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Reproductive Biology of the Critically Endangered Valcheta frog, *Pleurodema somuncurense* (Anura: Leptodactylidae), from Patagonia, Argentina

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Abstract. Amphibians exhibit a great diversity of reproductive modes. Nevertheless, reproductive biology is still unknown for several threatened Neotropical species, including the Critically Endangered microendemic Valcheta frog, *Pleurodema somuncurense*. This paper looks at the reproductive biology of this species, describes its behavior, amplexus, spawning, clutches, embryonic development and breeding microhabitats, and discusses differences and similarities between it and the other Patagonian species included in the *P. thaul* clade: *P. bufoninum* and *P. thaul*. Fieldwork was conducted from 2013–2015 in the headwaters of Valcheta Stream (northern Patagonia, Argentina), over the whole known range of *P. somuncurense*. We also added information from an *ex situ* survival colony of this species. *Pleurodema somuncurense* has prolonged seasonal reproductive activity during spring–summer and presents features of both explosive and prolonged breeders. The Patagonian species of the *P. thaul* clade all have inguinal amplexus and lay pigmented eggs in gelatinous strings. During inguinal amplexus, the male *P. somuncurense* arches its body, bringing its vent closer to the female's, while contracting its body muscles rhythmically and moving its pelvic girdle up and down while the female spawns the eggs. We occasionally recorded mating calls, release calls, and warning vibrations. Although there are similarities among the three species, *P. somuncurense* has some specific features, such as scramble competition, which has not yet been recorded for any other species in the genus, and the mating call, although better records in natural conditions are needed for detailed analysis and more robust comparison. Because the breeding microhabitats used by *P. somuncurense* are currently under disturbance from livestock, their protection should be a matter of concern.

Keywords. Clutches; *Pleurodema bufoninum*; *Pleurodema thaul*; Reproductive behavior; Reproductive pattern; Somuncura Plateau.

INTRODUCTION

Knowledge of reproductive biology is highly relevant to understanding the natural history of a species. It provides building blocks for evolutionary theories and for developing conservation strategies for endangered species (Vitt, 2013). Among the tetrapods, amphibians exhibit a wide variety of reproductive modes (Gómez-Mestre et al., 2012; Toledo et al., 2012), with more than 39 types described to date (Haddad and Prado, 2005; Iskandar et al., 2014; Crump, 2015). Nevertheless, reproductive biology is still unknown for many Neotropical species, partly because of the difficulty in gathering field data, but also due to the difficulty in publishing studies on natural history in several scientific journals (Crump, 2015).

The genus *Pleurodema* Tschudi, 1938 is part of Leptodactylidae Werner, 1896 (1838) (Frost, 2017) and includes 15 species that are widely distributed in the Neotropical region. Most of them inhabit arid, seasonal environments and are explosive breeders (*sensu* Wells, 1977). The genus exhibits several reproductive modes and at least three clutch structures have been described: foam nests, floating gelatinous mass and eggs laid in floating, and submerged gelatinous strings (Faivovich et al., 2012; Ferraro et al., 2016). However, information on

reproduction is still unknown for *Pleurodema alium* Maciel and Nunes, 2010, *Pleurodema marmoratum* (Duméril and Bibron, 1840) and *Pleurodema somuncurense* (Ceí, 1969) (Faivovich et al., 2012).

The Valcheta frog, *Pleurodema somuncurense*, is a microendemic species that only occurs in the headwaters of Valcheta Stream, at the northern edge of the Somuncura Plateau (Río Negro, Argentina). It is an almost wholly aquatic species that only inhabits the thermal springs from which the stream flows (Ceí, 1969b). Ecological knowledge of this species is restricted to a few isolated observations (Ceí, 1969b, 1970, 1980; Velasco et al., 2016) and the main features of its reproductive biology have not yet been studied.

The conservation of the Valcheta frog is of major concern worldwide. Its restricted distribution range (< 10 Km²), the existence of several human-related threats and the continuous decline in wild populations and habitats (Velasco et al., 2016) have led specialists to list *Pleurodema somuncurense* as Critically Endangered in the International Union for Conservation of Nature Red List (IUCN SSC Amphibian Specialist Group, 2016) and as Endangered in the Argentinean Red List (Vaira et al., 2012). Management activities have been conducted since 2013 to protect the species and its habitat (IUCN SSC

Amphibian Specialist Group, 2016). Within this framework, information on its reproductive biology would be important to identify key breeding habitats to be considered within conservation strategies.

The taxonomic status of *Pleurodema somuncurens* has been discussed in-depth (Lynch, 1978), and based on DNA analysis, a possible synonymy between *P. somuncurens* and *P. bufoninum* Bell, 1843 has been suggested (Faivovich et al., 2012). Information on the reproductive biology of the Valcheta frog might yield supporting data to establish ecological relationships among this and phylogenetically related species (Vitt, 2013), including better understanding of the evolution of clutch structure within the genus (see Faivovich et al., 2012).

The primary aim of this work was to study the reproductive biology of *Pleurodema somuncurens*. We describe its reproductive behavior, type of amplexus, spawning, clutches, embryonic development, and breeding microhabitats. We also discuss ecological relationships between *P. somuncurens* and the other Patagonian species included in the *P. thaul* clade on the basis of reproductive traits (Faivovich et al., 2012): *P. bufoninum* and *P. thaul* (Schneider, 1799), neither of which occur in Valcheta Stream. Finally, we identify and

describe breeding microhabitats to be considered for conservation.

MATERIALS AND METHODS

Study site

We conducted this study at “Paraje Chipauquil,” Valcheta Department, Río Negro Province, in northern Patagonia, Argentina. The area belongs to the filtration floors of the volcanic Somuncura Plateau (Ceï, 1969a). This plateau covers 29,000 Km² and encompasses the Monte region of plains and plateaus to the north and the Patagonian Steppe region to the center and south (sensu Burkart et al., 1999). The climate is arid and extreme, with annual temperature ranging from -25 to 35°C (Wegrzyn et al., 1992).

Valcheta Stream, where the Valcheta frog occurs, is one of the main watercourses in the region. It flows from thermal springs located 500–800 m above sea level on the northern slope of the plateau and ends in an endorheic lagoon located 80 Km downstream. We gathered reproductive data from the entire known range of the Valcheta frog, which includes the headwaters of this stream (Velasco

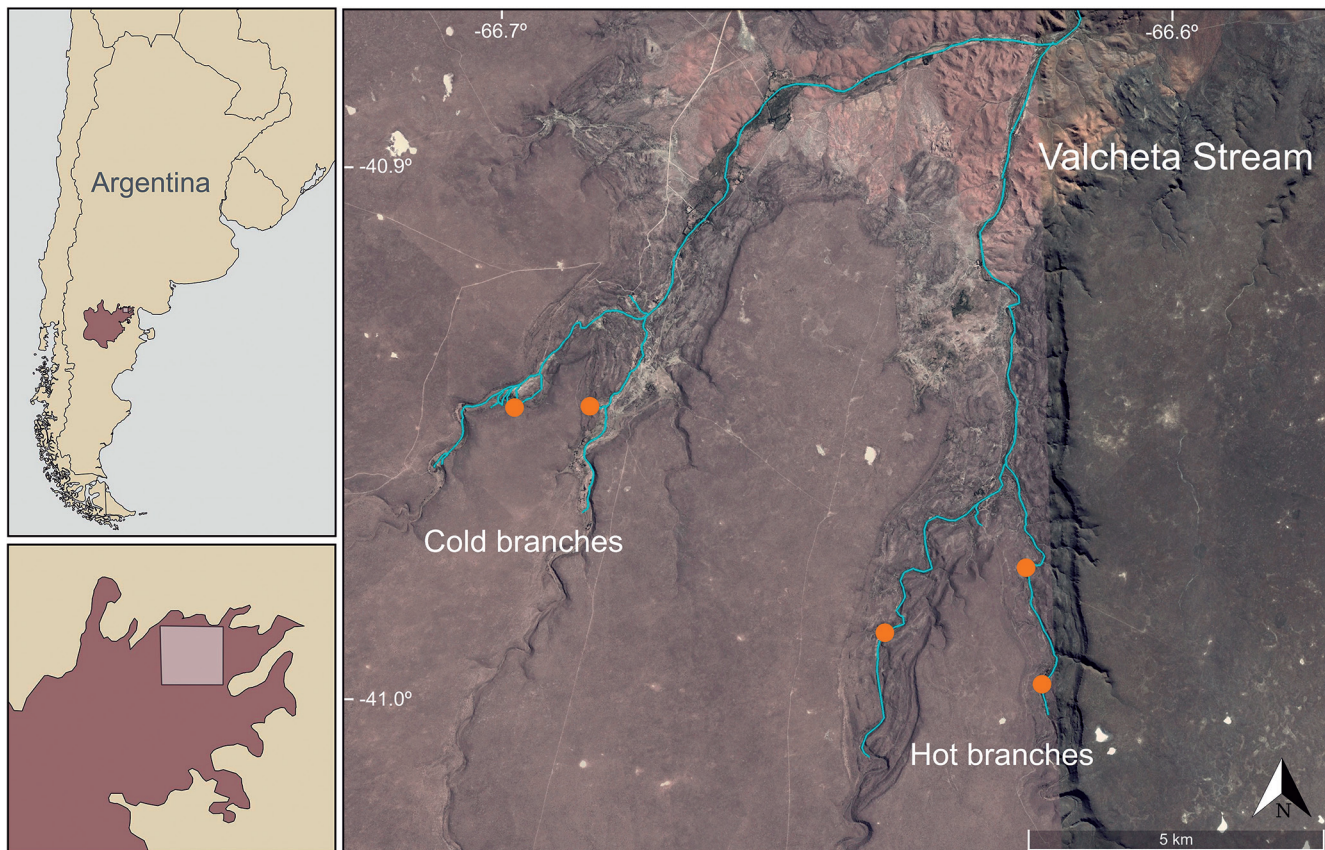


Figure 1. Map of Somuncura Plateau, showing the location of Valcheta Stream (above, right) and detail of the headwaters (below) and the sites where field data were recorded (orange circles). Images obtained from Google Earth Pro.

et al., 2016; Fig. 1), where temperatures are always higher than 19°C, even in winter (Ortubay et al., 1997).

Field surveys

We performed fieldwork during late spring and summer, including November 2013, February, November, and December 2014 and January, February, March, and December 2015, with a total sampling effort of 548 person-hours. We used the Visual Encounter Survey method (Crump and Scott, 1994) along the stream bank during the day and at night, searching for reproductive events, i.e., individuals displaying reproductive behaviors and the presence of clutches and tadpoles.

Whenever we detected individuals displaying reproductive behaviors, we followed them over time, recording displays and type of amplexus, until they hid from view or left the breeding site. We decided not to manipulate individuals in order to avoid affecting their reproductive activities; however, in a previous demographic study of the species conducted at the same sites, we had measured (snout–vent length [SVL], to the nearest 1 mm), sexed, and marked 99 frogs with fluorescent visible implant elastomers (VIE), and during reproductive events we were able to identify a few of these marked individuals by illuminating them with a UV flashlight, which enabled us to know their sex and SVL. For each clutch, we recorded oviposition mode, shape, number and diameter of eggs with and without capsule (using a caliper with an accuracy of 0.01 mm) and observed embryonic development for consecutive days until they hatched. For tadpoles, we recorded presence, stage (Gosner, 1960) and size at hatching (using a caliper with an accuracy of 0.1 mm) at breeding sites and non-breeding sites. We also added occasional records obtained from an *ex situ* colony of 40 individuals of this species housed at the Herpetology Laboratory of La Plata Museum.

In order to describe breeding microhabitats, we measured the following variables at each point where reproductive events were observed: water temperature, pH, depth and velocity (as lotic or lentic waters), substrate, distance to the bank, vegetation cover and main plant species. We also qualitatively recorded occurrence and intensity of rains in order to determine whether reproductive events were related to weather variables. We present quantitative data as $\bar{X} \pm SD$.

RESULTS

Breeding pattern and behavior

We observed clutches in the field from mid-spring to summer (November–February), indicating that breeding

activity lasts up to five months per year. We observed no association between breeding activity and rains. We found tadpoles and metamorphs from December to late March. We observed reproductive behavior only at night, between 22:00–2:00 h, since the frogs are concealed during daylight hours.

In December 2014 and 2015, we observed seven independent breeding aggregates composed of 6–15 individuals displaying reproductive behaviors in the warm eastern branch and the cool western branch of the stream (Fig. 1). We followed one of these groups for three consecutive nights, observing 8, 11, and 15 individuals each night, respectively. The first two nights, we identified six VIE marked frogs, indicating that at least 50% of individuals in the group were males (SVL = 38 ± 6 mm). On day three, we were only able to detect three of these marked frogs, indicating that at least 25% of all the males in the group stayed at the same breeding site during consecutive nights.

The males in the groups were continually active, moving in or around a small area of less than 2 m² and attempting to clasp other moving frogs. When a male clasped another frog (male or female), the others in the group would move towards the couple and climb on top of them. We counted up to eight individuals on top of the first amplexed pair. On six occasions, we observed females being amplexed by more than one male (multiple amplexus). When the amplexus ended, all the individuals moved to the periphery of the area. The observed behavior can be assigned to scramble competition *sensu* Wells (1977). Scramble competition and active searches were also observed at the *ex situ* facilities in September and October 2016, when we also recorded males emitting mating calls before, during, and after behavioral displays.

Although not all detected amplexus events were observed in the context of scramble competition (at some sites we only detected a single pair in amplexus), all of them occurred in the water, in similar microhabitats (see below). During amplexus, the pair remained floating on the water surface, with the female using her forelimbs to grasp a rock or aquatic vegetation and the male clasping the female. We recorded eight single amplexus events (i.e., comprising only one pair of individuals) in the field, four amplexus events in semi-captivity (when individuals were retained in water containers during the marking process), and 32 amplexus events among individuals of the *ex situ* colony. We also recorded six events of multiple amplexus (more than one male with the same female) among the individuals involved in the scramble competition and another 12 at the *ex situ* colony.

Single amplexus was generally inguinal, and rarely axillary (one recorded in the field and two in the *ex situ* colony). During multiple amplexus, one male grasped the female inguinally and the others grasped her in several unusual positions (by the head or by the belly). We only

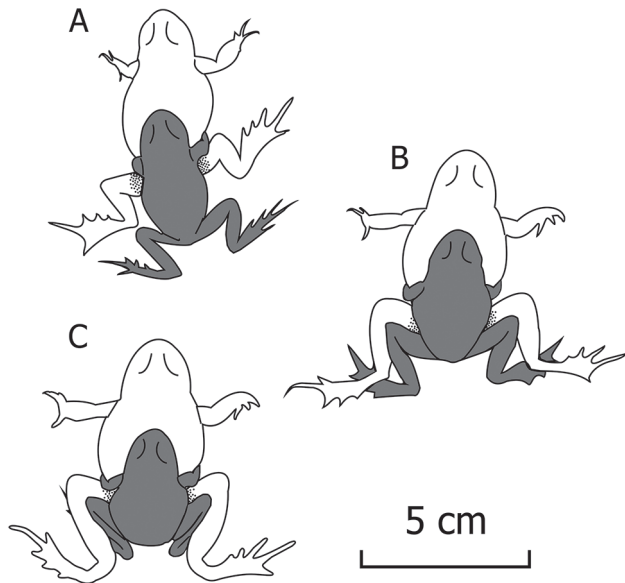


Figure 2. Sequence of events observed during single amplexus events. (A) Male (grey) amplexing the female (white), (B) male hooking its hind limbs to the limbs of the female, (C) male and female arching their backs in opposite directions, bringing the vents closer together. See text for detailed description of events.

recorded spawning in one case of a single pair in amplexus, during which the male moved its tibiotarsal joint on the ventral side of the female's tibiofemoral joint, achieving wider aperture of the female hind limbs. Then the male arched his body convexly and the female arched her body in the opposite direction, bringing the vents closer together (facilitating fertilization). At this time, the male began rhythmic contractions of the body muscles, stimulating the female (Fig. 2), which began to lay a string of eggs. The amplexed female moved around the microhabitat, attaching the string of eggs to aquatic vegetation.

Clutches, embryonic development, and tadpoles

We recorded clutches at five breeding sites, located in three of the four branches of the stream (Fig. 1). The clutches consisted of a gelatinous string of irregular diameter, thin, initially clear, with one or two helicoidally placed eggs occupying the diameter of the string. Number of eggs per clutch in the wild was 78 ± 14 ($n = 13$; 56–113). One end of the string was attached to roots or stems of aquatic plants. Egg diameter was 1.8 ± 0.01 mm ($n = 30$; 1.8–2 mm), or up to 5 mm including the gelatinous capsule. Eggs were strongly pigmented, with a black animal pole and a pale vegetal pole that turned black over time. The string became moister and more glutinous over time, losing its original string shape and becoming a mass.

Embryonic development in the field lasted 72 h. Tadpoles left the gelatinous matrix approximately four days after the oviposition. We did not observe synchrony

in hatching among the tadpoles of a single clutch ($n = 7$), but found eggs and tadpoles at several stages together. Tadpoles hatched at stage 21, measuring 4.8 ± 0.5 mm total length ($n = 7$; 4–5.5 mm) and were strongly pigmented. In early stages, they remained at the site where the eggs were laid (breeding sites), alone or in aggregations, sharing the habitat with adults. At later stages, we observed tadpoles in a wider range of microhabitats, including deeper lotic waters and less vegetated sites. Tadpoles were active throughout the day. We found metamorphs from early December to late March (austral summer–autumn). No tadpole was observed during winter.

Breeding microhabitats

Almost all the recorded behaviors and clutches in the field occurred in warm ($21.8 \pm 1.23^\circ\text{C}$) shallow (8 ± 15 cm) waters, with 7.86 ± 0.38 pH and mud substrate. Although all reproductive events occurred in a lotic environment, the clutches and the first larval stages occurred in lentic waters within the stream no further than 10 cm from the stream bank and 10 m from a thermal spring. Breeding sites were characterized by 100% aquatic vegetation coverage, mainly *Cardamine cordata* Bernéoud, 1845 and *Hydrocotyle bonariensis* Lam, 1789. Exceptionally, we observed three single amplexus events and one clutch at less vegetated sites (vegetation coverage 30%).

DISCUSSION

Biological aspects

Pleurodema somuncurense can be classified as mode I among the 39 reproductive modes described in Haddad and Prado (2005), and mode VII among the 16 reproductive modes described for Argentinean species (Lavilla and Rougés, 1992; Lavilla, 2004), albeit with some differences: eggs submerged and attached to aquatic vegetation instead of laid at the bottom of the body of water. The Valcheta frog exhibits features of both prolonged and explosive breeders (Wells, 1977). A breeding season longer than five months plus the absence of an association between reproduction and rains are typical of prolonged breeders, whereas scramble competition and active searches by males are typical of explosive breeders.

The Patagonian species of the *Pleurodema thaul* clade usually employ inguinal amplexus. However, axillary amplexus has been observed in *P. thaul* populations in southern Argentina (Ceï, 1962) and central Chile (Duellman and Veloso, 1977), as well as in *P. somuncurense*, in which it was observed rarely, when males and females were similar in size (females are usually larger). However, we do not yet know whether this kind of amplexus is viable. Release

calls and warning vibrations are present in both sexes of all three species (Cei and Espina-Aguilera, 1957; Penna and Veloso, 1982) but have not yet been described for *P. somuncurens*. The mating call has only been described for *P. thaul* (Duellman and Veloso, 1977; Penna et al., 2008). It was recently recorded for *P. somuncurens* (not yet described), and is unknown but inferred as absent in *P. bufoninum* (Duellman and Veloso, 1977; Cei, 1980).

All three species produce clutches consisting of gelatinous cylindrical strings with helicoidally arranged eggs. *Pleurodema thaul* and *P. somuncurens* attach one end of the string to aquatic vegetation (Úbeda, 1998), whereas the strings float freely in mid-water in *P. bufoninum* (Weigandt et al., 2004). In *P. bufoninum*, the strings can be long (30–50 cm) or short (9–15 cm), with the diameter of the helicoid usually occupied by up to four eggs (Weigandt et al., 2004). In contrast, in *P. somuncurens* we recorded up to two eggs per helicoid diameter. Gelatinous strings of eggs laid by these species are consistent with the most parsimonious prediction of polarity, proposed by Faivovich et al. (2012).

The eggs of all three species are strongly pigmented, but there are some differences among them regarding size and the number of eggs per string. In *Pleurodema somuncurens* in nature, egg number is 56–113 and diameter is 1.8–5 mm including the capsule. In *P. thaul*, egg number ranges from 350 to ca. 1,200 (Jara and Perotti, 2009; Perotti et al., 2013), while diameter is 2.4–8 mm including the capsule (Úbeda, 1998). The most variable is *P. bufoninum*, in which egg number can vary from 300–3,000 and diameter from 1.26–2.94 mm and 2.69–5.46 mm including the capsule (Weigandt et al., 2004).

Both *Pleurodema thaul* and *P. somuncurens* have asynchronous development, with several cohorts during one season, while *P. bufoninum* has synchronous development, with only one cohort per season (Perotti et al., 2011). A continuous annual sexual cycle was assumed for *P. somuncurens* (Cei, 1980), which may be related to the stable environmental conditions. In contrast, *P. thaul* presents a partially continuous cycle (Díaz-Páez and Ortiz, 2001), whereas *P. bufoninum* presents a discontinuous, self-regulated spermatogenic cycle (Cei, 1961).

The reproductive biology of *Pleurodema somuncurens* shows some similarities with *P. bufoninum* and *P. thaul*. However, some traits only present in *P. somuncurens* warrant special comments since they may represent specific features related to reproductive isolation. The existence of scramble competition is a novelty for *Pleurodema*; thus, further studies of the other species in this genus will be needed to determine whether this behavior is only present in *P. somuncurens*. The mating call is another specific feature, since at present it is unknown in *P. bufoninum* and differs between *P. thaul* and *P. somuncurens*. However, better records in natural conditions are needed for detailed analysis and more robust comparison.

Implications for conservation

Clutches and larval development in the three species occur in lentic waters. However, *Pleurodema somuncurens* was found to use a narrow range of microhabitats for oviposition (warm, shallow waters, with vegetation cover of almost 100%), in contrast to *P. bufoninum* and *P. thaul*, for which a wider range of microhabitats has been described (Úbeda, 1998; Weigandt et al., 2004). This could be related to lower plasticity in *P. somuncurens*, and thus to high sensitivity to disturbances and changes in these microhabitats.

The occurrence of several human-related threats at current breeding sites (Chebez and Diminich, 2008) should be a matter of concern to the managers of the area. During dry periods, insufficient pastures drove livestock to feed and trample on the few breeding sites, strongly affecting them by destroying key vegetation used for oviposition, concealment, and feeding sites for early-stage tadpoles, and serve as the setting for adult reproductive behavior.

There is a remarkable difference between the low number of clutches ($n = 13$) recorded over 3 a of surveys along 100 m of a stream that supports a population of more than 100 adults (MAV, unpublished data) and the number of clutches produced by 40 individuals in captivity over 1 a ($n = 14$). This may indicate scarcity of good breeding sites or appropriate conditions for reproduction in nature. Within this context, preventive protection of known breeding microhabitats should be prioritized to ensure the long-term viability of the Valcheta frog. The same recommendation applies to any other similar sites within the range of the species where management activities could be conducted, e.g., a reintroduction program for the species.

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