



Prey selection and dietary nutrient composition of an endangered amphibian, the Titicaca water frog (*Telmatobius culeus*)

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Abstract. It is important to understand the foraging strategies of an endangered species, in this way to evaluate the possible consequences of ecological changes. This study describes the availability of food resources for the Endangered Titicaca water frog *Telmatobius culeus* in one locality in Titicaca Lake and shows how potential prey abundance can be affected by season or depth of water. Insights about the diet composition and prey selection are provided together with nutritional information of its potential prey items. Substrate traps were installed during different seasons and different depths to collect potential prey items, gut content analysis was carried out in 44 frogs and nutritional properties of potential prey items was obtained. Thirteen species of potential prey items were recorded. For some of the species such as *Hyalella* spp., *Heleobia* sp. and *Biomphalaria* sp., the data indicated a seasonal and spatial variation of the prey items. Amphipod *Hyalella* spp. and snails *Heleobia* sp. and *Biomphalaria* sp. were the main prey items consumed by frogs. Diet did not differ between males and females. The diet of small frogs was dominated by *Hyalella* spp., in contrast to the diet of large frogs which consumed a greater diversity of prey types. *Telmatobius culeus* selected *Heleobia* sp. from all the available prey items from its environment. Calcium:phosphorus ratios, that when inverse can contribute to metabolic bone disease, were high in *Hyalella* spp. but this prey item was relatively low in crude protein and gross energy compared with other species besides *Heleobia* sp. and *Biomphalaria* sp. This study provides ecological information about *Telmatobius culeus* that can be useful for in-situ and ex-situ conservation efforts: (1) the identification of prey items will allow monitoring and eventually managing the ecosystem, (2) it indicates which prey items need to be cultured in captivity, and (3) how captive diets should be adapted to optimally mimic the natural diet of *Telmatobius culeus*.

Key words. Amphibia, Anura, Telmatobiidae, foraging strategies, prey availability, habitat, season, gut content.

Introduction

The Titicaca water frog, *Telmatobius culeus* (GARMAN, 1875) is a fully aquatic frog (BRUNETTI et al. 2017, PADOVA et al. 2024) with a maximum snout–vent length of 120 mm in males and 138 mm in females (PÉREZ 1998). This species inhabits Lake Titicaca in Bolivia and Peru and other nearby Peruvian small lakes (DE LA RIVA 2000, FLORES 2013). It is listed as Endangered in the IUCN Red List of Threatened Species (IUCN 2024), and as Critically Endangered in the Bolivian red book of Bolivian vertebrates (AGUIRRE et al. 2009), in DE LA RIVA & REICHLÉ (2014) and Peruvian list of threatened species approved by Presidential Decree. In 2016 the species was also included in the Appendix I of CITES (CITES 2016). This frog used to be common, but it has experienced serious population declines (IUCN 2024, DE LA RIVA & REICHLÉ 2014, MUÑOZ-SARAVIA et al. 2018) and in recent years, local massive die offs (MOLINA et al.

2017) have been reported. The species is facing at the moment a variety of threats such as those listed below.

(1) Pollution from agriculture, mining and urban activities, that for the last two decades has been affecting the trophic chain and interactions of fauna and flora in the lake (IUCN 2024, GAMMONS et al. 2006, AGUAYO 2009, DE LA RIVA & REICHLÉ 2014, MONROY et al. 2014, MOLINA et al. 2017). (2) Overharvesting for human consumption. Although it seems that previously *T. culeus* was not used for human consumption (ALLEN 1922). Nowadays, the frogs are intensively harvested for human consumption, previously in the 70s and 80s as source of protein and tourist attraction, and lately for an increasing demand of frogs for frog juice, presumed to have medicinal properties (EDERY et al. 2021, KOLBY 2024), all of which are illegal as it is a protected species (ANGULO 2008, STUART et al. 2008, READING et al. 2011, DE LA RIVA & REICHLÉ 2014). (3) Exotic fish species such as the rainbow trout *Oncorhynchus*

mykiss have been introduced to the lake at the beginning of the 1940s; the Argentinian silverside *Odontesthes bonariensis* was introduced in the same period in Lake Poopó, at the south in Oruro department, and invaded Titicaca Lake and surrounding rivers almost ten years later (DEJOUX 1991). Both predatory species are now having a big impact on the lake's ecosystem and food web and are preying on native species, including the frog itself (IUCN 2024). (4) Emerging infectious diseases such as chytridiomycosis have been reported in several areas of the lake. Some Bolivian samples have a high prevalence of infection around 84.6% (BURROWES & DE LA RIVA 2017a, BURROWES & DE LA RIVA 2017b) and also in the Peruvian side Bd has been reported (BERENGUEL et al. 2016, ZEVALLOS et al. 2016). Ranavirus is another disease present in the lake, reported in the small side of the Bolivian Lake "Lago Menor" (A. MUÑOZ unpubl. data).

Due to its critical situation, *T. culeus* has been recommended for captive propagation programs as one of the options to safeguard this species from extinction (IUCN 2024, BURROWES & DE LA RIVA 2017b). There have been several efforts to keep and breed this species in captivity, yet mostly with no positive results and usually for meat production purposes (PEREZ 1998, PEREZ 2005). In the last years, the species has been bred successfully in captivity in Bolivia, Peru and in United States and recently in Europe. These breeding programs were started as study projects and some of them are now with conservation purposes (MUÑOZ 2013, DE LA RIVA & REICHLER 2014, WEAVER 2017).

Not only for these captive breeding programs, but in general and with other related and non-related species, nutrition has been one of the main issues to maintain amphibians in good condition in captivity (FERRIE et al. 2014). Different disorders, syndromes and diseases have been reported in amphibians linked to an inadequate diet and have shown to have an impact on the success in these captive breeding programs (WRIGHT & WHITAKER 2001, FERRIE et al. 2014, SHARIFIAN-FARD et al. 2014).

There is some basic information about the diet of *T. culeus* in the wild, but it is very limited and mainly based on short-term studies such as those carried out by GARMAN (1875), PEREZ (1998) and ALT (2000). Although it is recommended that the study of a species' trophic niche should include prey availability (SOLÉ & RÖDDER 2010), no such information was published to our knowledge for *T. culeus*. This information is very important to understand the foraging ecology of the species and predator-prey interactions (GILLESPIE 2013). This information needs to be combined with knowledge of its ecology and foraging strategies, which is essential for habitat managers to understand, to correctly focus in situ conservation efforts and to assess the effects of ecological disturbances such as habitat modification, species introductions or global climate change (GILLESPIE 2013). For amphibians, studies analyzing the chemical composition of diets in the wild are currently scarce; such data could however help us to better understand the nutrient requirements for this taxonomic group

and could be used in captive breeding programs (BROOKS 1982, BARKER et al. 1998). Even though it will be difficult to mimic the prey composition in captivity, this nutrient composition from the wild diet could help to complement and supplement the nutrients needed in captivity. Nutrient requirements for amphibians are mostly unknown and few research model amphibian species are available in commercial production such as *Lithobates catesbeiana*, *L. perezii*, *Bufo woodhousei*, *Rana temporaria* and *Xenopus laevis*. In general, when recommending nutrient intakes for species with unknown requirements, the published nutrient requirements of related species are used (National Research Council, NRC). For amphibians, suggested species models for preliminary nutrient requirement recommendations are dogs, cats or fish (FERRIE et al. 2014). More importantly, this dietary information from threatened species such as *T. culeus* is a necessary component in designing management and conservation programs, especially captive breeding programs for these species (SOLÉ & RÖDDER 2010).

Information about the nutrient composition of wild diet in amphibians could help to improve the nutritional health of captive amphibians, having an impact in the success of these programs (LI et al. 2009). If those aspects are not understood, it could cause nutritional deficiencies, syndromes and diseases (WRIGHT & WHITAKER 2001, LI et al. 2009, FERRIE et al. 2014). Some of these most commonly recognized nutritionally related disorders are the metabolic bone disease due to an imbalance in the ingested dietary levels of calcium phosphorus and vitamin D₃, causing poorly mineralized bones, tetany, and weakness (WRIGHT & WHITAKER 2001, FERRIE et al. 2014). Calcium concentrations and especially Ca:P ratio has been recognized as a factor that could cause several problems in captive amphibians (WRIGHT & WHITAKER 2001, DIERENFELD & KING 2008, FERRIE et al. 2014), such as membrane function, muscle contraction, nervous system and for regulation of enzymes or storing of calcium in the bone (MCWILLIAMS 2008). It was also reported by VENESKY et al. (2012) that dietary protein restriction impairs growth, immunity and disease resistance of tadpoles and it is known that corneal lepidosis is related to incorrect levels of fatty acids and fats (WRIGHT & WHITAKER 2001). There is hardly any information about trace elements metabolism in amphibians but it is also known that they affect the condition of amphibians, and a varied diet, where multiple different feeder insects are provided, are advised to avoid deficiencies of trace elements (SHARIFIAN-FARD et al. 2014).

To characterize the dietary niche and nutrient composition of the wild diet of *T. culeus*, we investigated (1) the diversity and availability of potential prey items in the benthic habitat in one locality of Lake Titicaca; (2) variation of the availability of potential prey items for *T. culeus* due to seasonality and depth; (3) effects of sex and body size on diet composition at one locality in Lake Titicaca; (4) the basic nutritional composition of the wild diet of *T. culeus*.

Materials and methods

Study site

Fieldwork was carried out on one population of *Telmatobius culeus* over the course of 20 months in 2014 and 2015 at Isla de la Luna (16°2'42.87" S, 69°4'2.34" W), located in the Bolivian side of Titicaca lake at 3,810 meters above sea level in the Manco Kapac Province of La Paz department (Fig. 1). The underwater habitat in the south side of the island, where studies were carried out, includes rocky and sandy areas, in some cases covered by vegetation and a combination of all these substrates in different amounts. The vegetation of these areas is dominated by *Myriophyllum elatinoides*, *Elodea potamogeton*, *Potamogeton* spp. *Schoenoplectus californium* and the cosmopolitan species *Zannichellia palustris* and *Ruppia maritima* (DEJOUX 1991, LANSDOWN 2016, GBIF 2017). Water temperature fluctuates from 11 to 17°C (MUÑOZ-SARAVIA et al. 2018).

Prey availability

To estimate the prey availability in the frog's habitat, we sampled the benthic macroinvertebrate community with a

total of six artificial substrate basket traps of 50 cm long and 25 cm diameter (DICKSON et al. 1971) filled with the same rocks as the habitat and installed at a depth of 2 m from January 2014 to December 2014. Apart from these traps, to obtain data about the availability by depth of potential prey items, 15 other traps were installed at depths of 0.5 m, 1.5 m and 3 m with five traps per depth from November 2016 to January 2017. All these traps – once installed – were checked and cleaned once a month during the study period and returned to the same place. All macroinvertebrate individuals were obtained through filtering and separating from the trap for identification and counting. All samples were dried at a temperature of 60°C for five days and afterwards sorted by species for future analysis. Additionally, the presence of different fish species in the area was recorded and in some cases, collected, but no data about relative abundances was obtained.

Collection of frog individuals

Surveys were conducted every month; ten transects of 100 m were carried out with snorkelling on the surface or scuba diving was carried out near the bottom, in which in-

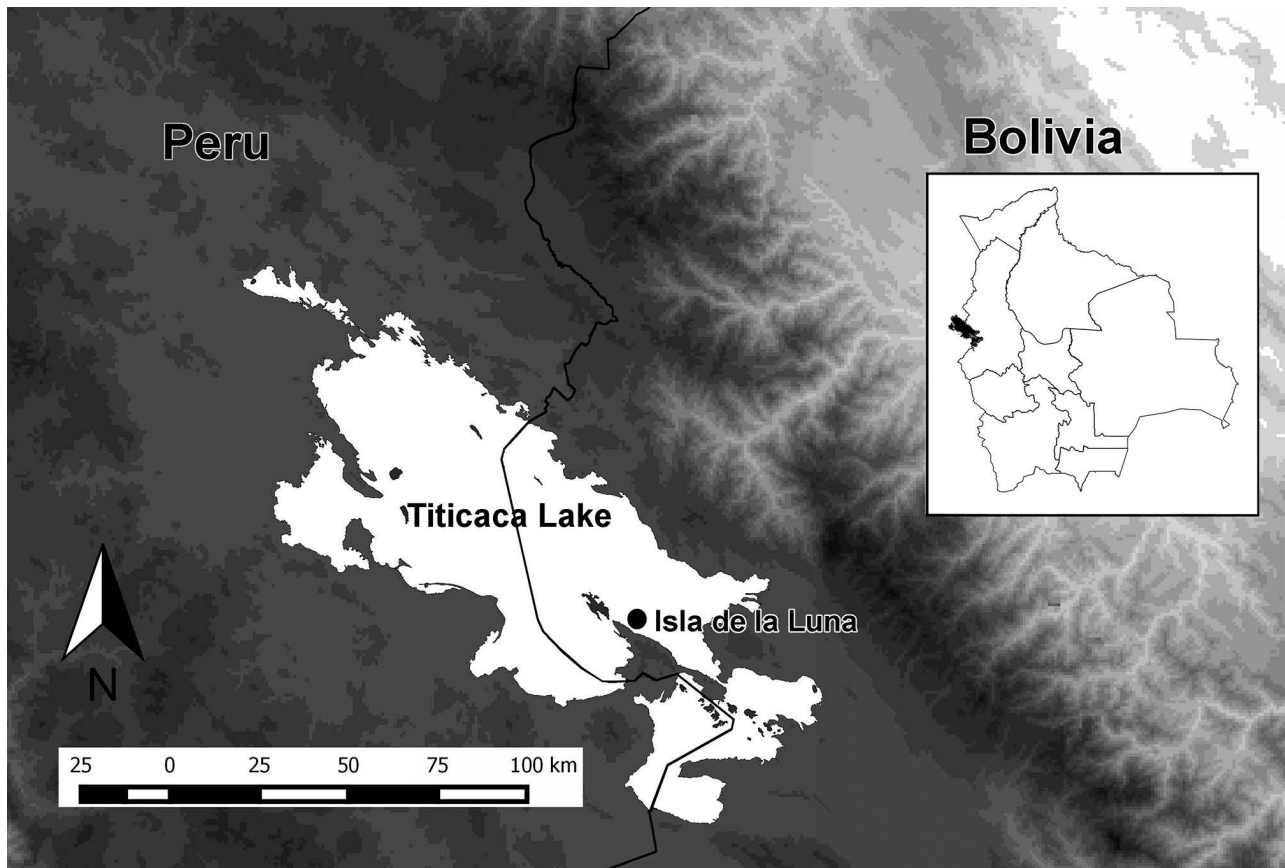


Figure 1. Map of the study area. The inset shows the location of the study region of Lake Titicaca within Bolivia. The main map highlights Isla de la Luna (black dot) in Lake Titicaca, Bolivia; map based on CGIAR SRTM (JARVIS et al. 2008) elevation model (light = low, dark = high elevations).

dividuals were recorded. Because we were working with a threatened species, for ethical reasons, we did not collect live specimens specifically for this study. In some cases we found fresh dead individuals within the transects or injured and dead frogs in fishing nets that were collected in addition to fresh dead individuals that were already obtained by fishermen. These frogs contained identifiable remains in different sections of the digestive tract, in some cases even in the mouth. Therefore, these individuals could be used as a representative sample of the population in the area. These samples were used for several other studies and afterwards deposited in the Museo de Historia Natural Alcide d'Orbigny, Cochabamba (for codes see Supplementary material). For the injured individuals that needed to be euthanized, we used tricaine methanesulfonate (MS-222) solution of 5 g/L (CECALA et al. 2007) and a post mortem analysis was carried out where different morphometrics, sex, body condition was noted, and tissue samples were obtained.

Gut content sampling

Immediately after obtaining the frog individuals, the digestive tract was isolated and separated by portions: stomach, small intestine and large intestine. Afterwards all the content was extracted, counted and preserved in 90% ethanol for later analyses. All potential prey items and stomach contents were identified to the lowest possible taxonomic level using descriptions of DEJOUX (1991).

Nutrient analysis

Subsamples of potential prey items were dried in an oven at 60°C for five days and afterwards homogenized. Proximate analysis was carried out: ash contents were determined as described by BOGUT et al. (2007) burning the samples at 550°C for 4 h in a muffle furnace. Crude protein (CP), calculated with nitrogen content in the sample according to Kjeldahl $\times 6.25$ methods using the Kjeld-Foss and crude fat (CF) content by Soxhlet methods. Sample preparation for mineral analysis consisted of microwave digestion with 10 ml HNO₃ in closed vessels, followed by filtration of the residue. Samples were analysed for Na, K, P, S, Ca, Mg, Cu, Fe, Mn, Zn concentrations, using inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista MPX radial, Varian, Palo Alto, CA, USA) and Se and Hg via inductively coupled plasma mass spectrometry (ICP-MS) (Elan DRC-e, Perkin Elmer, Sunnyvale, CA, USA).

Statistical analysis

Prey availability by depth and season

With the information from the traps, relative abundance of the potential prey items was obtained per month and per water depth. We calculated relative abundance of potential

prey items in each month by dividing the number of individuals of a single species of invertebrate divided by the number of individuals of all species on each date or each date and depth (GILLESPIE 2013). Temperature information for each month was obtained with data loggers installed during the fieldwork period in the habitat at a depth of 2 m.

To see if there was a relationship between water temperature in each season and the abundance of potential prey items, a simple linear regression was carried out with the average temperatures measured during the study period and the relative abundances of the different prey items. To show the association between these two parameters, a graph was built with water monthly temperatures and average numbers of the most common consumed prey item by *T. culeus*.

To compare the number of potential prey items at different depths (0.5, 1.5 and 3 m) one-way ANOVA analysis was conducted using RStudio version 3.1.1. A post hoc Tukey Honest Significant Difference (HSD) test was carried out to determine which depth was significantly different from the others.

Diet composition

In most dietary studies, information about number, frequency and volume and/or mass of the prey items is presented (SOLÉ & RÖDDER 2010). For the numbers and frequencies we counted all the prey items captured during this period in all the traps and digestive tract from frogs. For the mass, potential prey items were dried out as recommended by SOLÉ & RÖDDER (2010) in an oven at 60°C for five days and dry matter was obtained from each individual from a subsample of 30 samples per species with an analytical balance. To calculate volume of the potential prey items, the most commonly used formula for ellipsoid bodies was used (COLLI & ZAMBONI 1999):

$$V = \frac{4\pi}{3} \frac{L}{2} \left(\frac{W}{2}\right)^2,$$

where V is volume, L is length, and W is width of prey item. For this, measurements of the individuals from the subsample were carried out under stereoscope with a digital caliper with a precision of 0.01 mm.

The index of relative importance (IRI) for each potential prey item was calculated. This is a measure that reduces bias in descriptions of animal dietary data (HART et al. 2002). Introduced by PINKAS et al. (1971) and PIANKA (1973), it is mainly used currently to describe fish diets but also starting to be used in other taxonomic groups (SOLÉ & RÖDDER 2010, WATSON et al. 2017):

$$IRI_t = (PO_t)(PI_t + PV_t),$$

where PO_t is the percentage of occurrence (100 \times number of stomachs containing t item/total number of stomachs), PI_t is the percentage of individuals (100 \times total number of individuals of t in all stomachs/total number of individuals of all taxa in all stomachs), and PV_t is the percentage of volume (100 \times total volume of individuals of t in all stomachs/total volume of all taxa in all stomachs).

Table 1. Potential prey items for *T. culeus* in benthic habitat at 2 m depth at Isla de la Luna, Titicaca Lake, Bolivia from January to December 2014; frequency of prey items of *T. culeus*, number of individuals per month per trap collected (parentheses, percentage).

Prey species	Prey species	Common name	Habitat	Trap	Frequency
Arthropoda: Insecta, Coleoptera, Elmidae	<i>Austrelmis</i> sp.	Beetle	x	x	14 (5%)
Arthropoda: Insecta, Coleoptera, Elmidae	<i>Austrelmis</i> sp. (Larvae)	Beetle larvae	x	x	2 (0.8%)
Mollusca: Gastropoda: Sphaeriidae	<i>Sphaerium</i> sp.	Bivalve	x	x	1 (0.4%)
Mollusca: Gastropoda: Planorbidae	<i>Anisancylus</i> spp.	Mollusk	x	x	27 (9.6%)
Mollusca: Gastropoda: Cochliopidae	<i>Heleobia</i> sp.	Snail	x	x	4 (1.3%)
Mollusca: Gastropoda: Planorbidae	<i>Biomphalaria</i> sp.	Snail	x	x	6 (2%)
Arthropoda: Malacostraca: Amphipoda	<i>Hyalella</i> spp.	Shrimp	x	x	209 (74%)
Arthropoda: Insecta, Diptera	Chironomidae	Chironomidae	x	x	2 (0.8%)
Arthropoda: Insecta: Trichoptera	Trichoptera	Caddisfly	x	x	1 (0.4%)
Arthropoda: Insecta: Hemiptera, Corixidae	Corixidae	Water boatmen	x		–
Platyhelminthes: Tricladida: Dugesiidae	<i>Girardia</i> sp.	Flatworm	x	x	4 (1.4%)
Annelida: Rhynchobdellida: Glossiphoniidae	<i>Helobdella</i> sp.	Leech	x	x	13 (4.5%)
Chordata, Actinopterygii, Siluriformes, Trichomycteridae	<i>Trichomycterus</i> sp.	Catfish	x	x	1 (0.4%)
Chordata, Actinopterygii, Cyprinodontiformes, Cyprinodontidae	<i>Orestias ispi</i>	Ispi	x		–
Chordata, Actinopterygii, Cyprinodontiformes, Cyprinodontidae	<i>Orestias cf. luteus</i>	Carachi amarillo	x		–
Chordata, Actinopterygii, Cyprinodontiformes, Cyprinodontidae	<i>Orestias cf. olivaceus</i>	Carachi enano	x		–
Chordata, Actinopterygii, Atheriniformes, Atherinopsidae	<i>Odontesthes bonariensis</i> (juvenile)	Pejerrey	x		–
Chordata, Actinopterygii, Salmoniformes, Salmonidae	<i>Oncorhynchus mykiss</i> (juvenile)	Trout	x		–
Chordata, Amphibia, Anura, Telmatobiidae	<i>Telmatobius culeus</i>	Titicaca water frog	x	x	1 (0.4%)

Prey selection

To determine what diet items the frogs might be selecting from their environment, we performed Pearson's chi-square test of independence, with statistical significance when $p < 0.05$. Post hoc tests were used to determine which taxa were significantly different between numbers present in the habitat and numbers consumed. For this, Pearson's chi-square tests of independence for pairwise comparisons were made, with Bonferroni correction of the p-value ($0.05/11 = p < 0.0031$; 11 individual tests were run) (WATSON et al. 2017). All statistical analyses were conducted in the R statistical environment version 3.1.1.

To compare the number of prey items consumed among the different groups (males and females) one-way ANOVA analysis was conducted using RStudio version 3.1.1. A post hoc Tukey Honest Significant Difference (HSD) test was carried out to determine which prey item was significantly different from the others.

To find out if there was any relationship between the size of the frogs and the number of prey items found in stomach contents, a simple linear regression was carried out with size of the frog as the independent variable and proportion of prey items as the dependent.

Results

Prey availability

A total of 19 species (12 macroinvertebrates and seven vertebrates) of potential prey items were recorded at Isla de la Luna, in the habitat where *T. culeus* is more abundant. Taking into account only the benthic species that were collected in the traps, we found 11 species of macroinvertebrates, one species of fish and a frog (Table 1). Other species of fish were not captured with the traps but *Orestias ispi*, *O. cf. luteus*, *O. cf. olivaceus* and juveniles of exotic species *Oncorhynchus mykiss* and *Odontesthes bonariensis* were observed or captured in the surrounding nets from local fishermen (Table 1). From all 13 trap-collected species, we found that the amphipod *Hyalella* was the most common taxon, followed by *Anisancylus* spp., *Austrelmis* sp., *Helobdella* sp. and *Biomphalaria* sp. as the most abundant taxa (Table 1).

Prey availability by depth and season

Relative abundances of the most common prey were associated with water temperature of different seasons. We

observed a positive correlation between the relative abundances of *Hyaella* spp. with water temperature at a depth of 2 m ($F = 13.6$; $R^2 = 0.58$; $P < 0.01$), where numbers of this species decreased with lower temperatures during winter between July and August (minimum temperature of 11.47°C in June 2014), and increased with temperature during January to March (maximum temperature of 17.61°C in December 2014) (Fig. 2). The other taxa that showed a correlation were *Biomphalaria* sp. and Trichoptera but in this case the correlation was negative ($F = 22.99$; $R^2 = 0.6969$; $P < 0.01$) for *Biomphalaria* sp. and ($F = 7.35$; $R^2 = 0.4$; $P = 0.02$) for Trichoptera. For the rest of the species, we found no clear correlation between relative abundances and water temperatures (Supplementary Fig. 1).

The diversity of potential prey at various depths between 0.5 to 3 m did not differ, but the relative abundance in some species did. A very clear example was the relative abundance of the most abundant genus, *Hyaella*, that had a higher number of individuals at 0.5 m with an average

of 411 individuals per trap, compared with the depth of 1.5 m with an average of 106 individuals per trap ($P < 0.05$) and with 3 m with an average of 70 individuals per trap ($P < 0.05$), but no difference was found between 1.5 and 3 m depth ($P > 0.05$). The other species that followed the same pattern was the larvae of the beetle *Austrelmis*, with higher relative abundances at 0.5 m compared with 1.5 m ($P < 0.05$) and 3 m ($P < 0.05$). The rest of the species had some differences but did not follow any apparent pattern (Supplementary Table 1, Table 2).

Gut content and diet composition

We examined a total of 44 stomach contents of *T. culeus*. From these, 37 contained identifiable remains of prey items, 32 with macroinvertebrates and fish with a total of 544 prey items. From all stomachs, 10 prey species were identified. Nine species was the maximum number of prey items found in the stomach content per frog, with an av-

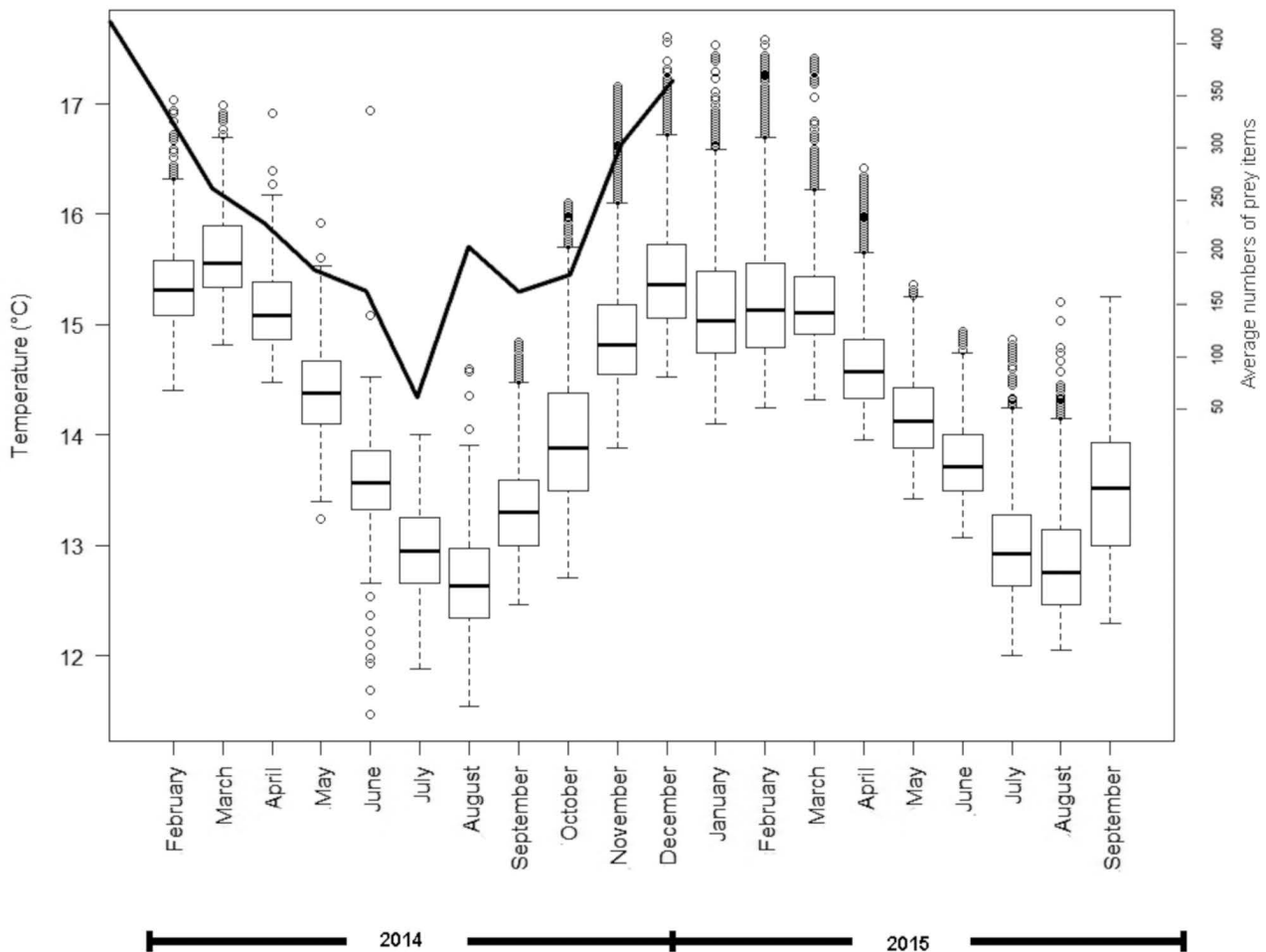


Figure 2. Box plot of monthly temperatures at Isla de la Luna in Lake Titicaca, Bolivia at 2 m depth (data from February 2014 to September 2015) and relative abundance variation of *Hyaella* spp. (black line) from February to December 2014.

Table 2. Frequency of prey items of *T. culeus*, number of stomachs in which prey items were found (parentheses, percentage); numbers of prey items found (parentheses, percentage); total mass of item species found; total volume of item species found; Index of relative importance IRI_m based on mass; Index of relative importance IRI_v based on volume.

Prey	Frequency	Total No.	Mass (g)	Volume	IRI m	IRI v
<i>Austrelmis</i> sp.	3 (9.38)	3 (0.55)	0.003	9.58	6.13	5.66
<i>Austrelmis</i> sp. Larvae	4 (12.5)	10 (1.84)	0.007	23.19	25.78	24.58
<i>Anisancylus</i> spp.	2 (6.25)	3 (0.55)	0.014	13.13	6.29	3.90
Chironomidae	2 (6.25)	2 (0.37)	0.000	1.41	2.31	2.35
<i>Hyalella</i> spp.	23 (71.88)	308 (56.62)	0.974	8319.02	6353.90	7302.05
<i>Heleobia</i> sp.	15 (46.88)	179 (32.9)	0.732	1921.65	2662.19	2031.10
<i>Orestias ispi</i>	2 (6.25)	2 (0.37)	0.884	6794.48	182.03	231.36
<i>Biomphalaria</i> sp.	11 (34.38)	31 (5.70)	0.438	1356.08	686.14	447.68
Trichoptera	2 (6.25)	5 (0.92)	0.022	100.99	10.17	9.16

erage of 3 ± 2 prey items. The most frequently items in the stomach content were *Hyalella* spp., *Heleobia* sp. and *Biomphalaria* sp. accounting for 76.8% of the total number of prey items, 62.5% in volume and 69.8% in mass. Other than the macroinvertebrates and fish, vegetal remains were found in 13 frog individuals (29.5%) (Table 2). Besides these species, we have records of frogs eating *Orestias* cf. *luteus* and *O. cf. olivaceus* even smaller conspecific individuals of the frogs on the basis of observation and photographic material.

Prey selection by sex and size

To investigate if there was a difference of prey selection by sex, a total of 39 frogs containing prey items in the gut were analyzed (25 females and 14 males). The total numbers of species consumed by the frogs did not differ considerably between males (7 species) and females (10 species), with an average of 3 species per individual in females and 2 in males. The proportions of macroinvertebrate individuals did not differ considerably between males and females (Supplementary Table 3, Table 4).

Analyzing the prey selection by size, a positive correlation was found between body size and the number of prey species found in the gut content ($F = 6.5$; $R^2 = 0.14$; $P = 0.01$), where small frogs consumed less prey taxa than larger individuals (Supplementary Fig. 2). For some prey items, we found a correlation between size of frogs and the proportion of prey items (Fig. 3). A negative relationship was found with *Hyalella* spp. that seemed to be consumed more by small frogs than by large individuals ($F = 7.77$; $R^2 = 0.49$; $P = 0.02$). In frogs smaller than 55 mm, around 90% of the diet of this group was constituted of this amphipod, while with frogs larger than 80 mm an average of 18% of the items were *Hyalella* spp. In the case of *Heleobia* sp. the relationship was positive, where small frogs hardly consumed this snail species. Yet, when grown to larger size, they started to use more of this resource ($F = 6.75$; $R^2 = 0.46$; $P = 0.03$). With the rest of the species no differenc-

Table 3. Numbers of potential prey (no. present) compared to prey consumed by *Telmatobius culeus* (no. consumed) and p-values from the post hoc Pearson's chi-square tests of independence for pairwise comparisons for each potential prey vs. all others, with Bonferroni correction of the p-value ($0.05/14 = 0.00357$). Percentages (%) are shown in parentheses. Bold values indicate significance ($p < 0.00357$). (–) data not available (*) numbers not noted.

Prey species	Number individuals	No. consumed	p-value
<i>Austrelmis</i> sp.	1906 (7.3)	3	< 0.001
<i>Austrelmis</i> sp. Larvae	505 (1.9)	10	1
<i>Sphaerium</i> sp.	16 (0.1)	0	0.604
<i>Anisancylus</i> spp.	1524 (5.9)	3	< 0.001
<i>Heleobia</i> sp.	910 (3.5)	179	< 0.001
<i>Biomphalaria</i> sp.	398 (1.5)	31	0.024
<i>Hyalella</i> spp.	16661 (64.1)	308	0.145
Chironomidae	1232 (4.7)	2	< 0.001
Trichoptera	210 (0.8)	5	1
Corixidae	5 (0.0)	0	< 0.001
<i>Girardia</i> sp.	1332 (5.1)	0	< 0.001
<i>Helobdella</i> sp.	1279 (4.9)	0	< 0.001
<i>Trichomycterus</i> sp.	4 (0.0)	0	0.604
<i>Orestias ispi</i>	0	2	1
Plants	–	*	–

es were found between the numbers of prey items in relation to size. In some cases a correlation appeared (such as *Biomphalaria* sp.), but this needs confirmation with larger sample size.

Nutrient composition of wild diet

Carrying out an analysis of nutrients in dry matter, we found that most of the prey items had higher crude protein levels than 50%, *Hyalella* spp. and Trichoptera with medium

Table 4. Nutrient composition of potential prey items for *T. culeus* at Isla de la Luna, Titicaca Lake, Bolivia, based on a dry matter basis (DMB).

Species	CP		GE		Ash		Lipid %	Ca mg/kg	Mg mg/kg	P mg/kg	S mg/kg	K mg/kg	Na mg/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg	Se µg/kg	Hg µg/kg
	%		kJ/g	%	%	%													
Trichoptera	50.3	18.5						15392	1652	7785	5770	4960	2698	<10	2090	67	1613	320	1175
<i>Austrelmis</i> sp.	62.0	19.6						3810	1640	4771	3930	1970	1152	22	478	51	2322	193	146
<i>Helobdella</i> sp.	65.3	20.6						3968	1262	8510	7746	4391	5349	<10	1349	18	6762	2659	308
<i>Girardia</i> sp.	58.2	20.8						4801	1435	12542	8392	5626	1377	<10	1578	11	1157	2256	125
Chironomidae								7266	2118	5285	4741	1895	1236	<10	2388	59	2754	169	77
Corixidae								2775	1301	11302	9273	6578	5011	14	237	13	153	382	125
<i>Hyaella</i> spp.	43.0	13.0	29.0	28.0	93426	2159	1363	6497	2072	3501	14	457	17	192	925	<65			
<i>Orestias ispi</i>	56.9	20.9	13.3	29.8	35955	1332	2483	8580	7145	2466	<10	79	8	118	1504	74			
<i>Orestias olivaceus</i>	64.2	21.4	14.0	21.8	38341	1569	2759	10928	9886	3424	<10	80	8	98	900	82			
<i>Trichomycterus</i> sp.	48.9	22.4	11.6	39.5	28088	1079	1871	6940	2514	1098	<10	188	10	196	1100	322			
<i>Orestias luteus</i>	52.6	15.9	18.3	29.1	52774	1622	3291	9176	10977	3610	<10	89	7	85	1099	192			
<i>Heleobia</i> sp.	15.6	3.4	79.1	5.2	3015	1009	7107	6775	3364	3740	<10	898	14	3931	2223	251			
<i>Biomphalaria</i> sp.	12.9	3.4	77.1	10.0	302128	833	2307	2386	636	1415	<10	1139	90	3766	386	<65			

percentages and the snails with the lowest values (Table 4). Most of the prey items had gross energy values higher than 20 kJ/g, *Hyaella* spp. with medium values around 20 kJ/g and finally with the lowest values the two snails *Heleobia* sp. and *Biomphalaria* sp. (Table 4). The same table shows

information about the minerals and other nutrients present in the different potential prey species. Plotting all potential items in a graph with crude protein (CP) on one axis and gross energy (GE) values on the other, demonstrates how the different taxa are divided: snails (*Heleobia* sp. and *Biom-*

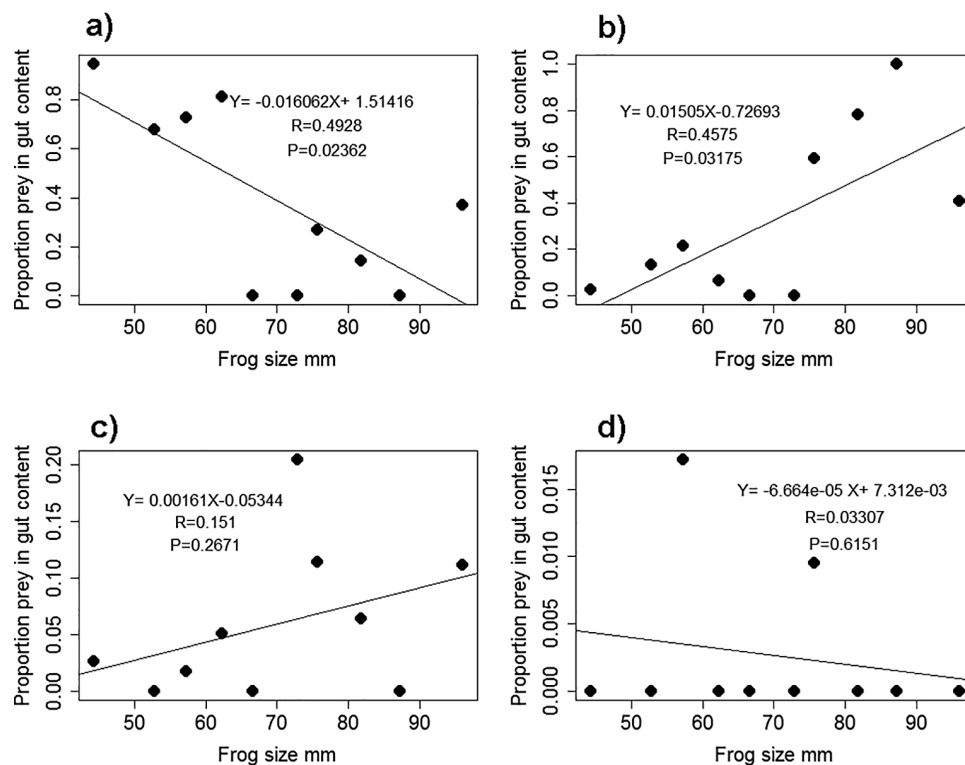


Figure 3. Correlation of the proportions of numbers of prey items in gut content found in *T. culeus* by size of frogs at Isla de la Luna, Lake Titicaca, Bolivia; (a) *Hyaella* spp., (b) *Heleobia* sp., (c) *Biomphalaria* sp., (d) *Austrelmis* sp.

phalaria sp.) were the items with lower concentrations of energy and protein accessible for the frog, in the middle was *Hyaella* spp., that has an average concentration of CP and GE, and in the top of this group are all other fish species, together with flatworms, leeches and Coleoptera (Fig. 4). The snail *Biomphalaria* sp. and the amphipod *Hyaella* had the highest calcium concentrations, followed by the different fish species of the genus *Orestias* and finally all other macroinvertebrates. Remarkably, the snail *Heleobia* sp. had the lowest calcium concentration. The calcium:phosphorus ratio followed the same pattern. Mercury concentrations in the different prey items were also very different depending of the species: Trichoptera had the highest concentrations of Hg and one of the main prey items, *Hyaella* spp. had undetectable concentrations (Table 4).

Discussion

Prey availability

This study linked prey availability with prey nutritive value and possible prey selection of *Telmatobius culeus*. We found that *Hyaella* spp., *Anisancylus* spp. and *Austrelmis* sp. respectively were the dominant prey species, whereas DEJOUX (1991) reported mollusks and *Hyaella* spp. respectively as the prevalent taxa. Although changes may have occurred in the structure of benthic fauna, likely, effects of season and sampling methods can be the reason of these differences. Relative abundances in some taxa such as *Hyaella* spp., *Heleobia* sp. and *Biomphalaria* sp. showed a seasonal variation associated with water temperature, but DEJOUX (1991) associated these changes mainly with water level fluctuations in the lake. Anyhow, it appears that the availability of main diet items can substantially vary in time and is not constant. Another source of variation of the prey abundances seems to be water depth in species

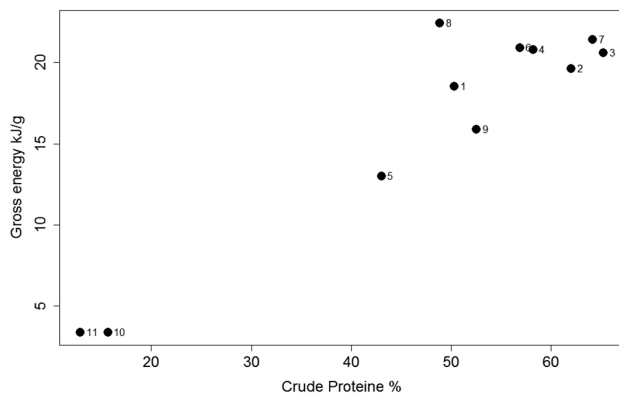


Figure 4. Correlation of crude protein and gross energy of potential prey items of *T. culeus* at Isla de la Luna, Lake Titicaca, Bolivia. 1 = Trichoptera, 2 = *Austrelmis* sp., 3 = *Helobdella* sp., 4 = *Girardia* sp., 5 = *Hyaella* spp., 6 = *Orestias ispi*, 7 = *Orestias* cf. *olivaceus*, 8 = *Trichomycterus rivulatus*, 9 = *Orestias* cf. *luteus*, 10 = *Heleobia* sp., 11 = *Biomphalaria* sp.

such as *Hyaella* spp. whose relative abundances decrease markedly with depth. DEJOUX (1991) provides information about depth distribution of the most abundant species such as *Littoridina*, *Biomphalaria* sp. and *Hyaella* spp., but the scale of depth makes direct comparisons impossible but did report changes in numbers due to water depth.

Gut content

The amphipod *Hyaella* was the most commonly taxa found in the gut content of frogs, followed by mollusks (*Heleobia* sp. and *Biomphalaria* sp.), whereas historical data report “worms” (probably nematode parasites), amphipods as the main preys and aquatic insects, tadpoles and fish of the genus *Orestias* in lower numbers (GARMAN 1875, ALLEN 1922). More recent study from PEREZ (1998) have shown mollusks (*Heleobia* sp. and *Biomphalaria* sp.), followed by *Hyaella* spp. as main items, and also reported vegetal remains, Coleoptera and some other minor groups. This historical data compared with our results show a common pattern, where *Hyaella* spp., *Heleobia* sp. and *Biomphalaria* sp. are the most consumed macroinvertebrate prey. Although some differences in proportions of prey items were observed between our data and these previous studies, it seems that *T. culeus* has been using basically the same main food resources for the last century. We need to be aware that soft bodied taxa such as *Helobdella* or *Girardia* could be potential prey items that were underrepresented because they were likely digested very fast, hence difficult to find in the gut content as indicated by MCKECHNIE (2004) and GILLESPIE (2013). Our methodology may thus have created a bias because it probably overemphasizes species with a hard or resistant exoskeleton. The natural abundance of stable isotope ratios could help us to improve the accuracy of measuring dietary changes in this frog (SOLÉ & RÖDDER 2010).

Similar data of a very closely related species is available, the Junin's frog (*Telmatobius macrostomus*), based on nine individuals, they reported nine taxonomic families in the frogs' stomachs, with snails (Physidae) and *Hyaella* spp. as the most common prey items (WATSON et al. 2017). Another study on *Telmatobius dankoi* reported *Hyaella*, Chironomid larvae and snails as main items (LOBOS et al. 2016). Although historical data include the native fish *Orestias* for the first species, WATSON et al. (2017) found no vertebrates during their study. It draws attention to the fact that the benthic fish *Trichomycterus* was never reported as a prey for this or any other *Telmatobius* (GARMAN 1875, ALLEN 1922, PEREZ 1998, LOBOS et al. 2016, WATSON et al. 2017), maybe because this fish shares the same habitat with the frog and has evolved an antipredator strategy that protects the species against very potential predators (ARRATIA, 2003).

Prey selection

PEREZ (1998) suggested that *T. culeus* is an opportunistic species, taking the prey items that are more abundant

from its habitat. We used foraging strategy definition sensu SINGER (2000), where an opportunist species feeds on a wide range of prey items, but the diet can change or switch to take advantage of temporary availability of profitable resources or to cope with temporary loss of main resources (GILLESPIE 2013). We found that *Hyalrella* spp., *Austrelmis* sp., *Anisancylus* spp., *Girardia* sp. and *Helobdella* were the most dominant taxa in the habitat, whereas gut content analysis showed that only *Hyalrella* spp. was commonly consumed by frogs. Yet, *Heleobia* sp. and *Biomphalaria* sp. were abundant in the gut content even though they were much less abundant in the habitat. This patterns suggests that the frogs are just eating the most abundant prey item (*Hyalrella* spp.) because it is easily accessed, but they are complementing the diet or choosing *Heleobia* sp. and *Biomphalaria* sp. for certain nutrients or properties that these species could be offering them.

No information is available from other aquatic anurans about prey selection based on sex, but in other big frogs such as the terrestrial Mountain chicken frog *Leptodactylus fallax*, just slightly differences were found (BROOKS 1982). The sex differences reported by MANEYRO et al. (2004) in the diet of *Leptodactylus ocellatus* only become apparent depending on sex in combination with season, so nutrient requirements might only differ between males and females in the breeding season. In our case no clear sex-specific differences were found.

Taking into account the size of frogs, we found that small frogs were mainly specialized in *Hyalrella* spp., and once the frogs were larger they started to diversify the diet. It seems that this ontogenic change of diet is common in big species such as in *L. ocellatus* (MANEYRO et al. 2004) or *L. fallax* (BROOKS 1982) where a diet shift according to the frog size was observed. This change of prey size could have an effect in the in the nutrient intake (BROOKS 1982) with a corresponding impact in the fitness and reproduction that eventually on the population (LI et al. 2009). Although a diet shift is observed in large frogs, they still retain a feeding response when presented with small prey items (BROOKS 1982, HOUSTON 1973, LABANICK 1976, BERRY 1966). Thus, in areas where food is a limiting factor, young or smaller frogs are probably still in competition for food resources with larger individuals (BROOKS 1982). In the case of *T. culeus* we saw this shift in the diet in larger frogs: these frogs still use small prey items such as *Hyalrella* spp. and competition of small individuals with larger conspecifics could be avoided with juveniles choosing other habitats (shallow waters or under rocks) than adults (deeper waters). In this sense they probably avoid competition and even conspecific predation (MUÑOZ-SARAVIA et al. 2018, 2020).

Based on direct observations we know that *T. culeus* eats fish relatively often, especially in the season when *Orestias ispi* was abundant in the area. Gross energy, protein and lipids that were higher in fish could probably explain this apparent opportunistic switch of diet, making these prey items nutritionally more interesting. This is probably the reason of some adaptations in the frog such as the shape

and size of the mouth that would allow them capture large items as observed with *L. fallax* by BROOKS (1982) or *Telmatobius* by BARRIONUEVO (2016). For a species with a very low metabolic rate (HUTCHISON et al. 1976) eating these items, the cost benefit of food intake and energy expenditure would be very beneficial and frogs would not need any other food sources for a long period.

Nutrient composition of wild diet

We found that the fat, protein and gross energy content differed substantially between *Hyalrella* spp. and other prey sources, as well as some of the mineral contents. This means that the proportion of *Hyalrella* spp. in the diet largely determines the overall nutrient intake. This is especially important at early stages when small and juvenile frogs are mainly dependent on this taxon. Knowing that exoskeleton of most arthropods is primarily composed of protein and chitin (FINKE 2013), we can assume that the 46% CP found in *Hyalrella* spp. will be not just protein. Protein is considered as energy source for metabolism and building stones for anabolic processes (VENESKY et al. 2012), it has an important function in the defense against pathogens maintaining the immune system (SCHMID-HEMPEL 2003, VENESKY et al. 2012). In this case *Hyalrella* spp. probably fulfills the protein requirements for the frogs, but probably any options with higher levels of protein as fish for example will be beneficial.

Calcium values were very high in *Hyalrella* spp., but it does not mean that all of that is accessible; Ca is mainly located in the exoskeleton and could be nutritionally unavailable if the exoskeleton is not digested. The frog should thus have strategies to access these resources. DIERENFELD & KING (2008) showed an example where soldier fly larvae with mineralized exoskeleton with high calcium content was fed to *L. fallax*, and where the calcium digestibility of whole soldier fly larvae was only 44% compared to 88% for soldier fly larvae that had been "mashed". Finding rests of *Hyalrella* spp. in gut of *T. culeus* in different degrees of digestion we can probably assume the frog is breaking down chitin. Studies about digestibility such as the study of DIERENFELD & KING (2008) would help to see if *T. culeus* has the capacity to break down and digest possible nutrients from the exoskeleton. The snails were often found in the frog's gut content, despite their low little nutritional value per se. We also saw that frogs were unable to destroy the shell, so that their presence in the gut cannot be explained as a Ca source. We therefore hypothesise that snails are helping in some way to break down the exoskeleton of *Hyalrella*. If not, even just slowing down digestion, gives the frog more time to digest and absorb the different nutrients. More studies should be done to see if those shells play a role in the amphibian digestion or if it has any effect in the passage rate of food in the amphibian digestive tract. With the high Ca:P ratio in the main prey *Hyalrella* spp. and different fish species and even the high carbonate levels dissolved in the water (MUÑOZ-SARAVIA et al. 2018), captive

breeding programs should think in providing enough Ca to avoid any issues (MCWILLIAMS 2008, FERRIE et al. 2014).

With *Hyaella* spp. as a main prey item, *T. culeus* should have a strategy to get access to the nutrients from within the exoskeleton. The presence of chitinase has been reported in gastric glands of *Xenopus laevis* and *Rana catesbeiana*, (FUJIMOTO et al. 2002), and in the pancreas of *Bufo japonicus*, being 4.5 times stronger than commercial chitinase (OSHIMA et al. 2002). In this group of vertebrates, in which arthropods constitute one of the main food items, chitinase could be a common strategy to break down glycosidic bonds in chitin to make simpler molecules ready to be used as energy source or other metabolic purposes and also release the calcium that is trapped in the exoskeleton of the prey (FUJIMOTO et al. 2002). This strategy and its high availability would make *Hyaella* spp. an interesting and good food resource for the species.

Metal concentrations such as Fe, Zn, Hg and to a lesser extent Cu and Mn seemed to vary with species, which likely has to do with the benthic versus pelagic habitat where the species lives, a pattern also observed by MONROY et al. (2014) who reported higher metal concentrations in benthopelagic species of the Peruvian side of the lake. As possible external sources of some metals, they indicated human sources such as domestic wastewaters, the run-off from adjacent agricultural systems and mining activities. All these minerals in some way will also enter the trophic web in the lake and as observed in Table 4, we assume that benthic species are more exposed to these higher concentrations of minerals. The only species with Hg concentration below detection limit was *Hyaella* spp.

Despite the higher energy and protein concentrations in other species such as beetles, *Helobdella* leeches or *Girardia* sp. observed in Figure 4, *Hyaella* spp. probably has the benefit of high abundance, accessibility and size. Therefore the energy investment required to get these other prey items is higher. Opportunities to get items that are more nutritious and are bigger than *Hyaella* such as *Orestias* will provide the frog more protein and energy, but they will also depend on the season. Now with threats such as the introduction of exotic species, habitat destruction, pollution, climate change (IUCN 2024) and even massive mortalities (MOLINA et al. 2017) of different organisms, the structure of trophic web in the lake has most likely been affected and this could have an important impact not just on the frog's health and its fitness but in the entire ecosystem.

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Supplementary data

The following data are available online:

Supplementary Table 1. Average number of individuals of potential prey items for *Telmatobius culeus* in benthic habitat at different depths at Isla de la Luna, Titicaca Lake, Bolivia, from November 2016 to January 2017.

Supplementary Table 2. ANOVA Tukey's Honest Significance Test of Abundances of potential prey items measured underwater at different depths in Isla de la Luna, Titicaca Lake, Bolivia, from November 2016 to January 2017.

Supplementary Table 3. Average number of consumed prey items individuals by *Telmatobius culeus* per sex at Isla de la Luna, Lake Titicaca, Bolivia.

Supplementary Table 4. ANOVA Tukey's Honest Significance Test of Abundances of consumed prey items by *Telmatobius culeus* per sex at Isla de la Luna, Titicaca Lake, Bolivia.

Supplementary Table 5. Museum numbers for the collected frog specimens during the study at Titicaca Lake, Bolivia.

Supplementary Figure 1. Correlation between average temperature and relative abundance of different prey items of *Telmatobius culeus* at Isla de la Luna, Lake Titicaca, Bolivia.

Supplementary Figure 2. Correlation showing the number of prey species found in individuals of *Telmatobius culeus* of different body size at Isla de la Luna, Lake Titicaca, Bolivia.