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INVASIVE SPECIES II

The rainbow trout is affecting the occupancy of native amphibians in Patagonia

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Abstract In this work, we assessed the occupancy of two native amphibian species of the Valcheta stream in the Somuncura Plateau, northern Patagonia: the Valcheta frog, *Pleurodema somuncurense* and the Argentine common toad, *Rhinella arenarum*. We hypothesized that the occupancy of both amphibian species will be shaped by the presence of trout; we also expected a decline in occupancy assuming that the new predator in the system will affect native amphibian populations. Between December 2014 and March 2015, we conducted surveys in 148 sites along the headwaters of Valcheta stream. We modelled the occupancy by including the presence of trout, the temperature of water and the coverage of rocks and vegetation as site-habitat covariates. Models including trout were among the best ranked for both amphibian species. The presence of trout reduced the occupancy of both species and the magnitude of this reduction was much higher in the Valcheta frog than in the Argentine common toad. Overall, our results are of great relevance for land managers considering that the Valcheta frog is among the only three IUCN Critically Endangered amphibians in Argentina.

Keywords Exotic invasive species · Occupancy model · *Oncorhynchus mykiss* · *Pleurodema somuncurense* · *Rhinella arenarum*

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Introduction

Native amphibian populations are declining and experiencing local extirpation from native ranges due to several causes, including the spread of non-native predators (Kats & Ferrer, 2003). Among introduced fish species, trout have caused the major reduction in several native amphibians (e.g. Guillespie 2001; Matthews et al., 2001; Pilliod & Peterson, 2001; Martín-Torrijos, 2016). Specifically, the rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) is considered as one of the 100 invasive exotic species more harmful of the world due to its ability to become the apex predator in many aquatic habitats where it was introduced (Lowe et al., 2004; Buria et al., 2007; Crawford & Muir, 2008; Habit et al, 2010).

In Argentina, *O. mykiss* was introduced in 1904 for fishing, first by individuals coming from the United States (Welcomme, 1988; Pascual et al., 2002; McDowall, 2003) and then with individuals coming from Europe (Pascual et al., 2002; Riva Rossi et al., 2004). In Patagonia, rainbow trout was firstly released in lakes and rivers of austral forest, and latter in rivers and lagoons of the steppe. By the 1950s, almost every lake and river of Patagonia was colonized by rainbow trout (Pascual et al., 2002; McDowall, 2003; Riva Rossi et al., 2004). The impact of trout on Patagonian native macroinvertebrates and fishes was extensively studied, but the impact on amphibian populations remains poorly described (Ortubay et al., 2006; Buria et al., 2007; Vigliano et al., 2009).

The Valcheta stream is one of most singular watercourses of Patagonia. This 80-km endorheic stream originates from thermal springs in the northern edge of the Somuncura plateau, resulting in a rare condition for a Patagonian watercourse, where the first 10 km maintains a perennially warm water temperature (20–26°C, Ortubay et al., 1997). Native aquatic vertebrates of Valcheta stream include one fish, the naked characin, *Gymnocharacinus bergi* Steindachner, 1903 and three amphibians, the escuercito, *Odontophrynus occidentalis* (Berg, 1896), the Argentine common toad, *Rhinella arenarum* (Hensel, 1867) and the Valcheta frog, *Pleurodema somuncurense* (Cei, 1969a, b). The escuercito is regionally widespread, but is very uncommon in the area and barely uses the stream (MV, obs. pers). The Argentine common toad is also regionally widespread, occupying the stream and other aquatic and terrestrial habitats in the surroundings. The Valcheta frog is an almost exclusively aquatic anuran (Velasco et al., 2016), micro-endemic from the headwaters of the Valcheta stream, and currently listed as Critically Endangered, because it is threatened by livestock, chytrid fungus and rainbow trout, among other threats (Velasco et al., 2016; IUCN, 2017).

The rainbow trout were introduced in 1928 in the Valcheta stream (Macchi & Vigliano, 2014), and currently is widely distributed along this watercourse (Quiroga et al., 2017). The rainbow trout rapidly became a serious threat to the aquatic native fauna of the Valcheta stream and it established as the main threat of the naked characin (Ortubey & Cussac, 2000; Quiroga et al., 2017). However, for several decades, the thermal condition would have restricted the access of rainbow trout to headwaters of the Valcheta stream (Ortubay & Cussac, 2000). Unfortunately, several trouts were recently recorded in headwaters confirming that thermal barrier is not as effective as previously observed (Quiroga et al., 2017).

In this work, we described the occupancy of Valcheta frog and Argentine common toad in the headwaters of the Valcheta stream and we evaluated if the presence of trout is disturbing them. We hypothesized that trout shapes the occupancy of these native amphibians. We expected a decline in occupancy assuming that the new predator in the system will affect native amphibian populations.

Materials and methods

Study area

Our study site was the headwaters of the Valcheta stream, and encompassed the entire distributional range of the Valcheta frog (Fig. 1; Velasco et al., 2016). These headwaters are located in northern Somuncura Plateau (Argentinean Patagonia), and they arise in four distinctive branches, grouped in two pairs, which are locally known as the cold and the hot branches, respectively (Cei, 1969b). The cold branch has 13 km length and the water temperature oscillates between 20.5 and 22.5°C, while the hot branch has 17 km length and the water temperature oscillates between 22 and 26°C (Ortubay et al., 1997).

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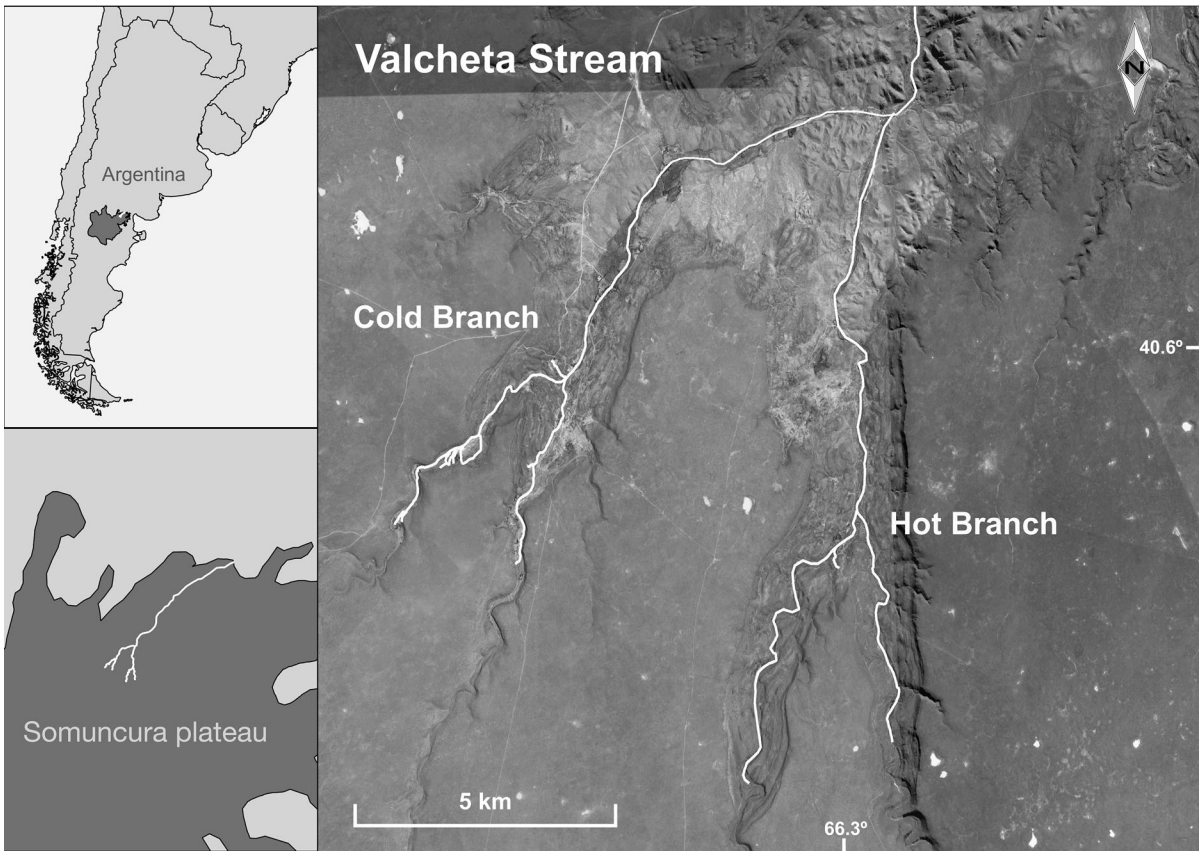

Fig. 1 Map showing the location of the Valcheta stream in the Somuncura plateau (left side) and the branches that conform the headwaters of the Valcheta stream (Patagonia, Argentina)

Field sampling

During the summer season, between December 2014 and March 2015, we conducted 15 daily surveys. We visited 78 sites along the cold branch and 70 sites along the hot branch. We defined a survey site as a 12 m^2 quadrant. We placed quadrants along the bank of the stream. The geographic position of the first site was randomly selected at each headwater and the following sites were placed systematically every 50 m downstream. Every site was visited in three different opportunities, and always by two observers, during the peak of activity for both species (22:00–02:00; Velasco et al., 2016), and under similar and typical weather conditions. At each visit, we conducted a Visual Encounter Survey (VES, Crump & Scott, 1994), which included registering the number of adults or juveniles of Valcheta frog and Argentine common toad.

For each surveyed site, we quantified five potential site covariates that seemed likely to influence the occupancy of both amphibian species: (a) water temperature at surface (measured at night during the first visit to each site, with a precision of 0.1°C); (b) rock and (c) vegetation coverages on the bank (as percentages); (d) rock and (e) vegetation coverages in the water surface (as percentages, Table 1). Coverage covariates were measured during the day prior to the first visit. We hypothesized that amphibians will occupy sites with high water temperature, assuming that these sites allow a more efficient thermoregulation. We also hypothesized that amphibians will occupy sites with high rock and vegetation coverage as they provide more opportunities of refuge against predators and, in the case of the Valcheta frog, are preferred for egg laying (Velasco et al., in press). Finally, to test the main hypothesis of this study, we included a sixth covariate, (f) the presence of rainbow

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Table 1 Variables and hypothesis proposed in the modelling of occupancy for the Valcheta frog (*Pleurodema somuncurense*) and the Argentine common toad (*Rhinella arenarum*) at Valcheta stream, Somuncura plateau, Argentina

Covariate	Type	Mean ± SD (range)	Hypothesis
Water temperature at surface	Continuous	21.8 ± 2.7°C (13.9–25.8°C)	Thermoregulation (+)
Vegetation coverage in the bank	Continuous	76 ± 30% (0–100)	Prey and shelter availability (+)
Vegetation coverage in the water surface	Continuous	39 ± 36% (0–100)	Prey and shelter availability (+)
Rock coverage in the bank	Continuous	23 ± 33% (0–100)	Shelter availability (+)
Rock coverage in the water	Continuous	30 ± 31% (0–100)	Shelter availability (+)
Presence of trout	Dichotomous	Presence ($n = 38$)/absence ($n = 110$)	Predation (−)

Symbols indicates a positive (+) or (−) relationship between the covariate and the occupancy

trout. We used current information from an ongoing study to determine the presence of rainbow trout on each site (Quiroga et al., 2017). To avoid including correlated covariates, we conducted pairwise comparisons using Pearson's correlation coefficient; all comparisons showed low coefficient values (i.e. $r \leq 0.7$). Values are showed as average ± SD (range) (Table 1).

Analysis

We estimated the naïve occupancy as the number of sites where the species was detected over the number of surveyed sites. We used single-season occupancy modelling (MacKenzie & Royle, 2005; Bailey et al., 2014) to identify which covariates better explained the occurrence of both amphibian species. We defined occupancy (ψ) as the proportion of survey sites occupied by each species, and detection (P) as the probability that a species would be detected within a sample area given that it was present within that sample area.

We developed a set of candidate models to assess whether the covariates better explained occupancy of both amphibians. To select which model should be included in the set, we followed a forward selection. This selection procedure starts with a simple model and adds variables systematically, checking whether they improved it (Draper & Smith, 1981), thus is useful to assess how explanatory such variables are. In our case, for each species, we started with the constant-occupancy model in which both detection and occupancy probabilities were assumed to be constant across all sites [denoted as $\psi(.)$ $P(.)$] and we add covariates one at time. We used Akaike's Information Criterion (AIC) to compare among models (Burnham & Anderson, 2003) and kept all models that outperformed the constant-occupancy model (i.e. if the addition of one covariate resulted in a model with a lower or within two AIC units [$\Delta AIC < 2$] with respect to the constant model, then we removed from the set). We repeated the selection process by adding a second, and latter a third covariate.

For each model, we calculated the estimates of parameters (β), and their standard errors. The occupancy (ψ) for each species was estimated based on the best-ranked model. We performed all occupancy models with *unmarked* package (Fiske & Chandler, 2011) in software *RStudio* (RStudio Team, 2015).

Results

We detected the Valcheta frog in 41% of sites ($n = 148$ surveyed sites). The naïve occupancy of Valcheta frog in sites without trout ($n = 110$ sites) was 10 times higher than sites with trout (54 vs. 5%). We recorded the highest number of Valcheta frogs during the second of the three visits, counting a total of 77 individuals (87%) at sites without trout and only 12 individuals (13%) at sites with trout. On average, during this visit, in sites without trout we detected 0.73 ± 1.51 (0–10) Valcheta frogs per site, while in sites where the presence of trout was confirmed, we found 0.24 ± 0.76 (0–2) Valcheta frogs per site.

We detected the Argentine common toad in 70% of sites. The naïve occupancy of Argentine common toads in sites without trout ($n = 110$ sites) was almost twice higher than sites with trout (77 vs. 47%). During the visit of major record of individuals at sites without trout, we counted a total of 306 Argentine common toads (81%), while only 73 individuals (19%) were counted at sites with trout. On average, during this

Table 2 Single-season occupancy models and coefficients of parameters (β) ± SD for the Valcheta frog (*Pleurodema somuncurense*) and the Argentine common toad (*Rhinella arenarum*) in the Valcheta stream, Patagonia, Argentina

Model	ΔAIC	*n*	−LogLik	*P*	Intercept	Temp	Trout	Bank rock	Water rock
Valcheta frog									
Ψ (Temp + trout + bank rock) *P* (.)	0.00	5	168.55	0.52 ± 0.07	0.19 ± 0.78	1.20 ± 0.44	− 2.75 ± 1.43	1.46 ± 1.23	
Ψ (Temp + bank rock) *P* (.)	9.98	4	174.54		− 0.53 ± 0.29	1.31 ± 0.31		1.11 ± 0.39	
Ψ (Temp + trout) *P* (.)	11.26	4	175.19		− 0.12 ± 0.28	1.32 ± 0.30	− 2.48 ± 0.79		
Ψ (Temp) *P* (.)	24.29	3	182.70		− 0.64 ± 0.24	1.53 ± 0.30			
Ψ (Bank rock) *P* (.)	36.14	3	188.62		− 0.35 ± 0.23			1.16 ± 0.29	
Ψ (Trout) *P* (.)	39.32	3	190.22		0.11 ± 0.21		− 2.90 ± 0.76		
Ψ (Water rock) *P* (.)	62.00	3	201.56		− 0.39 ± 0.19				0.40 ± 0.19
Ψ (.) *P* (.)	64.61	2	203.86		− 0.38 ± 0.19				
Argentine common toad									
Ψ (Temp + trout + bank rock) *P* (.)	0.00	5	112.40	0.89 ± 0.02	1.35 ± 0.37	1.72 ± 0.30	− 1.28 ± 0.51	0.84 ± 0.39	
Ψ (Temp + trout) *P* (.)	3.48	4	115.14		1.19 ± 0.32	1.87 ± 0.30	− 1.46 ± 0.51		
Ψ (Temp + bank rock) *P* (.)	4.42	4	115.61		0.88 ± 0.29	1.73 ± 0.29		0.93 ± 0.39	
Ψ (Temp) *P* (.)	10.21	3	119.51		0.67 ± 0.24	1.91 ± 0.29			
Ψ (Bank rock) *P* (.)	60.20	3	144.51		0.71 ± 0.24			1.19 ± 0.35	
Ψ (Trout) *P* (.)	66.27	3	147.54		0.93 ± 0.22		− 1.69 ± 0.41		
Ψ (.) *P* (.)	82.42	2	156.61		0.47 ± 0.17				

Models are ranked by their AIC scores. The probability of detection (*P* ± SD) is reported for the best model of each species

Temp temperature of the water, *Trout* presence of trout, *Bank rock* rock coverage on the bank of the stream, *Water rock* rock coverage in the water

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visit, in sites without trout we detected 2.9 ± 3.82 (0–27) Argentine common toads per site, while in sites where the presence of trout was confirmed, we found 1.74 ± 4.19 (0–22) Argentine common toads per site.

Four covariates explained the occupancy of Valcheta frog and three covariates explained the occupancy of the Argentine common toad (Table 2). Top models in both species included a positive relationship with water temperature and coverage of rock in the bank, and a negative relationship with trout presence. In the case of Valcheta frog, a positive relationship between occupancy and coverage of rock in the water was also observed. In both species, the increase of water temperature predicted a higher occupancy and the presence of trout reduced the occupancy (Fig. 2). This reduction in occupancy due to the presence of trout was more dramatic in the Valcheta frog than in the Argentine common toad (Fig. 2).

Discussion

As we predicted, the presence of rainbow trout negatively shaped the occupancy of both native amphibians of Valcheta stream. This effect was apparent given our results demonstrate lower occupancy of amphibians at sites where trout was present. Other studies have reported similar result in Neotropics, showing the impact of rainbow trout in amphibians of the Andes (Martín-Torrijos et al., 2016) and other regions of Southern South America (Miloch et al., 2015), including Patagonia (Úbeda et al., 2010). In our case, we assume that this effect is related with direct predation of amphibians by trout. Although we did not record amphibians eaten by trout, we think this is very probable considering that trout is an apex predator and no other predator fishes existed in the stream previous to its introduction. A similar situation was inferred for *Atelognathus patagonicus*, an endemic species from Patagonian, where the main subpopulation is believed to have been extirpated through predation by introduced fishes (Úbeda et al., 2010). However, we do not discard other potential effects of trout besides direct predation, like a high stress on tadpoles or even as vectors of the pathogen fungus *Saprolegnia diclina* Humphrey, 1893 as was reported in the Neotropical toad *Engystomops petersi* Jimenez de la Espada, 1872 by Martín-Torrijos et al. (2016).

Overall, the negative relationship between occupancy of amphibians and presence of trout was

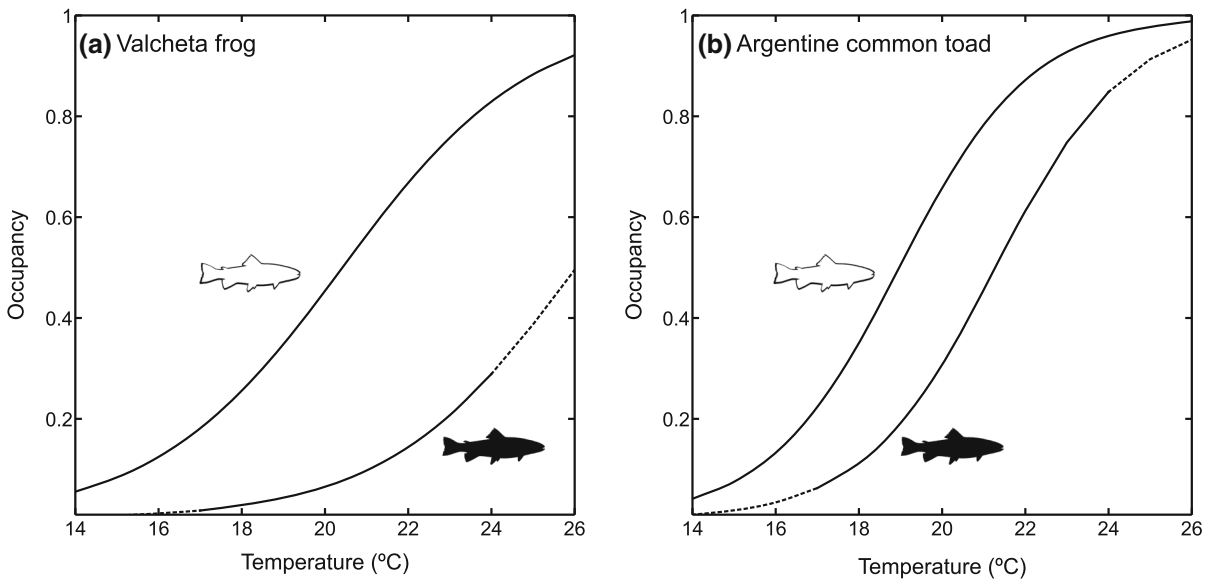

Fig. 2 Predicted occupancy in relation to water temperature in two hypothetical scenarios: without trout (white silhouette) and with trout (black silhouette); for the two native species of the Valcheta stream (Patagonia, Argentina): **a** the Valcheta frog (*Pleurodema somuncurense*) and **b** Argentine common toad (*Rhinella arenarum*). Lines represent occupancy estimated using beta parameters from the best-ranked model of each species incorporating temperature of the water, average value of rock coverage on the bank of the stream and the presence of trout (Table 2). Dotted lines denote the water temperatures where trout has not been recorded (i.e. below 17°C, and above 24°C)

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strongest for Valcheta frog than for Argentine common toad. We assume that this might be related with a higher likelihood of predation by trout related to the aquatic habits of Valcheta frog, which permanently inhabits in the stream, having more chances of contacting trout. Otherwise, the Argentine common toad is a more terrestrial species that can be found in and outside the stream in the ground or in temporal ponds. Further studies should be done to test the underlying causes to this pattern.

Besides the negative effect of trout, the occupancy of both amphibian species showed a positive relationship with coverage of rocks at the bank of the stream and water temperature. More rocks on the bank of the stream could be providing a larger number of shelter for amphibians and their prey. The same reasoning follows in the case of percentage of rock coverage in water, which was positively related with the occupancy of Valcheta frog. The species is almost entirely aquatic and typically remains hidden under these rocks during the day (Cei, 1969a).

In the case of the relationship between occupancy and water temperature, we have two possible explanations. In first place, this relationship could be reflecting a microhabitat selection, where frogs and toads prefer warmer water. A second, and non-exclusive hypothesis, would be that, due to the difficulty of trout to access to warmer waters of the stream (Ortubay, 1998; Ortubay & Cussac, 2000), many amphibian populations face lower pressure from trout. Unfortunately, rainbow trout are infiltrating warmer waters in headwaters of Valcheta stream where an increasing number of individuals are being recorded each year (Quiroga et al., 2017), indicating that this thermal barrier is not as effective as in the past. If this trend continues, an expansion of trout will not only negatively affect Valcheta frog and Argentine common toad, but also other sympatric native species like the Endangered naked characin, as well as endemic invertebrates.

Even though this work indicates a negative effect of rainbow trout over occupancy of both amphibian species, it is very difficult to understand and estimate the magnitude of this threat because of the absence of detailed information related to the historical range of Valcheta frog and Argentine common toad. Given the critical conservation status of the Valcheta frog an urgent action plan is needed. A management strategy based on the removal of rainbow trout should be implemented in the area. This kind of management has demonstrated to work under various scenarios (Kats & Ferrer, 2003) and can be an effective option for recovery of the native populations of vertebrates and invertebrates, including amphibians (Taylor et al., 2001; Vredenburg, 2004; Knapp et al., 2007). As observed with other amphibian species (Funk & Dunlap, 1999; Eaton et al., 2005; Knapp et al., 2007), we expect that this overall strategy can contribute to an effective population recovery of native amphibians of the Valcheta stream.

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