REPORT OF WATER AND SEDIMENT CONTAMINANTS OR POLLUTANTS AND THEIR POSSIBLE RELATION

WITH TELMATOBIUS CULEUS DIE-OFF IN APRIL AND MAY 2015

WATER QUALITY

Water quality is very important for amphibians, especially for the Titicaca Water frog that is completely aquatic, and different parameters can affect the metabolism of the frog in different ways.

Both quantity and quality of water are important considerations and are among the most important factors aiding an amphibian's survival (Zippel, 2012). Unlike reptiles and birds, amphibians do not have a shelled (amniotic) egg and the embryo is exposed to different water quality issues that can affect the survival of the individuals. Amphibians are perhaps even more sensitive to water quality than many fish (Zippel, 2012)

As a result, waterborne toxins including ammonia, nitrite, heavy metals, disinfectants, pesticides, chlorine, and chloramine are of particular concern when keeping amphibians. Due to the tremendous diversity of species and the variable tolerance of individual specimens, some amphibians exposed to water of poor quality may be unaffected while others become ill or die (Wright and Whitaker, 2001). Amphibians absorb a significant portion of their oxygen through their skin and this is the case of t. culeus, where lungs are reduced and the frog never exists the water. Unfortunately, an amphibian's amazing adaptations and strong ties to an aquatic environment also mean that they are particularly sensitive to changes in water quality and quantity (Zippel, 2012).

For that reason we measured different parameters of water quality. Measurements of water quality were taken in 39 localities of the lake (see annex); the parameters that we use are those that are recognized to be important for amphibians (Whitaker, 2001, Zippel, 2009, Zippel et al. 2011) such as pH, Ammonia, Ammonium, Nitrite, Nitrate, Phosphate, dissolved oxygen, hardness, and alkalinity. We also measured some heavy metals such as Chromium, Copper, Iron, Nickel and Zinc. These measurements were obtained with a Palintest 7500 photometer, an instrument with dual light source photometer offering direct-reading of pre-programmed test calibrations, Absorbance and Transmittance, with an accuracy of ±1.0% these measurements were obtained once a month per locality during one year period and in others less times or once during the study. We measured these different water parameters in a total of 47 sites on both sides of the lake (Figure 1-3)

We monitored water temperature with a HOBO water temperature Pro v2 Data logger –U22-001 with an accuracy of 0.21 °C. These data loggers were active at all times in the three localities at 2.5 meters of depth, where most of the frogs are present. The data was downloaded once a month and temperatures were saved every hour. This data was obtained over one year.

(For more detail of the values of each parameter please see Annex water quality)

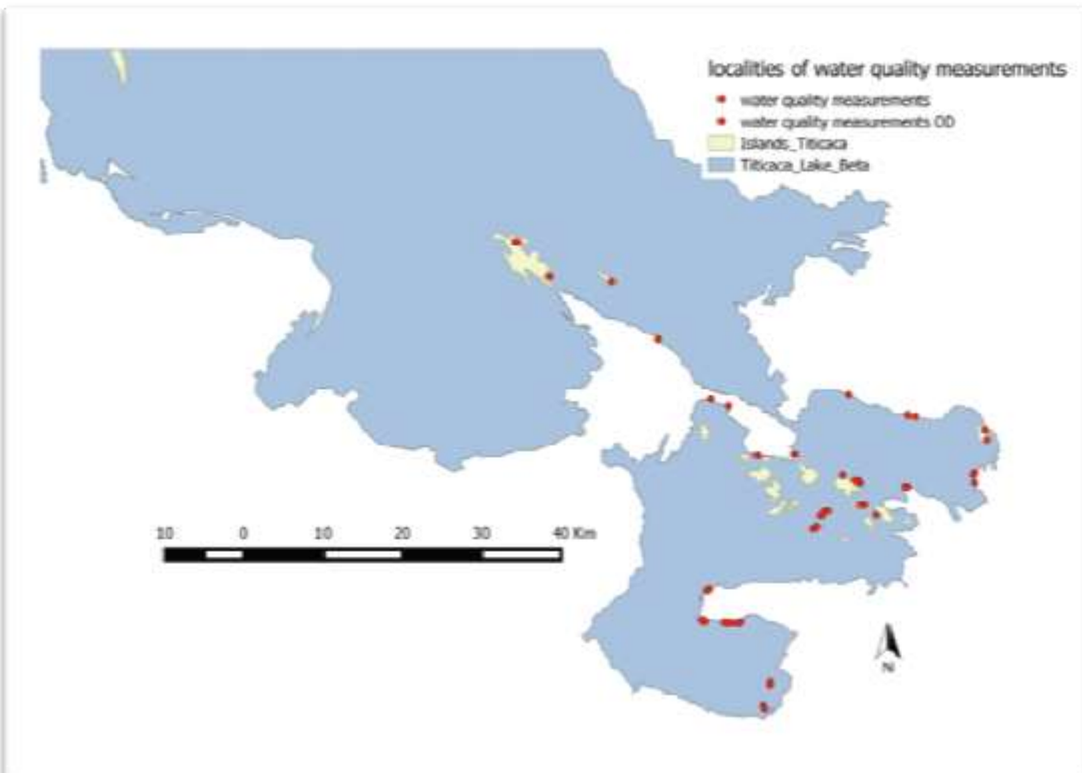

Figure 1 Points of water quality measurements in Titicaca Lake

Figure 2 Water measurements of different parameter in the Lake with the Palintest photometer

Figure 3 Dissolved oxygen measurements in the Lake

Water temperature

Values of water temperatures do not change too much in Lago Mayor within the two localities where dataloggers were installed (Isla de la Luna and Chachapolla). In the case of Chicharro, in Lago Menor we can see a bigger variation of temperatures during the day and the night. This probably because in Lago Menor the depth is not so great like in Lago Mayor and that causes a big variation of temperatures from 10 °C to temperatures above 20 in some cases. This can also cause some stress to the frogs and also can make them more susceptible to some diseases such as Chytridium. More studies would be recommended in this subject and to see the effects of temperature on the species because one aspect we need to take in account would be climate change.

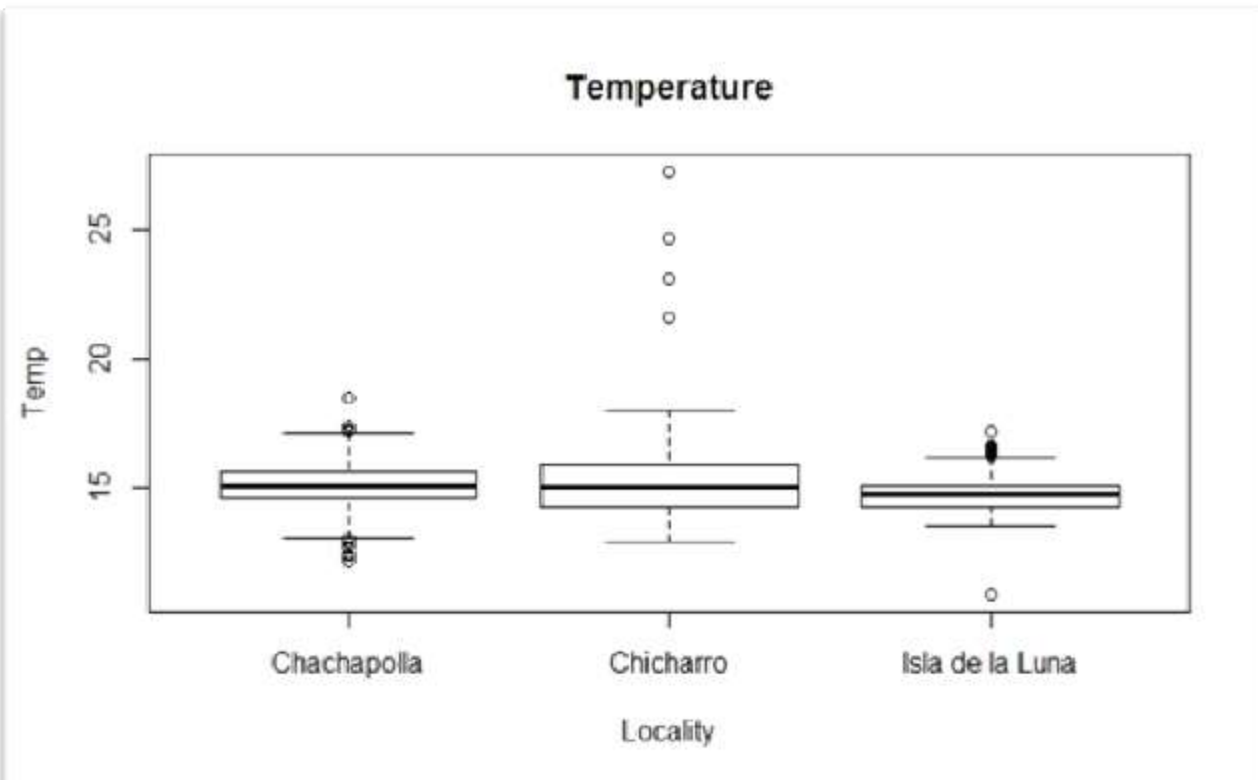

Figure 4 Temperatures recorded in three localities of Lake Titicaca during one year.

Ammonia/ Ammonium – NH_3/NH_4 N

Ammonia occurs as a breakdown product of nitrogenous material in natural waters. It is also found in domestic effluents and certain industrial waste waters. Ammonia is harmful to fish and other forms of aquatic life (Palintest, 2016). It originates as metabolic waste product Ammonia/ammonium ratio is pH and temperature dependent. For amphibians it is considered as very toxic and it is established that values <0.2 mg litre^{-1}, N as unionized ammonia, are acceptable (Odum and Zippel, 2012).

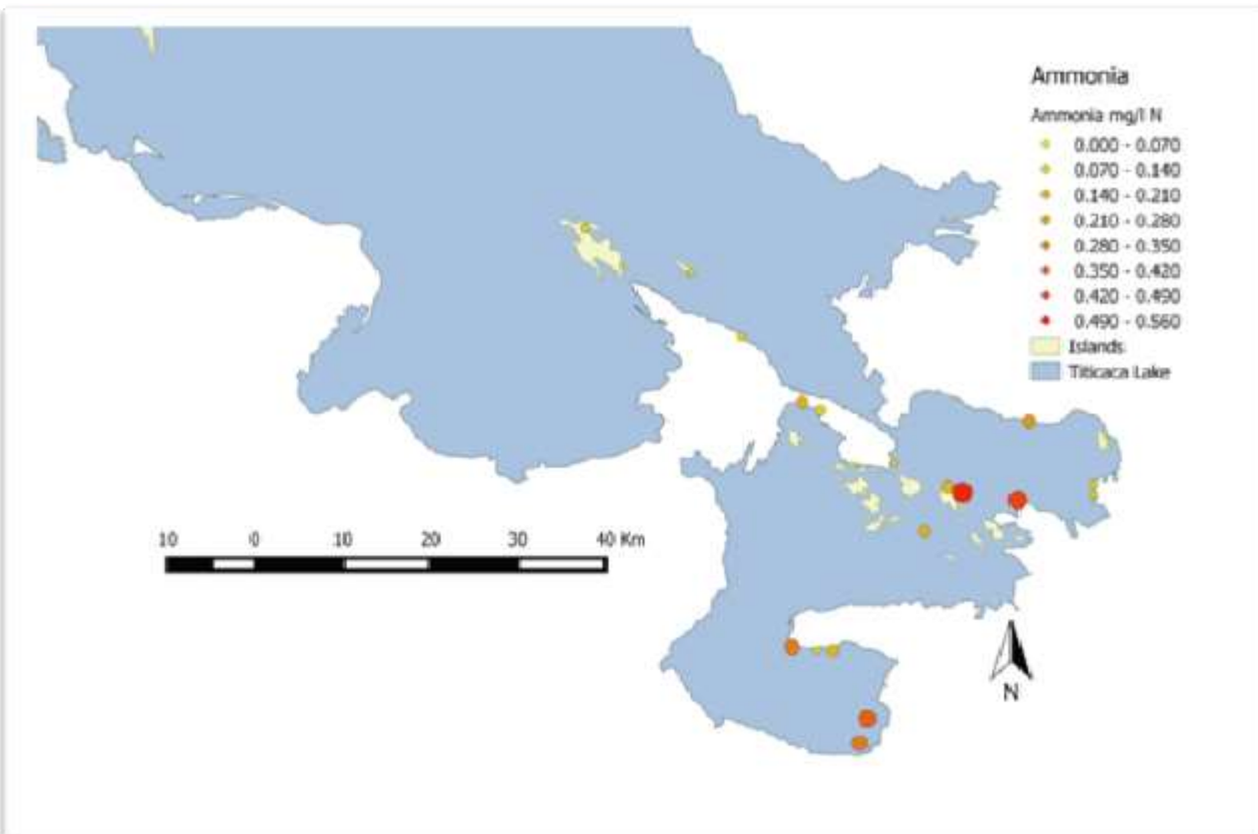

Figure 5 Ammonia values in Titicaca Lake

Ammonium/ammonia level in the lake normally are low, especially in the Lago Menor where most of the best populations are present, in figure 1 we can see that the levels are higher in Lago Menor with the highest values around Suriqui island and Patapatani. We can see that several localities present higher values than 0.2 mg litre^{-1}, N, being dangerous for amphibians, this occurring in the area of Guaqui and Suriqui. Here we can see a pattern that is happening in Lago Menor and we think that we should monitor the situation during the different seasons of the year. We think that these high values, much higher that the acceptable for amphibians, are having an effect on the population of *T. culeus.*

Nitrites NO_2^-

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The nitrate ion (NO_3^-) is the stable form of combined nitrogen for oxygenated systems. Although chemically inert, it can be reduced by microbial action. The nitrite ion (NO_2^-) contains nitrogen in a relatively unstable oxidation state. Nitrite is a product of aerobic biological action on ammonia NH_3/NH_4^+ It is considered a toxic substance and values <1.0 mg liter $^{-1}$ are acceptable, but ideally zero will be desired for amphibians (Odum and Zippel, 2012). When nitrites contact blood plasma, they transform hemoglobin to methemoglobin, decreasing the oxygen carrying capacity of the blood (Williams, 1994).

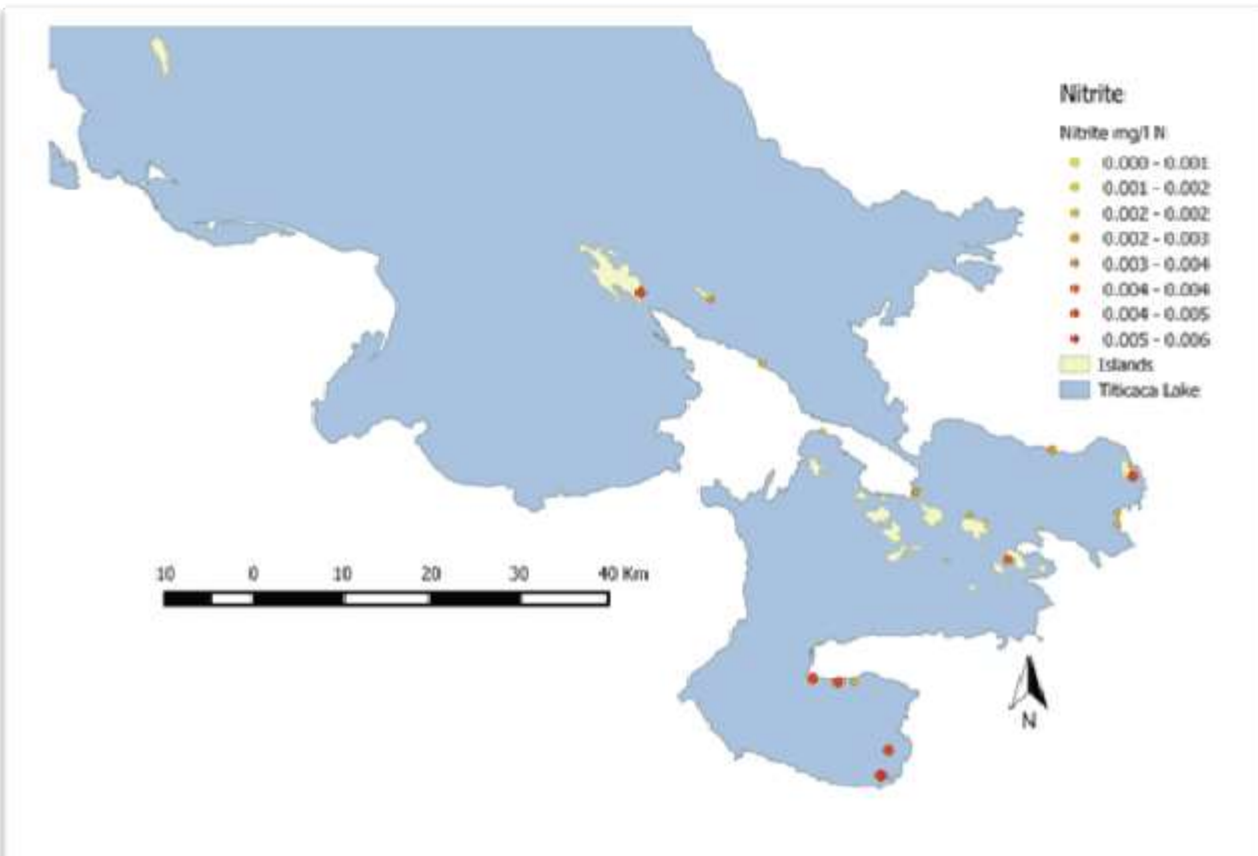

Figure 6 Nitrite values in Titicaca Lake

Nitrite levels in the lake are relatively low, not reaching the limit value of 1.0 mg liter $^{-1}$ that is dangerous for amphibians. But we can see a pattern that the south east part of the Lago Menor, the levels are higher than the other areas, these values should be monitored in different seasons of the year, because it also affect the oxygen carrying capacity.

Nitrates N/NO_3

Nitrates are normally present in natural, drinking and waste waters. Nitrates enter water supplies from the breakdown of natural vegetation, the use of chemical fertilizers in modern agriculture and from the oxidation of nitrogen compounds in sewage effluents and industrial wastes. The level of nitrate is an important control test for water supplies. Drinking waters containing excessive amounts of nitrates can cause methaemoglobinaemia in bottle-fed infants (blue babies). The EEC has set a recommended maximum level of 25 mg/l NO_3 (5.7 mg/l N) and an absolute maximum of 50 mg/l NO_3 (11.3 mg/l N) for nitrate in drinking water (Palintest, 2016). For amphibians nitrates are slightly toxic and values lower than 50.0 mg litre^{-1} are acceptable for amphibians (Odum and Zippel, 2012).

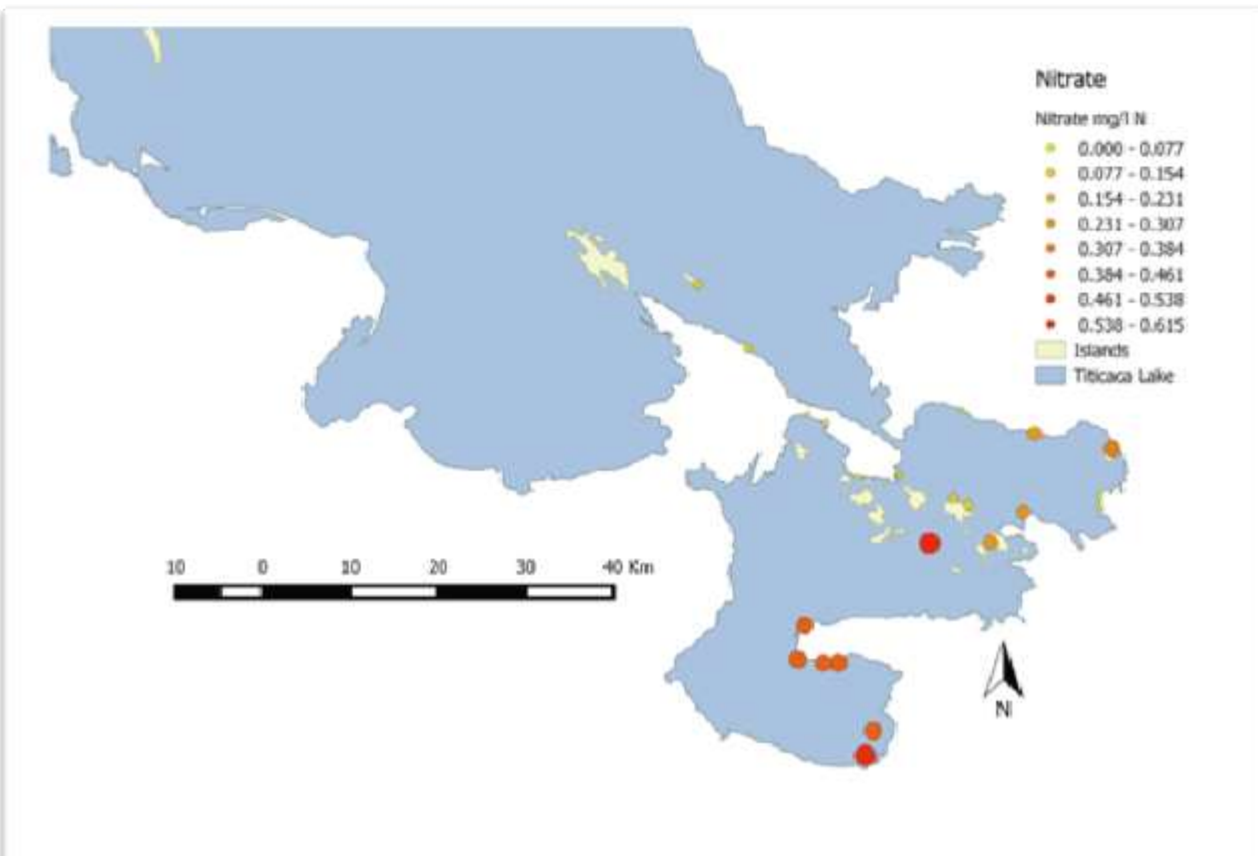

Figure 7 Nitrate values in Titicaca Lake

Values of nitrates in the lake do not reach the dangerous levels of 50 mg litter^{-1} but with the measurements we can also see a pattern that the values in the Lago Menor are higher, especially in the area of Guaqui to Santa Rosa at the south area of the lake.

Phosphates PO^4 /P

Phosphates are extensively used in detergent formulations, in food processing and in industrial water treatment processes. These phosphates may be in the form of orthophosphates, or are broken down to orthophosphates in the process concerned. Agricultural fertilizers normally contain phosphate minerals. Phosphates also arise from the breakdown of plant materials and are found in animal wastes. Phosphates can therefore enter water courses through a variety of routes, particularly domestic and industrial effluents and run-off from agricultural land. In particular, phosphates are associated with eutrophication of water and with rapid, unwanted plant growth in rivers and lakes (Palintest, 2016).

Phosphates are toxic to many animals and interfere with calcium metabolism. Toxicity may be species specific. EPA limits PO_4 to 10 mg $litre^{-1}$ (Odum and Zippel, 2012). For drinking water the EEC has set a guide level of 0.5 mg/l PO_4 (0.4 mg/l P_2O_5) and a maximum admissible concentration of 6.7 mg/l PO_4 (5 mg/l P_2O_5) (Palintest, 2016).

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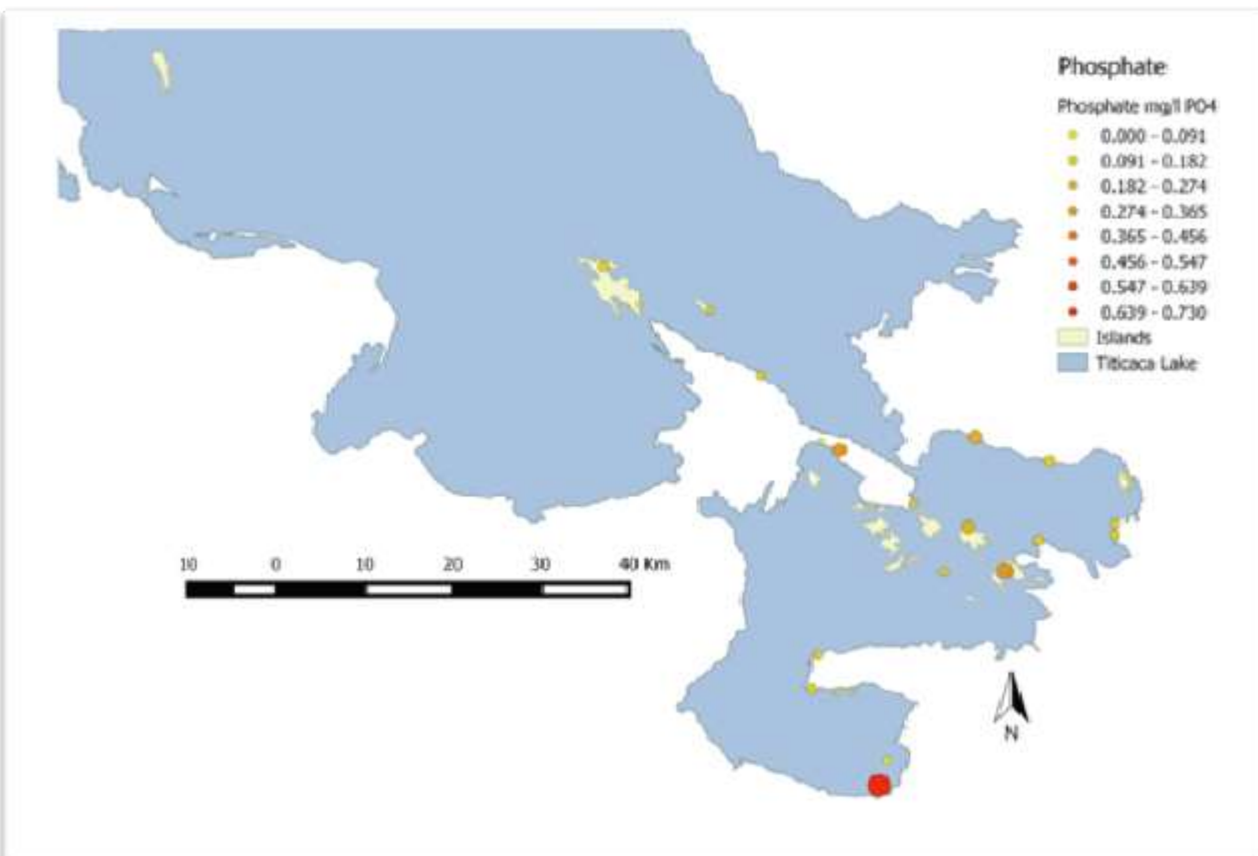

Figure 8 Phosphate values in Titicaca Lake

Phosphates can provide information about organic pollution in the lake but we saw that values of phosphates in the lake do not reach the dangerous levels of 10 mg $litter^{-1}$ but with the measurements we can also see Guaqui has the highest value even passing the maximum levels for drinking water established by EEC of 0.5 mg/l PO_4.

pH

The pH of water is determined by the proportion of hydrogen (H^+) and hydroxide ions (OH^-) present. Each pH unit represents a 10-fold change in the number of hydrogen ions. At a pH of 7, hydrogen ions equal hydroxide ions. Water becomes more acidic with increasing hydrogen ions resulting in a lower pH. While many amphibians are able to tolerate wide swings in pH for short periods of time, this can be stressful (Wright and Whitaker, 2001). Inadequate pH can cause metabolic problems if not within acceptable range for species, disrupting ion exchange. It is species dependent, but usually near neutral. pH below 6 and above 8 are potentially a problem (Odum and Zippel, 2012). It is important to remember that higher pH values favor the more harmful form of some potential toxins, such as ammonia. Nitrification also produces acid which, unless buffered, will lower pH (Wright and Whitaker, 2001).

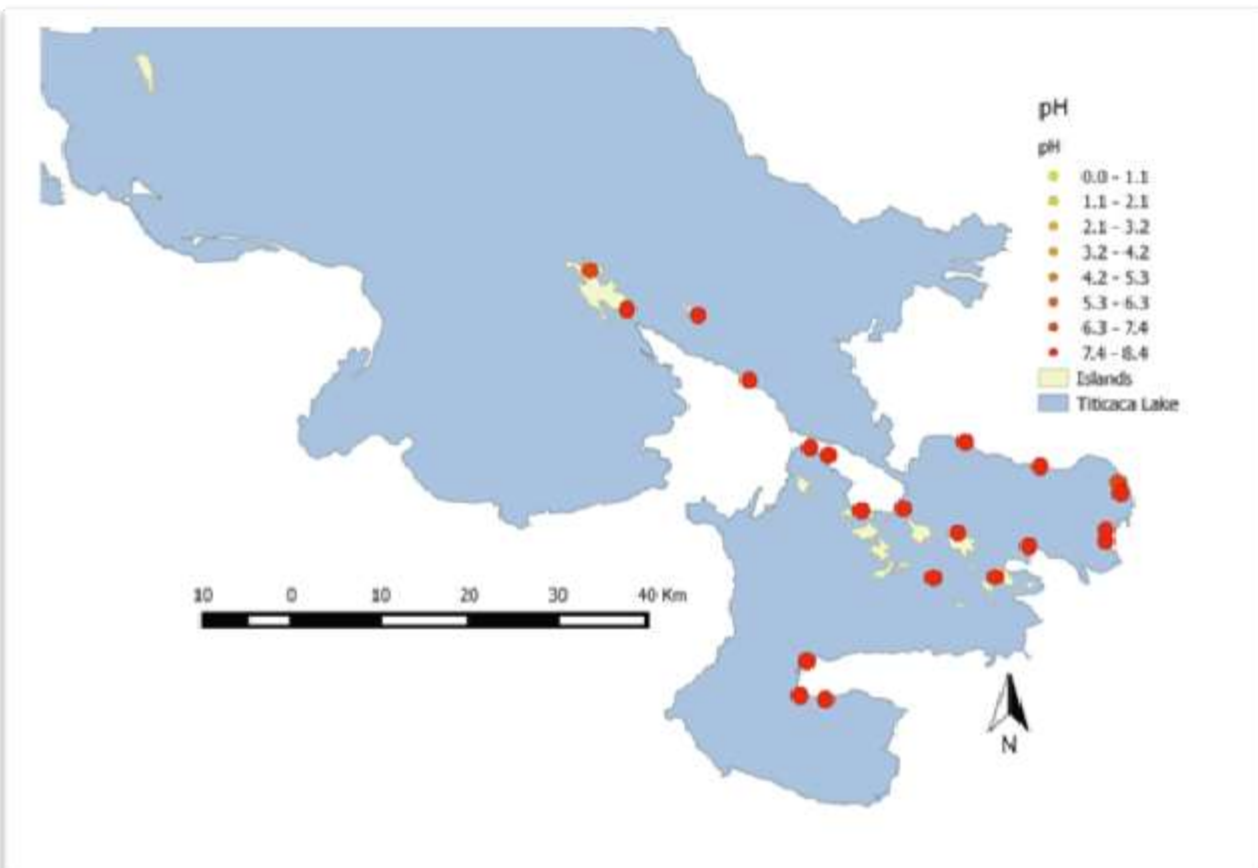

Figure 9 pH values in Titicaca Lake

pH values in the entire lake are relatively high, which is the normal situation for the lake and the value ranges from 7.05 to 8.45. In all the localities we did not find any place with values under 7.

Calcium hardness (dissolved Ca and Mg salts)

Calcium hardness is caused by the presence of calcium ions in the water. Calcium salts can be readily precipitated from water and high levels of calcium hardness tend to promote scale formation in water systems (Palintest, 2016). In amphibians hard water can cause skin problems in some species by disrupting normal osmotic regulation of the amphibian. Most show a preference for 'soft water' but this can be species dependent, 0.75 mg liter $^{-1}$ (ppm) of $CaCO_3$ for animals that require soft water >100 mg liter $^{-1}$ should be considered hard water for an amphibian. In the case of T. culeus it is known that the species inhabit waters with high values of calcium hardness above 100 mg liter $^{-1}$.

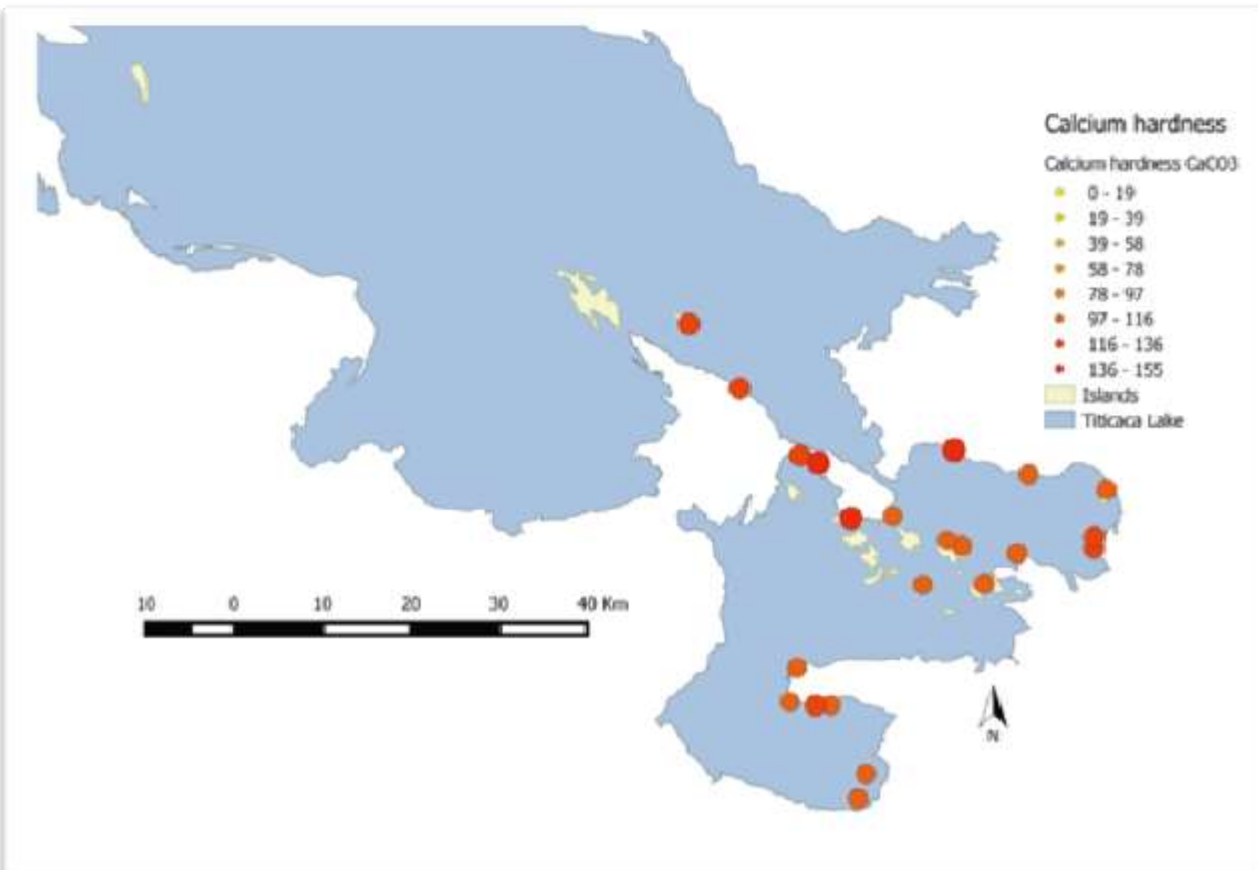

Figure 10 Calcium hardness values in Titicaca Lake

Calcium hardness in the lake seems to present the normal values because the kind of substrate we have in the lake makes this a lake with relatively high values, above 100 mg liter $^{-1}$. In the map (figure 6), we can see that most of the areas show high values.

Alkalinity

The Alkalinity of water is caused by the presence of alkaline substances such as hydroxides, carbonates, bicarbonates and, to a lesser extent, silicates and phosphates. Quantitatively, alkalinity is the capacity of the water to react with acid to a specified pH end point. The value obtained will depend on the pH indicator used (Palintest, 2016). For amphibians this affects the stability of the water, which will prevent stress in the species.

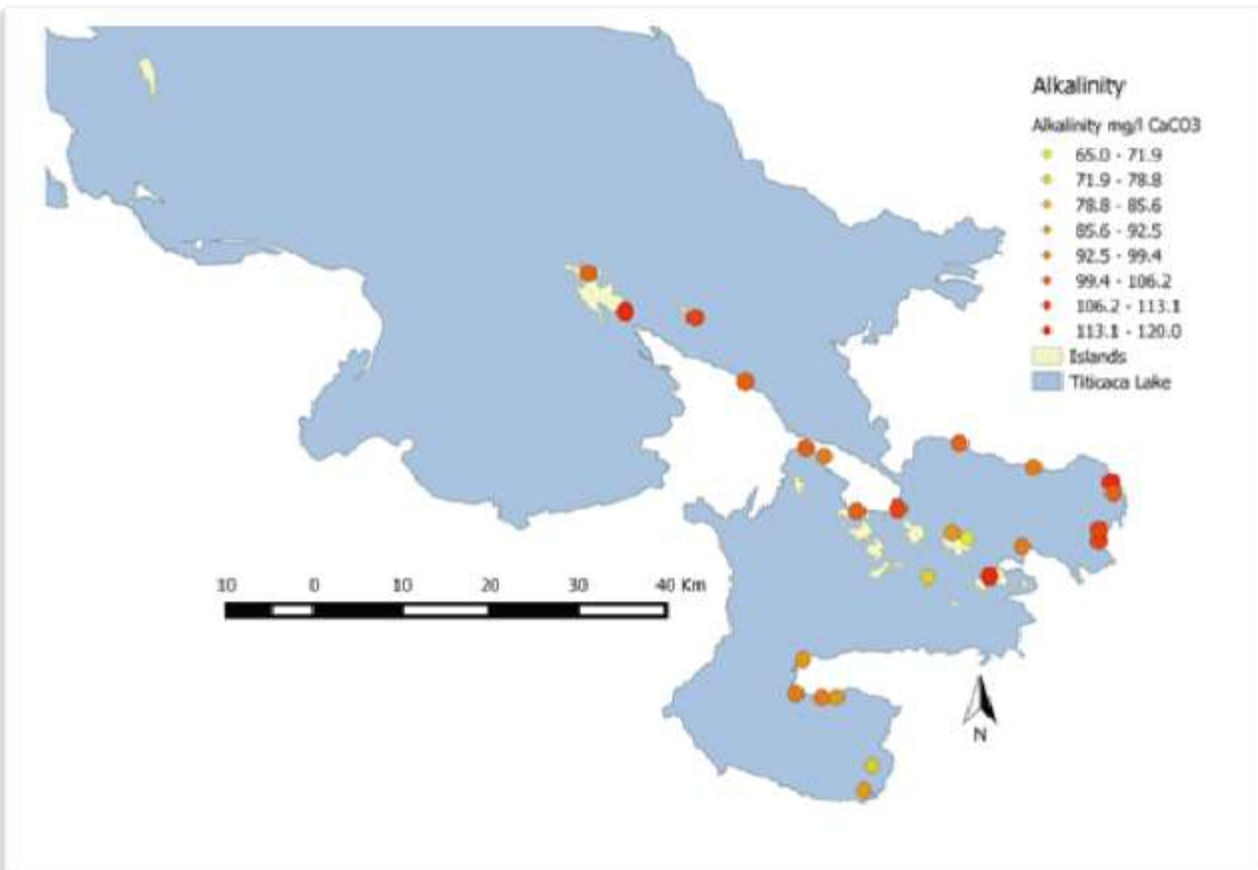

Figure 11 Alkalinity values in Titicaca Lake

Alkalinity values in the lake are normally high, approaching 100, but we can see points around Suriqui Island and Guaqui that have very low levels like 65 mg liter $^{-1}$ or 75 mg liter $^{-1}$

Copper (Cu)

Copper is a heavy metal that occurs naturally in many waters and may also result from corrosion of pipes and fittings. The presence of copper in drinking water can give rise to discoloration or an astringent taste. Chelated copper compounds are extensively used as algaecides (Palintest, 2016). Copper is considered toxic for amphibians and values <0.05 mg liter^{-1} is acceptable (Odum and Zippel, 2012).

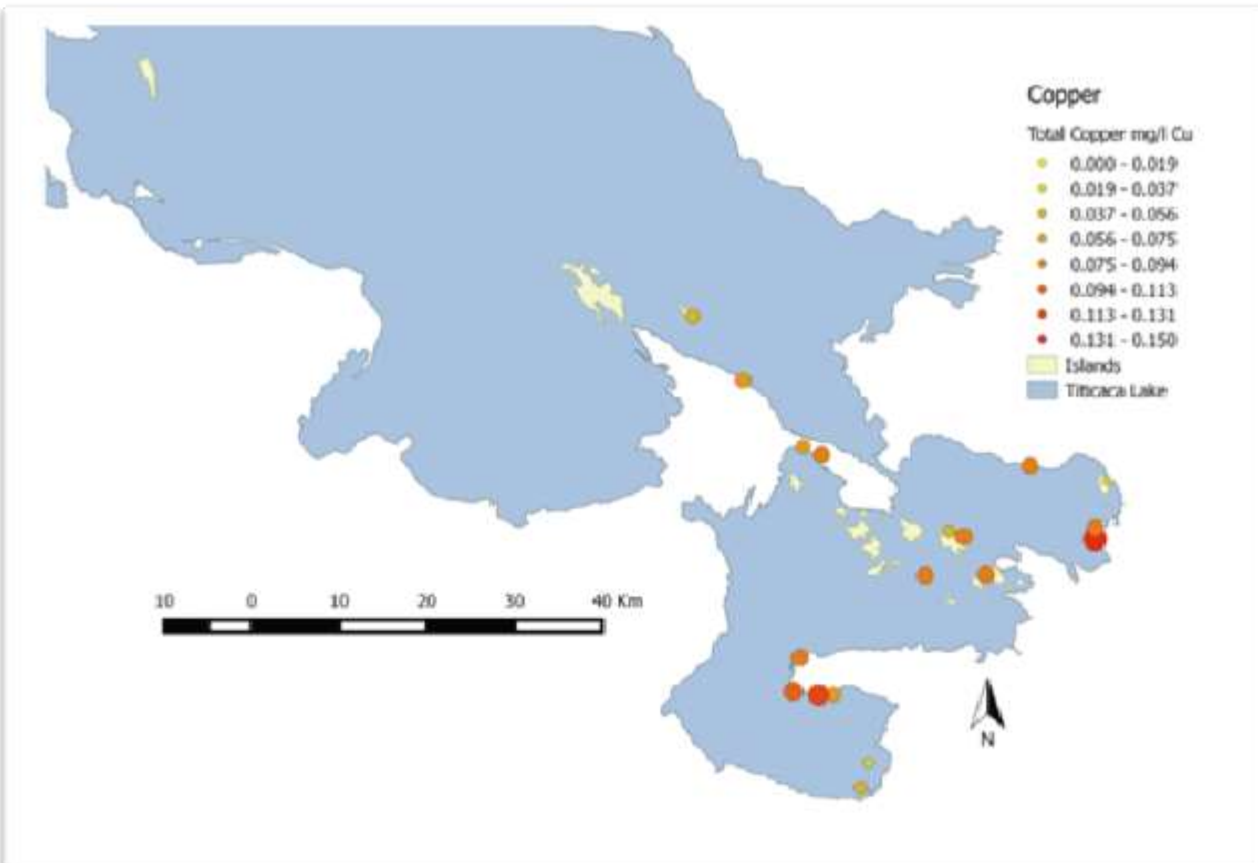

Figure 12 Copper values in Titicaca Lake

Values of copper that we found in the lake were normally under the normal values. Just three localities, Apillani, Santa Rosa and Cachilaya, had values above the safe levels proposed by Odum and Zippel (2012). All these localities that are present in Lago Menor.

NICKEL Ni

Nickel does not occur naturally in water but is found in many industrial waste waters, such as those from the steel and plating industries. It is considered an undesirable constituent of water, and hence requires close and careful monitoring. The EC maximum admissible concentration for drinking water (MAC) is 0.05 mg/l (Palintest, 2016).

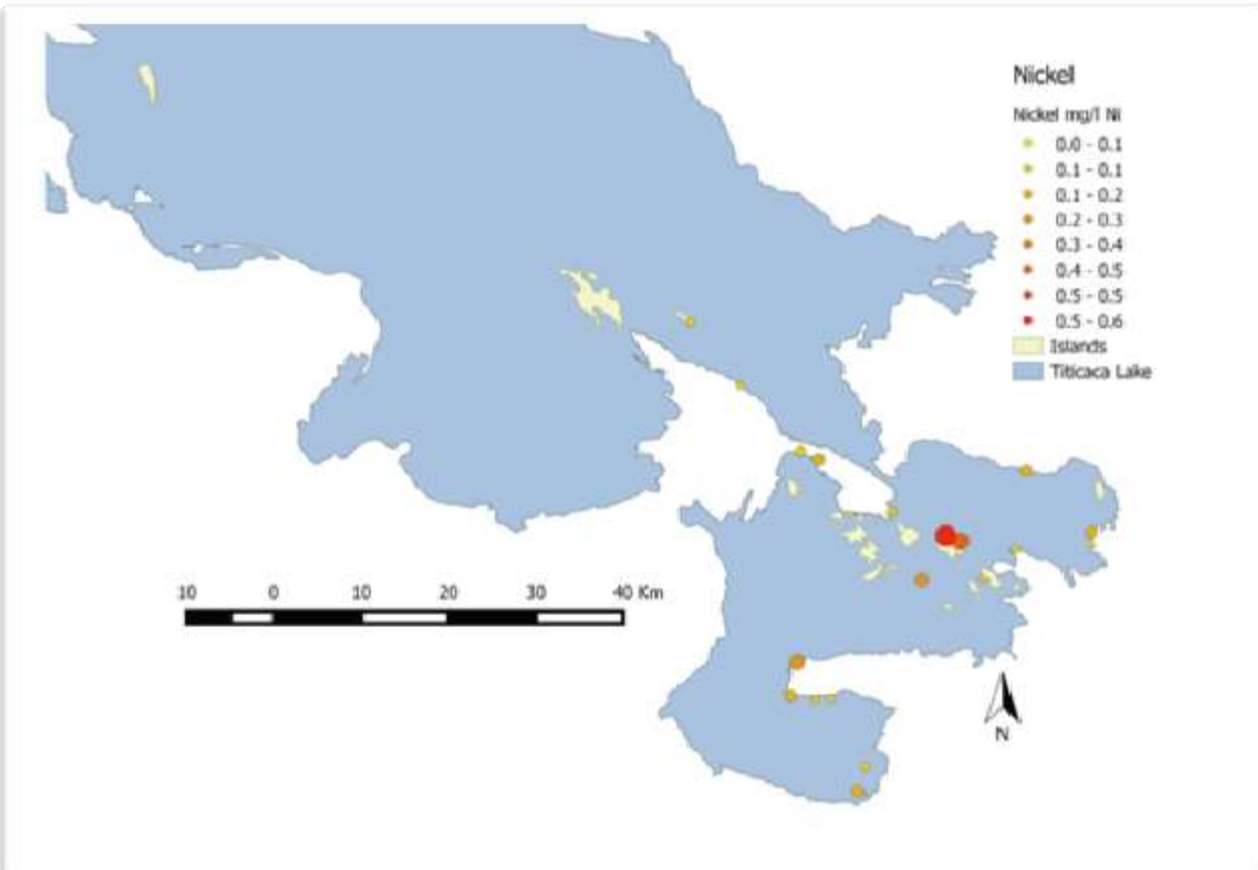

Figure 13 Nickel values in Titicaca Lake

With the values found in the lake we can see that nickel values are relatively high considering that is an element that should not be found in nature. Almost all of them pass the values recommended by the MAC of o.o5 mg/l, and we can see an extreme in Suriqui Island with values of 0.4 mg/l to 0.6 mg/l.

ZINC Zn

Zinc compounds are used as corrosion inhibitors in industrial cooling water systems and similar applications. Control of the zinc level is an important aspect of corrosion control in such systems. Zinc and zinc containing alloys are widely used in industry and zinc salts are commonly found in industrial effluents (Palintest, 2016).

Figure 14 Zinc values in Titicaca Lake

Values of Zinc in the lake are normally low but we can see that three localities, Cachilaya, Calauta and Santa Rosa, respectively, present the highest levels of Zinc, with values of 0.05 mg/l to 0.08 mg/l. All of these localities are in Lago Menor.

Iron

Iron occurs widely in nature and is found in many natural and treated waters. Iron is an objectionable constituent in both domestic and industrial water supplies. The presence of iron affects the taste of beverages and causes unsightly staining of laundered clothes, plumbing fittings, swimming pool surfaces and the like. The formation of insoluble iron deposits is troublesome in many industrial applications and in agricultural uses such as drip feed irrigation. Iron is an important test for effluents, waste waters and industrial water samples. The sources of iron in such samples are many and varied and include the corrosion of plant and equipment and waste from industrial processes (Palintest, 2016).

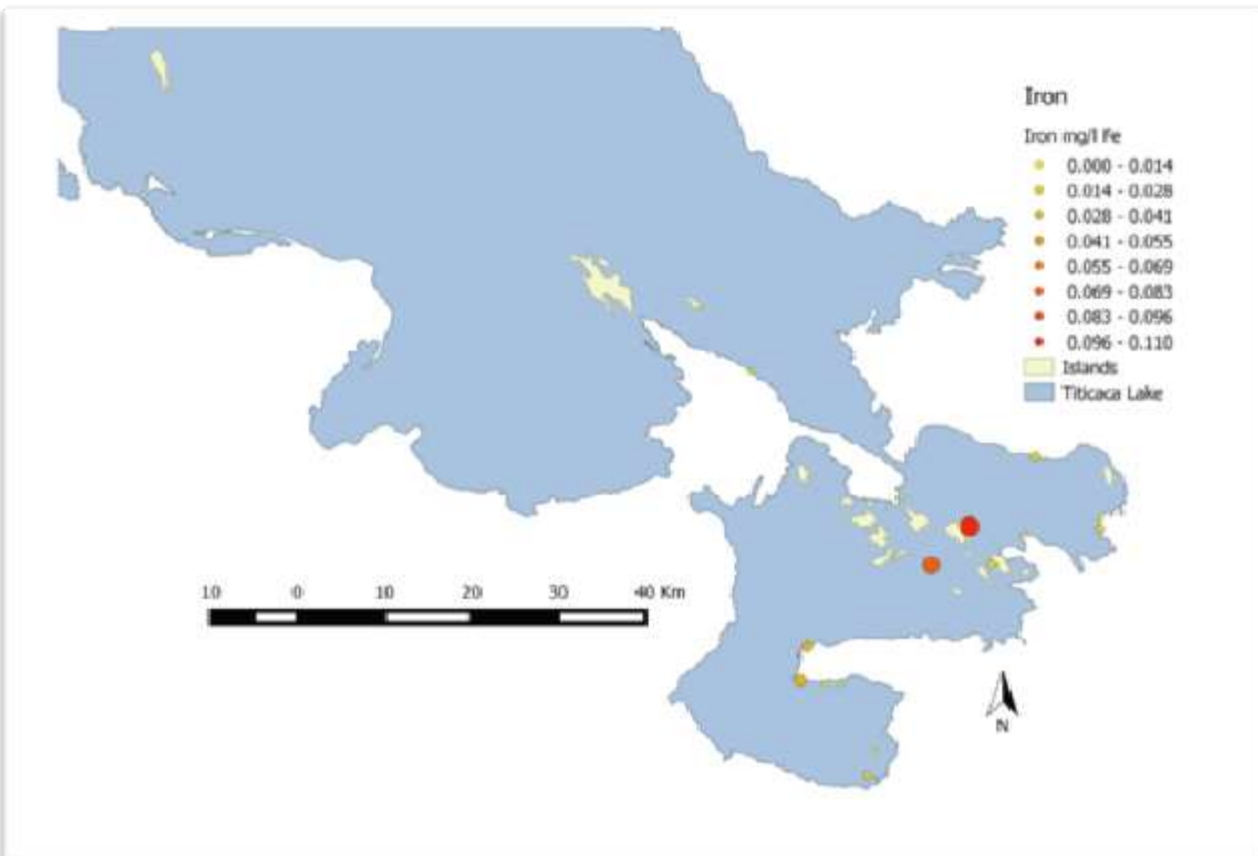

Figure 15 Iron values in Titicaca Lake

Iron levels in the lake normally were low, found in just two localities. One IRD1 that was in the middle of the lake showed values of 0.08 mg/l, and then the highest recorded value was in Suriqui Island with a value of 0.11 mg/l.

CHROMIUM/10

Hexavalent Chromium (Chromium - VI) is not normally found in natural waters. However chromates and dichromates are widely used in industrial processes such as tanning, coating, and water treatment. Hexavalent chromium is therefore commonly found in many effluents and industrial waste waters. Hexavalent chromium is regarded as a particularly objectionable constituent in water supplies. Careful monitoring of industrial effluents and waste waters is therefore necessary in order to ensure conformity to consent discharge limits and to prevent hexavalent chromium entering the aqueous environment (Palintest, 2016).

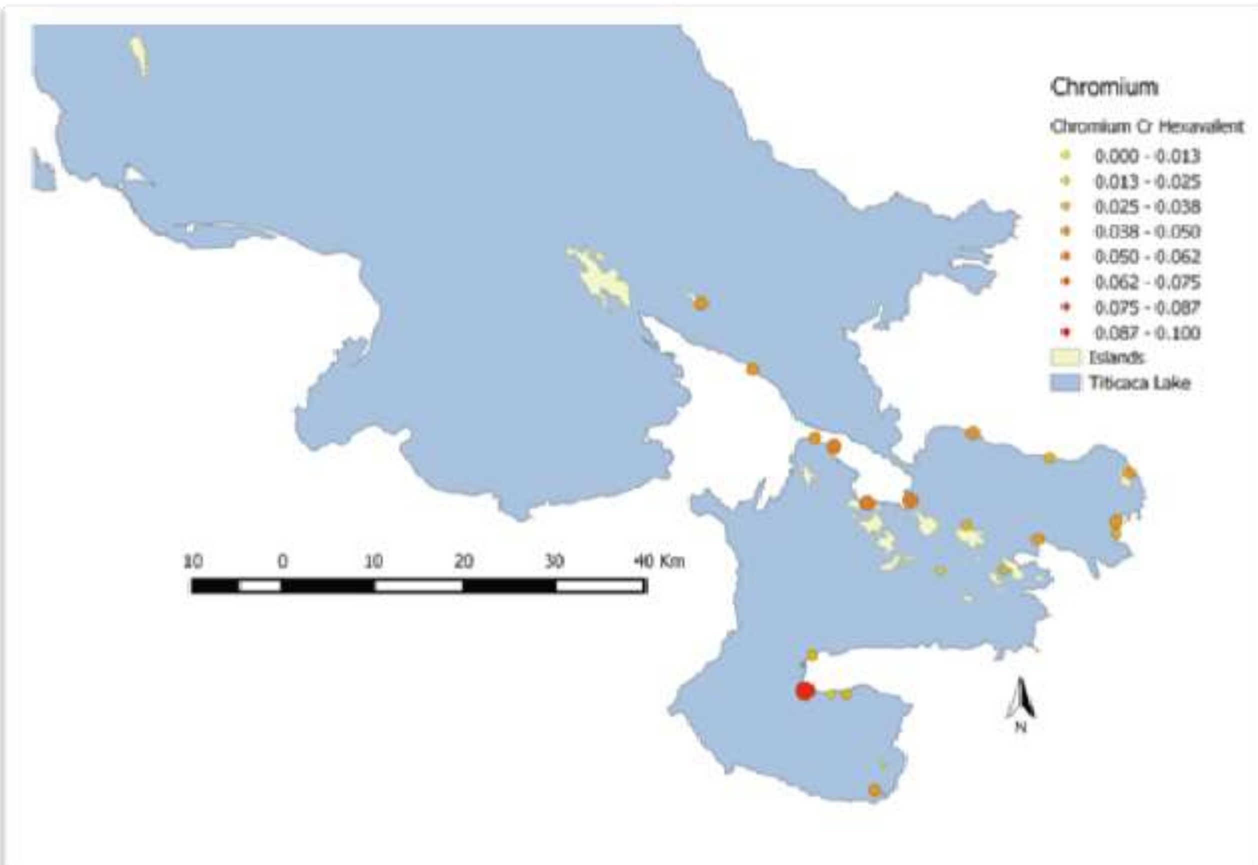

Figure 16 Chromium values in Titicaca Lake

Chromium values in the lake were normally low. Just one locality, Santa Rosa, had a high value with 0.1 mg/l.

Dissolved oxygen as O2 OD

Oxygen is an element that is vital for aerobic organisms to carry out their metabolic process. It is needed for amphibian respiration and aerobic processes. In the case of Titicaca water frog, it is very important the concentrations of dissolved oxygen in the water because this species never goes out from the water and low levels of OD will cause stress in the frog. If levels are very low it can cause the death of the individual. Values of OD with >80% saturation is recommended for amphibians, but it is also known that some anurans and salamanders may be able to tolerate very low levels of oxygen (Odum and Zippel, 2012).

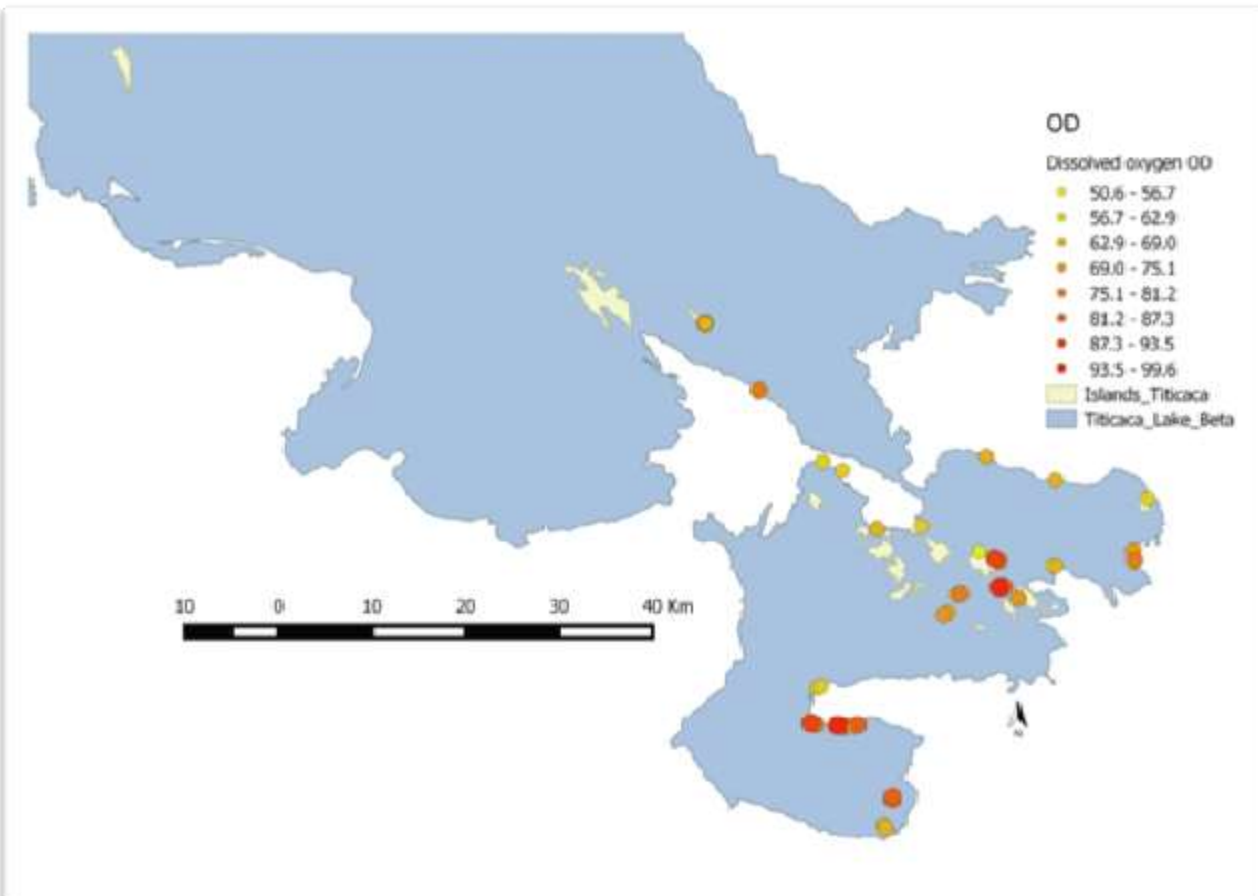

Figure 17 Dissolved oxygen in Titicaca lake

Values of OD found in the lake fluctuate from 50.6% to 99.6%, but all of these measurements were carried out during the transects or between these, thus during the day from 10:30 to 16:40. All the values are relatively acceptable for the frogs. We do not have OD measurements from the days of the massive deaths but data from Instituto de ecologia that that is monitoring the different parameters with automatic systems have recorded values close to 0% very close to the surface. If this is the case this can be one of the causes of the massive deaths where an anoxic environment is present in the lake which is not an adequate habitat for the frogs. These big fluctuations can be due to the presence of phytoplankton, small algae that exist in very high concentrations. Lately, in some months of the year, one can see very big areas with very green water where before it was completely transparent (Figures 17 and 18). One can even see in satellite photos these green spots in the water (Figure 19). We observed in months previously and during the massive deaths that in some areas it was not even possible to dive because the visibility was so bad. This can cause very high levels of OD during the day hours when the algae carry out photosynthesis and produce a lot of oxygen that fills the water. However, the situation is the opposite during the night hours, where the algae consume the oxygen and take all the oxygen from the water, which can cause very low levels of OD in the water. That was the situation in the lake were we found in some areas levels of 0% of OD. This can be one of the causes of the massive deaths where not just frogs were affected but other aquatic organisms that died in a short period of time. This coincides with the postmortem analysis where we did not find any lesions in the body, and that it seems frogs were in good condition and even eating before death.

Figure 18 Normal conditions and visibility of water in the lake

Figure 19 Green water observed in some areas of the lake during the massive deaths

Figure 20 Satellite imagen from Lago Menor where it is possible to see the green spots of water in the south side

Sulphide

Natural waters containing dissolved hydrogen sulphide and other sulphides are found in certain parts of the world, particularly in areas having hot springs. Sulphides are constituents of many industrial wastes such as those from tanneries, gas plants and chemical works. Sulphides can be toxic to fish and aquatic life; and their presence in water supplies gives rise to undesirable tastes and odours (Palintest, 2016).

A product of sulphide is hydrogen sulphide, H_2S, a very toxic and corrosive gas characterized by a typical odor of rotten egg. Hydrogen sulfide often results from the prokaryotic breakdown of organic matter in the absence of oxygen gas, such as in swamps and sewers; this process is commonly known as anaerobic digestion.

Unfortunately we were not able to measure this parameter during the massive deaths, but data from Insituto de Ecologia shows that levels were very high even in the surface. If this is the case we think this was the main reason of the massive deaths of the frogs and other organism such as fish, and birds. This is a very toxic gas that probably was originated at the bottom of the lake due the high levels of organic matter that is present over there.

Conclusions

Based on all of the water parameters we measured and data we obtained we can discern a pattern that has been playing a role in the massive deaths of the frogs. We think that very big variations of dissolved oxygen, from 0% to 100%, can be a condition that could have played a role in the bloom of green algae that created anoxic waters that were no longer viable for the frogs, and that could have caused the massive deaths at least on a local scale. Another thing that we need to take into account is that frogs cannot migrate like fish, and this group is more sensitive to this kind of changes. Another aspect is the very high levels of sulphide and sulphidric acid that were recorded during the days of the massive deaths. This could be the main reason for the massive deaths, where frogs were basically killed by these very high levels that even during our transects we noticed the smell of rotten eggs in very large areas of Lago Menor.

Now, what caused this situation? We think that all the organic material that we have in this area at the bottom of the lake creates an anoxic environment where this gas is produced. Normally this would not be a problem, but due to heavy rains that bring a lot of water in a short time and removes the bottom of the lake, these gases are exposed and liberated, and makes all the column of the water a toxic habitat for the frogs. We have been also recording smaller, local massive deaths of frogs and these are connected with very strong winds and small tornados that remove and mix the bottom of the lake where depths are not so big and this cause in the same way of the heavy rains the exposure of this gas in the water column.

Another aspect we saw during our transects is the very high amount of organic material at the bottom of the lake where in other areas this is not the situation. Also in the days of the massive deaths we saw big blocks of substrate or lake bottom floating on the surface of the water, including decomposing vegetation that was attached to the substrate. This shows that large amounts of organic material were removed from the bottom of the lake, and this affected the quality of the water. Local communities reported also that in previous days the water was completely brown, which probably caused the vegetation at the bottom to be unable to photosynthesize, and after a couple of weeks in this situation it died, increasing the problem.

Lately we have observed this situation as a regular phenomenon; big green spots of water that moves in the lake, heavy rains that brings a lot of material and removes the bottom of the lake, and local deaths of amphibians. We think that this side of the lake is becoming more unstable due to the large amounts of organic material being deposited at the bottom of the lake, among other things.

Special attention will be needed to monitor parameters such as ammonia, nitrites, nitrates, phosphates and some heavy metals that can also play a role, if not in a short term, but in all the ecologic process of the lake. Below we can see some images of some of the findings related to water quality and substrate.

Figure 21 water visibility in some areas was not so good, in picture one of the best sites where visibility was good during massive deaths

Figure 22 Pieces of substrate of the bottom of the lake floating in the surface after some days of the massive deaths

Figure 23 Pieces of substrate of the bottom of the lake floating in the surface after some days of the massive deaths

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