Temporal Gap Between Knowledge and Conservation Needs in High Andean Anurans: The Case of the Ascotán Salt Flat Frog in Chile (Anura: Telmatobiidae: Telmatobius)

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5 authors, including:

Gabriel Lobos
University of Chile

Nico Rebolledo
University of Chile

Carlos Canales-Cerro
Lemu

Jorge Perez-Quezada
University of Chile

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Temporal Gap Between Knowledge and Conservation Needs in High Andean Anurans: The Case of the Ascotán Salt Flat Frog in Chile (Anura: Telmatobiidae: *Telmatobius*)

Gabriel Lobos[1,2,*], Nicolás Rebolledo[2], Mariela Sandoval[2], Carlos Canales[2], Jorge F. Perez-Quezada[3]

[1] *Centro de Gestión Ambiental y Biodiversidad, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile. Santiago, Chile.*
[2] *Ecodiversidad Consultores. Santiago, Chile.*
[3] *Departamento de Ciencias Ambientales y Recursos Naturales Renovables, Facultad de Ciencias Agronómicas, Universidad de Chile. Santiago, Chile.*
[*] *Corresponding author. Email: galobos@ug.uchile.cl*

Abstract. The natural history of most species of *Telmatobius* that live in the Altiplano of the Andes is unknown due to the difficulty of performing long-term studies and the logistics of working in these remote areas with extreme environmental conditions. One of these anurans inhabits the springs of the Ascotán salt flat of Chile. Here, we provide for the first time information on its distribution, habitat, microhabitat, density, diet and reproductive activity. Suitable habitat for the specie is restricted to a few springs that drain into the salt flat. Amphibian density was variable among springs and seasons and diet was composed mainly of bottom-dwelling invertebrates. We also found evidence of selection for some benthonic prey. Reproductive activity occurred mainly at night. Larvae were found during the whole year, which suggests a long larval developmental period. The salt flat is under strong anthropic pressure, although so far this anuran has managed to persist in spite of habitat perturbations. Our biological and ecological data might increase our ability to act and protect this high Andean anuran species.

Keywords. Altiplano; Density; Diet; Habitat; *Telmatobius;* Reproduction.

Resumen. La historia natural del género *Telmatobius* en el altiplano de los Andes es prácticamente desconocida, debido a lo difícil que resulta la realización de estudios de largo plazo, tanto por aspectos logísticos como por las extremas condiciones ambientales del área. Uno de estos anuros habita en las vertientes del Salar de Ascotán en Chile. Nosotros entregamos por primera vez antecedentes sobre la distribución, hábitat, microhábitat, densidad, dieta y actividad reproductiva para una especie altoandina de este género. El hábitat adecuado para la especie se restringe a unas pocas vertientes del salar. La densidad de anfibios varió entre vertientes y estaciones del año y su dieta estuvo compuesta principalmente por invertebrados bentónicos. También evidenciamos la selección sobre algunas de estas presas. La actividad reproductiva ocurre durante la noche. Las larvas estuvieron presentes a lo largo de todo el año, lo que indica un largo periodo de desarrollo. El salar se encuentra bajo una fuerte presión antrópica, sin embargo la especie ha sido capaz de persistir. Nuestros datos sobre la biología y ecologia pueden incrementar la habilidad de actuar y proteger esta especie de anuro altoandino.

INTRODUCTION

The high elevation environments of southern South America are the habitat for anurans of the genus *Telmatobius* Wiegmann, 1834. These amphibians are specialized to live in conditions of high elevation, solar radiation, and aridity (Capurro, 1954; Lavilla, 2005; Veloso, 2006). Species of this genus live in wetlands, bogs, lakes, rivers, and wetlands associated with salt flats between 5° and 27° S latitude (from Ecuador to northern Argentina). Up to now, 63 species of *Telmatobius* are recognized (Frost, 2015), 10 of which inhabit Chile (Lobos et al., 2013). These are completely aquatic and highly endemic (Veloso, 2006; Méndez and Correa, 2008; Correa et al., 2011). Most of the life history of these amphibians is unknown, especially for the species of the high Andes (Díaz-Páez et al., 2002); it has been suggested that the genus diverged in the Pleistocene (Sáez et al., 2014).

The Andean salt flats are the result of the drying of large paleolakes in the Pliocene–Pleistocene (Lavenú, 1995; Placzek et al., 2011), resulting in saline deposits in flat watersheds at high elevation. Most of the Altiplano salt flats are fed only by scarce rainfall and subterranean water, which makes them vulnerable to anthropic disturbances (Keller and Soto, 1998; Teillier and Becerra, 2003). Our study focuses on the Ascotán salt flat, located in the administrative commune of Ollagüe, Antofagasta Region, where borax is extracted from the salt flat and water is drained by the mining industry (Contreras, 2002). The frog that inhabits the adjacent springs has been referred to *Telmatobius philippii* Cuevas and Formas, 2002 (Mella and Peñaloza, 2005) and also *T. halli* Noble, 1938 (Nuñez and Gálvez, 2015). A recent molecular study recognized the taxon from Ascotán as part of the *T. hintoni* group, which includes species of the southeast Altiplano of Chile and Bolivia such as *T. philippii, T. fronteriensis* Benavides

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et al., 2002 and *T. huayra* Lavilla and Ergueta-Sandoval, 1995 (Sáez et al., 2014). Although the taxonomy of the species of this geographic region is not yet clear and discussion will continue, it is clear that amphibians of this part of the Chilean Altiplano face multiple threats. Based in the work of Sáez et al (2014) here we refer to the Ascotán frog as *Telmatobius* cf. *philippii,* as it is nested inside a clade whith *T. phillippii*.

In this study we provide information on the biology of a population of *Telmatobius* cf. *philippii* in the Ascotán salt flat with the goal of adding to the knowledge of these frogs that live in extreme environments of the Andes. We emphasize the serious threats that might impact this species due to the large gap between life-history information and conservation needs.

MATERIALS AND METHODS

Study area

The Ascotán salt flat is located in the Chilean Altiplano at an elevation of 3,720 m in the Antofagasta Region, close to the border with Bolivia (Fig. 1). The salt flat has eleven springs on the eastern side that are isolated from one another by an arid matrix of other watercourses. The area has a tropical continental climate (Di Castri and Hajek, 1976) with a high degree of aridity and precipitation concentrated in the austral summer (called the Altiplano Winter). Mean precipitation is 67 mm/year and the potential evaporation is 1,630 mm/year (Schlumberger Water Services, 2009). The salt flat has an area of 243 km², of which water covers 18 km²; the total area of the springs (less brackish water associated with bogs) is only 0.035 km².

We made six expeditions to the salt flat in 2005–2014 (January and March 2005, December 2012, May and November 2013, and June 2014), including visits in the wet season after the rains (March–June) and the dry season before the rainy season (November–January). An additional visit was made in December 2014, to evaluate aspects related to reproductive activity.

Distribution and habitat

Due to the small surface area of the springs, we established a 5 m transect along the littoral zone in each spring

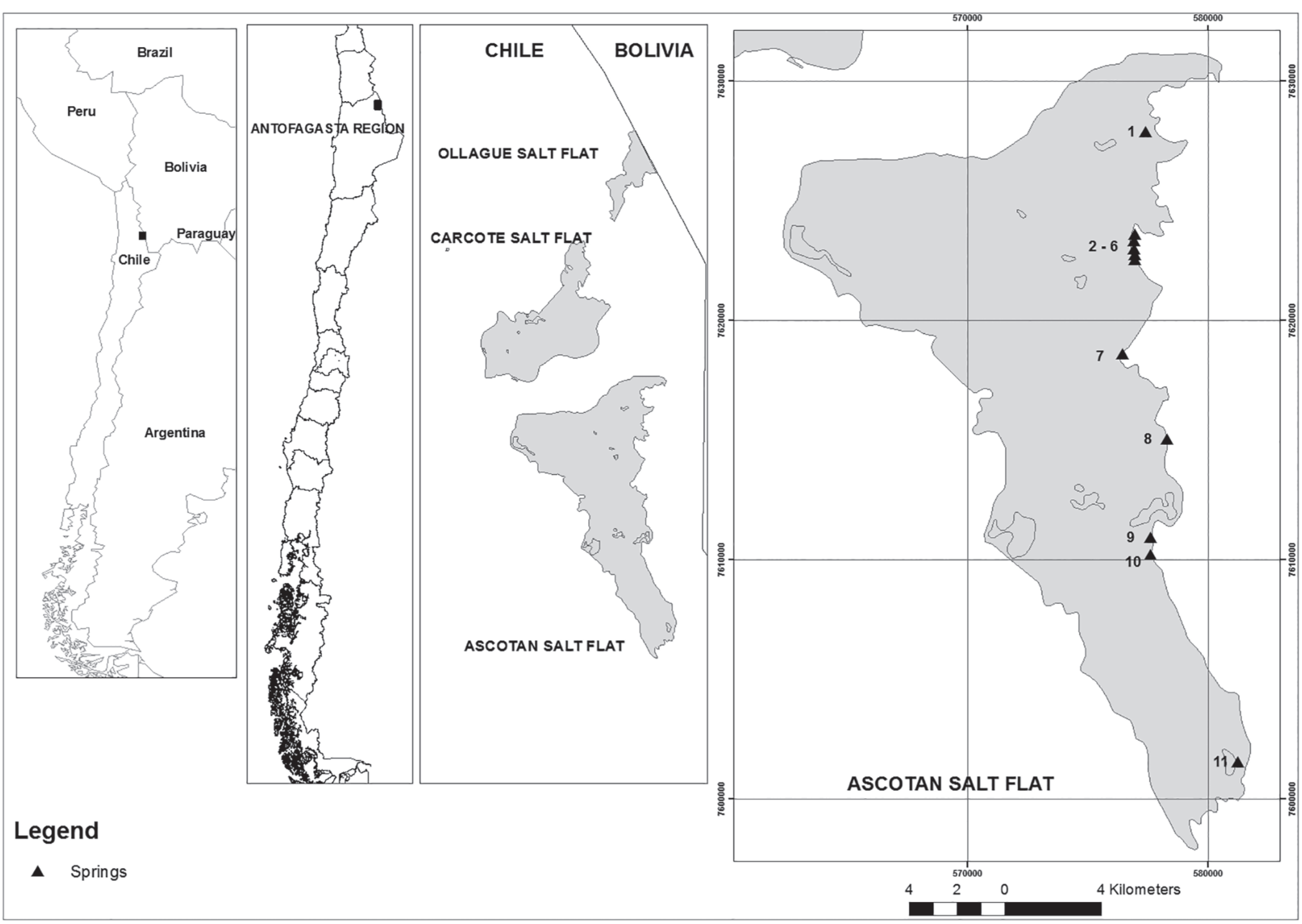

Figure 1. Study area of *Telmatobius* in the Ascotán salt flat, Antofagasta Region, Chile.

where we surveyed amphibians using visual searches and dip nets. The transects were established near the upwelling zones of the springs where there is greater plant cover and potential refuges. In these springs, the vegetation is concentrated around the upwelling, with the rest of the water body appearing more like the physiognomy of the low-depth brackish lagoons, no greater than 5 cm.

Assessing habitat parameters

In May and November 2013, in each transect we evaluated the dissolved oxygen and water temperature (portable dissolved oxygen meter Hanna Instruments, Romania, model HI 9146), pH, electrical conductivity, and total dissolved solids (portable pH/EC/TDS meter model Hanna HI 991301), turbidity (portable turbidity meter model Hanna HI 93703), and air temperature (portable thermometer model Hanna HI 98509-1). Other measurements included water body depth and the length and width of the hollows (refuges) in the banks using a tape measure. The quadrat-point method (Faúndez and Escobar, 2007) was used to estimate the percentage cover of azonal vegetation of each transect, using an extension of 3 m and 20 measurements per line (one measurement every 15 cm). Quadrants of 0.125 m^2 (three replications) were used to evaluate the percentage cover of aquatic vegetation in each transect.

Density and biometry

In all expeditions, we searched an area of 1 m × 5 m (Lobos et al., 2016) at each spring. Additionally, in May and November 2013 we recorded the sex, mass and snout–vent length (SVL) of all captured frogs and total length for tadpoles. We calculated a body condition index (BCI) using the residuals of a linear regression of log-mass and log-SVL (Bancila et al., 2010). Differences between population means were analyzed with *t*-tests. ANOVA was used in other cases, after confirming normal distribution of residuals using the Shapiro-Wilk test. Significant differences ($P < 0.05$) among means were evaluated with the Tukey *a posteriori* test, performed in Infostat (Di Rienzo et al., 2004). To explore which variables were correlated with the BCI, we constructed a generalized linear model (normal distribution). The BCI data for the individuals was utilized as a response variable of the sample season, sex and springs. To find the most parsimonious model, we used a stepwise backward selection approach and applied Akaike's Information Criterion (Harrel, 2001). To develop the model in R (R Core Team, 2016), we used the Rcmdr package (Fox, 2005). Individuals were handled using a biosecurity protocol for emergent diseases (Lobos et al., 2011).

Diet

Sampling was performed in May and November 2013 in Springs 2, 6, and 7. Each captured frog was placed in a separate plastic bag with enough water to avoid dehydration. Stomach contents were obtained using the stomach flushing technique (Solé et al., 2005); the collected contents were stored in Eppendorf tubes with 50% ethanol for later analysis. Frogs were immediately returned to the water. Prey availability was estimated in each of the capture sites by sampling macroinvertebrates using a Surber net, sampling an area of 0.09 m^2 with three replications (Ramírez, 2010).

Prey items were identified under a stereomicroscope using the taxonomic classification of Fernández and Domínguez (2001). The length and width (in mm) of each prey individual were measured to estimate the volume using the equation for a sphere (Barreto-Lima, 2009), thus obtaining the percentage contribution of each item to the total prey volume. Other calculations included the frequency of occurrence (%FO; proportion of frogs that had the food item) and the numerical percentage (proportion of the species of the total prey items). This information was used to evaluate an index of relative prey importance (IRI; Pinkas et al., 1971). To determine whether these frogs are selective or generalist predators, we used a modified Chi-squared test (Jaksic, 1979) in which the lack of statistical significance ($P > 0.05$) implies a strong relationship between the environmental supply and prey consumption (generalist) and significance implies that they are selective towards some food items.

Reproductive activity

In November 2013 and June and December 2014, we captured larvae in the same sites where adults were sampled. We recorded the Gosner (1960) stage, mass, and total length of larvae in the field before returning them to the springs. Also, in December 2014 we installed an automated recording device (SongMeter SM2+, WildlifeAcoustics, Inc.) at Spring 7, programmed to record for a 10-minute interval each hour from 18:00–15:00 h. This device also recorded the environmental temperature during the sound recordings. The acoustic activity was expressed as the percentage of time occupied by the records obtained in each recording period.

RESULTS

Distribution and habitat

Our focal species of *Telmatobius* cf. *philippii* was the only species of amphibian encountered and was only

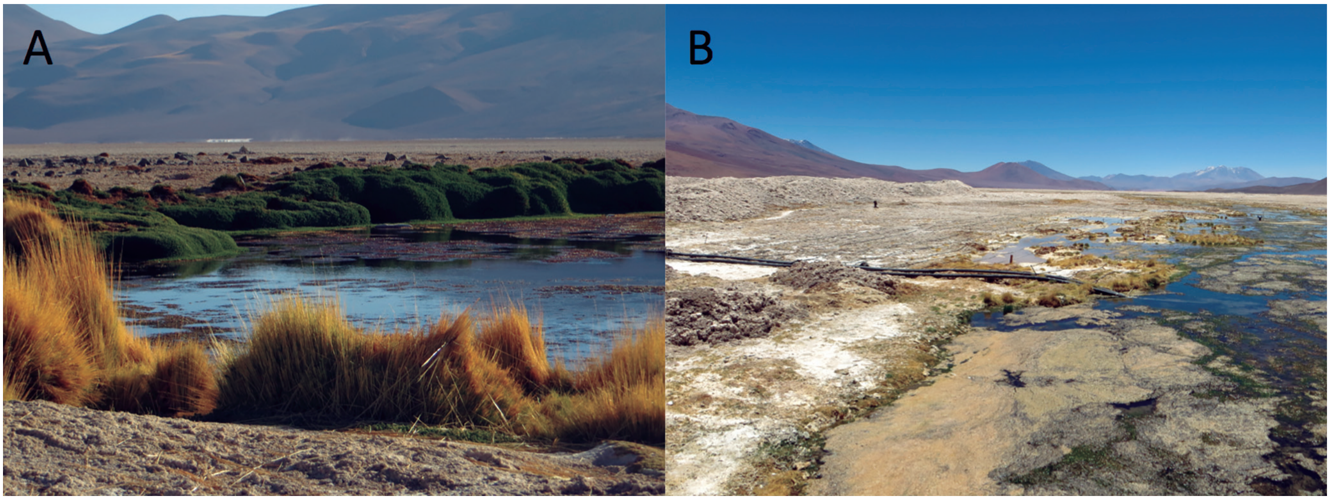

Figure 2. Habitat of *Telmatobius* in the Ascotán salt flat. **(A)** Spring 7 and **(B)** Spring 11.

found in Springs 2, 3, 5, 6, 7, and 11 (Fig. 1). The physiognomy of the springs was that of small, clear water lagoons surrounded by a bog with macrophytes. Away from the spring the vegetation gave way to salt crusts, macrophytes disappeared and the water body transitioned into a shallow, brackish lagoon (Fig. 2). Spring 1 differs from other springs in that it was a brackish lagoon. Frogs were captured in hollows on the banks of the springs, both underwater and within aquatic vegetation, and few individuals were observed outside these refuges during the day. Adults, larvae, and eggs were collected from the hollows.

Habitat parameters

Spring water had medium to high temperatures compared to the air; water temperature was 10.83–23.5°C in the wet season and 11.75–29.2°C in the dry season, while air temperature ranged from 10.20–17.20°C in the wet season and 7.90–23.00°C in the dry season (Table 1). Dissolved oxygen was variable (5.05–11.5 mg/L in the wet season and 5.83–10.73 mg/L in the dry season), pH was neutral to alkaline (7.33–8.60 wet season; 6.38–8.44 dry season), conductivity was high (2.80–9.04 mS/cm wet season; 3.01– 10.91 mS/cm dry season), total dissolved solids was also high (1.40–4.52 g/L wet season; 1.02–6.58 g/L dry season) and fluctuating water turbidity ranged from 0.73–9.79 NTU in the wet season and 0.64–16.41 NTU during dry season).

The terrestrial plant cover was highly variable among springs (41.33–85.00% in the wet season, 33.00–90.00% in the dry season). The aquatic plant cover varied from 19.58–76.39% in the wet season and 36.33%–83.33% in the dry season. The depth to the upwelling in the refuge zone was 10.00–56.67 cm in both samples and refuge width was 3.50–40.00 cm in the wet season and 3.33–24.44 cm in the dry season. Refuge length was 8.25–32.87 cm in the wet season and 6.67–36.11 cm in the dry season.

Comparison among springs with and without *Telmatobius* (excluding the highly disturbed Spring 11) indicated that suitable habitat for the species is restricted to sites with 42–90% terrestrial plant cover, 36–83% aquatic plant cover, 28–57 cm water column depth (sufficient for refuges to remain submerged), refuge opening 12.5–40 cm width and 10–33 cm length, water with high oxygenation (7.2–12.2 mg/L), alkaline pH (8.0–8.7), and electrical conductivity around 3.00 mS/cm.

Density and body size

The densities recorded in each spring are reported in Fig. 3. ANOVA found significant differences among springs ($F_{5,5} = 5.6$; $P = 0.0002$) and sampling seasons ($F_{5,35} = 7.75$; $P = 0.018$). The Tukey test indicated a low-density group (Springs 3, 5, and 11, with 0–2.8 individuals/m^2). Spring 7 showed the highest frog density (1.8–9

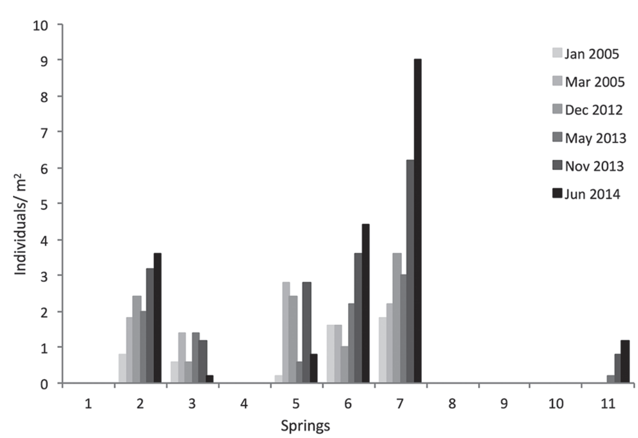

Figure 3. Amphibian density by spring and collection date.

Table 1. Microhabitat variables recorded in the springs (numbered 1–11) of the Ascotán salt flat (May/November 2013).

Variable	1	2	3	4	5	6	7	8	9	10	11
Water Temperature (°C)	12.23/11.75	14.90/19.87	13.03/17.8	10.83/18.90	13.57/25.50	11.80/15.63	16.10/19.20	17.13/17.40	17.70/20.93	19.57/22.67	23.50/29.20
Air Temperature (°C)	10.27/16.05	14.53/16.30	11.47/7.90	12.37/13.57	11.57/17.90	10.33/14.77	10.20/23.00	17.20/9.70	13.70/13.93	15.00/17.53	16.10/22.10
Dissolved Oxygen (mg/L)	6.21/5.83	7.24/8.78	10.61/7.63	7.81/7.62	11.55/7.51	7.69/8.10	12.19/6.41	9.55/6.64	5.05/8.19	7.68/10.37	12.50/10.73
pH	7.65/6.38	8.72/8.33	8.60/8.07	8.53/8.15	8.38/8.44	8.23/8.30	8.18/7.97	8.38/8.32	7.82/8.15	7.33/8.30	7.85/8.32
Conductivity (mS/cm)	9.04/10.91	3.39/3.61	2.89/3.23	2.90/3.07	2.90/3.01	2.98/3.40	2.88/3.29	2.80/3.19	4.50/4.74	4.52/5.77	3.63/2.06
Total Dissolved Solids (g/L)	4.52/6.58	1.72/1.72	1.77/1.63	1.47/1.53	1.46/1.55	1.50/1.71	1.44/1.65	1.40/1.59	2.25/2.37	2.26/2.89	1.81/1.02
Turbidity (NTU)	0.86/0.64	3.14/2.14	9.79/1.11	3.01/4.97	2.58/1.43	2.74/16.41	0.73/4.54	7.65/2.45	2.77/2.88	6.40/5.83	3.16/0.66
Terrestrial Plant Cover (%)	43.33/33.00	85.00/90.00	81.67/70.00	68.33/80.00	41.67/76.67	73.33/70.00	65.00/56.67	66.67/66.67	78.33/56.67	70.00/58.33	62.50/62.50
Aquatic Plant Cover (%)	29.61/36.33	50.61/80.00	51.67/38.33	54.39/40.00	36.06/60.00	51.67/56.67	45.83/83.33	70.55/63.33	37.22/46.67	76.39/56.67	19.58/42.92
Depth (cm)	10.00/10.00	33.33/33.33	33.33/33.33	40.00 ± 40.00	53.33/53.33	27.67/26.67	56.67/56.67	45.00/43.33	35.00/25.00	52.50/33.33	27.50/32.50
Mean refuge width (cm)	5.00/5.67	25.83/24.17	14.00/12.50	9.44/15.17	13.50/21.44	16.67/19.50	40.00/24.44	16.94/7.52	27.80/12.11	20.50/4.56	3.50/3.33
Mean refuge length (cm)	23.28/6.67	16.83/27.33	19.94/10.17	12.78/16.17	19.00/19.51	26.67/23.58	31.94/33.06	24.78/15.79	32.87/36.11	22.47/10.93	8.25/6.83

individuals/m^2), followed by Springs 2 and 6, with intermediate densities (0.8–4.4 individuals/m^2). The *a posteriori* test for sampling seasons showed only significant differences between samples from January 2005 and June 2014.

According to all field observations in water bodies, our focal frogs are associated exclusively with springs. Extrapolating the mean densities from springs to the entire optimal habit area (in the most positive scenario) yielded estimations of 18,425 frogs for Spring 2, 2,306 for Spring 3, 12,725 for Spring 5, 5,490 for Spring 6, 32,542 for Spring 7, and 18 for Spring 11, giving a total mean estimate of 69,506 individuals for the entire salt flat. These values indicate that Spring 7 concentrates nearly half (47%) of all frogs present in the system.

Table 2 summarizes the biometric measurements of the *Telmatobius* cf. *philippii* specimens recorded in the two expeditions of 2013. No significant differences were found between springs or sampling dates for the body size or mass (*t*-tests, $P > 0.05$), although females were larger than males (the largest female was 59 g and 85 mm SVL, whereas an adult male was 25 g and 58 mm). Body condition differed only between springs. The BCI (Table 3) showed that the lowest values were recorded in Spring 11, while the highest values were found in Springs 3 and 6.

Diet

The index of relative importance of prey (IRI) consumed in Springs 2 and 7 was highest for Hydrobiidae Troschel, 1857, whereas in both samples from Spring 6 it was highest for Hyalellidae Bulycheva, 1957, followed by Hydrobiidae. There were differences between springs in environmental prey availability. There was low prey consumption in Springs 6 and 7 in the dry season, perhaps influenced by reproductive activity. The Chi-square analysis revealed significant differences, suggesting that food consumption is not related to the abundance of the available prey (except in the dry season, with frogs from Spring 6 showing generalist feeding preferences). The consumed prey items are detailed in Table 4.

Reproductive activity

The Gosner stages in Spring 7 in November were mainly advanced stages, with low representation of early stages. The proportion of early stages was greater in December, with fewer later stages. In winter (June) there were mostly individuals of Gosner stages 30 or greater (Fig. 4). The larvae were large, especially in stages 37–39 (Fig. 5), being larger than post-metamorphic individuals. The nocturnal breeding activity was indicated by the greatest frequency of calls from 02:00–05:00 h, when the

Table 2. Sizes recorded (mean ± SD) as snout–vent length (SVL; mm), mass (g), and Body Condition Index (BCI) by sampling date, spring, and sex. F: female, M: male, *n:* number of individuals, *t:* Student's *t* test statistic, *P:* probability under the null hypothesis.

Sample	Spring	Sex	*n*	SVL	*t*	*P*	Mass	*t*	*P*	BCI	*t*	*P*
May	2	F	3	57.33 ± 3.79	2.74	0.11	23.67 ± 2.31	1.56	0.16	0.05 ± 0.02	-0.18	0.863
		M	7	51.29 ± 0.76			19.43 ± 4.35			0.06 ± 0.09		
	3	F	6	52.17 ± 7.03	0.63	0.54	20.50 ± 5.01	0.92	0.38	0.08 ± 0.03	1.01	0.343
		M	4	49.50 ± 5.69			17.75 ± 3.86			0.06 ± 0.01		
	5	F	0									
		M	3	50.33 ± 1.53			14.00 ± 2.00			-0.05 ± 0.04		
	6	F	5	55.00 ± 8.28	1.47	0.18	19.20 ± 5.89	1.6	0.15	-0.01 ± 0.04	0.45	0.66
		M	5	49.20 ± 3.03			14.60 ± 2.61			-0.02 ± 0.03		
	7	F	11	47.27 ± 4.45	-1.02	0.32	12.45 ± 2.34	0.15	0.88	-0.05 ± 0.07	1.59	0.135
		M	4	49.75 ± 3.10			12.25 ± 2.06			-0.10 ± 0.04		
	11	F	0									
		M	0									
	Totals	F	25	51.20 ± 6.79	0.72	0.477	17.08 ± 5.73	0.65	0.517	0.004 ± 0.071	0.08	0.933
		M	23	50.13 ± 2.91			16.13 ± 4.15			0.002 ± 0.085		
November	2	F	7	58.29 ± 10.39	1.67	0.14	20.86 ± 9.53	1.8	0.12	-0.04 ± 0.09	0.53	0.609
		M	6	51.50 ± 2.66			14.33 ± 1.03			-0.06 ± 0.04		
	3	F	1	55.50 ± 13.44			18.50 ± 9.19			-0.04 ± 0.00		
		M	2	55.00 ± 0.00			21.00 ± 0.00			0.04 ± 0.00		
	5	F	27	49.48 ± 8.93	0.89	0.38	16.15 ± 9.61	1.44	0.16	0.00 ± 0.07	1.17	0.24
		M	21	47.81 ± 3.59			13.38 ± 2.46			-0.03 ± 0.08		
	6	F	6	56.33 ± 9.31	1.87	0.12	25.00 ± 10.55	1.65	0.16	0.07 ± 0.07	-0.01	0.994
		M	11	49.09 ± 2.30			17.82 ± 2.09			0.07 ± 0.06		
	7	F	22	49.55 ± 3.84	-1.31	0.2	16.00 ± 2.02	0.29	0.78	0.02 ± 0.05	1.45	0.159
		M	9	50.78 ± 1.39			15.78 ± 1.79			-0.01 ± 0.06		
	11	F	1	49.25			13.75					
		M	0									
	Totals	F	64	51.18 ± 8.29	1.78	0.078	17.29 ± 8.12	2.17	0.032	0.003 ± 0.072	0.79	0.4318
		M	48	49.27 ± 3.23			15.13 ± 2.85			-0.004 ± 0.082		

Table 3. Best generalized linear model for the evaluation of the BCI in the Ascotán Sat flat frog. *B:* coefficient of multiple regression, *t:* Student's *t*-test statistic, *P:* probability under the null hypothesis, AIC: Akaike's information criterion.

Variable	*B*	*t*	*P*	AIC
(Intercept)	-0.005894	-0.321	0.7485	-388.08
Season[T.dry]	0.019731	1.428	0.1554	
Sex[T.male]	-0.013059	-1.106	0.2703	
Spring[T.03]	0.059512	2.334	0.0209	
Spring[T.05]	-0.021997	-1.169	0.2441	
Spring[T.06]	0.044196	2.163	0.0321	
Spring[T.7]	-0.015329	-0.819	0.4140	
Spring[T.11]	-0.080331	-2.012	0.0460	

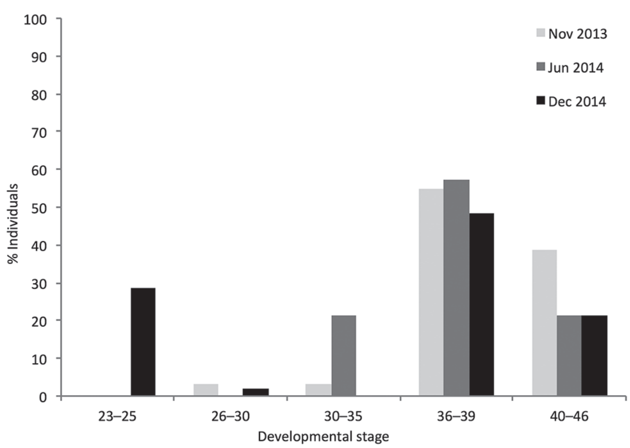

Figure 4. Gosner (1960) developmental stages for collections in November 2013, June 2014 and December 2014.

Table 4. Percentages of frequency of occurrence (%FO) and Index of Relative Importance (IRI) of the prey found in samples of stomach contents of *Telmatobius* cf. *philippii* in Springs 2, 6, and 7.

Order	Family	Wet season						Dry season					
		Spring 2		Spring 6		Spring 7		Spring 2		Spring 6		Spring 7	
		%FO	IRI	%FO	IRI	%FO	IRI	%FO	IRI	%FO	IRI	%FO	IRI
Amphipoda	Hyalellidae	20.00	130.72	60.00	6550.81	20.00	86.16	80.00	3884.38	40.00	3433.80	20.00	555.82
Bivalvia	Sphaeriidae	0.00	0.00	10.00	94.43	20.00	66.24	0.00	0.00	0.00	0.00	0.00	0.00
Coleoptera	Elmidae	10.00	7.49	40.00	490.58	40.00	337.70	60.00	259.50	20.00	402.72	10.00	99.20
Diptera	Chironomidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.00	135.87	10.00	65.05
	Muscidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	164.44
	Simuliidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	37.08	10.00	138.95
	Syrphidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	362.87	0.00	0.00
Gastropoda	Hydrobiidae	60.00	10885.55	30.00	1017.97	80.00	12069.47	90.00	12356.87	20.00	739.52	10.00	984.76
	Physidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	102.54	10.00	90.53
	Planorbidae	30.00	251.46	30.00	1055.42	60.00	1984.14	40.00	392.87	0.00	0.00	20.00	358.31
Hemiptera	Corixidae	20.00	23.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Macroveliidae	10.00	5.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tricoptera	Hydroptilidae	20.00	22.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

air temperature of the salt flat was low (0–4°C, Fig. 6), with only occasional calls during the day.

DISCUSSION

Distribution and habitat

All the springs we sampled showed evidence of past and recent mining activity, including the construction of canals to measure flow, vehicle tracks and the presence of infrastructure inside and outside the springs. Spring 11 was most critical, drying completely in 2005 due to water extraction. Currently, there is an artificial water supply that is provided by pipelines from wells in mountainous areas near the salt flat. As part of a restoration plan for this bog, a system of drip irrigation (similar to an agricultural field) was established, which involved the destruction of the riverbank zones where the hollows that the amphibians use are located.

Among the springs without frogs, only Spring 1, with high conductivity values and scarce plant cover, does not have adequate habitat conditions. The others (Springs 4, 8, 9, and 10) all have similar physiognomy and

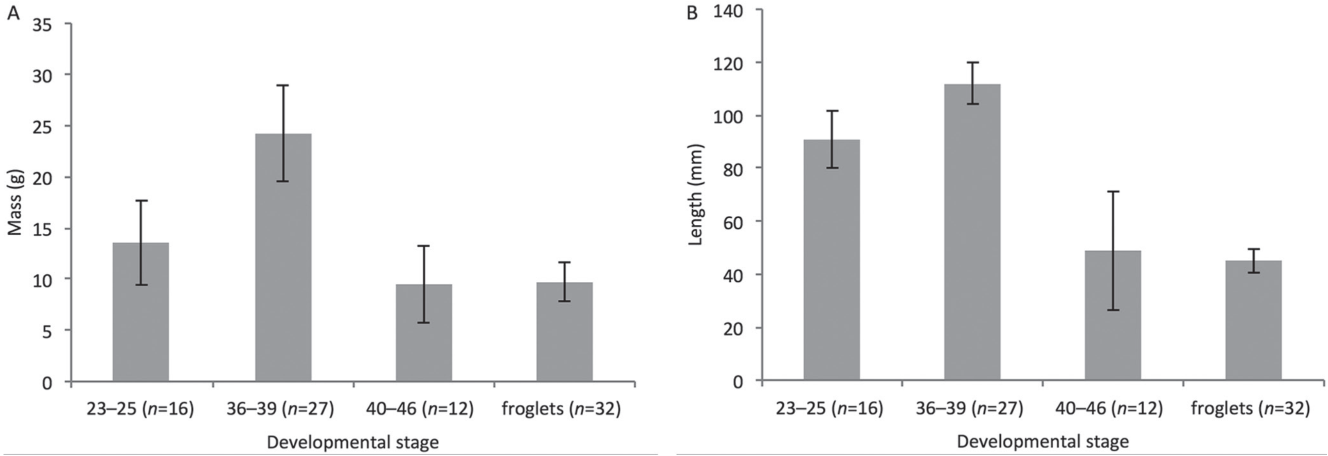

Figure 5. (A) Mass and **(B)** Total length of larvae in different Gosner (1960) stages and SVL for froglets of Spring 7 (number of samples in parentheses) for collections in December 2014.

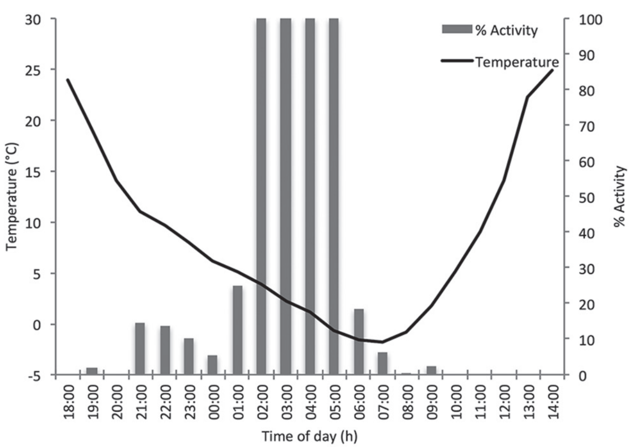

Figure 6. Records of times of calling activity and air temperature (°C) in Spring 7.

are located between springs occupied by the species. Technical reports (CEA, 2010) indicated the presence of frogs in Springs 9 and 10 in the 2006 and 2010, respectively. Environmental incidents with runoff of mineral waste were documented for these springs, which is a likely cause of *Telmatobius* extinction in these springs.

Habitat parameters

The habitat of these frogs is limited due to the small area that the springs occupy within the salt flat and the lack of connectivity between springs (physical barriers due to the salinity). Water electrical conductivity is often an important predictor of frog occupancy; our results showed a similar pattern to that described for the fish *Orestias ascotanensis* Parenti, 1984, which live in these same springs (Keller and Soto, 1998). The electrical conductivity values explain why the frogs are only found near the upwelling of the springs of the salt flat, which restricts them to 1.44% of the surface area of the salt flat. According to local inhabitants there is only one connection among springs (at least visually) in events of catastrophic precipitation.

Density and body size

Frog density varied between seasons. Only Springs 3, 5, and 11 maintained low density, the last spring due to anthropic disturbance. The species has a restricted distribution in all the springs where it was present, which may reach up to 9 animals/m² (Spring 7), this pattern was reported in another congener with restricted habitat, *Telmatobius dankoi* Formas et al., 1999 (Lobos et al., 2016), in which the densities are overestimated due to the confinement of the frogs. There was a tendency towards greater population sizes in Springs 2, 6, 7 and 11 (Fig. 3), which also appeared to be less disturbed. Over the last couple of decades, water has been extracted from wells drilled near the salt flat; however, the effect of drilling on the ecosystem is unknown. Another sensitive aspect is the concentration of half of the total estimated frog population in one spring (Spring 7), which makes it a high priority for environmental conservation. No sexual dimorphism was observed; however, this might be the expression of a population strongly supported by young individuals, since older (large) individuals were scarce and there was a tendency for females to be larger.

Diet

The diet is composed mainly of aquatic invertebrates of the families Hyalellidae and Hydrobiidae. No differences were found between seasons of the year, but there were differences in consumption among springs, explained by the differential availability of prey and the geographic isolation of the springs. The feeding preference in Springs 2 and 7 was towards Hydrobiidae, although the most abundant prey was Elmidae Curtis, 1830, while in Spring 6 the preferred prey was Hyalellidae, with higher consumption than environmental availability. Consumption was not proportional to the availability of prey, but in the dry season frogs from Spring 6 were the only ones showing generalist feeding preferences. This species of *Telmatobius* appears to be a selective predator, which was also reported for a congener from northern Chile (Lobos et al., 2016). Although the species inhabits an extreme environment, it does not have a highly generalist diet. Species showing specialized diets often dedicate much time to searching or waiting for the best prey in terms of net energy (Bozinovic and Medel, 1988; Litvaitis, 2000). Many species of anurans feed on a large variety of live organisms without appearing to discriminate among prey species (Díaz-Páez and Ortiz, 2003). However, it has been reported that attributes of size, movement, palatability, and nutritious value of prey can affect predator selection (Anderson and Mathis 1999; Anderson et al., 1999). Diet analysis indicated that *Orestias ascotanensis,* which lives with the frogs in the refuges, is not consumed by them. The association of *Telmatobius* and species of the genus *Orestias* Valenciennes, 1846 has also been reported for other salt flats and high-altitude creeks. Body condition fluctuated among seasons for the springs, reflecting the high environmental trophic variability of the springs.

Reproductive activity

The presence of larvae in advanced stages of development in winter, along with the overlapping of early and late development stages in other seasons of the year, might indicate that larvae require more than 1 year to reach metamorphosis. This idea is reinforced as well by the large size attained by the tadpoles, as has been reported for other high Andean amphibians (Corbalán et al., 2014). Calling activity was greatest at the time that the environmental temperature in the salt flat was low, from 02:00–05:00 h. One possible explanation is that the springs are thermal, which favors the maintenance of an optimum and stable temperature of the springs. In the majority of the springs there was a great difference between the air and water temperatures (e.g., at Spring 3 the air temperature was 7.9°C in November, whereas the water temperature was 17.8°C). It should be noted that winter air temperature in the salt flat reaches -20°C (Keller and Soto, 1998).

Temporal gap between threats and knowledge

The temporal gap between knowledge of the species (taxonomy, biology, ecology) and anthropic threats is a relevant risk factor in the conservation of the amphibians of the Chilean Altiplano, where accelerated industrial growth threatens various species. Salts and water for mining have been extracted from the Ascotán salt flat since at least 1883 (Chong et al., 2000). These amphibians were first collected in 1996, and all specimens were deposited in the Museo Nacional de Historia Natural de Chile and assigned to *T. halli* (Nuñez and Gálvez, 2015). The same population was later referred to *T. philippii* (Mella and Peñaloza, 2005), and most recently it has been recognized as part of the *T. hintoni* group (Sáez et al., 2014). The taxonomic confusion in this area of the Antofagasta Region began with the description of *T. halli,* which was described on the basis of a type series collected near Ollagüe in 1935, 25 km from the Ascotán salt flat (Formas et al., 2003). It must be noted that the Ollagüe salt flat no longer has surface water. There have been no subsequent collections of this species, and recently two new species were described from near the type locality of *T. halli: T. fronteriensis* from the locality of Puquios at a distance of 25 km (Benavides et al., 2002) and *T. philippii* in the Amincha and Quebrada del Inca area only 9 km from the type locality of *T. halli* (Cuevas and Formas, 2002).

As a consequence of this taxonomic confusion, the taxon present in Ascotán (and others such as the Carcote salt flat) have not been incorporated in the Classification Rules for Species of the Ministry of the Environment of Chile (RCE, for its initials in Spanish). Further taxonomic work is needed to include this species in de IUCN red list, which would allow the creation of official management plans for this species (Soto-Azat et al., 2015). Paradoxically, *Telmatobius halli* is listed as Critically Endangered by the RCE, and Data Deficient by IUCN (2012), although it has not been collected since its original description in 1938. The recently described species *T. fronterensis* and *T. philippii* have been listed as Critically Endangered due to their restricted distributions and high anthropic threats (Soto-Azat et al., 2015).

In addition to all the threats mentioned above for the Ascotán salt flat frogs, a large part of the creek in Amincha (*Telmatobius philippii*) has been diverted into pipes for industrial water use. At the head of the creek in Puquios (*T. fronteriensis*) a pond was constructed to accumulate water and the creek flows beside a sulfur mining operation and in the Carcote salt flat (*T.* cf. *philippii*). Trout was also introduced and in one site where a pond has been constructed. Thus, it is prudent to consider that anthropogenic threats generate local pressures (Grant et al., 2016) for all these taxa. There is evidence that frogs from our site at Ascotán historically occupied a larger portion of the springs, and we emphasize that half of its population lives in only one of the springs and that it is a highly specialized species in terms of habitat and diet preferences. Thus, we argue that it is imperative for environmental protection agencies to consider the aforementioned biological information and threat assessment to safeguard this amazing endemic amphibian species.

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