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Status and population dynamics of the Critically Endangered Valcheta frog *Pleurodema somuncurense* on the Patagonian Somuncura Plateau

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ABSTRACT: Amphibian populations are declining globally. In this scenario, detailed information on their ecology is crucial to determine the status and extinction risk of their populations and, therefore, better plan conservation activities. The Valcheta frog *Pleurodema somuncurense* is a Critically Endangered species, which is endemic to the Valcheta Stream, Patagonia, Argentina. We assessed the status of this species at the metapopulation level, which we consider is a key step in planning management activities. We conducted visual encounter surveys from 2013 to 2019 to determine the distribution range of each local population of the Valcheta frog. During 2015, we also applied a capture-recapture survey to estimate the density and apparent survival of 1 local population. We used POPAN models for density estimation and the classic model of Cormack-Jolly-Seber to estimate apparent survival, using snout–vent length as a covariate. The metapopulation of the Valcheta frog is composed of 9 isolated local populations: 7 extant and 2 extinct. The extant populations have population sizes ranging from 243 to 4516 individuals. The small size of the smaller local populations makes their long-term viability in isolation unlikely. We found a positive correlation between the apparent survival and the snout–vent length of individuals. Our results showed that management strategies should be aimed at ensuring the long-term survival of this species, with a focus on the recovery of extinct populations, the restoration of disturbed habitats and the improvement of connectivity between local populations.

KEY WORDS: Local populations · Density · Apparent survival · Capture-mark-recapture

1. INTRODUCTION

Amphibian populations are declining globally, and several species have already become extinct. With more than one-third of the total species within the IUCN threat category, amphibians are among the most imperiled vertebrates in the world (Pimm et al. 2014). The lack of ecological information is limiting the advance and implementation of solid action plans for several endangered species, particularly for most Neotropical amphibians, for which accurate information on their natural history and population ecology is still scarce (Gascon et al. 2007). Therefore, to determine the current conservation status and extinction risk, there is an urgent need for detailed information on the ecology of amphibian populations (Zug et al. 2001, Mills & Lindberg 2002, Reed et al. 2003, Luja et al. 2015).

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This lack of ecological information is even more concerning for the species distributed in the Patagonian steppe of Argentina, where amphibians are significantly threatened (Vaira et al. 2017). Among the Patagonian species, the Valcheta frog *Pleurodema somuncurense* (Cei 1969a), is one of the most threatened species in this region and is listed as Critically Endangered by the IUCN (IUCN SSC Amphibian Specialist Group 2016). The Valcheta frog is also included among the top 100 EDGE amphibians in the world, being the only Argentinean species within this rank. An EDGE is an Evolutionarily Distinct and Globally Endangered species, a rank that considers not only conservation status but also phylogenetic importance (EDGE 2018).

The Valcheta frog is a micro-endemic species restricted to the hot headwaters of the Valcheta stream in the Somuncura Plateau. This frog faces a combination of threats, including habitat degradation (Chebez & Diminich 2008, Velasco et al. 2016), direct predation by the invasive rainbow trout *Onchorynchus mykiss* (Úbeda & Grigera 2007, Basso et al. 2012, Velasco et al. 2018), potential trophic resource competition with the introduced characid fish *Cheirodon interruptus* (Velasco et al. 2018) and infection by the chytrid fungus *Batrachochytrium dendrobatidis* (Arellano et al. 2017a). All these threats are negatively affecting the Valcheta frog by promoting the isolation and decline of local populations, with at least 2 cases of local extinctions in recent decades (Velasco et al. 2016).

In 2013, a group of specialists concerned about the status of *P. somuncurense* developed and initiated a conservation action plan for the recovery of this species and conducted the first attempts to reintroduce individuals into a restored habitat that was previously occupied (Arellano et al. 2017b). The action plan for the Valcheta frog recognised that a deep understanding of its ecology would help to better allocate conservation efforts. In this work, we provide a detailed description of the metapopulation of the Valcheta frog. Our specific aims were to map the known local populations of this species, describe their structure and estimate population sizes and survival of individuals. Finally, based on our results, we discuss management options that aim to improve the long-term survival of this species.

2. MATERIALS AND METHODS

2.1. Study area

This study was conducted at Chipauquil, Department of Valcheta, Río Negro Province, northern Patagonia, Argentina. This area belongs to the filtration floors of a volcanic plateau called Somuncura (Cei 1969a). One of the main watercourses in the area is the Valcheta Stream, which is 80 km long (Wegrzyn et al. 1992) and is formed by the confluence of 2 pairs of tributaries, the western pair, locally called Rama Fría, and the eastern pair known as Rama Caliente (Fig. 1). All currently known populations of the Valcheta frog *Pleurodema somuncurense* inhabit this stream (Velasco et al. 2016).

2.2. Local populations

Visual encounter surveys (Crump & Scott 1994) were carried out along the 4 tributaries of the headwaters of the Valcheta Stream from 2013 to 2019 (Fig. 1), covering the pre-reproductive, reproductive and post-reproductive seasons of the Valcheta frog. A team of at least 2 observers searched for frogs intensively (survey effort > 2000 men h^{-1}) at night, during their peak of activity and under similar weather conditions. Our distributional records were

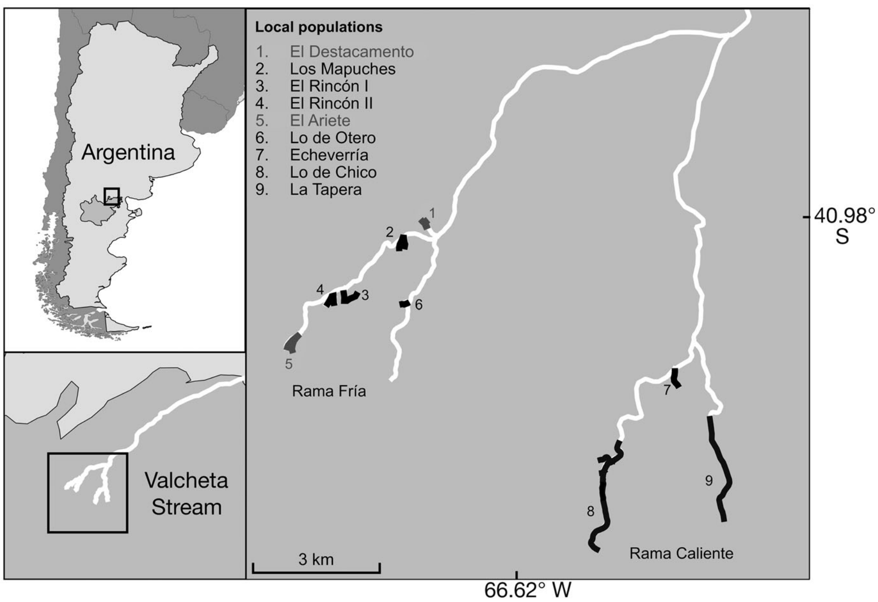

Fig. 1. Distribution range and local populations of the Valcheta frog *Pleurodema somuncurense*, including extant (black lines) and extinct populations (light grey lines). A reintroduction program is being developed in the local population of 'El Destacamento'. White lines indicate sites currently occupied by trout and without frogs' records

corroborated by Cei (1969a,b, 1970, 1980) and Diminich (2006).

The population structure of the Valcheta frog was established by considering the total number of individuals that inhabit the headwaters of Valcheta Stream. Local populations were identified as each group of individuals found in restricted patches of suitable habitats within this area; due to the mainly aquatic lifestyle of this species (Cei 1969a), suitable habitats include stream sections, banks, and associated vegetation (Velasco 2018). We used the information on habitat features and frog records to define the effective suitable area for each local population. The coverage of aquatic vegetation that this species requires for feeding, reproduction, and shelter was considered as a key habitat feature (Velasco 2018). Since most individuals were recorded at no farther than 1 m from the stream bank, even in its wider sections, the suitable habitat was defined as the total length of the watercourse occupied by individuals within 1 m on each side of the stream bank.

2.3. Density and apparent survival

Between January and March 2015, we conducted a capture-mark-recapture survey in the local population of 'El Rincón' (Fig. 1). We made 3 visits of 5 consecutive days each to this population, each visit separated by 30 d (i.e. primary periods of 30 d and secondary periods of 5 consecutive days). At each visit, we captured frogs manually and marked them with an individual code using visible implant elastomers, a low-risk marking technique that does not interfere with the biology of the individuals (Hale & Gray 1998, Fitzgerald et al. 2004, Olsen et al. 2004). For each marked individual, we measured the snout–vent length (SVL, with an accuracy of 1 mm), and in the case of adult individuals, we also determined the sex, based on external secondary sexual features (Cei 1980). We classified individuals <28 mm as juveniles since this was the minimum SVL at which we recognized secondary sexual features.

We employed the POPAN model (Schwarz & Arnason 1996) and the classic model of Cormack-Jolly-Seber (CJS) to estimate density and apparent survival, respectively, using the program MARK 9.0 (White & Burnham 1999). For density estimation, we developed a set of candidate models that consider temporal variation and group effect (juveniles, adult males and adult females). In the case of CJS, we modelled time and SVL as covariates of the apparent survival (POPAN does not allow tests of the effect of covariates on survival; Cooch & White 2004). In both cases, models were ranked according to the Akaike criterion (AIC) (Akaike 1976, Burnham & Anderson 2003), and the fit of the data was tested through the *c-hat* obtained by Fletcher's method (Cooch & White 2004). We determined *a priori* that an acceptable fit for the most general model to the data among the candidate set of models was a *c-hat* value between 1 and 2 (Cooch & White 2004). Finally, we used the best AIC-ranked model among the candidate set to estimate population parameters.

We estimated the size of each local population by extrapolating the average (and 95% confidence intervals) density of the local population of El Rincón (where we conducted the capture-mark-recapture study) multiplied by the effective suitable area occupied by each local population. We then estimated the adult population or the number of individuals that can contribute offspring to the next generation (i.e. all the breeding frogs or all mature individuals).

3. RESULTS

The distribution of the Valcheta frog *Pleurodema somuncurense* is composed of 7 local populations that are extant and 2 populations that are extinct (Fig. 1, Table 1). Each of the local populations is isolated, and the frog displays a metapopulation structure. The total effective suitable habitat occupied by the extant local populations is 1.8 ha, of which 0.3 ha are occupied by 4 local populations distributed in Rama Fria, and 1.5 ha are occupied by the 3 local populations in Rama Caliente. This last stream section contains almost 80% of the known distribution range of the species.

The best POPAN models were the ones in which N varied among adults and juveniles (age models), with juveniles as the most abundant group and without major differences between the number of adult males and females. Of the 99 marked individuals, 58.6% were adults, and 41.4% were juveniles; males represented 35.4% of the adults (n = 35), and the remaining 23% (n = 23) were females. The estimated density of the local population of El Rincón was 0.63 ± 0.15 ind. m^{-2} (average ± SD), with a 95% confidence interval between 0.46 and 1.11 ind. m^{-2}. In the case of the breeding population, the estimated density was 0.24 ± 0.04 ind. m^{-2}, with a 95% confidence interval between 0.19 and 0.34 ind. m^{-2}.

The size of the extant populations extrapolated from the estimated density ranged from 8261 to 19925 individuals. However, when considering only

Table 1. Population size and breeding population size (95% confidence intervals) of each local population of the Valcheta frog *Pleurodema somuncurense*, indicating status (extant/extinct) and effective area of occupied habitat. ID indicates localization (see Fig. 1 for references). Population 1 is being recovered through a reintroduction programme, and it is currently possible to find individuals there

ID	Population	Status	Area (m^2)	Population size	Breeding population size
Rama Fría					
1	El Destacamento	Extinct	388	243 (178–431)	93 (74–132)
2	Los Mapuches	Extant	870	544 (400–966)	209 (165–296)
3	El Rincón I	Extant	946	591 (440–1.050)	227 (180–317)
4	El Rincón II	Extant	968	605 (445–1074)	232 (184–324)
5	El Ariete	Extinct	1148	718 (528–1274)	276 (218–390)
6	Lo de Otero	Extant	352	220 (162–391)	84 (67–120)
Rama Caliente					
7	Echeverría	Extant	678	424 (312–753)	163 (129–231)
8	Lo de Chico	Extant	7226	4516 (3323–8021)	1734 (1373–2421)
9	La Tapera	Extant	6910	4319 (3179–7670)	1658 (1313–2315)

the breeding population size, the number of adult frogs varied from 3411 to 6024. Between 2815 and 4967 adult individuals (almost 80%) were estimated to inhabit the Rama Caliente habitats, and 596 to 1057 individuals were estimated to inhabit the Rama Fría habitats (Table 1). The average number of adult individuals in the extant local population was 188 in Rama Fria and 1185 in Rama Caliente.

With regard to the apparent survival estimates, the best AIC-ranked models were the ones with constant probabilities of capture. The SVL showed a positive correlation with survival that was stabilized after individuals reach 35 mm (Fig. 2, Table 2). The Valcheta frog shows moderate type III survival curve characteristic of an 'r strategist', in which juveniles have lower survival than adults (Begon et al. 1996).

4. DISCUSSION

We recorded 7 extant local populations of the Valcheta frog *Pleurodema somuncurense*. We did not record any individuals at the locations of 2 historic populations of El Destacamento and El Ariete (Diminich 2006), and these were considered extinct. The last record of individuals in El Destacamento was in 2004 (Diminich 2006). Although the factual causes of the absence of individuals in this population are difficult to establish, it is likely that they are associated with anthropogenic disturbance. The dam constructed in El Destacamento in 2009 not only caused the stagnation of water but also promoted considerable pressure on the native vegetation by the livestock (Arellano et al. 2017b), negatively affecting the stream habitats (Velasco et al. 2016). Although exhaustive surveys were conducted between 2013 and 2017, no more individuals were recorded after the dam was constructed. The small size of the known habitat area and the high detectability of this frog (Velasco et al. 2016) make the non-detection of individuals at El Destacamento unlikely, and thus, we agree with the local population extinction hypothesis. Although a reintroduction program is currently underway to recover the local population of El Destacamento, we decided not to consider this population as extant until the reestablishment is effectively confirmed.

In the case of El Ariete, the last record of individuals was in 1969 (Cei 1969b). After more than a decade of searching (Diminich 2006), we found only 1 isolated individual at this site in February 2015 (Velasco et al. 2016). However, no more individuals were recorded during the following 3 yr, even after applying a high survey effort (Velasco 2018). We assumed that the presence of this isolated adult male might be indicative of an early recolonization pro-

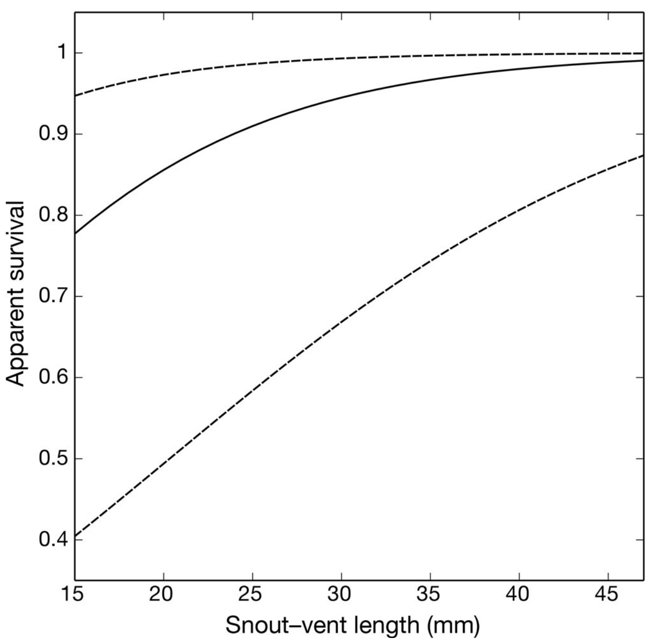

Fig. 2. Monthly apparent survival (solid line) and standard error (dashed lines) by snout–vent length (SVL) for the Valcheta frog *Pleurodema somuncurense*

Table 2. Set of models used for density estimation (POPAN) and apparent survival (Cormack-Jolly-Seber [CJS]). Models are ranked according to their Delta AIC values. Num. Par.: number of modelled parameters. Phi: apparent survival; p: capture probability; Pent: probability of entry (see Cooch & White 2004 for details); N: population size; age: models that differentiate adults from juveniles; t: time variation; g: group variation; svl: snout–vent length

	Delta AIC	AIC weights	Model likelihood	Num. Par.
POPAN				
Phi(age) p(age) Pent (t+age) N(age)	0.00	0.79	1.00	22.00
Phi(age) p(age) Pent(t+age) N(g)	2.84	0.19	0.24	23.00
Phi(g) p(g) Pent(t + g) N(g)	8.26	0.01	0.02	27.00
Phi(.) p(.) Pent(t) N(.)	11.76	0.00	0.00	7.00
Phi(g) p(g) Pent(t) N(g)	20.05	0.00	0.00	18.00
Phi(t) p(t) Pent(t) N(g)	68.20	0.00	0.00	40.00
Phi(t+g) p(t+g) Pent(t+g) N(t+g)	5969.02	0.00	0.00	132.00
CJS				
Phi(svl) p(.)	0.00	1.00	1.00	3.00
Phi(.) p(.)	11.17	0.00	0.00	2.00
Phi(.) p(t)	16.32	0.00	0.00	15.00
Phi(t) p(.)	19.28	0.00	0.00	15.00
Phi(t) p(t)	42.83	0.00	0.00	27.00

cess, an extremely low local population, or a case of a vagrant individual. In any case, a non-viable local population is highly expected at El Ariete. Therefore, we decided to support the hypothesis of local extinction for the population of El Ariete, following Lacy & Pollak (2017), who consider that in sexual organisms, the presence of only 1 individual indicates an extinct population.

The largest local populations of the Valcheta frog were observed at 2 sites locally known as Lo de Chico and La Tapera in Rama Caliente. In these local populations, frogs were present not only at the hot springs but also all along the stream, even several kilometres away from the stream headwaters. In contrast, the local populations of the Rama Fría were small and restricted to stream sections located up to 1 km away from the hot springs (Velasco et al. 2016). These differences might be due to the presence of a high number of threats at Rama Fría, including a higher number of sites occupied by the invasive rainbow trout *Onchorynchus mykiss* (Quiroga et al. 2017), which is a well-known predator of native amphibians worldwide (e.g. Gillespie 2001, Matthews et al. 2001, Pilliod & Peterson 2001, Martín-Torrijos et al. 2016). Velasco et al. (2018) found a negative effect of the trout on the occupancy of native amphibians, including Valcheta frogs, in Valcheta Stream. Importantly, the sites currently inhabited by the Valcheta frog at Rama Fría are inaccessible for this exotic predator due to the presence of natural waterfalls, which might explain why frogs are restricted to this stream section, even when some intermediate habitats have suitable reproductive habitat (M. Velasco pers. obs.). In any case, this pattern of frog absence in sections with trout presence was observed for the entire stream headwaters, indicating that local populations of Valcheta frogs are probably isolated because trout are occupying intermediate habitats, which are the interconnecting stream habitats between the populations, and are likely to be responsible for the observed metapopulation structure.

The Valcheta frog showed values of intermediate adult survival compared to other amphibian species, for which the annual survival varied from 0.3 to 0.78 (e.g. Bull & Williamson 1996, Biek et al. 2002, Anholt et al. 2003, Luja et al. 2015, Alzate-Lozano et al. 2018). The lower survival of juveniles with respect to adults, as in other r strategists, could be related to the juveniles being more prone to predation and/or extreme environmental factors (Ray 1958). However, it is difficult to know whether the lower values in juveniles are due to mortality, emigration or a combination of both. The real survival of the Valcheta frog is difficult to determine because CJS models only give values of apparent survival (i.e. do not differentiate between mortality and emigration). It is likely that a considerable number of individuals are effectively moving beyond the surveyed area since it is not closed. Even so, as discussed below, we assume it unlikely these frogs can reach other local populations.

The Valcheta frog is almost entirely aquatic (IUCN SSC Amphibian Specialist Group 2016) and, therefore, we expect most emigrations to occur through the stream. In the 6 yr of intensive on-ground surveys of the Valcheta frog, we have never observed individuals in terrestrial habitats (M. Velasco pers. obs.). In this study, we did not find marked individuals from El Rincón in nearby populations. We postulate that the dispersal in the stream habitats is prevented by the occurrence of rainbow trout (Velasco et al. 2018). Although some individuals might move outside their habitat, we do not expect that they would reach other local populations. Radio-tracking studies are necessary to test this postulate and determine if individuals are able to move between local populations or not.

The term 'extinction' is used in this study in a metapopulation sense and is considered as the absence of records of viable local populations in sites where the occurrence of the species was previously known. Therefore, we do not discard a potential recolonization by the emigration of individuals from a neighbouring local population. However, as discussed above, the connectivity between local populations is likely to be low or even null because rainbow trout currently occupy all stream corridors. Therefore, the metapopulation dynamics of the Valcheta frog appear to be severely affected due to isolation or very low connectivity between local populations. Considering that some extant local populations are of very small size (<400 individuals on average), a decrease in the long-term viability due to isolation is likely in cases of low or null connectivity (Velasco 2018).

Our results represent a key step in the development of conservation strategies and monitoring protocols for this species. The management of this frog should be oriented to the long-term survival of populations, which involves the recovery of wild populations and the restoration of habitats. To achieve this, it is important to start alleviating the main threat, that is, to remove the trout from the stream headwaters. This action is essential to restore safe corridors that allow the exchange of individuals between local populations of the Valcheta frog and, therefore, recovery of its metapopulation dynamics. However, since some local populations are currently extinct or have very small sizes, a reintroduction programme might help their rapid recovery until viable numbers are reached. However, the restoration of reproductive habitats through avoiding livestock pressure on frogs' habitat (as seen by Arellano et al. 2017b) must be a pre-condition for reintroductions. This study provides valuable information that could help improve the current action plans for the Valcheta frog. Finally, population viability models should be developed to evaluate the proposed management activities with the aim of improving the status of this species.

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