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Prolonged larval development in the Critically Endangered Pehuenche's frog *Alsodes pehuenche*: implications for conservation

Valeria Corbalán[1,2,*], Guillermo Debandi[2], Flavio Martínez[2,3], Carmen Úbeda[4]

Abstract. Overwintering is an anuran strategy to survive in cold-temperate climates. Those aquatic species that withstand harsh conditions and short growing seasons are candidates for having long larval periods. Prolonged larval development, which includes overwintering for more than two years, has been reported for North-American and Euro-Asiatic species, but this strategy has been poorly studied in the Southern Hemisphere. *Alsodes pehuenche* is an endemic frog from the high Andes mountains of Argentina and Chile, recently categorized as Endangered by the Asociación Herpetológica Argentina (AHA) and as Critically Endangered by the International Union for Conservation of Nature (IUCN). We studied egg laying in this species and its larval development by marking tadpoles with elastomers. We found that eggs are laid in clumps at the beginning of summer. The larval cycle includes four winters, although a fifth winter should not be ruled out. This is the first study that demonstrates a long larval development (four winters) in South-American species and has important implications for conservation biology.

Keywords: Central Andes, egg laying, endemism, life cycle, overwintering tadpoles.

Introduction

Several environmental factors influence growth and development rates in anurans (Saha and Grupta, 2011). Among them are temperature (Kaplan, 1980; Saidapur and Hoque, 1995), photoperiod (Saidapur, 1989), rainfall (Lynch and Wilczynski, 2005), food quality (Alvarez and Nicieza, 2002) and hydroperiod (Ryan and Winne, 2001). Typically, anurans in temporary ponds go through complete metamorphosis in several weeks, whereas those inhabiting permanent ponds or streams undergoing longer periods (Wilbur and Collins, 1973; Denver, 1997; Buchholz and Hayes, 2002, 2005; Wells, 2007; Richter-Boix, Tejedo and Rezende, 2011). Tadpoles of many species may even respond to pond drying by accelerating their development (Crump, 1989; Newman, 1992; Loman, 1999; Richter-Boix, Llorente and Montori, 2006; Székely, Tudor and Cogalniceanu, 2010).

Anurans withstanding harsh conditions and short growing seasons, instead, are suitable for having long larval periods (Brown, 1990; Bury and Adams, 1999). Studies on *Ascaphus truei* demonstrated that in a relatively mild climate (coastal sites or nearby) larval development occurs in one year, but in inland or high elevation areas with short growing seasons, larval cycle is completed in 3 or 4 years (Brown, 1990; Bury and Adams, 1999).

Overwintering is a strategy displayed by post-metamorphosed anurans that live under seasonal periods of low temperatures. However, it has been recognised that overwintering frogs are not strictly torpid, since they are capable of reacting to stimuli and moving at temperatures as low as 0.6-4°C (Logares and Úbeda, 2006; Tattersall and Ultsch, 2008). Most species are known to hibernate on land, but aquatic or semiaquatic species can do it underwater (Tattersall and Ultsch, 2008). North American adult ranid frogs usually hibernate underwater, and

1 - IADIZA-CCT Mendoza-CONICET, Av. Ruiz Leal s/n, Parque Gral. San Martín, 5500 Mendoza, Argentina
2 - BIOTA (Asociación para la Conservación de la Diversidad Biológica Argentina)
3 - Dirección de Recursos Naturales Renovables de la Provincia de Mendoza, Parque Gral. San Martín, 5500 Mendoza, Argentina
4 - Departamento de Zoología, Centro Regional Bariloche, Universidad Nacional del Comahue, Quintral 1250, 8400 Bariloche, Argentina
*Corresponding author;
e-mail: corbalan@mendoza-conicet.gob.ar

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several species also have tadpoles that overwinter (Collins and Lewis, 1979).

The presence of overwintering tadpoles is an important feature of the life history of species inhabiting cold-temperate climates, such as *Rana* (= *Lithobates*) *muscosa*, *R. catesbeiana*, *R. clamitans*, and *Alytes obstetricans* in the Northern Hemisphere (Martof, 1956; Bradford, 1983; Thiesmeier, 1992; Hulse, McCoy and Censky, 2001; Tattersall and Ultsch, 2008; Tobler, Garner and Schmidt, 2013). Tattersall and Ultsch (2008) remark that tadpoles of those North-American species are better suited physiologically to overwintering in hypoxic conditions than adults, because of their smaller size and a higher surface area to volume ratio.

In South America, larval overwintering was reported only for austral species such as *Alsodes gargola* (Logares and Úbeda, 2004, 2006), *A. tumultuosus* and *A. montanus* (Díaz and Valencia, 1985), as well as for *Atelognathus nitoi* (Úbeda et al., 1999) and *A. patagonicus* (Cuello and Perotti, 2006; Cuello et al., 2014). In addition, overwintering of tadpoles was suggested for *Chaltenobatrachus grandisonae* (Basso et al., 2011) and *A. pehuenche* (Corbalán, Debandi and Úbeda, 2008) based on the coexistence of tadpoles of different sizes and stages. Long larval periods were reported also for *Batrachyla* spp. (8 or more months, including one winter), *Hylorina sylvatica* (one year; Formas and Pugín, 1978; Úbeda, 1998) and *Calyptocephalella gayi* (over one year; Cei, 1962; Formas, 1979; Vélez, 2013).

Although it was suggested that the larval development of the endemic frog *A. pehuenche* includes more than two winters (Corbalán, Debandi and Úbeda, 2008), no study has been conducted to determine the total length of the larval period. Thus, the aim of this contribution was to evaluate aspects related to the breeding biology of *Alsodes pehuenche* by 1) determining the time of egg laying and 2) demonstrating a long larval development by using marking techniques. We discuss the importance of the results in terms of biological conservation.

Materials and methods

The study was conducted in the Pehuenche Valley in the Central Andes of Argentina (WGS 84, 35.97342°S, 70.38181°W, 2523 m a.s.l.), the type locality of *Alsodes pehuenche* (Cei, 1976) (fig. 1). The weather in the region is attenuated periglacial (Capitanelli, 1999). The nearest available climatic data come from Valle Hermoso (WGS 84, 35.14°S, 70.20°W, 2250 m a.s.l.) (Ortíz Maldonado, 2001) and from studies conducted at the Peteroa Volcano (WGS 84, 35.25°S, 70.58°W, 3489 m a.s.l.; Trombotto Liaudat, Penas and Aloy, 2014). Mean minimum air temperature for the coldest month (August in Valle Hermoso and July in Peteroa) is −6.2°C and −11.3°C, respectively. Mean maximum air temperature for January (the warmest month) is 21.5°C in Valle Hermoso (Ortíz Maldonado, 2001). The area is influenced by westerlies and the South Pacific cyclone, with heavy snowfalls prevailing in winter (Trombotto Liaudat, Penas and Aloy, 2014). The study site is covered with snow about six months in the year (from April-May to September-October).

The estimated distributional range of *Alsodes pehuenche* is 9 km^2, and the species occurs in Argentina and Chile (Corbalán, Debandi and Martínez, 2010) (fig. 1). It is considered Endangered in the current Red List for Argentinean amphibians (Corbalán et al., 2012; Vaira et al., 2012) and Critically Endangered by the IUCN (IUCN SSC Amphibian Specialist Group, 2013). The main threat faced by this species is habitat alterations because the broadening and paving of a minor road that is being turned into an international road that cuts across the five Argentinean streams inhabited by the frog (Corbalán, Debandi and Martínez, 2010). *Alsodes pehuenche* is a medium-size frog [mean ± SD, Snout-Vent Length in adults: 54.51 ± 4.07 mm (n = 154); Weight: 32.76 ± 3.89 g (n = 155)]. Both tadpoles and adults occupy the permanent snowmelt streams and ponds formed on meadows ("vegas") (Corbalán, Debandi and Úbeda, 2008). All streams flow into the wider and deeper Pehuenche's River. Slow snow melting during the warmer season causes water infiltration that supplies water to these little streams (Corbalán, Debandi and Martínez, 2010). Thus, the water of the streams is transparent, with pH ranging from 6.08 to 7.66, very low conductivity (7.3-66.6 μS/cm), and very high dissolved oxygen concentrations (72-108%) (Corbalán, Debandi and Martínez, 2010). In summer, water temperature could be very low in early morning (2-3°C), and we frequently found ice hanging down from the edges of streams. At the midday, water temperatures could reach 19°C.

A preliminary visit to the area was in February 2007 (austral summer), the time at which tadpoles of different sizes were found along the streams (Corbalán, Debandi and Úbeda, 2008). A second visit was carried out in December 2007, when we were able to observe one egg laying. In order to keep track of larval development, in December 2008 we captured with aquarium nets a total of 153 tadpoles. Most of the tadpoles (59.48%) belonged to two relatively confined ponds (fig. 1) where tadpoles were concentrated and were easy to find and capture. The remaining 40.52% of tadpoles were captured at different points along the streams. At each

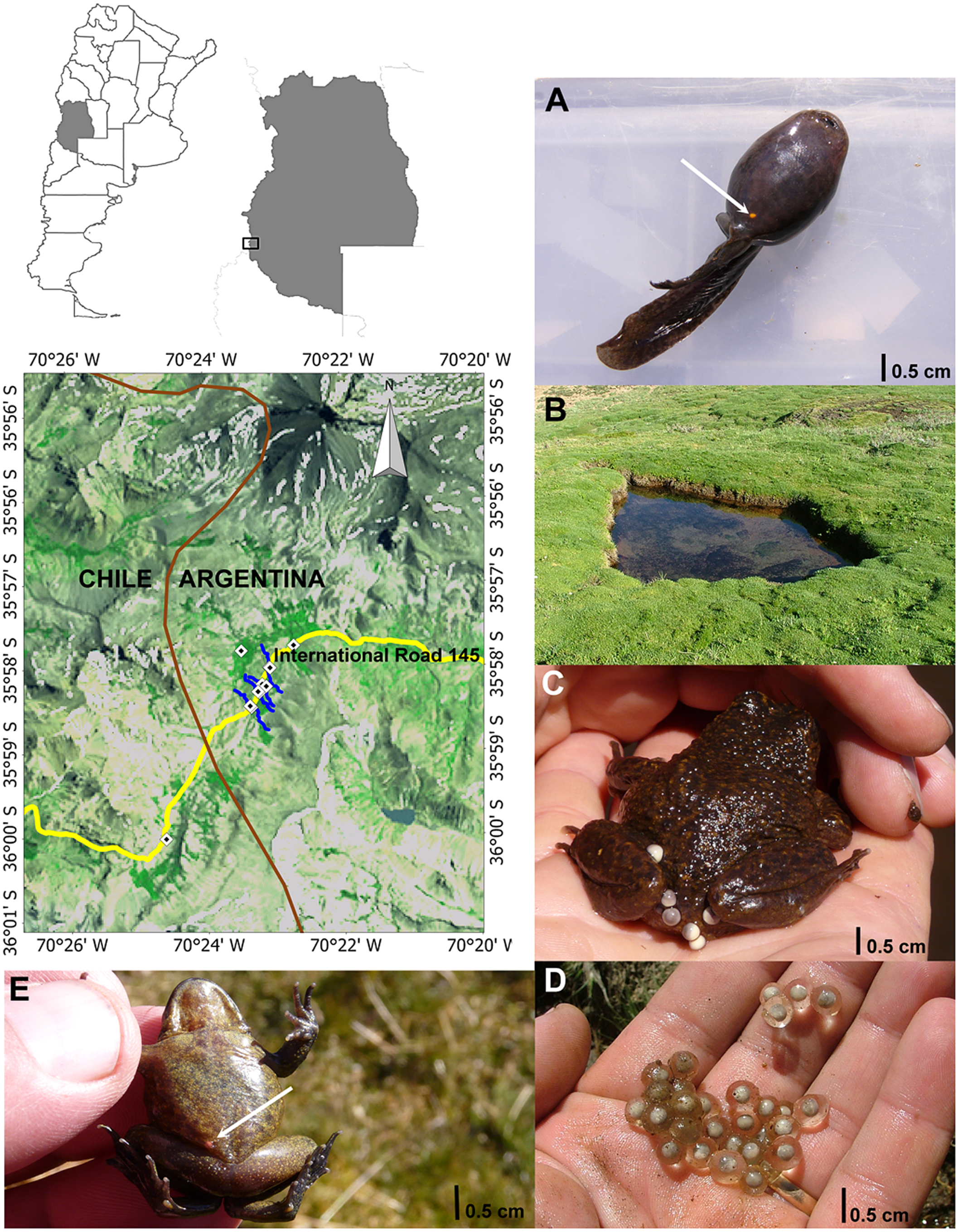

Figure 1. Study area (Paso Pehuenche valley). Points indicate sites where authors confirmed the presence of *Alsodes pehuenche* (see Corbalán, Debandi and Martínez, 2010). (A) Tadpole marked with elastomer (the arrow indicates the mark), (B) pond where tadpoles were marked, (C) female releasing eggs during handling, (D) egg clumps, (E) recaptured individual conserving the mark. This figure is published in colour in the online version.

site, tadpoles were classified into classes, kept in containers with water, marked with Visible Implant Elastomer (VIE) tagging system (Northwest Marine Technology, Inc., 2003), and then released at the capture site. Although sometimes VIE tags might be lost (Davis and Ovaska, 2001; Beausoleil, Mellor and Stafford, 2004), this technique is suitable for animals with transparent skin, like amphibians. Moreover, it is relatively inexpensive and it has been shown to be advantageous over toe-clipping (Davis and Ovaska, 2001). For practical purposes, we separated the different stages of tadpoles into six classes based on their sizes and development of limbs. Class 0: recently hatched larvae with yolk; Class I: tadpoles without yolk, but very small (snout-vent length $\leqslant$ 15 mm); Class II: tadpoles with null or early development of hind limbs and small size (15 to 20 mm); Class III: tadpoles with early development of hind limbs and medium size (21 to 25 mm); Class IV: tadpoles with well-developed hind limbs and larger (>25 mm), and Class V: individuals with forelimbs.

Tadpoles were marked subcutaneous and ventrally (fig. 1) using the 0.3 cc injection syringe of the elastomer kit. Recently hatched larvae (class 0) and very small tadpoles (class I) were not marked to avoid deaths from handling. Classes II to V were marked with four different colours (class II: green; class III: orange; class IV: pink, and class V: yellow). Length of tadpoles for each class and number of marked individuals are given in table 1. All sites with marked specimens were georeferenced so they could be found during the next sampling periods. As marks were made on the ventral side, we assume that they represent no risk of tadpole exposure to aerial predators. Moreover, marks on the belly remain visible throughout the entire life period, and individuals can be monitored even after their metamorphosis has been completed. We returned to the area in December 2009 (12 months later), April 2010 (16 months later), November 2010 (23 months later) and December 2012 (four years later) to determine the progress of larval development. For this purpose, we caught all tadpoles that were seen in the ponds and at the georeferenced points along the streams. So, those already marked were assigned to the current corresponding class. In order to estimate growth, we measured the recaptured larvae (about 10 from each class when possible). After that, all tadpoles were released to their respective sites. During the visit to the area in December 2009, one pond and all streams were covered with snow, and only few tadpoles were recaptured on that occasion.

Table 1. Total of marked tadpoles of *Alsodes pehuenche* in December 2008 and measurements (mean and SD) for each class. SVL: snout-vent length, TL: total length.

	Total marked	SVL (mm)	TL (mm)
Class II	64	17.68 ± 1.72 ($n = 14$)	41.24 ± 3.95 ($n = 14$)
Class III	60	21.82 ± 1.01 ($n = 10$)	48.61 ± 1.91 ($n = 10$)
Class IV	28	26.71 ± 1.51 ($n = 11$)	58.40 ± 3.12 ($n = 11$)
Class V	1	28.05 ($n = 1$)	63.93 ($n = 1$)

To increase the sample size and follow a newly recruited group, in April 2010 we marked 101 new tadpoles of class II in one of the two ponds. In this case, we used yellow to distinguish them from previously marked tadpoles. This pond was covered with 2 cm of ice which was broken to catch larvae. This site was re-visited in November 2010 and December 2012. Unfortunately, during our last visit, this pond was completely dry (and therefore larvae marked in 2008 and 2010 were no recaptured). Moreover, no marked larvae were found this time in the other pond. However, because larvae of different stages were marked with different colours, we could reconstruct the complete cycle based on larval development progress for each stage across consecutive sampling periods.

Results

Egg laying

According to the terminology used by Altig and McDiarmid (2007), eggs are laid in clumps (a three-dimensional arrangement). Eggs are individually surrounded by a protective jelly (fig. 1), and adjacent jellies remain distinct even if melded. Each egg in the clump is clear in colour, like those described for *Alsodes gargola* (Gallardo, 1970). Egg clumps are very difficult to find and were seen only once (in December 2007), adhered to the wall of a long tunnel on the edge of a stream. In addition, in November 2010 a female released her eggs spontaneously during handling, indicating that this is the time when eggs are ready to be laid (fig. 1D). Just hatched tadpoles had remaining yolk in the belly and lighter dorsal pigmentation.

Larval development

Total recapture success for marked larvae in December 2008 was better in ponds (15.38% in December 2009, 15.38% in April 2010 and 12.09% in November 2010). Only two tadpoles of the 62 marked outside the ponds (3.22%) were recaptured in November 2010, whereas no recaptures were made on the other dates. Of those 101 individuals marked with yellow elastomer in the pond in April 2010, we recaptured 12 individuals in November 2010 (11.88%).

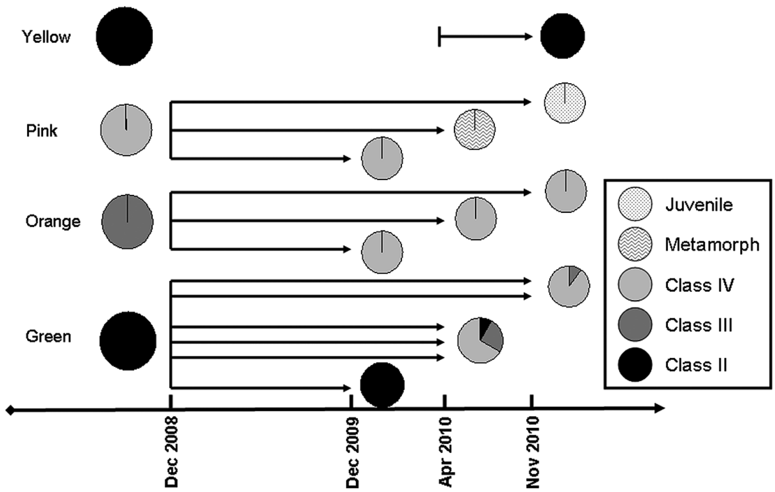

Figure 2. Development progress of marked larvae of *Alsodes pehuenche* throughout the two years sampling period.

Based on all recaptures, we could infer the time that class II tadpoles expend in becoming metamorphs. Most class II larvae marked in December 2008 (austral summer) were found in class IV after 24 months (two winters; fig. 2). Those marked in April 2010 (autumn) were found in the same class in November 2010 (spring), indicating that no progress in larval development occurs during the overwinter period. However, growth in size may occur over the winter, since tadpoles marked in April 2010 were longer in November 2010 (mean ± SD = 25.67 ± 2.42, $n = 13$; mean ± SD = 28.07 ± 2.10, $n = 10$ for April and November respectively; t-test: $t_{21} = -2.49$, $P < 0.05$). Tadpoles marked as class III in December 2008 were found in class IV 12 months later, but class IV tadpoles remained in this class for at least one year (fig. 2). The only individual marked as class V was not recaptured, but class IV tadpoles marked in December 2008 were found completely metamorphosed in April 2010. Taking into account that eggs were seen in December (early summer), and that at the beginning of January only a few class 0 and I larvae were recorded, we can infer that most of the hatching occurs at least in mid January. Probably, these larvae overwinter as class I, such as occurs in *A. gargola* (Úbeda, unpub. data), although we could not confirm it. During the next summer, class I tadpoles may develop into class II, a stage at which they overwinter again (second winter as tadpoles). They develop into class III during the next summer, and overwinter in this class (third winter as tadpoles). They develop into class IV during the summer to overwinter in this class (fourth winter as tadpoles). However, it is possible that some class IV tadpoles remain in this class for one more year (fifth winter as tadpoles). Development from class IV into class V and into metamorphs occurs during the next favourable season. So, based on our results, we can assert that the larval development of *A. pehuenche* includes four winters, but a fifth winter should not be ruled out. Just metamorphosed individuals could overwinter at this stage, since juveniles can be found at the beginning of the next summer.

Discussion

Several factors influence the growth and metamorphosis of amphibians. For example, in newts, experimental studies have demonstrated that permanent water, low density and high food level all favour a paedomorphic ontogenetic pathway, whereas drying, high density and fasting induce metamorphosis (Denoël and Poncin, 2001; Denoël, 2003). Anurans that inhabit high elevation areas and are forced to withstand harsh conditions and short growing seasons are candidates for having long larval periods, such as occurs in *Ascaphus truei* in North America (Brown, 1990; Bury and Adams, 1999). Moreover, these authors demonstrated that this species shows some degree of plasticity which allows it to adapt to environmental conditions, completing the larval cycle in one or four years, depending on the climate of the region they inhabit. In the case of micro-endemic species, such as *A. pehuenche*, it is not possible to know whether the species could express different development times under different natural conditions. However, some tadpoles of the congeneric *A. gargola* were kept in the laboratory under warmer conditions than in nature (10-20°C), and larval development remained incomplete after five years (Úbeda, unpub. data).

Overwintering tadpoles were reported for several species from the Northern and Southern Hemispheres, including the genera *Rana* (= *Lithobates*), *Ascaphus*, *Alytes*, *Alsodes*, *Atelognathus*, *Batrachyla*, *Hylorina*, *Calyptocephalella*, *Chaltenobatrachus* and *Polypedates* (Martof, 1956; Bradford, 1983; Díaz and Valencia, 1985; Thiesmeier, 1992; Úbeda, 1998; Úbeda et al., 1999; Hulse, McCoy and Censky, 2001; Logares and Úbeda, 2004, 2006; Cuello and Perotti, 2006; Tattersall and Ultsch, 2008; Navas et al., 2010; Basso et al., 2011; Hsu, Kam and Fellers, 2012). Instead, a long larval development that includes more than one winter was demonstrated only for *Ascaphus montanus* and *A. truei* (Brown, 1990; Bury and Adams, 1999) in North America. In the Southern Hemisphere this strategy was suggested for *Alsodes montanus* and *A. tumultuosus* (Díaz and Valencia, 1985) and observed for *A. gargola* (Casanovas and Úbeda, unpub. data) and for *Calyptocephalella gayi* (Vélez, 2013). This study is the first demonstrating a long larval development period lasting more than two years using marking techniques. The VIE tagging system was useful for following the larval development of *A. pehuenche*. However, the low recapture rate could be attributed, at least in part, to loss of marks (Davis and Ovaska, 2001). As no injection hole or scarring is evident in animals that lost their marks (Davis and Ovaska, 2001), we cannot determine whether the low recapture rates are due to mortality, migration or mark loss.

Assuming that tadpoles overwinter the first winter as class I, the results of this study indicate that the larval period of *A. pehuenche* involves at least four winters as tadpoles. These results are concurrent with preliminary studies on *Alsodes gargola* (Úbeda et al., unpub. data). Because the growing season 2009-2010 was very short (in December 2009, the austral summer, water bodies were still covered with snow, and in April 2010 the pond was covered by ice), probably some individuals took longer to develop (five winters). This was supported by the fact that in two years (from December 2008 to November 2010) some tadpoles showed little progress in their development (class II to class III; class III to class IV). However, the low recapture success did not allow us to confirm it. We noted that not all tadpoles showed similar larval development progress. For example, not all individuals marked as class II were found as class III when they were recaptured one year later (i.e. they remained at the same class II). This may be because each class includes different stages of development (i.e. different degree of hind limb development *sensu* Gosner, 1960), and transition from one class to another depends on the originally marked stage. The low probability to find class V tadpoles may indicate that this stage is brief, and once forelimbs emerge (Gosner's stage 42), tadpoles suddenly develop into metamorphs.

During the summer months tadpoles grow, and during the winter months they overwinter submerged in ice or snow-covered water bodies. It is most likely that they feed on periphyton (as was reported for *A. gargola*; Baffico and Úbeda, 2006) during this period, since growth in size was observed for tadpoles marked in April and recaptured in November 2010. Water temperature under ice layer in April 2010 was −0.5°C. Logares and Úbeda (2006) in simulated overwintering experiments (at 1-3°C) reported that frogs and tadpoles of *A. gargola* were always active, fed continuously and presented at least some degree of movement. Possibly the same occurs in *A. pehuenche*, since we observed the tadpoles moving beneath ice layer.

Long-term immersion of tadpoles and adults in very low conductivity water (7.3-66.6 μS/cm) under overwintering conditions is very puzzling, since amphibians must normally cope with constant osmotic influx into the body because of their permeable skin (Boutilier, Stiffler and Toews, 1992; Logares and Úbeda, 2006). Moreover, once surface ice forms and persists, frogs must endure extended periods of apnea, becoming dependent upon the surrounding water for exchanges of respiratory gases, ions, and water (Tattersall and Ultsch, 2008). Studies of *Rana muscosa* have demonstrated that tadpoles are better adapted to severe hypoxia than adults, thus serving as a population reservoir (allowing a population to persist without the need to immigrate after a winterkill) (Tattersall and Ultsch, 2008). Logares and Úbeda (2006) suggested that *Alsodes gargola* could be among the anurans with the highest capacity to overwinter submerged in very low conductivity waters. The adaptive strategy of *A. gargola* to tolerate long-term immersions is related with low values of osmolarity (220 ± 30 mOsm/kg, $n = 5$) of internal fluids and low skin permeability (7.6 ± 2.7 mg H_2O h^{-1} $(cm^2)^{-1}$, $n = 3$) in their adults (Logares, 2003). Curiously, tadpoles of *A. gargola* are less tolerant of freezing water than other Patagonian species with no overwintering strategy (Navas et al., 2010). Experimental studies on physiology of *A. pehuenche* should be made in the future to understand the role of osmolarity, temperature and dissolved oxygen in microhabitats where tadpoles overwinter, and how they can survive under the extreme conditions that prevail in the high Andes mountains.

The prolonged larval development of *Alsodes pehuenche* has significant implications for conservation, since larvae of different cohorts (hatched in different years) coexist at the same microsites (ponds or backwater sections of the streams), and a drastic change in habitat conditions could affect several tadpole generations at the same time. Furthermore, an unusually short favourable season (such as the one recorded in the summer 2009-2010) may be disadvantageous for laying eggs or for larvae growth. In addition to natural catastrophic events (such as storms with severe runoff or the drought recorded in December 2012) there is human disturbance, such as the modification of the courses of two streams by paving works in December 2008. This event dried the original stream producing the mortality of individuals present (about 25% of the total population), including metamorphosed individuals and different-age tadpoles (Corbalán, Debandi and Martínez, 2010). It is known that species with long life spans and delayed reproductive maturity are more sensitive to changes in their habitats (Congdon et al., 1994). Using simulation models, Harper, Rittenhouse and Semlitsch (2008) found that the spotted salamander had a much higher probability of extinction than the wood frog when terrestrial habitat was reduced. They attributed their results to the clutches and longevity of species; clutch sizes of salamander are small (100-300 eggs) and salamander may live as long as 32 years, while wood frogs lay one thousand eggs and are short-lived (most individuals breed only once or twice in a lifetime). As was reported for *Alsodes gargola* (about 30-40 eggs; Gallardo, 1970) the clutches of *A. pehuenche* are relatively small (probably less than 100 eggs), and individuals take at least four years to complete larval development. They probably do not reach sexual maturity until several years later. The alteration of the water quality is the most serious problem that *A. pehuenche* must face in the near future. Polluting particles from vehicular traffic, and salt and chemicals used for melting snow on the road during winter are the main threats (Corbalán, Debandi and Martínez, 2010). Road de-icers are used in temperate regions worldwide when harsh climatic conditions affect road traffic (Denoël et al., 2010). Sodium chloride, the active agent of most of road de-icers, affects the nervous system, and the skin of amphibians is particularly permeable to ions (Boutilier et al., 1992; Hillyard et al., 2007). The negative effect of high concentrations of chloride is particularly important in species that inhabit water throughout their entire life cycle (tadpoles and adults), such as *Alsodes pehuenche*. It has been demonstrated that de-icer agents can decrease amphibian survival (Viertel, 1999; Dougherty and Smith, 2006; Karraker et al., 2008) altering their physiology (Hillyard et al., 2007) and behavioural patterns (Denoël et al., 2010). Because of natural slope of the area, the lower reaches of the streams (below the line of the road) will be the most affected by de-icer agents. Preliminary results obtained in the area indicated that these reaches of streams have the highest proportion of tadpoles, suggesting that upon completion of the paving work we must focus on these sections to assess water quality and monitoring population abundance of the Pehuenche frog. Actions to prevent or mitigate this kind of pollution and preserve the population of *A. pehuenche* are underway, but success is not yet guaranteed.

Since the description of the species (Cei, 1976), and until few years ago, no studies had been made on the biology of *A. pehuenche* (Corbalán, Debandi and Martínez, 2010). In this study we were able to demonstrate important aspects of the breeding biology of the species such as time of egg deposition, overwintering and length of the larval period. Knowledge of these aspects of the natural history of the species is very valuable as a tool for managing strategies and conservation decisions.

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References

Altig, R., McDiarmid, R.W. (2007): Morphological diversity and evolution of egg and clutch structure in amphibians. Herpetol. Monogr. **21**: 1-32.

Álvarez, D., Nicieza, A.G. (2002): Effects of temperature and food quality on anuran larval growth and metamorphosis. Func. Ecol. **16**: 640-648.

Baffico, G.D., Úbeda, C.A. (2006): Larval diet of the frog *Alsodes gargola* (Leptodactylidae: Telmatobiinae) and some ecological considerations on its role in alpine and mountain aquatic environments in Patagonia. Amphibia-Reptilia **27**: 161-168.

Basso, N.G., Úbeda, C.A., Bunge, M.M., Martinazzo, L.B. (2011): A new genus of neobatrachian frog from southern Patagonian forests, Argentina and Chile. Zootaxa **3002**: 31-44.

Beausoleil, N.J., Mellor, D.J., Stafford, K.J. (2004): Methods for Marking New Zealand Wildlife: Amphibians, Reptiles and Marine Mammals. Department of Conservation, Wellington.

Boutilier, R.G., Stiffler, D.F., Toews, D.P. (1992): Exchange of respiratory gases, ions, and water in amphibious and aquatic amphibians. In: Environmental Physiology of the Amphibians, p. 81-124. Feder, M.E., Burggren, W.W., Eds, The University of Chicago Press, Chicago.

Bradford, D.F. (1983): Winterkill, oxygen relations and energy metabolism of a submerged dormant amphibian, *Rana muscosa*. Ecology **64**: 1171-1183.

Brown, H.A. (1990): Morphological variation and age-class determination in overwintering tadpoles of the tailed frog, *Ascaphus truei*. J. Zool. **220**: 171-184.

Buchholz, D.R., Hayes, T.B. (2002): Evolutionary patterns of diversity in spadefoot toad metamorphosis (Anura: Pelobatidae). Copeia **2002**: 180-189.

Buchholz, D.R., Hayes, T.B. (2005): Variation in thyroid hormone action and tissue content underlines species differences in the timing of metamorphosis in desert frogs. Evol. Devel. **7**: 458-467.

Bury, R.B., Adams, M.J. (1999): Variation in age at metamorphosis across a latitudinal gradient for the tailed frog, *Ascaphus truei*. Herpetologica **55**: 283-291.

Capitanelli, R.G. (1999): Climas de la Provincia de Mendoza. Meridiano **7**: 29-40.

Cei, J.M. (1962): Batracios de Chile. Santiago de Chile, Ediciones de la Universidad de Chile.

Cei, J.M. (1976): Remarks on some Neotropical amphibians of the genus *Alsodes* from Southern Argentina. Atti Soc. Ital. Sci. Nat. Mus. Civ. Stor. Nat. **117**: 159-164.

Collins, J.P., Lewis, M.A. (1979): Overwintering tadpoles and breeding season variation in the *Rana pipiens* complex in Arizona. Southwest. Nat. **14**: 371-373.

Congdon, J.D., Dunham, A.E., Van Loben Sels, R.C. (1994): Demographics of common snapping turtles (*Chelydra serpentina*): implications for conservation and management of long-lived organisms. Am. Zool. **34**: 397-408.

Corbalán, V., Debandi, G., Úbeda, C. (2008): *Alsodes pehuenche*. Larval biology. Herpetol. Rev. **39**: 457-458.

Corbalán, V., Debandi, G., Martínez, F. (2010): *Alsodes pehuenche* (Anura: Cycloramphidae): Past, present and future. Cuad. Herpetol. **24**: 17-23.

Corbalán, V., Úbeda, C., Martinazzo, L., Basso, N. (2012): *Alsodes pehuenche* Cei, 1976. Ranita del Pehuenche. In: Categorización del estado de conservación de la Herpetofauna de la República Argentina. Ficha de los Taxones. Anfibios. Cuad. Herpetol. **26**(Suppl. 1): 187-188.

Crump, M.L. (1989): Effect of habitat drying on developmental time and size at metamorphosis in *Hyla pseudopuma*. Copeia **1989**: 794-797.

Cuello, M.E., Perotti, M.G. (2006): *Atelognathus patagonicus*. Overwintering tadpoles. Herpetol. Rev. **37**: 441.

Cuello, M.E., Úbeda, C.A., Bello, M.T., Perotti, M.G. (2014): Plastic patterns in larval development of Endangered endemic *Atelognathus patagonicus*: implications for conservation strategies. Endang. Species Res. **23**: 83-92.

Davis, T.M., Ovaska, K. (2001): Individual recognition of amphibians: Effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. J. Herpet. **35**: 217-225.

Denoël, M. (2003): How do paedomorphic newts cope with lake drying? Ecography **26**: 405-410.

Denoël, M., Poncin, P. (2001): The effect of food on growth and metamorphosis of paedomorphs in *Triturus alpestris apuanus*. Arch. Hydrobiol. **152**: 661-670.

Denoël, M., Bichot, M., Ficetola, G.F., Delcourt, J., Ylieff, M., Kestemont, P., Poncin, P. (2010): Cumulative effects of road de-icing salt on amphibian behaviour. Aquat. Toxicol. **99**: 275-280.

Denver, R.J. (1997): Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. Am. Zool. **37**: 172-184.

Díaz, N.F., Valencia, J. (1985): Microhabitat utilization by two leptodactylid frogs in the Andes of central Chile. Oecologia **66**: 353-357.

Dougherty, C.K., Smith, G.F. (2006): Acute effects of road de-icers on the tadpoles of three anurans. Appl. Herpetol. **3**: 87-93.

Formas, J.R. (1979): La herpetofauna de los bosques temperados de Sudamérica. In: The South American Herpetofauna: Its Origin, Evolution, and Dispersal, p. 341-369. Duellman, W.E., Ed., Monograph N^{O} 7. Museum of Natural History, The University of Kansas, Kansas.

Formas, J.R., Pugín, E. (1978): Tadpoles of *Hylorina sylvatica*, *Eupsophus vittatus*, and *Bufo rubropunctatus* in Southern Chile. Herpetologica **34**: 355-358.

Gallardo, J.M. (1970): A propósito de los Telmatobiinae (Anura, Leptodactylidae) patagónicos. Neotropica **16**: 73-85.

Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**: 183-190.

Harper, E.B., Rittenhouse, T.A.G., Semlitsch, R.D. (2008): Demographic consequences of terrestrial habitat loss for pool-breeding amphibians: predicting extinction risks associated with inadequate size of Buffer zones. Cons. Biol. **22**: 1205-1215.

Hillyard, S.D., Viborg, A., Nagai, T., Hoff, K.V. (2007): Chemosensory function of salt and water transport by the amphibian skin. Comp. Biochem. Physiol. A **148**: 44-54.

Hsu, J.L., Kam, Y.C., Fellers, G.M. (2012): Overwintering tadpoles and loss of fitness correlates in *Polypedates braueri* tadpoles that use artificial pools in a lowland agroecosystem. Herpetologica **68**: 184-194.

Hulse, A.C., McCoy, C.J., Censky, E.J. (2001): Amphibians and Reptiles of Pennsylvania and the Northeast. Cornell University Press, New York.

IUCN SSC Amphibian Specialist Group (2013): *Alsodes pehuenche*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1. www.iucnredlist.org. Downloaded on 12 August 2013.

Kaplan, R.H. (1980): The implication of ovum size variability for offspring fitness and clutch size within several populations of Salamanders (*Ambystoma*). Evolution **34**: 51-64.

Karraker, N.E., Gibbs, J.P., Vonesh, J.R. (2008): Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. Ecol. Appl. **18**: 724-734.

Logares, R. (2003): Aspectos ecofisiológicos de *Alsodes gargola* Gallardo 1970 (Anura: Leptodactylidae) en ambientes nordpatagónicos de tipo alpino. Tesis de grado, Universidad de Comahue, San Carlos de Bariloche.

Logares, R., Úbeda, C.A. (2004): *Alsodes gargola* (Rana del Catedral). Overwintering tadpoles. Herpetol. Rev. **35**: 368-369.

Logares, R.E., Úbeda, C.A. (2006): First insights into the overwintering biology of *Alsodes gargola* frogs and tadpoles inhabiting harsh Andean-Patagonian alpine environments. Amphibia-Reptilia **27**: 263-267.

Loman, J. (1999): Early metamorphosis in common frog *Rana temporaria* tadpoles at risk of drying: an experimental demonstration. Amphibia-Reptilia **20**: 421-430.

Lynch, K.S., Wilczynski, W. (2005): Gonadal steroids vary with reproductive stage in a tropically breeding female anuran. Gen. Comp. Endocrinol. **143**: 51-56.

Martof, B. (1956): Growth and development of the green frog, *Rana clamitans*, under natural conditions. Am. Midl. Nat. **55**: 101-117.

Navas, C.A., Úbeda, C.A., Logares, R., Jara, G.F. (2010): Thermal tolerances in tadpoles of three species of Patagonian anurans. South Amer. J. Herpetol. **5**: 89-96.

Newman, R.A. (1992): Adaptive plasticity in amphibian metamorphosis. BioScience **42**: 671-678.

Ortíz Maldonado, A. (2001): Distribución geográfica de los elementos meteorológicos principales y adversidades de Mendoza. Córdoba, Triunfar.

Richter-Boix, A., Llorente, G.A., Montori, A. (2006): A comparative analysis of the adaptive developmental plasticity hypothesis in six Mediterranean anuran species along a pond permanency gradient. Evol. Ecol. Res. **8**: 1139-1154.

Richter-Boix, A., Tejedo, M., Rezende, E.L. (2011): Evolution and plasticity of anuran larval development in response to desiccation. A comparative analysis. Ecol. Evol. **1**: 15-25.

Ryan, T.J., Winne, C.T. (2001): Effects of hydroperiod on metamorphosis in *Rana sphenocephala*. Am. Midl. Nat. **145**: 46-53.

Saha, B.K., Grupta, B.B.P. (2011): The development and metamorphosis of an endangered frog, *Rana leptoglossa* (Cope, 1868). I.J.A.B.R. **1**: 67-76.

Saidapur, S.K. (1989): Reproductive cycle of amphibians. In: Reproductive Cycle of Indian Vertebrates, p. 166-224. Saidapur, S.K., Ed., Allied Publisher, New Delhi.

Saidapur, S.K., Hoque, B. (1995): Effect of photoperiod and temperature on ovarian cycle of the frog *Rana tigrina* (Daud.). J. Bio. Sci. **20**: 289-310.

Székely, P., Tudor, M., Cogalniceanu, D. (2010): Effect of habitat drying on the development of the Eastern spadefoot toad (*Pelobates syriacus*) tadpoles. Amphibia-Reptilia **31**: 425-434.

Tattersall, G.J., Ultsch, G.R. (2008): Physiological ecology of aquatic overwintering in Ranid frogs. Biol. Rev. **83**: 119-140.

Thiesmeier, B. (1992): Daten zur Larvalentwicklung der Geburtshelferkröte *Alytes o. obstetricans* (Laurenti, 1768) im Freiland. Salamandra **28**: 34-48.

Tobler, U., Garner, T.W.J., Schmidt, B.R. (2013): Genetic attributes of midwife toad (*Alytes obstetricans*) populations do not correlate with degree of species decline. Ecol. Evol. **3**: 2806-2819.

Trombotto Liaudat, D., Penas, P., Aloy, G. (2014): Impact of volcanic processes on the cryospheric system of the Peteroa Volcano, Andes of southern Mendoza, Argentina. Geomorphology **208**: 74-87.

Úbeda, C.A. (1998): Batracofauna de los bosques templados patagónicos: un enfoque ecobiogeográfico. Tesis Doctoral, Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Buenos Aires.

Úbeda, C., Zagarese, H., Diaz, M., Pedrozo, F. (1999): First steps towards the conservation of the microendemic Patagonian frog *Atelognathus nitoi*. Oryx **33**: 59-66.

Vaira, M., Akmentins, M., Attademo, A., Baldo, D., Barrasso, D., Barrionuevo, S., Basso, N., Blotto, B., Cairo, S., Cajade, R., Céspedez, J., Corbalán, V., Chilote, P., Duré, M., Falcione, C., Ferraro, D., Gutierrez, F., Junges, P., Lajmanovich, R., Lescano, J., Marangoni, F., Martinazzo, L., Marti, L., Moreno, L., Natale, G., Pérez Iglesias, J., Peltzer, P., Quiroga, L., Rosset, S., Sanabria, E., Sánchez, P., Schaefer, E., Úbeda, C., Zaracho, V. (2012): Categorización del estado de conservación de los Anfibios de la República Argentina. Cuad. Herpetol. **26**(Suppl. 1): 131-159.

Vélez, C.M. (2013): Manejo en cautiverio de la rana grande chilena *Calyptocephalella gayi* (Duméril and Bibron, 1841). Santiago de Chile, Ediciones Universidad Santo Tomás.

Viertel, B. (1999): Salt tolerance of *Rana temporaria*: spawning site selection and survival during embryonic development (Amphibia, Anura). Amphibia-Reptilia **20**: 161-171.

Wells, K.D. (2007): The Ecology and Behavior of Amphibians. University of Chicago Press, Chicago.

Wilbur, H.M., Collins, J.P. (1973): Ecological aspects of amphibian metamorphosis. Science **182**: 1305-1314.

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