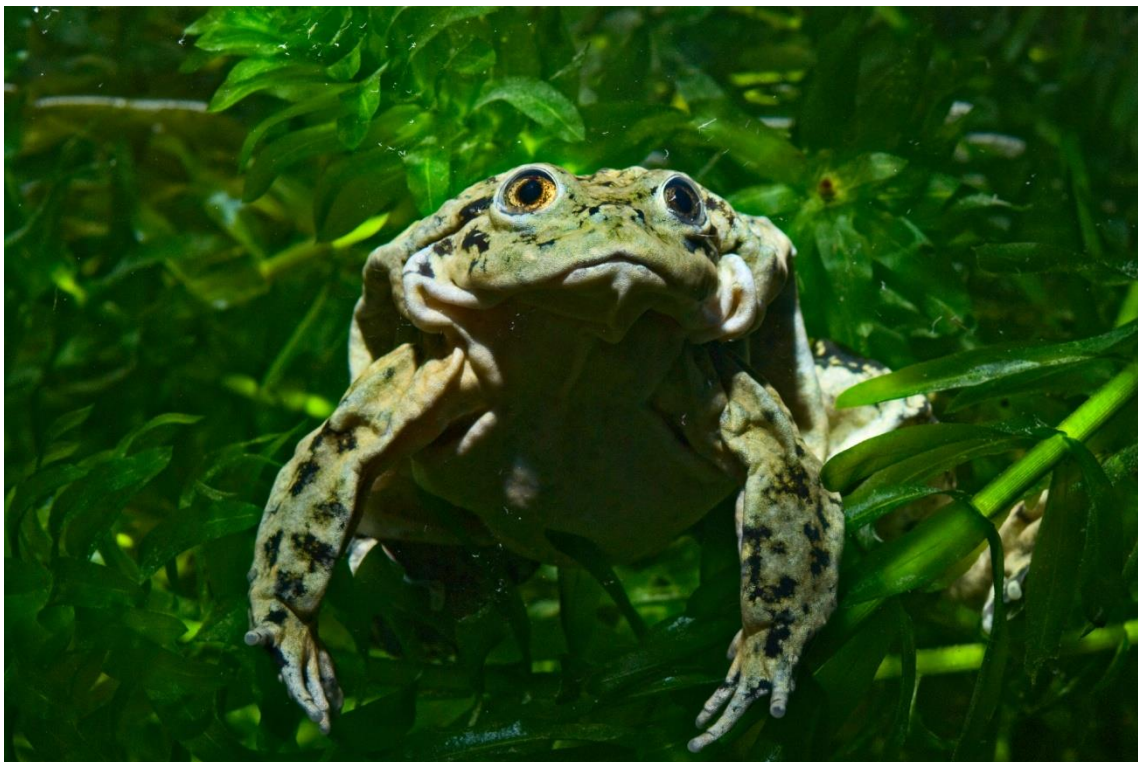


# **Titicaca Water Frog Conservation Project**

## **Final report**



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## INTRODUCTION

More than 30% of the 2000+ species of amphibian in South America are currently threatened with extinction and at least four species have already gone extinct. The most significant threats facing amphibians in the region are habitat loss and infectious disease. Combined with the impacts of over-harvesting, invasive species, pollution and climate change it is likely that many more amphibians within the region will go extinct before long-term solutions to these threats can be found.

The Titicaca water frog (*Telmatobius culeus*) is endemic to Lake Titicaca which spans the borders of Peru and Bolivia at an altitude of 3,810masl. Once common throughout the lake the species has undergone dramatic population declines estimated as being more than 80% over the last three generations, because of that *T. culeus* is considered as a Critically endangered amphibian (De la Riva, 2005, IUCN 2014, Perez, 2002, Perez 2005, Genova, 2011 ). A number of factors are thought to have played a role in this including over-harvesting of adults for human consumption, before mainly for restaurants and personal use, but lately is more used for frog juice that is offered in local markets as a potion that can cure everything; assumed predation of larvae by introduced trout; water extraction and pollution and more recently the deadly fungal disease Chytridiomycosis.

This species is under a high risk of extinction and almost nothing is known about its actual and real numbers, about its ecology, reproduction or other basic information

For this reason we want with this project to increase the knowledge about this species and in a short term to be able to take actions based in all the information we will gather.

In this way we are monitoring three areas in Titicaca Lake to compare the dynamics of the species in these three different areas.

## OBJECTIVE

- Carry out surveys in three Bolivian localities as a first step towards establishing a population monitoring program of at least three years to determine the seasonal and between-year variation in the density/abundance as well as overall population trends of the Titicaca Water Frog in Lake Titicaca.

## METHODS

### Study area

At first stage we carried out assessments in 19 localities (table 1) in the Bolivian side of Titicaca Lake to find the best localities for the research that we wanted to carry out.

Table 1. Localities where we carried out our assessments (\* monitored localities)

Departament	Province	Locality	Latitude	Longitude
La Paz	Manco Kapac	Sicuani	-16.08980	-69.11168
La Paz	Manco Kapac	Yampupata	-16.058554	-69.13248
La Paz	Manco Kapac	Isla del sol	-16.031328	-69.168089
La Paz	Manco Kapac	Titicachi	-16.103247	-69.098787
La Paz	Manco Kapac	Weko	-16.134526	-69.088577
La Paz	Manco Kapac	Copacabana	-16.180347	-69.115358
La Paz	Manco Kapac	Challapampa	-15.992694	-69.185719
La Paz	Manco Kapac	El Sapo	-16.160349	-69.094074
La Paz	Manco Kapac	El Suto	-16.06305	-69.11329
La Paz	Manco Kapac	Isla de la luna norte	-16.031098	-69.082206
La Paz	Manco Kapac	Isla de la luna sur*	-16.046373	-69.063347
La Paz	Manco Kapac	Chachapolla*	-16.118320	-69.007820
La Paz	Manco Kapac	Collasuyo, Belen	-16.115377	-69.009505
La Paz	Manco Kapac	Isla suriqui	-16.29723	-68.75398
La Paz	Manco Kapac	Isla lago menor	-16.280794	-68.816034
La Paz	Manco Kapac	Lago menor I	-16.268135	-68.837052
La Paz	Manco Kapac	Sahuina	-16.204349	-69.107933
La Paz	Manco Kapac	Chicharro*	-16.235664	-68.833234
La Paz	Manco Kapac	sicuani	-16.08980	-69.11168

Three localities were chosen for the monitoring of the species, Isla de la Luna, Chachapolla and Chicharro (Figure 1), this was based on the purpose to compare three localities, one in a good status (Isla de la Luna), a second one in medium conditions (Chachapolla) and the third one in bad conditions (Chicharro) talking about densities and also body conditions of the frogs.



Figure 1 Three localities where the study was carried out (from north to south: Isla de la luna, Chachapolla and Chicharro)

### **Water quality**

Measurements of water quality were taken at different sites of the lake; the parameters that we used are those that are recognized to be important for amphibians in captive breeding programs (Whitaker B. 2001, Zippel, 2009, Zippel et al. 2011) such as pH, Amonia, Amonium, Nitrite, Nitrate, Phosphate, hardness and alkalinity. These measurements are being obtained with a Palintest 7500 photometer and one per locality per month.

### **UV light**

UV radiation is an important parameter that we are also monitoring in the lake and for this we are using Solarmeter 6.2 UV meter, with an irradiation range response from 280 -322 nm and Solarmeter 6.5 UV Index meter with an peak sunlight response bandwidth from 290 -298 nm and a total solar response from 290-400 nm, diffey. This measurement is measured just once for all localities because the altitude and latitudinal differences are not so big.

### **Water temperature**

We are monitoring the water temperature during all the time of the research with a hobo water temperature Pro v2 Data logger –U22-001 with an accuracy of 0.21 °C. This data logger is activated since January 2014 in one of the study localities at 2.5 meters depth where most of the frogs are present. The data is downloaded once a month and temperatures are saved every one hour.

## Line transect method

Because Titicaca water frog is entirely aquatic and lives at the bottom of the lake and there is no set methodology for monitoring such kind of anuran species, we used an adaptation of a method used for monitoring coral reefs and fish. The transects were done at the approximately from 2 to 20 meters from the shore and maximum depth of 5 meters as visibility decrease at deeper areas and the chance to detect animals is lower.

One of the objectives of the current research is to estimate the density of *T. culeus* in three locations in Titicaca Lake. For this we used line transect method that it is one of the most effective methods used for estimating density and diversity of amphibian species (Hsu, M.Y., *et.al.*, 2005). This method is suitable for the needs for the current research as no individual data is required.

The transects consist in a line of 100 meters that we swim in the surface of the water, following a centreline located at the bottom of the lake. Each transect was divided in sections (10 sections of 10 meters) where we took note about the habitat in the area ( $10\text{ m}^2$ ) for this we used three categories (rock, plants and sand) we also took note of the average depth in this section with a hondex digital depth sounder with an accuracy of  $\pm 1\%$  and finally the vegetation present in the area.

Once we found a frog we took note of the depth where the frog was found, also we calculated distance from the frog to the centreline and associated data with the frog such as substrate, behaviour, size and when possible sex and age groups based in size. In some cases frogs were caught, measured and weighted, body conditions, number of ectoparasites and deformations were recorded and we also collected some samples of swabs of the skin for Chitridium analysis. After this process, the frogs were returned to the original place where they were found.

We also collected most of the dead individuals we found in the area to be stored in the Natural history museum Alcide d'Orbigny.

Sometimes we dive in the different areas to obtain more in detail information of the species mainly about its natural history.

The monitoring is being carried out every month covering the different seasons of the year. This research still being carried out but the information that we present here is from June 2013 to June 2014.

## RESULTS

### Water quality

The water quality in the lake was stable during our research and we can see that there are not so big differences in the different parameters among localities. With the data we can say that the water of the lake has high levels of pH and total hardness and alkalinity. The values of ammonia nitrite and phosphates are relatively low for amphibian levels. Following we present the values of the water in these three localities.

**Table 2. values of water quality in the three localities in Titicaca lake**

Locality	pH	(NH <sub>3</sub> )	(NH <sub>4</sub> )	(N)	(N)	(NO <sub>2</sub> )	(CaCO <sub>3</sub> )	(PO <sub>4</sub> )	(P)	(CaCO <sub>3</sub> )	(HCO <sub>3</sub> )	(CO <sub>3</sub> )
Chachapolla	8.10	0.35	0.13	0.1	0.004	0.056	200	0.11	0.04	110	135	60
Chicharro	8.10	0.08	0.09	0.07	0.002	0.007	200	0.09	0.03	100	125	60
Isla de la Luna	7.650	0.12	0.13	0.1	0.017	0.056	225	0.11	0.04	110	135	65

### UV light

UV radiation was measured during the different hours of the day we can see that in this season (winter) the highest values are around 400 at noon, comparing with values found in the rest of the world we can say that the values we found are in the high side comparing with other localities <http://www.uvguide.co.uk/uvinnature.htm> we also have some other readings where we got readings of 622 that is much higher than other measurements registered in different areas of the world. We also took some readings at that time under water and we got values of 240 nm at a depth of 1 meter. Making us to think that possibly the species use UV light in the synthesis of vitamin D.

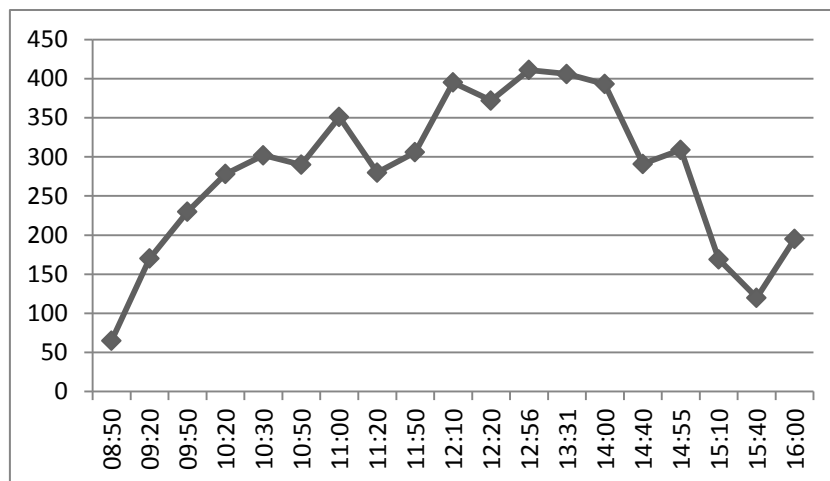


Figure 2 UV radiation Values during the day

## Temperature

Temperature is being measured constantly at the depth of 2 meters and we are building a database of the temperatures for all the seasons, we saw that the water temperature during 24 hours do not change too much, we have an average of 1-1.5 °C difference between night and day time. We also found that the differences between seasons are not so big at that depth with an average of 2-2.7 °C difference between seasons in the maximum temperatures recorded in one locality. We also think that would be interesting to use more dataloggers to measure the temperature at different depths and also different microhabitats in different areas.

In figure 2 we can find the temperature variation of the month of February at the depth of 2 meters.

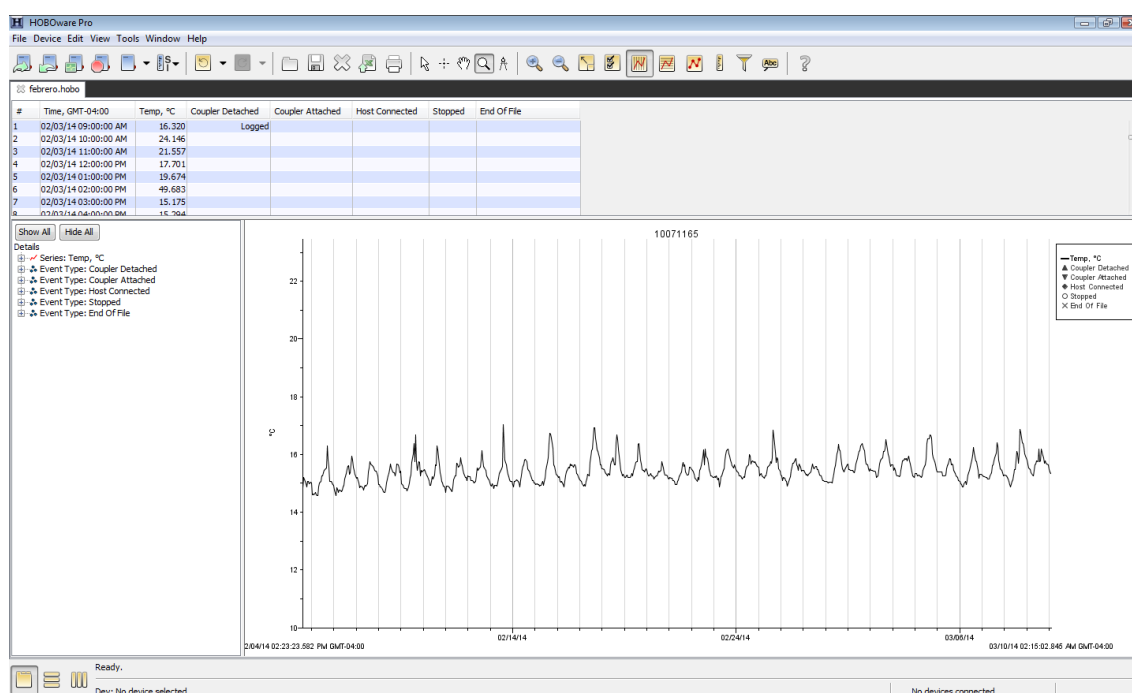


Figure 3 Temperature variation in Isla de la Luna in February

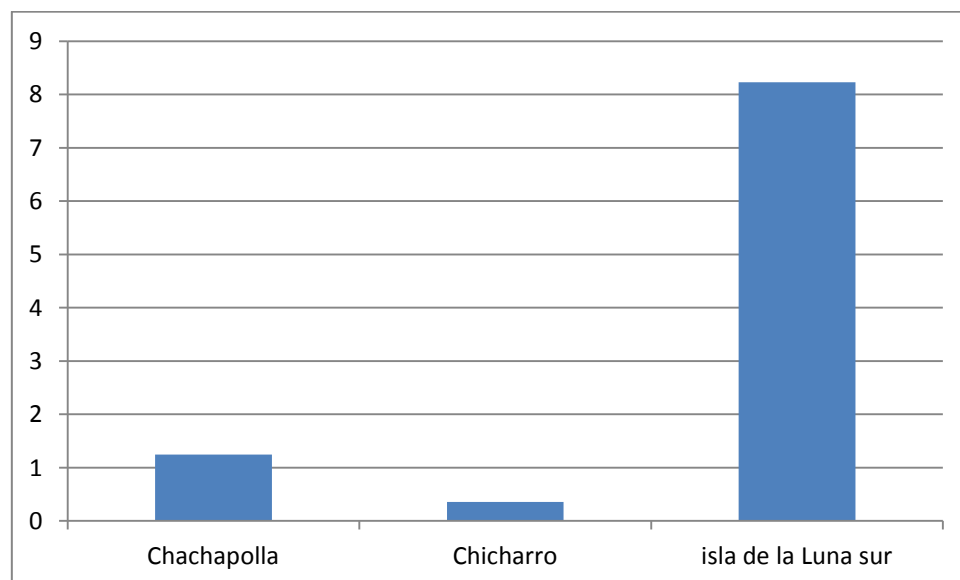


### Densities of *T. culeus*

A total of 19 localities were surveyed in the Bolivian side of Lake Titicaca, from them, 3 localities were chosen by the conditions they presented in mummies of frogs and also of the health status. From all of them we took Isla de la Luna, Chachapolla and Chicharro.

Comparing the densities among these three localities we can see that Isla de la Luna is de area with higher densities 8.23 ind per transect, followed by Chachapolla with 1.24 individuals per transect and finally Chicharro 0.35 individuals per transect. For this reason we think that the best locality talking about numbers of frogs is Isla de la Luna with very high numbers of frogs comparing with the other localities (figure 2).

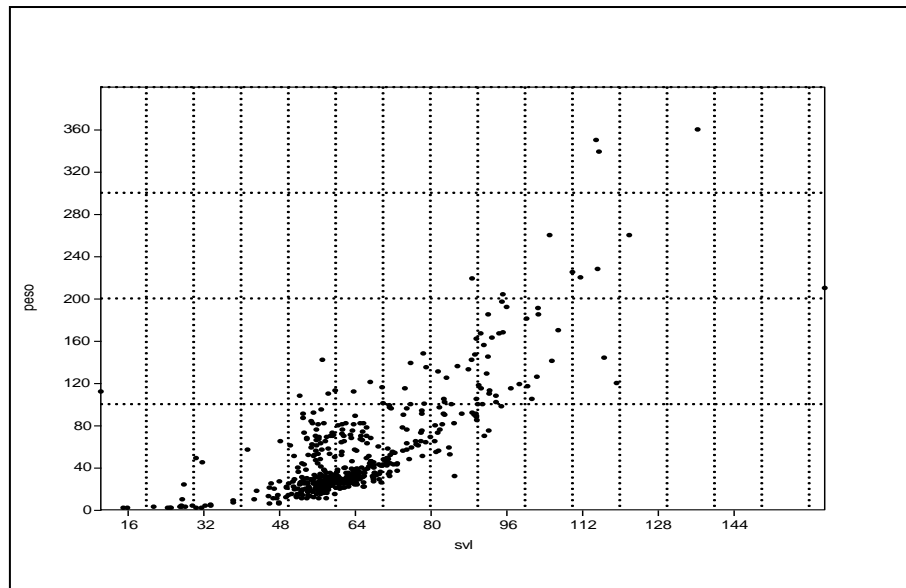
At the moment we do not have enough data to run them in Distance because the low numbers we found in the locality of Chicharro. If we want to analyse this data now, it is not going to be a good quality analysis for this reason we prefer to wait and until now to present the information with the basic analysis.



**Figure 2 Densities of *T. culeus* per transect**

### Body conditions and health

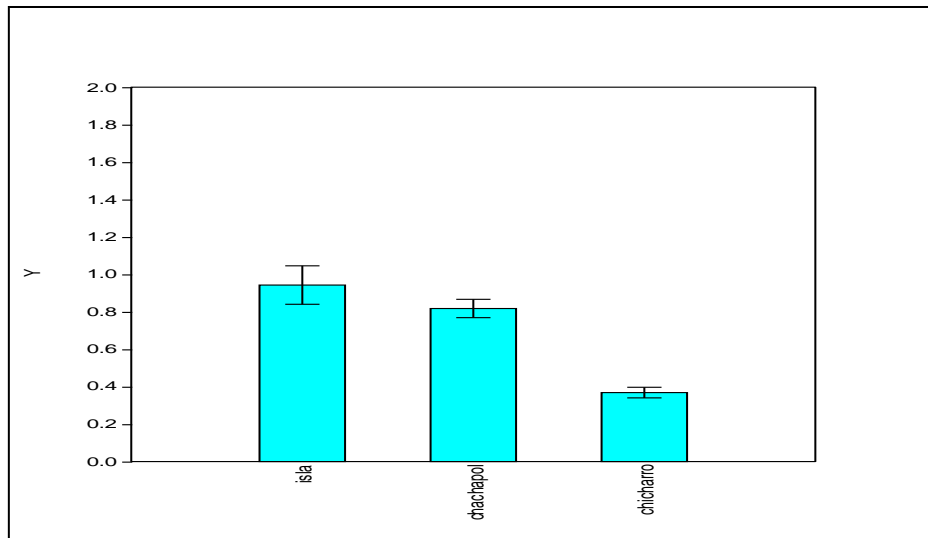
From all the frogs that we captured and measured we obtained the length and the weight and with this data we obtained a graph that can represent the body conditions of the species in the lake where most of the population is around 45 to 80 cm long and 15 to 80 grams, in figure 3 we can see in more detail the distribution of the body conditions of *T. culeus* in all the lake.



**Figure 3 svl/weight ratio in *T. culeus* in all localities**

Comparing body conditions of the different three localities, we can also see a difference where frogs from Isla de la Luna are in better conditions (more mass in relation of the size) than Chachapolla and Chicharro. The difference between Isla de la Luna and Chachapolla is not so big, but if we compare these with Chicharro, the difference is very big (figure 4). In Chicharro we found several individuals that were very skinny and in several occasions were sick or even dead. In one of the assessments we found more than 80% of the individuals dead in this locality and others very sick (figure 5), this was alarming and we are trying to see what happened that time, since then we are finding very few individuals in the area and also sick or dead ones, very few in good conditions.

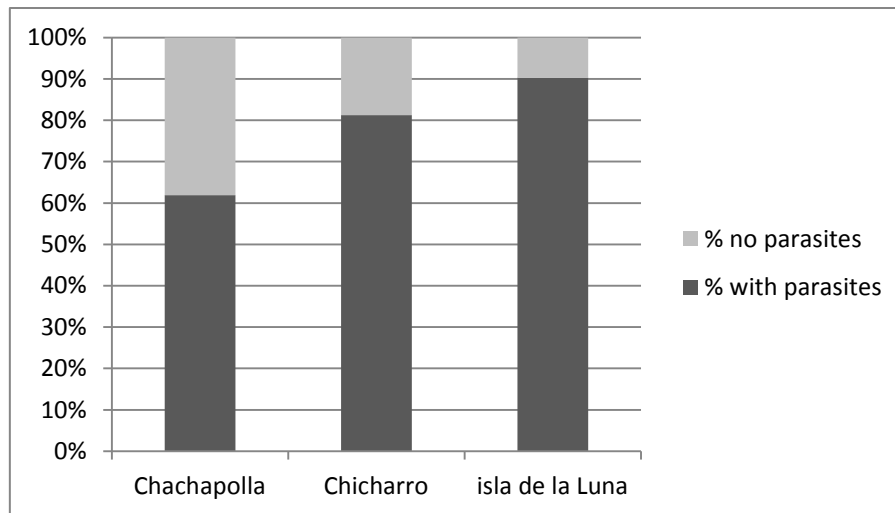
Talking about parasites we analyzed until now just the data from ectoparasites where we found that a big percentage of the frogs are infected by parasites. The endoparasites we found until now in the frogs are nematode, trematode and helminths, mainly in the stomach and near cloacae. The ectoparasites that are infecting *T. culeus* that we found are just hirudinea from the family Glossiphoniidae, we can see in figure 6 that 62% of the frog population is infected in Chachapolla and 81.2% and 90.3% in Chicharro and Isla de la Luna respectively.



**Figure 4 body conditions in the 3 localities**



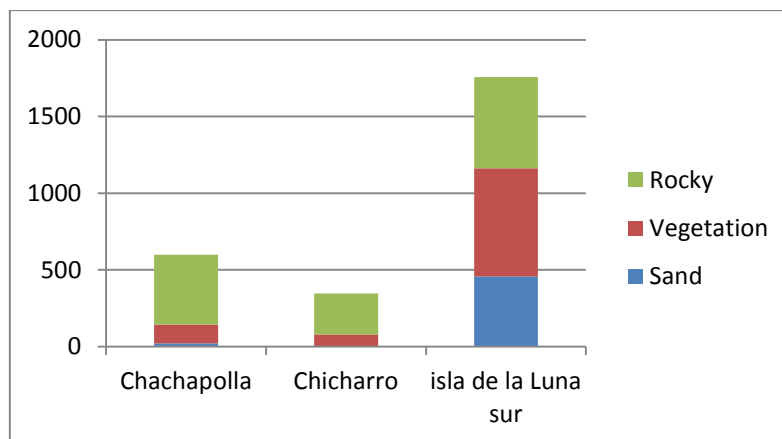
**Figure 5 One of the dead frogs found in Chicharro**



**Figure 6** The % of Population with and without ectoparasites

### Microhabitat use

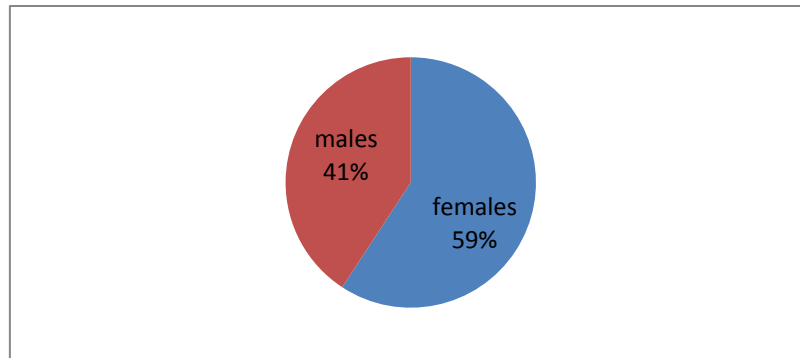
For the analysis of microhabitat use of *T. culeus* we divided in three main components, rocky areas, vegetation and sand, for this analysis we used the predominant substrate when it was a mix of these three components to provide the microhabitat name. In figure 7 we can find that rocky areas are the most used by the frogs with exception of Isla de la Luna where the main microhabitat used is vegetation, but we need to clarify that most of this area was a mix of vegetation and rock as a second dominant substrate.



**Figure 7** Microhabitat use of *T. culeus*

### Sex ratio

In the different localities we captured different individuals and tried to identify the sex of the individuals, in some cases was possible and in others not, for this reason we just take the data of the frogs that we were sure of the sex. We can find that almost 60% of the population we captured are female and 40% males (figure 8).



**Figure 8 Sex ratio of *T. culeus* in the three localities of Titicaca lake**

During our research we were also able to get some information about the reproduction of the frogs and we found individuals in amplexus (figure 9) since August until May so we think the frogs breed during all the year. We also were able to see for the first time the nest of *T. culeus* in the wild, nobody before was able to find the eggs of this species. We also found some strategies of the species to take care of the nest that was unknown for the species.



**Figure 9 Amplexus of a couple of frogs in the wild underwater**

## CONSERVATION

During the period of this project we did not take just the data needed for this project, we also collected information about the natural history, reproduction, foraging strategies among others, we also worked with capacity building, because during this time we were able to train a number of young biologists and students in the work in research and conservation of the species, following we present a list of these people that were trained and worked during the project.

Name	Position	Organization
Andrea Fuentes	Environmental sciences	Universidad Catolica and Museo de

		Historia Natural Alcide d'Orbigny
Ramiro Estrada	Young biologist	Universidad San Simon and Museo de Historia Natural Alcide d'Orbigny
Veronica Flores	Young biologist	Universidad San Andres
Sophia Barron	Biology student	Universidad San Simon and Museo de Historia Natural Alcide d'Orbigny
Gabriel Callapa	Biology student	Universidad San Simon and Museo de Historia Natural Alcide d'Orbigny
Rene Carpio	Biology student	Universidad San Simon and Museo de Historia Natural Alcide d'Orbigny
Jaime Salamanca	Environmental sciences student	Universidad Simon y Patiño

From this group of people some of them are carrying out also other specific research and thesis that is going to help to understand this species.

A lot of this information is being useful for different purposes such as the amphibian need assessment of Bolivian amphibians that we organized together with Amphibian Ark. Also information very useful is obtained for a captive breeding program that we are running under another project. All this information is helping us to understand better the situation of this species and to obtain data that was hided for a long time even several research studies were carried out in the area.

We are also working with local communities along this time that are supporting the project during the fieldwork and we are involving them with different activities and also with educational activities and material that we are developing to be distributed in the near future, this will help us with the conservation activities we want to develop in the area for the conservation of this critically endangered frog.

## Expenses

Following we present the expenses of the resources from Stiftung Artenschutz, we made other trips to the field with other resources too but for report purposes here in the expenses report we just put the funds obtained by Stiftung Artenschutz (4800 USD and 4000 Euros).

Personnel	Expenses Bolivianos	Expenses USD	Expenses Euros
Pago project coordinator		1600	1333
Pago project assistant		450	375
<b>Travel Expenses</b>			
trip 1			
bus	400	57.97	48
food and lodging	1380	200.00	167
boat	1400	202.90	169
bus	530	76.81	64
trip 2			
bus	400	57.97	48
food and lodging	1380	200.00	167
boat	700	101.45	85
bus	400	57.97	48
trip 3			
bus	400	57.97	48
food and lodging	1380	200.00	167
boat	700	101.45	85
bus	400	57.97	48
trip 4			
bus	400	57.97	48
food and lodging	1380	200.00	167
boat	700	101.45	85
bus	400	57.97	48
<b>Equipment and Supplies</b>			
Miscellaneous office supplies			
print cartridge	172.5	25	21
Paper	28	4.06	3
Photocopies	68	9.86	8
usb	80	11.59	10
Miscellaneous field equipment and supplies			

alcohol	450	65.22	54
malla, alambre	113	16.38	14
net	75	10.87	9
wings	395	57.25	48
<b>Communication</b>			
cellphone credit	270	39.13	33
<b>Contingencies</b>			
rental dive equipment		120	100
<b>Institutional Overhead (15%)</b>			
museum overhead		620	517
<b>TOTAL</b>	14001.5	4819.202899	4016

Note with 4 to 5 people per campaign during five days



## Appendix



Figure 10 carrying out transects in Titicaca lake



Figure 11 monitoring Titicaca water frog



Figure 12 Male of *T. culeus* taking care of nest



Figure 13 Team monitoring *T. culeus* at isla de la luna with local community members

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