Research Article

Vulnerability of *Xenopus laevis* to *Gambusia holbrooki*: Can the larval phase of the African clawed frog be the Achilles heel in its invasive potential?

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Abstract

Xenopus laevis has been present in the Chilean Mediterranean region since at least the 1970s. Studies of the global invasion of the African clawed frog have paid little attention to its early life stages, whose environmental requirements and threats are different to those of the adults. This study provides evidence of the vulnerability of *X. laevis* to *Gambusia holbrooki*, as well as information about its phenological development. Reproduction of *X. laevis* in Chile occurs between October and the end of April (austral spring to autumn). An overlap of early and late stages of larvae indicated variability within a clutch. The more recent establishment of *G. holbrooki* in Antumapu irrigation reservoir has resulted in a collapse in the reproduction and adult populations of *X. laevis* at this site.

Key words: Common platanna, larvae, development, competition, mosquitofish, Chile

Introduction

Xenopus laevis (Daudin 1802) is one of the most widely distributed amphibians in the world; it has successively invaded the USA (McCoid and Fritts 1980a, b; Crayon 2005; Krysko et al. 2011), South Wales, UK (Measey 1998; Measey and Tinsley 1998; Measey 2001), France (Fouquet and Measey 2006), Italy (Lillo et al. 2005, 2011), Portugal (Rebelo et al. 2010), Japan (Kobayashi and Hasegawa 2005; Arao and Kitano 2006; Kokuryo 2009), China (Wang et al. 2019) and Chile (Lobos et al. 1999; Lobos and Measey 2002; Lobos and Jaksic 2005). Its distribution includes four continents and global ecological modeling predicts that its distribution should continue to expand, with Mediterranean areas highly vulnerable (Measey et al. 2012).

Xenopus laevis has been recognized since the 1930s as a species with great versatility for use in research (Gurdon and Hopwood 2000; van Sittert and Measey 2016), due to the ease of manipulating it and maintaining it in captivity (adults and larvae), which no doubt explains its presence in laboratories around the world. However, it is its use as a pet that currently represents the most serious threat in its movement globally (Measey 2017). An important part of its natural history is unknown; almost all the knowledge of its larval phase comes from laboratory studies (e.g. Wassersug 1996). Several authors have indicated it may have environmental impacts in invaded areas (Lillo et al. 2011; Kumschick et al. 2017; Courant et al. 2018).

Organisms with complex life cycles such as amphibians (Wilbur 1980) have different requirements in each stage; larvae are sensitive to predation and environmental restrictions such as drought (Alford and Harris 1988). Laboratory studies have indicated that larvae of *X. laevis* perform well in a wide range of temperatures (Walsh et al. 2008). However, little is known about their interactions with the environment (Kruger et al. 2019).

Invasive fish are a relevant agent in the global decline of freshwater aquatic fauna (Casal 2006; Helfman 2007). In this way the presence of exotic fishes have caused a reduction in amphibians' fitness, reduced growth, changes in behavioral activity and greater avoidance behaviour (Nunes et al. 2019). One of their important impacts is the predation of larvae, which has been related to the absence of anti-predator behaviors (Griffiths et al. 2000; Kats and Ferrer 2003; Kruger et al. 2019). Few studies in South America explore the effect of invasive fish on amphibians (Martín-Torrijos et al. 2016). *Gambusia holbrooki* (Girard, 1859) (mosquitofish) has been present in Chile for at least 88 years (Iriarte et al. 2005). Iriarte et al. (2005) indicate that *Gambusia* is widely distributed in the country, along a latitudinal gradient of 2481 km, although recent findings (G. Lobos *pers. obs.*) in the northernmost region of the country (Administrative Region of Arica and Parinacota) indicate a possibility for further expansion in its distribution to 3078 km. Over this wide distribution, the mosquitofish has invaded diverse environments (deserts, Mediterranean and temperate areas); and its distribution overlaps completely with those of *X. laevis* (Mora et al. 2019).

In order to test the hypothesis of vulnerability of *X. laevis* to *Gambusia*, a sampling of tadpoles was conducted in an irrigation reservoir with presence of *Xenopus*, before and after being invaded by mosquitofish; the phenology of the larvae was characterized (seasons in which it is developed on the study site); the susceptibility to predation by *Gambusia* was evaluated through laboratory assays; and finally, from a historic perspective, the effects on the population size at the study site were evaluated.

Materials and methods

Density of Xenopus *larvae, fish and physical parameters of the water*

This study was performed near the city of Santiago, in the Antumapu irrigation reservoir (33°34.027S; 70°37.896W; 623 m elevation). The reservoir is rectangular, with an area of 400 m^2 and 0.8 m of deep, fed by a network of irrigation canals from the Maipo River. The reservoir is one final node of part of this network, since no canals lead from it. The population of African clawed frogs that inhabit it have been studied previously (Lobos 1998; Lobos et al. 1999; Lobos and Measey 2002).

Samples were collected weekly between September 2006 to May 2008, and from September 2017 to March 2018; between 2009 to 2016 sporadic sampling was undertaken in November and December. Four sample sectors were selected in the reservoir, located in the four extremes (2 × 15 m). To estimate *X. laevis* larvae and fish abundance, sampling points were determined by dividing the quadrant length into sampling points located every 1 meter. Then, a dipnet of 1 m diameter was used from the shore; three randomly sweeps in one minute were performed in each of the four sectors, covering an estimated surface area of 2 m^2 each (Shaffer et al. 2000). In each sample dissolved oxygen and water temperature (Hanna HI 9146), pH and conductivity (Hanna HI 991301) were measured.

Larval phenological development of X. laevis

For this purpose forty larvae were selected in each weekly sampling. The first 10 captured in the first sampling of each quadrant in 2006–2007, which were brought to the laboratory in order to establish their larval stages under a stereoscopic microscope (Nieuwkoop and Faber 1967). The larvae were grouped into five strata following the proposal of Walsh et al. (2008): E < 48 (free-living), E 48–51 (small hind-limb buds), E 52–55 (differentiation and flattening of hind feet), E 56–59 (separation of toes and development of hind legs) and E > 59 (mid-metamorphosis).

Gambusia holbrooki *predatory role*

To evaluate the predatory role of *Gambusia holbrooki* on *Xenopus* larvae, trials were performed in plastic containers (30 × 40 × 35 cm) in 20 L of dechlorinated water, where smaller-scale experiments have been proposed in order to analyze both competition and predation relationships on larvae of anurans (Skelly 1994; Kupferberg 1997; Komak and Crossland 2000). The predator fish used was collected in the Santuario de la Naturaleza Quebrada de Córdova (33°26.515S; 71°39.662W), Region de Valparaíso, about 120 km from the city of Santiago (range size 28–40 mm). To standardize the trials, all larvae used (stages 48–51, range size 20–29 mm; Nieuwkoop and Faber 1967) were obtained in the laboratory using a protocol development by Fischer et al. (2000). Fish and larvae were fed daily with commercial food, which was suspended 24 hours before the beginning of each trial. The experiment considered four treatments, taking into account the estimated densities in the dam. Control groups we carried out with either two anuran larvae (17 individuals/m^2) alone or two fish alone (17 individuals/m^2); to evaluate possible interactions, one fish and two larvae (8.3 individuals/m^2 for predator, 17 individuals/m^2 for prey), and two fish and two larvae (17 individuals/m^2 for predator, 17 individual/m^2 for prey), were used. Random treatments were performed and each treatment was replicated four times, which were evaluated every 24 hours

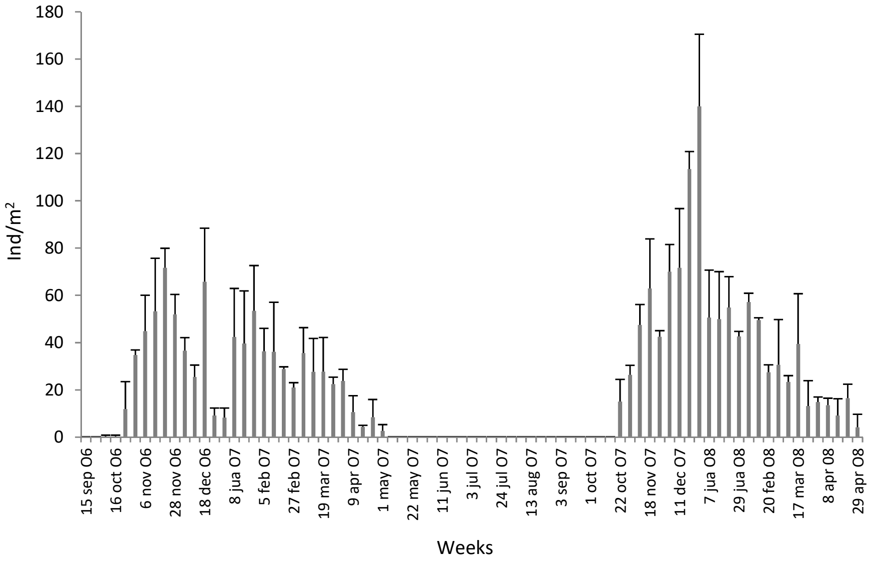

Figure 1. Seasonal fluctuation (mean ± SE) of larval density of *Xenopus laevis* in 2006–2008. Density (ind/m^2) is represented by grey bars.

for a period of 7 days, replacing preyed larvae. Individuals preyed on were replaced by others with similar characteristics (size and stages), to maintain the density relation in all treatments. Mortality, dividing the total number of replaced individuals by the initial number of larva, was also estimated.

Adult population of Xenopus laevis

A study by Lobos and Measey (2002) was repeated in 2014 and 2018, using the same sampling conditions. Adult clawed frogs were captured using simple funnel traps; five traps were set at dusk and collected at dawn at approximately two week intervals (during March and April; three samples). All *X. laevis* caught were sexed using external morphological features (protruding labial lobes in females and formation of nuptial pads on the forearms of males). Animals were then marked with an Passive Integrated Transponder (Biomark Inc). A closed population model using the Rcapture program in R package (R Core Team 2016) was assumed. Lobos and Measey (2002) did not report the mass of the amphibians, so the results were compared to those of Lobos (1998).

Statistical analysis

Differences between means were analyzed with *t*-tests. ANOVA was used in other cases, after testing the normality of the data using the Shapiro-Wilk test. Significant differences ($p < 0.05$) among means were evaluated with the Tukey *a posteriori* test, performed in Infostat (Di Rienzo et al. 2004).

Results

Density of Xenopus *larvae, fish and physical parameters*

Xenopus larvae were observed beginning in October (austral spring) and persisted in the reservoir until the end of April, both in 2006–2007 and 2007–2008 (Figure 1). The highest mean densities were recorded in November,

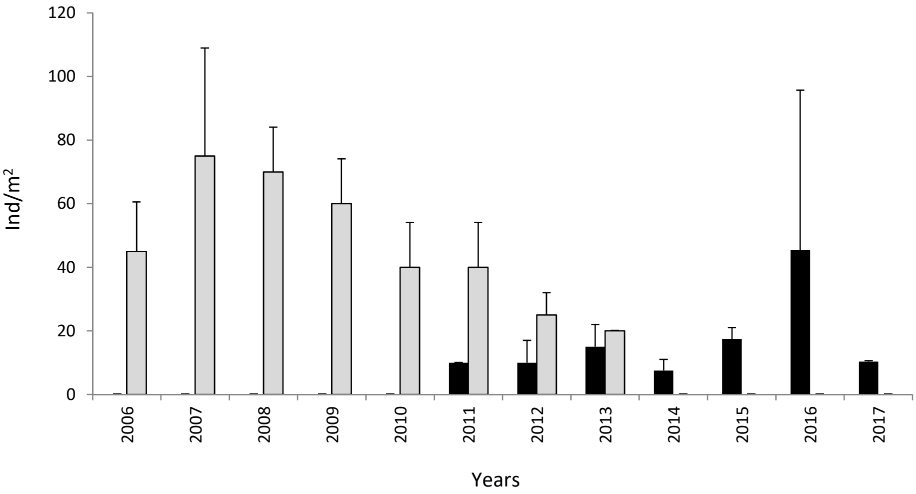

Figure 2. Fluctuation of *Gambusia holbrooki* (black bars) and *Xenopus laevis* larvae (grey bars). Average density (mean ± SE) in 2006 to 2017 for spring and summer in the Antumapu reservoir. The graph demonstrates the replacement of *Xenopus* larvae by the invasive fish in the study site.

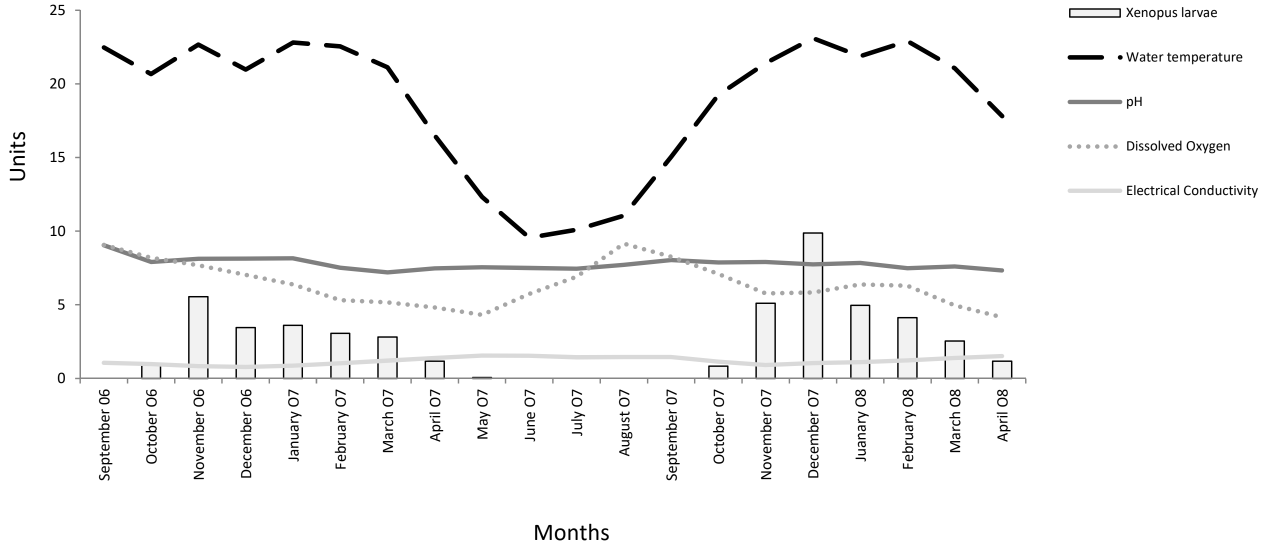

Figure 3. Monthly fluctuation of larval density (ind/m^2), shown in grey bars, in relation to electrical conductivity (mS*cm^{-1}), pH, dissolved oxygen (mg*L^{-1}) and water temperature (°C). Density was divided by 10 for graphic representation.

December, January and February in both periods (late spring and summer). The only significant difference was in December, with mean density being greater in 2007–2008 (*t*-test, $T_{(6;\ 0.95)} = -3.08$, $p = 0.03$). The difference in mean density over the entire sampling period was not significant between years (*t*-test, $T_{(14;\ 0.95)} = 1.01$, $p = 0.33$). Subsequently, until 2013, larvae were observed in the reservoir. From 2011, the dam began to be colonized by *Gambusia*. The samples from 2006 to 2017 indicate clearly the absence of larvae of the African clawed frog in the study site, and its replacement by *Gambusia holbrooki* (Figure 2).

Figure 3 shows the relationship between water parameters and larval density (Table 1). For the development period of *Xenopus* larvae (September to March), the ANOVAs comparing environmental variables for the 2006–2007, 2007–2008 and 2017–2018 (absence of amphibian larvae) found

Table 1. Mean monthly values of physical parameters of the water and larval density of *Xenopus laevis* in the Antumapu resevoir.

Months	Water temperature (°C)	pH	Dissolved oxygen (mg l^{-1})	Conductivity (*u*S cm^{-1})	Density (Ind/m^2)
September 2006	22.47 ± 0.00	9.03 ± 0.00	9.07 ± 0.00	1.06 ± 0.00	0.00 ± 0.00
October 2006	20.67 ± 3.4	7.91 ± 0.30	8.20 ± 1.71	0.98 ± 0.05	9.43 ± 15.07
November 2006	22.66 ± 2.68	8.13 ± 0.36	7.68 ± 1.70	0.83 ± 0.09	55.56 ± 11.06
December 2006	20.98 ± 2.68	8.13 ± 0.35	7.03 ± 1.38	0.78 ± 0.05	34.48 ± 24.22
January 2007	22.81 ± 1.23	8.15 ± 0.27	6.37 ± 1.46	0.86 ± 0.05	36.00 ± 19.40
February 2007	22.55 ± 0.85	7.51 ± 0.27	5.31 ± 1.02	1.03 ± 0.08	30.58 ± 7.28
March 2007	21.13 ± 1.02	7.21 ± 0.28	5.17 ± 0.81	1.20 ± 0.04	28.06 ± 5.59
April 2007	16.48 ± 1.07	7.46 ± 0.26	4.82 ± 1.17	1.38 ± 0.08	11.71 ± 11.71
May 2007	12.32 ± 0.99	7.54 ± 0.27	4.30 ± 1.17	1.55 ± 0.03	0.52 ± 0.00
June 2007	9.55 ± 2.84	7.49 ± 0.30	5.75 ± 0.98	1.54 ± 0.10	0.00 ± 0.00
July 2007	10.10 ± 2.93	7.45 ± 0.22	6.91 ± 0.97	1.43 ± 0.03	0.00 ± 0.00
August 2007	11.05 ± 2.75	7.71 ± 0.21	9.15 ± 0.40	1.45 ± 0.04	0.00 ± 0.00
September 2007	15.04 ± 2.72	8.04 ± 0.21	8.26 ± 0.26	1.45 ± 0.12	0.00 ± 0.00
October 2007	19.23 ± 2.92	7.87 ± 0.19	7.10 ± 0.38	1.14 ± 0.13	8.33 ± 12.07
November 2007	21.41 ± 0.84	7.91 ± 0.19	5.77 ± 0.92	0.91 ± 0.07	51.08 ± 10.83
December 2007	23.11 ± 1.17	7.74 ± 0.33	5.84 ± 0.82	1.03 ± 0.06	98.75 ± 34.44
Juanary 2008	21.88 ± 1.42	7.85 ± 0.34	6.38 ± 0.77	1.11 ± 0.04	49.52 ± 5.03
February 2008	22.92 ± 1.99	7.48 ± 0.29	6.30 ± 0.67	1.23 ± 0.06	41.17 ± 14.42
March 2008	21.06 ± 2.31	7.60 ± 0.18	4.95 ± 1.48	1.39 ± 0.04	25.31 ± 13.15
April 2008	17.81 ± 2.39	7.33 ± 0.15	4.14 ± 1.42	1.52 ± 0.13	11.73 ± 4.90
September 2017	12.06 ± 1.57	7.61 ± 0.24	6.08 ± 1.31	1.43 ± 0.04	0.00 ± 0.00
October 2017	15.83 ± 1.97	7.65 ± 0.41	5.93 ± 0.79	1.30 ± 0.13	0.00 ± 0.00
November 2017	18.30 ± 0.91	8.05 ± 0.16	6.89 ± 1.65	1.13 ± 0.13	0.00 ± 0.00
December 2017	21.33 ± 2.40	7.92 ± 0.15	5.83 ± 0.37	0.91 ± 0.05	0.00 ± 0.00
Juanary 2018	20.42 ± 1.38	7.76 ± 0.23	5.56 ± 1.10	1.16 ± 0.10	0.00 ± 0.00
February 2018	19.07 ± 1.36	7.89 ± 0.14	9.06 ± 0.70	1.21 ± 0.19	0.00 ± 0.00
March 2018	17.99 ± 1.02	7.93 ± 0.16	9.67 ± 2.34	1.46 ± 0.18	0.00 ± 0.00

significant differences in temperature between months (ANOVA $F_{(6,73)} = 12.6$, $p < 0.001$) and years (ANOVA $F_{(2,73)} = 18.2$, $p < 0.001$); Tukey tests found that the means for September and October were lower than the later months, and that 2017–2018 had lower values (median = 17.85, SE ± 0.42) than 2006–2007 (median = 21.38, SE ± 0.42) and 2007–2008 (median = 20.25, SE ± 0.44). No significant difference in dissolved oxygen was found for months (ANOVA $F_{(6,73)} = 4.25$, $p = 0.28$) or years (ANOVA $F_{(2,73)} = 0.43$, $p = 0.65$). The pH varied significantly between months (ANOVA $F_{(6,73)} = 4.25$, $p < 0.001$), but not between years (ANOVA $F_{(2,73)} = 0.68$, $p = 0.52$); the Tukey test found that pH in March was significantly lower than in September, October and November. Electrical conductivity was significantly different among months (ANOVA $F_{(6,73)} = 29.4$, $p < 0.001$) and years (ANOVA $F_{(2,73)} = 57.7$, $p < 0.001$); November and December had lower values than September, October and March, and 2006–2007 had lower values than 2007–2008 and 2017–2018.

Larval development

The earliest larval stages (less than stage 48) were present for at least three months (Figure 4), while larvae of stages 48–51 were recorded until late summer (February). Later larval stages (above stage 59) were recorded for at least five months. Larvae were not recorded from the beginning of autumn through winter.

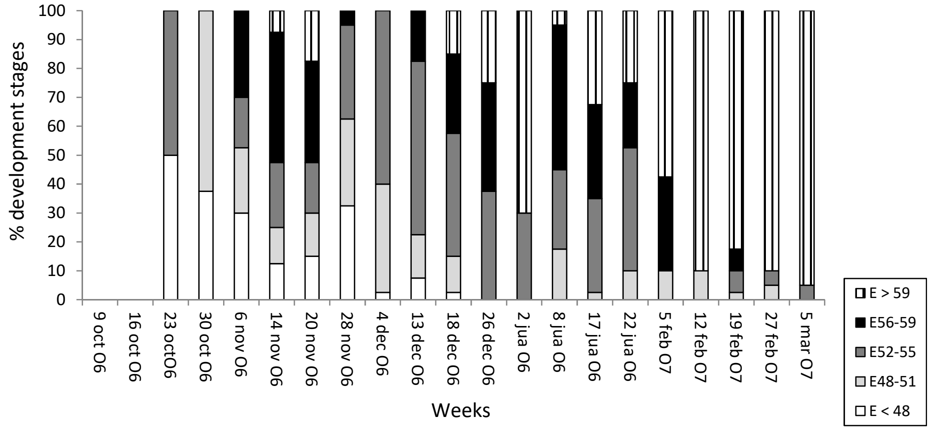

Figure 4. Weekly larval development of *Xenopus laevis* using the criteria of Nieuwkoop and Faber (1967), in the Antumapu reservoir shows differential development of the larvae. The proportions in states are represented in a color scale proposed by Walsh et al. (2008), which distinguished four classes of states: E < 48 (free-living), E 48–51 (small hind-limb buds), E 52–55 (differentiation and flattening of hind feet), E 56–59 (separation of toes and development of hind legs) and E > 59 (mid metamorphosis).

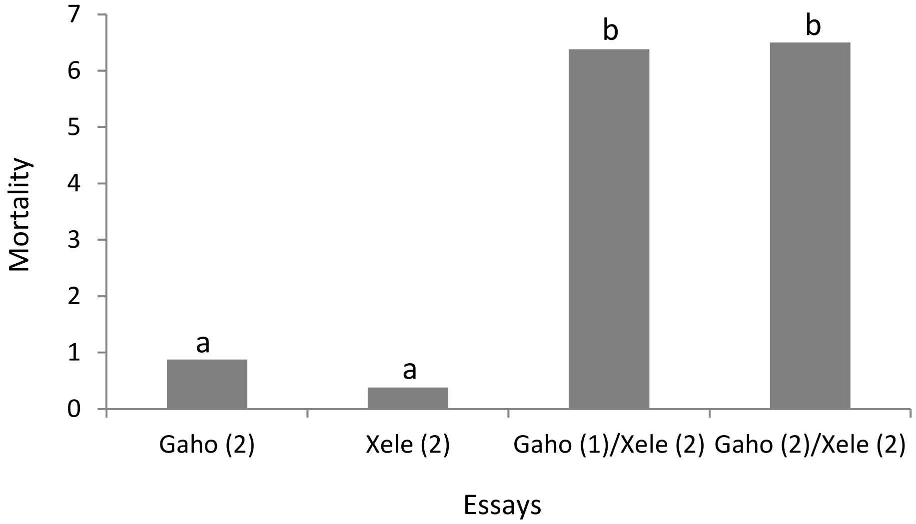

Figure 5. Laboratory trials to evaluate the mortality in controls (larvae and fish alone) and predatory role of *Gambusia* on larvae of *Xenopus*. The trials showed significant differences in the mean mortality (shown by letters above the grey bars) in the treatments with fish, both with one fish and two African clawed frog larvae and with two fish and two larvae. Gaho = *Gambusia holbrooki* and Xele = *Xenopus laevis*.

Gambusia holbrooki *predatory role*

The laboratory trials (Figure 5) show a high predation rates of *Gambusia* on *Xenopus* larvae (ANOVA $F_{(3, 12)} = 82.7$, $p < 0.001$), both in the treatments with low and high density of predators. The control groups, with larvae and fish alone, did not show any evidence of interference within the assays (stress in the larvae or cannibalism between fish).

Adult population of Xenopus laevis

The population size of *X. laevis* was estimated to be 43 (range = 20.1–98.4) in 2001 (Lobos and Measey 2002). The estimation for 2014 was similar

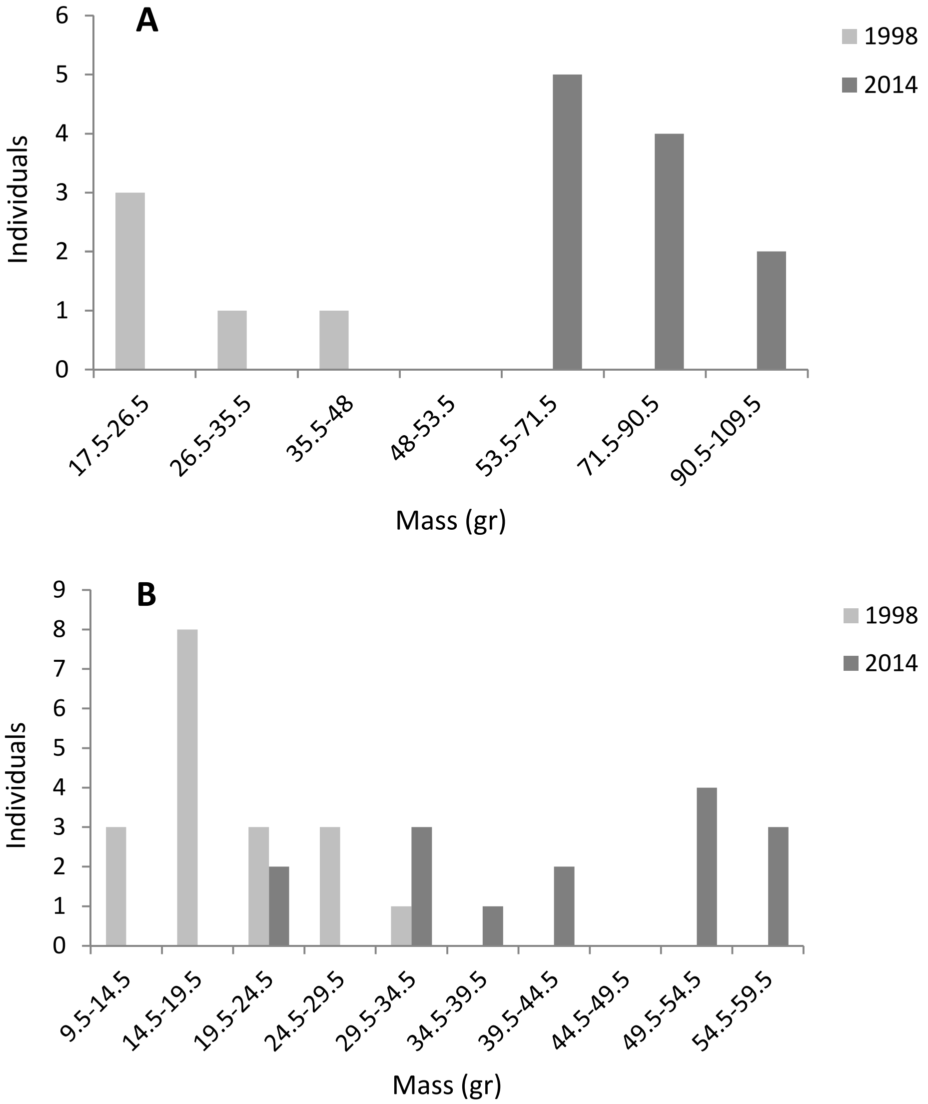

Figure 6. Comparison of mass (grams) of individuals collected in 1998 and 2014, showing greater representation of heavier individuals (assumed to be older) in 2014. A) Females: the lack of individuals in the lower classes is notable; B) Males: there are individuals in the lower classes, there are larger size classes not present in 1998, which suggests an ageing population that declined in 2018. Weight ranges were those defined by Lobos (1998).

(mean = 44.3, SD ± 13.3). However, no adults were captured in 2018. There were differences between sexes and between years in the mass recorded in 1998 and 2014 (Figure 6A, B).

Discussion

The global invasion of *Xenopus laevis* provides an interesting model for biological invasions, especially for its exposure to different demographic, environmental and evolutionary patterns (Facon et al. 2006). However, such models require information on the pre-adult stages (larvae, premetamorphic, postmetamorphic), which have received little attention. Information on the larvae is especially important for *X. laevis*, whose larvae are highly specialized compared to other anurans; they have a microphagous diet, aerial respiration, and specialized caudal myology and spinal cord (Wassersug 1996).

One of the few studies of the tadpoles of *Xenopus* in an invaded area (Moreira et al. 2017) found that lentic environments produce a greater number of metamorphic, larger tadpoles and metamorphic, and earlier-maturing metamorphic larvae than lotic environments; however, lotic environments may also contribute to the recruitment of individuals, in addition to the more favorable lentic sites.

Larval development is restricted to spring and summer in Chile, as has been observed in other places in the invaded area of Chile (G. Lobos *pers. obs.*). The complete development of *X. laevis* from egg to metamorphosis would last from two to three months (Nieuwkoop and Faber 1968; Wu and Gerhart 1991). The presence of early stages (less than 48) for at least three months in the reservoir and the presence of more-developed larvae for at least five months (overlapping of different stages), likely indicate a high developmental variability within a clutch (J. Measey *pers. comm.*). This may result from differential growth due to food availability and environmental conditions; then, fluctuations in abundance represent mortality events. Several studies have evaluated the effect of environmental conditions on the swimming speed of larvae and capacity for movement in the juvenile stage (van Buskirk and Saxer 2001; Alvarez and Nicieza 2002). It has also been shown that the body size of larvae and environmental temperature are related to the duration of metamorphosis (Downie et al. 2004; Walsh et al. 2008), a stage considered to be high risk due to vulnerability to predation (Williams 1966). At 21 °C, metamorphosis lasts about 8 days (Huang et al. 2001), but Walsh et al. (2008) showed that at lower temperatures (18 °C) it lasted more than twice as long as at high temperatures (24 and 30 °C).

The autumn and winter of the Chilean Mediterranean region were unfavorable for the reproduction of *X. laevis* (mean = 9.55 °C, SD ± 2.84 for June 2007, mean = 15.04 °C, SD ± 2.72 for September). The mean annual temperatures for 2017–2018, when larvae were not recorded from the reservoir, were lower than those of 2006–2007 and 2007–2008; although the summer temperatures (December mean = 21.33, SD ± 2.44, January mean = 20.42, SD ± 1.38 and February mean = 19.07, SD ± 1.36) were within the optimum range for *Xenopus* development. The absence of reproduction was noted at least since 2014, after *Gambusia holbrooki* invaded the reservoir in 2011; this species was not found in the reservoir or in the stomach contents of *X. laevis* in earlier studies (Lobos et al. 1999; Lobos and Measey 2002). Invasive fish affect anurans by consuming eggs, larvae and/or adults (Webb and Joss 1997; Bull and Marx 2002; Reshetnikov 2003; Welsh et al. 2006; Jara and Perotti 2006). In relation to smaller-scale experiments, these have been conceived as a methodology to explore competence relationships (Kupferberg 1997) and predation on anuran larvae (Skelly 1994; Komak and Crossland 2000). The laboratory trials showed a clear predatory tendency (almost 100% predation); in spite of the small size of the fish (not greater than 4 cm) (Pérez-Bote and López 2005), they readily consumed the larvae. Interestingly in California (USA), a low predatory capacity of *Xenopus* adults over *Gambusia* has been reported (Lafferty and Page 1997; McCoid and Fritts 1980b). In a global compilation of adult diets of *X. laevis* (Courant et al. 2017), both in native as well as on invasive populations, it was highlighted that the presence of fish was incidental.

Anti-predator behavior in anurans (Griffiths et al. 2000; Kats and Ferrer 2003; Nunes et al. 2019) has been associated with a genetic component (Kats et al. 1988) and an acquired component (Mirza et al. 2006). Defensive mechanisms developed by larvae include the search for refuges (Mirza et al. 2006; Ferrari et al. 2008), cryptic coloration (Wassersug 1971) and toxins with disagreeable taste (Brodie and Formanowicz 1987). Other aspects influencing larval survival are related to its level of activity (Skelly 1994; for predatory risk by dragonfly), age classes and size of predators (Kupferberg 1997; for impact evaluation by bullfrog) and ontogenetic stage of prey (Komak and Crossland 2000; for predation by *Gambusia*). In the laboratory assays, it was observed that fish devoured all larvae that were presented to them, excluding the presence of either toxins or cryptic behaviors in front of this kind of predator. In Australia (Komak and Crossland 2000), *Gambusia* were significant predators of all aquatic life-history stages of a native anuran and preyed only upon hatchling and early stages of invasive *Rhinella marina*. In this context, it has been reported that *Xenopus laevis* larvae are capable of reducing their activity (movement) in the presence of invertebrate predators (Kruger et al. 2019). This mechanism appears inadequate for more active predators such as *Gambusia*.

Adult population size was not different between 2002 and 2014, and when compared to 1998, the population of 2014 showed a greater representation of heavier individuals (assumed to be older). In relation to adults, they can again invade the reservoir by occurrence of overland migrations (De Villiers and Measey 2017). In this context, the absence of captures in 2018 is also notable; lack of reproduction in the presence of non-native fish was also reported in another area of central Chile (Lobos et al. 2014). Several studies have referred to the potential invasiveness of *X. laevis* (Measey et al. 2012; Lobos et al. 2013; Barbosa et al. 2017); biotic and abiotic restrictions on the larvae of this anuran may be critical for the success of its advance globally.

In the Chilean Mediterranean region, over almost 50 years, *X. laevis* has had to adjust to the bioclimatic region, which has allowed lessons to be learned about the threats for its early life stages. In this way, this study recognizes that the presence of non-native fish can be a factor that modulates the expansion and distribution of *Xenopus laevis* in the invaded areas.

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References

Alford RA, Harris RN (1988) Effects of larval growth history on anuran metamorphosis. *The American Naturalist* 131: 91–106, https://doi.org/10.1086/284775

Alvarez D, Nicieza A (2002) Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. *Oecologia* 131: 186–195, https://doi.org/10.1007/s00442-002-0876-x

Arao K, Kitano T (2006) *Xenopus laevis* from Hamamatsu City, Shizuoka Prefecture, Japan. *Bulletin of the Herpetological Society of Japan* 2006(1): 17–19

Barbosa FG, Both C, Araújo MB (2017) Invasive American bullfrogs and African clawed frog in South America: high suitability of occurrence in biodiversity hotspots. *Zoological Studies* 56: 28, https://doi.org/10.6620/ZS.2017.56-28

Brodie ED, Formanowicz DR (1987) Antipredator mechanisms of larval anurans: protection of palatable individuals. *Herpetologica* 43: 369–373, https://www.jstor.org/stable/3892504

Bull EL, Marx DB (2002) Influence of fish and habitat on amphibian communities in high elevation lakes in northeastern Oregon. *Northwest Science* 76: 240–248

Casal C (2006) Global documentation of fish introductions: the growing crisis and recommendations for action. *Biological Invasions* 8: 3–11, https://doi.org/10.1007/s10530-006-1833-0

Courant J, Vogt S, De Villiers A, Marques R, Measey J, Secondi J, Rebelo R, De Busschere C, Ihlow F, Backeljau T, Rödder D, Herrel A (2017) Are invasive populations characterized by a broader diet than native populations? *PeerJ* 5: e3250, https://doi.org/10.7717/peerj.3250

Courant J, Secondi J, Vollette J, Herrel A, Thirion JM (2018) Assessing the impacts of the invasive frog, *Xenopus laevis*, on amphibians in western France. *Amphibia-Reptilia* 39: 219–227, https://doi.org/10.1163/15685381-17000153

Crayon JJ (2005) Species account: *Xenopus laevis*. In: Lannoo MJ (ed), Amphibian Declines: The Conservation Status of United States Species, Berkeley (CA). University of California Press, pp 522–525

De Villiers FA, Measey J (2017) Overland movement in African clawed frogs (*Xenopus laevis*): empirical dispersal data from within their native range. *PeerJ* 5: e4039, https://doi.org/10.7717/peerj.4039

Di Rienzo JA, Balzarini M, Gonzalez L, Casanoves F, Tablada M, Robledo CW (2004) InfoStat, version 2004. Infostat Group, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina

Downie JR, Bryce R, Smith J (2004) Metamorphic duration: an under-studied variable in frog life histories. *Biological Journal of the Linnean Society* 83: 261–272, https://doi.org/10.1111/j.1469-7998.2007.00367.x

Facon B, Genton BJ, Shykoff J, Jarne P, Estoup A, David P (2006) A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution* 21: 130–135, https://doi.org/10.1016/j.tree.2005.10.012

Ferrari MC, Messier F, Chivers DP (2008) Larval amphibians learn to match antipredator response intensity to temporal patterns of risk. *Behavioral Ecology* 19: 980–983, https://doi.org/10.1093/beheco/arn056

Fischer WJ, Koch WA, Elepfandt A (2000) Sympatry and hybridization between the clawed frogs *Xenopus laevis laevis* and *Xenopus muelleri* (Pipidae). *Journal of Zoology (London)* 252: 99–107, https://doi.org/10.1111/j.1469-7998.2000.tb00824.x

Fouquet A, Measey GJ (2006) Plotting the course of an African clawed frog invasion in western France. *Animal Biology* 56: 95–102, https://doi.org/10.1163/157075606775904722

Griffiths RA, Blumstein DT, Evans CS (2000) Training Captive-Bred or translocated animals to avoid predators. *Conservation Biology* 14: 1317–1326, https://doi.org/10.1046/j.1523-1739.2000.99326.x

Helfman GS (2007) Fish conservation: a guide to understanding and restoring global aquatic biodiversity and fishery resources. Island Press, 600 pp

Gurdon JB, Hopwood N (2000) The introduction of *Xenopus laevis* into developmental biology: of empire, pregnancy testing and ribosomal genes. *International Journal of Developmental Biology* 44: 43–50

Huang H, Cai L, Remo BF, Brown DD (2001) Timing of metamorphosis and the onset of the negative feedback loop between the thyroid gland and the pituitary is controlled by type II iodothyronine deiodinase in *Xenopus laevis*. *Proceedings of the National Academy of Sciences USA* 98: 7348–7353, https://doi.org/10.1073/pnas.131198998

Iriarte JA, Lobos GA, Jaksic FM (2005) Invasive vertebrate species in Chile and their control and monitoring by governmental agencies. *Revista Chilena de Historia Natural* 78: 143–154, https://doi.org/10.4067/S0716-078X2005000100010

Jara FG, Perotti MG (2006) Variación ontogenética en la palatabilidad de los renacuajos de *Bufo spinulosus papillosus*, Philippi, 1902 (Anura, Bufonidae). *Cuadernos de Herpetologia* 19(2): 37–42

Kats LB, Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions* 9: 99–110, https://doi.org/10.1046/j.1472-4642.2003.00013.x

Kats LB, Petranka JW, Sih A (1988) Antipredator Defenses and the Persistence of Amphybian larvae with Fishes. *Ecology* 69: 1865–1870, https://doi.org/10.2307/1941163

Kobayashi R, Hasegawa M (2005) Can the African clawed frog *Xenopus laevis* become established in Japan?-An inference from recent distribution records in the Kanto plain. *Bulletin of the Herpetological Society of Japan* 2: 169–173

Kokuryo Y (2009) A survey of feral populations of African clawed toad in Shizuoka Prefecture. *Bulletin of the Herpetological Society of Japan* 2: 103–106

Komak S, Crossland MR (2000) An assessment of the introduced mosquitofish (*Gambusia affinis holbrooki*) as a predator of eggs, hatchlings and tadpoles of native and non-native anurans. *Wildlife Research* 27: 185–189, https://doi.org/10.1071/WR99028

Kruger N, Measey J, Herrel A, Secondi J (2019) Antipredator strategies of the invasive African clawed frog *Xenopus laevis* to native and invasive predators in western France. *Aquatic Invasions* 14: 433–443, https://doi.org/10.3391/ai.2019.14.3.03

Krysko KL, Burgess JP, Rochford MR, Gillette CR, Cueva D, Enge KM, Somma LA, Stabile JL, Smith DC, Wasilewski JA, Kieckhefer III GN, Granatosky MC, Nielsen SV (2011) Verified non-indigenous amphibians and reptiles in Florida from 1863 through 2010: outlining the invasion process and identifying invasion pathways and stages. *Zootaxa* 3028: 1–64, https://doi.org/10.11646/zootaxa.3028.1.1

Kumschick S, Vimercati G, De Villiers FA, Mokhatla MM, Davies SJ, Thorp CJ, Rebelo AD, Measey GJ (2017) Impact assessment with different scoring tools: how well do alien amphibian assessments match? *NeoBiota* 33: 53–66, https://doi.org/10.3897/neobiota.33.10376

Kupferberg S (1997) Bullfrog (*Rana catesbeiana*) Invasion of a California River: The Role of Larval Competition. *Ecology* 78: 1736–1751, https://doi.org/10.2307/2266097

Lafferty KD, Page CJ (1997) Predation on the endangered tidewater goby, *Eucyclogobius newberryi*, by the introduced African clawed frog, *Xenopus laevis*, with Notes on the frog’s parasites. *Copeia* 3: 589–592, https://doi.org/10.2307/1447564

Lillo F, Marrone F, Sicilia A, Castelli G, Zava B (2005) An invasive population of *Xenopus laevis* (Daudin, 1802) in Italy. *Herpetozooa* 18: 63–64

Lillo F, Faraone FP, Lo Valvo M (2011) Can the introduction of *Xenopus laevis* affect native amphibian populations? Reduction of reproductive occurrence in presence of the invasive species. *Biological Invasions* 13: 1533–1541, https://doi.org/10.1007/s10530-010-9911-8

Lobos G (1998) Antecedentes ecológicos del sapo africano *Xenopus laevis* (Pipidae) en la zona central de Chile. Memoria para optar al título de Médico Veterinario. Facultad de Ciencias Veterinarias y Pecuarias. Universidad de Chile, 49 pp

Lobos G, Jaksic FM (2005) The ongoing invasion of African clawed frog (*Xenopus laevis*) in Chile: causes of concern. *Biodiversity and Conservation* 14: 429–439, https://doi.org/10.1007/s10531-004-6403-0

Lobos G, Measey GJ (2002) Invasive populations of *Xenopus laevis* (Daudin) in Chile. *Herpetological Journal* 12: 163–168

Lobos G, Cattan P, López M (1999) Antecedentes de la ecología trófica del sapo africano *Xenopus laevis* en la zona central de Chile. *Boletín del Museo Nacional de Historia Natural de Chile* 48: 7–18

Lobos G, Cattan P, Estades C, Jaksic F (2013) Invasive African clawed frog *Xenopus laevis* in southern South America: key factors and predictions. *Studies on Neotropical Fauna and Environment* 48: 1–12, https://doi.org/10.1080/01650521.2012.746050

Lobos G, Hernández J, Jaksic F (2014) Antropización del paisaje e invasión de vertebrados exóticos en una quebrada de Chile central. In: Jaksic F, Castro S (eds), Invasiones biológicas en Chile: causas globales e impactos locales. Ediciones Universidad Católica de Chile, pp 393–414

Martín-Torrijos L, Sandoval-Sierra JV, Muñoz J, Diéguez-Uribeondo J, Bosch J, Guayasamin JM (2016) Rainbow trout (*Oncorhynchus mykiss*) threaten Andean amphibians. *Neotropical Biodiversity* 2: 26–36, https://doi.org/10.1080/23766808.2016.1151133

McCoid MJ, Fritts TH (1980a) Observations of feral populations of *Xenopus laevis* (Pipidae) in southern California. *Bulletin of the Southern California Academy of Sciences* 79: 82–86

McCoid MJ, Fritts TH (1980b) Notes on the diet of a feral population of *Xenopus laevis* (Pipidae) in California. *Southwestern Naturalist* 25: 272–275, https://doi.org/10.2307/3671256

Measey GJ (1998) Diet of feral *Xenopus laevis* in South Wales, UK. *Journal of Zoology (London)* 246: 287–298, https://doi.org/10.1111/j.1469-7998.1998.tb00159.x

Measey GJ (2001) Growth and ageing of *Xenopus laevis* (Daudin) in South Wales, UK. *Journal of Zoology (London)* 254: 547–555, https://doi.org/10.1017/S0952836901001054

Measey GJ (2017) Where do African clawed frogs come from? An analysis of trade in live *Xenopus laevis* imported into the USA. *Salamandra* 53(3): 398–404

Measey GJ, Tinsley RC (1998) Feral *Xenopus laevis* in South Wales. *Herpetological Journal* 8: 23–27, https://doi.org/10.2307/1447816

Measey GJ, Rödder D, Green SL, Kobayashi R, Lillo F, Lobos G, Rebelo R, Thirion JM (2012) Ongoing invasions of the African clawed frog, *Xenopus laevis*: a global review. *Biological Invasions* 14: 2255–2270, https://doi.org/10.1007/s10530-012-0227-8

Mirza RS, Ferrari MCO, Kiesecker JM, Chivers DP (2006) Responses of American toad tadpoles to predation cues: behavioural response thresholds, threat-sensitivity and acquired predation recognition. *Behaviour* 143: 877–889, https://doi.org/10.1163/156853906778017926

Mora M, Pons DJ, Peñafiel-Ricaurte A, Alvarado-Rybak M, Lebuy S, Soto-Azat C (2019) High abundance of invasive African clawed frog *Xenopus laevis* in Chile: challenges for their control an update invasive distribution. *Management of Biological Invasions* 10: 377–388, https://doi.org/10.3391/mbi.2019.10.2.11

Moreira FD, Marques R, Sousa M, Rebelo R (2017) Breeding in both lotic and lentic habitats explains the invasive potential of the African clawed frog (*Xenopus laevis*) in Portugal. *Aquatic Invasions* 12: 565–574, https://doi.org/10.3391/ai.2017.12.4.12

Nieuwkoop PD, Faber J (1967) Normal table of development of *Xenopus laevis* (Daudin). North-Holland Publications, Amsterdam, 188 pp

Nunes AL, Fill JM, Davies SJ, Louw M, Rebelo AD, Thorp CJ, Vimercati G, Measey J (2019) A global meta-analysis of the ecological impacts of alien species on native amphibians. *Proceeding of the Royal Society B* 286, https://doi.org/10.1098/rspb.2018.2528

Pérez-Bote JL, López MT (2005) Life-history pattern of the introduced eastern mosquitofish, *Gambusia holbrooki* (Baird & Girard, 1854), in a Mediterranean-type river: the River Guadiana (SW Iberian Peninsula). *Italian Journal of Zoology* 72: 241–248, https://doi.org/10.1080/11250000509356678

R Core Team (2016) R: a language and environment for statistical computing. Version 3.3.1. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rebelo R, Amaral P, Bernardes M, Oliveira J, Pinheiro P, Leitão D (2010) *Xenopus laevis* (Daudin 1802), a new exotic amphibian in Portugal. *Biological Invasions* 12: 3383–3387, https://doi.org/10.1007/s10530-010-9757-0

Reshetnikov AN (2003) The introduced fish, Rotan (*Perccottus glenii*) depresses populations of aquatic animals (macroinvertebrates, amphibians, and a fish). *Hydrobiologica* 510: 83–90, https://doi.org/10.1023/B:HYDR.0000008634.92659.b4

Shaffer HB, Alford RA, Wooward VD, Richards SJ, Altig RG, Gascon C (2000) Quantitative sampling of amphibian larvae. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayek LA, Foster MS (eds), Measuring and monitoring biological diversity, standard methods for amphibians. Smithsonian Institution Press, pp 130–141

Skelly DK (1994) Activity level and the suceptibility of anuran larvae to predation. *Animal Behavioral* 47: 465–468, https://doi.org/10.1006/anbe.1994.1063

van Buskirk J, Saxer G (2001) Delayed costs of an induced defense in tadpoles? Morphology, hopping, and development rate at metamorphosis. *Evolution* 55: 821–829, https://doi.org/10.1554/0014-3820(2001)055[0821:DCOAID]2.0.CO;2

van Sittert L, Measey, GJ (2016) Historical perspectives on global exports and research of African clawed frog (*Xenopus laevis*). *Transactions of the Royal Society of South Africa* 71: 157–166, https://doi.org/10.1080/0035919X.2016.1158747

Walsh PT, Downie JR, Monaghan P (2008) Plasticity of the duration of metamorphosis in the African clawed toad. *Journal of Zoology (London)* 274: 143–149, https://doi.org/10.1111/j.1469-7998.2007.00367.x

Wang S, Houg Y, Measey J (2019) An established population of African clawed frog, *Xenopus laevis* (Daudin, 1802) in mainland China. *BioInvasions Records* 8: 457–464, https://doi.org/10.3391/bir.2019.8.2.29

Wassersug R (1971) On the comparative palatability of some dry-season tadpoles from Costa Rica. *American Midland Naturalist* 86: 101–109, https://doi.org/10.2307/2423690

Wassersug R (1996) The biology of *Xenopus* tadpoles. In: Tinsley RC, Kobel HR (eds), The biology of *Xenopus*. Clarendon press, Oxford, pp 195–211

Webb CE, Joss J (1997) Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? *Australian Journal of Zoology* 30: 316–324, https://doi.org/10.7882/AZ.1997.007

Welsh HH, Pope KL, Boiano D (2006) Sub-alpine amphibian distributions related to species palatability to non-native salmonids in the Klamath mountains of northern California. *Diversity and Distributions* 12: 298–309, https://doi.org/10.1111/j.1366-9516.2006.00254.x

Wilbur HM (1980) Complex life-cycles. *Annual Review of Ecology, Evolution and Systematics* 11: 67–93, https://doi.org/10.1146/annurev.es.11.110180.000435

Williams G (1966) Adaption and natural selection. Princeton, NJ: Princeton University Press, 328 pp

Wu M, Gerhart J (1991) Raising *Xenopus* in the laboratory. *Methods in Cell Biology* 36: 3–18, https://doi.org/10.1016/S0091-679X(08)60269-1