

Necrops Rescuing *Telmatobius culeus* populations at Lago Menor, Lake Titicaca, Bolivia Final report
**y report during massive deaths of *Telmatobius culeus* in April and
 May 2015 in Titicaca Lake**

Animal information	
Species:	<i>Telmatobius culeus</i>
Number in group	106
Age	juvenile and adults
Sex	males and females
Number of dead:	106
Number of sick:	0
Locality information	
Department	La Paz
Locality:	Puerto Perez , Lake Titicaca
Coordinates	-16.283593° S and -68.602309° W
Altitude	3810
Collection date	3 May 2015
Report data and details	
Report date:	13 December 2015
Report responsible:	Arturo Munoz
Contact information	
Collector:	Arturo Munoz, Sophia Lavayen, Patricia Mendoza, Juan Pablo Vargas
Responsible and contacts :	Arturo Muñoz Hyla_art@yahoo.com 591-71701676 0032485860742
Required analysis	
<i>Batrachochytrium dendrobatidis</i> <i>Batrachochytrium salamandrivorans</i> Ranavirus Chlamydeales Histology ecotoxicology	
History: (include clinical signs, significant lab results, etc.)	
<p>On May 3rd the team carried out a campaign to monitor the massive deaths in Titicaca lake, where 106 frog samples were collected on the Bolivian side of Titicaca Lake in the locality of Puerto Perez and surroundings (-16.283593°S and -68.602309°W). Samples were fixed and stored in 94% alcohol and stored in the Museo de Historia Natural Alcide d'Orbigny in Cochabamba, Bolivia.</p>	

All of these frogs were found at the shore of the lake floating in the water close to emergent vegetation. They were collected one week after the beginning of the massive die off. No frogs were found alive in the area and the samples were found in different stages of decomposition. From these samples 24 postmortem analyses were carried out *in-situ*, mainly in the fresher samples (n=27) though some of them were old samples (n=6)

Data of water quality and other parameters were noted (attached in water quality file)

- **Skin:** The skin had a normal color and texture in the fresh individuals, no external lesions were observed. In some individuals (76) a layer of white sticky membrane was observed. In the individuals that were dead for about 4 to 7 days the condition of the skin was decomposing. No individuals were found with ectoparasites (Leaches). Some individuals showed signs of predation, possibly by birds (n=8).
- **Eyes:** Normal and with no lesions in fresh samples, some of them had white eyes due the time of death.
- **Primary incision:** Subcutaneous condition normal, fat content also normal, ventral and dorsal musculature without any sign of lesions or abnormalities. Some individuals showed signs of possible predation by birds (n=8)
- **Body cavities:** Normal color in fresh individuals and several frogs had signs of edemas in the cavities.
- **Liver:** Color was normal, most of the samples present normal size, some of them were bigger than the normal size.
- **Heart:** Normal shape, size and color, no parasites were present in the internal parts.
- **Genital system:** In adults the genital organs were well developed, from the examined females 70% had well-developed embryos.
- **Fat bodies:** Fat body amounts were normal and in good condition.
- **Respiratory system:** Individuals had lungs of normal size and no abnormal colors or texture, no presence of parasites.
- **Gastrointestinal tract:** All the components of the gastrointestinal tract were normal compared with other healthy individuals, no parasites were found in the different components. Gut content in several individuals (n=16) filled by a muddy substance of brownish color, it looked like the individuals ingested mud. In some other individuals the stomach and other gastrointestinal components were empty or just contained this muddy substance. In few samples (n=3) we found snails or crustaceans that were ingested before the dead of the frogs.
- **Bones:** No fractures or abnormalities were found in the bones of the examined animals.
- **Analisis Results:**
 - Analysis from specimens were as follow
 - Clamideaes- Negative
 - Ranavirus – positive
 - *Batrachochytrium salamandrivorans* - Negative
 - *Batrachochytrium dendrobatidis* –positive

The histological analysis was not successful because the condition of the samples was not so good. All these analyses were carried out in the Laboratory of Veterinary Bacteriology and Mycology at the Faculty of Veterinary Medicine, Ghent University.

Photos



Figure 1 Collection locality of samples, Puerto Perez and surroundings



Figure 2 Dead frog found in Lake Titicaca in Lago Mayor



Figure 3 Dead frogs found in Lake Titicaca, Puerto Perez



Figure 4 In-situ postmortem analysis of dead individuals found in Puerto Perez



Figure 5 Adult female with the different organs and the eggs almost ready to be laid

Conclusion

Collected individuals were in different decomposition stages, some of them seem to be in good conditions and with no lesions both, externally or internally, also due to the conditions of the different organs and eggs founded in the females it seems the frogs died in a short time of period.

One interesting aspect was the muddy substance found in several individuals in the different parts of the gastrointestinal tract. In some cases the amount was very large; probably coinciding with the brownish and muddy waters present in the area days before the collection that probably was the substrate of the lake removed by heavy rains. Local people said the water was very brown and it was not possible to see the bottom of the lake or even very shallow depths. The frogs probably ingested this muddy substance during this period.

Fat bodies in most of the individuals were normal and it shows that the individuals were in good conditions; in some individuals we also found different items in the gut content.

We found Chytridium and Ranavirus in some of the samples, this cannot be the cause of the deaths at this scale and also so fast, but it is also one aspect that future research needs to take in account.

The causes of the deaths was probably something in the environment that killed the frogs very fast, and that needs to be studied. Some possible explanations are explained by the water quality information and other aspects occurred in the area days and months before the massive deaths (see water quality report).