ASSESSING THE IMPACT OF AQUACULTURE ON THE SEVEN LAKES OF SAN PABLO, PHILIPPINES, USING PALAEOLIMNOLOGY

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ABSTRACT

In the Philippines, aquaculture in freshwater lakes contributes significantly to its economy, food security and employment. However, intensive aquaculture often leads to degradation in lake ecosystem integrity because of nutrient fertilisation resulting in harmful algal blooms (HABs), eutrophication and degradation of water quality. The few limnological and palaeolimnological studies carried out on Philippine lakes demonstrate a link between aquaculture activity and degraded water quality but there is a lack of information to help define how lakes have reacted over time to changing intensities of aquaculture and other catchment effects. This research attempts to redress this imbalance by using two different palaeolimnological approaches to assess the impact of aquaculture on six of the Seven Lakes of San Pablo (Luzon Island). Both approaches used multiple proxies, specifically, chlorophyll and carotenoid pigments, carbon and nitrogen isotopes ($\delta^{13}C$, $\delta^{15}N$) and C/N ratios to help disentangle lake-specific effects of aquaculture from the impacts of regional drivers. The first approach used generalised additive models (GAMs) to assess responses of lakes along an aquaculture disturbance gradient and hence whether lake response was proportional to the level of aquaculture. The second approach endeavoured to quantify the extent to which aquaculture (and other drivers) led to change in algal communities using variance partitioning analysis. Using this whole-ecosystem "experimental design" across individual lakes allowed for a more critical interpretation of the pigment and isotopic records. Both approaches concluded that there was no proportional relationship between changes in the proxy record and level of aquaculture disturbance, suggesting aquaculture is not the main driver of change. Land use changes (since 1950) explained the greatest proportion of variance and corresponded to periods of significant temporal change indicating it was a more dominant driver of change in algal communities. As aquaculture intensity increased, lakes became more eutrophic and anoxia increased due to nutrient enrichment from a combination of anthropogenic (urbanisation, coconut plantations and aquaculture) and climatic factors. In the majority of the lakes, changes in the proxy records predate the introduction of aquaculture, providing evidence that aquaculture exacerbated pre-existing change especially in the lakes with the highest aquaculture disturbance. Furthermore, each lake had a distinctive response to aquaculture due to the impact of multiple stressors (climate, land use and aquaculture) especially on the high disturbance lakes, and the modification by individual lake characteristics such as hydro-morphology. Mitigation strategies, therefore, need to be specifically developed for each lake, with consideration given

to their distinct morphological features and how this influences their complex response to aquaculture and other environmental pressures.

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LIST OF ABBREVIATIONS

Bchls….Bacteriochlorophylls
C…. Carbon
Ca^{2+}…. Calcium
$CaCO_3$…. Calcium Carbonate
CA:LA…. Catchment area to lake area ratio
CH_4…. Methane
Chls…. Chlorophylls
Cl^-…. Chloride
CO_2…. Carbon dioxide
CoV….Coefficient of Variance
C/N ratio…. Carbon to nitrogen ratio, in weight %
CRS…. Constant rate of supply model
DCA…. Detrended correspondence analysis
DCM…. Deep chlorophyll maximum
DIN…. Dissolved inorganic nitrogen
DO…. Dissolved Oxygen
EDF…. Estimated Degrees of Freedom
ENSO…. El niño southern oscillation
GAMs….Generalised Additive Models
GDP.... Gross Domestic Product
GLM…. Generalised Linear Models
GPS…Global Positioning Software
H_2O_2…. Hydrogen Peroxide
HABs…. Harmful algal blooms
HPLC…. High performance liquid chromatography
IPCC…. Intergovernmental Panel on Climate Change
ITCZ…Intercontinental tropical convergence zone
K^+…. Potassium
LLDA…. Laguna Lake Development Authority
MEI…. Multivariate index
Meq…. Miliequivalents
Mg^{2+}…. Magnesium
N…. Nitrogen
Na^+…. Sodium
NH_3…Ammonia
NH_4^+…. Ammonium
NO_3^-…. Nitrate
NO_x…. Nitrogen oxides
OM…. Organic matter
P…. Phosphorus
PCA…. Principal component analysis
PDA…. Photodiode Array
PO_4^{3-}….Phosphate
SRP…. Soluble Reactive Phosphorus
TP…. Total phosphorus
TN…. Total nitrogen
R^2…. Pearson's correlation coefficient
RDA…. Redundancy Analysis

S…Sulphur
SO_2…. Sulphur Dioxide
SO_4^{2-}…. Sulphate
USD…United States Dollar (Currency)
VIF…. Variance Inflation Factor

95% CI….95% confidence interval
SE….standard error
^{137}Cs…. Caesium-137 radioisotope
^{206}Pb…. Lead-206 radioisotope
^{210}Pb…. Lead-210 radioisotope
^{222}Rn…. Radon-222 radioisotope
^{226}Ra…. Radium-226 radioisotope
^{241}Am…Americium-241 radioisotope
^{210}Po…. Polonium-210 radioisotope
^{238}U…. Uranium-238 radioisotope
$\delta^{15}N_{org}$…. Ratio of ^{15}N:^{14}N in bulk organic matter
$\delta^{13}C_{org}$…. Ratio of ^{13}C:^{12}C in bulk organic matter
C/N Ratio….Ratio of %C over %Nitrogen
LOI…. Percentage loss-on-ignition
%... Percentage
±…Plus or minus

1. CHAPTER 1 - INTRODUCTION AND CONTEXT

Tropical freshwater lakes are under increasing threat from anthropogenic activities and climate change. In order to develop appropriate management strategies, it is vital to understand how tropical lakes are reacting to these stressors. Sediment cores can be used to decipher the effects of multiple drivers (e.g. climate change, aquaculture, deforestation) but the problem is ascribing what change recorded in lake sediments is due to natural variability or climate and which are caused by anthropogenic drivers (Simpson and Anderson, 2009), as they often cause a similar response within lake systems (Mills *et al.*, 2017). For example, rising temperatures and eutrophication can cause increased anoxia, algal productivity, nutrient loading and nutrient recycling. This is further complicated by the fact that these changes often occur simultaneously (Moss *et al.*, 2011). Lakes can also vary in their sensitivity to different drivers depending on how they processes environmental perturbations. There is also evidence that anthropogenic drivers have reduced the sensitivity of lake systems to climate change (Ryves *et al.*, 2011). The Philippines is heavily dependent on its aquaclture industry for economic development and the employment and food security it provides. A suitable strategy, therefore, is needed to evaluate how aquaculture impacts ecosystem functioning, so that these important freshwater systems can continue to provide the goods and services on which stakeholders depend.

1.1. Socio-economics of the Philippines

The Philippines currently has one of Asia's fastest growing economies with a current Gross Domestic Product (GDP) of 303.8 billion United States Dollar (USD). It is classified as a developing country which is defined as a poor agricultural country that is seeking to become more advanced, both economically and socially (The World Bank Group, 2019). It is an emerging market transitioning from a primarily agricultural to a services and manufacturing based economy (primarily assembling components for foreign multi-national companies). The country is, however, still heavily dependent on agriculture which accounted for 9.7% of GDP and employed 25% of the country's workforce in 2018 (The World Bank Group, 2019). The main agricultural products are coconut, rice, corn, sugar and fruit. The Philippines is also one of the major fish producing countries in the world with aquaculture contributing 1.1% to GDP and employing over a million people in 2018 (Bureau of Fisheries and Aquatic Resources, 2019). Despite the impressive growth rate in its economy in recent years, the Philippines has major problems associated with wide income and growth disparities between the different regions and socioeconomic classes. Over 21.6% of the population live below poverty line (defined as living off less than 1.90 USD a day) (Asian Development Bank, 2019). Developing

and exploiting its major resources, such as its aquaculture industry, is an important part of redressing this imbalance.

1.2. Aquaculture in the Philippines

An increasing awareness among consumers of the health benefits offered by seafood has seen the aquaculture sector become one of the fastest growing food producing sectors globally. It accounted for over 202 million tonnes of the fish produced worldwide in 2016 (Bureau of Fisheries and Aquatic Resources, 2019) with an average growth rate annually of >12% (1976 to 2012) (Table 1.1; Krause *et al.*, 2015). The Philippines' aquaculture production of fish, crustaceans and molluscs amounted to over 1.79 billion USD in 2018 (Bureau of Fisheries and Aquatic Resources, 2019)

The Asia Pacific market is expected to grow due to increased demand from Australia, India, Japan and multiple South East Asian countries (FAO, 2016). However, there is concern that the Philippines will not be in a strong position to take advantage of this, as unsustainable management of its fisheries has caused depleted fish stocks, degraded habitats and over exploitation of its resources (FAO, 2016). The Philippines has seen its global ranking in terms of aquaculture fall from an historical position of supplying 5% of global farmed fish supply in 1991 to 1.0% (volume) in 2016 (FAO, 2016). As well as contributing to GDP the development of aquaculture has also been found to benefit the poorest inhabitants; farmed fish having a high nutritional content (Prein and Ahmed, 2000) and providing secure employment in areas where this is often difficult to find (Gonzalez, 1998). Irz *et al.*, (2007) found in a study of certain coastal areas of the Philippines that aquaculture income reduced inequality. There is, therefore, a pressing need for the Philippines to plan and implement initiatives to ensure the future growth and sustainability of its aquaculture industry.

Table 1.1: Production and trade data for fisheries and aquaculture industry in the Philippines (1980 - 2017) (FAO, 2019)
**capture fishery is the harvesting of naturally occurring living resources in aquatic environments*

Year	1980	1990	2000	2010	2011	2012	2013	2014	2015	2016	2017
Production (1000s tons)	1576	2209	2290	3356.5	3150.5	3113.7	3571.9	4125.6	4649.3	4355.8	4312.1
Inland	276.4	318.7	263.8	493.7	498.8	505.9					
Marine	1299.6	1890.3	2026.2	2862.8	2631.7	2607.9					
Aquaculture	199.9	379.9	393.9	744.7	767.3	790.9	815.0	788.0	781.8	796.4	822.5
Inland	32.1	81.9	112	308.3	306.9	310.1					
Marine	167.8	298	281.8	436.4	460.4	480.8					
Capture fisheries*	1376	1829.1	1896.1	2611.8	2363.2	2322.9	2199.3	2246.3	2.151.5	2.024.8	1887.1
Inland	244.3	236.8	151.8	185.4	191.9	195.8	200.9	215.5	203.4	159.6	162.5
Marine	1131.7	1592.2	1744.4	2426.4	2171.3	2127.0	1998.3	2032.8	1948.1	1865.2	1724.5
Trade (USD Millions)											
Import	36.6	84.8	108.5	134	183.6	246.9	265.4	252.6	358.4	385.9	580.4
Export	141.6	396	406.9	638.6	649.4	808.8	1143.9	997.9	775.9	720.8	869.2

1.2.1. The development of aquaculture in the Philippines

Aquaculture has been a traditional practice in South East Asia for over 500 years (Eng *et al.*, 1989). Historically it was characterised by small-scale operations with poor management and low yields (FAO, 2016)**.** The earliest fishponds were milkfish (and shrimp) grown in brackish water using naturally occurring fry from tidal waters (FAO, 2016). Further species were introduced into the Philippines during the early twentieth century including as Common carp (*Cyrpinus carpio*; introduced 1915), oysters (*Crassostrea iredalei*; 1931), Nile Tilapia (*Oreochromis niloticus*) and Seaweeds (*Kappaphycus* and *Eucheuma* spp.; 1950s) (FAO, 2016). Aquaculture has expanded significantly in the Philippines since 1973 when a decree by President Marcos provided tax exemptions and the promotion of research into fisheries development (Malacañang Records Office, 1973).

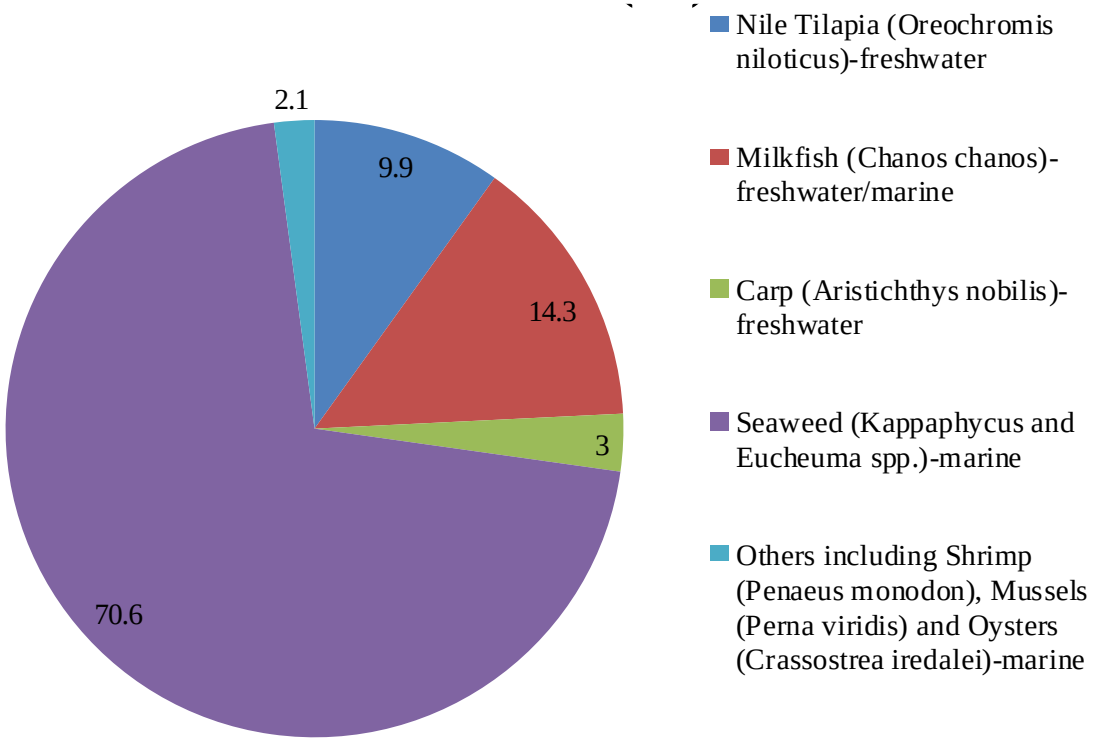


Figure 1.1: Contribution (in %) of seaweed and the different fish species towards total aquaculture production (including freshwater and marine) in the Philippines in 2017, (totaling 100 %; Bureau of Fisheries and Aquatic Resources, 2019)

Aquaculture in the Philippines is carried out in numerous diverse ecosystems, the largest is open coastal water (mariculture, a specialised branch of aquaculture) making up 75% of total volume (in the Philippines), which is divided into shellfish, finfish and seaweed culture (Figure 1.1; FAO, 2016). The second largest culture environment is brackish water areas (12.5% of total volume) (FAO, 2016), which comprises mangrove swamps, estuarine areas and inter-tidal zones for the cultivation of mainly milkfish and shrimp. The final culture environment is freshwater (12.5% of total volume) (FAO, 2016) which is the focus of this research.

1.2.2. Freshwater aquaculture

Freshwater aquaculture utilises lakes (and other freshwater environments) to raise and breed aquatic organisms for economic purposes (FAO, 2016). In the Philippines, freshwater aquaculture began with the introduction of the Mozambique tilapia, *Oreochromis mossambicu,* in 1950. Aquaculture expanded in the 1970s due to the ability to rear milkfish (*Chanos chanos)* without the use of additional fish food and the successful cultivation of tilapia in lakes, dams and reservoirs in Laguna de Bay (Southern Luzon). The development of freshwater aquaculture was also aided by a number of extensive and continuous programmes of aquaculture research (by the Philippine Bureau of Fisheries and Aquatic Resources (BFAR), Central Luzon State University (CLSU) and the Southeast Asian Fisheries Development centre (SEAFDEC)) undertaken in the Philippines since the 1970's. Today, Nile tilapia, (*Oreochromis niloticus*), milkfish (*Chanos chanos)*, carp (mainly bighead carp, *Aristichthys noblis* and common carp, *Cyprinus carpio)*, are the main species cultivated in freshwater aquaculture (López *et al.*, 2008). The methods of cultivation vary from fish corrals/pens, fish tanks, fish cages and earthen ponds. Levels of technology employed vary from low levels of technology in the cultivating of milkfish in fish pens and cages to the genetic manipulation of tilapia.

1.3. Impacts of aquaculture in freshwater environments

The rapid expansion of the aquaculture sector globally over the last 30 years (from 5 million to 63 million tonnes; FAO, 2016) has led to a rise in a number of environmental problems in freshwater environments. These include water quality degradation, a reduction in water clarity, contamination of drinking water and the food chain (Lalumera *et al.*, 2004), eutrophication (Páez-Osuna, 2001; Anderson *et al.*, 2002) and the introduction of invasive species (Arismendi *et al.*, 2009) (Figure 1.2).

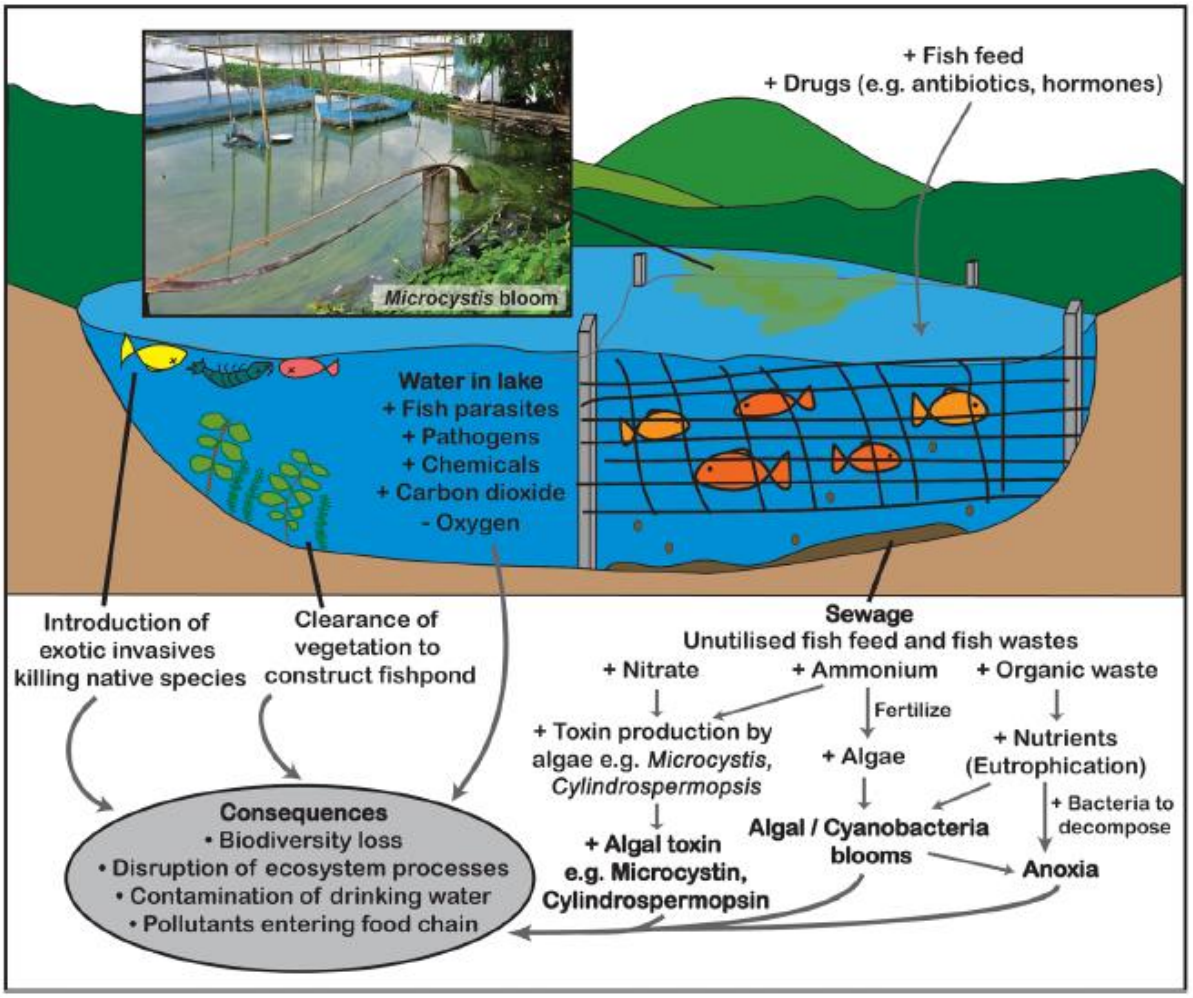


Figure 1.2: Schematic representation of aquaculture and its impacts on the environment (Legaspi et al., 2015)

1.3.1. Eutrophication and HABs

Eutrophication is defined as the enrichment of nutrients (such as P and N) stimulating algae and plant productivity resulting in the depletion of oxygen (Glibert *et al.,* 2010). Aquaculture causes increased nutrient inputs in the form of effluent from both the fish themselves and from the high nutrient fish feed (Holmer, 2002), which leads to nutrient enrichment. It is estimated that up to 85% of phosphorus and 52 to 95% of nitrate entering the fish culture system as feed is lost to the environment through waste and excretion (Southgate and Lucas, 2003). Increased nutrient enrichment leads to eutrophication and deterioration in water quality, which may have negative impacts on productivity and yield of aquaculture in freshwater lake environments (FAO, 2016).

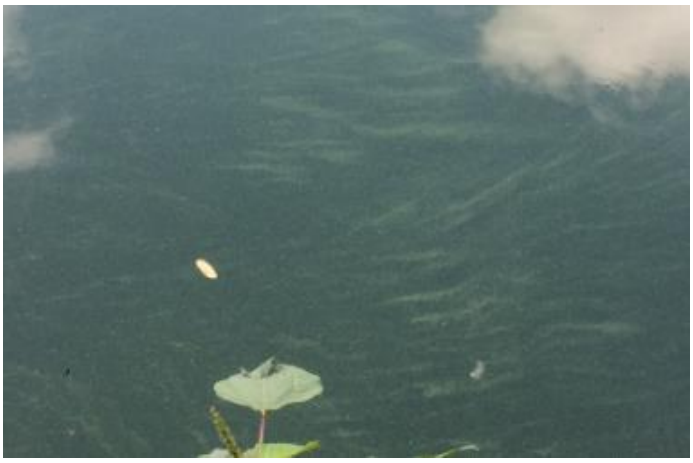


Figure 1.3: Picture of algal bloom from Lake Mohicap

Increased nutrients (eutrophication) can also lead to the development of harmful algal blooms (HABs; Figure 1.3) caused by cyanobacteria (Anderson *et al.,* 2002). Eutrophication causes increased hypolimnetic dissolved oxygen (DO) consumption, progressively lowering DO concentrations in the water column and enhancing diurnal DO fluctuations (Kalff, 2002). Continued eutrophication can result in the complete exhaustion of DO and the formation of hypoxic or anoxic conditions (in the hypolimnion) which can have a negative impact on aquatic organisms. In the Philippines, eutrophication has not only had environmental impacts but economic consequences too, through reduced revenue from the loss of habitat suitable for aquaculture (Szekielda *et al.,* 2014). Furthermore, nutrient enrichment and stoichiometric imbalances can lead to the formation of HABs, further deteriorating water quality and introducing toxic compounds detrimental to human health (Paerl *et al.*, 2001; 2014). HABs are

mostly composed of cyanobacteria in freshwaters (Paerl *et al.*, 2001), with the final phase of eutrophication experiencing permanent cyanobacterial dominance (Dokulil and Teubner, 2000). This has been attributed to their physiological adaptations such as the ability of certain species of cyanobacteria to fix nitrogen (Dokulil and Teubner, 2000). Anthropogenic nutrient loading reduces the N:P ratio which favours cyanobacteria, specifically heterocystous species (which can fix nitrogen) (Dolman *et al.*, 2012). It is common for primary production in most tropical freshwater lakes to be N limiting (Abell *et al.*, 2012; Bannister *et al.*, 2019). Since many species of cyanobacteria are able to fix N biologically, increased delivery of nutrients particularly P through fish food alters the N:P ratio, increasing the likelihood of the development of HABs. However, each lake system responds differently to nutrient loading, therefore, mitigation strategies have to take into account factors such as water residence times, hydrology, and the length and severity of eutrophication. Shallower tropical lakes generally have quicker recovery times (from eutrophic conditions) than deep tropical lakes due to shorter water residence times and are less impacted by stratification and internal nutrient loading (Jeppesen *et al.*, 2005).

A complex range of factors such as the intensity of the aquaculture, lake water retention time, the algal species present, meteorological conditions, and the abundance of grazers influence the propensity of HABs to form (Alonso-Rodrıguez and Paez-Osuna, 2003). Cyanobacteria compete well in lakes with high retention times and a stratified water column as they have slower growth rates compared with other algal groups (Paerl and Huisman, 2008). These conditions allow HABs to regulate their buoyancy permitting them to maintain an optimum position in the water column for maximum growth (McGowan, 2015). Cyanobacteria are less competitive in lakes that have experienced increased precipitation and wind-driven turbulence. A lack of a stratified water column results in the dispersion of cyanobacteria cells as the buoyancy mechanism means they cannot control their position in the water column. Increased precipitation may also enhance the transport of terrestrial OM (from increased run-off) and lead to an increase in HABs (Miller *et al.*, 2006). When periods of enhanced precipitation (which occurs during the monsoon season in tropical climates) is followed by a dry period, it makes for ideal conditions for HABs to form (McGowan *et al.*, 2012). This means that lakes can react differently to the same nutrient loads depending on the time of year and the type of environment. In addition, the effect of nutrient enrichment on HABs has been found to be species specific (Anderson *et al.*, 2002). Detailed knowledge of the current algal species occupying each lake is necessary to understand the nature of HABs.

Numerous studies have found a link between increased nutrient loading from aquaculture and the expansion of large biomass blooms (Legaspi *et al.*, 2015; Bannister *et al.*, 2019). This has been detrimental for the fisheries industry (Anderson *et al.*, 2002) and led to public health concerns (from contaminated drinking water). One of the most severe cases is that of Lake Taihu in China, (Glibert, 2013) which serves as a drinking reservoir for over 2 million people. This lake becomes clogged annually with toxic cyanobacteria blooms of *Microcystis aeruginosa*, a species which makes several toxic compounds including microcystin. The blooms were so severe in 2007, it necessitated the closure of the water supply for remediation. The development of HABs has also been linked to 'fish kills' (a localised die-off of fish populations) which impacts the industry through a loss of revenue (Landsberg *et al.*, 2009). Fish kills are an ecological phenomenon mainly caused by annual stratification or upwelling in the lake, in which the bottom water loaded with toxic substances (e.g. hydrogen, sulphides and ammonia) is brought up to the surface (Araullo, 2001). The fresh supply of nutrients (from the deep water) encourages the development of HABs (Kalff, 2002).

1.3.2. Chemical compounds

Many chemical compounds are used in aquaculture including antibiotics (to prevent and treat disease) and hormones (to accelerate growth; Lalumera *et al.*, 2004). Antibiotics enter the lake and the surrounding environment through leaching from fish faeces and uneaten antibiotic feed. Up to 75% of the antibiotics used in aquaculture are transported to the surrounding environment and accumulate in the lake sediment (Halling-Sørensen *et al.*, 1998; Lalumera *et al.*, 2004). Studies by Herwig *et al.*, (1997) concluded that aquaculture caused increases in the concentrations of oxytetracycline (an antibiotic), and oxytetracycline-resistant bacteria in lake sediments. In addition, there is also evidence that antibiotics can cause a reduction in microbial action within the sediments leading to rapid degradation of lake water quality (Southgate and Lucas, 2003).

1.3.3. Invasive species

Many non-native invasive species have been introduced into freshwater ecosystems in the Philippines, threatening their structure and function (Townsend, 1999). It has been estimated that over 170 non-native fish species have been introduced to Philippine inland waters since 1900 (Mendoza *et al.*, 2019). This can lead to a decline in native fish populations and increased competition for habitats and food, leading in some cases to the extinction of native fish species. In North Patagonian lakes in Chile, Arismendi *et al.*, (2009) investigated the impacts of escaped cultured salmonids from aquaculture on native fish populations. They found that escaped fish

from fish farms led to a reduction in native fish species due to predation from the salmonids. The increase in non-native fish species used in aquaculture has also led to the introduction of non-native parasites and pathogens (Beyer *et al.*, 2005) which, are harmful to native fish (Brown and Grenfell, 2001). Beyer *et al.*, (2005) found that parasites (such as, *Neoergasilus japonicus*), from non-native fish species spread from their original introduction site, freshwater lakes in Somerset, United Kingdom, to other water bodies, raising concerns that this rapid dispersal would negatively affect native fish species.

1.4. Other drivers of environmental change in tropical lakes

1.4.1. Anthropogenic drivers

In addition to aquaculture, a variety of other anthropogenic factors impact tropical lakes (Figure 1.4). One of the main drivers of change is deforestation and the associated change in land use including agriculture (e.g. coconut) and urbanisation. In the Philippines, 37% of the total water pollution originates from agriculture, such as animal waste and the use of fertilizer and pesticide (Szekielda *et al.,* 2014). Fertilisers used in agriculture result in the runoff of synthetic N and P into lake waters causing eutrophication (Smith and Schindler, 2009). Often increased urbanisation and expansion of the transport infrastructure leads to further runoff and an increased volume of human effluent, which fuels nutrient enrichment and HABs (McGrane, 2016).

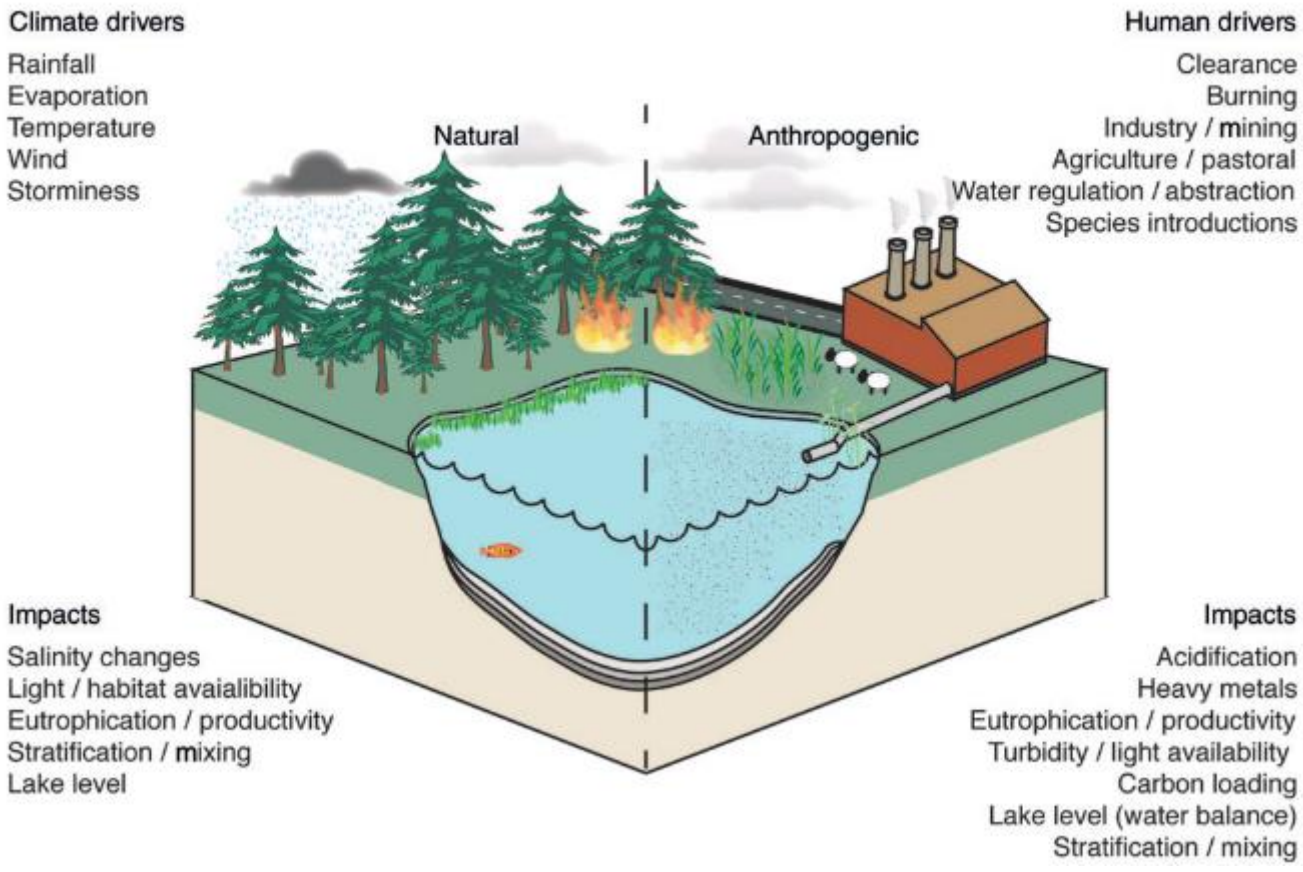


Figure 1.4 : Schematic representation of how climatic and anthropogenic drivers impact lake systems (Mills et al., 2017)

In South-East Asia, anthropogenic emissions of SO_2, NO_x, and NH_4 have risen dramatically since the 1950s, driven by economic development such as increased fossil fuel combustion and agricultural activities (Duan *et al.*, 2016). Nitrogen deposition, especially of NH_4, is of increasing concern in Asia due to its impacts on nitrification and nitrate leaching in N-limiting ecosystems (which is common in tropical lakes, see above). There is evidence that atmospheric deposition is influencing water quality in the Philippines causing an acidification of freshwater aquatic systems (Sia Su, 2008). Furthermore, the supply of biologically reactive forms of N is fuelling HABs, enhancing eutrophic conditions and hastening oxygen depletion (Palani *et al.*, 2007). Kim *et al.*, (2014) found that atmospheric N deposition contributed approximately 20% to annual biological production in the South China Sea, which borders the Philippines. This suggests atmospheric N deposition could be an important nutrient source for algae in tropical lakes.

1.4.2. Climatic drivers

Tropical lakes are experiencing warming trends, with an increase of 1°C since 1850 (Williamson *et al.*, 2009; O'Reilly *et al.*, 2015). The Philippines has seen a rise of 0.68°C in the annual mean temperature since 1950 (Cinco *et al.*, 2018). Increased lake temperatures have resulted in prolonged thermal stratification, reduced delivery of DO to the hypolimnion and increased the extent and/or duration of anoxic conditions (Wilhelm and Adrian, 2007). Anoxia in the hypolimnion can result in the internal release of P from the sediments fuelling eutrophication (Hou *et al.*, 2013). In African Rift Lakes, a greater temperature difference between the deeper hypolimnion and upper epilimnion has increased the stability of the thermocline and shallowed the mixing depth, reducing its strength (Vollmer *et al.*, 2005; Verburg and Hecky, 2009; Katsev *et al.*, 2014). Reduced mixing increases nutrient limitation, diminishes algal productivity and increases water transparency (Williamson *et al.*, 2009). In Lake Tanganyika, increased climatic warming and reduced nutrient supply led to a reduction in aquatic ecosystem productivity, due to a lack of mixing in the euphotic zone (O'Reilly *et al.*, 2003). This resulted in a 20% decrease in phytoplankton and a 30% drop in fish yield.

Significant changes to rainfall patterns including severe flooding and extended droughts throughout the 20th century have had a profound effect on tropical lake systems (IPCC, 2015). Extended droughts cause a reduction in lake level through changes to the inflows/outflows and evaporation. Hydro-morphological modifications caused by droughts increases mean water retention times and when coupled with warmer temperatures enhances the effects of thermal stratification. For example, a decline in precipitation resulted in a shallowing of Lake Chad

from 25,000km^2 (1960) to 1,350km^2 in 1990 (Coe and Foley, 2001). Hydrological variability (combined with temperature fluctuations) alters catchment vegetation and the delivery of OM into lakes (Adrian *et al.*, 2009). Severe flooding can increase erosion rates in the catchment and lead to a rapid influx of allochthonous material into the lakes. Furthermore, increased precipitation can cause increased lake levels, through the increased input of water (from inflows and the catchment). There is evidence that extreme storm events (which are becoming more intense as a result of climate change; Stuecker *et al.*, 2018) causes short lived mixing of the water column (Lewis Jr, 1984). This results in the release of toxic compounds (e.g. CH_4) from the hypolimnion and subsequent fish kills and algal blooms (Beadle, 1966). The IPCC (2015) predicts that in the 21st century the tropics will see an increase in the frequency of heavy rain due to a rise in the number of storm events.

1.5. Disentangling the impacts of anthropogenic drivers from climate change

Various approaches can help disentangle the impacts of anthropogenic and climatic drivers in tropical lake systems. A multi-proxy palaeolimnological approach can be useful when deciphering complex sedimentary records (Mills *et al.*, 2017). This approach uses multiple proxies (e.g. photosynthetic pigments, stable isotopes, diatoms) to simultaneously reconstruct ecosystem and biotic responses in the lake, the terrestrial catchment and the regional landscape over time. Due to the complex network of interactions within lake systems, it is desirable to study as many proxies as possible in order to gain a wider overview of the situation (Birks and Birks, 2006). The use of a multi-proxy approach is helpful in identifying the effect of a specific driver (such as aquaculture) on lake ecosystems. This is because it provides a number of independent lines of enquiry which allows the reconstruction of past environmental conditions with greater certainty (Lotter, 2003). Furthermore, it is an effective strategy for determining how the influence of different drivers has changed over time.

In addition, studying lakes along a disturbance gradient allows the response of 'pristine' or low disturbance lakes (influenced by climate) to be compared with high disturbance lakes (influenced by climate and human drivers) to separate out anthropogenic impacts. Inter-lake comparisons can also be useful when studying highly impacted regions that have been exposed to a variety of stressors (Moorhouse *et al.*, 2014) and to separate local from regional drivers (Mills *et al.*, 2014). Trebitz *et al.*, (2007) measured coastal wetlands along an agricultural gradient determining that agriculture has a prominent impact on algal productivity, nutrient recycling and water clarity. Combined with a statistical approach, this can help to quantify

individual drivers (e.g. aquaculture, urbanisation, and climate) and determine if individual, or a combination of environmental drivers, are the cause of ecosystem changes in tropical lakes.

1.6. Aims and Objectives

The aim of this research is to use two approaches **to quantitatively assess the ecological response of six lakes, on the island of Luzon in the Philippines, to aquaculture practices**. The sedimentary records from six lakes were investigate to determine whether lake response was proportional to the level of aquaculture disturbance. The specific research aims and objectives are:

1. Determine how algal communities and organic matter properties vary along an aquaculture disturbance gradient
    - Collect contemporary limnological samples to determine the current environmental and ecological conditions of the six lakes to aid the interpretation of down-core records
    - Reconstruct changes in primary producers and HABs using sedimentary chlorophyll and carotenoid pigments
    - Assess changes in lake organic matter sources and cycling using $\delta^{15}N_{org}$, $\delta^{13}C_{org}$ and C/N ratios
2. Determine the drivers of change in algal communities and organic matter properties using various statistical approaches
    - To determine periods of significant temporal change in algal communities and organic matter properties using GAMs
    - Determine the relative importance of regional and local-scale environmental drivers of lake ecosystem change using variance partitioning and end member analysis
    - Assess how landscape characteristics have altered lake response to aquaculture

1.7. Thesis outline

The structure of this thesis, addresses the aims and objectives outlined in section 1.6. Chapter 2 outlines the main factors which shape the physical environment of the study lakes (Seven Lakes of San Pablo) such as geology, climate and land use and provides a detailed description of each of the individual lakes. Sample collection and the subsequent laboratory and statistical analyses used are described in Chapter 3. Chapter 4 evaluates the contemporary limnology of the study lakes to aid in the interpretation of the individual palaeolimnological records, which follows in Chapter 5. Chapter 5 addresses if the key timings of algal community change, correspond to changes in environmental drivers (aquaculture, climate, land use) in order to support and interpret the results of the regional synthesis. Chapter 6 uses and combines the two approaches to determine if, and the extent to which, aquaculture drove change in the study lakes, whilst considering individual lake hydro-morphology. Chapter 7 summarises the main findings of this thesis and suggests future studies to build on the work presented here.

2. CHAPTER 2 – REGIONAL CONTEXT

2.1. Introduction

A major factor influencing the ecosystem functioning of tropical lakes is their position within the landscape (Nilssen, 1984). The legacy of past geological, climatic and anthropogenic changes can have a profound effect on lake ecology, so it is vital to understand how these characteristics have changed over time. Consideration of the physical characteristics and human modifications of the lake catchment is important in understanding how aquaculture and other drivers have influenced lake processes. This chapter provides context for interpretation of the proxy record which follows in Chapter 5.

2.2. Geology

The Philippines is in the 'Pacific Ring of Fire', a horse-shoe shaped region of high seismic and volcanic activity surrounding the Pacific Ocean. Its location makes it highly susceptible to a range of natural hazards such as volcanic eruptions, destructive earthquakes and tsunamis (Tupper *et al.*, 2004). The tectonic setting (of metamorphic terranes, magmatic arcs, ophiolitic complexes and sedimentary basins) heavily influences its geology. The island of Luzon, is part of the Philippine Mobile Belt; a stretch of crust situated between two subduction zones, the Manila Trench and the Philippine trench (Figure 2.1). The tectonic forces compressing the region cause the bisection of Luzon by the N-S trending Philippine Fault System. The ophiolitic complexes (which underlay the magmatic arcs), consist of tectonised periodities, that progress to layered gabbros, sheeted dike complexes, pillow basalts and finally to pelagic sedimentary rocks. The study area (the Seven Lakes of San Pablo, Luzon) is part of the South Western Luzon Volcanic Field which consists of Cenozoic volcanic rocks erupted 65 to 2.58 Ma and is associated with subduction processes from the Manila Trench. Geochemical studies on the Taal Volcano (located in the same volcanic field) show that lava composition ranges from calc-alkaline to iron enriched (Milkius *et al.*, 1991).

2.3. Climate

The Philippines has a tropical climate with wet and dry seasons heavily influenced by its topography, location and the tropical Pacific Ocean. This has resulted in a complex pattern of sub climatic zones, differentiated by the distribution of rainfall (Figure 2.2). Despite this regional diversity, temporal rainfall fluctuations across the country are uniform, certainly over inter-annual time scales. This appears to be driven by the relationship between large scale atmospheric dynamics and anomalies in sea surface temperatures in the Pacific Ocean (Lyon and Camargo, 2009).

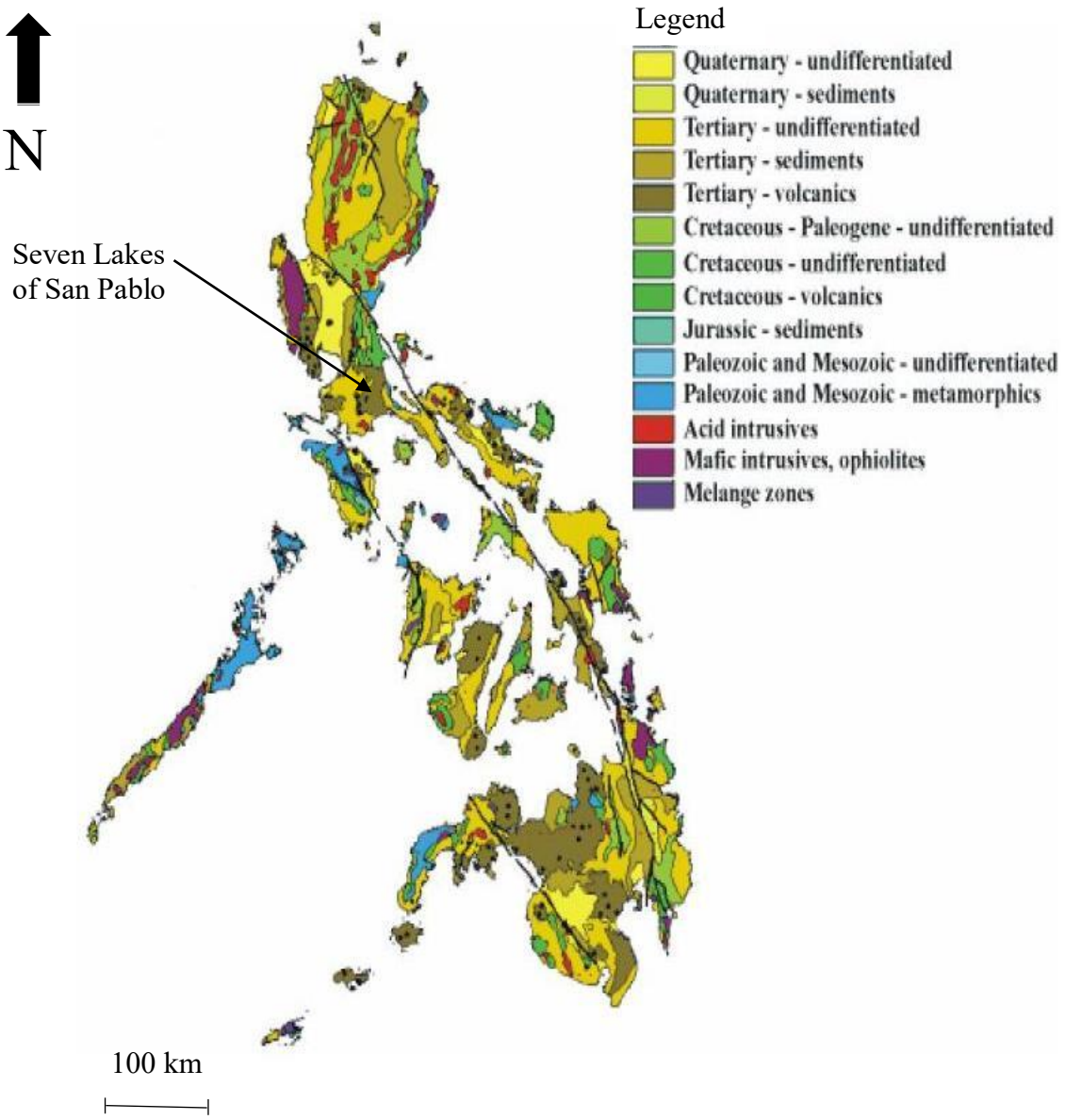


Figure 2.1: Geological map of the Philippines with the study area (Seven Lakes of San Pablo) highlighted. The main fault lines are indicated with black lines (Mines and Geosciences Bureau, 2010)

The climate of the archipelago can be divided into four distinct climatic types which vary with respect to the amount and distribution of precipitation (Figure 2.2). There are considerable variations between the climatic types with annual rainfall being as high as 5,000 mm in the mountainous east coast section but less than 1,000 mm in some of the sheltered valleys (Philippine Meteorological Institute, 2016). In comparison to rainfall, there is little variation in temperature throughout the country (mean maxima 28°C, daily variation 21°C to 32°C). The study area is located on the border of climatic types 1 and 3 (Figure 2.2) with average precipitation of 3178 mm per annum, a drier season from January to June (averaging 124 mm

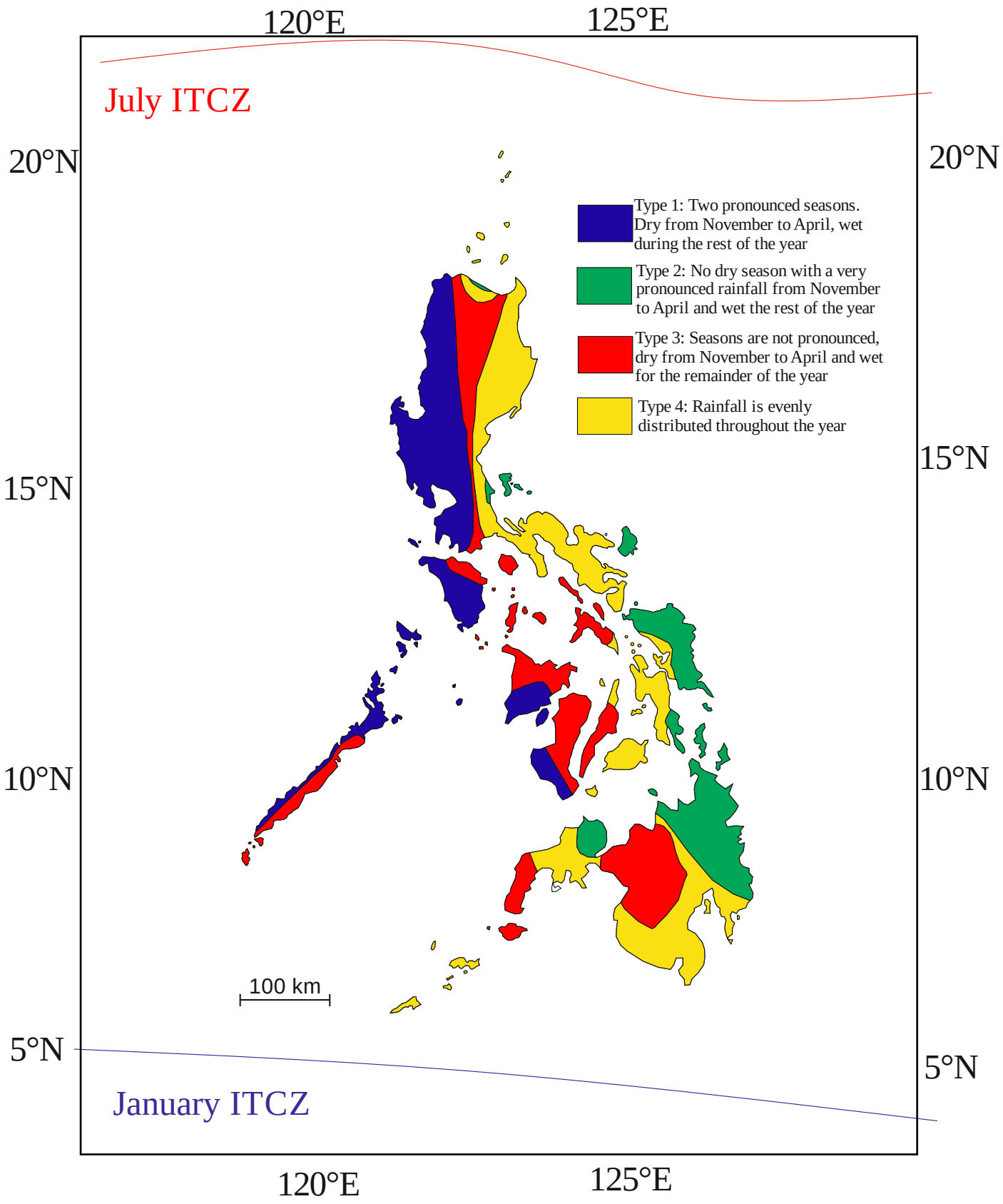


Figure 2.2: Map of the Philippines showing the four different climatic types and the changing position of the ITCZ (adapted from the Philippine Meteorological Institute, 2016)

per month) and a wet season from September to December (381 mm per month). The average annual temperature is c. 26.5ºC and humidity is c. 88% (1901-2017) (Harris *et al.*, 2014).

Rainfall in the Philippines is seasonally bimodal, driven by the biannual migration of the Inter Tropical Convergence Zone (ITCZ) between 5° N and 5°S of the equator, which brings moisture from both the Pacific and Indian Oceans (Figure 2.2; Polunin, 2008)). The annual cycle of the ITCZ sees it move northward and southward as it follows the thermal equator (Figure 2.2). These changes in the position and timing of the ITCZ have major consequences for the onset and duration of the monsoon season. High pressure over the cold northern hemisphere land masses, and areas of low pressure over the warm southern hemisphere push the ITCZ south. Conversely, when the northern hemisphere land masses heat up during the summer, the temperature anomaly between the land and the ocean pushes the ITCZ northward. This results in a bimodal rainfall pattern with northern and southern belts of monsoonal climates confining a humid equatorial zone (Gasse, 2000). The Philippines experiences two wet seasons, the first is the Northeast monsoon which occurs between October to January and the second is the Southwest monsoon from June to October. The majority of the precipitation falls as short, intense storms associated with the main wet season (June to October; Cordero and Baldia, 2015).

2.3.1. Fluctuations in the El Niño Southern Oscillation (ENSO)

In the Philippines, ENSO is associated with wetter and drier periods. ENSO refers to a naturally occurring phenomenon that originates in the Pacific Ocean and consists of the El Niño (ocean component) and the Southern Oscillation (atmospheric component) (Lyon and Camargo, 2009). Long-term research showed that tropical climates oscillate at irregular time intervals (3-7 years), usually between El Niño and La Niña phases (Table 2.1). In a normal year, the warm water that builds up in the Pacific Ocean is pushed westward by strong easterly trade winds, which causes the formation of rain in the North Western Pacific region. However, during El Niño this easterly wind becomes weakened, so it is unable to push the warm water westwards. The result is that the rain migrates eastwards, causing enhanced rainfall in the Eastern Pacific region and reduced rainfall in the Western Pacific. With respect to the Philippines, research has shown that El Niño years are, therefore, associated with an increased chance of drier conditions, with droughts and stresses on water resources and agriculture (Hilario *et al.*, 2009; Lyon and Camargo, 2009). In comparison, La Niña is associated with a change to wetter conditions and excessive rainfall (Hilario *et al.*, 2009).

Table 2.1: List of El Niño and La Niña events since 1950 as determined by the Oceanic Niño Index. A 3 month running mean SST anomaly was used for the region 5°N-5°S, 120°-170°W. They are defined as five consecutive 3 month periods greater than +0.5° for warm (El Niño) events and less than -0.5 for cold (La Niña) events. The threshold is further broken down into Weak (with a 0.5 to 0.9 SST anomaly), Moderate (1.0 to 1.4), Strong (1.5 to 1.9) and Very Strong (≥ 2.0) events (NOAA, 2019)

El Niño				La Niña		
Weak	Moderate	Strong	Very Strong	Weak	Moderate	Strong
1952-53	1951-52	1957-58	1982-83	1954-55	1955-59	1973-74
1953-54	1963-64	1965-66	1997-98	1964-65	1970-71	1975-76
1958-59	1968-69	1972-73	2015-16	1971-72	1995-96	1988-89
1969-70	1986-87	1987-88		1974-75	2011-12	1998-99
1976-77	1994-95	1991-92		1983-84		1999-00
1977-78	2002-03			1984-85		2007-08
1979-80	2009-10			2000-01		2010-11
2004-05				2005-06		
2006-07				2008-09		
2014-15				2016-17		
2018-19				2017-18		

El Niño events cause the trade winds to shift the West Pacific Warm Pool to the east, causing high sea surface temperatures (SSTs) in the eastern equatorial Pacific. The West Pacific Warm Pool is situated in the western Pacific Ocean and contains the warmest seawaters in the world ranging from 27-30 °C (Hansen *et al.,* 2006). Once the La Niña begins, the heat in the Pacific is drawn west by the North Equatorial Current recharging the warm pool. There is evidence that the extent and temperature of the warm pool varies on a decadal cycle (Partin *et al.*, 2007). Since these waters are warm enough to drive heat and moisture high into the atmosphere its position has a large influence on climate in the Philippines, particularly rainfall.

Table 2.2: List of typhoons that made land in southern Luzon, Philippines post 1950 (PAGASA, 2019). Most records prior to 1950 were unavailable

Date	Name	Category
1881	Haiphong Typhoon	Unknown strength
1950	Typhoon Fran	1
1951	Supertyphoon Iris	5
1951	Typhoon Lois	1
1952	Typhoon Trix	3
1953	Supertyphoon Betty	4
1956	Typhoon Karen-Lucille	2
1956	Supertyphoon Kit	5
1960	Typhoon Olive	1
1960	Typhoon Lola	1
1964	Typhoon Winnie	3
1964	Supertyphoon Cora	5
1964	Typhoon Elsie	3
1964	Supertyphoon Opal	5
1966	Typhoon Irma	4
1967	Supertyphoon Emma	5
1970	Supertyphoon Joan	5
1970	Supertyphoon Patsy	4
1971	Typhoon Dinah	2
1972	Typhoon Dan	1
1974	Typhoon Ivy	2
1974	Typhoon Alice	1
1975	Typhoon Olga	3
1975	Typhoon Ruby	4
1977	Typhoon Kim	4
1980	Typhoon Betty	4
1985	Supertyphoon Dot	5
1989	Typhoon Dan	1
1993	Typhoon Ira	4
1993	Typhoon Lola	3
2000	Typhoon Xangsane	2
2000	Typhoon Bebinca	2
2006	Typhoon Durian	4
2006	Tropical Xangsane	4
2008	Typhoon Fengshen	3
2013	Supertyphoon Yolanda	5
2014	Typhoon Glenda	5
2015	Typhoon Koppu	4
2015	Typhoon Melor	4
2016	Typhoon Sarika	4
2016	Typhoon Nock-ten	5
2017	Typhoon Doksuri	2
2019	Typhoon Kalmaegi	2
2019	Typhoon Kammiru	4
2020	Typhoon Vongfong	3
2020	Supertyphoon Goni	5
2020	Typhoon Vamco	4

ENSO dynamics influences large scale atmospheric circulation resulting in seasonal changes in the global climatic system. It also heavily influences the severity and frequency of tropical cyclones in the Philippines (Lyon and Camargo, 2009). During the summer of El Niño events, tropical cyclone activity is intensified in the West Pacific due to the increase of mid-level atmospheric moisture and sea surface temperatures, with the converse being seen during La Niña (Kubota and Chan, 2009; Lyon and Camargo, 2009). There are statistically significant differences between landfall rates in El Niño and La Niña years, with La Niña years being characterised by more intense typhoons making landfall on the island of Luzon (Saunders *et al.*, 2000; Elsner, 2003; Lyon and Camargo, 2009). Approximately 70% of the typhoons that formed over the north-west Pacific between 1945 and 2011 passed near to or over the Philippines (Table 2.3; Cinco *et al.*, 2016). In a typical year, 15 typhoons (or tropical cyclones) enter the Philippines with five to six making landfall (Table 2.3; Kubota and Chan, 2009).

2.4. Land Cover

The major feature of land cover change within the Philippines is the reduction of forest cover which has declined considerably from 17 million hectares in 1917, (>50% of the total land coverage in the Philippines), to 6.8 million hectares (27.8% of total land area) in 2018 (Philippine Statistical Authority, 2019). Of this, only 861,000 (12.7%) hectares is primary forest. This gives a current deforestation rate of c.2.1% per annum (USAID, 2017). The main cause of forest degradation in the Philippines is over-exploitation from logging, rapid population growth and increased conversion of forest land for agricultural, residential and commercial uses. However, despite the overall decline, there has been an improvement in recent years with an increase in forest cover from 22.0% of total land coverage (1990) to 27.8% (2018) (Philippine Statistical Authority, 2019), due to wide scale replanting efforts by local communities.

2.4.1. Agricultural shifts in the Philippines

The Philippines' main agricultural crops are corn, coconut, sugarcane, banana, and palay (rice), accounting for 75,349,464 (90.3 %) out of total of 83,462,550 metric tons produced in 2014 (Philippine Statistics Authority, 2015). Since 2010, the production of sugarcane, palay and corn has increased by 11.6%, 4.7% and 5.1% respectively. The high increase in yield for sugarcane is due to a more efficient use of fertiliser. Research by Jahn and Asio, (1998) found low concentrations of P in Philippine soils, so it is a limiting factor for terrestrial plants, highlighting the need for fertiliser use for agriculture. Coconut and banana production has declined by 1.3% and 0.5% respectively due to destruction of crops by Typhoon Yolanda

(2013; Table 2.2) and heatwaves (Philippine Statistics Authority, 2015). Even though production of these agricultural crops (sugarcane, palay and corn) has increased overall since 2010, the land used for agriculture has stabilised at 13.5 million hectares (c.25% of total land area) (Philippine Statistical Authority, 2019), highlighting the use of more efficient farming processes.

In Laguna province (where the study area is located; Figure 2.3) the top four crops produced (as of June 2018) were:

1. Palay – There has been an increase 1.3% per annum (since 2010) resulting in the production of 130,094 metric tons in 2014
2. Coconut - Annual decline in coconut production of 2.1% from 107,216 (2010) to 96,110 metric tons produced (2014)
3. Banana - Production has remained constant at 17,500 metric tons per annum since 2010
4. Corn - Production has remained constant at 2,700 metric tons per annum since 2010

The main agricultural crop in the study area is coconut (Figure 2.3) and its impacts will be investigated further in Chapters 5 and 6.

2.5. A brief history of San Pablo City

San Pablo city is the nearest urban centre to the study site and its development is important and directly impacts the Seven Lakes. The first historical evidence of the existence of San Pablo City, was the arrival of the Spanish in 1571 (Table 2.3). Between 1571 and 1815 trade was restricted by the Spanish, resulting in minimal exploitation of Philippine land for agriculture. This changed in 1834 with the lifting of trading restrictions. This culminated in wide scale conversion of land for agricultural purposes specifically tobacco, coconut, abaca and sugar, which further expanded after the opening of the Suez Canal in 1869.

The longest and most direct anthropogenic impacts on the study area have been from human settlements and agriculture, which began before the Spanish Colonisation in the 16th century (Table 2.3). Conversion of land around San Pablo City began in the 17th century for coconut (Migrino, 2017). It started as small scale deforestation until the conversion of San Pablo City into a ‘first class city’ in 1950, characterised by the building of many tourist and commercial properties.

*Table 2.3: Timeline of historical events that occurred in the Philippines from 1521 to 1950 *anthropogenic events post 1950 are discussed in Chapter 5 (Abinales and Amoroso, 2017)*

Year	Event
50,000 BC	Stone age settlements present
25,000-2,000 BC	Waves of migration of Negritos, Indonesians and Malays. They constructed large villages and used agriculture, mining, fishing and trading for their livelihoods.
1521	First Europeans reached the Philippines
1564	Discovery of a route to Mexico led to the development of the Manila Galleon Trade.
1565	The Philippines becomes a Spanish colony. The Spanish organised communities into towns under the rule of the Spanish colonial government and the Roman Catholic Church. The indigenous populations as a result were pushed into the mountains and more remote regions of the Philippines.
1571	Spanish arrived at Sampaloc (later renamed San Pablo City). Sampaloc was converted into a parish
1571- 1647	Sampaloc became a municipality and was renamed San Pablo City
17th Century	Conversion of land around San Pablo City for coconut plantations
1565-1815	Manila-Acapulco Galleon trade was the main source of income during this period. The Galleon trade brought silver from Mexico, which was used to purchase Asian goods. During this time there was little exploitation of indigenous material
1830	Port of Manila opened (restrictions lifted)
1834	Tariff free trade was formally recognised
Mid-19th Century onwards	Opening of the Philippines to trade
1869	Suez canal opened which allowed steamship service to be established between the Philippines and Europe
1872	Cavite mutiny, an uprising of 200 Filipino military personnel of Fort San Felipe. Believed to be the beginning of Filipino nationalism that would lead to the Philippine Revolution of 1896
1873	Additional ports opened in the Philippines for foreign commerce
1881- 1896	Civil and armed campaign for independence from Spain begins
1898	Spanish-American War is fought between April 21, 1898 – August 13, 1898 resulting in Philippines ceasing to be a Spanish colony
1899-1902	Philippine American War resulting in American colonisation. This led to the death of 200,000 Filipinos mostly from famine and disease The Laguna de Bay campaign (8th-17th April 1899) saw the Americans push into Laguna province
1901-1935	Insular Government. It was during this period that construction of highways/roadways were expanded in the Philippines
1941	Japan invades the Philippines. Battle of the Philippines (fought 8th December 1941 to 8th May 1942).
1944	US forces retake the Philippines
1944-onwards	Rehabilitation and construction of roads occurred after WW2 due to their destruction during the war
1946	The Philippines gains full independence
1950*	Conversion of San Pablo City into a 'first class city'

2.6. Study Lakes - The Seven Lakes of San Pablo

The study area, the Seven Lakes of San Pablo, consists of seven circular, freshwater volcanic crater lakes (Lakes Bunot, Sampaloc, Calibato, Palakpakin, Mohicap, Yambo and Pandin) situated in the southernmost region of Laguna, on the island of Luzon, Philippines (Figure 2.3). Lake Calibato was not analysed for this thesis. The study lakes were formed by a phreatic eruption, when shallow lava from Mount San Cristabel reacted with groundwater, causing the formation of craters which then in-filled with water over 700 years ago (LLDA, 2009). The nearest urban centre to the study area is San Pablo City with roughly 9.2 per cent of Laguna's population residing here (NSO, 2013). The city covers an area of 200 km^2, with a population of c. 266,000 (2015) (Philippines Statistic Institute, 2017) (Figure 2.3). Built up areas steadily

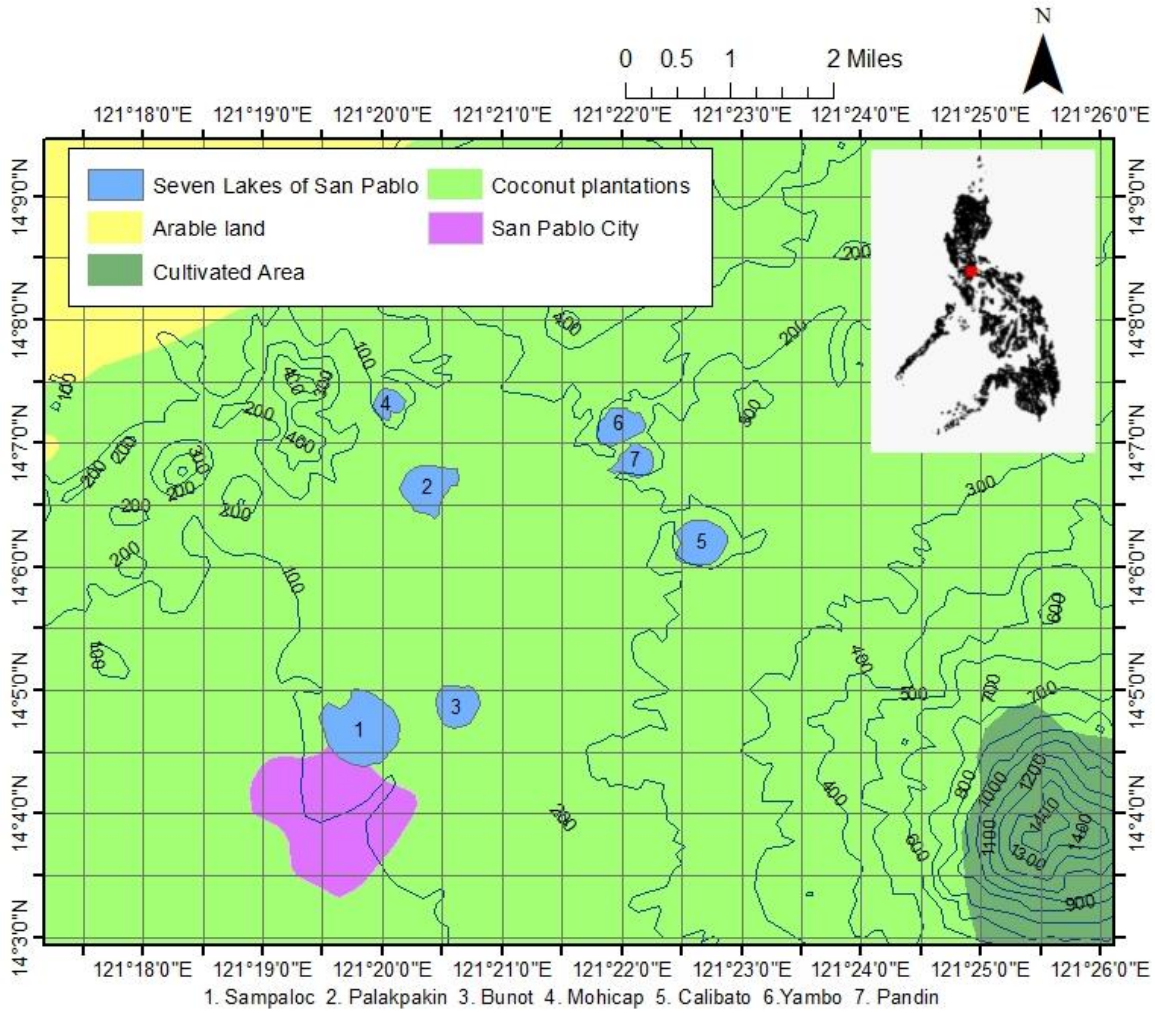


Figure 2.3: Map of the study area (Seven Lakes of San Pablo) with the main land uses highlighted. Insert shows the location of the study area in relation to the remainder of the Philippines. Geographic coordinate system:GCS_WGS_1984, Data source: Arc GIS Open Data, ESRI

increased between 1988 and 2010 in San Pablo City (Quintal *et al.*, 2018). Since 2003, much of the area north-east of the city centre around Lakes Mohicap, Bunot and Palakpakin has also been urbanised. Coconut (*Cocos nucifera L.*) is the main crop in Laguna province and was introduced around San Pablo, towards the end of the 17th century (Migrino, 2017) (Figure 2.3). The local population uses the Seven Lakes of San Pablo for a variety of social and economic activities, which have substantially impacted on water quality and the ecological integrity of the lakes (Brillo, 2015a). Enhanced nutrient input from anthropogenic activities has led to problems such as eutrophication and HABs on all of the lakes. This has resulted in six out of the seven lakes being classified as threatened (moderate pollution, sedimentation and ecological stress) (DENR-ENB, 2014) with Lake Yambo not yet classified (Table 2.4).

2.6.1. Aquaculture in the Seven Lakes of San Pablo

Aquaculture was first introduced to Lake Bunot in 1976 before spreading to the remainder of the Seven Lakes over the next 13 years (Figure 2.4). Tilapia (*Oreochromis niloticus*) is the main species farmed in the Seven Lakes with the use of floating cages, due to its adaptability and its relative resistance to poor water quality and disease (FAO, 2016). Cages are constructed of bamboo and empty oil drums for buoyancy (Figure 2.10). Each lake has been impacted to a different extent, with Lakes Palakpakin, Bunot and Sampaloc considered the most polluted and Lake Pandin the most pristine (Brillo, 2015a, 2016c). This is due to a number of factors such as location and the extent to which they are utilised for aquaculture and other human activities (Table 2.4). All lakes have experienced algal blooms, fish kills and a deterioration in water quality which the fisherman believe is linked to aquaculture (see appendix A). Concerns over the management of the lake bodies led to the establishment, in 1975, of the Laguna Lake Development Authority (LLDA), a specialised management body for the Seven Lakes of San Pablo, Laguna de Bay and Lake Taal. The LLDA is responsible for carrying out environmental management by preventing undue ecological deterioration of the water bodies and promoting balanced growth within the Laguna region (LLDA, 2009). The LLDA is also responsible for issuing permits and managing the aquaculture resources of the Seven Lakes (LLDA, 2009). The LLDA has made some attempt to mitigate these problems by limiting aquaculture to 10 % of the lake surface area (RA 8550 Fishery Code). In reality, however, this has not been enforced due to the large number of illegal fish farms in operation. The deterioration in water quality led to the LLDA undertaking monthly water quality monitoring in all lakes from 1996 to 2016 (this is discussed in further detail in Chapter 4).

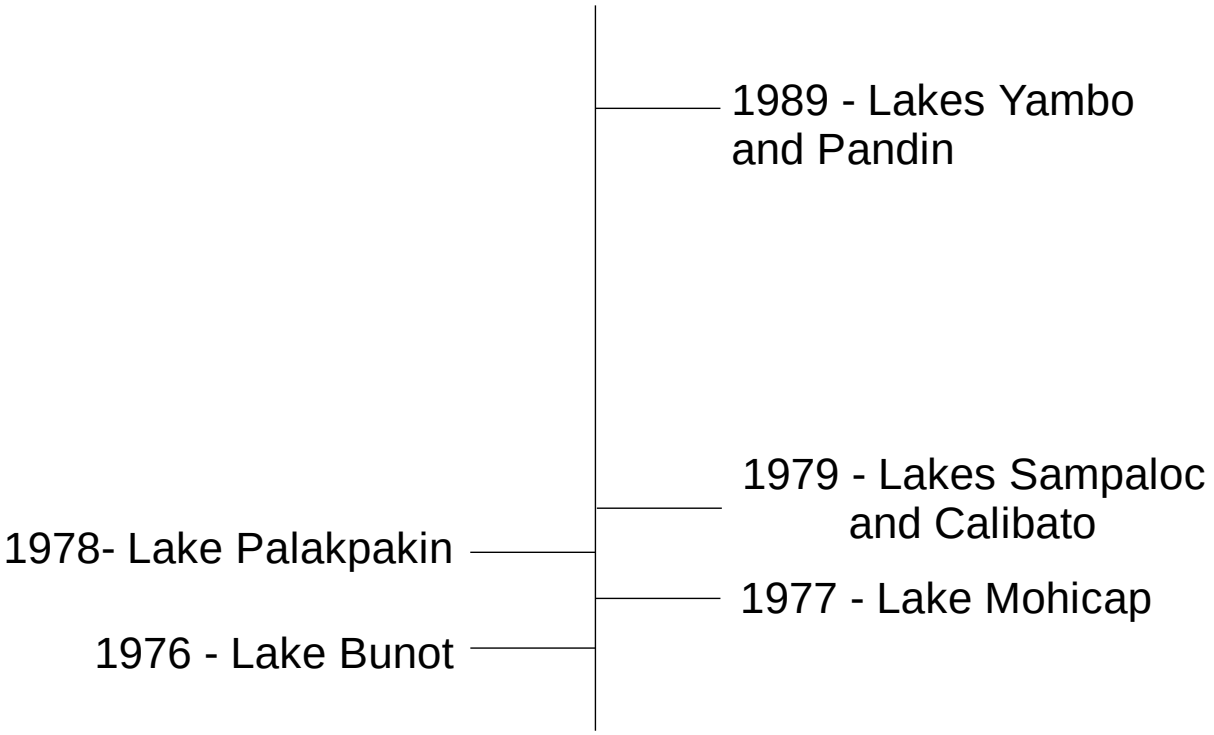


Figure 2.4: Railway plot showing the different years that aquaculture was introduced to the Seven Lakes of San Pablo

Table 2.4: Main morphological characteristics of the Seven lakes of San Pablo (Brillo, 2015; 2016a, 2016b, 2016c; 2016d, 2016e, 2016f). The information from Lake Calibato was not included due to lack of data
**As determined by the LLDA (LLDA, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2009)*

Lake	Maximum Depth (m)	Surface Area (ha)	Elevation above sea level (m)	Inflows	Outflows	Services	Condition of the lake*
Pandin	62	20.5	207	Precipitation Surface Runoff Underwater Springs	Seepage Evaporation Prinsa Creek	Aquaculture (sparsely) Tourism	Threatened
Yambo	27	30.5	207	Precipitation Surface Runoff Kali-e Creek	Seepage Evaporation	Aquaculture (sparsely) Tourism	Not Classified
Mohicap	30	23	80	Precipitation Surface Runoff Underwater Springs	Seepage Evaporation Pagbuga stream	Aquaculture (low levels) Tourism	Threatened
Sampaloc	27	104	106	Precipitation Surface Runoff Underwater Springs	Seepage Evaporation Sabang Creek	Aquaculture Tourism Settlements	Threatened
Bunot	23	30.5	110	Precipitation Surface Runoff Underwater Springs	Evaporation Seepage Sabang Creek	Aquaculture Settlements	Threatened
Palakpakin	7.5	43	100	Precipitation Surface Runoff Underwater Springs Pagbuga Stream	Seepage Evaporation Padparan Stream	Aquaculture Settlements	Threatened

2.6.2. Justification for the choice of the Seven Lakes of San Pablo as the study area

One of the major challenges of this research lies in distinguishing whether lake ecological changes were initiated by aquaculture or by the numerous other environmental perturbations that exist in this region (e.g. nitrogen deposition, climate change including extreme events, volcanic eruptions, mining and land use conversion). The Seven Lakes of San Pablo are ideal for this study as the impacts of many of these regional perturbations are to some extent mitigated by the fact that the lakes are within a small geographical area (<16 km^2). In addition, these lakes have different timings and intensities of aquaculture along a disturbance gradient. They range from 'more developed' lakes such as Lakes Palakpakin and Bunot to the 'pristine' lakes Yambo and Pandin, and as such offer the potential to help to identify, isolate and quantify whether changes in the proxy record were driven by aquaculture or other stressors. In addition, the Seven Lakes are a priority for research in the Philippines, and are designated a key biodiversity area. Their importance comes from the fact they are a critical resource, in terms of their biodiversity, in supporting the livelihoods of local communities and more widely contributing to the country's economy. This research adds to the understanding of how increasing anthropogenic pressures (including aquaculture), have jeopardised the delivery of vital ecosystem services, to aid the development of mitigation strategies and sustainable policies.

2.6.3. Individual Lake Descriptions

2.6.3.1. Lakes Yambo and Pandin

The twin lakes of Yambo (14.1160° N, 121.3670° E) and Pandin (14.1160° N, 121.3670° E) are the most remote and considered the most 'pristine' (Figure 2.5). These lakes have the highest elevation of (the Seven Lakes) of 207 m (Table 2.4). Lake Yambo is the only lake without an in/outflow. It is fed by natural springs and, consequently, its surface area can vary substantially, depending on the season (Figure 2.5; Brillo, 2016c). Both lakes have been utilised for aquaculture, however, its spread is limited. This is because both lakes are oligotrophic (nutrient poor, low OM content, high dissolved oxygen (DO) in the hypolimnion) and so have low phytoplankton biomass (Brillo, 2016). This results in a prolonged culture period for fish stocks, making fish farming less financially viable (due to the cost of additional fish food). The number of fish cages in Lake Yambo reached their peak of 10% of the lake's surface area, in the 1990s, but since then cage numbers have decreased to only three active cages in 2012 (Brillo, 2016c). Cage numbers in Lake Pandin never reached the 10% limit and, as of 2005, only 3% of the lake was occupied by fish cages (LLDA, 2006d). Lake Pandin has had a substantial tourism industry since the mid-2000s, run by a local community group, which uses hand pulled rafts to ferry tourists across the lakes (Figure 2.6). Tourism did not begin until 2018 in Lake Yambo.

Lakes Yambo (top), GR: 14.1160° N, 121.3670° E
Pandin (bottom), GR: 14.1160° N, 121.3670° E

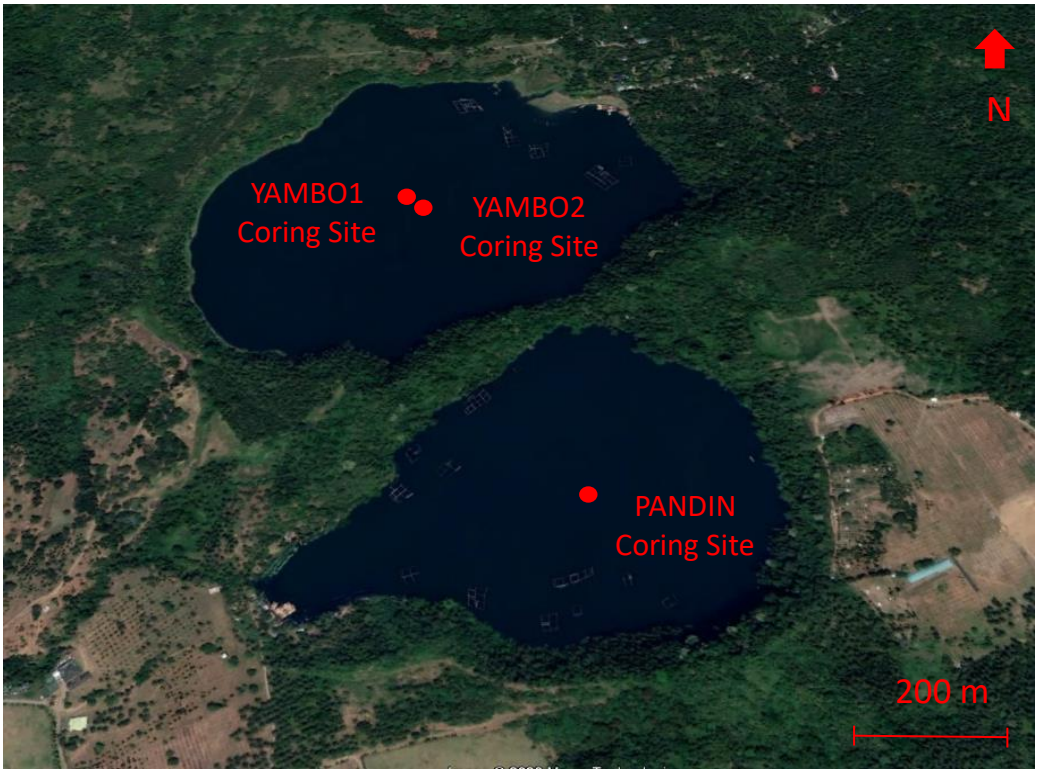


Figure 2.5: Map of Lakes Yambo and Pandin showing coring location (Adapted from Google Earth imagery, 2019)

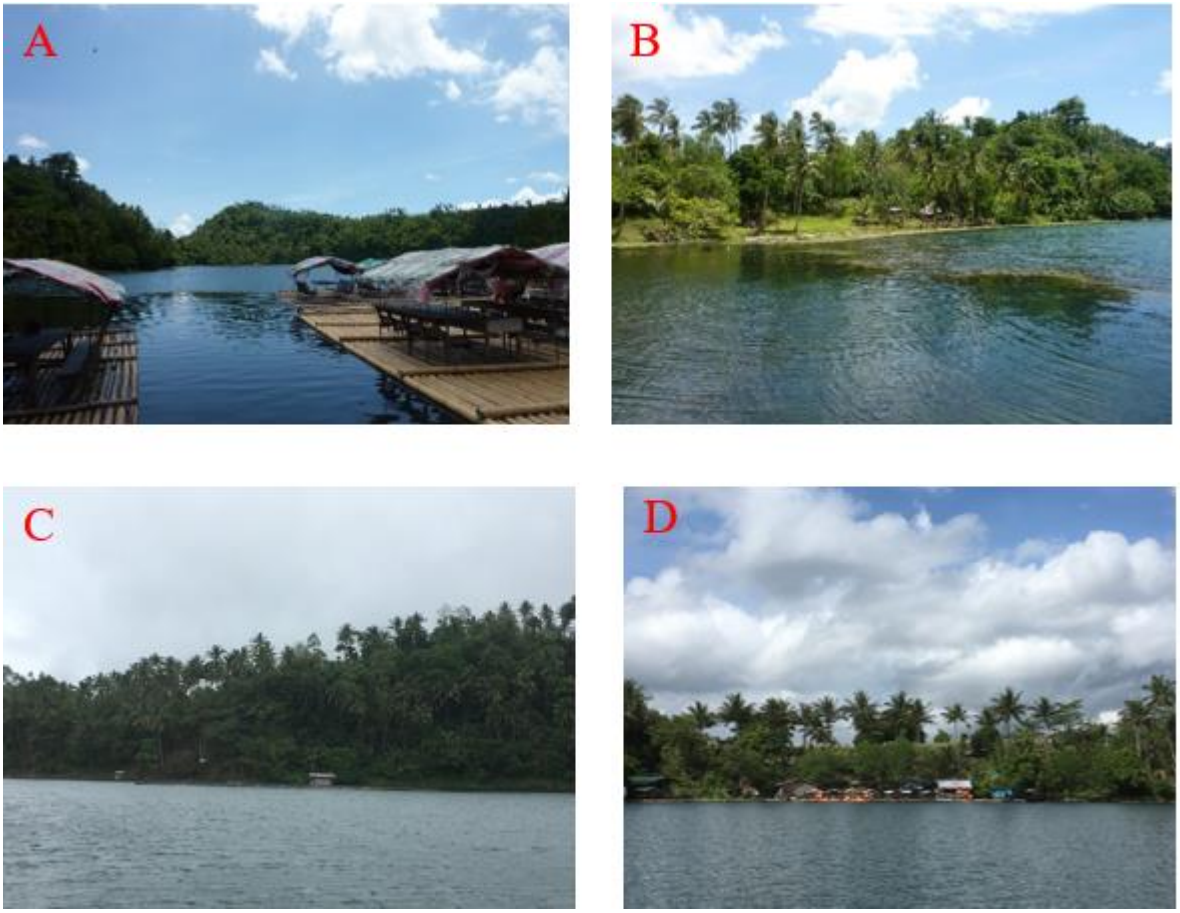


Figure 2.6: Photographs of Lakes Yambo and Pandin

A. *Bamboo rafts used for tourism at Lake Pandin*
B. *Coconut plantations at Lake Pandin*
C. *Coconut plantations at Lake Yambo*
D. *Bamboo rafts used for tourism at Lake Yambo with coconut plantations in the background*

2.6.3.2. Lake Mohicap

Lake Mohicap (Figure 2.7; 14.1033° N, 121.3775° E) is the second smallest of the study lakes with a surface area of just 23 hectares (Table 2.4). Even though it has a low number of illegal settlements (due to the steep slopes of the catchment making it unfavourable for building), and a limited number of fish cages (Figure 2.8), it is still a relatively polluted lake (Brillo, 2015a). Despite having fewer eutrophication pressures than the other lakes, it has still experienced fish kills (as reported by the fisherman and the LLDA). To prevent further degradation of the water quality, there has been a reduction in aquaculture cage numbers, to allow for the development of tourism (which began in 2018), which, is seen as a more viable financial to aquaculture (Fisherman, pers comm). The high pollution in this lake could be a result of a delayed reaction to the decline in fish cages, the problem of surface runoff from the steep catchment and/or the lack sewage systems for the settlements (Brillo, 2015a).

Lake Mohicap, GR:14.1033° N, 121.3775° E



Figure 2.7: Map of Lake Mohicap showing coring locations (Adapted from Google Earth imagery, 2019)



Figure 2.8: Photographs of Lake Mohicap

A. *Fish cages with small huts. These huts are used as temporary homes for fishermen*
B. *Algal Bloom*
C. *Example of the settlements (laundry house) that surround the lake*
D. *Example of natural forests in the lake catchment*

2.6.3.3. Lake Sampaloc

Lake Sampaloc (Figure 2.9; 14.0791° N, 121.3299° E) is located within the urban centre of San Pablo City. This lake has the largest surface area out of the study lakes (104 hectares) and is considered one of the most developed and polluted (Brillo, 2016a). A number of structures, such as a boardwalk around the lake, houses and restaurants, were constructed in the catchment in the 1980-1990s (Figure 2.10; Brillo, 2016a). In addition, Lake Sampaloc is used for aquaculture, with 232 fish pens/cage operators occupying 16.4% of the lake in 2004 (Jose, 2005; Brillo, 2016a). The addition of fish farm waste, excessive use of fish feed and effluents from the city drainage system has caused the lake to become eutrophic with a deterioration in water quality (Brillo, 2016a). Increased nutrients led to a rise in excessive HABs and water hyacinth proliferations (covering almost 60% of the lake), fish kills, foul smelling water and inhibited growth in the tilapia population farmed in the fish cages (Laquinon, 1997; Brillo, 2016a).

Lake Sampaloc, GR: 14.0791° N, 121.3299° E



Figure 2.9: Map of Lake Sampaloc showing coring locations (Adapted from Google Earth imagery, 2019)

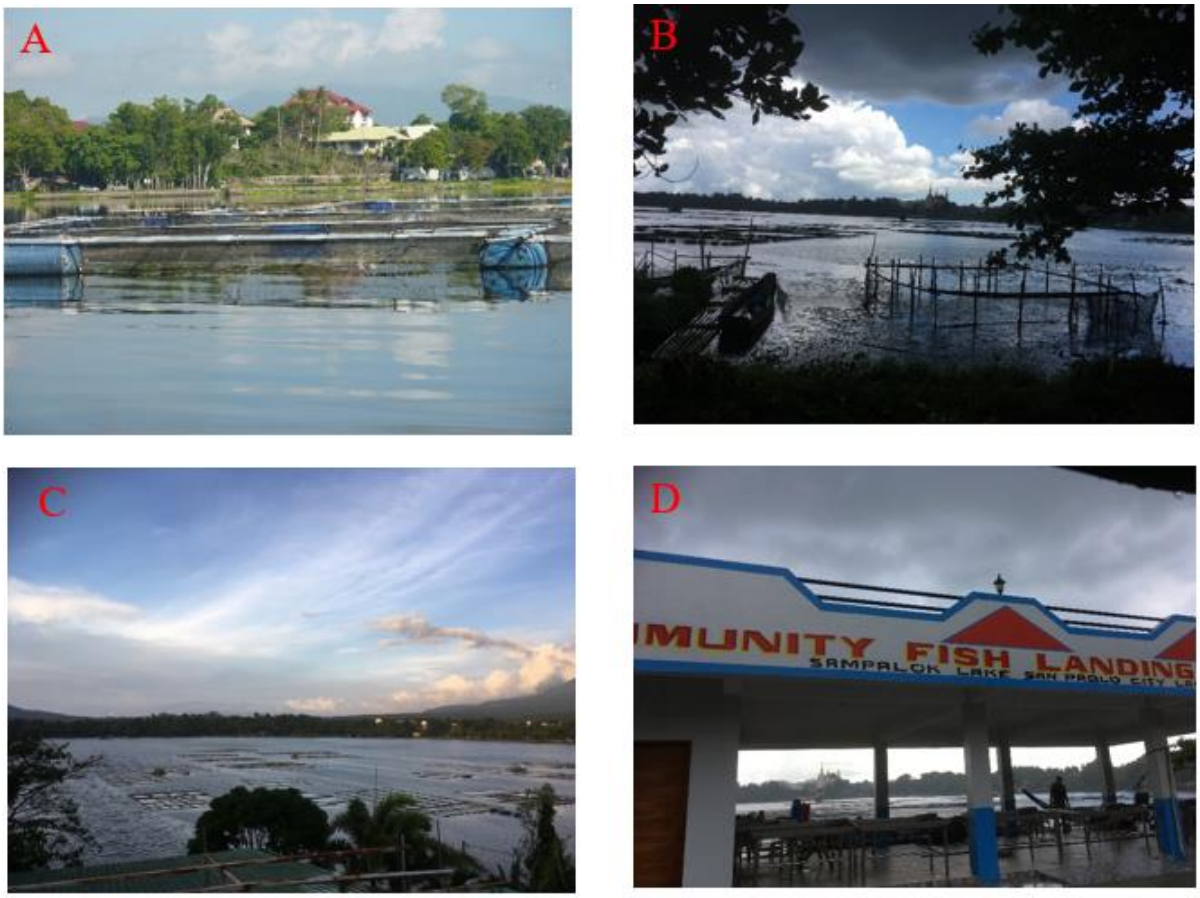


Figure 2.10: Photographs of Lake Sampaloc

A. *Fish cages built with bamboo, netting and oil drums to keep the cages afloat*
B. *Algal blooms visible in/around the fish cage*
C. *Fish cages*
D. *Example of the concreted lake catchment. Newly built community centre, not yet operational (April 2018)*

2.6.3.4. Lake Bunot

Lake Bunot (Figure 2.11; 14.0822° N, 121.3442° E) is the second closest lake to the urban centre of San Pablo (Table 2.4). Aquaculture expanded rapidly after its introduction in 1976, resulting in this lake having the second highest aquaculture intensity of all the cored lakes. As of 2006, 183 fish pens/cages were operational, covering 30.6% of the lake (Figure 2.12; Brillo, 2015b). This has resulted in the building of many illegal settlements, with two-thirds of the lake shore now occupied by structures (Figure 2.12; Brillo, 2015b). It the most congested of the study lakes and a dumping ground for human-related waste (Yap, 2004). A lack of drainage and sewage systems around Lake Bunot means waste is discharged directly into the lake.

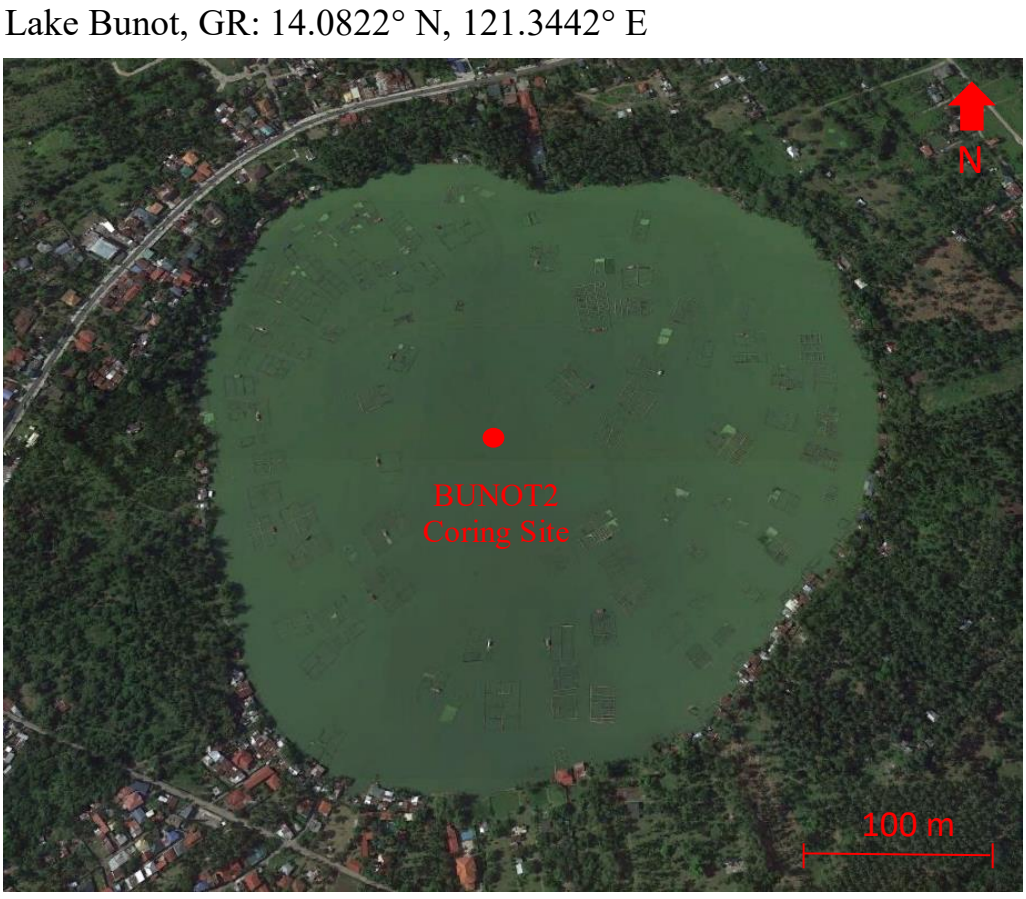


Figure 2.11: Map of Lake Bunot showing coring location. The green colour of the lake water suggests extension algal blooms (Adapted from Google Earth imagery, 2019)



Figure 2.12: Photographs of Lake Bunot:

A. *Fish cages. These are constructed using bamboo and netting*
B. *Eichornia spp. (water hyacinth), the dominant aquatic macrophyte in the lake. Mount San Cristobal is visible in the background*
C. *Example of the settlements that surround the lake*
D. *Coconut plantations*

2.6.3.5. Lake Palakpakin

Lake Palakpakin (Figure 2.13; 14.1113° N, 121.3384° E) is the shallowest (only 7.5 m deep) lake (Table 2.4). It is riverine system as a result of a strong current flowing in from the Pagbuga Stream and out via the Padparan Stream (with these two streams being more substantial in size than the in/outlets for the other lakes; Brillo, 2016b). The Pagbuga Stream connects Lake Palakpakin with Lake Pandin (which is upstream). Lake Palakpakin has short water residence times due to the strong water flow, allowing the lake to flush out toxins and pollutants quickly (Brillo, 2016b). The strong inflowing current has also led to the accumulation of soil and other particles, causing shallowing of the lake and the inlet (Caasi *et al.*, 2006). In the long term this could lead a shortening of the lake's life span and make the adjacent low lying areas susceptible to flooding (Brillo, 2016). This lake is primarily used for aquaculture (Figure 2.14), with 18% of the total surface area in 2008 being utilised for cages (Brillo, 2016b). The increase in fish farming in the last 30 years has caused an expansion in illegal settlements, largely on the northern tip of the lake (Figure 2.14). The lack of a sewage system means the majority of waste from the settlements (and the village) is directly discharged into the lake. The high volume of aquaculture has resulted in this lake having the highest aquaculture intensity out of all the cored lakes.

Lake Palakpakin, GR: 14.1113° N, 121.3384° E

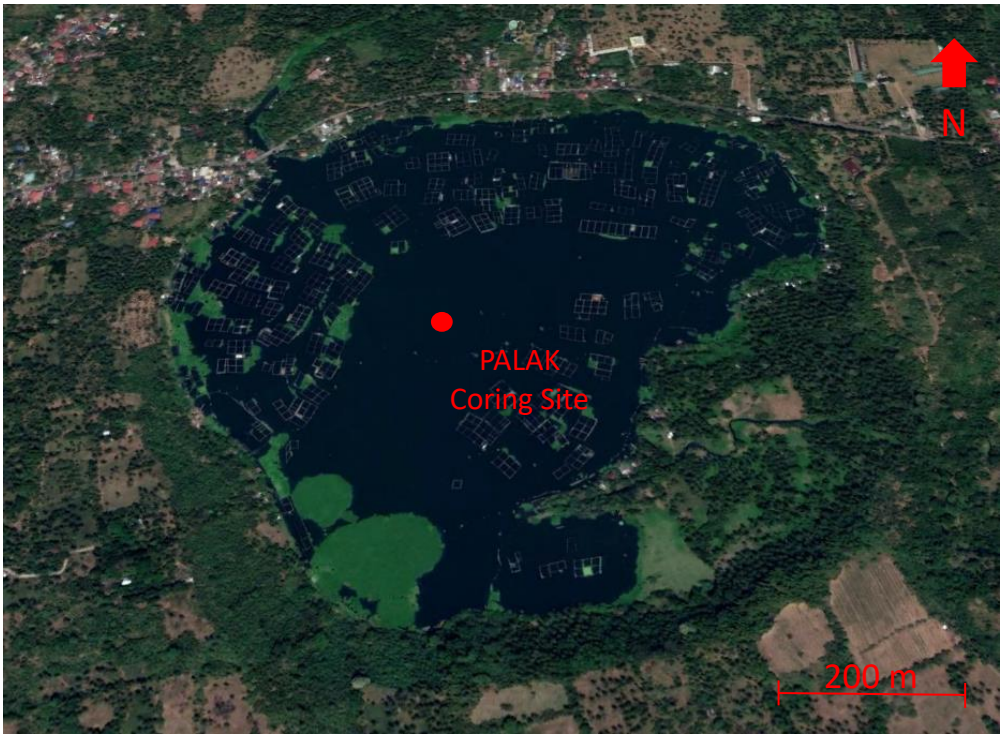


Figure 2.13: Map of Lake Palakpakin showing coring location. The green areas of the lake water suggests extensive algal blooms (Adapted from Google Earth imagery, 2019)

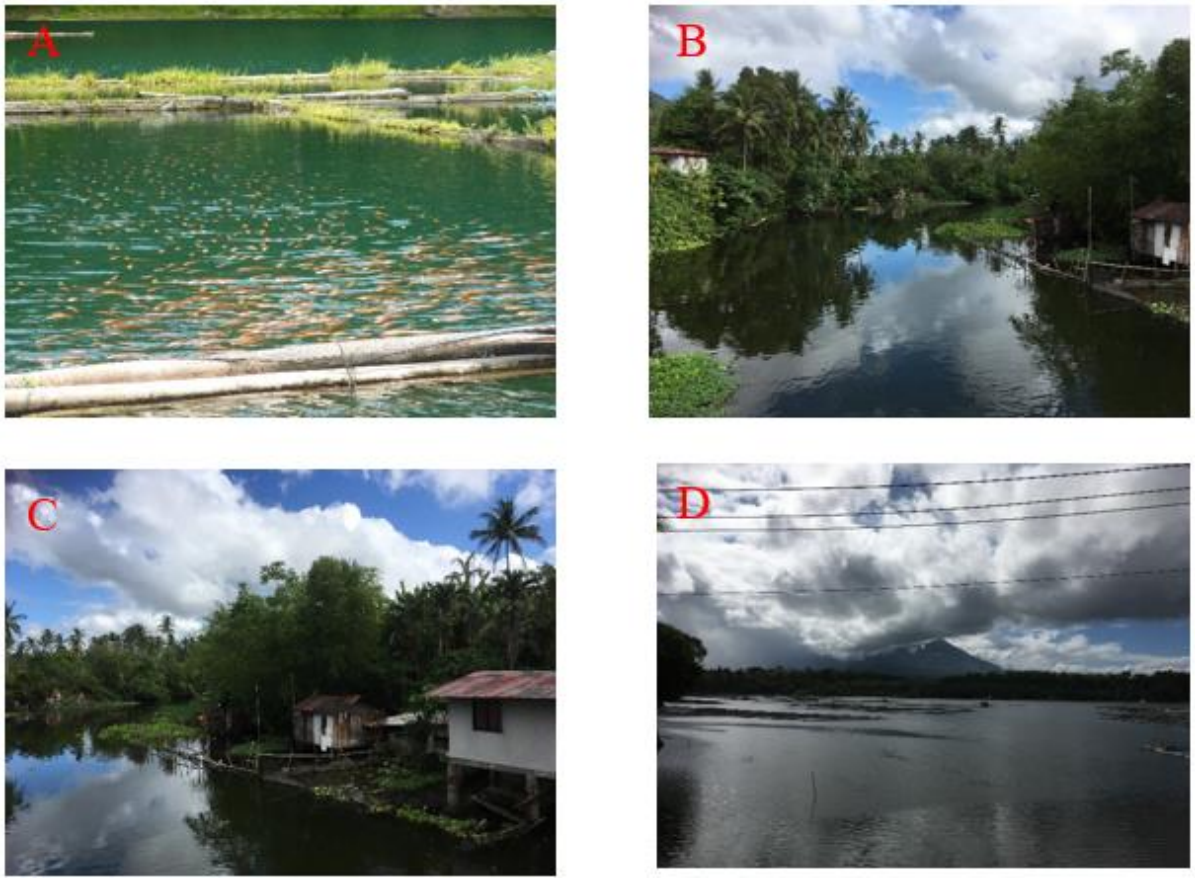


Figure 2.14: Photographs of Lake Palakpakin

A. *Tilapia in a fish cage*
B. *Pagbuga Stream (an inflow of Lake Palakpakin) surrounded by settlement. Coconut plantations are visible in the background*
C. *Example of the settlements that surround the lake*
D. *Fish cages with Mount San Cristobel in the background*

2.7. Previous Studies on the Seven Lakes

Limited research on the Seven Lakes has indicated a deterioration in water quality, which is related to the level of anthropogenic disturbance (Santiago and Arcilla, 1993; Zafaralla, 2010; Bannister *et al.*, 2019). The most impacted lakes (Bunot, Sampaloc and Palakpakin), have shown a deterioration in water quality as a result of aquaculture (and other anthropogenic drivers) resulting in eutrophication, fish kills, algal blooms and hyacinth proliferation in the last few years (Santiago and Arcilla, 1993; LLDA, 2006b, 2006c, 2006d, 2006e, 2006f, 2006a, 2009; Zafaralla, 2010; Solpico *et al.*, 2014). In Lake Mohicap, Cordero and Baldia (2015) found that aquaculture was causing a shift in phytoplankton communities (to a cyanobacteria dominated system) and a deterioration in water quality to a critical level (where a reversal back to the non-eutrophic state would not be possible). Studies suggest that even the most 'pristine' lakes (Lakes Yambo and Pandin) are susceptible to both anthropogenic (e.g. aquaculture) and climatic impacts (Zafaralla, 2010; Bannister *et al.*, 2019). These studies concluded that there was a greater tendency for algal blooms to form in lakes used for aquaculture even if only at low intensities.

In the last few years the publication of the first two palaeolimnological studies highlighted the important ecosystem services that the Seven Lakes provide and the sensitivity of tropical freshwater systems to disturbance pressures (Legaspi *et al.*, 2015; Bannister *et al.*, 2019). Legaspi *et al.*, (2015) was the first low resolution palaeolimnological study completed on Lake Mohicap (or any of the Seven Lakes) using diatoms, stable isotopes ($\delta^{13}C$), C/N ratios and photosynthetic pigments. This study showed that benthic diatom communities had disappeared in the youngest sediments due to high productivity in the epilimnion resulting in the shading of benthic habitats. This was examined in detail in Bannister *et al.*, (2019); the first high resolution palaeolimnological study of Lakes Sampaloc, Mohicap and Yambo. This study found that even though the lakes have differing intensities of aquaculture they all followed a common trajectory and timing of a potential regime shift, characterised by the replacement of *Fragilaria crotonensis* Kitton 1869 with *Aulacoseira granulata* (Ehrenb.) Simonsen and *Discostella* complex (Cleve and Grunow) Houk and Klee 2004.

3. CHAPTER 3 - MATERIALS AND METHODS – FIELDWORK, LABORATORY WORK AND STATISTICAL ANALYSES

3.1. Sample Collection

3.1.1. Introduction

It is important to develop a robust methodology that can effectively answer the aims of this research. The individual techniques chosen, need to complement each other to help disentangle the influence of various drivers that impact the study lakes. The methods described below have been selected to provide the data needed for the two approaches (see section 1.6), to determine if increased aquaculture intensity resulted in a proportional change in the proxy record.

3.1.2. Fieldwork - Sample collection

Two fieldwork excursions were carried out to the study area, the Seven Lakes of San Pablo (Lakes Mohicap, Sampaloc, Palakpakin, Bunot, Calibato, Yambo and Pandin), southern Luzon, Philippines. A single sediment core was collected (using a UWITEC (gravity) corer) from Lakes Sampaloc, Palakpakin, Bunot, and Mohicap in May 2017. Equipment malfunction of the gravity corer necessitated a second fieldwork excursion in April 2018 to collect the remaining cores from Lakes Yambo and Pandin (Table 3.1). No sediment core or water samples were collected from Lake Calibato due to equipment malfunction. A boat was used to collect a sediment core from the deepest part of each lake, identified using a Plastimo echotest II echo sounder and recorded using a Garmin etrex 10 GPS. Immediately after collection at the field site, core lengths were measured and descriptions taken, noting any colour and lithological changes and the presence of macrofossils. The cores were sectioned into 0.5 cm increments for the top 30 cm of the core and 1.0 cm increments for the remainder, with further subsampling (of approximately a third of the sample) into a separate sampling bag for pigment analysis. Due to a lack of sufficient freezer facilities on site, all samples were stored in air tight black bags in the fridge at 4°C to prevent deterioration. Once transported to the UK, samples for pigment analysis, were stored in the freezer at -20°C and the remainder stored in the refrigerator at 4 °C, to prevent deterioration. All laboratory work was completed at the University of Nottingham, the British Geological Survey and the Environmental Radiometric Facility, UCL.

3.1.3. Sampling of plants, soils and fish food

A variety of contemporary samples were collected from each lake (Table 3.2) for stable isotope analysis. Since identical products of ‘starter’ and ‘finisher’ fish food (Cutty Sark Fish Food) are used at all the lakes, only one sample of each was collected. The change from starter to finisher fish food occurs when the fish are 4 months old. Littoral mud samples were collected

from the study lakes, except Lake Sampaloc, by scooping a handful of mud into a labelled sample bag. At Lake Sampaloc, the construction of a raised promenade around the lake perimeter prevented sample collection. Samples were collected from the two most abundant aquatic plant species at each lake *Eichornia* spp. (water hyacinth) and *Pistia* spp. (water cabbage), determined by visual observation. In addition, terrestrial plant and bark samples were collected from coconut (*Cocos nucifera*) and banana (*Musa* spp.) trees found within the lake catchments. After drying at room temperature for three days, the plant samples were stored in a fridge at 4°C to prevent decomposition.

Table 3.1: Inventory of all sediment cores used in this thesis including date of collection and the team involved
**These sediment cores were used to cross correlate ^{210}Pb dates to the respective cores from the same lake*
UoN-University of Nottingham, UK
NUS- National University of Singapore, Singapore
UST-University of San Tomas, Manila, Philippines

Sediment Core Name	Lake	Sample Location	Date of Collection	Team involved with Collection
SAMP1*	Sampaloc	14°04′44″ N 121°19′48″ E	June 2015	NUS, UST
SAMP2	Sampaloc	14°04’44.90’’N 121°19’47.44’’E	May 2017	Author, UoN, NUS, UST
MOH1*	Mohicap	14°07′19″ N 121°20′03″ E	June 2015	NUS, UST
MOH2	Mohicap	14° 07'20.43"N 121°20'03.37"E	May 2017	Author, UoN, NUS, UST
YAMBO1*	Yambo	14°07.148' N 121°22.108' E	June 2016	NUS, UST
YAMBO2	Yambo	14° 07'08.32"N 121°21'58.64"E	April 2018	Author, UST
BUNOT2	Bunot	14°04’51.15’’N 121°20’36.95’’E	May 2017	Author , UoN, NUS, UST
PALAK	Palakpakin	14°06’37.63’’N 121°20’21.44’’E	May 2017	Author, UoN, NUS, UST
PANDIN	Pandin	14°06'51.30"N 121°22'05.20"E	April 2018	Author , UST

Table 3.2: List of the contemporary samples collected from the study lakes with the date of collection and sampling location shown

Lake	Sample Type	Sample Location		Date of Collection
Sampaloc	Water Cabbage (*Pistia* spp.)	14°04’45.10’’N	121°19’31.88’’E	May 2017
	Water Hyacinth (*Eichornia* spp.)	14°04’45.10’’N	121°19’31.88’’E	May 2017
	Coconut Leaf (*Cocos nucifera*)	14°04’45.10’’N	121°19’31.88’’E	April 2018
	Coconut Bark (*Cocos nucifera*)	14°04’45.10’’N	121°19’31.88’’E	April 2018
Palakpakin	Water Hyacinth (*Eichornia* spp.)	14° 06'39.57"N	121°20'17.40" E	May 2017
	Coconut Bark (*Cocos nucifera*)	14° 06'44.48"N	121°20'11.23"E	April 2018
	Coconut Leaf (*Cocos nucifera*)	14° 06'44.48"N	121°20'11.23"E	April 2018
	Grass	14° 06'44.48"N	121°20'11.23"E	April 2018
	Water Cabbage (*Pistia* spp.)	14° 06'41.32"N	121°20'15.28"E	April 2018
Bunot	Water Hyacinth (*Eichornia* spp.)	14°04’43.76’’N	121°20’34.57’’E	May 2017
	Water Cabbage (*Pistia* spp.)	14°04’43.76’’N	121°20’34.57’’E	May 2017
	Grass	14°04’40.74’’N	121°20’32.72’’E	April 2018
	Littoral Mud	14°04’40.74’’N	121°20’32.72’’E	April 2018
	Coconut Leaf (*Cocos nucifera*)	14°04’40.74’’N	121°20’32.72’’E	April 2018
	Coconut Bark (*Cocos nucifera*)	14°04’40.74’’N	121°20’32.72’’E	April 2018
	Water Cabbage (*Pistia* spp.)	14°04’40.74’’N	121°20’32.72’’E	April 2018
	Banana Bark (*Musa* spp.)	14°04’40.74’’N	121°20’32.72’’E	April 2018
	Banana Leaf (*Musa* spp.)	14°04’40.74’’N	121°20’32.72’’E	April 2018
Mohicap	Water Hyacinth (*Eichornia* spp.)	14° 07'22.76"N	121°20'07.26"E	May 2017
	Water Cabbage (*Pistia* spp.)	14° 07'23.24"N	121°20'09.86"E	May 2017
	Coconut Leaf (*Cocos nucifera*)	14° 07'23.26"N	121°20'10.65"E	April 2018
	Coconut Bark (*Cocos nucifera*)	14° 07'23.26"N	121°20'10.65"E	April 2018
	Grass	14° 07'23.26"N	121°20'10.65"E	April 2018
	Littoral Mud	14° 07'23.26"N	121°20'10.65"E	April 2018
	Water Cabbage (*Pistia* spp.)	14° 07'23.24"N	121°20'09.86"E	April 2018
Pandin	Banana Leaf	14° 06'44.52"N	121°21'54.33"E	April 2018
	Coconut Bark (*Cocos nucifera*)	14° 06'45.20"N	121°21'53.47"E	April 2018
	Coconut Leaf (*Cocos nucifera*)	14° 06'45.20"N	121°21'53.47"E	April 2018
	Grass	14° 06'45.20"N	121°21'53.47"E	April 2018
	Littoral Mud 1	14° 06'45.20"N	121°21'53.47"E	April 2018
	Littoral Mud 2	14° 06'44.52"N	121°21'54.33"E	April 2018
	Aquatic Macrophyte	14° 06'45.94"N	121°21'53.72"E	April 2018
	Water Cabbage (*Pistia* spp.)	14° 06'46.18"N	121°21'53.17"E	April 2018
Yambo	Coconut Bark (*Cocos nucifera*)	14° 07'15.08"N	121°22'10.27"E	April 2018
	Coconut Leaf (*Cocos nucifera*)	14° 07'15.08"N	121°22'10.27"E	April 2018
	Grass	14° 07'16.31"N	121°22'09.08"E	April 2018
	Littoral Mud	14° 07'16.31"N	121°22'09.08"E	April 2018
	Littoral Mud- 1m from shore	14° 07'16.04"N	121°22'08.66"E	April 2018
	Littoral Mud- away from tourist area	14° 07'15.96"N	121°22'05.12"E	April 2018
	Banana Leaf (*Musa* spp.)	14° 07'13.11"N	121°22'11.15"E	April 2018
N/A	Starter Fish Food	Sampaloc		May 2017
N/A	Finisher Fish Food	Sampaloc		May 2017

3.1.4. Water quality sampling

Measurements of the physicochemical parameters of the water column were taken at the same locations as the core collections. A YSI EXO1 multi-probe was used to collect pH, conductivity, chlorophyll *a*, DO and temperature data. To do this the multi-probe was slowly lowered over the side of the boat and a measurement taken every second. Water clarity was estimated using a Secchi Disk. Light intensity readings were collected using a LI Cor Sensor Light meter LI192, at 1 m intervals down the water column.

To calculate the euphotic depth (the depth at which 1% of incident irradiance is available to photosynthetic organisms), the following equation (equation 1) was used as per Degefu *et al.*, (2014):

Equation 1: $$(\text{Ɛ} = (\ln I_o - \ln I_z)/Z)$$

Vertical light extinction coefficient (Ɛ, m^{-1})

I_o is surface light irradiance

I_z is light irradiance at a certain depth Z, and Z is depth

$$\text{Euphotic depth (Zeu)} = 4.6/\text{Ɛ}$$

3.1.5. Water samples

Water samples were collected from the surface, mid and bottom depths (Table 3.3) of each lake, using a Van Dorn sampler. Acid-washed Nalgene bottles were used to collect 2 litres of water from each depth. Of this, 125 ml of unfiltered water was decanted into a Nalgene bottle for alkalinity, 250 ml of filtered water was retained for major ion, silicate, soluble reactive phosphorus (SRP) and ammonium analysis. Finally, 25 ml of unfiltered water was acidified with 1.5ml of 0.5 mol sulphuric acid for total phosphorus (TP) analysis.

A measured volume of water was filtered using GF/F Whatman glass-fibre filters (0.45µm) and a hand pump filtration unit, to collect sufficient filtrate for chlorophyll *a*, and photosynthetic pigment analysis. All filtrate samples were stored frozen, and water samples refrigerated at 4°C, to prevent degradation. Algal pigments are light sensitive, so were processed quickly under low light conditions to prevent photo oxidation.

Table 3.3: Details of water samples collected (in May 2017) showing depth and location

Lake	Depth		Sample Location	
Sampaloc	Surface	0.5 m	14°04’44.90’’N	121°19’47.44’’E
	Mid	10 m	14°04’44.90’’N	121°19’47.44’’E
	Bottom	20 m	14°04’44.90’’N	121°19’47.44’’E
Palakpakin	Surface	0.5 m	14°06’37.63’’N	121°20’21.44’’E
	Mid	3 m	14°06’37.63’’N	121°20’21.44’’E
	Bottom	6 m	14°06’37.63’’N	121°20’21.44’’E
Bunot	Surface	0.5 m	14°04’51.15’’N	121°20’36.95’’E
	Mid	10 m	14°04’51.15’’N	121°20’36.95’’E
	Bottom	20 m	14°04’51.15’’N	121°20’36.95’’E
Mohicap	Surface	0.5 m	14° 07'20.43"N	121°20'03.37"E
	Mid	10 m	14° 07'20.43"N	121°20'03.37"E
	Bottom	20 m	14° 07'20.43"N	121°20'03.37"E
Pandin	Surface	0.5 m	14°06'51.30"N	121°22'05.20"E
	Mid	18 m	14°06'51.30"N	121°22'05.20"E
	Bottom	30 m	14°06'51.30"N	121°22'05.20"E
Yambo	Surface	0.5 m	14° 07'08.32"N	121°21'58.64"E
	Mid	15 m	14° 07'08.32"N	121°21'58.64"E
	Bottom	30 m	14° 07'08.32"N	121°21'58.64"E

3.2. Sample Analysis

An outline of the analyses completed on the water samples are below.

3.2.1. Water Chemistry

3.2.1.1. TP and SRP

Prior to analysis, all glassware used was washed in 25% HCl and then rinsed with distilled water to remove any P adhered to the glass, to prevent contamination. 25ml of acidified unfiltered water (from the 25 ml Nalgene bottle collected specifically for this analysis) was analysed using the method described in Mackereth *et al.,* (1989). TP is a measure of all forms of phosphorus, dissolved or particulate. The first stage of the analysis for TP (adding 1 ml of 278:722 H_2SO_4) was completed in the field. Once in the laboratory, 0.7g of potassium persulphate powder was added to each sample and then a digestion was performed in a pressure cooker for 1 hour at 70°C. This was followed by the addition of ammonium molybdate (1 ml), potassium antimonyl tartrate (0.5 ml) and ascorbic acid (1 ml).

SRP is a measure of orthophosphate, the filterable (soluble, inorganic) fraction of phosphorus (the form directly taken up by algae and plants). For SRP, 50 ml of filtered water was used following the molybdenum blue method as described in Mackereth *et al.,* (1989). This involved adding 5 ml of the SRP mixed reagent (5 parts 140:900 H_2SO_4; 2 parts ammonium molybdate; 2 parts ascorbic and 1 part potassium antimonyl tartrate). After 0.5-12 hours, the absorbance of both mixtures was measured on a spectrophotometer at 885 nm. A number of calibration standards were also analysed with a known concentration of TP and SRP to ensure the validity of the results.

3.2.1.2. Dissolved Silicate

Silicate analysis was conducted on 4 ml of filtered water. Plastic bottles were used due to the presence of silicates in glassware. This analysis was completed using the molybdate yellow method described in Mackereth *et al.,* (1989).

3.2.1.3. Ammonium

50 ml of filtered water was analysed for ammonium using the method described in Mackereth *et al.,* (1989). This method involved the addition (in order) of phenol (2 ml), sodium nitroprusside (2 ml) and an oxidising solution (5 ml; 4 parts alkaline solution; 1 part sodium hypochlorite solution). After 1.5 to 12 hours in a low light environment, absorbance was measured on spectrophotometer at 640 nm.

3.2.1.4. Total Chlorophyll and Photosynthetic Pigments Analysis

The filter papers labelled for chlorophyll *a* were analysed using a photospectrometer as described in (Lorenzen, 1967) and Mackereth *et al.,* (1989).

The amount of chlorophyll *a* was calculated using equation 2 below:

Equation 2: Chlorophyll *a* (µg/l) = $(11.85(A_{665}-A_{750})-1.54(A_{645}-A_{750})-0.08(A_{630}-A_{750}))$ x E/V

Where E=volume of extraction solvent (ml) and V=total volume of water filtered

The filter papers labelled for photosynthetic pigment analysis were analysed using the method described in section 3.2.3.3. However, instead of using 0.2 g of freeze dried sample, the filter paper was cut into fine strips for extraction.

3.2.1.5. Alkalinity

Alkalinity, the ability of waters to buffer acid, was measured using 10 ml of unfiltered water (as described in Golterman *et al.*, (1978)). This analysis required a two stage titration in order to calculate the concentration of, firstly carbonate, (using 5 ml of phenolphthalein) and secondly, bicarbonate, (using 5 ml of BDH indicator). Both chemicals were titrated using sulphuric acid until the solution had turned grey in colour. During both steps, the volume of acid used in the titration was recorded.

To calculate alkalinity, the following equations (equation 3 and 4) were used:

Equation 3: Alkalinity in milliequivalents (mequiv/L)=10y/x

With x=volume of water in ml, with y = Bicarbonate alkalinity =yii (ml) or Phenolphthalein alkalinity= yi (ml).

Equation 4: Total alkalinity = yi+ yii (ml)

3.2.1.6. Ion Chromatography for major ion analysis

Ion Chromatography was used to quantify the concentrations of the major cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, NO^{3-}. SO_4^{2-}). 2 ml of filtered water (from each sample) was analysed using the Basic IC plus manufactured by Metrohm UK Ltd. The eluent (used to analyse each sample) consisted of 3.2 mmol/l of sodium carbonate and 1.0 mmol/l of sodium bicarbonate which was passed through a Metrosep A supp5 column 250 mm long and 40 mm wide, with a flow rate of 0.7 ml per minute. The IC calculated the concentration of each ion based on peak area.

In order to compare the ions and alkalinity, all values were converted into milliequivalents per litre (meq/l) using the following equations (equations 5 and 6).

For the alkalinity measurements:

Equation 5: Alkalinity in meq/l= 10x/y

x=volume of water, y= total alkalinity (ml)

For the ion concentrations:

Equation 6: Meq/l= x/ atomic weight

x=ion concentration in mg/l

3.2.2. Lake Trophic Status

Trophic state is defined as the total weight of biological biomass in a waterbody at a given time and place (Bekteshi and Cupi, 2014). It is a biological response to nutrient loading of an aquatic system and can be modified by various parameters such as (1) nutrient supply (geology, soil, vegetation, human land-use); (2) climate (sunlight, temperature, hydrology) and (3) basin morphometry (depth, volume, surface area) (Rast and Lee, 1978). The onset of lake eutrophication can invoke many water quality issues, including algal blooms, low DO concentrations (which has implications for the lake biota), fish kills, and the loss of water clarity (which affects the light penetration and therefore, photosynthesis) (Van Straten and Keesman, 1991; Müller and Stadelmann, 2004). Lake trophic status was obtained using the system developed by Vollenweider and Kerekes (OECD, 1982) and how it varied along the aquaculture disturbance gradient. The OECD classification is based on actual values of TP, chlorophyll *a* and Secchi depth using a fixed boundary system (Table 5.7). Higher values of TP and chlorophyll *a* and shallower Secchi depth readings suggest more eutrophic conditions and vice versa.

3.2.3. Sediment core analyses

All sediment cores were analysed for loss-on-ignition (LOI), photosynthetic pigments, stable isotope ($\delta^{13}C_{org}$, $\delta^{15}N_{org}$) analysis and C/N ratios. The chronology for each sediment core was obtained either directly using ^{210}Pb dating or by cross-correlation of ^{210}Pb dates with previously collected dated cores. The descriptions of the methodologies are below.

3.2.3.1. Loss-on-ignition

LOI was used to determine how dry density (LOI_{105}), OM (LOI_{550}) and carbonate (LOI_{925}) content has changed over time at the study lakes. For this research, LOI measurements were necessary as a precursor for multiple analyses. LOI_{550} was critical for the pigment analysis in order to calculate the relative pigment mass to the OM concentration. Expressing pigment abundance relative to the OM concentration minimises the dilution effects from minerogenic inwash. This correlates more strongly with the past biomass of phototrophs than when calculated relative to sediment dry weight (Leavitt, 1993). Dry mass density measurements (in this case LOI_{105}) were required to calculate dry mass accumulation rates for the ^{210}Pb constant rate of supply (CRS) modelling (see section 3.2.3.4) and to indicate the quantity of sample needed for $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ analysis.

3.2.3.1.1. Method

All six sediment cores were analysed at 1 cm intervals (due to the small sample volume in the top 30 cm of the core). Density corrected LOI was obtained using the method described in Heiri *et al.*, (2001). A 15 ml syringe (with the end cut off) was used to ensure only 1 cm^3 of each sample was analysed. In order to prevent cross contamination, the syringes were washed with distilled water after every sample. Wet samples (of 1 cm^3) were weighed and placed in a crucible and left in an oven overnight at 105°C (to remove moisture), samples were then reweighed before they were placed in a furnace and 'ignited' at 550°C for 5 hours to remove organic carbon. Following re-weighing the samples were returned to the furnace for 2 hours at 925°C (in order to remove carbonates). Bulk sediment was analysed for dry density (LOI_{105}), OM content (LOI_{550}), carbonate content (LOI_{925}*1.36), minerogenic content (100-(LOI_{550}+LOI_{925})) and bulk dry density (dry weight (g)/volume (cm^3)(Dean, 1974; Heiri *et al.*, 2001; Santisteban *et al.*, 2004)

3.2.3.2. Using %C, %N, C/N Ratios, and δ13C as a tool to understand sources of OM

Stable isotopic analysis of sedimentary OM allows for the assessment of the source of the OM, including whether it is from C_3 plants (such as vascular plants including cereal grains and trees)

C_4 plants (such as grasses, and some non-vascular aquatic macrophytes) or lacustrine algae (Figure 3.1). Most photosynthetic plants and lacustrine algae use the C_3 pathway (Calvin pathway), which preferentially takes up ^{12}C over ^{13}C, with an average $^{12}C/^{13}C$ ratio of –22 to –30‰. Plants that use the C_4 pathway (Hatch-slack pathway), produce a more positive $\delta^{13}C$ value of –4‰ to –6‰ (Meyers and Lallier-Vergès, 1999).

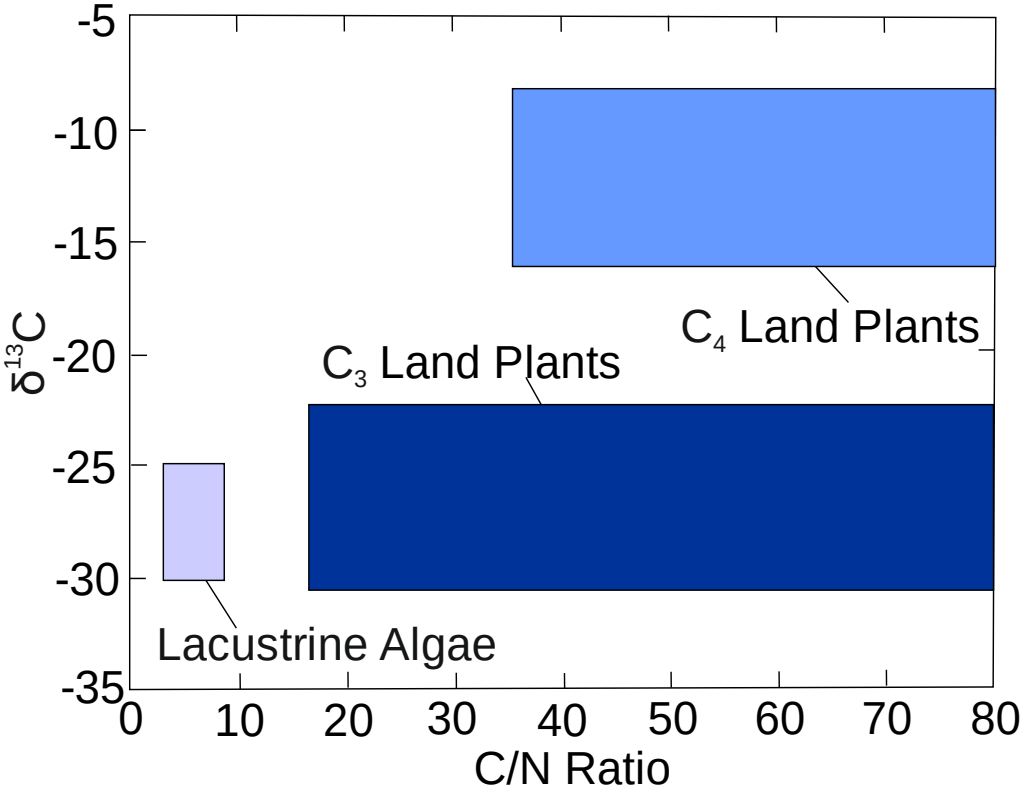


Figure 3.1: Typical ranges of C/N ratios and $\delta^{13}C$ isotopic composition of C_3 land plants, C_4 land plants and lacustrine algae (adapted from Meyers and Lallier-Vergès, 1999)

C/N ratio is the ratio of the mass of carbon to the mass of nitrogen in a sample. It is calculated by dividing %carbon (%C) by %nitrogen (%N) and is influenced by the presence/absence of the polysaccharide cellulose, the main component of plant cell walls (Meyers and Ishiwatari, 1993). Nonvascular aquatic plants have a C/N ratio of 4 to10, whereas, vascular land plants have a C/N ratio of >20 (Meyers and Ishiwatari, 1993). Therefore, if a lake has lower C/N ratios and $\delta^{13}C$ values of between –25 and –30‰ (Figure 3.1; Meyers and Ishiwatari, 1993),it suggests OM is dominated by lacustrine algal sources. A C/N ratio of 13 to 14 would suggest a mixture of non-vascular and vascular contributions, which is expected in most lakes (Meyers and Ishiwatari, 1993). Algal OM has a different $\delta^{13}C$ values compared to C_4 land plants or benthic algae. Lake derived OM produced by algae (which uses the C_3 pathway) utilises dissolved CO_2, which is in isotopic equilibrium with the atmosphere. However, dissolved CO_2

is isotopically indistinguishable from OM produced by C_3 land plants within the lake catchment (Meyers and Lallier-Vergès, 1999). One useful way to distinguish the OM from lacustrine algae from C_3 land plants, is by combining C/N ratios and $\delta^{13}C$ values (Figure 3.1).

Table 3.4: Factors likely to influence organic geochemistry in the sediments of the Seven Lakes of San Pablo away from the pre-aquaculture means of $\delta^{13}C_{org}$ values = −30.7‰ and $\delta^{15}N_{org}$ values = +2.9‰
**depends on the on species of aquatic macrophyte present*
*** can cause an increase in $\delta^{15}N_{org}$ if denitrification leads to a residual N pool becoming enriched in ^{15}N*
**** have high $\delta^{15}N$ values of 10 to 20‰*
***** have $\delta^{15}N$ values of −3 to 3‰*

Variable	Factor	Response	Source
$\delta^{13}C_{org}$	Increased anoxia	Decrease	Conrad *et al.*, 2011
$\delta^{13}C_{org}$	Increased algal productivity	Increase	Meyers and Lallier-Vergès, 1999
$\delta^{13}C_{org}$	Increased OM from aquatic macrophytes	Highly variable*	Conrad *et al.*, 2014
$\delta^{13}C_{org}$	Increase in soil erosion (allochthonous OM)	Increase	Melack and Coe, 2013
$\delta^{13}C_{org}$	Increase in methanogenesis	Decrease	Legaspi *et al.*, 2015
$\delta^{13}C_{org}$	Increase input of C4 plants	Increase	Meyers and Lallier-Vergès, 1999
$\delta^{13}C_{org}$	Presence of ^{13}C depleted phytoplankton	Decrease	Torres *et al.*, 2012
$\delta^{13}C_{org}$	Influx of littoral sediments	Decrease	Chen *et al.*, 2017
$\delta^{13}C_{org}$	Input of fish food	Increase	See Table 5.1
$\delta^{15}N_{org}$	Presence of dissolved nitrate	Increase	Meyers and Lallier-Vergès, 1999
$\delta^{15}N_{org}$	N_2 fixation	Decrease	Fogel and Cifuentes, 1993
$\delta^{15}N_{org}$	Utilisation of NO_3 or NH_4 by algae	Increase	Peters *et al.*, 1978
$\delta^{15}N_{org}$	Increase in trophic state	Increase	Peterson and Fry, 1987
$\delta^{15}N_{org}$	Increased proportion of NH_4^+	Increase	Barnes and Mann, 2009
$\delta^{15}N_{org}$	Increase in denitrification	Increase/Decrease**	Meyers and Teranes, 2001
$\delta^{15}N_{org}$	Increased algal productivity	Increase	Meyers and Lallier-Vergès, 1999
$\delta^{15}N_{org}$	Increased input of human effluent	Increase***	Mayer *et al.*, 2002
$\delta^{15}N_{org}$	Use of synthetic fertilisers	Decrease****	Finlay and Kendall, 2007
$\delta^{15}N_{org}$	Increased use of fish food	Increase	See Table 5.1

3.2.3.2.1. Organic carbon isotopic ratios

In warm, strongly stratified lakes, CO_2 exchange between the lake and the atmosphere is restricted and in-lake processing of organic carbon by bacteria and other heterotrophic

organisms is intense (Boehrer and Schultze, 2008). Microbial processes such as methanogenesis (which is the formation of methane by anaerobic microbes and is the dominant terminal OM degradation process in anoxic freshwater lakes) causes highly depleted $\delta^{13}C_{org}$ values of –40 to –60‰ due to the preferential uptake of ^{12}C by bacteria (Legaspi *et al.*, 2015). As the study lakes are strongly stratified tropical lakes, the influence of methane-producing microbial communities is an important consideration when interpreting the $\delta^{13}C_{org}$ record.

3.2.3.2.2. Organic nitrogen isotopic ratios

$\delta^{15}N_{org}$ can give important information about the sources of OM in a lacustrine environment (Meyers and Lallier-Vergès, 1999). The identification of different OM sources using $\delta^{15}N$ is possible due to variations between the inorganic N reservoirs available to land and aquatic plants as well as various algal groups. Dissolved nitrate has an isotopic ratio of +7 to +10‰, whereas, atmospheric nitrogen has a $\delta^{15}N$ of 0‰ (Meyers and Lallier-Vergès, 1999). This difference is in the distinct $\delta^{15}N$ isotopic ratios of algae (+8‰), C_3 land plants (+1‰) and nitrogen-fixing cyanobacteria (+1 to +2‰). N_2 fixing cyanobacteria assimilate atmospheric N_2, so algal assemblages with high abundances of this group have a lower sedimentary $\delta^{15}N_{org}$ of +1 to +2‰ (Fogel and Cifuentes, 1993). In comparison, algae utilise NO_3 or NH_4 from dissolved inorganic nitrogen (DIN) which has a higher $\delta^{15}N$ value (+7 to +10‰) (Peters *et al.*, 1978). $\delta^{15}N$ values of marine sources (such as the fish food used at the study area) can vary depending on the origin and trophic state of the source organism. Each trophic transfer increases the $\delta^{15}N$ value by +3 to +4‰ meaning heterotrophs have a higher $\delta^{15}N$ (Peterson and Fry, 1987). Fish food consisting of heterotrophic remains, would cause higher $\delta^{15}N$ values (+12 to +30‰).

The reduced form of nitrogen (NH_4^+) is considered the preferred form of N for phytoplankton uptake (Glibert *et al.*, 2016). The preference for NH_4^+ is due to the lower energy requirement for the cell and the ability to easily transport it across cell membranes compared to NO_3^- under N limiting conditions (common in tropical lakes). Anthropogenic activities such as aquaculture are a rapidly increasing source of NH_4^+ due to direct excretion and decomposition of undigested feed (Bouwman *et al.*, 2013). Increased input of reduced forms of N, combined with climatic change, is thought to impede NH_4^+ oxidation (to NO_3^-). The resulting stratification may increase the reduction of NO_3^- in surface waters leading to an increased availability of NH_4^+ (Huesemann *et al.*, 2002). HAB species are disproportionally favoured when N is available in

its reduced (NH_4^+) form rather than in its oxidised forms (NO_3). Lacustrine environments with an increasing proportion of NH_4^+ can cause a depletion in ^{15}N and negative $\delta^{15}N$ values.

Biogeochemical transformations of N can result in the substantial fractionation of $\delta^{15}N$ values and can indicate the processes that have taken place. Microbial communities can influence $\delta^{15}N$ values through denitrification, which is the reduction of NO_3 to N_2. Denitrification is stimulated by anoxia and high temperatures in sediments (Meyers and Teranes, 2001). Anoxia is more

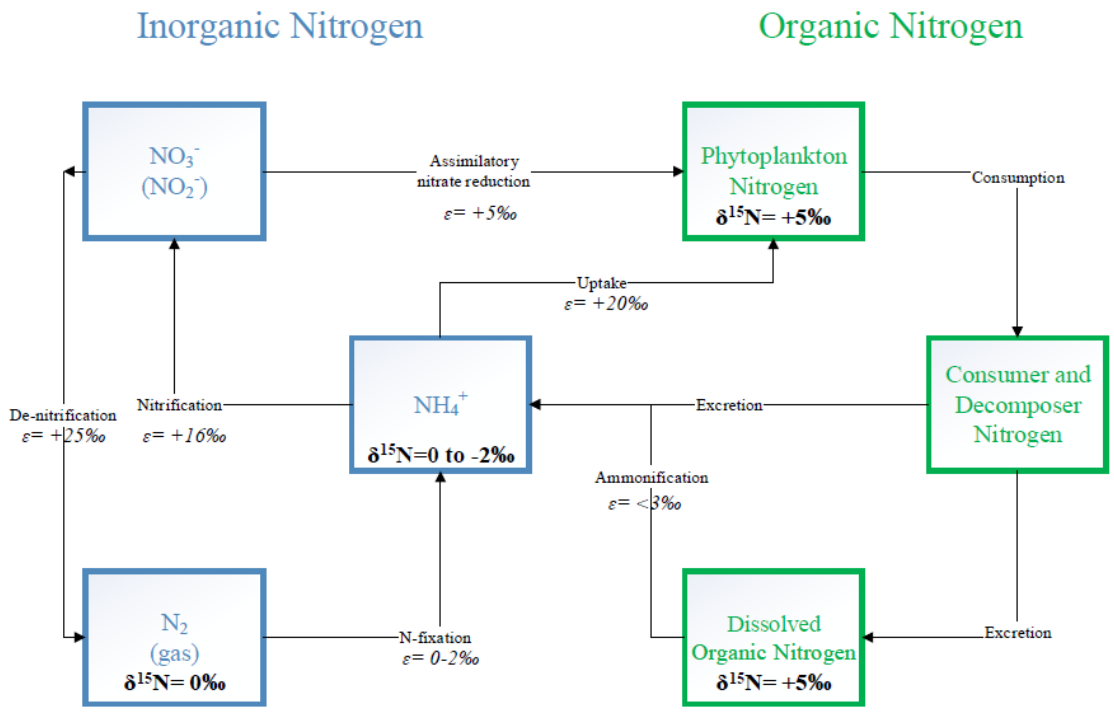


Figure 3.2: Simplified aquatic $\delta^{15}N$ cycle showing the different processes involved. At each stage, the $\delta^{15}N$ values of the beginning and end products and the degree of fractionation are shown (Barnes and Mann, 2009)

likely to occur and be more persistent in tropical lakes compared to temperate lakes. Even tropical lakes with low productivity will likely be anoxic during periods of extended stratification (Lewis, 2010). Virtually all stratified tropical lakes will lose a substantial portion of their N inventory on an annual basis through denitrification and, therefore, have a greater likelihood of N depletion by phytoplankton growing in the epilimnion. This process preferentially uses the lighter ^{14}N in the reduction of NO_3 to N_2, further enriching the residual ^{15}N in the DIN pool, resulting in isotopically light $\delta^{15}N$ values.

Lakes with higher N concentrations or loads generally have higher $\delta^{15}N$ values compared to pristine lakes because of anthropogenic pressures (Mayer *et al.*, 2002). Human and livestock waste have high $\delta^{15}N$ values of +10 to +20‰, whilst synthetic fertilisers have $\delta^{15}N$ values of –3 to +3‰ (Finlay and Kendall, 2007). Untreated sewage has $\delta^{15}N$ values of +10 to +20‰ and volatisation, nitrification and denitrification within septic systems can cause further enrichment of $\delta^{15}N$ values. NO_3^- in groundwater from septic systems can lead to $\delta^{15}N$ values of +10 to +20‰ in lake waters (Townsend *et al.*, 2001).

3.2.3.2.3. Stable Isotope Limitations

Numerous limitations are associated with interpreting $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$, as multiple lake processes can influence the isotopic signal. Robust interpretations of isotopic signatures requires a knowledge of the processes that control and modify their signals within the lake system (Leng and Marshall, 2004). For example, diagenetic processes can alter $\delta^{15}N$ ratios, as proteins and lipids decompose rapidly. In addition, it can be difficult to distinguish between exchangeable and fixed N, and inorganically bound N found in clay-rich lacustrine sediment (Talbot, 2002).

3.2.3.2.4. C/N limitations

Interpreting sources of OM can also be difficult as C_3 plants have a similar carbon isotopic ratio to lacustrine algae, but this difficulty is mitigated with the use of C/N ratios, as lacustrine algae and C_3 plants have different values (Meyers and Lallier-Vergès, 1999). Soil OM may have a high N content (with C/N values close to that of planktonic algae OM) due to the presence of N_2 fixing microorganisms around plant roots, exacerbating the difficulty of interpreting OM sources (Talbot, 2002). Furthermore, the significant processing of these ratios on land, prior to deposition in the lakes, can lead to difficulty in interpretation. A further limitation of interpreting C/N ratios is that OM undergoes degradation in the water column as well as during diagenesis, which can modify elemental composition and C/N ratios in the sedimentary record (Meyers and Lallier-Vergès 1999). C/N ratios in algal derived OM increases, as it sinks in the water column, due to the selective degradation of N rich proteins, especially prevalent in lakes with high productivity (Meyers and Lallier-Vergès, 1999). However, this change is not large enough to significantly influence C/N differences between algae, non-vascular and vascular plants.

3.2.3.2.5. C and N mass, $\delta^{15}N_{org}$ and $\delta^{13}C_{org}$ isotopic laboratory preparation and analyses

All sediment core samples (at 0.5 cm resolution for the top 30 cm and 1 cm for the remainder of the core) were analysed for $\delta^{13}C_{org}$, $\delta^{15}N_{org}$ and C/N ratios. The contemporary samples listed in Table 3.2 were also analysed for stable isotopes. Samples were frozen, freeze dried and then subsampled, so that half was analysed for $\delta^{13}C_{org}$, C/N ratios, %C and %N and the other half for $\delta^{15}N_{org}$. Preparation for stable isotope analysis was carried out by the author at the University of Nottingham and completed by technical staff at the Isotope Geoscience Facilities, British Geological Survey.

$\delta^{13}C_{org}$ samples were pre-treated with 5% HCl to remove $CaCO_3$, washed three times with de-ionised water and dried at 40°C. Sediments were then ground to a fine powder using a mortar and pestle and weighed into tin capsules. The amount weighed depended on the percentage of OM in the sample (estimated from LOI_{550} values). $\delta^{13}C_{org}$, percentage total organic carbon (%TOC) and percentage total nitrogen (% TN) were analysed by sample combustion in a Costech Elemental Analyser (EA) coupled on-line to a VG TripleTrap and Optima dual-inlet mass spectrometer. $\delta^{13}C_{org}$ values were calibrated to the VPDB scale using within-run laboratory standards (BROC2 and SOILA) calibrated against NBS 18, NBS 19 and NBS 22, with an analytical reproducibility of ± <0.1‰ (1 SD). C/N ratios were reported as the atomic ratio with an analytical reproducibility of ± <0.1.

For $\delta^{15}N_{org}$, the samples were powdered, weighed and then placed in a glass vial. The amount needed for the analysis was dependent on the percentage of N in the sample (as determined by the %N). For $\delta^{15}N$, samples were analysed by the Thermo Finnigan DeltaplusXL with oxidative and reductive elemental analysers. $\delta^{15}N$ values were calibrated to a known international standard of atmospheric N_2 using within-run laboratory standards (BROC2 and SOILA) calibrated against NBS18, NBS-19 and NBS 22, with a laboratory precision of ± <0.1‰ (1 SD).

3.2.3.3. Photosynthetic Pigments

For over 60 years, palaeolimnologists have been examining fossil photosynthetic pigments in sediment cores as a way to reconstruct changes in taxonomic groups of phototrophs in freshwater lakes. Primary producers are organisms within an ecosystem that produce biomass from inorganic compounds (autotrophs) and consist almost entirely of photosynthetically active organisms (plants, cyanobacteria and a number of other unicellular organisms) (Cole and Weihe, 2015). Since most primary producers within freshwater environments have pigments, fossil pigments can give invaluable insight into the entire record of in situ biomass (Sanger, 1988a). The use of a palaeolimnology to analyse preserved photosynthetic pigments in sediment cores allows for the identification of changes in the different primary producer groups. This is determined by the particular combination and abundance of each pigment (McGowan, 2013). Pigments found in sediments are also strongly dependent on the environmental conditions at the time of deposition (McGowan, 2007) allowing for the reconstruction of short and long-term environmental changes within lakes (Soma *et al.*, 2007).

Pigments are chemical compounds, which reflect only certain wavelengths of visible light and as such are 'coloured'. In addition, pigments are the means by which autotrophs (organisms which make their own OM using photosynthesis) capture light energy during the light-dependent reactions of photosynthesis (Lichtenthaler, 1987). Since each pigment only reacts with a narrow range of the visible light spectrum, each autotroph evolves different types of pigments to broaden the range of wavelengths that can be used in photosynthesis to maximise capture of solar energy (Meyers and Ishiwatari, 1993).

Pigments can be divided into the following classes:

Chlorophylls. Chlorophyll *a* is found in all phototrophs and is fundamental to the process of photosynthesis (Figure 3.3; Milenković *et al.*, 2012). Chlorophyll *a* passes energised electrons onto molecules, which manufacture sugars in all oxygenic photosynthetic organisms, plants, algae (the main primary producer in lakes) and cyanobacteria (Sanger, 1988). Chlorophyll *a* is the primary pigment, absorbing energy from the wavelengths of violet-blue to orange-red light and reflecting green/yellow light (McGowan, 2013). Some oxygenic photosynthetic organisms possess additional chlorophylls such as chlorophyll *b* and chlorophyll *c*. Chlorophyll *b* is found mostly in land plants whilst chlorophyll *c* is found in certain marine algae, including the photosynthetic *Chromista* and dinoflagellates.

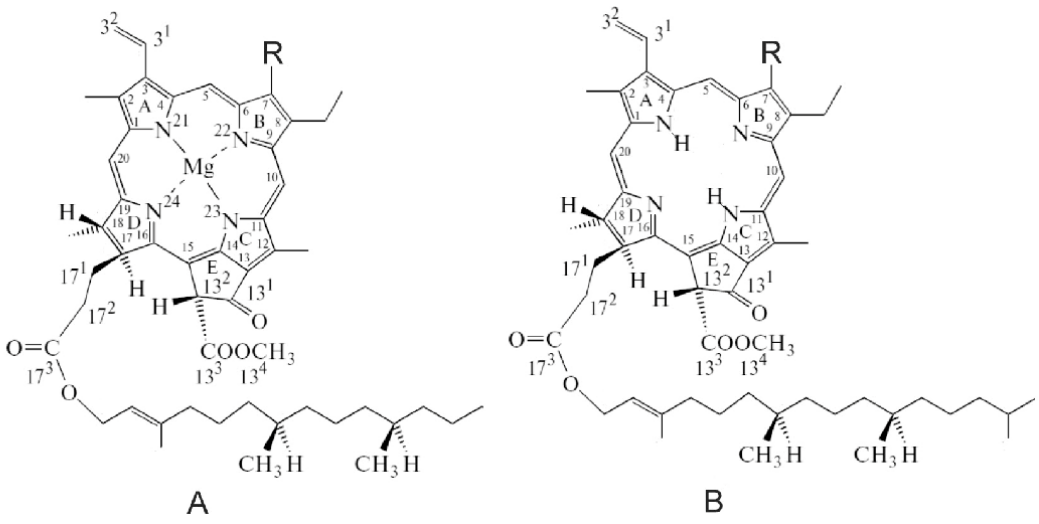


Figure 3.3: Chlorophyll (A) and pheophytin (B) structures (Milenković et al., 2012)

Carotenoids. Carotenoids are unable to transfer sunlight energy directly into the photosynthetic pathway so are known as accessory pigments. These are usually red, orange or yellow in colour and are composed of two small six-carbon rings connected by a 'chain' of carbon atoms. Carotenoids absorb light in the 400 – 500 nm wavelengths (violet to green light) (McGowan, 2013). They are found in the chloroplasts and chromoplasts of plants and other photosynthetic organisms such as bacteria and fungi (Sanger, 1988). There are over 600 known carotenoids divided into xanthophylls such as lutein and zeaxanthin (which contain oxygen) and carotenes, such as α-carotene, β-carotene and lycopene (which contain hydrocarbons and no oxygen) (McGowan, 2013).

Photosynthetic Bacteria. Bacteriochlorophylls (Bchl) *c*, *d* and *e* are the main light harvesting pigments found in green sulphur bacteria (Borrego and Garcia-Gil, 1993). They harvest far red light in the 720-755 nm range, whereas, purple sulphur bacteria use bchl *a* or *b* as the main light harvesting pigments, which absorb infrared radiation in the range of 800 to 1400 nm (Surette and Stearns, 2014). Carotenoids, chlorobactene (for green sulphur bacteria with green pigments), isorenieratene (for those with brown pigments) and okenone (for purple sulphur bacteria) aid light energy capture and accompany bchls. Okenone is best adapted to the yellow-green transition (520 nm), whilst isorenieratene and chlorobactene absorb green light between 520-550 nm on the visible light spectrum. Each anoxygenic phototroph occupies a different depth range within lacustrine environments. The presence of different pigments can, therefore, be useful indicators to constrain the depth of the oxic-anoxic zone over time.

Fossil pigments are excellent indicators of short-term change and can aid in identifying causes of eutrophication in freshwater lakes. Multiple studies conducted found increases in pigments from filamentous cyanobacteria (myxoxanthophyll, aphanizophyll) and siliceous algae (diatoxanthin, fucoxanthin) suggesting prolonged eutrophic conditions caused by anthropogenic activities (Hall *et al.*, 1997, 1999; McGowan *et al.*, 2012). The increases in these pigments all attest that greater algal production in the study lakes (during the last 50-60 years) was most likely the result of increased anthropogenic activities. However, lake morphology heavily influences pigment preservation (Figure 3.4), for example a greater lake depth combined with an irradiated water column, would lead to enhanced pigment degradation. Therefore, a lake's physical characteristics must be considered when interpreting pigment records.

Palaeolimnology studies using photosynthetic pigments in tropical freshwater lakes are fairly common. Studies by García-Rodríguez *et al.*, (2002) and Verschuren *et al.*, (1999) on African lakes used sedimentary pigments to investigate environmental change (specifically algal blooms and increased productivity) caused by natural and anthropogenic activities. Garcia-Rodriguez *et al.*, (2002) found that increases in pigment concentrations in 1986/87, most likely resulted from eutrophication caused by sediment input from a storm event in 1986. Verschuren *et al.*, (1999) found that between 1915 and 1945 algal abundance was between 100-300% higher than present day, which was characterised by unialgal blooms of filamentous cyanobacteria. However, the abundance of cyanobacteria declined between 1945 and 1970, due to a progressive reduction in nutrient concentrations.

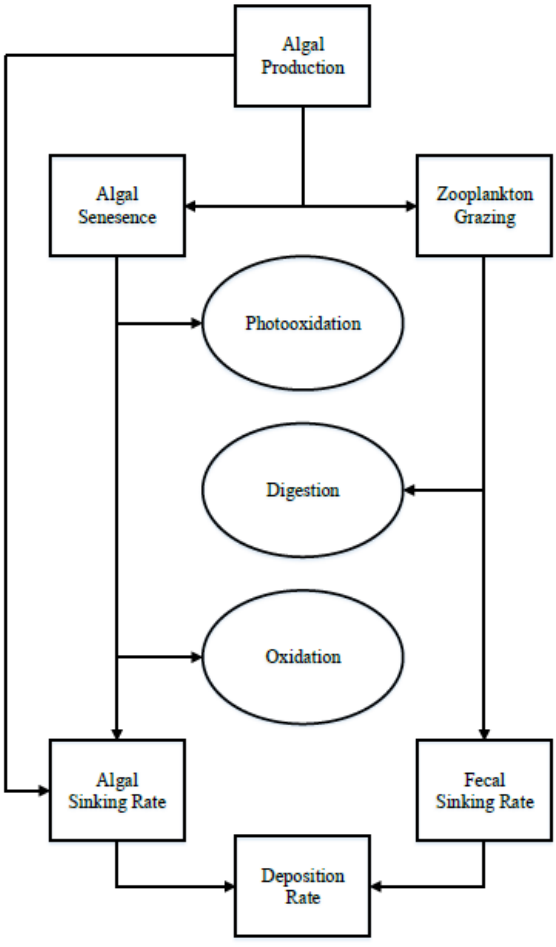


Figure 3.4: Pathways of pigment production, transformation and degradation within lakes (adapted from Cuddington and Leavitt, 1999)

3.2.3.3.1. Method for High Performance Liquid Chromatography (HPLC)

Analysis of photosynthetic pigments of the sediment core samples was completed using reversed-phase HPLC and online photo diode array (PDA) spectrophotometry (McGowan, 2013). HPLC is an analytical technique that can quickly isolate and separate biochemical markers such as photosynthetic pigments and therefore, has the ability to address the aims and objectives of this research. A modified approach of Chen *et al.*, (2001) was used for this study, as it is able to separate out the main indicator species of carotenoids and chlorophylls as well as their degradation products.

This analysis was carried out in the HPLC laboratory at the School of Geography at the University of Nottingham. Frozen sediments were freeze dried and 0.2g of freeze dried sample was transferred into a labelled vial for extraction in 5 ml of HPLC-grade acetone: methanol: water (80:15:5) over night in the freezer at -4°C. Extracts were decanted into a 50 ml beaker and the residue washed with solvent until all pigments were extracted. The extracts were filtered using a 0.22µm PTFE syringe filter to remove any sediment and then placed under a stream of N_2 gas until all the solvent was evaporated. Samples were stored in a freezer under N_2 gas before being redissolved in a measured amount of injection solution (acetone: ion pairing reagent consisting of 1.875g tetrabutyl ammonium acetate, 19.35 g ammonium acetate and 250 ml deionised water: methanol (70:25:5) (Chen *et al.*, 2001). The volume of injection solvent varied among samples in order to optimise the concentration for a good separation (which was assessed visually). The samples were then placed on the autosampler tray in the HPLC unit for analysis.

3.2.3.3.2. Separation of pigments in HPLC

Pigments were separated by reversed-phase HPLC, where the stationary phase is nonpolar and the mobile phase is polar (McGowan, 2013). HPLC separates pigments on the basis of polarity and mass, which is achieved by forcing solvents (mobile phase) under pressure (>2000 kPa) through a separating column consisting of fine (<10 µm) densely packed particles (stationary phase). As the pigment mixture travels through the separation column, polar pigments that have less affinity for the stationary phase travel through the column quicker, as they have a greater affinity for the solvent. The mass of each molecule (known as retention time) also aids separation (McGowan, 2013).

Table 3.5: Solvent separation gradients. Modified from Chen et al., (2001).

Time (minutes)	% Solvent A	% Solvent B	%Solvent C	Flow (ml/min^{-1})
0	100	0	0	1
4	0	100	0	1
38	0	25	75	1
39	0	25	75	1
43	100	0	0	1
52	100	0	0	1

The HPLC system contains an Agilent 1200 series quaternary pump, autosampler, and PDA detector, which detects UV and visible spectra data (of wavelength 300-750 nm) every 2 seconds. Each 52-minute run started and ended with a 'green' standard (a pigment extract from grass containing known pigments, prepared using the same method as the sediment core samples) to identify any drift in retention times and aid pigment identification. This method involved a mobile phase comprised of solvent A (80:20 Methanol: 0.5 m ammonium acetate), solvent B (90:10 acetonitrile: deionised water), solvent C (100% ethyl acetate) and a stationary phase consisting of an ODS Hypersil column (25x4.5 mm; 5µm particle size) (Table 3.5). To remove air bubbles, all solvents were degassed for 5 minutes prior to use.

3.2.3.3.3. Identification of sedimentary pigments

Chromatograms were analysed on the Agilent Chemstation, and the pigment's position, retention time and spectral characteristics on the chromatogram were used to identify the pigment, based on comparison with known pigment standards (Figure 3.5; McGowan, 2013). The relationship between pigment mass and peak area was determined by injecting known quantities of commercial pigment standards into the HPLC to develop pigment standards. The abundances of pigments were expressed as nano-moles (nmol) to account for different molecular weights and then calculated relative to OM (estimated by $\%LOI_{550}$). The lack of comparable pigment standards and low concentrations of bacteriochlorophylls, meant identification could not be definitive. They were therefore, excluded from any subsequent analyses.

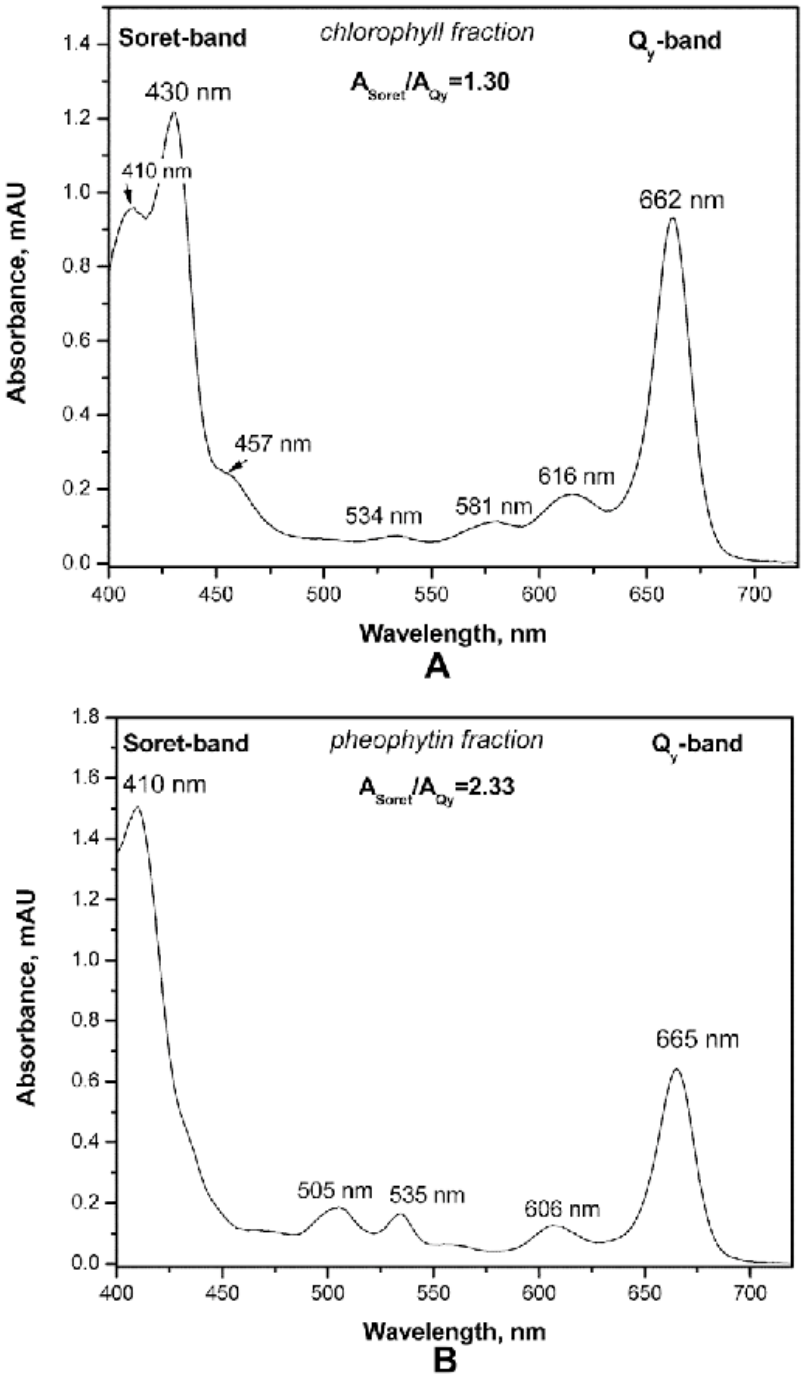


Figure 3.5: The absorption spectra of chlorophyll a (A) and pheophytin a (B) fraction in acetone. The positions of the absorption maxima (Soret and Qy) are displayed within the corresponding spectra. The positions of several observed small absorption bands (between the Soret- and Qy-) are also displayed within the corresponding spectra (Milenković et al., 2012)

3.2.3.3.4. Photosynthetic Pigment Limitations

There are a number of limitations in utilising photosynthetic pigments to infer past environmental conditions. One major problem is degradation before and after deposition on the lake bottom, which can result in pigments being lost from the sedimentary record. Different types of pigments are more prone to degradation than others, due to differences in their molecular structure. Carotenoids are generally more stable but xanthohylls (a type of carotenoid) can be prone to degradation due to the presence of a 5,6-epoxide end group. Where pigments have a molecular structure such as the C=C double bond and the presence of oxygen containing functional groups, they are susceptible to diagenetic alteration as this provide sites for attack from microbes (Meyers and Ishiwatari, 1993). Furthermore, the more labile pigments are vulnerable to degradation (after algal death), with the greater the lake depth (and longer period of time exposed to oxygen), the more substantial the pigment degradation. Differences in the degree to which different pigments degrade can hamper attempts to reconstruct shifts in algal abundances and communities. Chlorophyll pigments are especially prone to degradation. However, this problem is mitigated to some extent by the fact that chlorophylls produce recognisable and detectable breakdown products, which means they can still be identified in the sedimentary record (McGowan, 2013). One final limitation of using photosynthetic pigments is that the concentration and type of pigments observed in the sedimentary record can change in response to many different variables, such as productivity and preservation. It can, therefore, be problematic to disentangle the exact cause of the changes observed. This problem can be mitigated by the use of a multi-proxy palaeolimnological approach to provide information on a wide range of variables, to aid in the interpretation of past environmental conditions.

3.2.3.4. Sediment Chronologies- ^{210}Pb dating

Accurate sediment chronologies are based on the principles of radioactive decay and decay constants. They are crucial in palaeolimnology, so the timing of any observed changes in the sedimentary record can be correlated to environmental changes (Gell *et al.*, 2013). One of the most important and reliable methods of dating recent sediments less than 150 years old is ^{210}Pb dating (Last and Smol, 2002). ^{210}Pb (half-life of 22.3 years) is a naturally occurring daughter isotope of the ^{238}U decay series (Figure 3.6). A small proportion of ^{222}Rn atoms (the daughter isotope of ^{226}Ra) produced escapes from the soils into the atmosphere where it decays to ^{210}Pb (Last and Smol, 2002). This is then deposited into lakes by precipitation or catchment run-off and subsequently accumulates in the sediments on the lake bed (Noller *et al.*, 2000). This method provides important insights into different processes that occur/influence lake systems such as anthropogenic drivers and with-in lake processes (Appleby *et al.*, 2001).

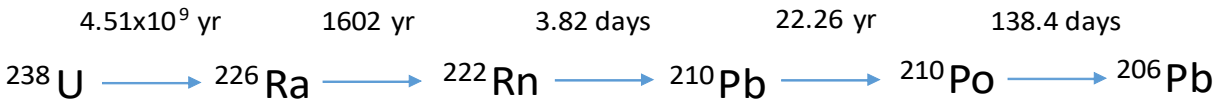


Figure 3.6: The main stages of the Uranium-238 decay series with the half-lives shown (adapted from Appleby, 2001)

Total ^{210}Pb activity in sediment has two components: supported ^{210}Pb, which is derived from in situ decay of the parent isotope ^{226}Ra, and unsupported ^{210}Pb, which is derived from atmospheric flux (Last and Smol, 2002). The supported component is measured using alpha or gamma spectrometry by assuming it is in radioactive equilibrium with ^{226}Ra (Last and Smol, 2002). As unsupported ^{210}Pb cannot be directly measured, it is calculated from subtracting supported ^{210}Pb activity from total activity (Appleby *et al.*, 2001). Total ^{210}Pb activity is measured via its granddaughter product ^{210}Po which it is assumed to be in equilibrium with (Appleby *et al.*, 2001).

$$Cuns = Cuns(0)e^{-\lambda t}$$

Equation 7

Where C_{uns}=present unsupported ^{210}Pb activity, $C_{uns}(0)$= estimated initial unsupported ^{210}Pb concentration, λ=decay constant , t=age of sediment

The constant rate of ^{210}Pb supply (CRS) model was applied to calculate chronologies for all sediment cores due to the non-monotonic features of the ^{210}Pb profiles (Appleby *et al.*, 2001). A chronology is calculated by comparing the unsupported ^{210}Pb, whose activity then becomes a function of its half-life from the upper, newly-deposited sediments, with the activity at lower depths (Equation 7; Noller *et al.,* 2000). This information is used to determine how sedimentation rates have varied over time (Appleby, 1998; Ruiz-Fernández *et al.*, 2007). This model assumes that changes in the sedimentation rate through time will result in change to the initial unsupported ^{210}Pb concentrations. Therefore, the dates of the older sediments are not calculated from present concentrations of ^{210}Pb but from the distribution of ^{210}Pb in the sediment record (Last and Smol, 2001). This model takes into account differences in sediment focusing, alterations due to various geochemical processes and those produced by catchment inputs (Appleby *et al.*, 2001).

To validate chronologies derived from ^{210}Pb models, artificial radionuclide activity can be measured. The atmospheric fallout of caesium-137 (^{137}Cs) and americium-241 (^{241}Am) from atmospheric testing of nuclear weapons (from 1953 to 1963) and the Chernobyl disaster (1986) can be used as stratigraphic markers (Appleby *et al.*, 2001). Further validation of the ^{210}Pb model can come from the use of marker horizons from well-documented events such as volcanic eruptions. The use of artificial radionuclides and marker horizons not only helps validate the ^{210}Pb chronology, but also to understand how atmospheric and sediment flux has changed prior to deposition.

3.2.3.4.1. Limitations

These techniques do have some limitations with respect to atmospheric deposition and storage within lake sediments. Variations in rainfall can lead to different delivery of atmospheric ^{210}Pb and other radioisotopes to a lake (Appleby *et al.*, 2001), but even in regions where wet deposition is similar, factors such as forest canopy and uneven topography have been shown to increase deposition (Branford *et al.,* 1998). Increased sediment accumulation from erosion caused by land use changes within the lake catchment and the transport of old sediments depleted in ^{210}Pb, can distort the ^{210}Pb signal making it difficult to interpret (Ruiz-Fernández and Hillaire-Marcel, 2009). Once deposited in the lake sediment, the radionuclides can be redistributed within the sediment column from turbidity currents, sediment slumps and mixing in or near the sediment water interface (Appleby *et al.*, 2001). Sediment mixing typically results in flattening of the ^{210}Pb activity vs. depth profile and degradation of ^{137}Cs and ^{241}Am peaks (Appleby *et al.*, 2001). ^{137}Cs is particularly susceptible to chemical advection or diffusion

within pore waters due to its higher solubility compared to ^{210}Pb. Furthermore, ^{210}Pb dating cannot be used on sediments over 150 years old when other dating methods such as radiocarbon dating would have to be used. Despite these limitations, the CRS model has been validated for use in the development of accurate chronologies, often supported by ^{137}Cs and ^{241}Am profiles (Appleby and Oldfield, 1978; Appleby *et al.*, 2001).

3.2.3.4.2. Methodology for ^{210}Pb dating

Due to financial constraints, only three sediment cores BUNOT2, PALAK and PANDIN were analysed for ^{210}Pb dating. A chronology was developed on the three remaining cores, SAMP2, MOH2 and YAMBO2, by correlating dates from previously collected cores from the respective lakes (Table 3.1).

Dried sediment samples from BUNOT2, PALAK and PANDIN were analysed for ^{210}Pb, ^{226}Ra, ^{137}Cs and ^{241}Am by direct gamma assay at the Environmental Radiometric Facility (University College London), using a ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. ^{210}Pb was determined via its gamma emissions at 46.5keV, and ^{226}Ra by the 295keV and 352keV gamma rays emitted by its daughter isotope ^{214}Pb following 3 weeks storage in sealed containers to allow radioactive equilibration. ^{137}Cs and ^{241}Am were measured by their emissions at 662keV and 59.5keV (Appleby *et al.*, 1986). The absolute efficiencies of the detector were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self-absorption of low energy gamma rays within the sample (Appleby, 1998).

3.2.3.4.3. Cross-correlation of cores for chronology development

The sediment cores SAMP2, MOH2 and YAMBO2 were correlated with dated ^{210}Pb cores collected from the same lakes in 2015 by project partners at the National University of Singapore (SAMP1, MOH1 and YAMBO1; published in Bannister *et al.*, (2019) using LOI_{550} and diatom relative abundances (Table 3.5)). Peaks and trends in the LOI_{550} profiles and the main diatom taxa were used to cross-correlate the core pairs. For SAMP2, MOH2 and YAMBO2, diatom analysis was completed at multiple intervals to capture the main trends/peaks in the five most abundant diatom species (*Aulacoseira granulata, Fragilaria crotonensis, Discostella stelligera, Discostella pseudostelligera* (Hustedt) Houl and Klee, 1939 and *Nitzschia palea* (Kützing) W. Smith; >90% of the total abundance in all three lakes).

3.2.3.4.4. Diatom Analysis

Samples were freeze-dried and diatom preparation followed the modified method of Renberg (1990), using 10% HCl treatment to remove carbonates, and 30% H_2O_2 to remove OM. The samples were left in the water bath at 90°C immersed in H_2O_2 until all OM had been removed. Rinsed samples were mounted onto microscope slides using Naphrax. A minimum of 300 valves were counted per sample at x1000 magnification using a Zeiss Axioskop 2 Plus microscope. The five main taxa were counted as they accounted for >90% of the total diatom abundance (as shown in Bannister *et al.*, 2019) and all other species were categorised together as 'other'. Krammer and Lange-Bertalot (1986-1991) assisted identification.

3.2.3.4.5. Developing a suitable age model

The CRS model was applied to calculate chronologies for all sediment cores. Since ^{137}Cs and ^{241}Am were detected in the sediments of PALAK and PANDIN, the CRS chronologies were validated with independent time markers. The decay rate and reduced accuracy of counting ^{210}Pb at low activities, means that before c.150 years, extrapolation of sediment chronologies to the bottom of the core (using the three earliest known sedimentation rates) were performed on SAMP2, MOH2 and YAMBO2. As not all levels were analysed for ^{210}Pb, a linear regression was used to fill in the gaps between dated samples to ensure each sample depth had a date assigned to it.

3.2.3.5. Historical data collection of local and regional environmental records

In order to understand the casual mechanisms of change within the sediment record it is necessary to compile a timeline of changes that have occurred at each of the lakes. Archival data from local and regional sources were used to determine changes in aquaculture timings and intensities, climate and urbanisation.

3.2.3.5.1. Interview data from stakeholders

The method and sources for the interview data are detailed in full in Bannister (2020). Full interview transcripts are shown in appendix A. Whilst completing fieldwork various stakeholders (fishermen, lake managers) were interviewed to aid in the collection of archival data. Varieties of different stakeholders were interviewed to collect first-hand accounts as limited information was available from scientific journals. The questions were related to changes in aquaculture timings/stocking densities as well changes to other anthropogenic activities that occurred within the lake catchment.

3.2.3.5.2. Meteorological data

Records from many meteorological stations in the island of Luzon are incomplete or cover a restricted timescale. Composite records can, however, provide a continuous dataset to allow comparisons over timescales relevant to the sedimentary record (1901-2018). The particular record used was taken from the Climate Research Unit's TS4.02 global dataset of annual mean temperature and precipitation records (Harris *et al.*, 2014). The dataset calculates records on grids of 0.5° x 0.5° and, for the study lakes, was centred on upon 121°21′00.0″E, 14°05′24.0″N at an altitude of 135 m. The gridding method used for Version 4 of this product is not documented in the Harris *et al.*, (2014), but can be accessed via the Google Earth Interface (TMP and PRE only) tool within the Release_Notes_CRU_TS4.00.txt. The gridding method is based on angular-distance weighting, which gives greater weighting to closer meteorological stations. This record was deemed most representative of the climate at the study area. Deviations from the 1901-2018 mean were calculated in order to highlight periods of cooler/warmer and wetter/drier weather.

A bimonthly record of the El Niño Southern Oscillation (ENSO), the Multivariate ENSO Index (MEI) was retrieved from the National Oceanic and Atmospheric Administration (NOAA) (https://www.esrl.noaa.gov/psd/enso/mei/). The MEI is based on the six main variables (sea-level pressure, the zonal and meridional components of sea surface wind, sea surface temperature, surface air temperature and total cloudiness fraction of the sky) observed over the

tropical Pacific (NOAA, 2018). This record was chosen due to its proximity to the study site (the record is of the NW Pacific) and due to the importance of ENSO in influencing, the climate of the Philippines (discussed in Chapter 2).

3.2.3.5.3. Urban Expansion

Historical information on occupied housing units in San Pablo City was obtained from the Philippine Statistics Bureau based on census data (https://psa.gov.ph/population-and-housing/node/129804) from 1960 to 2015. The annual number of occupied housing units was calculated by linear interpolation between census years. San Pablo City is the main urban centre (Figure 3.3) so, the distance from the centre of each lake to the edge of San Pablo city was used as a variable of urban expansion. This was done by measuring the distance (in km) using Google Earth imagery from the centre of each lake to the closest edge of San Pablo City (determined by visual inspection) annually from 1984 until present day.

3.2.3.5.4. Population

Historical information concerning population change in San Pablo City was obtained from the Philippine Statistics Bureau (based on census data) from 1950 to 2015. Annual population estimates were calculated by linear interpolation between census years. Census data was not available prior to 1950. A greater human population in a given area suggests increased human effluent and possible increased pressure on the surrounding aquatic systems.

3.2.3.5.5. Agriculture

Coconut plantations have been present in the catchments of all the lakes since the 17th century (Migrino, 2017; Bannister *et al.*, 2019). Visual inspection, historical records and interview data with stakeholders (appendix A), show it to be the most prevalent crop grown in the lake catchments and the surrounding area (Figure 2.3). However, the only available data is the area in hectares occupied by coconut plantations since 1990 in the province of Laguna collected from the Philippine Statistics Bureau (http://openstat.psa.gov.ph/PXWeb/pxweb/en/DB/DB__2E__CS/0072E4EAHO0.px/table/tableViewLayout1/?rxid=bdf9d8da-96f1-4100-ae09-18cb3eaeb313). In order to calculate the volume in hectares occupied by coconut plantation before 1990, a linear regression was applied to all data points between 1990 and 2018 to calculate the equation. This equation was then used to extrapolate the data back to 1960.

3.2.3.5.6. Aquaculture Cage Numbers

Multiple sources were used to create a high-resolution record of aquaculture cage numbers at the study area. A freedom of information request to the LLDA, the governing authority of the study lakes was used to acquire information on how aquaculture cage numbers have changed over time. This data is based on the number of fish pen permits issued at each of the lakes per annum. Further data on cage numbers was determined from the literature (Santiago and Arcilla, 1993; Santiago, 1994; Brillo, 2015a, 2015b, 2016a, 2016b, 2016c, 2016d). This was combined with landsat imagery, (Google Earth and USGS), which was used to count the number of individual aquaculture cages in each lake in the years 2009, 2010, 2012, 2015, 2016 and 2017. Three main cage sizes were counted in the study lakes, 10x10 m, 10x15m and 20x20 m (interview data; Appendix A). In order to account for the different cage sizes used for aquaculture, the average (cage size, 10x15 m; Google Earth, 2019) was used when calculating fish density. This was done for the years where Landsat imagery could not be used to determine cage numbers. This allowed the percentage of the total lake surface area occupied by aquaculture cages to be calculated. A linear regression was used to interpolate gaps in the time series. In order to standardise the data collected from different sources, aquaculture cage numbers were converted to a percentage of lake surface area (using the average cage size of 10x15 m). Data from multiple sources could then be combined to create high resolution records, to permit comparison between the different lakes.

3.3. Statistical Analysis

The objective of the statistical analyses is to quantitatively assess the ecological response of the six lakes to aquaculture practices, as described below.

3.3.1. Ordination methods to determine algal community change over time

Ordination methods are used to represent and summarise data along multiple axes (Legendre and Birks, 2012) in order to interpret patterns in species composition of ecological communities. Principal component analysis (PCA) and other forms of ordination reduce multiple dimensional datasets into two dimensions by rotating the data to explain maximum variability in axis 1, followed by axis 2 etc. (Legendre and Birks, 2012; Palmer, 2014). Therefore, PCA provides an overview of the linear relationships between species and samples. PCA axis 1 scores are used to describe variation in algal communities (Legendre and Birks, 2012). This is achieved by minimising the square of the distance from each point to the line that dissects the centroid (which is a vector of zero when species have been centralised and standardised) (Palmer, 2014). PCA axis 1 scores can, therefore, describe community change at each sample interval. The further these sample scores deviate from the centroid, the greater the variation of species at that interval, independent of whether the result is negative or positive. If two pigments are plotted close to one another, the samples have a similar species composition (Lepš and Šmilauer, 2003).

Redundancy analysis (RDA) combines both PCA and linear regression to extract and summarise the variation in a set of response variables (in this case photosynthetic pigments) that can be explained by a set of explanatory variables (Legendre and Birks, 2012). This is achieved by fitting regressions of multiple response variables onto various explanatory variables, with the resulting matrix of the fitted values subjected to PCA (Legendre and Legendre, 1998). The standard result of a RDA is the total variance of a data set partitioned into constrained and unconstrained variance. The constrained variance is the variance explained by the explanatory variables, whilst the unconstrained variance is the variance not explained by the explanatory variables. Therefore, if the constrained variance is higher than the unconstrained variance, most of the variance is accounted for by the explanatory variables (Ter Braak, 1994).

3.3.1.1. Method

The pigments used in this analysis were alloxanthin, diatoxanthin, lutein-zeaxanthin, canthaxanthin, echinenone, okenone (if present), chlorophyll *a*, chlorophyll *b*, pheophytin *a*, pheophytin *a*, phaeophorbide *a* and β carotene. Prior to analysis, the sedimentary pigment

abundances were standardised using a log(x+1) transformation to normalise the data and to reduce the asymmetry of distribution (Legendre and Birks, 2012). This approach was used to address the issue of differential preservation or difference in measured concentrations of the algal pigments between sites. This transformation was carried out on the 0.5 cm intervals (for the top 30 cm) and 1 cm intervals for the remainder of each core SAMP2, MOH2, YAMBO2, BUNOT2, PANDIN and PALAK.

The transformed sedimentary pigment records from each of the study lakes were analysed using the indirect ordination method of principal component analysis (PCA) using CANOCO 5. This analysis was carried out on individual lake sediment records to determine change in individual lake systems. To create a combined PCA plot, all pigment samples for all lakes were used to allow for a direct comparison between the lakes with high and low aquaculture intensity. The data was analysed using the linear ordination method of PCA with the PCA axis 1 scores then being extracted (Legendre and Legendre, 2012). PCA axis 1 scores were used to reduce the pigment assemblage data to a single summarising value that was interpreted as algal community change. This was necessary as linear regression and variance partitioning can only be used on a single response variable.

A RDA ordination (using CANOCO 5) was completed on the contemporary pigment samples, to determine if any of the water chemistry variables (see section 3.2.1) correlated with the changes observed. The explanatory variables were also standardised using a log(x+1) transformation prior to analysis. Single constrained ordination was completed on each environmental variable individually to test its significance, with only variables with a $p<0.005$ being included in the RDA.

3.3.2. Generalised Additive Models (GAMs) to determine trends in algal pigments

GAMs are a regression based method for estimating trends (Simpson, 2018). They are a statistical model in which the usual linear relationship between the response and predictors are replaced by several non-linear smooth functions, to model and capture the non-linearities in the data (Birks *et al.*, 2012). Unlike Generalised Linear Models (GLMs), GAMs are not limited to a defined mathematical function, as they replace a defined function with a non-parametric smoother to uncover existing relationships (Equation 8; Simpson, 2018).

Equation 8*:* $y=\beta 0+f(x1)+\varepsilon,\varepsilon\sim N(0,\sigma 2)$

Where $\beta 0$ is the constant term, $f(x1)$ is the smoothing term and $\varepsilon,\varepsilon\sim N(0,\sigma 2)$ is the linear predictor.

It also has regularisation of predictor functions to avoid overfitting and can handle the irregular spacing of samples in time. The main underlying assumptions are that the functions are additive and the components are smooth (Birks *et al.*, 2012).

3.3.2.1. Why GAMs are appropriate

GAMs can handle non-linear, linear and non-monotonic relationships between response and predictor variables. GAMs are ideal to use when assumptions cannot be made on the specific mathematical function needed for the distribution, when there is non-linearity in the residual plots, which may suggest semi-parametric modelling and when a prior hypothesis suggests non-linear or skewed relationship amongst variables. GAMs are, therefore, ideal for these data, as they use automatic smoothness selection methods to objectively determine the complexity of the fitted trend. This allows potentially complex, non-linear trends, proper account of model uncertainty and the identification of periods of significant temporal changes. This permits an accurate statistical analysis of trends in this data. The immediate advantage of the GAM is that models are not restricted to the shapes of trends fitted via global polynomial functions such as in GLMs. Instead, the shape of the fitted trend is estimated from the data itself. GAMs have been used in multiple studies to model the relationship between species response in relation to environmental variables (Simpson and Anderson, 2009).

3.3.2.2. Method

The GAMs were fitted using the R packages ‘ mgcv’, ‘scam’ and ‘gratia’ using the statistical package R (version 4.0.2) The exact R code used is as per Simpson, (2018). These three

packages calculated the GAM model for each proxy and created a graphic, which fitted the model onto the data. GAM models were applied to the individual PCA axis 1 scores from each of the cored lakes to assess if there were any trends/patterns shown in the algal communities over time. Furthermore, GAMs were fitted to determine if change in algal community composition was proportional to aquaculture disturbance gradient. The first step in developing an appropriate GAM model, is to choose an appropriate smoother to measure the 'wiggleness' of the data. The smoother chosen needs to find an optimal value of λ (the smoothing parameter) that balances the fit of the model with model complexity to avoid overfitting (Simpson, 2018). Furthermore, λ needs to minimise the prediction error of the model. For this thesis, both REML (Restricted Maximum Likelihood) and GCV smoothers were used to explore which smoother resulted in the optimal fit. After the GAM was fitted to each dataset, the summary() function was used to check the significance of the smooth terms, to determine if a trend was statistically significant ($p=<0.05$). A REML smoother was used for all lakes (except Lake Palakpakin) as it was less likely to over fit (which results in overly wiggly fitted splines) compared to GCV smoothers and minimise prediction errors. However, due to the 'wiggly' nature of the PCA axis 1 scores from Lake Palakpakin, a GCV smoother resulted in a more optimal fit as the REML smoother missed the underlying trends in the data.

Table 3.6: List of basis dimension (k values) used for the GAMs fitted to PCA axis 1 scores from individual lakes to ensure best fit of the smoothing function

Lake	k value
Pandin	75
Yambo	70
Mohicap	60
Sampaloc	80
Bunot	80
Palakpakin	50

To fit a GAM using the REML or GCV selection, the size/ rank of the basis expansion (k value) has to be selected manually. The k value is the upper limit of the degrees of freedom associated with the smoother (e.g. the number of data points in the model). The exact number of k is not critical however, it needs to be large enough to pick out the underlying trends (Simpson, 2018). The gam.check() function was used to check if the basis dimension (k value) for a smoother was adequate (Wood, 2017). As part of the output of the gam.check() function, the estimated degrees of freedom (EDF) value is given. Essentially, the larger the EDF value, the more wiggly the fitted model. Values of around 1 tend to be close to a linear term. This effectively determines if there is any additional nonlinearity or structure in the residuals that can be

explained by further smoothing (e.g. increasing the k value). For all datasets, GAMs were run with multiple k values to determine the optimum EDF value to ensure the best fit of the smoother. This resulted in the use of a different k value for each lake (Table 3.6). A 95% confidence interval (95% CI) was applied to assess the usefulness of each model and to identify periods of significant change (Simpson, 2018). The 95% CI was created from with +/- 2*standard error of the estimate trend at each sample in time (Simpson, 2018). Furthermore, the gam.check() function produces four diagnostic plots to check the validity of the model (Figure 3.7). These are:

1. Quantile-quantile plots of residuals to identify outliers and heavy tails. The upper left normal (Q-Q) plot is very close to a straight line, suggesting reasonable distributional assumption. If the 'tails' start to veer offline this suggests that outliers are influencing the model.

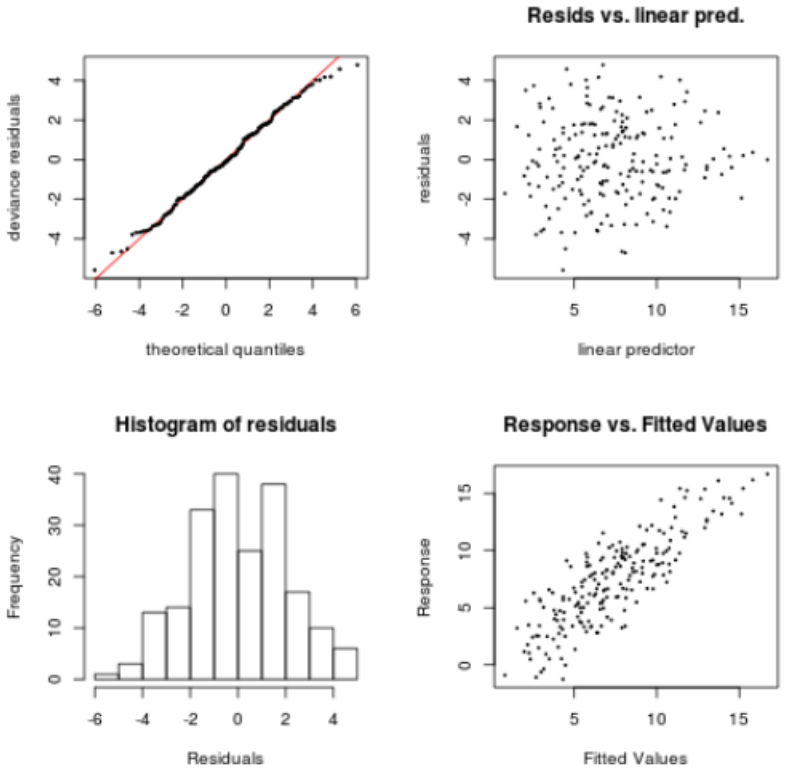


Figure 3.7: Ideal diagnostic plots for a fitted GAM. Top left) Q-Q plot, top right) Residuals vs. linear predictor, bottom left) Histogram of residuals and bottom right) Observed vs. fitted values

2. Histogram of residuals for detecting non-normality. If the histogram of residuals (lower left) is shaped like a normal distribution curve then the model is consistent with normality.
3. Residuals vs. linear predictor to find non constant error variances. If the data points in the upper right plot are scattered this suggests that variance is approximately constant as the mean increases.
4. Observed vs. fitted values to detect non-linearity, unequal error variances, and outliers to determine if there is any dependency within the data. A positive linear relation with a good deal of scatter (in the lower right plot) shows there is equal variance and no outliers skewing the model.

3.3.3. Determining periods of significant temporal change in GAM fitted PCA axis scores

GAMs were also used to determine periods of significant temporal change in the individual PCA axis 1 scores, as well as the pigment and isotopic records from each lake. This was done by determining if the first derivative at any time point of the fitted trend, is consistent with the null hypothesis of no change. This was done to see if the introduction/changes in aquaculture intensity corresponded with significant changes in algal communities. Confidence intervals are again calculated from the first derivative estimates using +/- 2*standard error. Periods of significant change are identified as those time points where the confidence intervals of the first derivative does not include zero. If the first derivative us indistinguishable from the value of zero that means there is no significant trend in the fitted model.

Determining periods of significant temporal change of the fitted GAMs was completed with the function ‘fderiv’ in the R package ‘mgcv’ (version 4.0.2).

3.3.4. Variance Partitioning

Variance partitioning involves partitioning the variance in a biostratigraphical sequence, such as photosynthetic pigments (Birks *et al.*, 2012) by the use of a series of direct gradient analyses (e.g. RDA). This technique attempts to estimate the fraction of variance explained by categories of measured explanatory variables (e.g. environmental) in relation to a response variable (e.g. algal community composition) (Borcard *et al.*, 1992; Hall *et al.*, 1999). Variation partitioning tests and determines the likelihood that these sets of explanatory variables explain patterns in a response variable (algal community composition)(Griffith and Peres-Neto, 2006).

This technique was ultimately chosen in order to quantify the extent to which algal pigment changes were caused by aquaculture and other variables (such as climate and land use). It allowed direct comparison between the study lakes to determine whether the variance explained by aquaculture, differs between the high disturbance lakes (Sampaloc, Bunot and Palakpakin) and the low disturbance lakes (Yambo and Pandin).

3.3.4.1. Variance Partitioning Method

Variance partitioning is sensitive to missing historical data (Hall *et al.*, 1999). Due to incomplete/missing historical data, variance partitioning was completed on post-1960 samples only. The analysis included the six stable carotenoids (β carotene, alloxanthin, diatoxanthin, lutein-zeaxanthin, canthaxanthin and okenone) (Table 3.7). However, since the number of carotenoids was less than the number of environmental variables, chlorophylls *a*, *b* and pheophytin *b* were also included. For Lakes Sampaloc and Mohicap, chlorophyll *a* and echinenone were reported as a single variable since they have a similar retention time and could not be separated out in the chronogram. The same pigments were analysed in all lakes wherever possible (Table 3.7). The one exception being the inclusion of okenone, an indicator for anoxia and stratification, which was not present in Lake Palakpakin.

Table 3.7: List of pigments used for variance partitioning in each lake
**coelutes with chlorophyll a*

	Lake					
List of pigments	Pandin	Yambo	Mohicap	Sampaloc	Bunot	Palakpakin
Alloxanthin (cryptophytes)	X	X	X	X	X	X
Diatoxanthin (siliceous algae)	X	X	X	X	X	X
Lutein-zeaxanthin (chlorophytes/cyanobacteria)	X	X	X	X	X	X
Canthaxanthin (cyanobacteria)	X	X	X	X	X	X
Okenone (purple sulphur bacteria)	X	X	X	X	X	
Chlorophyll b (chlorophytes)	X	X	X	X	X	X
Chlorophyll a	X	X	X	X	X	X
Pheophytin b (chlorophytes)	X	X	X	X	X	X
β carotene	X	X	X	X	X	X
Phaeophorbide *a*	X	X	X	X		
Echineone* (cyanobacteria)			X	X		

Table 3.8: Explanatory variables (post-1960) used in variance partitioning analysis on sedimentary pigments. Variables are listed according to the variable categories of climate, aquaculture and land use as described in the text

Variable category	
Climate (C)	ENSO Multivariate Index
	Annual precipitation (mm)
	Annual temperature (°C)
Aquaculture (A)	Surface area occupied by cages (%)
Land Use (L)	Population of San Pablo City
	Number of houses
	Area of coconut plantation (ha)
	Distance from San Pablo City (km)

Variance partitioning was carried out using the method described in Hall *et al.*, (1999) using CANOCO 5. Prior, to the analysis, all pigments and explanatory variables were log(x+1) transformed. The explanatory variables were split into three categories land use (L), aquaculture (A) and climate (C) (Table 3.8). As the focus of this thesis is to investigate the controls of long-term biological change rather than inter annual variability, fossil pigment and historical data were smoothed using a five-year running mean (2007-2011, 2012-2017 etc). As the sediment cores were collected from Lakes Yambo and Pandin a year later than the other cores, the historical data were smoothed using a five-year running mean using 2008-2012, 2013-2018 etc. As the number of samples inputted must match for both pigments and explanatory variables, the pigment sample inputted into the analysis were matched with the middle year of the five-year running mean, e.g. 2015 was matched with 2012-2017. Any empty data cells recorded as zeros and not missing values.

Constrained and partial constrained ordinations using direct gradient analysis (RDA; ter Blaak, 1988) were used to evaluate the relationship between the three categories of explanatory variables (C, A and L; Table 3.7) and changes in fossil pigments. Firstly, single constrained ordination was used for each variable separately to determine the % of variance explained and the p value for each individual variable. Only variables with a p value <0.005 were determined to cause significant change in fossil pigments and were included in the variance partitioning analysis. In the land use category, a number of the variables were strongly correlated. These

Table 3.9: List of variables used detailing which were removed at each stage, see table 3.5 for the full list

	Lake					
	Pandin	Yambo	Mohicap	Sampaloc	Bunot	Palakpakin
Original list of variables used	Population Housing Coconut Distance Aquaculture Temperature Precipitation ENSO	Population Housing Coconut Distance Aquaculture Temperature Precipitation ENSO	Population Housing Coconut Distance Aquaculture Temperature Precipitation ENSO	Population Housing Coconut Aquaculture Temperature Precipitation ENSO	Population Housing Coconut Distance Aquaculture Temperature Precipitation ENSO	Population Housing Coconut Distance Aquaculture Temperature Precipitation ENSO
Variables removed after single constrained ordination (e.g. p value >0.005)	Precipitation ENSO	Population Temperature ENSO	None of the variables were significant so variance partitioning was not completed on this lake	Precipitation ENSO	Precipitation ENSO	Population Precipitation ENSO
Variables removed due co-linearity	Housing Coconut Distance	Housing Coconut		Housing Coconut	Housing Distance Coconut	Housing Coconut
Final list of variables used	Population Aquaculture Temperature	Distance Aquaculture Precipitation		Population Aquaculture Temperature	Population Aquaculture Temperature	Distance Aquaculture Temperature

correlating variables needed to be removed, as their inclusion weakens the model, since each variable does not add new information but instead infuses the model with noise. This was completed using the CORRELL function in Microsoft excel, which determined that all land use variables were highly correlated ($r>0.95$). Therefore, only one variable from the land use category was included in the variance partitioning to best represent how sewage pressure from humans has impacted algal communities over time. Forward selection with Bonferroni correction was also completed on individual categories to determine if any of the variables were significantly correlated with the fossil pigment record. Bonferroni correction is used as sequential variables were measured to adjust the p value due to there being an increased risk of type I error (increased likelihood of a significant result by pure chance when there is not one) when multiple analyses are conducted on the same dependent variable (e.g. fossil pigment record). The variance inflation factor (VIF) was identified and checked it was <20 to make sure all collinearity within categories had been removed. For all analyses, 95% confidence limits were estimated using unrestricted permutations.

The next stage consisted of five steps (Hall *et al*., 1999). For all steps (Figure 3.9), a p value was used to determine the significance.

1) A canonical ordination, with no covariables was used to measure the total amount of variance (as the sum of all the canonical eigenvalues) attributed to all the explanatory variables (C+A+L; Figure 3.8) and the total of unexplained variance (100-(C+A+L)) in the fossil pigment records.
2) A number of partial canonical ordinations were used to calculate the variance explained by the effects of each individual category (C, A or L). This was completed by running ordinations of individual categories with the two other categories as covariables.
3) A series of partial canonical ordinations were used to calculate the pure effects plus first order interactions for each set of predictors (L+LA, L+LC, A+AC). For each run, one category of explanatory variable was paired with one of the remaining categories, acting as a covariable.
4) First order interactions (LC, LA, AC) were calculated by subtracting the values calculated during steps 2 and 3 (for example (LC= (L+LC)-L).
5) The second order interactions (LAC) were calculated as the difference between the sum of the variance explained in the first four steps (LAC=100-L-A-C-LC-LA-AC-unexplained variance) and 100%.

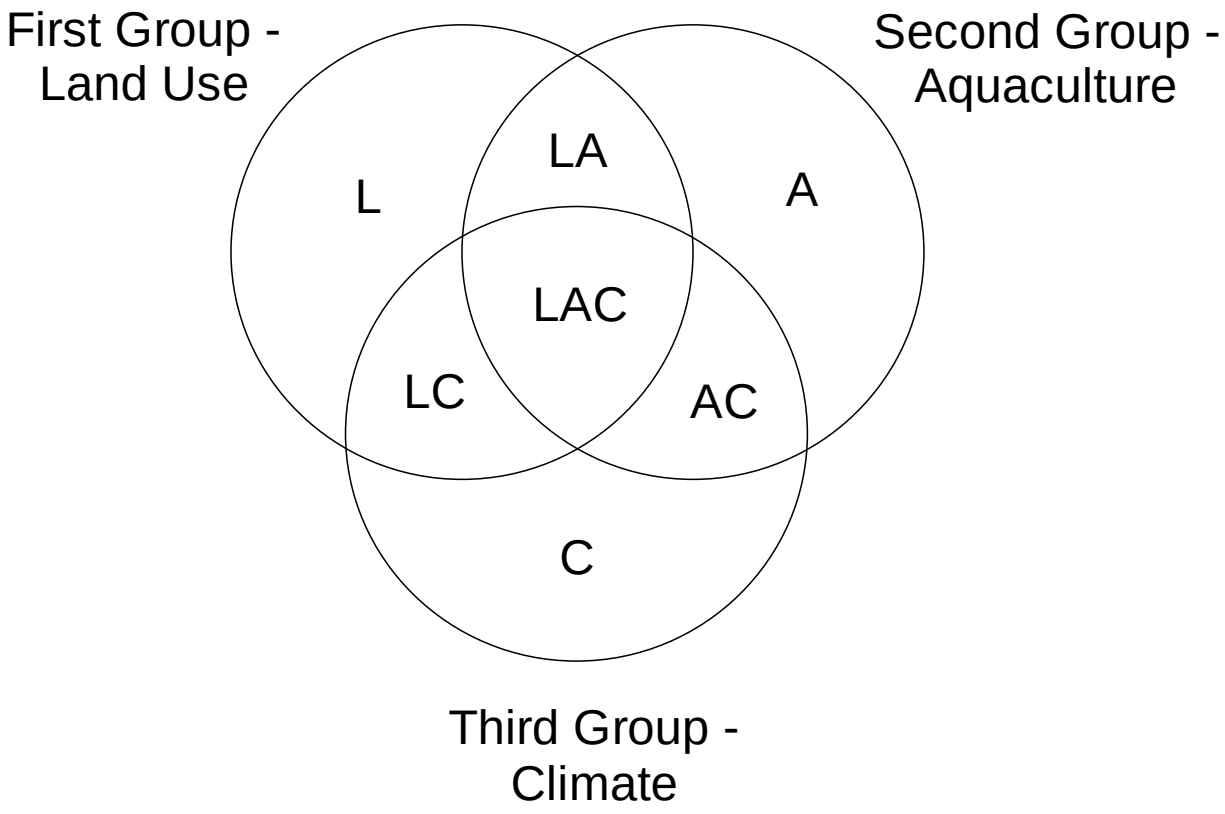


Figure 3.8: Example Venn diagram showing how variance is partitioned among three groups of explanatory variables. The letters correspond to the fractions (L, C, A) comprising of individual categories and first (e.g. LA) and second order interactions (e.g. LAC)

3.3.5. Linear Regression to determine the relationship between a lakes' physical characteristics and algal communities

Linear regression models describe the relationship between a response variable to a single regression models describe the relationship between a response variable to a single explanatory variable using a straight line, as shown in the equation below:

Equation 9 $Y=a+\beta_0+B_1*X$

Where: a is the intercept on the Y axis; β_0 is the slope which denotes the change in Y per unit change in the independent B_1 variable; X is the error term (McDonald, 2014).

Linear regressions are a useful step towards establishing environment-community relationships by producing a line of best fit (Legendre and Legendre, 2012). An adjusted R^2 value is used to test the strength of the relationship between an explanatory and a response variable, whilst taking into account the influence of sample size (Legendre and Legendre, 2012). The adjusted R^2 measures how close the data is to the fitted regression line, with a value of 1 meaning the values of the X variable can precisely predict the response in the Y variable (Legendre and Legendre, 2012). A p value (<0.005) shows a significant relationship between the two variables (McDonald, 2014). However, this analysis does assume the relationships are linear (Lepš and Šmilauer, 2003). Linear regression models have been used in many studies to explore the relationship between physical and physiochemical characteristics of lakes. Kraemer *et al.*, (2015) used linear regression models to determine which variables best predict lake stratification in multiple lakes worldwide. It determined that lake morphological variables and lake temperature are better predictors of lake stratification (as they explained a high % of variance) than lake warming rates.

Linear regressions were used to understand which physical site characteristics (lake depth, elevation, lake surface area, catchment area, CA:LA ratio) were important in determining change in algal communities (measured as Mann-Kendall coefficients of individual lake PCA axis 1 scores) and algal biomass (determined by mean β carotene concentrations from the sediment cores). Adjusted R^2 values and p values were used to determine the strength and significance of the relationship. The linear regressions for each of the five site characteristics was performed using the lm() function in the statistical package R (version 4.0.2).

3.3.6. Mann-Whitney U Test to determine the relationship between two categories of physical characteristics

Mann-Whitney U tests are a useful statistical technique to determine if two datasets are statistically different from one another, when the data is non-parametric (e.g. not normally distributed). Mann-Whitney U test assumes that the dependent variable is measured at an ordinal or continuous scale, that there are two categorical independent groups and the two variables are not normally distributed. This test can either determine the difference between the mean or the median of the two groups depending on the shape of the distributions. If the distributions have the same shape then the test will compare the medians rather than the means. The distributions had a different shape therefore, the Mann-Whitney U test was used to determine whether the means were statistically different. This test was carried out to on all proxies, pre- and post- aquaculture to determine if the introduction of aquaculture coincided with any significant changes in the proxy record. Mann-Whitney U tests were performed using the wilcox.test(y,x) formula in the R package 'pgirmess'. The p value was used to determine the significant difference between the two site characteristics.

4. CHAPTER 4 – LIMNOLOGY OF THE SIX LAKES

4.1. Introduction

This chapter describes the physical, chemical and biological aspects of the study lakes, to provide a better understanding of the contemporary functioning of these under-studied tropical lake systems.

4.2. Tropical Limnology

Lakes systems respond physically, chemically and biologically to environmental and climatic changes and as such, are excellent sensors of such change. However, climate and environmental changes influence lakes in many ways. Understanding these direct and indirect links can help to realise the potential of lake sediments as recorders of past climate and anthropogenic disturbance (Battarbee, 2000). The physical, chemical and biological characteristics of lakes, have been intensively studied in temperate regions, with long-term monitoring programmes (Beeton, 1963; Chapra, 1980; Muelbert *et al.,* 2019). In comparison, tropical lakes are less commonly studied, and the functioning of lakes at low latitudes (the tropics) is generally, poorly understood. Multiple studies have supported the idea that many (larger) tropical lakes do indeed show seasonality in mixing, stratification and phytoplankton succession (Talling, 1963; Cordero and Baldia, 2015). Although many processes are shared between temperate and tropical lakes, their physical, chemical and biological characteristics differ (Lewis, 1987; Danger *et al.,* 2009). Tropical lakes are potentially more sensitive to changes in temperature and nutrient availability than their cooler climate equivalents (Lewis, 1987; Adrian *et al.,* 2009), while N may be more limiting, and nutrient cycling more efficient (in tropical lakes), than previously thought (Abell *et al.,* 2012; Bannister *et al.,* 2019).

Deep tropical lakes (> 5 m deep) are usually only weakly thermally stratified with a much smaller temperature difference (thermocline) between the epilimnion and hypolimnion compared to temperate lakes (Kalff, 2002). This means that only limited changes in temperature and wind driven turbulence are needed to mix tropical lakes. Eutrophication further enhances the stability of stratification by increasing the concentrations of OM in the epilimnion, which sink to the hypolimnion, increasing the density of the deep water (Beadle, 1981; Singh *et al.,* 2019). The dissolved oxygen (DO) concentration of water is influenced by temperature, therefore, high temperatures in the hypolimnion of tropical lakes means there is less DO per litre than temperate lakes (Fukushima *et al.,* 2017). Higher productivity in the epilimnion of tropical lakes all year round can lead to a larger oxygen demand in the hypolimnion from decomposition of OM (that sinks from the surface waters). This results in

all deep tropical lakes becoming anoxic at depth (MacIntyre, 2012; Fukushima *et al.*, 2017). Even though tropical lakes experience less pronounced seasonal changes compared to temperate lakes, there is still a clear annual phytoplankton succession (in the Philippines, starting in January-February at the end of the wet season) of diatoms, chlorophytes, cyanobacteria and finally dinoflagellates (Lewis, 1986; Hubble and Harper, 2002), with nutrient and light availability being the two dominant controlling factors (Lewis, 1978; Ahmed and Wanganeo, 2015). However, there is evidence that eutrophication and climate change are influencing the phytoplankton succession in tropical lakes resulting in the dominance of cyanobacteria (Ferber *et al.,* 2004). Lakes in tropical East-Asia (TEA) are possibly the least studied and understood within the tropics, despite the region undergoing some of the highest rates of environmental change on Earth.

4.3. Water quality monitoring of the Seven Lakes of San Pablo

The Laguna Lake Development Authority (LLDA) began monitoring the surface water quality of the Seven Lakes of San Pablo in the 1980s in order to evaluate the impacts of anthropogenic activities on the lakes and to secure ecosystem services. Even though monitoring has taken place since the 1980s, only data collected from 1996-2008 was reported (by the LLDA) due to equipment malfunction and a lack of chemicals. This involved the monthly collection of DO, pH, total suspended solids (TSS), total dissolved solids (TDS), turbidity, chloride (Cl^-), nitrate (NO_3^-), ammonium (NH_4^+), inorganic phosphate (PO_4^{3-}), biochemical oxygen demand (BOD), chemical oxygen demand (COD) and chlorophyll *a* measurements. For Lake Yambo, water quality data was only collected between 2002 and 2008. Nitrate and inorganic phosphate concentrations have also been obtained from 2006-2016 for Lakes Yambo, Mohicap and Sampaloc (Bannister *et al.*, 2019). Bannister *et al.,* (2019) collected water column profiles of temperature, DO and pH quarterly (during February, May, September and November of 2017) from Lakes Yambo, Mohicap and Sampaloc to determine how these parameters change seasonally.

The LLDA monitoring determined that between 1996 and 2008:

NH_4^+ concentrations increased in all lakes (except Lake Palakpakin) with the largest increases observed in the Lakes Bunot and Sampaloc of up to 5000 and 2500 µg/l respectively (Figure 4.1). In comparison, there were modest increases in Lake Yambo and Pandin of 138 and 160 µg/l. Lake Palakpakin shows uniform NH_4^+ concentrations of 180 µg/l throughout the

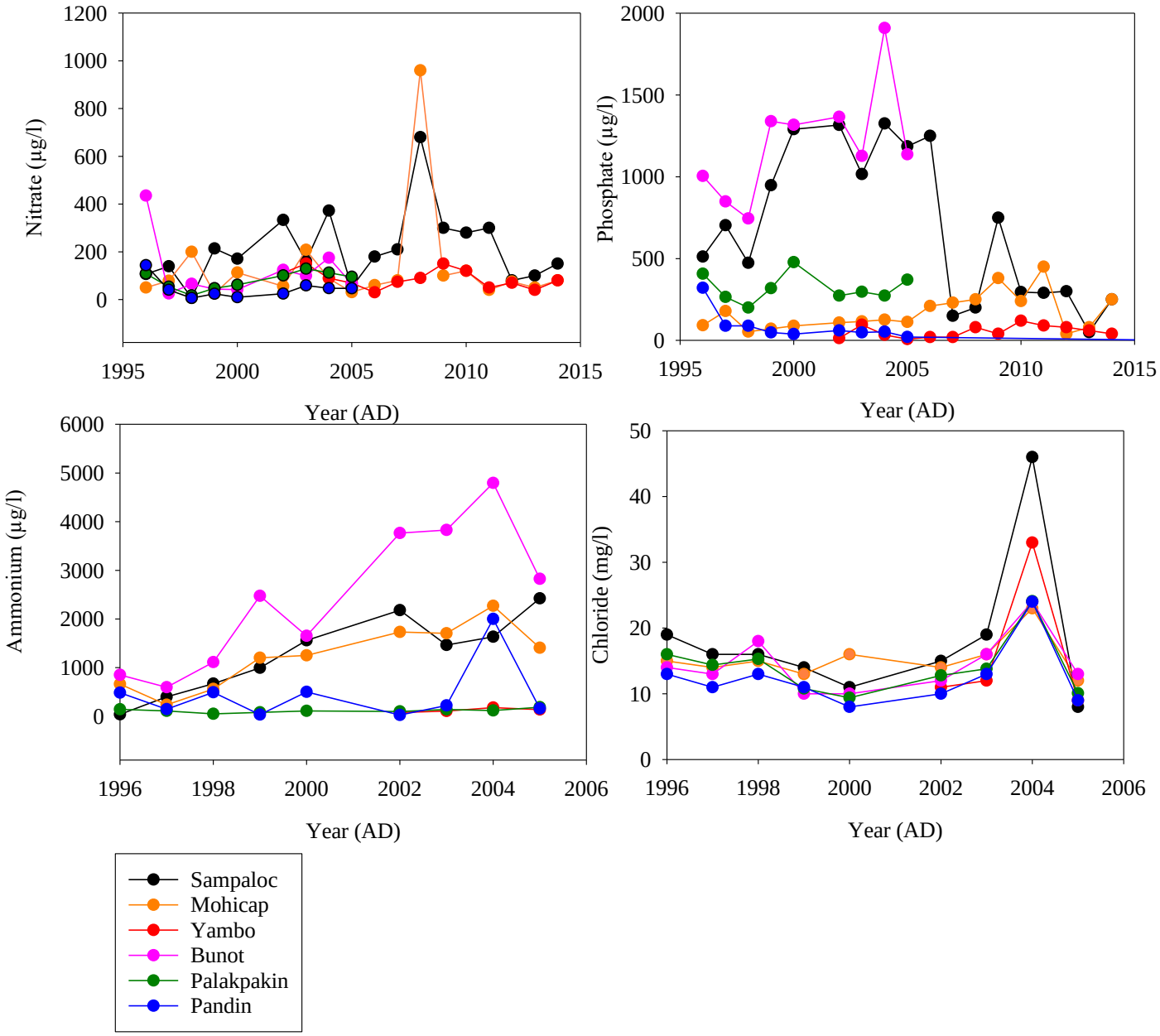


Figure 4.1: Mean annual values of nitrate, phosphate, ammonium and chloride from 1996 to 2006 (for ammonium and chloride) and from 1996 to 2016 (for nitrate and phosphate) from the study lakes (LLDA, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, , 2009; Bannister et al., 2019). All data from the LLDA, is presented as annual averages. These results will be compared with the measurements collected for this research, to determine temporal changes in the study lakes

monitoring period. There was an increase in PO_4^{3-} concentrations in Lakes Bunot and Sampaloc between 1996 and 2006, whereas there was no change in the other lakes. PO_4^{3-} concentrations declined in Lake Sampaloc to 250 µg/l in 2014. However, as the data for Lake Bunot is not available after 2006, it is not possible to determine if this lake follows the same trend. NO_3^- concentrations remained relatively constant for all lakes between 1996 and 2005 at <200 µg/l. Lakes Sampaloc and Mohicap exhibited a spike in values in 2008 to >600 µg/l. Finally, Cl^- concentrations remained relatively constant at 10 to 20 mg/l in all lakes. In 2004, Cl- concentrations spiked in all lakes (Figure 4.1), which could be due to equipment malfunction.

The data shows that during this period the least disturbed lakes (Yambo and Pandin) had lower levels of NO_3^-, NH_4^+ and PO_4^{3-}, compared to the more disturbed lakes (Bunot and Sampaloc). This observation is supported by the lake rankings in terms TP, chlorophyll *a* and secchi depth measurements (Table 4.1), with Lakes Bunot and Sampaloc again showing the highest values, in comparison to Lakes Yambo and Pandin. This suggests that Lakes Bunot and Sampaloc are the more productive and degraded lake systems than the relatively pristine Lakes Yambo and Pandin.

Quarterly temperature and DO profiles collected by Bannister *et al.*, (2019) do not show uniform values during the cold season (February), suggesting there is stable stratification and limited mixing in Lake Sampaloc. In this lake, DO concentrations reach anoxic condition (<2 mg/l) at a water depth <10 m. In Lakes Yambo and Mohicap, water column temperature measurements (of 25 and 26°C respectively) became uniform in February. This pattern was not observed in the DO profiles suggesting that an equal temperature between the epilimnion and hypolimnion did not result in mixing in these two lakes.

4.4. Aquaculture Intensity

Mean fish density was used to rank the lakes in terms of intensity of aquaculture. Annual data of aquaculture cage numbers was collated from a variety of sources (see section 3.2.3.5.4). Missing values were infilled using linear regression to ensure the data from each lake was at a similar resolution. For each lake, fish density was calculated for every year aquaculture was present (Table 4.1), as follows:

Equation 10:

No. of cages x no. of fish per cage* = total no. of fish in the lake per annum

* based on an average fish cage size of 10x15m with 5000 fish per cage (interview data, Appendix A).

Equation 11:

Total no. of fish in the lake per annum / Lake surface area (hectares) = Fish density (no. of fish/hectare) per annum

To calculate a mean value for cumulative aquaculture intensity over recent years, the following calculation was performed.

Equation 12:

Sum of annual fish density values / number of years aquaculture was present = mean fish density

This resulted in a disturbance gradient from lowest to highest mean fish density of:

Lake Pandin → Yambo → Mohicap → Sampaloc → Bunot→ Palakpakin (Figure 4.2).

Table 4.1: Ranking of the lakes according to mean aquaculture density since aquaculture was introduced to the lakes

Aquaculture criteria		
Mean Fish density (number of fish per hectare)*	Standard Deviation	Rank (lowest to highest)
10076	7721	Pandin
14787	13963	Yambo
56172	47886	Mohicap
56206	44075	Sampaloc
76343	50186	Bunot
85170	53656	Palakpakin

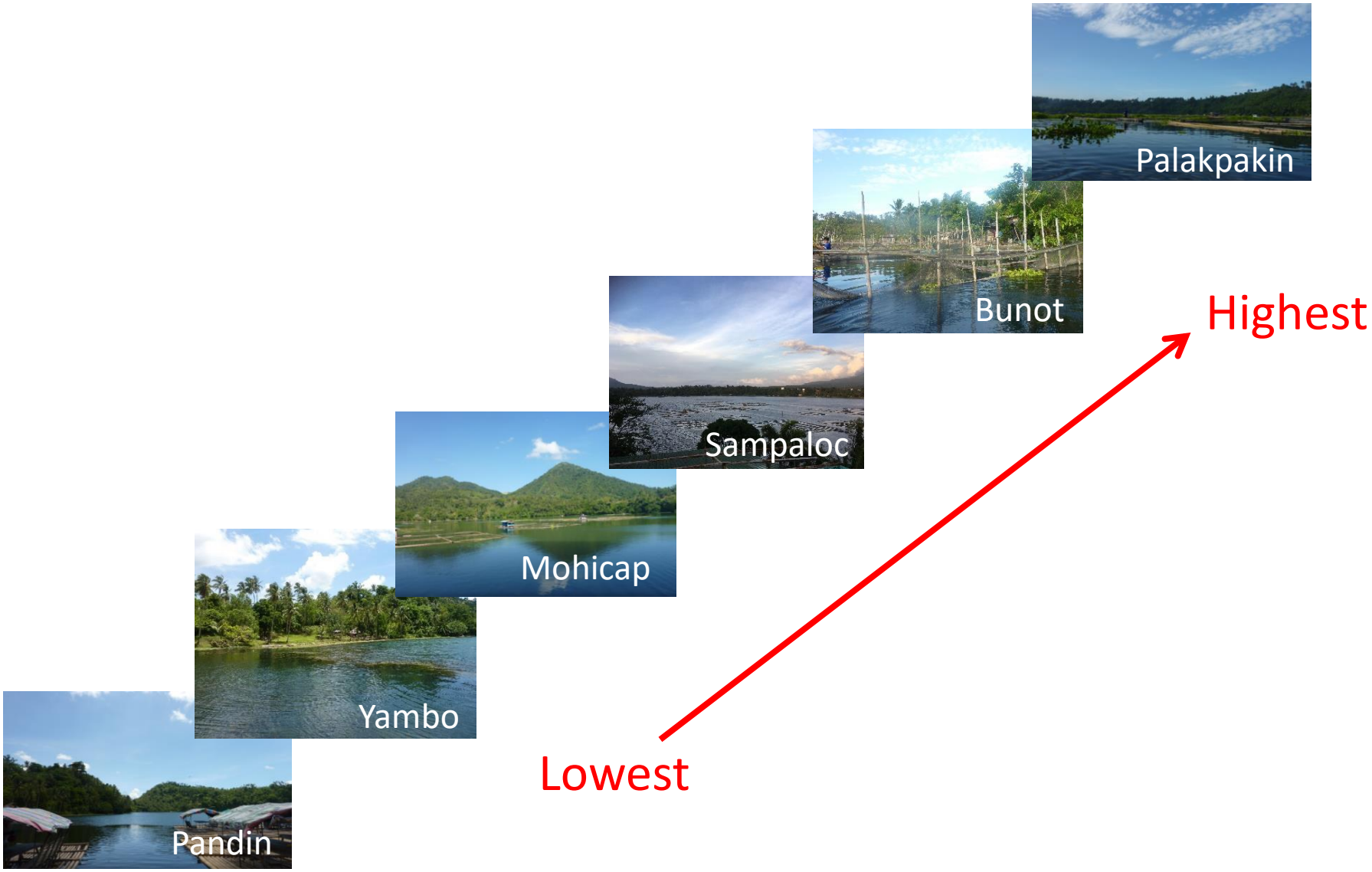


Figure 4.2: The study lakes ranked in order of aquaculture intensity (based on the results listed in Table 4.1)

4.5. Methods for contemporary sample collection and analysis

Water quality sampling took place at the study lakes in May 2017 (the end of the hot dry season). A YSI EXO1 multi-probe was used to collect water column profiles of pH, electrical conductivity, chlorophyll *a*, DO and temperature data in all lakes except Lake Calibato (see section 3.1.4). Water samples were collected from the surface, mid and bottom depths (Table 3.3) of each lake with a Van Dorn sampler. These samples were analysed for TP, SRP, NH_4^+, silicates, chlorophyll *a* and photosynthetic pigments (see section 3.1.5). For a description of the full methodology for each analysis, see Chapter 3.

Table 4.2: Morphological characteristics of the study lakes. Depth of the thermocline, anoxia (or near anoxic conditions**), Secchi depth, euphotic depth*** and the depth at which conductivity (chemocline) increases, are also stated*
** defined as the thin but distinct layer in which there is a substantial drop in temperature compared to the layers above and below*
***defined as DO concentrations <2.0 mg/l (Nürnberg, 1995; 2004)*
**** defined in section 4.6.2*

Lake	Date	Elevation above sea level (m)	Depth (m)	Surface Area (km^2)	Catchment Area (km^2)	CA:LA Ratio	Secchi Depth (m)	Euphotic Depth (m)***	Average Surface Water Temperature (°C)	Thermocline (m)*		Anoxia (m)**	Conductivity (m)
										Top	Bottom		
Pandin	April 2018	160	62	0.24	0.48	2.0	3.10	7.16	36.1	7.1	11.5	8.2	23.9 (shows an inversion between 7.1 and 23.9)
Yambo	April 2018	160	27	0.305	7.5	24.5	2.53	22.20	36.6	7.7	15.5	12.2	7.9 (shows a decline)
Mohicap	May 2017	80	30	0.23	6.8	29.5	1.58	4.28	35.6	3.9	7.0	5.3	5.3
Sampaloc	May 2017	106	27	1.04	10.7	10.3	0.98	5.25	36.8	2.7	7.4	3.9	5.3
Bunot	May 2017	110	23	0.305	20.9	68.6	0.67	2.21	37.2	2.0	9.0	4.5	14.7
Palakpakin	May 2017	100	7.5	0.48	10.05	20.9	1.73	2.47	36.2	0.3	6.1	3.9	5.0

4.6. Results

4.6.1. Morphometric and catchment characteristics

The study lakes have a variety of morphometric characteristics (Table 4.2). Lake depth ranges from 7.5 m (for the shallowest lake, Lake Palakpakin) to 62 m (for Lake Pandin). Lake Palakpakin is the only lake strongly influenced by rivers, with the Pagburga Steam (as an inflow) and the Padparan Stream (as an outflow). Lake catchments ranged in size from 0.48 km^2 (Lake Pandin) to 20.91 km^2 (Lake Sampaloc), with an average catchment size of 7.13 km^2. The catchments for Lakes Sampaloc, Bunot and Palakpakin were very flat (as shown by the digital elevation model; Figure 2.3) whereas the catchment sizes of Lakes Yambo, Pandin and Mohicap were smaller and characterised by steep slopes.

4.6.2. Physico-chemical characteristics

Along the aquaculture intensity gradient, secchi depths, the thermocline and euphotic zones shallowed (Figure 4.3). In comparison, electrical conductivity increased with depth in the high aquaculture lakes, but showed a decline in the low disturbance lakes (Figure 4.4). **S**ecchi depth (Table 4.2) varied between 0.67 m in Lake Bunot, to 3.1 m in Lake Pandin, correlating well with level of aquaculture (Figure 4.3). The euphotic depth (the depth at which net photosynthesis occurs at a light intensity of 1% of that of the surface) ranged from 2.21 m (Bunot) to 22.20 m (Yambo), following a similar trend to Secchi depth measurements (Figure 4.3). The order of light penetration (the depth of the water column at which light penetrations; from the deepest to shallowest) was Lake Yambo → Lake Pandin → Lake Sampaloc → Lake Mohicap → Lake Palakpakin → Lake Bunot.

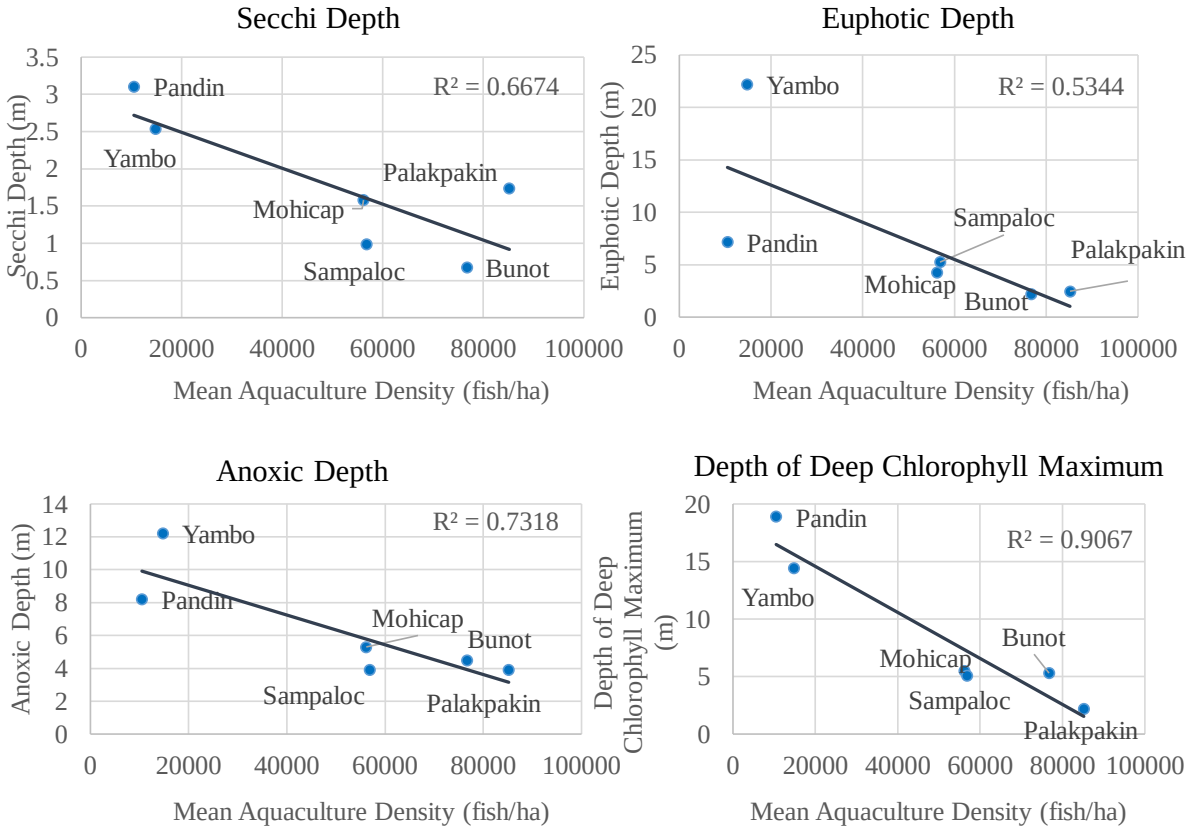


Figure 4.3: Scatterplots with a linear regression fitted showing the relationship between the secchi, euphotic, anoxic depth and the depth of the deep chlorophyll maximum against mean aquaculture intensity for the study lakes

Surface water temperatures ranged from 35.6°C (Lake Mohicap) to 37.2°C (Lake Bunot) with a median temperature of 36.5°C (Table 4.2; Figure 4.4). Temperature declined with depth in all lakes, declining most rapidly at the thermocline in all lakes (except Lake Palakpakin; Table 4.2; Kalff, 2002). The temperature profile of Lake Palakpakin declined more gradually with depth in comparison to the other lakes. The bottom temperatures of the lake waters varied from 30.5°C (depth 22 m, Mohicap) to 32.1°C (depth 28 m, Sampaloc) (Figure 4.5). In comparison, the shallowest lake, Lake Palakpakin had a warmer bottom temperature of 35.1°C (depth 6 m) (Figure 4.5).

All lakes showed anoxic or near anoxic conditions in the deeper waters, even the shallow lake, Palakpakin, at the sediment-water interface (Figure 4.5). DO concentrations of the surface waters varied between 14.3 mg/l (Lake Bunot) to 6.5 mg/l (Lake Palakpakin) with a median of 7.5 mg/l. DO concentrations declined with depth in all lakes, with a proportional relationship to aquaculture intensity (Figure 4.3) and followed the same pattern as the respective temperature profiles. DO concentrations declined in Lake Sampaloc at a depth of 3.5m, resulting in anoxic conditions (classified as <2 mg/l; Nürnberg, 1995; 2004) at depth 3.9 m (Figure 4.4). In comparison, the decline in DO in Lakes Pandin and Yambo was observed at a greater depth (Figure 4.5), with anoxia starting at a depth of 8.2 and 12.2 m respectively in the water column. The only deviation from this pattern was Lake Palakpakin, which had a more gradual reduction in DO concentration from 6.3 mg/l at the surface to 0.3 mg/l at the lake bottom.

The electrical conductivities of surface waters ranged from 208 µS/cm (Lake Yambo) to 408 µS/cm (Lake Sampaloc) (Figure 4.5), with the largest values occurred in the deep lakes with the highest levels of aquaculture (Lakes Mohicap, Sampaloc and Bunot). The electrical conductivity increased slightly with depth in all the lakes, except for Lakes Yambo and Pandin. The electrical conductivity profile gradually increased in Lake Sampaloc to the highest conductivity of 1019 µS/cm at the lake bottom. Lakes Mohicap, Bunot and Palakpakin had a sharp increase in electrical conductivity at the water-sediment interface. This increase was most likely reflecting measurements of the sediment-water interface. Lakes Pandin and Yambo had the lowest conductivities, which varied little throughout the water column, remaining stable at 226 and 208 µS/cm respectively.

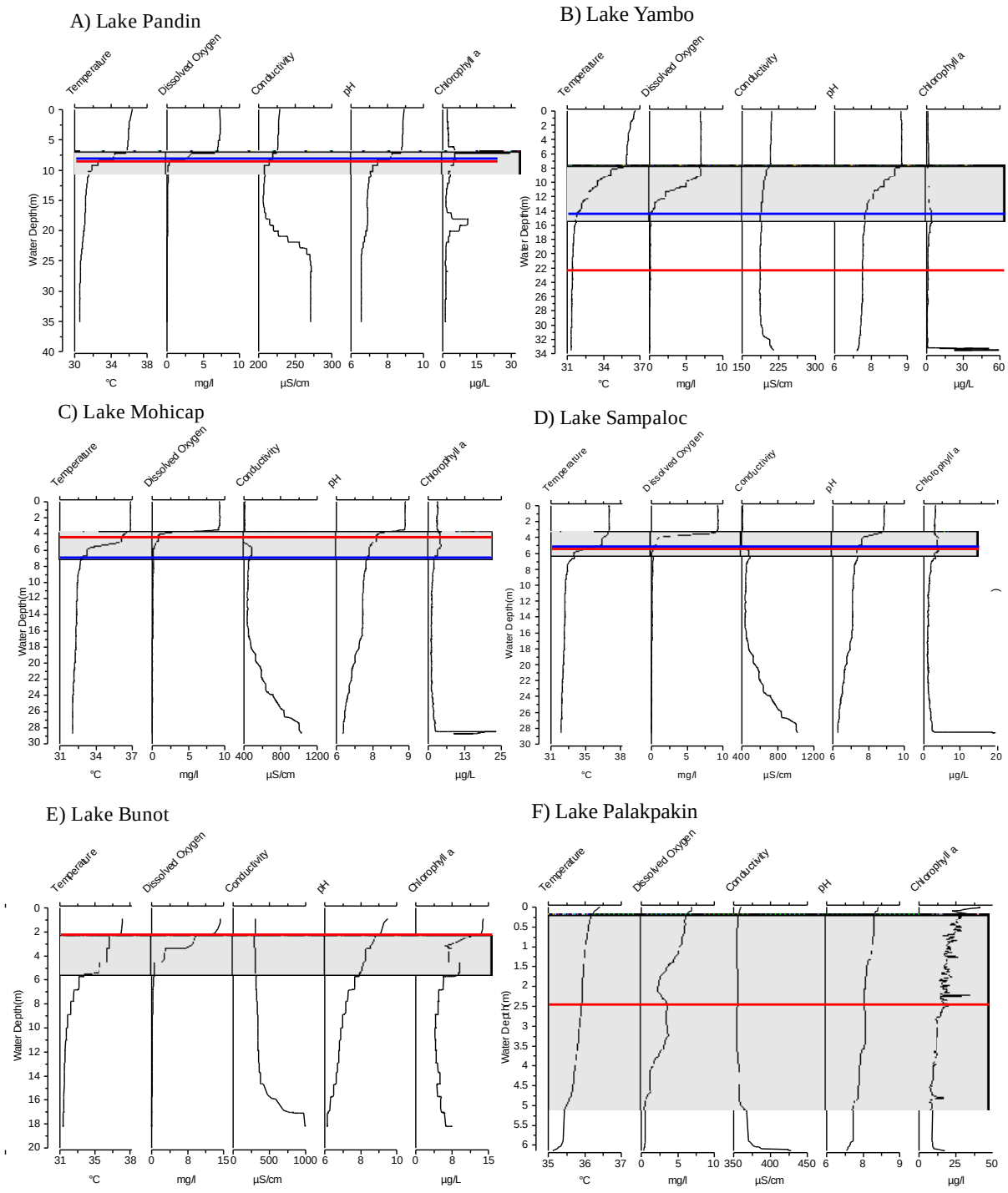


Figure 4.4: Limnological profiles showing temperature, dissolved oxygen, conductivity, pH and chlorophyll a concentrations for the study lakes. The light grey box highlights the location of the thermocline in each lake. The red and blue lines show the euphotic depth and depth of the bacterial plate respectively. This shows the uncoupling of the anoxic layer and the euphotic depth as the disturbance level increased. Depths of the thermocline and chemocline for each lakes are shown in Table 4.1

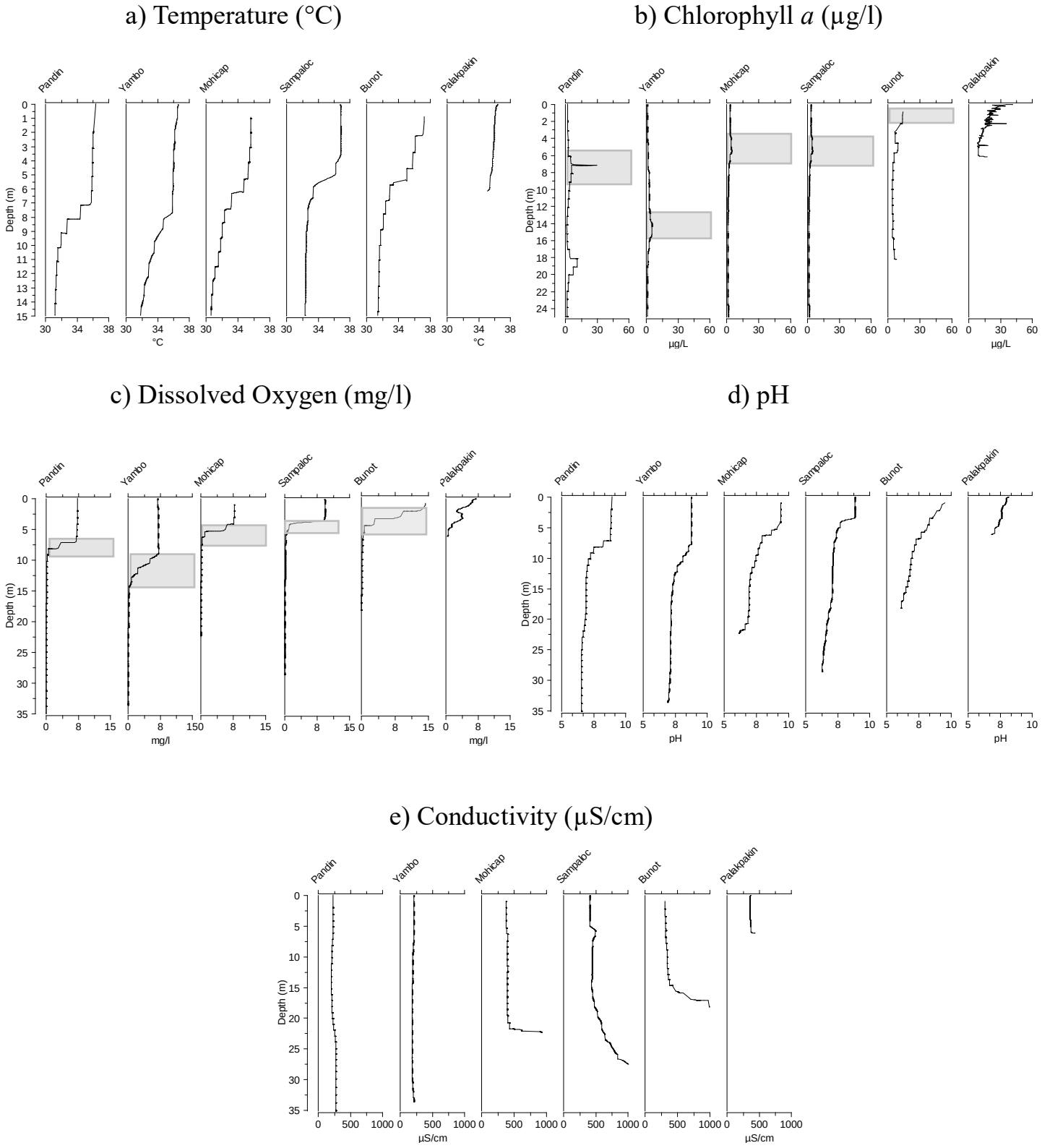


Figure 4.5: Limnological profiles showing temperature, dissolved oxygen, conductivity, pH and chlorophyll a concentrations on the same scale for the study lakes. The grey boxes show the shallowing of the anoxic layer (in the DO profiles) and the area where chlorophyll a is concentrated in each lake, which both shallow as the level of aquaculture increases

Surface waters lakes were alkaline, with pH in ranging from 7.97 (Lake Palakpakin) to 9.51 (Lake Bunot; Figure 4.4), while pH in the bottom waters ranged from 6.1 (Lake Bunot) to 6.91 (Lake Yambo). pH declined in all lakes with depth indicating a change towards more acidic conditions in the hypolimnion. Lake Bunot had the highest pH of 9.51, which declined to 6.28 in the lake bottom. Lakes Yambo and Pandin had less of a difference between the surface and lake bottom pH, with a difference of 1.82 and 2.37 units of pH respectively.

4.6.3. Ionic Composition

The highest total alkalinity (the concentration of CO_2^{3-} and HCO^- ions) was found in Lake Bunot (6.8 meq/l, depth 18 m) (Figure 4.6), whilst Lakes Yambo and Pandin had the lowest overall total alkalinity of <2.9 meq/l. Lakes Sampaloc, Bunot and Pandin had similar values throughout the water column of 3.5 and 2.1 meq/l respectively. Lakes Palakpakin and Mohicap showed high variability down the water column (Table 4.3; Table 4.4). Lakes Yambo and Pandin had the lowest Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-} out of all the study lakes and the concentration of these ions displayed an equal concentration throughout the water column (Figure 4.6). In the remaining lakes (Sampaloc, Mohicap, Bunot and Palakpakin) the concentrations of Mg^{2+} and Ca^{2+} were similar at c. 0.4 meq/l. Na^+ and K^+ concentrations were also similar in Lakes Sampaloc, Mohicap, Bunot and Palakpakin.

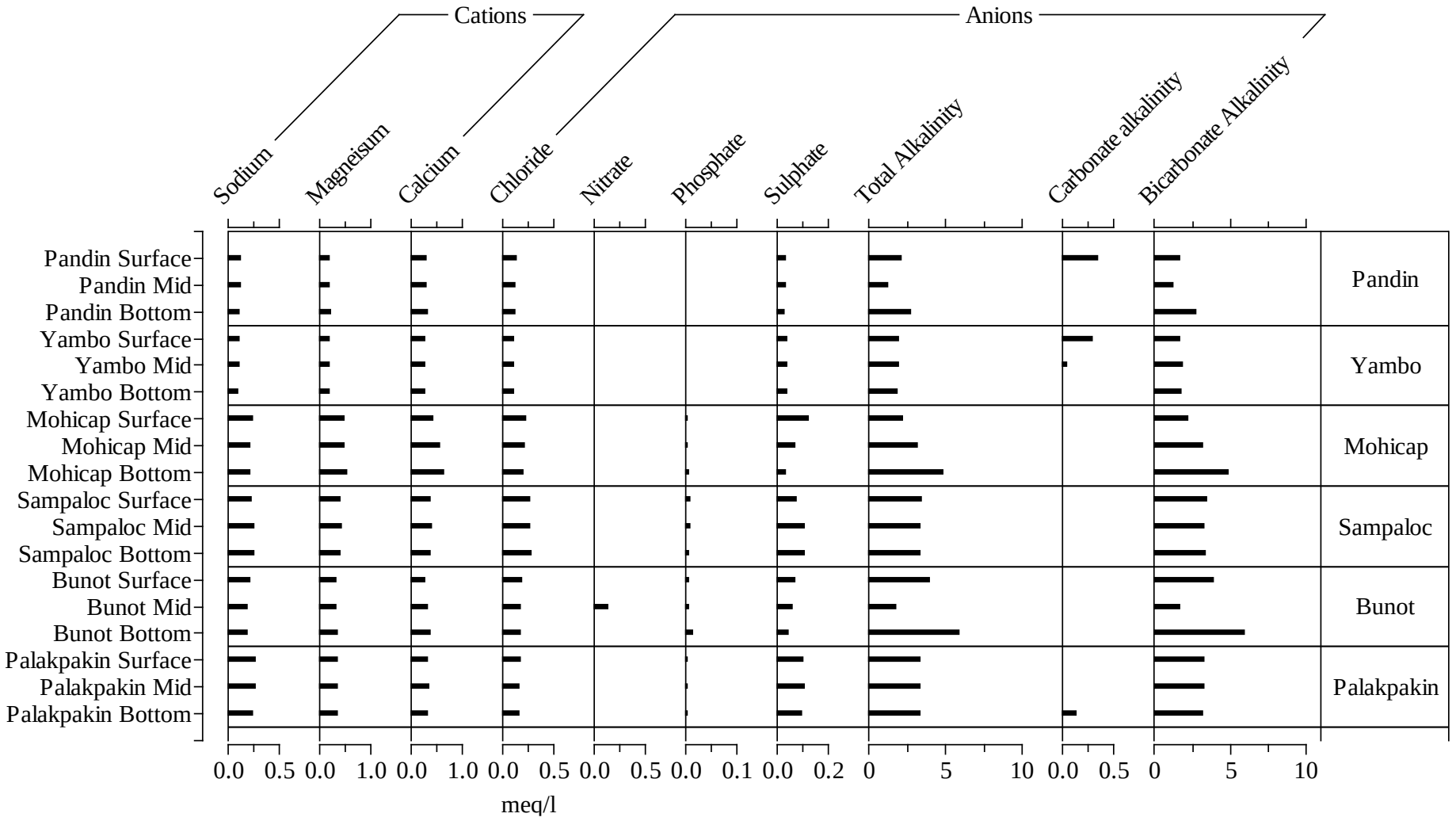


Figure 4.6: Measured ionic composition for the study lakes separated into anions and cations. As ammonium and phosphate do not analyse well on the IC, the values were removed

Table 4.3: Measured conductivity, pH and ionic composition for the study lakes including anions, cations and alkalinity in mg/l unless stated otherwise. BDL is abbreviated for Below Detection Limit (- missing data/not collected)

Lake	Depth (m)	Conductivity (µs/cm)	pH	Cations (mg/l)					Anions (mg/l)						
				Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Total Alkalinity (meq/l)	Carbonate Alkalinity (meq/l)	Bicarbonate Alkalinity (meq/l)
Pandin	0.5	227.1	8.88	3.168	BDL	1.489	2.609	6.338	5.017	BDL	0.008	1.822	2.15	0.35	1.8
	18	210.3	6.92	2.936	0.004	1.51	2.518	6.562	4.537	BDL	0.084	1.695	1.3	0	1.3
	37	270.8	6.56	2.817	1.923	1.589	2.775	6.982	4.794	BDL	0.0333	3.907	2.85	0	2.85
Yambo	0.5	210.6	8.74	2.599	BDL	1.267	2.501	6.009	4.358	BDL	0.014	1.957	2.05	0.3	1.75
	15	190.0	7.28	2.633	BDL	1.297	2.473	5.936	4.289	BDL	0.007	1.926	2	0.05	1.95
	35	214.2	6.91	2.520	BDL	1.225	2.505	5.574	4.228	0.155	0.013	1.921	1.9	0	1.9
Mohicap	0.5	407.8	8.85	5.638	BDL	1.797	6.009	9.003	8.305	BDL	0.028	6.202	4.1	0.3	3.8
	10	439.6	7.12	5.015	1.010	1.730	5.909	11.329	7.651	BDL	0.024	3.438	4.25	0	4.25
	25	769.2	6.40	5.013	1.970	1.956	6.688	13.185	7.394	BDL	0.092	2.184	6.75	0	6.75
Sampaloc	0.5	407.8	8.85	5.356	1.929	2.706	5.102	8.086	9.554	BDL	1.086	3.907	3.5	0	3.5
	10	439.6	7.12	5.959	BDL	2.817	5.227	8.184	9.777	0.257	0.900	5.416	3.4	0	3.4
	18	475.0	6.94	5.998	0.123	2.949	5.205	7.732	10.141	0.345	0.814	5.392	3.45	0	3.45
Bunot	0.5	309.6	9.51	5.235	BDL	2.498	4.122	5.750	6.795	BDL	0.748	3.503	4	0	4
	10	344.2	6.90	4.548	0.219	2.512	4.000	6.586	6.476	8.604	0.888	3.017	1.8	0	1.8
	18	990.4	6.15	4.571	1.961	2.797	4.464	8.117	6.326	BDL	1.656	2.178	6	0	6
Palakpakin	0.5	356.6	7.97	6.384	BDL	1.86	4.478	6.998	6.328	BDL	0.251	5.161	3.4	0	3.4
	3	356.2	7.62	6.400	BDL	1.832	4.541	7.183	6.028	BDL	0.255	5.198	3.4	0	3.4
	6	376.4	6.95	5.737	0.244	1.901	4.536	7.093	6.023	BDL	0.323	4.847	3.4	0.15	3.25

Table 4.4: Measured conductivity, pH and ionic composition for the study lakes including anions, cations and alkalinity in meq/l. BDL is abbreviated for Below Detection Limit (- missing data/not collected)

Lake	Depth (m)	Conductivity (µs/cm)	pH	Cations (meq/l)					Anions (meq/l)				Total Alkalinity	Carbonate Alkalinity	Bicarbonate Alkalinity
				Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}			
Pandin	0.5	227.1	8.88	0.138	BDL	0.038	0.217	0.317	0.143	BDL	0.000	0.038	2.15	0.35	1.8
	18	210.3	6.92	0.128	0.000	0.039	0.210	0.328	0.130	BDL	0.001	0.035	1.3	0	1.3
	37	270.8	6.56	0.122	0.107	0.041	0.231	0.349	0.137	BDL	0.000	0.033	2.85	0	2.85
Yambo	0.5	210.6	8.74	0.113	BDL	0.032	0.208	0.300	0.125	BDL	0.000	0.041	2.05	0.3	1.75
	15	190.0	7.28	0.114	BDL	0.033	0.206	0.297	0.123	BDL	0.000	0.040	2	0.05	1.95
	35	214.2	6.91	0.110	BDL	0.031	0.209	0.279	0.121	0.003	0.000	0.040	1.9	0	1.9
Mohicap	0.5	407.8	8.85	0.245	BDL	0.050	0.501	0.450	0.237	BDL	0.003	0.127	4.1	0.3	3.8
	10	439.6	7.12	0.218	0.056	0.044	0.492	0.566	0.219	BDL	0.006	0.072	4.25	0	4.25
	25	769.2	6.40	0.218	0.109	0.050	0.557	0.659	0.211	BDL	0.009	0.035	6.75	0	6.75
Sampaloc	0.5	407.8	8.85	0.233	0.107	0.069	0.425	0.404	0.273	BDL	0.011	0.081	3.5	0	3.5
	10	439.6	7.12	0.259	BDL	0.072	0.436	0.409	0.279	0.004	0.009	0.113	3.4	0	3.4
	18	475.0	6.94	0.261	0.007	0.076	0.434	0.387	0.290	0.006	0.008	0.112	3.45	0	3.45
Bunot	0.5	309.6	9.51	0.228	BDL	0.064	0.344	0.287	0.194	BDL	0.008	0.073	4	0	4
	10	344.2	6.90	0.198	0.012	0.064	0.333	0.329	0.185	0.139	0.009	0.063	1.8	0	1.8
	18	990.4	6.15	0.199	0.109	0.072	0.372	0.406	0.181	BDL	0.017	0.045	6	0	6
Palakpakin	0.5	356.6	7.97	0.278	BDL	0.048	0.373	0.350	0.181	BDL	0.003	0.108	3.4	0	3.4
	3	356.2	7.62	0.278	BDL	0.047	0.378	0.359	0.172	BDL	0.003	0.108	3.4	0	3.4
	6	376.4	6.95	0.249	0.014	0.049	0.378	0.355	0.172	BDL	0.003	0.101	3.4	0.15	3.25

4.6.4. Nutrients

The total phosphorus (TP) concentration in surface waters ranged from 40 µg/l (Yambo) to 1574 µg/l (Sampaloc; median value of 174 µg/l) (Figure 4.7). TP concentrations increased with depth in all lakes except the shallowest Lake Palakpakin, where the TP concentration remained constant at all depths at c. 400 µg/l (Figure 4.7). The TP concentrations in the hypolimnia varied from 43 µg/l (Yambo) to 3034 µg/l (Bunot; median value of 1241 µg/l). Soluble reactive phosphorus (SRP; a component of TP) ranged from 14 µg/l (Yambo) to 1086 µg/l (Sampaloc; median value of 138 µg/l; Figure 4.5; Table 4.1). SRP increased with depth in all lakes except Lake Palakpakin, where the SRP concentration remained constant at c. 300 µg/l following an identical pattern to the TP concentrations. Both TP and SRP concentrations increased along the aquaculture disturbance gradient, with Lake Palakpakin the exception to the pattern (Figure 4.8). The SRP concentrations in the hypolimnion varied from 13 µg/l (Yambo) to 1656 µg/l (Bunot; median value of 333 µg/l) (Figure 4.8). All lakes except for Lake Yambo had detectable concentrations of ammonium (NH_4^+). The highest concentrations of NH_4^+ (c.1900 µg/l) were found in the deepest measured depths of Lakes Bunot, Mohicap and Pandin (Figure 4.7; Table 4.4). Lake Sampaloc was the only lake to have NH_4^+ in its surface waters with a concentration of 1929 µg/l. The silicate concentrations in the surface waters across all sampled lakes ranged from 3.5 µg/l (Sampaloc) to 9.4 µg/l (Mohicap, median value of 6.8 µg/l) (Figure 4.7), with no proportional relationship to the aquaculture disturbance gradient (Figure 4.8). In all lakes, silicate concentrations generally increased with depth ranging from 1.9 µg/l (Lake Sampaloc) to 10.4 µg/l (Lake Mohicap, with a median of 9.0 µg/l).

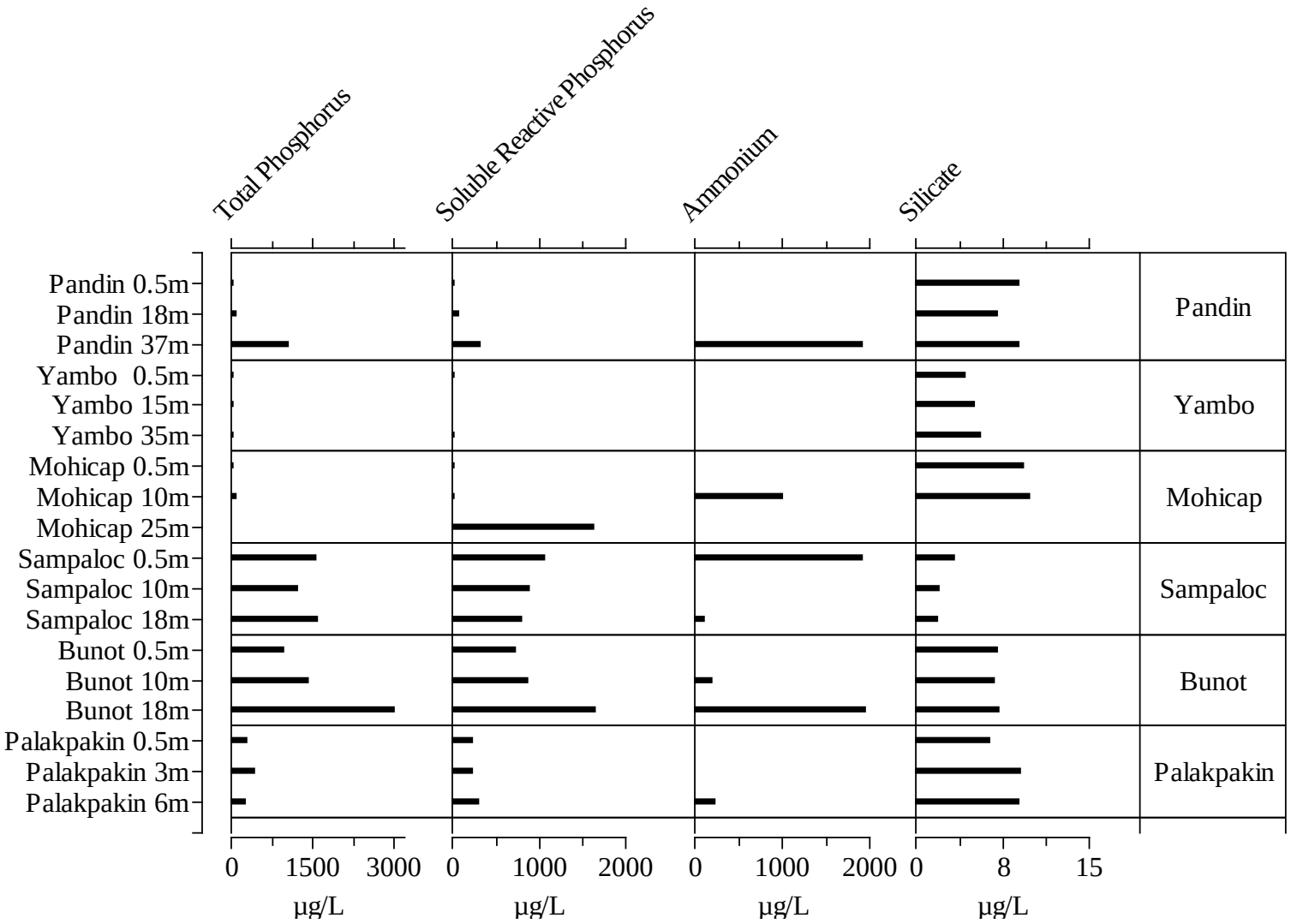


Figure 4.7: Plot showing changes in concentration of total phosphorus, soluble reactive phosphorous, ammonium and silicate, collected in May 2017 at three different depths from the water columns of the study lakes

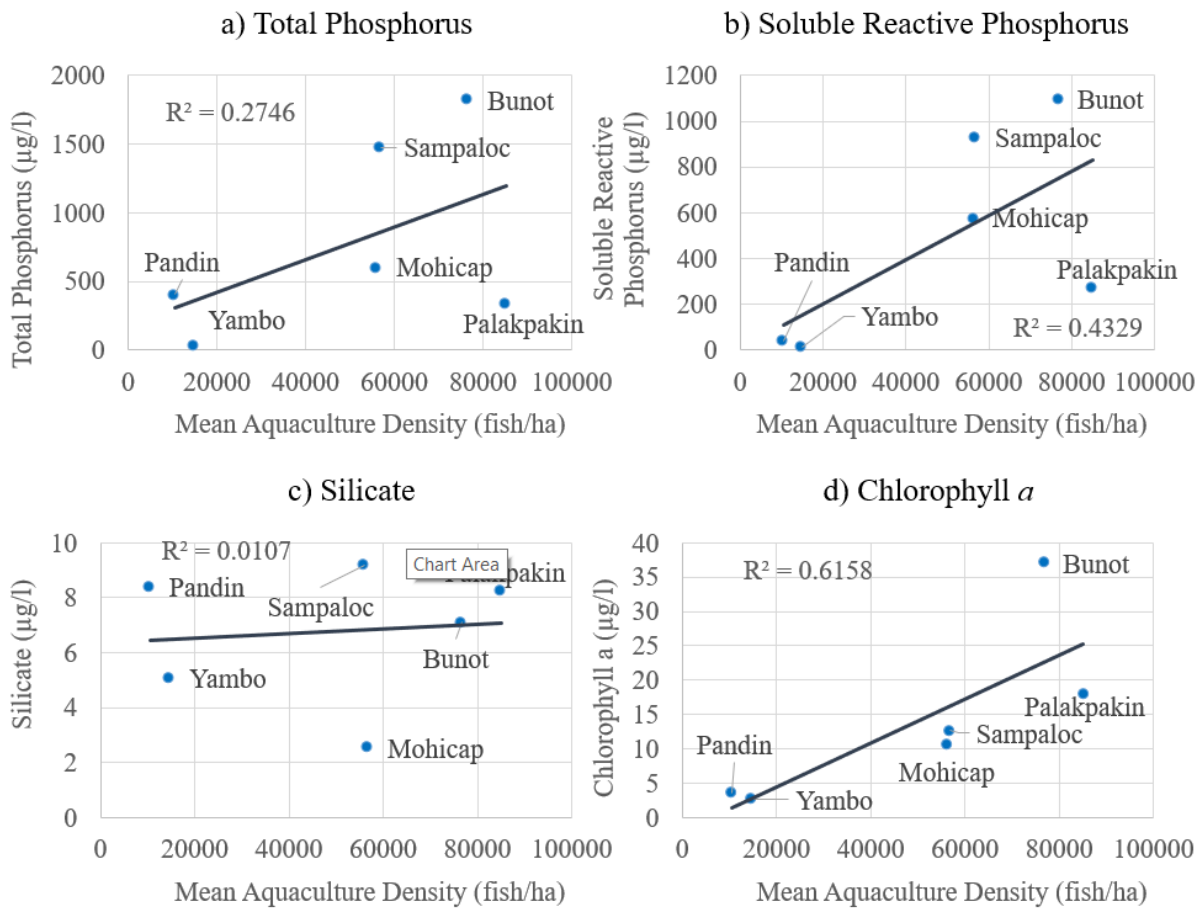


Figure 4.8: Scatterplots with a linear regression fitted showing the mean concentrations of a) total phosphorus, b) soluble reactive phosphorus, c) silicate and d) chlorophyll a in the study lakes against aquaculture intensity

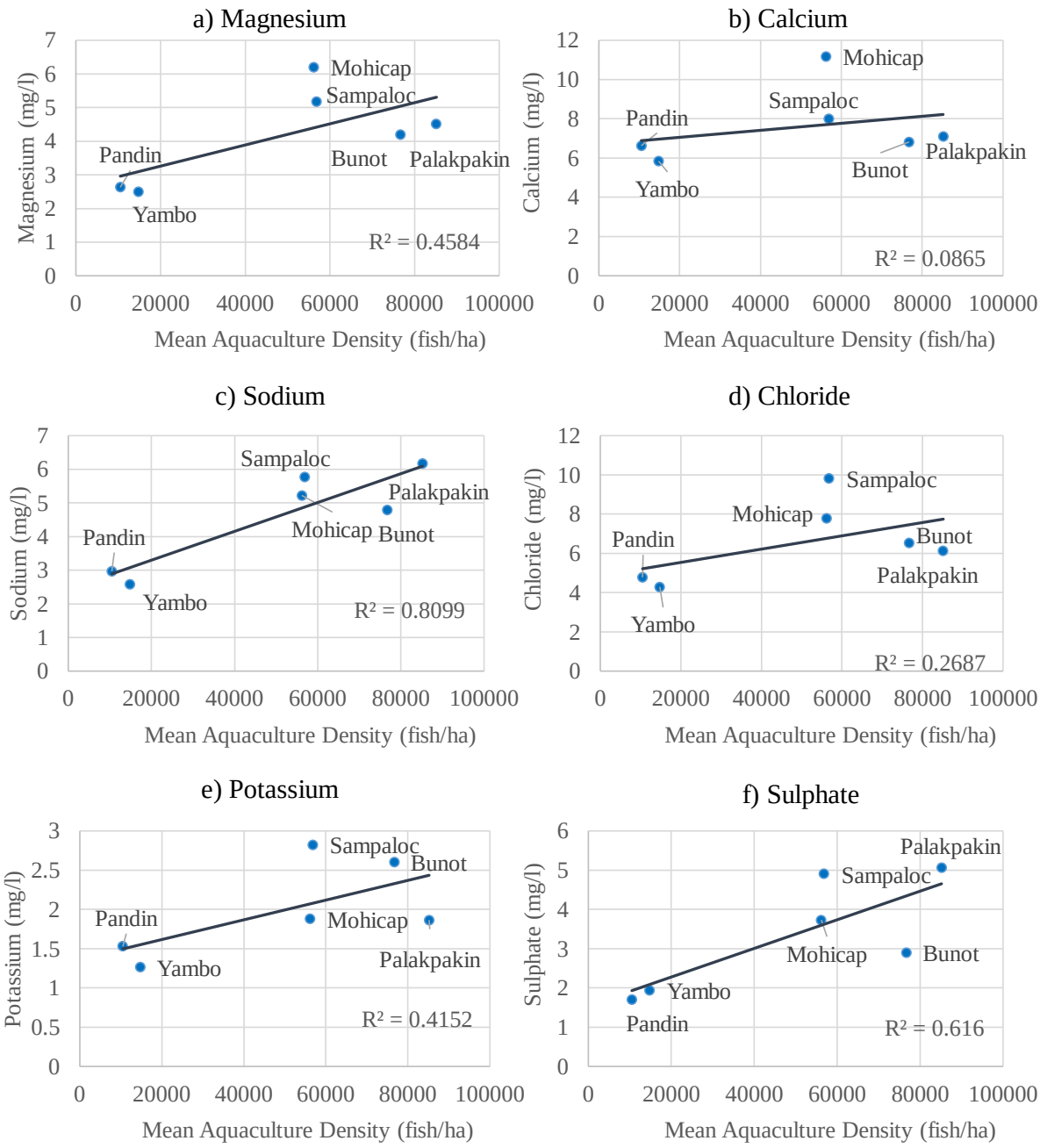


Figure 4.8 continued: Scatterplots with a linear regression fitted showing the mean concentrations of a) magnesium, b) calcium, c) sodium, d) chloride, e) potassium and f) sulphate in the study lakes against aquaculture intensity

4.6.5. Biological Characteristics: Chlorophyll *a*

The chlorophyll *a* water column profiles (taken by the YSI EXO1 multi-probe; Figure 4.4) showed that the shallowest lake, Lake Palakpakin had the highest chlorophyll *a* concentrations of all the study lakes of 41.5 µg/l at the surface, before gradually declining to 7.0 µg/l at the lake bottom (Figure 4.5). In the other lakes, the chlorophyll *a* depth profiles followed a different pattern, with concentrations of >3.0 µg/l at the surface (except Lake Bunot which had a higher surface value of 13.8 µg/l) and peak with values of 10.0 µg/l at the depth which coincides with the thermocline and oxycline. The peak in chlorophyll *a* concentrations is most likely the depth of the deep chlorophyll maximum (DCM) in the water column (Table 4.5). The depth of the DCM showed a proportional decline along the disturbance gradient (Figure 4.3). Lake Pandin exhibited two spikes of chlorophyll *a* concentrations (at depths 5.7 m and 11.1 m). The anomalously high chlorophyll *a* values observed at the deepest point of the water column profiles are most likely reflecting measurements in the sediment.

Table 4.5: Depth of deep water chlorophyll maximum in the study lakes

Lake	Depth of DCM (m)
Pandin	18.9
Yambo	14.4
Mohicap	5.5
Sampaloc	5.1
Bunot	5.3
Palakpakin	2.2

4.6.6. Photosynthetic Pigments

Pigment concentrations remained low in all lakes at all depths (Figure 4.9). Only low concentrations <0.03 nmol $g^{-1}$of β carotene, diatoxanthin (siliceous algae), chlorophyll *b* and pheophytin *b* were observed in Lakes Yambo and Pandin. In comparison, Lakes Sampaloc, Palakpakin and Bunot showed a wider variety of pigments from algal groups including cyanobacteria (canthaxanthin and myxoxanthophyll), siliceous algae (diatoxanthin and diadinoxanthin), and chlorophytes (lutein and chlorophyll *b*). These three lakes also had the highest levels of chlorophyll *a* and β carotene (indicators of total algal production) with the highest value found in the surface waters of Lake Bunot (3.2 and 0.5 nmol g^{-1} respectively). Concentrations of all algal pigments increased along the aquaculture disturbance gradient (Figure 4.10), with chlorophyte taxa showing a proportional change to the level of aquaculture.

Okenone (from purple sulphur bacteria) was only observed at the mid and bottom depths of the deepest lake, Pandin (Figure 4.9. The presence of okenone indicates anoxic conditions. Purple sulphur bacteria require illuminated anoxic conditions and, therefore, are often found at the bottom of oxyclines in high water clarity lakes, as this type of bacteria cannot grow under oxic conditions (Kalff, 2002). Purple sulphur bacteria require reduced forms of sulphur (e.g. S^{2-}) for anoxygenic photosynthesis and as a result are not found in oxygenated or dark waters. Many species of purple sulphur bacteria have flagellae for motility and gas vacuoles for buoyancy, allowing vertical migration in the water column for optimal nutrient and light conditions (Kalff, 2002). These characteristics mean that purple sulphur bacteria are likely to proliferate in stratified water columns that are undergoing eutrophication (due to the readily available supply of reduced sulphur from OM decomposition). However, algae in the epilimnion (which also become more prevalent under eutrophic conditions) can shade purple sulphur bacteria out, resulting in a decline in bacterial communities.

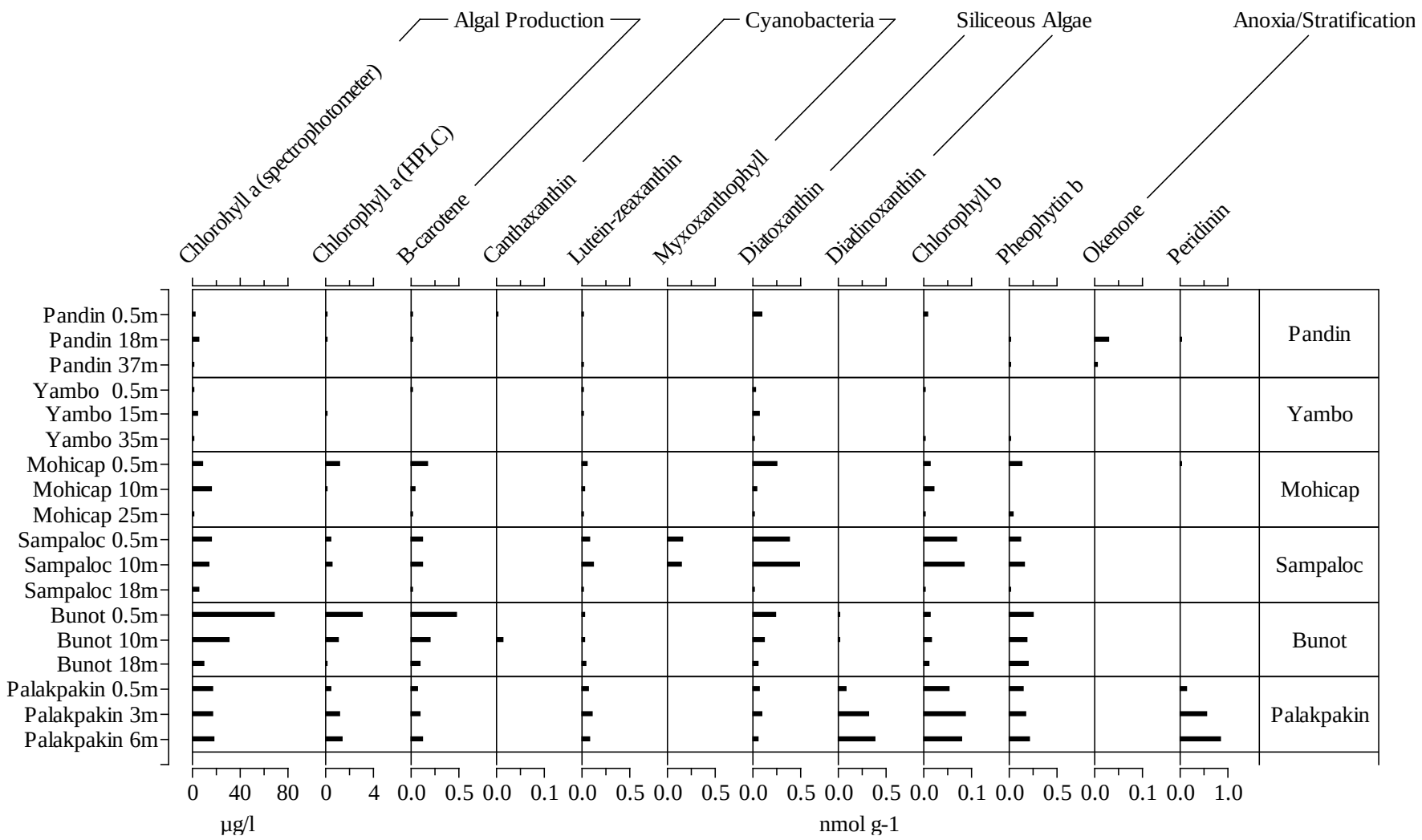


Figure 4.9: Measured photosynthetic pigment concentrations (completed using HPLC) for surface, mid and bottom depths from each of the study lakes. Chlorophyll a concentrations, determined using a spectrophotometer, are also shown. The pigments are grouped to show different algal groups/ indicators

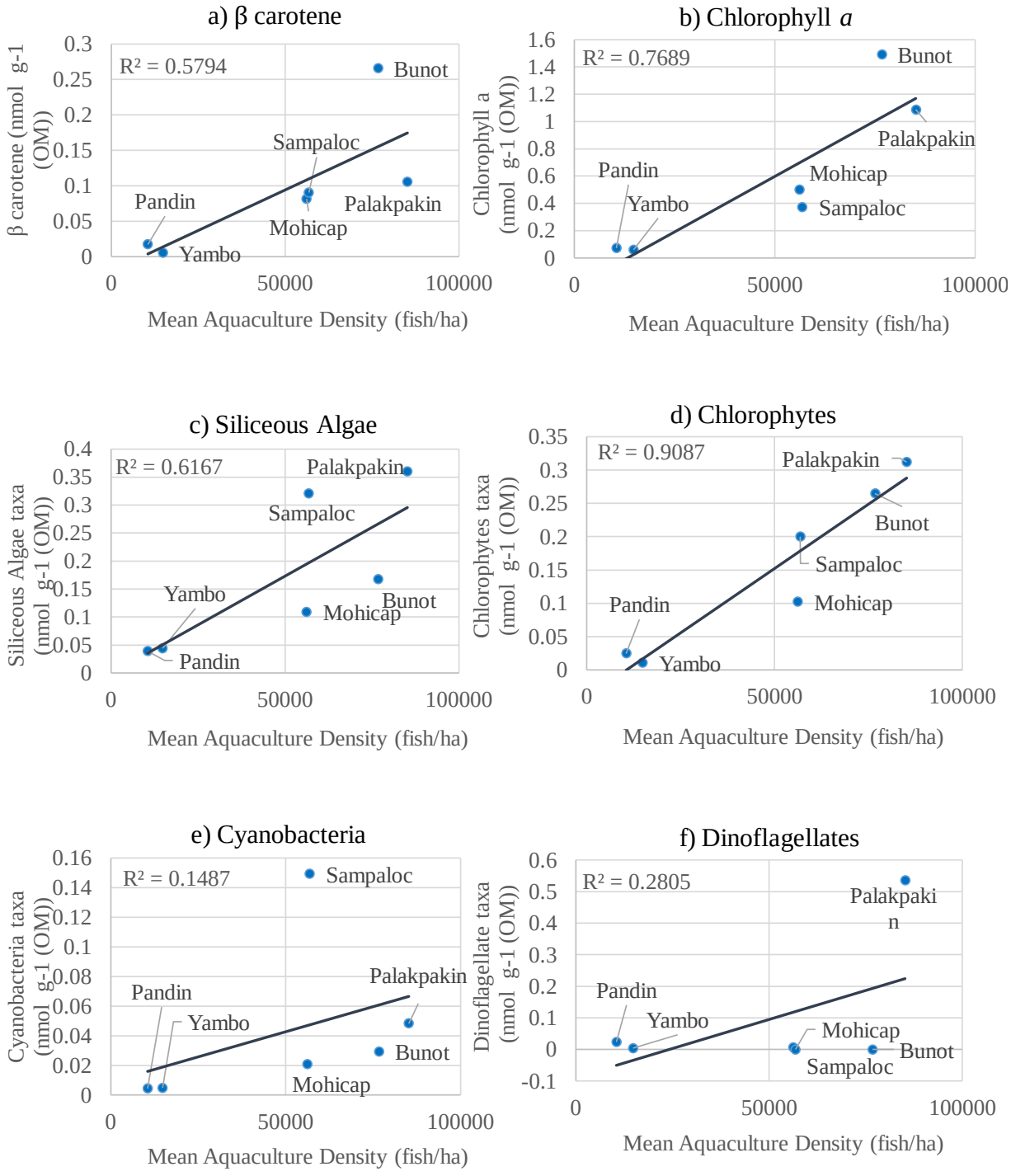


Figure 4.10: Scatterplots with a linear regression fitted showing the mean concentrations of a) β carotene, b) chlorophyll a, c siliceous algae (diatoxanthin and diadinoxanthin), d) chlorophytes (50% of lutein-zeaxanthin abundance, chlorophyll b and pheophytin b), e) cyanobacteria taxa (canthaxanthin, myxoxanthophyll and 50% of lutein-zeaxanthin abundance) and f) dinoflagellates (peridinin) in the study lakes, against aquaculture intensity

PCA was carried out on the water column pigment assemblages, to identify trends or clusters in the data (Figure 4.11a). Axis 1 explained 44.93% of the variance and axis 2 explained 19.4% of the variance. Several groupings were identified in the PCA. The first group consisted of samples from Lake Palakpakin, which were distinguished by high abundances of peridinin (dinoflagellates) and diadinoxanthin (siliceous algae including diatoms). The second group consisted of the surface and mid depth samples from Lakes Bunot and Sampaloc and the surface sample from Lake Mohicap. These samples were characterised by the presence of chlorophyll *a*, β carotene, the degradation product of chlorophyll *b*, pheophytin *b* and cyanobacterial pigments (canthaxanthin, myxoxanthophyll). The third and largest cluster of samples consisted of all the remaining samples, which consisted of mostly bottom water samples. This cluster was associated with the presence of okenone (purple sulphur bacteria) and low concentrations of chlorophyll *a*, β carotene and cyanobacteria (canthaxanthin and lutein-zeaxanthin). As okenone was only present in the mid and bottom samples from Lake Pandin, a second PCA (Figure 4.11b) was created, without okenone, to see if it led to a realignment of the remaining pigments. However, it led to minor change suggesting okenone was not driving the change in algal communities along axis 1.

RDA was completed on all samples and the 16 measured environmental variables (Figure 4.12), to explore correlations between water chemistry and pigment (algal) assemblages. Single constrained ordination was carried out on each variable individually to determine the % of variance explained by each variable and the p value. None of the variables were less than $p<0.005$, suggesting these environmental variables were not highly significant in explaining change in the contemporary pigment data. Only temperature (15.5%, p=0.02), Na^+ (15.9%, p=0.024), K^+ (15.9%, p=0.02), Mg^{2+} (14.7%, p=0.024) and SO_3^{2-} (4.1%, p =0.042) were included in the RDA. This suggests that temperature, Na^+, Mg^{2+} and SO_3^{2-} are most likely driving changes in algal communities.

There was a discrepancy in the two chlorophyll *a* measurements collected by HPLC and the spectrophotometer. These are due to the differences in analytical technique. HPLC measures pure chlorophyll *a* and therefore does not include other phorbins or degradation products (which are given separately), within the measurement compared to spectrophotometric measurements (Meyns *et al.*, 1994). To ensure consistency and reduce confusion, the chlorophyll *a* measurement from HPLC was used for any further discussion.

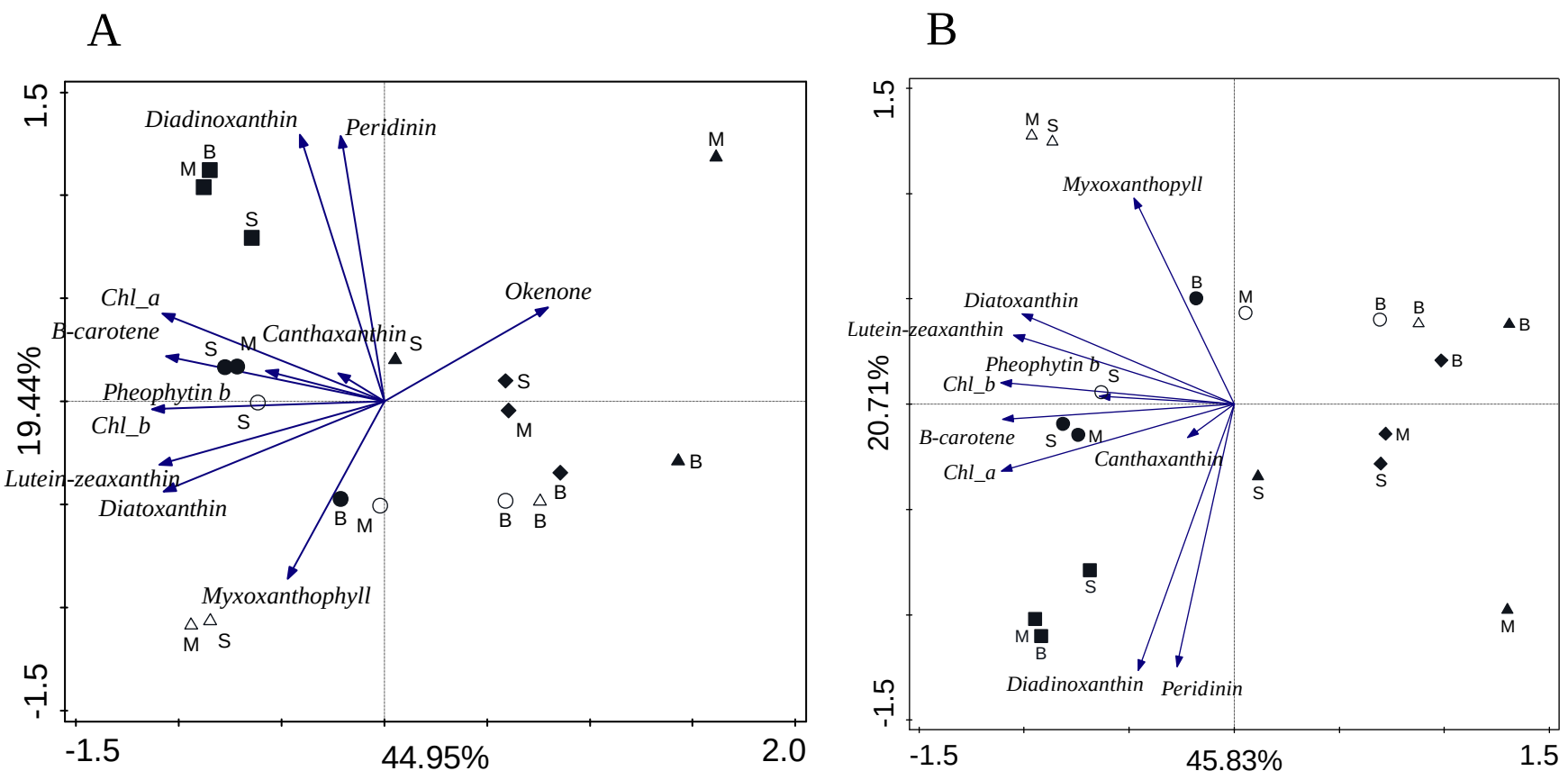


Figure 4.11: PCA biplot of the first and second principal components of algal community variability in the study lakes with A) all pigments and B) without okenone. The samples from each lake are shown with a different symbol (see legend) and further separated into surface (S), mid (M) and bottom (B) dictating where in the water column they were collected.

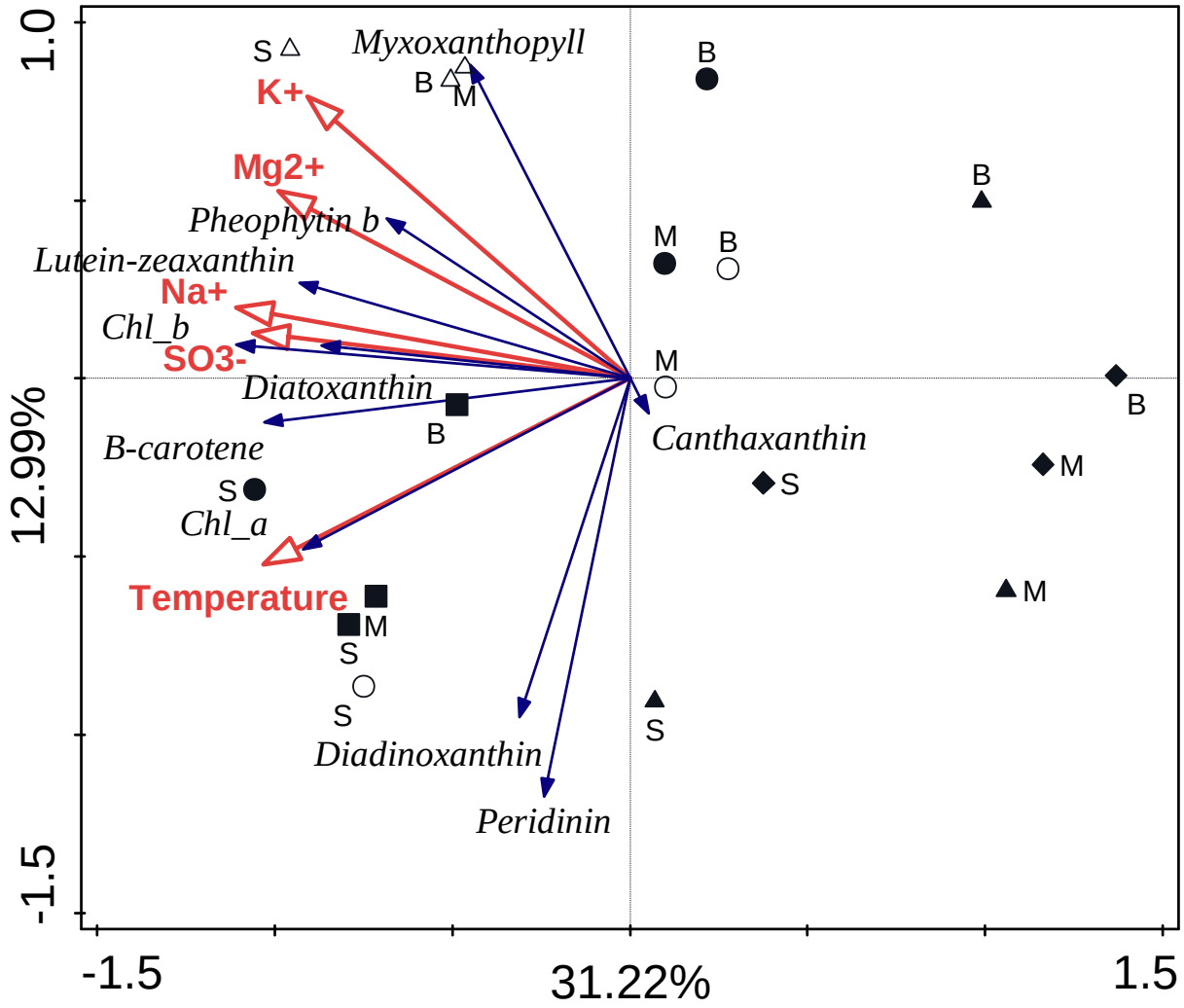


Figure 4.12: RDA biplot of the first and second principal components of algal community variability against environmental variables in the study lakes. The samples from each lake are shown with a different symbol (see legend) and further separated into surface (S), mid (M) and bottom (B) dictating where in the water column they were collected

4.7. Discussion

There are two outstanding characteristics of the study lakes. Firstly, they show limnological variability, even though they occupy a small geographical area. Secondly, they show a clear change associated with aquaculture intensity that indicates progressive eutrophication as aquaculture increases.

4.7.1. Physicochemical conditions in the water column

The vertical structure of physiochemical profiles changed along the aquaculture gradient (Figure 4.4: Table 4.2). The lakes with low aquaculture, Yambo and Pandin had the deepest thermoclines, oxyclines and euphotic depths in comparison to the lakes with high aquaculture (Bunot and Sampaloc). DO concentrations declined with depth in all lakes and followed the same pattern as the respective temperature profiles in each lake. This suggests oxygenation of the water column is mainly driven by lake mixing. In the low disturbance lakes, Lakes Yambo and Pandin, diffusion of oxygen from the atmosphere combined with high light irradiance, high water transparency, limited mixing and algal photosynthesis, meant the epilimnion was supersaturated with respect to DO. The high irradiance and water transparency allows phytoplankton to photosynthesise at a much deeper depth in the water column, so lower disturbance lakes have a deeper oxycline compared to high disturbance lakes (Figure 4.5). Furthermore, considerable OM input from the epilimnion in the high disturbance lakes resulted in elevated respiration and oxygen demand in the hypolimnion, progressively lowering DO concentrations, causing a shallowing of the anoxic layer (Kalff, 2002). In more eutrophic conditions, the dense phytoplankton blooms may get restricted closer to the surface, due to a lack of light penetration at depth from self-shading (Burson *et al.*, 2018). This is because phytoplankton must balance opposing gradients of light from above, with the availability of nutrients (Descy *et al.*, 2005; Leach *et al.*, 2018). This results in a shallowing of the anoxic layer and the thermocline (as sunlight can no longer penetrate as deep into the water column) and restricts the water volume for aquaculture. In Lake Bunot (which had the second highest level of aquaculture), the anoxic layer and euphotic depth did not intersect, resulting in a lack of suitable conditions for the growth of purple sulphur bacteria (Figure 4.4). The high pH measured in the surface waters of high disturbance lakes, likely results from increased photosynthetic productivity (Bwanika *et al.*, 2004; Henny and Nomosatryo, 2016). High algal productivity would further enhance stratification through the release of ions during decomposition in the hypolimnion. The large changes (of >100%) in the conductivity at depth observed in the high disturbance lakes (Bunot, Sampaloc and Mohicap), especially acute in

Lake Sampaloc, would aid density stratification of the lake waters, further preventing potential overturn that may occur due to thermal instability (Halfman, 1993). Lake Palakpakin has a different water column profile compared to the other lakes because it is a shallow polymictic lake (Figure 4.4), rather than having a steep thermocline (Table 4.2).

Deep tropical lakes (>10 m depth) tend to be monomictic (mix once per year) or meromictic and spend most of the year with stable stratification. In contrast, shallow tropical lakes tend to undergo mixing multiple times a year and are, therefore, more likely to be polymictic (Lewis, 1984; 2000). Multiple studies have shown that shallow tropical lakes undergo regular mixing of the water column (Talling, 2001; Lopes *et al.*, 2005; Xing, 2011) with water temperature being a major driver of changes in algal communities in these lakes. Lake Palakpakin exhibits a gradual decline in temperature, with DO following a similar pattern. Its shallower depth (7.5 m), high flushing rates (Brillo, 2016b) and shallow sided catchment, have resulted in constant mixing of the water column. Talling (2001) determined that in unsheltered shallow tropical lakes, wind induced circulation, prevents the formation of a persistent thermocline except during calmer periods. There is evidence at the time of sampling (May 2017, the end of the hot dry season), that there were anoxic conditions at the sediment-water interface. This was unlikely to have been caused by temporary stratification (due the lake's shallow depth) but was most likely caused by degradation of OM in the sediments drawing down the oxygen (Sobek *et al.*, 2009). These differences in conditions between Lake Palakpakin and the other lakes will have implications for interpreting the sedimentary record at this lake.

The lack of mixing in the water column resulted in anoxic conditions in the hypolimnion of the study lakes (at the time of sampling, May 2017), except in Lake Palakpakin (Nürnberg, 2007). Anoxia is widespread in many tropical freshwaters, especially given low-light conditions and reduced mixing (Chapman *et al.*, 1998), though it has been shown that the DO content of shallow lakes, can vary with seasonal rainfall (Chapman and Kramer, 1991). As a consequence, the hypolimnia all of the study lakes, except Palakpakin, are unlikely to mix completely as a result of any diurnal or seasonal mixing. A study by Bannister *et al.*, (2019), examined the temperature depth profiles of Lakes Mohicap and Yambo, and found that during the cooler months (December to February), the temperature between the epilimnion and the hypolimnion becomes uniform. However, the DO profiles showed distinct differences between the epilimnion and hypolimnion suggesting that this temperature differences does not cause mixing. Seasonal depth profiles were not collected from other lakes, however, it could be suggested that the other lakes with high aquaculture (Lake Bunot) could show the same trend.

The mixing of tropical lakes is predominantly controlled by wind, rainfall, and humidity rather than seasonal temperature changes, as seen in temperate lakes and lakes at higher altitudes (Beadle, 1966; 1981; Wood *et al.,* 1976). It is likely that increased eutrophication has intensified stratification of the high disturbance lakes through the associated impacts on light penetration (Mazumder and Taylor, 1994) and the chemocline (as discussed above; Camacho, 2009). It is probable that some form of 'seasonal' mixing of the upper waters (but not the bottom waters) occurs in most lakes (most notably after periods of heavy rains during the wet seasons). The steep-sided nature of many of the crater lakes, affords some protection from the wind (Gunkel and Beulker, 2009) and would reduce the likelihood of mixing in the water column. Although, it is likely that complete mixing in the deeper crater lakes (as suggested by the high electrical conductivity in the hypolimnion; Figure 4.4) is rare (lakes >5 m; (Beadle, 1966; Melack, 1978).

The timings of the seasonal partial breakdown in thermal stratification, in the colder months of December to February, coincided with fish kills and algal blooms (as reported by the fishermen; Appendix A). It is possible that the mixing caused the release of nutrients and/or toxic substances such as hydrogen sulphate and other reduced compounds (such as CH_4, NH_4^+, Fe^{2+}). These compounds accumulate in the hypolimnion during stratification and after mixing (Figures 4.7; 4.8), consumes most of the DO in the mixed layer (Wilhelm and Adrian, 2007). This could account for the major fish kills (interview data, appendix A) with the fresh supply of nutrients (from the deep water), encouraging the development of algal blooms (Kalff, 2002). This phenomenon has also been seen in a number of lakes of volcanic origin in the Philippines (such as Lake Lanao; Lewis, 1996). One possible explanation of the presence of fish kills and algal blooms could be due to the partial breakdown of stratification and mixing of the water column on an annual basis during the cold season (Beadle, 1966).

4.7.2. Major ion chemistry and the relationship to geology and other factors

The ion composition and electrical conductivity of the study lakes showed two distinct groupings along the aquaculture disturbance gradient (Figure 4.8). The first group consisted of the two low disturbance lakes Yambo and Pandin (characterised by lower ion concentrations) and the second group by the high disturbance lakes (characterised by higher ion concentrations; Figure 4.8). The main drivers of change to ion composition are geology, climate, vegetation and anthropogenic impacts (Kalff, 2002). As the lakes occupy a small geographical area (<16 km^2), it is assumed that changes in rainfall and temperature are uniform across the study area. The underlying geology may show some variation and could account for some of the

differences in alkalinity (Figure 4.6; see below). However, human disturbance within the individual lake catchments are the most likely cause of the majority of the differences between the two groups. Human waste, agricultural runoff and aquaculture increase the conductivity in lakes due to the addition of Cl^-, (Bhateria and Jain, 2016). The higher concentrations of Cl^- in the high disturbance lakes (Figure 4.9) could indicate the input of non-point sources such as human waste, from the heavily urbanised catchment (Marti *et al.*, 2004; Wu and Chen, 2013). Furthermore, LLDA monitoring data (LLDA, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2009) showed consistently higher Cl^- values in the high disturbance lakes (Palakpakin, Bunot, Sampaloc) between 1996 and 2005 (Figure 4.1). Kazi *et al.*, (2009) and Xu *et al.*, (2012) found that in Manchar Lake (Pakistan) and Zhangweinan River Basin (China), sewage input from domestic and industrial sources caused an increase in Cl^- in the sediments. The difference in ion concentrations between the lakes with low and high levels of aquaculture, could be as a result of increased sewage input from an expanding human population (the population of San Pablo City increased by 40,000 inhabitants during this period) (Bhateria and Jain, 2016).

The dominant anion in the study lakes was HCO_3^- from weathering and decomposition of OM (Chakrapani *et al.*, 2009) (Table 4.3). The concentration of HCO_3^- generally increased with depth, due to the build-up of OM in the hypolimnion. The predominance of Ca^{2+} and Mg^{2+} characterised by high (>0.8) $(Ca^{2+}+Mg^{2+}) / (Na^{+}+K^{+})$ equivalent ratios suggests the prevalence of carbonate weathering in the lakes, caused by the underlying geology (calc-alkaline to iron enriched volcanic rocks; section 2.2). The lower Ca^{2+} values of 0.29 meq/l in the surface waters of Lake Bunot could be due to the uptake of Ca^{2+} by phytoplankton (Ross and Gilbert, 1999). In comparison, the higher Ca^{2+} values in Lake Mohicap of 0.45 meq/l could be attributed to the release of additional Ca^{2+} by decaying phytoplankton combined with weathering processes (Khadka and Ramanathan, 2013). Low $(Na^{+}+K^{+})/Tz^{+}$ (Tz^{+} is the sum of base cationic charge) ratios of <0.4 suggests a low contribution of cations from aluminosilicate weathering (which is consistent with the local geology (Milkius *et al.*, 1991)). The high alkalinity (>2.0 meq/l) was probably due to leaching from the underlying bedrock of calc-alkaline to iron enriched rock (Milkius *et al.*, 1991).

4.7.3. Nutrients and eutrophication

Lakes with the highest levels of aquaculture had the largest observed concentrations of TP and SRP (Figure 4.8), with Lake Palakpakin being the exception to this pattern. Higher concentrations of TP (including SRP) are found in lakes which have undergone eutrophication due to increased inputs of P from anthropogenic sources (Kitkata *et al.*, 2002). The high levels

of SRP (>250 μg/l) in Lakes Bunot and Sampaloc (the lakes with the third and second highest aquaculture disturbance respectively) gives an indication that a greater concentrations of P is available for algae to utilise for photosynthesis (Grover, 2000). These lakes have experienced expanding human populations within their respective lake catchments, as well as high levels of aquaculture (see Chapter 5). In comparison, Lakes Mohicap, Yambo and Pandin have had lower levels of aquaculture and limited human settlement. The shallow Lake Palakpakin had uniform TP values throughout the water column (< 400 μg/l) and lower P values even though it has the highest aquaculture intensity (Figure 4.8) providing further evidence that this lake is well mixed. It also has high flushing rates (Brillo, 2016b) and, therefore, a shorter retention time, which removes P from the lake system at a faster rate, combined with low P delivery from Lake Pandin. The differences in P concentrations between the study lakes are probably caused by prolonged anoxic conditions (causing internal P loading), combined with increased anthropogenic P inputs.

Higher concentrations of both TP and SRP (in the bottom waters) in the deepest of the study lakes, may be partly due to the anoxic conditions and lack of light in the hypolimnion (Figure 4.75; Figure 4.8). Under low light conditions, SRP is not utilised by growth of photosynthetic organisms. Furthermore, the decomposition of algae could also release P back into the water column. TP (and SRP) concentrations would further increase in lakes with more pronounced anoxia, as this causes the release of iron bound P into the water column (Kowalczewska-Madura *et al.,* 2018). Kowalczewska-Madura *et al.,* (2018) found that SO_4^{2-} production (from bacterial sulphate decomposition) may exert an influence on P availability in sediments. SO_4^{2-} reduction can bind to iron in the sediment under anoxic conditions. P mobilisation and SO_4^{2-} reduction in anoxic sediment would generate a pH increase that would further inhibit P absorption (Clavero *et al.,* 1997). The lake with high aquaculture (Sampaloc) with abundant purple sulphur bacterial communities (which use S^{2-} to produce SO_4^{2-}) has the highest SO_4^{2-} in the mixolimnion as a result of SO_4^{2-} production from sulphur bacteria. These lakes also have a lower concentration of SO_4^{2-} in the hypolimnia suggesting that SO_4^{2-} could be binding to Fe^{2+} in the sediments (Figure 4.6). In Lake Bunot, there are lower SO_4^{2-} concentrations in the mixolimnion, which indicates that purple sulphur bacterial growth are limited due to shading from algal communities at the surface. Therefore, SO_4^{2-} is probably used as an electron acceptor by the host sulphate reducing bacteria (which thrive in anaerobic, unilluminated conditions) resulting in lower SO_4^{2-} concentrations and the production of S^{2-}. This indicates that lakes with

more abundant bacterial communities have less P being bound into the sediments, which, could be contributing to high levels of P in the hypolimnion of the high disturbance lakes.

A majority of the N observed in the study lakes was in the reduced form (NH_4^+) rather than its oxidised form (NO_3^-), could be due to a number of reasons including the assimilation of NO_3^- and the liberation of NH_4^+ during metabolism and decomposition by algae (Wetzel, 2001). The high concentrations of NH_4^+ in the surface waters of Lake Sampaloc, could have resulted from the direct discharge of sewage (as the lake is located in the centre of the city with an approximate population of 40,000 (Dimzon *et al.*, 2018)), from runoff from the concreted catchment and high levels of microbial decomposition in the epilimnion (Hampel *et al.*, 2018). NH_4^+ can be discharged directly into the water column as a component of human sewage, animal waste and industry (González *et al.*, 2004). NH_4^+ is toxic to fish at concentrations >2000 µg/l (Karasu Benli and Köksal, 2005), so high concentrations in the epilimnion has implications for aquaculture. NH_4^+ occurs in water bodies from the microbiological decomposition of N compounds in OM and has long been thought to be the preferred form of nitrogen for phytoplankton uptake (Patricia M Glibert *et al.*, 2016). As NH_4^+ is the preferred form of N, the lack of NH_4^+ in the surface waters of the study lakes, (except in Lake Sampaloc) could be due to phytoplankton uptake. The hypolimnion of Lakes Bunot, Mohicap and Pandin were characterised by high levels of NH_4^+, resulting from ammonification in the sediments and the intense decomposition and mineralisation of nitrogenous OM (Collos *et al.*, 2001; González *et al.*, 2004) at the oxic-anoxic interface (Kalff, 2002). Lake Yambo had a well-oxygenated epilimnion (which reached to a depth of 14 m) which would result in NH_4^+ concentrations remaining low as the NH_4^+ produced would be quickly oxidised to NO_3^-. This could explain why the low disturbance Lake Yambo contained a smaller concentration of ammonium (<2.0 µg/l), compared to the other lakes.

The concentrations of nutrients such as NH_4^+ and PO_4^{3-} in deep anoxic waters generally exceed their levels in the epilimnion of the study lakes, therefore, the fluxes of these nutrients from lower waters probably have an important influence on productivity in the epilimnion (Figure 4.7; Gulati *et al.*, 2017). This is because anoxia stimulates the release of P from sediments (see above). Combined with the removal of N through denitrification, this causes low N/P ratios in deep waters (Table 4.6). Mixing of these waters into the epilimnion is most likely caused by the episodic disruption of the thermocline resulting in the transfer of nutrients (Lewis, 2010). This has been observed in Lake Lanao, Philippines where mixing doubled the nutrient supply to the epilimnion (Lewis, 1996). However, all lakes do not have high values of P in the

hypolimnion. Lakes Sampaloc and Palakpakin have relatively uniform P values throughout the water column. Lake Palakpakin is a well-mixed shallow tropical lake that lacks a stratified anoxic layer, which could explain the uniform values. Lake Sampaloc is a stratified lake with a well-developed anoxic layer (Figure 4.4) and therefore the uniform P values could be explained by the input of P from urban sources within the catchment (which would also explain the high NH_4^+ values in the surface waters of this lake).

The increases in chlorophyll *a* observed in all lakes (relative to the concentration observed in the water depths above and below it) could correspond to the deep chlorophyll maxima (DCMs) in lakes (Table 4.5). DCMs vary between lakes and usually consist of the in situ growth of metalimnetic phototrophs including cyanobacteria, chlorophytes and diatoms (Camacho, 2006). Thermal stratification determines DCM depth because it can regulate nutrients mixing from deep waters into the euphotic zone (White and Matsumoto, 2012) and is an important factor in determining the depth of strong vertical gradients in nutrients. The DCMs of the study lakes also correspond to the light extinction depths (Table 4.1). This shows that DCMs form because phytoplankton must balance opposing gradients of light from above with the availability of nutrients, which often increase with depth under stratified conditions (Descy *et al.*, 2005; Leach *et al.*, 2018). The differing depths of the thermocline and, therefore, the increased availability of nutrients from mixing between the metalimnion and the hypolimnion, influences the depth of the DCMs at the study lakes (Figure 4.4).

4.7.4. Relationships between N, P and Chlorophyll *a*

Nutrient ratios in the lakes suggest that N rather than P probably limits phytoplankton growth in the study lakes. The relationship between TP and chlorophyll *a* was investigated. The results showed an insignificant relationship between the two variables (r^2=0.27, p=0.03) in the surface waters of the study lakes in 2017 (Figure 4.13), with the Chl:TP ratio ranging from 0.01 (Lake Sampaloc) to 0.22 (Lake Mohicap) (Table 4.6). This suggests that TP is important in determining chlorophyll *a* biomass. However it is commonly accepted that Chl:TP ratios >0.9 suggest a strong P limitation, whereas low Chl:TP ratios (<0.2) indicate some factor other than P is limiting phytoplankton biomass (Spears *et al.*, 2013). The lack of nitrogen in the surface waters of the lakes suggests N (Table 4.6) limits phytoplankton growth in the study lakes.

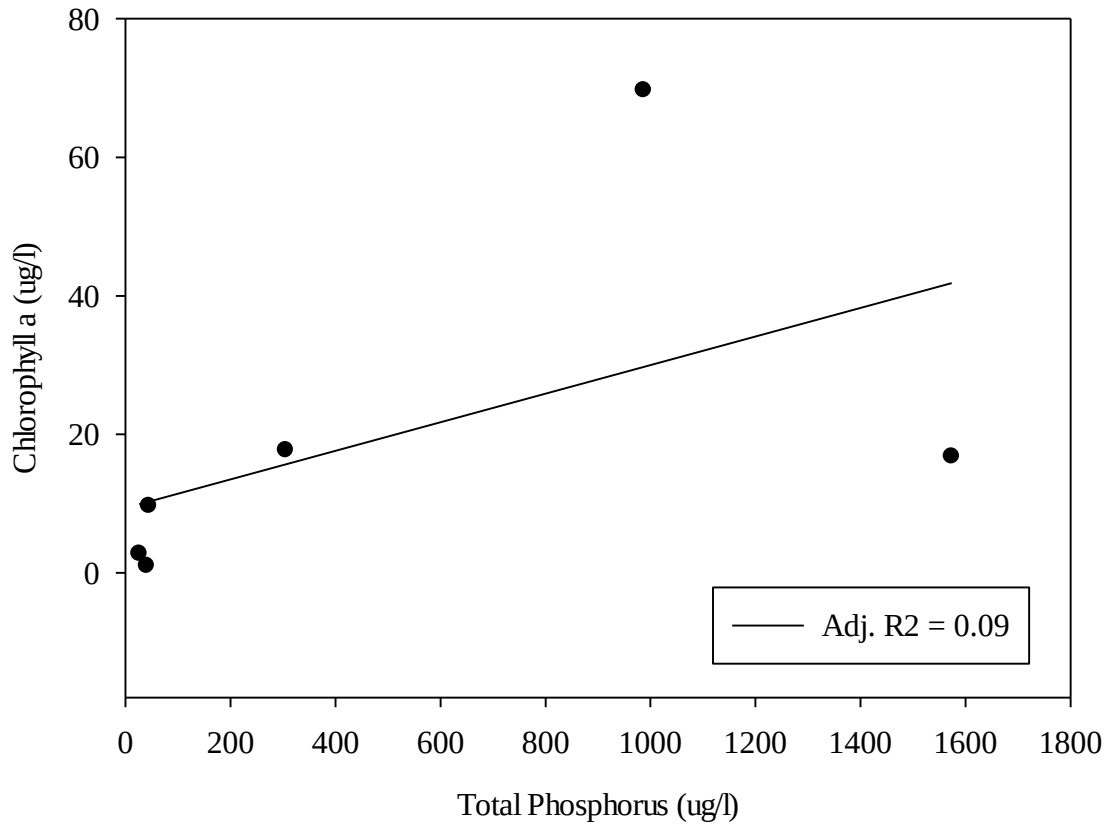


Figure 4.13: Total phosphorus vs. Chlorophyll a of the study lakes. The solid line represents the trend line with the R^2 value shown

Table 4.6: List of nutrient concentrations for the study lakes based on chl: TP and DIN: SRP indictaing the limiting nutrient . DIN is the sum of nitrate and ammonium

Lake	TP (µg/l)	Chlorophyll *a* (µg/l)	Chl:TP Ratio	Nitrate (µg/l)	Ammonium (µg/l)	SRP (µg/l)	DIN: SRP Ratio	Limiting Nutrient
Pandin	26.25	2.77	0.11	0	0	8.93	0	N
Yambo	40.00	1.03	0.02	0	0	13.91	0	N
Mohicap	44.38	9.65	0.22	0	0	25.71	0	N
Sampaloc	1573.75	16.77	0.01	0	0	747.5	0	N
Bunot	986.88	69.66	0.07	0	1929	1086	1.78	N
Palakpakin	305.00	17.69	0.06	0	0	251.1	0	N

The ratio between bioavailable P and N, SRP:DIN (when DIN = nitrate and ammonium) was calculated as a general guide to understand whether N is the limiting nutrient at the study lakes. This is not ideal, as DIN misses out species of N such as NO_2^-, NH_3 (which were not measured as part of this study) and therefore underestimates the concentration of dissolved N in the surface waters. The majority of the samples had no detected nitrate or ammonium in the surface waters except for Lake Sampaloc (which has a DIN:SRP ratio of 1.78) (Figure 4.14). The ratio of <7, suggests that N is the limiting nutrient in this lake (Downing *et al.,* 2001). This is consistent with the presence of myxoxanthophyll (from N_2 fixing and filamentous cyanobacteria) in Lake Sampaloc (Huszar *et al.,* 2006), which bloom in low N concentrations (Gulati *et al.*, 2017). It is likely that one of the major sources of P is from anthropogenic sources especially in the lakes with high aquaculture (Palakpakin, Bunot and Sampaloc). This combined with a greater internal N loss at higher temperatures via denitrification (Lewis, 2000; 2002), could explain the observed N-limitation. Combined with the high variability in Chl:TP ratios, this evidence suggests that N, not P, was the limiting nutrient in all of the study lakes at

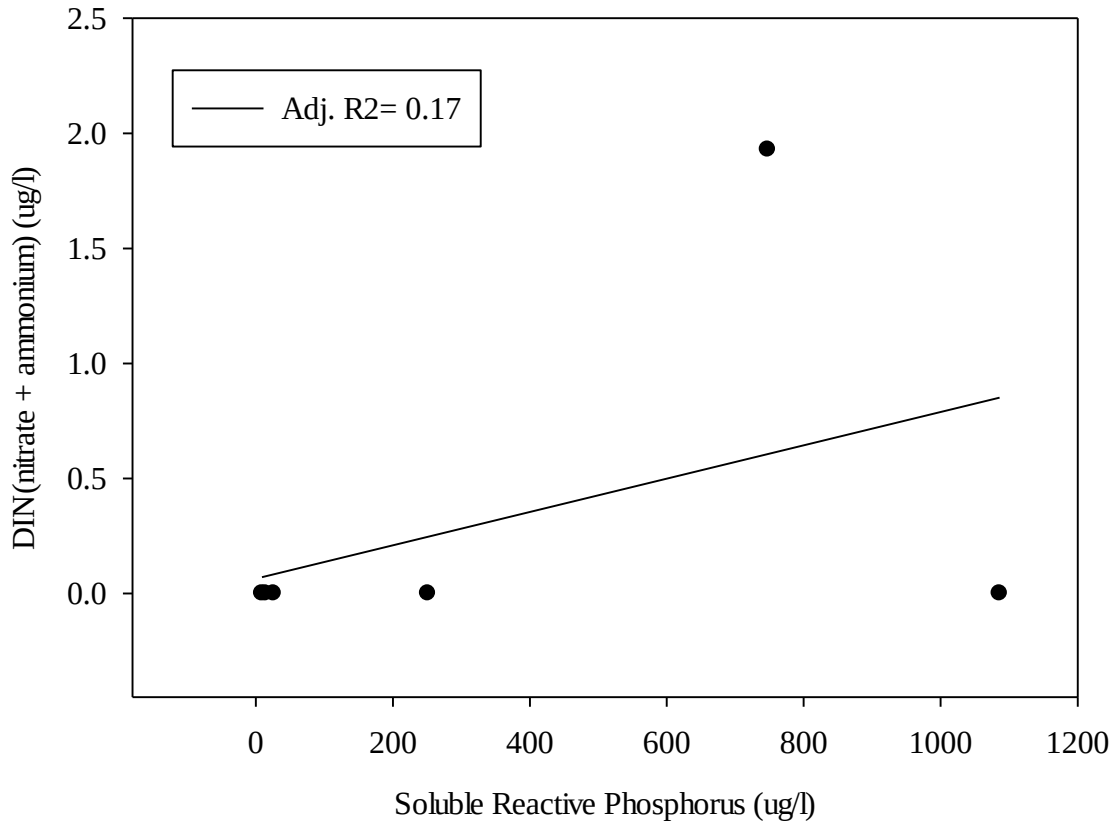


Figure 4.14: Soluble reactive phosphorus vs. Nitrate + Ammonium of the study lakes. The solid line represents the trend line with the R^2 value shown

the time of sampling. These findings provide further credence that N limitation of phytoplankton is common in tropical lakes. It is possible that N limitation has been exacerbated by anthropogenic inputs of P.

DIN:SRP ratios calculated from nutrient data collected by the LLDA (section 4.3), suggested that Lakes Sampaloc, Bunot and Palakpakin have been N limited since 1996, whereas Lakes Mohicap, Yambo and Pandin were P limited between 1996 and 2005. This suggests a 'shift' in the limiting nutrient in Lakes Mohicap, Yambo and Pandin from P to N between 2006 and 2017. One explanation is that the limited human settlements and increasing levels of aquaculture (which input P into the lake) in these low disturbance lakes finally reached the required level to cause a switch of the limiting nutrient (Jia *et al.*, 2015). Earlier inputs of P from anthropogenic sources prior to 1995, including human sewage and fish food, could explain why nutrient limitation patterns in Lakes Sampaloc, Bunot and Palakpakin did not change between 1996-2005. This shift in nutrient limitation in Lakes Mohicap, Yambo and Pandin could have resulted from increased inputs of P from fish food used in aquaculture and from human effluent (from the limited human settlements (López and Dávalos-Lind, 1998)). Furthermore, the increase in temperature observed (see section 4.7.1) promotes increased rates of denitrification (a major sink of N in lakes) and release of P from sediment (Kolzau *et al.*, 2014). The minimal anthropogenic pressures at Lakes Yambo and Pandin combined with increased warming could have accounted for the switch from P to N limitation. In the following chapter, analysis of the fossil pigment record will be used to determine the timing of these 'switches'.

The input of PO_4^{3-} into the lake systems has almost certainly increased in the recent past (Figure 4.1, in accordance with the findings from the LLDA (2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2009)) due to the observed catchment disturbance through land clearance for urbanisation, coconut plantations and from aquaculture. Periods of intense rainfall combined with steep sided craters, would aid the transport of nutrients into the lakes. Nutrient limitation tends to be stronger in tropical lakes compared to temperate aquatic systems (Lewis Jr., 2002). The greater efficiency of nutrient recycling in the epilimnion and monimolimnion also makes tropical lakes more sensitive to external nutrient inputs (Gulati *et al.*, 2017), with those situated within urban environments such as Lake Sampaloc (Figure 4.1) receive extremely high nutrient loads (Meriläinen *et al.*, 2003). Human impacts such as urbanisation can result in a massive response in aquatic systems due to modification of catchment hydrology (Miller and Hutchins, 2017) and through nutrient loss from erosion and runoff. The Philippines has undergone

substantial land conversion due to rapid population growth and migration (Malaque and Yokohari, 2007; Bravo, 2017). The resulting urbanisation could explain the rise in P inputs into the study lakes.

4.7.5. Contemporary lake phototrophs inferred from pigments

The composition of the algal communities in each lake changes from chlorophytes to siliceous algae, as aquaculture disturbance intensity increases (Figure 4.11). The PCA shows that siliceous algae were associated with the high disturbance lakes (Bunot, Sampaloc and Mohicap) and chlorophytes (green algae) to the low disturbance lakes (Yambo and Pandin) (Figure 4.12). González and Roldán, (2019) also observed the dominance of chlorophytes in the oligotrophic section of Lake Titicaca (the deep Lago Mayor) as a result of minimal eutrophication. Multiple studies have found that the dominance of siliceous algae and cyanobacteria as a result of eutrophication from anthropogenic sources (Soares *et al.*, 2012; González and Roldán, 2019). Siliceous algae, especially freshwater diatoms (which are abundant in the study lakes (Bannister *et al.*, 2019)) have a faster growing rate under non inhibiting conditions (Berg *et al.*, 2017). Feng *et al.*, (2019) found significant differences in phytoplankton communities between a eutrophic and oligotrophic lake in China with phytoplankton density higher in the eutrophic lake compared to the oligotrophic lake. This was observed in the low disturbance lakes (Yambo and Pandin) having a reduced density compared to the high disturbance lakes, which had undergone eutrophication. As eutrophication increased along the aquaculture gradient, higher nutrient concentrations (specifically N, P and Si (Figure 4.8)) favoured the faster growing diatoms over green algae. However, as nutrient enrichment continues, these blooms are likely to be eventually replaced with cyanobacteria due to changes in the nature of resource limitation (Ferber *et al.*, 2004). The timing of the sampling, at the end of the hot dry season (May 2017) and the 'single point' sampling may have influenced the patterns of phytoplankton composition observed, and mean that stages of the phytoplankton succession could have been missed. There is evidence of high concentrations of cyanobacterial blooms in Lake Mohicap (Figure 1.3) but were not captured by the water sampling because they are all concentrated towards the very surface of the lake.

Lake Palakpakin has high abundances of peridinin (dinoflagellates) and diadinoxanthin (siliceous algae including diatoms) (Figure 4.11). Diatoms bloom during periods of high nutrient (N, P, Si) concentrations as they have an enhanced storage capacity for N, particularly reflected by their intrinsic growth rates (Cordero and Baldia, 2015). As nutrients become depleted, dinoflagellates (which are mixotrophic) start to bloom (Lewis, 1978; Hubble and

Harper, 2002). The fact that peridinin (dinoflagellates) were only observed at Lake Palakpakin is possibly due to the timing of sampling, missing the dinoflagellate bloom in the other lakes. Further evidence of this succession was found in a yearlong study of Lake Mohicap from April 2013 to March 2014 (Cordero and Baldia, 2015). The phytoplankton succession in Lake Mohicap was correlated (Pearson's correlation) with nutrients ($r=0.472$, $p=0.001$), water transparency ($r=-3.90$, $p=0.001$) and temperature ($r=-0.518$, $p=0.000$). This study found that dinoflagellate blooms appeared in July 2013, after the diatom bloom and favoured the seasonal depletion of nutrients. The dinoflagellate blooms were then replaced by cyanobacteria during the rainy season (August to November), followed by chlorophytes and diatoms in the cool wet season (December to February). The timing of sampling (May 2017), at the end of the hot dry season would have most likely captured the bloom of dinoflagellates in the succession when Lake Palakpakin would have been seasonally depleted, prior to the beginning of the rainy season in June. Lake Lanao, Philippines, has a clear annual succession starting at the end of the wet season (January-February) from diatoms and cryptomonads to chlorophytes followed by cyanobacteria and finally dinoflagellates (Lewis (1978). Furthermore, a study completed on the eutrophic temperate lake, Rostherne Mere (United Kingdom) found that the dominance of phytoplankton groups changed as a response to light limitation and timing of thermal stratification (Reynolds and Bellinger, 1992). In Rostherne Mere, dinoflagellates dominated during the warmer summer months unless there was high water clarity, which would promote cyanobacterial blooms. This suggests the dominance of dinoflagellates in Lake Palakpakin could be in part due to its polymictic nature and eutrophic state preventing high transparency in the water column. Dinoflagellates sit at the end of this successional sequence due to their ability to use a variety of nutrient sources and motility (Lewis, 1978). However, this sequence can be regularly interrupted. In Lake Palakpakin, the lack of cyanobacterial pigments (canthaxanthin, myxoxanthophyll and zeaxanthin) could be the result of wind driven turbulence and high flushing rates from the inflowing Pagbuga Stream preventing the proliferation of cyanobacteria. These factors may prevent the formation of a stratified water column which is needed to maintain their buoyancy (Stroom and Kardinaal, 2016).

The similar PCA scores for Lakes Yambo and Pandin and low pigment abundances suggests a similarity between the algal and bacterial communities in these two lakes (Figure 4.11). Furthermore, phytoplankton showed no significant vertical changes in species composition but there was a shift in abundance (Figure 4.10). High light penetration in these two lakes of 22.20m and 7.16m (for Lakes Yambo and Pandin respectively; Table 4.2) and substantial lake

depth especially in Lake Pandin (62 m) would inhibit pigment preservation and promotes degradation, especially of the more labile pigments such as chlorophyll *a* after algal death (Leavitt and Hodgson, 2006). This could explain the low pigment concentrations from all algal groups found in these two lakes. Feng *et al.*, (2019) found that oligotrophic lakes have a lower density of both bacterial and algal communities due to a lack of nutrients. The lack of nutrients in these two lakes (Figure 4.8) combined with poor pigment preservation could explain the near-identical pigment profiles of these two lakes.

Okenone was only observed in the deepest lake (Pandin) at depths >15m due to the fact that the sampling depth did not capture this narrow depth interval in the other lakes. Populations of phytoplankton and photosynthetic bacteria in lakes are restricted to narrow depth intervals (called the bacterial plate) (Volkman *et al.*, 1988). A bacterial 'plate' is found in lake systems where light penetrates to the anoxic layer and consists of phototrophic sulphur bacteria found just beneath the oxic-anoxic interface (Fenchel *et al.*, 2012). Generally, the upper layers of this plate consists of purple sulphur bacteria, whilst green sulphur bacteria occur in the lower levels. This is due to the more efficient light harvesting mechanisms in green sulphur bacteria (Kalff, 2002). As photosynthetic bacteria require illuminated anoxic conditions, this leaves a small window in the water column where these bacteria can thrive (Figure 4.4).

N_2 fixing cyanobacterial pigments were only detected in Lake Sampaloc suggesting the lack of N in the surface waters of this lake may have favoured this type of cyanobacteria. N_2 fixing cyanobacteria was most likely abundant at the time of sample collection due to low N:P ratio and low DIN (Ferber *et al.*, 2004; Figure 4.14). Additionally, the increase in P loading from aquaculture and sewage input from the settlements can temporarily stimulate the growth of N_2 fixing cyanobacteria (as recorded by the presence of myxoxanthophyll (McGowan *et al.*, 2012)). At the time of sampling, the water column was stratified, therefore, there would have been limited interaction between the epilimnion and hypolimnion. This would have restricted the upwelling of nutrients from the hypolimnion.

4.7.6. Lake Trophic Status

There are some differences in the trophic states of each lake depending on the criteria used. The OECD classification (Table 4.7) uses three different variables (TP, secchi depth and

Table 4.7: Lake trophic status as defined by OECD (Vollenweider and Kerekes,1982)

Trophic State	TSI (0-100 scale)	OECD		
		TP (µg/l)	Chl. a (µg/l)	Secchi Depth (m)
Ultra-Oligotrophic	--	<4	<1	>12
Oligotrophic	<40	<10	<2.5	>6
Mesotrophic	40-50	10-35	2.5-8	6-3
Eutrophic	50-70	35-100	8-25	3-1.5
Hyper-eutrophic	>70	>100	>25	<1.5

chlorophyll *a* concentrations) to determine trophic state. The results from the study lakes using chlorophyll *a* indicates Lake Yambo is oligotrophic, Lake Pandin is mesotrophic, Lakes Sampaloc, Palakpakin and Mohicap are eutrophic and Lake Bunot is hypereutrophic (Table 4.8). In comparison, TP and secchi depth measurements suggest all lakes are eutrophic or hyper-eutrophic. Some of these discrepancies could be a result of the lakes having TP and chlorophyll *a* values very close to the OECD boundaries (Table 4.7; Table 4.8). As chlorophyll *a* values are more likely to provide a good estimate of algal biomass, they are a better indicator of lake trophic state. The low trophic states calculated by chlorophyll *a* in Lakes Yambo and Pandin is likely a testament to the relatively low algal biomass in the photic zone. Whereas, Lakes Bunot and Mohicap had a chlorophyll *a* TSI (trophic state indicator) that indicated eutrophic conditions reflecting the pronounced cyanobacterial algal blooms (from visual observations) and higher algal biomass in these two lakes. However, the estimated trophic states must be used with care as the index was originally developed for temperate lakes.

Table 4.8: Lake trophic status calculated using the parameters defined in Vollenweider and Kerekes (1982)

Lake Name	Secchi Depth (m)	TP (µg/l)	Chl. a (µg/l)	Secchi Depth	Total Phosphorus	Chlorophyll *a*
Pandin	3.10	26.25	2.77	Meso	Meso	Meso
Yambo	2.53	40.00	1.03	Eutro	Eutro	Oligo
Mohicap	1.58	44.38	9.65	Eutro	Eutro	Eutro
Sampaloc	0.98	1573.75	16.77	Hyper	Hyper	Eutro
Bunot	0.67	986.88	69.66	Hyper	Hyper	Hyper
Palakpakin	1.73	305.00	17.69	Eutro	Hyper	Eutro

Fundamental differences between tropical and temperate lakes can lead to simplifications with regard to trophic state (Salas and Martino, 1991). However, it can be used to compare lakes within a small geographical area such as the study lakes. The chlorophyll *a* trophic gradient shows good correlation with the aquaculture disturbance gradient (Table 4.1; Table 4.8; Figure 4.10).

4.8. Summary of limnological changes along the aquaculture disturbance gradient

The study lakes showed distinct physical, chemical and biological changes along the aquaculture gradient. As aquaculture increased, there was a shallowing of the thermocline, the oxycline, euphotic zone, chlorophyll *a* and pH profiles (Figure 4.4; Table 4.2). The RDA shows that temperature and a number of cations were correlated with pigments ($p=<0.05$; accounting for 31.2% of the variance), providing evidence of concordant shifts in biota and physicochemical conditions (Figure 4.12). Along the aquaculture gradient there was a proportional decrease in hypolimnetic DO, which implies oxygen was being consumed in respiration and microbial degradation leading to a widening of the anoxic layer and an associated increase in algal biomass (Kalff, 2002; Rhodes *et al.*, 2017). This occurs in the more disturbed lakes as these lakes have increased nutrient loading, fuelling increased algal blooms (as shown by the TP:Chl ratio; Figure 4.13). The sinking of these blooms into the hypolimnion and their subsequent degradation, results in greater consumption of DO leading to a shallowing of the anoxic layer. In contrast, the low aquaculture lakes, such as Lakes Yambo and Pandin have a reduced algal biomass and greater water transparency. This allows phototrophic algae to live deeper in the water column, deepening the anoxic layer. Hypereutrophic conditions can lead to increases in epilimnetic algal biomass, which results in light limitation of benthic and bacterial communities from shading by algae at the surface (Burson *et al.*, 2018). As algae photosynthesise, they are restricted to the depth of the euphotic zone. However, as the algal biomass increases, it consumes DO and reduces light availability with depth. This results in a thinning of the euphotic zone and DO profiles (Figure 4.4; Kalff, 2002). At the advanced stages of eutrophication, algae can only occupy the surface of the water column, which receives oxygen via diffusion with the atmosphere. Although an increase in eutrophication initially causes an expansion in algal biomass, this is not sustained in prolonged hypereutrophic conditions. This is because of self-shading by algae, which reduces light limitation forming 'dead zones' in the water column.

As Lake Palakpakin is a shallow polymictic lake, it has different algal assemblages compared to the other study lakes. It is classified as eutrophic and lacks an anoxic layer at depth. Jeppesen

et al., (2010) determined that shallow lakes respond differently to eutrophication compared to deeper tropical lakes, due to a lack of stratification and anoxia. Furthermore, this lake has a substantial submerged macrophyte community (determined by visual observation) compared to the deeper lakes, where they are restricted to near-shore areas. Once submerged macrophytes become abundant, they can greatly prevent wave resuspension of sediments resulting in a less turbulent water column (Scheffer and Van Nes, 2007). Conversely, due to the high flushing rates (Brillo, 2016b) in Lake Palakpakin, the presence of submerged macrophytes would be unlikely to influence the turbulence of the water column. The lack of light at the bottom of the water column suggests that high turbulence resulted in oxygenated bottom waters. The high turbulence could prevent the development of anoxia and permanent stratification.

The concentration of chlorophyll *a* was similar in all the study lakes (c. 10 µg/l) however, the depth at which chlorophyll *a* was observed in the water column decreases along the aquaculture disturbance gradient (Figure 4.3 Figure 4.5). In the most eutrophic lakes, the area of chlorophyll *a* is concentrated in a smaller depth of the water column, so these lakes appear more productive in the surface waters. Examples of a similar phenomenon happening in freshwater lakes found increased phytoplankton population density reduced the depth of the euphotic zone as a consequence of self-shading (Bindloss, 1974). Silsbe *et al.*, (2006) found that a shallowing of chlorophyll *a* profiles among different bays of Lake Victoria occurred due to increased thermal stratification, which, reduced mixing and led to light limitation. Increased eutrophication results in an increase of hypolimnetic DO consumption leading to a widening of the anoxic layer and light limitation (Rhodes *et al.,* 2017). As the depth of epilimnion shallows it also prevents access to nutrients in the anoxic layer (Kalff, 2002), which could explain the similar chlorophyll *a* concentrations in all the study lakes regardless of trophic state.

It is possible that changes in fish abundance have influenced phytoplankton biomass via 'trophic cascades', especially in the lakes with the highest fish density. A trophic cascade is caused by the addition/removal of the top predator and the reciprocal changes in the relative populations of other organisms in the food change (Steneck, 2012). Meerhoff *et al.*, (2007) found that warming influences fish community structure through increased density and smaller size (from enhanced reproduction). Furthermore, this study found that fish predation pressure was stronger in subtropical lakes in comparison to temperate lakes, due to stronger predation pressures on zooplankton with warming (Mehner, 2000). Mazumder *et al.,* (1990) also hypothesised that low abundances of planktivorous fish were associated with high abundances of zooplankton, low algal biomass and improved water clarity. In the study lakes, the fish used

for aquaculture are predominantly fed using fish food, however, fishermen (see interview data; Appendix A) have also used algal blooms as a source of food. As trophic cascades control species composition and nutrient recycling this could explain why Lakes Bunot and Sampaloc have similar concentrations of chlorophyll *a* to those in Lake Yambo and Pandin. Usually an increase in nutrient loading along the aquaculture gradient would cause an increase in algal biomass and production (Huszar *et al.*, 1998; Gons *et al.*, 2008), however this was not observed at the study lakes. As a lake becomes hypereutrophic, pigment concentrations might not increase with eutrophication due to light limitation because of self-shading (as observed in Lake Bunot). The shallowing of the photic zone would also result in a smaller water volume in which to conduct aquaculture. However, Cordero and Baldia, (2015) observed, these lakes undergo substantial seasonal variability and therefore, the single (point) sampling could have missed the period of high algal blooms.

4.9 Summary of key findings

The six lakes showed distinct physical, chemical and biological changes along the disturbance gradient:

- There were significant differences between the lakes with high (Palakpakin, Bunot, Sampaloc and Mohicap) and those with low aquaculture (Yambo and Pandin) indicating the combined impact of aquaculture and other drivers on anoxia, nutrient cycling, ion composition and algal communities.
- The hypolimnion of all the deep lakes were anoxic, with the depth of the anoxic layer and DCM shallowing along the aquaculture disturbance gradient. In comparison, the shallow lake Palakpakin shows evidence of temporary stratification at the sediment-water interface at the time of sampling (at the end of the hot dry season). This suggests that oxygenation of the water column is driven by mixing conditions
- Human disturbance (urbanisation, aquaculture) is the most likely cause of differences in ion composition and electrical conductivity within the six lakes
- The six lakes are N limiting, however, evidence suggests that this was not always the case. Lakes Mohicap, Yambo and Pandin have exhibited a shift from P to N limitation since 2006. The increasing use of fish food could be a contributing factor with all lakes becoming N limited by 2017
- There was a shift in algal communities from chlorophytes to siliceous algae and cyanobacteria, providing evidence of a major restructuring of algal communities due to enhanced nutrient loading from fish food and excretion. Lake Palakpakin has different

algal assemblages (dominated by dinoflagellates) compared to the other lakes as a result of it being a shallow polymictic lake

- Chlorophyll *a* profiles show a shallowing along the eutrophication gradient yet the concentration of chlorophyll *a* stays relatively constant. As the anoxic zone shallows, it concentrates the chlorophyll *a* into a smaller water volume. There is evidence that in the most eutrophic lake (Bunot) algal biomass has progressed to the stage where dead zones have formed in the water column. This suggests that pigment concentrations might not increase with eutrophication due to self-shading.

An understanding of how lake systems respond physically, chemically and biologically to environmental and climatic changes is necessary to interpret core records correctly. The contemporary limnology will have implications for how pigments and OM are preserved in the sediment record. A shallower and more pronounced anoxic layer would reduce degradation in the water column and result in a higher concentration of pigments being deposited in the sediments. However, this also reduces the water volume in which autotrophs can photosynthesis in the water column. The limnological data provides a contemporary 'snapshot' against which the impacts of aquaculture and interpretation of the core records can be made in Chapter 5.

5. CHAPTER 5 – RESULTS AND INTERPRETATIONS OF INDIVIDUAL LAKE SEDIMENT RECORDS

5.1. Introduction

The aim of this research is to quantitatively assess the ecological response of the Seven Lakes of San Pablo to aquaculture practices. However, disentangling the impact of aquaculture on tropical lakes is problematic as they are under threat from a variety of climatic and anthropogenic pressures (not just aquaculture). Separating out the impact of aquaculture is more difficult in lakes experiencing eutrophication and increased temperature, as both drivers can produce similar responses, such as increased anoxia, algal productivity, nutrient loading and nutrient recycling (Figure 5.1) (Marti *et al.*, 2004; Verburg and Hecky, 2009; Moss *et al.*, 2011; Levine *et al.*, 2012; Jeppesen *et al.*, 2014). To complicate matters further, catchment and lake characteristics have the ability to modify the response of algal communities to both aquaculture and other drivers (Moorhouse *et al.*, 2018). This can result in the dilution or confounding of the impacts of aquaculture in the sedimentary record.

This thesis attempts to assess the impact of aquaculture by studying lakes at different points along a disturbance gradient, to assess whether lake response was proportional to the level of aquaculture disturbance (Figure 5.2). This chapter takes an initial look at how individual lake responses compare to the timings of change in the proxy and historical records, to identify possible drivers of algal community change in the study lakes.

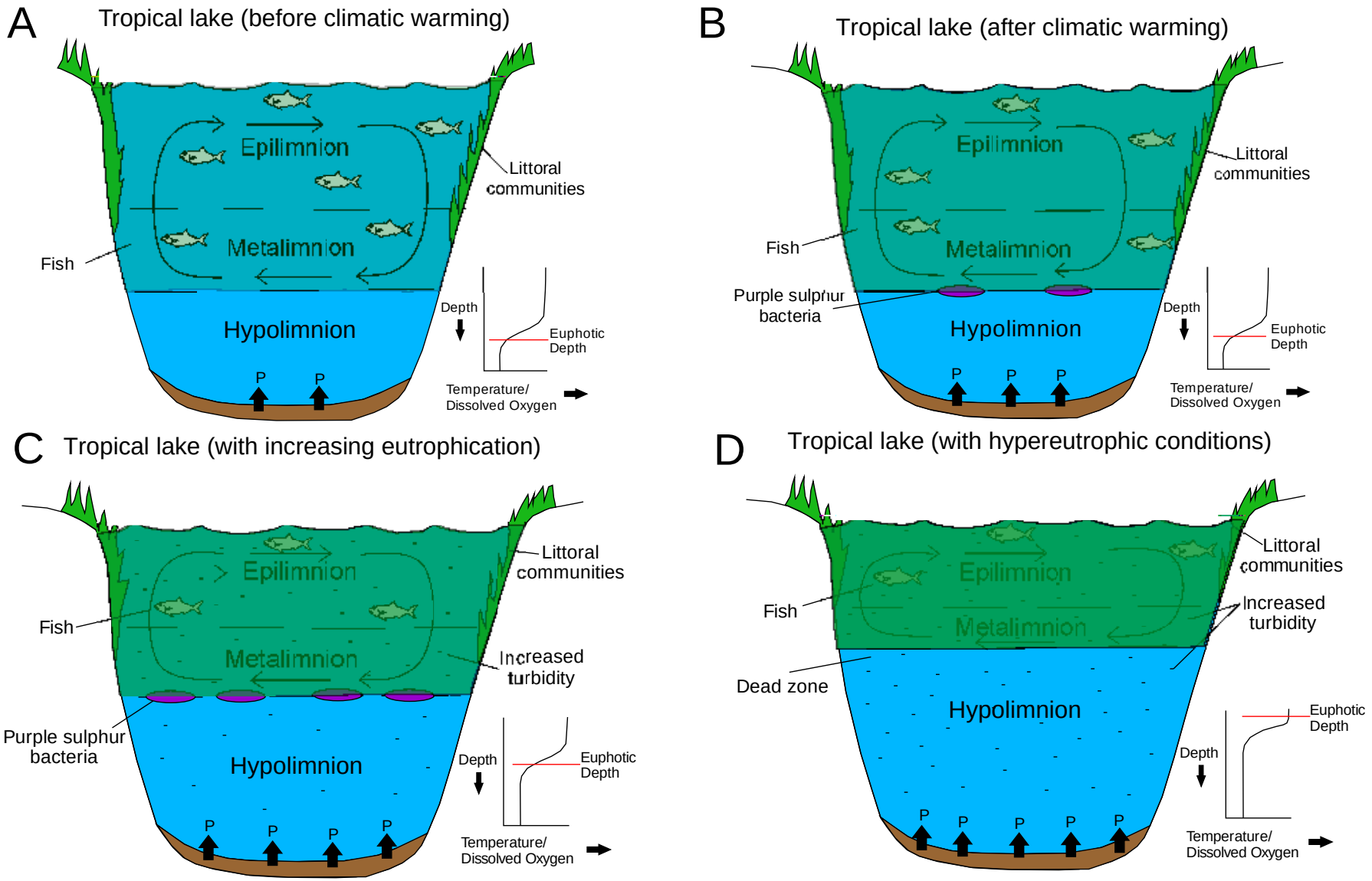


Figure 5.1: Conceptual diagrams proposing how a deep (>10 m) tropical lake responds to climatic warming and eutrophication. A) prior to climatic warming, with weak thermal stratification, limited anoxia, abundant littoral algae and macrophytes and the absence of purple sulphur bacteria. B) influenced by climatic warming showing more stable stratification, hypolimnetic anoxia, purple sulphur bacteria and greater rates of internal loading of phosphate. C) impacted by both climatic warming and eutrophication showing stable stratification, shallowing of the anoxic layer, abundant purple sulphur bacterial, reduction of littoral community abundance and greater rates of internal P loading. D) hypereutrophic conditions resulted in further shallowing of the anoxic layer, substantial self-shading of phytoplankton and aphotic dead zones without purple sulphur bacteria, reduced littoral communities and a smaller volume of water which is habitable for fish. The intensity of green shading signifies algal production. The arrows indicate mixing of the water column. Temperature/dissolved oxygen water column profiles show a shallowing of the anoxic and euphotic depth as eutrophication progresses.

5.2. Brief Summary of Materials and Methods

Single sediment cores (< 1m in length) were collected from Lakes Bunot, Sampaloc, Palakpakin and Mohicap in May 2017 and Lakes Yambo, and Pandin in April 2018 (see section 3.1.2). Each sediment core was analysed for photosynthetic pigments, loss-on-ignition (LOI), stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and C/N ratios (see section 3.2.3). To acquire a chronology for each core, two different methods were used. For sediment cores from Lakes Bunot, Palakpakin and Pandin, ^{210}Pb dating was used (see section 3.2.3.4). For the three remaining cores (from Lakes Sampaloc, Mohicap and Yambo), an age model was developed by correlation with dated cores previously collected from the respective lakes (Table 3.1; Chapter 3). Detailed records of aquaculture cage numbers, population, housing numbers, coconut cultivation and meteorological data (mean annual temperature and precipitation) were compiled (see section 3.2.3.5). Mean fish density was used to rank each lake along by aquaculture density (Figure 5.2; section 4.1.4) to aid in the interpretation of the individual lake records (see section 4.4).

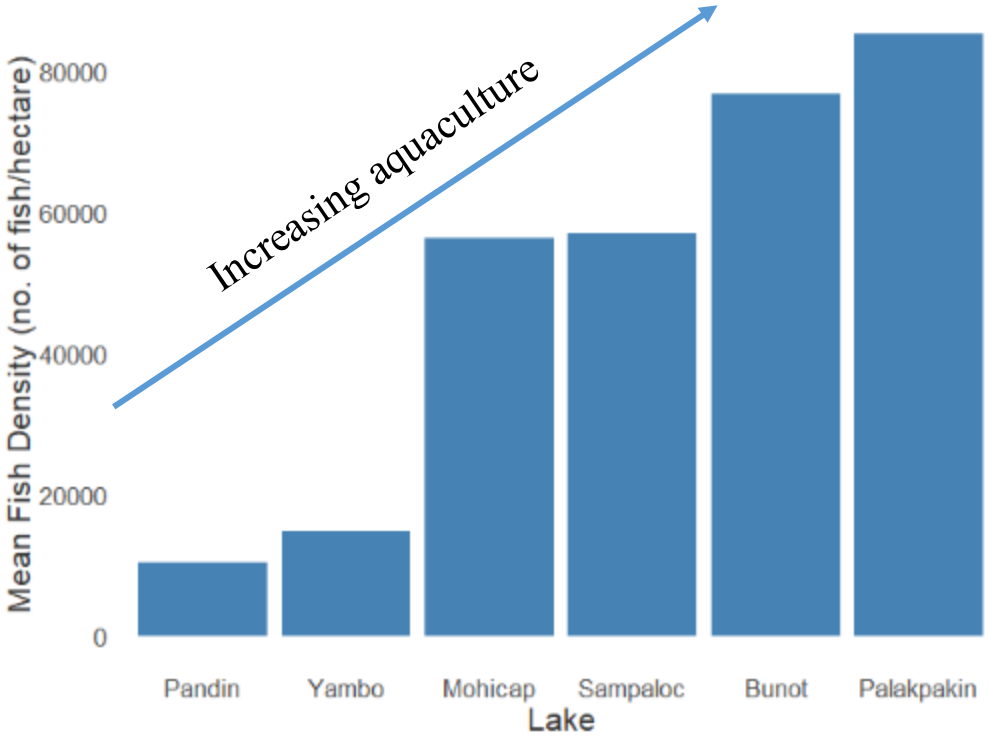


Figure 5.2: Aquaculture intensity calculated using mean fish density (no. of fish/hectare) in order of increasing disturbance (from left to right). For each lake, mean fish density was calculated using all years aquaculture was present

5.3. Results

5.3.1. Meteorological Data from 1901-2018

As records from many meteorological stations on the island of Luzon are incomplete or cover a restricted timescale, a composite record was used. The particular record used was taken from the Climate Research Unit's TS4.02 global dataset of annual mean temperature and precipitation records (Harris *et al.*, 2014). The dataset calculates records on grids of 0.5° x 0.5° and, for the study lakes, was centred on upon 121°21′00.0″E, 14°05′24.0″N at an altitude of 135 m. The gridding method is based on angular-distance weighting, which gives greater weighting to closer meteorological stations.

Mean annual temperature record, showed a gradual increase throughout the 20th and early 21st century from 26.5°C (1901) to 27.5°C (2016). This was punctuated by a more rapid increase in temperature between 1918 and 1938 and between 1973 and 2018, as summarised by the 5 year smoothing (Figure 5.3). The mean precipitation record showed greater variability with a gradual increase in the early 20th century from 1733 mm (1911) to 3055 mm (1930), followed by a slight decline to 2103 mm in 1938. Beyond that, precipitation stabilised at ca. 2200 mm per annum until 1973, before gradually increasing throughout the remainder of the 20th and the beginning of the 21st century, peaking at 3301 mm (1999) (Figure 5.3).

5.3.2. Urban Variables

Historical information on population and occupied housing units in San Pablo City were obtained from the Philippine Statistics Bureau (based on census data).

5.3.2.1. Area Planted/Harvested with Coconut in Laguna province (Hectares)

Historical data on the area occupied by coconut plantations were collected from the Philippine Statistics Bureau:

(http://openstat.psa.gov.ph/PXWeb/pxweb/en/DB/DB__2E__CS/0072E4EAHO0.px/table/tableViewLayout1/?rxid=bdf9d8da-96f1-4100-ae09-18cb3eaeb313). Since 1990, there has been a decline in the area utilised for coconut from 66,631 hectares (1990) to 59,980 hectares (2019) (Figure 5.3). To calculate the area utilised for coconut prior to 1980 a linear regression was applied to the whole dataset, suggesting that 72,953 hectares of coconut were present in Laguna province in 1960 (assuming plantation rates have remained constant). This is consistent with a decline in coconut plantation rates and an increase in forest cover since 1960 in Laguna (Philippine Statistical Authority. 2019; see section 2.4).

5.3.2.2. Population

The population of San Pablo showed a gradual increase from 22,612 (1901) to 50,435 inhabitants (1948). This was followed by a more rapid increase in population for the remainder of the 20th and early 21st century to 276,285 in 2018 (Figure 5.3).

5.3.2.3. Occupied Housing Units in San Pablo City

The number of occupied housing units in San Pablo showed a linear increase in values from 12,358 (1960) to 60,788 (2015) (Figure 5.3).

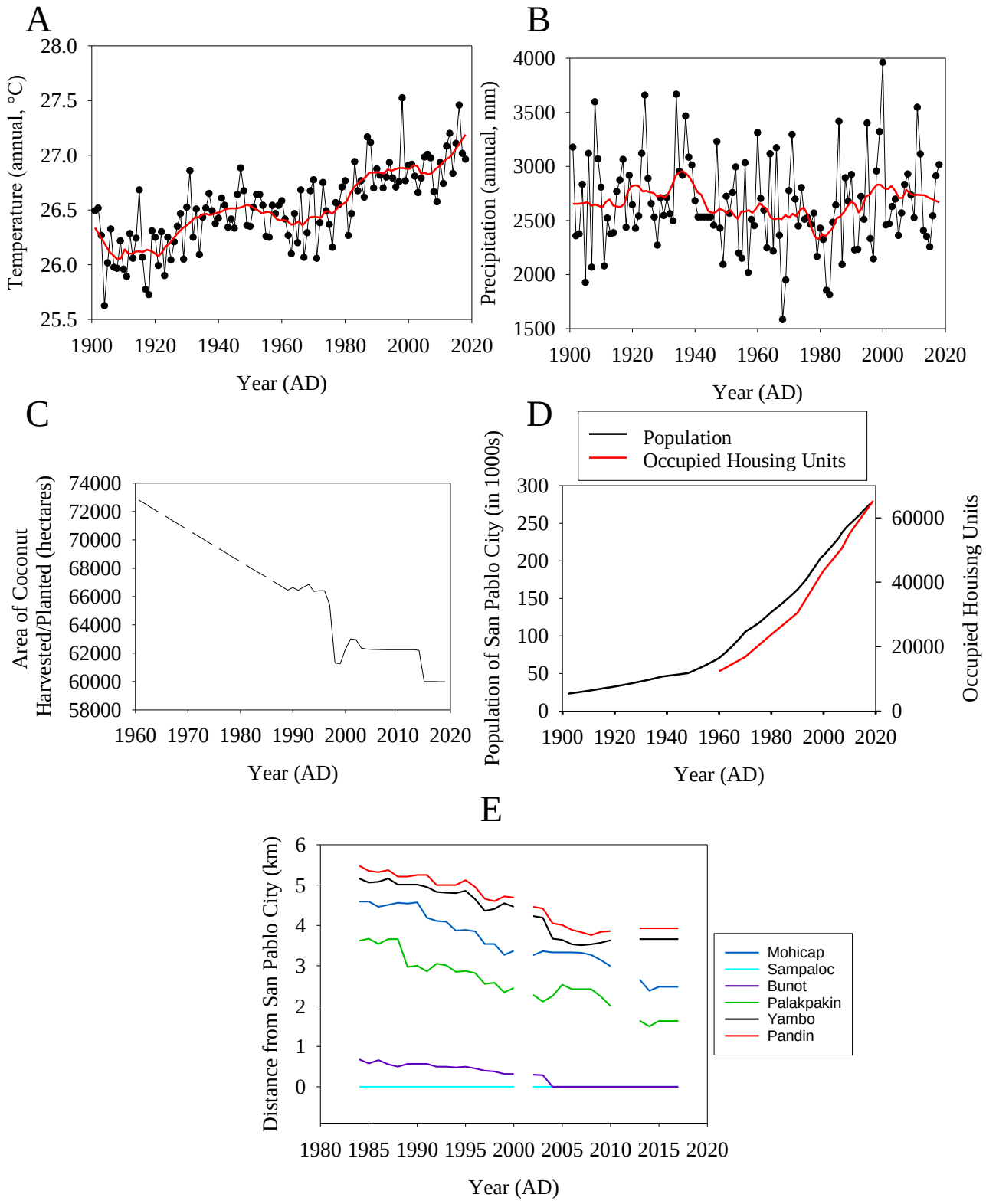


Figure 5.3: A); Annual mean air temperature (°C), B) annual mean total precipitation (mm) from 1901 to 2016 based on summing and averaging mean monthly values from four synoptic weather stations on the island of Luzon. A 5 year running average shows moving averages (in red), C) area of coconut harvested/ planted in Laguna province (in hectares) from 1960 to 2018. Values between 1960 and 1990 were determined by linear extrapolation (dashed line) using the rate of change between 1990 and 2018 (Philippine Statistical Bureau, 2019), D) population of San Pablo City from 1901 to 2018 (red) and the number of occupied housing units from 1960 to 2018 (black) and E) Distance of each lake from the edge of San Pablo City (in km) since 1984 to 2018. The missing data points from 2001 are due to cloud coverage (Google Earth, Imagery, 2019)

5.3.2.4. Distance from the edge of San Pablo City (km)

San Pablo City is the main urban centre (Figure 3.3), so the distance from the centre of each lake to the edge of San Pablo city was used as a proxy for urban encroachment onto each site (see section 3.2.3.5.4). Since 1984, the boundary of San Pablo City has gradually encroached on the catchment of all cored lakes, except Lake Sampaloc (Figure 5.3). As Lake Sampaloc is located within the centre of San Pablo City it has had an urbanised catchment since at least 1984 (i.e. distance of the lake from the edge of the city is 0 km). Lake Bunot (the second closest lake to the centre of San Pablo City) was 0.68 km away from the city boundary in 1984, with the urban area reaching the lake catchment in 2004. In comparison, the two low disturbance lakes Yambo and Pandin (which are situated furthest from the city boundary) have seen the city boundary encroach from 5.48 and 5.16km (1984) to 3.93 and 3.63 km (2018) respectively.

5.3.3. Isotopic analysis of contemporary "end member" samples

There was a wide range in the $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of the contemporary samples collected from the lakes and their catchments (Table 5.1). Due to machine failure $\delta^{15}N$ values are missing for some of these samples. The most notable result is the high $\delta^{15}N$ value (+33.9‰) for the starter fish food. This is due to the origin of the food which comprises fish meal/oils, plant proteins, minerals and vitamins, and comes from primarily marine sources. This 'uniquely' high value (compared to the other contemporary samples) may be a useful aid in identifying the presence of aquaculture in the sedimentary record. The $\delta^{13}C$ values for water cabbage (*Pistia* spp.) were highly variable, with the highest –19.2‰ (Lake Sampaloc) and the lowest –33.1 ‰ (Lake Mohicap) (Figure 5.4; Figure 5.5). The high variability in $\delta^{15}N$ values of *Pistia* spp., ranged from +2.3 to +8.5‰ (Lake Sampaloc) to +2.3‰ (Lake Mohicap). $\delta^{13}C$ values for water hyacinth (*Eichornia* spp.) ranged between –28.4 ‰ (Lake Bunot) and –32.6 ‰ (Lake Mohicap) (Figure 5.4; Figure 5.5). Again, these two lakes showed the greatest variation in $\delta^{15}N$ values for water hyacinth (*Eichornia* spp.), at +12.2‰ (Lake Bunot) and +1.3‰ (Lake Mohicap). The mean isotopic values for the coconut bark was –29.0‰ for $\delta^{13}C$ and +4.0‰ for $\delta^{15}N$, with limited variability between lakes. In comparison, $\delta^{13}C$ isotopic ratios of coconut leaf fluctuated between –26.6‰ (Lake Bunot) and –33.9‰ (Lake Mohicap) (Figure 5.4). The C/N ratios showed a clear distinction between wood and aquatic macrophytes, with the wood samples having values >50 and aquatic macrophytes having values between 6.9 to 18.9 (Figure 5.5). When combined with the C/N ratios, $\delta^{13}C$ isotopic ratios are consistent with plants that use a C_3 photosynthetic pathway.

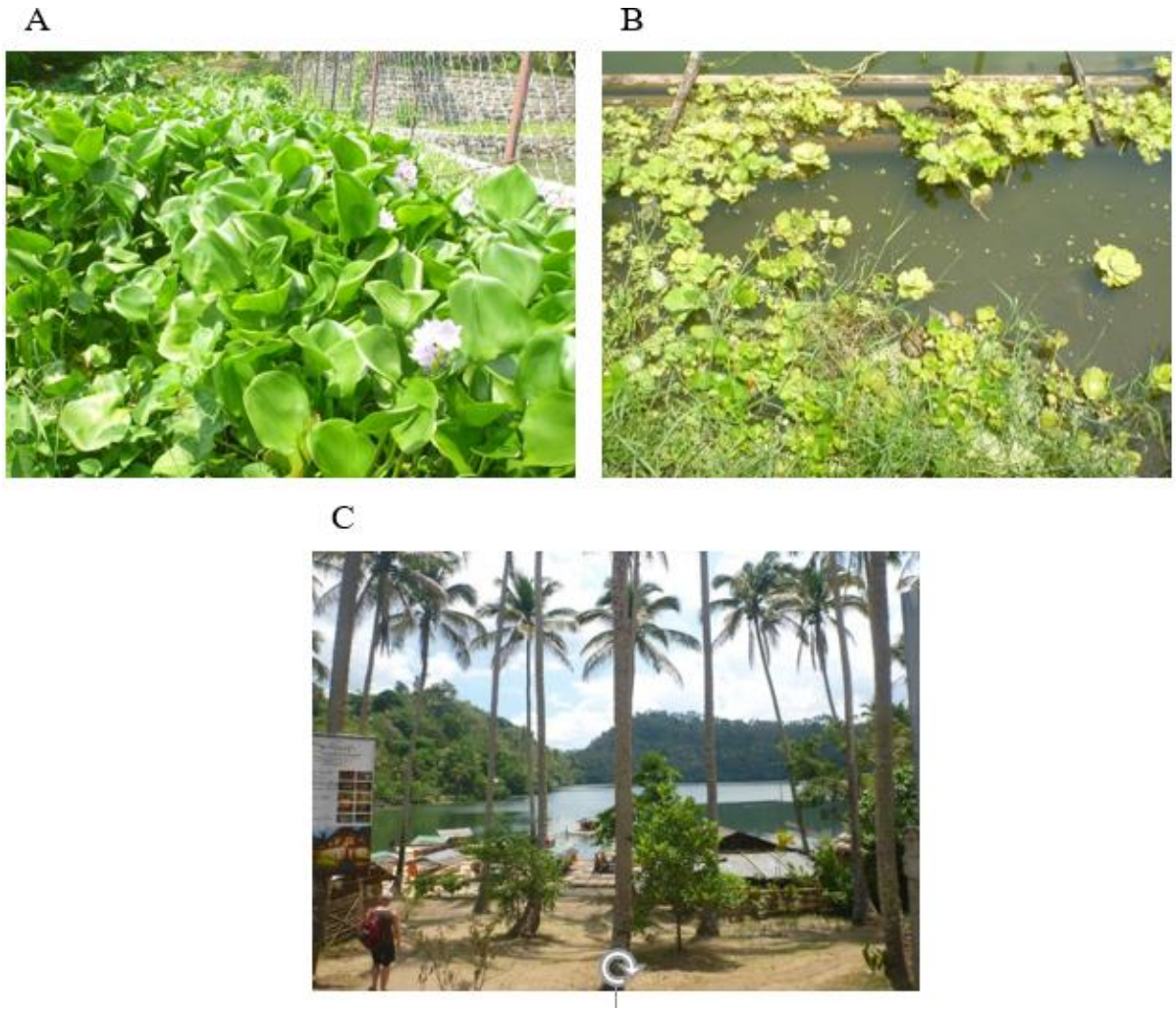


Figure 5.4: Photographs of (A) Water Hyacinth (Eichornia spp.) (B) Water Cabbage (Pistia spp.) and (C) Coconut plantation (Cocos nucifera) (photo credit: Suzanne McGowan)

Table 5.1: Stable isotopic ratios ($\delta^{13}C$, $\delta^{15}N$), %C, %N and C/N ratios for contemporary samples collected from the cored lakes and their catchments.

Lake	Sample Type	$\delta^{13}C$	$\delta^{15}N$	%C	%N	C/N Ratio
Sampaloc	Water Cabbage (*Pistia* spp.)	–19.2	+18.0	35.3	5.1	6.9
	Water Hyacinth (*Eichornia* spp.)	–32.5	+11.4	43.8	4.2	10.5
	Grass	–16.35	+10.19	14.49	0.74	19.5
	Coconut Bark (*Cocos nucifera*)	–27.87	+4.11	48.68	0.75	64.9
Palakpakin	Water Hyacinth *(Eichornia* spp.)	–29.4	+5.4	44.3	2.9	15.4
	Coconut Bark (*Cocos nucifera*)	–28.45		40.26	0.32	124.6
	Coconut Leaf (*Cocos nucifera*)	–30.06		34.60	2.06	16.8
	Grass	–14.03	+2.22	31.69	1.96	16.2
	Water Cabbage (*Pistia* spp.)	–30.48	+7.54	37.11	3.53	10.5
Bunot	Water Hyacinth (*Eichornia* spp.)	–31.3	+7.4	44.1	3.3	13.2
	Water Cabbage (*Pistia* spp.)	–28.4	+12.2	43.0	2.8	15.3
	Grass	–14.04	+8.25	36.41	2.26	16.1
	Littoral Mud	–27.76	+7.03	4.13	0.32	13.1
	Coconut Leaf (*Cocos nucifera*)	–26.85		41.88	2.99	14.0
	Coconut Bark (*Cocos nucifera*)	–28.82		48.82	0.52	93.5
	Water Cabbage (*Pistia* spp.)	–30.37	+7.39	36.09	3.15	11.5
	Banana Bark (*Musa* spp.)	–24.23		39.62	0.79	50.1
	Banana Leaf (*Musa* spp.)	–26.91		39.01	1.89	20.7
Mohicap	Water Hyacinth (*Eichornia* spp.)	–32.6	+1.3	46.6	5.9	7.8
	Water Cabbage (*Pistia* spp.)	–32.5	+2.7	46.4	5.9	7.8
	Coconut Leaf (*Cocos nucifera*)	–33.95		37.18	1.97	18.9
	Coconut Bark (*Cocos nucifera*)	–29.33		47.48	0.47	100.6
	Grass	–32.09	+3.49	33.40	3.03	11.0
	Littoral Mud	–27.48	+5.01	2.61	0.19	14.0
	Water Cabbage (*Pistia* spp.)	–33.07	+2.70	32.61	1.91	17.1
Pandin	Coconut Leaf (*Cocos nucifera*)	–28.38	+4.46	45.27	1.21	37.4
	Coconut Bark (*Cocos nucifera*)	–28.09	+4.15	45.33	0.62	72.6
	Grass	–16.35	+8.2	14.49	0.74	19.5
	Littoral Mud 1	–25.50	+3.84	0.13	0.01	13.0
	Littoral Mud 2	–26.10	+3.38	0.25	0.02	13.3
	Aquatic Macrophyte (species unknown)	–21.34	+8.67	24.80	2.02	12.3
	Water Cabbage (*Pistia* spp.)	–26.67	+2.31	34.05	3.92	8.97
Yambo	Coconut Bark (*Cocos nucifera*)	–26.81		40.37	0.39	104.9
	Coconut Leaf (*Cocos nucifera*)	–28.97	+2.78	43.73	1.41	31.0
	Grass	–13.16	+6.36	34.28	1.76	19.5
	Littoral Mud	–23.32	+3.31	0.43	0.03	17.2
	Littoral Mud- 1m from shore	–24.82	+3.29	0.58	0.03	18.2
	Littoral Mud- away from tourist area	–22.81	+3.03	1.73	0.14	12.8
N/A	Starter Fish Food	–26.2	+33.9	40.8	4.2	9.7
N/A	Finisher Fish Food	–26.5	+2.5	46.0	5.7	8.0

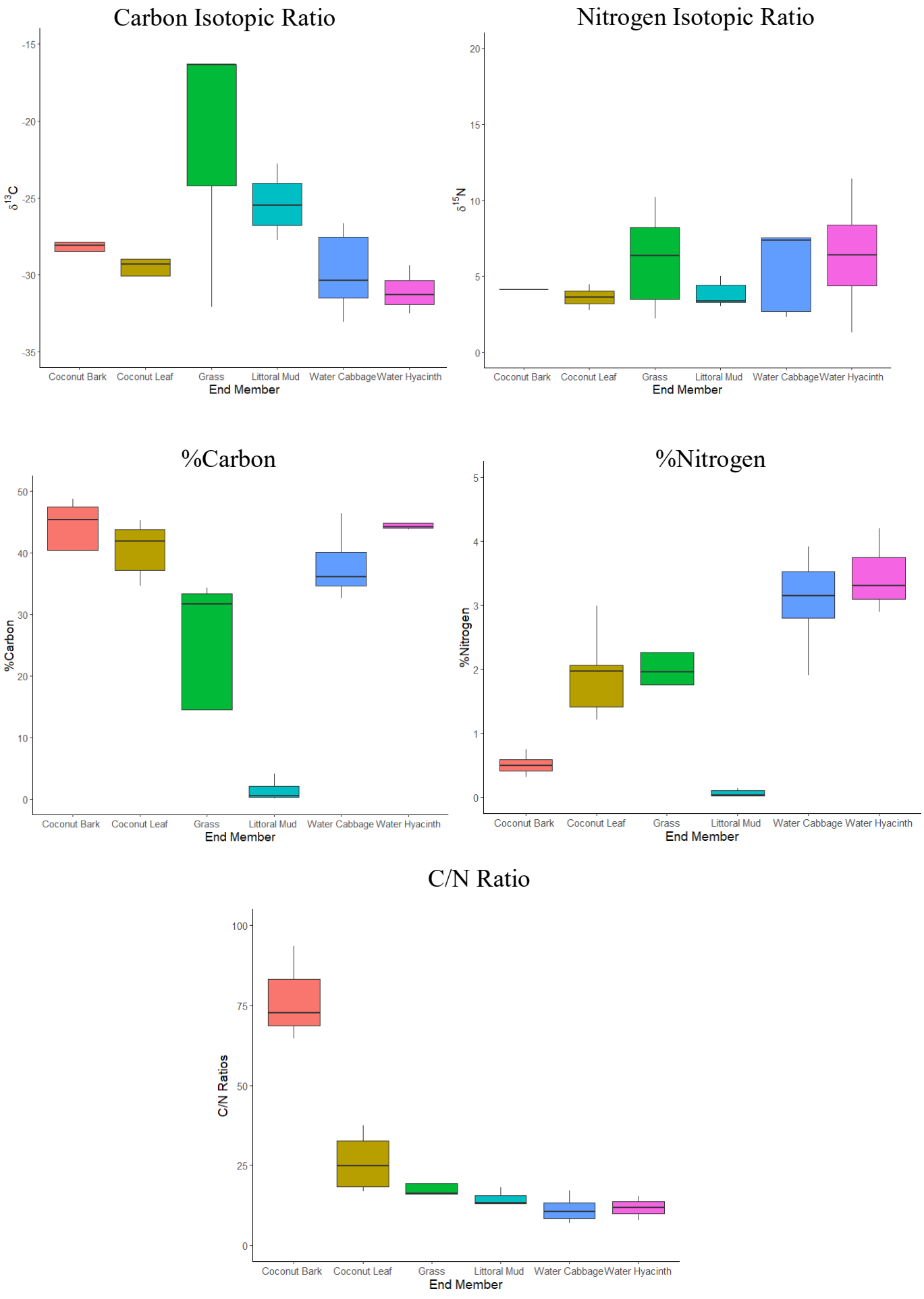


Figure 5.5: Boxplot showing the variety in the Stable isotopic ratios ($\delta^{13}C$, $\delta^{15}N$), %C, %N and C/N ratios for contemporary samples, water hyacinth (Pistia spp.), water cabbage (Eichornia spp.), grass, coconut leaf and bark (Cocos nucifera) collected from the cored lakes and their catchments.

5.4. Lake Pandin

5.4.1. Field Core Description

From 47 to 15 cm, sediment core PANDIN consisted of consolidated dark brown OM-rich gyjtta (Table 5.2). Between 15 cm and 0 cm, sediment colour changed from a dark to light brown unconsolidated gyjtta.

5.4.2. Sediment Chronology

In the core, total ^{210}Pb activities did not reach an equilibrium with supported ^{210}Pb activities (Figure 5.6; Appendix B). Total ^{210}Pb activity at the top of the core was 240.29 Bq Kg^{-1} increasing to 292.97 Bq Kg^{-1} at 10.25 cm and decreasing to 61.28 Bq Kg^{-1} at 46.5 cm. Unsupported ^{210}Pb activities, declined irregularly with depth, permitting the use of the CRS model to develop the core chronology (Appleby and Oldfield, 1978; Appleby, 2001). ^{137}Cs activities in the sediments were low and only detected between 30.5 and 43.5 cm (Figure 5.5). The CRS age-depth model placed 1963 between 32.5 and 35.5 cm, in agreement with the ^{137}Cs record (Figure 5.6). The CRS age–depth model suggested that sediment accumulation rates could be divided into three stages: <0.069 g cm^{-2} yr^{-1} before 1940, 0.138 g cm^{-2} yr^{-1} between the 1940s and 1980s, and 0.082 g cm^{-2} yr^{-1} after the 1990s. These stages are divided by two increased sediment accumulation rates in the 1940s and 1980s, suggesting that the two events that caused the increased rates at the times have changed the sedimentation processes in the coring location. The calculated age model indicated that the 48 cm core covered the past c. 130 years (starting from 1890).

Table 5.2: List of sediment cores (including descriptions) from the six cored lakes used in this thesis
**Sediment cores collected by Wayne Bannister and team from the National University of Singapore. These cores were not analysed as part of this project but were used to tie-in dates for the ^{210}Pb chronology*

Lake	*Core Name*	*Date Collected*	*Coring Depth (m)*	*Geological Remarks*	
Pandin	PANDIN	April 2018	62	0-15 cm	Light brown unconsolidated gyjtta, high organic matter content
				15-47 cm	Consolidated dark brown gyjtta, high organic matter content
Yambo	YAMBO2	April 2018	27	0-27 cm	Water
				27-33 cm	Light brown unconsolidated gyjtta, high organic matter content
				33-35 cm	Dark brown layer of gyjtta with wood fragments
				35-80 cm	Light brown consolidated gyjtta, with high organic matter content
Yambo	YAMBO1*	June 2016	27	0-104 cm	Unconsolidated sediment core. Sediments were fine-grained. Slight changes in colour occurred when organic matter levels were lower. Many plant macrofossils present.
Mohicap	MOH2	May 2017	30	0-6 cm	Unconsolidated gyttja
				6-36 cm	Water
				36-79 cm	Dark brown gyttja, high organic matter content, macro rich (high volume of leaves present)
				79-120 cm	Lighter brown gyttja, high organic matter content (abrupt colour change from dark to light brown at this boundary)
Mohicap	MOH1*	June 2015	30	0-88 cm	Unconsolidated sediment core. Sediments were fine. Mostly black and watery throughout the core. Colour is consistent.
Sampaloc	SAMP2	May 2017	27	0-30 cm	Dark brown , organic rich gyttja
				30-35 cm	Water
				35-64 cm	Light brown, organic rich gyttja

Sampaloc	SAMP1*	June 2015	27	0-81 cm	Unconsolidated sediment core. Sediments were fine. Slight changes in colour when organic matter levels were lower. Many plant macrofossils present.
Bunot	BUNOT2	May 2017	23	0-30 cm	Unconsolidated, dark brown gyttja with high organic matter content
				30-57 cm	Consolidated, dark brown gyttja with high organic matter content
				57-82 cm	Light brown, consolidated gyttja with high organic matter content, laminated, contains macrofossils (leaves)
Palakpakin	PALAK	May 2017	7.5	0-12 cm	Dark brown gyttja, high organic matter content
				12-70 cm	Light brown gyttja, high organic matter content, well-laminated, contains macrofossils (leaves and wood fragments) (abrupt colour change at this boundary from dark to light brown)
				70-74 cm	Dark brown gyttja, high organic matter content, well laminated

Table 5.3: Comparison of the mean and standard deviations (S.D.) of lithological, isotopic and β carotene values of the six cored lakes

Lake	LOI_{550} (OM content) (%)			LOI_{925} (carbonate content) (%)			$\delta^{13}C_{org}$ isotopic ratio (‰)			$\delta^{15}N_{org}$ isotopic ratio (‰)			β carotene (nmol g^{-1} (OM))		
	Mean	S.D.	CoV (%)	Mean	S.D.	CoV (%)	Mean	S.D.	CoV (%)	Mean	S.D.	CoV (%)	Mean	S.D.	CoV (%)
Pandin	14.3	5.2	36	2.7	1.7	63	–31.0	1.8	6	+1.3	0.6	50	21.2	24.5	116
Yambo	18.5	9.1	49	5.4	1.9	36	–30.9	0.9	3	+1.8	0.3	18	8.1	9.4	117
Mohicap	17.7	8.2	46	13.8	4.9	36	–32.2	2.4	7	+1.2	0.8	65	197.0	102.8	52
Sampaloc	30.3	5.9	19	5.8	3.8	65	–28.1	2.3	8	+5.7	0.5	8	457.9	345.5	75
Bunot	27.8	8.1	29	16.4	17.9	109	–30.1	2.3	8	+3.1	1.4	44	132.4	122.6	92
Palakpakin	12.2	3.2	26	7.8	2.1	27	–31.6	0.7	2	+4.5	0.9	20	144.7	115.2	80

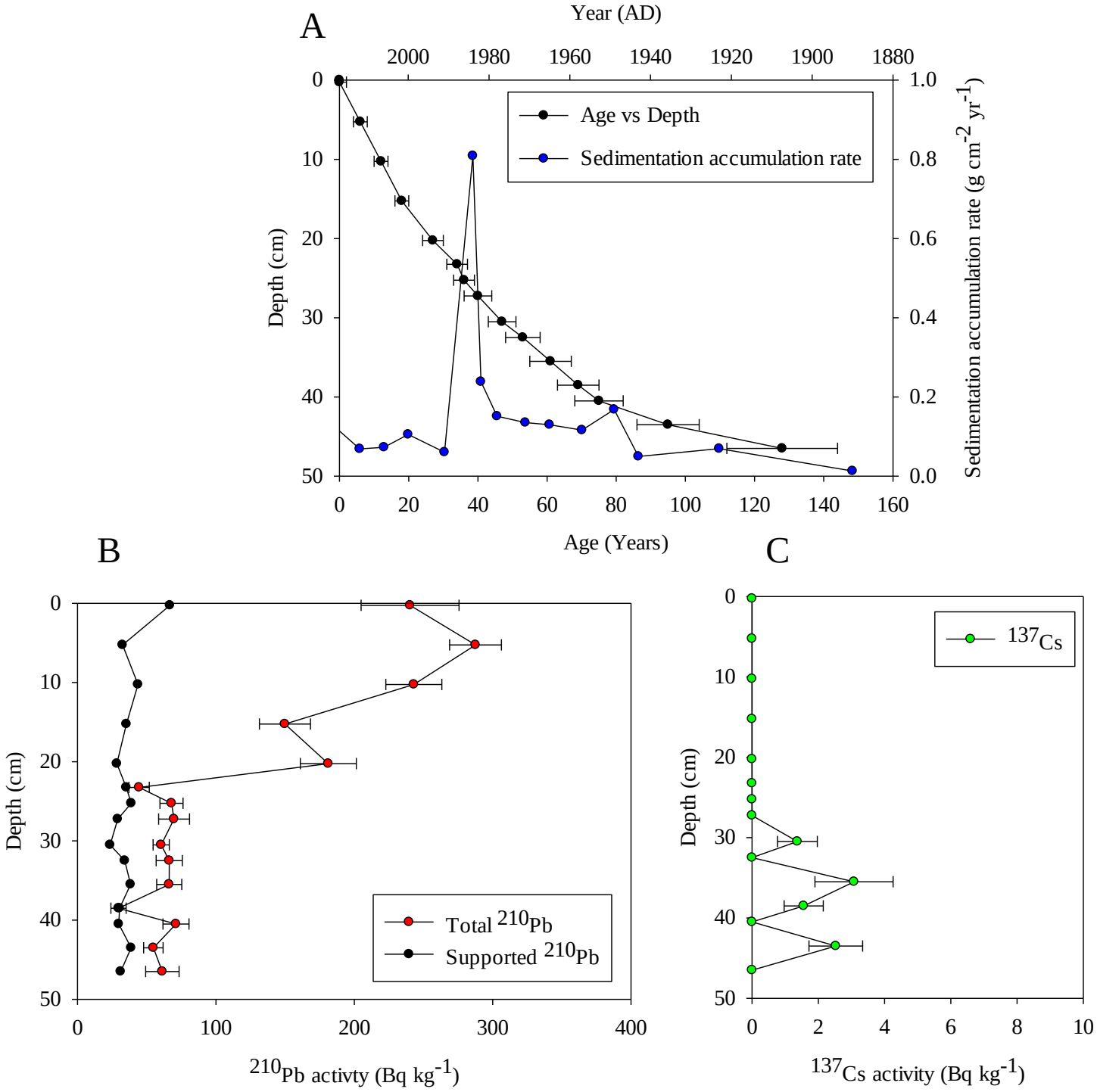


Figure 5.6: A) age-depth model and sediment accumulation rates for PANDIN sediment core, B) changes in total and supported ^{210}Pb and C) changes in ^{137}Cs activity for the PANDIN sediment core

5.4.3. Sediment Lithology

PANDIN had the second lowest mean and standard deviation of OM of 14.2% and 5.2% respectively, of all the cored lakes (Table 5.3). It also had the lowest mean and standard deviation for carbonate content of 2.7% and 1.7%. PANDIN also has the third highest CoV for both proxies. The OM and carbonate content increased gradually towards the youngest sediments to their maximum values of 21.0% (2000) and 8.0% (2000) respectively (Figure 5.7). In comparison, the water content declined in the youngest sediments from 87.0% (pre-1900) to 76.6% (2018±2). Bulk dry density was highly variable pre-1988, with values fluctuating between 0.23 and 0.92 g/cm^3. After 1988±3, the bulk dry density values declined and remained constant at c.0.10 g/cm^3.

5.4.4. Sediment Stable Isotopes and C/N Ratios

The mean and standard deviation for $\delta^{13}C$ was in the middle range of values yet this lake had the second lowest mean and third lowest standard deviation for $\delta^{15}N$ values (Table 5.3) and the second highest CoV for both proxies. The $\delta^{13}C$, C/N ratios, %C and %N records remained constant at –29.0‰, 13.0, 6.0% and 0.4% respectively until 1980 (Figure 5.7). After 1980, the $\delta^{13}C$ and C/N ratios gradually declined towards the youngest sediments to –34.0‰ and 10.0 respectively. The %C and %N values increased during the same period to 10.0% and 0.8% respectively. The $\delta^{15}N$ record remained constant at +0.8‰ until 2000, when the values started to increase, reaching a maximum of +3.3‰ in 2018±2.

5.4.5. Sedimentary Pigments

Sedimentary pigments in PANDIN showed high variability throughout the core (Figure 5.7) reflected in the second highest CoV value for β carotene. Concentrations of β carotene, diatoxanthin (siliceous algae) and diadinoxanthin (the precursor pigment of diatoxanthin) were relatively stable until 1910, followed by a decline to their lowest values by 1960, as summarised in the PCA axis 1 scores (41.7% variance explained; Figure 5.8). This also coincided with significant temporal change in algal communities (Figure 5.8). The concentrations of chlorophyll *a*, canthaxanthin (colonial cyanobacteria), okenone (purple sulphur bacteria), lutein-zeaxanthin (chlorophytes, cyanobacteria) and alloxanthin (cryptophytes) were low/undetectable until the late 1980s, when the concentrations of these pigments started to increase to their peak values. In 2003±2 and 2006±2, there were two sharp peaks in the concentrations of all pigments except chlorophyll *a* and diadinoxanthin.

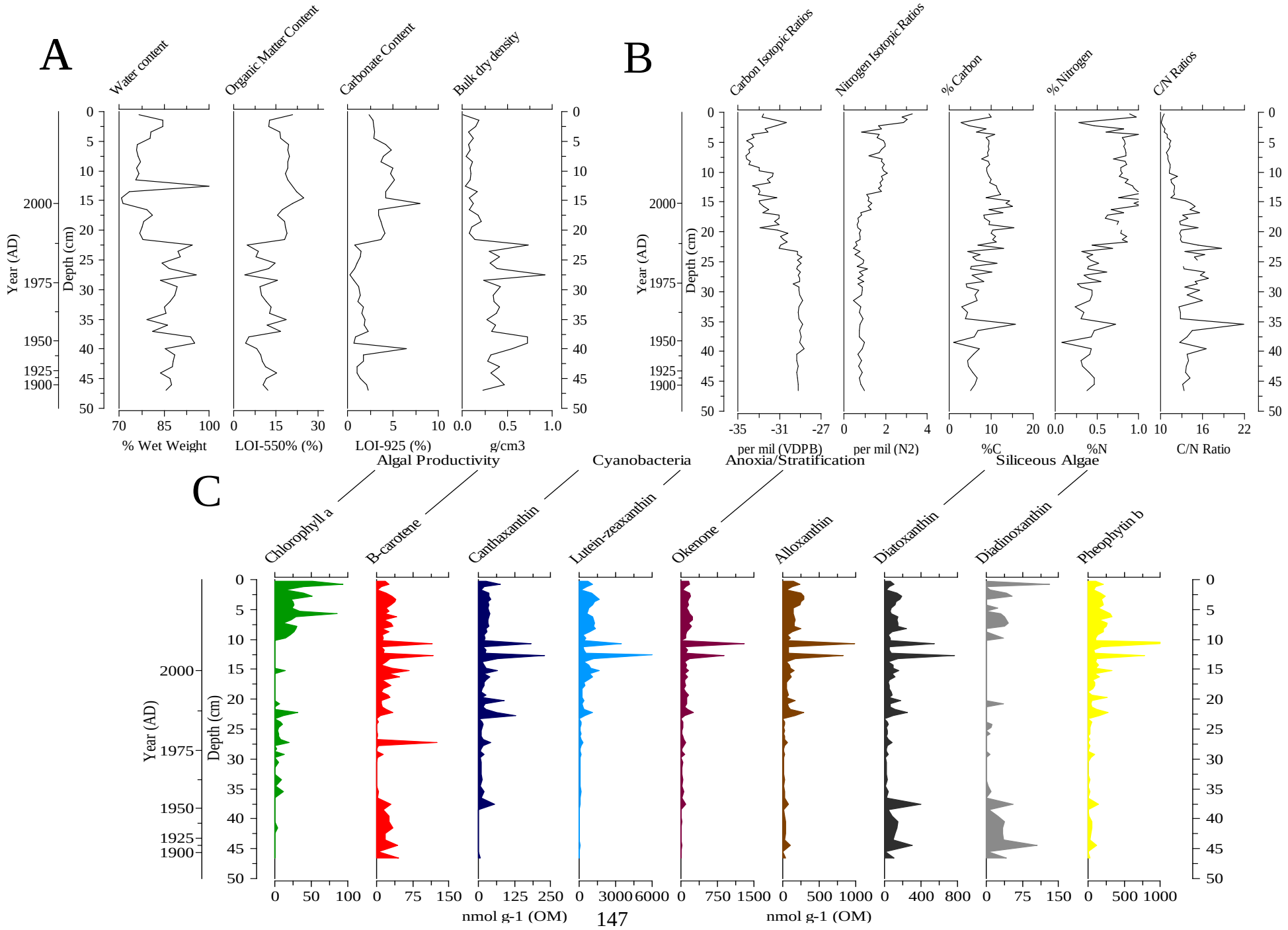


Figure 5.7: Sediment proxy results from PANDIN a) sediment lithology, b) stable isotope results ($\delta^{13}C_{org}$, $\delta^{15}N_{org}$) and C/N ratios and c) sedimentary pigment results

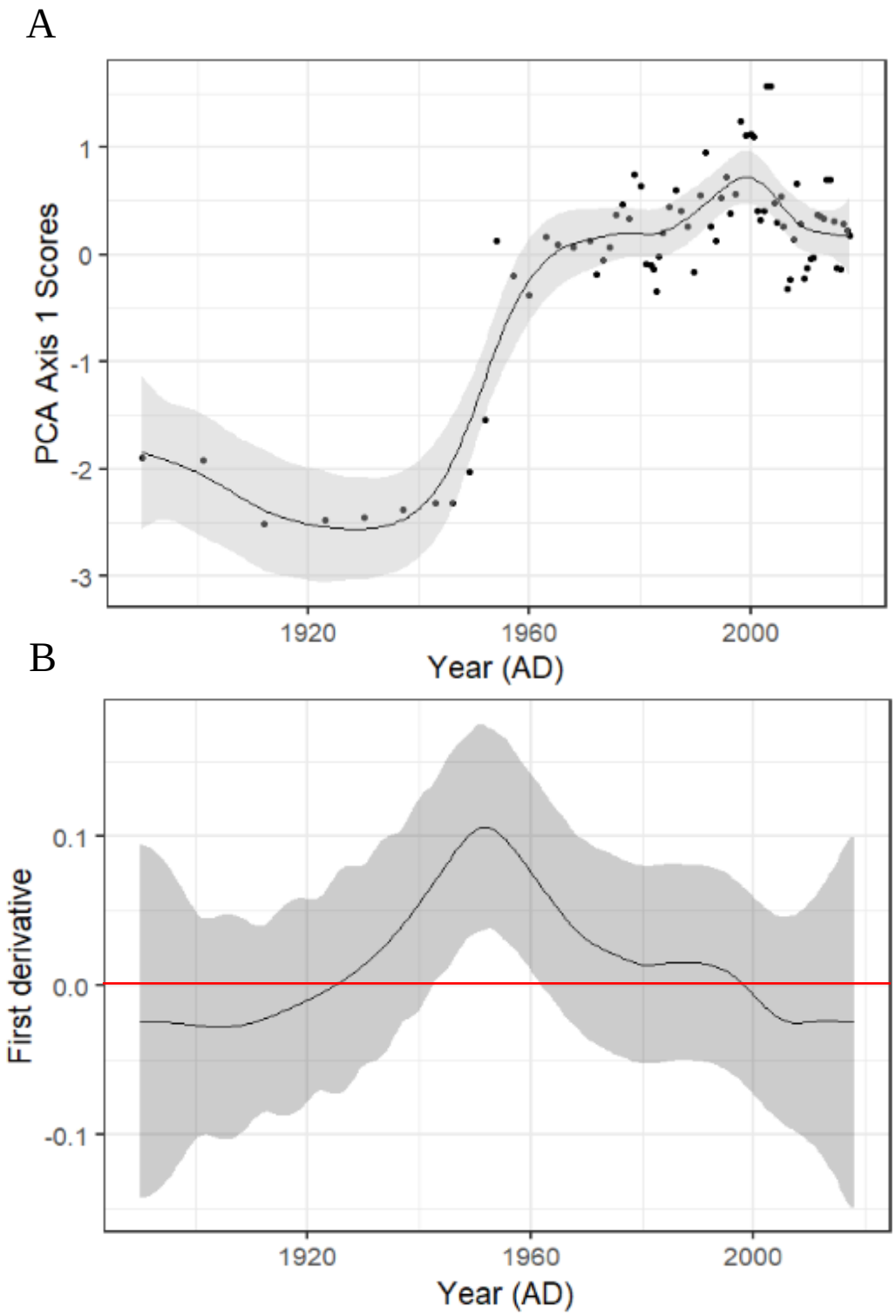


Figure 5.8: A) Generalised additive model fitted to the pigment PCA axis 1 scores from PANDIN. B) Estimated first derivatives (black line) of the GAM trend fitted to the PCA axis 1 scores from PANDIN. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. In both figures, the grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

5.4.6. Historical Records

The timings of notable events are shown in Table 5.4:

Table 5.4: Timeline of the main anthropogenic changes within the catchment of Lake Pandin with data source shown.

Year		Source
Pre-1989	Coconut plantations introduced. The exact date is unknown	Interview Data
1989	Aquaculture introduced. However it remained limited due to Pandin being oligotrophic (nutrient poor, low OM content, high DO concentrations in deeper waters) resulting in low phytoplankton biomass. This prolonged the culture period of fish stocks, requiring more food to grow, making it more expensive. The residents also stuck to the 10% rule (see Chapter 2).	Brillo, 2016d
2000	Aquaculture cage numbers peak at 1.7 hectares (7.08% of lake surface)	Brillo, 2016d
2001	1.54 hectares of cages (6.41% of lake surface) (maximum cage coverage)	LLDA
2003	Beginning of tourism on the lake	Brillo, 2016d
2005	SKMBLP established by residents to directly manage the ecotourism enterprise 3% of the surface area used for aquaculture	Brillo, 2016d
2006	0.64 hectares of cages (2.67% of lake surface)	Interview Data
2008	0.64 hectares of cages (2.67% of lake surface)	Interview Data
2010	0.64 hectares of cages (2.67% of lake surface)	LLDA
2012	12,108 tourists reported to have visited the lake (11,022 locals and 246 foreigners) Last reported algal bloom (they occurred annually prior to 2012, although the date they first appeared is unknown) 0.74 hectares of cages (3.08% of lake surface)	Brillo, 2017 LLDA
2013	14 registered fish cages	Brillo, 2017
2014	0.74 hectares of cages (3.08% of lake surface)	LLDA
2015	0.71 hectares of cages (2.96% of lake surface)	LLDA
2016	0.43 hectares of cages (1.79% of lake surface)	LLDA
2017	0.43 hectares of cages (1.79% of lake surface)	LLDA
2018	0.43 hectares of cages (1.79% of lake surface)	LLDA

5.4.7. Interpretation of Palaeolimnological Records

Low nutrient levels (Figure 4.8), depressed algal productivity (Figure 4.9), high water transparency (Table 4.2) and limited aquaculture (Table 5.4) suggests Lake Pandin is 'pristine' compared to the other lakes. The small standard deviations of $\delta^{13}C_{org}$, $\delta^{15}N_{org}$ and β carotene Table 5.3) suggests the impacts of aquaculture and other anthropogenic activities were minimal. Yet the high CoV (in comparison to its position in the aquaculture disturbance gradient) suggests this lake has high variation in the proxy record. High water transparency would cause limited algal productivity and high degradation of pigments prior to deposition in the sediment record (Cuddington and Leavitt, 1999). This is supported by the low β carotene concentrations (<30 nmol g^{-1}), OM values (<15%; Table 5.3) and high pheophytin *a*/chlorophyll *a* ratio (Reuss *et al.*, 2005). The morphological features of this lake are important in moderating its response to environmental change. Lake Pandin is the deepest of all the cored lakes (62 m), with the second deepest euphotic depth (7.2 m) and oxygen penetration depth (8.2 m; Table 4.2) making it more susceptible to a loss of pigments prior to deposition, than the other lakes. Pigment depositional fluxes in the sediment decline with lake, euphotic and

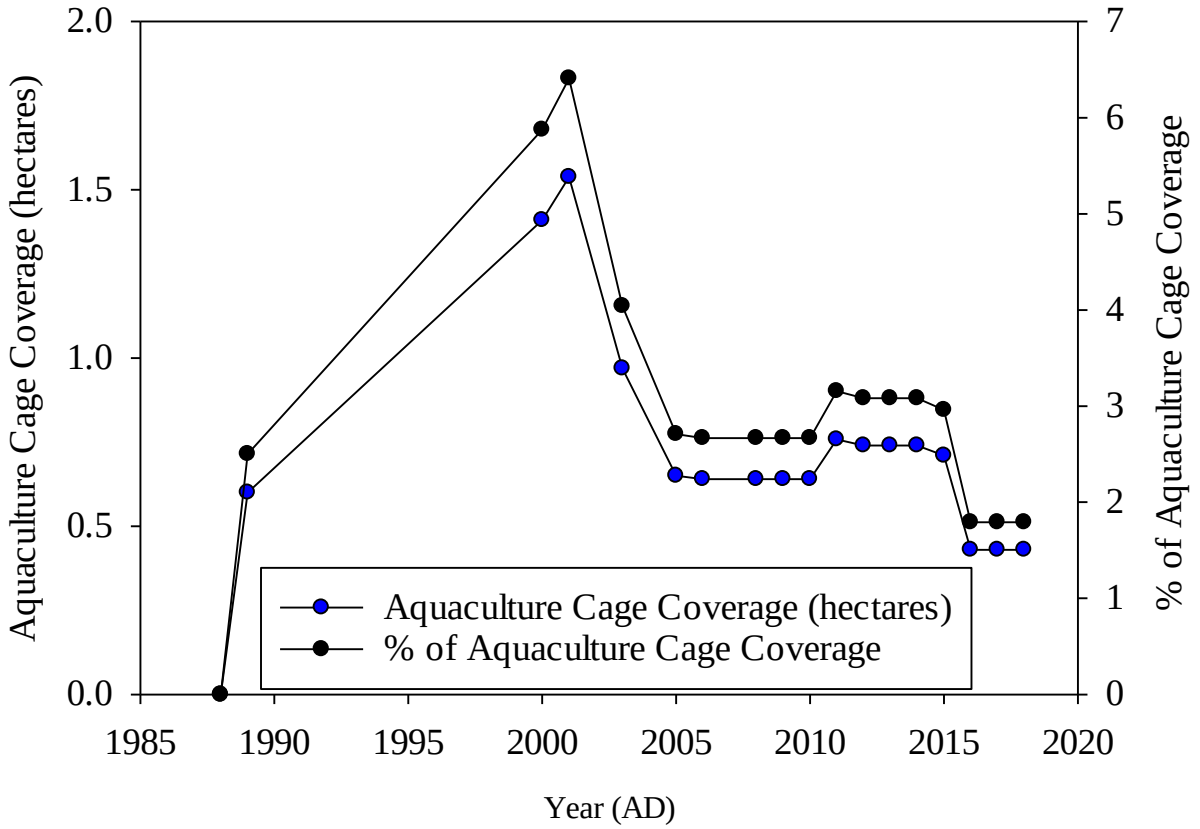


Figure 5.9: The surface area of Lake Pandin utilised for aquaculture cages in hectares (blue) and % (black). Source of each data point is listed in Table 5.4

oxygen penetration depth; there is a greater exposure to oxygen and light in a deeper water column which causes bleaching of the pigments (Cuddington and Leavitt, 1999). Furthermore, Lake Pandin has the smallest CA:LA ratio of all the cored lakes (Table 4.2), meaning the catchment is likely to have a relatively limited influence on in-lake processes (Walter *et al.*, 2020). Lake Pandin has been influenced by its 'pristine' condition and morphological features, which need to be considered when interpreting lake responses.

There are two major shifts in the proxy record of Lake Pandin. The first period of significant change between 1930 and 1970 (as determined by shifts in the GAM; Figure 5.8), was characterised by a decline in all pigments (most pronounced in the 1950s; Figure 5.7), a significant change in algal community composition (Figure 5.8) and a higher sedimentation rate (0.15 g cm^{-2} yr^{-1}). After 1980, pigments did not change significantly, but there was a continuous decline in $\delta^{13}C$ values from (–29 to –34‰), an increase in OM, a spike in sedimentation rates, the appearance of okenone (from purple sulphur bacteria) and declining C/N ratios. There was also high variability in the isotopic records, which coincided with a higher sampling resolution (see section 3.1.2). Since 2000, $\delta^{15}N$ isotopic ratios increased from +0.8 to +3.3‰.

Prior to 1930, the lack of change in the proxy record, specifically, high $\delta^{13}C$ values of –29‰ and the lack of anoxic indicators (e.g. okenone) (Figure 5.1a) suggests lake conditions were characterised by low algal productivity, limited anoxia and unstable stratification, preventing the formation of a purple sulphur bacteria layer (Beadle, 1966). The changes noted in algal communities and sedimentation rates between 1930 and 1970 were most likely the result of resuspension and focusing of sediment (Reuss *et al.*, 2005). The focusing of previously deposited material from the sides of the lake (evidenced by a spike in sedimentation rate at this time) (Sanger, 1988b) into the deep depositional site, could dilute the pigment concentration from settled phytoplankton (Engstrom *et al.*, 1985; Conley and Bianchi, 2005; Hily *et al.*, 2008). This could also explain the spike in the sedimentation rate in 1980. The period of change in the proxy record post 1980 suggests the onset of stratification and anoxic conditions in Lake Pandin which would explain the concordant increase in pigment concentrations (suggesting enhanced preservation), appearance of okenone and decline in $\delta^{13}C$ values. There are two possible explanations to explain the decline in C/N ratios. One explanation is increased contribution of algal derived OM (Meyers and Lallier-Vergès, 1999,) supported by higher β carotene and chlorophyll *a* concentrations. Alternatively, the decline in C/N ratios could be due to the influence of newly deposited material, which contains easily degraded components from

algae (with a C/N ratio of 6-8) (Meyers and Teranes, 2001; Gälman *et al.*, 2008). More prevalent anoxia, would cause enhanced preservation of OM, and the relative preservation of N in the sediment record. Lehmann *et al.*, (2002) determined seasonally anoxic Lake Lugano (Philippines) had a 30% lower net loss of C and N compared to oxic lakes. The best explanation of increasing $\delta^{15}N$ isotopic ratios (from +0.8 to +3.3 ‰) and the lack of change in algal indicators, since 2000, is changes in N cycling in the hypolimnion. Most mechanisms of N loss (such as nitrification, denitrification, anammox) are selective for ^{14}N (Bedard-Haughn *et al.*, 2003; Hamersley *et al.*, 2009), so preferential loss of excess ^{14}N from a system over time leaves the residual N pool enriched in ^{15}N (Högberg, 1990), causing increased $\delta^{15}N$ values. It could be hypothesised that a larger and more persistent anoxic hypolimnion caused increased denitrification (Hecky e*t al.*, 2001; Martin et al., 2010). Contemporary limnological data also suggests a shift in the limiting nutrient from P to N between 2006 and 2017. All these changes to the N cycle would promote the loss of N from the lake system, further enhancing N limitation in the epilimnion.

The limited nature and timings of anthropogenic activities and aquaculture in Pandin suggests climatic warming and/or other processes most likely drove changes in the proxy records. Human activities consist of the partial conversion of the lake catchment for coconut and nominal settlements (Figure 5.10; Table 5.4). This is due to the remote nature of the lake (the only access is via a 1 km trek from the nearest road) and a desire by the local inhabitants to preserve the lake for tourism (Figure 5.10; interview data; Brillo, 2017). Aquaculture was introduced in 1989, only occupying a limited volume (reaching a maximum extent of 7.1% of the lake's surface area in 2001). Due to a mismatch in timings, anthropogenic activities or aquaculture cannot be driving the concordant decline in pigments noted in the proxy record between 1930 and 1970. The most likely explanation is that its small CA:LA ratio (Ojala *et al.*, 2000) combined with intense typhoon activity in the early 1950s (Table 2.2) enhanced wind-induced wave turbulence and resuspension of sediment resulted in a reduction in pigment concentrations. The second period of change in the proxy record occurred post-1980 (Figure 5.7), again this does not coincide with the timings of anthropogenic activities, which did not start in the catchment until a decade later. Climatic warming is a more likely explanation. Historical meteorological records indicate a warming trend at the study area since 1900 with a faster rate of increase since 1980 (Figure 5.1b). Increased thermal stratification of the water column, more pronounced anoxic layer and enhanced preservation (Branco *et al.*, 2009) (Figure 5.7b; Wang *et al.*, 2012) would cause an increase in all pigments, the appearance of okenone

and a decline in $\delta^{13}C$ and C/N ratios. There are two possible explanations for the change in $\delta^{15}N$ values. The first is the combined pressure of low levels of aquaculture and the influence of tourists. The second explanation could be atmospheric N deposition contributing to the change in %N and $\delta^{15}N$ values (see section 1.4.1). Lake Pandin has lowest disturbance and, therefore, is most likely responding to climate rather than aquaculture or other anthropogenic drivers.

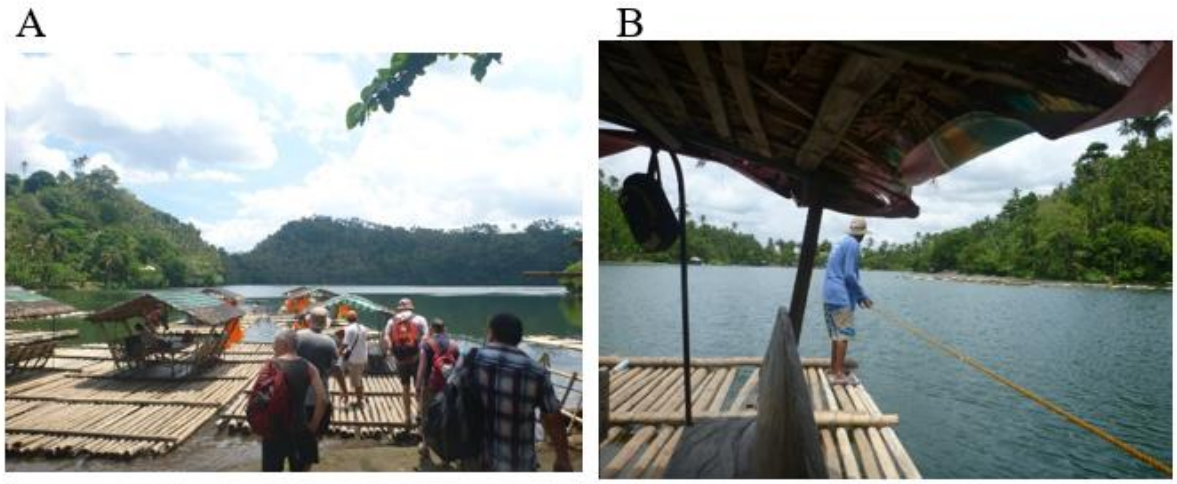


Figure 5.10*: Photographs of Lake Pandin (photo credit: Suzanne McGowan)*

A. *Foreground) bamboo rafts and background) relatively untouched catchment with a small number of coconut trees and man-made structures*
B. *A fisherman pulling the rafts via a rope tied to trees either side of the lake*

5.5. Lake Yambo

5.5.1. Field Core Description for YAMBO1

The sediment core YAMBO1 consisted of 104 cm of unconsolidated fine-grained sediment with abundant remains of aquatic macrophytes (Table 5.2).

5.5.2. Sediment Chronology for YAMBO1

In YAMBO1, total ^{210}Pb activities reached equilibrium with supported ^{210}Pb at a depth of 69.5 cm (Figure 5.11). Total ^{210}Pb activity decreased from 210.23 Bq Kg^{-1} (at the top of the core) to 27.91 Bq Kg^{-1}at 79.5 cm (Appendix C). Unsupported ^{210}Pb activities, declined irregularly with depth, permitting the use of the CRS model to develop the core chronology (Appleby and Oldfield, 1978; Appleby, 2001). Activities of ^{137}Cs and ^{241}Am were too low in the samples analysed to validate the chronologies based on ^{210}Pb data and the CRS model. The CRS age–depth model suggested that sediment accumulation rates increased from 0.11 to 0.22 g cm^{-2} yr^{-1} between the base of the core and 14.5 cm (1998±2), which is the peak accumulation. A drop in sediment accumulation rates followed this to 0.15 g cm^{-2} yr^{-1} at 0.75 cm (2015±2). The calculated age model indicated that the 104 cm core covered the past c. 350 years, however, ages for the basal sediments were extrapolated beyond the lowermost ^{210}Pb date using a constant sedimentation rate. The published chronology for YAMBO1 is found in Bannister *et al.*, (2019).

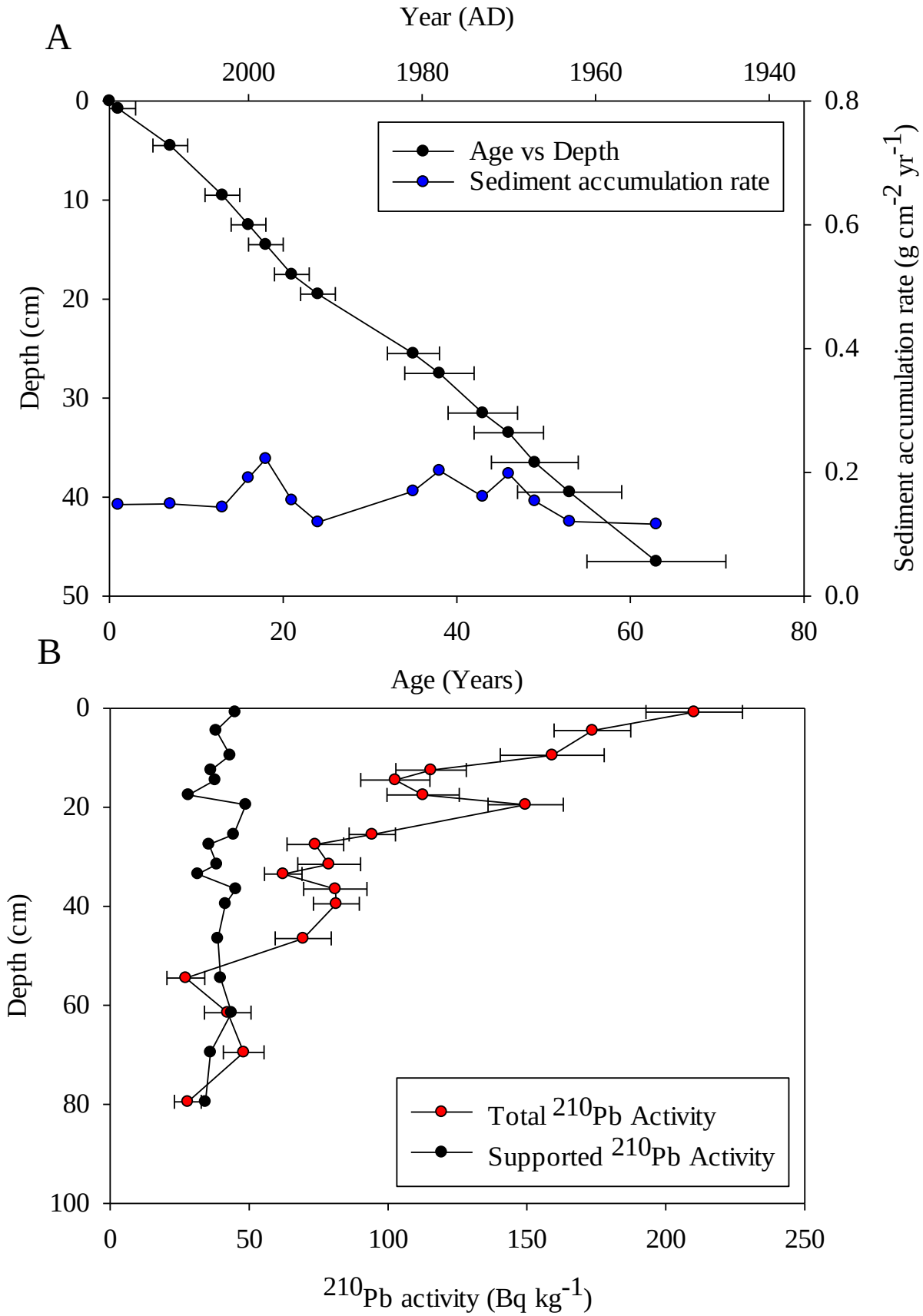


Figure 5.11: A) Age-depth model and sediment accumulation rates and B) changes in total and supported ^{210}Pb and for YAMBO1 sediment core

5.5.3. Field Core Description for YAMBO2

The 53 cm sediment core YAMBO2 consisted of light brown unconsolidated gyjtta with a high OM content (Table 5.2). The core was intersected by a layer of dark brown gyjtta with woody fragments at 35-33cm. Above 27 cm, the sediment core was very floccy with a high water content.

5.5.4. Sediment Chronology for YAMBO2

There was a reasonable match between the LOI_{550} profiles of sediment cores, YAMBO1 and YAMBO2, indicating good potential for cross correlation (Figure 5.12). Two tie-in points were determined through the ^{210}Pb-dated portion of core YAMBO1, with sediment core YAMBO2, based on % organic matter (LOI_{550}) (Table 5.5). The diatom profiles between YAMBO1 and YAMBO2 were also similar, so to further constrain the age model, eight further tie-in points were determined by matching shifts in diatom relative abundances from the ^{210}Pb-dated portion of core YAMBO1, with sediment core YAMBO2 (Figure 5.13; Table 5.5).

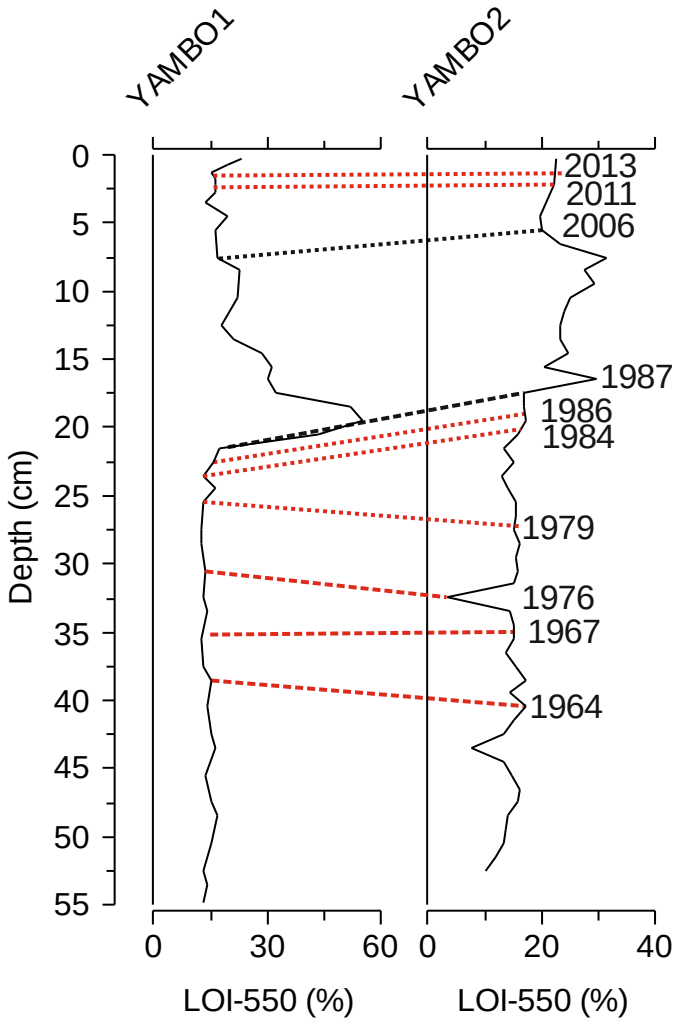


Figure 5.12: Correlations in % organic matter (LOI_{550}) between ^{210}Pb dated sediment core YAMBO1 and sediment core, YAMBO2. ^{210}Pb-derived dates (Year AD) corresponding to tie-points from YAMBO1 are indicated. The diatom tie-points are shown with a red dashed line and the LOI tie-points with a black dashed line.

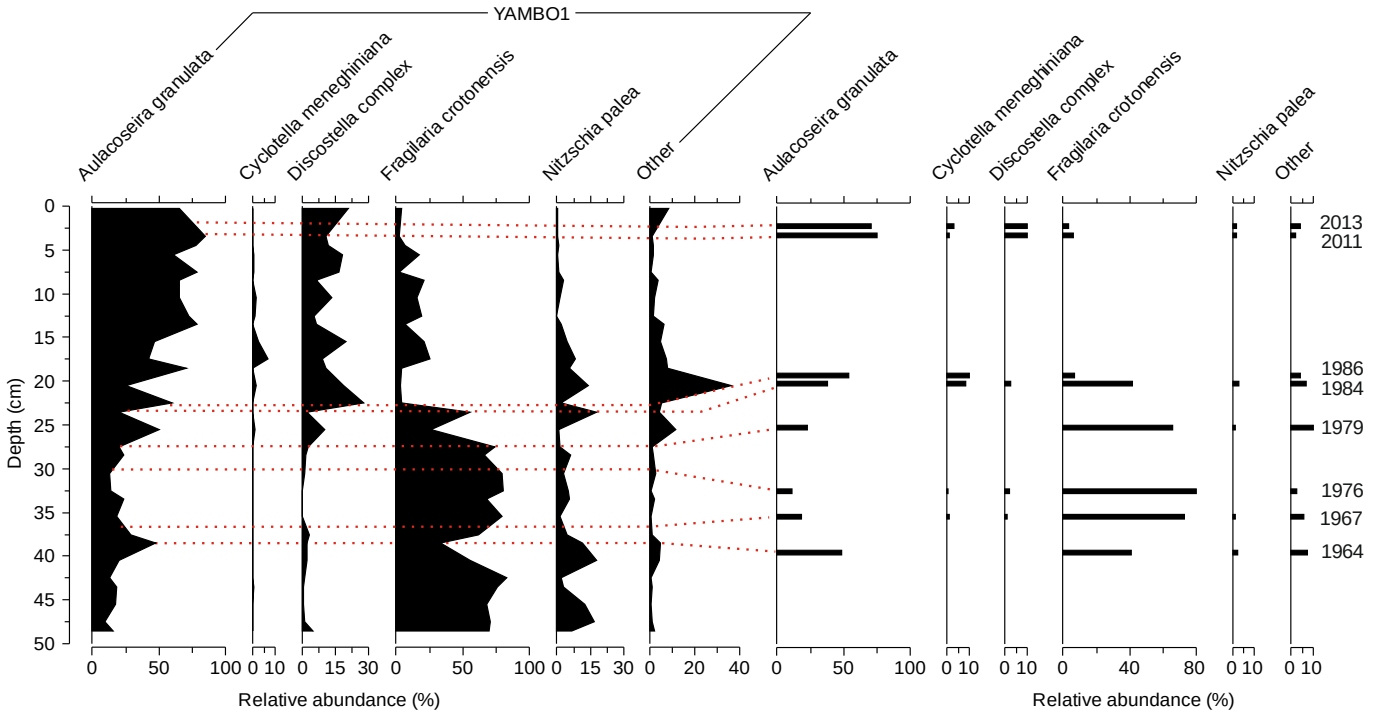


Figure 5.13: Correlations in % relative diatom abundance between ^{210}Pb dated sediment core YAMBO1 and sediment core, YAMBO2. ^{210}Pb-derived dates (Year AD) corresponding to tie-points from YAMBO1 are indicated by red dashed lines.

5.5.4.1. Justification for selection of tie-points based on diatom profiles

The two tie-points at depths 2.25 and 3.25 cm were vital for the development of the chronology of YAMBO2 as they captured the decline in *Aulacoseira granulata* between depths 0-5 cm in YAMBO1. Combined with the LOI tie points at depth 6.25 cm, this constrained the ^{210}Pb dates for the youngest sediments of YAMBO2. In addition, the LOI tie point at depth 17.25 cm matched with the peak in LOI_{550} in YAMBO1. The two tie points at depths 19.25 and 20.25 cm were crucial as they captured the sudden drop in *Fragilaria crotonensis* at depths 22-25 cm in YAMBO1. The four oldest tie points at depths 25.25, 32.5, 35.5 and 39.5 cm were necessary as they captured the earliest peak in *Aulacoseira granulata* and its subsequent decline in abundance. These tie points also captured the first drop in *Fragilaria crotonensis* abundance (and its recovery) between depths 25-40 cm in YAMBO1. This helped to constrain the age model of YAMBO2 back to the earliest ^{210}Pb date from YAMBO1.

Table 5.5: List of tie-points between YAMBO1 and YAMBO2 correlated using % organic matter (LOI_{550}) and diatoms

YAMBO1 (Collected in 2015)		YAMBO2 (Collected in 2018)		Method used
Depth (cm)	Date	Corresponding Depth (cm)	Corresponding Date	
2.5	2013	2.25	2013	Diatom Analysis
3.5	2011	3.25	2011	Diatom Analysis
7.5	2006	6.25	2006	LOI_{550}
10.5	2002	12.25	2002	Diatom Analysis
21.5	1987	17.25	1987	LOI_{550}
22.5	1986	19.25	1986	Diatom Analysis
23.5	1984	20.25	1984	Diatom Analysis
27.5	1979	25.5	1979	Diatom Analysis
30.5	1976	32.5	1976	Diatom Analysis
35.5	1967	35.5	1967	Diatom Analysis
38.5	1964	39.5	1964	Diatom Analysis

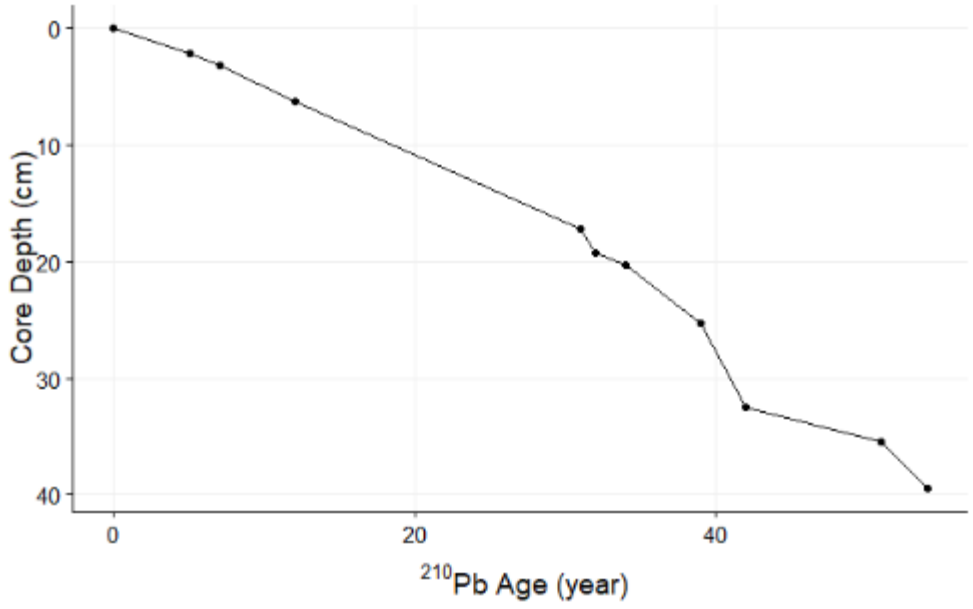


Figure 5.14: Age-depth model for core YAMBO2 using cross-dating methods

5.5.4.2. Age Model for YAMBO2

Any dates below 39.5 cm in YAMBO2 were extrapolated using the mean of the three earliest sedimentation rates from core YAMBO1 (at depths 49.5, 53.3 and 63.3 cm) to account for the uneven sedimentation rates amongst cores collected from the same lake (Appendix C). This resulted in the deepest sample from YAMBO2 at 53.5 cm having an extrapolated date of 1945. An age-depth model (Figure 5.14) was created for YAMBO2 based on the cross correlation with ^{210}Pb dated constant rate of supply (CRS) model (Appleby, 2001) established for YAMBO1.

5.5.5. Sediment Lithology

YAMBO2 had the third highest mean OM content (18.5%) yet the highest standard deviation (9. 15%) and CoV (49%). This sediment core also had the second lowest mean carbonate content (5.4%), standard deviation (1.9%) and second lowest CoV (36%) of the cored lakes (Table 5.3). The water, OM and carbonate content remained relatively constant throughout the length of the core except for a dip in values in 1971, most likely due to an influx of mineralogical material (as shown by an increase in the residual component of LOI; Figure 5.15). The bulk dry density fluctuated between 0.3 g/cm^3 and 0.03 g/cm^3 along the whole core, with two spikes to 0.5 g/cm^3 in 1971 and 2017, most likely from in-wash events.

5.5.6. Sediment Stable Isotopes and C/N Ratios

YAMBO2 had the second lowest standard deviation for δ^{13}C and lowest for δ^{15}N of 0.9‰ and 0.3‰ respectively. YAMBO2 had the second lowest CoV for δ^{13}C and δ^{15}N of 3% and 18%. The δ^{13}C record declined towards the present day from –29.0‰ (1945) to –30.9‰ (2005) followed by a sharp decline to -32.5‰ (2018) (Figure 5.15). The δ^{15}N record remained relatively constant throughout the whole core, only fluctuating slightly between +1.3 and +2.7‰. The %C and %N records remained constant at c.6.0% and 0.5% until 1990 when the values started to increase rapidly to their maximum extent of 19.0% and 1.8% (1994). This was followed by a decline in %C and %N values to c.6% and 0.6% respectively in 2018. Compared to the other geochemical records from YAMBO2, the C/N values were variable, fluctuating between 19 and 11.5, pre-1980 (Figure 5.15). After 1980, the values remained constant at 11.5 until 1994, when there was a sharp increase in values to 15.1 (2004). In the youngest sediments, there was a decline in values to 11.6 (2018).

5.5.7. Sedimentary Pigments

YAMBO2 had the lowest sedimentary pigment concentrations and standard deviation of β carotene of all the cored lakes (Table 5.3) yet the highest CoV for β carotene (117%). Pigment concentrations of chlorophyll *a*, β carotene, canthaxanthin (cyanobacteria), okenone (purple sulphur bacteria), lutein-zeaxanthin (chlorophytes, cyanobacteria), pheophytin *b* (chlorophytes) alloxanthin (cryptophytes) and diatoxanthin (diatoms) remained low until the early 2000s when concentrations started to increase (Figure 5.15). There was also a peak in concentrations of chlorophyll *a*, canthaxanthin, okenone, pheophytin *b*, alloxanthin and diatoxanthin in the early 1970s. Unlike the other lakes, echinenone (cyanobacteria) was not detected in Lake Yambo. Concentrations of all pigments sharply increased until 2010 and then declined to the present day as summarised in the PCA axis 1 scores (29.0% variance explained) which coincides with significant temporal change in algal communities (Figure 5.16).

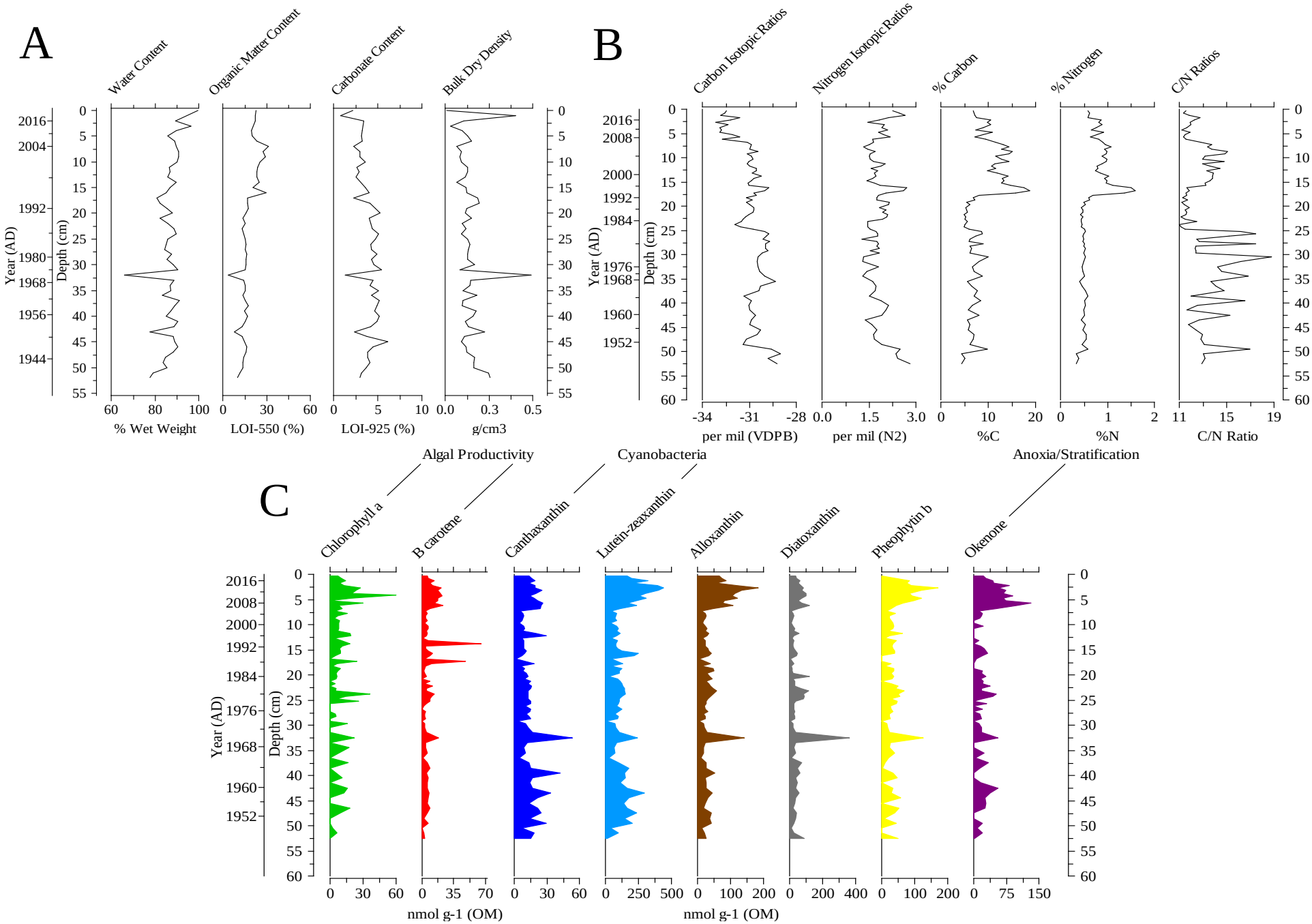


Figure 5.15: Sediment proxy results from YAMBO2 a) sediment lithology, b) stable isotopes ($\delta^{13}C_{org}$, $\delta^{15}N_{org}$) and C/N ratios and c) sedimentary pigments

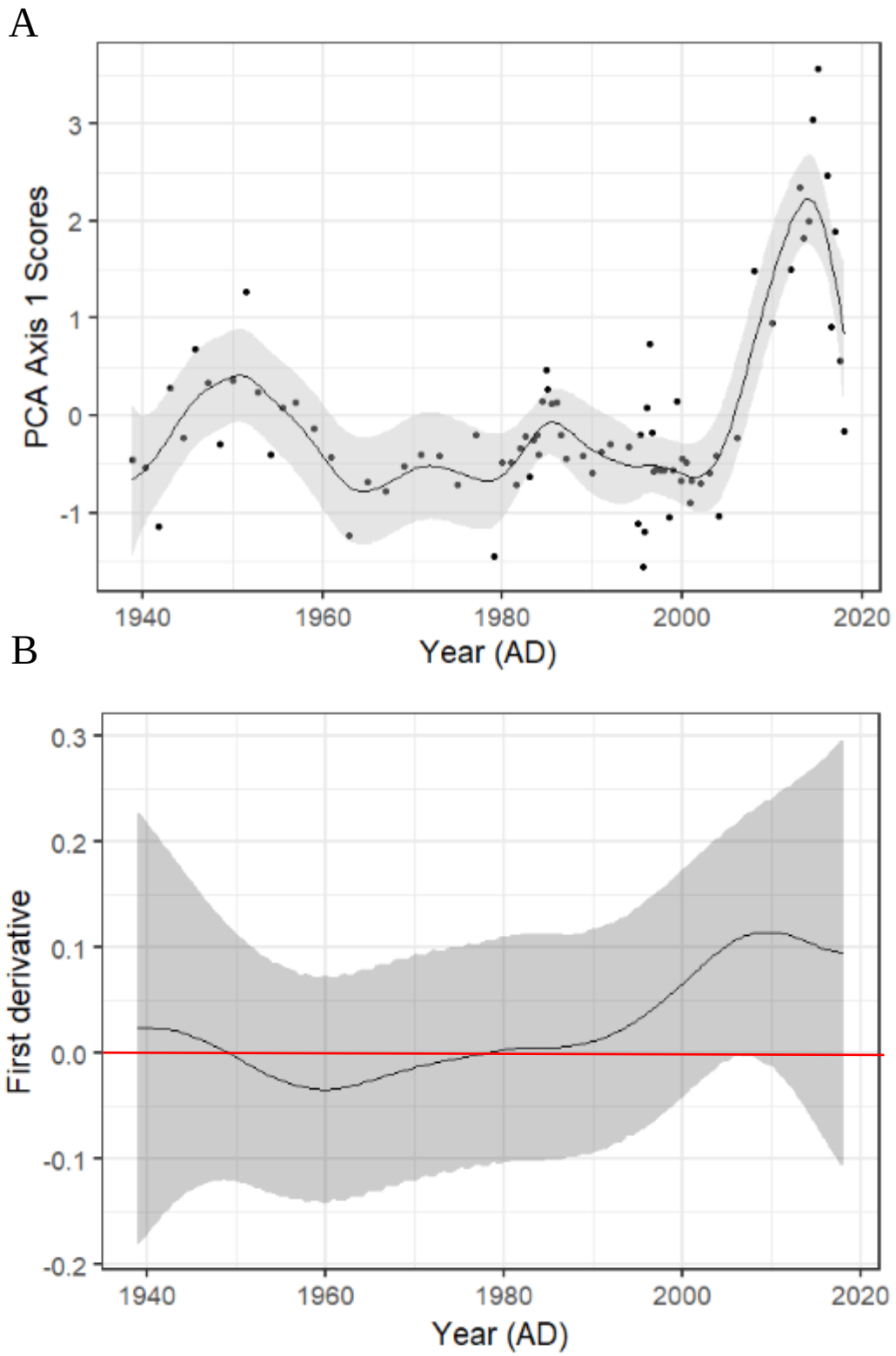


Figure 5.16: A) Generalised additive model fitted to the PCA axis 1 scores from YAMBO2. B) Estimated first derivatives (black line) of the GAM trend fitted to the PCA axis 1 scores from YAMBO2. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. In both figures, the grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

5.5.8. Historical Records

The timings of notable events are shown in Table 5.6:

Table 5.6: Timeline of the main anthropogenic changes within the catchment of Lake Yambo from 1956 onwards with data sources shown

Year		Source
1956	First coconut plantations	Interview Data
1980s	Algal blooms occur annually in January	Interview Data
1989	Aquaculture introduced	Interview Data
	Colour of the lake water changed from blue to green	
1996	2.6 hectares of cages (8.52 % of lake surface)	Interview Data
1997	LLDA introduced tilapia fishlings	Brillo, 2016c
1999	Highest number of fish kills reported (4th February)	Interview Data
	Aquaculture cage numbers reached its peak	
2000	2.1 hectares of cages (6.89 % of lake surface)	Interview Data
2001	2.059 hectares of cages (6.75% of lake surface)	LLDA
2002	Road built to the edge of Lake Yambo, now the lake is accessible by road	Interview Data
2004	Fish kills observed in February	Interview Data
	1.6 hectares of cages (5.25% of lake surface)	
2006	1.2 hectares of cages (3.93% of lake surface)	Interview Data
2009	0.8 hectares of cages (2.62% of lake surface)	Interview Data
2010	0.74 hectares of cages (2.43% of lake surface)	LLDA
2012	3 fish cages operational	Interview Data
	Fish kill event reported	
	0.64 hectares of cages (2.10% of lake surface)	LLDA
2013	0.74 hectares of cages (2.43% of lake surface)	LLDA
2014	0.71 hectares of cages (2.43% of lake surface)	LLDA
2015	Tourism increased	Interview Data
	3 Fish pen cages operational	
	0.71 hectares of cages (2.33% of lake surface)	LLDA
2016	0.64 hectares of cages (2.10% of lake surface)	LLDA
2017	0.21 hectares of cages (0.69 % of lake surface)	LLDA
	Tourism became more established	Interview Data
2018	Only 1 fish pen operational (600m^2 allocated for aquaculture)	Interview Data
	Establishing new fish pens is prohibited	LLDA
	0.15 hectares of cages (0.49% of lake surface)	

5.5.9. Interpretation of Palaeolimnological Records

Lake Yambo had the lowest mean β carotene (8.1 nmol g^{-1}; Table 5.3) of all the sampled lakes, suggesting either low algal production or enhanced decomposition of algal pigments prior to deposition in the sediments. The low pigment concentrations across all algal groups could be due to two factors. Firstly, Lake Yambo had the deepest euphotic depth of 22.2 m (Table 4.2) and a water column depth of 27 m (Table 2.4). This would inhibit pigment preservation as evidenced by low concentrations of β carotene. Combined with high irradiance in the water column, this would promote degradation, especially of the more labile pigments, such as chlorophyll *a* (Leavitt and Hodgson, 2006). Secondly, low pigment concentrations could be due to low levels of nutrients in the lake (Figure 4.9). Feng *et al.*, (2019) found that a lack of nutrients in oligotrophic lakes caused a lower density of both bacterial and algal communities. This is supported by low Chl:TP ratio (of 0.02) showing low chlorophyll *a* production in this lake. In Lake Yambo, the low concentrations of β carotene were probably due to a combination of both factors. Since 2000, concordant increases in all pigments, and a decline in both the pheophytin *a*/chlorophyll *a* (c. 1.5) and C/N ratios, provide evidence of improved preservation conditions (Gälman *et al.*, 2008).

Prior to 2000, there was minimal change in algal communities and lithological records, despite the high variability in the C/N record (from 1950), a continuous decline in $\delta^{13}C$ values from -29.0 (in 1950) to -31.5‰ (in 1980) and a colour change of the lake water from blue to green (Table 5.6). There was a notable spike in pigment concentrations and bulk dry density in 1970. After 2000, there was a major shift in algal communities composition (as shown by the PCA axis scores; Figure 5.16) and a change in the limiting nutrient from P to N (see Chapter 5). This change is characterised by concordant increases in all pigment concentrations and OM content and the continued decline in $\delta^{13}C$ values to –33‰. Throughout the sequence, the $\delta^{15}N$ record remained constant at c. +2‰.

Palaeolimnological evidence suggests that prior to 2000, Lake Yambo had a well-mixed water column, limited hypolimnetic anoxia and extensive OM degradation prior to deposition in the sediment record (Guilizzoni *et al.*, 1986). Post 2000, a significant change in algal communities (characterised by a concordant increase in all pigments) indicates enhanced preservation conditions (Reuss *et al.*, 2005), and a decline in both the pheophytin *a*/chlorophyll *a* ratio (c. 1.5) and C/N ratios (Gälman *et al.*, 2008) suggests stable stratification and pronounced anoxia conditions. Low nutrient levels in the epilimnion (determined by water monitoring data

(LLDA, 2006f) and contemporary limnology) indicates increased productivity was not responsible.

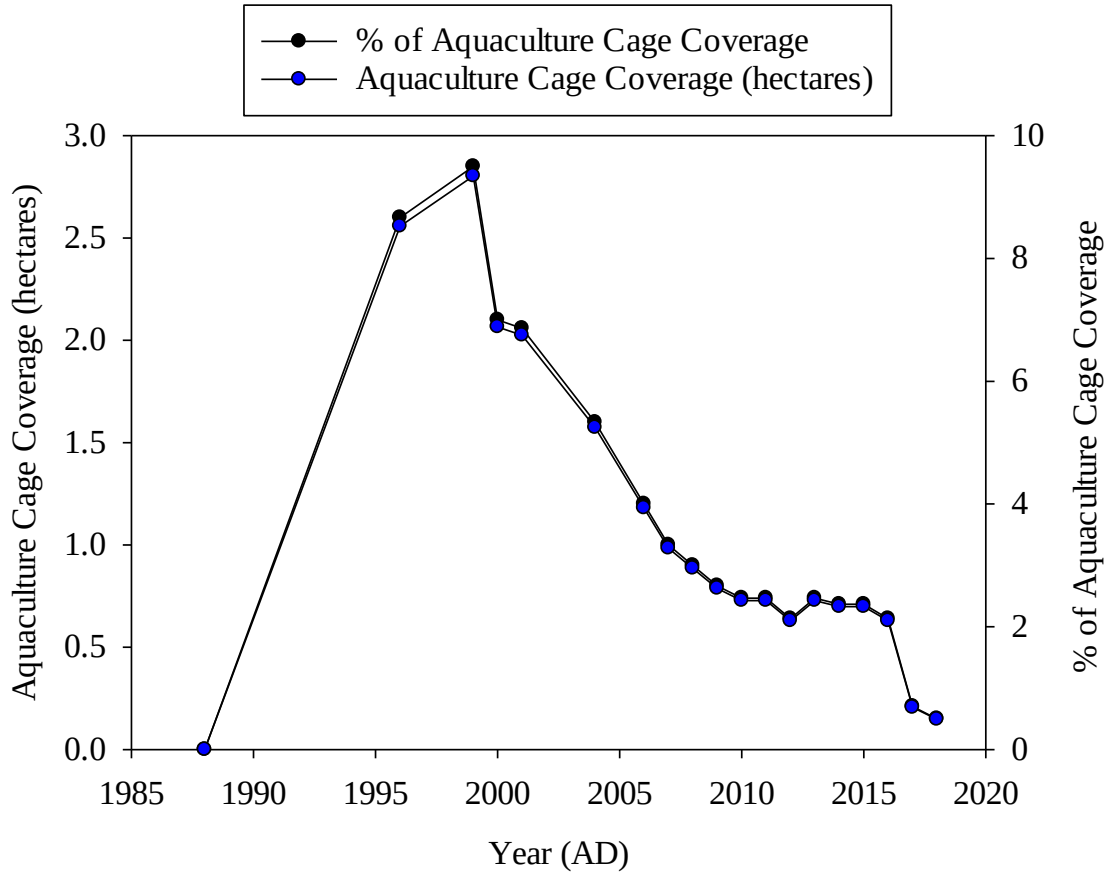


Figure 5.17: The surface area of Lake Yambo utilised for aquaculture cages in hectares (blue) and % (black). Source of each data point is listed in Table 5.6

The high variability in the C/N record from 1950 to 1980 coincides with intensive deforestation within the lake catchment. The first coconut plantations were planted in 1956 before spreading to a large proportion of the catchment by the present day (see interview data in Appendix A; Figure 5.18). Deforestation can cause increased soil erosion, with a larger proportion of terrestrial OM entering the lake (Rhodes and Davis 1995; Kaushal and Binford, 1999). Combined with the steep sided catchment, this would result in the lake being supplied with allochthonous material, especially during the wet season. The influx of the terrestrial end members with high C/N ratios (>15 (Table 5.1) could be a possible cause of the high variability in the sediment record (Meyers and Lallier-Vergès, 1999). The spike in pigment concentrations and bulk dry density in 1970 was probably due to a large influx of minerogenic material. Although aquaculture was introduced in 1989 (Figure 5.17), along with the construction of

human settlements (Figure 5.18), the low intensity and mismatch of timings (between aquaculture and algal community change) suggest they are unlikely to be driving the changes seen in algal communities, post 2000. A lack of change in the $\delta^{15}N$ record (remaining constant at c. +2‰) supports the view that aquaculture had minimal influence on this lake, as fish food and human waste have high $\delta^{15}N$ values, of +33‰ and +10 to +20‰ respectively (Townsend-Small *et al.*, 2007). A more likely explanation is that an accelerating rate of increase in climatic warming, since 1980 caused more prevalent anoxia and enhanced preservation of algal pigments (Figure 5.7b; Branco *et al.*, 2009). Increased anoxia would cause internal loading of P and could account for the shift in limiting nutrient and the colour change of the lake water from blue to green (most likely suggesting the presence of HABs; Table 4.6).

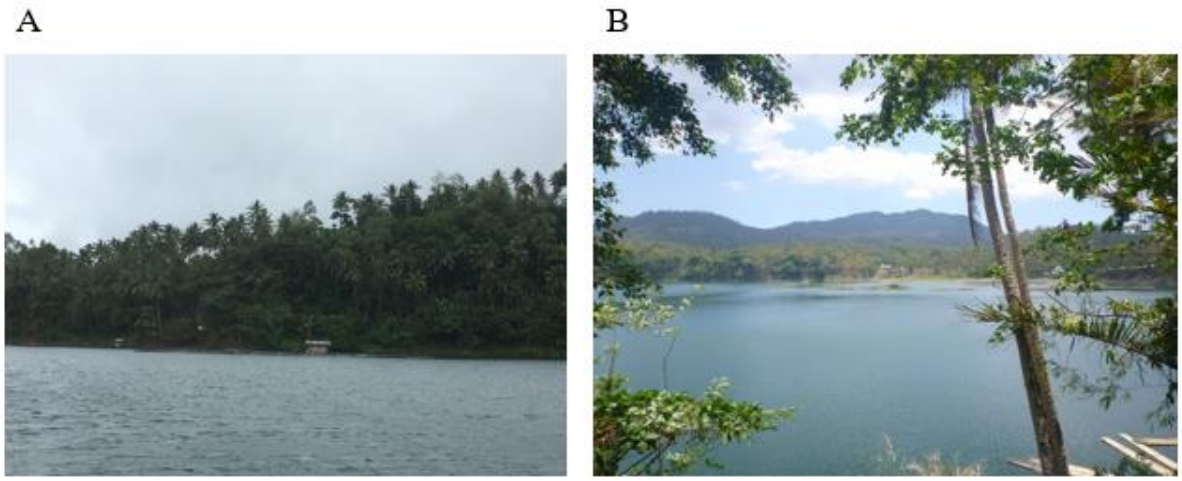


Figure 5.18: Photographs of Lake Yambo

A. *Man-made structures and coconut plantations*
B. *Overview of Lake Yambo showing minimal aquaculture cages (photo credit: Suzanne McGowan)*

5.6. Lake Mohicap

5.6.1. Field Core Description for MOH1

The 88 cm sediment core MOH1 consisted of unconsolidated watery and fine-grained black coloured sediment (Table 5.2).

5.6.2. Sediment Chronology for MOH1

In MOH1, total ^{210}Pb activity (Figure 5.19) reached equilibrium with supported ^{210}Pb at a depth of 46.7 cm. Total ^{210}Pb activity decreased from 186.45 Bq Kg^{-1} (at the top of the core) to 33.67 Bq Kg^{-1}at 47.5 cm. Unsupported ^{210}Pb activities, declined irregularly with depth, permitting the use of the CRS model to develop the core chronology (Appleby and Oldfield, 1978; Appleby, 2001). Activities of ^{137}Cs and ^{241}Am were too low in the samples analysed to validate the chronologies based on ^{210}Pb data and the CRS model (Appendix D). The CRS age-depth model suggested that sediment accumulation rates increased from 0.06 to 0.25 g cm^{-2} yr^{-1} between the base of the core and 2.0 cm (2012±2), which was peak accumulation. The calculated age model indicated that the 95 cm core covered the past c. 260 years (1715), however ages for the basal sediments were extrapolated beyond the lowermost ^{210}Pb date using the earliest known sedimentation rate (0.0653 g cm^{-2} yr^{-1}). The published chronology for MOH1 is found in Bannister *et al.*, (2019).

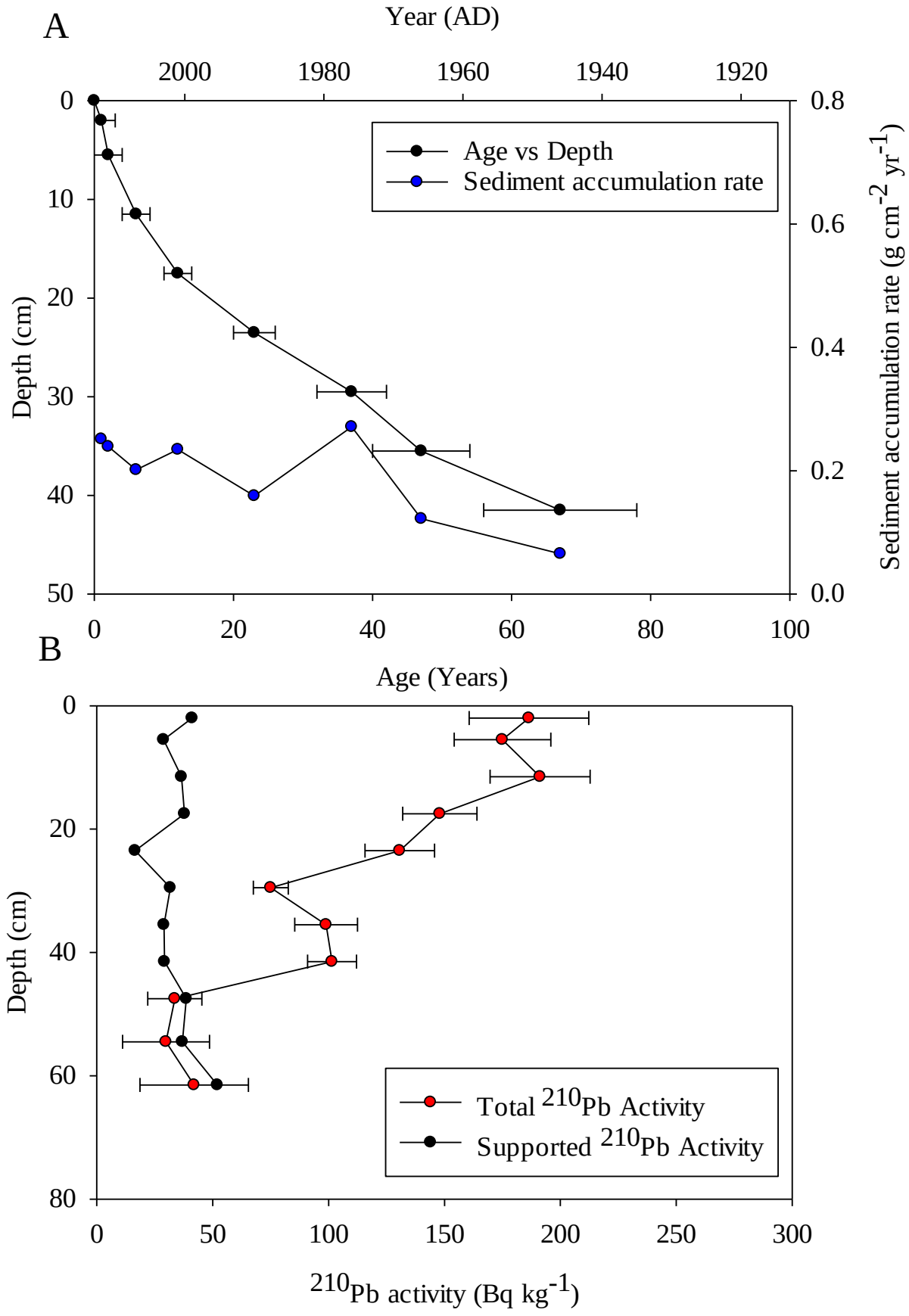


Figure 5.19: A) Age-depth model and sediment accumulation rates and B) changes in total and supported ^{210}Pb for the MOH1 sediment core

5.6.3. Field Core Description for MOH2

Between 120 and 79 cm, sediment core MOH2 consisted of light brown gyttja with a high OM content (Table 5.2). This was followed by an abrupt colour change at 79 cm from light to dark brown gyttja. The sediment between 79-36 cm was characterised by a high abundance of leaves from aquatic macrophytes. The less consolidated sediment from 36 to 0 cm, consisted of very floccy sediment with a high water content and gyttja in the top 6 cm.

5.6.4. Sediment Chronology for MOH2

The profiles of LOI_{550} of the sediment cores, MOH1 and MOH2 did not match well (Figure 5.20), so diatom analysis was used between the depths of 5-40 cm to help establish the stratigraphic relationship between the cores. The diatom profiles between MOH1 and MOH2 were similar, allowing for cross correlation between the cores (Figure 5.21). Seven tie-points were determined through cross correlation of diatom relative abundances (Table 5.7).

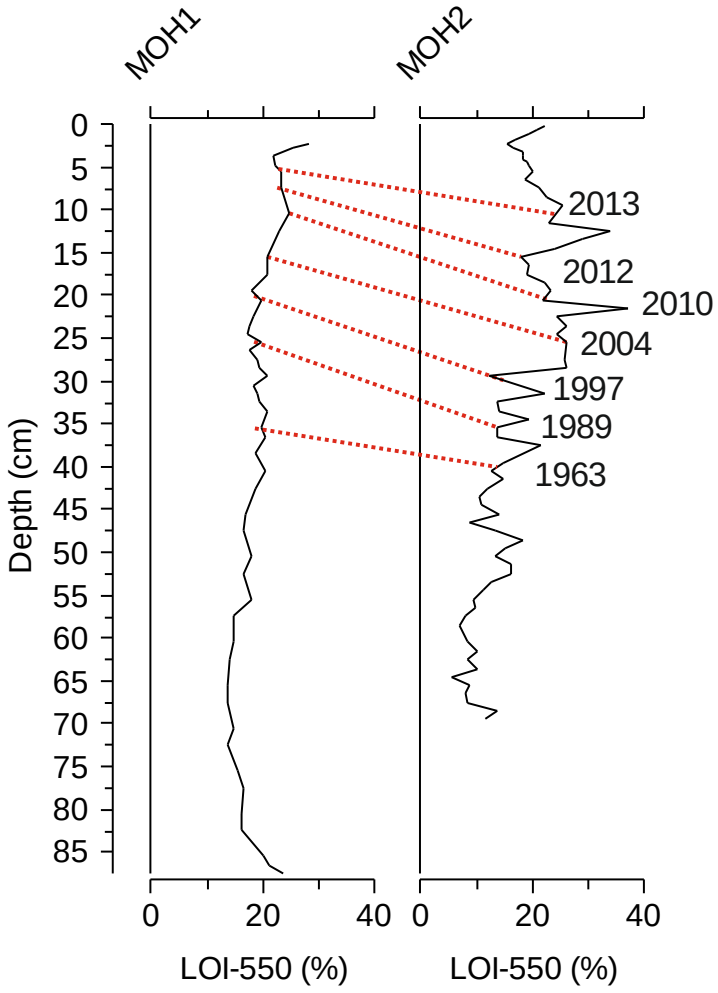


Figure 5.20: Correlations in % organic matter (LOI_{550}) between ^{210}Pb dated sediment core MOH1 and sediment core, MOH2. ^{210}Pb-derived dates (Year AD) corresponding to diatom tie-points from MOH1 are indicated by the red dashed lines

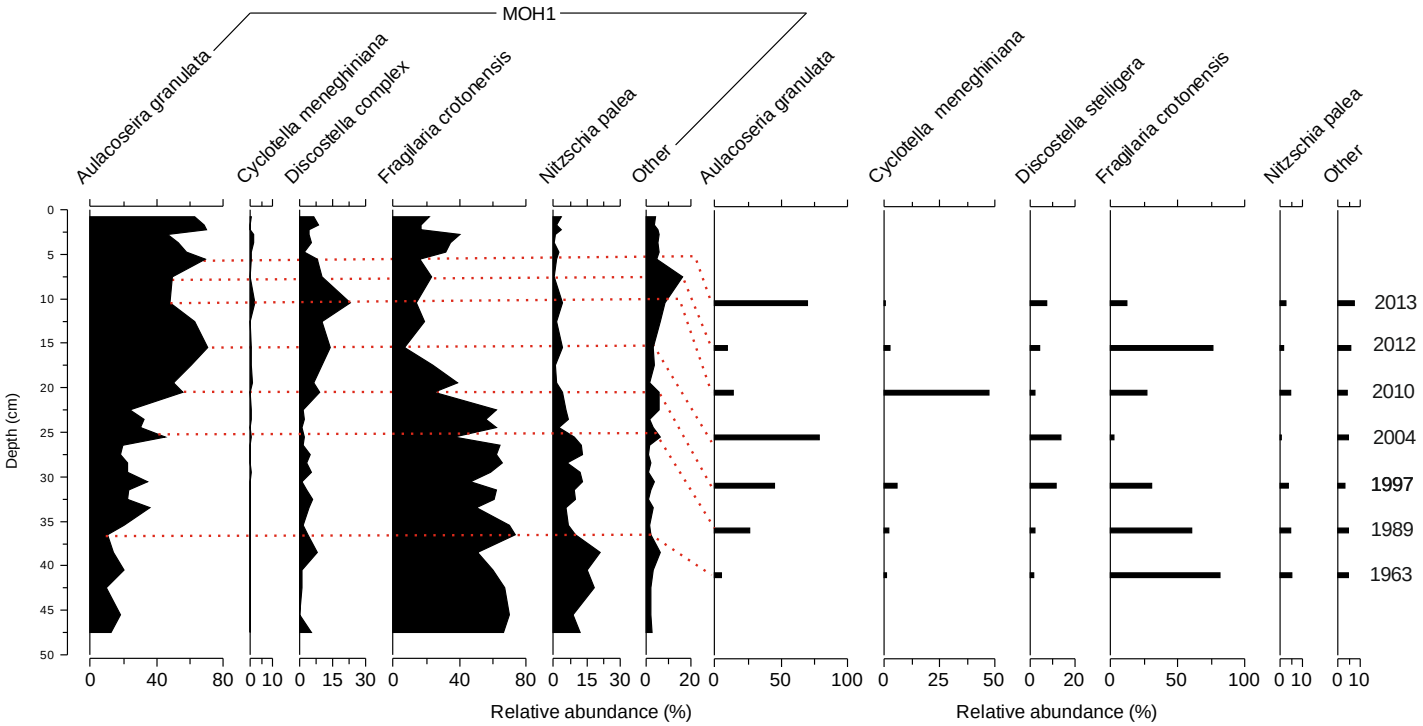


Figure 5.21: Correlations in % relative diatom abundance between ^{210}Pb dated sediment core MOH1 and sediment core, MOH2. ^{210}Pb-derived dates (Year AD) corresponding to tie-points from MOH1 are indicated by red dashed lines

5.6.4.1. Justification for selection of tie-points based on diatom profiles

No tie-points were based on LOI_{550} in MOH2 due to the poor match between LOI_{550} profiles for MOH2 and MOH1. The diatom tie-points at depths 10.25, 15.25 and 20.25 cm were crucial for the development of a chronology for MOH2 as they captured the fluctuating trend in *Aulacoseira granulata* and *Fragilaria crotonensis* abundance between the depths of 5 to 15 cm on MOH1. The tie-points at depths 25.25, 30.5, 35.5 and 40.5 cm were necessary as they captured the decline in *Fragilaria crotonensis* and the increase in *Aulacoseira granulata* and *Discostella* complex abundance between the depths of 15 to 40 cm on MOH1. This helped to constrain the age model of MOH2 back to the earliest ^{210}Pb date from MOH1.

5.6.4.2. Age Model for MOH2

Below 40.5 cm, dates in MOH2 were extrapolated using the three earliest known sedimentation rates from core MOH1 (at depths 29.5, 35.5 and 41.5 cm, Appendix D). The three earliest values were used to account for the uneven sedimentation rates amongst cores collected from the same lake (Appendix D). This resulted in the deepest sample from MOH2 at 69.5cm having an extrapolated date of 1773. An age model (Figure 5.22) was created for MOH2 based on the

cross correlation with ^{210}Pb dated constant rate of supply (CRS) model (Appleby, 2001) established for MOH1.

Table 5.7: List of tie-points between MOH1 and MOH2 correlated using % organic matter (LOI_{550}) and diatom analysis

MOH1 (Collected in 2015)		MOH2 (Collected in 2017)		Method used
Depth (cm)	Date	Corresponding Depth (cm)	Corresponding Date	
5.5	2013	10.25	2013	Diatom Analysis
7.5	2012	15.25	2012	Diatom Analysis
10.5	2010	20.25	2010	Diatom Analysis
15.5	2004	25.25	2004	Diatom Analysis
20.5	1997	30.5	1997	Diatom Analysis
25.5	1989	35.5	1989	Diatom Analysis
36.5	1963	40.5	1963	Diatom Analysis

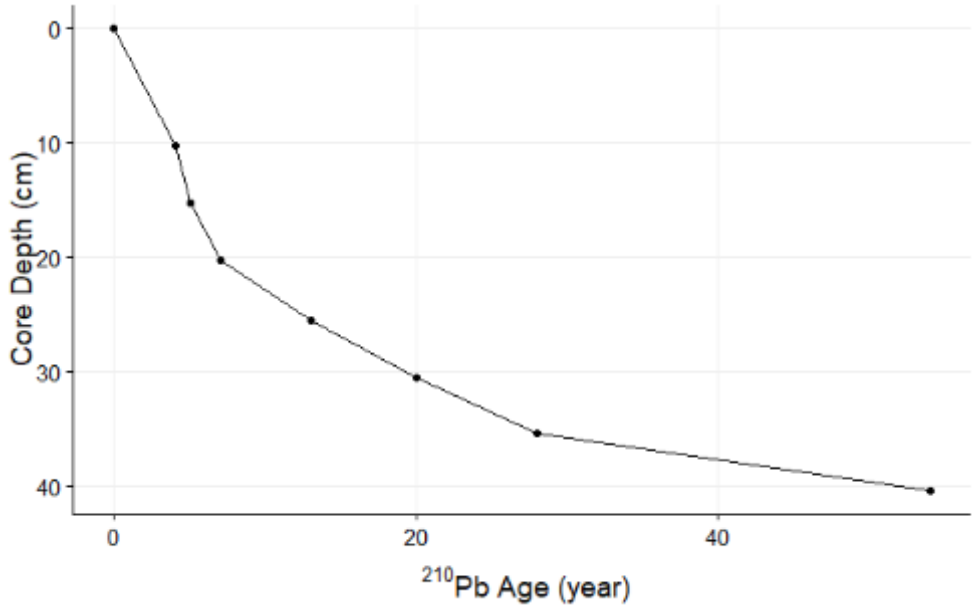


Figure 5.22: Age-depth model for core MOH2 using cross-dating methods

5.6.5. Sediment Lithology (LOI)

MOH2 had the fourth highest mean OM content of 17.7% and the second highest mean carbonate content of 13.8% of all the cored lakes (Table 5.3). MOH2 also had the second highest standard deviation and the highest CoV for OM content of 8.22% and 49 % respectively (Table 5.3), suggesting high variability throughout the sediment core. The OM content remained constant at 11.0% until 1970, when there was a peak in values to 54.0% (Figure 5.23), followed by a gradual decline in values to 22.5% (2017). The water content showed a decline in values in the oldest sediment, reaching a low of 78.0% in the 1830s, followed by a subsequent increase in values to 95.0% in 1930. The values then plateaued at c.90.0%. The carbonate content and bulk dry density showed a decline in values throughout the core from 17.2% and 0.1815 g/cm^3 (1776) to 13.9% and 0.0873 g/cm^3 (2017) respectively. The carbonate content record showed high variability throughout.

5.6.6. Sediment Stable Isotopes and C/N Ratios

The %C and %N values fluctuated between 5-16% and 0.4-1.4% respectively until 1990 (Figure 5.23). During the same period, both $\delta^{13}C$ and $\delta^{15}N$ values declined from –27.1‰ and +1.7‰ to –36.4‰ and +0.4‰ respectively. In 1980, the $\delta^{13}C$, %C, %N and $\delta^{15}N$ values increased to -–30.8‰, 16.0%, 1.6% and +2.5‰ followed by a decline in values in the youngest sediments. MOH2 also had the highest standard deviation for $\delta^{13}C$ of 2.4‰ and the highest CoV for $\delta^{15}N$ (65%) suggesting high variability throughout the sediment core. Unlike the other proxies, the C/N record remained constant until the early 2000s when there was a rapid increase in values from 10.5 to its maximum value of 17.3, suggesting a sudden influx of allochthonous OM. This was followed by a sharp decline in values for the remainder of the core.

5.6.7. Sedimentary Pigments

The sedimentary pigment record of MOH2 was highly variable (Figure 5.22) as shown by the standard deviation of 102.8 nmol g^{-1} for β carotene (Table 5.3). Post-1990, the high variability may be partly explained by the greater sampling resolution (0.5 cm). Chlorophyll *a* was not present in detectable concentrations until 1923 as it was overshadowed by a high abundance of echinenone (cyanobacteria). Chlorophyll *a* has a similar retention time as echinenone and could not be separated in the chromatograph. However, it was still possible to determine if both pigments were present from visual inspection of the spectra (Figure 5.23). Myxoxanthophyll (filamentous/N_2-fixing cyanobacteria) appeared in 1870 reaching a peak of 195.6 nmol g^{-1} before disappearing from the record by 1900. Concentrations of β carotene, lutein-zeaxanthin

(chlorophytes, cyanobacteria), canthaxanthin (colonial cyanobacteria) and echinenone (cyanobacteria) gradually declined throughout the length of the core, as summarised by the PCA axis 1 scores (30.0% variance explained; Figure 5.24). In comparison, the concentrations of diatoxanthin (siliceous algae), alloxanthin (cryptophytes), pheophytin *b* (chlorophytes) increased. Okenone (purple sulphur bacteria) first appeared in 1820, after which the concentration sharply increased to 491.8 nmol g^{-1}(1831). The concentrations of okenone then fluctuated over the remainder of the core.

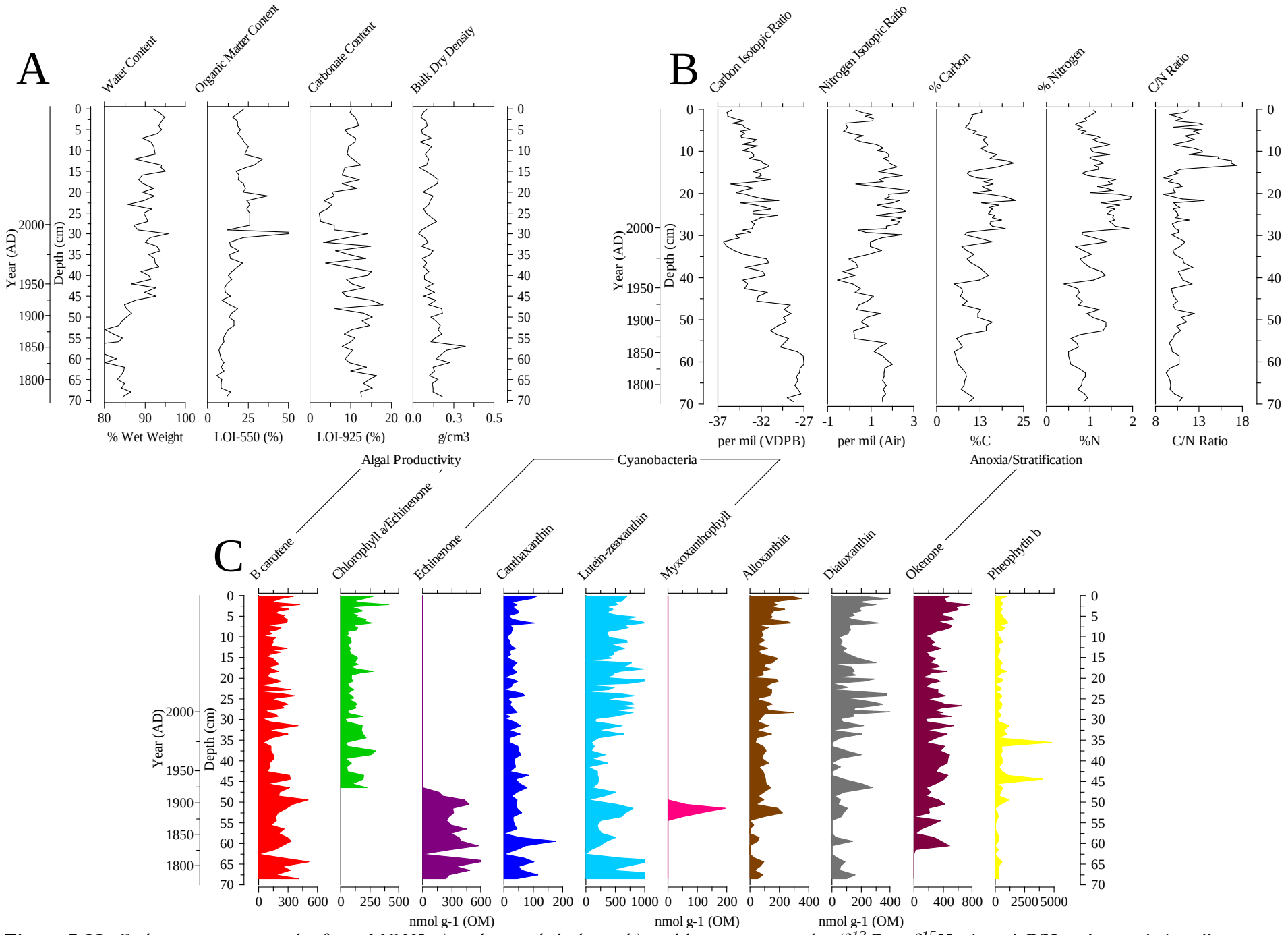


Figure 5.23: Sediment proxy results from MOH2 a) sediment lithology, b) stable isotope results ($\delta^{13}C_{org}$, $\delta^{15}N_{org}$) and C/N ratios and c) sedimentary pigment results

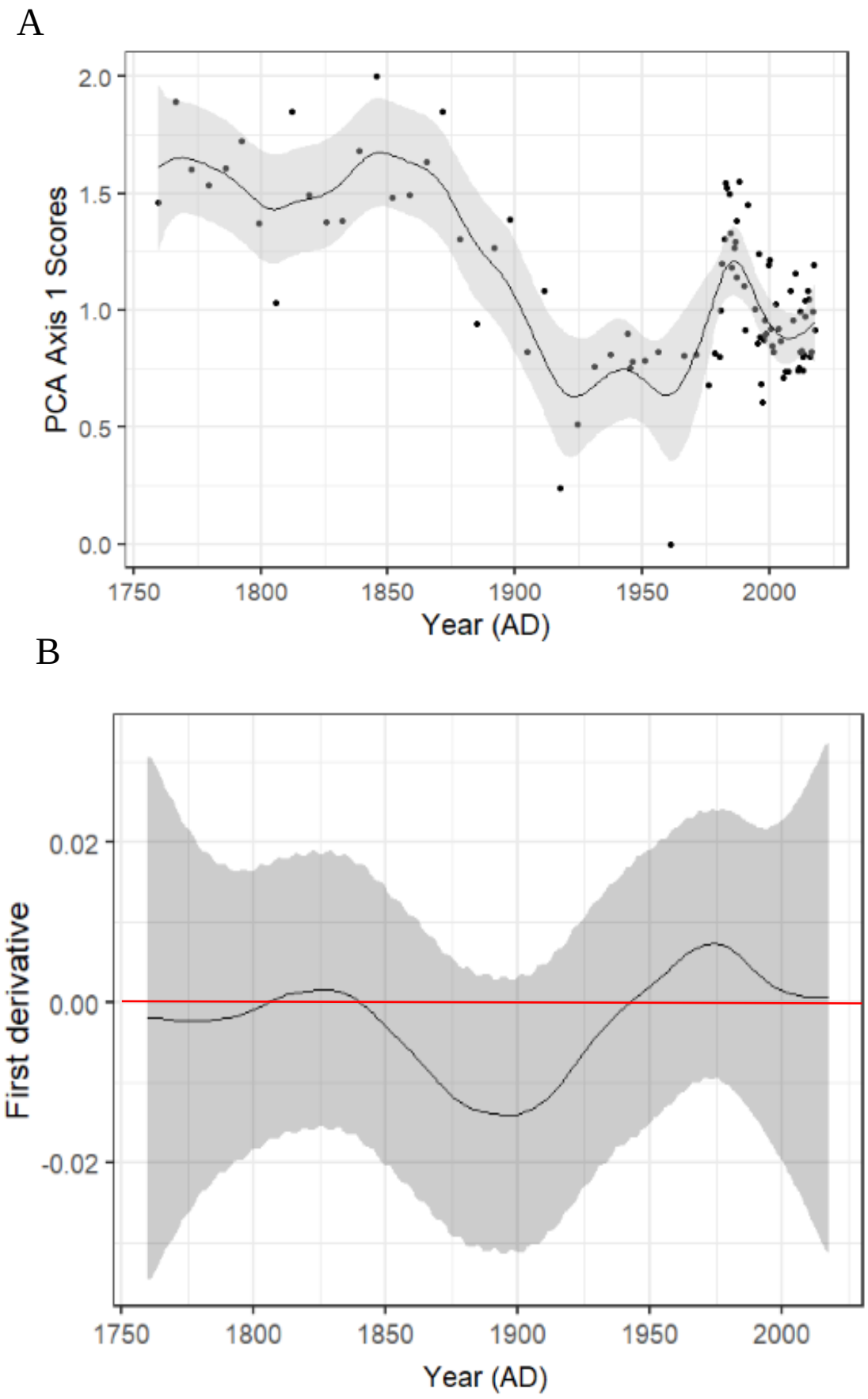


Figure 5.24: A) Generalised additive model fitted to the pigment PCA axis 1 scores from MOH2. B) Estimated first derivatives (black line) of the GAM trend fitted to the PCA axis 1 scores from MOH2. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. In both figures, the grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

5.6.8. Historical Records

The timings of notable events are shown in Table 5.8:

Table 5.8: Timeline of the main anthropogenic changes within the catchment of Lake Mohicap with data source shown.

Year		Source
Pre-1940s	Coconut plantations already present when the fisherman was born. Unknown when first planted	Interview Data
1977	Aquaculture introduced, the main species were tilapia reared in floating cages	Interview Data
1980s	Water clarity at a depth of 4.5 m	Interview Data
1990s	Switch in the type of fish food used from floating fish food to sinking fish food 10 Philippine peso per bag cheaper)	Interview Data
1990	Aquaculture cage numbers reach peak coverage of 7 hectares (30.43% of lake surface)	Interview Data
1991	Fish cages and illegal structures destroyed by typhoon 7.0 hectares of cages (30.43% of lake surface)	Interview Data
1994	6.3 hectares of cages (27.39% of lake surface)	Interview Data
1996	6.0 hectares of cages (26.13% of lake surface)	Interview Data
1999	5.3 hectares of cages (23.00% of lake surface)	Interview Data
Post-2000	Beginning of remediation efforts by the LLDA Switch to floating fish food (in line with the other lakes) due to the harmful effects of the sinking fish food	LLDA Interview Data
2001	4.29 hectares of cages (18.63% of lake surface)	LLDA
2004	LLDA reports 80 fish cages operating occupying an area of 36000m^3 (15.65 % of lake surface)	Brillo, 2015a
2006	3.2 hectares of cages (13.84% of lake surface)	Interview Data
2010	2.5 hectares of cages (10.87% of lake surface)	LLDA
2012	1.0 hectares of cages (4.35% of lake surface)	LLDA
2013	Only 25 registered fish pens operating Last reported fish kill event 0.9 hectares of cages (3.91% of lake surface)	Brillo, 2015a LLDA LLDA
2014	0.8 hectares of cages (3.48% of lake surface)	LLDA
2015	0.7 hectares of cages (3.04% of lake surface)	LLDA
2016	0.5 hectares of cages (2.17% of lake surface)	LLDA
2017	0.5 hectares of cages (2.17% of lake surface)	LLDA
2018	Fish cages decreasing by 10 a year 15 fish pens operating but there are plans to reduce the number further 0.87 hectares of cages (3.78% of lake surface)	Interview Data LLDA

5.6.9. Interpretation of Palaeolimnological Records

Compared to the other lakes, Lake Mohicap ranks in the middle for OM content and β carotene concentrations (Table 5.3) coinciding with its position along the aquaculture gradient, yet has the highest CoV for $\delta^{15}N$ (65%) suggesting this lake has the highest variation in this proxy. It had the lowest mean $\delta^{13}C_{org}$ values (–32.2‰) providing evidence of extensive purple sulphur bacterial communities and pronounced anoxia in the hypolimnion (Brown *et al.*, 1983). Moreover, the first observed appearance of chlorophyll *a* in the 1920s (without being overwhelmed by echinenone (cyanobacteria)) and the low pheophytin *a*/chlorophyll *a* ratio indicates there is enhanced preservation in the water column. This is in part due to Lake Mohicap having a steep sided catchment, which would reduce the impact of wind driven turbulence (MacIntyre, 2012).

There are two main periods of change in the proxy record of Lake Mohicap, yet they did not coincide with any significant temporal change in algal communities. The first was between 1830 and 1930, with PCA axis scores indicating a gradual shift in algal communities (Figure 5.24). This period saw the extirpation of myxoxanthophyll (filamentous and N_2 fixing cyanobacteria), the first appearance of okenone (purple sulphur bacteria) and the beginning of a decline in $\delta^{13}C_{org}$ values. The second period of change, post 1980, is characterised by a shift in sediment colour from light to dark brown gyjtta and a rapid increase (followed by a gradual decline post-2000) in all isotopic records and OM content. There was also a concordant increase in all pigments (Figure 5.23) reflected in a sharp peak in PCA axis scores (Figure 5.24). A decade after this (2000s), there was a spike in C/N ratios (from 10.5 to 17.3) followed by a sharp decline.

Prior to 1830, the lack of okenone (and labile chlorophyll *a*) and high $\delta^{13}C$ values of –28‰ in the sediment record suggests the water column in Lake Mohicap was well-mixed and lacked a permanent anoxic layer (Figure 5.1a). In 1830, the appearance of okenone (purple sulphur bacteria) and the beginning of the decline in $\delta^{13}C$ values from –28.0‰ (1830) to –36.4‰ (1978) indicates the development of anoxic conditions (Kalff, 2002; Conrad *et al.*, 2014). The disappearance of myxoxanthophyll from the sedimentary record (in 1900) coupled with a decline in $\delta^{15}N$ values indicates the replacement of N_2-fixing taxa. This suggests an increase in N:P ratios, as non-N_2 fixing blooms prefer high N and P levels (Paerl *et al.*, 2001). After 1980, there was an increase in $\delta^{13}C$ (from –36.4 to –30.8‰), $\delta^{15}N$ (from +0.4 to +2.5‰),

cyanobacteria (lutein-zeaxanthin) and a shift in sediment colour, most likely due to a rise in productivity (Paerl *et al.*, 2001). This is supported by increases in nutrient concentrations (N and P; LLDA, 2006a). The shift in PCA axis scores during this period suggests this caused a change in algal community composition (Figure 5.24). The spike in C/N ratios in 2000 (from 10.5 to 17.3) indicates a large influx of N at this time.

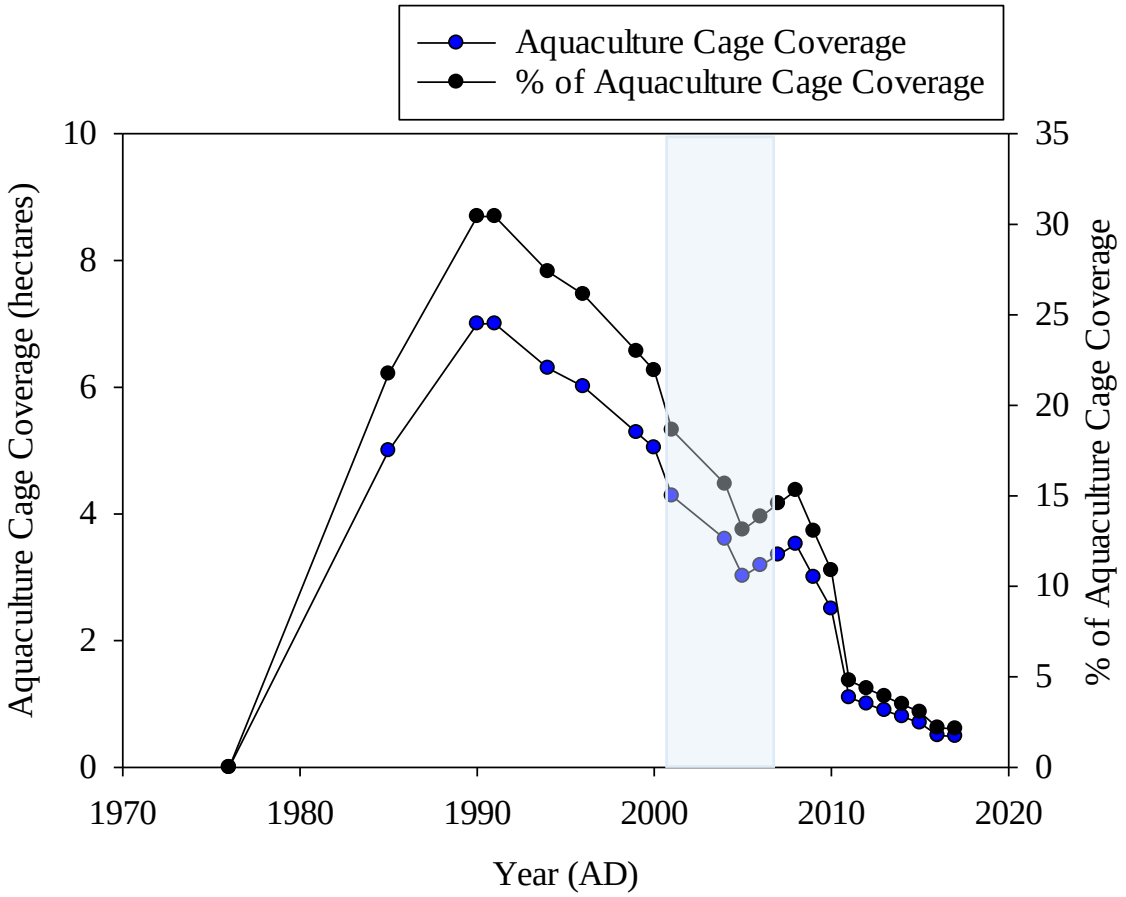


Figure 5.25: The surface area of Lake Mohicap utilised for aquaculture cages in hectares (blue) and % (black). The blue shading corresponds to when sinking fish food was used for aquaculture. Source of each data point is listed in Table 5.8

The first appearance and proliferation of okenone (purple sulphur bacteria) in 1820 and the beginning of a decline in $\delta^{13}C_{org}$ values coincides with the onset of a warming period in this region. Sea surface temperatures reconstructed using corals from the western Pacific show the beginning of a general warming trend from 1800 (Tierney *et al.*, 2015). The steep sided catchment would have sheltered the lake from wind driven turbulence and increased the likelihood of stratification and anoxia in the water column (Figure 5.1b; Jean-Philippe *et al.*, 2015). The second period of change, post 1980 coincides with the introduction of aquaculture

in 1977 and its subsequent rapid increase to its peak intensity (30.43%, 1989; Figure 5.25). This period also saw a rise in the construction of man-made structures and coconut/banana plantations within the lake catchment (Figure 5.26). The change in pigments and the increase in the isotopic records could be attributed to the use of additional fish food and fish excrement fuelling algal productivity (Kibria *et al.,* 1997). Furthermore, change in the 2000s from floating to the more harmful sinking fish food (as it was 10 Philippine peso per bag cheaper, see above) could have caused the spike in C/N ratios (Figure 5.23). The subsequent switch back to the floating fish food in the mid-late 2000s also correlates with the decline in C/N ratios (Table 5.1). After 2000, the decline in $\delta^{13}C$ and $\delta^{15}N$ corresponds with remediation efforts by the LLDA (in response to the deteriorating water quality and HABs; Figure 5.26). The indicated declines in algal productivity and anthropogenic loading could be explained by the reduction of the number of fish cages within the lake (to 5% in the present day) and the demolition of illegal structures.

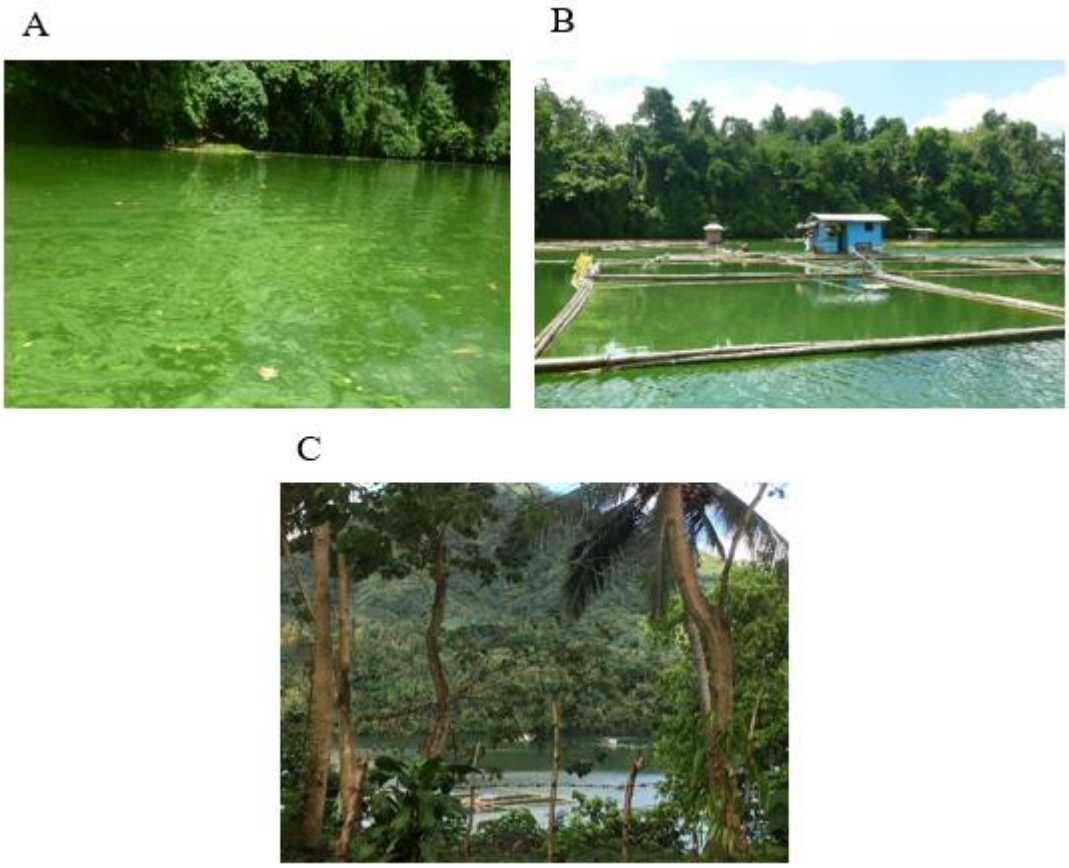


Figure 5.26: Photographs of Lake Mohicap

A. *Extensive algal blooms*
B. *Fish cages with man-made structure*
C. *Mixture of coconut and native forests in the lake catchment with cages in the background*

5.7. Lake Sampaloc

5.7.1. Field Core Description of SAMP1

The sediment core SAMP1 of 81 cm consisted of unconsolidated fine grained sediments rich in aquatic plant macrofossils (Table 5.2).

5.7.2. Sediment Chronology for SAMP1

In the SAMP1 core, total ^{210}Pb activities reached equilibrium with supported ^{210}Pb at a depth of 46.5 cm (Figure 5.27). Total ^{210}Pb activity increased from 189.61 Bq Kg^{-1} (at the top of the core) to 212.88 Bq Kg^{-1} (19.5 cm), which was subsequently followed by a decline to 38.22 Bq Kg^{-1}at 46.5 cm (Appendix E). Unsupported ^{210}Pb activities, declined irregularly with depth, permitting the use of the CRS model to develop the core chronology (Appleby and Oldfield, 1978; Appleby, 2001). Activities of ^{137}Cs and ^{241}Am were too low in the samples analysed to validate the chronologies based on ^{210}Pb data and the CRS age-depth model. The CRS age–depth model suggested that sediment accumulation rates increased from 0.02 to 0.15 g cm^{-2} yr^{-1} between the base of the core and 14.5 cm (1994±3). This was followed by a drop to 0.07 g cm^{-2} yr^{-1} at 0 cm (2015±2). The calculated age model indicated that the 81 cm core covered the past c. 700 years (1290), based on the date of the oldest sediment. The published chronology for SAMP1 is found in Bannister *et al.,* (2019).

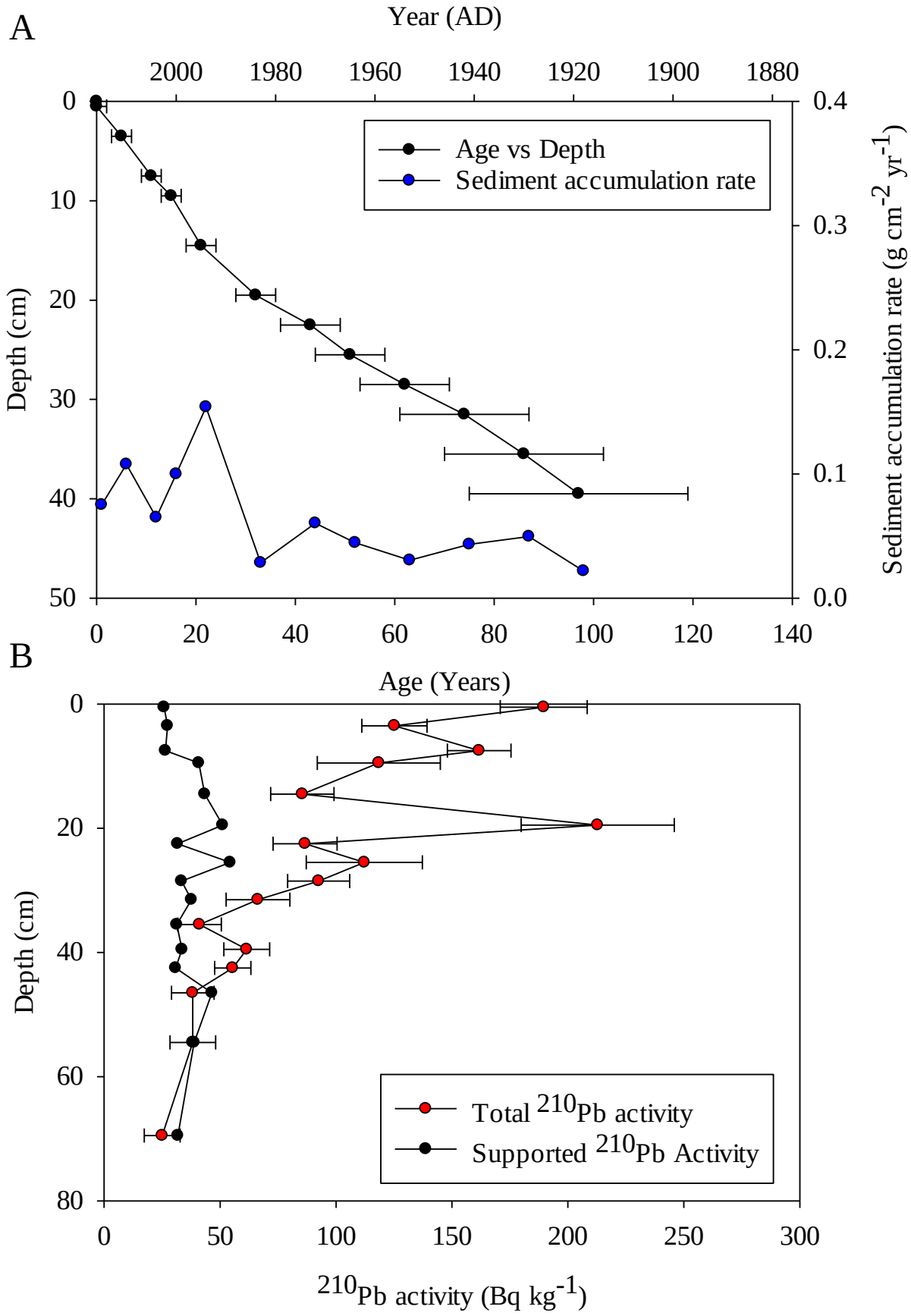


Figure 5.27: A) age-depth model and sediment accumulation rates and B) changes in total and supported ^{210}Pb for the SAMP1 sediment core

5.7.3. Field Core Description of SAMP2

The 64 cm sediment core SAMP2 consisted of light brown, organic rich gyjjta until 30 cm when there is a colour shift to dark brown gyjjta for the remainder of the core (Table 5.2). This is intersected by a watery layer, between 35-30 cm.

5.7.4. Sediment Chronology for SAMP2

LOI_{550} profiles of SAMP1 and SAMP2 were similar (Figure 5.28) indicating a good potential for cross correlation. LOI_{550} profiles from both cores were compared using tie points, which identified distinctive changes; thus allowing the correlation for dates at three tie-points down the core (Table 5.9). The diatom profiles between SAMP1 and SAMP2 were also similar, so to further constrain the age model, seven additional points were determined by matching diatom relative abundances from the ^{210}Pb-dated portion of core SAMP1, with sediment core SAMP2 (Figure 5.29; Table 5.9).

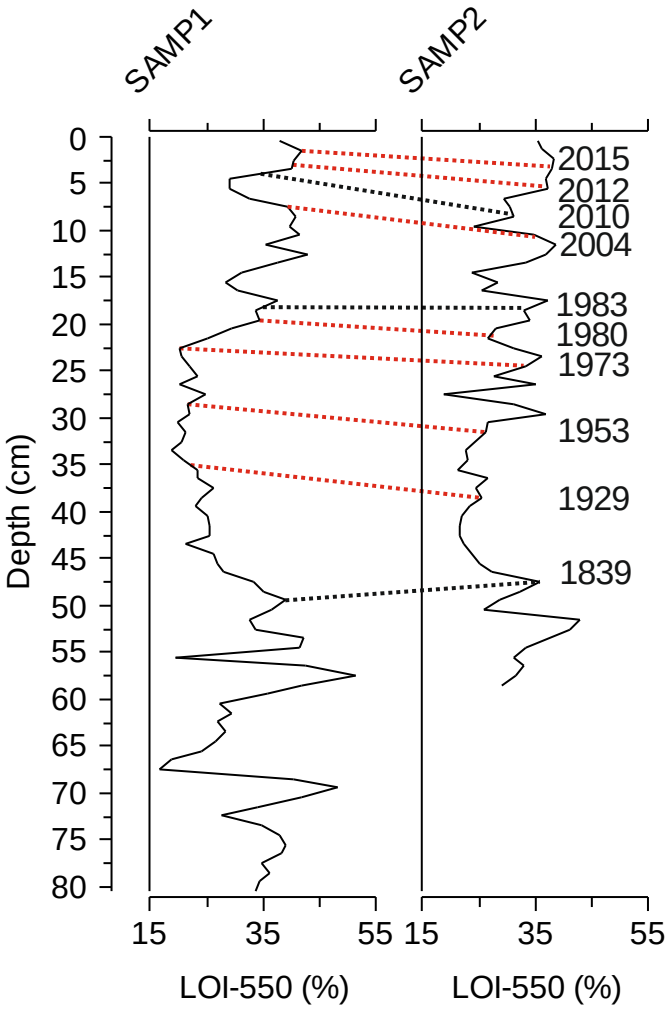


Figure 5.28: Correlations in % organic matter (LOI_{550}) between ^{210}Pb dated sediment core SAMP1 and sediment core, SAMP2. ^{210}Pb-derived dates (Year AD) corresponding to tie-points from SAMP1 are indicated. The diatom tie- points are shown with a red dashed line and the LOI tie points with a black dashed line.

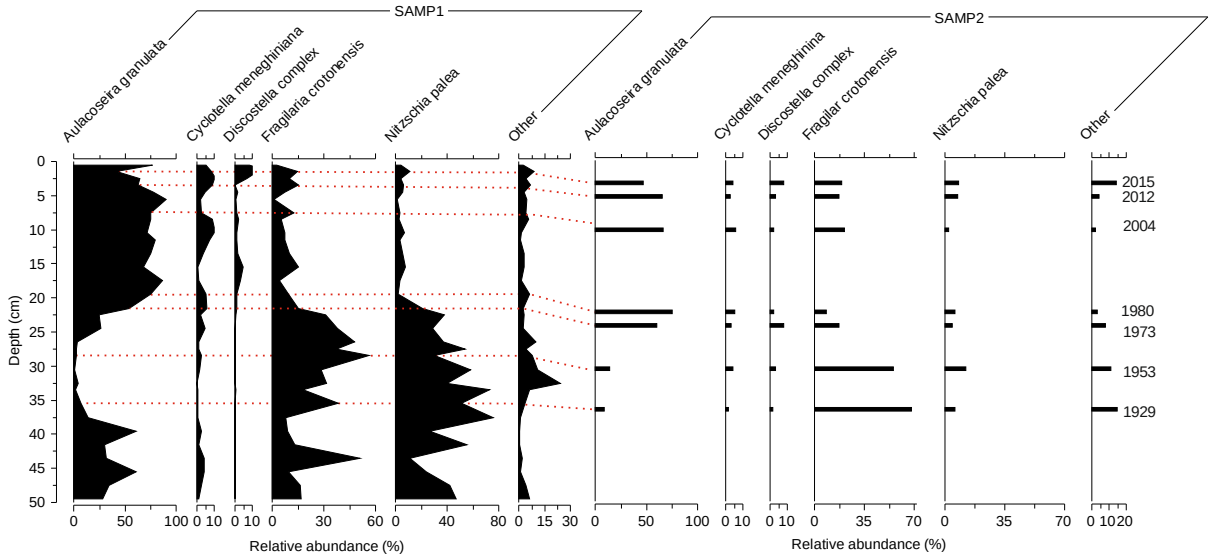


Figure 5.29: Correlations in % relative diatom abundance between ^{210}Pb dated sediment core SAMP1 and sediment core, SAMP2. ^{210}Pb-derived dates (Year AD) corresponding to tie-points from SAMP1are indicated by red dashed lines.

5.7.4.1. Justification for selection of tie-points based on diatom profiles

In order to constrain the ^{210}Pb dates for the youngest sediments in SAMP2, two tie points at depths 3.25 and 5.25 cm were used which captured the decline in abundance of *Aulacoseira granulata* observed in the top 6 cm of SAMP1 (Figure 5.29). Furthermore, the tie-point at 10.25 cm was necessary to capture the spike in *Fragilaria crotonensis* abundance and the plateau in abundance of *Aulacoseira granulata* observed in SAMP1 between depths 5-10 cm. The four oldest tie points at depths 22.25, 24.25, 30.5 and 37.5 cm were necessary as they captured the increasing trend in *Aulacoseira granulata* and the decline in *Fragilaria crotonensis* and *Nitzschia palea* observed in SAMP1 between depths 20-35 cm. This was combined with a final LOI_{550} tie point at depth 46.5 cm. This helped to constrain the age model of SAMP2 back to the earliest ^{210}Pb date from SAMP1.

5.7.4.2. Age Model for SAMP2

Below 37.5 cm, dates in SAMP2 were extrapolated using the three earliest known sedimentation rates from core SAMP1 (at depths 31.5, 35.5 and 39.5 cm). The three earliest values were used to account for the uneven sedimentation rates amongst cores collected from the same lake (Appendix E). This resulted in the deepest sample from SAMP2 at 58.5 cm having an extrapolated date of c. 1790. An age model (Figure 5.30) was created for SAMP2 based on the cross correlation with the ^{210}Pb dated CRS model (Appleby, 2001) established for SAMP1.

Table 5.9: List of tie-points between SAMP1 and SAMP2 correlated using % organic matter (LOI_{550}) and diatom analysis

SAMP1 (Collected in 2015)		SAMP2 (Collected in 2017)		Method Used
Depth (cm)	Date	Corresponding Depth (cm)	Corresponding Date	
1.5	2015	3.25	2015	Diatom Analysis
3.5	2012	5.25	2012	Diatom Analysis
4.5	2010	7.25	2010	LOI_{550}
7.5	2004	10.25	2004	Diatom Analysis
18.5	1983	18.25	1983	LOI_{550}
19.5	1980	22.25	1980	Diatom Analysis
22.5	1973	24.25	1973	Diatom Analysis
28.5	1953	30.5	1953	Diatom Analysis
35.5	1929	37.5	1929	Diatom Analysis
49.5	1839	46.5	1839	LOI_{550}

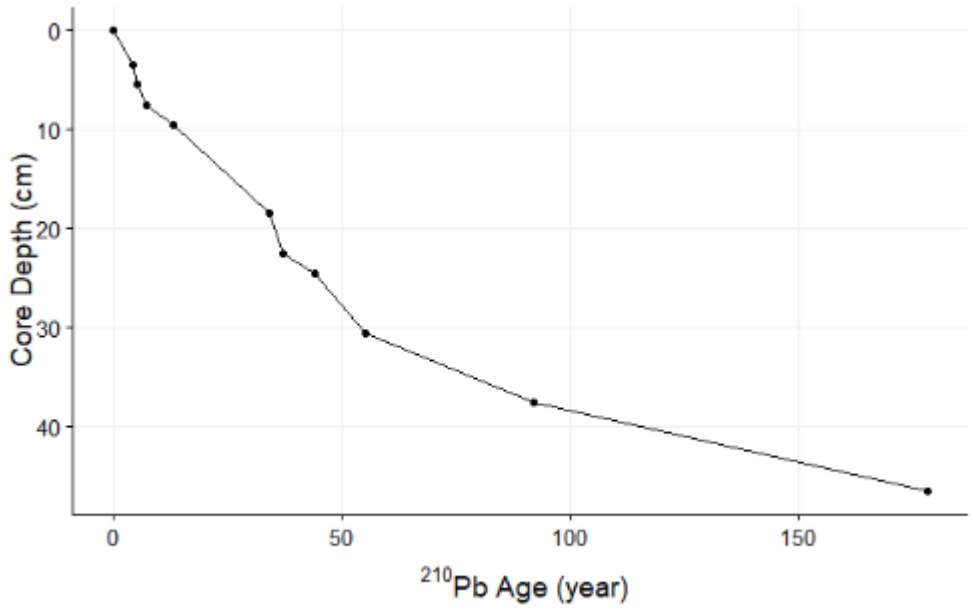


Figure 5.30: Age-depth model for core SAMP2 using cross-dating methods

5.7.5. Sediment Lithology

SAMP2 had the highest mean OM content of 30.3% (yet only the fourth highest standard deviation of 5.9%, lowest CoV of 19%) and the third lowest carbonate content of 5.78% (Table 5.3). Water, OM and carbonate content remained relatively constant until 1950 at c. 90%, 20% and 2% respectively, when large fluctuations were observed in all lithological records (Figure 5.31). A noticeable feature was the peak in the carbonate content of 18% in the 1960s. Bulk dry density stayed constant at 0.15 g/cm^3 throughout the core.

5.7.6. Sediment Stable Isotopes and C/N Ratios

SAMP2 had the highest mean $\delta^{13}C$ and $\delta^{15}N$ values of all the cored lakes (Table 5.3). The $\delta^{13}C$, %C, %N and C/N values all remained constant at –26.0‰, 12.0%, 2.0% and 11.0 respectively until 1950 (Figure 5.31). Between 1950 and 1980, $\delta^{13}C$ values declined to –30‰ and %C, %N, $\delta^{15}N$ and C/N values increased to 22%, 2%, +5‰ and 12.7, respectively. After 1980, the $\delta^{13}C$, $\delta^{15}N$, %C and %N showed high variability, with fluctuations in $\delta^{13}C$ record (of between –34‰ and –26‰) particularly prominent (standard deviation of 2.3‰; Table 5.3). This coincided with the onset of higher sampling resolution. The C/N ratios declined rapidly in 1980 and then stayed constant at 8.2 until 2017.

5.7.7. Sedimentary Pigments

SAMP2 had the highest concentrations of all sedimentary pigments (Table 5.3) of all the cored lakes. Pigment concentrations of β carotene, okenone (purple sulphur bacteria), lutein-zeaxanthin (chlorophytes, cyanobacteria), alloxanthin (cryptophytes) and diatoxanthin (siliceous algae) remained low until 1950. Chlorophyll *a* was not present in detectable concentrations until 1900 as it was overshadowed by a high abundance of echinenone (Figure 5.31). Myxoxanthophyll (filamentous/N_2-fixing cyanobacteria) reached a peak of 250.7 nmol g^{-1} (1840) before it disappeared from the record by 1870. Concentrations of chlorophyll *a*, echinenone, canthaxanthin (colonial cyanobacteria), lutein-zeaxanthin, pheophytin *b* (chlorophytes), alloxanthin and diatoxanthin all started to increase in 1950, before peaking in the late 2000s (as summarised by the PCA axis 1 scores; 39.3% variance explained). This coincided with the highest rate and only period of significant temporal change in algal communities (Figure 5.32). Okenone started to increase in 1950 from 74.8 nmol g^{-1} to 154.3 nmol g^{-1} until 1982 when it suddenly decreased to 43.9 nmol g^{-1}. After 1982, okenone concentrations steadily increased. All pigments were highly variable after 1970, which coincided with the depth of higher sample resolution. This was also reflected in SAMP2 which had the highest standard deviation for β carotene of 345.5 nmol g^{-1} of all the cored lakes (Table 5.3).

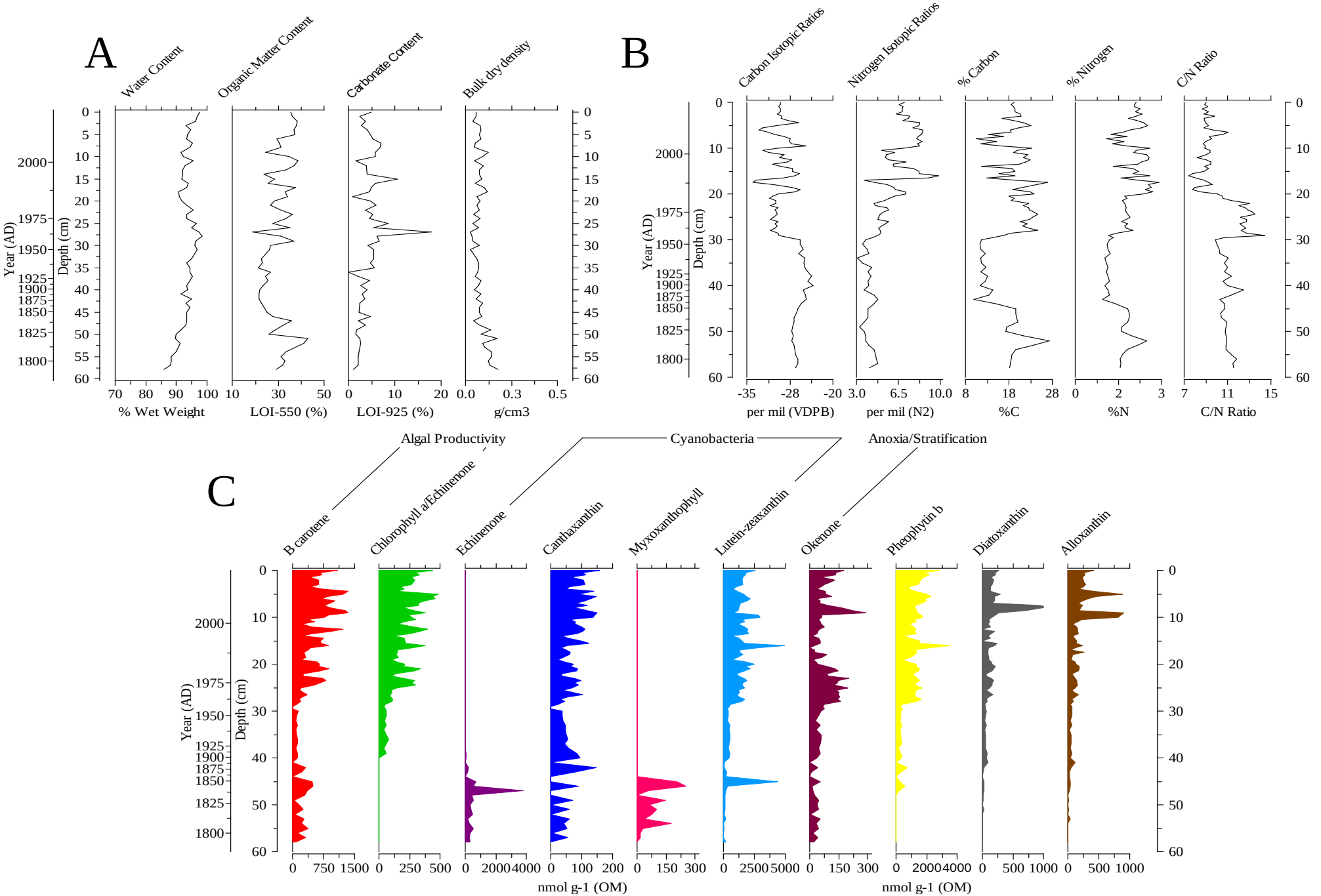


Figure 5.31: Sediment proxy results from SAMP2 a) sediment lithology, b) stable isotopes ($\delta^{13}C_{org}$, $\delta^{15}N_{org}$) and C/N ratios and c) sedimentary pigments

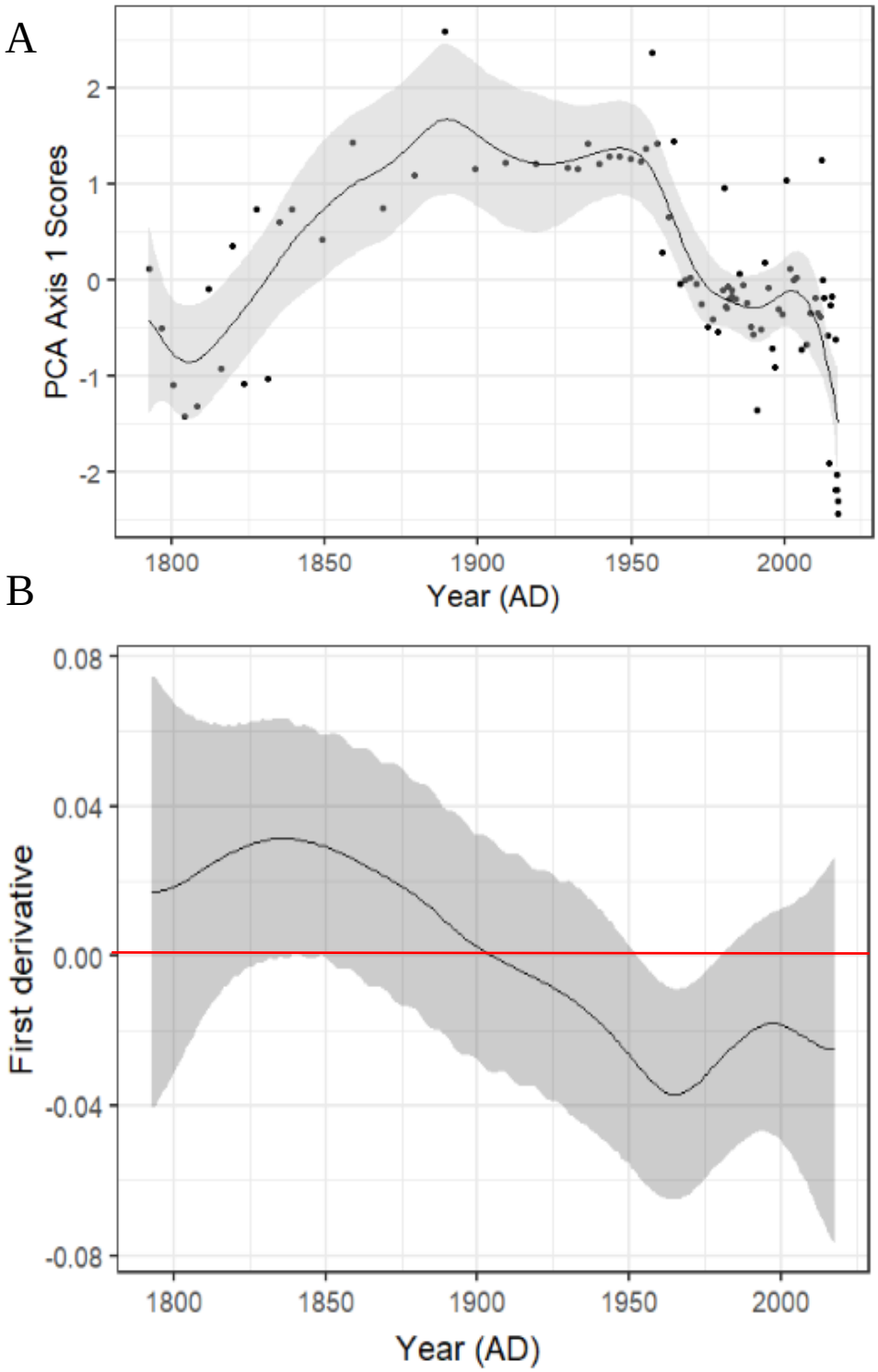


Figure 5.32: A) Generalised additive model fitted to the pigment PCA axis 1 scores from SAMP2. B) Estimated first derivatives (black line) of the GAM trend fitted to the PCA axis 1 scores from SAMP2. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. In both figures, the grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

5.7.8. Historical Records

The timings of notable events are shown in Table 5.10:

Table 5.10: Timeline of the main anthropogenic changes within the catchment of Lake Sampaloc from 1950 onwards with data source shown.

Year		Source
1950	Conversion into a 'first class city' characterised by large scale development	Interview Data
1975	Water is still clear	Interview Data
1979	Aquaculture started intensively	Santiago and Arcilla, 1993
1980	6 hectares of cages (5.77% of lake surface)	Santiago and Arcilla, 1993
1981	6 hectares of cages (5.77% of lake surface)	Santiago and Arcilla, 1993
1982	10 hectares of cages (9.62% of lake surface)	Santiago and Arcilla, 1993
1983	12 hectares of cages (11.54% of lake surface)	Santiago and Arcilla, 1993
1984	16 hectares of cages (15.38% of lake surface)	Santiago and Arcilla, 1993
1985	16 hectares of cages (15.38% of lake surface)	Santiago and Arcilla, 1993
1986	21 hectares of cages (20.19% of lake surface)	Santiago and Arcilla, 1993
1987	24 hectares of cages (23.08% of lake surface)	Santiago and Arcilla, 1993
1988	25 hectares of cages (24.04% of lake surface)	Santiago and Arcilla, 1993
1989	28 hectares of cages (26.92% of lake surface) Floating cages for tilapia expanded	Santiago and Arcilla, 1993
1990	33 hectares of cages needing 6000 ton of fish food annually (maximum cage coverage)	Santiago, 1994
1998/99	Major fish kills in colder months.	Interview Data
2000	Big fish kill event - Stakeholders believe that increased aquaculture caused the deterioration in water quality since the 1980s and is responsible for the algal blooms and fish kills (which occurred annually in November and December respectively)	Interview Data
2001	23 hectares of cages (22.12% of lake surface)	LLDA
2002	17.1 hectares of cages (16.44% of lake surface)	Jose, 2005
Early 2000s (2003-2006)	Reduction in fish cages to 12 hectares (11.54% of lake surface)	Brillo, 2016a
2005	More fish kills	Interview Data
2010	10.55 hectares of cages (10.14% of lake surface) Big fish kill event	LLDA Interview Data
2012	163 fish pens operating 10.03 hectares of cages (9.64% of lake surface)	Brillo, 2016a LLDA
2013	10.03 hectares of cages (9.64% of lake surface)	LLDA
2014	Promenade constructed along lake edge	Brillo, 2016a

	10.47 hectares of cages (10.07% of lake surface)	LLDA
2015	10.47 hectares of cages (10.07% of lake surface)	LLDA
2016	8.4 hectares of cages (8.08% of lake surface)	LLDA
2017	400 families of illegal settlers discharge household waste directly into the lake	Interview Data
	8.46 hectares of cages (8.13% of lake surface)	LLDA
2018	8.96 hectares of cages (8.62% of lake surface)	LLDA

5.7.9. Interpretation of Palaeolimnological Records

Lake Sampaloc had the highest mean $\delta^{13}C_{org}$, $\delta^{15}N_{org,}$ β carotene values, OM content and high nutrient levels (of both PO_4^{3-} and NO_3^-) in the water column (Figure 4.1) suggesting this is the most productive of the cored lakes (Table 5.3). High $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ values can be attributed to high productivity due to the selective removal of ^{12}C and ^{14}N from the DIC and DIN pools (Meyers and Lallier-Vergès, 1999). The majority of the lake catchment is urbanised and historical records state this has been the case since at least 1984 (see section 5.3.2.3). Removal of stabilising forest cover from a lake catchment can amplify soil erosion, escalating inputs of minerogenic material into the lake (Enters *et al.*, 2010), and fuelling algal productivity (Melack and Coe, 2013). Low pheophytin *a*/chlorophyll *a* ratios and a high pigment inventory also indicates good preservation in this lake. This lake also had the highest standard deviations for β carotene (345.50 nmol g^{-1}; Table 5.3) and CoV (75%) suggesting this lake has demonstrated the greatest pigment response, despite being ranked third on the aquaculture gradient.

There are three main periods of change in the proxy record of Lake Sampaloc (as shown in the PCA axis scores; Figure 5.31). The first period between 1800 and 1900, was characterised by the extirpation of myxoxanthophyll and minimal change in the isotopic and lithological records. The second period from 1950 to 1980 saw the beginning of an increase in all pigment concentrations and $\delta^{15}N$ isotopic ratios. There was also a sharp decrease in $\delta^{13}C$ values (from –26 to –30‰) and C/N ratios (12.7 to 8.5) and a rapid rise in okenone (74.8 to 159.3 nmol g^{-1}). The final period was after 1980, characterised by a sharp decline in okenone in 1980 (to 43.9 nmol g^{-1}) with a recovery in values post-1990. This period also saw a sharp decline in C/N ratios, high variability in $\delta^{13}C$ values and a concordant increase in total productivity and cyanobacterial pigments.

The presence of okenone in the earliest dated sample (c.1790) (Figure 5.31) suggests anoxic conditions and enhanced preservation, throughout the sediment record (Friedrich *et al.*, 2014). The gradual changes in algal communities between 1800 and 1900 can be explained by additive

nutrient sources (from an increasing population) increasing N concentrations in the water, causing the replacement of N_2 fixing with non-N_2 fixing cyanobacteria (Paerl *et al.*, 2001). The sharp jump in okenone (purple sulphur bacteria) and increases in algal productivity indicators (chlorophyll *a*, β carotene, %N and %C), in 1950, provides further evidence of a rapid influx of nutrients, fuelling purple sulphur bacterial productivity. The sudden drop in okenone concentrations in 1980 suggests algal biomass at the surface increased to such an extent that it reduced light penetration and water transparency (Melack and Coe, 2013), resulting in a decline in bacterial communities deeper in the water column (Brown *et al.*, 1983; Cohen *et al.*, 1993; Yang *et al.*, 2008). Elevated productivity combined with anoxia could explain the high variability in the $\delta^{13}C$ record, after 1980.

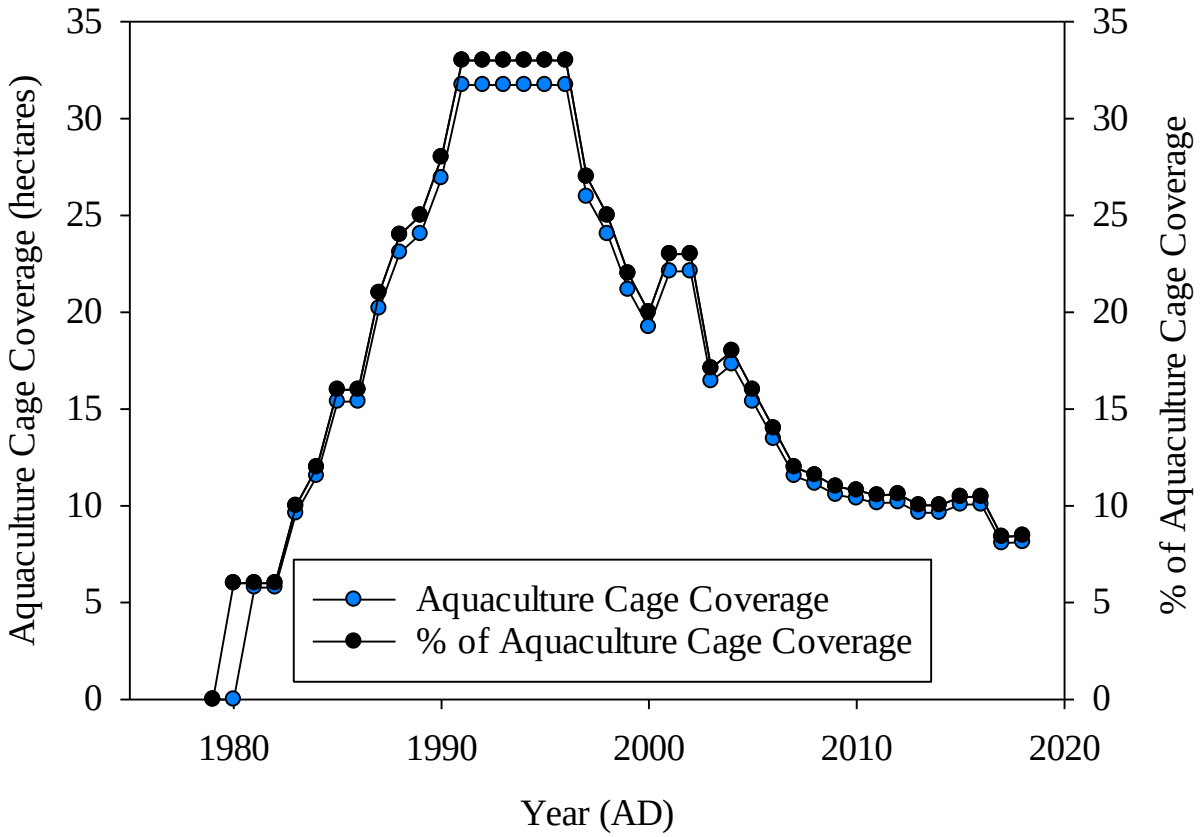


Figure 5.33: The area of Sampaloc occupied by aquaculture cages in hectares (blue) and % (black). Source of each data point is listed in Table 5.10

The presence of okenone in the sediment record, in 1792, predates the onset of climatic warming in this region (which began in 1800; section 5.6.9), suggesting anoxic conditions were driven by anthropogenic activities. Anthropogenic activities have been present at Lake Sampaloc since the 17th century, due to its location in the centre of San Pablo City, (Table 2.2; Figure 5.34). Multiple studies of tropical lakes have shown that nutrient inputs (N and P) from

anthropogenic activities can lead to anoxic conditions in the hypolimnion (Hecky *et al.*, 1994; Hayami *et al.*, 2008; Jean-Philippe *et al.*, 2015; Fukushima *et al.*, 2017). The increase in nutrient sources from the expansion of San Pablo City probably drove the changes in algal communities, between 1800 and 1900, (Table 2.2; Figure 5.32). Expanding population and its associated infrastructure would have increased the input of untreated domestic waste (with a $\delta^{15}N$ value of +5 to +25‰; Teranes and Bernasconi, 2000) causing the extirpation of myxoxanthophyll. The second period of significant change, 1950 to 1980, corresponds with large-scale deforestation and increased urbanisation, as San Pablo was transformed into a first class city in 1950. This would result in extensive erosion (Karamage *et al.*, 2016) and a large



Figure 5.34: Photographs of Lake Sampaloc (photo credit: Suzanne McGowan)

A. *Bamboo structure at the lake's edge*
B. *Foreground) Fish cages and background) man-made structures in the catchment*
C. *Bamboo rafts used for transport on the lake. The sediment core BUNOT2 is held by the author prior to subsampling*
D. *Foreground) Bamboo rafts used for transport on the lake and background) fish cages with man-made structures*

input of allochthonous OM (causing the change in C/N ratios and $\delta^{13}C$ values; Figure 5.31) and fuel the rapid increase in okenone and algal productivity indicators (Melack and Coe, 2013).This would have been further exacerbated by the introduction of aquaculture in 1979 (Figure 5.33). Interview data suggests that over 50% of the nutrients added to fish cages through feed (with a $\delta^{15}N$ of +33‰) are lost to the surrounding environment (Gondwe *et al.*, 2012), contributing to the increased $\delta^{15}N$ values in 1980 (Figure 5.31). The variability in the $\delta^{13}C$ record in 1980 is explained in part by a higher sampling resolution (0.5 cm compared with 1.0 cm for the older samples). However, higher phototrophic productivity, changes in allochthonous sources and the development of anoxia and methanogenesis (Legaspi *et al.*, 2015) are likely to also be contributing factors. Increased eutrophication caused by the introduction of aquaculture and anthropogenic pressures probably reached a stage, in 1980, where increased algal biomass reduced light penetration and water transparency (Cohen *et al.*, 1993) causing the decline in okenone. After 1990, the secondary increase in okenone (Figure 5.31) suggested a recovery in the lake system. This corresponds with remediation efforts by the LLDA which saw a reduction in aquaculture cage numbers (from 31.73% in 1991 to 8.62% in 2018 (Figure 5.33)).

5.8. Lake Bunot

5.8.1. Field Core Description

The bottom 14 cm (82-57 cm) of the sediment core BUNOT2 (total core length of 82 cm) consisted of consolidated well-laminated gyttja with a high OM content and macrofossils (aquatic macrophyte leaves) (Table 5.2). The top 57 cm of sediment was consolidated, dark brown gyttja with a high OM content, which became unconsolidated between 30-0 cm.

5.8.2. Sediment Chronology

In BUNOT2, total ^{210}Pb activities did not reach equilibrium with supported ^{210}Pb activities (Figure 5.35). Total ^{210}Pb activity at the top of the core was 104.34 Bq Kg^{-1}, increasing to 136.62 Bq Kg^{-1} at 24.25 cm and decreasing to 49.01 Bq Kg^{-1} at 80.5 cm (Appendix F). Unsupported ^{210}Pb activities, declined irregularly with depth, permitting the use of the CRS model to develop the core chronology (Appleby and Oldfield, 1978; Appleby, 2001). Artificial fallout radionuclides (^{137}Cs and ^{241}Am) were not detected in the sediments, therefore, the CRS chronologies could not be validated by any independent time marker. The CRS age–depth model suggested that the sediment accumulation rate was c.0.1 g cm^{-2} yr^{-1}, with some periods of increased sedimentation rates in the 1960s, 1970s and the 2010s. The calculated age model indicated that the 82 cm core covered the past c. 88 years (1932).

5.8.3. Sediment Lithology (LOI)

BUNOT2 had the second highest mean and third highest standard deviation for both OM content (of 27.8% and 8.1%) and highest carbonate content (16.4% and 17.9%) of the cored lakes (Table 5.3). The water content stayed constant at 90%; however, there were dips in the record in 1965±6, 1970±5 and 2010±2 to 85%. The OM content remained at 25% until 1980, when there was a marked increase to 42%. Values then stayed c.40% until 1993±3 when they started to decline (Figure 5.36). Carbonate values remained low (<6%) until 1993±3, when there was a sudden increase to >30%. After 1990, the carbonate content fluctuated slightly, peaking at 57% in 2017±2. The increase in carbonate content occurred 20 years after the increase in OM suggesting this change was not because of increased productivity but more likely a dilution effect. The bulk dry density stayed relatively constant throughout the core at 0.15g/cm^3.

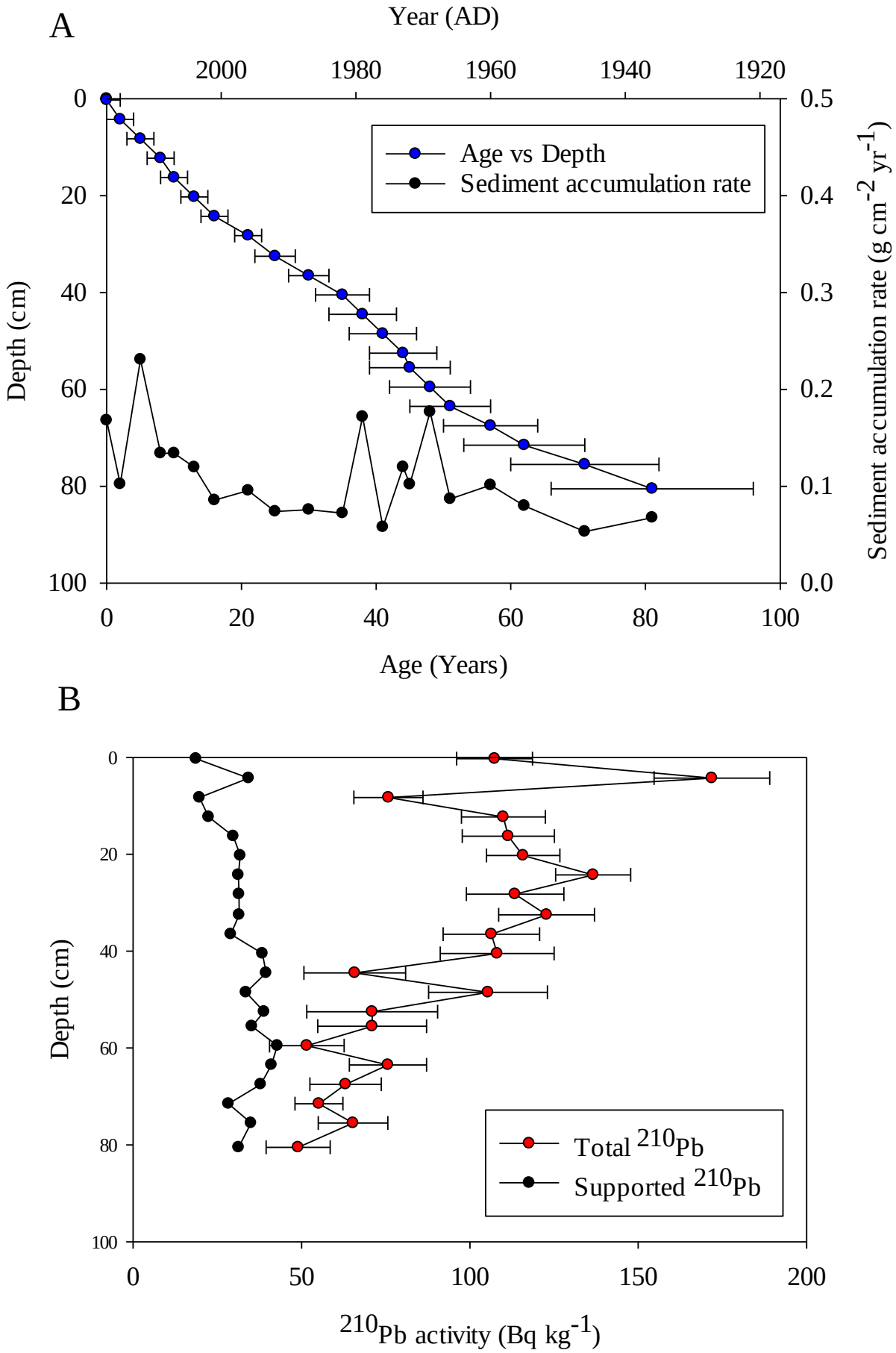


Figure 5.35: A) Age-depth model and sediment accumulation rates and B) changes in total and supported ^{210}Pb for the BUNOT2 sediment core

5.8.4. Sediment Stable Isotopes and C/N Ratios

BUNOT2 had the highest standard deviation and second highest CoV for $\delta^{15}N$ (of 1.4‰ and 44% respectively) of all the cored lakes suggesting high variability in this record. $\delta^{13}C$ values remained constant at –25‰ until 1956±9, when they started to decline to –33‰ in 1983±4 (Figure 5.36). After this, the $\delta^{13}C$ values started to fluctuate, with the highest variability after 1997±3. $\delta^{15}N$ values remained constant at +2‰ until 1997±3, after which $\delta^{15}N$ started to increase to +6‰ (however, it demonstrated high variability; Figure 5.34). The higher variability after 1997±3, could partly be explained by a higher sampling resolution. The %C and %N records both followed a similar pattern, with records remaining constant (at 10% and 1%) until 1978±5. In 1978±5, both records increased (to 25% and 2.5%) until 1983±4, when values started to steadily decline to 15% and 1.7% respectively in 2017±2. The C/N ratio showed a gradual decline in values from 11 to a minimum of 7.5 in 2016.

5.8.5. Sedimentary Pigments

BUNOT2 had the highest concentrations of sedimentary pigments after Lake Sampaloc (Table 5.3) and the third highest CoV of 92%. Pigment concentrations remained constant until 1975 (excluding okenone) when they started to increase, as summarised in the PCA axis 1 scores (45.5% variance explained; Figure 5.37). Between 1970 and 1995 also coincided with significant temporal change in algal communities (Figure 5.37). β carotene, lutein-zeaxanthin, pheophytin *b*, alloxanthin, and diatoxanthin reached their peak concentration between 1975 and 1990 before declining in the youngest sediments. Okenone started to increase in the oldest sediments of the core reaching a peak of 177 nmol g^{-1}in 1960, followed by a fluctuation in values until 1983±4. After 1983±4, okenone was not detected. The concentrations of chlorophyll *a* and canthaxanthin (colonial cyanobacteria) remained constant at c. 70 and 40 nmol g^{-1} throughout the core. All pigments showed high variability after 1995±3 (Figure 5.36).

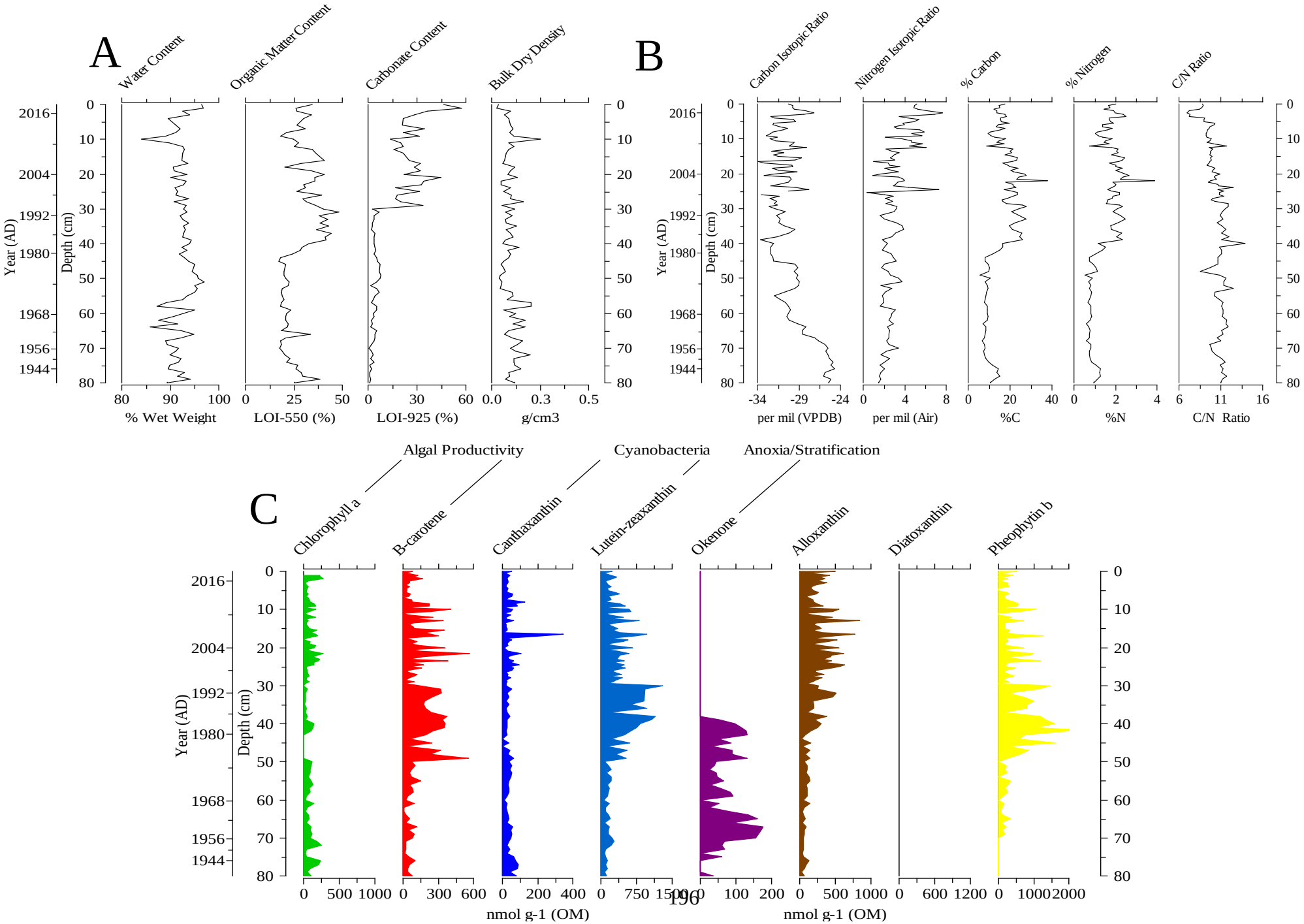


Figure 5.36: Sediment proxy results from BUNOT2 a) sediment lithology, b) stable isotope results ($\delta^{13}C_{org}$, $\delta^{15}N_{org}$) and C/N ratios and c) sedimentary pigment results

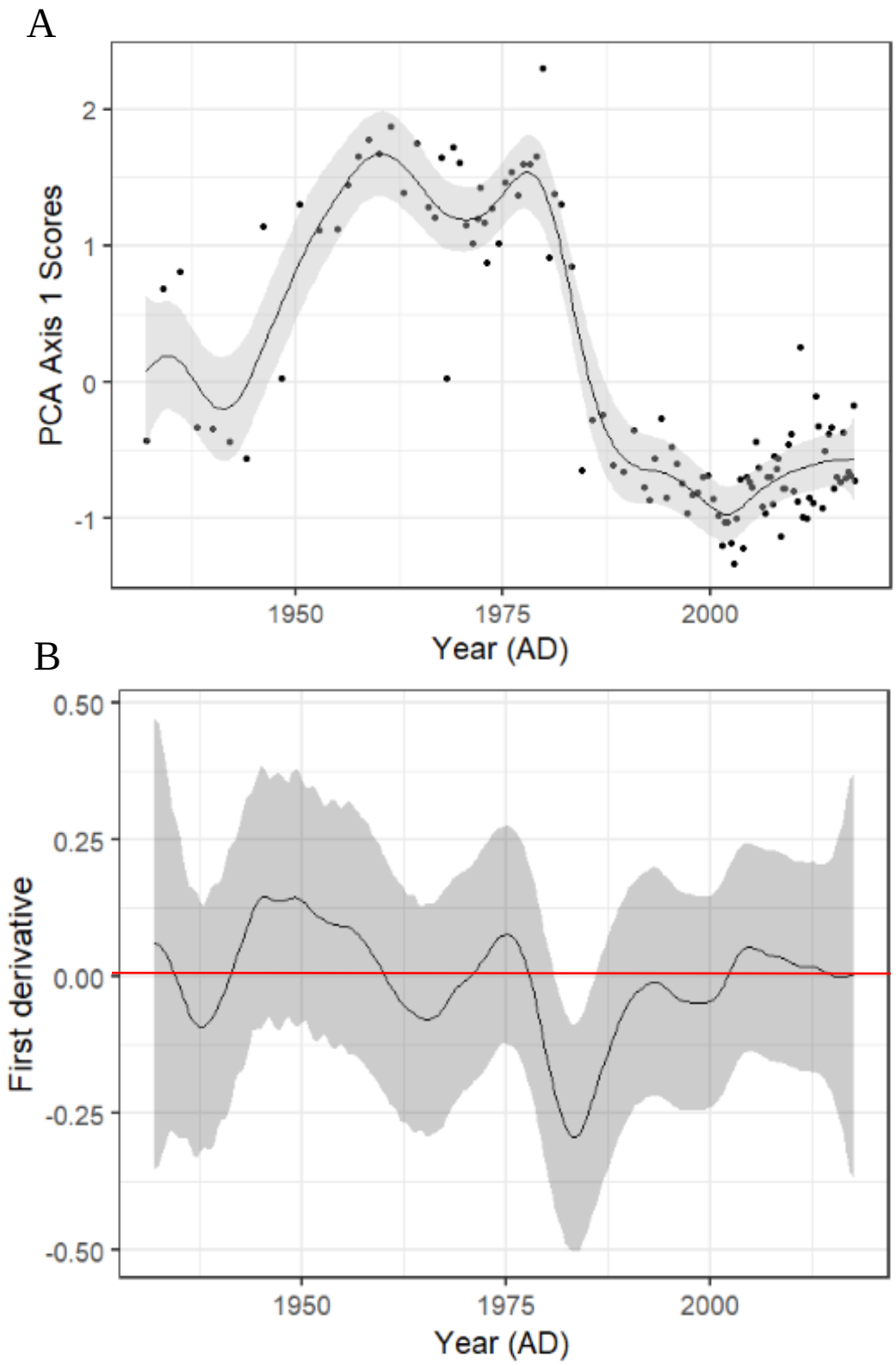


Figure 5.37: A) Generalised additive model fitted to the PCA axis 1 scores from BUNOT2. B) Estimated first derivatives (black line) of the GAM trend fitted to the PCA axis 1 scores from BUNOT2. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. In both figures, the grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

5.8.6. Historical Records

The timings of notable events are shown in Table 5.11:

Table 5.11: Timeline of the main anthropogenic changes within the catchment of Lake Bunot from 1950s onwards with data source shown.

Year		Source
1950s to 1970s	Water is very clear	Interview Data
1976	Aquaculture established. Beginning of a population increase	Interview Data
1980	Floating cages established. Water is still clear 4 hectares of cages (13.11% of lake surface)	Interview Data
1984	6 hectares of cages (19.67% of lake surface)	Interview Data
1989	8 hectares of cages (26.23% of lake surface)	Interview Data
1995	10.48 hectares of cages (34.36% of lake surface)	Interview Data
1998	Aquaculture cage number reach maximum coverage of 11.58 hectares (37.97% of lake surface) Decrease in fish farming	Interview Data
2001	10.48 hectares of cages (34.36% of lake surface)	LLDA
2005	Fish cages occupy 26.23% of the total surface area of the lake (equivalent to 8 hectares)	Coronado, 2010
2006	LLDA reported 183 cages occupying 30.6% of Bunot	Coronado, 2010
2009	4.58 hectares of cages (15.02% of lake surface)	Google Earth
2010	7.8 hectares of cages (25.57% of lake surface)	LLDA
Post-2010	Up to 150 fish cages operate on the lake	Brillo, 2015b
2012	Big fish kill and algal bloom event. Algal blooms are seen as positive (by the fishermen) as they become a source of food for the fish (used for aquaculture) 7.6 hectares of cages (24.92% of lake surface)	Interview Data LLDA
2013	Last reported fish kill (previously occurred in January) 6.9 hectares of cages (22.62% of lake surface)	Interview Data LLDA
2014	4.17 hectares of cages (13.67% of lake surface)	LLDA
2015	4.17 hectares of cages (13.67% of lake surface)	LLDA
2016	3.8 hectares of cages (12.46% of lake surface)	LLDA
2017	3.8 hectares of cages (12.46% of lake surface)	LLDA
2018	65 fish pens operational (10 fish cages have been removed in the last year) 2.8 hectares of cages (9.18% of lake surface)	Interview Data LLDA

5.8.7. Interpretations of Palaeolimnological Records

Lake Bunot had the second highest aquaculture disturbance of the cored lakes (Table 4.1), yet the OM, β carotene or $\delta^{13}C_{org}$ values nor the standard deviations or CoV (Table 5.3) do not correspond to its position on the disturbance gradient. Low values for the algal productivity indicators suggest light limitation (evidenced by a euphotic depth of 2.21m; Table 4.2) was restricting the depth of the photic zone in the water column available to algae for photosynthesize, depressing production on a whole ecosystem scale (see section 3.7.1) (Burson *et al.*, 2018). Lake Bunot has the largest CA:LA ratio of 68.6 meaning the catchment exerts a greater influence on in lake processes than in the other lakes. In lakes with a larger drainage area relatively more nutrients and sediments derive from allochthonous sources (compared to within the lake) (Walter *et al.*, 2020). A partially urbanised catchment also amplifies soil erosion, escalating the input of minerogenic material into the lake which fuels algal productivity (Melack and Coe, 2013).

There were two rapid periods of change in algal communities in Lake Bunot (as shown by the PCA axis scores) (Figure 5.37). The first period change occurred between 1940 and 1960 (Figure 5.36) and was characterised by an increase in okenone (especially after 1950) without any accompanying increase in algal productivity indicators and the beginning of a decline in $\delta^{13}C$ from –25 to –33‰. The second period of significant change occurred between 1980 and 1990. This was characterised by the sharp decline and the subsequent disappearance of okenone. There was also a rapid increase in β carotene, lutein-zeaxanthin and pheophytin *b* concentrations followed in 1990 by a gradual decline in all pigments. Furthermore, this period saw a change in the isotopic and lithological records; an increase in %C, %N, $\delta^{15}N_{org}$, OM content and the beginning of a decline in C/N ratios.

An increase in okenone between 1940 and 1960 suggests a shift to deeper light penetration and increased water transparency, which resulted in an enlargement of photosynthetic bacterial communities (Gemerden, 1995; Overmann and Van Gemerden, 2000). The lack of an accompanying increase in algal production indicators, cyanobacterial pigments and OM content suggests this increase was not fuelled by nutrient enrichment (Lürling *et al.*, 2017). The decline in $\delta^{13}C$ values from –25‰ (1940) to –33‰ (1970) (Figure 5.36) provides evidence of the increasing influence of anoxic conditions. The disappearance of okenone from the sediment record in the 1980s suggests hypereutrophic conditions have caused light limitation (from algal self-shading), creating dead zones in the water column (Conroy *et al.*, 2011). Water quality measurements collected since the 1990s by the LLDA, show an increase in nutrients,

particularly phosphate, (from 1.0 mg/l (1996) to 1.9 mg/l (2004)), and nitrate concentrations (from 0.02 mg/l (1997) to 0.17 mg/l (2006)). Usually an increase in nutrient availability would lead to an increase in primary production, but this is not the case here. The declining values in the majority of the pigments suggests the shallowing of the anoxic layer is limiting the depth at which phototrophs can photosynthesise (Figure 4.4).

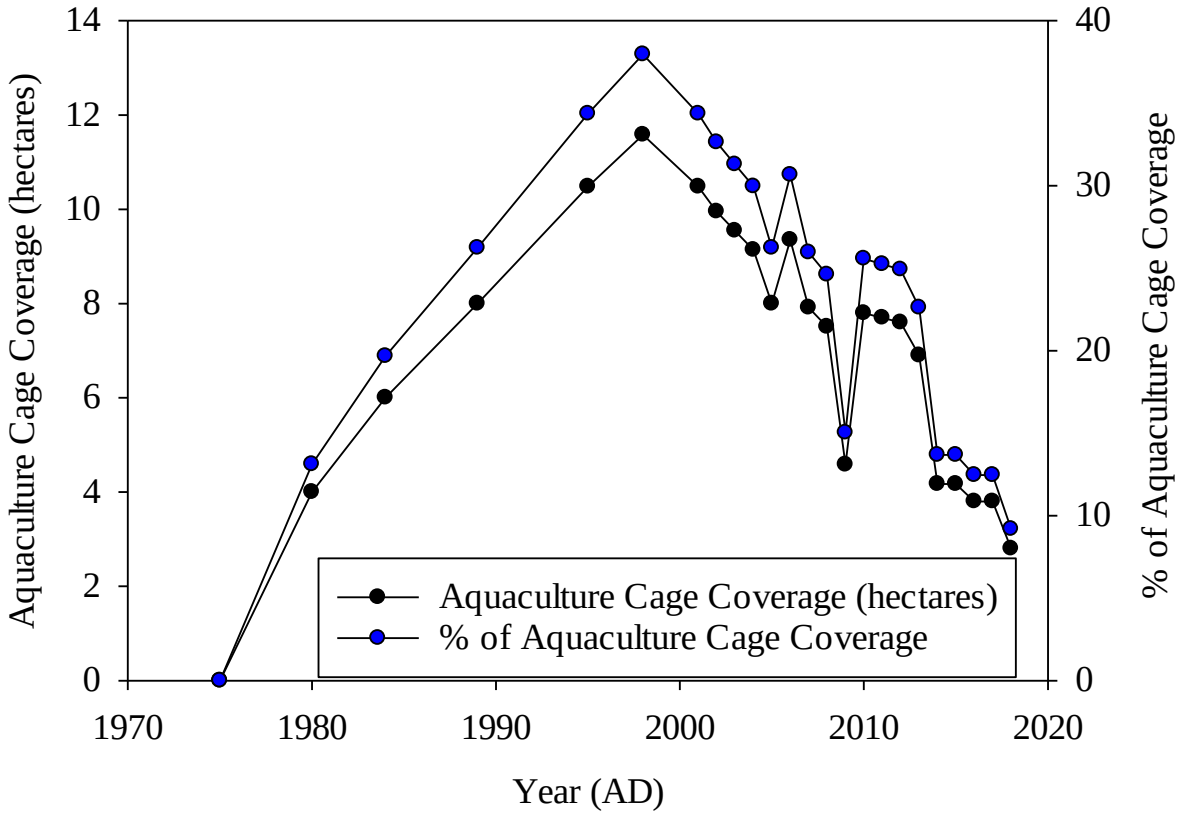


Figure 5.38: The surface area of Lake Bunot utilised for aquaculture cages in hectares (blue) and % (black)

The first period of change identified in the proxy record (between 1940 and 1960), corresponds to a period of limited anthropogenic activity at this lake, so increasing temperatures, anthropogenic or other non-anthropogenic factors are most likely the cause of change in algal communities (Figure 5.1d). Climatic warming would have enhanced anoxic conditions allowing purple sulphur bacteria to bloom and explain the decline in $\delta^{13}C$ values (Figure 5.36). The shift in the majority of the proxy records between 1980 and 1990 corresponds to the introduction of aquaculture in 1976 (which increased to its peak value of 37.97% of lake surface area, in 1998; Figure 5.38) and a concordant increase in human settlements (Figure 5.39). This development would intensify eutrophication (resulting in algal self-shading) and cause a

shallowing of the anoxic layer (Figure 5.8d) (Fukushima *et al.*, 2017). The increased input of human effluent (with $\delta^{15}N$ values of +10 to +20‰) and fish food (with a $\delta^{15}N$ values of +33‰) causing the rise in $\delta^{15}N$ values since the 1990s (Gondwe *et al.*, 2012). Interestingly, a reduction in cage numbers (from the 2000s) has not resulted in a response in algal communities.



Figure 5.39: Photographs of Lake Bunot (photo credit: Suzanne McGowan)

A. *Extensive algal blooms*
B. *Fish cages and bamboo rafts used for transport around the lake*
C. *Man-made structures*
D. *Extensive macrophyte coverage*

5.9. Lake Palakpakin

5.9.1. Field Core Description

The total length of the sediment core PALAK was 74 cm. Between 74 and 70 cm, the sediment consisted of dark brown well-laminated gyttja with a high OM content (Table 5.2). This was followed by a layer of lighter brown well-laminated gyjjta, rich in macrofossils (aquatic macrophyte leaves and wood fragment), between 70 and 12 cm. The top 12 cm consisted of unconsolidated dark brown gyjjta.

5.9.2. Sediment Chronology

In the PALAK core, total ^{210}Pb activities did not reach equilibrium with supported ^{210}Pb activities (Figure 5.40). Total ^{210}Pb activity at the top of the core was 56.19 Bq Kg^{-1} increasing to 122.37 Bq Kg^{-1} at 8.25 cm and decreasing to 66.00 Bq Kg^{-1} at 71.5 cm (Appendix G). Unsupported ^{210}Pb activities, declined irregularly with depth, permitting the use of the CRS model to develop the core chronology (Appleby and Oldfield, 1978; Appleby, 2001). The fact that the unsupported ^{210}Pb activity in the surface sediments reached zero suggests disturbance in the sedimentation process. ^{137}Cs activities in the sediments were low, and only detected between 62.5 and 65.5 cm (Figure 5.39). The CRS age-depth model placed 1963±8 at 65 cm, in agreement with the ^{137}Cs record (Figure 5.39). The CRS age–depth model suggested that the sedimentation accumulation rate increased from 0.03 g cm^{-2} yr^{-1} (1942±15) to 0.11 g cm^{-2} yr^{-1} (1980±5). There were considerable fluctuations in sedimentation accumulation rates in the last thirty years, with sharp peaks in the 1980s, 2000s and the surface sediments. The calculated age model indicated that the 74 cm core covered the past c. 75 years (starting in 1942±15).

5.9.3. Sediment Lithology (LOI)

PALAK had the lowest mean and standard deviation for OM content (of 12.2% and 3.15%) and the third highest carbonate content (7.8%) of all the cored lakes (Table 5.3), but has the highest aquaculture density. The water content remained constant between c. 85% throughout the core except for two falls to 75% in 1975±6 and 1988±4. OM content peaked at 18.6% in 1942±15 and then declined until 1990, when there was a slow rise in OM content from 8.1% to 16.4% (2017) (Figure 5.41). The carbonate content remained constant at c. 6.0% except for a large peak in values of 14.0% in 1976±5. The bulk dry density steadily decreased from 0.15 g/cm^3 (1943±15) to 0.05g/cm^3 (2017±2).

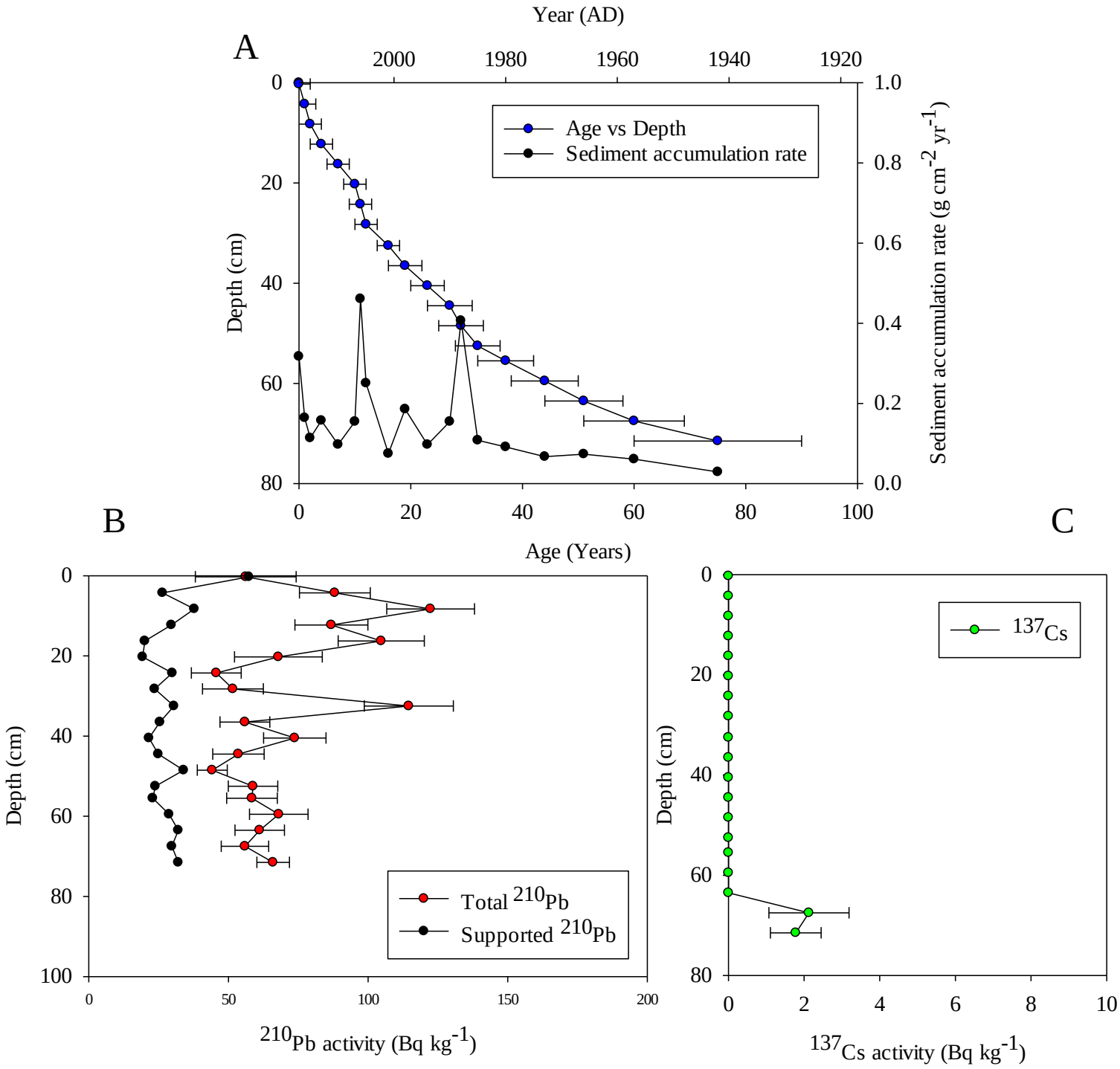


Figure 5.40: A) Age-depth model and sediment accumulation rates, B) changes in total and supported ^{210}Pb and C) ^{137}Cs activity for PALAK sediment core

5.9.4. Sediment Stable Isotopes and C/N Ratios

PALAK had the second lowest mean and lowest standard deviation and CoV for $\delta^{13}C$ of –31.5‰, 0.7‰ and 2%, yet had the second highest mean (4.5‰) and standard deviation (0.9‰) for $\delta^{15}N$ of all the cored lakes (Table 5.3). The $\delta^{13}C$ record from PALAK was relatively stable, with the values fluctuating slightly between –31‰ and –33‰ (Figure 5.41). The $\delta^{15}N$ record was constant at c.+3‰ until 1995±3, when the values started to increase to reach a peak of +6‰, which was maintained to the present day, except for a dip in 2016±2 to +3.5‰. The %C and %N follow a similar pattern with a steady increase in values to 10% and 1% respectively in 1968±7, then a decline towards relatively constant percentages of 5% and 0.6% respectively, for the reminder of the core. C/N ratios were stable at 12 until 1970±6, when they continuously declined to 8 (2017±2).

5.9.5. Sedimentary Pigments

Compared with the other lakes, the concentrations of sedimentary pigments in PALAK remained relatively constant throughout the whole core (Figure 5.41), even though this lake has a standard deviation for β carotene of 115 nmol g^{-1} and the third lowest CoV (80%; Table 5.3). Pigment concentrations of chlorophyll *a*, canthaxanthin (colonial cyanobacteria), lutein-zeaxanthin (chlorophytes, cyanobacteria) and alloxanthin (cryptophytes) all remained constant until the early 2000s (Figure 5.41). Concentrations of β carotene, and diatoxanthin (siliceous algae) increased slightly between 1940 and 1960, followed by high variability until 2000. Concentrations of pheophytin *b* (chlorophytes) were relatively stable (at 500 nmol g^{-1}) until 1985±4, when values rapidly increased to 1000 nmol g^{-1}. Subsequently, pheophytin *b* concentrations declined to c. 600 nmol g^{-1} before plateauing. This is summarised by the PCA axis 1 scores, which show continuous change throughout the core (55.8% variance explained). After 2005, concentrations of chlorophyll *a*, β carotene, canthaxanthin, alloxanthin, and diatoxanthin all showed an increase and high variability (which coincided with the higher sampling resolution). This coincided with significant temporal change in algal communities (Figure 5.42).

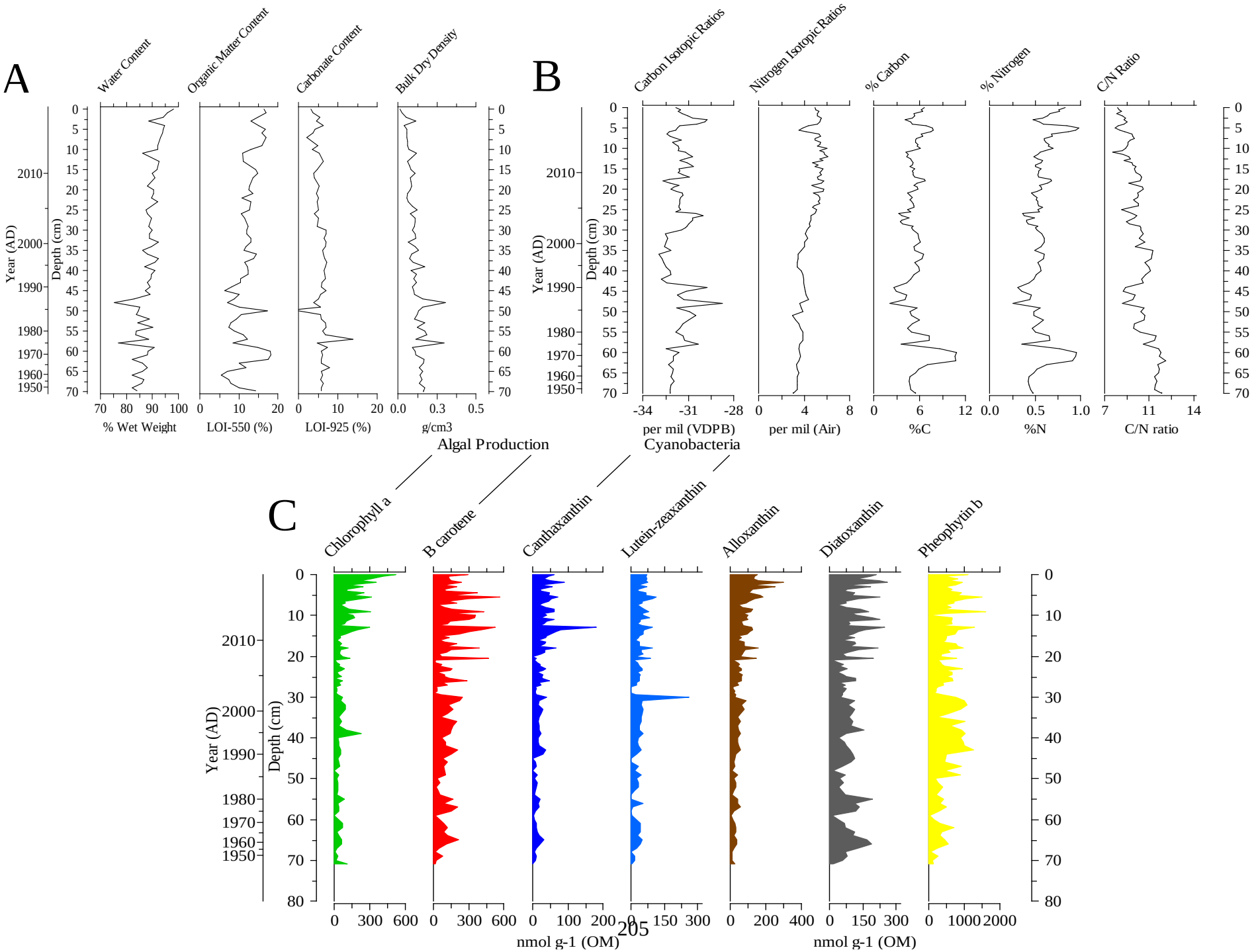


Figure 5.41: Sediment proxy results from PALAK a) sediment lithology, b) stable isotope results ($\delta^{13}C_{org}$, $\delta^{15}N_{org}$) and C/N ratios and c) sedimentary pigment results

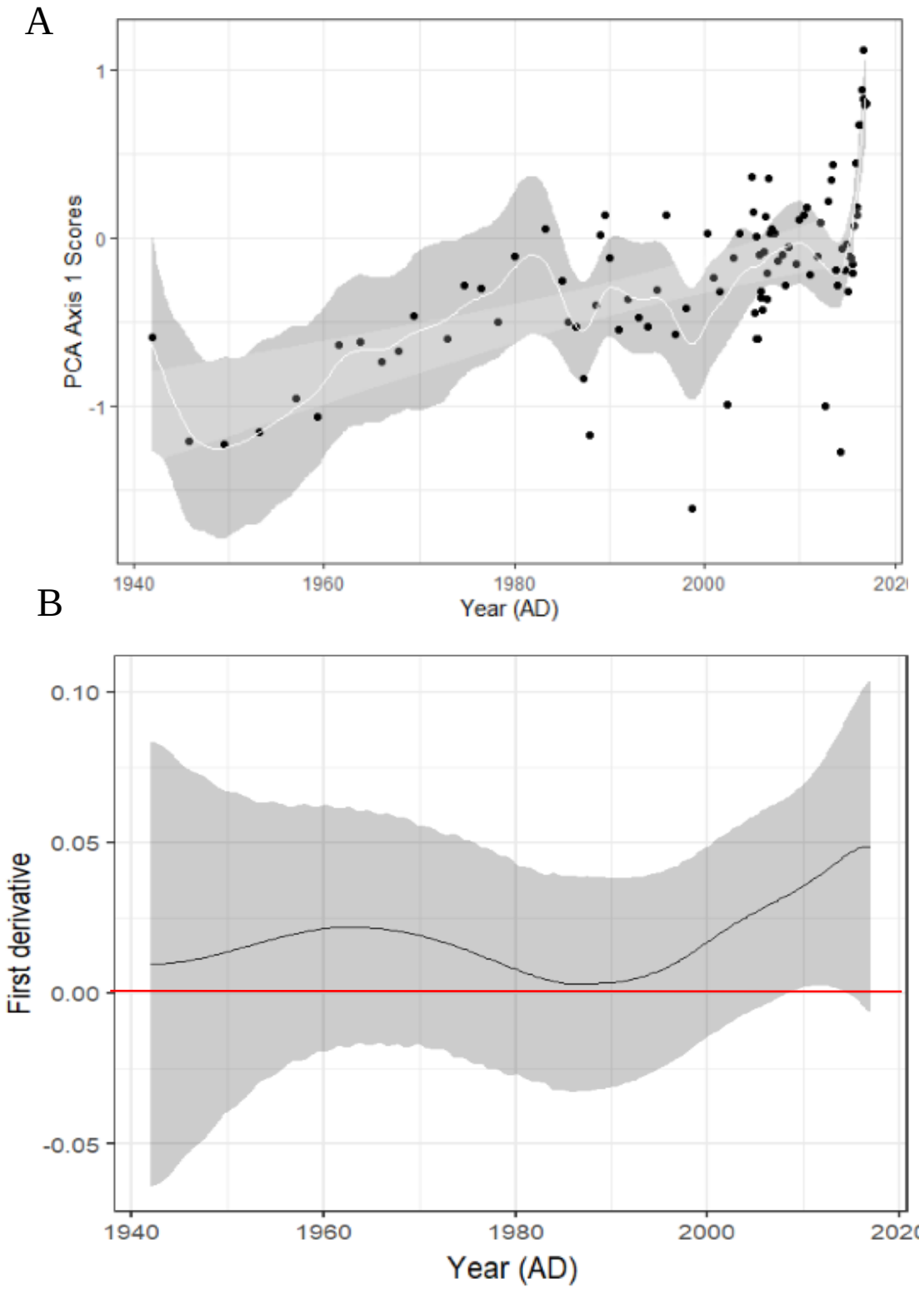


Figure 5.42: A) Generalised additive model fitted to the pigment PCA axis 1 scores from PALAK. B) Estimated first derivatives (black line) of the GAM trend fitted to the PCA axis 1 scores from PALAK. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. In both figures, the grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

5.9.6. Historical Records

The timings of notable events are shown in Table 5.12:

Table 5.12: Timeline of the main anthropogenic changes within the catchment of Lake Palakpakin from 1960s onwards with the data source shown.

Year		Source
1978	Intensive aquaculture first introduced	Interview Data
1986-1990	Floating cages introduced 17 hectares used for aquaculture (maximum cage coverage) (35.42% of lake surface)	Brillo, 2016b
1996	16 hectares of cages (33.33% of lake surface)	Interview Data
2001	15.89 hectares of cages (33.11% of lake surface)	LLDA
2005	13.5 hectares of cages (28.13% of lake surface)	Interview Data
2007	12.30 hectares of cages (25.63% of lake surface)	Interview Data
2008	Aquaculture structures occupied 85000m^2 (c.18% of the total surface area)	Palanca-Tan, 2016
2009	5.22 hectares of cages (10.88% of lake surface)	Google Earth
2010	10.51 hectares of cages (21.90% of lake surface)	LLDA
2012	9.8 hectares of cages (20.42% of lake surface)	LLDA
2013	8.2 hectares of cages (17.08% of lake surface)	LLDA
2014	7.95 hectares of cages (16.56% of lake surface)	LLDA
2015	6.77 hectares of cages (14.10% of lake surface)	LLDA
2016	4.796 hectares of cages (9.99% of lake surface)	LLDA
2017	93 cages (4.566 hectares of cages)	LLDA
2018	4.372 hectares of cages (9.11% of lake surface)	LLDA

5.9.7. Interpretation of Palaeolimnological Records

Lake Palakpakin is 'distinct' in comparison to the other lakes as it is a shallow (<7.5 m), polymictic lake, and as such, lacks a stratified water column and a permanent anoxic layer (see Chapter 5). This lake had the lowest mean OM content of all the cored lakes (Table 5.3) and second lowest CoV (26%) suggesting low variability in OM content in Lake Palakpakin. Combined with low pigment values and the absence of chlorophyll *b*, but high abundance of its degradation product pheophytin *b*, this suggests poor conditions for pigment preservation. Two factors explain this. Firstly, Lake Palakpakin is a shallow lake system, influenced by wind, river flow and high flushing rates (Brillo, 2016b), so mixing in the water column (illustrated by the limnological data; section 4.7.1) prevents anoxia from developing in the hypolimnion. Secondly, pigments undergo photodegradation when exposed to light. A euphotic depth of 2.7 m (Table 4.3), allowed photosynthesis to maintain DO at depth, inhibiting fossil pigment

preservation before permanent burial (García-Rodríguez *et al.*, 2002; Leavitt, 1993). The lack of okenone, relatively stable $\delta^{13}C$ values (–32.0‰) and the presence of DO (0.5 mg/l) in the deepest waters suggests the absence of sustained anoxia (Deevey *et al.*, 1963). Furthermore, the contemporary limnology data showed that Lake Palakpakin had the highest abundances of chlorophyll *b* (>0.1 nmol g^{-1}) and pheophytin *b* (>0.5 nmol g^{-1}) (Figure 4.9) of all the cored lakes. Today, this lake has abundant floating macrophytes (which produce chlorophyll *b*) (Figures 5.44). The irregular removal of water hyacinth (*Eichornia* spp.) by the fishermen to make room for fish cages shows this lake has a problem with prolific aquatic macrophytes (supported by the high abundance of lutein-zeaxanthin and pheophytin *b* since the 1980s; Figure 5.44).

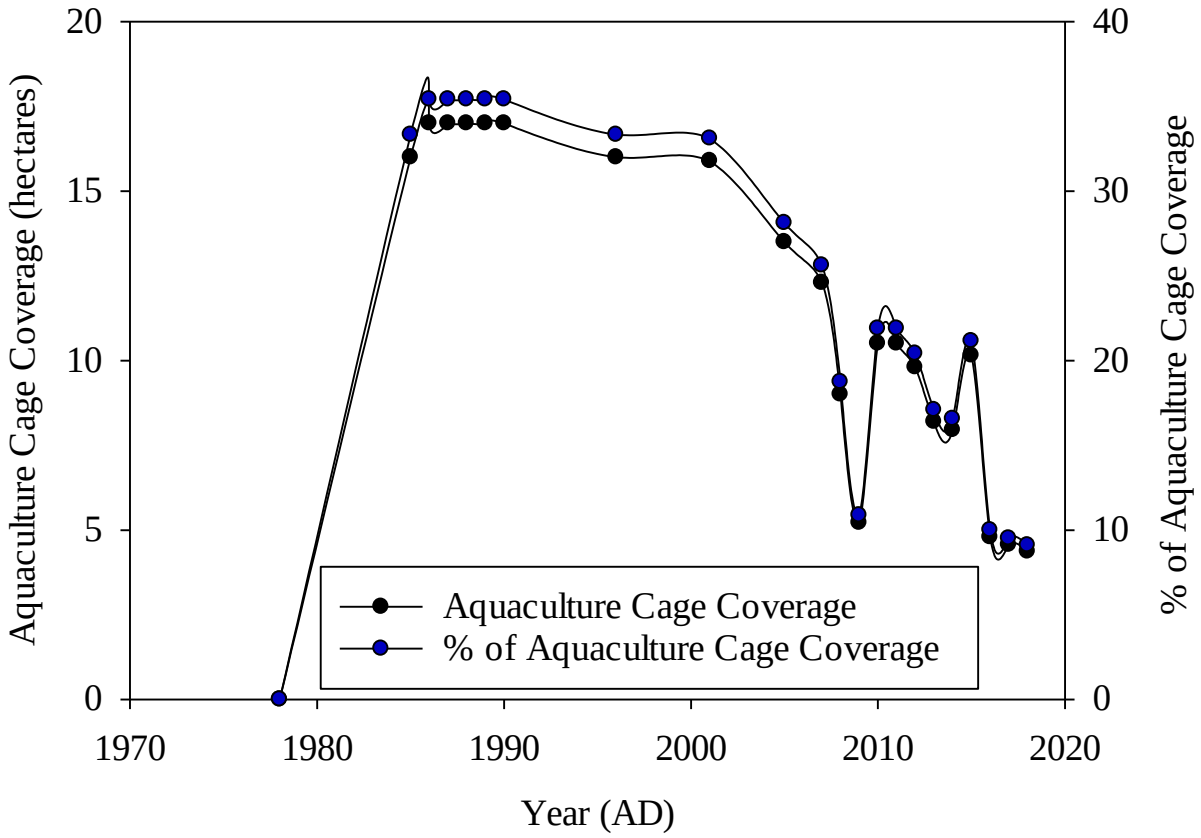


Figure 5.43: The surface area of Lake Palakpakin utilised for aquaculture cages in hectares (blue) and % (black). Source of each data point is listed in Table 5.12

Prior to 1970, there was minimal change in the isotopic and lithological records, but afterwards the $\delta^{13}C_{org}$ record started to fluctuate, and C/N values declined from 12.5 to 8. Pigment concentrations remained stable until 2005, after which variability was high (corresponding to a higher sampling resolution). Two spikes in sedimentation rates in 1990 and 2005

corresponded to peaks in $\delta^{13}C_{org}$. Consistently high $\delta^{15}N_{org}$ values further increased (from +4 to +6‰) after 1995.

The slight increase in β carotene and diatoxanthin throughout the sedimentary record, indicated increased algal production (Figure 6.21). Since 1970, declining C/N ratios, increased OM content and contemporary limnology (Figure 4.1) provided evidence of a shift in algal groups from diatoms → chlorophytes → cryophytes → cyanobacteria (Chapter 4). High nutrient loading (supported by water monitoring data; Figure 4.1; Figure 4.7) has led to the proliferation of *Eichornia* spp. (Brendonck *et al.*, 2003; Yu *et al.*, 2019). The dominance of aquatic macrophytes would cause increased littoral production (supported by high pheophytin *b*), inputting high quantities of OM into the water column (Reitsema *et al.*, 2018), stimulating aquatic productivity. Increases in productivity would normally result in higher $\delta^{13}C$ values (Torres *et al.*, 2012), but Lake Palakpakin displayed fluctuating $\delta^{13}C$ values. This indicates, either the presence of ^{13}C depleted phytoplankton or OM from aquatic macrophytes influenced the ^{13}C of organic carbon in the sediments. Furthermore, $\delta^{13}C$ and $\delta^{15}N$ values of the two main aquatic macrophytes (*Eichornia* spp. and *Pistia* spp.; Table 5.1) suggests they may be contributing towards the fluctuating $\delta^{13}C$ values and the consistently high $\delta^{15}N$ values (Figure 5.44).

Lake Palakpakin had the highest aquaculture density (Figure 5.43) of the studied lakes, yet records limited changes in the proxy record (compared to the other cored lakes) suggesting a lack of bottom-water anoxia and riverine influence has 'dampened' its response to anthropogenic and climatic drivers. This lake had the lowest OM and $\delta^{13}C$ standard deviations providing further evidence that a lack of anoxia has diminished the lake's response to disturbance. Changes in precipitation could be responsible for fluctuations in the $\delta^{13}C$ record (Figure 6.1), as trends show a slightly drier period between 1970 and 1990. Reduced precipitation in this shallow lake would have led to lake level drawdown, leading to desiccation, decomposition and erosion of littoral sediments, and their subsequent deposition at the coring site (Chen *et al.*, 2017). It would have increased sediment resuspension from littoral areas and the deposition of minerogenic sediments (supported by spikes in the bulk dry density and sedimentation rates). Human activities expanded considerably after 1970, with the introduction of aquaculture in 1978, rapidly increasing to peak density in the late 1980s (35.42% of the lake surface area). The 1970s also saw an increase in the number of human settlements, with a rise in the number of coconut plantations and the encroachment of San Pablo City into the lake catchment (Figure 5.44). These pressures could account for the low C/N

ratios, observed from 1970 onwards and for the fluctuations in all pigments (post 2000s), especially, when combined with oxygenated conditions to aid pigment preservation. The C/N ratios of the starter and finisher fish food are 9.7 and 8.0 respectively, therefore, their increased use would cause a decline in C/N ratios.



Figure 5.44: Photographs of Lake Palakpakin (photo credit: Suzanne McGowan)

A. *Chen Qinqin Christina on a boat holding a sediment*
B. *Foreground) fish cages and background) coconut*
C. *Extensive macrophyte coverage and man-made structures*
D. *Fish cages surrounded by macrophytes*

Often up to 50% of the fish food is not consumed by the fish (interview data; Gondwe *et al.*, 2012) leaving the remainder to be utilised by algae (Kong *et al.*, 2020) which could be a contributing towards the fluctuating pigment values. After 1995, the small increase in $\delta^{15}N$, from +4 to +6‰, indicates there was an input of anthropogenic N sources into the lake. The concordant increases in the concentration of canthaxanthin and lutein-zeaxanthin (indicators for cyanobacteria) suggests anthropogenic sources are fuelling cyanobacteria blooms. The introduction of aquaculture, over a decade prior to these changes, shows other human sources such as deforestation for coconut plantations and human settlements must be responsible.

5.10. Summary

The analysis of sediment records has identified how sedimentation conditions vary between lakes and has helped to understand how the individual morphological features of each lake are modifying their response to environmental change. A comparison of the timing of changes in the proxy record with those in the historical records has allowed an initial identification of the possible drivers of these changes. This will be explored in detail in Chapter 6, which attempts to quantify the extent to which aquaculture is driving lake responses.

6. CHAPTER 6 – IS LAKE RESPONSE DRIVEN BY AQUACULTURE?

6.1. Introduction

Lake sediments demonstrate continuity, environmental and seasonal sensitivity, and as such provide high resolution records of past environmental and climatic changes (Hodell *et al.*, 1999). Lakes can act as sentinels for both climate change and human impacts directly, or indirectly, through modifications of the catchment (Battarbee, 2000; Adrian *et al.*, 2009). Climatic warming has resulted in tropical lakes having more stable stratification, reduced mixing and more prevalent anoxia in the hypolimnion (Lewis Jr, 1984). Reduced mixing increases nutrient limitation and diminishes algal productivity (Williamson *et al.*, 2009), which can have an acute impact on pelagic and benthic communities. To complicate matters, tropical lakes are becoming increasingly stressed by human activities such as urbanisation, agriculture and aquaculture (McGrane, 2016). Anthropogenic eutrophication leads to nutrient loading and increased algal productivity, which further enhances anoxic conditions (Figure 5.1). This results in the shallowing of the hypolimnion, restricting the volume of water available for aquaculture (Kalff, 2002). Increased nutrient loading is seen as one of the main causes of changes to phytoplankton succession, resulting in the dominance of cyanobacteria (Ferber *et al.*, 2004; Cordero and Baldia, 2015). Despite its association with surface algal blooms, increased nutrient loading does not always result in net higher algal productivity on a whole lake basis, as eutrophication can progress to the stage where dead zones form in the water column, due to light limitation from self-shading (Figure 5.1; Bhateria and Jain, 2016).

Tropical freshwater lakes are under increasing threat from both anthropogenic activities and climate change, but disentangling and separating out these two drivers is problematic as they both can cause a similar response within lake systems (Mills *et al.*, 2017). Separating out these impacts is most difficult in lakes experiencing eutrophication because increasing temperature may produce similar responses, such as increased anoxia, algal productivity, nutrient loading and nutrient recycling (Moss *et al.*, 2011). One approach is to use lakes that are located within a small geographical area, as it can be assumed that the pressures exerted by regional drivers (such as climate) will be similar on all lakes. By accounting for the impact of such regional drivers it is reasonable to attribute any further observed changes in lake communities to local (catchment-scale), probably anthropogenic, activities (Mills *et al.*, 2017), although these impacts would be shaped by individual lake morphology. Furthermore, studying lakes along a ‘disturbance gradient’, such as aquaculture, allows comparisons to be made between relatively ‘pristine’ lakes (subjected to climate drivers only) and the more disturbed lakes. Examining

how increased aquaculture disturbance relates to the development of features, such as eutrophication and anoxia, helps understand how these lakes will evolve over decadal timescales.

6.2. Brief Summary of Statistical Materials and Methods

This thesis used two methodological approaches to determine if aquaculture has driven change in the lake district of the Seven Lakes of San Pablo. The first approach assessed whether lake response was proportional to the level of aquaculture disturbance. This approach used GAMs fitted to the PCA axis 1 scores (from individual PCA) to determine patterns/trends and periods of significant temporal change in algal communities (see sections 3.3.2 and 3.3.3). Correlations between periods of significant temporal change for the individual isotopic and pigment records and the introduction of aquaculture were also noted. Furthermore, the difference between the pre and post aquaculture means was used to assess the response of each proxy to the introduction of aquaculture and to determine if there was a proportional response to aquaculture intensity. Mann-Whitney U tests were used to evaluate differences between pre and post aquaculture values for $\delta^{13}C$, $\delta^{15}N$ and C/N ratios (see section 3.3.7) to help determine the influence of fish food on these records.

The second approach attempted to quantify the extent to which aquaculture drove change in algal communities and OM properties in comparison to other drivers of change. This approach used variance partitioning to quantify the extent to which aquaculture and other variables (such as climate and land use) were responsible for algal community change (see section 3.3.4). Finally, linear regression was used to understand the relationship between the lakes' physical characteristics, algal community change (measured as Mann-Kendall coefficients of PCA axis 1 scores) and algal biomass (determined by mean β carotene concentrations from individual sediment core records), to assess whether the lakes' physical characteristics had modified their response to aquaculture (see section 3.3.6).

6.3. Approach 1 - Summarising change in the palaeolimnological records

Lakes Yambo, Pandin, Sampaloc, Bunot and Palakpakin demonstrated significant temporal change in algal communities (Figure 6.1), however, the timing and scale of these shifts (assessed by GAMs) differed considerably among lakes (Figure 6.2). There were no periods of significant temporal change in Lake Mohicap. The periods of significant temporal change pre-dates aquaculture in Lakes Pandin and Sampaloc and post-dates aquaculture in Lakes Yambo and Palakpakin. Only in Lake Bunot, does the introduction of aquaculture coincide with significant temporal change in algal communities.

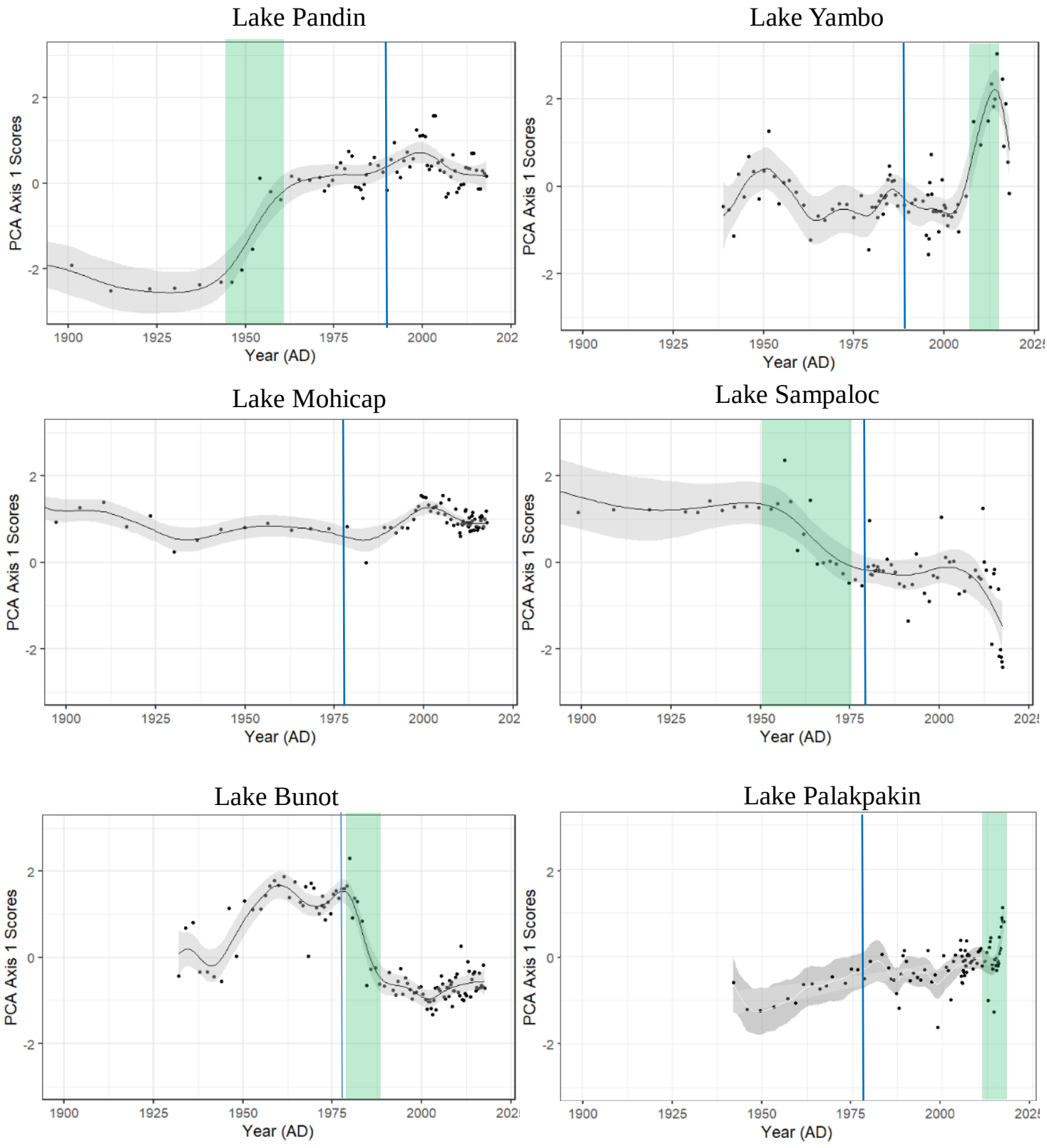


Figure 6.1: Generalised additive model fitted to the PCA axis 1 scores from 1900 onwards for the six lakes. The grey area corresponds to the 95% confidence intervals. The models are presented in order of aquaculture disturbance from lowest (Lake Pandin) to highest (Lake Palakpakin). The blue line signifies the year in which aquaculture was introduced to each lake whilst the green shaded area corresponds to periods of significant temporal change (see Chapter 5)

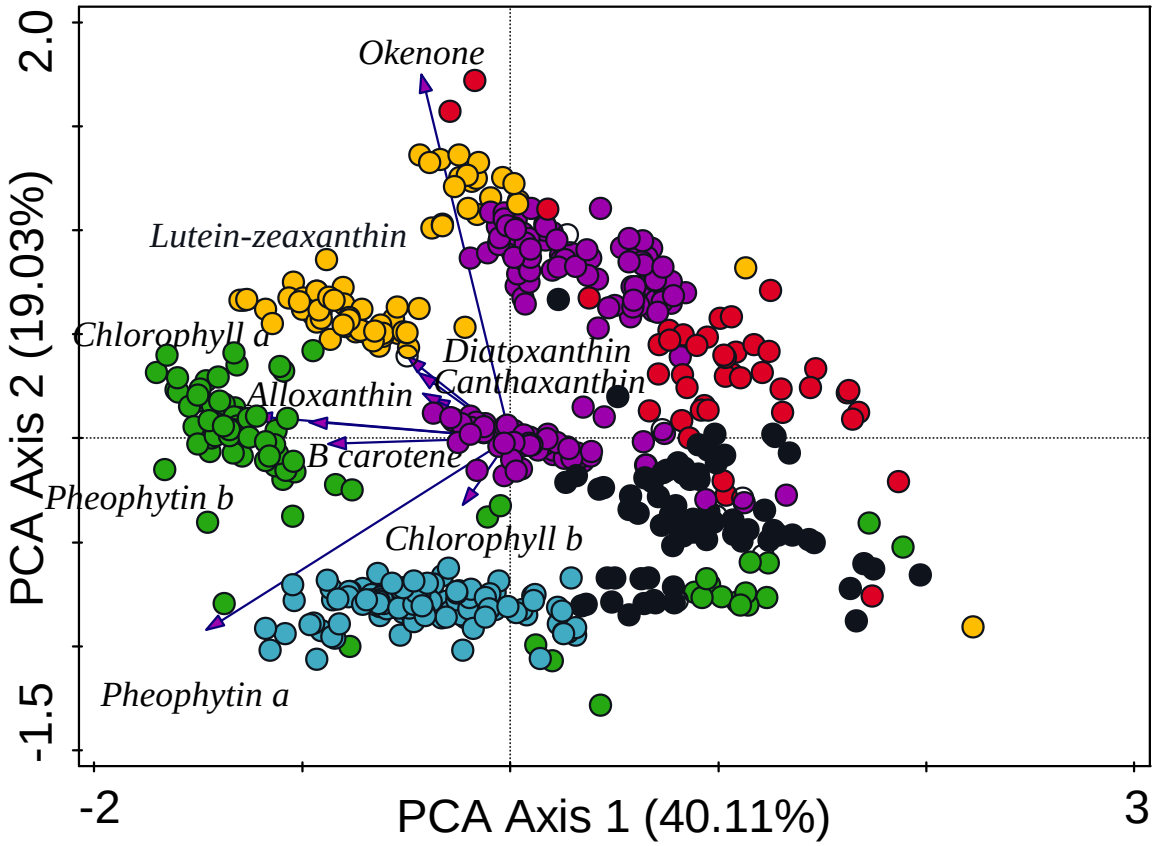


Figure 6.2: PCA biplot with all samples from the six lakes. The coloured circles correspond to different lake groupings. Black= Lake Pandin, Red= Lake Yambo, Yellow=Lake Mohicap, Green=Lake Sampaloc, Purple=Lake Bunot and Blue= Lake Palakpakin

The majority of the isotopic records from all lakes showed a deviation from the pre aquaculture mean (Figure 6.3). There was no proportional relationship in the difference between the pre and post aquaculture means for $\delta^{13}C$, $\delta^{15}N$, %C, %N or C/N ratios and the level of aquaculture (Figure 6.4). The $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios showed a significantly smaller decline/increase in the low disturbance lakes (Yambo and Pandin) compared to the high disturbance lakes (Mohicap, Sampaloc and Bunot), with Lake Palakpakin the exception (Figure 6.3). The C/N ratio demonstrated a deviation from the pre-aquaculture mean for all lakes, yet each displayed a distinct response (Figure 6.3). The timing of the introduction of aquaculture only coincides with significant temporal change in %C and %N records in Lake Bunot. The introduction of aquaculture does not match with any discernible deviation in $\delta^{13}C$, $\delta^{15}N$, %C and %N in any of the other lakes. Lake Sampaloc showed significant change in the isotopic records in 1950, prior to the introduction of aquaculture, whilst the other lakes demonstrated significant change post aquaculture.

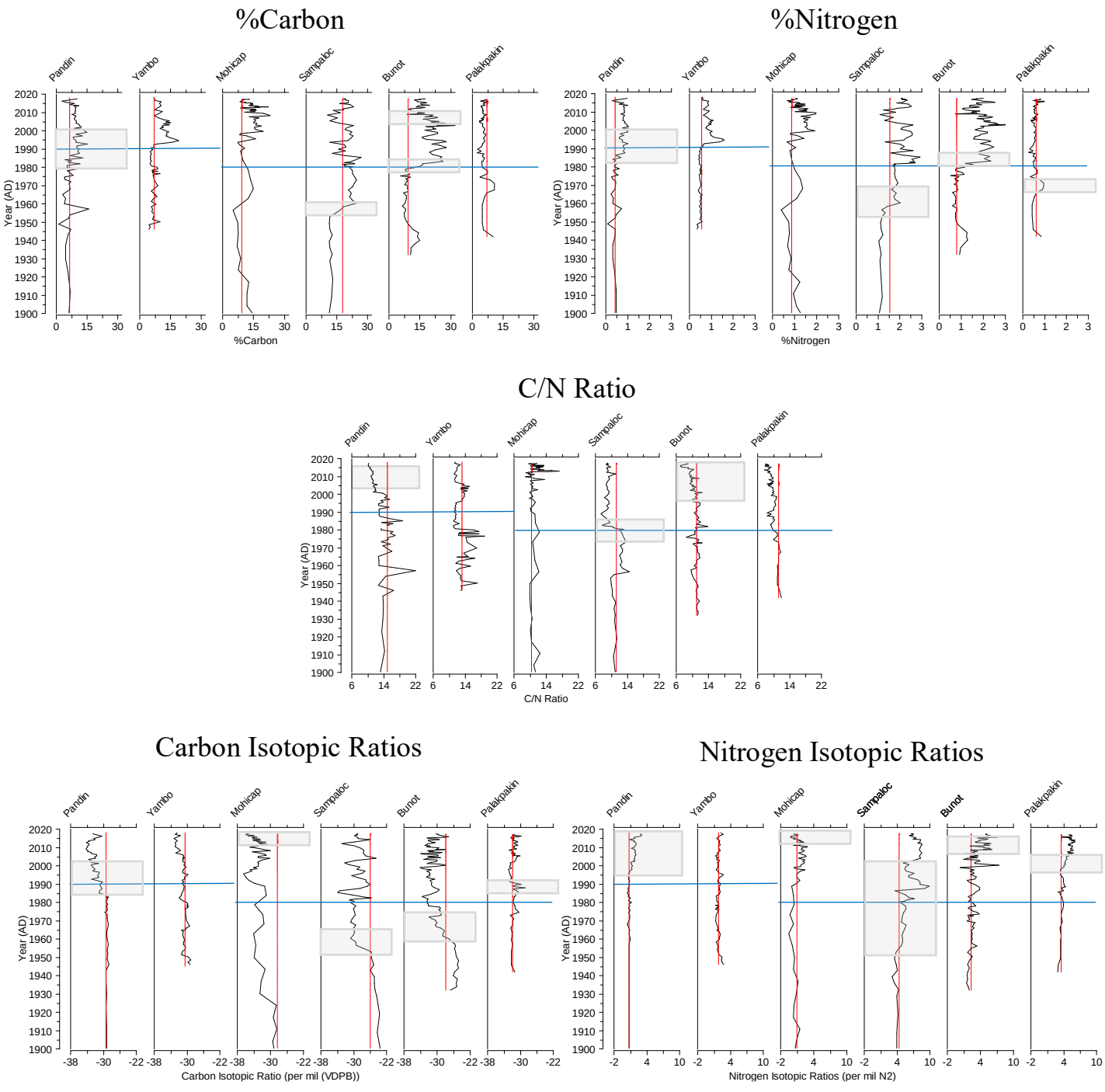


Figure 6.3: %Carbon, %Nitrogen, C/N Ratios, $\delta^{13}C_{org}$, $\delta^{15}N_{org}$ values on the same scale for the six lakes. The blue line represents the year aquaculture was introduced. The red line represents the pre-aquaculture baseline to help determine deviations from the mean. The grey shading corresponds to periods of significant temporal change as assessed by GAMs (see Appendix J)

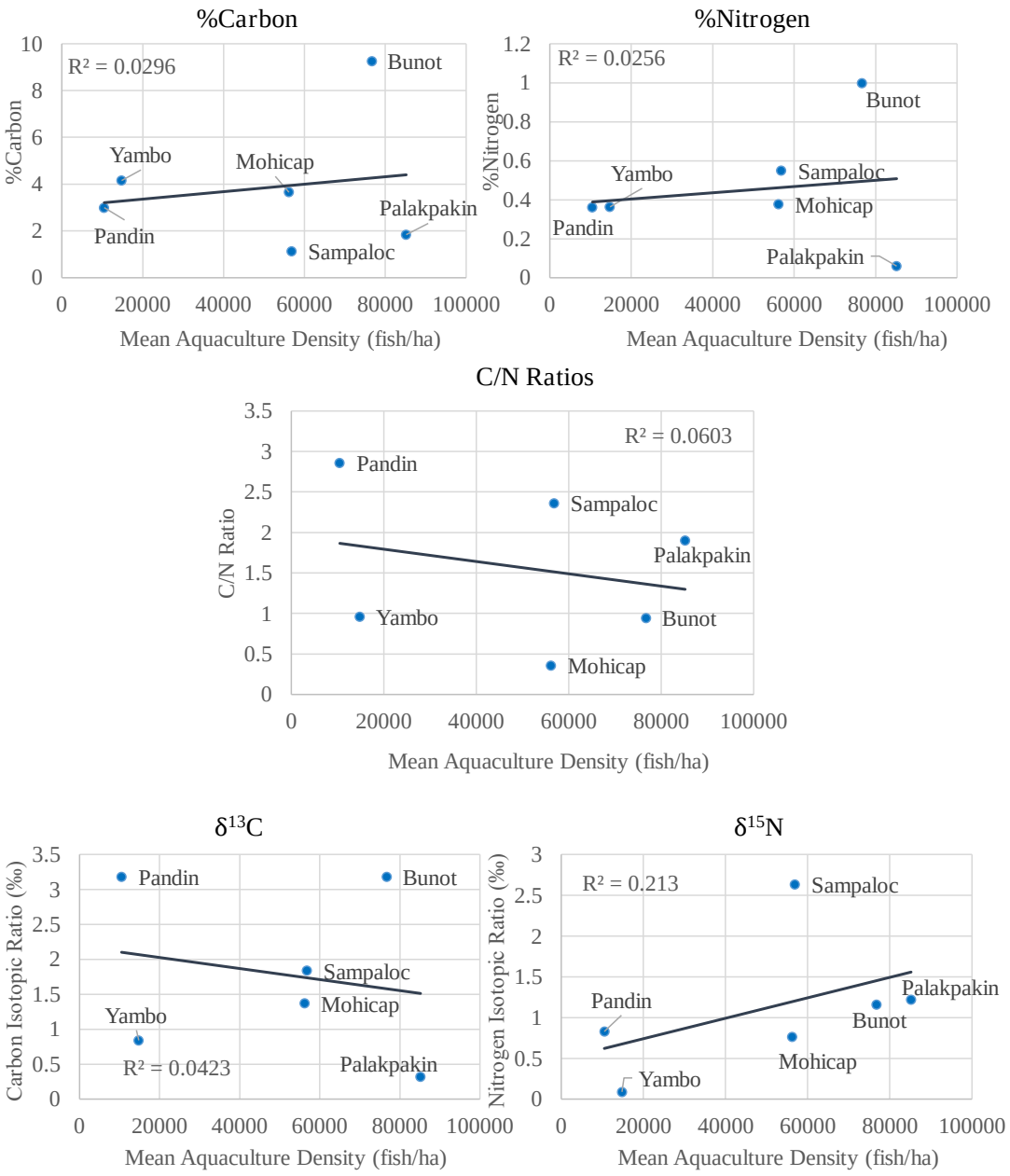


Figure 6.4: Scatterplot demonstrating the relationship between the impact of aquaculture and %C, %N, C/N ratios, $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$. The values plotted on the y axis are the differences between the pre and post aquaculture means for each variable, which was compared with the mean aquaculture fish density in each lake. The strength of the relationship is indicated by the R^2 value. The difference between the pre and post aquaculture means is used to show how each proxy has responded to aquaculture.

In the high disturbance lakes (Bunot and Sampaloc), pigment concentrations were generally higher and showed a greater deviation from the pre-aquaculture mean, in comparison to the low disturbance lakes (Yambo and Pandin; Figure 6.5). There was a greater change in algal communities (R^2=0.47) as the level of aquaculture increased (as determined by the Mann-Kendall coefficient of the PCA axis 1 scores), yet it was not a proportional relationship (Figure 6.6). The increases in the different algal groups, alloxanthin (cryptophytes), diatoxanthin (siliceous algae), chlorophytes (lutein, pheophytin b) and cyanobacteria (zeaxanthin, canthaxanthin, myxoxanthophyll), were not proportional to the level of aquaculture. This pattern was also observed in the other pigments (okenone, chlorophyll *a* and β carotene; Figure 6.7), as Lake Sampaloc had the highest concentration in all these pigments, yet only had the third highest aquaculture disturbance. The periods of significant temporal change were unique to each lake and did not correspond with the introduction of aquaculture. For Lake Sampaloc significant temporal change in chlorophyll *a* and β carotene occurred pre aquaculture. In Lakes Pandin, Yambo, Mohicap, Bunot and Palakpakin, periods of significant temporal change occurred post aquaculture.

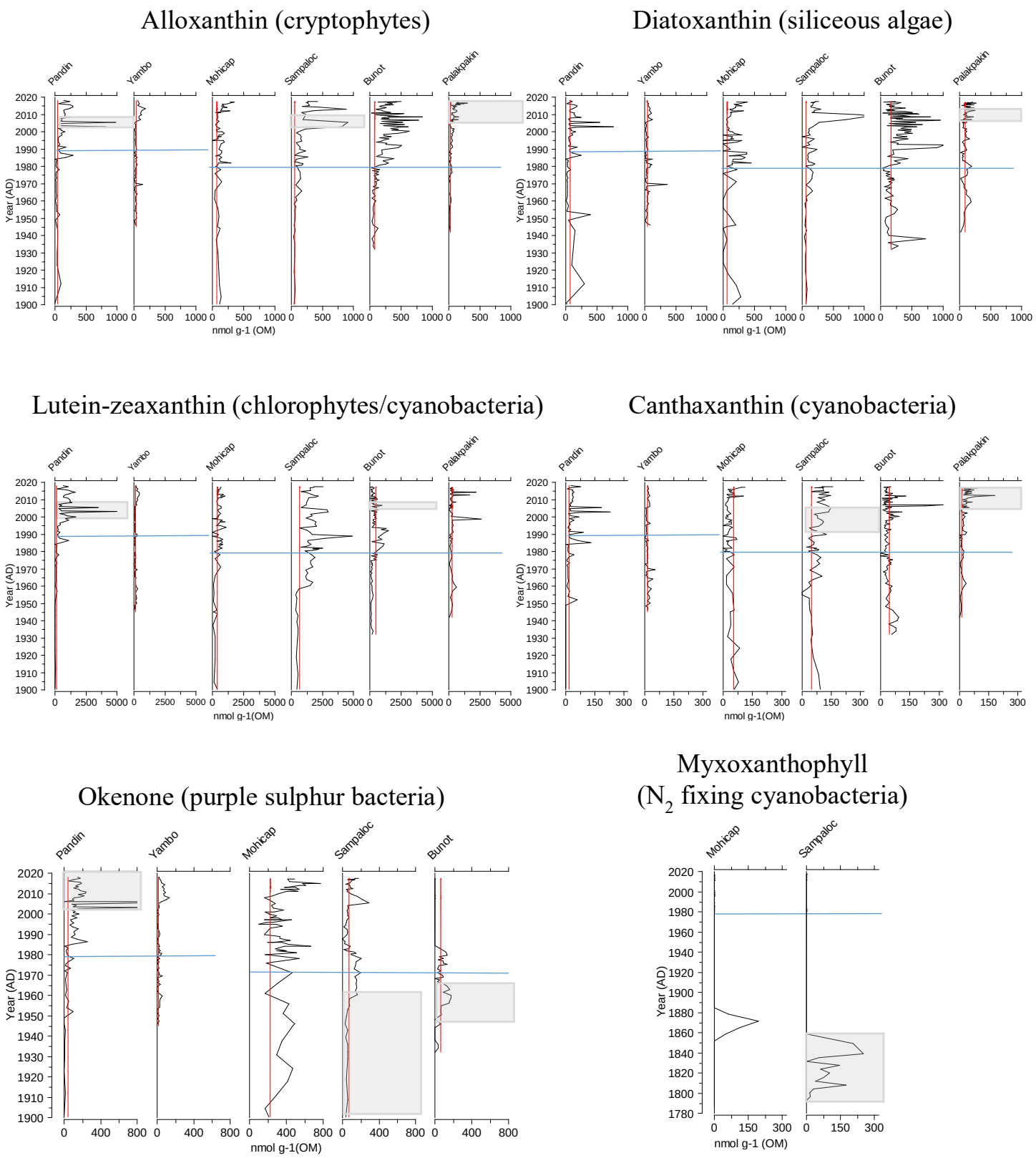


Figure 6.5: Stratigraphic profiles showing alloxanthin, diatoxanthin, lutein-zeaxanthin, canthaxanthin and okenone concentrations on the same timescale for the study lakes. The blue line represents the year aquaculture was introduced. The red line represents the pre-aquaculture baseline to help determine deviations from the mean. The grey shading corresponds to periods of significant temporal change as assessed by GAMs (see Appendix J). Note-the scale for myxoxanthophyll is from 1780 to present day, as this pigment was only detected in the pre-1900 sample.

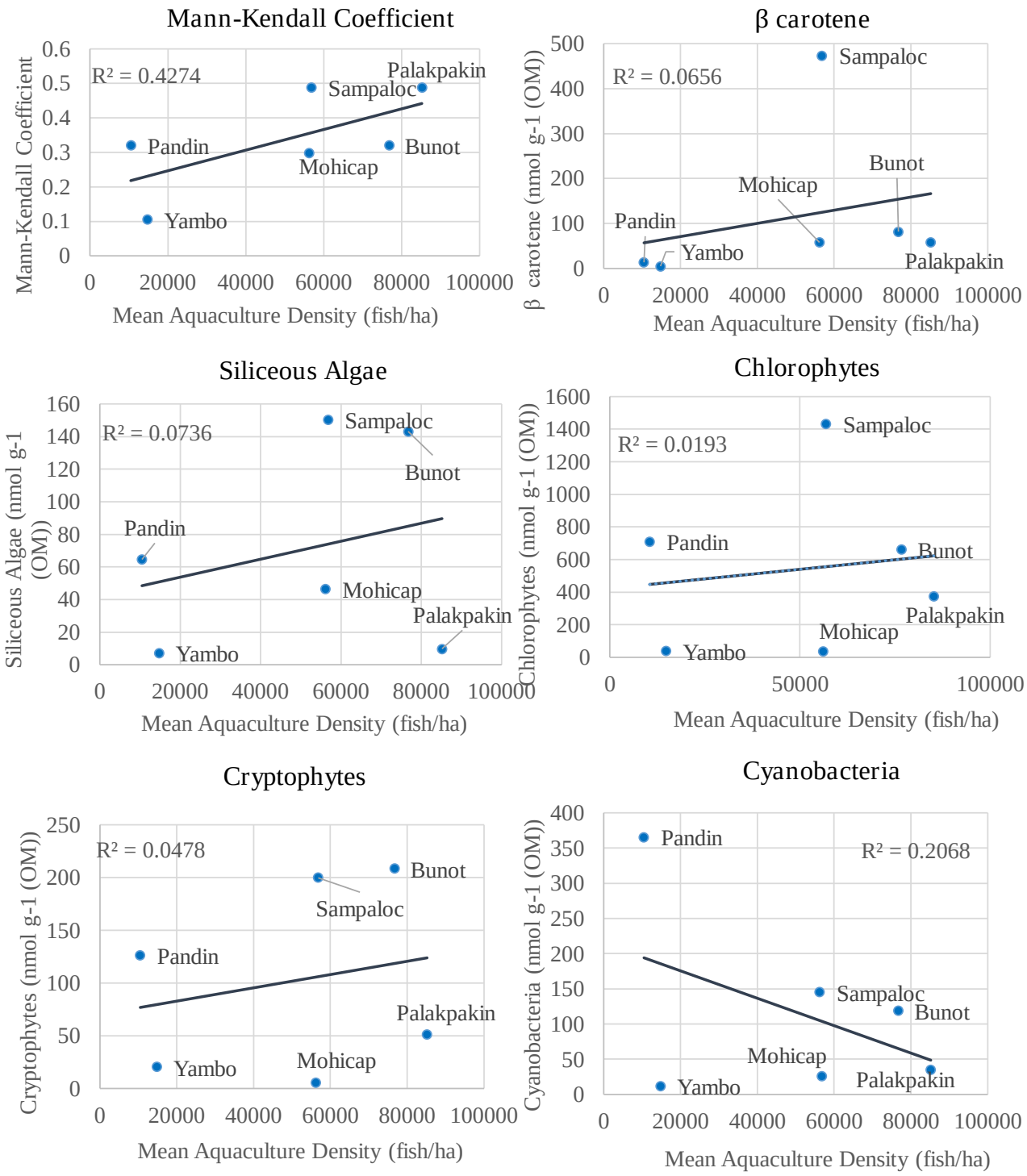


Figure 6.6: Scatterplot demonstrating the relationship between aquaculture and the different algal groups, β carotene and the Mann-Kendall coefficient (a single summarising variable for algal community change). The different algal groups shown are siliceous algae (diatoxanthin), sum of all chlorophyte taxa (chlorophyll b, pheophytin b and lutein-zeaxanthin), cryptophytes (alloxanthin) and sum of all cyanobacteria pigments (canthaxanthin, myxoxanthophyll, echinenone and lutein-zeaxanthin). The values plotted on the y axis are the differences between the pre and post aquaculture means for each variable (except the Mann-Kendall coefficient which uses absolute values), compared with the mean aquaculture fish density in the study lakes. The strength of the relationship is indicated by the R^2 value. The difference between the pre and post aquaculture means is used to show how each proxy has responded to aquaculture.

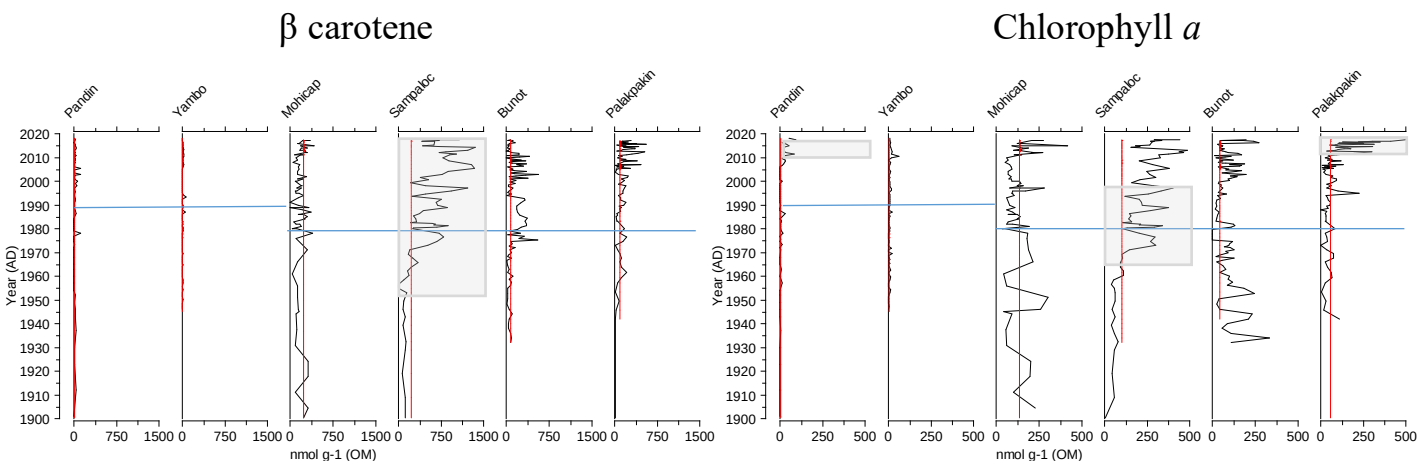


Figure 6.7: Stratigraphic profiles showing β carotene and chlorophyll a concentrations on the same scale for the six lakes. For Lakes Sampaloc and Mohicap, as echinenone and chlorophyll a coelute, both pigments are included in the chlorophyll a values presented. The red and blue lines represent the pre-aquaculture mean and the year aquaculture was introduced respectively. The grey shading corresponds to periods of significant temporal change as assessed by GAMs (see Appendix J)

6.4. How do the responses differ between lakes with high and low aquaculture disturbance?

Overall, sediment $\delta^{15}N_{org}$ and %N values have shown considerable disruption to N cycling in the study lakes, since 1980 (Figure 6.3). All lakes demonstrated a deviation from the %N baseline after 1980, yet only the deep, high disturbance lakes (Mohicap, Sampaloc and Bunot), saw an increase in $\delta^{15}N$ values. Lakes Pandin and Palakpakin, saw a delay in the shift in $\delta^{15}N$ until 2000, and there was no change in Lake Yambo. Since these changes do not correspond to the introduction of aquaculture (even considering the age model uncertainty; Appendix B), and is the lack of proportional relationship (Figure 6.4), suggests aquaculture is not the cause of change in $\delta^{15}N_{org}$ and %N values. The delayed response in these two lakes is unlikely to be due to a lake's resilience to change as these two lakes are on opposite ends of the aquaculture disturbance gradient. Furthermore, Lake Palakpakin, which lacks an anoxic layer, has been subjected to other anthropogenic pressures (urbanisation), therefore, it is unlikely to be demonstrating a muted/delayed response to aquaculture. These variations could, therefore, be attributed to differences in the trophic conditions of these systems, the prevalence of anoxia and/or the relative influence of anthropogenic N loading (Table 3.4; Botrel *et al.*, 2014). Anoxia is more likely to occur and be more persistent in tropical lakes compared to temperate lakes. Even tropical lakes with low productivity (such as Lakes Yambo and Pandin) will likely be anoxic during periods of extended stratification (Figure 4.4; Lewis, 2010). Biogeochemical transformations of N can result in the substantial fractionation of $\delta^{15}N$ values and, therefore, $\delta^{15}N$ can indicate the processes that have taken place (Meyers and Ishiwatari, 1993). Virtually

all stratified tropical lakes will lose a substantial portion of their N inventory on an annual basis through denitrification and, therefore, there is a greater likelihood of N depletion by phytoplankton growing in the epilimnion (Lewis, 2002). Microbial communities can influence $\delta^{15}N$ values through denitrification, which is the reduction of NO_3 to N_2. This process preferentially uses the lighter ^{14}N in the reduction of NO_3 to N_2, further enriching the residual ^{15}N in the DIN pool, resulting in isotopically light $\delta^{15}N$ values. Denitrification is a process stimulated by anoxia and high temperatures in sediments (Meyers and Teranes, 2001). Lake Palakpakin lacks an anoxic layer (as determined by contemporary limnological data). In Lake Pandin, anoxia did not set in until the 1980s (determined by the increase in okenone and decline in $\delta^{13}C$ values), which when combined with limited anthropogenic N loading would explain the delayed response in this lake. The response in the shallow Lake Palakpakin helps support the idea that lake depth/anoxia drove change in the study lakes.

The $\delta^{13}C_{org}$ records showed a complex response to environmental drivers (Table 3.4). All lakes (except Lake Palakpakin) showed a decline in $\delta^{13}C_{org}$ and C/N values at some point (Figure 6.3). This was accompanied by a concordant increase in pigment concentrations suggesting enhanced hypolimnetic anoxia or productivity, was responsible for the decline in values (Conrad *et al.*, 2011). However, the high disturbance lakes deviated from this pattern, post 1980, with each lake showing a unique response after this time (Figure 6.3). Freshwater carbon cycling is complex with a wide range of factors such as trophic level, productivity and biogeochemical cycling influencing $\delta^{13}C_{org}$ isotopic compositions (Guiry, 2019) making interpretations difficult. This indicated that lake responses have been influenced by different drivers and/or tempered by catchment modifications and physical characteristics (see Chapter 5) (Moorhouse *et al.*, 2018). In Lake Mohicap, $\delta^{13}C_{org}$ increased, suggesting enhanced algal productivity was the most likely cause (Meyers and Teranes, 2001), supported by low C/N ratios (10). In comparison, the high variability in $\delta^{13}C$ in Lake Sampaloc, suggests a combination of drivers were influencing the record, including higher phototrophic productivity, changes in allochthonous sources and the development of anoxia and methanogenesis (Legaspi *et al.*, 2015). This is supported by low C/N ratios (8), increases in algal productivity indicators (chlorophyll a, β carotene) and concordant increases in the rest of the pigments (indicating increased preservation due to enhanced anoxic conditions). The post 1980 change in $\delta^{13}C$ in the high aquaculture lakes corresponded with the introduction of aquaculture. However, each lake showed a unique response in the $\delta^{13}C_{org}$ record indicating individual lake catchment modifications could be mediating lake response to aquaculture.

All pigments increased and deviated from the baseline after 1950 (Figure 6.5; Figure 6.7). There are three possible explanations for this. Firstly, in the high disturbance lakes urbanisation and high levels of aquaculture enhanced nutrient enrichment (Deshpande *et al.*, 2014). Multiple studies have found that increased nutrient loading increases algal biomass (Legaspi *et al.*, 2015; Ding *et al.*, 2019) in the initial stages of eutrophication (Figure 5.1). Secondly, shallowing of the anoxic layer in the high disturbance lakes would have enhanced the preservation of pigments, with higher concentrations preserved in the sediment record (see Chapter 5; Figure 4.5). Thirdly, the low disturbance lakes (Yambo and Pandin) have the deepest euphotic depths (Table 5.2), which is detrimental to pigment preservation (Leavitt, 1993). Photosynthesis is possible further down the water column, resulting in a deeper oxygenated layer and chlorophyll maximum and enhanced pigment degradation (section 5.4.7). Less pronounced anoxia combined with greater depth (and increased sinking times) in the low disturbance lakes, resulted in high degradation in the water column, meaning the actual pigment abundance was potentially underestimated (Deshpande *et al.*, 2014). It is likely that a combination of these three factors were responsible for the increase in algal pigments.

The low disturbance lakes, Yambo and Pandin have low levels of aquaculture yet demonstrated different responses. Even though these lakes are in close proximity, they vary in both the timing and scale of change observed in the proxy record. The timing of change in algal communities and abundance was first observed in Lake Pandin in 1940±7 (pre-aquaculture) but not until post 2000 in Lake Yambo (Figure 6.1), with pigment abundances consistently higher in Lake Pandin. The chronological offset in Lake Yambo, has most likely resulted in uncertainty with regards to the timing of change in algal communities. Despite the offset, the change in algal communities, is unlikely to coincide with the introduction of aquaculture. Both lakes show a decline in $\delta^{13}C$ and an increase in $\delta^{15}N$ values, yet the scale of change is greater in Lake Pandin (Figure 6.3). It seems reasonable to suggest that since these lakes have limited aquaculture and other anthropogenic pressures, that despite the difference in the timing of the response, climate must somehow be responsible for the change in algal communities. Furthermore, differing lake management strategies could be a contributing factor in the disparity between these two lakes. There is evidence that Lake Pandin had a structured management strategy which included a community group, which actively promoted a switch from aquaculture to ecotourism in the early 2000s (Anastacio *et al.*, 2016). The lack of documented evidence about any similar management strategy at Lake Yambo (Brillo, 2017) and the fact that ecotourism did not commence in this lake until 2018 (from visual observation) suggests less active intervention.

The most likely explanation is that individual lake characteristics are modifying the extent and timing of the response (Adrian *et al.*, 2009; Jeppesen *et al.*, 2014). Lake Yambo is shallower and has a larger surface area than Lake Pandin (Table 4.2). Lake depth has been found to control the intensity of the impact from climatic warming (Blenckner, 2005). Deeper tropical lakes have a longer hydrologic residence time and therefore the impact of climate is 'stored' for longer in the lake system (Talling, 1963). Furthermore, the impact of precipitation, wind and storm events is reduced in lakes with a longer residence time (Blenckner, 2005). Therefore, Lake Yambo would be more susceptible to wind driven turbulence and less likely to have stable stratification (compared to Lake Pandin). In Lake Pandin, stratification most likely developed earlier (due to climatic warming; Figure 6.3) leading to more prevalent anoxia (Rabalais *et al.*, 2010). As previously discussed (in Chapter 5), increased anoxia would result in enhanced preservation of algal pigments and a greater decline in the $\delta^{13}C$ record. This could explain why Lake Pandin has a similar community composition to Lake Yambo, yet has a higher abundance of algal pigments (as less is being lost to degradation in the water column). This highlights that differing lake morphologies can mediate a lake's response to both climatic and anthropogenic drivers.

6.4.1. The role of anoxia

As observed in the contemporary limnological data, the palaeolimnological records showed distinct physical, chemical and biological changes along the aquaculture gradient (Figure 6.4; Figure 6.6). Movement along the aquaculture gradient was characterised by progressive eutrophication and enhancement of anoxic conditions, leading to the appearance and increase in okenone (purple sulphur bacteria). N_2 fixing cyanobacteria (myxoxanthophyll) also disappeared as nutrient loading increased (Figure 6.5). As the level of aquaculture increased, more oxygen was consumed in respiration and microbial degradation leading to a widening of the anoxic layer and an associated increase in algal biomass (Kalff, 2002; Rhodes *et al.*, 2017). This resulted in concordant increases in all pigments from enhanced preservation. Although an increase in aquaculture initially caused an expansion in algal biomass (including okenone; Maheaux *et al.*, 2016), this is not sustainable in prolonged hypereutrophic conditions, as demonstrated in Lake Bunot. There was a decline in pigment concentrations in Lake Bunot even though nutrient inputs of NO_3^- and PO_4^{3-} increased (LLDA, 2006a; 2009). This is because increased oxygen consumption (by algae) resulted in a shallowing of the photic layer, reducing the area in the water column where algae can photosynthesise, confining algae to the surface. This caused the disappearance of okenone because of shading by the algae, reduced light penetration, ultimately creating 'dead zones' in the water column. Maheaux *et al.*, (2016) determined that okenone concentrations increased with eutrophication, yet were ultimately constrained by light limitation (Lami, 2000). However, Lake Palakpakin has the highest level of aquaculture but lacks significant temporal change in the proxy record (Figure 6.1) suggesting the impact of anoxia is pivotal in the preservation of pigments and had 'dampened' the lake's response to aquaculture (see section 6.4).

Although, the introduction of aquaculture antedates the appearance of okenone, it does coincide with a significant reduction of okenone in the deep, high disturbance lakes, Sampaloc and Bunot. Even considering dating errors of ±4 years at 1980, the introduction and subsequent increase in aquaculture lead to a reduction, and in the case of Lake Bunot, the disappearance of okenone. This suggests aquaculture increased nutrient availability (from fish food and excretion) leading to increased algal biomass (supported by increased chlorophyll a, %C and, %N; Figure 6.3; Figure 6.7), reduced light penetration, negatively impacting okenone concentrations. The lakes with high aquaculture also have high levels of other disturbance (see Chapter 5) which would exacerbate anoxia, therefore, it could be hypothesised that aquaculture pushed already stressed lake systems to a state where the conditions were unfavourable for

purple sulphur bacteria. Anoxia plays an important role in aquaculture, limiting the space available for fish to live in the water column (see section 4.7) and a reduction in fish quality. Low DO concentrations cause a substantial reduction in appetite, poor food ingestion and nutrient absorption leading to less energy for growth (Dong *et al.*, 2011). Attempts in the high disturbance lakes to optimise productivity by increasing stocking densities in the 1980s, would have caused water quality degradation and negatively impacted fish used for aquaculture. Each fish species responds differently to stocking densities, yet the adaptability of Nile Tilapia (the main fish species farmed at the study lakes) to poor water conditions (FAO, 2016) could have resulted in some resilience to low DO. However, the annual fish kills and declining quality of fish, especially in the high disturbance lakes (interview data, Appendix A) suggests declining DO is having a drastic impact on aquaculture in the study lakes.

6.4.2. Cyanobacteria and HABs

Surprisingly, myxoxanthophyll (N_2-fixing cyanobacteria), the main HAB indicator, was not present in post 1900 samples (Figure 6.5) but was detected in low abundance in the contemporary record (Figure 4.9). Photographic evidence (Figure 5.26) showed extensive blooms, yet this was not reflected in the pigment record. Surface bloom pigments (such as myxoxanthophyll) are generally not incorporated well into the sediment record due to extensive photo-oxidation (from the light at the surface) and because the pigments have further to sink prior to deposition (Freiberg *et al.*, 2011). The shallowing of the anoxic layer, which aids pigment preservation (especially in the lakes with high aquaculture) does not seem to have increased the preservation of myxoxanthophyll, due to the effect of sunlight on pigment degradation at the surface. All of the study lakes were N limiting at the time of sampling (May 2017) providing ideal conditions for N_2 fixers to bloom (Havens *et al.*, 2003). It is most likely that the low N concentrations (caused by greater P loading from aquaculture) provided the ideal conditions for N_2-fixing cyanobacteria to bloom (McGowan *et al.*, 2012), yet high irradiance resulted in a lack of preservation.

There was a slight increase in cyanobacterial pigments relative to the other pigments in the high disturbance lakes, post aquaculture (Figure 6.8). The enrichment of surface waters with nutrients (N and P) is a key driver in the formation of cyanobacterial blooms (Watson *et al.*, 1997). Furthermore, research has shown that physiological (more rapid growth rates) and physical (enhanced stratification) factors promote the proliferation of cyanobacteria (O'Neil *et al.*, 2013) under warming conditions (Paerl and Huisman, 2008). Lürling *et al.*, (2017) determined that eutrophication and warming act in synergy to stimulate algal and

cyanobacterial biomass with eutrophication the more dominant factor. This suggests the combined effects of warming and eutrophication (from aquaculture) could have fuelled the increase in cyanobacteria, particularly in the high disturbance lakes. Furthermore, traits such as high-affinity PO_4^{3-} uptake system activated at low P concentrations and the ability to store P (Cottingham *et al.*, 2015) allows certain cyanobacteria taxa to access P stored in lake sediment or trapped in the hypolimnion (that is not available to other algal groups) (Xie, 2006). In stratified tropical lakes, P builds up in the hypolimnion from internal P loading (Figure 5.1) even in lakes with low disturbance. These traits combined with warming and eutrophication could explain the increase in cyanobacterial taxa in the six lakes (Figure 6.6).

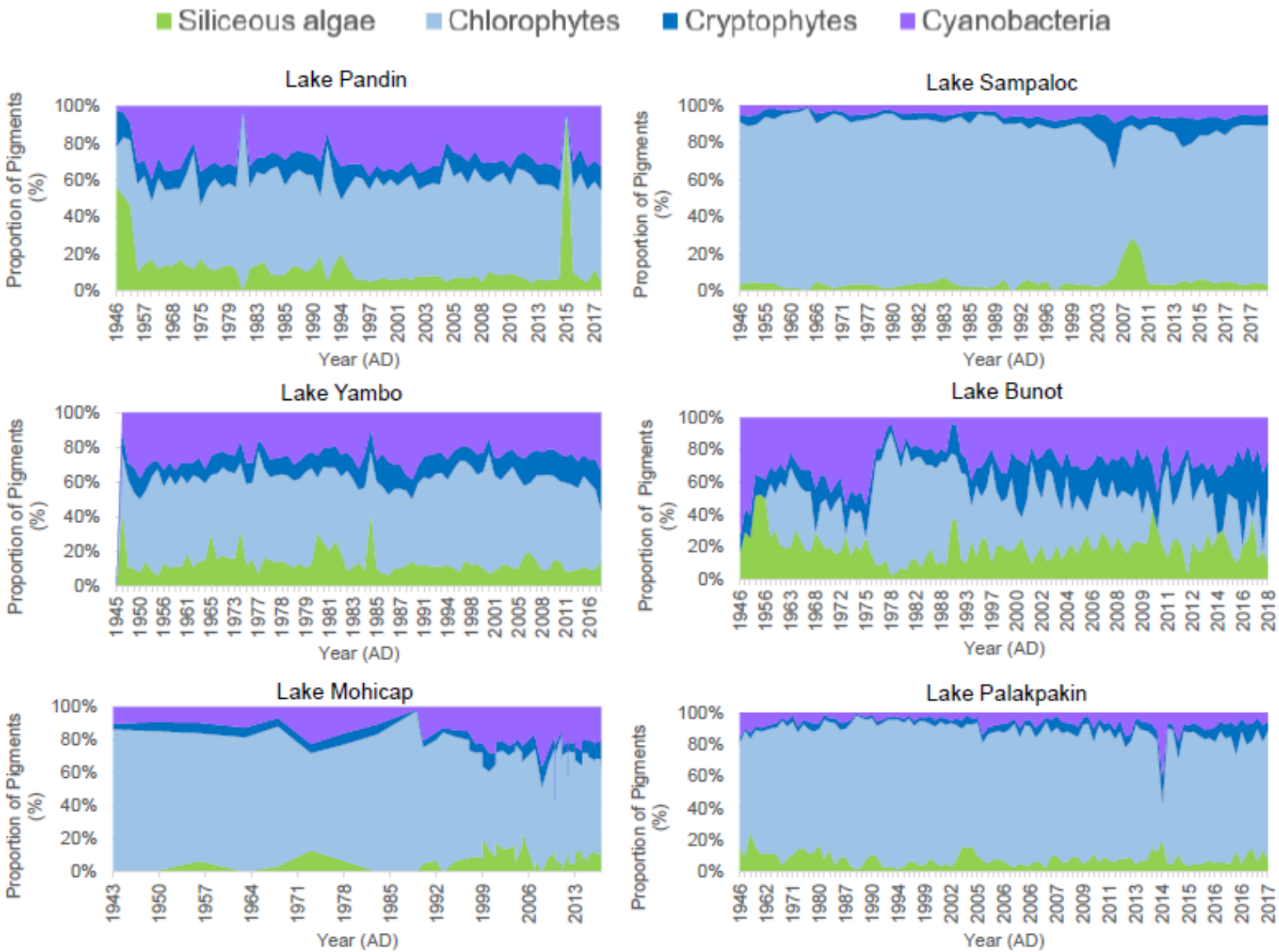


Figure 6.8: Changes in the proportion of preserved pigments indicated by the four different algal groups, siliceous algae (diatoxanthin), chlorophytes (lutein-zeaxanthin, pheophytin b), cryptophytes (alloxanthin) and cyanobacteria (canthaxanthin, echinenone, myxoxanthophyll, lutein-zeaxanthin) since 1945. As the pigments lutein and zeaxanthin coelute, 50% of the total concentration was used to calculate chlorophyte abundance and the other 50% was used to calculate the abundance of cyanobacteria

The different proportion of algal groups in the sediment records of the individual lakes could be the result of the addition of different forms of N, from a variety of anthropogenic activities. Since N limitation of phytoplankton is generally more common in tropical than temperate lakes, lake responses to changes in N concentrations are to be expected (Lewis Jr., 2002). The source and intensity of N inputs (which is greater in the high disturbance lakes from aquaculture and urbanisation) apparently resulted in a higher abundance of chlorophytes and cyanobacteria (at the expense of siliceous algae and cryptophytes) (Figure 6.8). The addition of differing forms of N can influence the algal community composition and favour non-N_2 fixing cyanobacteria. Previous research suggests that effects of N on algal productivity and composition may depend on the source and chemical composition of N added to lakes (Berman and Chava, 1999). Donald *et al.*, (2011) found that the magnitude of N effects on P-rich lakes varies with the chemical form of N, which changes between different types of human pollution. For example, N from urban wastewaters is composed of mainly NH_4 (Savage *et al.*, 2004). Therefore, under these conditions, urban pollution of P-rich lakes should increase phytoplankton production by selectively stimulating growth of non-N_2 fixing cyanobacteria and chlorophytes (lutein-zeaxanthin, canthaxanthin) to the detriment of N_2 fixing taxa (myxoxanthophyll). This was observed in the Qu'Appelle catchment, where non–N_2 fixing taxa increased 500% as a result of NH_4 influx from the City of Regina (Leavitt and Hodgson, 2006). NO_3^- from diffuse nutrient sources can also cause an increase in potentially toxic cyanobacteria although this would be to a lesser extent than an influx of NH_4 (Berman and Chava, 1999; Bunting *et al.*, 2007). In aquaculture, NH_4 and urea are the two major forms of N that are produced and excreted to the surrounding environment (Zimmer et al., 2017). Urea stimulates production of toxic cyanobacteria (Finlay *et al.*, 2010). Donald *et al.*, (2011) further demonstrated that N pollution escalates eutrophic conditions and algal toxicity in lakes with elevated concentrations of SRP and low N:P, and that the magnitude of these effects depends on the chemical form, and the source of N. It could be hypothesised high levels of aquaculture and urbanisation (consisting of mainly NH_4 and urea) magnified eutrophication and HABs in the high disturbance lakes.

6.5. Approach 2 - Correlating multiple drivers and algal responses

6.5.1. Variance Partitioning – Quantifying the impact of drivers on algal communities

Land use changes (and its combined interactions) explained the highest significant variance (19.5 to 24.8%, p=<0.005; Figure 6.9) in Lakes Pandin. Yambo and Sampaloc. In Lakes Bunot and Palakpakin, land use changes did not explain any significant variance. Aquaculture only explained marginally significant variance in Lake Sampaloc (13.0%, p=0.008). These results were consistent with past studies on the Seven Lakes and other tropical lakes, which show catchment disturbances and aquaculture are linked to enhanced P delivery, accelerated eutrophication, increase in algal abundance and a deterioration in water quality (Cordero and Baldia, 2015; Bannister *et al.*, 2019). Annual mean temperature, precipitation, and ENSO did not explain any significant variance in algal communities in any lake. The unexplained variance ranged from 34.5 (Lake Bunot) to 63.4% (Lake Yambo; Table 6.2) suggesting some explanatory variables were missed in this analysis (Borcard *et al.,* 1992). Furthermore, the absence of significant explanatory variables in Lake Mohicap indicates that short term fluctuations in algal community composition were regulated by factors not included in this analysis (e.g. macrophyte coverage). It could be inferred that the low sample size, shorter time series (post-1960s, n=12) and chronological offsets resulted a less robust analysis, explaining the lack of significance. Furthermore, the validity of the analysis is constrained by the choice of explanatory variables for each lake and a lack of catchment specific data for land use changes (population, housing numbers and coconut). This means trends are generalised and may not accurately reflect actual change in individual lake catchments. The variance partitioning results should be therefore, be interpreted with caution and only be used as an indication of possible causal links.

Table 6.1: Variance partitioning results for the six lakes showing the percentage of variance explained by land use, aquaculture, climate and a combination of first (Land Use –Aquaculture, Land Use – Climate and Aquaculture – Climate), second order interactions (Land Use – Aquaculture – Climate) and unexplained variance
** significant p<0.005, ** marginally significant P<0.01*
**** these fraction are negative which indicates that two variables together explain the variance in fossil pigments better than the sum of the individual effects of these variables. As no partial regression can be computed for the negative interactions, it cannot be tested for significance (so lacks a p value).*

Component	Lake Pandin		Lake Yambo		Lake Mohicap		Lake Sampaloc		Lake Bunot		Lake Palakpakin	
	% variance explained	P value	% variance explained	P value	% variance explained	P value	% variance explained	P value	% variance explained	P value	% variance explained	P value
Total	61.5	0.002*	36.6	0.014	None of the variables were significant so variance partitioning was not completed on this lake		56.8	0.002*	65.5	0.002*	50.3	0.008**
Land Use	19.9	0.005*	19.5	0.005*			24.8	0.002*	6.7	0.068	17.6	0.18
Aquaculture	2.0	0.254	14.4	0.026			13.0	0.008**	6.9	0.034	9.8	0.062
Climate	-1.4***		1.4	0.364			0.7	0.46	-2.3***		12.4	0.154
Land Use - Aquaculture	10.9	0.002*	-8.9***				-5.2***		4.6	0.016	-5.1***	
Land Use - Climate	-0.4***		4.9	0.014			0.3	0.006**	0.3	0.014	-1.5***	
Aquaculture - Climate	3.2	0.264	11.6	0.086			8.2	0.126	4.7	0.204	14.5	0.126
Land Use - Aquaculture - Climate	26.7		-16.4***				15.0		44.5		2.7	
Unexplained	38.5		63.4				43.2		34.5		47.7	

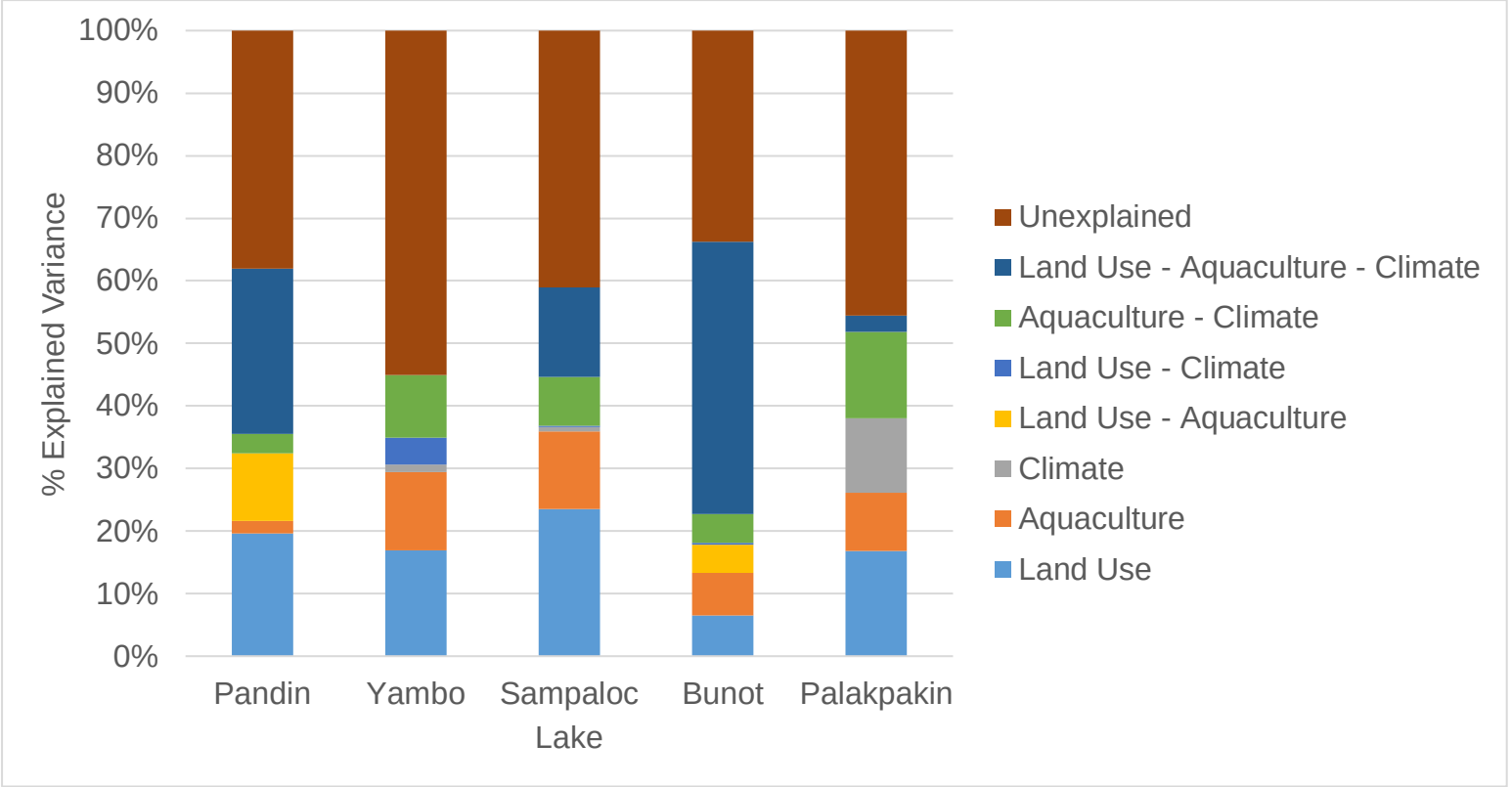


Figure 6.9: Stacked bar chart showing the percentage of variance explained by land use, aquaculture, climate and a combination of first (Land Use – Aquaculture, Land Use – Climate and Aquaculture – Climate), second order interactions (Land Use – Aquaculture – Climate) and unexplained variance in the six lakes. The results from Lake Mohicap were not included due to the lack of significant explanatory variables

6.5.2. Do lake physical characteristics influence sedimentary pigment deposition and the degree of pigment assemblage change?

Two separate variables were used (Mann-Kendall and mean β carotene) to see if the lake's physical characteristics were associated with change in algal community and/or algal biomass. Mann-Kendall coefficients of PCA axis 1 scores (which provides a single variable for algal community change for each lake) were used to determine if any of the five physical variables were responsible for the change in algal community composition. In comparison, the mean β carotene (which provides a single variable for algal biomass for each lake), was used to determine if any of the five physical variables influenced algal biomass. For example, this would help determine if lake depth influenced preservation conditions.

In terms of community compositional change, linear regression revealed no significant correlation between the degree of algal community change (Mann-Kendall coefficients of PCA axis 1 scores) and the five physical variables (Table 6.2, Figure 6.11). Furthermore, none of the physical variables showed a significant correlation with algal biomass (Figure 6.10).

Table 6.2: Linear regression models of mean β carotene concentrations and Mann Kendall Coefficients for the six lakes against the five physical proxy variables with adjusted R^2 and p values shown.

Linear Regression Model	Adjusted R^2 Value	P value
β carotene ~Elevation	-0.22	0.20
β carotene ~Depth	-0.14	0.57
β carotene ~Surface Area	0.68	0.03
β carotene ~Catchment Area	-0.16	0.60
β carotene ~CA:LA	0.23	0.81
Mann Kendall coefficient ~Elevation	-0.19	0.22
Mann Kendall coefficient ~Depth	-0.12	0.53
Mann Kendall coefficient ~Surface Area	0.06	0.45
Mann Kendall coefficient ~Catchment Area	0.09	0.48
Mann Kendall coefficient ~CA:LA	0.16	0.60

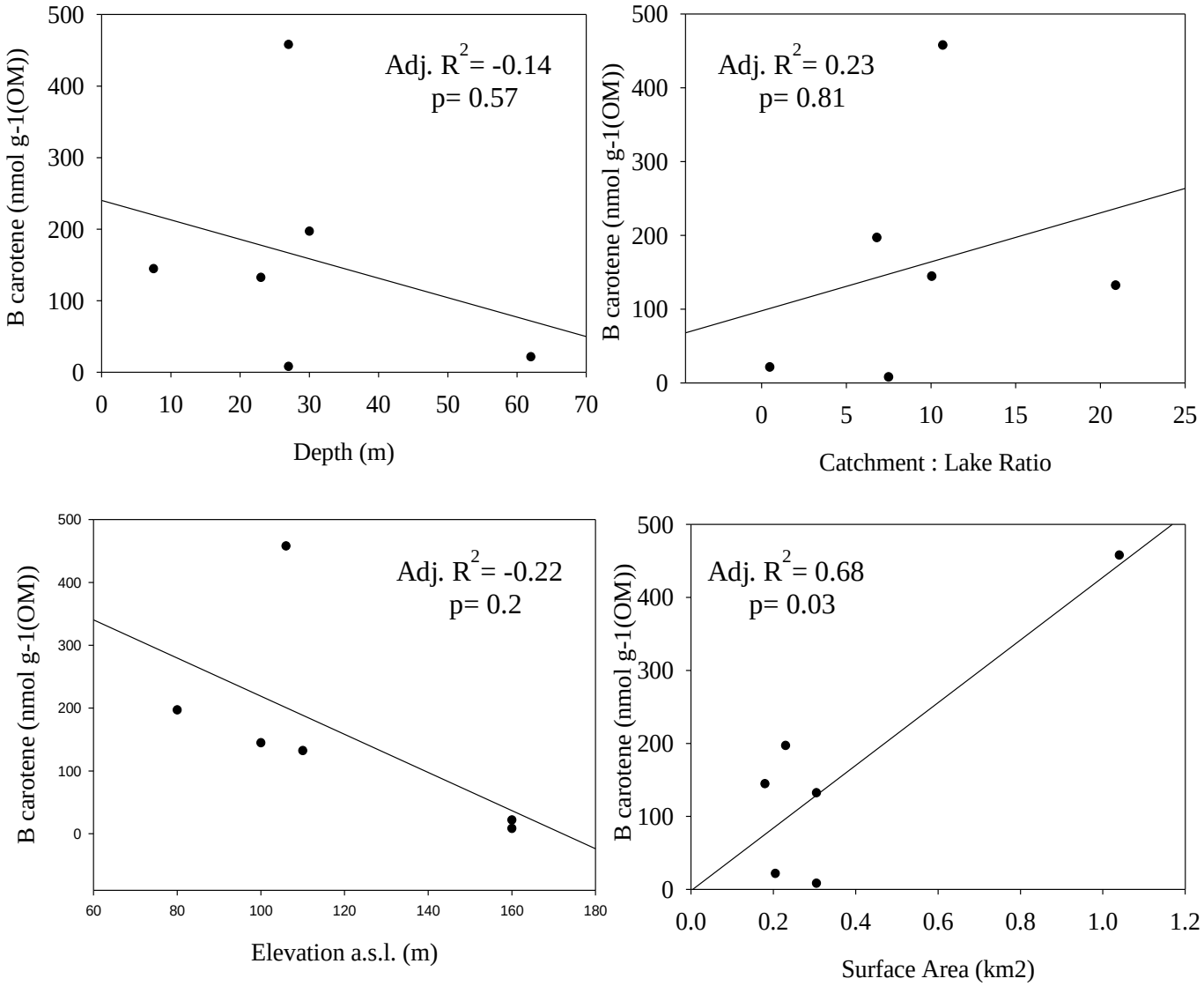


Figure 6.10: Linear regression showing the relationship between mean β carotene concentrations (calculated from individual sediment core records) and four physical variables (depth, catchment: lake ratio, elevation and surface area)

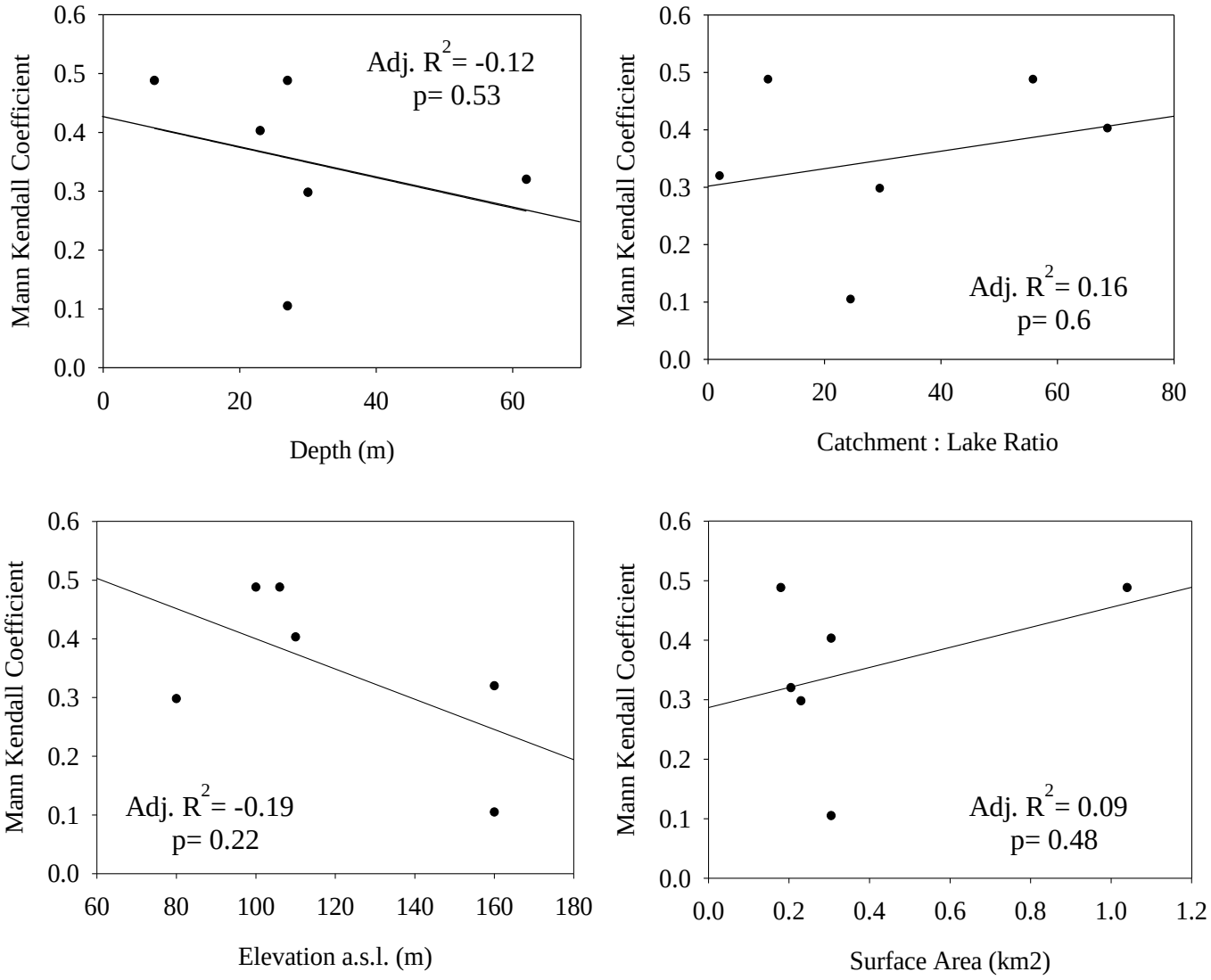


Figure 6.11: Linear regression showing the relationship between mean β carotene concentrations (calculated from individual sediment core records) and four physical variables (depth, catchment: lake ratio, elevation and surface area)

6.6. Does climate explain pre-aquaculture change?

Surprisingly, annual mean temperature and precipitation did not explain any significant variance in algal communities. It would be expected that the low disturbance lakes have the same amount of variance explained by climate as the other lakes but that it would constitute a larger proportion of the total variance explained. This is because fewer anthropogenic factors were influencing algal communities in Lakes Yambo and Pandin. Bannister *et al.* (2019) suggested that climatic warming had exerted a strong influence on algal communities at Lakes Sampaloc, Mohicap and Yambo, since the 1980s because of enhanced stratification and anoxia. Warming enhanced stratification leads to longer periods of hypolimnetic anoxia (as determined by monthly DO measurements) and the appearance/increase in okenone (Foley *et al*., 2012). Limnological data (section 4.6.2), the decline in $\delta^{13}C$ values and increase in okenone concentrations in the low aquaculture disturbance lakes provided evidence that warming has led to increased stratification and enhanced anoxia. Additionally, these anoxic conditions could have led to the release of P and NH_4 from the sediments, which enhanced eutrophication and promoted algal growth (as shown by high concentrations of cyanobacterial pigments), increasing the potential for enhanced pigment preservation. Furthermore, Bannister *et al.*, (2019) determined the changes in diatom communities (the co-occurrence of *Aulacoseira granulata*, *Cyclotella meneghiniana* and *Discostella* spp.) at Lakes Yambo, Mohicap and Sampaloc reflected enhanced eutrophication superimposed on the effects of climatic warming. It could be hypothesised that climatic change in the late 20th century accentuated the symptoms of eutrophication, particularly in the high disturbance lakes, making algal communities more receptive to nutrient loading.

Interestingly, ENSO did not explain any significant variance in algal communities (Figure 6.9). However, this analysis was only completed on post 1960 samples, so it is possible that ENSO was a driver of pre-aquaculture change in Lakes Bunot and Yambo. Figure 6.2 suggests PCA axis 2 scores correspond to changes in the concentration of okenone. Stuecker *et al.,* (2018) determined that ENSO is the dominant climatic influence on inter annual timescales and has a pronounced effect on rainfall and temperature variability in the Philippines. Lyon and Camargo (2009) determined that precipitation (from typhoons and storm events) was significantly higher during El Niño compared to La Niña events. El Niño events enhance nutrient run-off into lakes and when combined with long water residence times provide ideal conditions for blooms of purple sulphur bacteria (Phlips *et al.,* 2020). In Lake Yambo, the lack of an in/outflow results in considerable seasonal variation in the lake's surface area compared to the other lakes (Table

2.4), so the associated influence ENSO has on temperature and precipitation could be a contributing factor (Okonkwo *et al.*, 2014). However, since Lake Yambo has a CA:LA ratio of 24.5, this suggests catchment size is not limiting the volume of rain inwashed into the lake. Lake Bunot has the largest CA:LA ratio of 68.6 which means it has a large catchment in relation to the lake's surface area and is, therefore, more susceptible to changes in flushing rates and precipitation. Lake catchments with gentle relief receive proportionally greater amounts of OM, whereas steep catchments are supplied with a greater proportion of mineralogical material (Hall and Smol, 1993). The nutrient run-off from the large catchment could have fuelled nutrient enrichment in Lake Bunot and aided the formation of dead zones in the water column (Figure 5.36).

6.7. Do lake physical characteristics modify their response to aquaculture?

In terms of community compositional change, there was no significant relationship between algal community change (Mann-Kendall coefficients of PCA axis 1 scores) and the five physical variables (Table 6.2, Figure 6.10). The relationship between algal community change/algal biomass and the physical variables appeared to be driven predominantly by the outlier sample from Lake Sampaloc, which could explain the lack of significant relationships (except for surface area and algal biomass; Table 6.2; Figure 6.11). The lack of a significant relationship could also be explained by a lack of samples (e.g. lakes; n=6), resulting in the analysis not being sufficiently powerful to detect a significant relationship between the physical variables and algal community change (Nayak, 2010). Spatial differences could have arisen from variations in the level of nutrient input from within-lake, catchment activities and/or other morphological features (Tammelin and Kauppila, 2018). The results suggest the modifications of past algal community change were not systematically driven by the physical characteristics of the study lakes. Despite this, it is important to consider the individual morphological features of each lake when interpreting change in the proxy record (see Chapter 5). Lake Palakpakin demonstrated a distinctive response compared to the other lakes (Figure 6.3; Figure 6.5; Figure 6.7) due to its shallow depth and lack of anoxia, providing clear evidence that lake morphometry was dictating its response to change.

6.8. Do inputs of fish food lead to changes in the stable isotopic record?

The lack of a proportional relationship between pre and post aquaculture $\delta^{15}N$ values and the level of aquaculture disturbance, suggested aquaculture is not driving the change in this record (Figure 6.4; Figure 6.12). Since starter fish food has a very high $\delta^{15}N$ value of +33‰, it would be expected that lakes with higher levels of aquaculture would have shown a greater proportion

increase in $\delta^{15}N$ values pre and post aquaculture. Other studies have shown fish food used in aquaculture exhibits a distinct isotopic signature, relative to local resources and biota (Grey, 2006), which allows for the identification of relative contributions from natural and fish food sources in shrimp and mussel farming (Yokoyama *et al.*, 2006). This should result in a distinct aquaculture signal in the sediment record of the study lakes. If fish food was driving the change there would be a positive relationship between $\delta^{15}N$ values and trophic state (Botrel *et al.*, 2014), but this is not the case. The lack of a proportional relationship could be due to other factors. In the case of Lake Sampaloc, its location in the centre of the city and the resultant high input of sewage ($\delta^{15}N$ values of +5 to +25‰; Meyers and Lallier-Vergès, 1999) into the lake, could explain why it has the highest $\delta^{15}N$ values yet is ranked third in the aquaculture gradient. Lake Bunot has a higher aquaculture disturbance than Lake Sampaloc but showed a lower proportional increase $\delta^{15}N$ values (Figure 6.12). Lake Bunot is considered

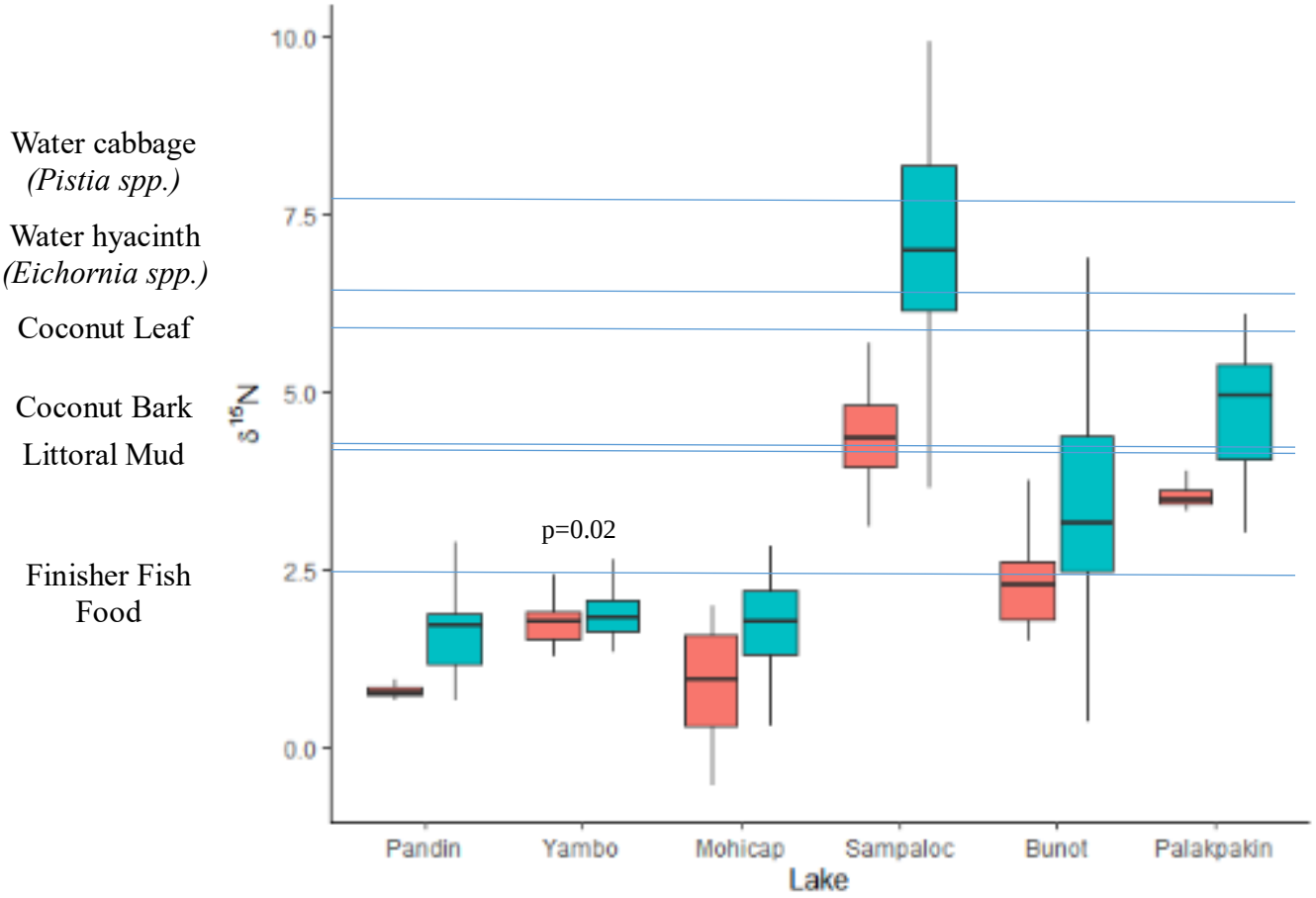


Figure 6.12: Boxplot showing $\delta^{15}N_{org}$ values pre- and post- aquaculture for the six lakes. There was significant difference between the two groups of $p<0.05$ unless stated otherwise. Lakes are presented in order of disturbance from lowest (left) to highest (right). The mean $\delta^{15}N_{or}$ values of the end members are indicated. The end member for starter fish food is not shown as the mean value (+33.9‰) is out of range of the y axis

hypereutrophic (Table 4.8) and under these conditions, N became limiting (Table 4.6), resulting in the rise of N_2 fixers (with $\delta^{15}N$ values of +1 to +2‰), leading to a reduction in $\delta^{15}N$ values (Gu, 2009). The lack of myxoxanthophyll in contemporary water samples in Lake Bunot, suggests the decline in $\delta^{15}N$ values was not due to N_2 fixation. However, Lake Bunot also has the shallowest anoxic layer (as discussed above and in Chapter 5) and would undergo extensive denitrification which would cause isotopically light $\delta^{15}N$ values (Ekberg *et al.*, 2004; Yokoyama *et al.*, 2006). Therefore, pronounced anoxia could have made microbial activity the dominant driver on N cycling in Lake Bunot.

There was a decline in C/N ratios along the aquaculture disturbance gradient post aquaculture, most likely driven by the low C/N values in the fish food end members (8.0 and 9.7; Table 5.1), yet this does not seem to be the case for the $\delta^{13}C_{org}$ records (Figure 6.4). If aquaculture was the cause, the high $\delta^{13}C$ values of the starter and finisher fish food (of –26.2 and –26.5‰ respectively; Table 5.1) would cause an increase in $\delta^{13}C$ values, but the opposite occurs. The differing timings and lack of change in Lake Palakpakin (Figure 6.13) suggests anoxia is most likely the cause of the decline. Low disturbance lakes typically have higher C/N ratios from reduced autochthonous production and greater in-wash of terrestrial OM (Figure 6.13; Meyers and Teranes, 2001), which could explain the higher C/N ratios observed in Lake Yambo and Pandin. Gondwe *et al.*, (2012) determined the incorporation of fish cage waste into the food webs acts as a food source for algae in lakes. The enhanced use of fish food would have stimulated autochthonous (algae) production particularly in the high disturbance lakes, lowering C/N ratios (Figure 6.13). Furthermore, in Lake Mohicap, a switch in the type of food used (see section 5.8.7) could have caused the spike in C/N ratios in the 2000s. However, only Lake Sampaloc had a significant change in C/N ratio following the introduction of aquaculture (Figure 6.3) providing some evidence aquaculture is driving the change in C/N ratios.

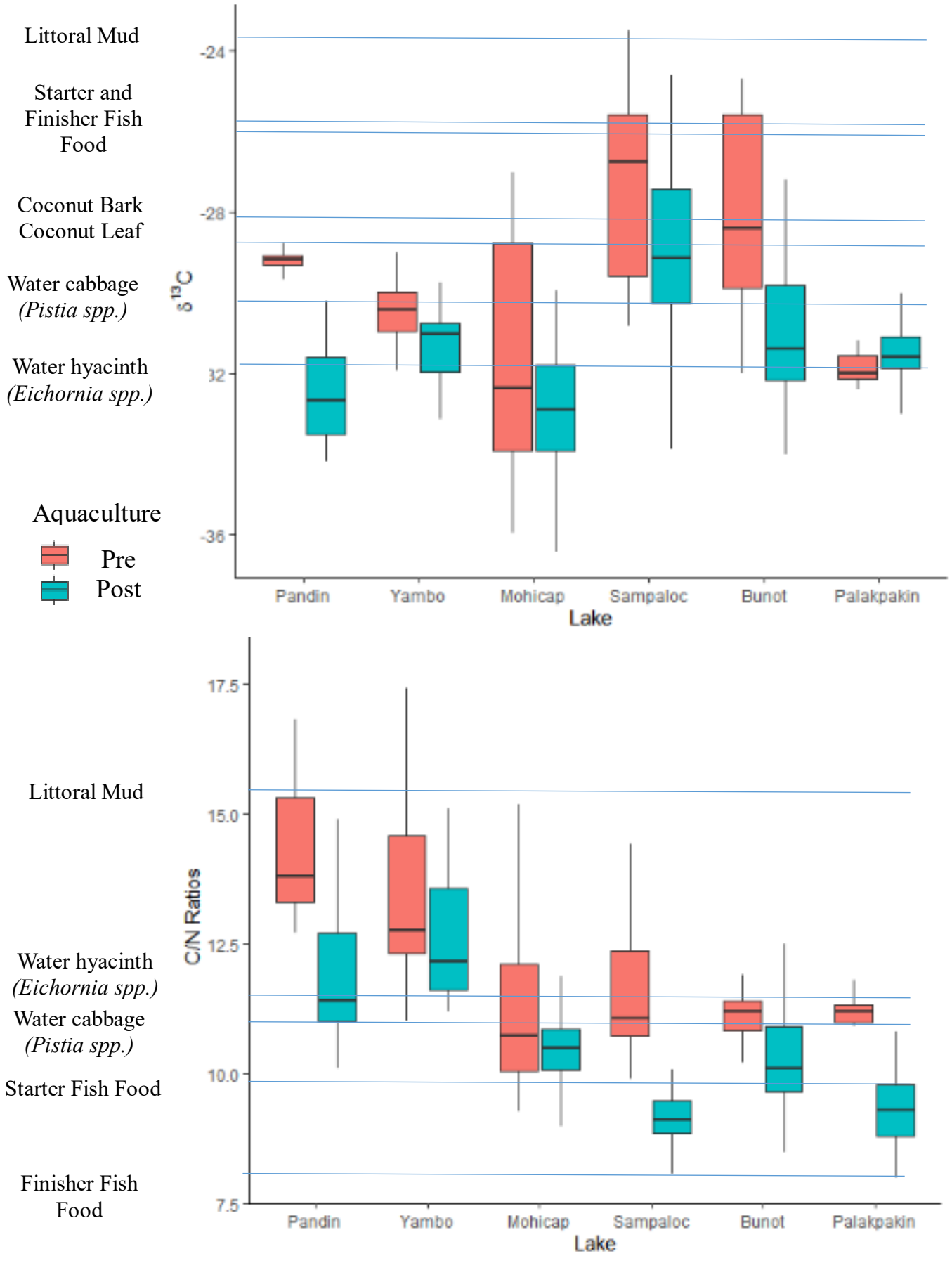


Figure 6.13: Boxplot showing top) $\delta^{13}C_{org}$ values and bottom) C/N ratios pre- and post- aquaculture for the six lakes. There was significant difference between the two groups of $p<0.05$ unless stated otherwise. Lakes are shown in order of aquaculture disturbance from lowest (left) to highest (right). The mean $\delta^{13}C_{org}$ and C/N values of the end members are indicated. The end members, coconut bark (93.5‰) and leaf (23.6‰) are not shown on the top plot as the mean values are out of the range of the axis

6.9. To what extent is aquaculture responsible for changes in the proxy record?

The % of variance explained in algal communities by aquaculture does not rise with increased aquaculture intensity. Surprisingly, aquaculture only caused marginal significant change in Lake Sampaloc (13.0%, p=0.008) despite this lake not having the highest level of aquaculture. In contrast, in the high disturbance lake (Palakpakin), aquaculture was not significant and explained a lower amount of variance (9.5%, p=0.062). The lack of significant change as a result of aquaculture could be due to chronological offset and dating inaccuracies (especially in Lakes Yambo and Mohicap). This inconsistency could also be partly explained by the fact that all variables (e.g. aquaculture, land use and climate) changed concordantly post 1980, making the results statistically indistinguishable. This inconsistency between changes in aquaculture density and algal community change was most likely due to continuous nutrient loading from an expanding human population combined with more prevalent anoxia masking the impacts of aquaculture (Folke *et al.*, 2004; OECD, 2012). Pronounced anoxia would lead to internal P loading from the sediments (Orihe *et al.*, 2017) further aggravating eutrophic conditions. The higher proportion of variance attributed to factor interactions rather than direct effects suggests the effects of the aquaculture were mediated by the other variables especially in Lake Bunot, but this was also found in the other lakes to a lesser extent.

Land use explained the highest % of significant variance in a majority of the lakes, suggesting the gradual encroachment of the urban centre had the greatest influence on algal communities (Figure 6.9). The high value recorded for Lake Sampaloc (24.8%, p=0.002) was expected as it is located in the centre of San Pablo City. In comparison, Lake Pandin (19.9%, p=0.005) is remote (located the furthest from the urban centre), with a limited number of settlers and structures within its catchment. However, the ecotourism industry in Lake Pandin has expanded substantially, with >12,000 visitors in 2012 (Brillo, 2017) and the resulting input of waste into the lake could be a possible reason for the high % variance explained by land use. The high value of unexplained variance in the low disturbance lake provides further evidence that these lakes have a low threshold for disturbance effects (Bannister *et al.*, 2019). In Lake Sampaloc, the expansion of the human population, replacement of native forest and the conversion of San Pablo into a 'first class' city in 1950 (see Chapter 5) coincided with significant temporal change in algal communities (Figure 6.5; Figure 6.7). However, the other lakes do not show a significant change in 1950 (Figure 6.3; Figure 6.5; Figure 6.7). The high % variance suggests that the continuous additive nutrient sources are driving change in algal communities. Urbanisation and the resulting deforestation would lead to a run-off of allochthonous OM

(suggested by the increase in C/N ratios and a decline in $\delta^{13}C$; Costa-Böddeker *et al.*, 2012) fuelling algal productivity (indicated by an increase in β carotene, chlorophyll *a*, cyanobacterial pigments and okenone) (Figure 6.5; Figure 6.7). These results are consistent with previous studies on the Seven Lakes and other tropical lakes which show urbanisation enhanced the influx of nutrients fuelling eutrophication and increases in algal abundances (Wu *et al.*, 2006; Townsend-Small *et al.*, 2007; Costa-Böddeker *et al.*, 2012; Bannister *et al.*, 2019).

6.10. What is the role of aquaculture?

Both approaches showed no proportional relationship between change in the proxy record and level of aquaculture disturbance, with the changes along the aquaculture gradient driven by increased eutrophication and enhanced anoxia. The variance partitioning concluded there was no relationship between the % variance explained by aquaculture and level of aquaculture disturbance. There is evidence that aquaculture exacerbated pre-existing change, especially in lakes higher up the aquaculture disturbance gradient (which also equated to greater anthropogenic disturbance in general). Generally, the high disturbance lakes showed greater change in the proxy record compared to the low disturbance lakes (Figure 6.4; Figure 6.6). Land use changes explained the greatest % of significant variance in a majority of the lakes, suggesting it is a more dominant driver than aquaculture (Table 6.1). This is supported by the significant temporal change, recorded post aquaculture, particularly in the high disturbance lakes (Figures 6.1). This suggests nutrient enrichment (particularly the type of N in fish food) combined with enhanced anoxia magnified HABs, eutrophication and changes in N cycling. There is some indication that aquaculture is one of the contributing factors towards nutrient enrichment but not the sole driver.

Both approaches produced similar results suggesting that aquaculture is not the dominant driver of change but is exacerbating it. This highlights the usefulness of a dual approach, in meeting the aims of this thesis, to quantitatively assess the ecological response of the study lakes to aquaculture. Sediment cores are useful in determining long term change, yet rely on robust chronologies in order to match timings of change in algal communities with those in the historical records. Using tie in dates from previously dated cores to determine chronology could have introduced inconsistencies. In addition, although this thesis used the best available historical data, it may not be truly representative of the environmental change in the lake catchments, or be mismatched with the spatial and temporal scales of this study. For instance, the land use records were at a city or province level rather than at lake catchment scale and the climatic data consisted of a collection of weighted records from around the Philippines. The

lack of high resolution and/or annual records of an appropriate length made it difficult to quantify the relative importance and impact of each factor (population, coconut etc.) on the pigment record. These limitations were mitigated by the use of a multiple lake study. Single sediment cores may be representative of an individual lake system but make it difficult to determine if palaeolimnological changes present regional or local impacts. Using a multi-lake approach, allows the impact of regional changes (climate) and/or local drivers (aquaculture) to be determined, whilst taking into account the influence of lake morphology (Mills *et al.*, 2017).

The continuous pressure of climatic warming combined with the encroachment of San Pablo City, could explain why each lake is responding differently to aquaculture (supported by the high % variance explained by the factor interactions). In the case of the study lakes, there was evidence that warming drove pre-aquaculture change (see Chapter 5). In addition, urbanisation (since 1950) and the introduction of aquaculture post 1970, would have exerted greater pressure on already stressed systems (especially in the high disturbance lakes). Although evidence from both methodological approaches suggests the low disturbance lakes are still responding to the effects of urbanisation and aquaculture (Table 6.1). This attests to the sensitivity of deep, warm lakes to disturbance (Bannister *et al.*, 2019). The distinct response to aquaculture by each lake, could be due to the impact of multiple stressors on the study lakes. Research has shown that climate and land-use change drive a suite of stressors that shape ecosystems and interact to yield complex ecological response (Birk *et al.*, 2020). Recent large-scale analyses have shown that freshwater ecosystems are exhibiting novel ecological responses to different stressors (Richardson *et al.*, 2018). Furthermore, a lake's response to multiple stressors varies greatly with lake type (level of stratification, physical characteristics; Richardson *et al.*, 2018). Evidence from the study lakes showed lake's physical characteristics influenced pigment preservation, the timings of response and the impact of aquaculture. A combination of these factors could explain the lack of a unified regional response to aquaculture.

7. CHAPTER 7 – CONCLUSION

The limnological and palaeolimnological records show distinctive physical, biological and chemical changes along the aquaculture disturbance gradient. Although all lakes demonstrated an individual response to aquaculture and other external drivers (land use changes, climate), in terms of timing, direction and speed of change in the proxy record, there are some similarities. These include a change in algal communities with an increase in pigment concentrations (including HABs; Figure 6.5) and $\delta^{15}N$ values, and a decline in $\delta^{13}C$ and C/N ratios; yet these changes were not proportional to the aquaculture disturbance gradient (Figure 6.4; Figure 6.6). The timing of the change occurred earlier in the high disturbance lakes suggesting nutrient loading from anthropogenic activities (including aquaculture) lead to more pronounced anoxic conditions (highlighted by the proportional shallowing of the anoxic layer). Lake responses show a limited regional coherence to the same drivers, highlighting the role played by differing lake morphologies in moderating the response to aquaculture.

The contemporary limnology of the Seven Lakes of San Pablo helps understand the ecosystem functioning of these under-studied tropical lake systems. The significant difference between lakes with high (Palakpakin, Bunot, Sampaloc and Mohicap) and those with low aquaculture (Yambo and Pandin) indicates the combined impact that aquaculture and other drivers had on anoxia, nutrient cycling, ion composition and algal communities. The hypolimnion of all the deep lakes were anoxic, with the depth of the anoxic layer and DCM shallowing along the aquaculture disturbance gradient. The shift in algal communities, from chlorophytes to siliceous algae and cyanobacteria (Figure 4.11; Figure 6.8), provides evidence of a major restructuring of algal communities due to enhanced nutrient loading from fish food and excretion. The use of fish food could be a contributing factor towards the shifts in the limiting nutrient from P to N, since 2006, in Lake Mohicap, Yambo and Pandin, with algal productivity in all lakes becoming N limited by 2017. Enhanced anoxia is predicted to worsen due to the continued impact of climatic warming (predicted to increase by 2.5 to 4.1°C by 2100; Cinco *et al.,* 2018) and the pressure of an ever-expanding population (Jean-Philippe *et al.*, 2015).

Only Lake Bunot had significant temporal change in algal communities at the introduction of aquaculture. In the high disturbance lakes, pigment concentrations were generally higher and showed a greater deviation from the pre-aquaculture mean in comparison to low disturbance lakes (Figure 5). This suggests a combination of more pronounced anoxia and nutrient loading from aquaculture enhanced preservation potential and increased algal biomass. The limnological and palaeolimnological data provides evidence that pigment concentrations do

not increase proportionally with eutrophication, with Lake Bunot demonstrating a reduction of algal biomass due to self-shading of algal communities in the surface waters. Even though the shallow Lake Palakpakin has the highest aquaculture density, it does not show the greatest proportional response in the proxy record; its response to aquaculture tempered by its hydro-morphology.

Many of the lakes show a decline in $\delta^{13}C$ values at some point, consistent with the influence of anoxia. The decline in $\delta^{13}C$ values was initially caused by pronounced stratification induced by warming and further enhanced by nutrient loading (from anthropogenic sources), especially in the high disturbance lakes. These changes also coincided with an increase in pigment concentrations from enhanced preservation conditions in the water column and the appearance/increase in okenone. The lack of proportional change in $\delta^{15}N$ values with respect to aquaculture (Figure 6.4) demonstrated not just the influence of anoxia (stimulating denitrification) on N cycling but also the relative input of anthropogenic N from sewage and fish food (which has isotopically heavy $\delta^{15}N$ values). Anthropogenic N loading could also explain the different proportions of algal groups among the study lakes. NH_4 and urea produced by high levels of aquaculture and urbanisation could be a cause of the proliferation of non-N_2 fixing cyanobacterial pigments (canthaxanthin, lutein-zeaxanthin) and chlorophytes at the expense of myxoxanthophyll (N_2 fixing cyanobacteria), siliceous algae and cryptophytes in the proxy record.

The variance partitioning showed that aquaculture is not the dominant driver of change in the Seven Lakes of San Pablo. There was a lack of a proportional increase in the % variance explained by aquaculture along the gradient. Evidence suggests that urbanisation is the dominant driver of change in algal communities, with aquaculture aggravating pre-existing change in the palaeolimnological records. This is supported by the lack of a relationship between the fish food end members and the proportion of change between the pre and post aquaculture means in the isotopic records (Figure 6.4). The continuous additive sources from urbanisation and the resulting deforestation would lead to a run-off of allochthonous OM (supported by an increase in C/N ratios and a decline in $\delta^{13}C$) fuelling algal productivity (evidenced by an increase in cyanobacterial pigments, okenone, β carotene and chlorophyll *a*) (Figure 6.4; Figure 6.6). The more prevalent anoxic conditions would further intensify eutrophic conditions due to the internal loading of P. The effects of multiple stressors on these tropical lake systems could have altered individual lake response to aquaculture. This research supports previous work highlighting that lakes are exhibiting novel ecological responses due

to the interaction of multiple drivers (Richardson *et al.*, 2018; Birk *et al.*, 2020). Even though there was no significant relationship between the physical characteristics and algal biomass/community change, evidence from the study lakes suggests it played an important role in pigment preservation.

The research presented in this thesis fills a gap in understanding the contemporary functioning and ecosystem change of under-studied tropical lake systems. It has attempted to quantify the impacts of individual drivers on algal communities. Further investigation into land use changes (the dominant driver of change in algal communities) and particularly, the collection of more robust records would aid understanding of how the delivery of allochthonous material, altered geochemical cycles and influenced algal productivity in the Seven Lakes of San Pablo. The impact of atmospheric N deposition is relatively unknown in tropical lake systems, therefore the use of compound-specific isotope analyses of $\delta^{15}N$ would help determine the direct fertilisation effects of atmospheric N deposition (Enders *et al.*, 2008). Continued monitoring of these threatened lake systems is vital to understand how the Seven Lakes of San Pablo are responding to sustained environmental pressures to allow for tailoring of lake specific management strategies.

8. REFERENCES

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APPENDICES

APPENDIX A – INTERVIEW TRANSCRIPTS

LAKE PANDIN

Collected in April 2018

Interview 1

- No algal blooms in the last 5 years but annual before that.
- There are coconut plantations in the lake catchment but the fisherman are not sure when they were first planted
- <3% of the lake used for aquaculture in the late 2000s
- Only 3 fish cages present in 2018. The reason for this is due to tourism being the more profitable venture. Aquaculture was having a negative impact on the condition of the lake.
- Aquaculture began in 1989 but cage numbers were always kept low due to unfavourable conditions
- Tourism is the preferred industry

LAKE YAMBO

Collected in April 2018

Interview 1

- Aquaculture cages spread rapidly (reaching c.8-10% of surface area in the mid-1990s) after its introduction, however, it was financially less viable than the other Seven Lakes, due to the lake conditions
- Algal blooms have occurred every year since 1980s (in January)
- No Banana but lots of coconut in the lake catchment
- Coconut plantations since 1956 which gradually increased until covering the whole catchment
- Only one fish cage now, reduced from 3 in 2017. They see tourism as the more profitable industry and aquaculture was having a detrimental effect on it.
- Raft pulled tourism started in 2018

Collected by Wayne Bannister in May 2017

Interview 2

- 3 active fish pens
- Fish pen = 6 m depth
- Fish pens are 10 x 20 square area
- 5000 fingerlings = 100 bags x 25 kg
- 600,000 fingerlings provided by BFAR.
- Fish kills occur during November – December, when the weather is colder. Water becomes white, but it happens intermittently.
- Algal blooms occur in January.
- 4 months of fish farming takes place every year.

- In February 4th 1999, the highest number of fish kills were reported with the highest number of fish cages present of >10% (past the LLDA 10% limit).
- Fishermen began fishing 30 years ago, in approximately 1987.
- In 2012, a fish kill event was reported but the fish were relatively unaffected.
- Aquaculture considered to be responsible for the loss of water clarity.
- The fish farmers do not use floater type feed unlike in Sampaloc.
- Tilapia farmed on the lake.
- Establishing new fish pens here is now prohibited.

Interview 3

- In 2004, fish kills were observed in Feburary.
- 600 m^2 currently allocated for fish pens.
- 3 active fish pens, which are considered expensive to maintain.
- LLDA introduced tilapia fingerlings about 20 years ago.
- Fish pens introduced 30 years ago.
- Changed from blue to green after introduction of fish pens.
- Floater type feeds are not used due to their expensive nature.
- Tourism takes place here: promoting Yambo as a place to eat; tour guide can take tourists over to Pandin.
- Tourism picked up about 2 years ago.

Interview 4

- Farming tilapia.
- December – January fish kills.
- Aquaculture was established in 1989

In December, lake annually turns white.

LAKE MOHICAP

Collected in April 2018

Interview 1

- Floating cages introduced in the 1990s - they were already here when the fishermen moved in
- Aquaculture cage number peaked in early 1990s when the fishermen moved in
- Algal blooms occur in January. Cage numbers slowly decline after this
- The last algal bloom occurred three years ago
- Aquaculture was first introduced in 1977
- The number of cages is decreasing by 10 a year
- They were coconut/banana plantations here long ago- only the coconut is still visible
- The fisherman was born in 1940

It seems that since I was last here in May 2017 the focus of the lake has switched to tourism, with the creation of a new set of stairs down to the lake and the building of tourism bamboo rafts (like those on Yambo and Pandin). The fishermen said aquaculture is having a negative effect on tourism so is being reduced.

Collected by Wayne Bannister in May 2017
Interview 2

- Fish pen 10 x 10 m^2, 10 x 15 m^2, 20 x 20 m^2 area.
- 1000 fingerlings = 10 bags x 25 kg
- 5000 fingerlings per fish cage
- Net 10 x 20 m^2 and can reach 10 m depths.
- Before aquaculture began, density of fish pens was not as much.
- Due to expensive feeds, fish pens decreased in number.
- 1 bag of feed = 700 Philippine Pesos.
- 6 months of farming takes place per year.
- Pens belong to the residents.
- Fish kills have not been reported for 4 years. No insurance on the pens.
- LLDA incurred tax on pens; 60 PhP per 10 x 10 m^2 area of fish pens.
- San Pablo is the market.
- In 1991, there was a big fish kill event. The locals think that because of the origin of the lake, the chemicals killed the fish.

Interview 3

- Currently 5-6 month harvest of fishes, previously 4 months.
- Fish kills take place in January during the cold season.
- Fish kills December – January.
- 9 operational fish cages. Many of the cages in the lake are not operational.
- Impacted by typhoon in 1991.
- Tilapia introduced to Mohicap.
- During the 1980s, water clarity said to reach 15 ft depth.
- Every lake experiences fish kills but they do not occur at the same time.

Floating pellets were suspended in the 1990s because they are more expensive. At Mohicap, they are using cheaper feed that sinks. Difference of 100 PhP, but the effects of the cheaper feed are more harmful. However, they switched back to floating fish food in the 2000s due its less harmful effects

LAKE SAMPALOC

Collected by Wayne Bannister in May 2017
Interview 1

- 10 x 10 m^2 pens: 5000 fingerlings x 50 bags
- 10 x 15 m^2 pens: 5000 fingerlings x 60 bags.
- 20 x 20m^2 pens: 7000 fingerlings x 70 bags
- Fish pens are 5 m deep.
- Illegal before >50% cleared pens.
- December 2010 big fish kill event.
- Feeding starts in February.
- LLDA enforce taxes.
- More fishes present before than now.

- In 1976 fish farming was established.
- Overfeeding takes place.
- Floater feeds have been used since 2010.

Interview 2

- Tilapia introduced in 1953.
- Extensive aquaculture occurring in 1960s.
- The local thinks fish feeding became a problem.
- Fish kills occurring annually; before there were more kills but the number has decreased.
- Algal blooms have not increased in frequency.

Interview 3

- Tilapia farmed in 1965.
- The lake was clear before 1965.
- A lot of fish kills took place in 1998 and 1999.
- Fish kills take place during the summer.
- Fish kills and algal blooms occurred every year but not recently.
- The lake was very clear in the 1960s.
- The locals predict algal blooms by the seasons. They predict blooms occur when it is summer.

Interview 4

- Algal blooms occur in November.
- Fish kills in December.
- In 2000, there was a big fish kill event.
- Aquaculture started in 1979.
- There were more fish cages in the past compared to the present.
- Lake Sampaloc is more polluted now than before.
- Aquaculture seen as the reason for water pollution.
- In 2005, there were a lot of fish kills.
- The locals believe the colder waters are the reason for fish kills.
- Aquaculture affects water quality.
- Fishing in open water was industry before intensive aquaculture.

Interview 5

- Water clear in 1975.
- During December, fish kills take place when water is colder.
- Fish kills and algal blooms are not frequent.
- There used to be a lot of restaurants along the shoreline.
- Tilapia and black bass have been farmed since 1985.
- In the 1960s aquaculture was not yet established.

Local community predict algal blooms by seasons

LAKE BUNOT

Collected in April 2018

Interview 1

- Big fish kills occur every year in January. But no fish kills in the last 5 years
- The number of fish cages changes every year. In the last year 10 fish cages have been removed.
- It is unknown when the first banana/coconut plantations appeared
- Cage numbers have increased rapidly since aquaculture was introduced in 1976. Reaching 4 hectares in 1980 and 8 hectares by the late 1980s
- Aquaculture reached its peak in the late 1990s of c. 40% of the lake's total surface area

Collected by Wayne Bannister in May 2017

Interview 2

- Lake Bunot was clear before 1970s.
- 10 x 10 m sq area fish pens
- 4000 – 5000 fingerlings: 50 bags x 25 kg.
- 10 x 10 m sq area: 5000 fingerlings
- 10 x 15 m sq: 5000 fingerlings.
- 20 x 20 m sq: 7000 fingerlings
- Used logs in the past instead of bamboo for fish pens.
- In 2010 fish pens were cleared. Approximately 50% of pens cleared.
- Fish farming takes place each year for 4 months; 5 harvestings in two years.
- 4.5 m deep fish pens.
- Fish kills not seen as a problem. Fish kills are higher in November and December.
- In 2012, there was a big fish kill event.
- Local admits that fish pens may be the reason for fish kills.
- Many fishes reach the shoreline before dying, so they are not being caught.
- Minimum 5 kg of fish kills per pen per year; maximum 10 kg of fish kills per pen per year.
- Lina algal blooms occurring every year during the summer.
- Algal blooms eaten by the fish.
- Algal blooms sometimes before November and December.
- Algal blooms seen as positive, because it's a source of food for the fish.
- Lina blooms deplete the oxygen.

Interview 3

- During the 1980s, water was clear.
- Aquaculture started in the 1970s.
- Water lilies growing on the lake.
- Fish kills occur every year between November and February.
- Annual algal blooms.
- Tilapia bred in the lake.
- Fish are harvested two times per year for 6 months.
- When aquaculture began, population in the settlement increased.

- Fish farming decreased when LLDA took over in 1998.
- The fish farmers are using sinking feed.
- 75 legal fish pen operators living here.
- The majority are illegal settlers.
- The local community leave the algae alone, as it is removed by typhoons and rain.

Interview 4

- Floating cages established in the 1980s.
- In the 1950s, the water was very clear.
- Fish farming takes place twice per year, for 6 months.
- White event takes place in December.
- Between October and February, water is very polluted.
- The local community can not predict the algal blooms.
- The local community do not do anything about fish kills and blooms. Dead fish are buried under the soil and not consumed.

LAKE PALAKPAKIN

Collected in April 2018

Interview 1

- Aquaculture first started in 1978
- Fish floating cages number=198 and floating cages where brought in the 1980s
- Fish pens and floating cages take up 9 hectares of space
- Tilapia 5000 per cage with majority of cages of 15 x10m^2
- The fish food used is B-meg I Tate, Biogreen (3 different types→ starter, grower and finisher)
- This lake uses floater feeds. Was 17 hectares is now 9 hectares
- Algal blooms occur annually and in January
- Cage numbers slowly declined after peaking at 17 hectares in the late 1980s to 18% of the total lake surface area in 2008

APPENDIX B

PANDIN

Table B1: ^{210}Pb concentrations in core PANDN taken from Lake Pandin.

Depth	Dry Mass	Pb-210 Total		Pb-210 Supported		Pb-210 Unsupp		Cum Unsupported Pb-210	
cm	g cm^{-2}	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq m^{-2}	±
0.25	0.0028	240.29	35.41	66.76	8.99	173.53	36.53	4.9	0.8
5.25	0.5784	287.6	18.74	32.55	3.85	255.05	19.13	1223.3	134.2
10.25	1.0156	242.97	20.25	43.7	4.74	199.27	20.8	2211.4	170
15.25	1.528	149.86	18.46	35.3	4.42	114.56	18.98	2995.5	201.4
20.25	2.2135	181.2	20.24	28.44	4.43	152.76	20.72	3905.5	241.4
23.25	3.3379	44.48	7.34	35.26	1.93	9.22	7.59	4480.4	303.7
25.25	4.3167	67.88	8.33	38.81	2.1	29.07	8.59	4649.6	314.1
27.25	5.0367	69.74	11.21	29.08	2.62	40.66	11.51	4898.3	322.6
30.5	6.0086	60.45	5.84	23.57	1.31	36.88	5.99	5274.8	336.2
32.5	6.7663	66.25	9.49	34.12	2.37	32.13	9.78	5535.8	341.2
35.5	7.7547	66.24	9.09	38.31	2.43	27.93	9.41	5832.1	353.2
38.5	9.1022	29.59	5.48	30.38	1.33	-0.79	5.64	6008.3	369
40.5	10.1449	71.15	9.39	29.78	2.27	41.37	9.66	6214.6	376.7
43.5	11.2802	54.79	7	38.75	1.74	16.04	7.21	6518.1	390.4
46.5	12.3106	61.28	12.06	31.19	3.23	30.09	12.49	6748.2	401.4

Table B2: Artificial fallout radionuclide concentrations in core PANDN.

Depth	Cs-137		Am-241	
cm	Bq Kg^{-1}	±	Bq Kg^{-1}	±
0.25	0	0	0	0
5.25	0	0	0	0
10.25	0	0	0	0
15.25	0	0	0	0
20.25	0	0	0	0
23.25	0	0	0	0
25.25	0	0	0	0
27.25	0	0	0	0
30.5	1.37	0.6	0	0
32.5	0	0	0	0
35.5	3.08	1.18	0	0
38.5	1.56	0.59	0	0
40.5	0	0	0	0
43.5	2.53	0.81	0	0
46.5	0	0	0	0

Table B3: ^{210}Pb chronology of core PANDN taken from Lake Pandin.

Depth	Drymass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2018	0				
0.25	0.0028	2018	0	2	0.1233	1.119	21.7
5.25	0.5784	2012	6	2	0.069	0.681	10
10.25	1.0156	2006	12	2	0.0729	0.768	12.9
15.25	1.528	2000	18	2	0.1055	0.881	18.6
20.25	2.2135	1991	27	3	0.0606	0.268	16.6
23.25	3.3379	1984	34	3	0.8091	1.924	82.9
25.25	4.3167	1982	36	3	0.2385	0.562	31.5
27.25	5.0367	1978	40	4	0.1515	0.47	30.5
30.5	6.0086	1971	47	4	0.1352	0.41	21.4
32.5	6.7663	1965	53	5	0.1299	0.372	34
35.5	7.7547	1957	61	6	0.1164	0.299	37.6
38.5	9.1022	1949	69	6	0.1684	0.352	42.5
40.5	10.1449	1943	75	7	0.0498	0.114	30.2
43.5	11.2802	1923	95	9	0.0695	0.193	51.8
46.5	12.3106	1890	128	16	0.0132	0.038	54.6

APPENDIX C

YAMBO1

Table C1: ^{210}Pb concentrations in core YAMBO1 taken from Lake Yambo in June 2016.

Depth	Dry Mass	Pb-210					
		Total		Supported		Unsupported	
cm	g cm^{-2}	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq Kg^{-1}	±
0.75	0.1188	210.23	17.38	44.97	4.19	165.26	17.88
4.5	1.0329	173.6	13.81	38.15	3.17	135.45	14.17
9.5	1.9378	159.12	18.7	43.17	4.14	115.95	19.15
12.5	2.446	115.47	12.66	36.25	3.18	79.22	13.05
14.5	2.7848	102.58	12.45	37.75	3.39	64.83	12.9
17.5	3.341	112.62	13	28.15	2.98	84.47	13.34
19.5	3.7118	149.55	13.55	48.8	3.7	100.75	14.05
25.5	5.2979	94.3	8.33	44.43	2.13	49.87	8.6
27.5	5.8124	73.8	10.19	35.54	2.75	38.26	10.55
31.5	6.8414	78.78	11.31	38.36	3.21	40.42	11.76
33.5	7.2229	62.26	6.77	31.53	1.78	30.73	7
36.5	7.7952	81	11.4	45.26	2.99	35.74	11.79
39.5	8.3674	81.38	8.27	41.49	2.18	39.89	8.55
46.5	9.507	69.45	10.09	38.75	2.81	30.7	10.47
54.5	10.787	27.21	6.82	39.71	2.03	-12.5	7.12
61.5	12.0589	42.3	8.39	43.69	2.97	-1.39	8.9
69.5	13.3613	48.04	7.33	36.11	2.57	11.93	7.77
79.5	16.7343	27.91	4.81	34.25	1.59	-6.34	5.07

Table C2: Radiometric ^{210}Pb chronology for sediment core YAMBO1

Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2016	0				
0.75	0.1188	2015	1	2	0.1481	0.645	12.1
4.5	1.0329	2009	7	2	0.1492	0.718	12.2
9.5	1.9378	2003	13	2	0.1438	0.814	18
12.5	2.446	2000	16	2	0.1912	1.129	18.2
14.5	2.7848	1998	18	2	0.222	1.24	21.4
17.5	3.341	1995	21	2	0.1551	0.837	18
19.5	3.7118	1992	24	2	0.1195	0.488	16.5
25.5	5.2979	1981	35	3	0.1697	0.646	20.9
27.5	5.8124	1978	38	4	0.2029	0.789	30.2
31.5	6.8414	1973	43	4	0.1609	0.684	32.4
33.5	7.2229	1970	46	4	0.1979	1.038	27.4
36.5	7.7952	1967	49	5	0.1536	0.805	36.8
39.5	8.3674	1963	53	6	0.1208	0.706	28.3
46.5	9.507	1953	63	8	0.1164	0.721	42.7

APPENDIX D

MOH1

Table D1: ^{210}Pb concentrations in core MOH1 taken from Lake Mohicap in December 2013.

Depth	Dry Mass	Pb-210					
		Total		Supported		Unsupported	
cm	g cm^{-2}	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq Kg^{-1}	±
2	0.1907	186.45	25.77	41.13	7.19	145.32	26.75
5.5	0.5337	175.01	20.89	28.81	5.66	146.2	21.64
11.5	1.3437	191.27	21.59	36.55	6.01	154.72	22.41
17.5	2.6547	147.99	16.05	37.82	4.4	110.17	16.64
23.5	4.8498	130.7	15	16.56	3.75	114.14	15.46
29.5	7.6704	75.04	7.55	31.72	2.04	43.32	7.82
35.5	9.5238	98.93	13.53	29.01	3.68	69.92	14.02
41.5	11.2548	101.49	10.56	29.24	2.69	72.25	10.9
47.5	15.9975	33.67	11.68	38.63	3.69	-4.96	12.25
54.5	23.5701	29.87	18.72	36.92	2.46	-7.05	18.88
61.5	29.5457	42	23.36	52.03	6.03	-10.03	24.13

Table D1: Radiometric ^{210}Pb chronology for a sediment core MOH1 taken from Lake Mohicap in December 2013.

Depth	Dry Mass	Chronology			Sedimentation Rate
		Date	Age		
cm	g cm^{-2}	AD	yr	±	g cm^{-2}yr^{-1}
0	0	2013	0		
2	0.1907	2012	1	6	0.2515
5.5	0.5337	2011	2	2	0.2393
11.5	1.3437	2007	6	2	0.2016
17.5	2.6547	2001	12	2	0.2345
23.5	4.8498	1990	23	3	0.1592
29.5	7.6704	1976	37	5	0.2713
35.5	9.5238	1966	47	7	0.1222
41.5	11.2548	1946	67	11	0.0653

APPENDIX E

SAMP1

Table E1: ^{210}Pb concentrations in core SAMP1 taken from Lake Sampaloc in June 2015.

Depth	Dry Mass	Pb-210					
		Total		Supported		Unsupported	
cm	g cm^{-2}	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq Kg^{-1}	±
0.5	0.031	189.61	18.76	25.84	3.89	163.77	19.16
3.5	0.4713	125.17	14.04	27.33	3.37	97.84	14.44
7.5	0.9571	161.75	13.73	26.41	3.24	135.34	14.11
9.5	1.2822	118.42	26.54	40.84	6.16	77.58	27.25
14.5	1.991	85.44	13.66	43.36	3.02	42.08	13.99
19.5	2.6652	212.88	33.03	51.12	7.85	161.76	33.95
22.5	3.0848	86.63	13.83	31.69	3.62	54.94	14.3
25.5	3.5043	112.18	25.03	54.37	6.42	57.81	25.84
28.5	3.9305	92.49	13.38	33.4	4.36	59.09	14.07
31.5	4.3566	66.32	13.75	37.64	4.63	28.68	14.51
35.5	4.9398	41.12	9.37	31.3	3.21	9.82	9.9
39.5	5.523	61.48	9.88	33.56	3.21	27.92	10.39
42.5	6.0576	55.44	7.79	30.8	2.47	24.64	8.17
46.5	6.7704	38.22	9.15	46.47	2.47	-8.25	9.48
54.5	8.4348	38.22	9.86	38.83	2.48	-0.61	10.17
69.5	11.7134	25.03	7.75	31.78	2.32	-6.75	8.09

Table E2: Radiometric ^{210}Pb chronology for a SAMP1 taken from Lake Sampaloc in June 2015.

Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2015	0				
0.5	0.031	2015	0	2	0.0751	0.558	14.7
3.5	0.4713	2010	5	2	0.1078	0.815	17.9
7.5	0.9571	2004	11	2	0.065	0.481	15.9
9.5	1.2822	2000	15	2	0.0999	0.676	37.1
14.5	1.991	1994	21	3	0.1538	1.112	36
19.5	2.6652	1983	32	4	0.0285	0.208	26
22.5	3.0848	1972	43	6	0.0603	0.431	32.9
25.5	3.5043	1964	51	7	0.0446	0.316	49.9
28.5	3.9305	1953	62	9	0.0305	0.215	38.7
31.5	4.3566	1941	74	13	0.0433	0.301	64.5
35.5	4.9398	1929	86	16	0.0496	0.34	76
39.5	5.523	1918	97	22	0.0218	0.136	79.9

APPENDIX F

BUNOT2

Table F1: ^{210}Pb concentrations in core BUNOT2 taken from Lake Bunot.

Depth	Dry Mass	Pb-210 Total		Pb-210 Supported		Pb-210 Unsupp		Cum Unsupported Pb-210	
cm	g cm^{-2}	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq m^{-2}	±
0.25	0.0085	107.34	11.29	18.67	2.62	88.67	11.59	7.5	0.8
4.25	0.2446	171.88	17.18	34.27	4.84	137.61	17.85	270.5	28.7
8.25	0.6456	75.8	10.28	19.69	3.09	56.11	10.73	634.8	66.7
12.25	1.1995	109.97	12.47	22.41	3.1	87.56	12.85	1026.2	91
16.25	1.4958	111.42	13.68	29.71	3.78	81.71	14.19	1276.9	102.9
20.25	1.8453	115.83	10.91	31.81	3.34	84.02	11.41	1566.5	113.4
24.25	2.1946	136.62	11.13	31.23	3.1	105.39	11.55	1895.8	121.4
28.25	2.606	113.42	14.49	31.44	3.68	81.98	14.95	2279.2	132.6
32.5	3.0071	122.78	14.24	31.49	3.94	91.29	14.78	2626.5	146.6
36.5	3.3441	106.37	14.34	29	3.66	77.37	14.8	2910	156.3
40.5	3.7079	108.1	16.93	38.37	4.3	69.73	17.47	3177.3	166.3
44.5	4.0847	65.8	15.08	39.5	3.95	26.3	15.59	3345.2	178.3
48.5	4.3359	105.37	17.66	33.5	4.43	71.87	18.21	3459	184.1
52.5	4.5399	70.98	19.43	38.86	5.24	32.12	20.12	3559.7	188.5
55.5	4.7341	70.97	16.16	35.27	4.39	35.7	16.75	3625.5	192.3
59.5	5.2441	51.58	11.08	42.8	3.15	8.78	11.52	3723.4	202.7
63.5	5.8133	75.65	11.45	41.08	3.49	34.57	11.97	3830.5	212.8
67.5	6.3065	63.09	10.59	37.9	3.13	25.19	11.04	3976.6	221.3
71.5	6.804	55.17	7.1	28.29	1.89	26.88	7.35	4106.1	227.2
75.5	7.408	65.28	10.32	35.02	3.13	30.26	10.78	4278.5	232.5
80.5	7.9756	49.01	9.51	31.3	3.01	17.71	9.97	4411.4	240.4

Table F2: Artificial fallout radionuclide concentrations in core BUNOT2.

Depth	Cs-137		Am-241	
cm	Bq Kg^{-1}	±	Bq Kg^{-1}	±
0.25	0	0	0	0
4.25	0	0	0	0
8.25	0	0	0	0
12.25	0	0	0	0
16.25	0	0	0	0
20.25	0	0	0	0
24.25	0	0	0	0
28.25	0	0	0	0
32.5	0	0	0	0
36.5	0	0	0	0

40.5	0	0	0	0
44.5	0	0	0	0
48.5	0	0	0	0
52.5	0	0	0	0
55.5	0	0	0	0
59.5	0	0	0	0
63.5	0	0	0	0
67.5	0	0	0	0
71.5	0	0	0	0
75.5	0	0	0	0
80.5	0	0	0	0

Table F3: ^{210}Pb chronology of core BUNOT2 taken from Lake Bunot.

Depth	Drymass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2017	0				
0.25	0.0085	2017	0	2	0.1682	2.922	14.5
4.25	0.2446	2015	2	2	0.1024	1.286	14.5
8.25	0.6456	2012	5	2	0.231	1.935	20.4
12.25	1.1995	2009	8	2	0.1341	1.262	16.5
16.25	1.4958	2007	10	2	0.1341	1.661	19.1
20.25	1.8453	2004	13	2	0.1197	1.37	16.1
24.25	2.1946	2001	16	2	0.0857	0.901	14.6
28.25	2.606	1996	21	2	0.0956	0.971	21.1
32.5	3.0071	1992	25	3	0.074	0.827	20.3
36.5	3.3441	1987	30	3	0.0759	0.867	23.6
40.5	3.7079	1982	35	4	0.0723	0.781	29.4
44.5	4.0847	1979	38	5	0.1718	2.189	61.6
48.5	4.3359	1976	41	5	0.0579	1.018	31.2
52.5	4.5399	1973	44	5	0.1199	2.108	65.5
55.5	4.7341	1972	45	6	0.1021	1.015	50.8
59.5	5.2441	1969	48	6	0.1769	1.311	50.3
63.5	5.8133	1966	51	6	0.087	0.655	41.1
67.5	6.3065	1960	57	7	0.1013	0.818	50.5
71.5	6.804	1955	62	9	0.08	0.581	40.3
75.5	7.408	1946	71	11	0.0533	0.409	51.7
80.5	7.9756	1936	81	15	0.0677	0.588	54.2

APPENDIX G

PALAK

Table G1: ^{210}Pb concentrations in core PALAK taken from Lake Palakpakin.

Depth	Dry Mass	Pb-210						Cum Unsupported Pb-210	
		Total		Supported		Unsupp			
cm	g cm^{-2}	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq Kg^{-1}	±	Bq m^{-2}	±
0.25	0.0032	56.19	18.05	57.32	6.05	-1.13	19.04	0	0.4
4.25	0.2252	88.08	12.68	26.4	3.95	61.68	13.28	67.2	26.2
8.25	0.4444	122.37	15.72	37.77	5.19	84.6	16.55	226.2	41.5
12.25	0.7305	86.85	13.08	29.56	4.42	57.29	13.81	426.7	60.3
16.25	1.0776	104.7	15.42	20.05	4.68	84.65	16.11	669.9	78
20.25	1.44	67.88	15.7	19.28	4.53	48.6	16.34	905.4	98
24.25	1.7304	45.65	8.91	29.91	2.95	15.74	9.39	990	108.5
28.25	2.087	51.6	10.9	23.58	3.82	28.02	11.55	1065.9	113.8
32.5	2.5424	114.56	15.95	30.5	5.18	84.06	16.77	1298.2	127.8
36.5	2.9305	55.89	8.93	25.47	3.07	30.42	9.44	1503.1	142.7
40.5	3.4036	73.74	11.17	21.55	2.97	52.19	11.56	1693.9	150
44.5	3.9142	53.56	9.2	24.88	2.62	28.68	9.57	1894.4	160.3
48.5	4.4251	44.19	5.36	33.97	1.58	10.22	5.59	1985.8	166.5
52.5	5.0235	58.79	8.87	23.85	2.42	34.94	9.19	2106.1	171.1
55.5	5.5043	58.41	9.07	22.89	2.65	35.52	9.45	2275.5	177.7
59.5	6.0322	68.02	10.48	28.76	2.94	39.26	10.88	2472.7	185.1
63.5	6.5046	61.19	8.85	32.04	2.53	29.15	9.2	2633.1	192.2
67.5	7.1374	55.91	8.49	29.74	2.33	26.17	8.8	2808	199.7
71.5	7.7823	66	5.83	32.04	1.67	33.96	6.06	3000.7	206.8

Table G2: Artificial fallout radionuclide concentrations in core PALAK.

Depth	Cs-137		Am-241	
cm	Bq Kg^{-1}	±	Bq Kg^{-1}	±
0.25	0	0	0	0
4.25	0	0	0	0
8.25	0	0	0	0
12.25	0	0	0	0
16.25	0	0	0	0
20.25	0	0	0	0
24.25	0	0	0	0
28.25	0	0	0	0
32.5	0	0	0	0
36.5	0	0	0	0
40.5	0	0	0	0

44.5	0	0	0	0
48.5	0	0	0	0
52.5	0	0	0	0
55.5	0	0	0	0
59.5	0	0	0	0
63.5	0	0	0	0
67.5	2.13	1.06	0	0
71.5	1.78	0.67	0	0

Table G3: ^{210}Pb chronology of core PALAK taken from Palakpakin.

Depth	Drymass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2017	0				
0.25	0.0032	2017	0	2	0.3175	5.993	
4.25	0.2252	2016	1	2	0.1642	2.978	22.9
8.25	0.4444	2015	2	2	0.1139	1.803	21.2
12.25	0.7305	2013	4	2	0.1573	1.987	25.6
16.25	1.0776	2010	7	2	0.0975	1.099	21.1
20.25	1.44	2007	10	2	0.1547	1.896	35
24.25	1.7304	2006	11	2	0.4611	5.701	60.4
28.25	2.087	2005	12	2	0.2506	2.546	42.4
32.5	2.5424	2001	16	2	0.0749	0.733	22.6
36.5	2.9305	1998	19	3	0.186	1.728	33.1
40.5	3.4036	1994	23	3	0.0971	0.789	25.5
44.5	3.9142	1990	27	4	0.1548	1.213	36.1
48.5	4.4251	1988	29	4	0.4067	2.933	56.6
52.5	5.0235	1985	32	4	0.1082	0.702	30.6
55.5	5.5043	1980	37	5	0.0916	0.636	32
59.5	6.0322	1973	44	6	0.0673	0.538	34.7
63.5	6.5046	1966	51	7	0.0734	0.532	40.1
67.5	7.1374	1957	60	9	0.061	0.382	46
71.5	7.7823	1942	75	15	0.0293	0.184	51.7

APPENDIX H – Chapter 5 - GAMS Diagnostic plots for individual lakes

LAKE PANDIN

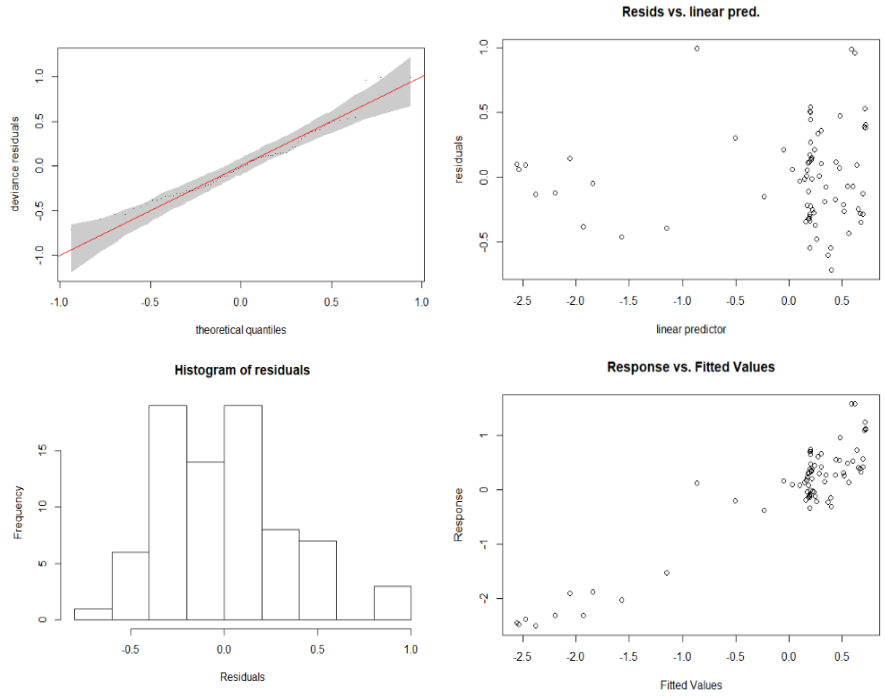


Figure H.1: Diagnostic plots for a fitted GAM of the PCA axis 1 scores from sediment core, PANDIN. Top left) Q-Q plot, top right) Residuals vs. linear predictor, bottom left) Histogram of residuals and bottom right) Observed vs. fitted values

LAKE YAMBO

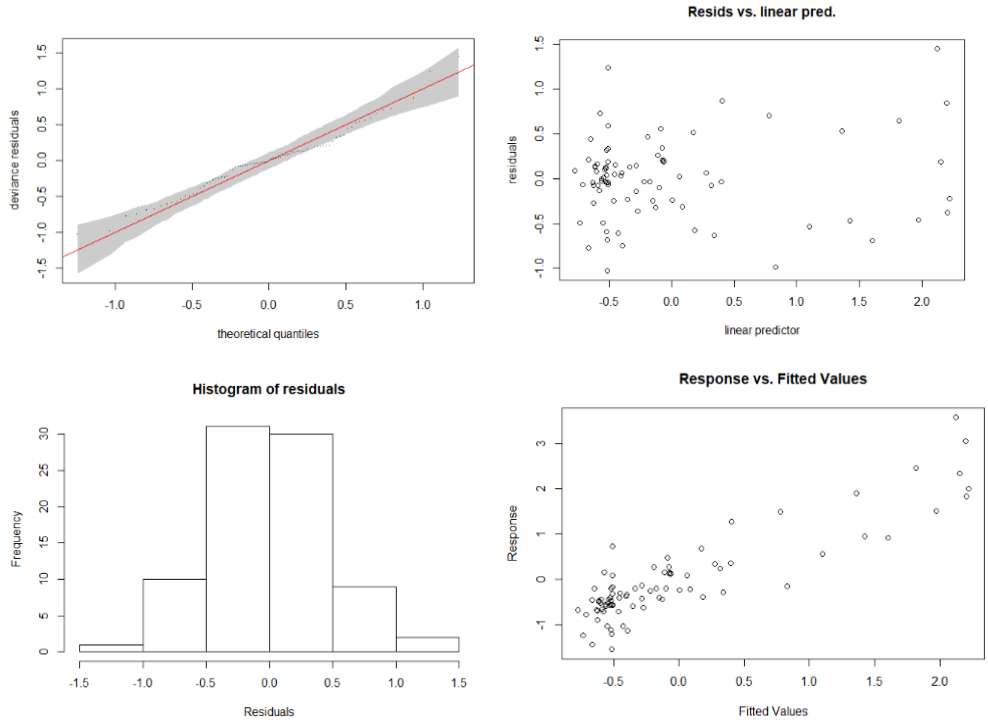


Figure H.2: Diagnostic plots for a fitted GAM of the PCA axis 1 scores from sediment core, YAMBO2. Top left) Q-Q plot, top right) Residuals vs. linear predictor, bottom left) Histogram of residuals and bottom right) Observed vs. fitted values

LAKE MOHICAP

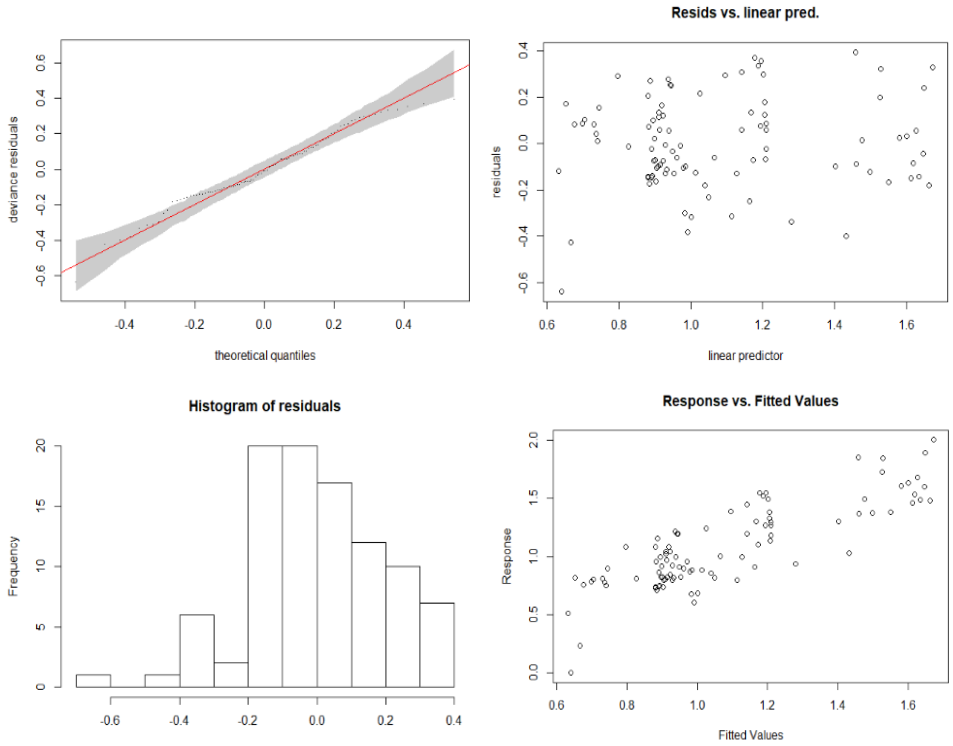


Figure H.3: Diagnostic plots for a fitted GAM of the PCA axis 1 scores from sediment core, MOH2. Top left) Q-Q plot, top right) Residuals vs. linear predictor, bottom left) Histogram of residuals and bottom right) Observed vs. fitted values

LAKE SAMPALOC

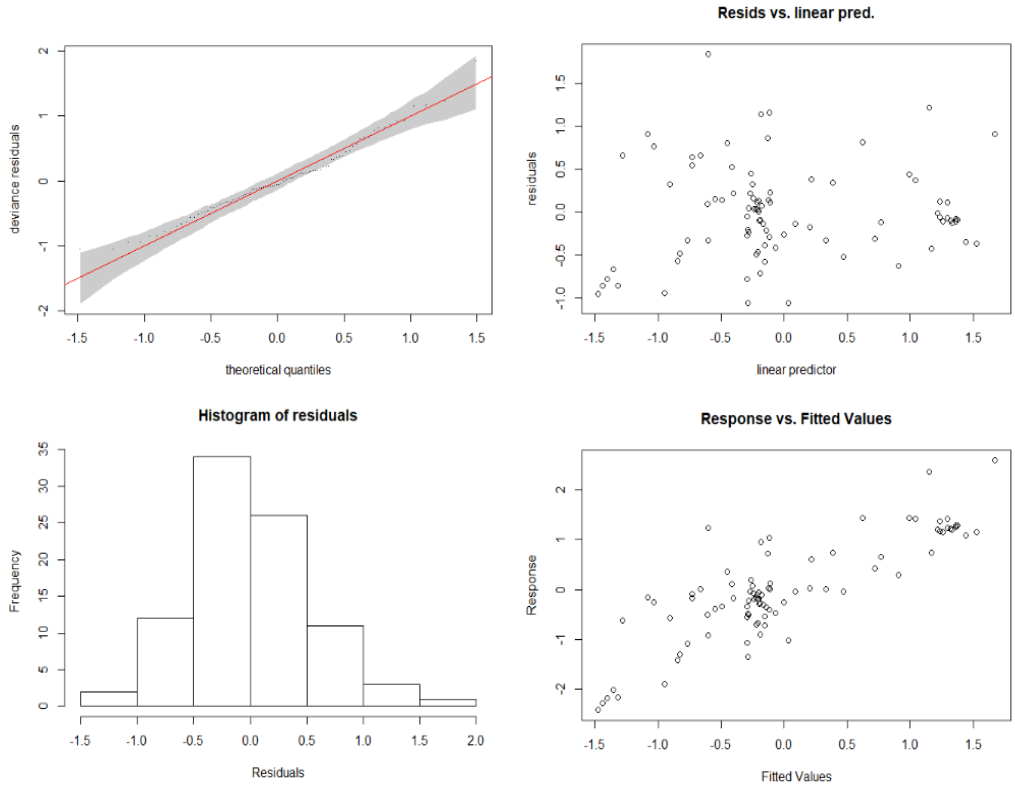


Figure H.4: Diagnostic plots for a fitted GAM of the PCA axis 1 scores from sediment core, SAMP2. Top left) Q-Q plot, top right) Residuals vs. linear predictor, bottom left) Histogram of residuals and bottom right) Observed vs. fitted values

LAKE BUNOT

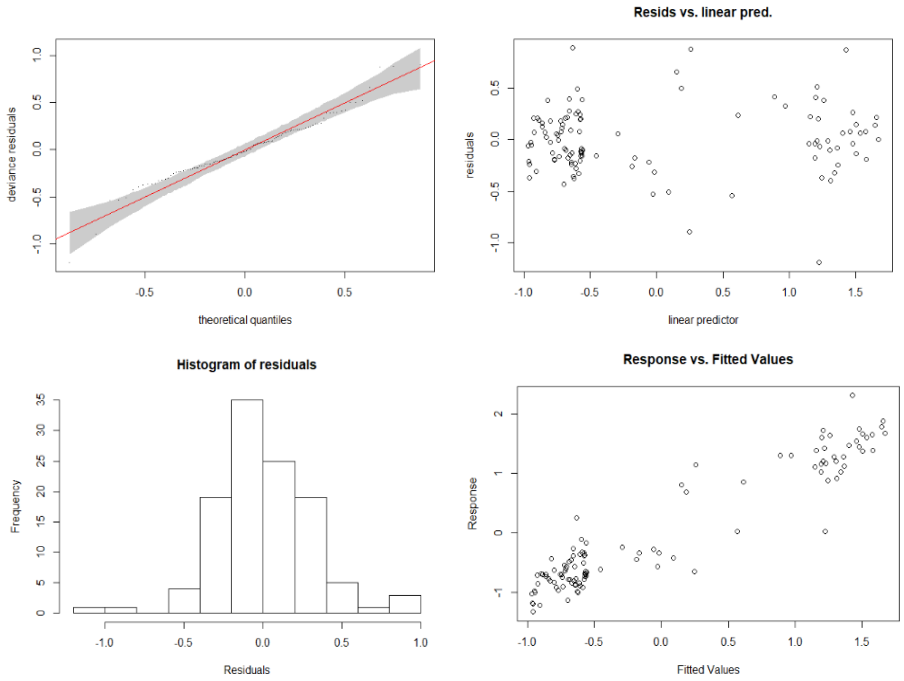


Figure H.5: Diagnostic plots for a fitted GAM of the PCA axis 1 scores from sediment core, BUNOT2. Top left) Q-Q plot, top right) Residuals vs. linear predictor, bottom left) Histogram of residuals and bottom right) Observed vs. fitted values

LAKE PALAKPAKIN

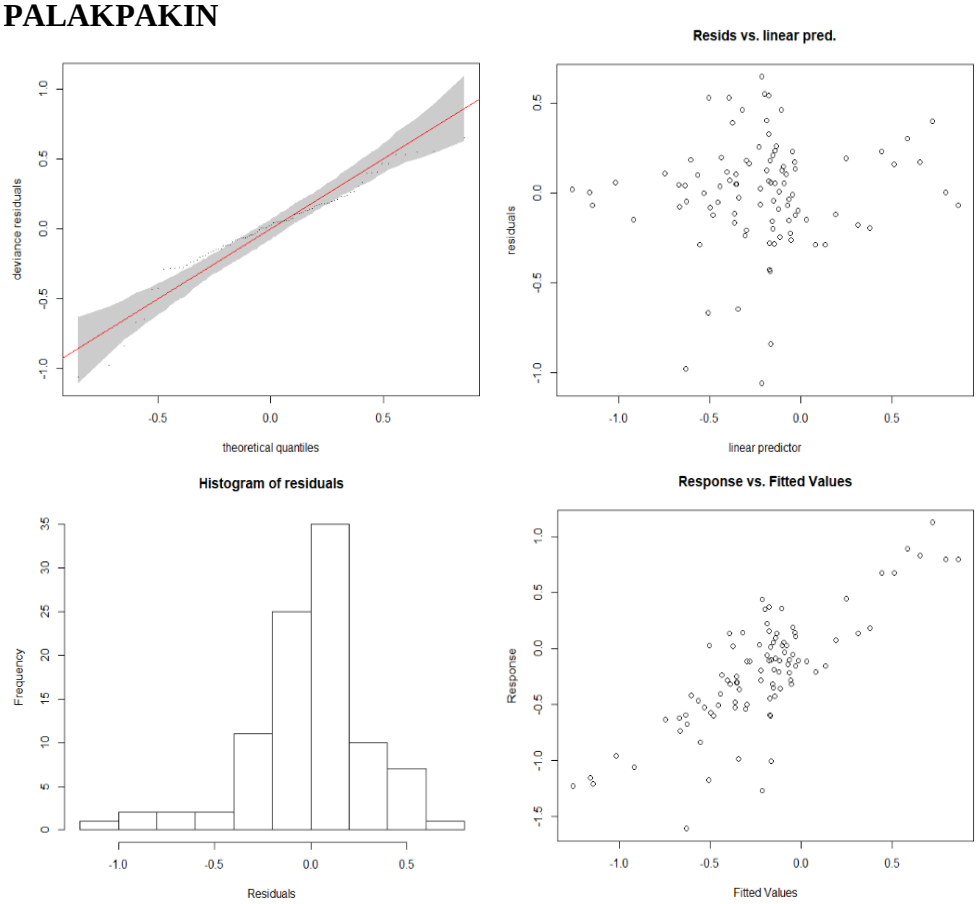


Figure H.6: Diagnostic plots for a fitted GAM of the PCA axis 1 scores from sediment core, PALAK. Top left) Q-Q plot, top right) Residuals vs. linear predictor, bottom left) Histogram of residuals and bottom right) Observed vs. fitted values

APPENDIX I – Chapter 6 - Temporal change figures for the individual proxies

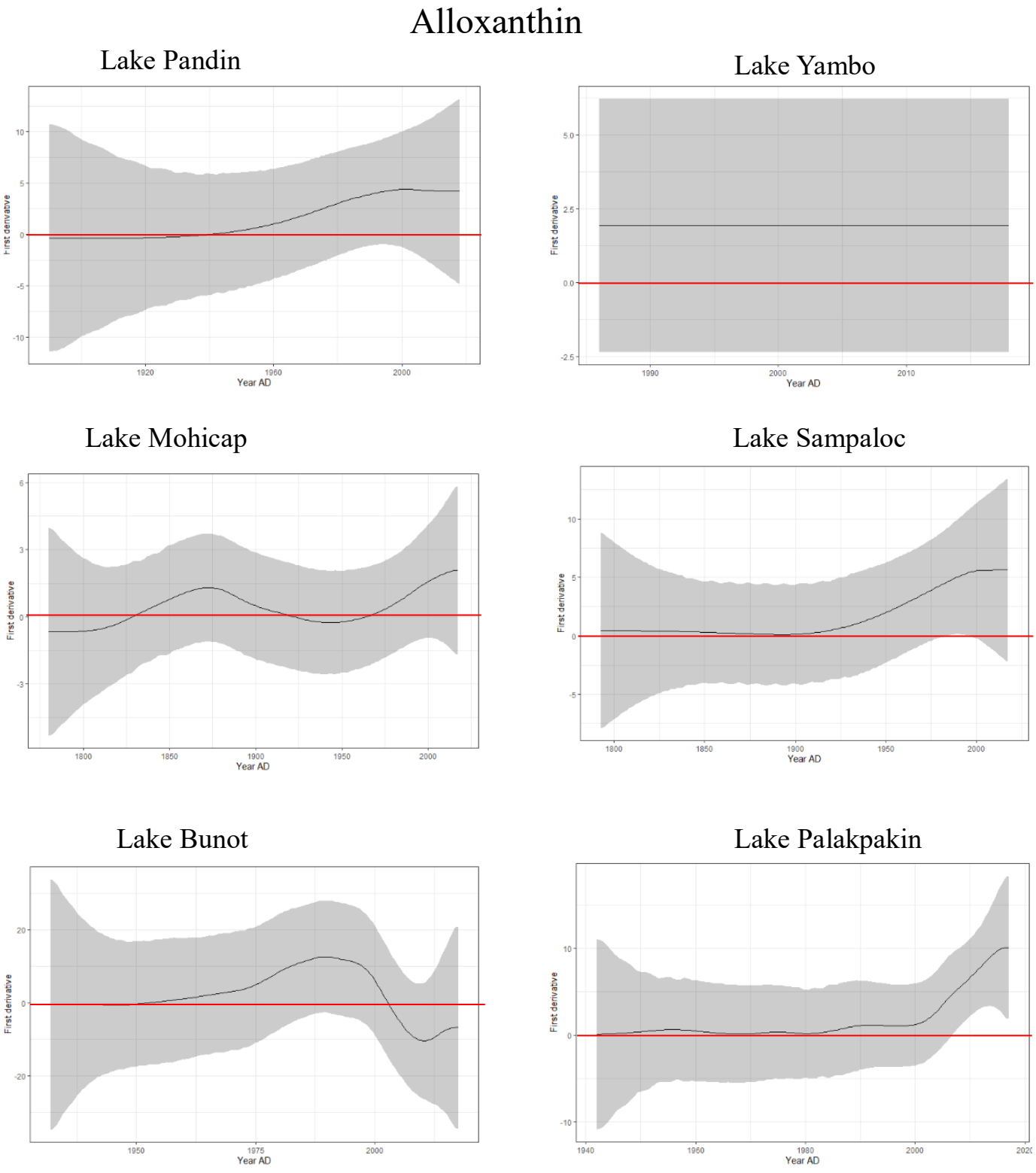


Figure I.1: Estimated first derivatives (black line) of the GAM trend fitted to the concentration of the pigment alloxanthin from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

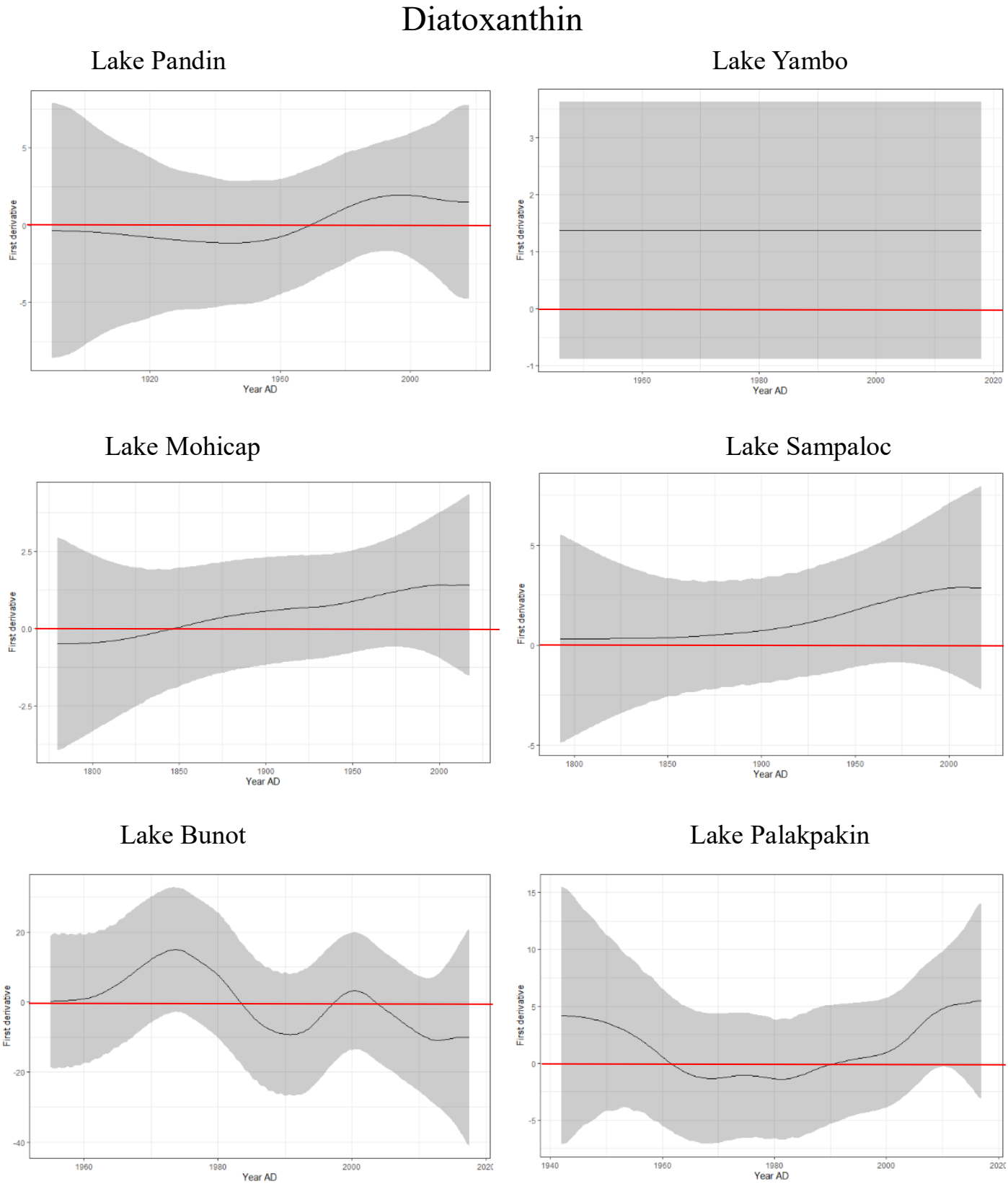


Figure I.2: Estimated first derivatives (black line) of the GAM trend fitted to the concentration of the pigment diatoxanthin from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

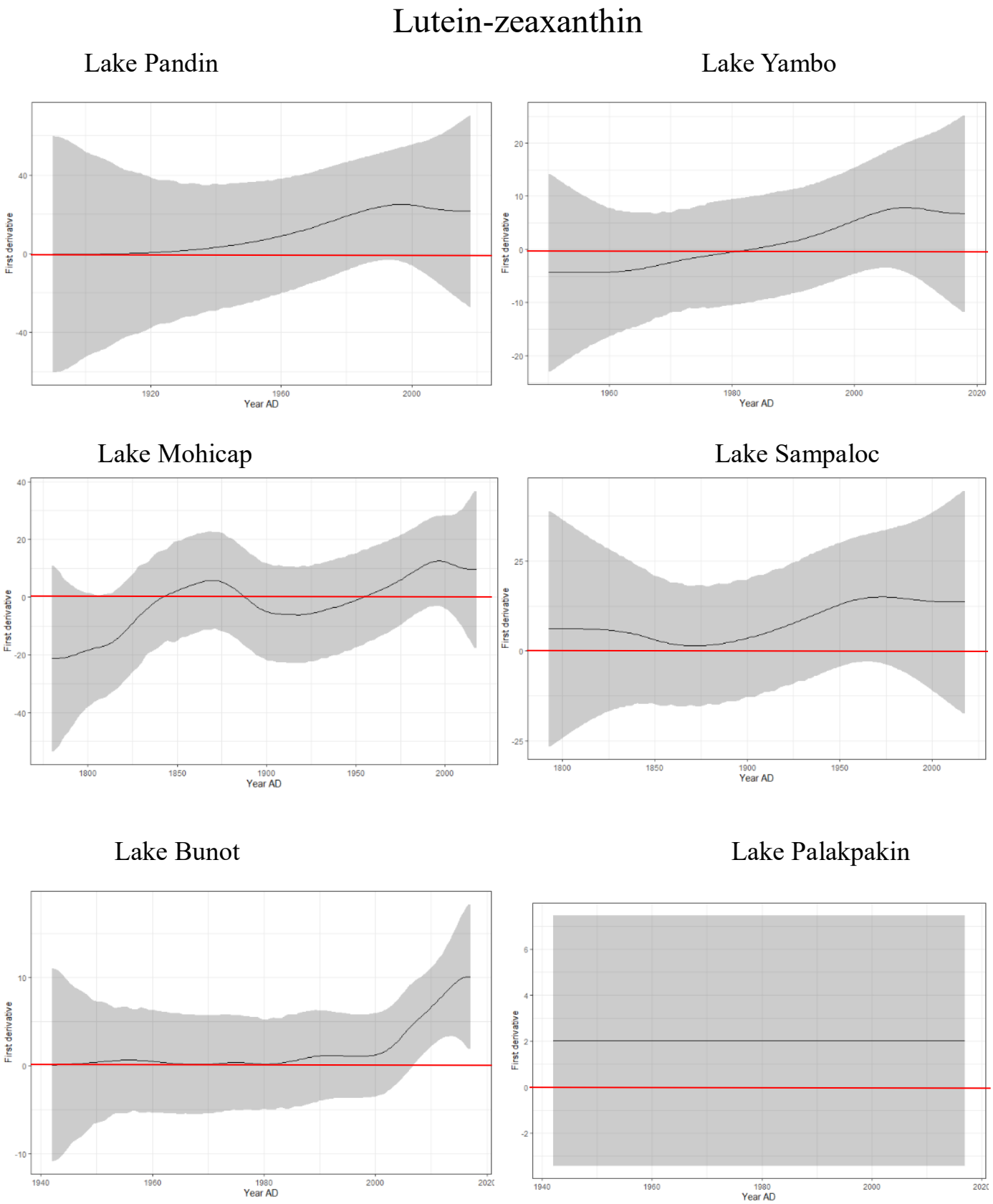


Figure I.3: Estimated first derivatives (black line) of the GAM trend fitted to the concentration of the pigment lutein-zeaxanthin from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

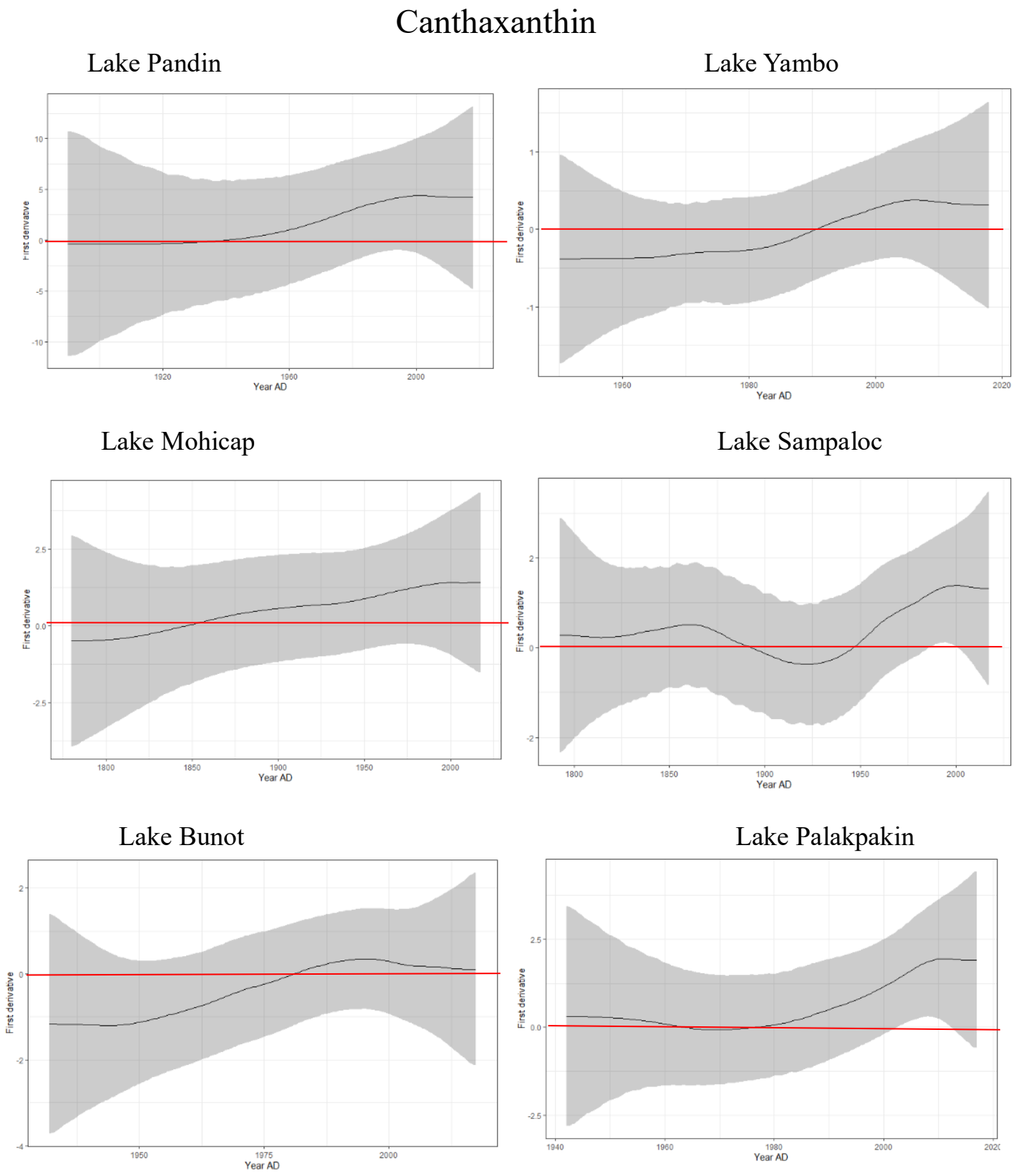


Figure I.4: Estimated first derivatives (black line) of the GAM trend fitted to the concentration of the pigment canthaxanthin from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

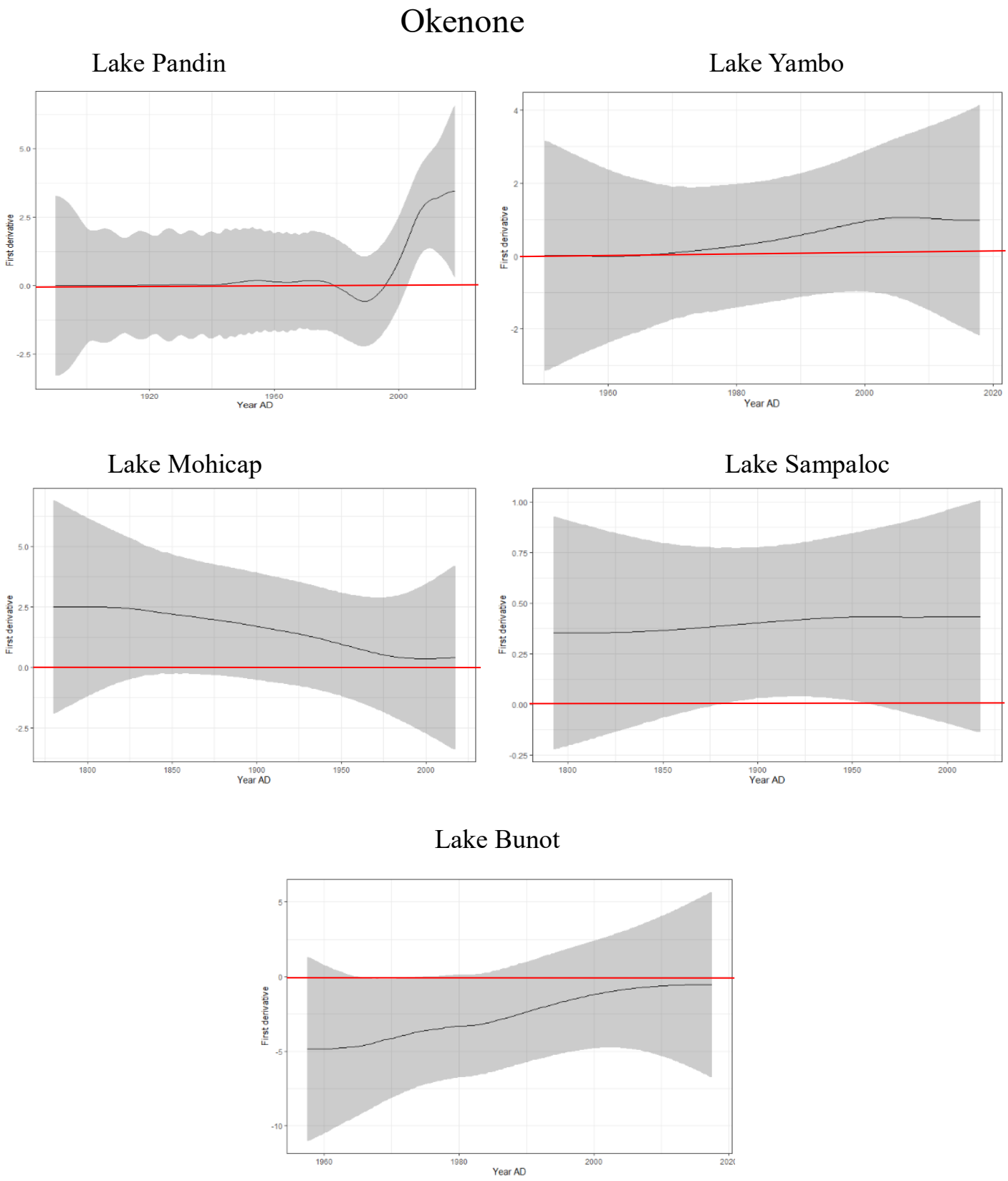
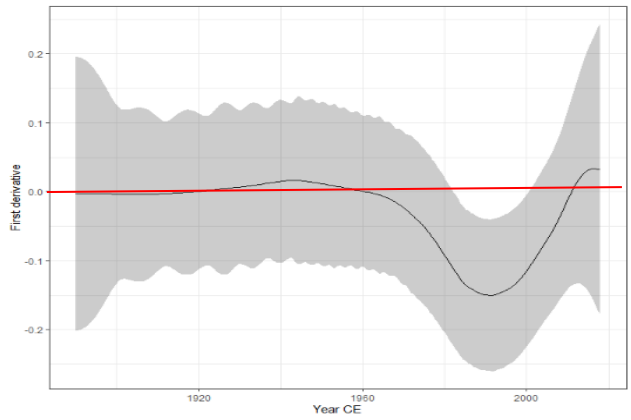


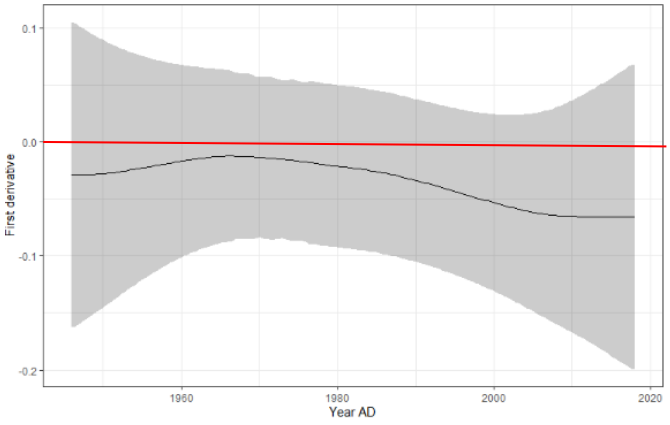
Figure I.5: Estimated first derivatives (black line) of the GAM trend fitted to the concentration of the pigment okenone from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

Carbon Isotopic Ratios

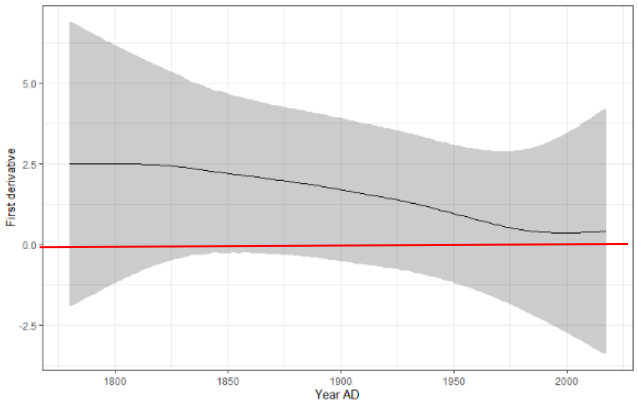
Lake Pandin



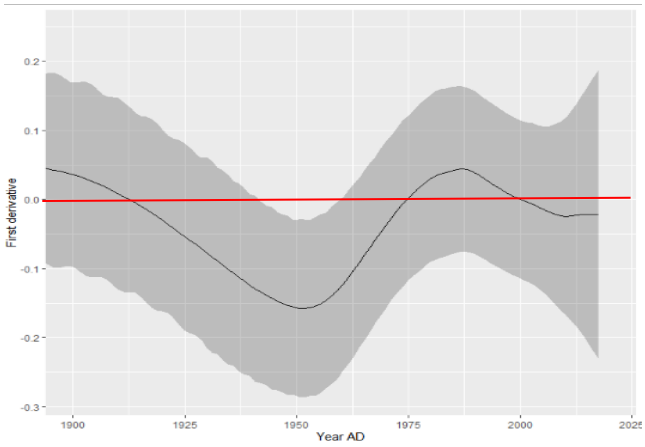
Lake Yambo



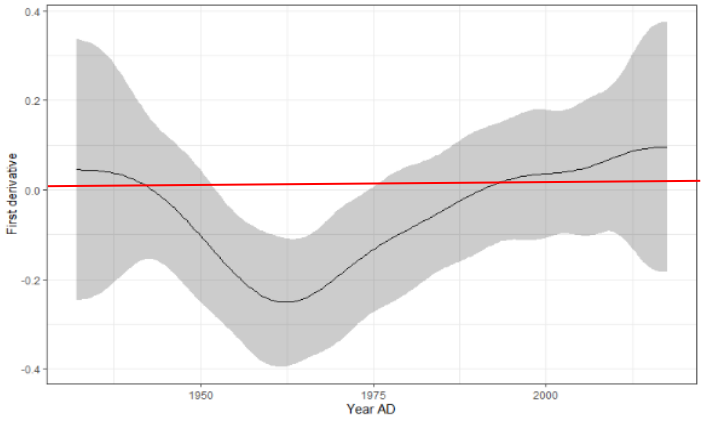
Lake Mohicap



Lake Sampaloc



Lake Bunot



Lake Palakpakin

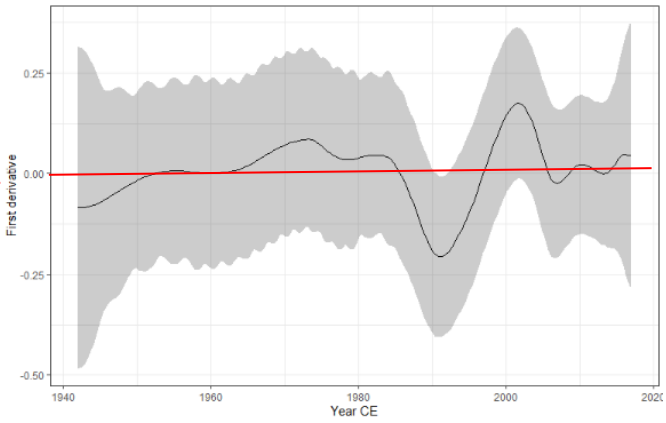


Figure I.6: Estimated first derivatives (black line) of the GAM trend fitted to $\delta^{13}C$ isotopic ratios from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

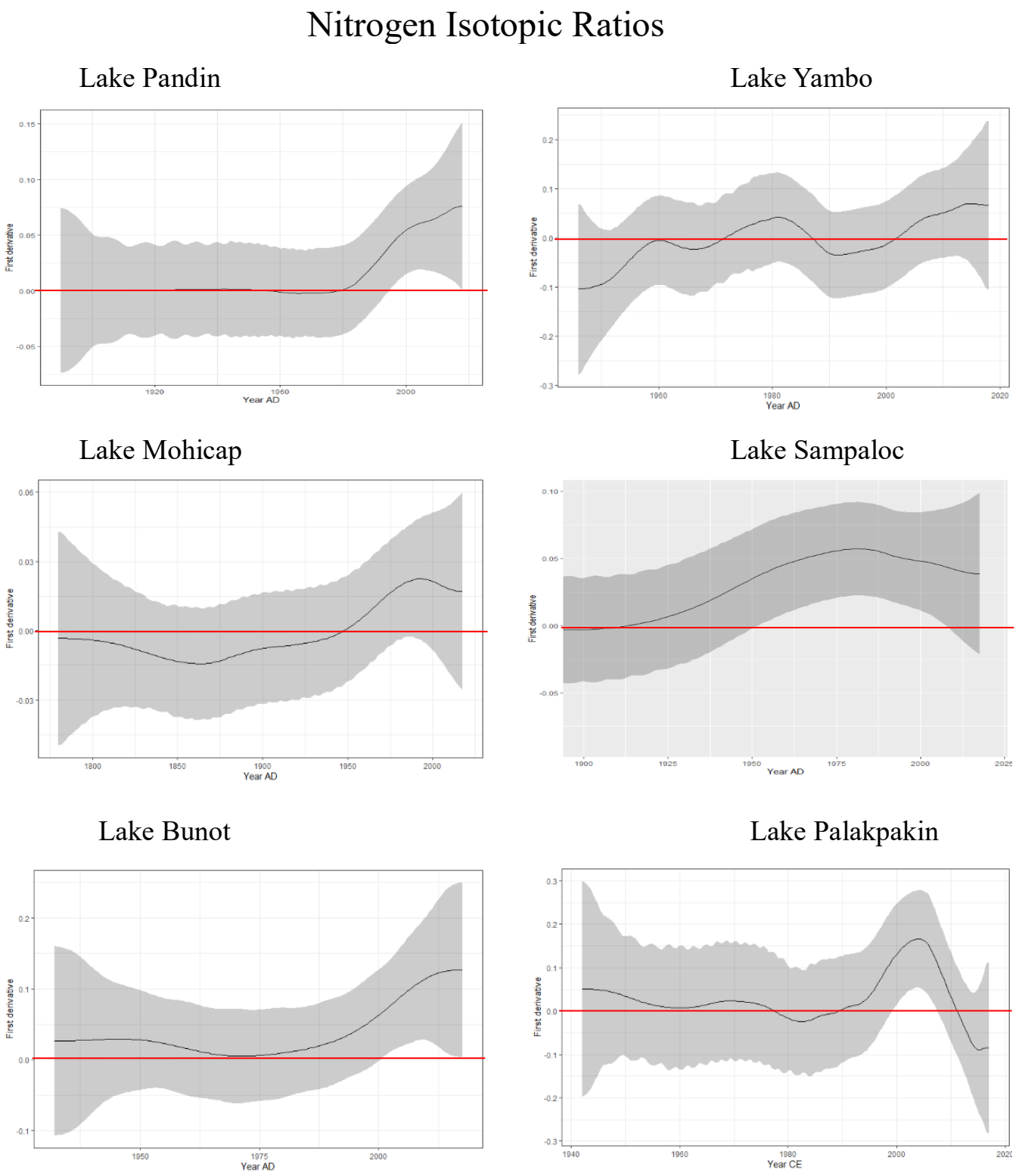
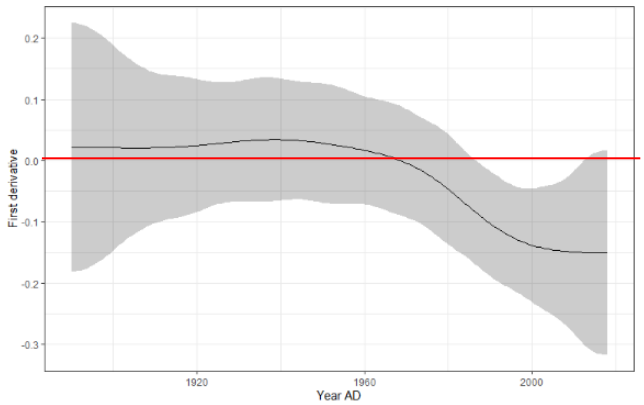


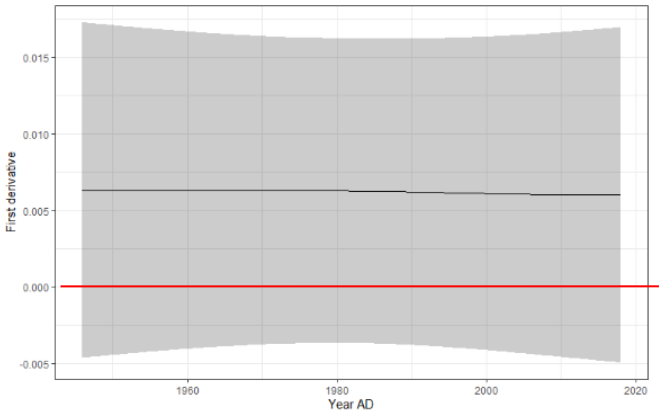
Figure I.7: Estimated first derivatives (black line) of the GAM trend fitted to $\delta^{15}N$ isotopic ratios from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

C/N Ratios

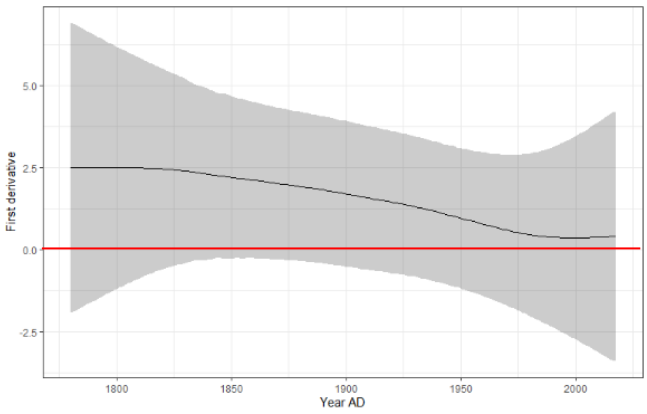
Lake Pandin



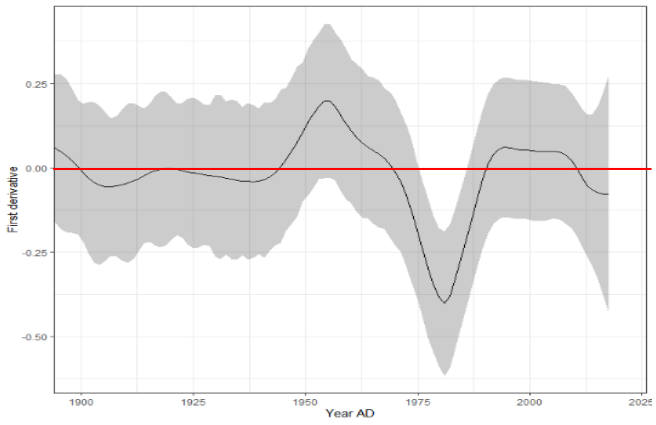
Lake Yambo



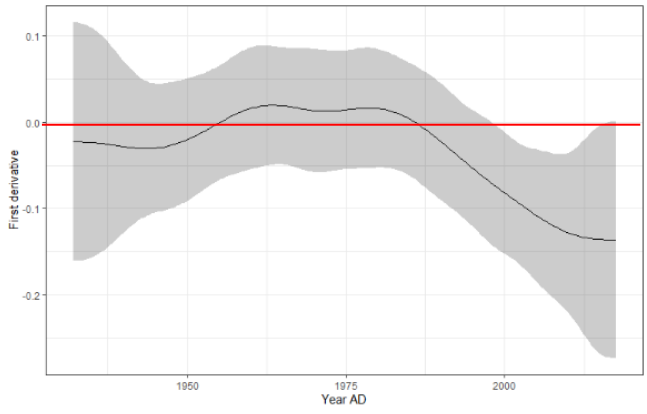
Lake Mohicap



Lake Sampaloc



Lake Bunot



Lake Palakpakin

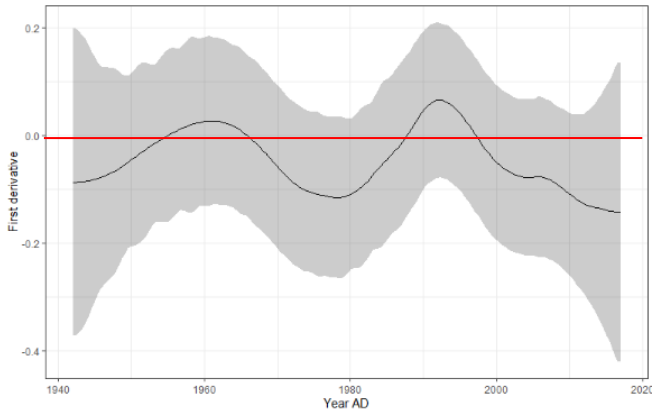


Figure I.8 Estimated first derivatives (black line) of the GAM trend fitted to C/N ratios from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

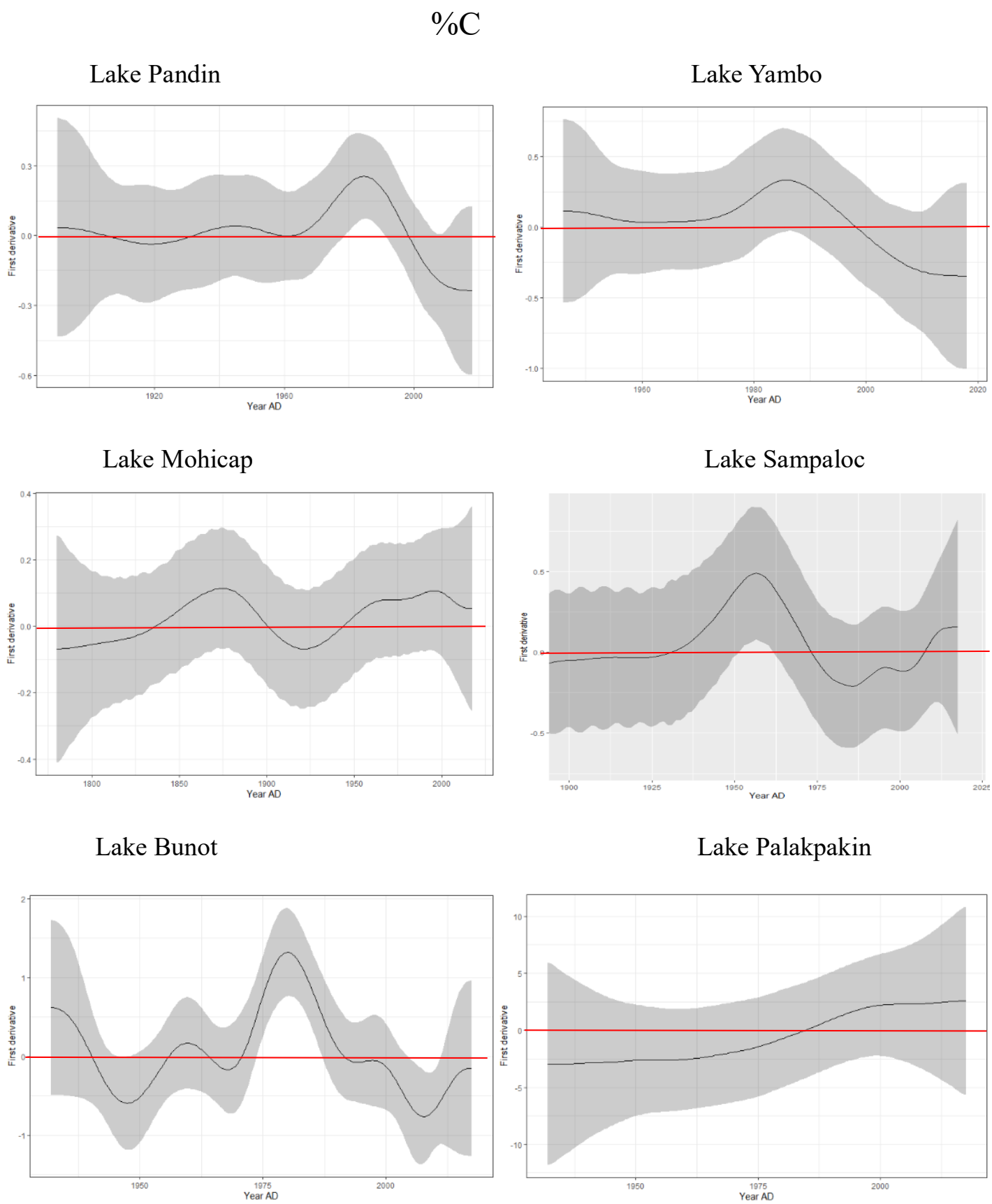


Figure I.9: Estimated first derivatives (black line) of the GAM trend fitted to the %C values from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

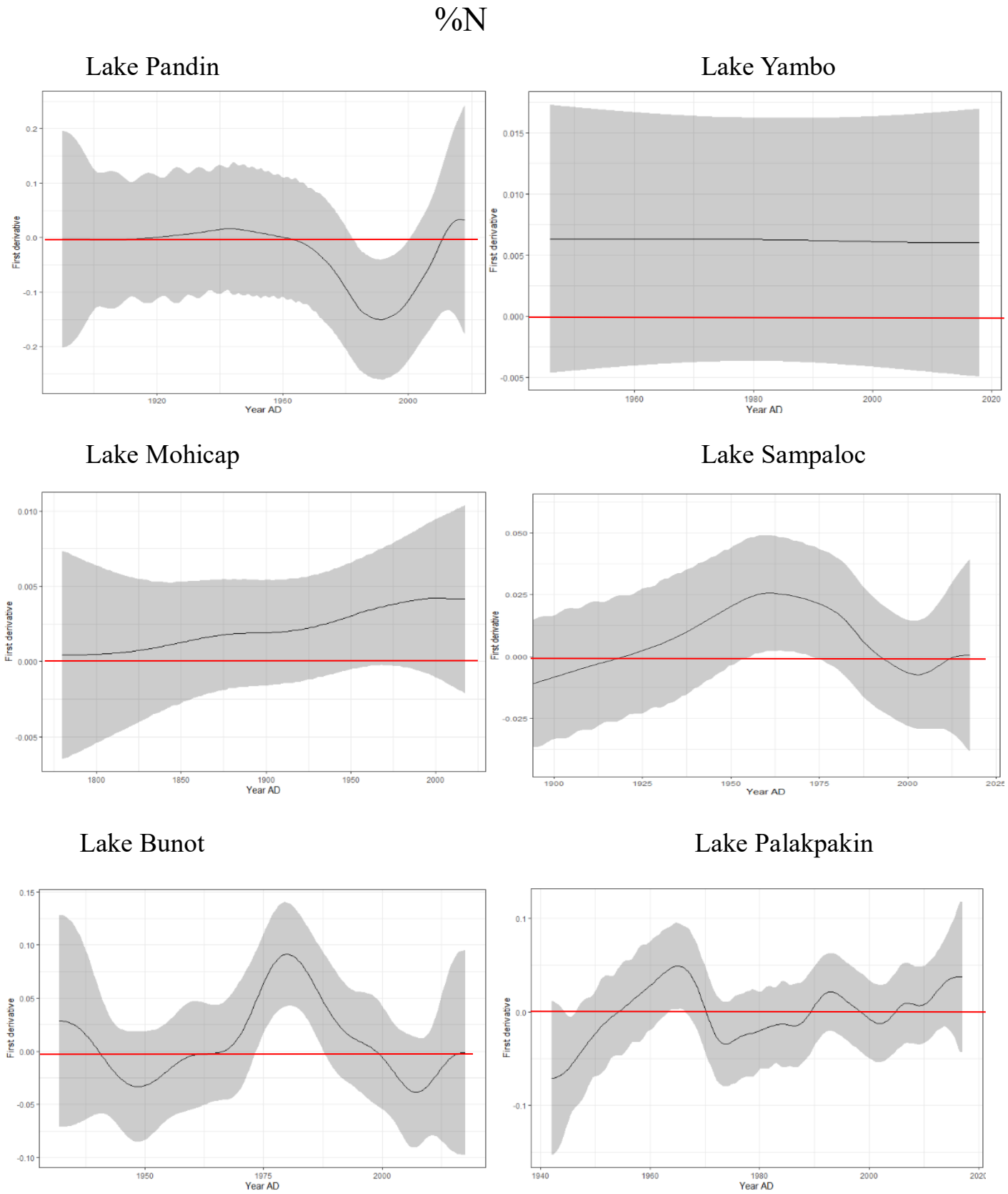


Figure I.10: Estimated first derivatives (black line) of the GAM trend fitted to the %N values from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

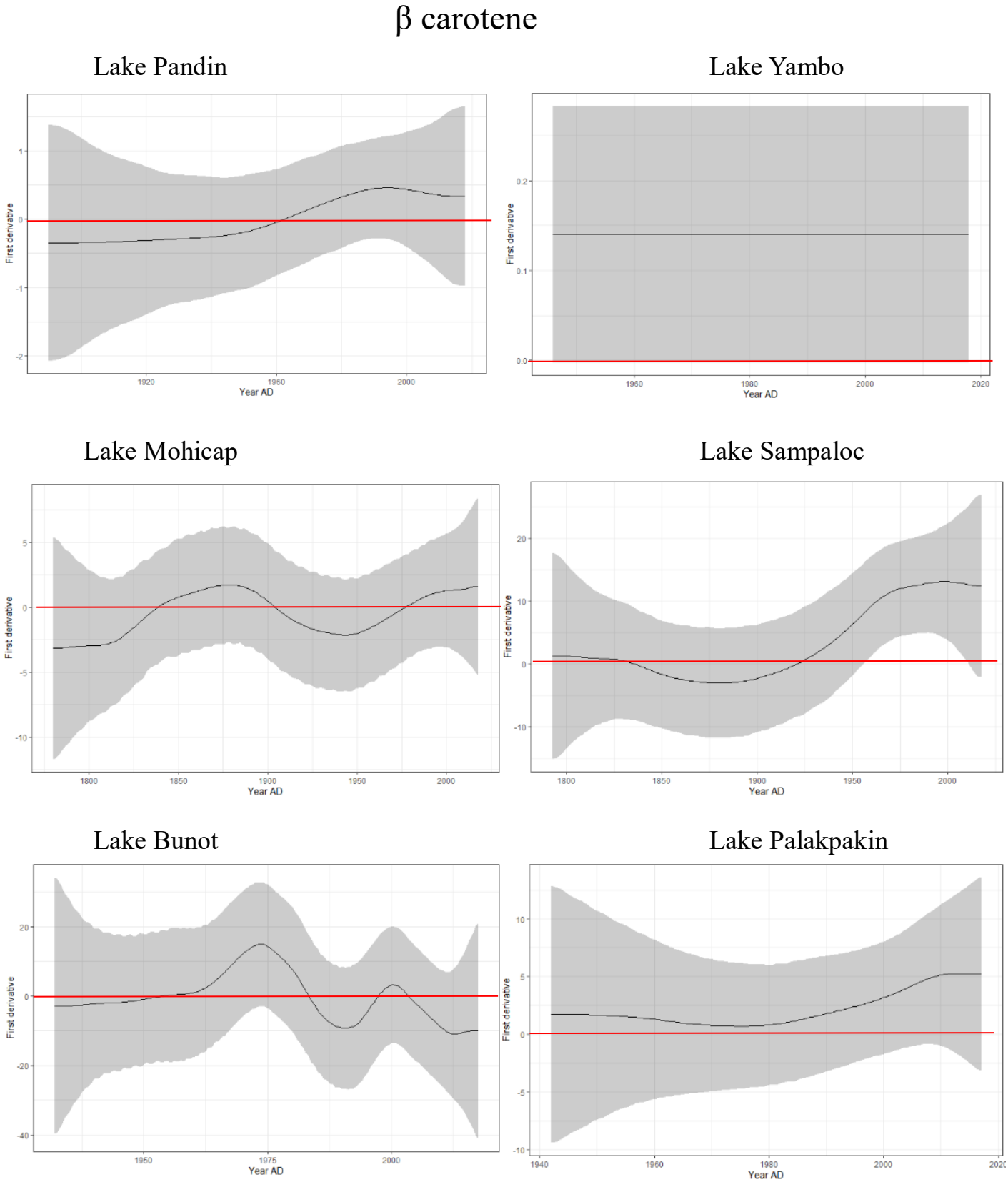


Figure I.11: Estimated first derivatives (black line) of the GAM trend fitted to the β carotene concentrations from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).

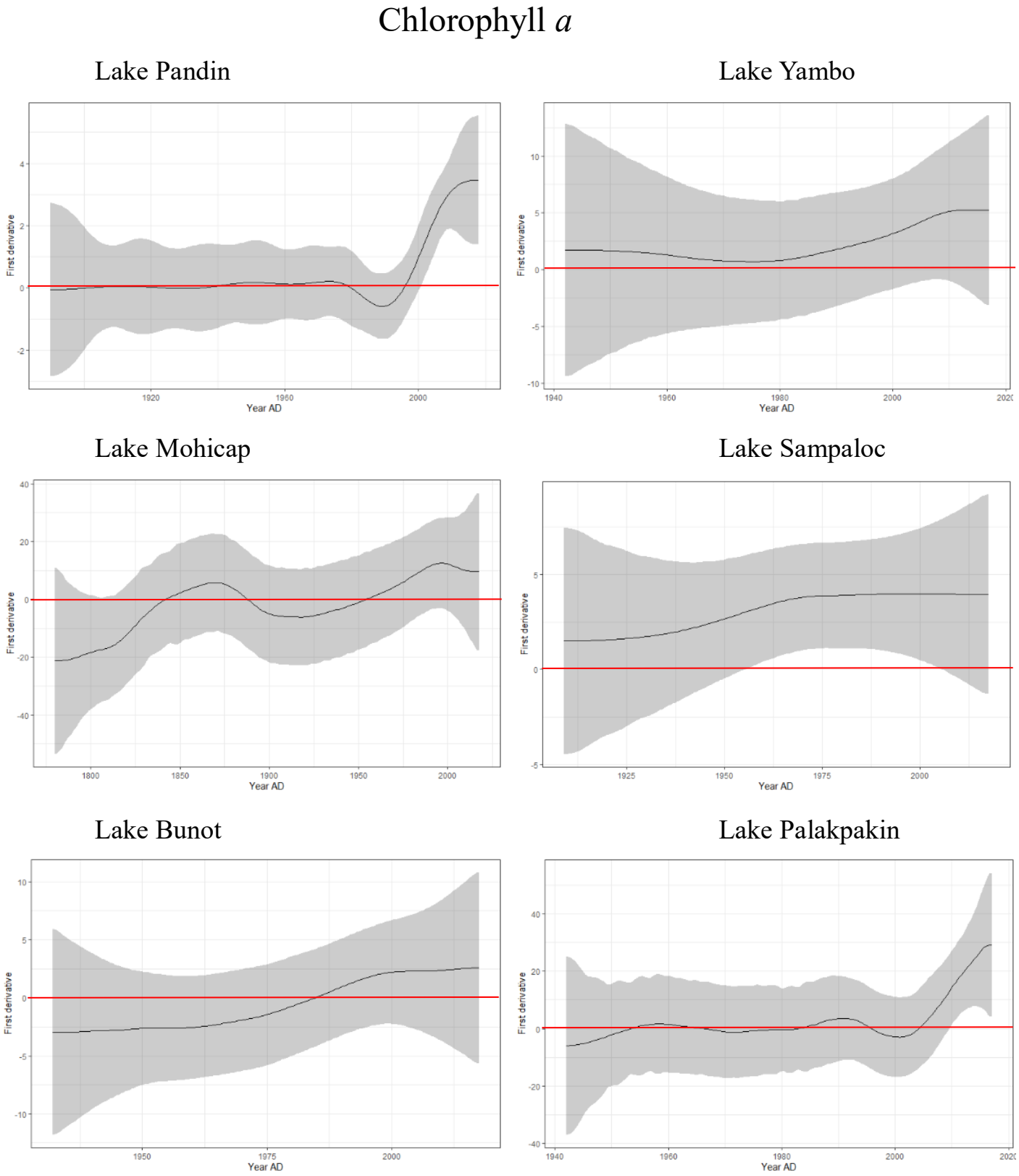


Figure I.12: Estimated first derivatives (black line) of the GAM trend fitted to the Chlorophyll a concentrations from the six lakes in order of disturbance. Areas where the confidence intervals have deviated from the red line signify periods of significant ($p<0.05$) temporal change. The grey shaded areas correspond to 95% confidence intervals (calculated ±2SE).