

Factors affecting algal blooms in high altitude lakes at low latitude.

A review and the case of Puno Bay (Lake Titicaca, Peru)

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Factors affecting algal blooms in high altitude lakes at low latitude

A review and the case of Puno Bay (Lake Titicaca, Peru)

Master of Science Thesis
by

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Abstract

Algal blooms are an increasing threat to lakes worldwide. This phenomenon has been studied worldwide but still a better understanding is needed of how algal bloom driving factors are affected by low-latitude and high-altitude conditions. Four regions were compared: Lakes at low-latitude and low-altitude; low-latitude and high-altitude; mid-latitude and low-altitude; and, mid-latitude and high-altitude. The influence of altitude in determining climate was more noticeable for low latitudes than for mid-latitude regions. For the deeper areas of a low-latitude lake, the latitudinal influence seems to be more relevant, as those lakes have a more stable stratification throughout the year. Anthropogenic activities (agriculture runoff, sewage discharge with low or no treatment and tourism) in the lake and its basin have a higher influence than the lake's position within the hydrological network on the trophic status of high altitude lakes. Water quality parameters were analysed in Puno Bay, as an example of high altitude lake at low latitude. The increasing trend of parameters related to turbidity in Inner Puno Bay seemed to indicate the shift to a more turbid state for this area of Puno Bay. The limited exchange between Inner Puno Bay (IPB) and Outer Puno Bay (OPB) along with a higher external loading of nutrients for IPB than for OPB may play a role for IPB to have significantly higher values of water quality parameters. As IPB is a shallow water body, it is more exposed to the effect of the wind and the effect of daily temperature variation. Moreover, the connection of OPB with Lago Mayor may also enhance the mixing and the dilution of nutrients in OPB.

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Abbreviations

ANA Autoridad Nacional del Agua [National Authority of Water]

ALT Autoridad Binacional del Lago Titicaca [Binational Authority of the Lake

Titicaca]

BOD₅ Biological oxygen demand

Chl-a Chlorophyll-a concentration

DO Dissolved oxygen concentration

EC Electrical conductivity

IMARPE Instituto del Mar del Perú [Sea Institute of Peru]

IPB Inner Puno Bay

IUCN International Union for Conservation of Nature

NO₃-N Nitrate-nitrogen concentration

OPB Outer Puno Bay

PAR Photosynthetically active radiation

PO₄-P Orthophosphate as phosphorus concentration

T Temperature

TDPS Lake Titicaca, Desaguadero River, Lake Poopó and Salar de Coipasa system

TN Total nitrogen concentration

TP Total phosphorus concentration

TSS Total suspended solids

UV-A Ultraviolet A radiation

UV-B Ultraviolet B radiation

UVR Solar ultraviolet radiation

Chapter 1

Introduction

Lakes are systems that provide important ecosystem services including water purification, carbon sequestration, biodiversity support (Zedler and Kercher 2005) microclimate regulation (Wong et al. 2017) and recreation (Völker and Kistemann 2011). Algal blooms are an increasing threat to lakes worldwide (Ho et al. 2019). These phenomena are caused by the rapid propagation of phytoplankton, which is the base of the aquatic food web. During algal blooms, the turbidity increases reducing the photic zone, the oxygen in the water can be depleted and even some species of phytoplankton may produce toxins (Van Dolah 2000). As a consequence, severe impacts to aquatic organisms and humans may occur (Romanowska-Duda 2002).

Several factors influence the proliferation of algae such as nutrient loading, solar radiation, temperature and community structure. Among these, the main factors are nutrients, solar radiation and temperature (Dou et al. 2019). Nutrients, mainly nitrogen (N) and phosphorus (P), have a positive influence on algal growth (Vollenweider 1968). Components of solar radiation are photosynthetically active radiation (PAR) and ultraviolet radiation (UVR). The former is used by autotrophic organisms to perform photosynthesis while the latter can inhibit the photosynthesis process (Kinzie et al. 1998). Although some species of algae have developed protection mechanisms to deal with relatively high levels of solar radiation (Kinzie et al. 1998). The increase in temperature affect positively the growth rate of phytoplankton (Lürling et al. 2013) and affects the dominant species (Yu et al. 2014). Solar radiation and temperature are influenced by latitude (Lewis 1996) and altitude (Barry 1981, as cited in Körner 2007). As a result, there are higher levels of solar radiation and small annual variation of temperature for low-latitude regions. And higher levels of solar radiation and lower temperature for highaltitude regions of the same latitude. The extent of the impact of solar radiation and temperature can be determined by lake-specific characteristics such as size and depth (Scheffer 1998; Woolway et al. 2017).

High-altitude lakes at low latitudes are exposed to particular conditions that affect light and temperature. Solar radiation level increases as the regions get closer to the equator and increase more at higher altitudes (Greenland 2005). For the same latitudinal regions, the temperature at high-altitudes is lower than at sea level (Körner 2007). High-altitude lakes are typically oligotrophic, but lakes Waiau, USA (Kinzie et al. 1998), Yahuarcocha, Ecuador (Van Colen et al. 2017) and Titicaca, Bolivia (Achá et al. 2018) have reported algal blooms. The main sources of nutrient pollution for these water bodies were agricultural runoff and sewage discharge with deficient or null treatment. These pressures along with the increase in global temperature may turn high-altitude lakes vulnerable to algal blooms (Burpee and Saros 2020).

Lake Titicaca is an example of a high-altitude (3810 m.a.s.l), low latitude (15° south of the equator) lake ecosystem. It is located in the Andes mountain range and is shared by Peru and Bolivia. Lake Titicaca is the largest freshwater lake in South America and 5 million people (Pouilly et al. 2014) rely, directly or indirectly, on its resources (e.g., fish and freshwater) and benefit from visiting tourists. Due to its importance for wildlife that live on its shoreline wetlands, this lake has been included as a Ramsar site of international importance (MINAM 2013). Despite its protected status, two of the most populated cities on the Peruvian side of the

lake discharge their effluents in Puno Bay (ALT 2014). Overall, Puno Bay waters are considered mesoeutrophic, and the area next to Puno city (Inner Puno Bay) is hypereutrophic. The rest of the lake, mainly in the deeper areas, remains oligotrophic (Lazzaro et al. 2016). Algal blooms in Inner Puno Bay were first reported in 1992 (Northcote 1992), while the first documented algal bloom in the southern part of the lake (Bolivia) occurred in 2015 after an intense rain period (Achá et al. 2018). Thorough studies about processes that drive eutrophication were done in the 1980s (Northcote 1992) and nowadays they are used as a reference for comparison with recent data (Lazzaro and Gamarra Peralta 2014). More recently, cyanobacteria species with the potential to develop algal bloom (Montoya et al. 2014) have been studied and a model to predict algal blooms has been developed (Duquesne et al. 2021).

1.1 Problem statement

Research about eutrophication and algal bloom driving factors for lakes has been done historically in mid-latitude regions, although since the mid-1970s, there has been an increasing amount of literature for aquatic ecosystems in low latitude regions (Talling and Lemoalle 1998). However, most of the studies on high-altitude lakes have been performed in temperate regions, and there is limited information for high-altitude lakes at low latitudes (Catalan and Donato Rondón 2016). For Puno Bay (Lake Titicaca), there is little knowledge about the ecological and biogeochemical functioning of the lake and specifically little understanding about the nutrient limitation and primary production (Lazzaro and Gamarra Peralta 2014). Although the specific characteristics of Lake Titicaca have been studied, the integration of this knowledge with the effect of latitude and altitude could help to improve understanding of algal bloom driving processes. Thus, this study is aimed to relate the effect of altitude and latitude for solar radiation and temperature and the interaction of these factors with the depth. Moreover, the nutrient loadings (N and P) are also considered.

1.2 General objective

To study the interaction of algal bloom driving factors within the environment in high altitude lakes at low latitude and to identify differences for lakes at different latitudes and altitudes. To achieve this goal, specific objectives have been defined and the type of data required to achieve them has been identified. The relationship between the specific objectives are shown in Figure 1.2-1.

1.2.1 Specific objectives

Specific objective 1: To construct a conceptual model with processes and environmental factors driving algal blooms in lakes.

Specific objective 2: To compare algal blooms at process level in lakes at different altitudes and latitudes.

Specific objective 3: To determine correlations between water quality parameters measured in Puno Bay for the period 2010-2020.

Specific objective 4: To identify similarities or differences between results for Puno Bay and findings for other high-altitude lakes at low latitude.

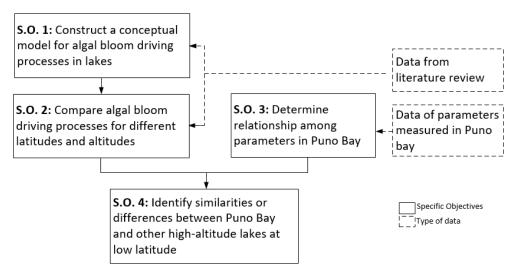


Figure 1.2-1 Relation between the specific objectives and type of data required

1.2.2 Research question

The numbering of each research question shows the link with the specific objectives:

Research question 1.1: How are process driving algal blooms related to solar radiation, nutrients and depth of the lake?

Research question 2.1: How latitude and altitude affect factors and processes that promote the growth of algal bloom?

Research question 3.1: What is the correlation between temperature, dissolved oxygen, total nitrogen, total phosphorus and chlorophyll-a in Puno Bay for the period 2010-2020?

Research question 4.1: What particular conditions in Puno Bay (human activities and natural conditions) have the potential to favour the processes that drive eutrophication in Puno Bay?

1.3 Justification

The study of factors that promote algal blooms in high-altitude lakes at low latitudes aims to contribute to the understanding of this phenomenon in Puno Bay and in similar systems that are affected by human activities. A better understanding of the factors and processes related to algal blooms for a specific region could help to develop models and to better inform the decision-makers. This understanding could contribute to the sustainable management of Lake Titicaca for the benefit of the people who live in the region. Thus, enabling the preservation and continuity of ecosystem services provided by this lake. In this manner, this research will contribute to the targets of goals 6 (Clean water and sanitation) and 14 (Life below water) of the Sustainable Development Goals.

In this chapter, the literature that gives the theoretical framework for this study is presented. The aim of this chapter is to present topics that are aligned with the objectives proposed in Chapter 1 (Section 1.2.1), which are going to be used during the interpretation of the results (Chapter 4) and for the discussion (Chapter 5). First, the algal bloom driving factors will be addressed, followed by a brief classification of lakes by their trophic status. Then, the occurrence of alternative stable states will be presented. Finally, the case study will be presented with a brief description of Lake Titicaca followed by the area of study: Puno Bay.

2.1 Algal bloom driving factors

It is common to find in the literature that eutrophication and algal blooms are referred to without distinction. However, eutrophication is the natural or anthropogenic enrichment of water bodies with nutrients, while algal blooms refer to the rapid increment and accumulation of microalgae biomass, often dominated by single species. Phytoplankton is free-floating microalgae that can be composed of bacteria, protists and unicellular plants. Green algae (chlorophytes and charophytes) and diatoms are considered to be ubiquitous photosynthetic organisms in lakes (Boney 1981; Agusti et al. 2015). While cyanobacteria can live under extreme conditions and can tolerate environmental fluctuations (Whitton 1992). Phytoplankton are primary producers and the base of the aquatic food chain. The magnitude and duration of algal blooms depend on a variety of factors such as solar radiation, temperature, nutrients and grazing.

2.1.1 Solar radiation

Lakes near the equator are exposed to higher levels of solar radiation than other latitudes. This is attributed to more direct exposure to sunlight and the thinner ozone layer at low latitudes (Gleason et al. 1993). Moreover, high altitude lakes receive a higher irradiance as the sunlight undergoes less absorption and scattering due to the shorter distance (Blumthaler et al. 1997). Thus, those high-altitude lakes over low latitudes receive much higher solar radiation levels than other lakes.

Components of solar radiation are photosynthetically active radiation (PAR) and ultraviolet radiation (UVR). Unlike mid-latitude lakes, where the photic conditions vary between an abundance of light in summer and low light intensity during the rest of the year. In low latitude regions, solar radiation is regular throughout the year. In both latitudinal regions, the altitude influences the photosynthetically active radiation (PAR). Higher altitudes mean higher PAR for the same latitudinal zone under clear conditions. While, under cloudy conditions, PAR decrease at higher altitudes (Wang et al. 2007). While higher altitudes contribute to greater exposure to ultraviolet radiation (UVR) in low (Liley and Mckenzie 2006) and mid-latitudes (Blumthaler et al. 1997). Although low latitudes receive the highest effect due to the proximity to the equator (Liley and Mckenzie 2006). In high-altitude lakes at low latitudes, a negative effect of UVR in photosynthesis was found (Kinzie et al. 1998). Given that high altitude lakes may have UVR levels up to 20 % higher than at sea level for a similar region, organisms that live there have evolved mechanisms of protection. Some algal mats seem to have some protection from UVR-

absorbing aminoacids. While planktonic cells on the surface of the lake seem to lack that protection as they exhibit the maximum photosynthesis inhibition at midday (Kinzie et al. 1998). Another mechanism to avoid UVR exposure used by species with motility is shading (Kinzie et al. 1998) and vertical displacement (Helbling et al. 2006).

Light availability affects the relationship between phytoplankton biomass and chlorophyll-a (Chl-a) concentration due to the higher concentrations of Chl-a per cell in conditions of low light availability (Osborne and Raven 1986). However, Corman et al. (2015) reported that Chl-a concentrations increased when increasing light availability for experiments under shifting light conditions.

2.1.2 Temperature

The effect of temperature on phytoplankton productivity has been widely studied. When light is not a limiting factor, higher temperatures lead to an increment in the photosynthetic activity of phytoplankton (Lewandowska et al. 2012). When nutrients are not a limiting factor, higher temperatures increase phytoplankton growth rate (Staehr and Sand-Jensen 2006). Although the temperature effect differs depending on the phytoplankton community composition due to differences in their optimal environmental conditions. For instance, the mean optimum growth temperature for chlorophytes (26.3 °C) is slightly lower than for cyanobacteria (27.2 °C). Moreover, at 20 °C, the growth rate of chlorophytes is significantly higher than those of cyanobacteria (Lürling et al. 2013). These differences influence the phytoplankton dominant population. As reported for warmer lakes where a higher percentage of cyanobacteria was found (Kosten et al. 2012).

The temperature also influences the characteristics of the zooplankton population. The size at maturity is reduced and the reproductive cycles are shortened (Hanazato and Masayuki 1985). Elevated temperatures reduce the ingestion efficiency of zooplankton (Rall et al. 2010) and increase their growth rates (Hall and Burns 2002). In the latter case, it might increase the grazing of phytoplankton. Moreover, increasing temperatures are causing the shift of the zooplankton genus *Daphnia* to *Bosmina* as the dominant one (Li et al. 2016).

2.1.3 Stratification and mixing

Stratification and mixing in lakes may influence algal blooms, as it allows the transportation of nutrients in the sediments to parts of the lake where light is available. Thermal stratification is the most common form of stratification and occurs due to differences in density. The warmer layer of water remains above until it gets cold either by diurnal or seasonal variation. For temperatures above 4 °C, as the upper layer gets colder its density increases. This change of density contributes to disrupt the stratification and mixing occur. As the relationship between water temperature and density is non-linear when the temperature increase in 1 °C, a larger change in density occurs for temperatures above 4 °C (Figure 2.1-1). Thus, stratification of warmer water would be more stable (Bade 2005).

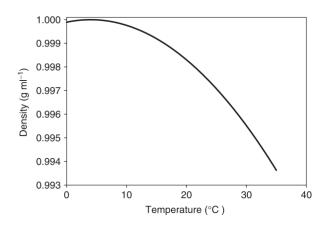


Figure 2.1-1 Non-linear relationship between water temperature and density (Bade 2005).

As the temperature is influenced by latitude and altitude, stratification and subsequent mixing in lakes is also influenced by latitude and altitude, as shown in Figure 2.1-2. Depending on the frequency that the layers of water intermix. In monomictic lakes, layers of water are mixed only once per year. Cold monomictic lakes are ice-covered most of the year and mixed when after ice melt. In these lakes, the temperature is always below 4 °C. While in warm monomictic lakes the water temperatures temperature never goes below 4 °C. In dimictic lakes, stratification occurs in summer and winter, and mixing periods occur during spring and fall. In polymictic lakes, mixing occurs several times per year. It occurs mainly in lakes no deep enough to develop thermal stratification. Amicitic lakes are covered permanently with ice and no mixing is expected. In oligomictic lakes, the water is generally warm and stratified, with occasional mixing. These lakes are found at low latitudes, where there is an almost regular temperature during the year (Wetzel 2001).

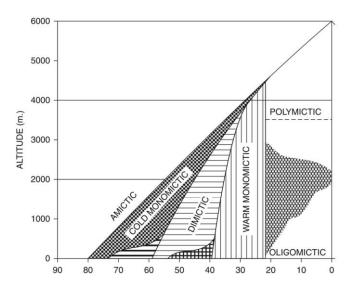
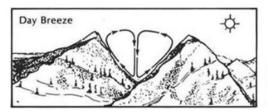


Figure 2.1-2 Thermal stratification in lakes according to altitude and latitude (Wetzel 2001).

No-labeled areas represent transitional regions. Broken vertical bars in between polymictic and oligomictic lakes represent mixed types of lakes, mainly warm monomictic.

Mixing of lakes may occur also by the effect of wind. The global wind direction varies depending on factors such as proximity to oceans and latitude (Lamb 1975). For high-altitude lakes, there is an additional interaction of the air mass with the valley slope. During the day,

the air close to the mountain slope gets warmer due to solar radiation. As the air gets warmer, it becomes less dense and tends to flow upslope (Figure 2.1-3). During the night the opposite occurs. These phenomena occur independently of the latitudinal zone and are most evident in calm and cloudless weather (Oliver and Fairbridge 2005). The cases of plateaus at high altitudes are different because of the lack of slope (Körner and Ohsawa 2005).



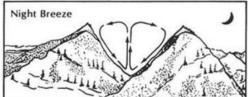


Figure 2.1-3 Wind circulation during the day (mountain wind) and night (valley wind) in mountains (Oliver and Fairbridge 2005).

2.1.4 Trophic lake classification

Eutrophication may occur naturally in a long term due to lakes aging (Carpenter 1981) or due to anthropogenic pressures, such as sewage effluents or agricultural runoff. The eutrophication caused by human activities is called cultural eutrophication. In the past, it was generally accepted that phosphorus was the main factor for eutrophication (Feuchtmayr et al. 2009) but nowadays It is now well established from a variety of studies that both phosphorus and nitrogen are involved (Elser et al. 2007; Dalu and Wasserman 2018). Table 2.1-1 shows the lake classification according to their trophic status using the Organization for Economic Cooperation and Development criteria (OECD 1982).

Trophic category	TP, mean (μg L ⁻¹)	Chl-a, mean $(\mu g \text{ Chl-a L}^{-1})$	Chl-a, max. $(\mu g \text{ Chl-a L}^{-1})$	Secchi depth, mean (m)
Ultra-oligotrophic	<4	<1	<2.5	>12
Oligotrophic	<10	<2.5	<8	>6
Mesotrophic	10–35	2.5–8	8–25	6–3
Eutrophic	35-100	8-25	25-75	3-1.5
Hypereutrophic	>100	>25	>75	<1.5

Table 2.1-1 Classification of the trophic status (OECD 1982).

2.2 Alternative stable states

The idea that communities can have several possible stable states was proposed in the late 1960s (Lewontin 1969). Alternative stable states theory proposes that for an ecosystem there are several self-reinforcing states. The dominant state will be the result of the current conditions and the previous state (Scheffer et al. 1993). The shift of state may be triggered by perturbation events that can be natural, e.g., seasonal changes, wind, internal nutrient loading (Bade 2006) or anthropogenic, e.g., sewage discharge, agriculture runoff (Janssen et al. 2014). The level of resistance to change (resilience) of each ecosystem may be reduced gradually as the disturbance

persists. Once the conditions have changed enough, an abrupt transition occurs as shown in Figure 2.2-1 (Scheffer et al. 2001). This change alters the structure and dynamics of the ecosystem and cannot be reversed by restoring the conditions before the perturbation (F2). Instead, further reverse in the conditions (F1) will be required to achieve a state similar to that before the perturbation (Scheffer et al. 2001). This phenomenon is known as hysteresis. Alternative stable states have been studied mainly for shallow lakes (Scheffer et al. 2001; Janssen et al. 2014). Two states were distinguished for shallow lakes: one with clear water dominated by macrophytes and the other with high turbidity dominated by phytoplankton (Scheffer et al. 1993).

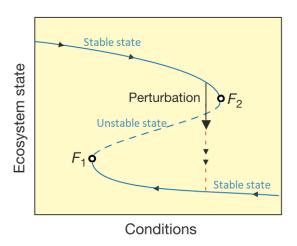


Figure 2.2-1 Alternative stable states and hysteresis. (Adapted from Scheffer et al. 2001); F2= environmental conditions before the shift; F1= another shift point.

2.3 Case study description

2.3.1 Lake Titicaca

Lake Titicaca is the highest navigable water body in the world. It is located in the Andean high plateau at 3810 m above sea level, in the southern border of Peru and northwest of Bolivia. This lake is comprised of two water bodies connected by the narrow Strait of Tiquina. The larger water body is located in the north and is called Lago Mayor or Chucuito. Puno Bay is part of Lago Mayor and is located in the northeast part of the lake. The second water body is in the south and is called Lago Menor or Huiñaimarca (Figure 2.3-1).

The water inflow to the lake is mainly through precipitation (55 %), rivers (44 %) and groundwater (1 %). While the main output is through evaporation (95 %) and a minor output is through the Desaguadero River (5 %). The main tributary is the Ramis River, followed in magnitude order by Coata, Ilave, Huancané and Suches. The inflow through these five rivers accounts for 85% of the river input. Lake Titicaca's only effluent is the Desaguadero River, which ends up in Lake Poopó, Bolivia (Martínez Gonzales and Zuleta Roncal 2007). The basins of Lake Titicaca, Desaguadero River, Lake Poopó and Lake Coipasa (*Salar de Coipasa*) comprise an endorheic hydrological system called the TDPS System (Martínez Gonzales and Zuleta Roncal 2007; ANA 2014a).

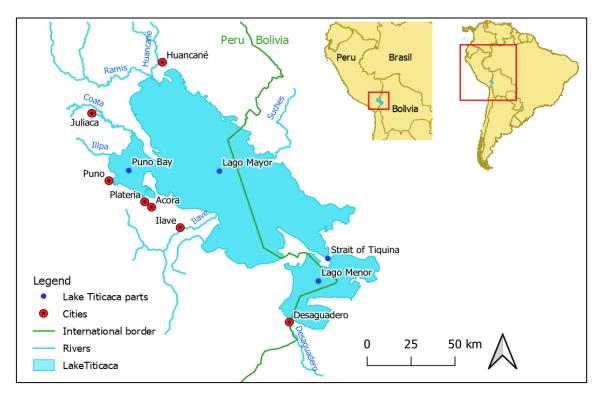


Figure 2.3-1 Lake Titicaca, Lago Mayor (North), Lago Menor (South) and main rivers. Map made with QGIS. Map data sources: OpenStreetMap, Google Satellite.

Lake Titicaca is an important water resource for five million people (Pouilly et al. 2014), being used for agricultural and domestic purposes. Lake Titicaca is also associated with cultural traditions dating back to the Inca civilisation and even before (UNESCO 2019). Moreover, Lake Titicaca as a touristic place attracts large public (Francisco Revollo et al. 2005). This lake is essential for the life of unique flora and fauna species with high endemism. To protect this biodiversity, the Titicaca Nacional Reserve was established in 1978. The part of the reserve located in Puno Bay (Figure 2.3-2) covers an area of 291 km² (INRENA 2002). This area is the habitat of endemic species such as fish of the genus Orestias, the aquatic bird Rollandia microptera, the suri enano (Pterocnemia pennata), the giant frog Telmatobius coleus and the totora (Schoenoplectus californicus, syn Scirpus californicus) (Monroy et al. 2014; Lazzaro et al. c2016; INRENA 2002). The totora is emergent vegetation used not only as a shelter for fish, amphibians and birds but also provides construction material for building floating homes and boats by the Uros community. A buffer area around the Titicaca Nacional Reserve has been established to guarantee the conservation of the protected area. The buffer area covers completely Puno Bay. To ensure the preservation of Lake Titicaca and the water bodies related to the lake, the Binational Authority of the Lake Titicaca (Autoridad Binacional del Lago Titicaca [ALT]) was created in 1996. This institution was created by the governments of Peru and Bolivia who agreed on the joint management of resources of the lake and its basin. Since then, the ALT is responsible for water management in the TDPS system. Besides, national and international regulations have been approved in an attempt to ensure the preservation of this ecosystem (Appendix C). However, anthropogenic pressure without control is applied to this system nowadays.

According to Lazzaro et al. (2016), in the period 1970-1990, the waters of Lago Mayor and Lago Menor presented high transparency and nitrogen as limiting nutrient. Both water bodies were then oligotrophic. However, nowadays only the deep region of Lago Mayor is oligotrophic

while Lago Menor and the shallow parts of Lago Mayor are increasing their eutrophication (Table 2.3-1).

Waterbody	Surface (km²)	Mean depth (m)	Max. depth (m)	Trophic status
Lake Titicaca ¹	8559	171	283	Mesotrophic
Lago Mayor ²	7131	125	284	Ultraoligotrophic
Lago Menor ²	1428	9	42	Mesotrophic
Puno Bay ³	589	14	37	Mesotrophic
Inner Puno Bay ⁴	16.1	2.7	8	Hypereutrophic

Table 2.3-1 Characteristics of water bodies in Lake Titicaca

2.3.2 Puno Bay

Puno Bay (also known as Chucuito Bay) is located in the northwestern part of Lake Titicaca, in the Peruvian section of the lake. Its depth is lower than Lago Mayor from which is separated in a way that reduces their interaction. Puno Bay is divided geographically into two sections: Inner Puno Bay (IPB) and Outer Puno Bay (OPB) (Figure 2.3-2). Inner Puno Bay is shallow, with low flow, and is located next to Puno city. The high input of organic matter coming from urban sewage of Puno city combined with its depth and morphology made hypereutrophic this part of the Bay (Northcote 1992; Beltrán Farfán et al. 2015). For this reason, Puno Bay has been identified as one of the most polluted and the most prone to algal blooms in the previous years. The other shallow part of the lake is Lago Menor and faces similar issues in terms of pollution and its duration (Northcote 1992; Molina et al. 2017).

Inner Puno Bay is considered to have the major problem of and eutrophication in the whole lake (ALT 2014). Although, high levels of eutrophication have also been reported for Cohana Bay in Bolivia (Molina et al. 2017). The direct discharge of sewage in Puno Bay is worsening the condition of hypereutrophic of some areas of this Bay, and this fact has been reported in studies and official reports (ALT 2014; Beltrán Farfán et al. 2015). While Outer Puno Bay is deeper than Inner Puno Bay, the latter share some characteristics of shallow lakes such as the depth and frequent mixing of water. The evaporation rate is higher in this lake is 95 % of total water outflow (Martínez Gonzales and Zuleta Roncal 2007), which might increase its eutrophication tendency as there is no outflow of water containing nutrients.

¹⁾ Richerson et al. (1992); 2) Iltis (1992); 3) Montoya et al. (2014); 4) Beltrán Farfán et al. (2015).



Figure 2.3-2 Puno Bay and its main sections

Map made with QGIS. Map data sources: OpenStreetMap, Google Satellite.

Climate

The climate in the region where Lake Titicaca is located is semi-dry and cold. Phenomena such as freezing occur commonly with a higher incidence in winter (June to August). Despite the frequent occurrence of freezing periods, the lake itself and the area surrounding the lake are the less affected areas due to regulating effect of the water. Areas around the lake have less than 150 days of freezing per year, while areas away from the lake can have more than 300 days of freezing in a year (Martínez Gonzales and Zuleta Roncal 2007).

Due to proximity to the Equator, the air temperature in the north is warmer than in the south of the lake (Martínez Gonzales and Zuleta Roncal 2007). The air temperature measured by the closest station to Puno Bay shows that there is a low interannual variation for the period 2010 – 2012 (Figure 2.3-3). However, variation within the year is visible for the temperature during the night, which can descend up to -2 °C during winter (June – September). The mean diurnal variation for the period December – February is 10 °C, while for June - August it increases to 15.7 °C.

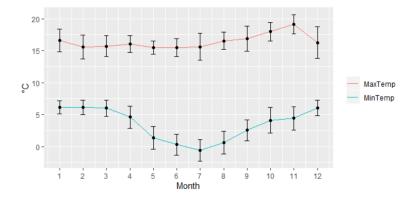


Figure 2.3-3 Air temperature for Puno Bay during the period 2010 – 2012 (SENAMHI 2012)

The sunshine near Lake Titicaca is 3000 h year⁻¹ in Puno. The minimum value is 180 hours in January and February, during the maximum rainfall, and the maximum value is 296 hours in mid-winter (ANA 2013). In Puno, the mean number of sunshine hours per day changes from 9.6 in July to 6.0 in January (Martínez Gonzales and Zuleta Roncal 2007). While the precipitation in the north of Lake Titicaca is higher than in the south. With mean precipitation of 650 mm/year and a maximum of 800 to 1400 mm in the centre of the lake (Roche et al. 1992). In Puno Bay, almost 80 % of the rainfall occurs from December to March (Figure 2.3-4). During this period the precipitation can go up to 240 mm month⁻¹. The period with minimum precipitation from May to August, although some years without precipitation during this period.

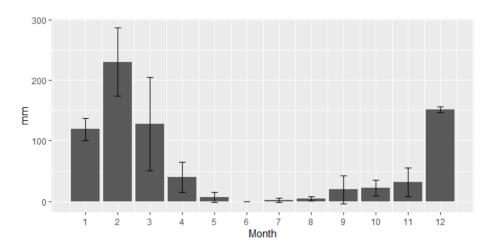


Figure 2.3-4 Precipitation for Puno Bay during the period 2010 – 2012 (SENAMHI 2012)

Local relief patterns influence surface wind direction. In Lake Titicaca, the circulation of wind is also influenced by the temperature differences between the lake and the land. In the daytime, the land becomes warmer than the lake, then the air goes from the lake towards the shore. At night, the air from the land becomes cooler and denser than the lake, then it flows towards the lake (Oliver and Fairbridge 2005; Martínez Gonzales and Zuleta Roncal 2007). In the northern sector of Lake Titicaca, calm winds predominate throughout the year. The speed values may go up to 4.8 m s⁻¹. The wind speed is usually higher in winter than in summer by 1 m s⁻¹ (Martínez Gonzales and Zuleta Roncal 2007). Rainy months (summer) are usually companied by dominant winds from the north-east during the rainy season and from the west to the south-west the rest of the year (ANA 2013).

Hydrology

The water mean residence time in Lago Mayor is about 75 years (Gonfiantini 2002). While, Inner Puno Bay is a semi-enclosed water body, with scarce water renewal, due to the presence of barriers formed by *totora* that separate it from the Outer Puno Bay. It has very low water surface currents (Figure 2.3-5) with velocities between 0.54 to 19.31 cm s⁻¹. The highest speed for water surface current was recorded near the navigation channel and with a predominant direction towards the southwest.

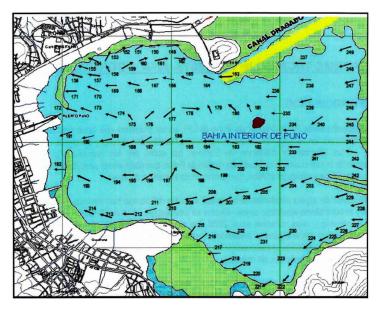


Figure 2.3-5 Map of currents in Inner Puno Bay during May 2002 (PELT 2002, as cited in ANA 2013)

External nutrient sources

Puno Bay is close to the main cities in the Peruvian side of Lake Titicaca: Puno and Juliaca. Puno city has a stabilization pond for sewage treatment that is oversaturated and in a deficient operational condition (ALT 2014). Thus, the sewage is discharged in Inner Puno Bay (Martínez Gonzales and Zuleta Roncal 2007; Beltrán Farfán et al. 2015). A similar situation occurs in Juliaca city, a deficient stabilization pond discharges the sewage in Coata River (ALT 2014). This river then ends up in the north part of Outer Puno Bay (Figure 2.3-6a). Besides, urban centres and rural areas have a poor capacity for solid waste collection and treatment. For this reason, solid waste is thrown away in waterways and the lake shorelines (Figure 2.3-6b). For the population around the lake, it has been estimated that 100 kg of solid waste per inhabitant per year is dumped around the lake (PNUMA 2010, as cited in Agreda-Corrales 2015).

Aquaculture using floating cages in Lake Titicaca started in 1977. This consists of metallic nets where trout fingerlings are sowed. The main species of farmed fish is the rainbow trout (*Oncorhynchus mykiss*) (Chura and Mollocondo 2009). The conversion rate is approximately 2 kilos of feed per 1 kilo of fresh trout production. In this regard, the Binational Master Plan (Peru – Bolivia) recommended to avoid the installation of the floating cages in shallow areas to reduce eutrophication risk due to the feeding process (INTECSA-AIC-CNR 1993). Figure 2.3-6 shows that the floating cages are located in Outer Puno Bay where the mean depth is 14 m (Table 2.3-1). In the outer part of the Bay, the Uros community is located in the water current called Rio Willy. There are several groups within the community and the total population arises to 681 people (MINCUL 2019). The main activity is the tourism to the floating islands and the subsequent transport. Other activities performed are *totora* extraction and fishing.

Another important activity in the region is mining. Gold mining is the main source of mercury contamination with exploitation centres located close to the Ramis River (Guédron et al. 2017). Moreover, acid mining drainage is released from closed mines, enabling the lixiviation of metals. As a result, silverside fish (*Basilichtys bonaerensis*) from the Puno Bay have been found with concentrations of mercury and arsenic exceeding the limits for human consumption (Martínez Gonzales and Zuleta Roncal 2007). According to Guédron et al. (2017), the eutrophication process may facilitate the methylation of mercury, enabling its accumulation in the present biota. Monroy et al. (2014) conducted a study in the main lake fishing harbours and found concentrations of mercury, cadmium and lead of four fish species were above the limit

for human consumption established by the Food and Agriculture Organization, World Health Organization and European Commission. On the Peruvian side, 59 % of Lake Titicaca basin has herbaceous vegetation with subsequent livestock grazing. While agriculture activity occupies 14 % for temporary crops cultivation (Laqui Vilca 2019). Thus, Ramis River basin is affected by livestock grazing (Martínez Gonzales and Zuleta Roncal 2007) and mining, while Coata River is polluted with urban and industrial waste.

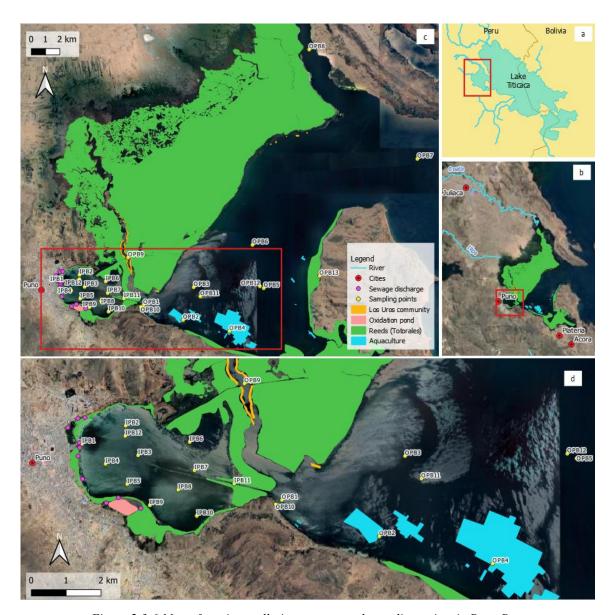


Figure 2.3-6 Map of nutrient pollution sources and sampling points in Puno Bay.

a) Lake Titicaca map; b) Puno Bay map; c) Nutrient pollution sources and sampling points for Inner Puno Bay (IPB) and Outer Puno Bay (OPB); d) Close view of sampling points in IPB and OPB. ANA sampling points (IPB1-11, OPB1-9), IMARPE sampling points (IPB12, OPB10-13). Map made with QGIS. Map data sources: OpenStreetMap, Google Satellite, ANA, IMARPE, Municipality of Puno.

Algal species and algal blooms

Green algae of genus *Chara* spp. were abundant in some shallow areas of Lake Titicaca in the 1980s (Collot et al. 1983, as cited in Talling and Lemoalle 1998). In the last twenty years, accelerated growth of water lentils (*Lemna* sp.) being able to form large floating spots have

been reported for Inner Puno Bay (Martínez Gonzales and Zuleta Roncal 2007; ALT 2011; Lazzaro and Gamarra 2014). For Puno Bay, Montoya et al. (2014) found cyanobacteria species (*Microcystis aeruginosa*) forming blooms, which was not reported previously by other authors. During the algal bloom that covered a large fraction of the southern part of Lake Titicaca in March–April 2015, the dominant species was *Carteria* sp. (Acha et al. 2018). This algal bloom was replicated by Duquesne et al. (2021) using a nutrient–phytoplankton–zooplankton–detritus (NPZD) model.

3.1 Data collection for conceptual model and lake comparison

Specific objective 1 was to construct a conceptual model for algal bloom processes in lakes and their interaction with environmental factors, and specific objective 2 was to compare algal blooms processes for different altitudes and latitudes. To address these objectives, academic journals, thesis, technical reports, government websites and websites of research institutes were consulted. Primary and secondary data were searched in electronic databases (Springer Link, Google Scholar and Science Direct) and electronic libraries from the United Nations Educational, Scientific and Cultural Organization (UNESCO-UNESDOC) and International Union for Conservation of Nature (IUCN). Depending on the expected information, a combination of several keywords was used. Combination of keywords regarding information of Puno Bay were searched in Spanish and English as shown in Table 3.1-1. The websites of government agencies and academic institutions that were consulted are shown in Table 3.1-2.

Table 3.1-1 Keywords for searching in databases

Expected information	Keywords
Factors and processes related to eutrophication in lakes	Lake, eutrophication, fertilization, nutrient enrichment, nutrient loads, limiting nutrient, interaction of light and nutrients, temperate climate, tropical climate, shallow lakes, deep lakes, stratification, temperature, seasonality, primary production, alternative stable states, top-down control, macrophytes, turbidity, phytoplankton, zooplankton, grazing, sediments
Algal bloom events in high- altitude lakes at low latitude	Algal bloom, phytoplankton, cyanobacteria, green algae, high altitude lake, mountain lake, limnology, tropical region, equatorial region
Algal bloom events in high- altitude lakes at mid latitude	Algal bloom, phytoplankton, cyanobacteria, high altitude lake, mountain lake, limnology, alpine lake, temperate region
Algal bloom driving processes in Puno Bay	Floraciones algales (algal bloom), fitoplancton (phytoplankton), nutriente limitante (limiting nutrient), eutrofización (eutrophication), celdas flotantes para aquicultura (culture cages), contaminación (contamination, pollution), descarga de efluentes (sewage discharge), limnología (limnology), actividades antrópicas (anthropogenic activities), monitoreo de calidad de agua (water quality monitoring), morfología (morphology), batimetría (bathymetry), hidrología (hydrology), clima (climate), estratificación térmica (thermal stratification), Lago Titicaca (Lake Titicaca), Bahía de Puno (Puno Bay), Bahía interior de Puno (Inner Puno Bay), Bahía exterior de Puno (Outer Puno Bay).

Table 3.1-2 Consulted websites and expected information

Website	Expected information
http://alt-perubolivia.org/web/	Land use, economic and ecological zonification of the TDPS system, reports of Lake Titicaca water quality scientific expeditions, pollution sources,
http://www.geotiticaca.org/web/	Land use, economic and ecological zonification of the TDPS system
http://siar.minam.gob.pe/	Hydrographic network, points of sewage discharge
http://satelite.imarpe.gob.pe/uprsig/ Titicaca/titicaca.html	Photosynthetically active radiation (PAR) and surface temperature of the lake
https://www.senamhi.gob.pe/	Maximum air temperature, minimum air temperature, precipitation, sunshine duration, wind direction and velocity
https://portal.munipuno.gob.pe/	Points of sewage discharge
https://pelt.gob.pe/	Land use, reports of Lake Titicaca water quality scientific expeditions, pollution sources,
https://www.undp.org/	Technical reports of pollution and water quality monitoring

3.2 Lake comparison

Low latitude regions are situated between 23.5°N and 23.5°S (Henry 2005), while middle latitude regions occupy regions up to 56°N and S (Perry 2005). Low-latitude and mid-latitude lakes were compared. Studies were selected to have lakes at high altitude (above 1500 m.a.s.l.) and low altitude (below 1500 m.a.s.l.) for each latitudinal zone. The selection of lakes was based on available studies that reported nutrients and chlorophyll. Since these parameters are indicators of trophic status, and this is strongly related to algal blooms. For each region (low-latitude and low-altitude, low-latitude and high-altitude, mid-latitude and low-altitude, mid-latitude and high-altitude) and depth (shallow and no shallow) at least two lakes were selected. Priority was given to articles containing the study of two or more lakes to reduce the variation in the methodology used. Only for Lake Victoria and Lake Titicaca more than one study was considered. For Lake Victoria, due to the availability of studies and for Lake Titicaca because it is the object of study.

First, latitude, altitude, depth, climatic classification, trophic aspects and reported algal blooms were compared. Each lake was assigned the first letter according to the Köppen-Geiger climate classification map (A = tropical, B = arid, C = temperate, D = cold, E = polar). Two letters were considered when two climates affected different regions of the same lake, according to the Köppen-Geiger map (Beck et al. 2018). Lake depth was considered as reported for the area of the lake that was studied. Shallow lakes were considered to have less than 5 m depth, while no shallow lakes were those with a depth higher than 5 m. Regarding trophic status and algal bloom, information reported by the studied lake was considered. Whether this information was not included, other studies were used to fill this information. The column of the main source of eutrophication and subsequent columns were filled only with information reported in the article cited in the first column.

Other aspects compared were total nitrogen, total phosphorus, total nitrogen to total phosphorus ratio and chlorophyll-a concentrations. The minimum and the maximum concentration reported by the studies were considered for each parameter. Data reported as mean \pm standard deviation was converted to a range. The minimum concentration was obtained by subtracting the standard deviation from the mean and the maximum concentration was obtained by adding the standard

deviation to the mean. When the same study reported the data for two areas of the lake, the different areas were considered separate.

3.3 Water quality data collection for Puno Bay

Specific objective 3 was to determine the relationship among water quality parameters measured in Puno Bay for the period 2010-2020. This objective was addressed by analysing water quality data for Puno Bay. The data was obtained from two Peruvian institutions: the Sea Institute of Peru (*Instituto del Mar del Perú* [IMARPE]; IMARPE 2020) and the National Authority of Water (*Autoridad Nacional del Agua* [ANA]; ANA 2013; ANA 2014b; ANA 2014c; ANA 2016a; ANA 2016b; ANA 2017; ANA 2018; ANA 2019a; ANA 2019b; ANA 2019c). Parameters and measurement methods provided by ANA and IMARPE are in Appendix D. Data collected during eleven years (2010 – 2020) was analysed to determine the relationship between the parameters. Sampling points from each institution are shown in Figure 2.3-6. Data provided by IMARPE was at different depths for Outer Puno Bay (OPB). Only data collected at 0.5 m deep by IMARPE was analysed together with the ANA data set.

3.4 Water quality data analysis

All statistical tests were performed using R version 3.6.1 (R Core Team 2019).

3.4.1 Data handling

Comparison of data from ANA and IMARPE

A preliminary assessment to compare data collected by ANA and IMARPE was done to evaluate whether the data was comparable and able to be used as a single data set. First, sampling points that were located as close as possible to each other were chosen for Inner Puno Bay (IPB) and Outer Puno Bay (OPB). Then, common parameters to those sampling points were selected. Finally, tests for difference between ANA and IMARPE sampling points were performed. Whether the parameters had normal distribution and homogeneous variance, a t-test was used, otherwise a Mann–Whitney U test was performed.

Estimation of values below the limit of quantitation

All values reported as below the limit of quantification (BLQ) were considered for analysis after taking half of each BLQ value. The substitution of BLQ values with half the value of BLQ was found to be better than substituting with zero (Beal 2001).

Estimation of missing values

Chlorophyll-a (Chl-a) data was missing for almost half of the observations of the combined dataset, as shown in Appendix E. To perform further analysis, those missing values were estimated from transparency (SD) values using a non-linear regression. First, Chl-a and SD values were linearized using log transformation. Then, a type-II regression was performed to

predict log-transformed chlorophyll-a concentrations based on log-transformed SD values. The Reduced Major Axis (RMA) regression method was applied due to the use of log-transformed data. This calculation was done using 'lmodel2' package version 1.7-3 (Legendre 2018).

3.4.2 Comparison of Inner and Outer Puno Bay

The datasets (ANA and IMARPE) were combined and a Mann–Whitney U Test for significance difference between parameters from Inner Puno Bay and Outer Puno Bay was performed. Moreover, the mean and range for each parameter was calculated. To compare a possible effect of depth, the parameters compared were temperature, pH, dissolved oxygen and conductivity. To compare a possible effect of sewage discharge and depth, the parameters compared were total phosphorus, total nitrogen, orthophosphate as phosphorus, nitrate-nitrogen, chlorophyll-a, biological oxygen demand, total suspended solids and total nitrogen to total phosphorus ratio.

3.4.3 Correlation analysis

A correlation analysis was performed to assess the relationships between variables. Given the significant differences found for IPB and OPB, separate correlation analysis were performed for each section of Puno Bay. For correlation analysis, variables common to as many sampling sites as possible were chosen and all the available observations were included. This analysis was done using the package 'PerformanceAnalytics' version 2.0.4. (Peterson and Carl 2020) which enables to obtain the significance p-value along with histogram for each variable and scatterplots for each bivariate correlation.

3.4.4 Factor analysis

For factor analysis, variables common to as many observations as possible were chosen. To be able to perform the analysis, the parameters with missing values were not considered.

Assumptions in factor analysis

All the statistical analyses were performed with a significance level of 0.05. To assess empirical normality, the Shapiro test was used. The assessment was done by combining information from the empirical (p-value) and graphical method (histogram). Whether the departure from normality was not extreme, the variable was used in its original form. Whether extreme departure from normality was found, the variable was transformed to achieve normality. To transform variables, square root, cube root, logarithms, square, and cube $(X^2 \text{ and } X^3)$ transformations were evaluated. In case normality was not achieved after a transformation the variable was removed. Then, Bartlett's Test of Sphericity was performed before proceeding with factor analysis to verify that a data reduction technique can be performed. It tested whether the correlation matrix (Pearson method) was significantly different from an identity matrix. Missing values were removed from the data set to perform factor analysis.

Extraction of factors

The number of factors to extract was determined using multiple decision criteria. First, the scree test criterion was used. This criterion states that the point where the curve first begins to become a horizontal line indicates the number of factors that should be extracted for the analysis. Then, the overall variance accounted for the number of factors suggested by the scree test criterion was verified.

Factor interpretation and factor scores

Once the matrix of factors was obtained, a guideline based on sample size was used (Table 3.4-1) to determine the significance of factor loadings. Using the factor loadings, the variables for each factor were identified. Then factor scores for each group of sampling points were calculated using the 'psych' package version 1.9.12.31 (Revelle 2019). Sampling points within IPB were grouped according to their proximity to areas with predominant characteristics as shown in Table 3.4-2. After obtaining score factors for each group of sampling points, trends for different locations within IPB were compared. Finally, for each factor score a significance test was performed to verify if they were different between sampling points. Normality and variance homogeneity were tested using Shapiro and Bartlett test, respectively. These tests were done before performing the analysis of variance (ANOVA). Whether the assumptions were not satisfied, a non-parametric test was applied.

Table 3.4-1 Significant factor loadings based on the sample size

Factor loading	Sample size needed for significance*
0.55	100
0.60	85
0.65	70
0.70	60
0.75	50

^{*}Significance is based on a 0.05 significance level, a power level of 80% and standard errors assumed to be twice than conventional correlation coefficients (BMDP Statistical Software Inc. 1993, as cited in Hair et al. 2010)

Table 3.4-2 Grouping sampling points in Inner Puno Bay (IPB)

Group	Characteristics	Sampling points
Open	Center of IPB where the depth was higher than in the shorelines	IPB2, IPB3, IPB4, IPB7, IPB8 and IPB12
Reeds	Presence of totora reeds	IPB6, IPB10 and IPB11
Sewage	Close to sewage discharge points	IPB1, IPB5 and IPB9

4.1 Conceptual model

The lake components, factors and processes illustrated in Figure 4.1-1 were selected to compare algal bloom driving factors at different latitudes and altitudes (Table 4.1-1). Solar radiation increases at high altitudes and with this the ultraviolet radiation (UVR) increases (Blumthaler et al. 1997). Temperature decreases with the altitude and it results in lower temperatures for high-altitude lakes than for low-altitude lakes at the same latitudinal region. In low-latitude regions, there is a seasonal pattern of precipitation (Gerard Rooney et al. 2018). While, midlatitude lakes have seasonality caused by temperature fluctuation throughout the year (Perry 2005). Independent of the latitude, the temperature effect may depend on the depth of the lake (Bade 2005). Temperature variation in deep lakes allows the formation of thermocline, which is a layer dividing the upper mixed layer and the bottom layer. While, shallow lakes are not able to form thermocline and the water column is frequently mixed by diurnal variation of temperature and wind. For high-altitude lakes, the topography usually induces winds to interact with the valley slope creating breezes (Greenland 2005).

Interaction between the lake elements was depicted with arrows in Figure 4.1-1. External nutrients are carried in with the water inflow. This contributes to the nutrient stock in the lake, as do so nutrients from sediment resuspension (Schallenberg and Burns 2004). Nutrients are taken up by macrophytes and phytoplankton (Hilt (nee Körner) and Lombardo 2010). In midlatitude lakes, the seasonality determines the duration of available light for phytoplankton to perform photosynthesis (Fernández et al. 2017). Among the factors that influence phytoplankton growth rates are temperature and grazing. The temperature also influences the characteristics of the zooplankton population, as the size at maturity is reduced and the reproductive cycles are shortened (Hanazato and Masayuki 1985). While for macrophytes, the availability of light is affected by the turbidity (Hilt et al. 2010), which may be increased by algal blooms and sediment resuspension. Sediment resuspension can be influenced by winds mainly in shallow lakes (Schallenberg and Burns 2004) but can be limited by vegetation present at the bottom of the lake (Scheffer 1999). The sediments are enriched by macrophytes, phytoplankton and zooplankton when they decay and with the excretion of phosphorus by zooplankton (Alimov and Golubkov 2014).

There is very little published research on the role of fish in the food web of Lake Titicaca (Vaux et al. 1988; Northcote 2000), therefore it was not included in the analysis. Of the environmental factors considered, only solar radiation and temperature are affected by latitude and altitude. But the temperature can influence all other processes and elements occurring in the lake. Therefore, the analysis focuses on solar radiation and temperature and does not consider other factors such as lake size (Woolway et al. 2017). Since the extent of influence of solar radiation and temperature is determined by the depth, this factor was also included in the conceptual model. Each of the processes nitrification, denitrification (Ngugi et al. 2011), nitrogen mineralization, nitrate ammonification (Zhu et al. 2020), phosphorus mineralisation (Maerki et al. 2009) and immobilization (Wang et al. 2012) were not illustrated in the model but they were considered to be related to nutrient resuspension.

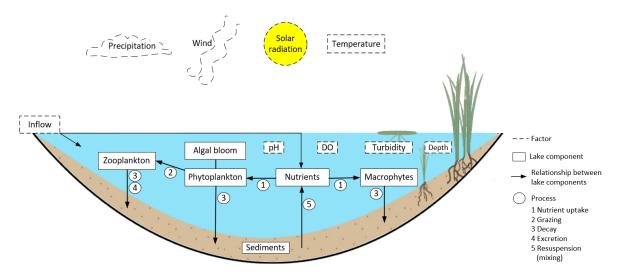


Figure 4.1-1 Conceptual model of algal bloom in lakes

Factor	Low-latitude low-altitude	Low-latitude high-altitude	Mid-latitude low-altitude	Mid-latitude high-altitude
UVR	High ¹	High ²	Low	High ³
Light availability	Constant	Constant	Variable	Variable
Temperature (annual)	Small range ¹	Small range ¹	Large range ¹	Large range ¹
Temperature (daily)	Small range ²	Large range ²	Large range	Large range
G	ъ.	ъ.	Temperature	Temperature

Rain

and light

and light

Table 4.1-1 Overview of factors for different latitudes and altitudes

High = 1) At low latitudes, the angle of solar radiation is nearly perpendicular. 2) At high altitudes, UVR is higher for systems at low and mid latitudes. 3) At high altitude, even at mid latitude, the UVR is high. **Low** = At mid latitudes, the solar radiation has a smaller incidence angle. At low altitudes, the radiation undergoes more absorption and scattering. **Constant** = At low latitudes, light availability and duration are regular throughout the year. **Variable** = At mid latitudes, light availability and duration vary throughout the year. **Small range** = 1) At low latitudes, the temperature has small variation throughout the year. 2) At low latitude and low latitude the diurnal variation of temperature is small. **Large range** = 1) At mid latitudes, the temperature has considerable variation throughout the year. 2) Low-latitude systems at high-altitude have considerable diurnal variation. **Rain** = At low latitude, there is a seasonal pattern of rain. **Temperature and light** = At mid latitude, there is a seasonal pattern for temperature and light duration.

4.2 Comparison of factors influencing algal blooms

Rain

Seasonality

Figure 4.2-1 illustrates the twenty-three lakes used for comparison and their location in mountain regions. Of which, low-altitude lakes at low latitude were 3: Malawi, Tanganyika and Victoria; high-altitude lakes at low latitude were 7: Atitlán (Guatemala), San Pablo (Ecuador), Tana (Ethiopia), Titicaca (Peru-Bolivia), Tota (Colombia), Waiau (United States) and Yahuarcocha (Ecuador); low-altitude lakes at mid latitude were 9: Chagan Lake (China), Como (Italy), Erie (Canada-United States), Garda (Italy), Iseo (Italy), Lugano (Italy), Maggiore (Italy), Superior (United States) and Taihu (China); high-altitude lakes at mid latitude were 4: Erhai (China), Sky Pond (United States), The Loch (United States) and Tianchi (China). The high-altitude lakes for comparison depicted in Figure 4.2-1 are located in the Rockies in North

America, the Andes in South America, Central American ranges, the Alpes in Europe, the Himalayas and Tibetan Plateau in Asia and the East African Highlands.

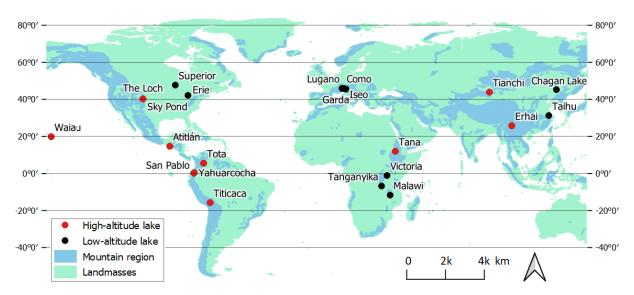


Figure 4.2-1 Location of compared lakes at different latitudes and altitudes. Map made with QGIS. Map data sources: OpenStreetMap, Körner et al. 2017.

Table 4.2-1 shows the morphologic characteristics, climatic classification, reported algal blooms and nutrient concentrations for the considered studies. The latitudinal location of lakes has the greatest influence on the climate. However, the altitude had a role to determine the local climate. According to the Köppen-Geiger climate classification, the climate of low-latitude lakes with altitudes between 468 and 1555 m.a.s.l. was classified as tropical (Atitlán, Malawi, Tana, Tanganyika, and Victoria). The climate of other low-latitude lakes with altitudes between 1555 and 3803 m.a.s.l. was classified as temperate (Atitlán, San Pablo, southern Titicaca, Tota and Yahuarcocha) and for those lakes between 3803 and 3970 m.a.s.l., the climate was classified as polar (Waiau and northern Titicaca). For mid-latitude lakes with altitudes between 3 and 1974 m.a.s.l., the climate was classified as temperate (Como, Erhai, Garda, Iseo, Lugano, Maggiore and Taihu), although for altitudes between 126 and 183 m.a.s.l. it was classified as cold (Chagan Lake and Erie). While for Lake Tianchi (1920 m.a.s.l.), the climate was classified as arid. For other mid-latitude lakes with altitudes between 3322 and 3048 m.a.s.l., the climate was classified as polar (Sky Pond and The Loch). For low latitudes the elevation ranged from 468 and 3970 m.a.s.l., while for mid latitudes the elevation ranged from 3 to 3322 m.a.s.l.

Table 4.2-1 shows no pattern for variation of chlorophyll-a (Chl-a) concentration depending on latitude or altitude. The concentration ranged from 0.1 to 6784 $\mu g \ L^{-1}$. In water bodies with a depth lower than 5 meters, Chl-a concentration ranged from 0.3 to 185 $\mu g \ L^{-1}$. In water bodies with a depth higher than 5 meters, it ranged from 0.03 to 78.5 $\mu g \ L^{-1}$, except Lake Erie (19 m mean depth) which had a maximum Chl-a concentration of 6784 $\mu g \ L^{-1}$. In lakes reported with trophic status mesotrophic, meso-eutrophic and eutrophic, the Chl-a concentration ranged from 0.05 to 78.5 $\mu g \ L^{-1}$. In lakes reported as oligotrophic and meso-oligotrophic, the Chl-a concentration ranged from 0.65 to 4.1 $\mu g \ L^{-1}$. As expected, the upper value of concentration ranges was higher for those lakes where the algal bloom was reported.

Similarly, no pattern was found for nutrients (TN and TP) concentration depending on latitude, altitude nor depth. Total nitrogen (TN) concentration ranged from 28.7 to 6440 $\mu g \, L^{-1}$ and total phosphorus (TP) range were from <1 to 2482 $\mu g \, L^{-1}$. In lakes reported with trophic status

mesotrophic, meso-eutrophic and eutrophic, the TP concentration ranged from 0 to 630 $\mu g \; L^{\text{-1}}.$ In lakes reported with trophic status oligotrophic and meso-oligotrophic, the TP concentration ranged from 3.13 to 20 $\mu g \; L^{\text{-1}}.$ The differentiation between the compared lakes might be related to the differences in sampling periods, land uses in the lake basin and morphologic characteristics of the lakes (depth and size). Moreover, water sample collection has been different among studies. All of them have focused on the epilimnion (upper water layer) but some samples were collected at 0.5 m deep while others were integrated samples collected up to 10 m deep. At low latitudes, the TN:TP ratio ranged from 0.5:1 to 35.7:1. The same range was for high altitude lakes at low latitude. According to this thesis, the range for Puno Bay was between 0.13:1 and 310:1. While for mid-latitudes, TN:TP ratio was generally not available. This data was available only for Lake Superior and Tianchi and the values ranged from 1:1 to 248:1. For Puno Bay, the TN:TP ratio by weight was higher for OPB (41:1) than for IPB (4:1). Which indicated that the limiting nutrient in IPB was nitrogen while in OPB the limiting nutrient was phosphorus.

Eleven high-altitude lakes were compared and nine of them have reported algal blooms. High-altitude lakes at low latitudes (Atitlán, Tana, Titicaca, Waiau and Yahuarcocha) and midlatitudes (Erhai, Sky Pond, The Loch and Tianchi) reported algal blooms. Five of the reported algal blooms were cyanobacterial and the other four were caused by green algae (chlorophytes and charophytes). Five of the reported algal blooms at high altitude occurred in water bodies with a depth less than 5 m, and the other three occurred where the depth was greater than 5 m. The trophic status of these high-altitude lakes was reported to be changing towards a more eutrophic state, mainly in the shallower areas (Table 4.2-1). The main driving factor for eutrophication for these lakes was the anthropogenic activities either in the catchment area or in the surroundings. This includes agriculture runoff, tourism and sewage discharge with low or no treatment. Similar problems are faced by low-altitude lakes, which have an additional factor that is their location in the basin.

Table 4.2-1 Comparison of climatic classification and trophic status of lakes

Lake	Part of the lake	Country	L-A	Depth	Elev. m.a.s.l.	K-G	SE	TS	AB	Period	EC μS cm ⁻¹	NO3-N μg L ⁻¹	PO4-P μg L ⁻¹	TN μg L ⁻¹	TP μg L ⁻¹	Chl-a μg L ⁻¹	TN:TP
	Nyanza Gulf	Kenya	L-L	S	1134	A			CB	1998	132-172	10 -128	18-80			6-61	
	Murchinson Bay,inner	Uganda	L-L	S	1134	A	S, A	Е	CB	2003-04		<10-114	13.7-53.3	874-1380	63-118.8	21.3-40.9	13.1:1
Malawi ³	.,	MMT	L-L	NS	468	A			CB^{20}	1990–93, 1997				65.8-207.2	3.1-15.5	0.03-18.7	5.5:1-22.5:1
Tanganyika ⁴	Kigoma Bay	Tanzania	L-L	NS	773	Α	S	M-E	CB	1998-99	608-650	37-85	1.12-7.56		8.8-20.6	1.25-3.59	
	Open waters		L-L	NS	773	A		O	CB	1998-99	628-650	29-59	1.09-3.37		3.13-9.79	0.65-2.45	
	Murchinson	Uganda	L-L	NS	1134	A			CB	2003-04		<10-17.6	7-32.2	612-1000	42.5-87.5	15-26.8	13.6:1
	Bay,outer	- 5															
	Northern basin	Uganda	L-L	NS	1134	Α		Е	CB	1990,				233.8-903	37-263	4.7-78.5	3.4:1-15.3:1
		- 6								1992-96							
Victoria ¹	Open Lake	Kenya	L-L	NS	1134	A	U, A		CB	1998	84-126	116	87			8	
	Inner Puno Bay	Peru	L-H	S	3803	Е	- /	H^{21}	CB ³⁰	1982		ND	4.6-18.6			10-30	
	Inner Puno Bay		L-H	S	3803	E				2010-20		2-788	15-934	5-9000	9-3500	0.2-118.9	0.13:1-192:1
Waiau ⁷	inner i uno buj	USA	L-H	S	3970	Ē		E^{22}	GB^{22}	1994	54-121	2,00	57.9	648.8	950.8	31	0.7:1
Yahuarcocha ⁸		Ecuador	L-H	S	2192	Č	A, T	E	CB	2014-15	0.121		1.3-7.5	1220-2730	36-98	30-185	0.7.1
Atitlán ⁹		Guatemala	L-H	NS	1555	A,C	A, S	M	CB	2008-09		1-46	3.3-6.5	145-469	25-95	1.8-19.4	6.5:1
San Pablo ¹⁰		Ecuador	L-H	NS	2660	C	S, A	M-E	СВ	1994	250-300	500-3000	6.5-48.9	1590	140-360	5.5-14.5	0.5:1-7:1
Tana ¹¹		Ethiopia	L-H	NS	1840	Ā	R, A	M-E	CB^{23}	2016-17	200 000	200 2000	0.5 10.5	10,0	36-630	0.05-40.4	0.0.1 7.1
	Lago Mayor	Peru	L-H	NS	3803	E,C	,	O^{24}	CD	1982		ND- ~63	~3-~25		20 020	~0.5-~6	
	Outer Puno Bay	Peru	L-H	NS	3803	E,C		M^{25}		1982		110 03	~3-~12.4			1-~5	
	Outer Puno Bay	reru	L-H	NS	3803	E		111		2010-20		0.2-398	3-145	24-2140	5-500	0.1-8	1:1-310:1
Tota ¹²	Outer I uno Bay	Colombia	L-H	NS	3015	Č	S,A,Q	M-E ²⁶		2014-15		0.2-370	3-143	800-2000	20-90	3.4-9.9	18:1-35.7:1
Chagan Lake ¹³		China	M-L	S	126	D	-,-,-,-	M-H		2018				850-2190	40-490	5.4 7.7	10.1 55.7.1
Taihu ¹⁴		China	M-L	S	3	C		E^{27}	HAB^{27}	2000-08				450-6440	1-194	<1-108.4	
Como ¹⁵		Italy	M-L	NS	198	C		L	CB^{28}	1997-99				430-0440	35-40	3.6-3.8	
	Western basin	Canada, USA	M-L	NS	173	D			HAB	2008-18			0.01-44		1.8-2482	0.1-6784	
Garda ¹⁵	Western basin	Italy	M-L	NS	65	C		O-M	CB	1998-00			0.01-44		20	3-4.1	
Iseo ¹⁵		Italy	M-L	NS	186	C		M-E	CB	1998-00					62-66	5.8-8.3	
	Northern basin	Italy	M-L	NS	271	C		M-E	СБ	1998-00					148-172	6.4-8.5	
Maggiore ¹⁵	1 TOTALCHI DASIII	Italy	M-L	NS	193	C		O-M	CB^{28}	1998-00					<20	2.8-3.6	
Superior ³		USA	M-L	NS	183	D		O-IVI	CB^{29}	1990-91				285.6-446.6	3.1-9.3	0.6-1.3	35.8:1-145:1
The Loch ¹⁷		USA	M-H	S	3048	E		0	GB	2015-17	10.6-15	100-300		200-400	7.3-10.3	0.3-4.1	33.0.1-143.1
Sky Pond ¹⁷		USA	м-п М-Н	S	3322	E		0	GB	2015-17	8.1-12.5	100-300		290-310	8.5-12.1	2.3-8.3	
Erhai ¹⁸		China	м-н М-Н	NS	3322 1974	E C	U	E	CB	2015-17	8.1-12.5 24.2-563	0-1080	0-52	40-1650	8.5-12.1 0-485	2.3-8.3 1-31.5	1:1-248:1
	Courthous osoo	China	м-п М-Н	NS NS	1974	В	G, T	M-E	GB	2011-12	24.2-303	0-1080	0-32	280	0-483 41	0.12-67.5	1.1-246.1
	Southern area		M-H	NS Altitude								14:4-1- (- 1500			41		

MMT = Malawi, Mozambique, Tanzania; **L-A** = Latitude - Altitude; **L-L** = Low latitude and low altitude (<1500 m.a.s.l.); **L-H** = Low latitude and high altitude (>1500 m.a.s.l.); **M-L** = mid latitude and low altitude; **M-H** = mid latitude and high altitude; **Depth** = depth of studied water body; **S** = shallow (< 5 m); **NS** = not shallow (> 5 m); **K-G** = First letter of the Köppen-Geiger climate classification; **A** = tropical; **B** = arid; **C** = temperate; **D** = cold; **E** = polar; **SE** = source of eutrophication; **A** = agriculture; **G** = grazing; **Q** = aquaculture; **R** = sediment resuspension; **S** = sewage; **T** = tourism; **U** = urban waste; **TS** = trophic status; **O** = oligotrophic; **M** = mesotrophic; **H** = hypereutrophic; **AB** = algal bloom occurrence; **CB** = cyanobacterial bloom; **GB** = green algae bloom; **HAB** = harmful algal bloom; **ND** = no detected; **EC** = electrical conductivity; **DO** = Dissolved oxygen; **NO**₃-**N** = Nitrogen content in the nitrate ion; **PO**₄-**P** = Phosphorus concentration; **TN**:**TP** = Total nitrogen to total phosphorus ratio by weight; **1)** Lung et al. (2011); **3)** Guildford and Hecky (2000); **4)** Chale (2003); **5)** Vincent et al. (1985); **6) This thesis**; **7)** Kinzie et al. (2019); **9)** Rejmánková et al. (2011); **10)** Gunkel (2000); **11)** Dersseh et al. (2020); **12)** Aranguren-Riaño et al. (2018); **13)** Liu et al. (2019); **14)** Li et al. (2016); **19)** Lu et al. (2016); **19)** Lu et al. (2017); **20)** Bootsma and Hecky (1999); **21)** Beltrán Farfán et al. (2015); **22)** Laws and Woodcock (1981); **23)** Goshu and Aynalem (2017); **24)** Iltis (1992); **25)** Montoya et al. (2014); **26)** Barrera et al. (2019); **27)** Duan et al. (2015); **28)** Bresciani et al. (2016); **29)** Kaeding (2020); **30)** Montoya et al. (2014).

4.3 Analysis of water quality data

4.3.1 Comparison of data from ANA and IMARPE

In Inner Puno Bay (IPB), sampling points that were located as close as possible to each other were IPB2 and IPB12. IPB2 was measured by the National Authority of Water (*Autoridad Nacional del Agua* [ANA]) and IPB12 was measured by the Sea Institute of Peru (*Instituto del Mar del Perú* [IMARPE]). The distance between these sampling points was 305 m. The parameters the sampling points had in common were dissolved oxygen (DO), temperature (T), total phosphorus (TP), total nitrogen (TN), orthophosphate as phosphorus (PO₄-P), nitratenitrogen (NO₃-N) and transparency (SD). There was a significant difference between ANA and IMARPE for DO, TP and TN, for the other parameters there was not a significant difference between ANA and IMARPE. The table with the corresponding p-values is in Appendix E.

In Outer Puno Bay (OPB), two sets of sampling points that were located as close as possible to each other were found: OPB1 (ANA) - OPB10 (IMARPE) and OPB5 (ANA) - OPB12 (IMARPE). The distance between OPB1 and OPB10 was 216 m, and the parameters they had in common were DO, pH, T, electrical conductivity (EC) and SD. Most of the compared parameters did not have a significant difference between ANA and IMARPE, except DO. For sampling points OPB5 and OPB12, the distance was 300 m, and the parameters they had in common were DO, pH, T, EC, PO₄-P, NO₃-N and chlorophyll-a (Chl-a). For all of the compared parameters, there was not a significant difference between ANA and IMARPE. The table with the corresponding p-values is in Appendix E.

From this comparison, it can be seen that data from ANA and IMARPE in IPB can be considered as one dataset. For IPB, significant differences were found for 3 of the 7 compared parameters. Still, it was decided to merge the two data sets to have a large time span with a more complete set of parameters.

4.3.2 Estimation of chlorophyll-a missing values

Chlorophyll-a (Chl-a) missing values were estimated from transparency values (SD) using non-linear regression (Section 3.4.1). The resulting equation is shown in Figure 4.3-1 along with the regression line (Model II regression, p-value< 0.05).

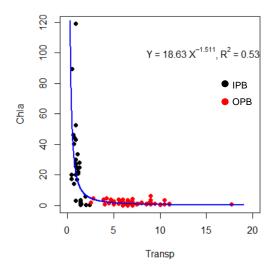


Figure 4.3-1 Regression line for chlorophyll-a estimation.

4.3.3 Comparison of Inner and Outer Puno Bay

Table 4.3-1 shows that the maximum Chl-a concentration in OPB (8 μg L⁻¹) was lower than IPB (118.9). The method used by ANA and IMARPE to determine the Chl-a concentration was based on SM 10200H (APHA 2005) as indicated in Appendix D. Similar differences were observed for parameters TN, TP, PO₄-P, NO₃-N and BOD₅. The maximum value of TSS was lower for OPB (8 mg L⁻¹) than for IPB (69 mg L⁻¹). The maximum temperature for OPB (18.94 °C) was lower than for IPB (20.5 °C). The mean TN:TP ratios for OPB (41:1) were higher than for IPB (4:1). All the parameters in IPB were significantly higher than in OPB. The table with the corresponding p-values is in Appendix E.

Table 4.3-1 Mean values and ranges of water quality parameters for IPB and OPB for the period 2010 – 2020.

Parameter	Unit	IPB	OPB	All
Chl-a	μg L-1	17.79 (0.2-118.9)	1.8 (0.1-8)	8.08 (0.1-118.9)
TN	$\mu g \ L^{1} \ N$	2171 (5-9000)	445 (24-2140)	1659 (5-9000)
TP	$\mu g \; L^{1} \; P$	959 (9-3500)	28 (5-500)	690 (5-3500)
PO ₄ -P	$\mu g \; L^{1} P$	382 (15-934)	23 (3-145)	184 (3-934)
NO ₃ -N	$\mu g \; L^{1} N$	103 (2-788)	21 (0.2-398)	59 (0.2-788)
TSS	mg L ⁻¹	14.01 (2.33-69)	1.96 (1-8)	10.49 (1-69)
BOD ₅	mg L ⁻¹	5.56 (1-21.14)	1.67 (1-5.06)	4.42 (1-21.14)
EC	$\mu S \ cm^{-1}$	1735 (1480-1933)	1524 (1041-1705)	1592 (1041-1933)
pН	-	9.21 (6.84-10.34)	8.73 (7.94-10.1)	8.89 (6.84-10.34)
T	°C	16.76 (11.7-20.5)	15.32 (11.67-18.94)	15.93 (11.67-20.5)
DO	mg L ⁻¹	8.09 (1.24-15)	6.8 (3.69-12.54)	7.35 (1.24-15)
TN:TP	-	4:1 (0.13:1-192:1)	41:1 (1:1-310:1)	15:1 (0.13:1-310:1)
n	-	197	265	462

Chl-a = chlorophyll-a concentration with estimated missing values; TN = total nitrogen concentration; TP = total phosphorus concentration; PO_4 -P = orthophosphate as phosphorus concentration; PO_3 -N = nitrate-nitrogen concentration; PO_4 -P = total suspended solids concentration; PO_5 = biological oxygen demand; PO_5 = electrical

conductivity; T = temperature; DO = dissolved oxygen concentration; TN:TP = Total nitrogen to total phosphorus ratio by weight; n = sample size; IPB = Inner Puno Bay; OPB = Outer Puno Bay.

4.3.4 Correlation analysis

The selected variables for correlation were DO, pH, T, EC, BOD₅, TSS, TP, TN, PO₄-P, NO₃-N and Chl-a. The highest significant correlation for IPB was between T and EC (0.55) (Figure 4.3-2). The significant correlation between TSS and TP (-0.13) showed an almost lack of relationship between these variables. A negative relationship was observed between several variables, most notably for the combination of PO₄-P and EC (-0.39). Values above the diagonal reveal that twenty-nine out of the fifty-five correlations were significant at the 0.05 level. From the scatterplot, no combination seemed to exhibit a non-linear relationship that would not be represented in a bivariate correlation. For BOD₅, an outlier was observed. The distribution of BOD₅, TN and Chl-a were skewed right.

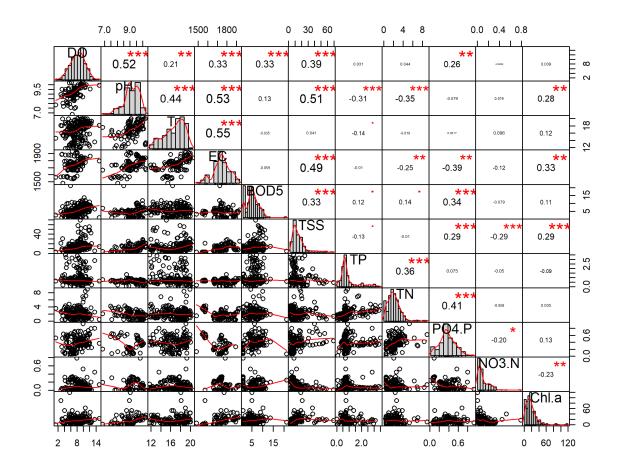


Figure 4.3-2 Matrix of bivariate relationships between variables selected for IPB.

Diagonal portrays the distribution of each variables. Values below the diagonal are scatterplots with the corresponding bivariate correlation above the diagonal (Pearson method). Each significance level (p-value) was associated to a symbol: "***" (<0.001), "**" (<0.01), "*" (<0.05), "." (<0.1) and " " (>0.1). DO = dissolved oxygen concentration; T = temperature; EC = electrical conductivity; BOD_5 = biological oxygen demand; TSS = total suspended solids; TP = total phosphorus concentration; TSS = orthophosphate as phosphorus concentration; TSS = nitrate-nitrogen concentration; TSS = concentration with estimated missing values.

For OPB, the highest significant correlation was between TP and PO₄-P (0.87), although it was heavily influenced by the outlier (Figure 4.3-3), without the outlier the correlation was 0.12. The other correlation that were higher than 0.5 and significant (p-value < 0.05) was between BOD₅ and TSS. Values above the diagonal reveal that eleven out of the fifty-five correlations were significant at the 0.05 level. From the scatterplot, it can be observed that the relationships related to BOD₅, TSS and TN are concentrated mostly in two values for each variable. This is because 44 %, 54 % and 37 % of the concentrations for BOD₅, TSS and TN, respectively, were estimated as half of the limit of quantification. The other scatterplots show that the dots are condensed and it was not possible to reveal linear or non-linear patterns.

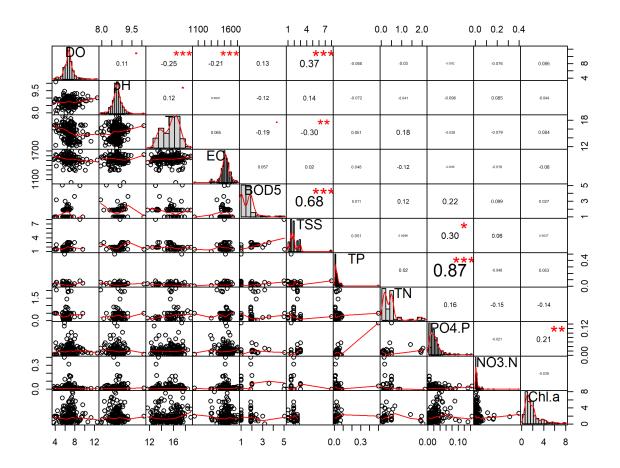


Figure 4.3-3 Matrix of bivariate relationships between variables selected for OPB.

Diagonal portrays the distribution of each variables. Values below the diagonal are scatterplots with the corresponding bivariate correlation above the diagonal (Pearson method). Each significance level (p-value) was associated to a symbol: "***" (<0.001), "**" (<0.01), "*" (<0.05), "•" (<0.1) and " " (>0.1). DO = dissolved oxygen concentration; T = temperature; EC = electrical conductivity; $BOD_5 = \text{biological oxygen demand}$; TSS = total suspended solids; TP = total phosphorus concentration; TN = total nitrogen concentration; PO_4 -P = orthophosphate as phosphorus concentration; NO_3 -N = nitrate-nitrogen concentration; Chl-a = chlorophyll-a concentration with estimated missing values.

4.3.5 Factor analysis

Assumptions in Factor Analysis

After omitting missing values, there were 147 observations (n=147) for each of the 9 variables. Due to more available parameters from ANA, only data from this source remained. This data was for 2013-14, 2016 and 2018-19. Table 4.3-2 shows the assessment of normality for variables in IPB. As observed from the distribution plots (Figure 4.3-2), BOD₅, TN and Chl-a presented the highest deviation from normality. Chl-a was transformed using the cube root and the normality was achieved. TN could not be transformed to improve in its distributional characteristics. This variable will be used in its original form, as its deviation from normality was not so extreme. For BOD₅, none of the transformations could improve its normality, not even removing the outlier in 2014. Thus, BOD₅ was excluded for factor analysis.

	Before transf	ormation	Data	After transformation		
	p-value	Description of the distribution*	transformation	p-value	Description of the distribution	
DO	0.014	Almost normal				
pН	0.015	Almost normal				
T	0.007	Slightly skewed left				
EC	0.057	Normal				
BOD_5	1.7 x 10 ⁻¹⁰	Skewed right	None			
TSS	8.7 x 10 ⁻⁵	Almost normal				
TP	0.002	Almost normal				
TN	1 x 10 ⁻⁴	Skewed right	None			
Chl-a	4.8 x 10 ⁻¹⁰	Skewed right	Cube root	0.08	Normal	

Table 4.3-2 Test for normality before and after transformation.

From Figure 4.3-2 and Figure 4.3-3, it can be observed that IPB and OPB are different samples. Whether the two samples are combined, the resulting correlations will be a poor representation of the unique structure of each group. Thus separate factor analysis would be better to perform for each group. It was verified that different factor structure was obtained when OPB was combined with IPB data. Since issues related to eutrophication and algal bloom occur mainly in IPB, factor analysis was performed only for this area. Data with the variables selected for IPB were suitable for a data reduction technique (χ^2 (86) = 203.7, p-value = 1.4 x 10⁻²⁸, d.f. = 28)

Extraction of factors

According to the scree test criterion, 3 variables may be retained (Figure 4.3-4). The fact that the three factors account for 72 % of the overall variance (Table 4.3-3), reassures the retention of three factors for further analysis.

^{*}Distribution of plots for variables in Inner Puno Bay (Figure 4.3-2)

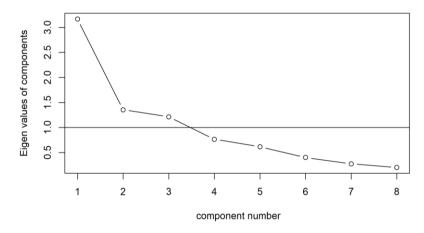


Figure 4.3-4 Eigenvalue Plot

Factor interpretation

A series of factor analyses were conducted and the most interpretable solution is shown in Table 4.3-3. Then, patterns of significant factor loadings were examined. Loading with values of ± 0.60 or above were considered significant based on the sample size (Table 3.4-2). Table 4.3-3 shows that each of the variables had a significant loading on only one factor. Factor loading of DO (0.58) was considered significant for Factor 1 as it was so close to the cut-off value.

The communalities are the proportion of variance in the variable explained by the extracted factors. Communalities for all variables were above 0.40. The eigenvalue represents the amount of variance accounted for by a factor. Factor 3 had an eigenvalue of 2.17, which accounts for 27 % of the variance in the data. Factor 1 had an eigenvalue of 2.11 and accounted for a further 26 % of the variance. The eigenvalue for Factor 2 was 2.74, accounting for a further 18 % of the total variance.

Table 4.3-3 Matrix of factor loadings with Varimax rotation.

(n=86)	. Significant f	factor 1	loadings	were	bolded.

Variable	Abbreviation	Factor 3	Factor 1	Factor 2	Communality
Dissolved oxygen	DO	0.20	0.58	-0.24	0.44
pH	pН	0.45	0.61	-0.34	0.69
Temperature	Temp	-0.03	0.84	0.15	0.73
Conductivity	EC	0.51	0.68	0.09	0.72
Total suspended solids	TSS	0.90	0.15	-0.10	0.84
Total phosphorus	TP	-0.10	0.22	0.83	0.74
Total nitrogen	TN	0.04	-0.39	0.75	0.71
Chlorophyll-a	Chl-a	0.92	0.13	0.00	0.87
Eigenvalue		2.17	2.11	1.46	
Proportion Variance		0.27	0.26	0.18	
Cumulative variance		0.27	0.53	0.72	

Factor 3 had significant loadings for TSS and Chl-a. The highest loading was for Chl-a (0.92) and both variables were positively related to the factor (positive sign). Both variables are related

to turbidity. Turbidity in water is caused by suspended and colloidal matter. Suspended solids include finely divided organic and inorganic matter, plankton, and other microscopic organisms. Factor 1 had significant loadings for DO, pH, T and EC. The variable with the highest factor loading was T (0.84). These physico-chemical parameters are strongly affected by the depth. Thus, higher values for sampling points close to shorelines would be expected. Factor 2 had TP and TN with high loadings. This factor was related to nutrients. The fact that TP and TN are part of the same factor, might indicate a relationship between these variables. This relationship might be explained by the pollution from urban and industrial wastewater. Organic nitrogen and ammonia may be present in domestic wastewater, where phosphates from household detergents can also be found. Agriculture in the surrounding area is limited, but this could also be a source of phosphates.

Factor scores

Case-wise factor scores were calculated for each factor and plotted over time in Figure 4.3-5. There was a significant increasing trend of Factor Score 3 (FS3) in all areas of IPB. The slopes for the linear regression of this factor were all significant (Appendix E). For Factor Score 1 (FS1) and Factor Score 2 (FS2), the regression lines are less steep and do not show a significant trend (Appendix E) for the period 2013-2019 in IPB.

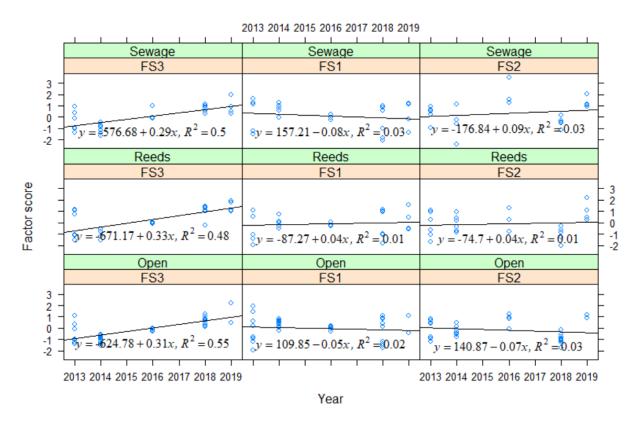


Figure 4.3-5 Factor scores for IPB for the years 2013-14, 2016, 2018-19.

The linear regression line was included with their corresponding coefficient of determination (R2). FS1 = Factor Score 1, FS2 = Factor Score 2, FS3 = Factor Score 3.

Since FS3 slopes of the three groups in IPB had close values, this could indicate that in all IPB areas the trend was almost the same. Boxplots in Figure 4.3-6 confirmed that the values are

similar for all sampling points in IPB, and are not significantly different (ANOVA, F value=0.373, p-value = 0.96). For FS1 (Figure 4.3-7), higher values for sampling points close to shorelines would be expected, but FS1 values had no significant difference between sampling points within IPB (ANOVA, F value=0.354, p-value = 0.962).

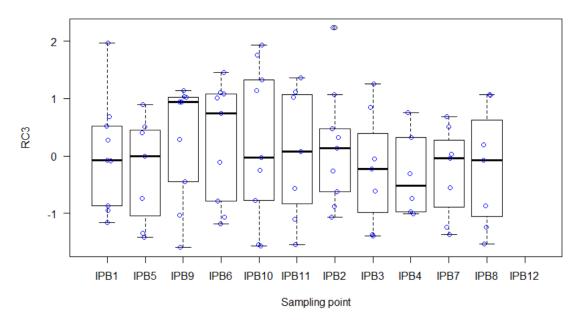


Figure 4.3-6 Factor Score 3 (turbidity) boxplots for each sampling point in Inner Puno Bay
Sewage = IPB1, IPB5 and IPB9; Reeds = IPB6, IPB10 and IPB11; Open = IPB2, IPB3, IPB4, IPB7, IPB8 and IPB12

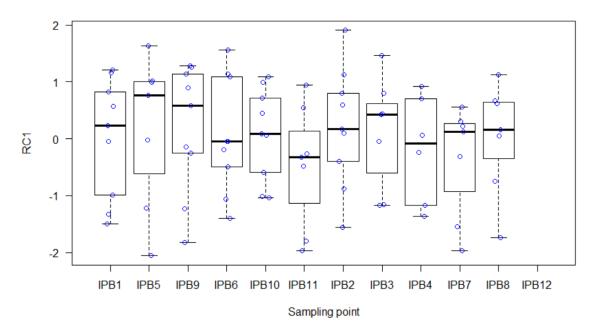


Figure 4.3-7 Factor Score 1 boxplots for each sampling point in Inner Puno Bay
Sewage = IPB1, IPB5 and IPB9; Reeds = IPB6, IPB10 and IPB11; Open = IPB2, IPB3, IPB4, IPB7, IPB8 and IPB12

A more detailed analysis was done to compare the 11 sampling points in IPB. As Puno city is next to IPB, it was expected that the sampling points in IPB close to sewage discharge had the highest nutrient-related factor score (FS2). However, no significant difference in FS2 values was found between sampling points within IPB (ANOVA, F value=1.875, p-value = 0.06). Since the p-value was close to 0.05, a posthoc test was performed. A significant difference was found for IPB9 and IPB6 (Tukey test, p-value = 0.058). This difference can be observed in Figure 4.3-8.

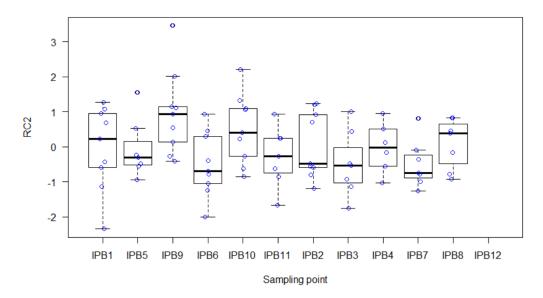


Figure 4.3-8 Factor Score 2 (nutrients) boxplots for each sampling point in Inner Puno Bay
Sewage = IPB1, IPB5 and IPB9; Reeds = IPB6, IPB10 and IPB11; Open = IPB2, IPB3, IPB4, IPB7, IPB8 and IPB12

5.1 Conceptual model

Factors directly related to algal blooms that are affected by latitude and altitude are temperature and solar radiation. While precipitation and wind are more related to the local characteristics of the lake such as proximity to sea or topography. The extent of influence of these climatic factors is determined by the depth. Regarding nutrients, lakes at different altitudes naturally differ in their location in the basin. That is why high altitude lakes are usually considered oligotrophic. Whereas lakes at low altitudes are more influenced by the inflow of the rivers they receive. The possible occurrence of nutrient input by anthropogenic activities in the lake or the surroundings is not necessarily related to the lake's position within the hydrological network. Anthropogenic activities do not occur in all the lakes and their intensity and type also differ. Thus, the conceptual model in Figure 4.1-1 illustrated both natural and anthropogenic in the "inflow" factor.

The factors considered in Figure 4.1-1, are in accordance with the components considered by Janssen et al. (2019) for algal bloom models. Climate change was considered by Janssen et al. (2019) as part of the environmental component. High altitude lakes might have a higher sensitivity to changes in precipitation and temperature by climate change. Since these lakes are usually oligotrophic with clear conditions, and the effects caused by climate change could be more catastrophic.

5.2 Algal bloom driving factors

5.2.1 Temperature

Table 4.2-1 showed that the influence of altitude in determining climate was most noticeable for low-latitude regions, despite the higher elevation range for mid-latitude regions. For low-latitude regions, the climates were classified from A to E (A, C and E), while for mid latitudes the climates were classified from B to E (B, C, D and E). This might be considered as evidence of the higher influence of altitude for low latitudes than for mid-latitudes. Wetzel (2001) pointed that at low latitudes, the altitude causes many exceptions in lake classification, although the emphasis was given to the highest elevations. From Table 4.2-1 it was observed that this variation was not necessarily for the highest elevations but also for the minimum elevation considered as high altitude (1500 m.a.s.l). The higher variation of climates as a function of altitude for low latitudes could highlight the importance of studying the algal bloom dynamics for this region.

For Puno Bay, the mean amplitude of daily variations of atmospheric temperature went up to 15.7 °C during the dry season. Its depth (average 14 m) does not allow a yearly stratification, although it occurs to a certain extent (Richerson 1992). As the atmospheric temperature influence the temperature of the lake by convection, this variation can result in approximately 4 °C mean amplitude of daily variation on lake surface temperatures (Duquesne et al. 2021). The mixing by the diel temperature variation is more relevant for shallow lakes or shallower areas of a lake, which is also influenced by the wind. Low-latitude regions do not present

seasonality due to small annual variation of temperature compared with mid-latitudes. This means that the stratification followed with mixing is limited. Thus, for the deeper areas of a low-latitude lake, the latitudinal influence seems to be more relevant, as those lakes have a more stable stratification throughout the year. Still, mixing in low-latitude regions occur to some extent due to alternation of the diurnal heating and the nocturnal cooling.

5.2.2 **Depth**

Table 4.2-1 showed no clear pattern for variation of chlorophyll-a (Chl-a) concentration depending on latitude or altitude. Although comparing by depth, shallow water bodies (< 5 m) had a higher maximum value for Chl-a. This may be explained by a higher impact of temperature (Herb and Stefan 2004) and mixing (Bade 2005) for shallow water bodies. The significantly higher Chl-a concentration and temperature for IPB than for OPB is likely to be related to the difference in depth. Besides, the barrier effect caused by totora between IPB and OPB and the circulation of water within IPB (Figure 2.3-5) seem to indicate that there is limited exchange between IPB and OPB. The other parameters TN, TP and TSS also had significantly higher concentrations for IPB than for OPB. Which also could be partially attributed to the difference in depth, as depth might have favoured the resuspension of pollutants entering IPB. Thus, the limited exchange between IPB from OPB along with a higher external loading of nutrients for IPB than for OPB may play a role for IPB having significant higher values of Chla, nitrogen and phosphorus. In addition to this, IPB, being a shallow water body, is more exposed to the effect of the wind and the effect of daily temperature variation. Therefore, the algal blooms for shallow water bodies at high altitudes showed in Table 4.2-1 might be the result of a more evident alternation of stable states that occur for shallow lakes. As noted by van Nes and Scheffer (2005), the shallower parts of a lake can undergo alternative stable states that differ in dynamics and time with the deeper parts. Although the shifts to turbid states in the shallow areas of deep lakes (Titicaca, Atitlan) might indicate also changes in the pelagic areas (Bruel et al. 2018).

5.2.3 Nutrients

High-altitude lakes used to be poorer in nutrient content than low-altitude lakes. However, the difference in nutrient concentration between lakes at low and high altitude regardless of the latitude was not evident in Table 4.2-1. The differences in studied periods and local activities might partially explain this fact. But it also might be explained by the difference in inflow and outflow. If the outflow is minimum and the main loss of water occurs through evaporation the sink characteristic of the lakes is accentuated. Such is the case of Lake Titicaca, which loses 95 % of its total water outflow through evaporation (Martínez Gonzales and Zuleta Roncal 2007).

Table 4.2-1 showed no clear pattern for variation of nutrients (TN and TP) concentration depending on latitude, altitude nor depth. This might be related to the difference in sampling period, land uses in the lake basin, morphologic characteristics of the lakes (depth and size) and water sample collection. Similarly, information in Table 4.2-1 was limited to compare lakes concerning their TN:TP ratio. Considering that the Redfield ratio C:N:P in grams is 41:7.2:1. High altitude lakes at low latitudes reported nitrogen as the limiting nutrient (Atitlan, San Pablo and Waiau). This is in accordance with Abell et al. (2012) who, based on latitudinal trends for TN:TP, suggested that N as a limiting nutrient in lakes prevailed more at low latitudes. For Inner Puno Bay, nitrogen as the limiting nutrient is likely to be related to sewage discharge.

The trophic status of high-altitude lakes compared in Table 4.2-1 was reported to be changing towards a more eutrophic state. Of the eleven high-altitude lakes compared in Table 4.2, only

The Loch, Sky Pond (Lubinski 2021) and the deep part of Lake Titicaca were reported to remain oligotrophic. The shift of status in the other high altitude lakes was influenced by anthropogenic activities either in the catchment area or in the lake itself. This included agriculture runoff, sewage discharge with low or no treatment and tourism (Gunkel 2000; Rejmánková et al. 2011; Li et al. 2016; Lu et al. 2017; Van Colen et al. 2017; Aranguren-Riaño et al. 2018). Similar problems are faced by low-altitude lakes, which have an additional factor that is their location in the basin. Although Lake Lugano, a low-altitude lake, has been subjected to management measures implemented by Switzerland and Italy to reduce the input of phosphorus, and reverting trophic status to less eutrophic (oligotrophication) (Lepori and Capelli 2021). Oligotrophication might be more difficult for deep monomictic lakes, whose layers of water are mixed only once per year and remain stratified the rest of the year (Atitlan, Titicaca, San Pablo), and for meromictic lakes, whose layers of water do not intermix (Tanganyika). Whereas shallow lakes have a shorter sediment cycle because they are resuspended by wind and diel stratification.

5.3 Algal blooms

Table 4.2-1 showed that cyanobacterial blooms were reported at different latitudes and altitudes, while the cases of green algae blooms were reported only for high altitude lakes. The presence of cyanobacteria blooms in the four regions compared might be related to the temperature. Since, for each latitudinal region, warmer conditions are found at low altitudes and cyanobacteria thrive in warmer temperatures than green algae (Kosten et al. 2012). Even when algal blooms are expected to be caused by cyanobacteria, it should be noted that half of the occurrence of algae blooms in high altitude lakes that were compared were caused by green algae (Waiau, Tianchi and the benthic chlorophyte blooms in The Loch and Sky Pond). In Lake Titicaca, a chlorophyte species, which is a type of green algae, caused the algal bloom reported in Lago Menor in 2015 (Achá et al. 2018). The southern part of Lake Titicaca, where Lago Menor is located, is also subjected to intense nutrient pollution by sewage (Molina et al. 2017). For high altitude lakes, considering that the photosynthetically active radiation (PAR) is not a limiting factor, the input of nutrients, in particular phosphorus, might have an important role to favour future cyanobacterial blooms, considering that cyanobacteria also thrives at low N:P ratios (Gunkel 2000). Beside, of the potentially forming algal blooms species reported by Montoya et al. (2014) for Lake Titicaca, the cyanobacterial species Microcystis aeruginosa have been reported to have compounds for protection against UV damage (Liu et al. 2004). Thus, the detriment for algal blooms formation caused by UVR might be lower compared to the benefit light availability, so that since light is not a limiting factor, availability of nutrients may create favourable conditions for algal blooms to occur.

5.4 Puno Bay

5.4.1 Data handling

The variability in the collected data might have been related to differences in sample collection, sampling frequency and measurement methods. The latter not only were different between institutions but even varied for the same institution over the years. The data was valuable as it enabled the analysis of ten years span. Still, it was necessary to estimate values below the limit of quantification and Chlorophyll-a missing values to be able to get the most out of the data set. The estimation of the Chlorophyll-a missing values was useful to fill the gaps and to perform

correlation, calculation of mean, ranges and factor analysis. While the estimated LOQ values were useful for comparing averages and ranges, these values could not be used to perform factor analysis, so another estimation method for values below LOQ could be evaluated. The factor analysis was useful to reduce the eight variables to three. After omitting missing values, only data from ANA remained, due to more available parameters from this source. While no significant differences were verified for the parameters that could be compared from ANA and IMARPE, other parameters and sampling points could not be compared due to lack of data. Thus, using only ANA for factor analysis might have helped to eliminate a possible source of variation or error.

Although the data has been analysed without considering the rainfall seasons, significant differences between IPB and OPB have been found. To analyse the variation of concentrations for water quality parameters and the likely effect of rainy and dry seasons in the correlation of variables, especially in IPB which is the most affected area, an analysis considering the seasons should be done.

5.4.2 Inner and Outer Puno Bay

The mean TN:TP ratio by weight was higher for OPB than for IPB. The mean values of TN:TP in Puno Bay indicate that nitrogen is the limiting nutrient in IPB, while for OPB phosphorus is the limiting nutrient. For IPB, this might be related to the waste discharge. Which was observed when the nutrient-related factor score (FS2) was compared for all the sampling points within IPB. The sampling points close to the discharge of the oxidation pond and other pipes of sewage discharge had higher values of FS2. This discharge of domestic and industrial wastes coupled with the shallow depth could be the reason that the nutrient inputs are available for phytoplankton. As no control on the nutrient pollution is being applied, this could explain the increasing trend of turbidity-related factor score (FS3) in IPB. In IPB, the availability of nutrients is associated with the propagation of duckweed (*Lemna* sp.) reported for the last 20 years, along with the occurrence of cyanobacterial blooms in more recent years (Montonya et al. 2014).

The greater depth for OPB could be the reason why the TN and TP values for sampling points close to aquaculture cages are no different from sampling points in the open water. Excess food and waste from these cages might be settling. OPB is not deep enough to have a metalimnion and is considered to be polymictic (Richerson 1992), which could facilitate the mixing of phosphorus present in the sediments causing the dilution effect and transporting to the reeds area. Moreover, the connection of OPB with Lago Mayor (Duquesne et al. 2021) may also enhance the mixing and the dilution of nutrients in OPB. The higher depth of OPB compared to IPB, might not be exempt it from a shift to a turbid state, as occurred in Lago Menor in the southern part of the lake in 2015 after an intense period of rainfall (Achá et al. 2018). As temperature and precipitation are factors related to global warming, the occurrence of more extreme climates might worsen the loss of resilience of the system already exposed to uncontrolled pollution.

For Puno Bay, no relationship was found between TP and Chl-a for IPB nor OPB, as reported by Corman et al. (2015) for Lake Atitlán (low latitude, 1555 m.a.s.l.). This supports the previous trend reported before regarding the relationship between total phosphorus (TP) and Chl-a concentrations, which seems to be more prevalent for temperate lakes and inexistent in tropical mountain lakes (Huszar et al. (2006).

For OPB, the highest correlation was between BOD₅ and TSS. This correlation should be considered carefully since it was calculated with estimated values for these parameters.

Nevertheless, this might indicate that the transport channel and other water transport mechanisms do allow interaction between these two sectors of Puno Bay, even when no much interaction between IPB and OPB is expected (ANA 2013). This correlation could also be related to the waste generated by the cage culture. There is not much information available about the currents in OPB to help understand water and sediment interaction. For OPB another important element related to nutrient cycling is the presence of reeds, which can help to retain nutrients. This could be helping to control the concentration of nutrients in this part of Puno Bay.

Factor score 3 had significant loadings for TSS and Chl-a. The highest loading was for Chl-a (0.92) and both variables were positively related to the factor (positive sign). Both variables are related to turbidity. Turbidity in water is caused by suspended and colloidal matter. Suspended solids include finely divided organic and inorganic matter, plankton, and other microscopic organisms. Factor 1 had significant loadings for DO, pH, T and EC. The variable with the highest factor loading was T (0.84). These physico-chemical parameters are strongly affected by the depth. Thus, higher values for sampling points close to shorelines would be expected. Factor 2 had TP and TN with high loadings. This factor was related to nutrients. The fact that TP and TN are part of the same factor, might indicate a relationship between these variables. This relationship might be explained by the pollution with urban and industrial wastewater. Organic nitrogen and ammonia may be present in domestic wastewater, where phosphates from household detergents can also be found. Agriculture in the surrounding area is limited, but this could also be a source of phosphates. The significant increasing trend of turbidity-related factor score (FS3) in all areas of IPB and the higher values of the sampling point close to the discharge of the oxidation pond seems to be related to the lack of control in sewage discharge that might affect OPB given the certain connection that exists between these two water bodies.

Although the nutrient–phytoplankton–zooplankton–detritus (NPZD) model used by Duquesne et al. (2021) for Lake Titicaca does not considered phosphorus, given that there is a significant difference of the limiting nutrient for Inner Puno Bay (IPB) and Outer Puno Bay (OPB), the inclusion of phosphorus in the box of nutrients (Figure 4.1-1) might help in a model for Puno Bay. Moreover, this thesis considered macrophytes in the conceptual model (Figure 4.1-1). The emergent macrophytes (*totoras*) cover approximately 50 % of Puno Bay surface area (INRENA 2002) and are efficient in the consumption of nitrogen and phosphorus (Chacón Nina 2014).

Chapter 6

Conclusion

The influence of altitude in determining climate was more noticeable for low latitudes than for mid-latitude regions. The higher variation of climates as a function of altitude for low latitudes could highlight the importance of studying the algal bloom dynamics specific for each region.

For the deeper areas of a low-latitude lake, the latitudinal influence seems to be more relevant, as those lakes have a more stable stratification throughout the year. Therefore at low latitude regions there is less mixing of water layers.

The limited exchange between Inner Puno Bay (IPB) and Outer Puno Bay (OPB) along with a higher external loading of nutrients for IPB than for OPB may play a role for IPB having significant higher values of water quality parameters. In addition to this, IPB, being a shallow water body, is more exposed to the effect of the wind and the effect of daily temperature variation. Moreover, the connection of OPB with Lago Mayor may also enhance the mixing and the dilution of nutrients in OPB.

Anthropogenic activities (agriculture runoff, sewage discharge with low or no treatment and tourism) in the lake and its basin have a higher influence than the lake's position within the hydrological network on the trophic status of high altitude lakes.

Cyanobacterial blooms were reported at different latitudes and altitudes, while the cases of green algae blooms were reported only for high altitude lakes. The effect of temperature increasing by global warming might favour the occurrence of more cyanobacterial blooms at high altitude lakes.

The increasing trend of parameters related to turbidity in Inner Puno Bay seemed to indicate the shift to a more turbid state for this area of Puno Bay.

Chapter 7

Recommendation

The inclusion of phosphorus in a conceptual model for Puno Bay may be relevant given the difference in limiting nutrient between IPB and OPB.

The inclusion of reeds in a conceptual model for Puno Bay may also be relevant given the presence of emergent macrophytes (*totoras*) that cover approximately 50 % of Puno Bay surface area, which have a role in the cycle of nutrients.

Algal bloom models might consider to include the quantification of nutrient inputs in high altitude lakes caused by extreme events (temperature and rain) of climate change.

To analyse the variation of concentrations for water quality parameters and the likely effect of rainy and dry seasons in the correlation of variables, an analysis considering the seasons should be done for Puno Bay.

Due to the increasing trend of parameters related to turbidity in Inner Puno Bay, it might become more relevant to monitor a possible shift in macrophytes species and fish species composition.

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Appendices

Appendix A. - Research Ethics approval



Research Ethics Committee IHE Delft Institute for Water Education

E ResearchEthicsCommitee@un-ihe.org

Date: 11 January 2021

To: Patricia Mahela Prieto Veramendi

MSc Programme:

Approval Number: IHE-RECO 2021-132

Subject: Research Ethics approval

Dear Patricia.

Based on your application for Ethical Approval, the Research Ethics Committee (RECO), IHE Delft RECO has been approved ethical clearance for your research proposal: "External nutrient loading and eutrophication in a high-altitude lake, Puno Bay (Lake Titicaca, Peru)".

This approval is valid until April 16, 2021. You need to notify the RECO of any modifications to your research protocol. If you do not complete your research by the specified date, you should to contact RECO to request an extension.

Please keep this letter for your records and include a copy in the final version of MSc. Thesis, together with your personal reflection. Additional information is available at https://ecampusxl.un-ihe.org/course/view.php?id=1555§ion=2.

On behalf of the Research Ethics Committee, I wish you success in the completion of your research.

Yours sincerely,

AMS

Dr. Angeles Mendoza Sammet **Acting Ethics Coordinator**

Copy to: Archive.

Appendix B. - Personal declaration

The author thanks a scholarship provided by the Orange Knowledge Programme (OKP), funded by the Netherlands' Ministry of Foreign Affairs and managed by Nuffic. This scholarship allowed the author to conduct the research free from the influence of potentially interested parties. The author declares that she has no relevant or material financial interests that relate to the research described in this thesis.

Appendix C. - Regulations for preservation of Lake Titicaca

Table 1. National regulations addressing water quality and preservation of resources in Lake Titicaca

Regulation	Aim	Source
Law No. 29338, Law of Water Resources	Issued 30 March 2009, repeals Decree-Law No. 17752-General Water Law (1969-07-24). Regulates the use and management of water resources. It includes surface, underground, continental water and the goods associated with it.	(ANA 2014a)
Supreme Decree No. 185/78 / AA (1978)	Declares the creation of the Titicaca National Reserve, as a protected natural area that is part of the lake to conserve the flora and wild fauna of the lake and support the socio-economic development of the populations that they live in the vicinity of the lake.	(INRENA 2002)
Supreme Decree No. 002-2008-MINAM	Approves the National Environmental Quality Standards for Water	(ANA 2014a)
Supreme Decree No. 023-2009-MINAM	Approves the provisions for the implementation of the Environmental Quality Standards for Water	(ANA 2014a)
Supreme Decree No. 001-2010-AG	Approves the Regulation of Law No. 29338	(ANA 2014a)
Resolution of Ministry No. 202-2010-ANA	Approves the Classification of Surface and Marine-Coastal Water Bodies.	(ANA 2014a)
Headquarters Resolution No. 182-2011-ANA	Approves the National Protocol for the Quality Monitoring of Surface Water Resources.	(ANA 2014a)
Law N° 29906	Declares of necessity and public utility the prevention and integral environmental recovery of Lake Titicaca and its tributaries	(ANA 2014a)
Supreme Decree No. 075-2013-PCM	Creation of the Multisectoral Commission for the prevention and environmental recovery of the Lake Titicaca basin and its tributaries.	(ANA 2014a)
Ministerial Resolution No. 301-2013-MINAM	Approves the Internal Regulations of the Multisectoral Commission for the prevention and environmental recovery of the Lake Titicaca basin and its tributaries.	(ANA 2014a)

Table 2. International regulations addressing water quality and preservation of resources in Lake Titicaca

D. J.		g.	
Regulation	Aim	Source	
Special Binational Project Lake Titicaca (Proyecto Especial Binacional Lago Titicaca, PELT)	Developed the Global Binational Master Plan, which is being implemented both in Peru and Bolivia, in the aspects of protection, flood prevention and use of the resources of Lake Titicaca, Desaguadero River, Lake Poopo and Lake Coipasa (TDPS System)	(MINAM 2013)	
Ramsar Convention on Wetlands	The Lake Titicaca was recognized by the Ramsar Convention on January 20, 1997, as a Wetland of International Importance, especially as a habitat for waterfowl.	(MINAM 2013)	
Indicative list of World Heritage of the UNESCO Convention of 1972	Lake Titicaca was registered as a mixed asset in 2005, as it represents at the same time a cultural space where tradition, ways of life, customs and ancestral values of its current inhabitants coexist.	(MINCUL 2008)	

Appendix D. - Water quality data for Puno Bay

Field measurements by the National Authority of Water (*Autoridad Nacional del Agua* [ANA]) for dissolved oxygen, pH, temperature and conductivity were done using multiparameters: PONSEL MESURE model ODEON SN-ODEON-1588 and HORIBA model U-50 SN 9EEXYHW6. Those measurements were done according to Chief Resolution (*Resolución Jefatural*) Nº 182-2011-ANA regarding the National Protocol for Monitoring the Quality of Natural Surface Water Bodies.

Field measurements by the Sea Institute of Peru (Instituto del Mar del Perú [IMARPE]) for dissolved oxygen, pH, temperature and conductivity were carried out with a HANNA multiparameter during the period 2010 - 2014, and with a YSI PROFESSIONAL PLUS multiparameter and a METTLER TOLEDO optical oximeter during the period 2015 – 2019. The determination of dissolved oxygen was checked with the Winkler method. For this measurement, the multiparameter probe was immersed in the surface (0.5 m depth) of the water body and water samples were extracted from the water column with a 5.0 L NISKIN bottle, the multiparameter probe was carefully immersed in it without generating turbulence. The measurements were carried out according to the procedures and instructions for the operation of the equipment, the IMARPE protocol and standard methods (APHA, 2005). Transparency measurement was done with a secchi disc by both institutions. The methods of quantification for other parameters are shown in Table 1, while the sampling frequency is shown in Table 2.

Table 1. Parameters provided by ANA and IMARPE and method of quantification

Parameter	Unit/format	Period	Measurement method by ANA	Measurement method by IMARPE
BOD ₅	mg O ₂ /L	(ANA) 2013 2014-15 2017	EPA 405.1. Quantification limit (QL): 2 mg/L. 5-Day BOD Test (QL: 2 mg/L). SM 5210 B 5-Day BOD Test. SMEWW-APHA-AWWA-WEF Part 5210 B, 22nd Ed. 2012	5-Day BOD Test. Simple dilution. INDECOPI, 2015.
TSS	mg/L	2018-19 2013	5-Day BOD Test. SMEWW-APHA-AWWA-WEF Part 5210 B, 23 rd Ed. 2017 Total suspended solids (QL: 5 mg/L). SM 2540-D	SST analysis protocol, IMARPE 2013; Standard Methods for the Examination of Water and Wastewater -2540-D, APHA.2005
		2014-15 2017-19	Total Suspended Solids Dried at 103-105°C (QL: 3 mg/L). SM 2540-D Total Suspended Solids Dried at 103-105°C. SMEWW-APHA-AWWA-WEF Part 2540 D, 23 rd Ed. 2017	, , , , , , , , , , , , , , , , , , , ,
PO ₄ -P	mg P/L	2013	Phosphates (0.008 mg/L). EPA 365.3	Phosphate analysis protocol, IMARPE 2013; Standard Methods for the Examination of Water and Wastewater -2500 -E, APHA 2005; EPA Method 365.2; STRICKLAND & PARSONS 1972.
		2014-15	Phosphorus. Ascorbic Acid Method (QL: 0.03 PO ₄ ⁻³ mg/L). SM 4500-P E	
TP	mg P/L	2013	Total Phosphorus (QL: 0.01 mg/L). EPA 365.3	Procedure manual HACH DR 2800. Method 8190 PhosVer 3, digested with acid persulphate (0.06 to 3.50 mg/L PO ₄ or 0.02 a 1.10 mg/L P)
		2014-15 2017 2018	Phosphorus. Ascorbic Acid Method (QL: 0.01 P mg/L). SM 4500-P E Phosphorus, all forms (Colorimetric Ascorbic Acid, Two reagent). EPA 365.3, 1983 Water Quality - Determination of orthophosphate and total phosphorus contents by flow analysis (FIA and CFA). ISO 15681-2. 1st. Ed. 2003	
		2019	Water Quality - Determination of orthophosphate and total phosphorus contents by flow analysis (FIA and CFA). ISO 15681-2:2018, 2 nd edition.	
NO ₃ -N	mg N/L	2014	Ultraviolet Spectrophotometric Screening Method (QL: 0.03 NO $_3$ -N mg/L). SM 4500-NO $_3$ - B. Nitrogen (Nitrate).	Nitrate analysis protocol, IMARPE 2013; Standard Methods for the Examination of Water and Wastewater - 2500 -NO ₃ -E, APHA.2005; EPA Method 353.3; STRICKLAND & PARSONS 1972.
		2017-19	Determination of inorganic anions in drinking water by Ion Chromatography. EPA 300.1 Rev. 1, 1997	
TN	mg N/L	2013	Total nitrogen (0.1 mg/L). EPA 351.3	Standard Methods, 2005. Examination of water and wastewater. Method 4500-Norg B. Total Kjeldahl nitrogen.
		2014-15 2017-19	Nitrogen (organic). Macro- Kjeldahl method (QL: 0.02 NH ⁴⁺ -N mg/L). SM 4500-Norg-B Determination of total nitrogen after UV digestion - Method using flow analysis (CFA and FIA) and spectrometric detection. ISO 29441, 1 st Ed. 2010	
Chl-a	mg/m ³	2013	Standard Methods for Examinations of Water and Wastewater. APHA-AWWA-WEF $21^{\rm st}$ Ed 2005. Chlorophyll (QL: 0.1 mg/L). SM 10200-H "Chlorophyll"	Jeffrey & Humphrey, 1975; Standard Methods for the Examination of Water and Wastewater-10200-H. APHA 2005; STRICKLAN & PERSON 1972.

DO = Dissolved oxygen concentration; pH = Potential of hydrogen; T = Temperature; EC = Electrical conductivity; BOD₅ = Biological oxygen demand; TSS = Total suspended solids concentration; PO₄-P = Orthophosphate as phosphorus concentration; TP = Total phosphorus concentration; NO₃-N = Nitrate-nitrogen concentration; TN = Total nitrogen concentration; Chl-a = Chlorophyll-a concentration; SD = Transparency.

Table 2. Parameters and sampling frequency by ANA and IMARPE.

Parameter	Source	Freq.	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020*
DO	A	T				✓	✓	✓	✓	✓	✓		
	I	D	\checkmark										
pН	A	T				\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark		
•	I	M	\checkmark	✓	✓								
T	A	T				\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark		
	I	D	\checkmark	✓	✓	\checkmark	\checkmark	✓	✓	✓	✓	✓	✓
EC	A	T				\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark		
	I	M	\checkmark	✓	✓	\checkmark	\checkmark	✓	✓	✓	✓	✓	✓
BOD ₅	A	T				\checkmark	\checkmark	✓	✓	✓	✓		
TSS	A	T				\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark		
PO ₄ -P	A	T				\checkmark	\checkmark	✓	✓				
	I	F	\checkmark	✓	✓	\checkmark	✓	✓	✓	✓	✓	✓	✓
TP	A	T				\checkmark	\checkmark	✓	✓	✓	✓		
NO ₃ -N	A	T				\checkmark	✓	✓	✓	✓	✓	✓	✓
	I	F	\checkmark	✓	✓	\checkmark	\checkmark	✓	✓	✓	✓	✓	✓
TN	A	T				\checkmark	✓	✓	✓	✓	✓		
Chl-a	A	T				✓	✓				✓		
	I	F	\checkmark	✓	✓	\checkmark	\checkmark	✓	✓	✓	✓	✓	✓
SD	A	T				✓	✓		\checkmark		✓	✓	✓
	I	F	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

^{*}Data collected by IMARPE from January to March. This table shows the overall availability by source (ANA, IMARPE) for each parameter, the availability was not the same for all sampling sites from the same source. 2020; A=ANA; I=IMARPE; Freq.= Sampling frequency; T = Twice per year; F = Four times per year; M = Monthly; D = Daily; DO = Dissolved oxygen concentration; pH = Potential of hydrogen; T = Temperature; EC = Electrical conductivity; BOD₅ = Biological oxygen demand; TSS = Total suspended solids concentration; PO₄-P = Orthophosphate as phosphorus concentration; TP = Total phosphorus concentration; NO₃-N = Nitrate-nitrogen concentration; TN = Total nitrogen concentration; Chl-a = Chlorophyll-a concentration; SD = Transparency.

Appendix E. - Some results from data comparison and statistical tests

Comparison of data from ANA and IMARPE

Table 1. Results of tests for difference between ANA and IMARPE sampling points (Section 4.3.1)

Sampling points	Parameter	test	d.f.	t value	U value	p-value
IPB2-IPB12	DO	U			211	0.003
	T	U			502.5	0.79
	TP	U			243.5	0.009
	TN	U			280	0.042
	PO ₄ -P	U			164	0.14
	NO_3 -N	U			413	0.75
	SD	U			240.5	0.99
OPB1-OPB10	DO	U			105	0.021
	pН	t	51	1.53		0.13
	T	U			260	0.23
	EC	U			159.5	0.42
	PO ₄ -P	U			102	0.34
	NO_3 -N	U			173	0.69
	Chl-a	U			78	0.77
OPB5-OPB12	DO	t	53	-1.5		0.14
	pН	U			276	0.066
	T	U			268	0.17
	EC	U			179	0.66
	SD	U			97.5	0.59

ANA sampling points = IPB2, OPB1, OPB5; IMARPE sampling points = IPB12, OPB10, OPB12; U = Mann–Whitney U Test; t = t-test

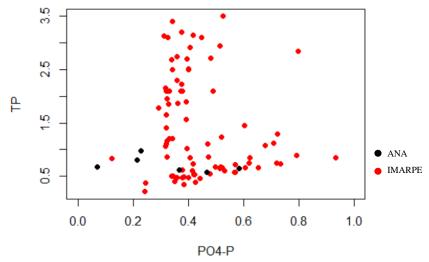


Figure 1. Bivariate plot for total phosphorus and dissolved phosphorus (PO_4 -P) in IPB2. (ANA, n=11; IMARPE, n=87)

Comparison of Inner and Outer Puno Bay

Table 2. Results of Mann-Whitney U Test for significance difference between parameters from IPB and OPB (Section 4.3.3)

Parameter	U value	p-value
Chl-a	34976	< 2.2 x 10 ⁻¹⁶
TN	15558	$< 2.2 \times 10^{-16}$
TP	15558	< 2.2 x 10 ⁻¹⁶
PO ₄ -P	28079	< 2.2 x 10 ⁻¹⁶
NO ₃ -N	26992	< 2.2 x 10 ⁻¹⁶
TSS	15633	< 2.2 x 10 ⁻¹⁶
BOD ₅	13726	< 2.2 x 10 ⁻¹⁶
EC	27073	< 2.2 x 10 ⁻¹⁶
pH	21673	2.8 x 10 ⁻¹⁶
T	37064	1.1 x 10 ⁻¹⁴
DO	36501	2.4×10^{-13}
TN:TP	1602	< 2.2 x 10 ⁻¹⁶

Comparison of factor scores between sampling groups within IPB

Table 3. Results of t-test for significance of the slopes in Figure 4.7 (Section 4.3.5)

	d.f.	t value	p-value
Factor 3 - Sewage	23	4.81	15 x 10 ⁻⁵
Factor 3 - Reeds	23	4.57	2.7 x 10 ⁻⁴
Factor 3 - Open	34	6.42	4.8 x 10 ⁻⁷
Factor 1 - Sewage	23	-0.81	0.86
Factor 1 - Reeds	23	0.52	1.22
Factor 1 - Open	34	-0.73	0.94
Factor 2 - Sewage	23	0.87	0.78
Factor 2 - Reeds	23	0.42	1.35
Factor 2 - Open	34	-1.10	0.56