



# Temperature exposure and possible thermoregulation strategies in the Titicaca water frog *Telmatobius culeus*, a fully aquatic frog of the High Andes

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**ABSTRACT:** Temperature has an important effect on amphibians, influencing virtually all physiological systems. Thermoregulation is used to manage unfavourable thermal conditions, but has been poorly studied in aquatic amphibians, and no information is available for adult aquatic anurans. We studied the temperatures to which the Critically Endangered and high Andean fully aquatic Titicaca water frog *Telmatobius culeus* is exposed and investigated possible thermoregulation strategies of the species in the wild. We measured water temperature in different seasons, microhabitats and depths at which *T. culeus* is most abundant. We established transects to obtain population densities and activity patterns of the species during the day. Seasonal and daily temperature variations as well as temperature variations among depths and microhabitats were noted. These variations were associated with densities of visible *T. culeus*, where adult densities were higher at depths with more stable temperatures. *T. culeus* behaviour associated with thermoregulation strategies in the aquatic habitat included depth selection and movement of individuals between different microhabitats. Selection of microhabitat depended on the temperature at a site and the hour of the day. These results indicate possible behavioural thermoregulation strategies such as movement to different sites or basking used by a highly specialized amphibian, and provide insights into how aquatic benthic organisms use strategies for thermoregulation in aquatic heterogeneous environments. These strategies are probably also used by other aquatic organisms from the tropical Andes. Knowledge of how these organisms respond to temperature could also help to understand the impact of global warming on their status.

**KEY WORDS:** Endangered amphibian · Lake Titicaca · Temperature · Thermoregulation · Behaviour

## INTRODUCTION

Temperature has a profound effect on biological processes, influencing the physiological performance and fitness of ectotherms (Christian & Tracy 1981, Hillman et al. 2009, Navas et al. 2013, Basson et al. 2017). An increase or decrease in body temperature can affect different parameters in amphibians such as metabolic rate, feeding, digestion, growth and immunity. These parameters will eventually have an effect on reproduction. In contrast, locomotion in some am-

phibian species is relatively temperature-independent over a narrow range of temperatures (Rome et al. 1992, Rojas et al. 2005, Raffel et al. 2006). Other processes, such as anaerobic metabolism, also undergo very little change with changing temperature (Bennett 1978, Wells 2007). The overall effect of these changes will determine amphibian ecology and eventually the fitness or success of the species and its distribution (Blouin-Demers & Nadeau 2005, Buckley & Jetz 2007).

Aquatic amphibians are generally buffered from the extremes of low and high temperatures that ter-

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restrial amphibians can experience (Navas 1997, Hillman et al. 2009), which gives them an advantage in extreme ecosystems such as the highlands. The high heat capacity and thermal diffusivity of water mean that an aquatic amphibian exchanges heat very rapidly with its environment, and so body temperature ( $T_b$ ) of the amphibian cannot differ much from the environmental temperature ( $T_a$ ) (Hillman et al. 2009, Duarte et al. 2012). In every habitat, temperature varies over time and location according to the balance of energy input and output. The Earth's surface experiences at least 3 temperature cycles at different time scales: daily, annually and infra-annually (Camacho 2012). Aquatic environments tend to be thermally stable over the daily cycle, especially large water bodies, but there are cases where temperatures in shallow, small water bodies and ephemeral ponds can still fluctuate markedly on a daily cycle (Hillman et al. 2009). At high altitudes, heating and cooling cycles can be very fast and the temperature can reach extremely low minimum values (Navas 1997, Wells 2007, Hillman et al. 2009). Such fast cycles decrease the amount of time it takes for the water to change temperature, which tends to have a greater impact on high Andean species (Navas 1997).

To maintain the required temperature for processes such as feeding, digestion, metabolic rate, growth, reproduction and immunity, ectothermic organisms must rely mainly on behavioural adjustments to regulate  $T_b$  (Navas 1997). This is particularly the case in amphibians, which have very limited capacity to alter their  $T_b$  physiologically (Wells 2007), and especially with small species that have a smaller thermal inertia (Hillman et al. 2009). Aquatic amphibians have limited physiological capacity for thermoregulation because they cannot use strategies such as evaporative water loss to decrease heat, and metabolic processes do not produce enough heat (Wells 2007, Hillman et al. 2009). Behavioural strategies that amphibians use to increase or decrease  $T_b$  include heliothermy: getting or gaining heat from the sun (from Greek 'helios' meaning 'sun'); and thigmothermy: getting heat into the body from contact with warm substrates in the environment (from Greek 'thigmo' meaning 'touch') (Wells 2007). As ectotherms, anurans depend primarily on external heat sources to regulate their body temperature. Basking is one of the main mechanisms by which most ectothermic organisms increase body temperature, exposing the whole or a part of the body to direct sunlight (Hillman et al. 2009, Centeno et al. 2015). Especially for amphibians, basking implies water loss through evaporation, and for these species, microhabitat selection with different temperatures is a strategy

to maintain desired temperature ranges (Wells 2007). Some aquatic species, such as the mudpuppy salamander *Necturus maculosus* (Beitinger & Anderson 1979), can reach their preferred  $T_b$  using efficient heat exchange through blood circulation in the gills.

Behavioural thermoregulation has benefits, as it allows an animal to select temperatures that are most favourable (Hillman et al. 2009); however, behavioural thermoregulation also has costs, such as increased exposure to predation, increased energy expenditure and decreased time available for other activities (Wells 2007, Bancroft et al. 2008, Basson et al. 2017).

Information about thermoregulation in adult aquatic amphibians is scarce. Not a single detailed study of thermoregulation in a fully aquatic adult frog has been published, and only a handful of such studies on the thermal ecology of larvae or on salamanders and newts are available (Wilson et al. 2000, Wells 2007, Catenazzi & Kupferberg 2017). Studying the temperature ranges to which a species is exposed, and identifying the possible behavioural strategies used to maintain its metabolic processes can help to understand how temperature fluctuations in large water bodies in highlands could affect an amphibian species, and to recognize the possible strategies the individuals use to maintain their preferred temperatures using thermoregulation. An understanding of these processes would also help to predict the impact of climate change on the species.

The Titicaca water frog *Telmatobius culeus* is listed as Critically Endangered (CR) on the IUCN Red List (Icochea et al. 2004). It has also been listed in the Red Book of Bolivian Vertebrates (Aguayo 2009), in De la Riva & Reichle (2014) and on Peru's Instituto Nacional de Recursos Naturales. In 2017 the species was also included in Appendix I of CITES (CITES 2016). The species is facing a variety of threats such as habitat destruction, overharvesting, introduced exotic species, climate change (Icochea et al. 2004) and emerging infectious diseases such as chytridiomycosis (Catenazzi et al. 2010, Cossel et al. 2014, Berenguel et al. 2016, Zevallos et al. 2016, Burrowes & De la Riva 2017a,b). *T. culeus* has experienced serious population declines (Icochea et al. 2004, De la Riva & Reichle 2014) and in the last few years, local massive die-offs have been reported (BAI 2015, Molina et al. 2017). This frog is endemic to Lake Titicaca and small surrounding lakes (De la Riva 2005) and is considered to be the world's largest fully aquatic frog. The species has unique adaptations such as extremely loose skin, which allows it to breathe underwater without needing to surface for air and is also an adaptation to high altitude (De la Riva 2005,

De la Riva & Lavilla 2008). Little information is available about this species, and particularly about the temperature ranges to which it is exposed. General publications about Lake Titicaca indicate that this species lives in cool (10°C) O<sub>2</sub> saturated waters (Hutchison et al. 1976), with superficial temperatures fluctuating between 11.25 and 14.35°C (Dejoux 1991, Perez 1998).

We observed differences in the frog's activity depending on the site and time of day, and found them basking on rocks under the water (Fig. 1) in hours with highest solar radiation (see Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/n037p091\\_supp.pdf](http://www.int-res.com/articles/suppl/n037p091_supp.pdf)). We thus investigated whether *T. culeus* adapts its behaviour to the temperature, and whether this is possibly an example of thermoregulatory behaviour of a fully aquatic amphibian living in a lower temperature range. To investigate the temperature to which *T. culeus* is exposed and determine if the frogs display a thermoregulation strategy, we examined (1) baseline information about seasonal temperature ranges in the habitat of *T. culeus* in one locality of Lake Titicaca, (2) compared the temperature ranges to which different stages of the species are exposed at different depths and microhabitats and (3) identified possible thermoregulation strategies and possible costs-benefits the species faces in these different microhabitats due to potential thermoregulation.

## MATERIALS AND METHODS

### Study site

The study site was adjacent to 'Isla de la Luna' (16° 2' 42.87" S, 69° 4' 2.34" W) in Lake Titicaca, 3810 m above sea level in the Provincia Manco Kapac of La Paz department, Bolivia (Fig. 2). This site has an average air temperature of 7 to 9°C and annual average rainfall of 500 to 1600 mm (Ibisch & Mérida 2003), and probably hosts one of the densest populations of *Telmatobius culeus*. The underwater habitat on the south side of the island, where this study was carried out, includes rocky and sandy areas, in some cases covered by vegetation. All of these substrates were present in combinations, with varying proportions of each substrate. The vegetation of these areas is dom-



Fig. 1. Female individual of *Telmatobius culeus* 'basking' on a rock at 14:54 h at a depth of 3 m at Isla de la Luna, Lake Titicaca, Bolivia (Photo: A. Muñoz)

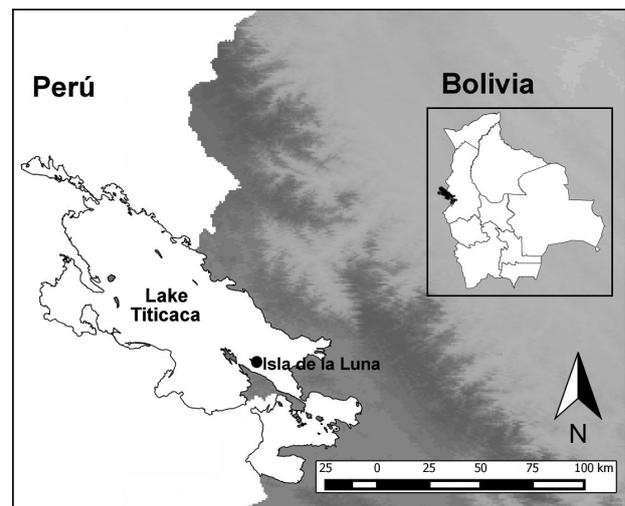


Fig. 2. Study area showing study site, Isla de la Luna, represented by black dot in Lake Titicaca; inset shows location of Lake Titicaca in Bolivia. Map based on CGIAR SRTM (Jarvis et al. 2008) elevation model (light = low, dark = high elevations)

inated by *Myriophyllum elatinoides*, *Elodea potamogeton*, *Potamogeton* spp., *Schoenoplectus californium* and the cosmopolitan species *Zannichellia palustris* and *Ruppia maritima* (Dejoux 1991, Lansdown 2016, GBIF Secretariat 2017). The water contains high levels of dissolved solids, like other areas in the lake, with a pH between 7.55 and 8.15, hardness of 225 to 285 mg l<sup>-1</sup> CaCO<sub>3</sub>, and total alkalinity of 90 to 145 mg l<sup>-1</sup> CaCO<sub>3</sub> (Fuentes 2014).

### Temperature measurements

To measure the temperature range over the year, we used HOBO Water Temperature Pro v2 data loggers (Onset) with an accuracy of  $\pm 0.21^\circ\text{C}$  and a resolution of  $0.02^\circ\text{C}$  at  $25^\circ\text{C}$ . A data logger was installed on a rocky area with short vegetation of *Zannichellia palustris* and *Myriophyllum elatinoides* for a period of 20 mo, at a depth of 2 m (where most adult frogs are present). This data logger was programmed to measure temperature automatically with intervals ranging from 5 min to 1 h. Once a month, the data were downloaded to a computer using HOBOWare Pro version 3.7.2 software.

To compare temperatures at different depths, we installed the same type of data logger at different depths at the bottom of the lake (Fig. 3). One data logger was positioned outside of the water at ground level to compare aquatic and terrestrial habitats (data logger A); one was placed in the water, under the rocks at a depth of 50 cm (data logger B); a pair of data loggers were installed together, one on a rock at a depth of 200 cm (data logger C) and the other under the same rock at 210 cm (data logger D); and another pair was installed in the same way at a depth of 300 cm and 310 cm (data loggers E and F respectively). All data loggers above the rocks were attached in a horizontal position directly on rocks of more or less the same size (about 20 cm diameter), with no shade present for any of the data loggers; all the data loggers under the rocks were under the same rocks used for the data logger above the rocks and these data loggers were not exposed to solar radiation. These data loggers were programmed to measure temperature every 5 min and measured temperatures for the

entirety of 3 consecutive sunny days (2 to 4 December 2015). After this period, data were downloaded using the software HOBOWare Pro version 3.7.2. We chose the microhabitats on the rock and under the rock because of the clear variations in densities of basking frogs observed previously. In sandy areas, and in areas with abundant vegetation, logistical issues and low frog densities prevented us from comparing densities within the same site.

### Sampling of frogs

To obtain *T. culeus* depth and substrate preference in order to correlate them with temperature ranges and possible thermoregulation strategies, we measured the density and activity of the visible frogs at different depths and hours. For the density analysis, we only considered adults, because the presence of juveniles and metamorphs in shallow waters and under the rocks would require other methods that would not allow comparisons. For adults, we carried out transects in Isla de la Luna every month from February 2014 until December 2015, resulting in a total of 22 surveys between 08:00 and 17:00 h. Transects were 100 m long, divided into sections of 10 m long and about 4 m wide. The duration of the surveys was about 30 min ( $\pm 5$  min), with variation arising from difficulties experienced in controlling the observer's speed due to wind or water currents. A total of 10 transects of 100 m were carried out every month. Locations for these transects were chosen randomly within the southeast side of the island covering different habitats and following more or less a parallel line from the shore, trying to cover all depths equally up to 7 m deep. We snorkelled on the

surface of the water, counting all individuals that could be observed at a depth between 0.5 and 7 m. The depth and hour of transect sampling was randomized so that all depths and transects would be sampled at all hours. Transects at these depths were used because previous studies had shown that most frogs occur at depths between 0.5 and 7 m (A. Muñoz pers. obs.), as also observed by Flores (2013). The species can be found in deeper areas (Perez 1998), but densities are much lower, and safety and logistical considerations prevented systematic SCUBA diving sampling. In most of the transects, the observer also noted behavioural information and breeding activity.

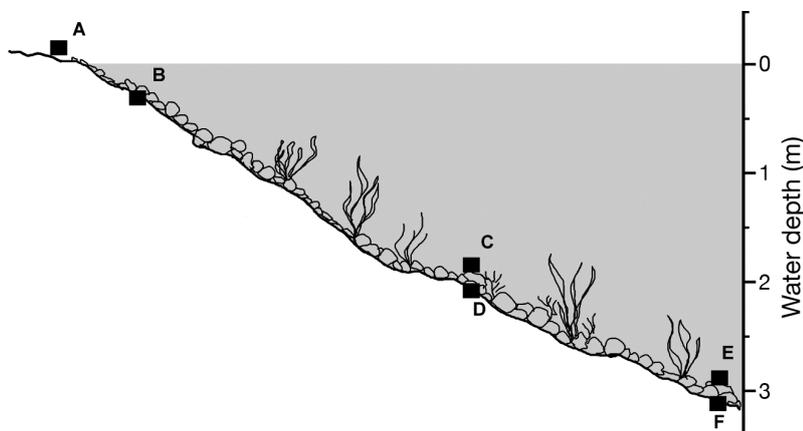


Fig. 3. Profile of the study site, Isla de la Luna, Lake Titicaca, Bolivia. Sites where data loggers were installed represented by black squares. A: outside water (ground level); B: 50 cm (under rock); C: 200 cm (on the rock) and D: 210 cm (under rock); E: 300 cm (on the rock) and F: 310 cm (under rock). Grey area: water

As well as snorkelling, SCUBA diving observations were carried out, where we randomly turned over rocks on the bottom to get more information regarding individuals possibly present under the rocks, and to note behaviour. These SCUBA diving observations were non-structured transects and were meant to obtain extra information such as presence of individuals of different stages under the rocks, behaviour of frogs at that moment, presence of individuals in amplexus and presence and stage of nests and eggs, among others. We were not able to carry out structured transects because it was difficult to dive for long periods due to altitude and logistical limitations with the diving equipment and staff.

To obtain more information about the depth distribution of *T. culeus* at least down to 7 m, we complemented the transects with quadrats along the shore of the lake, at depths of 0 to 0.5 m, where individuals were counted and captured by hand under the rocks; these data were not used for the density analysis but only to see if frogs were present at these depths, and if so, which age stages.

With captured individuals, the snout–vent length (SVL) and body mass of each individual were measured. Sex was established by the presence of black or brown nuptial pads found only on the thumb of males, present in the breeding season, or white and smaller traces of the nuptial pads found on male frogs when not breeding. The frogs were separated into 2 groups: (1) juveniles: individuals found under rocks in shallow water, sub-adult males with small or absent nuptial pads, and SVL  $\leq 51$  mm; and (2) adults: found mainly in open water or under rocks in deeper areas, males with nuptial pads or traces of these structures and SVL  $> 55$  mm.

### Data analysis

To compare the temperatures among the different depths and microhabitats (above and under rocks) we conducted 1-way ANOVA analyses using RStudio version 0.98.1087. A post hoc Tukey's honestly significant difference (HSD) test was carried out to determine which depths or microhabitats were significantly different. Comparisons were made for temperatures across the 24 h period, then for 'cooler' temperatures where solar radiation was absent (between 02:00 and 07:55 h) and finally for 'warm' hours where solar radiation was highest (between 10:00 and 15:55 h).

To determine the relationship between temperature and depth, we used a simple linear regression, with depth as the independent variable and temperature as the dependent variable.

To obtain the density of observed frogs at different hours and depths, we used the number of counted animals per unit area. Density can be estimated using the following equation:

$$D = n / (2 \times w \times L)$$

where  $D$  is animal density at a given interval of 1 h,  $n$  is the number of counted animals (abundance),  $w$  is effective half width strip of the transect line and  $L$  is the length of the transect line (Buckland et al. 2001). We took into account only frogs that were visible during transect sampling, excluding the detection probability (Genova 2011), because we were only interested in the numbers of actively basking frogs on the rocks and exposed sites at that hour or depth. No frogs were counted more than once because they were normally static or moved very short distances, allowing accurate information to be obtained.

To determine if frogs actively moved to microhabitats where temperatures are higher during hours of greater solar radiation (on rocks), we correlated the hour of the day with the temperature and the density of the frogs during this period (presence of frogs on rocks). We also correlated the density of basking frogs (frogs above rocks and exposed sites) at different hours of the day with the mean, maximum and minimum temperatures above the rocks during hours of greater solar radiation.

## RESULTS

### Seasonality of temperature

Average water temperature at Isla de la Luna at a depth of 2 m during the period studied was  $14.24^{\circ}\text{C}$ , with a total fluctuation of  $6.14^{\circ}\text{C}$  (min.  $11.47^{\circ}\text{C}$  in June 2014, max.  $17.61^{\circ}\text{C}$  in December 2014) (Fig. 4). Temperatures showed clear monthly variations, with the warmest months occurring between October and April and the coolest between May and September. We also observed higher temperature variation within months at the end of the cold seasons and beginning of the warm season between September and October ( $\text{SD} = 0.62$ ) compared to the more stable months of April and June ( $\text{SD} = 0.35$ ) (see Table S1 in the Supplement).

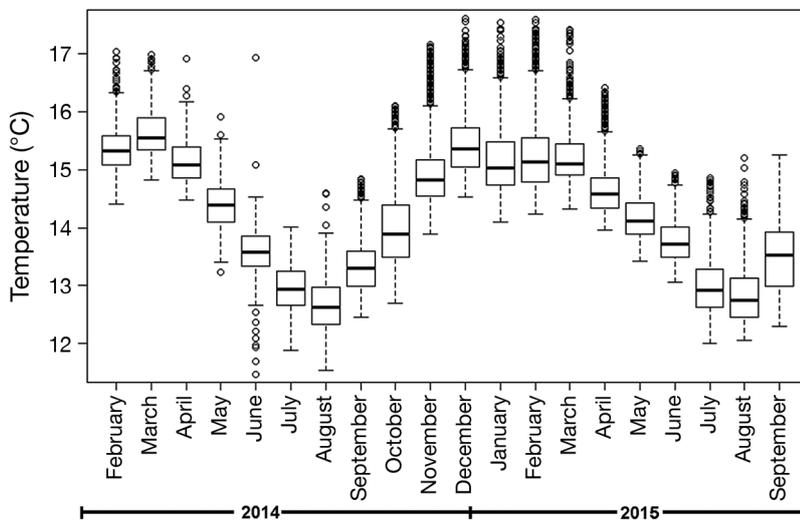


Fig. 4. Monthly temperatures for Isla de la Luna in Lake Titicaca, Bolivia, at 2 m (data from February 2014 to September 2015). Plots show monthly temperature median (horizontal line), 25th and 75th percentile (box), 5th and 95th percentiles (error bars), and outliers (open circles)

#### Terrestrial versus aquatic temperatures

Comparison of temperatures outside of the water and at different depths under the water showed that mean temperatures in both habitats were similar (terrestrial habitat:  $15.77^{\circ}\text{C}$ ; aquatic habitat:  $15.31^{\circ}\text{C}$ ), although more obvious differences were observed when standard deviations were used ( $9.40^{\circ}\text{C}$  in ter-

restrial habitat and  $0.51^{\circ}\text{C}$  in aquatic habitat). Temperature fluctuation or range differed between the 2 habitats, with a daily variation of  $39.50^{\circ}\text{C}$  in terrestrial habitats compared with  $3.56^{\circ}\text{C}$  in aquatic habitats (Fig. 5a, Table S2).

#### Temperatures at different depths and aquatic microhabitats

Comparing temperatures at different depths above rocks on 3 sunny days (Fig. 5b), there were significant differences (means  $\pm$  SD) between 2 m ( $15.49 \pm 0.58^{\circ}\text{C}$ ) and 3 m ( $15.35 \pm 0.39^{\circ}\text{C}$ ) ( $p \leq 0.001$ ). No difference was found in temperatures under rocks at different depths on the same days (0.5 m:  $15.24 \pm 0.70^{\circ}\text{C}$ ; 2.1 m:  $15.23 \pm 0.38^{\circ}\text{C}$ ; 3.1 m:  $15.23 \pm 0.33^{\circ}\text{C}$ ;  $p = 0.99$ ).

Average temperatures at the same depth but on different substrates (above the rock, exposed to solar radiation, i.e. at 2 or 3 m; and under rocks or surface not exposed to solar radiation, i.e. at 2.1 or 3.1 m) were significantly different, being  $15.49 \pm 0.58^{\circ}\text{C}$  and  $15.23 \pm 0.38^{\circ}\text{C}$  for 2 and 2.1 m, respectively ( $p < 0.001$ ) and  $15.35 \pm 0.39^{\circ}\text{C}$  and  $15.23 \pm 0.33^{\circ}\text{C}$  for 3 and 3.1 m, respectively ( $p < 0.001$ ) (Tables S3 & S4).

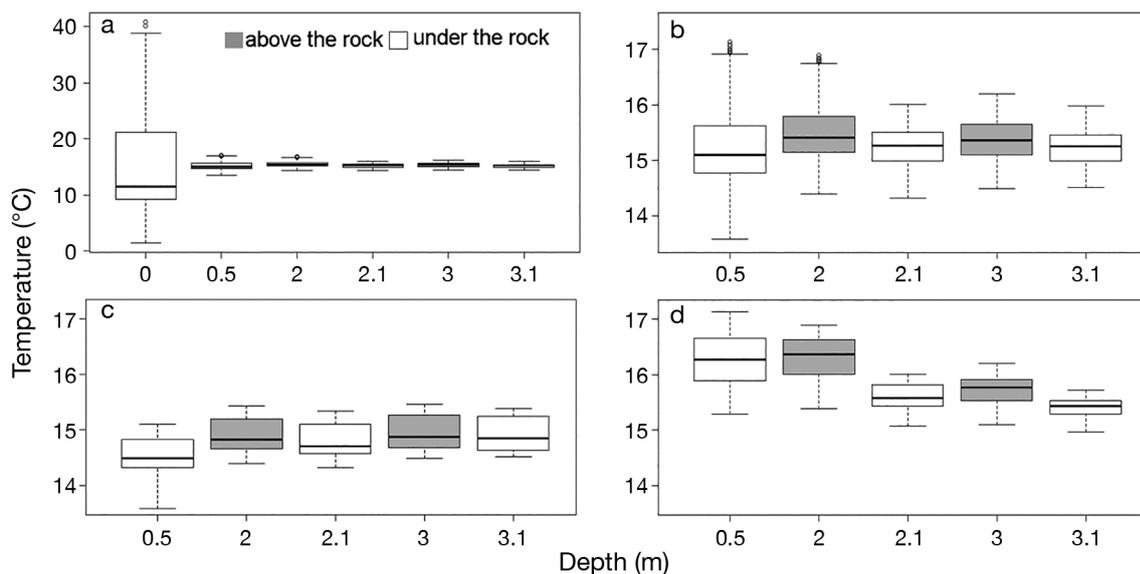


Fig. 5. Temperatures registered in Isla de la Luna in Lake Titicaca, Bolivia, in December 2015. (a) All temperatures outside water (ground level, on the beach of the lake) and underwater; (b) all temperatures underwater over 24 h throughout the study period (showing data for 0.5 to 3.1 m depth from (a) in more detail); (c) temperatures at cold hours underwater (02:00 to 08:00 h); (d) temperatures at hours with high solar radiation underwater (10:00 to 16:00 h). Depths correspond to data loggers: 0: data logger A; 0.5: data logger B; 2: data logger C; 2.1: data logger D; 3: data logger E; 3.1: data logger F. Box plots as in Fig. 4

Between 02:00 and 07:55 h, when air temperatures were lower and no solar radiation was present (Fig. 5c), shallower waters were generally colder than deeper areas. At 2 m, temperatures above rocks were no different ( $14.89 \pm 0.29^\circ\text{C}$ ) than at 3 m ( $14.94 \pm 0.30^\circ\text{C}$ ) ( $p = 0.62$ ), whereas temperatures under rocks differed among all depths (0.5 m:  $14.53 \pm 0.31^\circ\text{C}$ ; 2.1 m:  $14.81 \pm 0.29^\circ\text{C}$ ; 3.1 m:  $14.91 \pm 0.29^\circ\text{C}$ ;  $p < 0.05$ ). Temperatures above and under rocks at the same depth were not significantly different, being  $14.90 \pm 0.29^\circ\text{C}$  and  $14.81 \pm 0.29^\circ\text{C}$  ( $p = 0.07$ ) for 2 and 2.1 m, respectively; and  $14.95 \pm 0.30^\circ\text{C}$  and  $14.91 \pm 0.29^\circ\text{C}$  ( $p = 0.8$ ) for 3 and 3.1 m, respectively (Tables S5 & S6).

Between 10:00 and 15:55 h, when solar radiation was higher (Fig. 5d), temperatures on rocks were higher at 2 m ( $16.31 \pm 0.38^\circ\text{C}$ ) than at 3 m ( $15.72 \pm 0.28^\circ\text{C}$ ) ( $p < 0.001$ ). Findings were similar for temperatures under rocks (0.5 m:  $16.27 \pm 0.47^\circ\text{C}$ ; 2.1 m:  $15.59 \pm 0.24^\circ\text{C}$ ; 3.1 m:  $15.40 \pm 0.20^\circ\text{C}$ ;  $p < 0.001$ ). Temperatures above and under rocks during hours of more radiation showed greater differences than during hours of no solar radiation or over a 24 h period. Finally, temperatures at 2 and 2.1 m ( $16.31 \pm 0.38^\circ\text{C}$  and  $15.59 \pm 0.24^\circ\text{C}$ , respectively) showed a larger difference than temperatures at 3 and 3.1 m ( $15.72 \pm 0.28^\circ\text{C}$  and  $15.40 \pm 0.20^\circ\text{C}$ , respectively) ( $p < 0.001$ ) (Tables S7 & S8).

#### Temperature fluctuations in water correlated with depth

When comparing the fluctuation in temperature at different depths on 3 consecutive sunny days in the hottest month, we noticed that shallower waters were exposed to more extreme changes in temperature than deeper waters. A negative correlation ( $F = 12.84$ ,  $R^2 = 0.81$ ,  $p = 0.37$ ) between standard deviation and

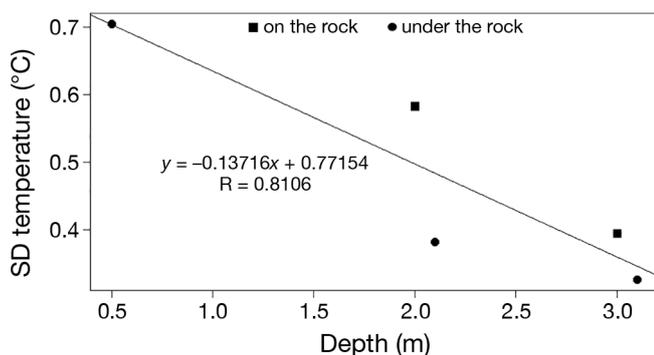


Fig. 6. Standard deviation of temperatures registered at different depths and underwater microhabitats at Isla de la Luna in Lake Titicaca, Bolivia, during 24 h over 3 d in December 2015

depth was revealed, with deeper waters having less temperature variation than shallow waters (Fig. 6): shallower water had a temperature fluctuation of  $3.56 \pm 0.70^\circ\text{C}$  whereas deeper waters (3 m) had a range of  $1.48 \pm 0.32^\circ\text{C}$ .

Temperature variation also differed between microhabitats at the same depth. At 2 m, variability was higher on rocks (2 m;  $\text{SD} = 0.38^\circ\text{C}$ ) than under rocks (2.1 m;  $\text{SD} = 0.24^\circ\text{C}$ ); during hours of more radiation, the difference in temperature between these 2 microhabitats was  $1.12^\circ\text{C}$ . At 3 m, the temperature difference was smaller ( $0.60^\circ\text{C}$ ), with a larger standard deviation on rocks (3 m;  $\text{SD} = 0.29^\circ\text{C}$ ) than under rocks (3.1 m;  $\text{SD} = 0.20^\circ\text{C}$ ) (see Figs. S2 & S3).

#### Depth preference of *Telmatobius culeus*

The transect observations showed that adults mainly used depths between 1.5 and 3 m, with higher densities of basking frogs at 1.5 m: 28% ( $D = 0.28 \text{ ind. m}^{-2}$ ); 2 m: 23% ( $D = 0.23 \text{ ind. m}^{-2}$ ); 2.5 m: 17% ( $D = 0.17 \text{ ind. m}^{-2}$ ); and 3 m: 12% ( $D = 0.12 \text{ ind. m}^{-2}$ ) of observed individuals. Other depths each accounted for <10% of the observations (Fig. 7). We also observed that all breeding individuals and those with eggs were at depths between 1.5 and 3 m. Other age stages, such as metamorphs and juveniles, were found in shallow waters  $\leq 0.5$  m, but never in deeper waters. No larvae were found in the snorkelling transects during this period; only 5 larvae in Gosner stage 25 to 26 were found during SCUBA diving, but all of

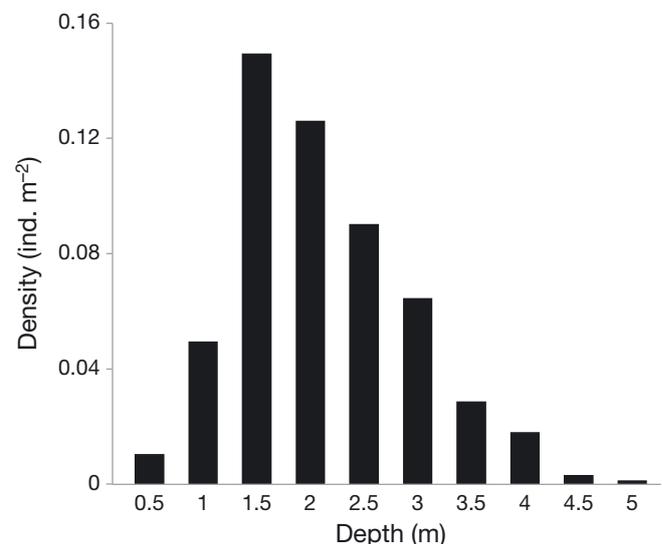


Fig. 7. Densities of individuals of *Telmatobius culeus* observed during transects at different depths at Isla de la Luna in Lake Titicaca, Bolivia, from February 2014 until December 2015

them were in poor body condition and were swimming erratically in the water column.

#### Daytime microhabitat preference of *T. culeus*

The number of individuals observed along transects changed throughout the day, with a clear increase in numbers of frogs during hours with more solar radiation (Fig. S6). During the day, most individuals (77.6%) were found between 11:00 and

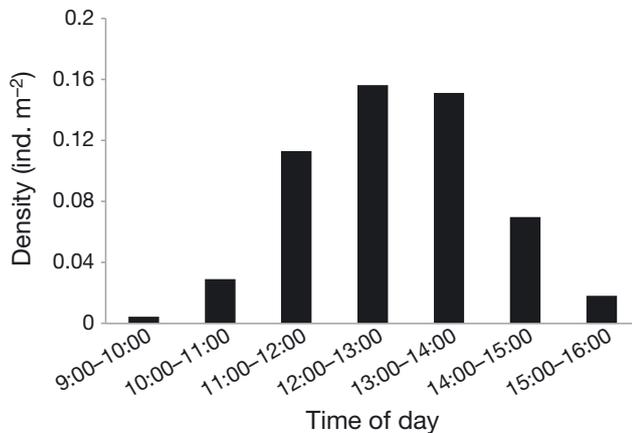


Fig. 8. Densities of individuals of *Telmatobius culeus* observed during transects at depths of 0.5 to 2.5 m at different hours at Isla de la Luna in Lake Titicaca, Bolivia, from February 2014 until December 2015

14:00 h above rocks or exposed sites (Fig. 8). Frogs that were found on rocks were always adults that were basking on exposed sites and, when disturbed, escaped to hide in more protected places (Figs. S3–S5). No juveniles were found basking during this study.

During the warmer months (November to March), densities of observed frogs (Fig. 9) at the beginning of the day (08:00 to 09:00 h) were relatively low (0.04 to 0.08 ind. m<sup>-2</sup>), increasing between 10:00 and 12:00 h (0.13 to 0.22 ind. m<sup>-2</sup>) above rocks when temperatures at these sites increased. Frog densities above rocks decreased between 13:00 and 15:00 h (0.17 to 0.08 ind. m<sup>-2</sup>), probably because individuals hid under the rocks. All months except March showed the same pattern: in March, frog numbers increased slowly and started to decrease from 14:00 h (Fig. 9).

Correlating temperatures with this relationship (Fig. 10), we found that densities of frogs above rocks started to increase during hours with more solar radiation (11:00 to 14:00 h), which were also the hours when temperatures above rocks were higher than temperatures under the rocks (Fig. 5d). From 13:00 h on, the numbers of frogs start to decrease.

#### DISCUSSION

Based on our data, *Telmatobius culeus* in Isla de la Luna occurs at water temperatures around 14°C with

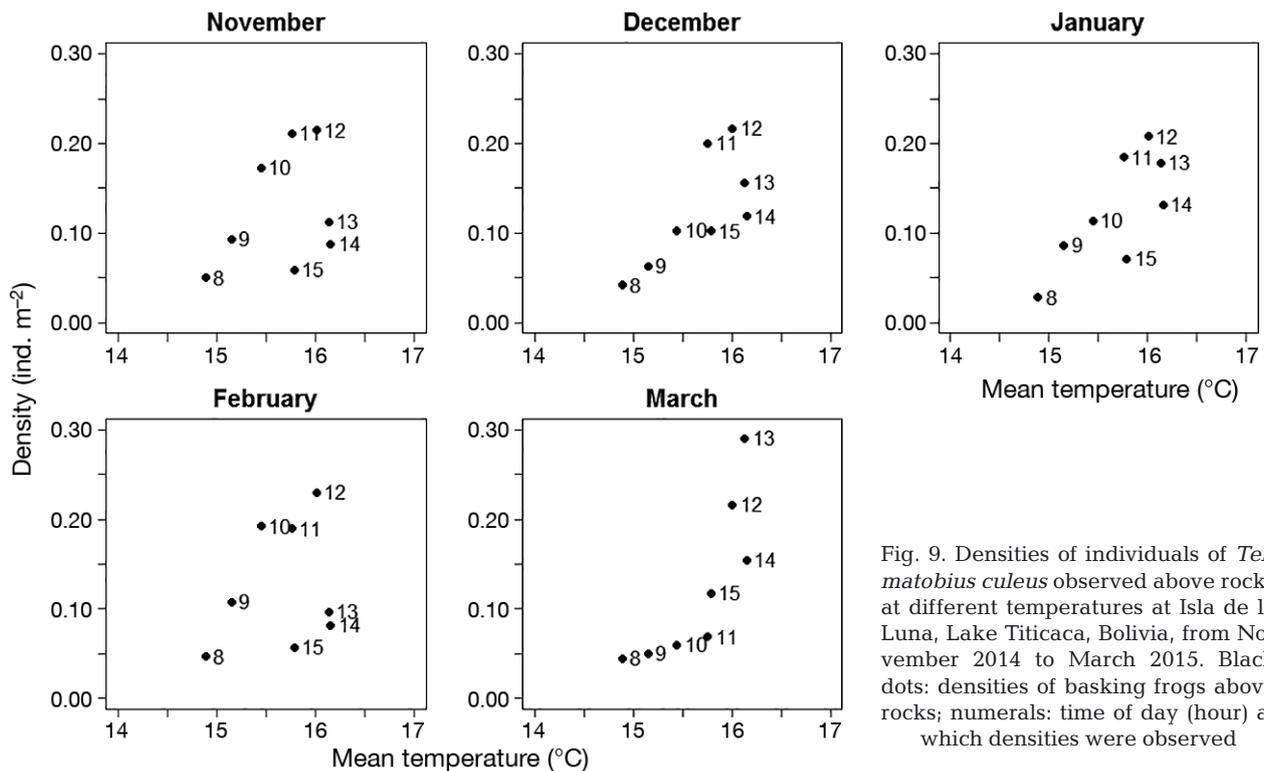


Fig. 9. Densities of individuals of *Telmatobius culeus* observed above rocks at different temperatures at Isla de la Luna, Lake Titicaca, Bolivia, from November 2014 to March 2015. Black dots: densities of basking frogs above rocks; numerals: time of day (hour) at which densities were observed

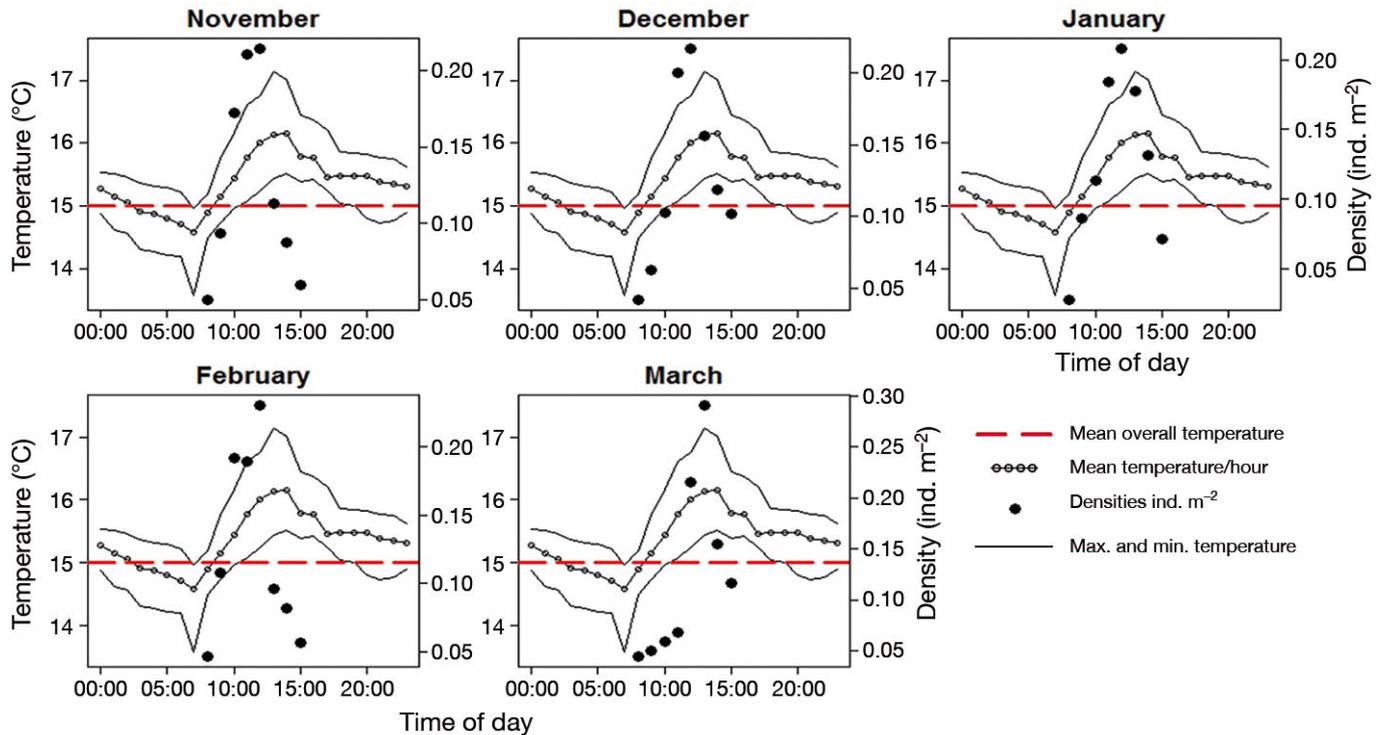


Fig. 10. Water temperatures at different hours of the day and densities of basking individuals of *Telmatobius culeus* above the rocks in Isla de la Luna, Lake Titicaca, Bolivia, from November 2014 to March 2015

a fluctuation of approximately 6°C. In contrast, temperature variation in the terrestrial habitat at Isla de la Luna (3810 m a.s.l.) was very high, with a daily fluctuation of 39.5°C. These large daily thermal ranges, which are normal for high neotropical Andean anurans inhabiting elevations >3000 m (Navas 1997, Wells 2007), cause terrestrial species to choose more stable microhabitats. For example, *Microkayla illimani* (3680 m a.s.l.) breeds and lays eggs in humid places under rocks or moss, where offspring experience daily temperature fluctuations of 18.7°C over period of 28 d (Willaert et al. 2016). By inhabiting the milder aquatic environments in the Andes, anuran species such as *T. culeus* can be active and breed throughout almost the entire year, as indicated by Perez (1998) and Brunetti et al. (2017). In addition, the depths at which *T. culeus* adults are most abundant provide access to better sources of nutrients than shallow waters, including small fish of the genus *Orestias*, which provide higher levels of proteins and lipids than the snails and small amphipods of the genus *Hyaella* that are more abundant in shallow waters (A. Muñoz pers. obs.).

It is important to note that the average water temperature and variation in temperature identified in the present study differ from those reported in earlier studies. Navas (1997), Hutchison et al. (1976) and Hutchison (1982) indicated that *T. culeus* was exposed to water temperatures ranging from 8 to

12.5°C, with Hutchison et al. (1976) showing that surface waters averaged 10°C with a <4°C annual range. However, we found that temperatures at the depths at which *T. culeus* was most abundant (between 1.5 and 3 m) were not homogenous throughout the day, with a daily fluctuation of >3°C, and we identified a variation in temperature between depths of 0 and 3 m. This provides *T. culeus* with a variety of thermal microhabitats. In addition, temperature variations among microhabitats were identified, with average temperatures under and above rocks being different when solar radiation was high. However, the temperature extremes showed clear variation among habitats, and could play a more important role than average temperatures (Camacho et al. 2015).

Although the observed water temperature variations appear relatively small, it is known that the heat capacity of water is approximately 3000 times greater than that of air; even in still water, heat is dissipated as rapidly as it reaches the surface from the body core (Erskine & Spotila 1977). Thus, small temperature differences can have a disproportionate impact on physiological functions, making a slightly warmer site potentially more beneficial for certain metabolic and physiological processes. Aquatic amphibians exchange heat with the environment through blood vessels just beneath the skin (Beitinger & Anderson 1979). *T. culeus* has high levels of blood vessels and

cutaneous capillaries that penetrate the outer layers of the skin (Hutchison et al. 1976). We postulate that this species is likely to use this strategy not only to obtain oxygen, but also to regulate temperature and absorb heat from rocks.

We postulate that the availability of a range of temperature zones promotes better physiological performance. Thermoregulation strategies are used by different amphibian species to maintain temperatures that allow optimal performance, and some amphibian species use mainly behavioural thermoregulation strategies such as heliothermy or thigmothermy by moving from one site to another with different temperatures (Hillman et al. 2009). However, balancing the positive and negative effects of temperature is important for amphibians, and this is probably also the case for *T. culeus*. For example, it is proposed that some amphibian communities (e.g. tropical montane salamanders with narrow distributions) are at risk of extinction (Wake & Vredenburg 2008), due in part to warming-induced physiological depression (Bernardo & Spotila 2006, Duarte et al. 2012).

Adult *T. culeus* are most abundant at depths of 1.5 to 3 m, where water temperature is relatively stable but temperature differences between microhabitats related to rocks provide opportunities for frogs to bask in warmer and exposed places (above rocks). In shallow waters, daily temperature variations are much larger. Juveniles of *T. culeus* are abundant in this environment and therefore are likely to experience greater thermal variation than adults. The degree to which a given species engages in active thermoregulation represents a balance between costs and benefits (Huey & Slatkin 1976, Huey 1982). When the environment imposes a high energetic cost, animals should thermoconform rather than thermoregulate (Basson et al. 2017); *T. culeus* juveniles may experience greater thermal variation than adults, but being under rocks in shallower waters, may be under lower pressure from predators that exist in deeper water such as fish (e.g. *Oncorhynchus mykiss* and *Odontesthes bonariensis*), birds (e.g. *Phalacrocorax brasilianus*) and even conspecifics that can depredate smaller individuals (A. Muñoz pers. obs.). Predation pressure exists in shallow waters from birds such as *Larus serranus* and *Nycticorax nycticorax*, but only if juveniles are active above rocks (A. Muñoz pers. obs.). For this reason, *T. culeus* juveniles probably stay under rocks for safety and are possibly more thermoconformist. Such a shift in behaviour or microhabitat use has been observed in other amphibian species such as the cane toad *Rhinella marina* (Pizzatto et al. 2008).

With respect to the embryonic stage, it is known that some species of *Telmatobius*, such as *T. laticeps*, *T. pisanoi* and *T. oxycephalus*, lay eggs under rocks to avoid predation (Lavilla & Barrionuevo 2005, A. Brunetti pers. comm.). Eggs of *T. culeus* were found in exposed sites close to areas in which the adults bask and at similar temperatures. This is probably a strategy to benefit from slightly higher temperatures that help in the development and hatching of their eggs. The adults' metabolism may also benefit from these sites; the increased risk of predation would then be compensated by parental care from the male (Brunetti et al. 2017).

Although anuran thermal physiology is phenotypically plastic and susceptible to adaptive evolution (Navas et al. 2008), it is not clear how temperatures and temperature fluctuations affect adults and juveniles, and how these frogs could be adapted to daily temperature changes. According to Wells (2007) and Hillman et al. (2009), no detailed study of thermoregulation in adult fully aquatic frogs is available.

The body temperature of amphibians dictates their metabolic activity, with higher metabolic rates at higher temperatures (Homyack et al. 2010, Kristín & Gvoždík 2014). With any event that creates a situation in which not all necessary thermal zones are available, the frog will no longer be able to display adequate thermoregulatory behaviour. In fish, even mild increases in water temperature can stimulate greater protein catabolism (Geda et al. 2012), which generally leads to decreased energetic efficiency. In the case of *T. culeus*, which has one of the lowest metabolic rates among amphibians (Hutchison 1982) and is fully aquatic, these apparently small temperature differences between microhabitats, and larger temperature variations between depths, could modulate their metabolic rate and protein catabolism. This in turn could affect body condition. As Geda et al. (2012) indicated, the combination of increased metabolic rate and decreased assimilation efficiency will lead to loss of body condition, which can be aggravated by the lack of specific nutrients derived from prey that are no longer available due to the shift in temperature. Poor body condition has been related to poor reproductive performance (Litzgus et al. 2008, Barnett et al. 2015). Thus, indirectly, the inability to adequately thermoregulate puts pressure on the population through body condition loss, and a higher sensitivity to disease is likely to compound the negative effects of restricted thermoregulatory behaviour (Barnett et al. 2015). Temperature effects on immunity have been described in newts (Raffel et al. 2006) and leopard frogs (Maniero & Carey 1997), and increased susceptibility

due to temperature changes has been implicated in outbreaks of the amphibian disease chytridiomycosis, with higher mortality at temperatures (17 to 25°C) that provide better conditions for the reproduction and spread of *Batrachochytrium dendrobatidis* (*Bd*) (Berger et al. 2004, Andre et al. 2008). *Bd* has been present in the lake for many decades (Burrowes & De la Riva 2017a) and have been found in different places in the Bolivian and Peruvian Andes (Burrowes & De la Riva 2017a,b, Catenazzi et al. 2017, Seimon et al. 2017). It seems *T. culeus* is not strongly affected. If, however, temperatures increase due to climate change, this would not just affect the metabolic rate or body condition of this population, but would also expose the species to higher temperatures than the average of 15°C recorded in this study. In these new temperature ranges, *Bd* could be more virulent, and thus this shift in temperature could make the frog more susceptible to this disease.

Laboratory studies would also need to take into account whether factors other than temperature could influence frog behaviour. For example, exposure to UV radiation could explain basking behaviour, because it is known that UV light is important for the synthesis of vitamin D3, as an intermediate step in the absorption of calcium (Wright & Whitaker 2001). Thus, absorption and scattering of UV light in the water column should be taken into account, to determine whether UV-B radiation penetrates to the depths where frogs are basking during the hours when this behaviour occurs. Sommaruga & Psenner (1997) reported a 10% attenuation of UV light at 9.6 m depth at the wavelengths of 305 nm in an oligotrophic Austrian lake located at 2417 a.s.l., but also reported changing values depending of the water transparency. The wavelengths between 290 and 315 nm are where vitamin D3 is synthesized (MacLaughlin et al. 1982), so we assume that *T. culeus* probably receives some UV radiation in shallow water. Häder et al. (2015) provided a complete review on the effects of UV radiation on aquatic ecosystems but little information was provided for aquatic amphibians. Potential prey items are mainly under the rocks during the hours in which basking behaviour is mainly observed, with the exception of snails, which are present in these microhabitats at all times. This suggests that access to food is not a reason why frogs are on the rocks at these hours.

Although we present information from just one location, these patterns can likely be extrapolated to other localities within the same depths in the large, more northerly part of the lake (Lago Mayor). The situation is slightly different in the small, more

southerly part of the lake (Lago Menor) where temperatures are, on average, higher and more variable (A. Muñoz pers. obs.). This could provide an opportunity to study the effects of global warming where an aquatic species is exposed to different temperature ranges, because shallower waters are likely to be the first to be impacted by global warming.

Information on the seasonal and daily temperature regime to which *T. culeus* is exposed has been and will be very useful for captive breeding programs. For example, the captive breeding program for the species in Bolivia started to have success with breeding the species when they simulated the seasonal temperature changes observed in the lake, suggesting that temperature change might be a trigger for reproduction in this species (Bolivian Amphibian Initiative pers. comm.). The same situation was observed in the captive breeding program in Denver Zoo in the USA (T. J. Weaver pers. comm.).

In conclusion, this study shows that *T. culeus* is exposed to both temporal and local temperature variations, and proposes that thermal zone preference and basking behaviour are 2 main strategies the species uses for thermoregulation. These findings warrant laboratory studies on temperature preference, tolerance and the effect of temperature on frog physiology. If pollution and global warming events reduce the ability of *T. culeus* to display thermoregulatory behaviour, this may negatively affect the population of this endangered species.

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