 29, 79-94.

Ramstack, J.M., Fritz, S.C. & Engstrom, D.R. (2004) Twentieth century water quality trends in Minnesota lakes compared with pre-settlement variability. Canadian Journal of Fisheries and Aquatic Sciences, 61 561-576

Rast, W. & Thornton, J.A. (1996) Trends in eutrophication research and control. Hydrological Processes, 10, 295-313.

Reed, J.M. (1998) A diatom-conductivity transfer function for Spanish salt lakes. Journal of Paleolimnology, 19, 399-416.

Reid, M. (2005) Diatom based models for reconstructing past water quality and productivity in New Zealand lakes. Journal of Paleolimnology, 33, 13-38.

Renberg, I. (1990). A procedure for preparing large sets of diatom slides from sediment cores. Journal of Paleolimnology 4, 87-90

Renberg, I. & Hellberg, T. (1982) The pH history of lakes in south western Sweden, as calculated from the subfossil diatom flora of the sediments. Ambio, 11, 30-33

Reynolds, C.S. (1986). The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge, 384 pp

Reynolds , C.S. & Davies, P.S. (2001) Sources and bioavailability if phosphorus fractions in freshwaters: a British perspective. Biological Review, 76, 27-64.

Richardson, J.L. (1968) Diatoms and lake typology in East and Central Africa. International Revue ges Hydrobiologia, 53, 299-338.

Richardson, J.L., Harvey, T.J. & Holdship, S.A. (1978) Diatom in the history of shallow East African lakes. Polskie Archiwum Hydrobiologii, 25, 341-353.

Rico, R., Vilaclara, G., Miranda, J. & Canetas, J. (1997) Origin of laminations in Tlaxcala Paleolake, México. Verhandlungen Internationale Vereinigung für Limnologie, 26, 383-841.

Rippey, B. & Anderson, N.J. (1996) Reconstructions of lake phosphorus loading and dynamics using the sedimentary record. Environmental Science and Technology, 30, 1786-1788.

Rippey, B., Anderson, N.J. & Foy, R.H. (1997) Accuracy of diatom-inferred total phosphorus concentrations and the accelerated eutrophictaion of a lake due to reduced flushing and increased internal loading. Canadian Journal of Fisheries and Aquatic Sciences, 54, 2637-2646.

Robin, C., Mossand, P., Camus, G., Cantagrel, J-M., Gourgaud, A. & Vincent, P.M. (1987) Eruptive history of the Colima volcanic complex (México). Journal of Volcanology and Geothermal Research, 31, 99-113.

Robson, J.N. & Rowland, S.J. (1988) Synthesis of a highly branced C30 sedimentary hydrocarbon: Tetrahedron letters, 29, 3837-3840.

Rosas, I., Velasco, A., Belmont, R., Baez, A. & Martinez, A. (1993) The algal community as an indicator of the trophic status of Lake Patzcuáro, México. Environmental Pollution, 80, 255-264.

Rosenmeier, M.F., Hodell, D.A., Brenner, M. & Curtis, J.H. (2002) A 4000-year lacustrine record of environmental change in the southern Maya lowlands, Peten, Guatemala. Quaternary Research, 57, 183-190.

Rosenmeier, M.F., Brenner, M., Kenney, W.F., Whitmore, T.J. & Taylor, C.M. (2004) Recent eutrophication in the sourthern basin of Lake Peten Itza, Guatemala: human impact on a large tropical lake. Hydrobiologia, 511, 161-172.

Rowland, S.J. & Robson, J.N. (1990) The widespread occurrence of highly branched acyclic C20, C25 and C30 hydrocarbons in recent sediments and biota - A review. Marine Environmental Research, 30, 191-216.

Ruley, J.E. & Rusch, K.A., (2002) An assessment of long-term post-restoration water quality trends in a shallow, subtropical, urban hypereutrophic lake. Ecological Engineering, 19, 265-280.

Round, F.E., Crawford, R.M. & Mann, D.G. (1990) The Diatoms: biology & morphology of the genera. Cambridge University Press, Cambridge, 747 pp.

Ryding, S.-O. & Rast, W. (1989) The control of eutrophication of lakes and reservoirs. UNESCO, Paris 250pp

Ryves, D.B., McGowan, S. & Anderson, N.J. (2002) Development and evaluation of a diatom-conductivity model from lakes in West Greenland. Freshwater Biology, 47, 995-1014.

Rzedowski, J. (1986) Vegetacion de México. Editorial Limusa, México DF.

Sakata, S., Hayes, J.M., McTaggart, A.R., Evans, R.A., Keckrone, K.J. & Togasaki, R.K. (1997) Carbon isotopic fractionation associated with lipid biosynthesis by a cyanobactirium: Relevance for interpretation of biomarker records. Geochimica et Cosmochimica Acta, 61, 5379-5389.

Sakamoto, M. (1996) Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. Archive fur Hydrobiologie 62 1-28

Sanchez, W.A. & Kutzbach, J.E. (1974) Climate of the American tropics and subtropics in the 1960s and possible comparisons with climatic variations of the last millennium. Quaternary Research, 4, 128-135.

Sanders, W.T. (1981) Cultural ecology of nuclear Mesoamerica. In: J.A. Graham (Ed.), Ancient Mesoamerica; selected readings, pp. 35-44. Peek Publishing, Palo Alto.

Saros, J.E. & Fritz, S.C. (2000) Nutrients as a link between ionic concentration/composition and diatom distributions in saline lakes. Journal of Paleolimnology, 23, 449-453.

Saros, J.E. & Fritz, S.C. (2002) Resource competition among saline-lakes diatoms under varying N/P ratio, salinity and anion composition. Freshwater Biology, 47, 87-95.

Sas, H. (1990) Lake Restoration of nutrient loadings: Expectations, experiences and extrapolations. Verhandlungen Internationale Vereinigung für Limnologie, 24, 247-251.

Sayer, C. (2001) Problems with the application of diatom-total phosphorus transfer functions: examples from a shallow eutrophic English lake. Freshwater Biology, 46, 743-757.

Sayer, C. & Roberts, N. (2001) Establishing realistic restoration targets for nutrient-enriched shallow lakes: linking diatom ecology and palaeoecology at the Attenborough Ponds, UK. Hydrobiologia, 448, 117-142.

Schelske, C. & Hodell, D.A. (1991) Recent changes in productivity and climate of lake ontario detected by isotopic analysis of sediments. Limnology and Oceanography, 35, 961-975.

Schelske, C. & Kenney, W.F. (2001) Model erroneously predicts failure for restoration of Lake Apopka, a hypereutrophic, subtropical lake. Hydrobiologia, 448, 1-5.

Schelske, C.L. & Stoermer, E.F. (1971) Eutrophication, silica depletion and predicted changes in algal quality in Lake Michigan. Science, 173, 423-424

Schindler, D.W. (1977) Evolution of phosphorus limitation in lakes. Science, 195, 260-262.

Schindler, D.W. (1987) Detecting ecosystem responses to anthropogenic stress. Canadian Journal of Fisheries and Aquatic Sciences, 44, 6-25.

Schubert, C.J., Villanueva, J., Calvert, S.E., Cowie, G.L., von Rad, U., Schulz, H., Berner, U. & Erlenkeuser, H. (1998) Stable phytoplankton community structure in the Arabina Sea over the past 200,000 years. Nature, 394, 563-566.

Sears, P.B. & Clisby, K.H. (1955) Palynology in southern North America part IV: Pleistocene climate in México. Bulletin of the Geological Society of America, 66, 521-530.

Seltzer, G.O., Rodbell, D.T., Baker, P.A., Fritz, S.C., Tapia, P.M., Rowe, H.D. & Dunbar, R.B. (2002) Early warming of Tropical South America at the Last Glacial-Interglacial Transition. Science, 269, 1685-1686.

Servant-Vildary, S. & Roux, M. (1990) Multivariate analysis of diatoms and water chemistry in Bolivian saline lakes. Hydrobiologia, 197, 267-290.

Shapiro, J., & Wright, D.I. (1984). Lake restoration by biomanipulation - round lake, Minnesota, the 1st 2 years. Freshwater Biology, 14, 371-383.

Sharpley, A.N., Smith, S.J., Jones, O.R., Berg, W.A. & Coleman, G.A. (1992). The transport of bioavailable phosphorus in agricultural runoff. Journal of Environmental Quality, 21, 30-35.

Silliman, J.E. & Schelske, C. (2003) Saturated hydrocarbons in the sediments of Lake Apopka, Florida. Organic Geochemistry, 34, 253-60.

Sims, J.T., Simard, R.R. & Joern, B.C. (1998) Phosphrous loss in agricultural drainage: Historical perspective and current research. Journal of Environmental Quality, 27, 277-293.

Siver, P.A. (1999) Development of paleolimnological inference models for pH, total nitrogen and specific conductivity based on planktonic diatoms. Journal of Paleolimnology, 21, 45-59.

Siver, P.A., Lott, A.M., Cash, E., Moss, J. & Marsicano, L.J. (1999) Century changes in Conneticut, USA, lakes as inferred from siliceous algal remains and their relatioship to land-use change. Limnology and Oceanography, 44, 1928-1935.

Sladecek, V. & Vilaclara, G. (1993) A water bloom in Lake Patzcuáro. Verhandlungen Internationale Vereinigung für Limnologie, 25, 431-435.

Sluyter, A. (1997) Regional, Holocene records of the human dimension of global change: sea-level and land-use change in prehistoric México. Global and Planetary Change, 14, 127-146.

Smith, M.E. (2003) The Aztecs. Blackwell Publishing, Oxford. 367 pp

Smith, V.H. & Shapiro, J. (1981) Chlorophyll-phosphorus relations in individual lakes. Their importance to restoration strategies. Environmental Science and Technology, 15, 444-451.

Smittenberg, R.H., Pancost, R.D., Hopmans, E.C., Paetzel, M. & Sinninghe Damste, J.S. (2004) A 400-year record of Environmental change in an euxinix fjord as revealed by the sedimentary biomarker record. Palaeogeography, Palaeoclimatology, Palaeoecology, 202, 331-351.

Smol, J.P. (1990) Are we building enough bridges between paleolimnology and aquatic ecology? Hydrobiologia, 214, 201-206.

Smol, J.P. (2002) Pollution of Rivers and Lakes - a palaeoenvironmental perspective. Arnold, London, 280 pp

Smol, J.P., Cumming, B.F., (2003) Tracking long-term changes in climate using algal indicators in lake sediment. Journal of Phycology, 36, 986-1011.

Sommer, U. (1984) Sedimentation of principal phytoplankton species in Lake Constance. Journal of Plankton Research, 6, 1-14.

Sommer, U. (1989) Nutrient status and nutrient competition of phytoplankton in a shallow hypertrophic lake. Limnology and Oceanography, 34, 1162-1173.

Sommer, U. (1993) Phytoplankton competitions in Plußsee: a field test of the resource-ratio hypothesis. Limnology and Oceanography, 38, 838-845.

Sommer, U. (1994) The impact of light intensity and day length on silicate and nitrate competition among marine phytoplankton. Limnology and Oceanography, 39, 1680-1688.

Sondergaard, M. (1989) Phosphorus release from a hypertrophic lake sediment: Experiments with intact sediment cores in a continuous flow system. Archive fur Hydrobiologie, 116, 45-49.

Sondergaard, M., Jensen, J.P. & Jeppesen, E. (1999) Internal phosphorus loading in shallow Danish lakes. Hydrobiologia, 408/409, 145-152.

Sondergaard, M., Jeppesen, E., Kristensen, P. & Sortkjaer, O. (1990) Interactions between sediment and water in a shallow and hypertrophic lake: a study on phytoplankton collapses in lake Sobygard, Denmark. Hydrobiologia, 191, 139-148.

Sondergaard, M., Jeppesen, E. & Sortkjaer, O. (1987) Lake Sobygaard: a shallow lake in recovery after a reduction in phosphorus loading. Geojournal, 14, 381-384.

Stauffer, J. (1998). The Water Crisis: Constructing Solutions o Freshwater Pollution. Earthscan Publications Ltd, London, 161 pp

Stockner, J.G. (1971) Preliminary characterization of lakes in the Experimental Lakes Area, Northwest Ontario using diatom occurrences in sediments. Journal of the Fisheries Research Board of Canada, 28, 265-275.

Stockner, J.G. & Benson, W.W. (1967) The succession of diatom assemblages in the recent sediments of Lake Washington. Limnology and Oceanography, 12, 513-531.

Stoermer, E.F. (1985) Phytoplankton assemblages as indicators of water quality in the Laurentide Great Lakes. Transactions of the American Microscopy Society, 97, 2-16

Stoermer, E.F. & Smol, J.P. (1999) The diatoms: applications for the environmental and earth sciences. Cambridge University Press, Cambridge 469 pp

Stoermer, E.F., Wolin, J.A., Schelske, C. & Conley, D.J. (1985) As assessment of ecologicla changes during the recent history of Lake Ontario based on siliceous algal microfossils preserved in the sediments. Journal of Phycology, 21, 257-276.

Straka, H. & Ohngemach, D., (1989) Late Quaternary vegetation history of the Mexican highland. Plant Systematics and Evolution, 162, 115-132.

Street-Perrott, F.A., Perrott, R.A. & Harkness, D.D. (1989) Anthropogenic soil erosion around Lake Patzcuáro, Michoácan, México, during the Pre-Classic and Late Post-Classic-Hispanic periods. American Antiquity, 54, 759-765.

Suarez, E., Segura, L. & Fernandez, M.A. (1986) Diversidad y abundancia del plancton en la Laguna de Catemaco, Veracruz durante un ciclo anual. Anales del Instituto Ciences del Mar y Limnologia, Universidad Nacional Automoma de México, 13, 313-316.

Suchlicki, J. (2001) México: from the Montezuma to the fall of the PRI. Brassey's Inc, Washington, D.C., 228 pp

Swain, E.B. (1985) Measurement and interpretation of sedimentary pigments. Freshwater Biology, 15, 53-75.

Talbot, M.R. (2001). Nitrogen Isotopes in palaeolimnology. In Tracking Environmental Change Using Lake Sediments.

Talbot, M.R. & Johannessen, T. (1992) A high-resolution paleoclimatic record for the last 27,500 years in tropical west africa from the carbon and nitrogen isotopic composition of lacustrine organic-matter. Earth and Planetary Science Letters 110, 23-37.

Talbot, M.R. & Lærdal, T. (2000) The Late Pleistocene - Holocene palaeolimnology of Lake Victoria, East Africa, based upon elemental and isotopic analyses of sedimentary organic matter. Journal of Paleolimnology 23, 141-164

Talling, J.F. (2001) Environmental controls on the functioning of shallow tropical lakes. Hydrobiologia, 458, 1-8.

Tilman, D. (1982). Resource competition and community structure. Princeton University Press, Princeton, USA, 296 pp

Tilman, D., Kilham, S.S. & Kilham, P. (1982) Phytoplankton community ecology: The role of limiting nutrients. Ann. Rev. Ecol. Syst. 13: 349-72.

Telford, R.J., & Lamb, H.F. (1999) Groundwater-mediated response to Holocene climatic change recorded by the diatom stratigraphy of an Ethiopian crater lake. Quaternary Research, 52, 63-75.

Tenzer, G.E., Meyers, P.A., Robbins, J.A., Eadie, B.J., Morehead, N.R. & Lansing, M.B. (1999) Sedimentary organic matter record of recent environmental changes in the St Mary's River ecosystem, Michigan-Ontario border. Organic Geochemistry, 30, 133-146.

ter Braak, C.J.F. & Prentice, I.C. (1988) A theory of gradient analysis. Advances in Ecological Research, 18, 271-317.

ter Braak, C.J.F. & van Dam, H. (1989) Inferring pH from diatoms: a comparison of old and new calibration methods. Hydrobiologia, 178, 209-223.

ter Braak, C.J.F. & Juggins, S. (1993) Weighted average partial least squares regression (WA-PLS): an improved method for reconstructing environmental variables from species assemblages. Hydrobiologia, 269/270, 485-502.

ter Braak, C.J.F. & Šmilauer, P. (2002) Canoco reference manual and CanoDraw for Windows user's guide: Software for canonical community ordination (version 4.5): Ithaca, NY, USA, Microcomputer Power, 500pp.

Teranes, J.L., McKenzie, J.A., Lotter, A.F. & Sturm, M. (1999) Stable isotope response to lake eutrophication: Calibration of a high-resolution lacustrine sequence from Baldeggersee, Switzerland. Limnology and Oceanography, 44, 320-333.

Teranes, J.L. & Bernasconi, S.M. (2000) The record of nitrate utilization and productivity limitation provided by D15N values in organic matter-A study of sediment trap and core sediments from Baldeggersee, Switzerland. Limnology and Oceanography, 45, 801-813.

Tavera, R. & Martinez-Almeida, V. (2005) Atelomixis as a possible driving force in the phytoplankton composition of Zirahuén, a warm-monomictic tropical lake: Hydrobiologia, 533, 199-208.

Thompson, R. & Oldfield, F. (1986) Environmental Magnetism. Allen & Unwin, London, 227 pp

Tibby, J. (2004) Development of a diatom based model for inferring total phosphrous in southeastern Australian water storages. Journal of Paleolimnology, 31, 23-36.

Tilman, D., Kiesling, R., Sterner, R., Kilham, S.S. & Johnson, F.A. (1986) Green, bluegreen and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Archive fur Hydrobiologie, 106, 473-485.

Townsend, R.F. (2000) The Aztecs. Thames and Hudson, London 232 pp

Tricart, J. & de los Rios Paredes, M. (1985) L'ancien lac de Zacapu, mise en evidence d'une transgression lactustre au quaternaire recent. TRACE, 8, 46-51.

Uinited Nations (2005). Millenium Development Goals Report 2005.

UNESCO (2003) The UN world Water Development Report: Water for People, Water for Life. UNESCO, Paris, pp 535

Van Dam, H., Mertens, A. & Sinkeldam, J. (1994) A coded checklist and ecological inidactor values of freshwater diatoms from the Netherlands: Netherlands Journal of Aquatic Ecology, 28, 117-133.

Van Donk, E. & Kilham, S.S. (1990) Temperature effects on silicon-and phosphorus-limited growth and competitive interactions among three diatoms. Journal of Phycology, 26, 40-50.

Van Tongeren, O.F.R., (1995) Cluster Analysis. In: R.H.G. Jongman, C.J.F. ter Braak & O.F.R. Van Tongeren (Eds.), Data analysis in community and landscape ecology p174-212 Cambridge University Press, Cambridge.

Vilaclara, G. (1989) Estrategias de Adaptation Ecologica del fitoplancton. Revista de la Sociedad Mexicana de Historia Natural, 40, 72-76.

Vilaclara, G., Chavez, M., Lugo, A., Gonzalez, H. & Gaytan, M. (1993) Comparitive description of crater-lakes basic chemistry in Puebla state, México. Verhandlungen Internationale Vereinigung für Limnologie, 25, 435-440.

Volkman, J.K., Barrett, S.M. & Dunstan, G.A. (1994) C25 and C30 highy branched isoprenoid alkanes in laboratory cultures of two marine diatoms. Organic Geochemistry, 21, 407-413.

Volkman, J.K., Barrett, S.M., Blackburn, S.I., Mansour, M.P., Sikes, E.L. & Gelin, F. (1998) Microalgal biomarkers: a review of recent research developments Organic Geochemistry, 29, 1163-1179.

Volkman, J.K., Barrett, S.M. & Blackburn, S.I. (1999) Eustigmatophyte microalgae are potential sources of C29 sterols, C22-C28 n-alcohols and C28-C32 n-alkyl diols in freshwater environments. Organic Geochemistry, 30, 307-318.

Wallen, C.C. (1955) Some characteristics of precipitation in México. Geografiska Annaler, 37, 51-85.

Wang, R. (1998) Acyclic isoprenoids - molecular indicators of archaeal activity in contemporary and ancient Chinese saline/hyperasline environments. Hydrobiologia, 381, 59-76.

Wang, R.L., Brassell, S.C., Scarpitta, S.C., Zheng, M.P., Zhang, S.C., Hayde, P.R. & Muench, L.M. (2004) Steroids from sediments from Zabuye Salt Lake, western Tibet: diagenetic, ecological or climatic signals? Organic Geochemistry, 35, 157-168.

Watts, W.A. & Platt Bradbury, J. (1982) Palaeoecological studies at Lake Patzcuáro on the west-central Mexican Plateau and at Chalco in the Basin of México. Quaternary Research, 17, 56-70.

West, R.C. (1948) The Cultural Geography of the Modern Tarascan Area. Smithsonian Institution Institute of Social Anthropology Publication No. 7. Greenwood Press, Westport Connecticut 77 pp + plates

West, R.C. (1964) Natural Environment & Early Cultures. University of Texas Press, Austin, 526 pp

Wetzel, R.G. (1992) Gradient-dominated ecosystems: sources and regulatory functions of dissolved organic matter in freshwater ecosystems. Hydrobiologia, 229, 181-198.

Wetzel, R.G. (1992) Clean water: a fading resource. Hydrobiologia, 243/244, 21-30.

Wetzel, R.G. (2001) Limnology: lake and river ecosystems. Academic Press, San Diego, 1006 pp

Whiteside, M.C. (1983) The mythical concept of eutrophication. Hydrobiologia, 103, 107-111.

Whitmore, T.J. (1989) Florida diatom assemblages as indicators of trophic state and pH. Limnology and Oceanography, 34, 882-895.

Whitmore, T.J., Brenner, M., Curtis, J.H., Dahlin, B.H. & Leyden, B.W. (1996) Holocene climatic and human influences on the lakes of the Yucatan Penninsula, México: an interdisciplinary, palaeolimnological approach. The Holocene, 6, 273-287.

Whitmore, T.M. (1991) A simulation of the sixteenth-century population collapse in the Basin of México. Annals of the Association of American Geographers, 8, 464-487.

Whitmore, T.M. & Turner, B.L. (1992) Landscapes of cultivation in Mesoamerica on the eve of the Conquest. Annals of the Association of American Geographers, 82, 402-425.

Wilkes, H., Ramrath, A. & Negendank, J.F.W. (1999) Organic geochemical evidence for environmental changes since 34,000 yrs BP from Lago di Mezzano, central Italy. Journal of Paleolimnology, 22, 349-365.

Wright, H.E. (1977) Environmental change and the origin of agriculture in the Old and new Worlds. In: C.A. Reed (Ed.), Origins of Agriculture, pp. 281-318. Mouton Publishers, The Hague.

Wurtsbaugh, W.A., Vincent, W.F., Alfaro Tapia, R., Vincent, C.L. & Richerson, P.J. (1985) Nutrient limitation of algal growth and nitrogen fixation in a tropical alpine lake, Lake Titicaca (Peru/Bolivia). Freshwater Biology, 15, 185-195.

Yang, J.-R., Pick, F.R. & Hamilton, P.B. (1996) Changes in planktonic diatom flora of a large mountain lake in response to fertilization. Journal of Phycology, 32, 232-243.

Young, A. (1976) Tropical Soils and Soil Survey. Cambridge University Press, Cambridge.

Zalat, A. & Vildary, S.S. (2005) Distribution of diatom assemblages and their relationship to environmental variables in the surface sediments of three northern Egyptian lakes. Journal of Paleolimnology, 34, 159-174.

Zeeb, B.A., Christie, C.E., Smol, J.P., Findlay, D.L., Kling, H. & Birks, H.J.B. (1994) Responses of diatom and chrystophyte assemblages in Lake 227 sediments to experimental eutrophication: Canadian Journal of Fisheries and Aquatic Sciences, 51, 2300-2311.

Zhang, H.C., Cao, Z.H., Shen, Q.R. & Wong, M.H. (2003) Effect of phosphate fertilizer application on phosphorus (P) losses from paddy soils in Taihu Lake region I. Effect of fertilizer rate on P losses from paddy soil. Chemosphere, 50, 695-701.

Zhang, Y. & Prepas, E.E. (1996) Regulation of the dominance of planktonic diatoms and cyanobacteria in four eutrophic hardwater lakes by nutrients, water column stability and temperature. Canadian Journal of Fisheries and Aquatic Sciences, 53, 621-633.

Zhou, Y., Li, J. & Zhang, M. (2001) Vertical variations in kinetics of alkaline phosphates and P species in sediments of a shallow Chinese eutrophic lake (Lake Donghu). Hydrobiologia, 450, 91-98.

Ziller, S. & Economou-Amilli, A. (1998) Freshwater algae from lakes in the lower Niger Delta system (Nigeria). Hydrobiologia, 368, 217-229.

Table of Appendices

Appendix 1
Dry season field data
Wet season field data
Dry season ionic data
Wet season ionic data
Dry season nutrient data
Wet season nutrient data

Appendix 2
Modern diatom species, codes and authorities
Diatom species counts for modern samples (% relative abundance)
TWINSPAN analysis
Diatom plates (fossil and modern material)

Appendix 3
Results of CCA and WA

Appendix 4
Structures of major lipids identified in the study

Table A1.1 Dry season field data

Site	State	Date sampled	Altitude (m.a.s.l.)	Depth (m)	Temp (C)	pH	EC (µS/cm)	TDS (mg/l)	Secchi depth (m)
Lago de Yuriria	Guanajuato	15/03/2003	1740	1.8	18	7.98	818	406	0.26
Piscina de Yuriria	Guanauato	15/03/2003	1730	0.2	18	9.46	1907	1452	0.08
Zapotlan	Jalisco	18/03/2003	1570	3.6	21.8	8.69	925	467	0.28
Atotonilco	Jalisco	19/03/2003	1367	1	27.8	8.39	1373	629	0.1
Los Negritos	Michoacan	04/05/2003	1566	7.8	28.1	6.67	3834	1880	0.75
Chapala	Jalisco	05/05/2003	1519	1.4	23.4	8.89	1214	605	0.2
Cajititlan	Jalisco	05/05/2003	1551	1.8	25.6	8.19	795	396	0.14
Cuitzeo (East)	Guanajuato	07/05/2003	1551	0.5	27.9	9.12	3808	1906	0.03
Tecuitlapa	Puebla	23/04/2003	2360	3	18.7	7.29	14	0	0.22
La Alberca	Michoacan	14/03/2003	1892	9.9	18	8.3	436	218.5	0.18
Laguna La Maria	Colima	18/03/2003	1240	21	23.5	7.88	629	315	0.7874
San Pedro Lagunillas	Nayarit	22/03/2003	1265	7.3	23.6	8.67	287	146	0.405
Tepitiltic	Nayarit	22/03/2003	1410	3	19.8	6.43	139.5	69.5	1.89
Lago de Pátzcuaro	Michoacan	11/04/2003	2044	5.1	20.9	8.71	1054	529	0.33
Laguna San Gregorio	Michoacan	11/04/2003	3100	1.8	18.1	6.98	22.1	10.5	0.47
Laguna Zacapu	Michoacan	12/04/2003	1980	3.6	20.7	8.35	148.1	74.3	0.62
Santa Maria del Oro	Nayarit	14/04/2003	700	53	26.9	8.87	1234	642	8.87
Camecuaro	Michoacan	06/05/2003	1551	1.9	21.6	6.87	200	91.2	1.9
Laguna Juanacatlán	Jalisco	20/03/2003	1995	22	17.7	6.87	127.2	63.1	2.28
La Hoya de los Espinos	Michoacan	12/04/2003	1980	40.1	21.4	8.73	1045	530	2.3
La Laguna	Michoacan	02/05/2003	1487	5.84	26.2	7.81	122.3	61.2	0.63
La Alberca (Tacambaro)	Michoacan	02/05/2003	1452	35	24.8	8.58	178.1	89.3	0.82
Laguna Zempoala	Morleos	01/06/2003	2808	4.75	19.3	7.0	87.5	43.1	1.7
Alchichica	Puebla	22/04/2003	2325	61	19.7	9.05	13740	8790	4.52
Quechulac	Puebla	22/04/2003	2344	40	19.26	8.7	796	400	5.45
Atexcac	Puebla	23/04/2003	2359	50	19.06	8.36	11201	6400	5.75
Lago del Sol	Estado de Mexico	31/05/2003	4200	11	8	6.9	62.1	30.1	8
Zirahuen	Michoacan	02/05/2003	2070	50	21	8.26	109.1	54.8	5.72
Lago de al Luna	Estado de Mexico	31/05/2003	4206	8	8.5	4.6	19.3	10.5	8
La Preciosa	Puebla	22/04/2003	2337	45	17.78	8.5	2122	1100	4.51

Table A1.2 Wet season field data

Lake	**Date Sampled**	**Temp (°C)**	**pH**	**EC (µS/cm)**	**SD (m)**
Lago de Yuríria	07/07/2004	20.2	8.8	496	0.12
Piscina de Yuríria	07/07/2004	23.7	9.57	2910	0.09
Zapotlan	23/08/2004	25.1	8.63	661	25.1
Atotonilco	23/08/2004	30	9.41	5200	0.02
Los Negritos	19/08/2004	29	8.31	4030	66
Chapala	27/07/2004	26.3	7.853	718.333	0.26
Cajititlan	27/07/2004	26.1	8.68	1043.6	0.2
Cuitzeo (East)	07/07/2004	28.3	9.48	1494	0.06
Tecuitlapa	02/09/2004	25.4	9.93	2790	0.15
La Alberca	08/07/2004	20.1	8.78	326	0.42
Laguna La Maria	24/08/2004	26.2	8.8	611	1.37
San Pedro Lagunillas	24/07/2004	27.3	9	371.3	0.37
Tepitiltic	24/07/2004	28.1	9.67	155.7	1.89
Lago de Pátzcuaro	18/08/2004	23.8	10.4	980	0.34
Laguna San Gregorio	09/07/2004	18.7	6.9	20	0.41
Laguna Zacapu	08/07/2004	20.8	8.64	144	1.32
Santa Maria del Oro	26/07/2004	33.4	8.67	1646	2.3
Camecuaro	19/08/2004	21.5	7.85	179.87	2.4
Laguna Juanacatlán	23/07/2004	23.3	7.94	122.83	2.95
La Hoya de los Espinos	08/07/2004	24.2	8.33	1014	8
La Laguna	09/07/2004	30.7	8.8	95	0.42
La Alberca (Tac)	09/07/2004	29.4	8.1	154.7	0.91
Laguna Zempoala	14/08/2004	18	7.98	70.8	2.43
Alchichica	09/08/2004	20.55	8.78	12680	6.49
Quechulac	01/09/2004	21.5	8.567	655	6.36
Atexcac	03/09/2004	20.5	8.5	12151	4
Lago del Sol	15/09/2004	14	6.9	56.23	4.6
Zirahuen	18/08/2004	23.3	8.55	119	3.14
Lago de al Luna	15/09/2004	12.6	5.807	14.6	7.7
La Preciosa	02/09/2004	22.467	8.473	1887.667	2.31

Table A1.3 Dry season ionic data

Site	**HCO_3^- + CO_3^{2-} Alk (meq/l)**	**SO_4^{2-} (meq/l)**	**Cl^- (meq/l)**	**Ca^{2+} (meq/l)**	**Mg^{2+} (meq/l)**	**K^+ (meq/l)**	**Na^+ (meq/l)**
Lago de Yuríria	7.2	0.15	1.69	2.40	2.24	0.76	5.51
Piscina de Yuríria	20.8	0.43	6.21	2.88	2.39	1.70	26.32
Zapotlan	8.4	0.17	2.82	1.88	0.24	0.95	4.74
Atotonilco	10.4	0.22	4.51	2.40	4.31	0.64	14.12
Los Negritos	6.4	0.13	20.88	1.28	1.04	2.09	0.37
Chapala	10.4	0.22	3.39	2.32	2.45	1.10	8.31
Cajititlan	8.4	0.17	1.97	1.84	2.64	0.98	5.08
Cuitzeo (East)	16.4	0.34	13.54	0.80	2.00	1.76	39.62
Tecuitlapa	26.4	0.55	3.95	1.04	1.76	1.83	25.49
La Alberca	6	0.12	0.56	1.52	2.36	0.45	1.77
Laguna La Maria	6.8	0.14	1.13	2.00	4.39	0.09	2.30
San Pedro Lagunillas	2.8	0.06	0.99	1.28	0.96	0.76	1.23
Tepitiltic	2	0.04	0.56	5.68	3.31	0.24	0.27
Lago de Pátzcuaro	10.4	0.22	1.41	0.96	3.20	1.14	6.94
Laguna San Gregorio	1.3	0.03	0.42	0.58	0.86	0.06	0.13
Laguna Zacapu	2.4	0.05	0.42	0.76	1.24	0.03	0.55
Santa Maria del Oro	9.2	0.19	8.46	1.64	5.35	0.48	7.86
Camecuaro	2	0.04	0.42	0.56	1.76	0.09	0.84
Laguna Juanacatlán	2	0.04	0.56	0.88	0.72	0.06	0.27
La Hoya de los Espinos	8	0.17	5.64	2.48	4.91	0.72	5.11
La Laguna	2	0.04	2.82	0.44	1.24	0.12	0.20
La Alberca (Tacambaro)	2	0.04	0.71	0.80	1.52	0.09	0.33
Laguna Zempoala	1	0.02	0.28	0.85	1.52	0.04	0.17
Alchichica	37.2	0.77	91.40	2.00	36.77	6.17	108.02
Quechulac	7.2	0.15	3.39	2.40	5.57	0.19	3.27
Atexcac	24	0.50	90.84	3.20	36.77	2.40	76.28
Lago del Sol	0.6	0.01	0.28	0.51	1.18	0.02	0.04
Zirahuen	1.4	0.03	0.42	0.76	1.00	0.13	0.27
Lago de al Luna	0.1	0.00	0.14	0.05	0.23	0.01	0.00
La Preciosa	14.4	0.30	10.72	0.88	16.86	0.45	7.93

Table A1.4 Wet Season ionic data (ND = no data)

Lake	**$HCO_3^- + CO_3^{2-}$ Alk (meq/l)**	**SO_4^{2-} (meq/l)**	**Cl^- (meq/l)**	**Ca^{2+} (meq/l)**	**Mg^{2+} (meq/l)**	**Na^+ (meq/l)**	**K^+ (meq/l)**
Lago de Yuríria	1.2	0.11	2.86	0.84	2.76	4.87	0.47
Piscina de Yuríria	16.4	0.85	16.68	2.56	ND	163.38	1.48
Zapotlan	6.8	0.33	3.42	0.92	1.24	5.60	0.51
Atotonilco	5.2	0.31	6.00	0.32	2.24	423.98	0.99
Los Negritos	6.4	0.33	36.78	0.80	0.96	560.19	1.62
Chapala	5.6	0.32	3.70	1.84	1.68	7.05	0.49
Cajititlan	10.4	0.21	5.85	2.64	1.44	16.83	0.96
Cuitzeo (East)	6.8	0.34	17.24	7.20	ND	56.14	0.70
Tecuitlapa	27.6	0.25	9.38	2.99	ND	0.31	0.19
La Alberca	4.4	0.14	0.92	1.00	0.20	2.19	0.31
Laguna La Maria	6.8	0.00	1.41	1.20	1.04	2.65	0.07
San Pedro Lagunillas	3.2	0.09	1.70	0.68	0.20	2.17	0.63
Tepitiltic	2.4	0.12	0.60	0.78	0.82	0.31	0.19
Lago de Pátzcuaro	9.2	0.11	2.54	1.28	0.16	11.15	0.80
Laguna San Gregorio	0.3	0.16	0.58	0.20	ND	0.08	0.05
Laguna Zacapu	1.95	0.09	0.75	0.48	ND	0.53	1.05
Santa Maria del Oro	8.4	0.08	17.97	1.60	5.12	22.33	0.47
Camecuaro	1.8	0.18	0.89	0.56	1.16	0.82	0.05
Laguna Juanacatlán	1.75	0.19	0.35	0.74	0.78	0.53	0.04
La Hoya de los Espinos	7.2	0.06	15.17	1.44	3.36	7.97	0.43
La Laguna	1.4	0.16	1.25	0.40	ND	2.50	0.17
La Alberca (Tac)	2.4	0.03	0.62	0.72	1.28	0.34	0.10
Laguna Zempoala	1.1	0.00	0.44	0.24	0.48	0.56	0.04
Alchichica	10	0.93	143.98	20.16	27.91	1761.39	2.68
Quechulac	6.8	0.18	4.74	1.80	3.62	5.31	0.17
Atexcac	29.6	0.86	174.79	25.95	24.41	985.25	2.09
Lago del Sol	0.6	0.07	0.27	0.20	0.80	0.07	0.56
Zirahuen	1.3	0.01	0.42	0.60	0.56	0.36	0.47
Lago de al Luna	0.1	0.01	0.25	1.16	0.64	2.04	0.03
La Preciosa	13.6	0.47	9.86	1.75	14.25	24.52	0.37

Table A1.5 Dry Season nutrient data (ND = No data)

Site	**TP (µg/l)**	**SRP (µg/l)**	**NO_3-N (mg/l)**	**NO_2-N (mg/l)**	**NH_4-N (mg/l)**	**SiO_2 (mg/l)**	**Chl-a (µg/l)**
Lago de Yuríria	500.000	1588.333	24.6837	0.0082	0.0034	80	33.413
Piscina de Yuríria	562	405.927	60.7826	0.0043	0.0102	100	27.323
Zapotlan	297.685	340.32	23.2167	0.0000	0.0032	3	15.986
Atotonilco	665.500	1312.2	299.5865	0.0348	0.0664	100	2.306
Los Negritos	376.289	403.2	5.1893	0.0013	0.0086	320	2.507
Chapala	426	391.65	12.7336	0.0023	0.0039	17	3.318
Cajititlan	600	666.66	33.8853	0.0022	0.0104	100	23.860
Cuitzeo (East)	870	952.925	78.5626	0.0515	0.0416	120	57.728
Tecuitlapa	208	181.65	27.6297	0.0012	0.0072	14	33.078
La Alberca	72.8	33.6	10.3625	0.0017	0.0040	180	42.956
Laguna La Maria	46.079	27.508	13.8909	0.0000	0.0037	100	
San Pedro Lagunillas	41.898	3.4057	7.5702	0.0000	0.0261	1	24.344
Tepitiltic	43.326	16.581	8.5587	0.0000	0.0048	10.5	19.556
Lago de Pátzcuaro	44.85	34.44	10.7798	0.0009	0.0037	12	5.200
Laguna San Gregorio	25.35	11.55	5.9923	0.0018	0.0069	5	118.145
Laguna Zacapu	23.4	16.8	0.0000	0.0000	0.0014	140	16.403
Santa Maria del Oro	27.9	13.65	0.0000	0.0001	0.0016	100	0.184
Camecuaro	13.5	0	0.0000	0.0024	0.0044	200	0.091
Laguna Juanacatlán	48.335	75.641	10.3154	0.0000	0.0017	13	4.458
La Hoya de los Espinos	40.3	39.9	2.4172	0.0000	0.0010	120	0.582
La Laguna	9.88	4.62	2.0941	0.0000	0.0033	15	14.809
La Alberca (Tacambaro)	6.8	5.25	0.3986	0.0001	0.0053	120	4.469
Laguna Zempoala	13.8	ND	0.0280	0.9800	0.0000	21	0.6613056
Alchichica	4.55	4.2	0.0000	0.0002	0.0013	2	1.285
Quechulac	0	0	0.3582	0.0000	0.0022	3	0.611
Atexcac	5.2	5.04	0.0000	0.0010	0.0161	100	0.555
Lago del Sol	6.6	ND	0.0420	1.4139	0.0000	4	0.1175104
Zirahuén	0	0	0.0000	0.0000	0.0031	0	0.206
Lago de al Luna	2.9	ND	0.2100	1.6239	0.0000	3	0.1810816
La Preciosa	3.12	0	0.0000	0.0000	0.0084	23	0.435

Table A1.6 Wet Season nutrient data

Lake	**TP (µg/l)**	**SRP (µg/l)**	**NO_3-N (mg/l)**	**NO_2-N (mg/l)**	**NH_4-N (mg/l)**	**SiO_2 (mg/l)**	**Chl-a (µg/l)**
Lago de Yuríria	669.82	5.3844	0.0303	0.0035	0.0358	60	117.35
Piscina de Yuríria	744.487	120.087	0.1827	0.0287	0.1057	100	227.37
Zapotlan	272.52	125.49	0.0271	0.0099	0.0124	40	53.431
Atotonilco	2899.8	2860.27	0.0000	0.1486	0.2231	40	47.712
Los Negritos	670	695.04	0.0102	0.0019	0.0063	160	19.978
Chapala	524.11	570.76	0.0648	0.0083	0.0170	20	13.37
Cajititlan	1480.7	1398.13	0.1087	0.0149	0.0334	28	41.77
Tecuitlapa	699.823	378.87	0.0000	0.0244	0.0437	160	1.294
Cuitzeo (East)	984.74	101.811	0.1729	0.0242	0.0657	80	104.47
La Alberca	60.467	7.954	0.0153	0.0023	0.0098	120	26.16
Laguna La Maria	74.92	42.32	0.0103	0.0001	0.0082	100	15.694
San Pedro Lagunillas	52.69	18.021	0.0099	0.0003	0.0079	50	62.95
Tepitiltic	120.8	84.524	0.0279	0.0000	0.0040	60	4.93
Lago de Pátzcuaro	98.213	59.192	0.0379	0.0063	0.0063	40	14.316
Lagunilla San Gregorio	54.813	7.774	0.0631	0.0029	0.0073	4	41.03
Laguna Zacapu	63.484	0	0.0037	0.0007	0.0022	80	6.72
Santa Maria del Oro	169.143	65.082	0.0068	0.0000	0.0104	80	46.67
Camecuaro	42.05	30.48	0.0000	0.0000	0.0000	55	0.804
Laguna Juanacatlán	20.085	0	0.0080	0.0000	0.0017	60	4.69
La Hoya de los Espinos	10.0885	0	0.0014	0.0008	0.0018	70	1.1
La Laguna	51.09	18.408	0.0631	0.0029	0.0073	60	25.42
La Alberca (Tacambaro)	13.832	12.652	0.0046	0.0007	0.0016	100	134.3
Laguna Zempoala	19.61	13.62	0.0000	0.0023	0.0021	16	4.408
Alchichica	13.534	6.664	0.0029	0.0000	0.0503	120	2.268
Quechulac	17.939	113.9	0.0000	0.0000	0.0019	130	2.795
Atexcac	11.115	153.44	0.0000	0.0000	0.0510	180	88.651
Lago del Sol	17.525	6.91	0.0000	0.0000	0.0000	3	1.62
Zirahuen	1.59	0	0.0000	0.0000	0.0000	2	3.272
Lago de al Luna	4.568	2.388	0.0000	0.0000	0.0000	3	0.89
La Preciosa	19.773	121.7333333	0.0000	0.0000	0.0073	200	1.15

Appendix 2a. Modern Diatom species, codes and authorities

Code	Name	Authority
	Achnanthes	
Aacar	*Achnanthes acares*	Hohn & Hellerman (Krammer &Lange-Bertalot)
AC016B	*Achnanthes delicatula*	(Kütz) Grun. In Cleve & Grun
AC168A	*Achnanthes delicatula*	(Grun) LB in LB & Rupple
AC008A	*Achnanthes exigua*	Grun. In Cleve & Grun
AC032A	*Achnanthes hungarica*	(Grun.) Grun in. Cleve & Grun
PTH031A	*Achnanthes lanceolata*	(Breb. Ex Kutz.) Grun. In Cleve & Grun.
AC031A	*Achnanthes lanceolata ssp. dubia f. minuta*	Grun. In Cleve & Grun
AC001R	*Achnanthes lanceolata spp. frequentissima*	Lange-Bertalot
AD009A	*Achnanthes minutissima)*	Kutz
AD009A1	*Achnanthes minutissima* var. *microcephela*	Lange Bertalot
AC028A	*Achnanthes oblogella*	Orst.
AC028A1	*Achnanthes saxonica*	Krasske in Hust.
AC042A	*Achnanthes subatomoides*	(Hust.) Lange-Bertalot & Archibald in Krammer & Lange Bertalot
AC160A	*Achnanthes thermilis*	Rabenhorst (Schoenfeld)
	Amphora	
AM006A	*Amphora coffeaeformis*	(Ag.) Kutz.
AM084A	*Amphora montana*	Krasske
AM001A	*Amphora ovalis*	(Kutz.) Kutz.
AM001D	*Amphora ovalis* var. *libyca*	(Ehrenb. Ex Kutz.) Cleve
AM001B	*Amphora pediculis*	Kutz.) Grun. Ex A. Schmidt
AM004A	*Amphora venta* var. *venta*	Kutz
	Anomoeoneis	
BR006A	*Anomoeoneis brachysira*	(Breb.) Grun.
AN009A	*Anomoeoneis sphaerophora*	(Ehrenb.) Pfitz
AN009D	*Anomoeoneis sphaerophora* f. *costata*	(Kutz.) Schmidt
BR001A	*Anomoeoneis vitrea*	Kutz.
	Aulacoseira	
AU002A	*Aulacoseira ambigua*	(Grun. In van Heurck) Simonsen
AU003D	*Aulacoseira granulata*	(Ehrenb.) Simonsen
AU003B	*Aulacoseira granulata* var. *angustissima*	(O. Mull.) Simonsen
AU001A	*Aulacoseira itallica*	(Ehrenb.) Simonsen
	Asterionella	
AS001A	*Asterionella Formosa*	Hassall
	Caloneis	
CA002A	*Caloneis bacillum*	(Grun.) Cleve
CA010A	*Caloneis schumanniana*	(Grun. In van Heurck) Cleve
CA012A	*Caloneis silicula*	(Ehrenb.) Cleve
	Camplylodiscus	
CP001A	*Campylodiscus clypeus*	(Ehrenb.) Ehrenb. Ex Kutz.
	Chaetocerous	
CH057A	*Chaetocerous muelleri*	Lemm.
	Cocconeis	
CO010A	*Cocconeis disculus*	(Schum.) Cleve
CO066A	*Cocconeis neodiminuta*	Krammer

CO001A	*Cocconeis placentula*	Ehrenb.
	Cyclotella	
CY057A	*Cyclotella choctawhatcheeana*	Prasad
CY007A	*Cyclotella glomerata*	Bachm.
CY003A	*Cyclotella meneghiniana*	Kutz.
CY006A	*Cyclotella ocellata*	Pant.
CY002A	*Cyclotella pseudostelligera*	Hust.
CY017A	*Cyclotella schumannii*	Bailey
CT004A	*Cyclotella stelligera*	(Cleve & Grun. In Cleve) Van Heurck
	Cymbella	
CM016A	*Cymbella amicephala*	Nageli ex Kutz.
CM015A	*Cymbella cesatii*	(Rabenh.) Grun. In A Schmidt
CM007A	*Cymbella cymbiformis*	Ag.
CM052A	*Cymbella descripta*	(Hust.) Krammer & Lange Bertalot
CM048A	*Cymbella gracilis*	(Rabenh.) Cleve
PI014A	*Cymbella lanceolata*	(Ag.) Ag.
CM032A	*Cymbella mexicana*	(Ehrenb.) Cleve
ENC01A	*Cymbella microcephela*	Grun. In van Heurck
EY011A	*Cymbella minuta* var. *silesica*	(Belisch ex Rabenh.) Reimer
EY016A	*Cymbella perpusilla*	A. Cleve
CM030A	*Cymbella proxima*	Reimer
	Denticula	
DE002A	*Denticula elgans*	Kutz.
DE003A	*Denticula kuetzingii*	Grun.
DE001A	*Denticula tenuis*	Kutz.
DE020A	*Denticula themilis*	Kutz.
	Diatoma	
DT002B	*Diatoma mesodon*	(Ehrenb.) Ehrenb.
	Dipolneis	
DP009A	*Dipolneis eliptica*	(Kutz.) Cleve
DP001A	*Dipolneis ovalis*	(Hilse.) Cleve
DP006A	*Dipolneis puella* var. *puella*	(Schum.) Cleve
	Entomoneis	
AI001A	*Entomoneis alata*	(Ehrenb.) Ehrenb.
	Epithemia	
EP007A	*Epithemia adnata*	(Kutz.) Rabenh.
EP003A	*Epithemia argus*	(Ehrenb.) Kutz.
EP023A	*Epithemia frickei*	Krammer
EP001A	*Epithemia sorex*	Kutz.
EP004B	*Epithemia turgida* var. *granulata*	(Ehrenb.) Brun
	Eunotia	
EU049A	*Eunotia* ssp. *Bilinearis*	(Ehrenb.) F.W. Mills
EU002B	*Eunotia minor*	(Kutz.) Grun. In Van Heurck
EU048A	*Eunotia naegelli*	Migula
	Fragilaria	
PS001A	*Fragilaria brevistriata*	Grun in Van Heurck
FR009A	*Fragilaria capucina*	Desm.
FR009K	*Fragilaria capucina* var. *capitellata*	(Grun.) Lange Bertalot
FR009J	*Fragilaria capucina* var. *perminuta*	(Grun.) Lange-Bertalot
FR009G	*Fragilaria capucina* var. *rumpens*	(Kutz.) Lange-Bertalot
SR001A	*Fragilaria construens* f. *construens*	(Ehrenb.) Grun.
FR002D	*Fragilaria construens* f. *exigua*	(W. Sm.) Schulz
FR002E	*Fragilaria construens* f. *subsalina*	Hust.
FR002C	*Fragilaria construens* f. *venta*	(Ehrenb.) Grun in Van heurck

FR008A	*Fragilaria crotonensis*	Kitton
FR057A	*Fragilaria fasciculata*	(Ag.) Lange-Bertalot *sensu lato*
FR004A	*Fragilaria hungarica*	Pant.
SS002A	*Fragilaria pinnata* var. *pinnata*	Ehrenb.
FR001D	*Fragilaria pinnata* var. *trigona*	(Brun & Herib. In Herin) Hust.
	Frustulia	
FU002A	*Frustulia rhomboides*	(Ehrenb.) de Toni
FU002B	*Frustulia rhomboides* var. *saxonica*	De Toni
	Gomphonema	
GO006A	*Gomphonema acuminatum*	Ehrenb.
GO020A	*Gomphonema affine*	Kutz.
GO003A	*Gomphonema angustatum*	(Kutz.) Rabenh.
GO019A	*Gomphonema augar*	Ehrenb.
GO029A	*Gomphonema clavatum*	Ehrenb.
GO004A	*Gomphonema graclie*	Ehrenb.
GO050A	*Gomphonema minutum*	(Ag.) Ag.
GM001A	*Gomphonema olivaceum*	Hornemann) Breb.
GO001C	*Gomphonema olivaceum var minutissimum*	Hust.
GO013A	*Gomphonema parvulum*	(Kutz.) Kutz.
GO030A	*Gomphonema subclavatum*	(Grun in Schneider) Grun. In Van Heurck
GO023A	*Gomphonema truncatum*	Ehrenb.
	Gyrosigma	
GY025A	*Gyrosigma scalproides*	(Rabenh.) Cleve
	Hantzschia	
HA001A	*Hantzschia amphioxys*	Ehrenb.
	Mastogloia	
MA001A	*Mastogolia smithii*	Thwaits Ex W. Sm.
MA001B	*Mastogolia smithii* var. *lacustris*	Grun.
	Melosira	
ME032A	*Melosira lineata*	(Dillw.) Ag.
	Navicula	
CV004A	*Cavinula pseudocutisformis*	(Hust.) Mann & Stickle
CI005A	*Craticula halophila*	(Grun. Ex Heurck) Mann
NA069A	*Navicula americana*	Ehrenb.
HP002A	*Navicula capitata*	Ehrenb.
NA066C	*Navicula capita* var. *lueneburgensis*	(Grun.) Patrick in Patr. & Reimer
NA051C	*Navicula cincta* var. *cincta*	Ehrenb. Ralfs in Prtich
LU022A	*Navicula cohnii*	(Hilse) LB
NA118A	*Navicula confervacea*	(Kutz.) grun in Van Heurck
NA007A	*Navicula cryptocephala*	Kutz.
NA007B	*Navicula cryptocephala* var. *veneta*	(Kutz.) Rabenh.
NA175A	*Navicula gerloffii*	Schimanski
HP001A	*Navicula hungarica*	Grun.
FA012A	*Navicula indifferens*	Hust.
NA044A	*Navicula krasskei*	Hust.
PI014A	*Navicula lanceolata*	(Ag.) Ehrenb.
NA156A	*Navicula leptostriata*	Jorgensen
NA030A	*Navicula menisculus* var. *menisculus*	Schum.
SL003A	*Navicula minima*	Grun. In Van Heurck
NA112A	*Navicula minuscula*	Grun. In Van Heurck
NA024A	*Navicula oblonga*	Kutz.
NA007F	*Navicula perminuta*	Grun in Van Heurck
FA001A	*Navicula pygmaea*	Kutz.
NA003A	*Navicula radiosa*	Kutz.

NA751A	*Navicula radiosa* var. *tenella*	(Breb. Ex Kutx.) Grun ex. Van Heurck
NA763A	*Navicula saxophila*	Brock ex. Hust
NA134A	*Navicula subminuscula*	Manguin
NA734A	*Navicula subrhynchocephala*	Hust.
NA168A	*Navicula vitabunda*	Hust
NA738A	*Navicula vitiosa*	Schimanski
SL001A	*Sellaphora puplua*	(Kutz.) Mereschkowsky
	Neidium	
NE036A	*Neidium ampliatum*	(Ehrenb.) Krammer
NE001A	*Neidium iridis*	(Ehrenb.) Cleve
	Nitzschia	
NI042A	*Nitzschia acicularis*	Kutz.) W. Sm.
NI014A	*Nitzschia amphibia*	Grun.
NI020A	*Nitzschia angustata*	(W. Sm.) Grun. In Cleve & Grun.
NI211A	*Nitzschia bacillum*	Hust. In A. Schmidt et al.
NI044A	*Nitzschia capitellata*	Hust
NI010A	*Nitzschia communis*	Rabh.
NI015A	*Nitzschia dissipata*	(Kutz.) Grun.
NI098A	*Nitzschia filiformis*	(W. Sm.) Van Heurck
NI008A	*Nitzschia frustulum* var. *frustulum*	(Kutz.) Grun. In Cleve & Grun.
NI017A	*Nitzschia gracilis*	Hantzch
TF014A	*Nitzschia granulata*	Grun.
NI008A	*Nitzschia inconspicua*	Grun.
NI044A	*Nitzschia intermedia*	Hantzsch ex Cleve & Grun.
NI203A	*Nitzschia liebetruthii*	Rabenhorst
NI045A	*Nitzschia ovalis*	Arnott ex Cleve & Grun
NI009A	*Nitzschia palea*	(Kutz.) W. Sm.
NI033A	*Nitzschia paleacea*	(Grun. In Cleve & Grun.) Grun. In Van Heurck
NI193A	*Nitzschia perminuta*	(Grun.) M. Perag.
NI152A	*Nitzschia pusilla*	Grun.
	Nitzschia sinuata	(Thwaits ex W Sm.) Grun in Cleve & Grun.
NI116A	*Nitzschia sociabilis*	Hust.
NI048A	*Nitzschia tubicola*	Grun in Cleve & Grun.
	Pinnularia	
PI016A	*Pinnularia divergentissima*	(Grun. in Van Heurck) Cleve
RH001A	*Pinnularia gibberula*	(Ehrenb.) Ehrenb.
PI011A	*Pinnularia microstauron*	(Ehrenb.) Cleve
PI022A	*Pinnularia subcapitata*	Greg.
PI007A	*Pinnularia viridis*	(Nitzsch) Ehrenb.
	Rhoicosphenia	
RC002A	*Rhoicosphenia curvata*	(Kutz.) Grun
	Rhopalodia	
RH010A	*Rhopalodia acuminata*	Krammer
RH009A	*Rhopalodia brebissonii*	Krammer
RH001A	*Rhopalodia gibba*	(Ehrenb.) O. Mull
RH003A	*Rhopalodia gibberula*	(Ehrenb.) O. Mull
RH006B	*Rhopalodia musculus*	(Kutz.) O. Mull
RH004A	*Rhopalodia operculata*	(Grun.) O. Mull
	Stauroneis	
SA001A	*Stauroneis anceps*	Ehrenb.
SA006A	*Stauroneis phoenicenteron*	(Nitzsch) Ehrenb.
SA008A	*Stauroneis producta*	Grun in an Heurck

	Stenopetrobia	
SU006A	*Stenopterobia delicatissima*	(Lewis) M. Perag
	Stephanodiscus	
ST011B	*Stephanodiscus astraea* var. *intermedia*	Fricke
ST019B	*Stephanodiscus astraea* var. *minutula*	(Kutz.) Grun.
ST003D	*Stephanodiscus asteroides* var. *intermedia*	Gasse
ST001A	*Stephanodiscus hantzschii*	Grun.
ST014A	*Stephanodiscus medius*	Håkansson
ST012A	*Stephanodiscus minutus* var. *minutus*	(Ehrenb.) Grun.
ST006A	*Stephanodiscus niagarae*	Ehrenb.
ST008A	*Stephanodiscus subtilis*	Van Goor
ST002B	*Stephanodiscus tenuis* f.minor	(Hust.) Gass
	Surirella	
SU073A	*Surirella bohemica*	Krammer & Lange-Bertalot
SU012A	*Surirella elgans*	Ehrenb.
	Surirella ssp. linearis	W. Sm
	Synedra	
SY003A	*Synedra acus*	Kutz.
SY011A	*Synedra acus* var. *delicatissima*	(W. Sm.) Grun.
EO017A	*Synedra biceps*	W. Sm
SY011A	*Synedra delicatissima*	W. Sm
SY001B	*Synedra delicatissima* var. *angistissima*	Grun. in Van Heurck
SY014A	*Synedra gourlardii*	Breb.
SU049A	*Synedra linearis*	Ehrenb.
FR059A	*Synedra radians*	Kutz.
SY002B	*Synedra rumpens* var. *familiaris*	(Kutz.) Hust.
FR060A	*Synedra tenera*	W. Sm
SY001A	*Synedra unla*	(Nitzsch) Ehrenb

Appendix 2b

Diatom species counts for modern samples

Lago de Yuriría	**Rock**	**Veg**	**Sed**
Achnanthes minutissima	0	0.8	0.19
Amphora veneta	0.9	0.3	0.19
Aulacoseira ambigua			0.75
Aulacoseira granulata	12.4	4.1	22.6
A. granulata var angustissima	10.3	8.7	26.55
Cocconeis placentula (agg)	0.8	6.8	0.94
Cyclotella glomerata	2.2	3.0	9.04
Cyclotella meneghiniana	3.7	3.0	14.31
Cyclotella pseudostelligera	6.0	6.3	6.4
Cyclotella stelligera			4.14
Fragilaria capucina	4.5	1.4	
Fragilaria capucina var. capitellata	0.1	0.0	
Fragilaria construens			0.56
Fragilaria crotonensis	0.5	1.1	0.38
Gomphonema affine			0.19
Gomphonema angustatum	0.0	0.8	
Gomphonema minutum			0.19
Gomphonema parvulum	1.2	1.9	0.94
Gomphonema truncatum	21.0	0.8	
Navicula cincta			0.38
Navicula confervacea			0.19
Navicula hungarica			2.5
Navicula indifferens	0.0	3.6	
Navicula Krasskei	4.8	7.7	
Sellaphora pupula			0.19
Navicula radiosa var. tenella	1.4	11.2	
Nav1			0.19
Navicula subminuscula			2.3
Nitzschia filiformis ?	0.1	0.0	
Nitzschia liebetruthii	8.9	7.4	
Nitzschia amphibia	0.9	2.5	3.39
Nitzschia communis			0.75
Nitzschia inconspicua	9.0	12.0	
Nitzschia palea	7.9	13.1	0.19
Nitzschia perminuta	0.5	1.6	1.32
Nitzschia spp (girdle view)	1.5	0.0	
Rhoicosphenia curvata	0.0	1.1	
Rhopalodia brebissonii	0.5	0.8	
Stephanodiscus astraea minutula			0.38
Stephanodiscus asteroides var intermedia			0.19
Synedra acus	0.9	0.0	0.38
Synedra rumpens var familiaris			0.19

La Piscina de Yuriría	Rock	Veg	Sed
Achnanthes minutissima			0.4
Amphora coffeaeformis			0.8
Amphora veneta	4	15.5	1.4
Anomoeoneis sphaerophora f costata	1.7	0	3.7
Aulacoseira ambigua			0.4
Aulacoseira granulata	1	0	
Aulacoseira granulata var angustissima	1.7	0	0.6
Chaetocerous muelleri			5.3
Cyclotella meneghiniana	0.5	0	1.0
Cyclotella pseudostelligera	0.3	0	
Denticula tenuis			1.6
Denticula thermalis			4.9
Eunotia spp1			0.2
Fragilaria crotonensis			0.4
Fragilaria construens var. venter	0.7	0	
Gomphonema	0	0.7	
Gomphonema affine	0	0.4	
Gomphonema clavatum	0.7	0	
Gomphonema parvulum	1	0	
Navicula capitata var capitata			0.4
Navicula cincta			1.6
Navicula cryptocephala	0.3	0	
Craticula halophila	2.7	9.2	56.1
Navicula radiosa var. tenella	39.6	5.9	
Nitzschia palea	2	0	1.2
Nitzschia communis	4	46.9	5.1
Nitzschia filiformis	2.8	1.11	
Nitzschia girdle	3.5	0	
Nitzschia inconspicua	5.5	0	2.3
Nitzschia liebetruthii	19.1	2.21	
Nitzschia ovalis	0	1.48	3.3
Nitzschia palea	0	0.37	
Nitzschia perminuta	2.5	4.43	2.5
Nitzschia pussila			0.6
Rhopalodia acuminatum			0.2
Rhopalodia gibberula	6.2	11.44	3.5
Stephanodiscus medius			0.2
Stephanodiscus tenuis f. minor	0.3	0	
Species 1			2.1

Zapotlan	**Sed**
Achnanthes exigua	0.61
Achnanthes minutissima	1.42
Amphora montana	0.2
Amphora veneta	0.2

Aulacoseira granulata var granulata	12.2
Aulacoseira granulata var angustissima	8.74
Chaetocerous muelleri	1.83
Cyclotella meneghiniana	20.73
Cyclotella pseudostelligera	8.33
Cyclotella stelligera	1.63
Diploneis elliptica	0.41
Diploneis ovalis	0.41
Epithemia adnata	1.63
Epithemia frickei	0.2
Fragilaria brevistriata	8.94
Fragilaria construens var venter	6.1
Fragilaria pinnata v. pinnata	5.29
Fragilaria pinnata var. trigona	8.94
Gomphonema affine	0.81
Gomphonema parvulum	2.24
Hantzschia amphioxys	0.41
Navicula capitata var capitata	0.61
Sellaphora pupula	0.2
Navicula radiosa var tenella	2.44
Nitzschia amphibia	2.24
Nitzschia palea	0.41
Rhopalodia gibba	1.22
Rhopalodia gibberula	0.4
Unknown 2	1.22

Atotonilco	**Veg**	**Sed**
Achnanthes lanceolata		0.68
Achnanthes minutissima		1.7
Achnanthes sp		0.34
Amphora veneta	36.3	3.74
Cyclotella meneghiniana		1.36
Cyclotella ocellata		4.08
Gomphonema clavatum	0.6	3.06
Gomphonema gracile		2.04
Gomphonema parvulum	0.3	6.8
Gomphonema truncatum	0.9	
Navicula confervacea		1.36
Navicula radiosa		2.04
Navicula radiosa var tenella	10.9	
Nitzschia amphibia	28.6	60.2
Nitzschia communis	1.7	
Nitzschia filiformis var. conferta	8	
Nitzschia inconspicua	8.3	3.74
Nitzschia liebetruthii	0.9	
Nitzschia perminuta	2.3	0.68
Pinnularia spp		1.36
Rhopalodia gibberula	0.6	
Rhopalodia musculus		1.36
Stephanodiscus astraea var intermedia		1.7
Stephanodiscus asteroides var intermedia		2.72
Stephanodiscus hantzschii	0.9	
Synedra ulna		1.02

Los Negritos	**Sed**
Achnanthes acares	0.29
Achnanthes exigua	3.39
Achnanthes minutissima	0.29
Achnanthes sp	0.44
Achnanthes thermalis	0.15
Amphora coffeaformis	0.15
Amphora montana	0.44
Amphora ovalis	6.05
Amphora ovalis var libyca	7.23
Anomoeoneis sphaerophora	0.15
Aulacoseira granulata var granulata	0.44
Aulacoseira granulata var angustissima	0.15
Chaetocerous muelleri	6.64
Campylodiscus clypeus	0.15
Cyclotella glomerata	0.88
Cyclotella meneghiniana	12.39
Cyclotella pseudostelligera	0.59
Cyclotella stelligera	0.29
Denticula elgans	0.59
Diploneis ovalis	0.59
Entomoneis alta	0.74
Fragilaria (girdle)	9.73
Fragilaria brevistriata	0.15
Fragilaria construens var venter	0.15
Gomphonema affine	0.44
Gomphonema auger	0.29
Gomphonema minutum	0.29
Gomphonema parvulum	0.15
Craticula halophila	0.74
Navicula leptostriata	2.8
Navicula radiosa var tenella	1.03
Navicula subminuscula	0.15
Nitzschia 2	4.13
Nitzschia amphibia	3.1
Nitzschia angustata	0.59
Nitzschia communis	0.15
Nitzschia frustulum	8.41
Nitzschia inconspicua	8.26
Nitzschia spp	0.59
Nitzschia palea	2.51
Nitzschia perminuta	11.65
Pinnularia spp	0.15
Rhopalodia gibberula	2.06
Unknown 4	0.29

Chapala	**Sed**
Amphora ovalis	0.67
Amphora ovalis var libyca	0.67
Amphora pediculus	0.22
Aulacoseira ambigua	0.22
Aulacoseira granulata var granulata	3.34
Aulacoseira granulata var angustissima	6.24
Cocconeis placentula agg	0.89
Cyclotella glomerata	9.8
Cyclotella meneghiniana	1.78
Cyclotella pseudostelligera	1.34
Cymbella mexicana	2.01
Epithemia turgida var granulata	0.22
Fragilaria capucina var capucina	0.89
Fragilaria construens	17.37
Fragilaria crotonensis	0.22
Fragilaria construens var venter	3.56
Fragilaria pinnata v. pinnata	1.11
Navicula radiosa var tenella	0.22
Nitzschia inconspicua	0.22
Rhoicosphenia curvata	0.45
Stephanodiscus astraea var intermedia	18.93
Stephanodiscus astraea minutula	12.92
Stephanodiscus minutus	12.25
Stephanodiscus niagarae	1.56
Stephanodiscus sp 2	2.45
Surirella elgans	0.22
Synedra acus	0.22

Cajititlan	veg	Sed
Achnanthes hungarica	0.3	
Achnanthes minutissima	1.3	
Amphora veneta	1.8	
Aulacoseira spp		4.9
Aulacoseira ambigua		4.1
Aulacoseira granulata var angustissima	9.1	19.7
Aulacoseira granulata		35.3
Cocconeis placentula	0	0.4
Cyclotella glomerata	5	4.1
Cyclotella meneghiniana	0.3	4.7
Cyclotella ocellata?	1	
Cymbella minuta		1.2
Epithemia turgida var granulata		1.2
Eunotia bilunaris	0.5	
Fragilaria capucina	6.3	0.2
Fragilaria capucina (girdle ?)	1.6	
Gomphonema affine		0.8
Gomphonema clavatum	3.1	
Gomphonema minutum	0.3	
Gomphonema parvulum	4.4	1.6
Navicula confervacea	0.3	2.4
Navicula indifferens	1.3	
Navicula krasskeii	1.3	
Navicula minuscula		1.8
Navicula vitriosa	0.3	
Nitzschia amphibia	30.7	2.2
Nitzschia filiformis conferta	3.7	
Nitzschia filiformis var filiformis	0.8	
Nitzschia granulata		0.2
Nitzschia inconspicua	0.8	0.8
Nitzschia intermedia	3.7	
Nitzschia palea	20.8	
Nitzschia perminuta		0.4
Pinnularia	0.5	
Pinnularia gibba	0.26	
Rhopalodia gibba	0.5	0.2
Stephanodiscus medius		0.2
Stephanodiscus 1		13.6
Synedra ulna	0.3	

Cuitzeo	
Achnanthes exigua	1.4
Amphora ovalis var libyca	1.2
Anomoeoneis sphaerophora	1
Chaetocerous muelleri	1.4
Cocconeis placentula agg	9.38
Cyclotella glomerata	0.2
Cyclotella meneghiniana	1.6
Cyclotella stelligera	0.4
Cymbella mexicana	0.4
Denticula elgans	0.6
Denticula tenuis	0.2
Epithemia adnata	1
Fragilaria brevistriata	16.37
Fragilaria construens	7.78
Fragilaria construens f. subsalina	9.78
Fragilaria construens var venter	11.18
Fragilaria pinnata v. pinnata	15.77
Gomphonema affine	0.4
Gomphonema angustatum	2.2
Gomphonema parvulum	1
Hantzschia amphioxys	0.2
Navicula capitata	1
Craticula halophila	0.2
Navicula menisculus v. menisculus	0.4
Navicula radiosa var tenella	3.79
Nitzschia amphibia	1
Nitzschia angustata	0.8
Nitzschia gracilis	0.2
Nitzschia palea	0.2
Nitzschia perminuta	2.2
Nitzschia pussila	3.99
Nitzs1	0.2
Pinnularia spp	0.4
Rhoicosphenia curvata	0.6
Rhopalodia gibberula	0.4
Rhopalodia musculus	0.4
Surirella brebissonii	0.2
Unknown 2	0.2
U3	0.2

Tecuitlapa	
Achnanthes minutissima	28.87
Amphora ovalis	3.63
Amphora ovalis var libyca	7.26
Amphora ovalis var pediculus	0.36
Amphora veneta	5.26
Cocconeis placentula agg	0.91
Cyclotella meneghiniana	22.32
Cyclotella stelligera	0.18
Cymbella cymbiformis	1.81
Cymbella mexicana	0.18
Cymbella microcephela	0.54
Cymbella minuta	0.36
Denticula elgans	0.91
Epithemia adnata	4.17
Epithemia sorex	0.36
Fragilaria brevistriata	0.18
Fragilaria pinnata v. pinnata	1.45
Gomphonema affine	0.73
Gomphonema parvulum	0.91
Sellaphora pupula	0.18
Navicula radiosa var tenella	0.73
Navicula saxophila	0.18
Nitzschia amphibia	15.97
Nitzschia palea	0.44
Nitzs1	0.36
Pinnularia spp	0.18
Rhopalodia gibba	1.09
Synedra ulna	0.36

Alberca Teremendo	**Sed**
Achnanthes saxonica	0.34
Achnanthes sp	2.4
Amphora montana	1.37
Amphora ovalis	1.71
Cocconeis placentula agg	1.37
Cyclotella meneghiniana	0.34
Fragilaria brevistriata	1.03
Fragilaria capucina var capucina	0.34
Fragilaria construens	3.08
Fragilaria construens var venter	1.03
Frustulia spp	0.34
Gomphonema angustatum	1.37
Gomphonema parvulum	54.45
Gomphonema subclavatum	1.37
Gomphonema parvulum (girdle)	11.3
Hantzschia amphioxys	1.37
Sellaphora pupula	0.34
Navicula pygmaea	0.34
Navicula radiosa var tenella	1.37
Nitzschia amphibia	2.05
Nitzschia communis	0.68
Nitzs1	4.45
Rhopalodia gibberula	0.68
Stephanodiscus astraea var intermedia	2.05
Stephanodiscus niagarae	0.68
Synedra ulna	4.11

La Maria	**Sed**
Achnanthes exigua	0.94
Achnanthes minutissima	0.59
Achnanthes sp	4.01
Amphora montana	0.24
Aulacoseira ambigua	1.18
Aulacoseira granulata var granulata	2
Aulacoseira granulata var angustissima	12.15
Cocconeis placentula agg	0.24
Cyclotella glomerata	10.61
Cyclotella meneghiniana	1.53
Cyclotella pseudostelligera	1.65
Cyclotella stelligera	10.61
Cymbella minuta	3.54
Fragilaria brevistriata	0.59
Fragilaria capucina var capucina	0.59
Fragilaria construens	13.09
Fragilaria construens var venter	14.15
Fragilaria pinnata v. pinnata	1.53
Gomphonema affine	2.36
Gomphonema auger	0.35
Gomphonema minuta	0.71
Gomphonema parvulum	1.65
Gomphonema truncatum	0.47
Navicula americana	0.12
Navicula capitata	0.24
Navicula menisculus v. menisculus	0.24
Sellaphora pupula	0.24
Navicula radiosa var tenella	0.24
Navicula spp	0.12
Nitzschia amphibia	12.5
Nitzschia inconspicua	0.24
Nitzschia perminuta	0.59
Rhopalodia brebissonii	0.35
Rhopalodia gibba	0.12
Synedra ulna	0.12
Synedra ulna var acus	0.12

San Pedro	**Veg**	**Sed**
Achnanthes minutissima	94.8	93.4
Aulacoseira granulata	1.9	4.9
Aulacoseira granulata var angustissima		0.2
Cocconeis placentula (Ehrenb.)	0.3	
Cyclotella meneghiniana		0.3
Fragilaria pinnata var pinnata		0.4
Gomphonema parvulum	0.6	
Nitzschia palea	0.6	
Stephanodiscus medius		0.2
Synedra tenera	1.9	

Tepetiltic	**Sed**
Achnanthes exigua	0.22
Achnanthes minutissima	12.53
Amphora montana	1.34
Aulacoseira ambigua	43.62
Aulacoseira granulata var granulata	1.12
Aulacoseira granulata var angustissima	1.12
Aulacoseira spp	4.25
Chaetocerous muelleri	0.67
Cyclotella glomerata	0.45
Cyclotella meneghiniana	1.34
Cymbella microcephela	1.12
Cymbella minuta	0.22
Eunotia ssp. bilunaris	2.01
Eunotia spp	0.22
Fragilaria capucina var capucina	1.79
Fragilaria construens	0.23
Fragilaria pinnata v. pinnata	1.57
Gomphonema affine	0.45
Gomphonema parvulum	3.13
Gomphonema truncatum	1.34
Sellaphora pupula	1.57
Navicula radiosa var tenella	3.8
Navicula saxophila	1.79
Nitzschia amphibia	3.13
Nitzschia paleacea	2.91
Nitzschia perminuta	0.45
Pinnularia subcapitata	4.92
Pinnularia spp	0.22
Stauroneis phoenicenteron	0.67
Surirella spp	0.22
Synedra acus delicatissima	1.12

Pátzcuaro (Cent.)	Veg	Sed
Achnanthes minutissima		2.08
Amphora ovalis var libyca		0.3
Amphora veneta	0.4	
Aulacoseira ambigua		10.09
Aulacoseira granulata	0.4	47.77
Aulacoseira granulata var angustissima		2.37
Cocconeis neodiminuta		0.3
Cocconeis placentula	0.4	8.6
Cyclotella glomerata		1.78
Cyclotella meneghiniana	0.6	
Cyclotella stelligera		2.08
Cymbella mexicana		0.3
Cymbella minuta var silesiaca	0.2	
Diploneis ovalis		0.3
Epithemia adnata		2.97
Epithemia sorex		0.89
Fragilaria brevistriata		0.3
Fragilaria capucina	0.4	
Fragilaria construens		0.89
Fragilaria construens var venter		2.08
Fragilaria crotonensis		0.59
Fragilaria pinnata v. pinnata		1.19
Gomphonema acuminatum		0.3
Gomphonema clavatum	19	
Gomphonema girdle	0.4	
Gomphonema minutum	1	0.3
Gomphonema parvulum	9.2	0.59
Gomphonema girdle		0.59
Sellaphora pupula		0.59
Navicula radiosa var tenella	11.9	0.3
Neidium ampliatum		0.3
Nitzschia amphibia		0.59
Nitzschia palea	25	
Nitzschia amphibia	1.2	
Nitzschia inconspicua	1.6	
Nitzschia perminuta	0.8	0.59
Nitzschia tubicola	4.3	
Pinnularia spp	0.3	0.3
Rhoicosphenia curvata	6.3	1.78
Rhopalodia gibba		0.3
Stephanodiscus astraea var intermedia		1.19
Stephanodiscus astraea minutula		2.97
Stephanodiscus asteroides var intermedia		0.3
Stephanodiscus niagarae		1.78
Stephanodiscus subtilis	0.2	
Synedra gourlardii	15.1	
Synedra gourlardii (var.)	1.4	
Synedra ulna		2.37

Pátzcuaro (S)	**Veg**
Nitzschia palea	39
Gomphonema clavatum	33.1
Synedra ulna	9.1
Gomphonema parvulum	10.6
Navicula radiosa var tenella	3
Synedra gourlardii	1.1
Rhoicosphenia curvata	1.7
Gomphonema minutum	0.9
Aulacoseira granulata	0.2
Cyclotella meneghiniana	0.2
Stephanodiscus	0.4
Cymbella sp	0.2
Amphora veneta	0.6

Zacapu	**Veg**	**Sed**
Achnanthes exigua	0.7	
Achnanthes lanceolata var frequentissima	2.2	
Achnanthes minutissima	9.4	2.4
Amphora ovalis var libyca	4.3	0.1
Amphora ovalis var libyca	11.5	
Amphora veneta	1.4	0.1
Aulacoseira ambigua		0.1
Aulacoseira granulata	0.7	1.4
Aulacoseira granulata var angustissima		3.7
Cocconeis disculus		0.1
Cocconeis placentula	0.7	4.1
Cyclotella glomerata	18.7	3.4
Cyclotella meneghiniana		0.1
Cyclotella pseudostelligera		2.6
Cyclotella stelligera		0.1
Cymbella proxima		0.5
Epithemia sorex		0.1
Fragilaria construens		0.3
Fragilaria pinnata	0.7	2.2
Frustulia rhomboides var rhomboides		0.1
Gomphonema minuta		0.7
Gomphonema parvulum	5	1.9
Navicula cryptocephala		0.3
Navicula cryptocephala var veneta		0.9
Navicula cryptotenella	2.9	
Navicula indifferens	4.3	
Navicula radiosa var tenella	17.3	
Nitzschia amphibia	0.7	0.6
Nitzschia communis	0.7	
Nitzschia inconspicua	0.7	
Nitzschia palea	2.9	11.2
Nitzschia perminuta		0.3
Nitzschia tubicola	0.7	
Nitzschia sociabilis		0.1
N supralitorea		1.2
Pinnularia sp.	1.4	
Pinnularia viridis	0.7	
Rhoicosphenia curvata		1.2
Stephanodiscus asteroides var intermedia		0.3
Stephanodiscus hantzschii		0.4
Stephanodiscus subtilis	11.5	30.3
Synedra acus		17.5
Synedra acus var		1.6
Synedra tenera		0.1
Synedra ulna		10.0
Synedra ulna var acus	0.7	

Sta Maria del Oro	Veg	sed
Achnanthes exigua		4.3
Achnanthes minutissima		3.38
Amphora ovalis		0.46
Amphora ovalis var libyca		0.15
Aulacoseira granulata var granulata		48.23
Aulacoseira monospina		4.3
Cocconeis placentula agg		2.61
Cyclotella meneghiniana		0.77
Cymbella mexicana		0.15
Epithemia adnata	0.3	0.15
Fragilaria brevistriata	0.3	1.08
Fragilaria capucina var capucina		0.15
Fragilaria crotonensis		0.15
Fragilaria fasciculata	23.2	
Fragilaria pinnata v. pinnata		2
Gomphonema minutum	0.3	0.31
Gomphonema parvulum	0.9	
Hantzschia amphioxyis	0.3	
Navicula capitata		0.46
Navicula lanceolata		1.08
Navicula radiosa var tenella	0.3	0.91
Nitzschia amphibia	0.3	23.19
Nitzschia angustata		0.15
Nitzschia granulata		0.15
Nitzschia inconspicua	0.3	1.08
Nitzschia palea	4.7	
Nitzschia perminuta		0.61
Rhoicosphenia curvata	0.6	0.61
Rhopalodia brebissonii	61.4	2.76
Rhopalodia gibba	1.3	
Rhopalodia musculus	3.1	
Synedra ulna	2.5	

Camecuaro	Veg	**Sed**
Achnanthes acares		12.4
Achnanthes exigua		1.5
Achnanthes lanceolata ssp dubia f minuta		2.5
Achnanthes minutissima	2.7	2.7
Amphora ovalis	0.25	
Amphora pediculus	1.2	6.2
Amphora veneta	0.3	0.3
Cocconeis disculus	0.6	
Cocconeis neodiminuta		2.5
Cocconeis pediculus		0.3
Cocconeis placentula	10	4.0
Cymbella lanceolata	1.8	
Cymbella mexicana		0.7
Cymbella proxima		3.5
Fragilaria brevistriata		0.3
Fragilaria capucina var capucina	0.6	1.0
Fragilaria capucina var capitellata		4.9
Fragilaria capucina var rumpens		2.5
Fragilaria construens		5.4
Fragilaria construens var venter		2.0
Fragilaria pinnata var pinnata	1.2	18.0
Gomphonema clavatum	0.9	3.2
Gomphonema parvulum	0.3	4.0
Melosira lineata	13	
Sellaphora pupula		2.2
Navicula radiosa var tenella	2.1	1.8
Nitzschia acicularis	0.3	10.9
Nitzschia amphibia	3.3	
Nitzschia dispata	1.5	
Nitzschia intermedia	0.6	
Nitzschia liebetruthii	49.9	
Nitzschia palea	8	1.8
Rhoicosphenia curvata	1.8	4.0
Surirella spp		0.7
Terpsinoe Spp		1.0
Synedra ulna	0.3	

Juanacatlan	**Veg**	**Rock**	**Sed**
Achnanthes lanceolata			2.7
Achnanthes minutissima	11.5	22	1.0
Aulacoseira granulata	0.5	3.5	
Aulacoseira granulata var angustissima			1.4
Cocconeis placentula	42.6	2.3	0.7
Cyclotella glomerata			2.2
Cyclotella pseudostelligera			1.7
Cyclotella stelligera			0.7
Cymbella microcephela	2.1	3.5	0.9
Cymbella minuta var minuta			0.2
Cymbella minuta var. silesiaca	0	0.4	
Epithemia adnata	2.1	0.8	
Epithemia sorex			0.2
Epithemia spp			0.2
Fragilaria brevistriata			3.3
Fragilaria capucina var capucina			0.2
Fragilaria construens var venta	0	0.8	22.9
Fragilaria construens var. construens	0.5	0	5.5
Fragilaria crotonensis	2.1	27	43.4
Fragilaria pinnata v. pinnata			9.2
Frustulia rhomboides	0.5	0	
Gomphonema angustatum	12	0	
Gomphonema clavatum	8.4	1.2	
Gomphonema parvulum	1.6	4.3	0.2
Gomphonema truncatum	0	0.4	0.3
Hantzschia amphioxys	0.5	0	
Navicula cryptocephala	4.2	6.2	1.2
Navicula glomus			0.2
Navicula laevissima			0.3
Sellaphora pupula			0.7
Navicula radiosa var tenella			0.2
Navicula saxophila	0	0.8	
Nitzschia amphibia	1.6	25.1	0.3
Nitzschia inconspicua	0.5	0	
Nitzschia palea	3.7	1.2	
Pinnularia subcapitata	0.5	0.4	
Stauroneis anceps	0	0.4	

La Hoya de Los Espinos	**Sed**
Achnanthes minutissima	5.9
Amphora montana	0.2
Amphora ovalis var libyca	0.2
Amphora ovalis var pediculus	5.4
Amphora veneta	0.2
Aulacoseira ambigua	3.9
Aulacoseira granulata var granulata	5.6
Caloneis schumanniana	0.2
Cocconeis disculus	0.3
Cocconeis placentula agg	8.3
Cyclotella meneghiniana	10.1
Cymbella mexicana	2.6
Diploneis ovalis	0.2
Epithemia turgida var granulata	0.2
Fragilaria construens	1.2
Fragilaria construens var venter	2.0
Fragilaria pinnata v. pinnata	0.5
Gomphonema affine	0.3
Gomphonema affine (girdle)	1.0
Hantzschia spp	0.2
Navicula capitata	0.2
Navicula radiosa var tenella	3.9
Navicula saxophila	0.7
Neidium iridis	0.2
Nitzschia amphibia	5.2
Nitzschia capitellata	2.9
Nitzschia palea	34.1
Nitzs1	1.7
Pinnularia spp	0.3
Rhoicosphenia curvata	2.2
Synedra ulna	0.5

La Laguna	**Sed**
Achnanthes exigua	0.1
Achnanthes minutissima	65.6
Amphora montana	0.4
Aulacoseira ambigua	6.8
Aulacoseira granulata var granulata	0.7
Aulacoseira granulata var angustissima	3.4
Cyclotella glomerata	0.4
Cyclotella pseudostelligera	2.5
Cyclotella stelligera	11.5
Cymbella minuta	0.1
Denticula spp	0.3
Gomphonema gracile	0.1
Gomphonema gracile (girdle)	0.6
Gyrosigma scalproides	0.1
Craticula halophila	0.1
Navicula radiosa var tenella	0.1
Navicula saxophila	0.1
Navicula sp	1.0
Navicula subrynconocephela	0.1
Nitzschia amphibia	0.1
Nitzschia inconspicua	0.6
Nitzschia paleacea	3.2
Nitzschia perminuta	0.6
Rhopalodia gibba	0.3
Synedra acus delicatissima	0.3

La Alberca (Tacambrao)	**Sed**
Achnanthes exigua	0.14
Achnanthes lanceolata	0.42
Achnanthes minutissima	82.98
Anomoeoneis vitrea	1.55
Aulacoseira ambigua	3.28
Aulacoseira granulata var granulata	0.28
Cocconeis placentula agg	0.42
Cyclotella glomerata	2.25
Cyclotella pseudostelligera	0.42
Cymbella descripta	0.14
Cymbella minuta	0.14
Denticula kuetzingii	0.56
Fragilaria crotonensis	1.69
Gomphonema spp	1.13
Nitzschia palea	0.84
Rhopalodia gibba var parallela	0.28
Synedra acus delicatissima	0.14
Synedra delicatissima var angustissima	3.24

Zempoala	**Veg**	**Sed**
Achnanthes exigua		0.2
Achnanthes lanceolata ssp lanceolata	0.4	2.3
Achnanthes lanceolata ssp frequentissima		1.6
Achnanthes minutissima	53.8	
Amphora pediculus		0.4
Asterionella formosa	15.8	3.1
Aulacoseira ambigua		0.8
Aulacoseira granulata var angustissima		10.86
Cocconeis placentula	4.6	1.4
Cymbella amphicephela		0.2
Cymbella descripta		0.2
Cymbella microcephela	6.7	
Cyclotella glomerata		0.2
Denticula kuetzingii		0.4
Diatoma spp		2.1
Diatoma mesodon		0.4
Fragilaria capucina	0.4	0.6
Fragilaria construens var construens		4.7
Fragilaria construens var venter		2.7
Fragilaria crotonensis	4.4	58.4
Fragilaria pinnata	8.9	
Frustulia rhomboides		0.2
Gomphonema parvulum	1	0.4
Navicula cryptocephala	0.2	0.2
Sellaphora pupula		0.2
Navicula saxophila		0.8
Nitzschia palea	2.4	1.4
Nitzschia perminuta		1.4
Nitzschia sinuata	1.2	
Pinnularia microstauron		0.6
Rhoicosphenia curvata	0.4	2.25
Rhopalodia gibba		0.6

Alchichica	**Veg**	**Sed**
Achnanthes delicatula	0.3	
Amphora ovalis v libyca	0.3	0.22
Amphora ovalis var pediculus		1.32
Amphora veneta	2.8	
Cocconeis placentula	17.2	0.22
Cyclotella choctawhatcheeana	0.6	70.25
Cyclotella meneghiniana		0.11
Cyclotella quillensis		23.27
Cymbella cymbiformis	0.3	
Diploneis ovalis		0.11
Epithemia spp		0.11
Fragilaria pinnata v. pinnata		0.11
Fragilaria faciculata	0.3	
Frustulia rhomboidies	0.3	
Gomphonema minuta		0.33
Mastogloia smithii	1.3	
Navicula capitata		0.33
Craticula halophila		0.11
Navicula radiosa var. tenella	0.6	
Navicula spp		0.44
Nitzschia bacillum		0.99
Nitzschia communis	0.9	
Nitzschia inconspicua	4.4	
Nitzschia liebetruthii	64	1.87
Nitzschia palea	5.3	
Nitzschia perminuta	0.6	
Rhopalodia musculus	0.6	0.22

Quechulac	**Veg**	**Sed**
Achnanthes lanceolata		10.8
Achnanthes minutissima	19.1	
Amphora ovalis var pediculus		3.5
Anomoeoneis sphaerophora		0.1
Aulacoseira ambigua		3
Aulacoseira granulata	0.4	0.1
Cocconeis neodiminuta		43.1
Cocconeis placentula	22.9	0.2
Cyclotella meneghiniana		0.3
Cyclotella ocellata		0.3
Cymbella cesatii	0.4	
Cymbella cymbiformis		0.1
Cymbella minuta	0.6	
Epithemia turgida		0.1
Epithemia sp		0.2
Fragilaria (girdle)		11.59
Fragilaria brevistriata		7.9
Fragilaria construens var venter		9.71
Fragilaria capucina var radians	28.5	
Fragilaria crotonensis	6.1	6.8
Fragilaria fasciculata	1.2	
Gomphonema olivaceum var minutissima	20.3	
Navicula cincta		0.1
Navicula minima		0.7
Navicula subrotundata		0.5
Neidium ampliatum		0.2
Rhoicosphenia curvata	0.4	0.1
Rhopalodia brebissonii	0.4	

Atexcac	**Veg**	**Sed**
Achnanthes sp		0.1
Achnanthes minutissima	38.5	
Amphora coffeaeformis	2.1	0.1
Amphora ovalis	0.5	0.1
Amphora pediculus	0.5	0.4
Amphora spp 1	11.8	
Cocconeis placentula	5.3	0.4
Cyclotella choctawhatcheeana	16.3	94.2
Cymbella gracilis	1.4	
Denticula elgans		1.4
Diploneis ovalis		0.2
Entomoneis alta	0.23	
Epithemia adnata	1.65	
Gomphonema clavatum	0.7	
Gomphonema minutum	0.2	
Gomphonema olivaceum	3.1	
Gomphonema sp (Girdle)		0.2
Mastogloia smithii	1.6	
Mastogloia smithii var lacustris	0.23	
Navicula cryptocephala	0.5	
Navicula radiosa var tenella	1.2	
Nitzschia communis	2.1	0.2
Nitzschia inconspicua	3.6	2.4
Nitzschia liebetruthii	8.5	
Nitzschia palea	0.9	
Synedra delicatissima	0.2	

Lago del Sol	**Veg**	**lit**	**Sed**
Achnanthes danoensis	0	0.5	
Achnanthes delicatula ssp hauckiana	0.4	0	
Achnanthes minutissima	39.4	7.1	8.6
Achnanthes oblongella	2.2	10.9	
Aulacoseira italica			16
Caloneis silicula			0.5
Cavinula pseudocutisformis	27.1	68.2	10.6
Cyclotella glomerata	0.9	0.8	
Cyclotella meneghiniana	0.2	0	
Cymbella minuta			12.1
Cymbella perpusilla	1.5	1	
Eunotia ssp bilinearis			1.5
Fragilaria (girdle)			0.7
Fragilaria brevistriata			4.9
Fragilaria construens var exigua			0.3
Fragilaria construens f. subsalina			0.3
Fragilaria capucina	5.5	0.8	
Fragilaria pinnata	2.6	4.3	1.5
Frustulia rhomboides	0.2	0	
Frustulia rhomboides var. saxonica			1.9
Gomphonema clavatum			0.2
Gomphonema gracile			0.5
Gomphonema parvulum	1.5	0.3	0.34
Gomphonema parvulum (girdle)			1
Navicula capitata	0.2	0	
Navicula cryptocephela	0	0.5	
Navicula gerloffii	3.3	0.5	
Navicula NTA			2
Navicula minima			9.4
Navicula vitabunda			12.7
Neidium ampliatum			0.5
Nitzschia palea	12.1	5.3	3.4
Pinnularia divergentissima			0.3
Pinnularia microstauron	0.7	0	2.5
Pinnularia spp			1
Stauroneis producta			0.2
Stenopetrobia delicatissima			1
Surirella linearis			0.2
Surirella bohemica			0.5
Synedra lunaris			2

Luna	**Sed**
Achnanthes subatomoides	24
Anomoeoneis vitrea	0.2
Aulacoseira italica	0.2
Caloneis bacillum	0.4
Caloneis silicula	1.1
Cymbella minuta	34.5
Cymbella minuta var silesiaca	5.2
Cymbella spp	13.7
Navicula NTA	13
Pinnularia microstauron	0.19
Stenoptreobia delicatissima	6.4
Surirella linearis	1.1

Zirahuén	Sed
Achnanthes minutissima	1.3
Achnanthes sp	0.3
Aulacoseira ambigua	1.9
Cyclotella meneghiniana	0.4
Cyclotella ocellata	67.1
Cyclotella schumannii	1.9
Cyclotella stelligera	1.2
Diploneis Elliptica	0.4
Epithemia spp	0.4
Fragilaria (girdle)	2.1
Fragilaria arcus	0.7
Fragilaria brevistriata	0.3
Fragilaria construens	1.5
Fragilaria construens var venter	2.8
Fragilaria crotonensis	16.6
Fragilaria pinnata v. pinnata	0.3
Gomphonema spp	0.7
Navicula radiosa	0.3
Nitzschia inconspicua	0.1

La Preciosa	**Veg**	**Sed**
Achnanthes exigua		0.3
Achnanthes minutissima	33	
Amphora ovalis var. lybica		0.9
Amphora pediculus		0.6
Amphora veneta	0.5	0.3
Amphora spp		1
Chaetocerous muelleri		0.6
Cocconeis placentula	2.3	1.8
Cyclotella meneghiniana	1.9	21.3
Cyclotella ocellata	0.2	
Cymbella cesatii	1.6	
Cymbella cymbiformis	5.9	0.45
Cymbella gracilis	0.5	
Cymbella mexicana	0.2	0.2
Cymbella microcephela		0.5
Cymbella spp		0.2
Diploneis elliptica		0.3
Diploneis puella	0.2	0.7
Entomoeoneis alata	0.2	
Epithemia adnata	9.5	
Epithemia argus		1.9
Epithemia turgida		1.2
Fragilaria brevistriata	6.7	9.8
Fragilaria capucina	5	
Fragilaria capucina var perminuta		0.5
Fragilaria construens		3.1
Fragilaria construens var venter		5.5
Fragilaria crotonensis		0.3
Fragilaria fasciculata	2.3	0.7
Fragilaria hungarica		0.7
Fragilaria pinnata	1	0.9
Gomphonema angustatum	0.2	
Gomphonema olivaceum	10.4	
Gomphonema olivaceum var minutissima	1.2	
Mastogloia smithii	5.7	2.4
Mastogloia smithii var lacustris	0.7	3.9
Navicula capitata		0.2
Navicula capitata var lueneburgemsis	0.2	
Craticula halophila		0.3
Navicula menisculus var. menisculus		0.2
Navicula minima var. minima		0.5
Navicula perminuta		0.5
Nitzschia amphibia	0.9	0.2
Nitzschia inconspicua		0.7
Nitzschia palea	4.7	32.8
Rhoicosphenia curvata		0.3
Rhopalodia gibba	0.4	0.45
Synedra biceps	0.2	
Synedra delicatissima	0.7	
Synerda radians	4.2	
Synedra ulna		1.9

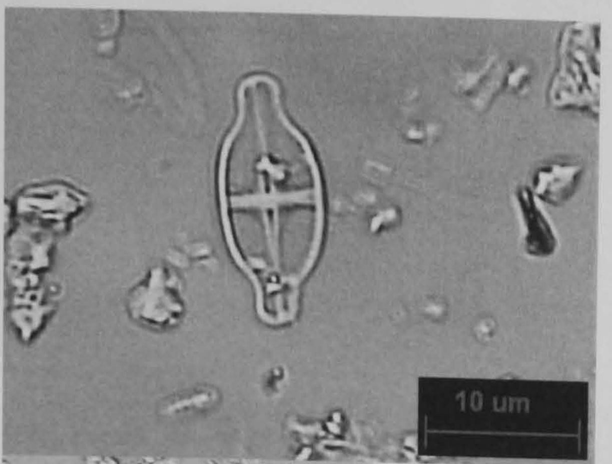
Appendix 2c - TWINSPAN results for all species identified in the surface sediment

```
                2211212         11111222231122
                279252319134567814789035806046

  9    AC 042A  --2---------------------------  00
 25    AU 001A  -2----------------------------  00
 29    CH 057A  1-----------1-----------------  00
 51    EY 016A  -23---------------------------  00
 52   EY0 16A1  --1---------------------------  00
 55     C YMB1  --2---------------------------  00
 99    CI 005A  4-2---------------------------  00
105    SL 003A  -1----------------------------  00
106    CV 004A  -2----------------------------  00
113    NA 168A  -2----------------------------  00
117    NI 010A  1-----------------------------  00
142    SU 006A  --1---------------------------  00
  6   AD0 09A1  -1-535243------------1--------  0100
 87    GO 013D  -----------1---------------4--  01010
 90    GO MPH1  ---------------------------2--  01010
 42    CY 004A  -------2--------2-------------  010110
  5    AD 009A  ----1-------------------------  010110
 22    AU 002A  ------31---------2------------  010110
 28     A ula1  ----1-------------------------  010110
 46    CM 052A  ----1-------------------------  010110
 13    AM 001D  --------1---1-----------------  010111
 15    AM 004A  --------1---------------------  010111
 37    CY 003A  --------222-2--------2---2----  0110
115    NI 014A  --------2--4----2-22-1--------  0110
125    NI 009A  ---------------------3---32---  01110
148    ST 008A  --------------------------3---  01110
152    SY 003A  --------------------------2---  01110
161    SY 001A  --------------------------2---  01110
  1     A ACAR  -------------------2----------  01111
  4    AC 001R  -----------------------2------  01111
 12   AM0 01A1  ------------1-----------------  01111
 14    AM 012A  -------------------1-1--------  01111
 23    AU 003D  ---------22---3--33--1--------  01111
 24    AU 003B  ---------31--12-2-----2-------  01111
 31    CO 066A  -----------------------3------  01111
 34    CO 001C  ---------------1-1---1--------  01111
 36    CY 007A  ---------1---1--2-------------  01111
 38    CY 006B  ------------------------4-----  01111
 39    CY 002A  ---------11-------------------  01111
 66       FRGR  ------------1----------2------  01111
 67    PS 001A  ----------1----2-------1-1----  01111
 71    SR 001A  -------------2-12--11---------  01111
 72    FR 002E  ---------------1--------------  01111
 73    FR 002C  ----------1----22---2--1-1----  01111
 74    FR 008A  --------------------3-412-----  01111
 77    SS 002A  ----------1----2---21---------  01111
 78    FR 001D  ----------1-------------------  01111
120    NI 008A  ------------1-----------------  01111
121   NI0 08A1  ------------1-----------------  01111
127    NI 193A  ------------2-----------------  01111
143    ST 011B  -------------2----------------  01111
144    ST 019B  -------------2----------------  01111
146    ST 012A  -------------2----------------  01111
149    St eph1  --------------2---------------  01111
 35    CY 057A  ----------------------------45  1
 40    CY 017A  ----------------------------2-  1

                000000000000000000000000000011
                0001111111111111111111111111
                   0000000000000000000000001
                   000000111111111111111111
                   000001000000000000000001
                   00011
```

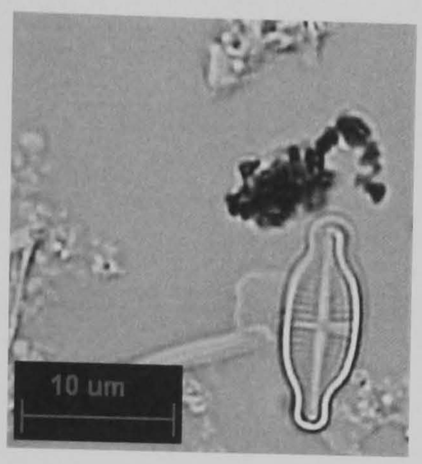
Diatom Plates

1. *Achnanthes exigua* San Pedro (modern material)
2. *Achnanthes exigua* (rapheless) valve Santa Maria del Oro (modern material)
3. *Achnanthes hungarica* La Alberca Teremendo (modern material)
4. *Achnanthes lanceolata* Cuitzeo (modern material)
5. *Achnanthes lanceolata* ssp *frequentissima* Quechulac (modern material)
6. *Achnanthes lanceolata* ssp *dubia* Camecuaro (modern material)
7. *Achnanthes lanceolata* Zirahuén (Core material 4 cm)
8. *Achnanthes lanceolata* Zirahuén (Core material 10 cm)
9. Achnanthes minutissima *San Pedro (modern material)*
10. *Achnanthes minutissima* San Pedro (modern material) – illustrating curvature at ends
11. *Achnanthes minutissima* (girdle view) Tecuitlapa (modern material)

1

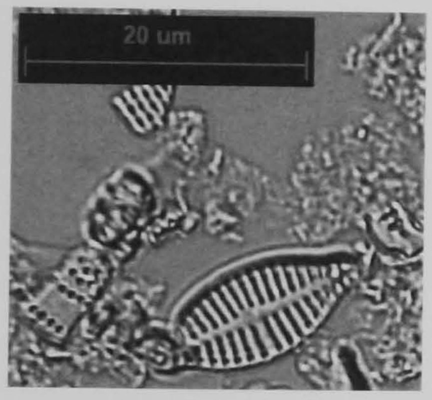


2

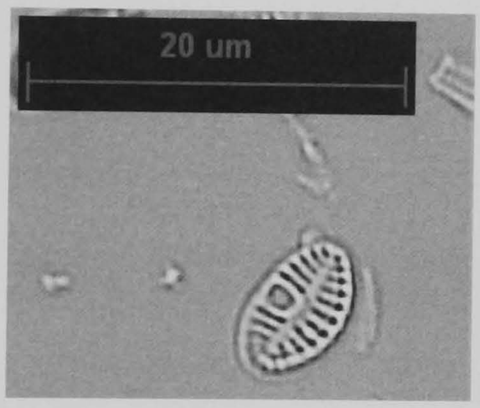


3

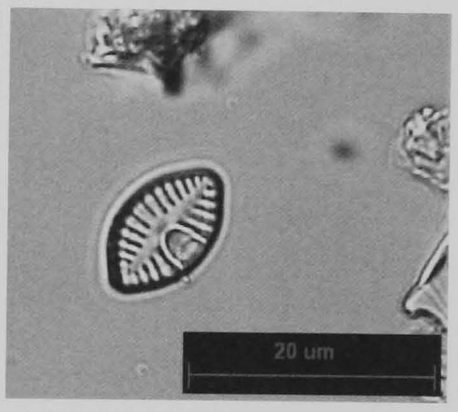
4



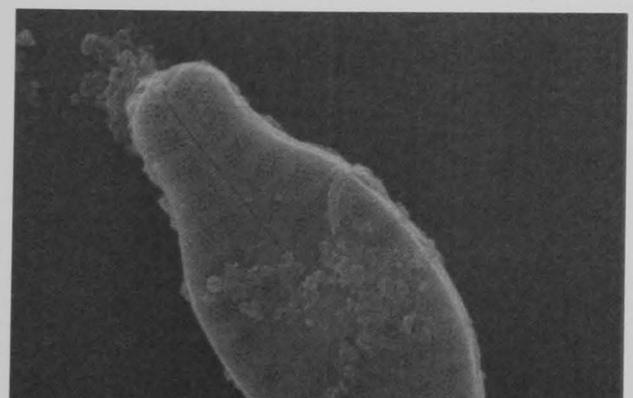
5



6



7

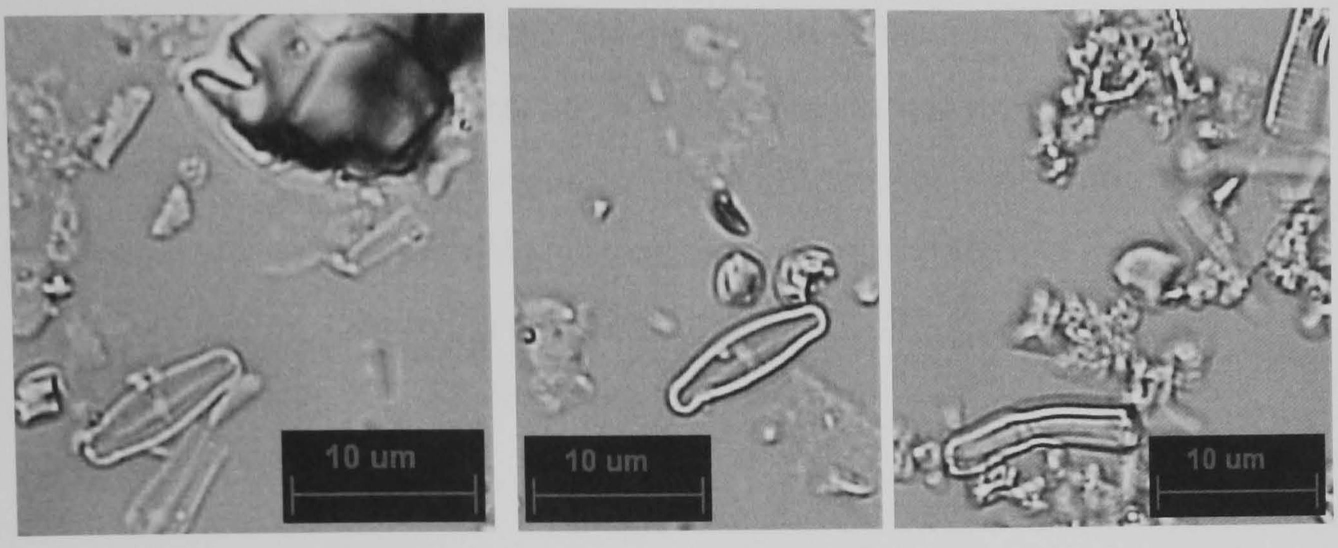


8

9

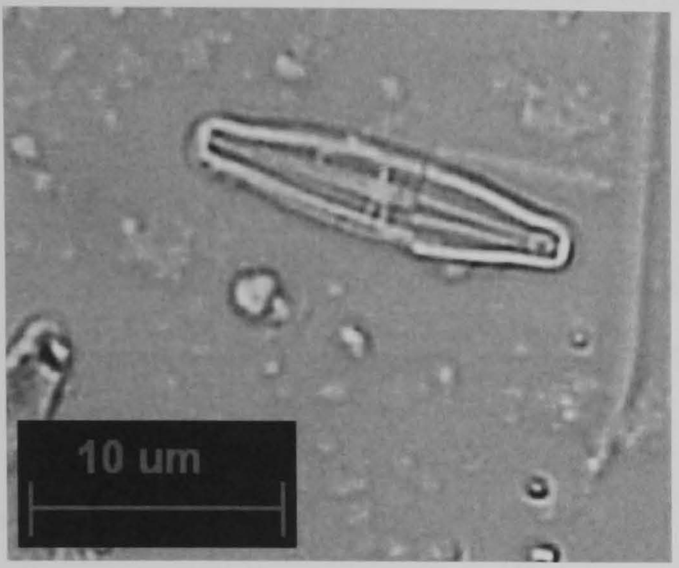
10

11

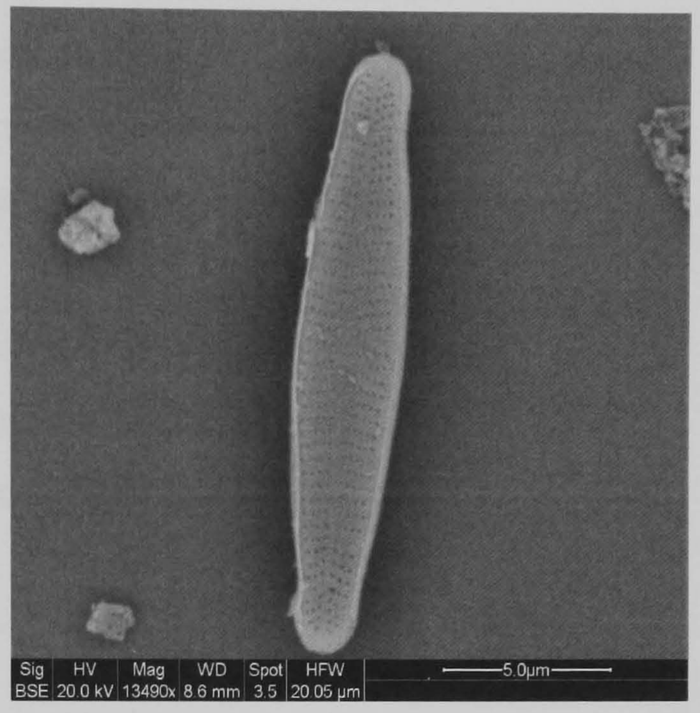

10 um
10 um
10 um

12. *Achnanthes minutissima* Zirahuén (Core material 24 cm)
13. *Achnanthes minutissima* (SEM) San Gregorio (modern material)
14. *Achnanthes subatomoidies* Lago de la Luna (modern material)
15. *Amphora coffeaeformis* La Piscina de Yuriria (modern material)
16. *Amphora montana* La Alberca Teremendo (modern material)
17. *Amphora ovalis* Cuitzeo (modern material)

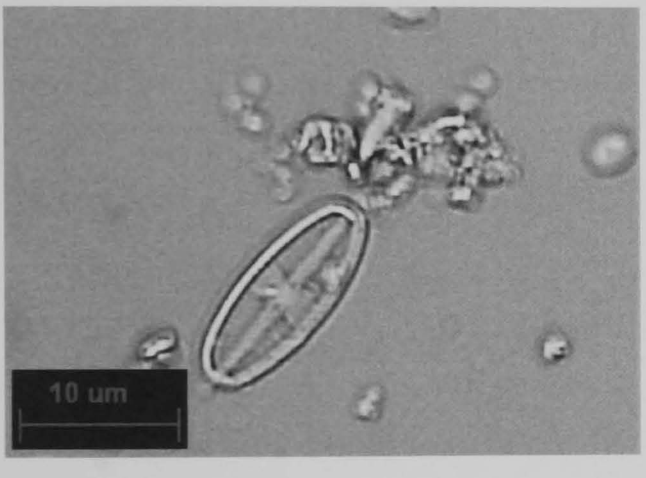
12



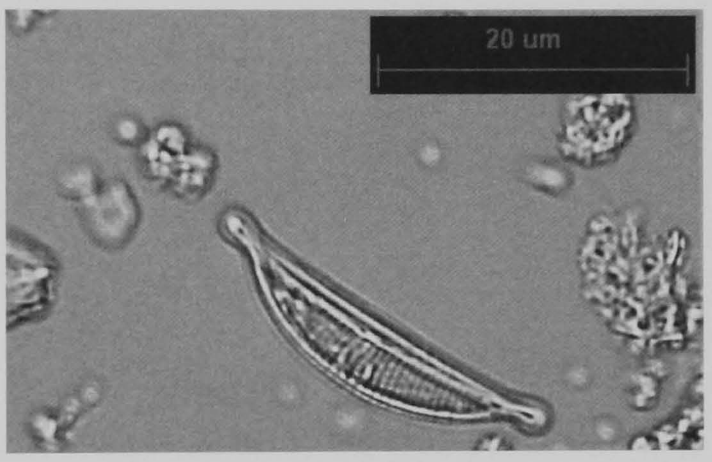
13



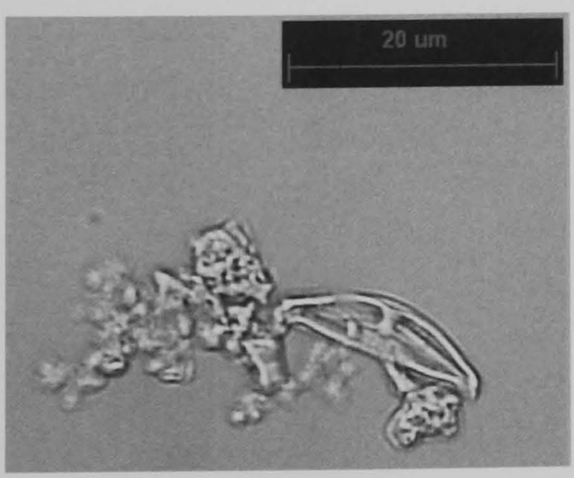
14



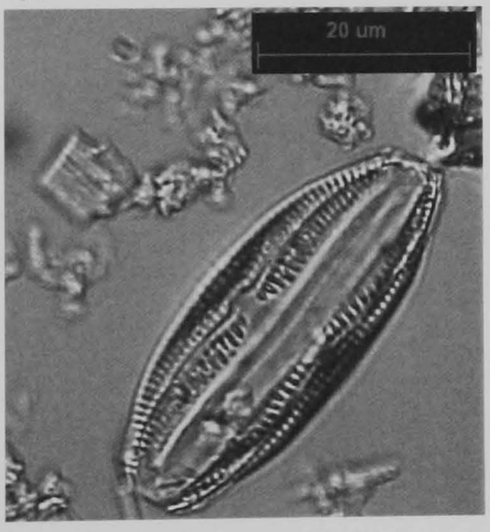
15



16

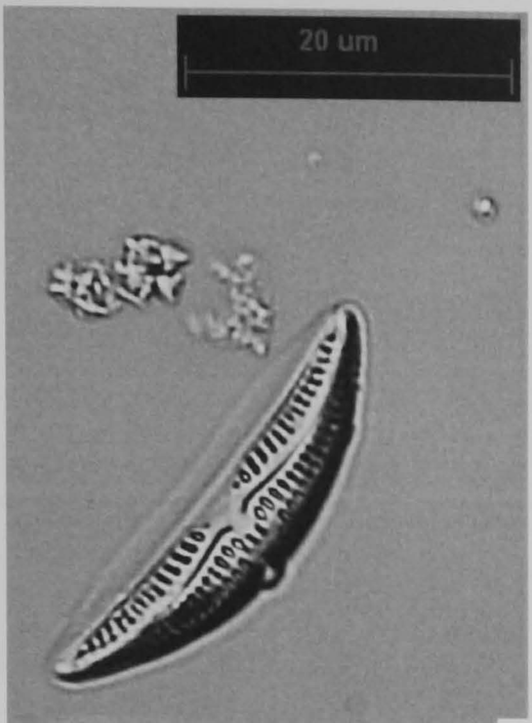


17

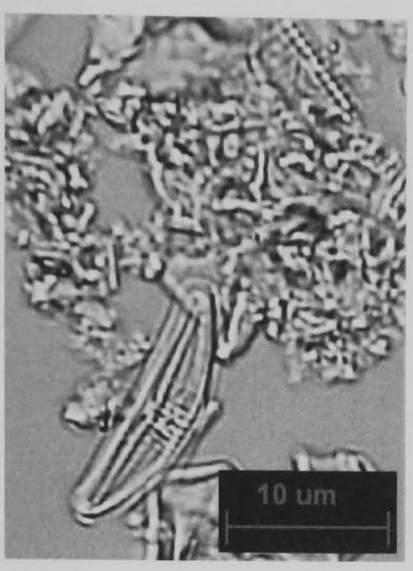


18. *Amphora ovalis* var. *lybica* Los Negritos (modern material)
19. *Amphora veneta* Tepetiltic (modern material)
20. *Amphora pediculus* Quechulac (modern material)
21. *Anomoeoneis sphaerophora* f. *costata* La Piscina de Yuriria (modern material)
22. *Anomoeoneis vitrea* La Alberca Tacambaro (modern material)
23. *Asterionella formosa* Zempoala (modern material)
24. *Aulacoseira ambigua* Zirahuén (core material 7 cm)

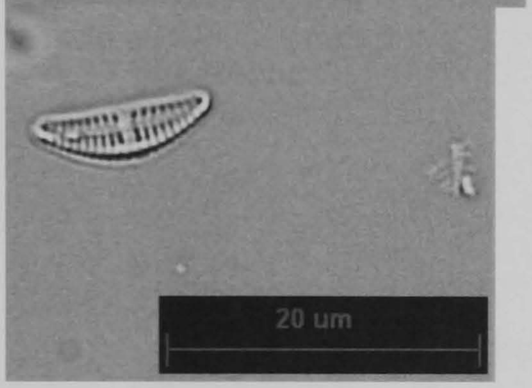
18



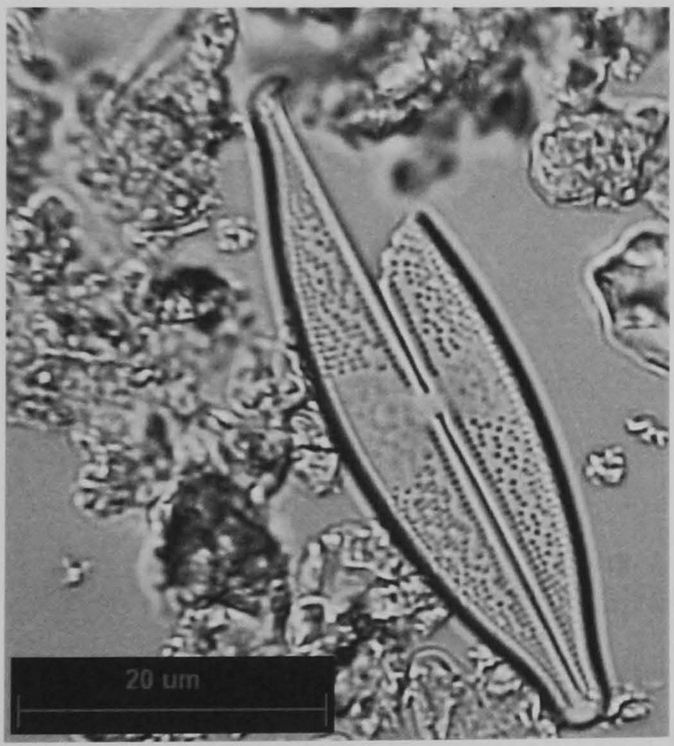
19



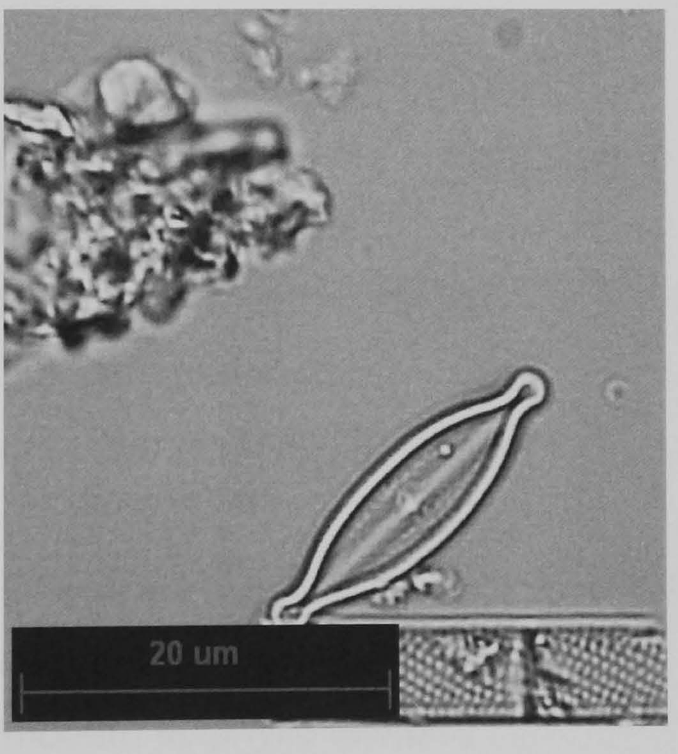
20



21

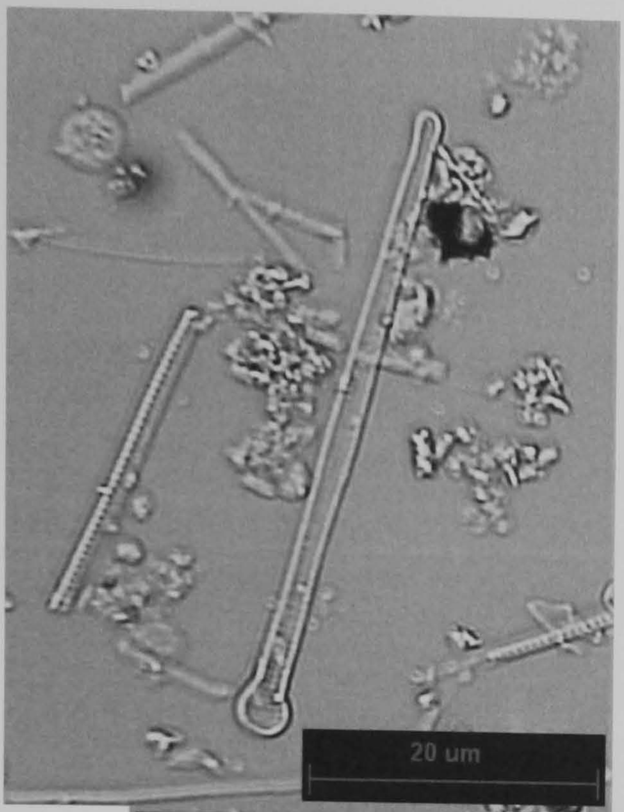


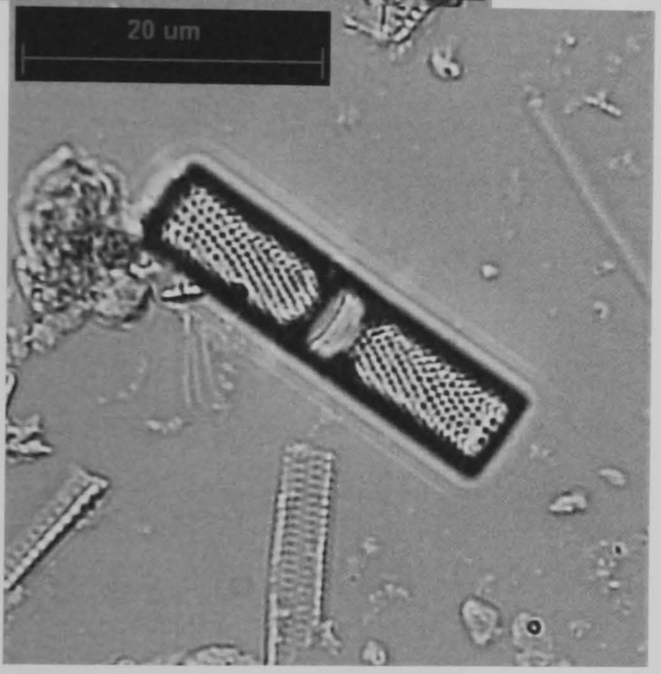
22



23

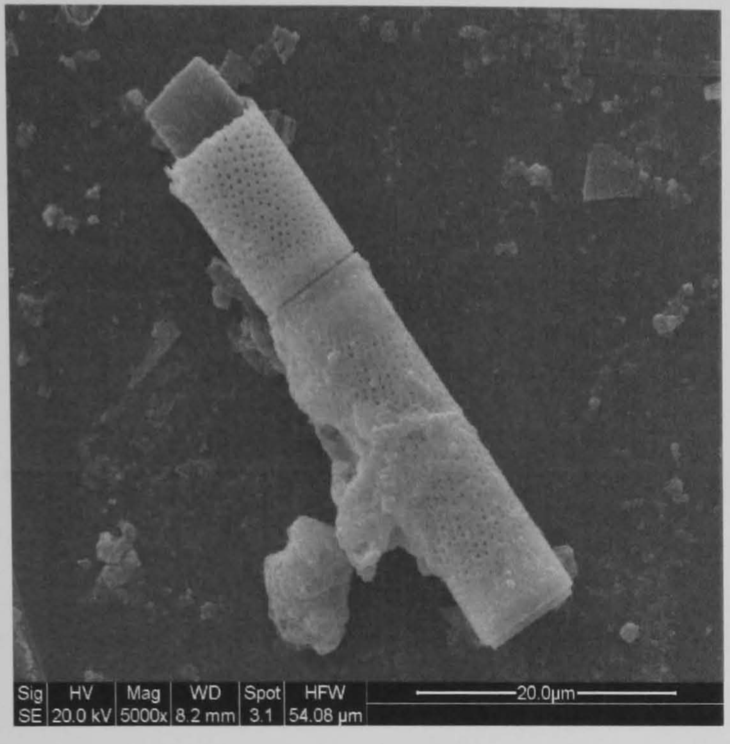
24



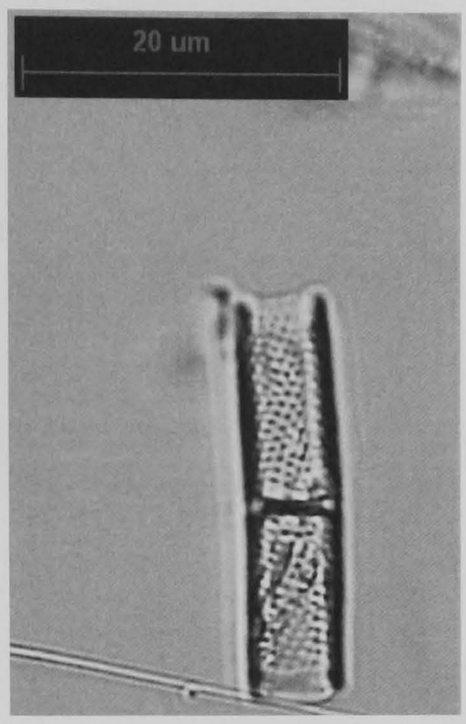


25. *Aulacoseira ambigua* (SEM) Zirahuén (core material 10 cm)
26. *Aulacoseira ambigua* Quechulac (modern material)
27. *Aulacoseira granulata* var. *graunulata* Santa Maria Oro (modern material)
28. *Aulacoseira granulata* var. *granulata* (SEM) Cajititlan (modern material)
29. *Aulacoseira granulata* var *angustissima* (Zapotlan)
30. *Aulacoseira italica* Lago del Sol (modern epiphytic sample)

31. 25

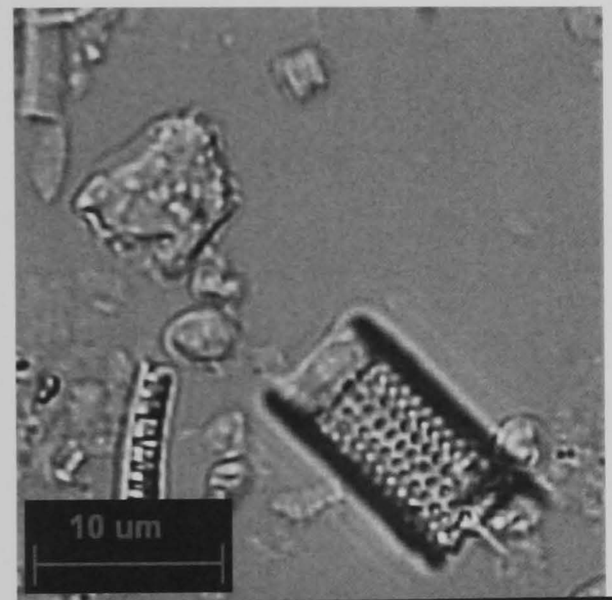


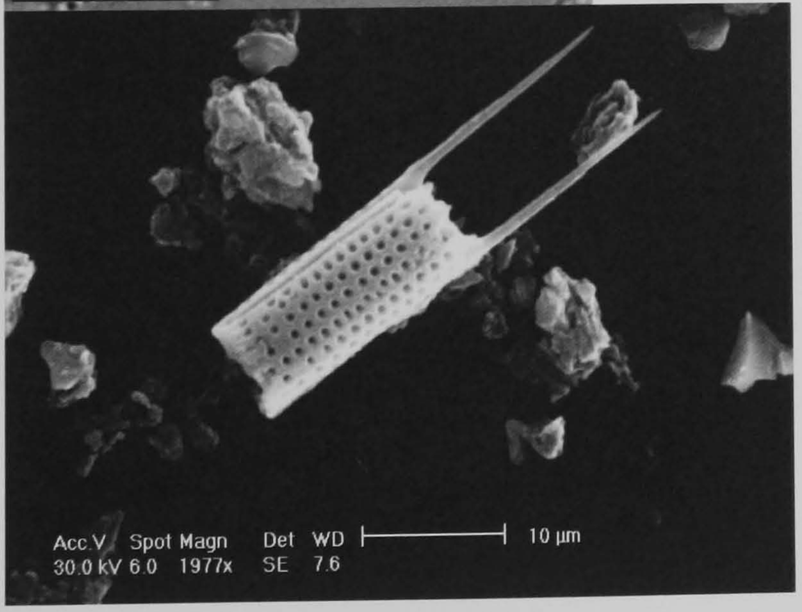
26



27

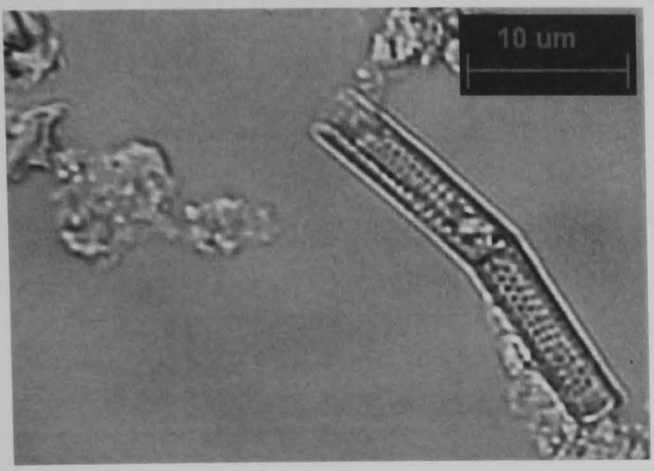
28

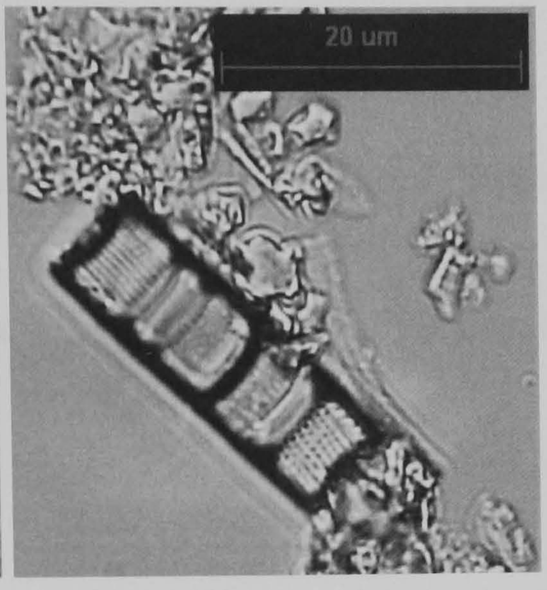




29

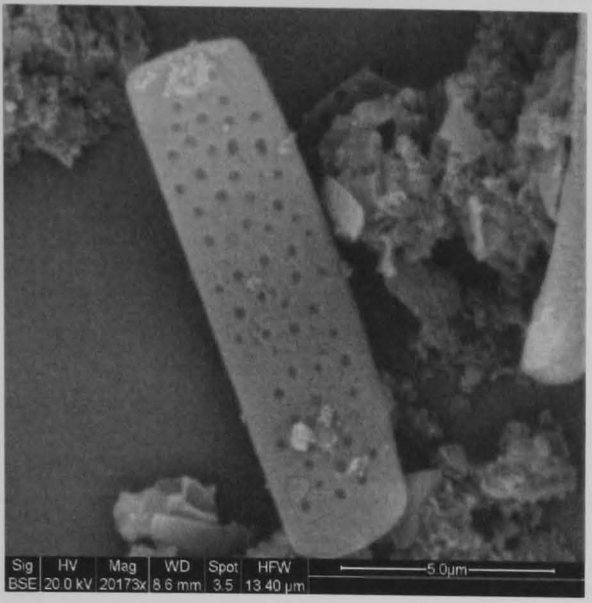
30


10 um

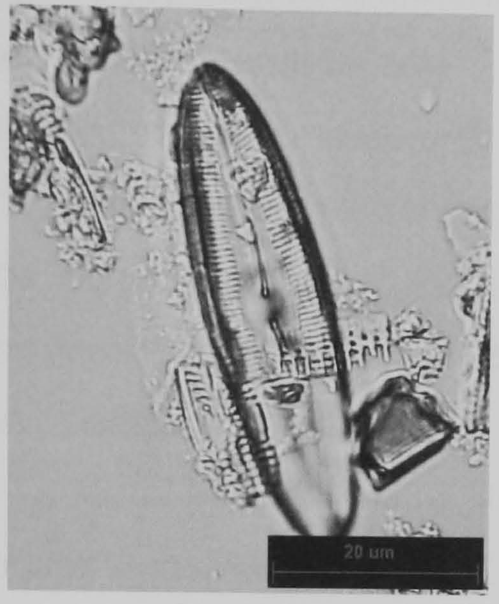

20 um

32. *Aulacoseira spp.* (SEM) San Gregorio
33. *Caloneis silicula* Cuitzeo (modern material)
34. *Cavinula* (= *Navicula) pseudoscutisformis* Lago del Sol (modern material)
35. *Cavinula* (= *Navicula) pseudoscutisformis* (SEM) Lago de la Luna (modern material)
36. *Chaetocerous muelleri* (resting spore) La Piscina de Yuriria (modern material)
37. *Cocconeis neodiminuta* Quechulac (modern material)

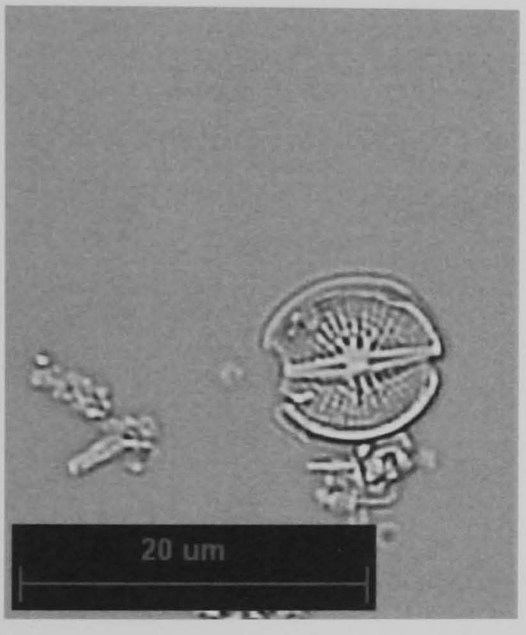
32



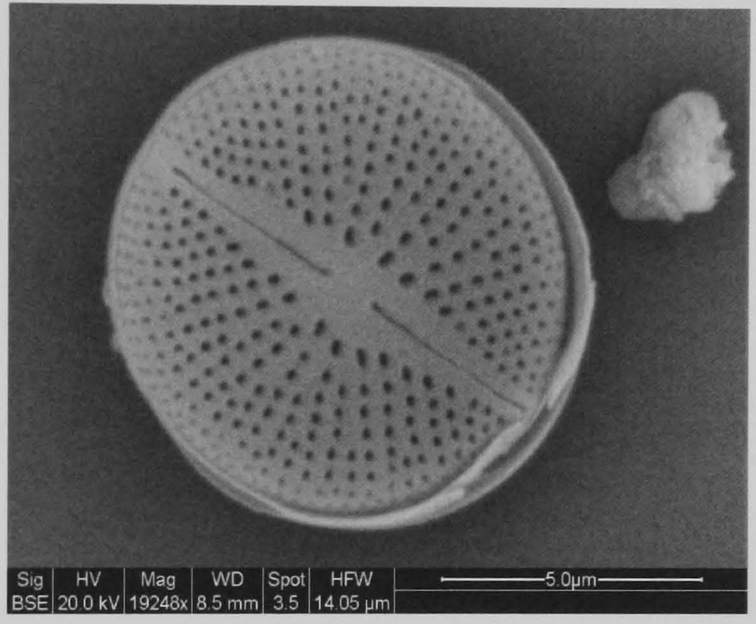
33



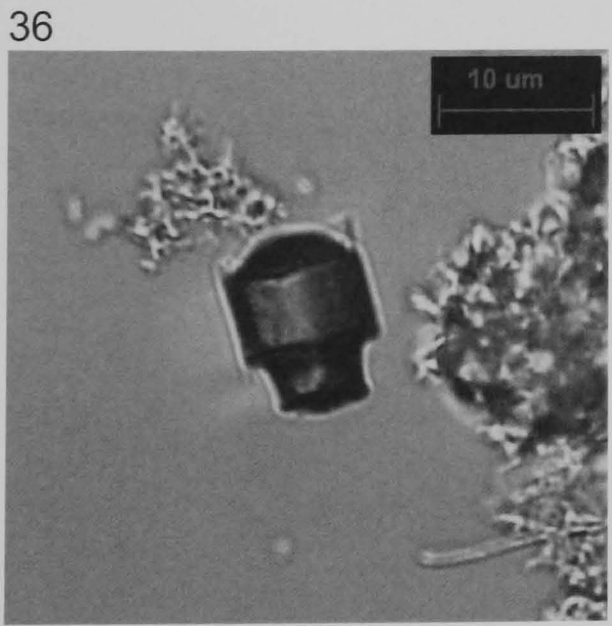
34



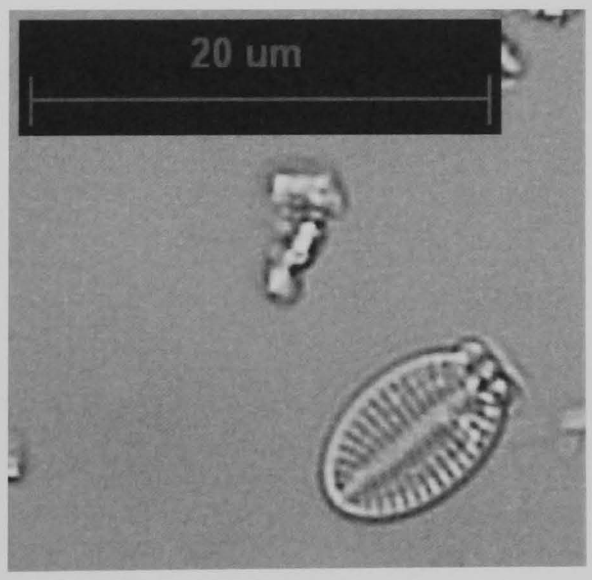
35



36



37



38. *Cocconeis placentula* (raphless valve) Cuitzeo (modern material)

39. *Cocconeis placentula* Zirahuén (core material 64 cm)

40. *Cocconeis placentula* (SEM) Zirahuén (core material 10 cm)

41. *Craticula* (=*Navicula*) *halophila* La Piscina de Yuriría (modern material)

42. *Cyclotella choctawhatcheeana* Alchichica (modern material)

43. *Cyclotella choctawhatcheeana* Atexcac (modern material)

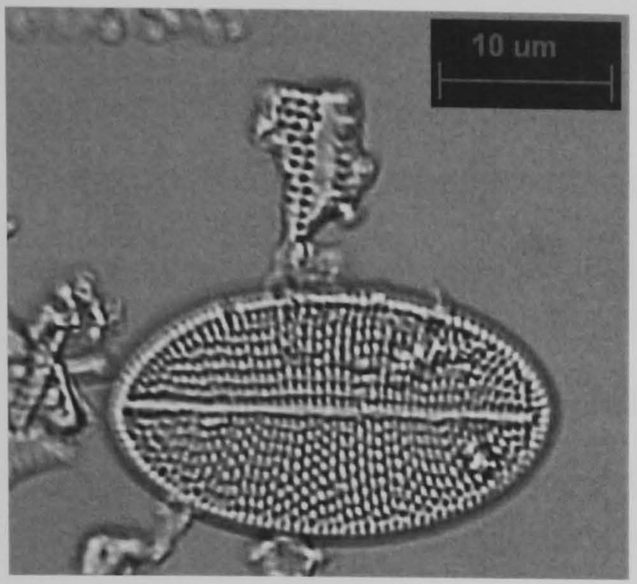
44. *Cyclotella glomerata* Zapotlan (modern material)

45. *Cyclotella glomerata* Laguna La Maria (modern material)

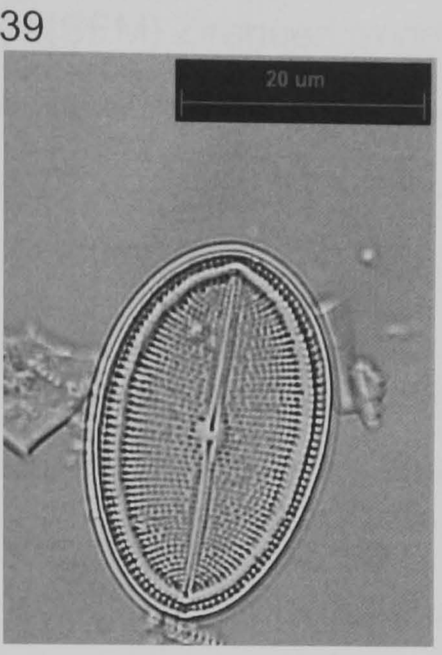
46. *Cyclotella meneghiniana* Zapotlan (moern material)

47. *Cyclotella ocellata* Zirahuén (modern material)

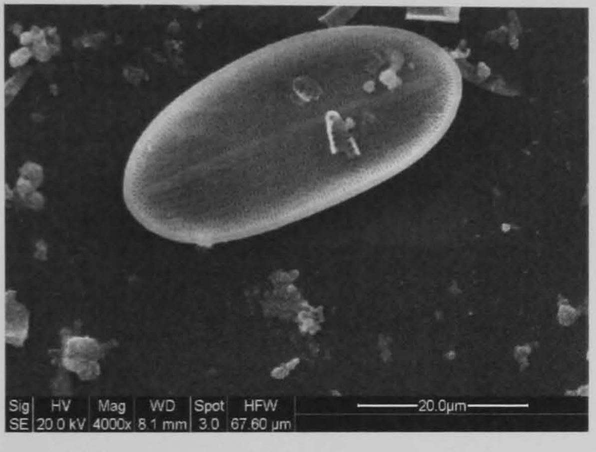
38



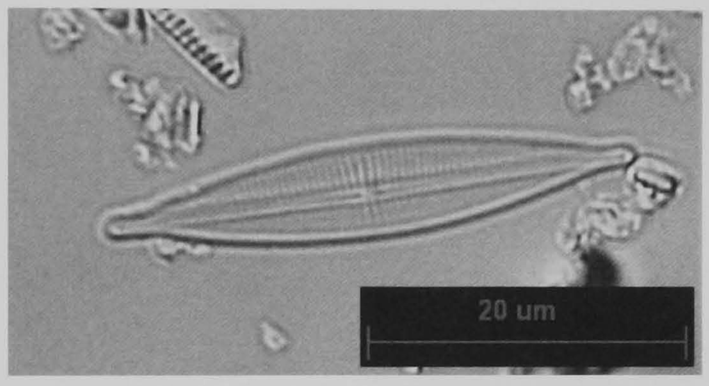
39



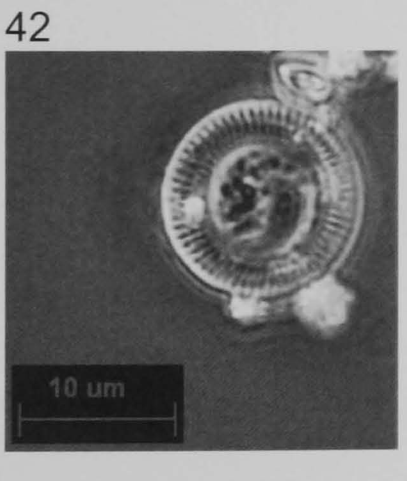
40



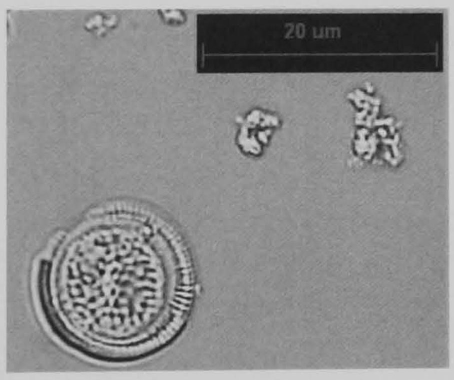
41



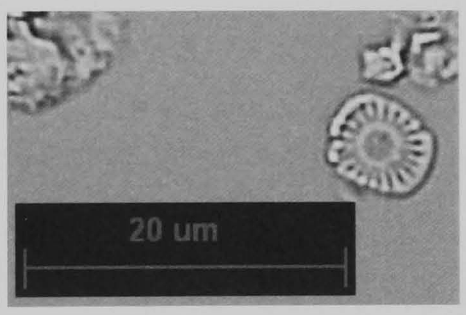
42



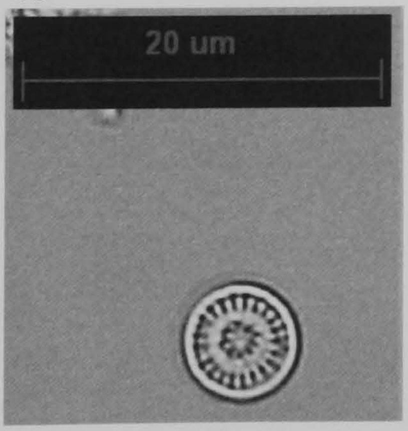
43



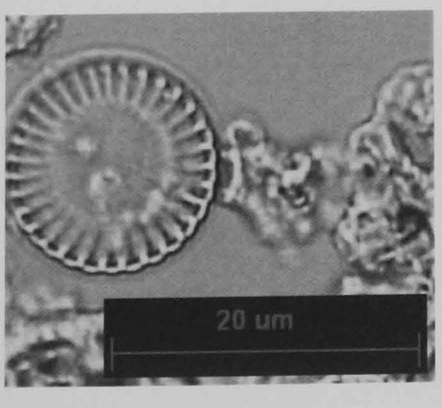
44



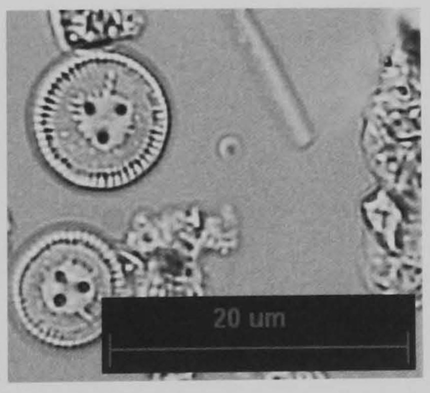
45



46

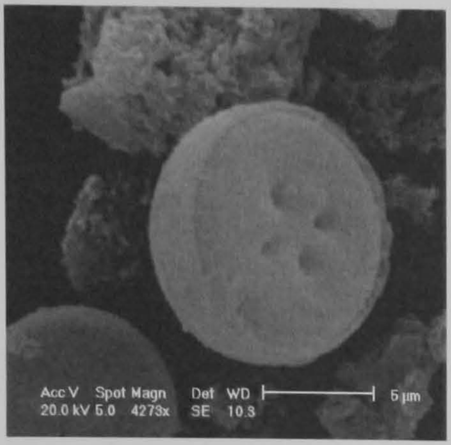


47

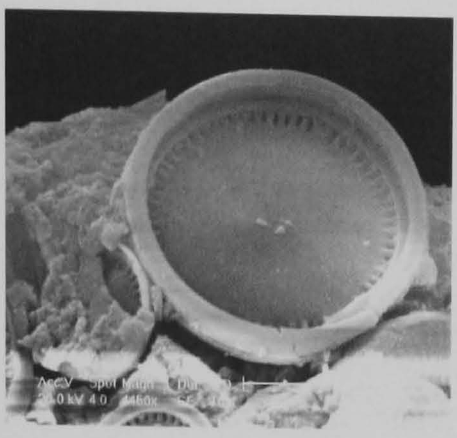


48. *Cyclotella ocellata* external view (SEM) Zirahuén (modern material)
49. *Cyclotella ocellata* internal view (SEM) Zirahuén (modern material)
50. *Cyclotella pseudostelligera* Laguna La Maria (modern material)
51. *Cyclotella quillensis* Alchichica (modern material)
52. *Cyclotella stelligera* Zapotlan (modern material)
53. *Cyclotella stelligera* (SEM) Juanacatlan (modern material)
54. *Cyclotella stelligera* (SEM) Zirahuén (core material 52 cm)
55. *Cyclotella stelligera* Zirahuén (core material 64 cm)
56. *Cymbella amphicephala* San Gregorio (modern material)
57. *Cymbella amphicephala* (SEM) San Gregorio (modern material)

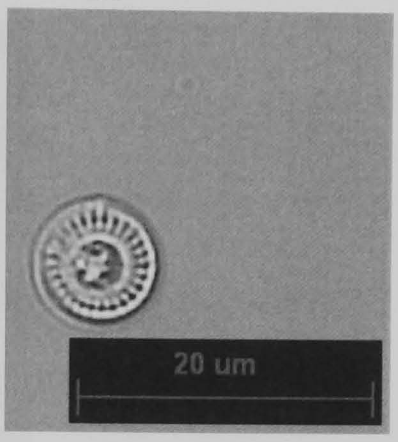
48



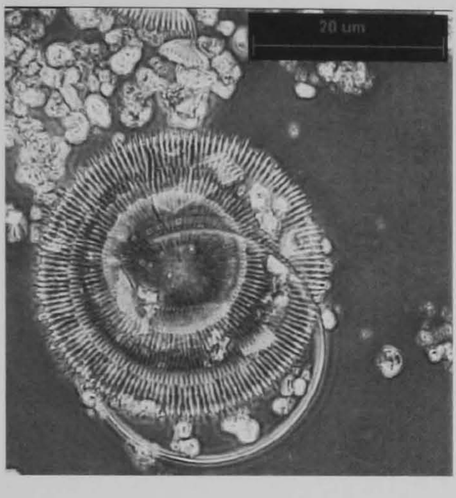
49



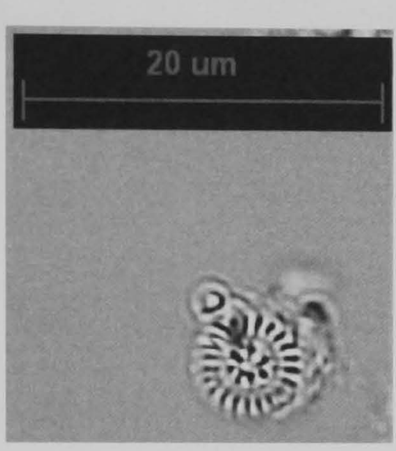
50



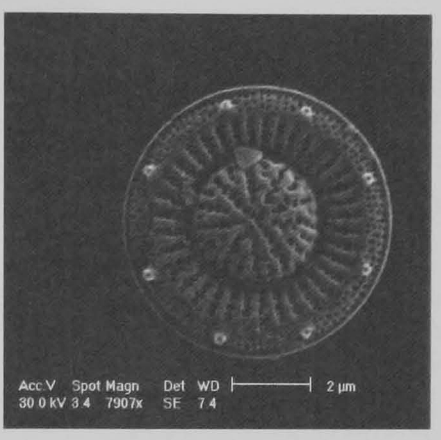
51



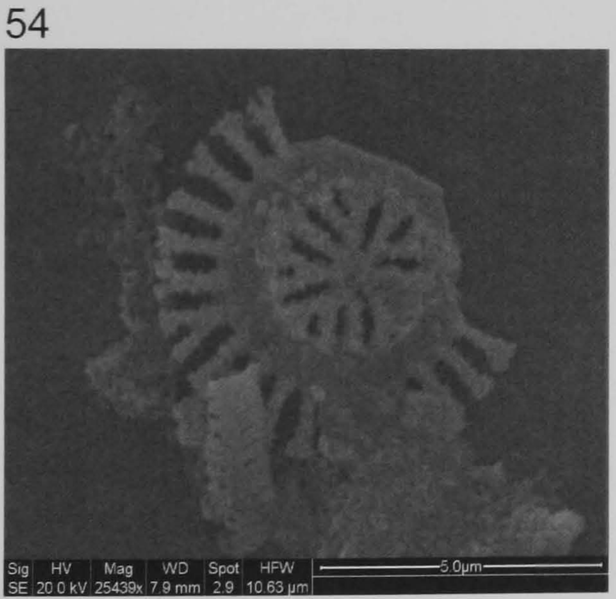
52



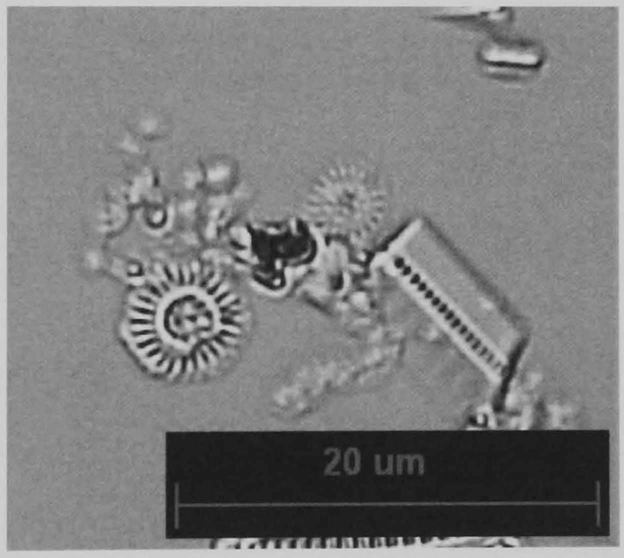
53



54

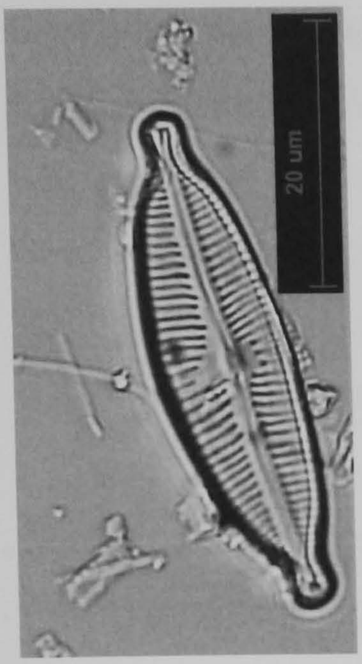


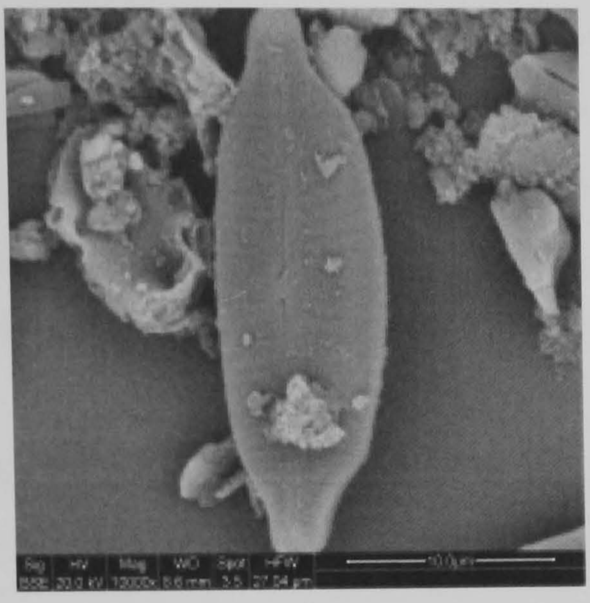
55



56

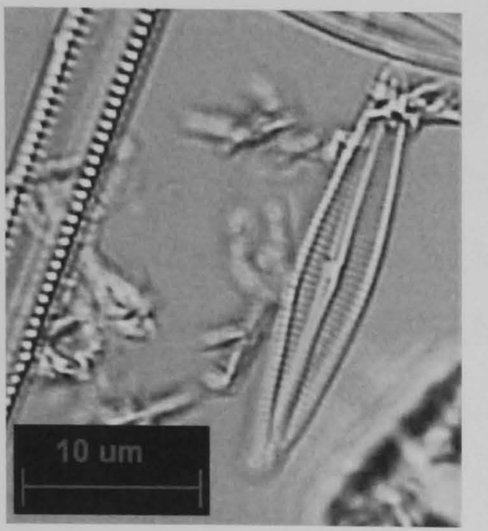
57


20 um

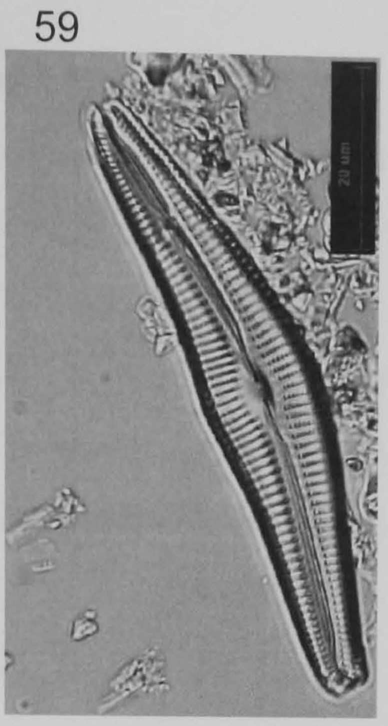

Sig
HV
Mag
WD
Spot
HFW
SSE
20.0 kV
10000x
8.6 mm
3.5
27.54 µm
10.0µm

58. *Cymbella cesatii* La Preciosa (epiphytic sample)
59. *Cymbella cymbiformis* Tecuitlapa (modern material)
60. *Cymbella descripta* San Gregorio (modern material)
61. *Cymbella descripta* Zirahuén (core material 0 cm)
62. *Cymbella descripta* internal view (SEM) Zirahuén (core material 16 cm)
63. *Cymbella descripta* external view (SEM) Zirahuén (core material 16 cm)
64. *Cymbella gracilis* La Preciosa (modern marterial)
65. *Cymbella mexicana* (central area) La preciosa (modern material)

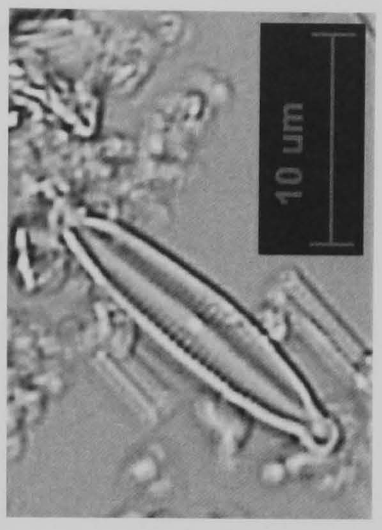
58



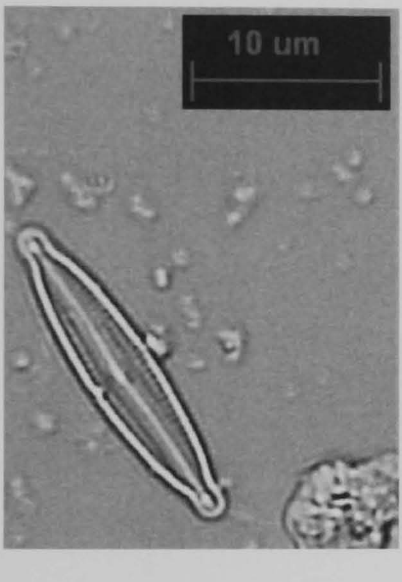
59



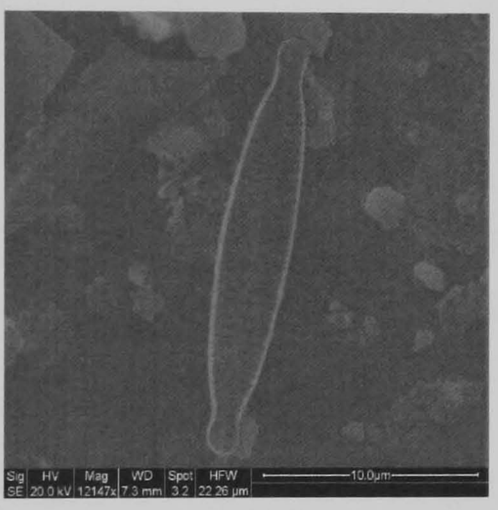
60



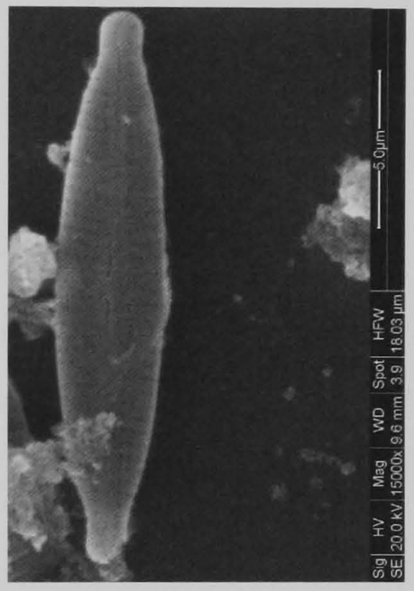
61



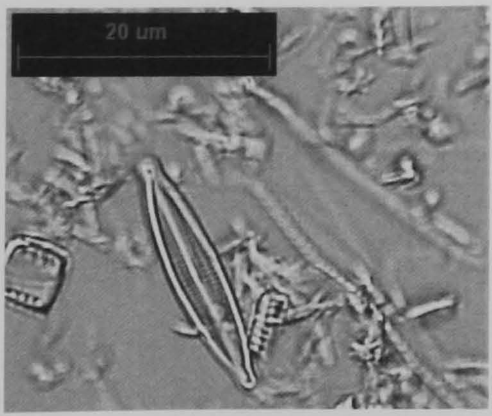
62



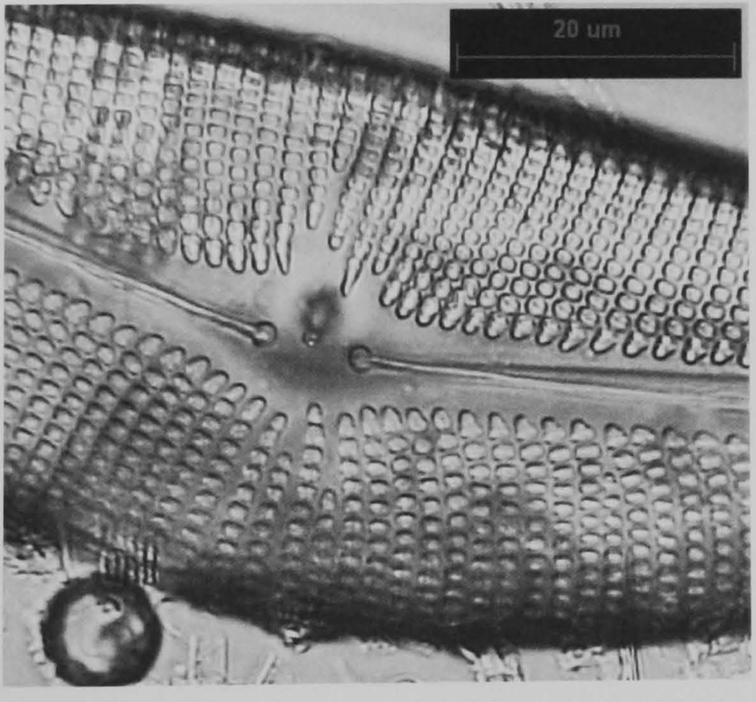
63



64

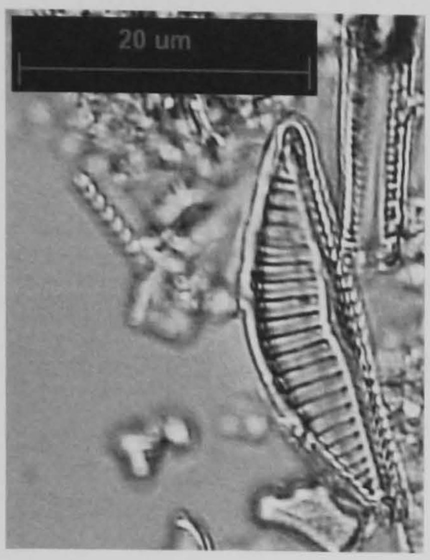


65

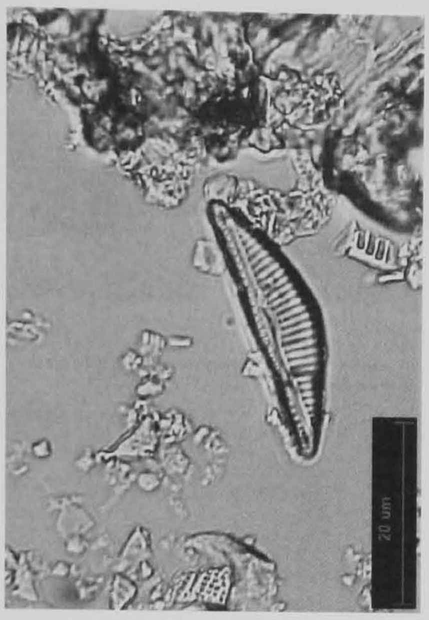


66. *Cymbella minuta* Laguna la Maria (modern material)
67. *Cymbella minuta* var. *silesiaca* San Gregorio (modern material)
68. *Cymbella minuta* Lago de la Luna (modern material)
69. *Cymbella spp (minuta?)* Lago de la Luna
70. *Cymbella minuta* (SEM) Lago de la Luna (modern material)
71. *Cymbella minuta* (SEM) (end) Lago de la Luna (modern material)
72. *Cymbella minuta* (SEM) (central area) Lago de la Luna (modern material)

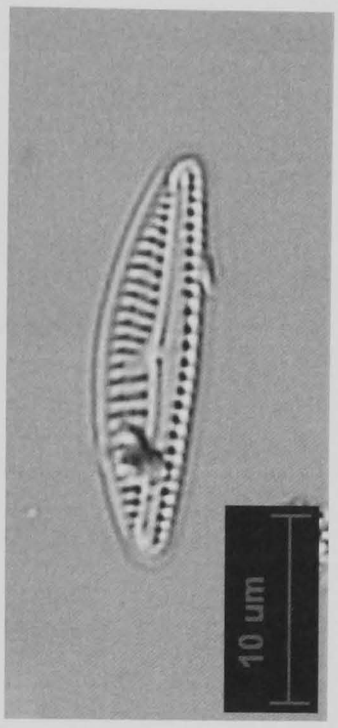
66



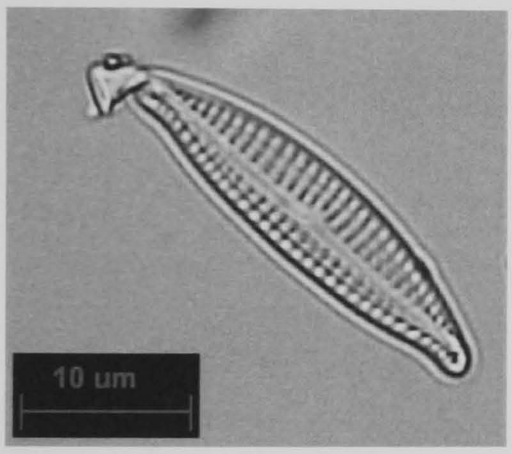
67



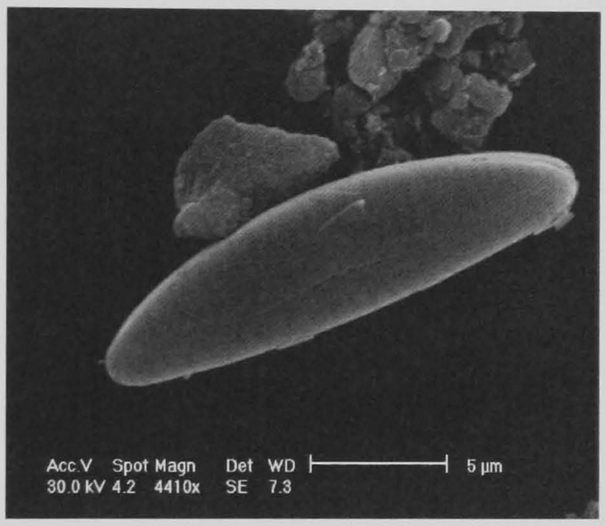
68



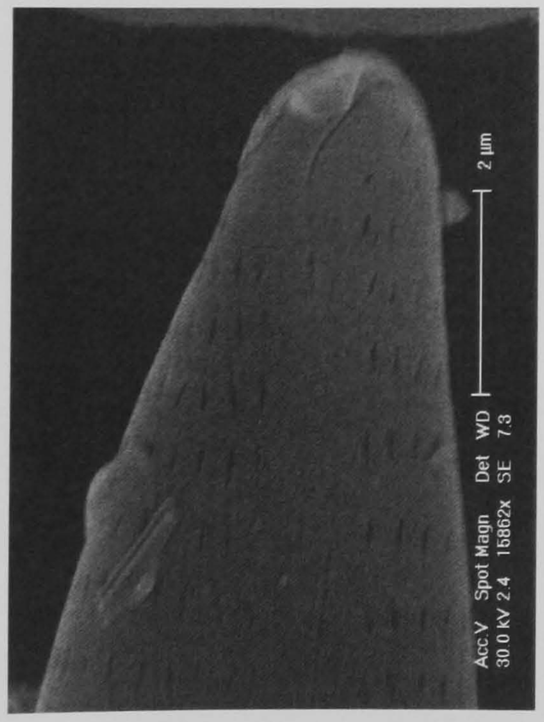
69



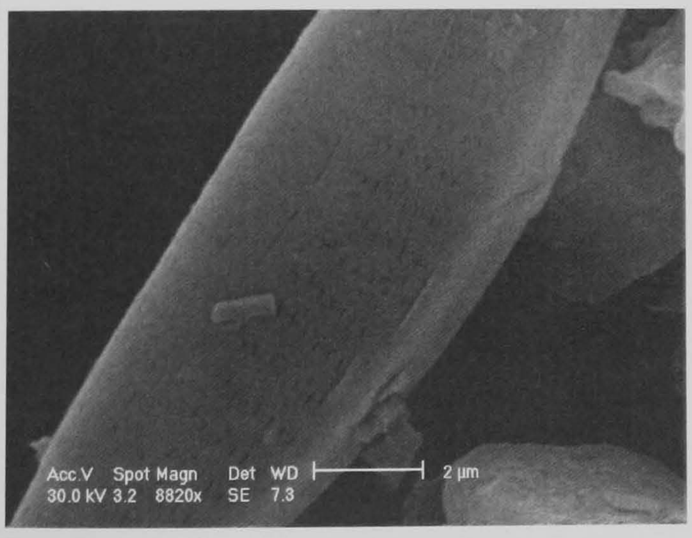
70



71

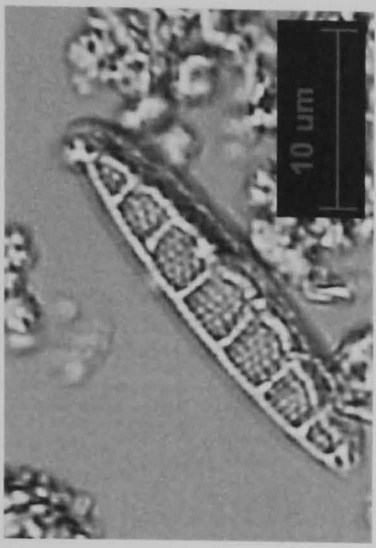


72

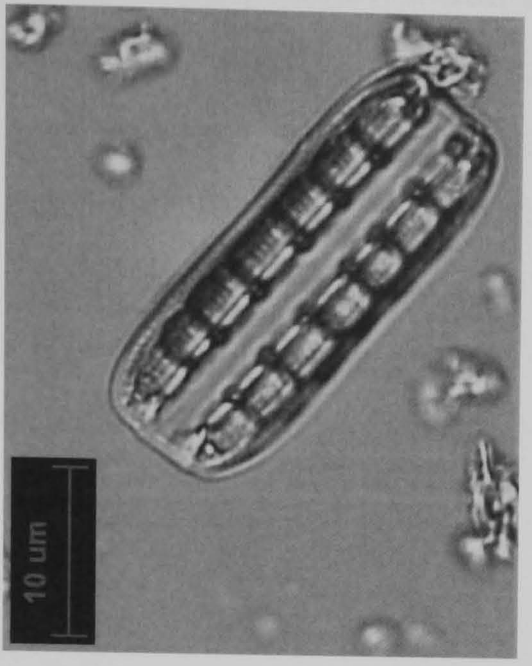


73. *Denticula thermalis* La Piscina de Yuriría (modern material)
74. *Denticula thermalis* (girdle view) La Piscina de Yuriría (modern material)
75. *Diatoma* spp Zempoala (modern material)
76. *Diploneis elliptica* Zirahuén (core material 24 cm)
77. *Diploneis elliptica* (SEM) Zirahuén (modern material)
78. *Diploneis ovalis* Zapotlan (modern material)
79. *Diploneis modica* Zirahuén (core material 7 cm)
80. *Diploneis modica* (SEM) Zirahuén (core material 40 cm)

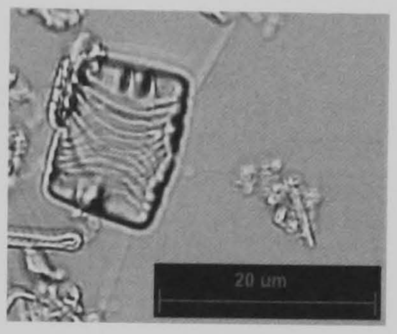
73



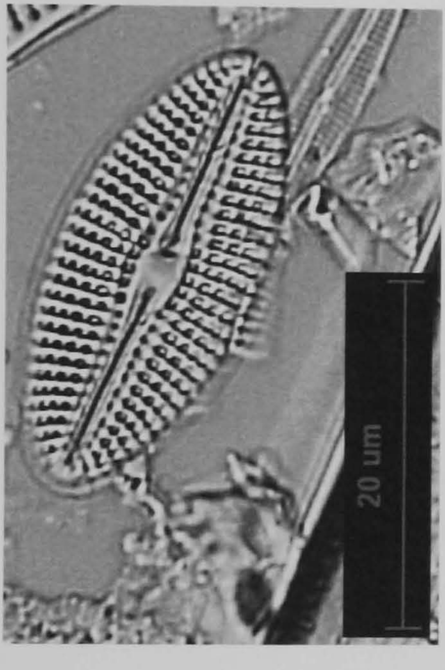
74



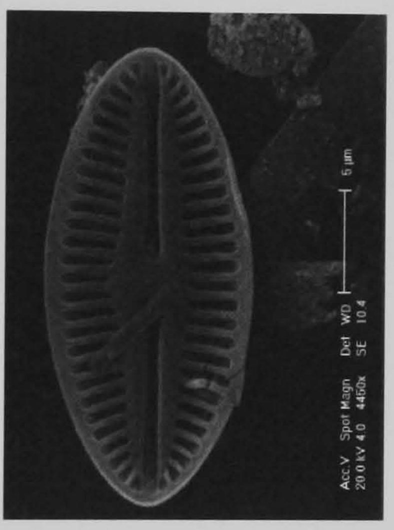
75



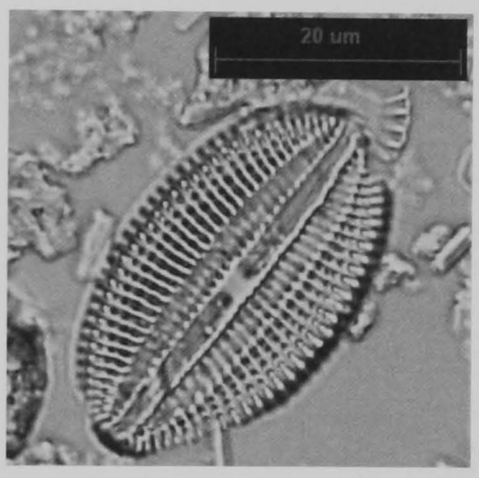
76



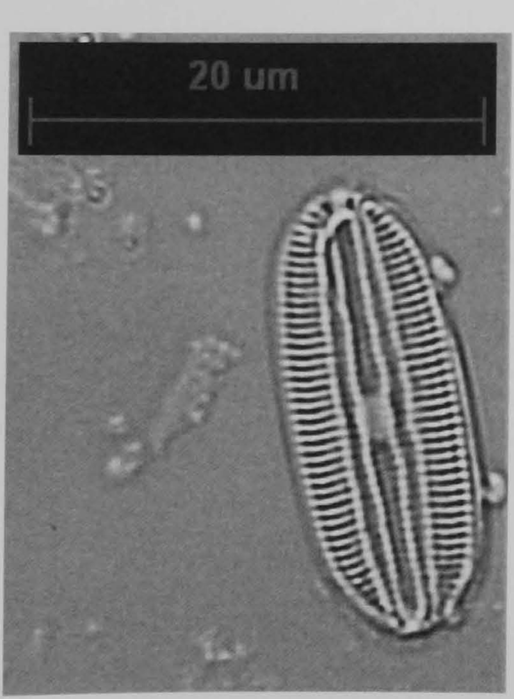
77



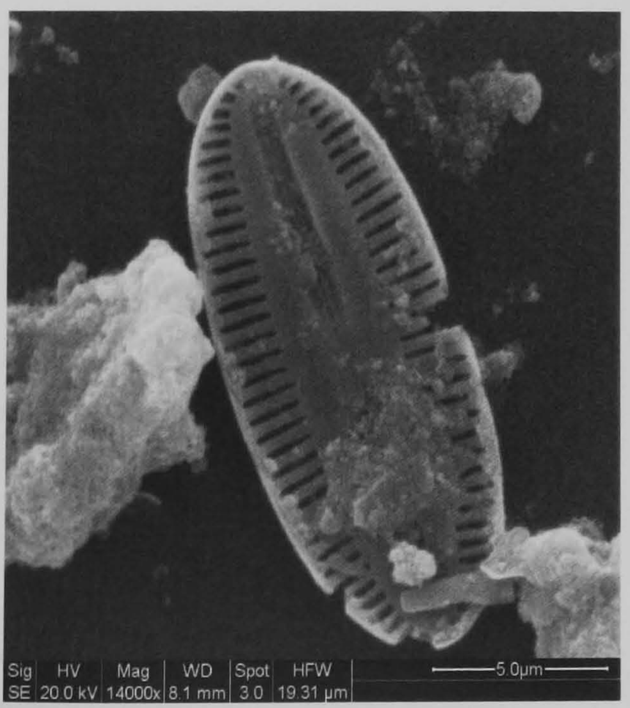
78



79



80



81. Epithemia adnata Tecuitlapa (modern material)

82. Epithemia adnata (SEM) Chapala (modern material)

83. Epithemia argus La Preciosa (modern material)

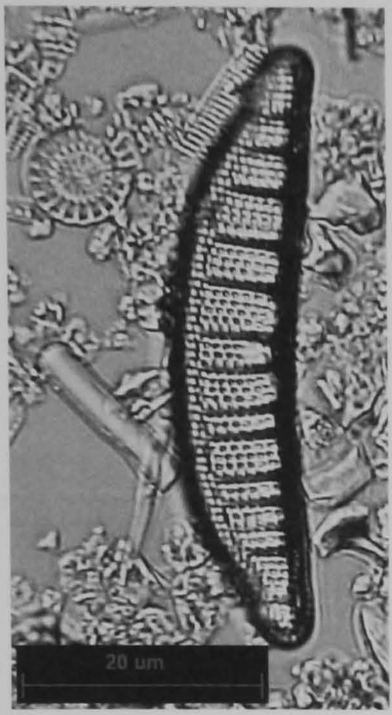
84. Epithemia sorex (SEM) Zirahuén (core material 0 cm)

85. Epithemia sorex Zirahuén (core material 5cm)

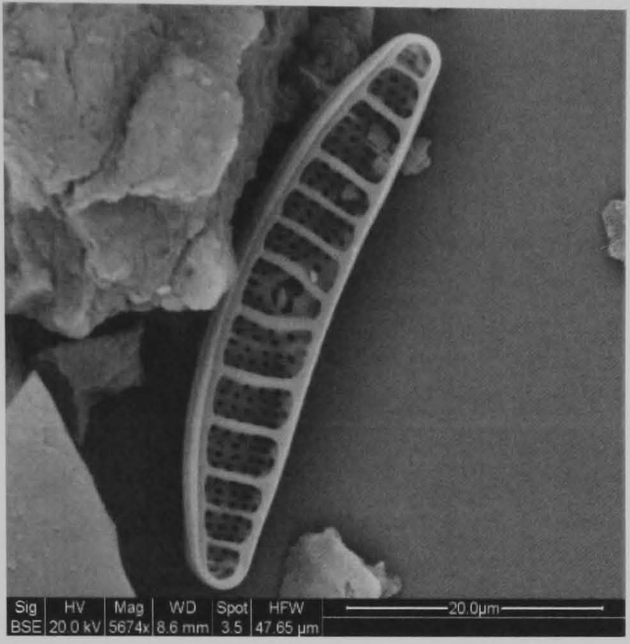
86. Epithemia turgida Cuitzeo (modern material)

87. Epithemia turgida (SEM) Zirahuén (core material 5 cm)

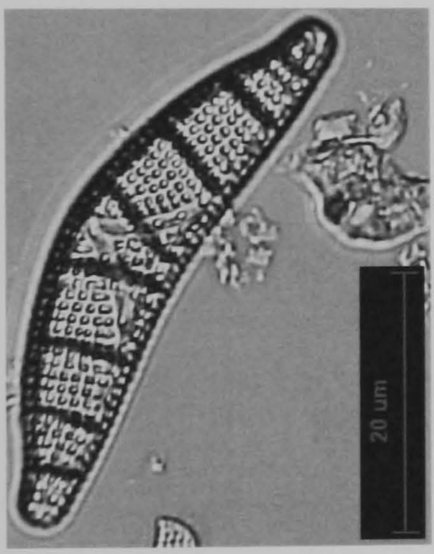
81



82

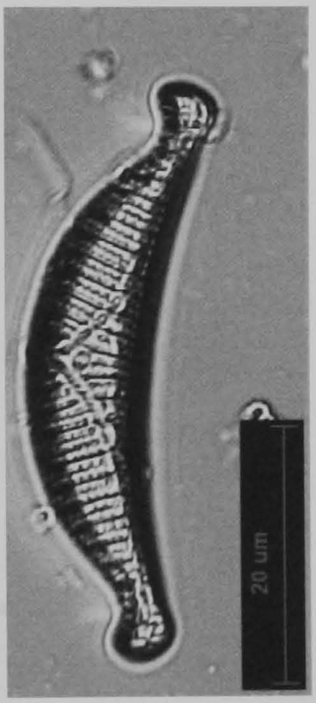


83

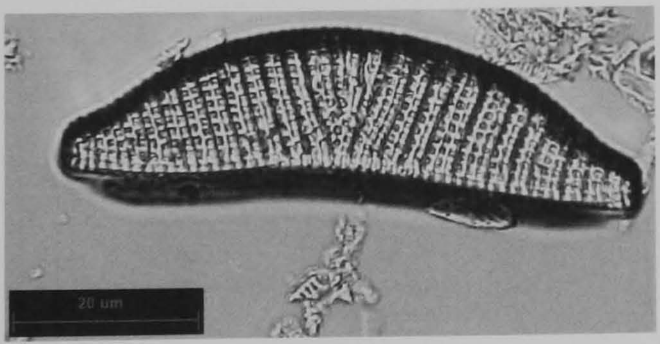


84

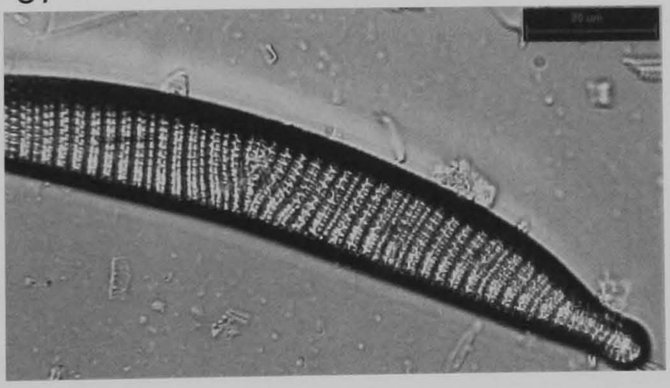
85



86



87



88. *Fragilaria brevistriata* La Preciosa (epiphytic sample)

89. *Fragilaria brevistriata* (internal view) Zirahuén (modern material)

90. *Fragilaria brevistriata* (SEM) Zirahuén (core material 34 cm)

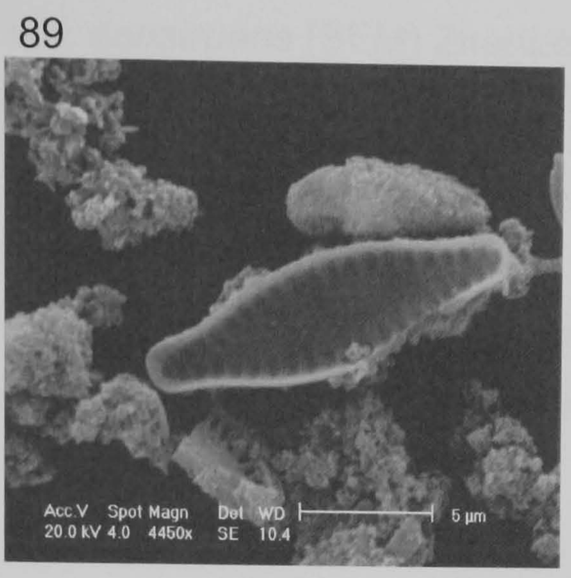
91. *Fragilaria capucina* Zirahuén (core material 0 cm)

92. *Fragilaria capucina* colony Zirahuén (core material 7 cm)

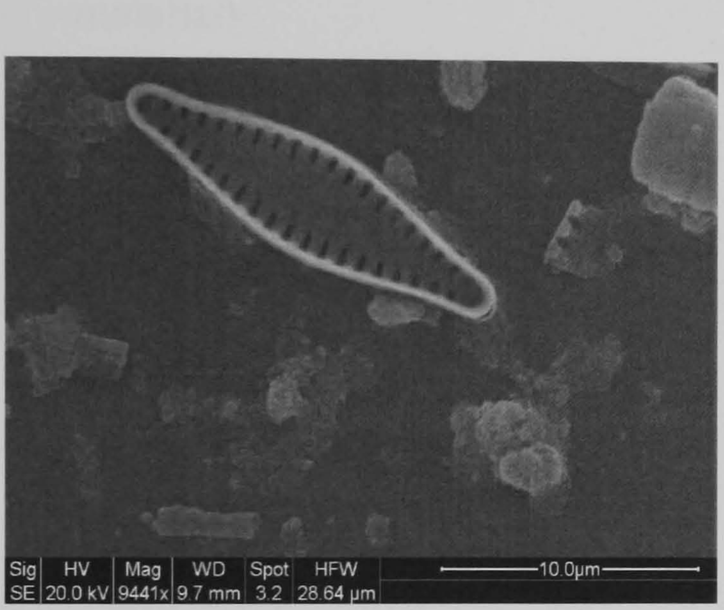
93. *Fragilaria construens* var. *construens* Los Negritos (modern material)

88

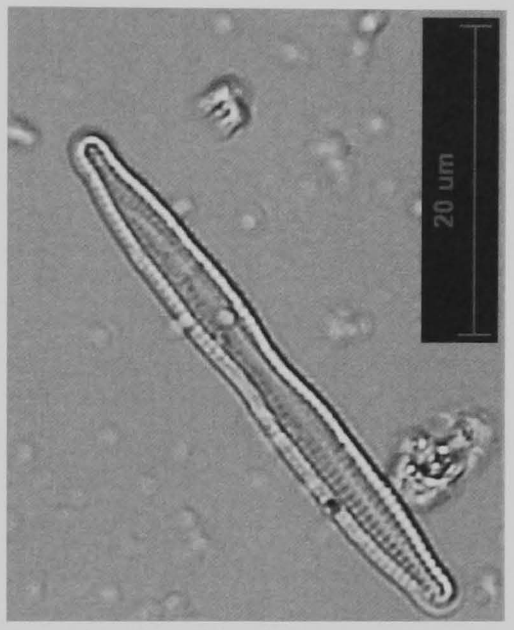
89



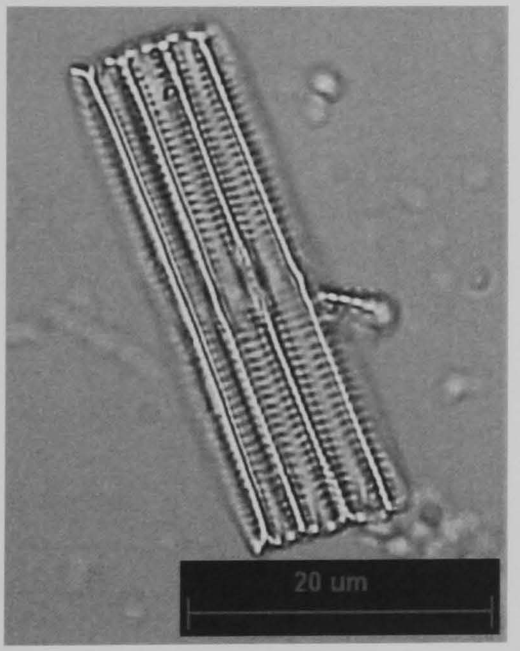
90



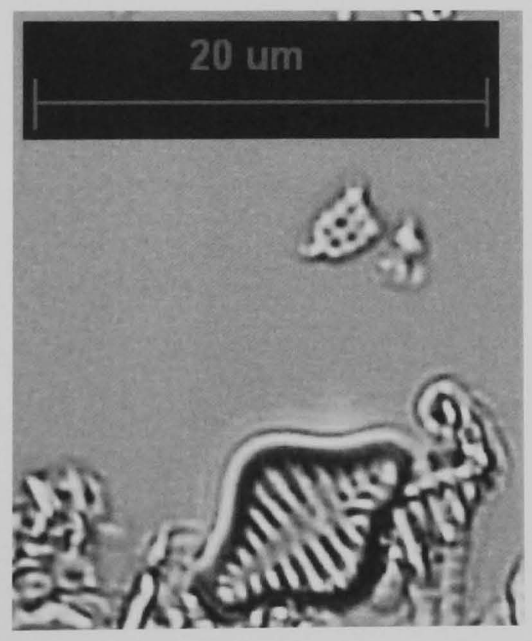
91



92

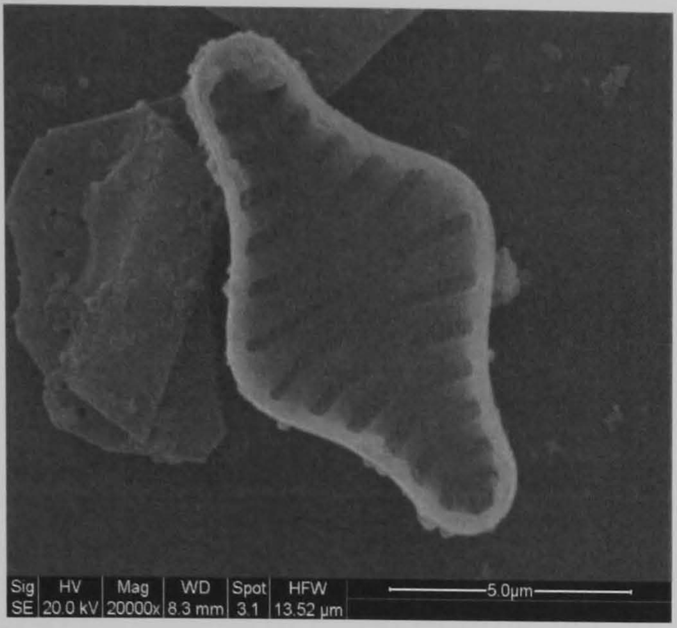


93

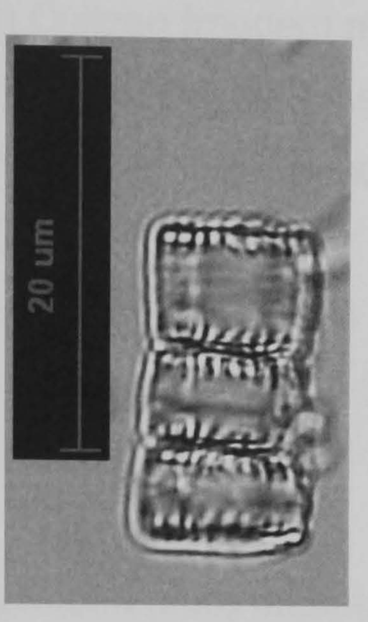


94. *Fragilaria construens* var. *construens* (SEM) Zirahuén (core material 0 cm)
95. *Fragilaria construens* var. *construens* colony (girdle view) Zirahuén (core material 0 cm)
96. *Fragilaria crotonensis* La Alberca Tacambaro (modern material)
97. *Fragilaria crotonensis* central area (SEM) Juanacatlan (modern material)
98. *Fragilaria fasciculata* Santa Maria del Oro (modern material)
99. *Fragilaria fasciculata* (girdle view) Santa Maria del Oro (modern material)

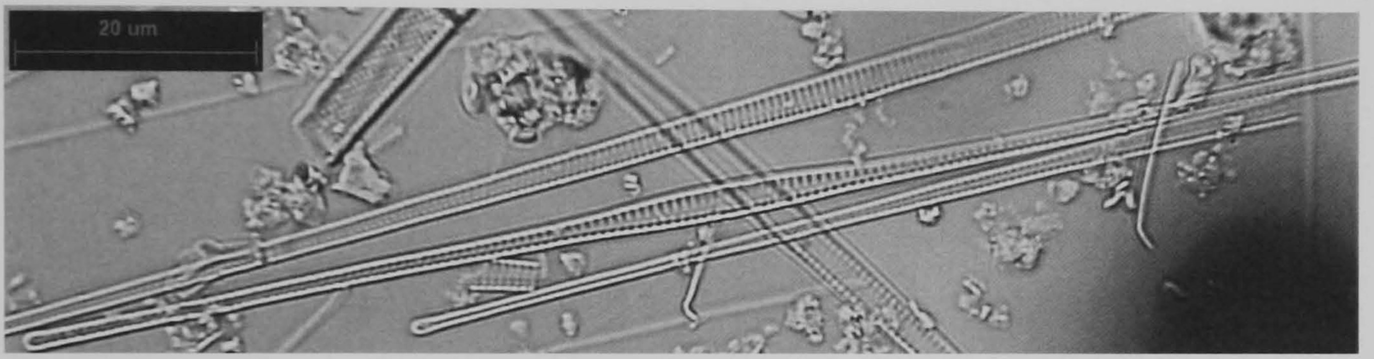
94



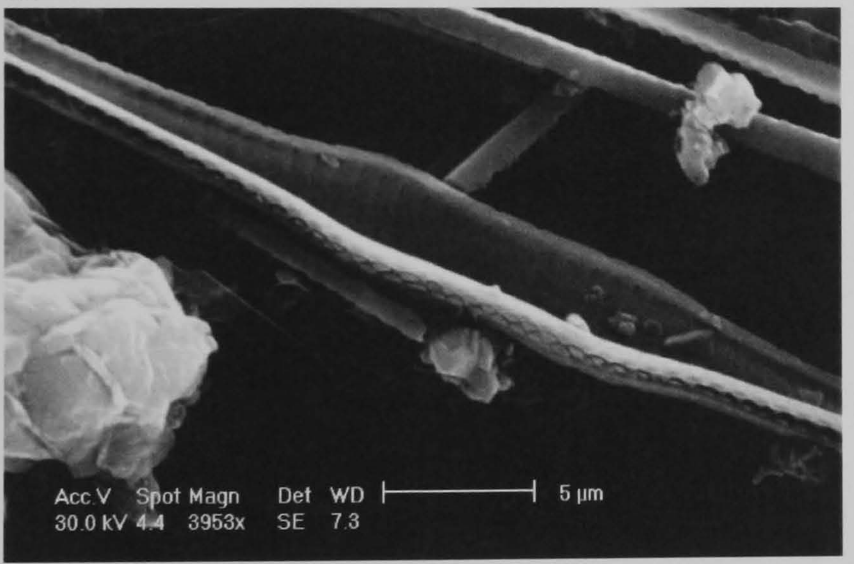
95



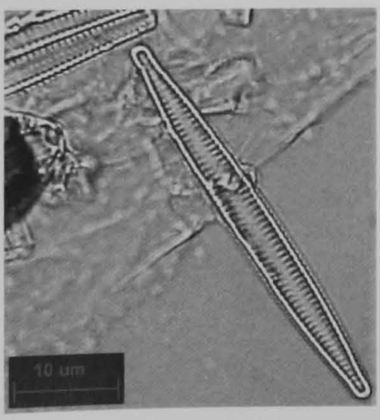
96



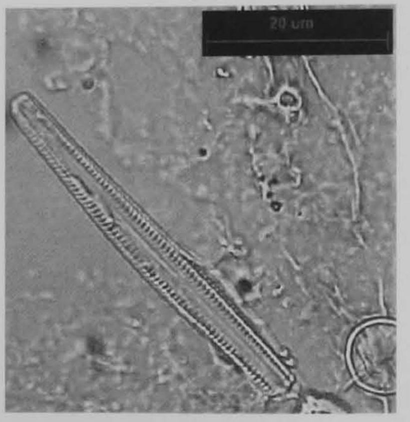
97



98



99



100. *Fragilaria pinnata* (girdle view) Cuitzeo (modern material)

101. *Fragilaria pinnata* (SEM) Juanacatlan (modern material)

102. *Fragilaria pinnata* colony (SEM) Juanacatlan (modern material)

103. *Fragilaria pinnata* Zirahuén (core material 0 cm)

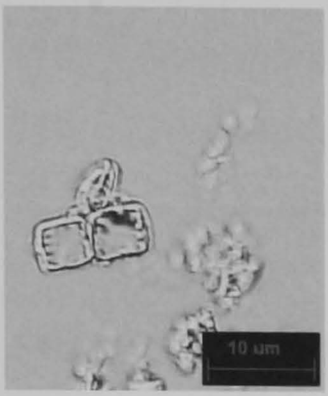
104. *Fragilaria pinnata* Zirahuén (core material 24 cm)

105. *Fragilaria pinnata* (SEM) Zirahuén (core material 0 cm)

106. *Gomphonema acuminatum* (internal view) (SEM) Zirahuén (core material 64 cm)

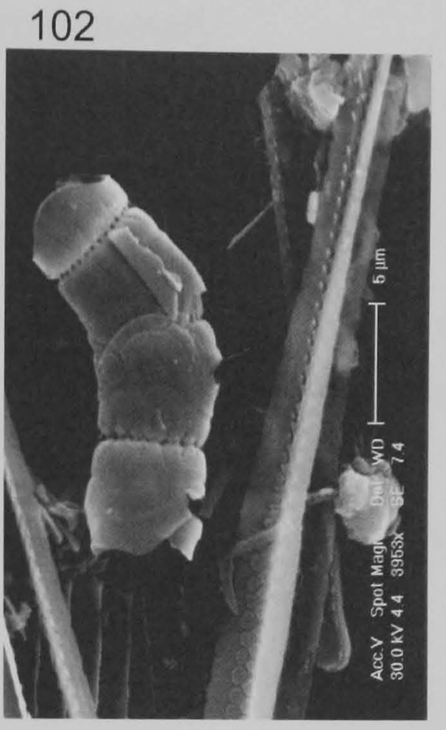
107. *Gomphonema acuminatum* Zirahuén (core material 64 cm)

100

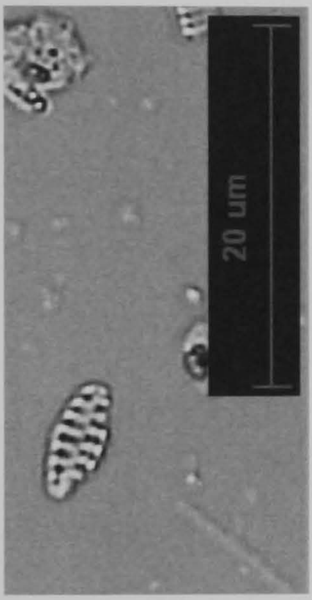


101

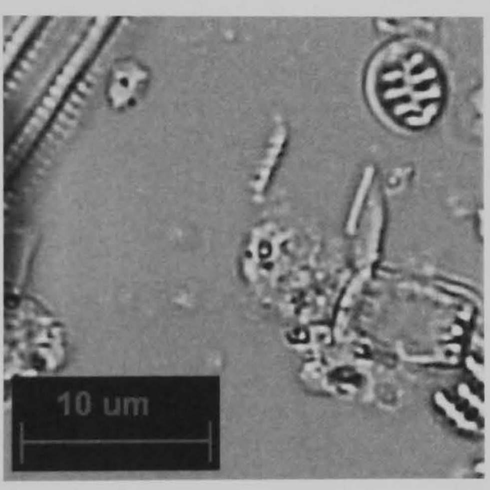
102



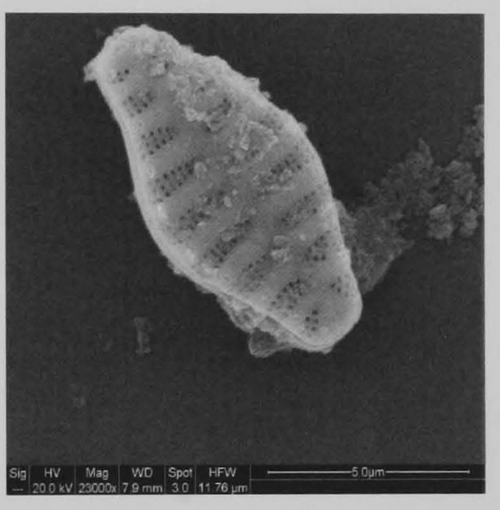
103



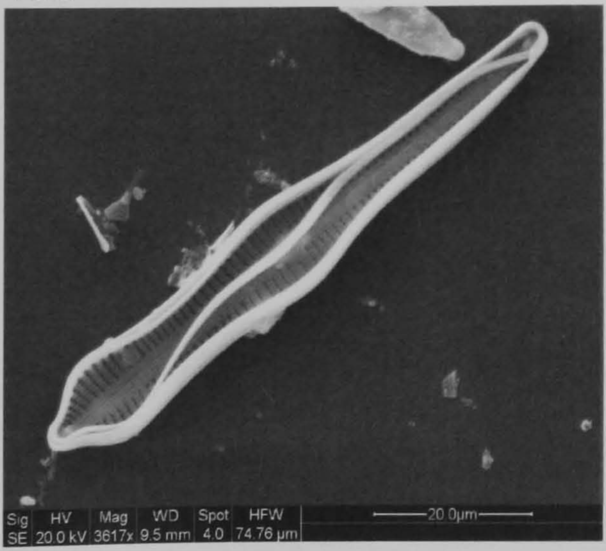
104



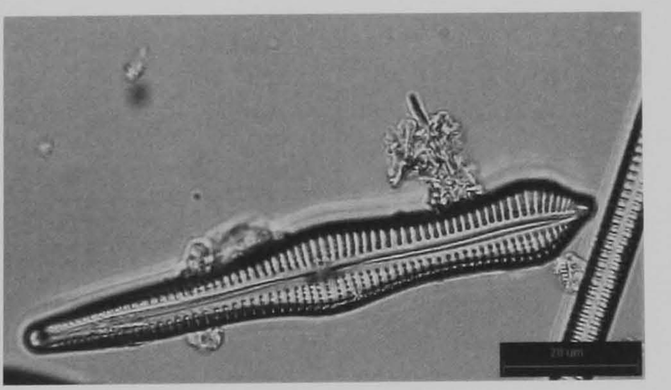
105



106



107



108. *Gomphonema affine* Tepetiltic (modern material)

109. *Gomphonema clavatum* Patzcuáro (epiphytic sample)

110. *Gomphonema gracile* Zirahuén (core material 64 cm)

111. *Gomphonema gracile* (SEM) Zirahuén (core material 40 cm)

112. *Gomphonema gracile* (girdle view) Zirahuén (Core material 0 cm)

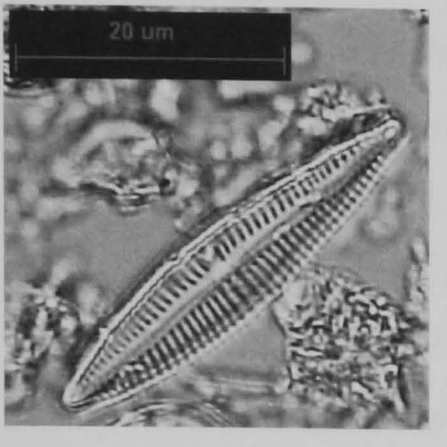
113. *Gomphonema parvulum* Santa Maria del Oro (modern material)

114. *Gomphonema olivaceum* Atexcac (modern material)

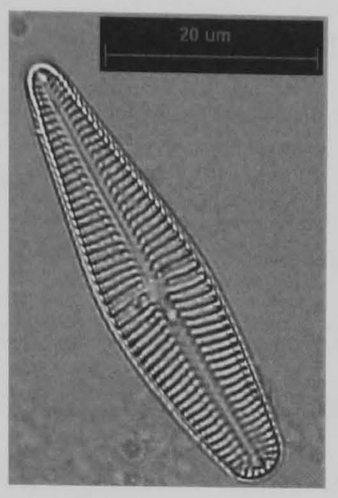
115. *Gomphonema olivaceum* var. *minutissimum* Atexcac (modern material)

116. *Gomphonema olivaceum* (girdle view) La Preciosa (modern material)

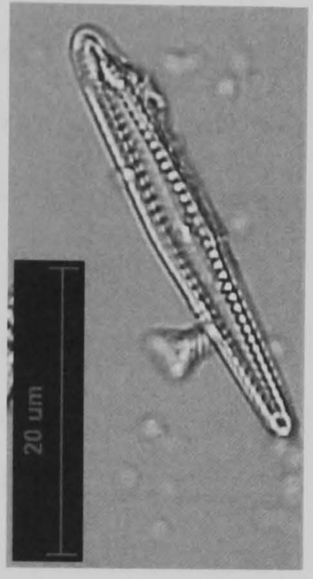
108



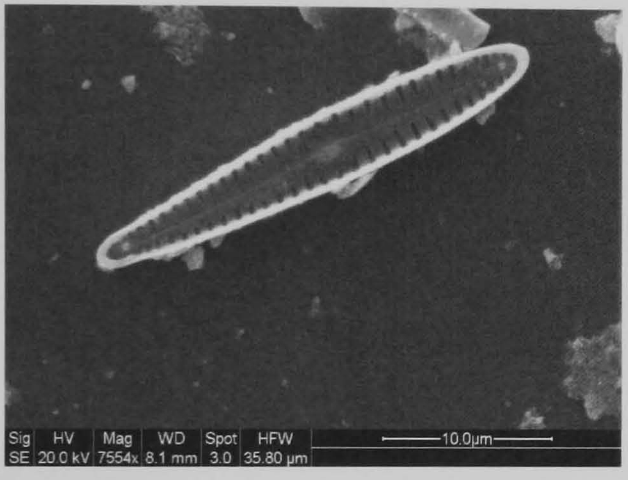
109



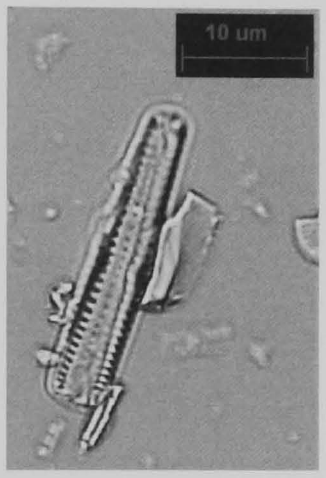
110



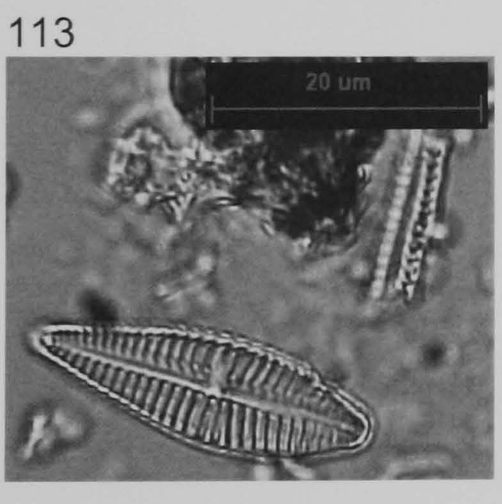
111



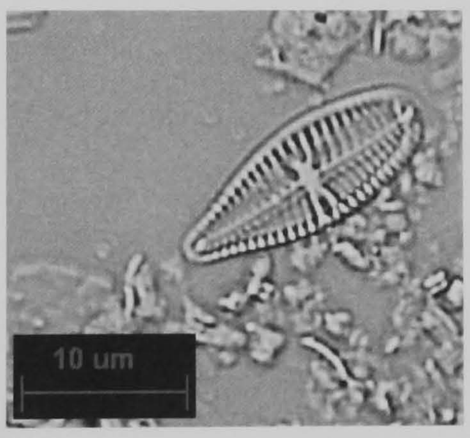
112



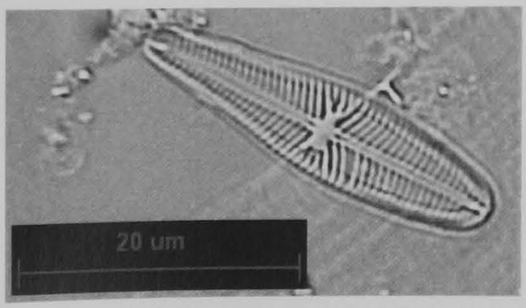
113



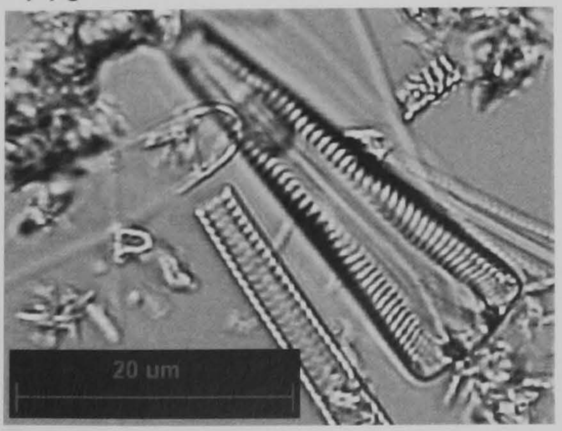
114



115

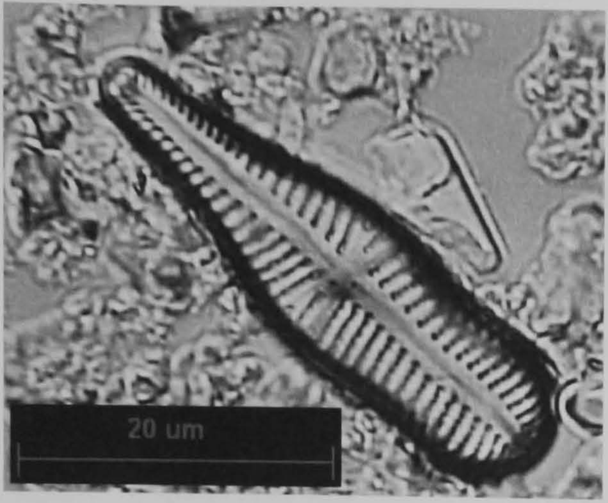


116

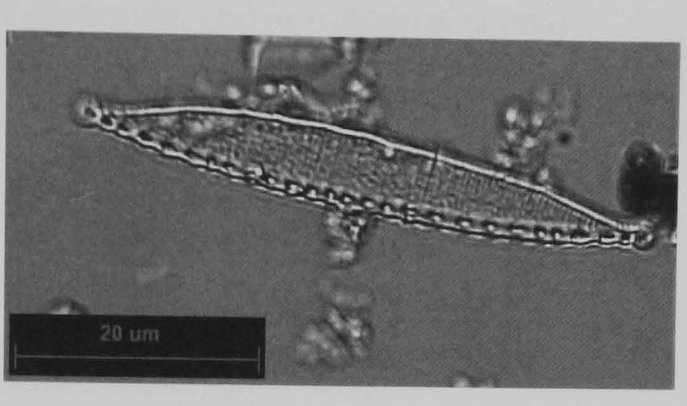


117. *Gomphonema truncatum* (Tepetiltic (modern material)
118. *Hantzschia amphioxys* Cuitzeo (modern material)
119. *Mastogloia smithii* var. *smithii* La Preciosa (modern material)
120. *Mastogloia smithii* var. *lacustris* La Preciosa (modern material)
121. *Navicula capitata* Cuitzeo (modern material)
122. *Navicula cincta* La Piscina de Yuriría (modern material)
123. *Navicula confervacea* Cajititlan (modern material)
124. *Navicula gerloffii* Lago del Sol (epiphytic sample)
125. *Navicula krasskei* Lago de Yuriría (modern sample)

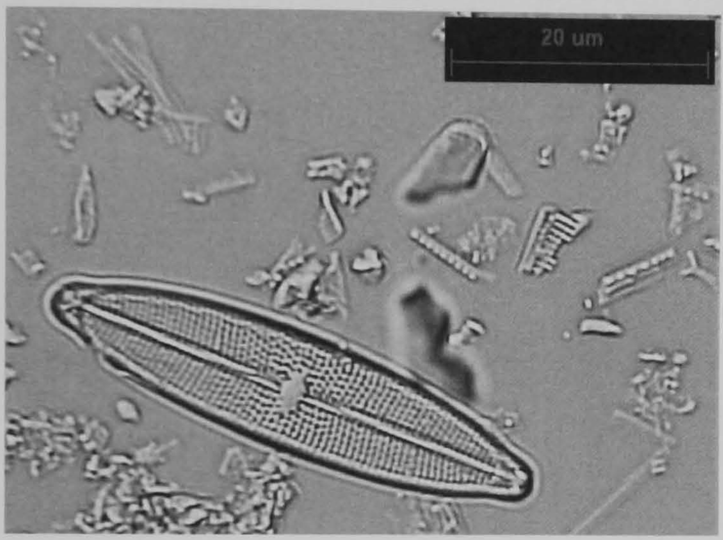
117



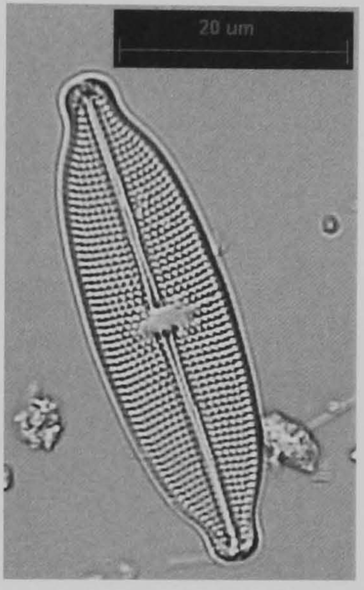
118



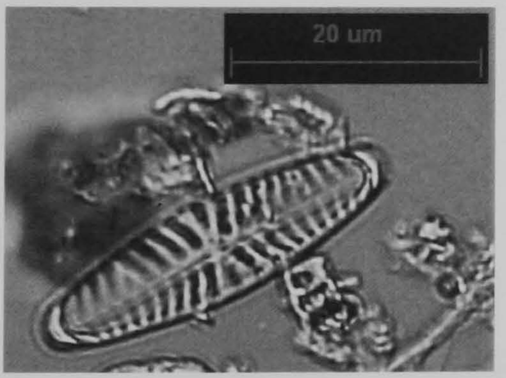
119



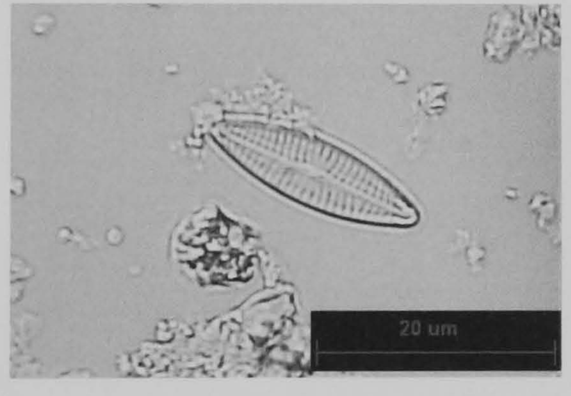
120



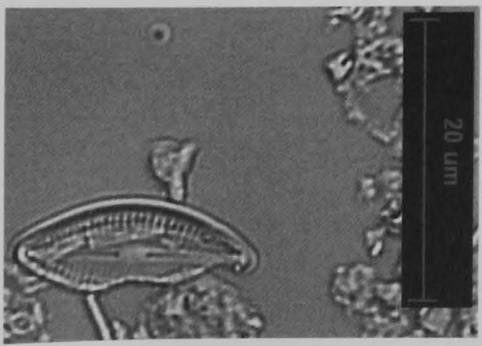
121



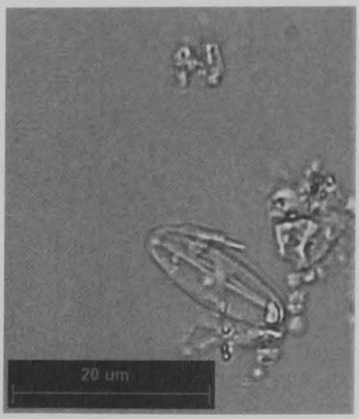
122



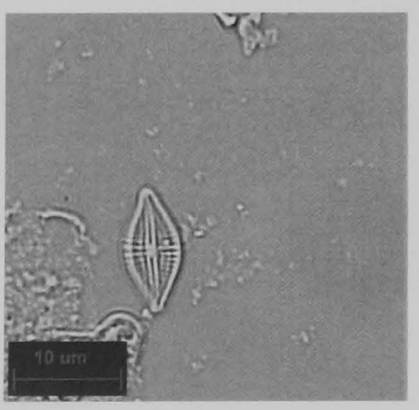
123



124



125



126. *Navicula radiosa* Cuitzeo (modern material)

127. *Navicula radiosa* var. *tenella* Zirahuén (core material 24 cm)

128. *Navicula* c.f. *rotunda* (SEM) Zirahuén (core material 0 cm)

129. *Navicula* c.f. *rotunda* (SEM) Zirahuén (core material 4 cm)

130. *Navicula* c.f. *rotunda* Zirahuén (core material 5 cm)

131. *Navicula* c.f. *rotunda* Zirahuén (core material 7 cm)

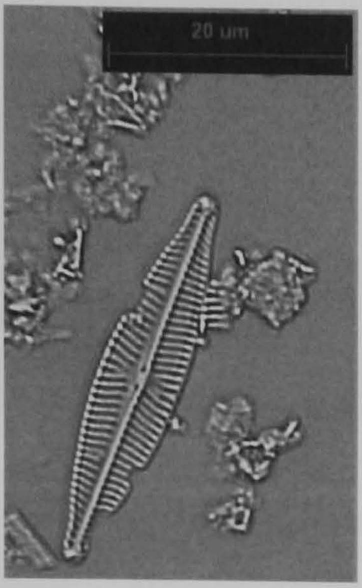
132. *Navicula saxophila* Tepetiltic (modern material)

133. *Navicula schudei* Zirahuén (core material 5 cm)

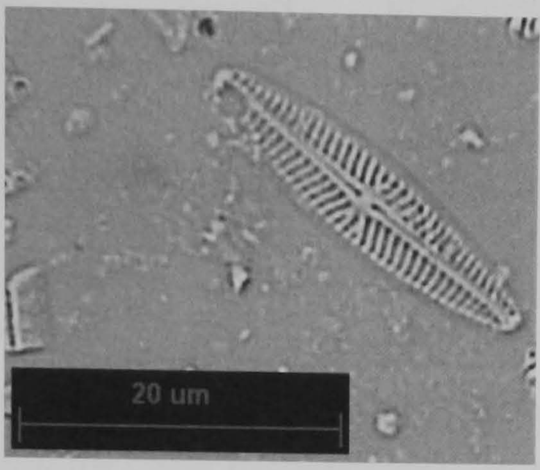
134. *Navicula seminulum* Zirahiuen (core material 5 cm)

135. *Navicula seminulum* (internal view) (SEM) Zirahuén (core material 16 cm)

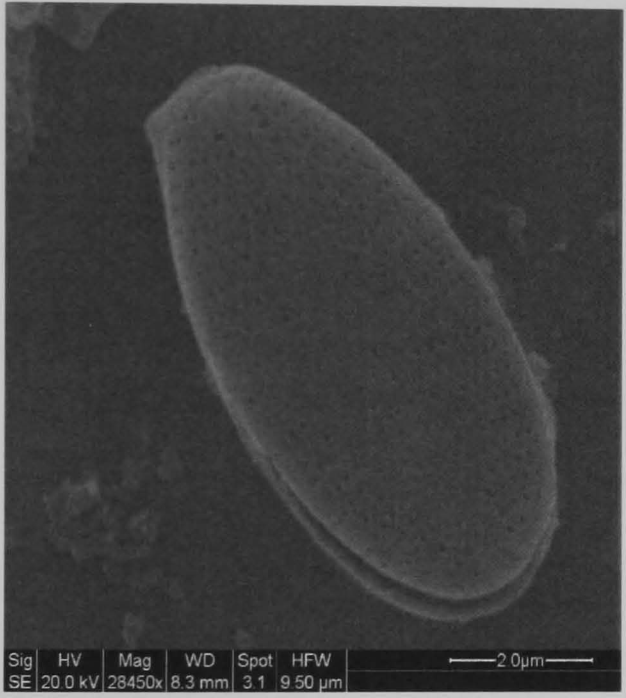
126



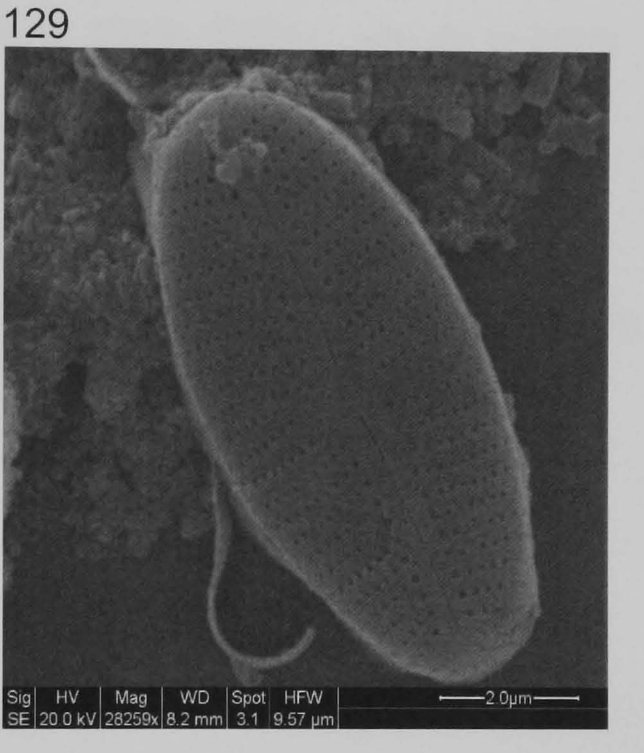
127



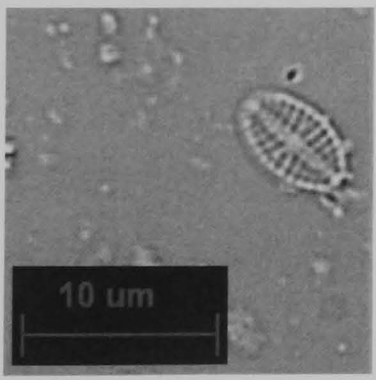
128



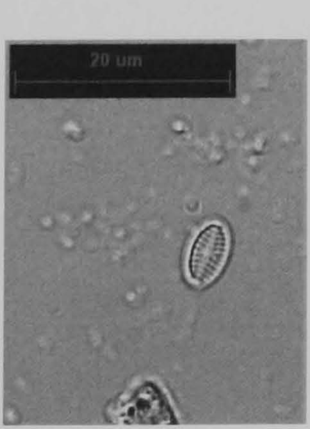
129



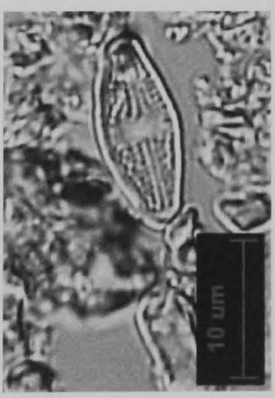
130



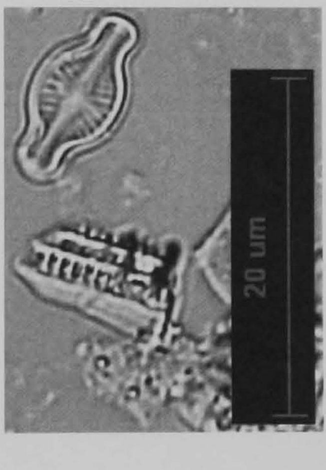
131



132

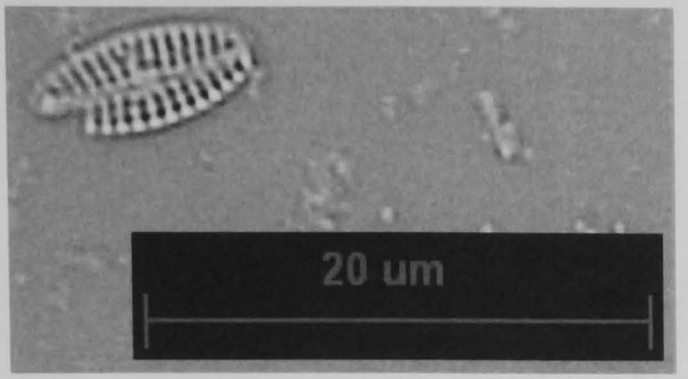


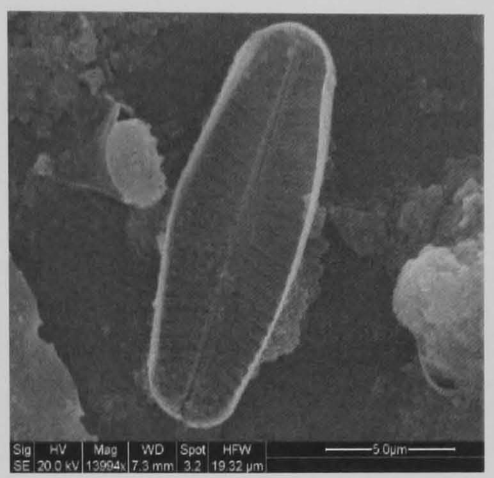
133



134

135





136. *Navicula viridula* Zirahuén (core material 0 cm)

137. *Navicula viridula* Zirahuén (core material 7 cm)

138. *Navicula vitabunda* Lago del Sol (modern material)

139. *Navicula NTA* (after Caballero, 1995) (SEM) Lago de la Luna (modern material)

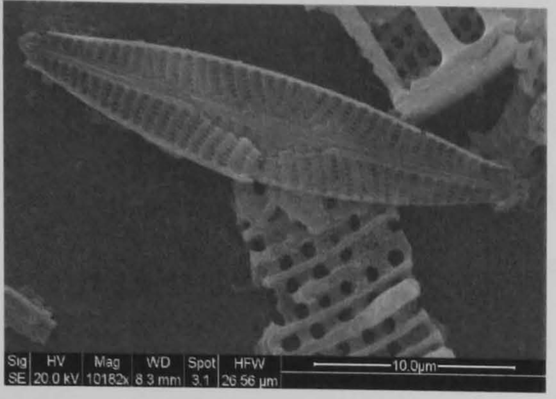
140. *Navicula NTA* Lago de la Luna (modern material)

141. *Nitzschia amphibia* Laguna la Maria (modern material)

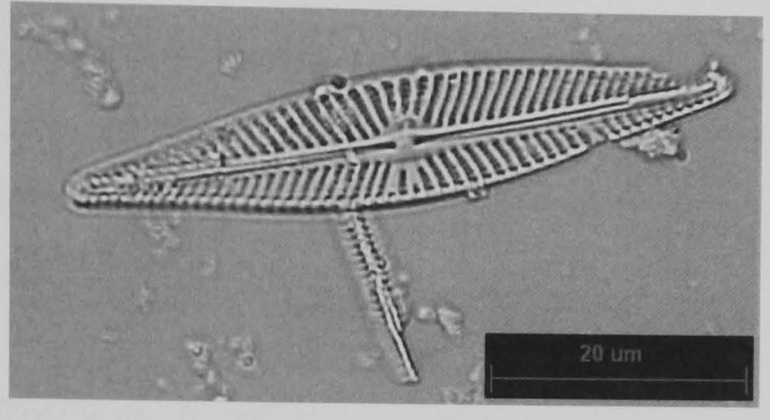
142. *Nitzschia amphibia* (girdle view) Zirahuén (core material 5 cm)

143. *Nitzschia amphibia* (SEM) Zirahuén (core material 64 cm)

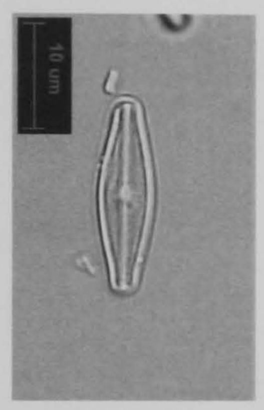
136



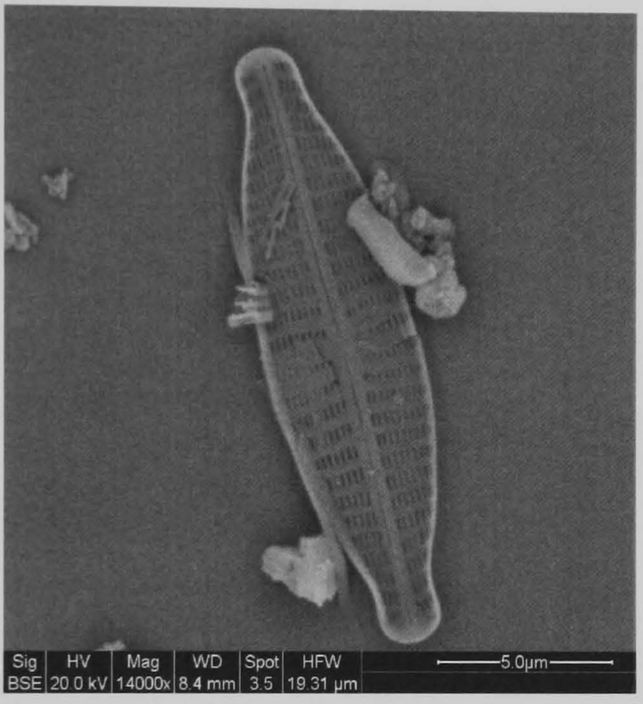
137



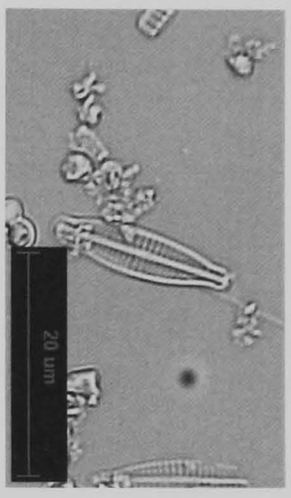
138



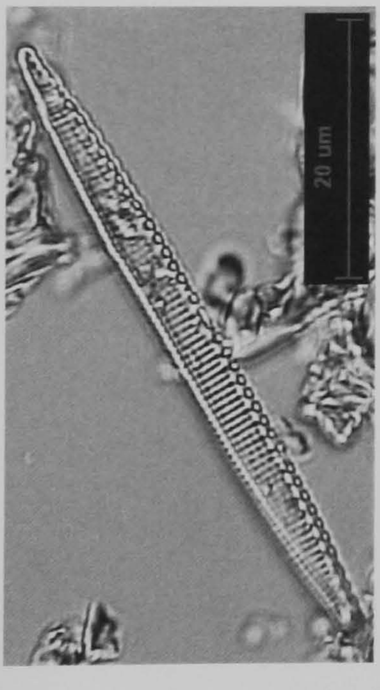
139



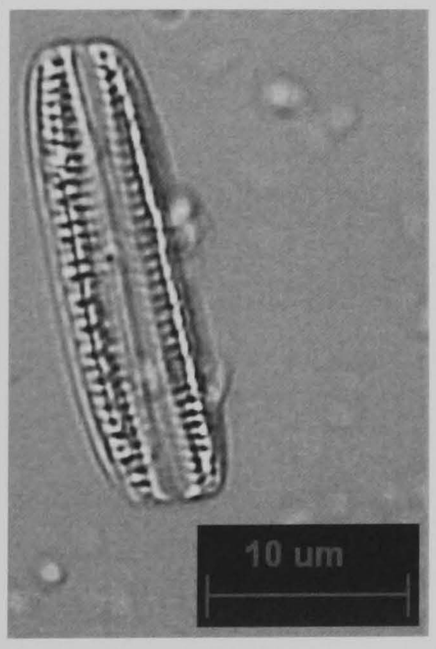
140



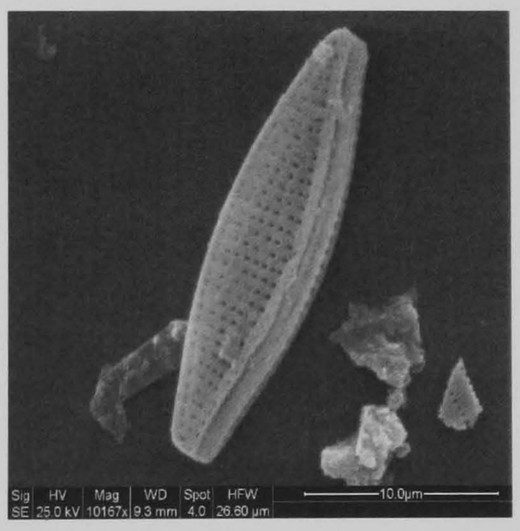
141



142



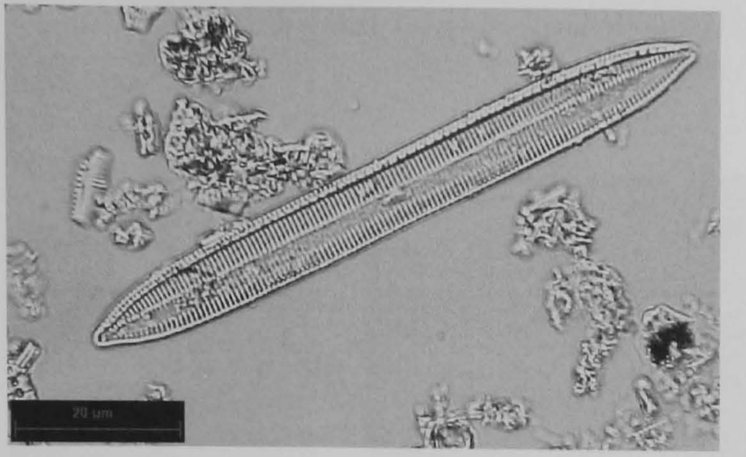
143



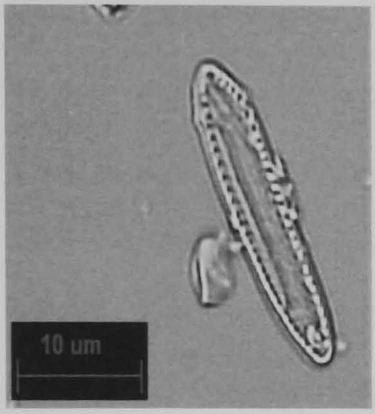
144. *Nitzschia angustata* Cuitzeo (modern material)

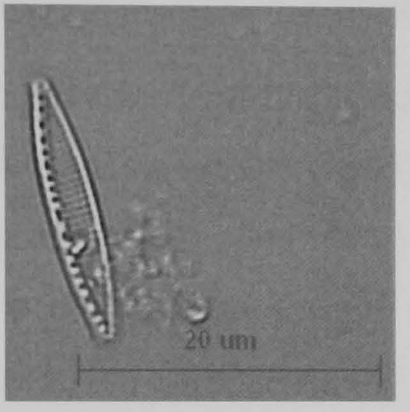
144. *Nitzschia communis* La Piscina de Yuriría (modern material)
145. *Nitzschia inconspicua* Lago de Yuriría (modern material)
146. *Nitzschia liebetruthii* (Alchichica)
147. *Nitzschia ovalis* La Piscina de Yuriría (modern material)
148. *Nitzschia palea*
149. *Nitzschia paleacea* La Preciosa (modern material)
150. *Nitzschia perminuta* Los Negritos (modern material)
151. *Nitzschia tubicola* Pátzcuaro (epiphytic sample)
152. *Pinnularia microstauron* Zempoala (modern material)

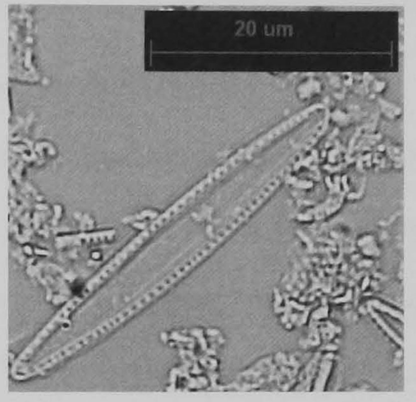
145



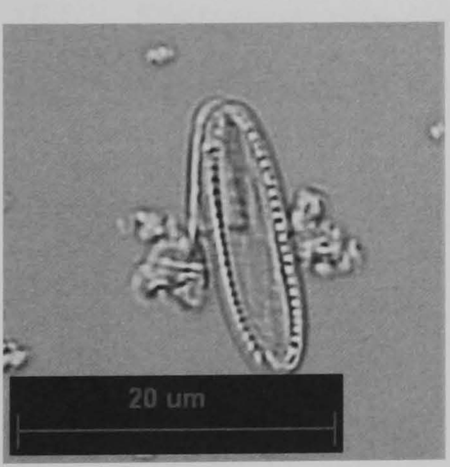
145 146 147

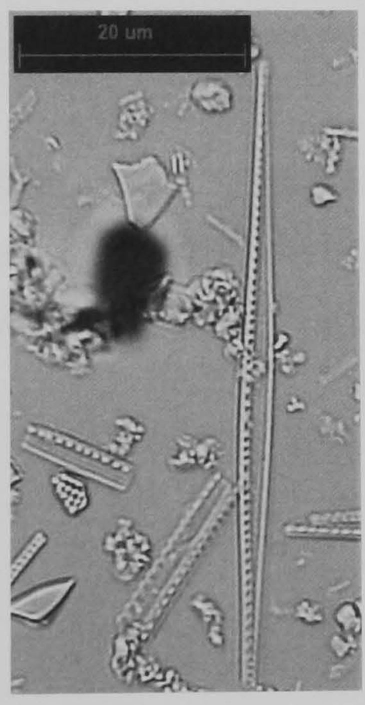


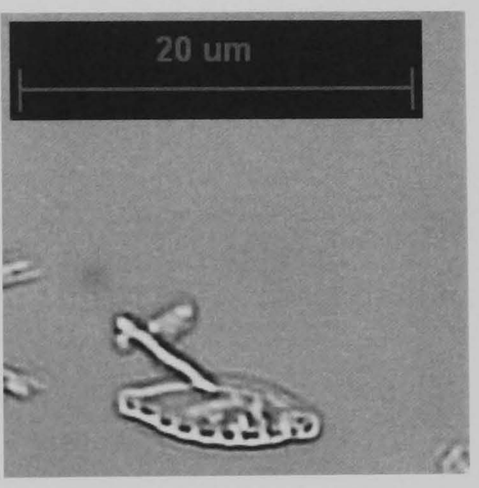




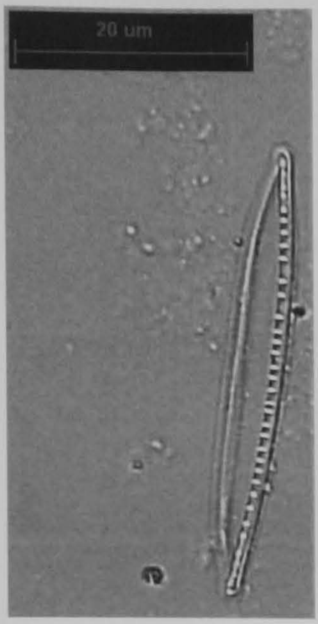
148 150 151

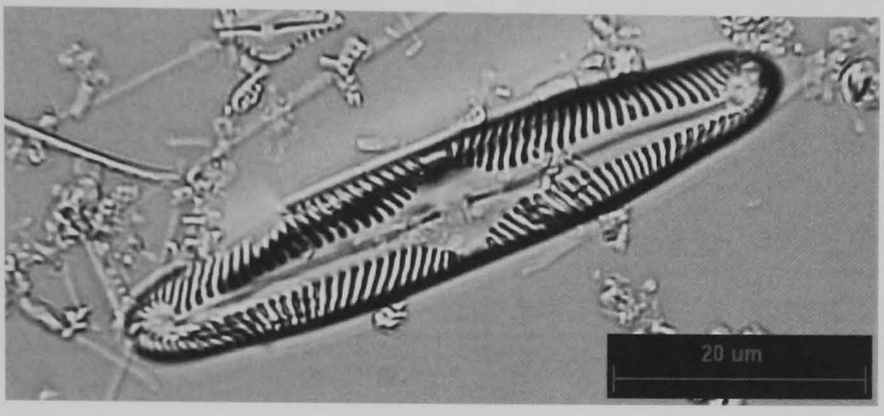






152 153





153. *Pinnularia subcapitata* Tepetiltic (modern material)

154. *Rhoicosphenia curvata* (girdle view) Cuitzeo (modern material)

155. *Rhoicosphenia curvata* (Calve view) Cuitzeo (modern material)

156. *Rhopalodia brebissonii* Santa Maria del Oro (epiphytic sample)

157. *Rhopalodia gibberula* La Piscina de Yuriría (modern material)

158. *Rhopalodia gibba* Santa Maria del Oro (modern material)

159. *Sellaphora* (=*Navicula*) *pupula* Santa Maria del Oro (modern material)

160. *Stauroneis anceps* San Gregorio (modern material)

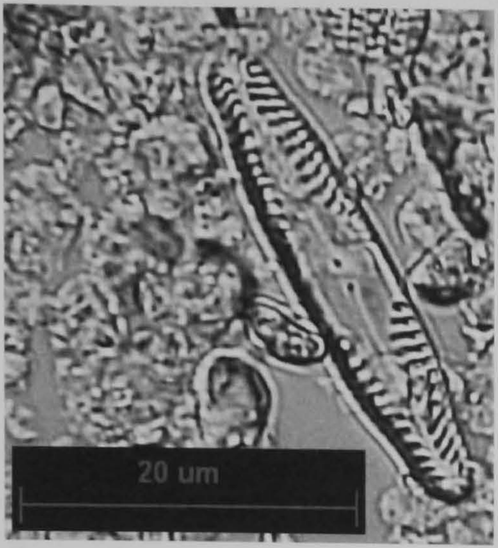
161. *Stenopetrobia delicatissima* Lago de la Luna (modern material)

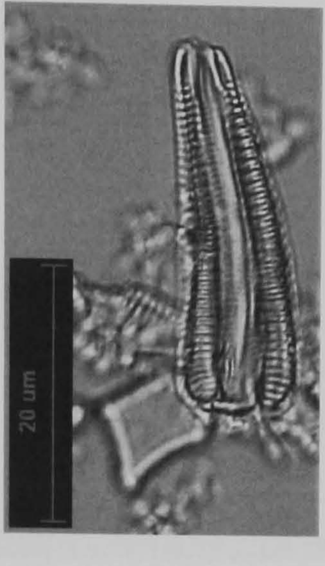
162. *Stenopetrobia delicatissima* (SEM) Lago de la Luna (modern material)

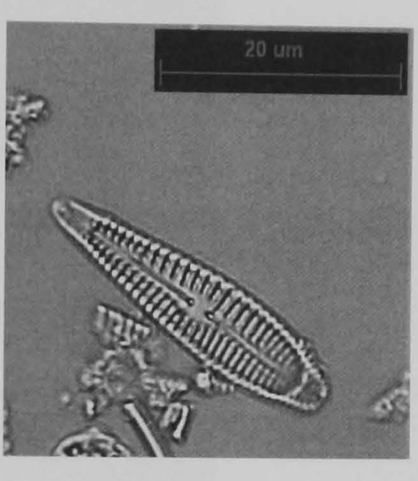
154

155

156

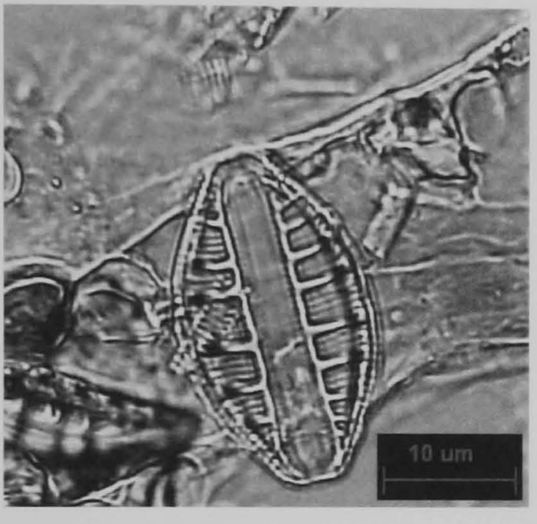


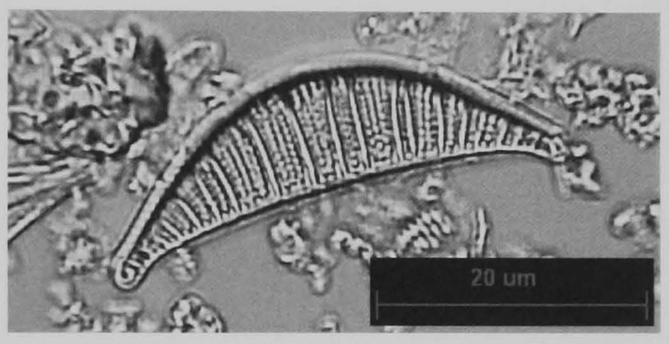




157

158

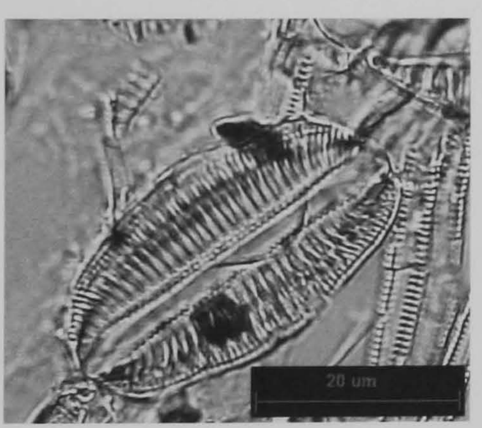


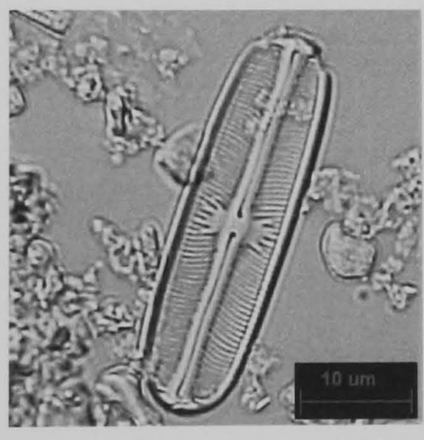


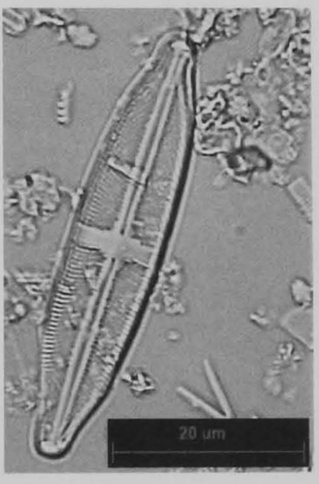
159

160

161

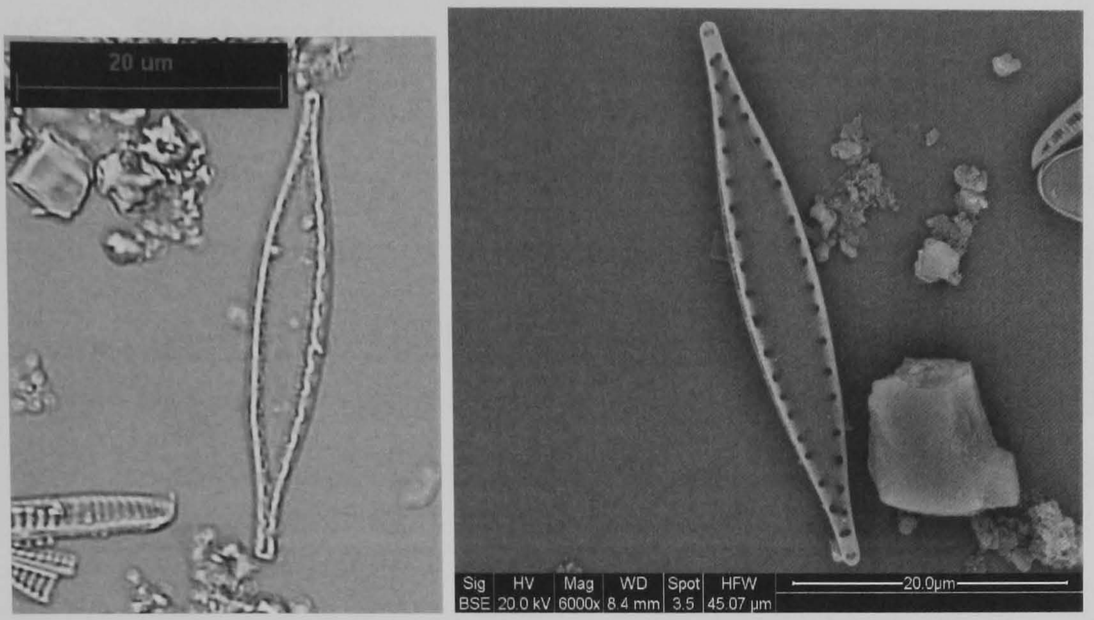






162

163


20 um
Sig
HV
Mag
WD
Spot
HFW
BSE
20.0 kV
6000x
8.4 mm
3.5
45.07 µm
20.0µm

163. *Stephanodiscus 1* Cajititlan (modern material)

164. *Stephanodiscus 1* Cajititlan (modern material)

165. *Stephanodiscus 1*(internal view) (SEM) Cajititlan (modern material)

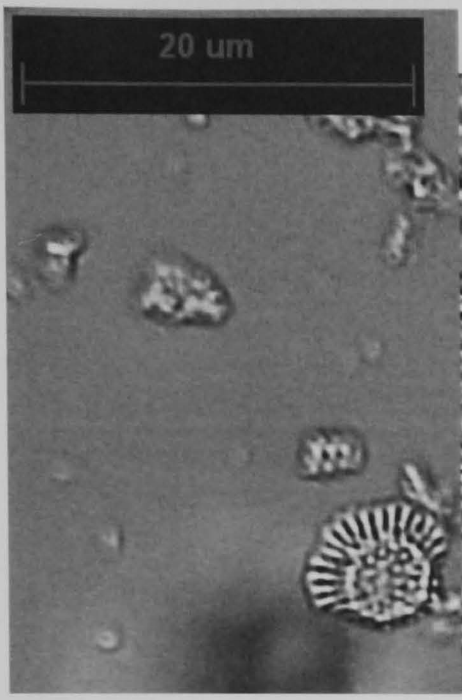
166. *Stephanodiscus 1* (external view) (SEM) Cajititlan (modern material)

167. *Stephanodiscus astraea* var. *intermedia*

168. *Stephanodiscus astraea* var. *minutula* Chapala (modern material)

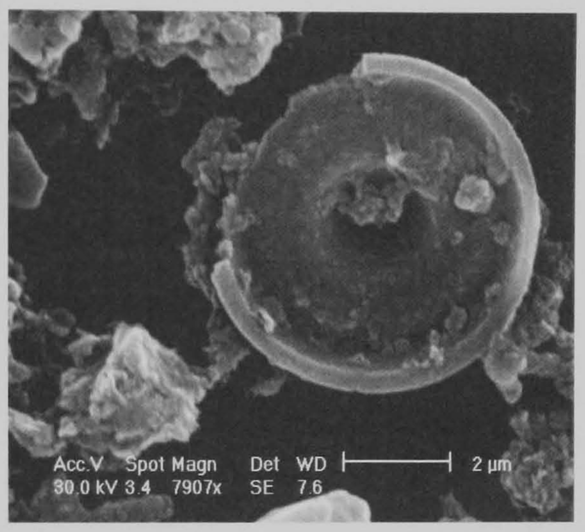
169. *Stephanodiscus astraea* var. *minutula* (SEM) Chapala (modern material)

164

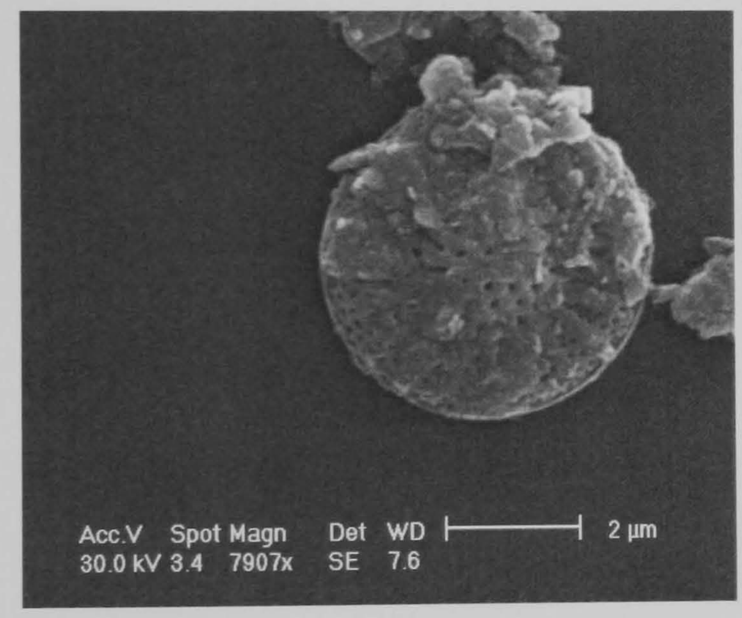


165

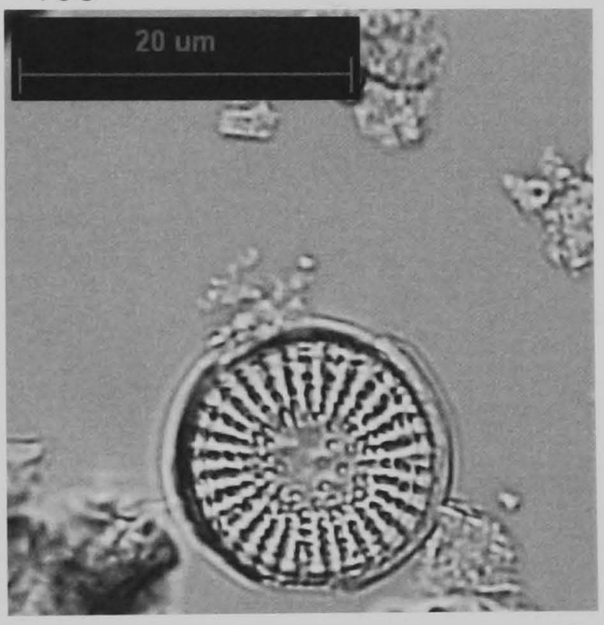
166



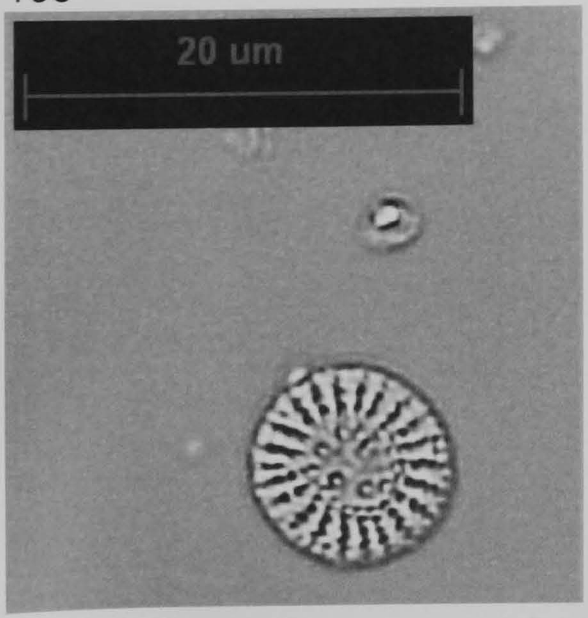
167



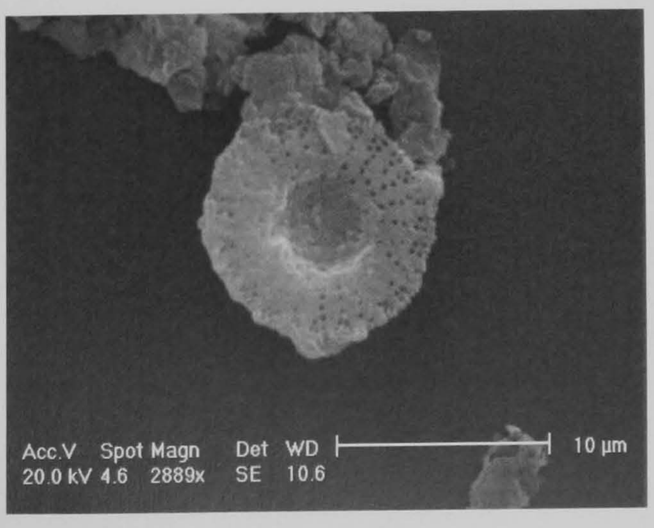
168



169



170



170. *Stephanodiscus minutus* Chapala (modern material)

171. *Stephanodiscus minutus* (SEM) Chapala (modern material)

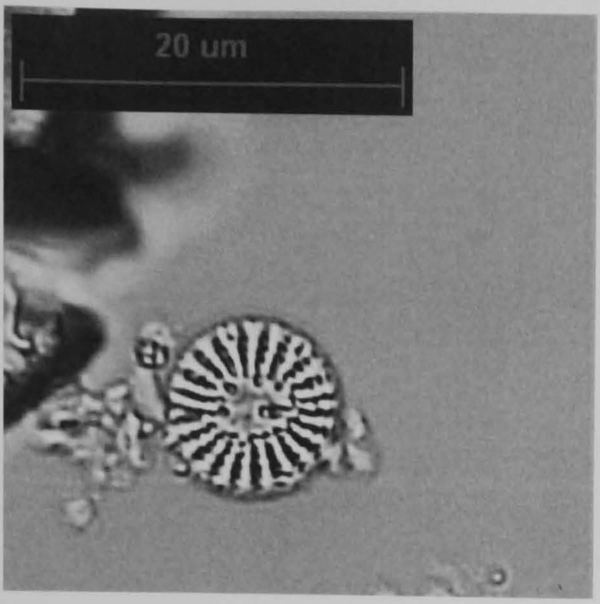
172. *Stephanodiscus minutus* (internal view) (SEM) Chapala (modern material)

173. *Stephanodiscus spp 2.* Chapala (modern material)

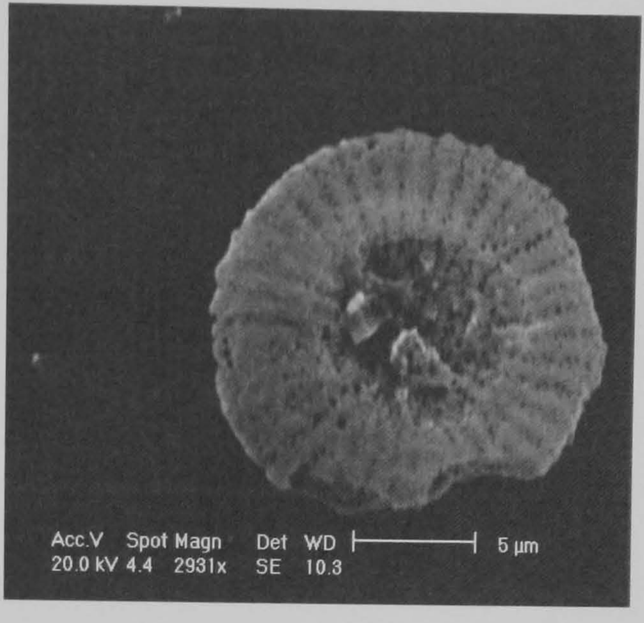
174. *Stephanodiscus subtilis* Zacapu (modern material)

175. *Surirella linearis* Lago del la Luna (modern material)

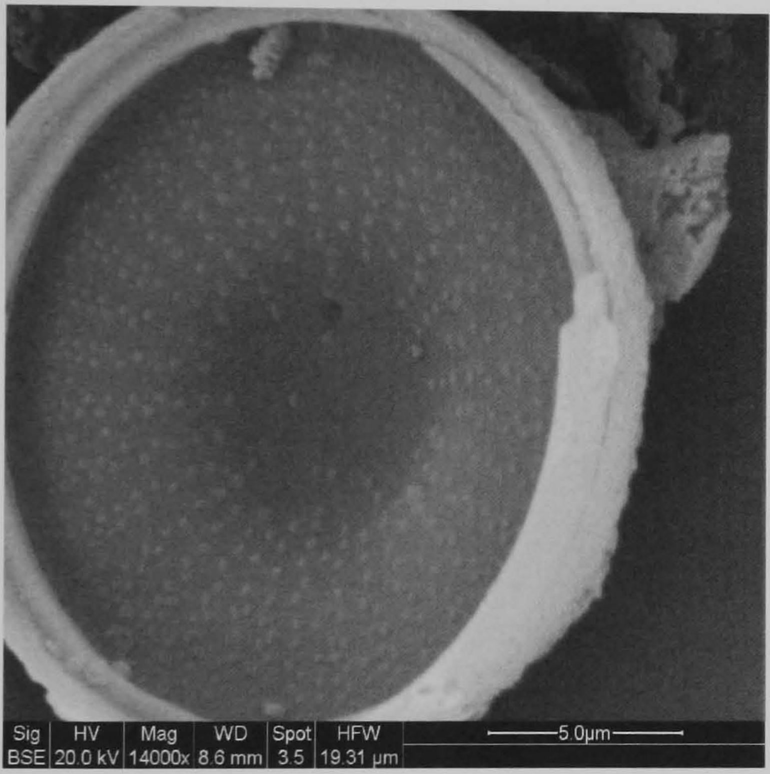
171



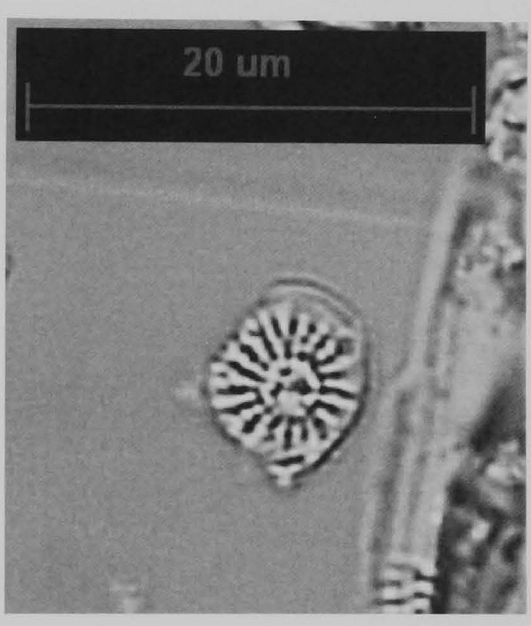
172



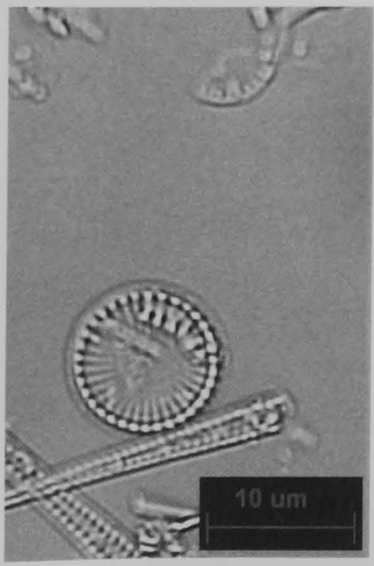
173



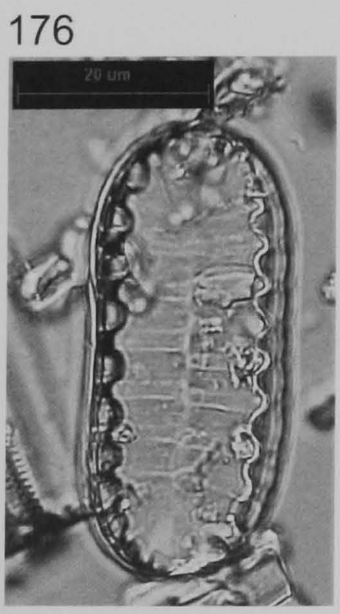
174



175



176



176. *Synedra acus* (central area) Zacapu

177. *Synedra goulardii* Pátzcuaro (epiphytic sample)

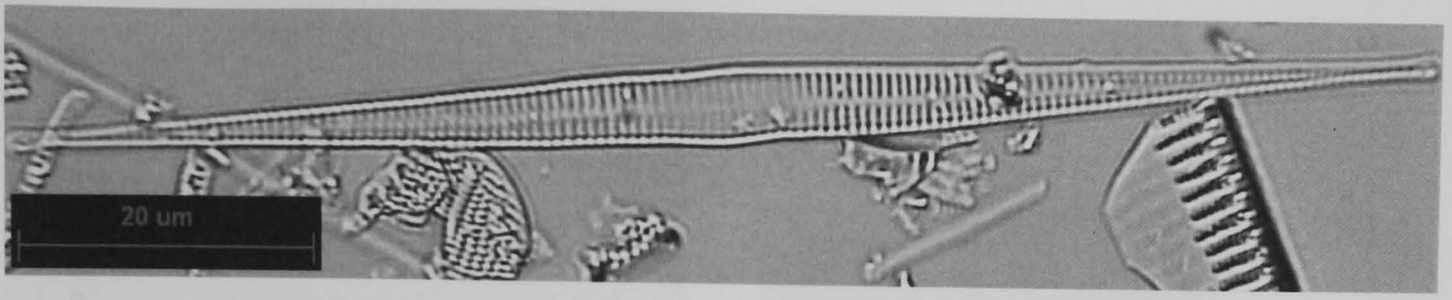
178. *Synedra goulardii* Pátzcuaro (modern sample)

179. *Synedra ulna* Zacapu (modern sample)

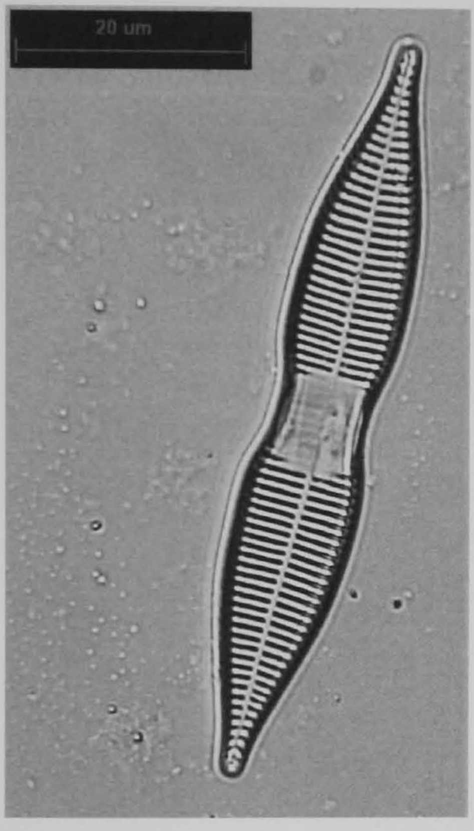
180. *Terpsinoe spp* Camecuaro

181. *Unknown 4* Los Negritos (modern material

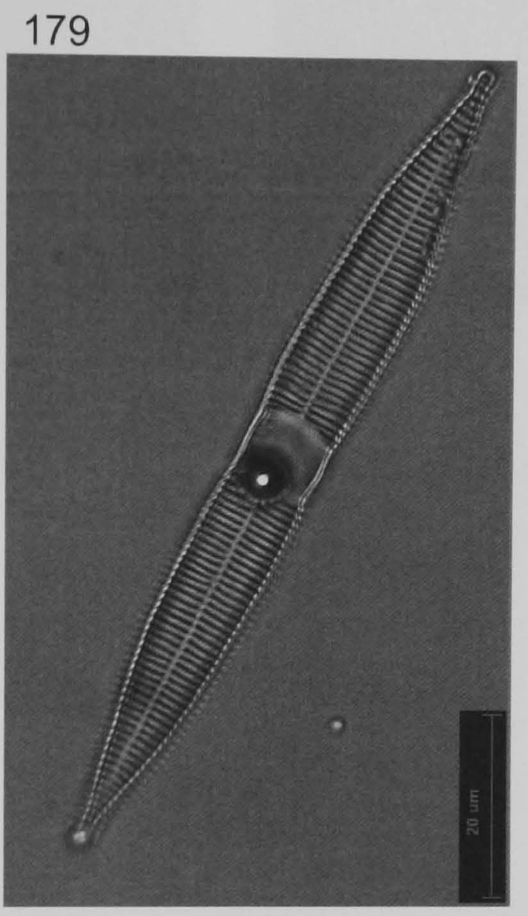
177



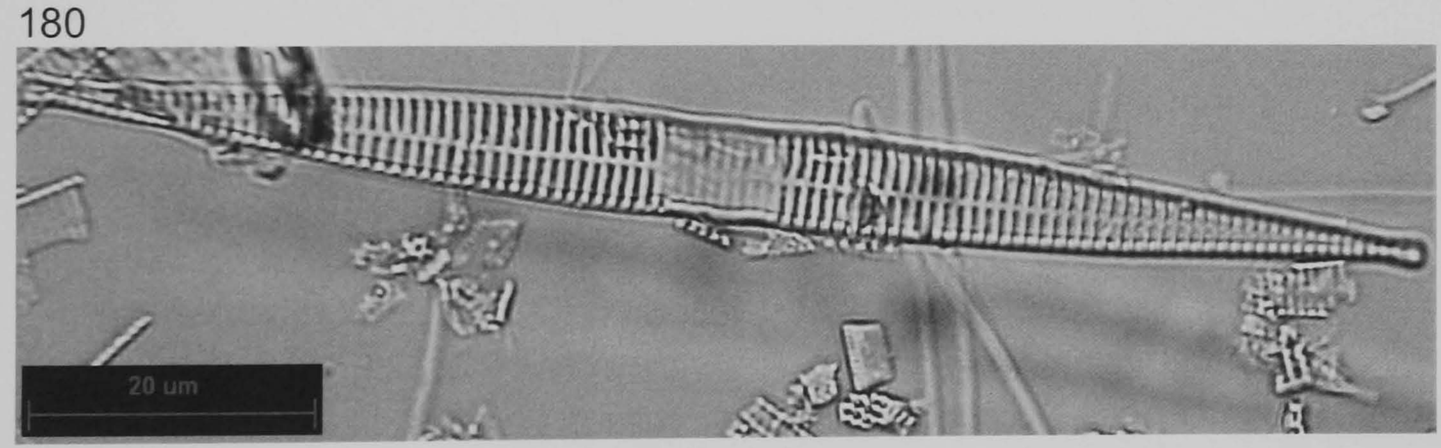
178



179

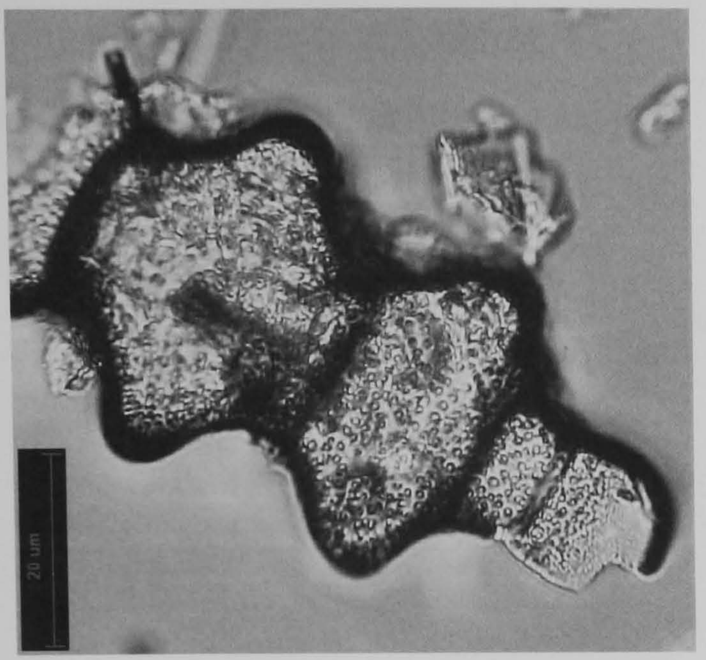


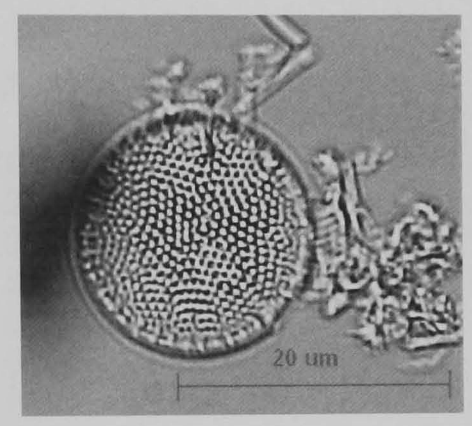
180



181

182


20 um


20 um

Appendix 3. Results of CCA and WA

CCA results – Variance Inflation Factors

All Environmental Variables

N	name	(weighted) mean	stand. dev.	inflation factor
1	SPEC AX1	0.0000	1.0394	
2	SPEC AX2	0.0000	1.0617	
3	SPEC AX3	0.0000	1.0647	
4	SPEC AX4	0.0000	1.0937	
5	ENVI AX1	0.0000	1.0000	
6	ENVI AX2	0.0000	1.0000	
7	ENVI AX3	0.0000	1.0000	
8	ENVI AX4	0.0000	1.0000	
1	Depth SQ	2.9676	2.0677	12.6593
2	Temp (c)	23.0242	3.9764	3.0919
3	EC LOG	2.7199	0.5809	21.0700
4	pH	8.3403	0.6711	5.6808
5	.HCO+CO	69.7667	15.8863	4.1322
6	. SO4	3.1795	2.8493	1.6507
7	. Cl	27.0623	15.5192	0.0000
8	. K+Na	62.9827	24.5709	23.9208
9	. Ca	18.8848	14.4018	12.1196
10	. Mg	18.1321	13.0794	0.0000
11	TP log	1.9528	0.7490	17.3751
12	TN LOG	-1.4910	0.6627	3.0898
13	SiO2 SQR	7.9847	3.3428	2.9991
14	Chl-a LO	1.0609	0.7035	8.2418
15	SD SQRT	1.0531	0.7167	11.8713

9 significant variables

N	name	(weighted) mean	stand. dev.	inflation factor
1	SPEC AX1	0.0000	1.0380	
2	SPEC AX2	0.0000	1.1012	
3	SPEC AX3	0.0000	1.0945	
4	SPEC AX4	0.0000	1.0745	
5	ENVI AX1	0.0000	1.0000	
6	ENVI AX2	0.0000	1.0000	
7	ENVI AX3	0.0000	1.0000	
8	ENVI AX4	0.0000	1.0000	
2	Temp (c)	23.0242	3.9764	1.6433
3	EC LOG	2.7199	0.5809	9.3131
4	pH	8.3403	0.6711	3.1504
8	. K+Na	62.9827	24.5709	7.5907
10	. Mg	18.1321	13.0794	8.6340
11	TP log	1.9528	0.7490	6.9574
12	TN LOG	-1.4910	0.6627	2.6333

```
 13   SiO2 SQR          7.9847        3.3428        2.6361
 14   Chl-a LO          1.0609        0.7035        3.1308
```

WA results for Chlorophyll-a model

WA no cross validation all sites and species

```
Model name                : wachla-all
Description               : Model 02
Model type                : Weighted Averaging
Date                      : 10 August, 2005: 15:26:38
Species data              : sed only diatoms
Environmental data        : water TN
Environmental variable    : Chl-a LOG
Total number of samples   : 30
Number of samples in model : 30
Total number of variables  : 139
Number of variables in model: 139
```

Taxa with only one occurrence have had their tolerances set to 0.60975

Deshrinking regression coefficients

```
 #           Id            WA_b0     WA_b1   WATOL_b0  WATOL_b1
 1  Inverse deshrinking  -0.80656   1.8592  -0.34519   1.3468
 2 Classical deshrinking  0.54342   0.42419  0.40452   0.5888
```

Model performance

```
 #     Id       WA_Inv      WA_Cla     WATOL_Inv   WATOL_Cla
 1    RMSE     0.34425     0.38764     0.34071     0.38261
 2     R2      0.78865     0.78865     0.79298     0.79298
 3  Ave_Bias  -1.41e-016  -2.04e-016   7.68e-017   1.67e-016
 4  Max_Bias   0.39635     0.28065     0.45771     0.37068
```

WA with jack-knife cross validation – all sites and species

```
Model name                : WA-chl-all-jack
Description               : Model 02
Model type                : Weighted Averaging
Date                      : 10 August, 2005: 16:59:58
Species data              : sed only diatoms
Environmental data        : water TN
Environmental variable    : Chl-a LOG
Total number of samples   : 30
Number of samples in model : 30
Total number of variables  : 139
Number of variables in model: 139
```

Taxa with only one occurrence have had their tolerances set to 0.59738

Deshrinking regression coefficients

```
 #           Id            WA_b0     WA_b1   WATOL_b0  WATOL_b1
 1  Inverse deshrinking  -0.80656   1.8592  -0.34519   1.3468
 2 Classical deshrinking  0.54342   0.42419  0.40452   0.5888
```

Model performance

```
#        Id          WA_Inv       WA_Cla       WATOL_Inv   WATOL_Cla
1       RMSE        0.34425      0.38764      0.34071       0.38261
2        R2         0.78865      0.78865      0.79298       0.79298
3     Ave_Bias     -1.41e-016   -2.04e-016    7.68e-017     1.67e-016
4     Max_Bias      0.39635      0.28065      0.45771       0.37068
5      Jack_R2      0.37828      0.39018      0.069986      0.081465
6 Jack_Ave_Bias  -0.0045067   -0.0070105   -0.078405     -0.098922
7 Jack_Max_Bias   0.76556      0.71324      1.4055        1.5826
8       RMSEP       0.59073      0.60098      0.78633       0.83218
```

Results for simple WA with no cross validation and Lago de la Luna, La Piscina de Yuriria and Alchichica removed

```
Model name                 : WA-py-lun-alch
Description               : Model 03
Model type                 : Weighted Averaging
Date                      : 15 October, 2005: 17:44:02
Species data               : sed only diatoms
Environmental data          : water TN
Environmental variable      : Chl-a LOG
Total number of samples     : 30
Number of samples in model  : 27
Total number of variables   : 139
Number of variables in model: 132
Fossil data                : % >1%
Total number of samples     : 32
Total number of variables   : 24
Number of variables in model: 15

The following training set samples have been excluded from the model:

  2  P Yur
 24  Alch
 29  Luna

The following training set variables have been excluded from the model:

  8  AC042A       * Achnanthes subatomoides
 29  CY017A       * Cyclotella quillensis
 43  EY016A       * Cymbella minuta var silesiaca
 48  DE020A       * Denticula thermilis
104  NI203A       * NI203A
105  NI045A       * Nitzschia ovalis
137  Spp1         * Species 1

The following fossil variables do not occur in the training set and have been excluded from
the reconstruction:

  5  DP009A        Diploneis elliptica
  6  DP067A        Diploneis modica
  8  EP001A        Epithemia sorex
 13  FR004A        Fragilaria hungarica
 18  NA090A        Navicula rotunda
 19  NA110A        Navicula schadei
 20  SL002A        Navicula seminulum
 21  NA114A        Navicula subrotunda
```

```
 22  NA027A         Navicula viridula
```

Taxa with only one occurrence have had their tolerances set to 0.64528

Deshrinking regression coefficients

```
 #               Id      WA_b0     WA_b1   WATOL_b0   WATOL_b1
 1   Inverse deshrinking   -1.3154    2.363   -0.97621    1.9306
 2 Classical deshrinking    0.6281    0.3513   0.59754    0.42554
```

Model performance

```
 #       Id         WA_Inv       WA_Cla      WATOL_Inv      WATOL_Cla
 1      RMSE       0.29099      0.31937      0.29825         0.32906
 2       R2        0.83014      0.83014      0.82155         0.82155
 3   Ave_Bias     6.74e-016    4.99e-016    9.25e-017       2.78e-016
 4   Max_Bias      0.34796      0.22609      0.47586         0.28744
```

Results WA with jack-knife cross validation and sites Lago de la Luna, La Piscina de Yuriría and Alchichica removed

```
Model name                  : WAjack-chl-py-alch-luna
Description                 : Model 03
Model type                  : Weighted Averaging
Date                        : 15 October, 2005: 17:50:50
Species data                : sed only diatoms
Environmental data          : water TN
Environmental variable      : Chl-a LOG
Total number of samples     : 30
Number of samples in model  : 27
Total number of variables   : 139
Number of variables in model: 132
Fossil data                 : % >1%
Total number of samples     : 32
Total number of variables   : 24
Number of variables in model: 15
```

The following training set samples have been excluded from the model:

```
  2  P Yur
 24  Alch
 29  Luna
```

The following training set variables have been excluded from the model:

```
   8  AC042A       * Achnanthes subatomoides
  29  CY017A       * Cyclotella quillensis
  43  EY016A       * Cymbella minuta var silesiaca
  48  DE020A       * Denticula thermilis
 104  NI203A       * NI203A
 105  NI045A       * Nitzschia ovalis
 137  Spp1         * Species 1
```

The following fossil variables do not occur in the training set and have been excluded from the reconstruction:

```
 5  DP009A          Diploneis elliptica
 6  DP067A          Diploneis modica
 8  EP001A          Epithemia sorex
13  FR004A          Fragilaria hungarica
18  NA090A          Navicula rotunda
19  NA110A          Navicula schadei
20  SL002A          Navicula seminulum
21  NA114A          Navicula subrotunda
22  NA027A          Navicula viridula
```

Taxa with only one occurrence have had their tolerances set to 0.62424

Deshrinking regression coefficients

```
#            Id            WA_b0     WA_b1  WATOL_b0   WATOL_b1
1  Inverse deshrinking    -1.3154    2.363  -0.97621    1.9306
2 Classical deshrinking    0.6281   0.3513   0.59754   0.42554
```

Model performance

```
#         Id        WA_Inv      WA_Cla     WATOL_Inv    WATOL_Cla
1        RMSE       0.29099     0.31937     0.29825      0.32906
2         R2        0.83014     0.83014     0.82155      0.82155
3     Ave_Bias     6.74e-016   4.99e-016   9.25e-017    2.78e-016
4     Max_Bias      0.34796     0.22609     0.47586      0.28744
5      Jack_R2      0.080053    0.090636    0.01131      0.0077791
6 Jack_Ave_Bias    -0.12208    -0.1462     -0.17386     -0.20753
7 Jack_Max_Bias     1.3694      1.3633      1.5832       1.7848
8       RMSEP       0.69398     0.7027      0.92681      0.99764
```

Results for WAPLS no cross validation and 3 sites removed

```
Model name                : WAPLS-3sites
Description               : Model 04
Model type                : Weighted Averaging Partial Least Squares
Date                      : 09 January, 2006: 16:05:12
Species data              : sed only diatoms
Environmental data        : water TN
Environmental variable    : Chl-a LOG
Total number of samples   : 30
Number of samples in model : 27
Total number of variables  : 139
Number of variables in model: 132
```

The following training set samples have been excluded from the model:

```
 2  P Yur
24  Alch
29  Luna
```

The following training set variables have been excluded from the model:

```
 8  AC042A        * Achnanthes subatomoides
29  CY017A        * Cyclotella quillensis
43  EY016A        * Cymbella minuta var silesiaca
48  DE020A        * Denticula thermilis
```

104	NI203A	* NI203A
105	NI045A	* Nitzschia ovalis
137	Spp1	* Species 1

Model performance

#	Id	Component 1	Component 2	Component 3	Component 4	Component 5
1	RMSE	0.29415	0.15733	0.081561	0.053777	0.027795
2	R2	0.83014	0.95042	0.9867	0.99425	0.99848
3	Ave_Bias	0.02076	-0.0064306	0.0025037	-0.0033631	-9.9715e-004
4	Max_Bias	0.32473	0.18981	0.11489	0.092748	0.041828

Results WAPLS with jack-knife cross validation and Lago de la Luna, Piscina de Yuriria and Alchichica removed

Model name : wapls-jack-3site
Description : Model 04
Model type : Weighted Averaging Partial Least Squares
Date : 09 January, 2006: 16:05:46
Species data : sed only diatoms
Environmental data : water TN
Environmental variable : Chl-a LOG
Total number of samples : 30
Number of samples in model : 27
Total number of variables : 139
Number of variables in model: 132

The following training set samples have been excluded from the model:

2	P Yur
24	Alch
29	Luna

The following training set variables have been excluded from the model:

8	AC042A	* Achnanthes subatomoides
29	CY017A	* Cyclotella quillensis
43	EY016A	* Cymbella minuta var silesiaca
48	DE020A	* Denticula thermilis
104	NI203A	* NI203A
105	NI045A	* Nitzschia ovalis
137	Spp1	* Species 1

Model performance

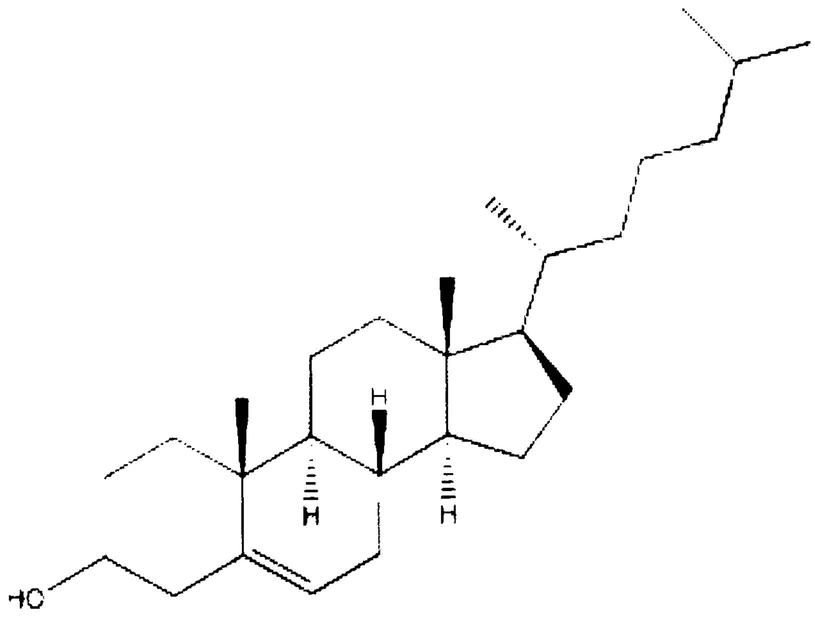
#	Id	Component 1	Component 2	Component 3	Component 4	Component 5
1	RMSE	0.29415	0.15733	0.081561	0.053777	0.027795
2	R2	0.83014	0.95042	0.9867	0.99425	0.99848
3	Ave_Bias	0.02076	-0.0064306	0.0025037	-0.0033631	-9.9715e-004
4	Max_Bias	0.32473	0.18981	0.11489	0.092748	0.041828
5	Jack_R2	0.12492	0.15152	0.14497	0.15429	0.168
6	Jack_Ave_Bias	-0.086969	-0.062331	-0.059492	-0.052382	-0.043452
7	Jack_Max_Bias	1.3517	1.2326	1.1892	1.1481	1.1203
8	RMSEP	0.66754	0.65983	0.66182	0.65728	0.6519

Appendix 4 – Structures of major lipids identified in this study

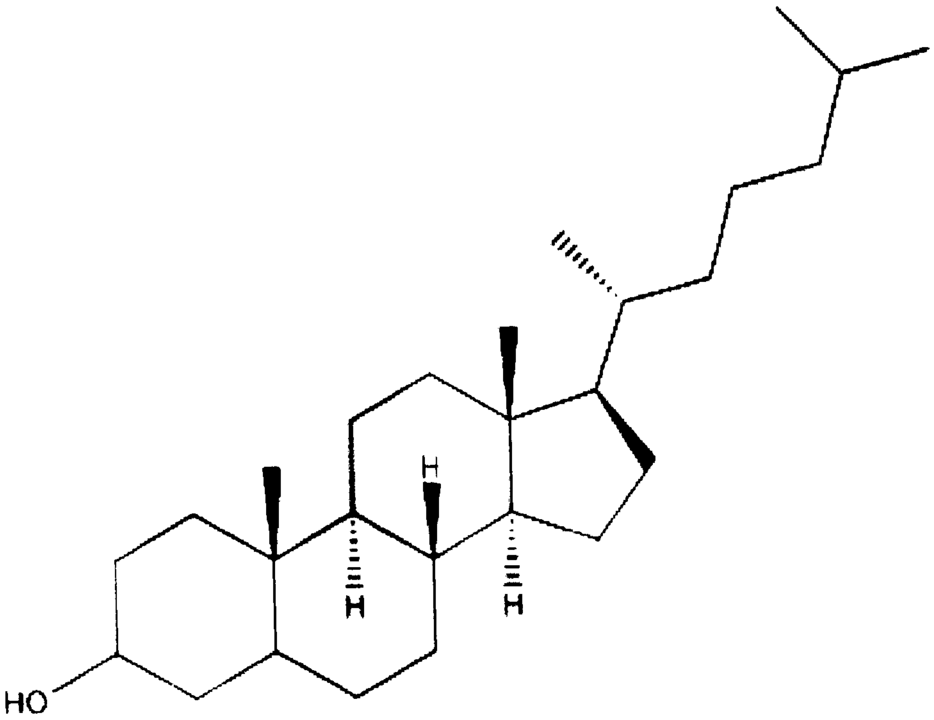
n-C_{17} $C_{17}H_{36}$

n-C_{19} $C_{19}H_{40}$

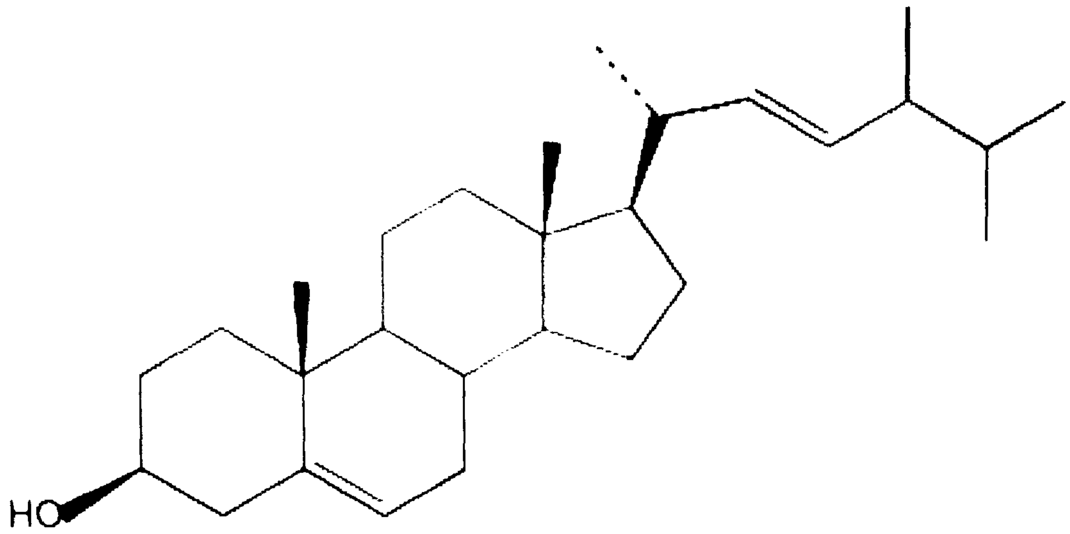
Cholesterol (Cholest-5-en 3β-ol)
$C_{27}\Delta^{5}$



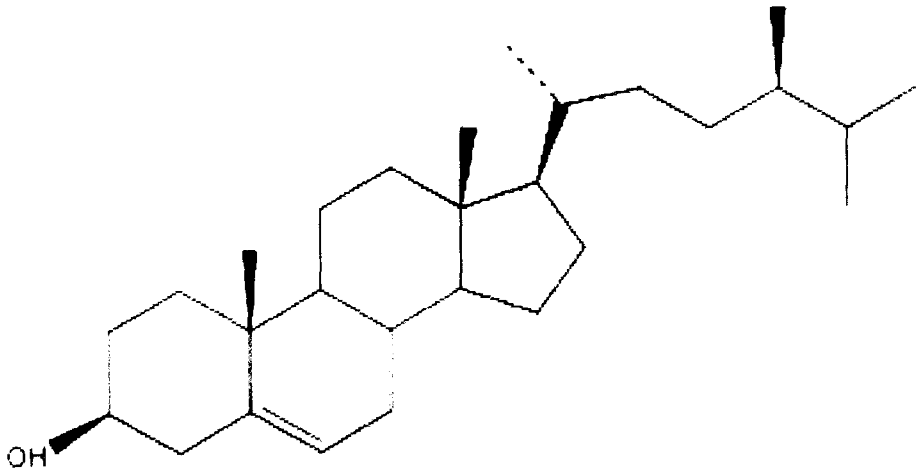
Cholestanol 5α-cholestan-3β-ol


H
H
H
HO

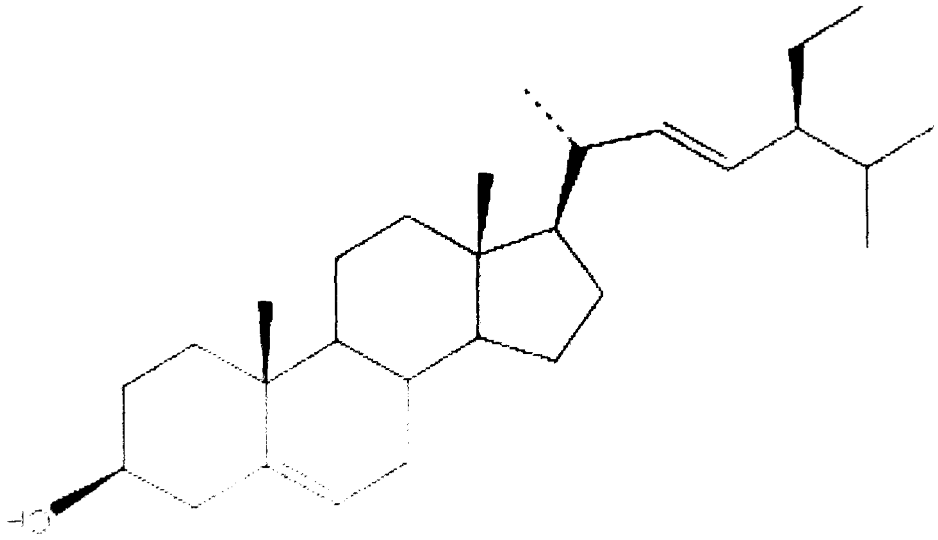
Brassicasterol (24-methylcholesta-5,22E-dien-3β-ol)
$C_{28}\Delta^{5,22}$



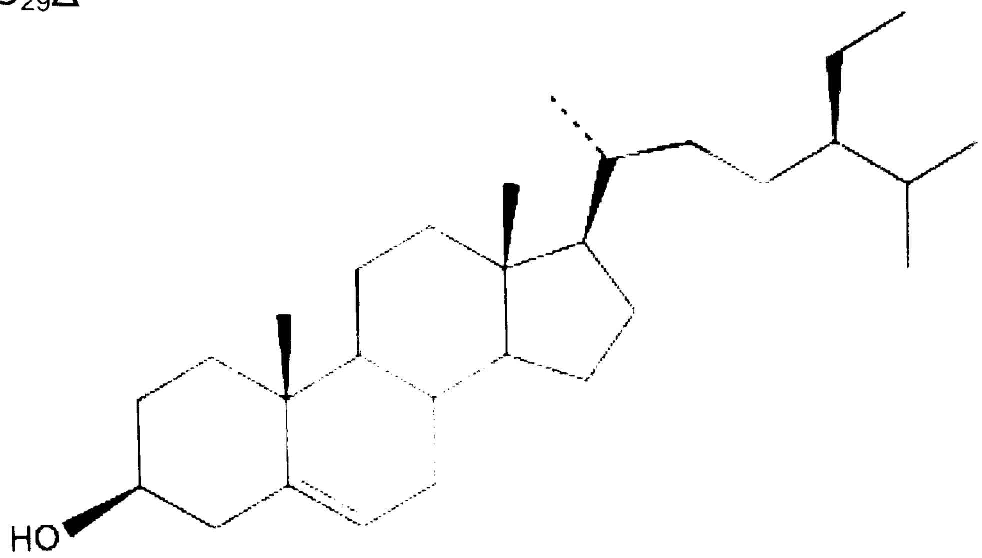
Campesterol (24-methylcholesterol-5-en-3β-ol)
$C_{28}\Delta^{5}$



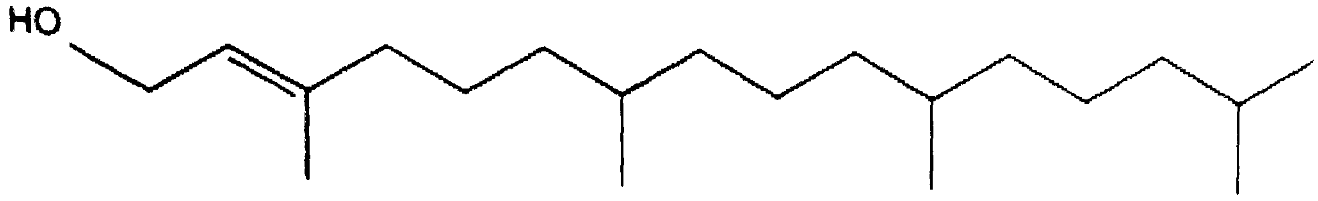
Stigmasterol (24-ethylcholesta-5,22,dien-3β-ol)
$C_{29}\Delta^{5,22}$



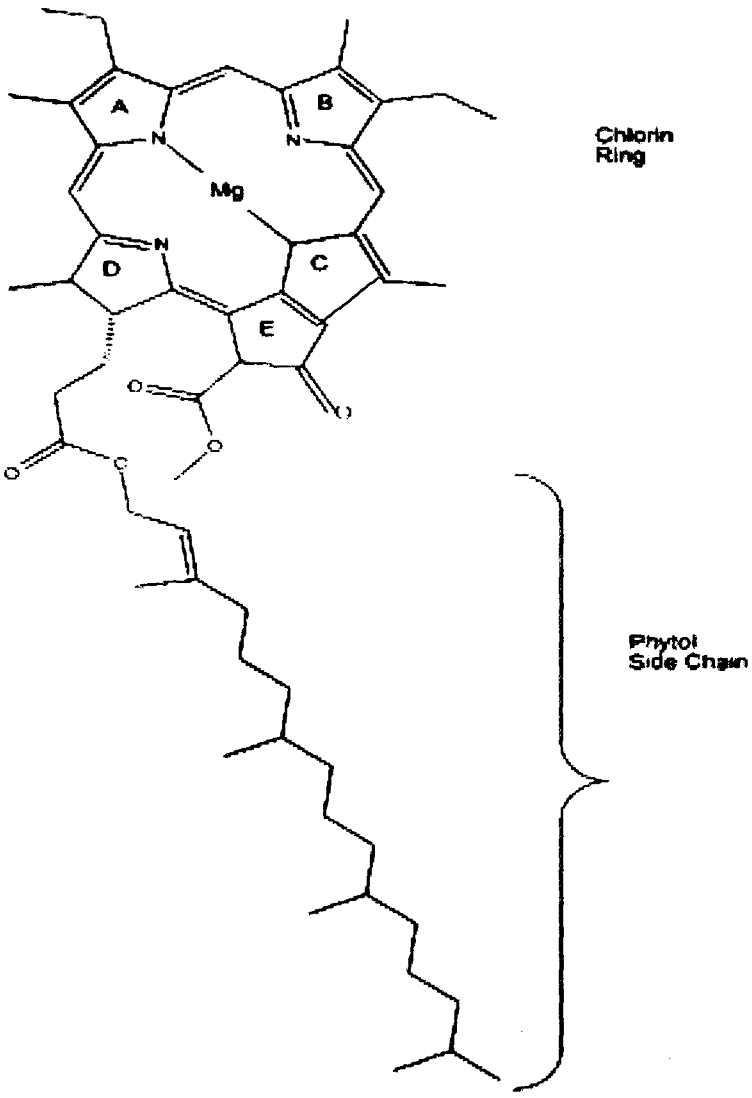
β-sitosterol (24-ethylcholest-5-en 3β-ol)
$C_{29}\Delta^5$



Phytol



Chlorophyll



Squalene (C_{30})