

Research Article

Breeding in both lotic and lentic habitats explains the invasive potential of the African clawed frog (*Xenopus laevis*) in Portugal

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Abstract

The African clawed frog is a successful invader in several countries throughout the world. Although it may be found in both lentic and lotic habitats, its use of both habitats for breeding has not been documented. Nonetheless, in its Portuguese invasive range this frog was found to breed in small streams, as well as in ponds. We recorded all the sites where *Xenopus laevis* bred during a 7-year period (2010–2016) in the entire Portuguese invasive range. In 2015 and 2016, we measured snout-vent length of 970 tadpoles from eleven sites, and the size and body condition at the end of metamorphosis of 91 metamorphs from four sites. To assess the size at which reproductive investment begins, we dissected and weighed the gonads of 409 juveniles and small adults. We found that the species can produce metamorphs in both habitats, but their numbers were much higher in lentic sites. Furthermore, tadpoles and metamorphs from lentic sites were much larger than those from lotic sites. Body condition of metamorphs was similar across all sites. Gonad development was size-dependent, and we estimate that larger metamorphs from lentic sites will mature sooner and may reproduce in the following year, while smaller metamorphs will need an extra year. Our results suggest that while lentic sites are most likely to be responsible for population booms, the hitherto unknown reproduction in lotic sites may contribute to the maintenance of the invasive population even in the absence of lentic sites.

Key words: Anura, gonad development, invasive species, larval growth, size at metamorphosis

Introduction

Climate matching and propagule pressure are two of the most important variables in predicting the establishment success and expansion of invasive species (Lockwood et al. 2007; Capinha et al. 2013). In invaded areas, factors that enhance reproductive success will contribute to an increase in density and impacts, and will foster spread to new areas. High quality propagules produced at favorable sites will thus play an important part in the invasion process, due to their higher survival and, after sexual maturation, higher reproductive output (Tejedo 1992; Lockwood et al. 2005; Cabrera-Guzmán et al. 2013a).

Invasive amphibian species have caused ecological and economic damage worldwide (Measey et al. 2016). The availability and overall quality of aquatic breeding sites is of particular importance for species that have a free-swimming larval stage. Growth and development of anuran tadpoles is highly plastic and different phenotypes can be induced by, for example, food quality and availability (Kupferberg et al. 1994; Álvarez and Nicieza 2002; San Sebastián et al. 2015), inter and intraspecific competition (Kupferberg 1997; Relyea 2002; Cabrera-Guzmán et al. 2013b), temperature (Álvarez and Nicieza 2002; Walsh et al. 2008a, c) or predator presence (Relyea 2002; Walsh et al. 2008b; Nunes et al. 2014). Body size variability is commonly found in most of these studies, and the

assumption that a large size at metamorphosis constitutes an advantage later in life is widely accepted. Larger metamorphic size increases adult survival and fitness in many anurans and has been associated with earlier maturity, larger adult sizes and higher fecundity in females (Smith 1987; Tejedo 1992; Altwegg and Reyer 2003; Cabrera-Guzmán et al. 2013a).

The African clawed frog, *Xenopus laevis* (Daudin, 1802), is native to sub-Saharan Africa and has become invasive in multiple countries throughout the world, mainly as a consequence of its common usage in laboratories and the pet trade (Measey et al. 2012). In Portugal, the species was discovered in Laje stream in 2006 and in Barcarena stream in 2008, both located in Oeiras County, about 20 km West of Lisbon (Rebelo et al. 2010). This population probably results from a single unintentional introduction in the winter of 1979/1980 in Laje stream, a hypothesis that was recently supported by genetic data (De Busschere et al. 2016). Although a large part of the country is climatically suitable for *X. laevis* (Measey et al. 2012; Ihlow et al. 2016), its distribution has been limited to these two streams for almost 40 years. An eradication program for this frog began in 2010 (Rebelo et al. 2015), and since then tadpoles have been caught and removed from several lotic and lentic sites. In 2014 there was a large invasion in one of the streams (Barcarena); almost 5000 specimens were caught in an approximately 5300 meter stretch (Rebelo et al. 2015). Several breeding sites were suspected to have contributed to this invasion, and in 2015 and 2016 there was a greater effort to find them. Although *X. laevis* can live in rivers (Tinsley et al. 1996), its use of lotic habitats is thought to be mostly for dispersion routes (Fouquet and Measey 2006; Faraone et al. 2008; Measey 2016). Nonetheless, its tadpoles have been discovered in the stomachs of adult *X. laevis* living in lotic habitats in Chile (Lobos and Jaksic 2005), California and Portugal (Courant et al. 2017), suggesting that this species attempts to reproduce in such habitats. The contribution of lotic sites to the maintenance and spread of *X. laevis* invasive populations is yet to be examined.

Almost all anurans display a preference for a particular type of reproductive habitat, with some favouring lotic while others prefer lentic habitats with different hydroperiods (e.g., Van Buskirk 2003; Johansson et al. 2016). Only a minority of species use both lentic and lotic habitats consistently (Johansson et al. 2016) of which some become invasive (e.g. *Rhinella marina* – Evans et al. 1996; *Xenopus laevis* – this work). Evidence of morphological differences in amphibian larvae of the same species developing in

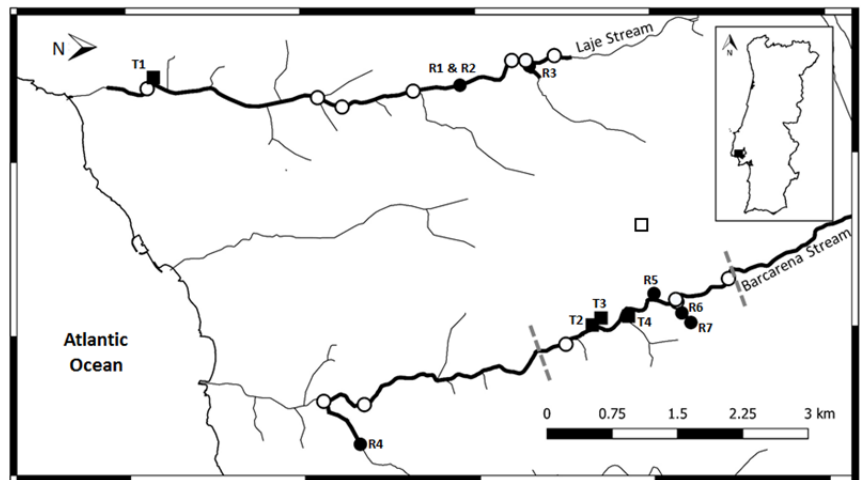
both environments is rare in the literature (e.g. Petranka 1984). However, understanding the comparative importance of lotic and lentic habitats to the recruitment and body size of newly metamorphosed specimens may aid in the management of invasive populations (Fuller et al. 2011; Sepulveda et al. 2015). In this study we compared the quality of lotic and lentic breeding sites by measuring *X. laevis* tadpole abundance, stage-specific size, metamorphic size, body condition, and time at which metamorphosis started. Furthermore, we estimated average body size at the onset of gonad development for both sexes and the difference in time required to reach that threshold for large and small metamorphs. Given that in its native range *X. laevis* breeds in ponds (Tinsley et al. 1996), we expected tadpoles that grow in lentic sites to develop and metamorphose with a larger size and a better body condition compared to those developing in lotic sites. We also expected to find a size threshold for gonad development, enabling the estimation of the time saved until first reproduction by metamorphosing at a larger size.

Methods

Study area and capture of Xenopus laevis

The study area is composed of two small river basins (Laje and Barcarena) that flow parallel to each other over the final 8 km of their course. The maximum known distribution of *X. laevis* stretches along 5.86 km of the main stream in Laje basin, plus one of its tributaries, and 6.39 km of main stream in Barcarena basin, plus three tributaries. The physiography of both river basins is very similar: i) natural lentic habitats are absent due to steep gradients, but there are several garden and cement irrigation ponds nearby, as well as golf course lakes; ii) both streams are permanent and approximately ten meters wide in most stretches and up to two meters deep in the summer in the deepest stream pools; iii) the tributaries occupied by *X. laevis* are also permanent and approximately one meter wide and up to one and a half meters deep in the summer. The mean water temperature between April and October 2010–2013 was 22.5 °C in Barcarena and 20.9 °C in Laje (SANEST unpublished data). The distribution of *X. laevis*, as well as the 25 sites where tadpoles were seen throughout the first seven years of the eradication program (2010–2016) and the section where it was most abundant in 2014 are depicted in Figure 1. Of the 18 lotic sites where large tadpole swarms were found, ten have been used by the species more than once (up to six years at one site in Barcarena between R5 and R6). This persistence occurred despite our removal efforts and is probably due to adult migration

Figure 1. Invaded part of Laje and Barcarena streams (thick black lines) and sites where tadpoles were seen throughout the eradication program. Circles correspond to lotic and squares to lentic sites. White symbols represent sites where tadpoles were seen only prior to 2015; black symbols depict the sites where the tadpoles used in this study were collected. Some circles are not clear due to overlay. Dashed grey lines in Barcarena stream define the highest invaded stream section in 2014 (where 99.6% of the captures took place).



from other parts of the stream, but is also an indication of its suitability as a reproduction habitat. Rarely did we find isolated tadpoles which were most likely carried downstream from known breeding sites. Only five breeding sites are lentic, and of these only one is in the Laje basin. During the 2015 and 2016 campaigns we visited several new sites (particularly artificial ponds) in addition to covering all the invaded stretches of the two streams and all sites where tadpoles had been seen during previous campaigns. In both years, visits took place twice a month from June to August and monthly in September and October. In 2015, groups of tadpoles were seen at 10 sites whereas in 2016 they were seen at six sites, two of which were visited for the first time in that year. In total, we found and captured tadpoles in 14 different sites, of which we selected those where more than 20 tadpoles were captured ($n = 11$). These are lotic ($n = 7$) and lentic ($n = 4$) and differ widely in dimensions as well as in tadpole abundances (Table 1). All lotic sites have permanent water inflow and outflow, but with slow water current. They are small stream pools located on the banks of the main stream and connected to either the main stream or small tributaries. All lentic sites are artificial ponds not connected but close to the main streams (20 to 110 meters). Except for T1, which is only fed by rainwater, lentic sites have a small water inflow, enough to maintain water levels but not enough to create a significant current. The number of tadpoles in each site was classified into four categories, according to the total number found throughout the year (Table 1).

Tadpoles were captured with the same technique used for adults, using an electrofishing device (SAMUS-752GN (PDC)) with frequency set to 30 Hz, coupled

with dipnet sweeps. After being caught, all specimens were euthanized by immersion in a buffered MS222 solution and then frozen. An exception was made for tadpoles caught between stages 62 and 64 of the Nieuwkoop and Faber (1994) classification table, which were kept alive until completion of metamorphosis to estimate metamorphic body size and condition. These tadpoles were placed in individual $14 \times 14 \times 5$ cm containers and maintained at constant 20°C under a 12h:12h photoperiod until metamorphosis was completed (i.e. complete tail re-absorption, stage 66). No food was supplied during that time. Typically, *X. laevis* tadpoles stop feeding at stage 61 (Naitoh et al. 1989), but since we had previously found tadpoles with their guts full in stage 61, only tadpoles from stages 62 to 64 were retained.

To determine the threshold body size at which gonad development begins, we used 409 frogs captured between June and October during the 2014 and 2015 campaigns.

Measurements

We measured the snout-vent length (SVL) to the nearest 0.1 mm using digital calipers, and determined tadpole developmental stage after Nieuwkoop and Faber (1994) of tadpoles from 11 breeding sites. A total of 1515 tadpoles were selected after excluding those damaged by thawing. For further analyses we considered only tadpoles from stages 50 to 57 ($n = 970$; Lotic: 401; Lentic: 569), which correspond to the formation and elongation of the hindlimb, from the stage where it is conical in shape and constricted at the base (stage 50), up to the stage before forelimbs break through (stage 57). The sample size for the different sites ranged from 20 to 222.

Table 1. Description of each site and number of tadpoles seen in 2015 and 2016, with each group of site/year used in figures 2 and 3 indicated with respective symbol in the top-right corner of each estimate cell. The last column indicates the first time a metamorphosing tadpole (stage 60 or later) was seen. Dashed cells indicate the site was not checked that year or stage 60 tadpoles were not seen.

Habitat	Site / Stream	Dimensions	Flow velocity	Turbidity	Vegetation	Main substrate	2015 Estimate	2016 Estimate	Stage 60 first seen
Lotic (R)	R1 / Laje	4×2×0.5 m	Very low	Clear	Rare; emergent	Rough gravel and cobble (1–30 cm)	■ 20–100	0	–
	R2 / Laje	4×1.5×0.5 m	Very low	Clear	Rare; emergent	Rough gravel and cobble (1–30 cm)	0	○ 100–250	08–Aug
	R3 / Laje	5×1×0.5 m	Low	Turbid	Submersed	Silt	■ 100–250	20–100	28–Aug
	R4 / Barcarena	15×7×1 m	Very low	Clear	Submersed	Sand and boulders (> 30cm)	–	▲ 100–250	04–Aug
	R5 / Barcarena	10×10×1,5 m	Low	Turbid	Submersed	Sand and boulders (> 30cm)	▲ 20–100	0	26–Aug
	R6 / Barcarena	8×7×1,5 m	Low	Turbid	Submersed	Silt and sand	□ >1000	>1000	–
	R7 / Barcarena	12×9×1,5 m	Low	Turbid	Absent	Silt and sand	● >1000	>1000	17–Jul
Lentic (T)	T1 / Laje	1×1×2 m	Absent	Turbid	High concentration of algae in water column	Concrete	□ 20–100	0	19–Jul
	T2 / Barcarena	11×6×0.7 m	Absent	Clear	Absent	Concrete	▲ >1000	10	29–Jul
	T3 / Barcarena	15×5×2.5 m	Absent	Turbid	High concentration of algae in water column	Concrete	–	● >1000	07–Jul
	T4 / Barcarena	25×10×1.5 m	Absent	Turbid	Low concentration of algae in water column	Concrete	■ >1000	100–250	07–Jul

Newly-metamorphosed individuals ($n = 91$, from four locations) were weighed and measured – SVL was determined using ImageJ 1.8.0 (Abràmoff et al. 2004) and estimated as the average of the SVL measured in three different digital photographs. Body condition was estimated using the scaled mass index proposed by Peig and Green (2009), which standardizes the mass of the froglets to an average SVL (23.33 mm, in our case) using the scaling relation between log mass and log SVL. We plotted the results of SVL and body condition index for each individual.

The SVL of the 409 juveniles and small adults captured in 2014 and 2015 was measured to the nearest 0.1 mm using digital calipers. The frogs were weighed to the nearest 0.01 g and sex was determined after dissection. When recognizable, gonads were removed and weighed to the nearest 0.001 g.

Statistical Analysis

For tadpoles, we used generalized linear models (GLZ) with a normal distribution to examine stage-specific differences in SVL among all sites. Stage was set as an ordinal covariate because tadpole size increases throughout the selected stages. For metamorphs, since our data did not meet the assumption of homoscedasticity (Levene test, $P < 0.05$), we used a Kruskal-Wallis test to analyze the differences in SVL and body condition among the three sites. We followed this with pairwise comparisons using Dunn's test; significance values were corrected with the false discovery rate (FDR). Individuals from lotic site R7 were not used in these comparisons due to a very small sample size ($n = 3$). The GLZ was performed using IBM SPSS Statistics version 22. We used R version 3.3.3 (R Core Team 2017) to perform

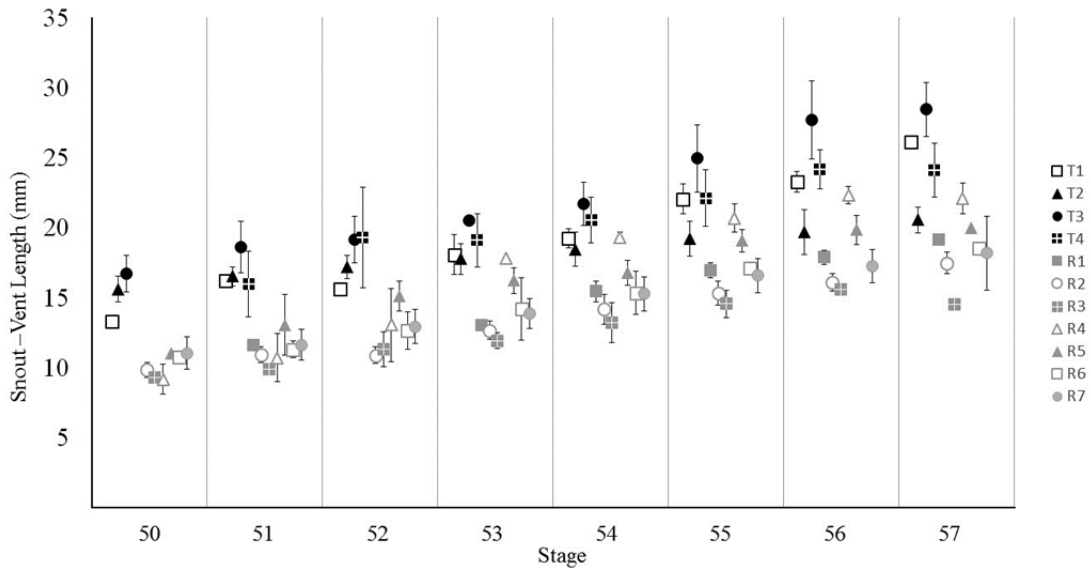


Figure 2. Tadpole average SVL (mm) ± SD per stage in Lentic (black) and Lotic (grey) sites.

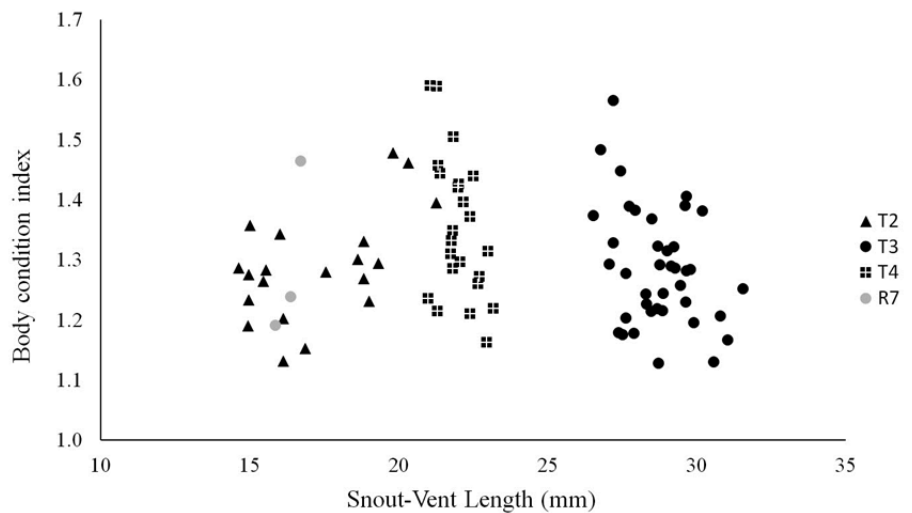


Figure 3. SVL (mm) and Body Condition Index of metamorphs from different sites.

the Kruskal-Wallis test using the native “stats” package, and pairwise comparisons using the “FSA” package version 0.8.13 (Ogle 2017).

Results

There was a large variability in the number of tadpoles found at each site; a high number of tadpoles (> 1000) was found in two lotic and three lentic sites (Table 1). While late stage tadpoles beginning metamorphic climax were found in most sites, they were first seen about 1 month earlier in lentic sites, on average on July 15, compared to August 10 in lotic sites (Table 1).

Given the developmental stages considered, there was a clear difference in the average SVL of tadpoles among sites (GLZ, Wald $\chi^2 = 2923.78$, $df = 10$, $P < 0.001$). Overall, average SVL of tadpoles from lentic sites was higher than that from lotic sites. However, the difference in average SVL was more obvious in the earlier developmental stages (stage 50/51) than in the latter stages (stage 56/57) (Figure 2). Considering only the final stages (stage 54 onwards), the lowest average SVL of a single lentic site (T2) was lower than the largest average SVL of a single lotic site (R4) (Figure 2). Additionally, there was a large variability within each type of habitat (Figure 2).

Tadpoles were only able to complete metamorphosis in four sites, of which three were lentic. There was a large size difference between the metamorphs from site T3 (average SVL = 28.76 mm) and those of other locations (Figure 3). The only three metamorphs from a lotic site (R7) were among the smallest measured, although their SVL is within the range of variation for the SVL of metamorphs from T2, a lentic site (Figure 3). The Kruskal-Wallis test showed differences in the average size at metamorphosis among sites ($\chi^2 = 39.808$, $df = 2$, $P < 0.001$), but no significant differences in metamorph body condition ($\chi^2 = 4.743$, $df = 2$, $P > 0.05$). Pairwise comparisons showed SVL to be greater in T3 than in the other two sites ($P < 0.001$).

The minimum SVL for females with distinctive gonads was 40.5 mm, while for males this value was 35.0 mm; all animals larger than these sizes showed gonad development (Figure 4).

Discussion

As expected, the importance of lentic sites for this species' reproduction was found to be much higher than that of lotic sites. However, to our knowledge this is also the first confirmation of successful reproduction in lotic habitats, and in fact the species may have maintained its population in one of the stream basins by breeding in small tributaries and low current stream pools.

Across the small Portuguese *X. laevis* invasive range, lotic breeding sites are more frequent than lentic breeding sites. This species is able to produce large tadpole swarms in both habitats. However, this work shows that lentic sites probably contribute more to the maintenance and expansion of this invader: i) tadpoles living in lentic habitats were consistently larger than tadpoles living in lotic habitats, with a carry-over effect from tadpoles to metamorphs; ii) tadpoles started metamorphosis about one month earlier in lentic sites; iii) the number of individuals completing metamorphosis in lentic sites was higher; iv) individuals that complete metamorphosis in lentic sites (with a large body size) may start reproducing before the ones from lotic sites. These findings have direct and important implications for the design and implementation of eradication campaigns, suggesting that directing efforts towards lentic sites could prevent the recruitment of large numbers of large-sized juveniles.

The difference in body size of tadpoles from the two habitats was evident early in development, as by stage 50 tadpoles from some lentic sites were twice as large as tadpoles from lotic sites. We cannot positively identify the reasons for the size differences

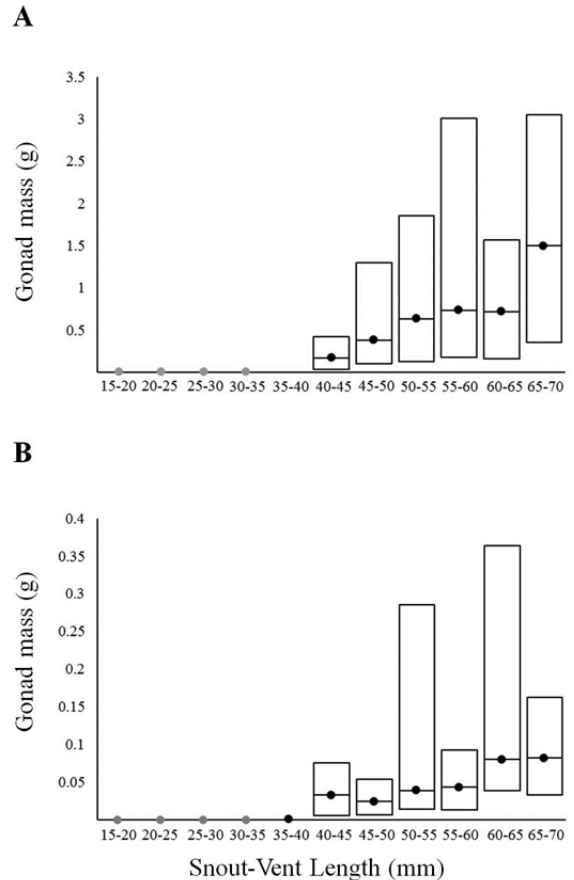


Figure 4. Female (A) and male (B) gonad mass (g) across size classes (mm). The first four classes represent individuals whose sex was not identified ($n = 16$). Circles and lines represent the average female and male gonad mass per size class ($n = 189$ and $n = 220$, respectively). Boxes represent minimum and maximum gonad mass values. Note that values on the vertical axes differ.

between habitats, but there are a few plausible hypotheses. The first concerns food availability. Petranks (1984) found that the small-mouthed salamander (*Ambystoma texanum*) attained larger sizes in ponds than in streams, which the author argues could be driven by the lack of food in lotic habitats. Since *X. laevis* tadpoles are suspension feeders (Wassersug 1996), the higher abundance of algae in lentic waterbodies (Stevenson 1996) should allow for higher growth rates, as reported for other species (e.g., Kupferberg et al. 1994; Álvarez and Nicieza 2002; San Sebastián et al. 2015). Additionally, the stream current may decrease feeding efficiency. During metamorphosis, *X. laevis* tadpoles greatly increase their swimming ability (Walsh et al. 2008a), which could increase their feeding efficiency and nullify disturbance from the water current. This increase may explain the lower difference in tadpole

size among habitats during the final developmental stages and suggests that tadpoles can partially compensate for the smaller size in earlier developmental stages.

Experiments under controlled settings showed that exposure to alarm cues can reduce growth, tadpole size, and size at metamorphosis of *Rhinella marina* (Hagman et al. 2009). Although predators are present in all sites (*X. laevis* adults, dragonfly larvae and fishes), lentic sites tended to have fewer predatory fish (they are all artificial ponds), which could also contribute to the larger sizes attained in those sites.

A temperature-related explanation seems unlikely in our study area. Following the general size-temperature rule of ectotherms (Atkinson 1994), the usually lower water temperature in lotic sites should promote an increase in stage-specific size of tadpoles, as found in numerous species (e.g., Smith-Gill and Berven 1979; Álvarez and Nicieza 2002), including *X. laevis* (Walsh et al. 2008c). Yet, the influence of temperature appears to be contrary to expected in this case. An effect of intraspecific competition also does not satisfactorily explain our findings. Several authors have shown that high abundances can hamper tadpole growth, either by increasing intraspecific competition and/or by increasing conspecific chemical cues (e.g., Travis 1980; Faragher and Jaeger 1998; Cabrera-Guzmán et al. 2011; Crossland and Shine 2012). However, we found no relationship between tadpole abundance and stage-specific size. In fact, the site with the highest stage-specific SVL (T3) maintained one of the highest tadpole abundances per estimated pond volume.

Our results suggest that tadpole survival is much higher in lentic sites, as we recorded only three individuals completing metamorphosis in lotic sites. This disparity may be associated with a greater exposure to stochastic events, such as discharges of pollutants happening upstream, higher predation, or lower temperatures that increase larval period and therefore also increase the effect of the previous two factors. Walsh et al. (2008a) found the time to metamorphosis of *X. laevis* tadpoles at 18 °C to be twice that at 24 °C or 30 °C. Rot-Nikcevic and Wassersug (2004) report body length in stage 56 to be similar to the body length at the end of metamorphosis (stage 66), and indeed our measured body sizes in stage 56 are similar to the average metamorph SVL at the same site (Figures 2 and 3). Therefore, looking at the average SVL at stage 56 across all sites it is probable that metamorphic size in sites where metamorphosis was not completed would not be much different to the smaller metamorphs measured. The possibility of tadpoles completing metamorphosis in other lotic sites besides R7 cannot be excluded, as there was an

effort to remove tadpoles during the eradication campaigns. Furthermore, we found isolated tadpoles downstream of known breeding sites on two occasions, so tadpoles may be being carried downstream and completing metamorphosis in another site. However, this type of situation is probably rare, due to the rarity of favourable lotic sites within the stream and to the presence of large predatory fish in the deepest stream pools.

Whether from lotic or lentic sites, the body sizes of wild-living tadpoles were within the range of, or larger than, those of tadpoles reared under laboratory conditions. In our study the largest measured SVL occurred in stage 56: 32.3 mm in lentic sites and 22.9 mm in lotic sites. Rot-Nikcevic and Wassersug (2004) reported “gigantic” tadpoles with arrested development between stages 53 and 56 to have an average SVL of 27 ± 1 mm, which was greater than the average SVL for “normal” tadpoles of the same stages (11.7 mm). Average SVL of stage 60 tadpoles in the study by Walsh et al. (2008a, b) varied between 18.1 and 19.5 mm. Metamorphs were also either within the range or larger than the ones reared in laboratory facilities, which varied between 13 and 23 mm (Pickford et al. 2003; Du Preez et al. 2008). This means that all the tadpoles found in this study would probably develop into common-sized (in lotic and some lentic sites) or very large (in most of the lentic sites) metamorphs.

Despite the similar body condition of metamorphs in all sites, an increase in fitness for larger individuals may be a result of “skipping” the first few months of post-metamorphic development and becoming sexually mature earlier, as described for other species. Smith (1987) showed that, while an equal proportion of different-sized metamorphs of *Pseudacris triseriata* survived the first two years, the survival of the largest metamorphs to first reproduction was higher due to the higher probability of reaching reproductive size within their first year of life. Female and male *X. laevis* can mature 10 and 6 months after metamorphosis, respectively (Kelley 1996). Our results show that both sexes started developing gonads upon reaching a similar size, and that no animal larger than this size failed to start that process, which is an indication that maturation is size-dependent in this population. These minimum sizes correspond to frogs in their second or third year of life, following the growth curves obtained from a skeletochronological study of the Portuguese populations (Ferreira 2014). Although an increase in gonad weight does not mean gonads are mature, it is indicative of an investment in reproduction. Indeed, we registered a 45.8 mm male with secondary sexual characters (nuptial pads). Since at

location T3 the average SVL of metamorphs was 28.76 mm (max. = 31.57 mm), it is possible that these individuals could have matured in the following year. For metamorphs from all of the other sites, this process would take one or two extra years.

Although a few models of the climatic suitability of different world regions for *X. laevis* have been developed (e.g., Capinha and Pateiro-López 2014; Ihlow et al. 2016), none of these models consider finer information, such as the availability and type/quality of breeding habitats. Being able to breed in different habitats can be a great advantage for an invasive species. *X. laevis* probably uses both types of breeding habitats in other invaded areas (McCoid and Fritts 1989; Lobos and Jaksic 2005), and invasive populations have had a much higher success in areas with higher availability of lentic habitats (Fouquet and Measey 2006; Faraone et al. 2008; Lobos et al. 2013). In Portugal *X. laevis* was able to establish a population in the Laje basin, but the lack of lentic sites may have hampered the invasion process; lotic sites were probably important in maintaining the population, but not enough to foster a rapid expansion. In the Barcarena basin the greater number and the higher quality of available breeding sites (both lentic and lotic) may explain a more efficient recruitment, as well as the 2014 invasion. Here, most adult frogs (99.6% of the 2014 invasion) were captured in stream stretches close to the known breeding sites (Figure 1). Moreover, average size and age of frogs caught in Barcarena have consistently been smaller than in Laje (Ferreira 2014).

Assessing lifetime individual traits such as fitness and survival in species with multiphasic life-histories and small body size is often impractical, particularly in natural conditions. Therefore, size at metamorphosis is often used as an indicator of fitness and post-metamorphic survival. Hence, to focus on lentic sites, where recruitment is higher and recruits are larger in size, seems to be the most effective strategy for eradicating the species from invaded areas. Govindarajulu et al. (2005) suggest the removal of tadpoles might not always be the most successful strategy, as it can be more time-efficient to remove adults and because partial removal of tadpoles may result in higher survival and developmental rates, as well as higher post-metamorphic survival of the remaining ones. Still, lentic breeding sites in our area and in other periurban areas are relatively small and manageable, and should be the main target of eradication efforts. Regardless of whether these efforts focus on removing adults, tadpoles or both, an effective control of source-sites will have a strong positive impact on eradication efforts.

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