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Research Article

High abundance of invasive African clawed frog *Xenopus laevis* in Chile: challenges for their control and updated invasive distribution

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Abstract

Invasive African clawed frog *Xenopus laevis* (Daudin, 1802) are considered a major threat to aquatic environments. Beginning in the early 1970s, invasive populations have now been established throughout much of central Chile. Between September and December 2015, we studied a population of *X. laevis* from a small pond in Viña del Mar, where we estimated the population size and evaluated the use of hand nets as a method of control. First, by means of a non-linear extrapolation model using the data from a single capture session of 200 min, a population size of 1,182 post-metamorphic frogs (range: 1,168–1,195 [quadratic error]) and a density of 13.7 frogs/m^2 (range: 13.6–13.9) of surface water were estimated. Second, based on 10 capture-and-removal sessions of 60-min and separated by approximately 2 weeks each, a total of 2,184 post-metamorphic frogs were removed, but the number of captured individuals did not significantly decrease over time. Additionally, using novel records of its occurrence, we updated the distribution range of *X. laevis* in Chile, estimated at 36,055 km^2, which is 1.7 to 3.5 times higher than previously assessed. Our results indicate that invasive *X. laevis* can reach extremely high densities, and removal of individuals in large numbers was not useful in reducing its abundance. This study shows that control and eradication of *X. laevis* from invaded areas proves extremely difficult, particularly when populations are well-established and/or expanding, as occurs in Chile. Our study reports the first record of *X. laevis* invading rivers of north Chile, 380 km north of previously reported. A management plan is urgently needed to prevent its further spread, and subsequent impacts on biodiversity. We propose our model and methodology as a tool to estimate and compare *X. laevis* densities, while suggesting the need to explore other control techniques in order to identify cost-effective strategies to contain the spread of *X. laevis* into new areas.

Key words: Amphibian, aquatic invasions, eradication, pest control, population size

Introduction

With laboratory colonies across the globe and invasive populations established on four continents, the African clawed frog (*Xenopus laevis* Daudin, 1802) is the world's most widespread amphibian (Measey et al.

2012). The discovery of the early human pregnancy diagnosis (or Hogben test) using *X. laevis* in the 1930s, its wide use as an animal model in biological research, and growing interest in this species as a pet has increased the range of *X. laevis* globally: initially from South Africa to the United Kingdom, but later to other regions across the globe via the scientific and pet trade networks (Van Sittert and Measey 2016). Escapes from captivity and deliberate releases of animals have allowed the successful establishment of invasive populations in the United Kingdom (perhaps now extinct; see Tinsley et al. 2015), United States, Chile, Portugal, France, Italy, Japan (reviewed in Measey et al. 2012) and Mexico (Peralta-García et al. 2014). Environmental suitability models predict an ongoing expansion of most known invasive populations and a high potential for colonization of new areas (Measey et al. 2012; Lobos et al. 2013; Barbosa et al. 2017; Rödder et al. 2017; but see Ihlow et al. 2016). Negative impacts of introduced *X. laevis* on aquatic organisms include competition (Lillo et al. 2011; Courant et al. 2017) and predation, with subsequent displacement of local fish and amphibians (Lafferty and Page 1997; Crayon 2005; Rebelo et al. 2010; Vogt et al. 2017; Courant et al. 2018). Also, hybridization with the Cape platanna (*Xenopus gilli*) occurs in much of its reduced range in southwestern South Africa (De Villiers et al. 2016). Due to its high trade, *X. laevis* has also been implicated in the spread of two amphibian diseases of conservation concern. Although tolerant to infection but resistant to developing clinical disease, *X. laevis* can act as reservoir maintaining chytridiomycosis and *Ranavirus* in invaded habitats (Weldon et al. 2004; Robert et al. 2007; Solís et al. 2010; Soto-Azat et al. 2010, 2016).

Its aquatic nature and secretive behavior make *X. laevis* difficult to detect in the wild (Measey et al. 2012). Reports of only a few successful control or eradication programs for *X. laevis* are described in the literature, and these are limited to restricted habitats (e.g., a pond), early-stage invasions or when occurring in sub-optimal environments (reviewed in Measey et al. 2012; Tinsley et al. 2015). In Chile, it is believed that this species was introduced in the early 1970's near the International Airport of Santiago, and since then, invasive populations have colonized an extensive area of central Chile following a dispersal rate of between 3.1 to 5.4 km/year (Lobos and Jaksic 2005). Today, most water bodies in which *X. laevis* have established show either a decline or absence of native fish and amphibians, while there is also evidence of *X. laevis* acting as vectors for chytridiomycosis and *Ranavirus* in the country (Solís et al. 2010; Soto-Azat et al. 2010, 2016; Bacigalupe et al. 2017). Consequently, effective programs to control or eradicate invasive *X. laevis* are required, in particular from areas under high invasion pressure (Measey et al. 2012). For control we refer to an intervention that limits the size of the population to low numbers that minimize their impacts (Lockwood et al. 2013). Central Chile has been

identified as a biodiversity hotspot, known for its high endemism of fish, amphibians and reptiles, many of which are under a high risk of extinction (Lobos and Jaksic 2005; Vidal et al. 2009). Based on population monitoring and a management intervention, we studied an invasive population of *X. laevis* from central Chile with the aim of: 1) estimating abundance of *X. laevis* using a theoretical population model, and 2) assessing the effectiveness of a method of control to prevent the spread of *X. laevis*. In addition, using new presence data we 3) estimated its current extent of occurrence and updated the distribution range of this invasive species in Chile.

Materials and methods

Study area

We conducted our study in an 86 m^2 pond located in the National Botanical Gardens, Viña del Mar, central Chile (Figure 1). The study area is characterized by a Mediterranean climate with dry summers and cool and wet winters. Aquatic environments in the area are dominated by lentic waters, highly modified for urban and agriculture purposes. The study pond drains through a small brook (30 cm wide and 5 cm deep) and is separated from upstream by a dam. Upstream three endemic and threatened aquatic vertebrates were identified: two fish (*Cheirodon pisciculus* and *Trichomycterus aerolatus*) and one frog (*Calyptocephalella gayi*), together with the widespread native amphibian *Pleurodema thaul*. No aquatic vertebrate, other than *X. laevis*, was recorded at this pond during the study.

Capture and abundance estimation

On 2nd September 2015, two herpetologists entered the pond and proceeded to capture *X. laevis* with the use of dip nets (see Figure 2) during 10 sessions of 20 min separated by 5 min intervals (total 200 min search effort, starting at 13.00 PM and finishing at 17.05 PM). The entire pond area was searched during each session. Frogs were captured and contained in 50 L containers (one per each 20-min session). Following search completion captured frogs were counted and released back to the pond. Animals were not tagged or marked in any way, and were not euthanized only because of phase II of the study (see below, population control assessment). Only post-metamorphic frogs were included in the analyses as they were the vast majority of captures and since the capture probability is likely different from that of larval stages (Wassens et al. 2016).

To estimate abundance, capture data was fitted to a non-linear extrapolation model following Medina-Vogel et al. (2015) given by

$$X(t) = N(1)\ (1\text{-}\exp(\text{-}k\ t)),$$

where X(t) is the cumulative amount of frogs captured up to the t-session, k is a measure of the efficiency of the trapping, and N(1) is the amount we

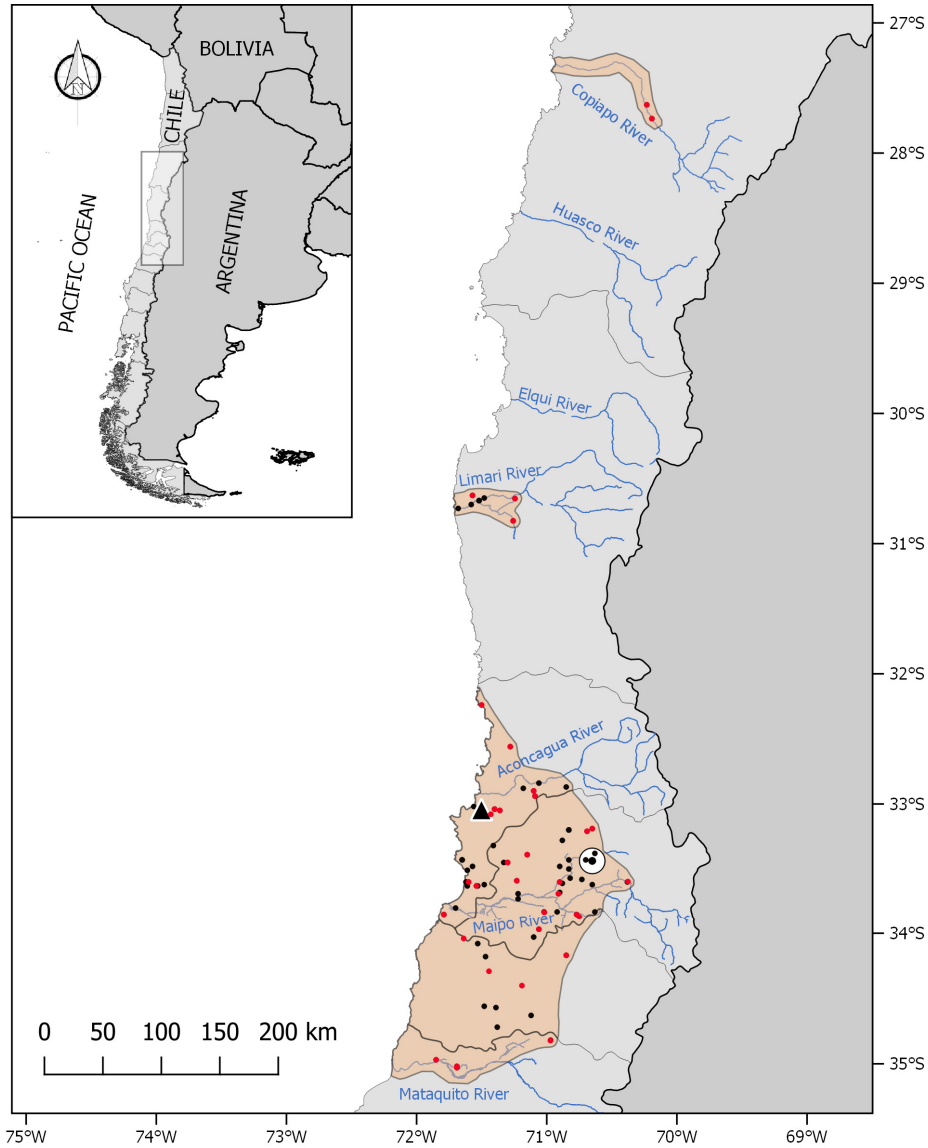

Figure 1. Map showing the estimated invasive distribution range of *Xenopus laevis* (shaded orange) in Chile and the location (black triangle) where abundance estimation and population control were carried out. Black dots represent historical records of *X. laevis* in Chile, and red dots new presence records reported in this study. White circle is the capital city of Santiago, original site for the introduction of *X. laevis* in Chile. For details see Supplementary material Table S1 (Localities in Chile with records of *X. laevis*).

are extrapolating for the abundance, namely the number of frogs in the pond at the beginning of the survey. The mathematical model assumes that each animal has an equal probability of being captured and that the population is closed during the survey period. Emigration, death, immigration of frogs, as well as newly developed post-metamorphs, was likely zero or negligible because of the short survey period (200 min). The model highlights some expected behavior, namely a decreasing capture rate as the number of sessions (or time t) increases, in particular the need of a huge amount of effort (or time t) to capture all the individuals from a site: this can be easily seen from the formula and its corresponding graph (see Supplementary material Figure S1).

Figure 2. Photograph of the studied pond at the Chile's National Botanical Gardens, Viña del Mar, showing capture of *Xenopus laevis* using dip nets. Inner box shows captured post-metamorphic *X. laevis* of different sizes. Bar = 5 cm. Photographs Claudio Soto-Azat.

Population control

During the subsequent 4 months, between 4th September and 30th December 2015, 10 capture and removal sessions of 60 min each, separated by 2-week intervals, were carried out (during daytime) at the same pond following the same capture methods described above (essentially two people with dip nets; Figure 2). This periodicity and type of capture was chosen based on park rangers' abilities and their availability of time for a potential *X. laevis* control campaign led by environmental authorities. Also, this period coincides with the arrival of spring, the end of the rains and therefore feasibility to implement water management practices. After each session, frogs were counted, snout-vent length (SVL) measured and euthanized by immersion in a buffered solution of 10 g/L of tricaine methanesulfonate (Dolical 80%, Centrovet). Previous studies considered adults those individuals reaching a 65 mm SVL (e.g. McCoid and Fritts 1980). A Spearman rank test correlation was calculated to assess the effect of frog removal on *X. laevis* abundance (dependent variable) over time (independent variable).

Distribution range and extent of occurrence

Between 2005 and 2019 extensive amphibian surveys were carried out in central Chile by the authors to detect and sample invasive *X. laevis* for disease surveillance (chytridiomycosis and *Ranavirus*). In order to assess the increase in distribution range, all sites with previous presence of *X. laevis*

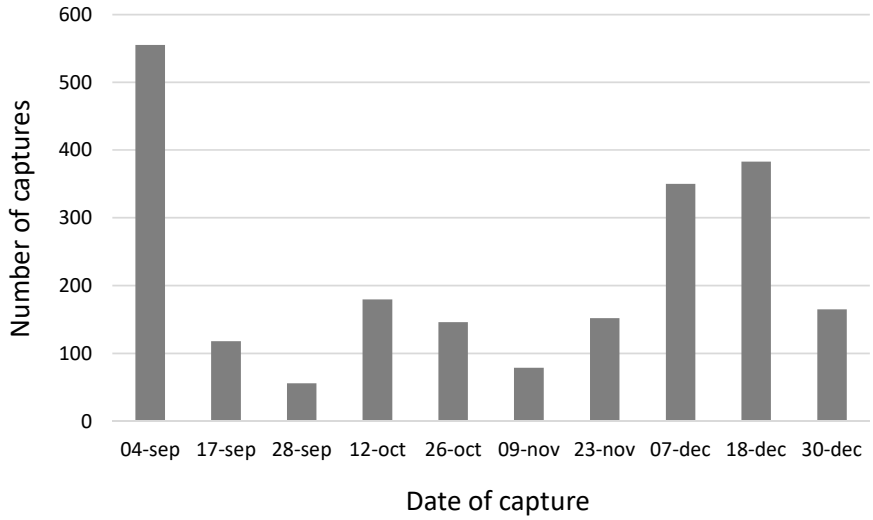

Figure 3. Number of *Xenopus laevis* caught and removed in a wild population from central Chile. Frogs were hand captured with dip nets by two people over 60-min sessions every 2 weeks from September to December 2015. The graph shows a no significant change in *X. laevis* abundance (Spearman rank test, $r_s = 132$, $P = 0.5$).

were visited and its occurrence confirmed if found. New records for *X. laevis* presence were collated along with records from the literature, to create a distribution range map so as to estimate the current extent of occurrence (EOO) of this species in Chile, following the standards of the IUCN (2012). An α-minimum convex polygon (α-convex hull) containing all localities, considering a 5 km buffer area for each point and shaping by suitable environment, namely lentic waters at low altitudes, that in Chile include river mouths, streams, agriculture channels, dams and wells (Lobos and Jaksic 2005; Lobos et al. 2013), was elaborated and the area covered by the polygon estimated using qGIS (QuantumGIS v2.2.0). Inclusion of suitable environment means expanding the distribution considering the knowledge of habitat preferences of the species, based on published information and expert knowledge (IUCN 2012).

Results

Abundance estimation

At the study pond a total of 819 post-metamorphic *X. laevis* were captured during the 200 min search and our non-linear extrapolation model estimated a population size of N(1) = 1,182 frogs (range: 1,168–1,195 [quadratic error]), giving an average density of 13.7 frogs/m^2 (13.6–13.9) of surface water. The efficiency of trapping factor (k) was 0.118. Cumulative captures and the fitted curve along the 10 capture sessions are presented in Figure S1.

Population control

During the 4-month period of 10 capture-and-removal sessions (60 min each) a total of 2,184 post-metamorphic *X. laevis* were removed from the study pond (Figure 3). Of these, 1,697 (77.7%) and 487 (22.3%) were categorized

as juveniles and adults, respectively. Average body size was 37.2 mm (range: 1.3–6.4) and 73.5 mm (6.5–10.1) of SVL in juveniles and adults, respectively. Number of post-metamorphic frogs did not significantly differ over time (Spearman rank test, $r_s = 132$, $P = 0.5$; Figure 3).

Distribution range and extent of occurrence

We created a range map of *X. laevis* in Chile based on 81 records, among 45 obtained from the literature and 36 reported in this study, increasing the invasive range to two new administrative regions in north (Atacama) and central-south (Maule) Chile. We estimated *X. laevis* to occupy an area of 36,055 km^2 of EOO in central and north Chile, divided into three separate populations, two at the lower parts of Copiapó (1,816 km^2) and Limarí rivers (1,304 km^2), and other more widespread from the coastal town of Los Molles to the Mataquito river (32,935 km^2; Figure 1). We found frogs to inhabit lentic waters in both urban and rural environments from sea level to 913 m. A full list including historical and new occurrence records for *X. laevis* in Chile can be found in the supplementary data (Table S1).

Discussion

Our results document the extremely high densities that *X. laevis* can reach in the wild. Based on baited funnel traps and capture-mark-recapture techniques, other studies have reported densities of invasive post-metamorphic *X. laevis* ranging from 0.25 to 8.9 frogs/m^2 of surface water (see below). Our abundance estimate (13.7 frogs/m^2) is considerably higher than those obtained in previous studies. Having differences in capture technique, effort, season and habitat, our results are not necessarily comparable with other density estimates. For example, near to our study site and during a 3-month monitoring period, Lobos and Measey (2002) estimated densities of 0.25 and 0.37 frogs/m^2 at a lagoon and a pond respectively, both within the Metropolitan Region. Also in Chile, Ross et al. (2015) reported a density of 0.18 frogs/m^2 for El Peral lagoon; however, mathematical methodology associated with population size estimation is not provided. McCoid and Fritts (1980) calculated densities of 0.8 and 4.6 frogs/m^2 at two ponds in southern California. Finally, an abundance of 8.9 frogs/m^2 was obtained during five years of monitoring a population in a small pond in south Wales by Measey and Tinsley (1998). Our high population density estimates may be explained in part due to the small size of the study pond, as lower population densities are expected at large water bodies (Wassens et al. 2016). Also, dip netting appeared to be very effective in removing frogs in our study site (small pond), thus allowing a more precise estimation of population size. It is possible that low capture rates, for instance obtained with funnel traps, may lead to an underestimation of true population size (Lockwood et al. 2013).

The total removal of post-metamorphic *X. laevis* from the pond (2,184 frogs during 4 months) was almost the double of our initial population size estimate (1,182 frogs). Therefore, considering that virtually no larval *X. laevis* were captured during the removal period (only one tadpole was captured), immigration of adult *X. laevis* by overland movement (Measey 2016) or by upstream entrance through the drainage brook (Fouquet and Measey 2006) occurred during the 4-month removal period, causing the failure to locally control *X. laevis*. Despite very useful, only a handful of *X. laevis* population control/eradication experiences have been reported (reviewed in Measey et al. 2012). For instance, in France ongoing eradication/mitigation efforts using massive capturing of frogs from ponds have been taking place over the past decade. However, these efforts have shown very little effect on the population density and the population continues to expand every year (Communauté de communes de l'Argentonnais – Agglomération du Bocage Bressuirais 2013). In contrast, successful control management attempts have been also reported. In California, complete extirpation of local isolated and small populations has been achieved by using a variety of techniques, including removal of frogs, gradual draining of ponds and poisoning (Tinsley and McCoid 1996; Crayon 2005). In Barcelona, Spain, *X. laevis* tadpoles in a public garden pond were successfully eradicated by drying the pond and subsequent addition of a solution of copper sulfate during refill to kill any remaining egg or tadpole (Measey et al. 2012). Long-term population management programs have also been successful. Since 2010, a 5-year study assessing the effect of *X. laevis* removal (by seining and trapping) on populations of the Endangered and sympatric *X. gilli* in the Western Cape Province in South Africa, have shown improved population abundance estimates for the endemic and threatened species (De Villiers et al. 2016). In the United Kingdom, an eradication program initiated in 2003 achieved the extirpation of *X. laevis* from Lincolnshire, Humberside by 2011 (Tinsley et al. 2015). Likewise, in Wales *X. laevis* has been apparently eradicated based on intensive trapping and pond management, however adverse weather seems to have played a contributing role in its local extinction, as described from areas with sub-optimal environments (i.e., years with exceptionally cold and dry winters; Tinsley et al. 2015). For instance, the invasion of *X. laevis* in the Isle of Wight, southern England, is believed to be extinct due to extreme weather conditions (Tinsley et al. 2015). More recently, a program to eradicate *X. laevis* from two streams in Portugal was initiated in 2010. After 7 years and using a variety of techniques, including hand netting, electrofishing and use of low concentrations of sodium hypochlorite to kill tadpoles living in cement tanks, the population has been effectively reduced to very low numbers (e.g., from 86 to eight caught adult frogs captured in 2016 in Lage). Due to its restricted distribution and the success so far, it is possible that this program will eradicate *X. laevis* from Portugal during the next few years (Rebelo et al. 2016).

Outside Africa, the most widespread wild populations of *X. laevis* are found in Chile and the Californian ecoregion of the United States and Mexico (Tinsley and McCoid 1996; Measey et al. 2012; Peralta-García et al. 2014). Both regions present Mediterranean climates optimal for the establishment of *X. laevis* (Measey et al. 2012). As a consequence, Mediterranean regions are particularly vulnerable to the impacts of *Xenopus* (De Villiers et al. 2016; Lafferty and Page 1997; Soto-Azat et al. 2016; Vogt et al. 2017; Courant et al. 2018), invasions that are expected to continue to grow according to projections from habitat-suitability models (Lobos et al. 2013; Ihlow et al. 2016; Barbosa et al. 2017; Rödder et al. 2017). Of particular concern is the potential arrival and spread of *X. laevis* in vast territories of Argentina, Australia, northern Africa and China, all under a high risk of invasion (Ihlow et al. 2016; Rödder et al. 2017).

Our estimation of the distribution range (EOO) of this invasive species in Chile (36,055 km^2) is between 1.7 to 3.5 times larger than previously calculated: 21,200 km^2 (Lobos and Jaksic 2005; Lobos et al. 2013) and 10,432 km^2 (Measey et al. 2012). Previous estimations have been done using less presence records of *X. laevis*, while its invasion continues to increase each year (see supplementary data). The new invasive populations described in north and central-south Chile are particularly alarming. The population of Copiapó river is 380 km north of its previous known northern range (Limarí river). Between Copiapó and Limarí, there are two main rivers: Huasco and Elqui and although evidence of *Xenopus* does not exist here, these rivers represent a high possibility of being invaded by these frogs (if not already). The rivers of north of Chile are intensively used for agriculture. It is likely that transportation of agriculture material and products between these rivers has served as main mechanism to the spread of *X. laevis*, and is where focus must be placed to prevent further invasions. In contrast, the populations of Mataquito river in the south, represent an extension of 35 km south than previously reported, and most likely this expansion respond to natural colonization of *X. laevis*. Our results suggest that both the abundance and occurrence of *X. laevis* as an invasive species is greater in Chile than anywhere else. The eradication of *Xenopus* is considered difficult and, with the exception of Portugal, no other management or control operations are currently in place in Mediterranean invaded environments (Rebelo et al. 2010, 2016).

Although early detection and rapid-response actions are key to eradicating alien species from new areas, population control is desirable when eradication is not feasible, particularly when invasive species threaten local biodiversity (Measey et al. 2012; Lockwood et al. 2013). Our study demonstrates that invasive *X. laevis* can reach high abundance in Chile and that removal of individuals in large numbers, was not useful in reducing its population size. We think that our model and methodology to

estimate abundance provide a fast, simple and systematic framework to estimate *X. laevis* density and that might be applicable to other organisms. Our study shows a rapid and ongoing expansion of *X. laevis* in Chile, in both directions north and south, including the first report of *X. laevis* in the north of Chile. Thus, invasive populations of *X. laevis* in Chile have become the largest outside their natural range in Africa, despite a comparable short history of introduction. If no control program is put in place by the Chilean government, is it foreseeable that new water bodies will be invaded faster than previously thought, with subsequent high impacts on aquatic biodiversity. Our results also highlight the difficulties and challenges managing invasive *X. laevis* when well established. However, further assessments on the efficacy of more intensive capture and other control methods, including a combination of mechanical, chemical, biological and/or habitat-management techniques is needed to inform cost-effective strategies to mitigate the impacts and contain the spread of *X. laevis*.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Localities in Chile with records of the African frog (*Xenopus laevis*).
Figure S1. Cumulative capture during 10 search sessions of 20-min each and graphical representation of the model used to estimate the size of our study population of *Xenopus laevis* in central Chile.

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