Oecologia
https://doi.org/10.1007/s00442-022-05240-6

PHYSIOLOGICAL ECOLOGY – ORIGINAL RESEARCH

Phenotypic variation in *Xenopus laevis* tadpoles from contrasting climatic regimes is the result of adaptation and plasticity

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Received: 25 August 2020 / Accepted: 6 August 2022

Abstract

Phenotypic variations between populations often correlate with climatic variables. Determining the presence of phenotypic plasticity and local adaptation of a species to different environments over a large spatial scale can provide insight on the persistence of a species across its range. Amphibians, and in particular their larvae, are good models for studies of phenotypic variation as they are especially sensitive to their immediate environment. Few studies have attempted to determine the mechanisms that drive phenotypic variation between populations of a single amphibian species over a large spatial scale especially across contrasting climatic regimes. The African clawed frog, *Xenopus laevis,* occurs in two regions with contrasting rainfall regimes in southern Africa. We hypothesised that the phenotypic variation of life-history traits of *X. laevis* tadpoles emerges from a combination of plastic and genetic responses. We predicted that plasticity would allow the development of tadpoles from both regions in each environment. We also predicted that local adaptation of larval traits would drive the differentiation of reaction norms between populations and lower survival in tadpoles reared away from their home environment. We measured growth, time to metamorphosis, and survival in a reciprocal transplant experiment using outdoor mesocosms. Supporting our prediction, we found that the measured variation of all traits was explained by both adaptation and plasticity. However, the reaction norms differed between populations suggesting adaptive and asymmetric plasticity. All tadpoles experienced lower survival when translocated, but only translocated tadpoles from the winter rainfall region matched survival of local tadpoles. This has implications for the dynamics of translocated *X. laevis* into novel environments, especially from the winter rainfall region. Our discovery of their asymmetric capacity to overcome novel environmental conditions by phenotypic plasticity alone provides insight into their invasion success.

Keywords Adaptive plasticity · Anuran · Evolution · Life history · Pipidae · Tadpole · Reaction norms

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Communicated by Howard Whiteman.

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Introduction

Local climates can differ remarkably across the distribution of many species, generating spatially heterogeneous selection pressures (Bozinovic et al. 2011; Blanquart et al. 2013). A non-random relationship between phenotype and climate has been found for traits as diverse as phenology, physiology and behaviour (Merilä and Hendry 2014). Historically, in the search for mechanisms that drive phenotypic variation across large distributions, local adaptation was the preferred explanation because gene flow was hypothetically reduced between distant populations (Richter-Boix et al. 2010; Richardson et al. 2014). However, this assumption is not necessarily met (Fourcade et al. 2016). Phenotypic plasticity, which is the capacity of a given genotype to express different phenotypes in response to different environmental conditions (Williams 1966), may be enough to maintain performance or fitness levels across a species' range and among populations, with the fitness effects being the ultimate reason behind the proximate pattern (Via and Lande 1985; Price et al. 2003). More realistically, geographic variation for any phenotypic trait is likely to result from the combination of phenotypic plasticity and local adaptation (Phillimore et al. 2010; Chevin and Lande 2011). Yet, their relative contributions depend on the trait and may determine the potential for a population to cope with novel conditions. Estimating these contributions in populations from contrasting climates over a large spatial scale can provide insight into the mechanisms that create geographical patterns of phenotypic variation and diversity (e.g., Yamahira and Conover 2002). It can also assist in explaining intraspecific phenotypic diversity and its distribution across ecologically heterogeneous ranges and predict the scale of potential evolutionary responses to environmental change (Williams et al. 2008).

For local adaptation of traits to occur, genetic modification is required resulting in an adaptive genotypic and phenotypic change (Kawecki and Ebert 2004; Hereford 2009; Radersma et al. 2020). This adaptive potential of populations is essential for their long-term persistence (Orizaola et al. 2010). Plasticity may be adaptive if heritable, and phenotypic plasticity can drive adaptation further by shifting phenotypes towards a local optimum (plasticity first evolution hypothesis: West-Eberhard 2003; Levis et al. 2018; Radersma et al. 2020). Phenotypic plasticity itself can evolve when populations of the same species experience heterogeneous environments in space and time (e.g., Scheiner 1993; Berrigan and Scheiner 2004). This situation can drive the evolution of the reaction norm of populations as a response to environmental change (Kingsolver and Huey 2007; Lind et al. 2011). Therefore, variation among populations can be due to genetic differences, plasticity, and their interaction and these effects can be adaptive, or not (Radersma et al. 2020). Investigating these effects is becoming increasingly relevant given the ongoing global change of the environment (Hoffmann et al. 2003; Thomas et al. 2004; Kellermann et al. 2006).

Ectotherms such as amphibians provide a good model for studies of phenotypic plasticity and/or local adaptation as they are especially sensitive to immediate climatic conditions (Li et al. 2013). A reciprocal transplant experiment is a common approach to investigate local adaption and phenotypic plasticity (Shaw 1991; Briggs and Walters 1997; Hereford 2009). In a reciprocal transplant experiment, two populations are reared in their own (home) environment, as well as the other (away) environment (hereafter home and away environments), allowing differentiation between genetically fixed and plastic traits. Then, both types of variation can be adaptive or not (Berrigan and Scheiner 2004). Many life-history and morphological traits in amphibians have been studied and mostly plasticity has been observed (Relyea 2001; Booth 2006; Urban 2010; Urban et al. 2014). The contribution of local adaptation has been found in survival and some other traits (e.g., developmental rate, and size at metamorphosis, Amburgey et al. 2012; Orizaola et al. 2013). Phenotypic differences in individuals originating from the same location but reared in the home and away environment are evidence of phenotypic plasticity. The observation of higher fitness in the local phenotype than in the translocated phenotype supports local adaptation (e.g., Smith and Van Buskirk 1995; Merilä et al. 2000; Relyea 2002; Urban et al. 2017; Bachmann and Van Buskirk 2021).

Temperature and precipitation are important climatic variables that influence how ectothermic species, including amphibians, regulate their body temperature mostly through heat, water and gas exchange (Feder and Burggren 1992). Amphibians are exposed to daily and seasonal environmental temperature variation and may rely on phenotypically plastic responses to cope with these fluctuations (Huey et al. 1999; Angilletta 2009; Seebacher and Franklin 2011). Climatic conditions, such as the rainfall and temperature regime, experienced by tadpoles can influence time to metamorphosis (Morand et al. 1997), larval survival (Blaustein et al. 2010) and traits affecting metamorphic survival like size at metamorphosis (Berven and Smith-Gill 1983; Semlitsch et al. 1988; Atkinson 1996; Walsh et al. 2008; Blaustein et al. 2010; Gomez-Mestre et al. 2010). The effect of temperature on larval survival is considered to be complex (Blaustein et al. 2010). For instance, the survival of Pacific treefrog (*Pseudacris regilla*) tadpoles depends on density at higher temperatures but not at lower temperatures (Govindarajulu and Anholt 2006). However, during reciprocal exchange experiments it is expected that survival will be higher in the home environment (Hereford 2009). Time to metamorphosis and body size at metamorphosis are also considered as relevant proxies for survival after metamorphosis in amphibians (Chelgren et al. 2006; Gomez-Mestre et al. 2010).

Time to metamorphosis is determined by plasticity and local adaptation (Ficetola and Bernardi 2005; Orizaola et al. 2010; Amburgey et al. 2012). Several studies that investigated heritability quantified additive genetic variance of larval traits including larval growth in amphibians (Uller et al. 2002; Laugen et al. 2005). They showed that larval traits also have a strong environmental variance (Laugen et al. 2005) and, in this regard, time to metamorphosis is strongly plastic (Richter-Boix et al. 2006; Michimae 2011). Higher temperatures shorten the larval period in many species (Morand et al. 1997; Buchholz and Hayes 2000) which is expected to increase survival probability of the progeny of individuals reproducing in ephemeral ponds and streams. However, this relationship is observed up to an optimal temperature beyond which increasing temperatures progressively reduce body mass at metamorphosis, which can lead to lower survival probability (Watkins and Vraspir 2006). Similarly, tadpole body size is smaller at higher temperatures (Morand et al. 1997) and larger with higher daily temperature fluctuation (Arrighi et al. 2013). As for larval size and morphology at metamorphosis, they are determined by phenotypic plasticity alone (Touchon and Warkentin 2010; Niehaus et al. 2012) or in combination with local adaptation (Laugen et al. 2005; Amburgey et al. 2012; Orizaola et al. 2013). As a consequence, differences in climatic conditions during larval development may determine the contribution of environmental and genetic factors on tadpole phenotype. Species or populations living in more permanent aquatic habitats, and more constant environments are expected to exhibit lower levels of phenotypic plasticity (Kulkarni et al. 2011).

Southern Africa is characterised by two principal rainfall regimes, winter and summer, that differ in precipitation and temperature (Chase and Meadows 2007). The winter rainfall region falls along the West coast resulting in generally cooler temperatures and rainfall during winter months. The summer rainfall region is a larger area characterised by summers with higher temperatures and increased rainfall. The native amphibian fauna is separated by these contrasting regimes into the respective regions (Poynton 1964; Poynton and Broadley 1978; Schreiner et al. 2013). However, some species like the African clawed frog, *Xenopus laevis*, straddle both climatic areas (Measey 2004; Matthews et al. 2016). Separate phylogeographic lineages of *X. laevis* occur in these two regions (Furman et al. 2015), and adults display diverse morphologies possibly due to the differing thermal regimes, resulting in shifts in breeding phenology aligning with contrasting rainfall patterns (Berk 1938; Kalk 1960; Du Preez et al. 2009). In the summer rainfall region, breeding takes place from September to mid-March from austral spring to summer (Balinsky 1969). Whereas, in the winter rainfall region frogs breed from July to September from austral winter to the onset of spring (Kalk 1960). Adults can breed up to twice a year when the conditions are favourable (Wood 1965). The spatial heterogeneity in temperature and rainfall in each area may have driven further genetic differentiation within either region (Grohovaz et al. 1996; Measey and Channing 2003). However, evidence of both local adaptation and phenotypic plasticity of tadpole locomotor traits (burst swimming speed) has been found in natural and captive bred populations of *X. laevis* (see Wilson et al. 2000; Wagener et al. 2021). Walsh et al. (2008) found a plastic response of metamorphic climax duration to environmental temperature in captive-bred *X. laevis*. However, there is no experimental evidence of phenotypic differentiation in survival and life-history traits in natural populations across the native distribution.

We investigated the relative contribution of phenotypic plasticity and local adaptation in the larval development of *X. laevis* originating from different climatic regimes in southern Africa. A complete overlap in the reaction norms of both populations would suggest that differences between populations are explained by phenotypic plasticity alone. In contrast, a lack of phenotypic variation between tadpoles reared in home or away environments would suggest a strong genetic effect on development. Finally, differentiation in the reaction norm whereby traits of tadpoles from a population are consistent regardless of the environment in which they are reared could be considered as evidence of local adaptation. To address these questions, we performed a reciprocal transplant experiment, with *X. laevis* populations from summer and winter rainfall regions to measure the variation of larval survival, time to metamorphosis, and morphology. We used outdoor mesocosms as they allow for the exposure to natural variation of the climatic parameters that influences adaptation to climate such as water temperature (Freidenburg and Skelly 2004), density (Govindarajulu and Anholt 2006) and hydroperiod (Amburgey et al. 2012). Large sample sizes of tadpoles can be used in mesocosms to increase statistical power to detect effects (Skelly and Kiesecker 2001).

We predicted that body size, and time to metamorphosis display a combination of phenotypic plasticity, as seen elsewhere in this species (Wilson et al. 2000; Walsh et al. 2008), and local adaptation, because this finding has been observed in the larval traits of amphibians where temperature and precipitation vary between populations (Ficetola and Bernardi 2005; Amburgey et al. 2012; Orizaola et al. 2013), and because the climatic differences between the two regions are large. Additionally, we predicted survival to be higher in tadpoles reared in their home environment than translocated individuals from the other population (Hereford 2009).

Materials and methods

Study species

The African clawed frog, *Xenopus laevis*, is native to southern Africa (Furman et al. 2015) from southernmost South Africa to Malawi (Measey 2004; Furman et al. 2015). It occurs in natural, but also artificial waterbodies within the different rainfall regimes (Measey 2004). Mitochondrial and nuclear DNA and phenotypic differentiation have been observed for *X. laevis* throughout its native range (Du Preez et al. 2009; Furman et al. 2015; De Busschere et al. 2016). It is likely that the region's geological and climatic features impacted the current population structure within *X. laevis* (see Measey and Channing 2003). The development of *Xenopus laevis* tadpoles from embryos to metamorphs is well described (Nieuwkoop and Faber 1994; Segerdell et al. 2008). Tadpoles of *X. laevis* are mid-water suspension filter feeders and are free-swimming around two days after hatching (Viertel 1992; Nieuwkoop and Faber 1994). The development of *X. laevis* from fertilisation to metamorphosis undergoes 66 Nieuwkoop and Faber (NF) stages (Nieuwkoop and Faber 1994) grouped into nine stage categories according to an anatomical ontology (Segerdell et al. 2008). The post-embryonic development (NF stage 45–57) includes limb bud development and toe differentiation. Overall, body size increases during pre- and prometamorphic stages and decreases during the metamorphic climax stage (NF stage 58–66) during which tail resorption occurs.

Sampling sites

We chose, Stellenbosch, Western Cape province, South Africa as a population locality in the winter rainfall region and Potchefstroom, North-West province, South Africa as a population locality in the summer rainfall region (Fig. 1). These sites are separated by approximately 1200 km (Euclidian distance) and are representative of each climatic region. Within each study area, two sites (ponds) separated by 10–40 km were sampled: summer rainfall region: Site 1 (1355 m a.s.l., 26° 40.9′ S, 27° 5.6′ E,); Site 2 (1347 m a.s.l., 26° 45.3′ S, 27° 3.6′ E); winter rainfall region: Site 3 (241 m a.s.l., 33° 57.8′ S, 18° 55.5′ E); and Site 4 (196 m a.s.l., 33° 49′ S, 18° 53′ E).

Adult collection, care and breeding

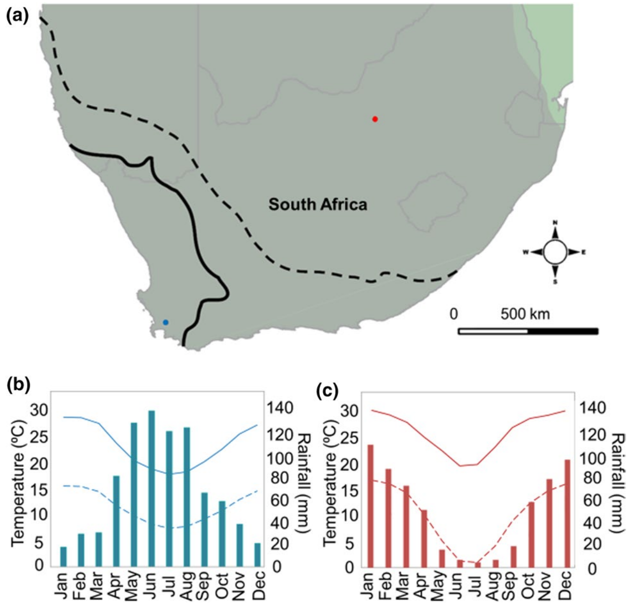

Fig. 1 **a** Localities of the sampling sites in the summer rainfall region (red dot) and in the winter rainfall region (blue dot). The grey area indicates the current distribution of *Xenopus laevis* in southern Africa. The area beneath the solid line illustrates the current winter rainfall region and the area to the right of the dashed line illustrates the current summer rainfall region. The area between the dashed and solid line illustrates the region where rainfall occurs throughout the year. Rainfall zones as indicated by Chase and Meadows (2007). **b** Ombrothermic diagram of maximum (solid blue line) and minimum (dashed blue line) monthly temperature (°C) and rainfall (mm) in the winter rainfall region (blue bars). **c** Ombrothermic diagram of maximum (solid red line) and minimum (dashed red line) monthly temperature (°C) and rainfall (mm) in the summer rainfall region (red bars) (Source: SA weather service 2020)

We collected two or three males and females of *X. laevis* at each locality and transported them to an open and secure field used as the experimental site in each region. The Amphibian Metamorphosis Assay (AMA) and the *Xenopus* Metamorphosis Assay (XEMA) recommend using human chorionic gonadotrophin hormone (HCG) to induce breeding in *X. laevis* (OECD 2008). We inspected adults to determine whether hormonal priming was necessary. We examined the cloaca to determine female reproductive status. If the cloaca was red and swollen animals were confirmed to be in the preferred reproductive state to induce (Balinsky 1969). We inspected the forearms of the males for the presence of black nuptial pads to determine their reproductive status. If the nuptial pads were hardly visible or grey, males were primed with hormones. Both males and females were primed with a subcutaneous injection of 50 international units (IU) HCG to ensure that adults were in the preferred reproductive state (Balinsky 1969). On the third day, males were injected with 250 IU HCG and females with 500 IU HCG (dosages as prescribed by Van Wyk and Du Preez 1984; Wlizla et al. 2018). Injection of hormones took place shortly after collection. Individuals were kept separate until the final injection on the third day and placed together in a plastic aquarium (7–10 L). A plastic mesh (3 mm) was placed in the bottom of the tank to prevent adults from consuming eggs. Schultz and Dawson (2003) and OECD (2008) recommend that the preferred temperature for rearing *X. laevis* tadpoles is between 18 and 24 °C. The daily mean temperature in the winter and summer rainfall region falls within the 21 °C and 24 °C margin. Therefore, the breeding and subsequent holding temperature of eggs were chosen to be 22 °C to prevent favouring the growth of one population more than the other. Adults were kept at 22 °C to ensure that when eggs were laid, they were all at the same temperature. Frogs were removed from the aquaria the following morning. Eggs were left to hatch in their aquaria maintained at 22 °C and well aerated until they developed into free-swimming tadpoles, approximately two days later. The mesh was removed after hatching and unfertilised eggs were syphoned out to prevent fouling of the water. Free swimming tadpoles ($N=200$) from each laboratory bred clutch were introduced into outdoor mesocosms.

Staging

The dataset was divided according to Segerdell et al.'s (2008) pre-defined stage categories. Individuals from the pre- and prometamorphosis stage categories (NF stage 45–57) are collectively referred to as 'larvae'. These were analysed separately from the metamorphic climax stage category (NF stage 58–66) where body size decreases.

Experiment

The experiment in the summer rainfall region was conducted from 02/11/2017 to 10/01/2018 and the experiment in the winter rainfall region was conducted from 19/11/2018 to 26/01/2019, each for a period of 10 weeks. Tadpoles from parents originating from the summer rainfall region were reared in the summer rainfall region (SR_{Home}), and in the winter rainfall region (SR_{Away}). Tadpoles from parents originating from the winter rainfall region were reared in the winter rainfall region (WR_{Home}), and the summer rainfall region (WR_{Away}). We used 1000 L non-toxic plastic tanks as mesocosms (110 cm L × 130 cm W × 50 cm H, $N=10$). The experimental sites were located in rural open habitats (Potchefstroom: 26° 40.5′ S, 27° 06.4′ E; Stellenbosch: 33° 56.5′ S, 18° 52′ E), close to collection sites of adult frogs. Ten mesocosms were arranged in a matrix and treatments were randomly allocated to each mesocosm. We added 200 L of tap water and allowed this to age for at least one month prior to the onset of the experiment. The water level was maintained at a volume of 200 L. In addition, five temperature data loggers (HOBO K8® Temperature/Alarm (Waterproof) Data Logger—UA-001-08, Onset Computer, Bourne, Mass, USA) were placed in mesocosms that were selected randomly (in *Excel* Microsoft, Redmond, WA, USA) to determine variation in water temperature. The temperature loggers were attached to a floating piece of polystyrene and suspended 15 cm under the water to avoid differences in surface temperatures. We calculated the coefficient of variation to determine the dispersion of data points around the mean to assess the daily temperature variation in each experimental venue. We found that the summer rainfall region had the highest average maximum temperature in all mesocosms compared to the winter rainfall region (Table 1) throughout the 10 weeks of the experiment. The winter rainfall region had the lowest average minimum temperature compared to the summer rainfall region (Table 1). A larger coefficient of variation of temperature from the mean was observed in the summer rainfall region than the winter rainfall region (Table 1).

Mesocosms had full exposure to sunlight to enhance algal growth and after the first week algae grew naturally in each mesocosm. At the onset of the experiment, each mesocosm was covered with 20% shade mesh to avoid predation from birds, prevent dragonflies from depositing eggs and to prevent overheating. Tadpoles were fed dissolved Sera-Micron (1 g) per mesocosm per week. This was enough food and algae for a filter feeder to eat. We introduced individuals from a single clutch with an initial density of 200 free swimming larvae (NF stage 45) in each mesocosm. We followed the FETAX protocol which requires no more than four tadpoles per litre (Dumont et al. 1983). When kept at higher densities the effect of density and subsequent competition may affect the growth of tadpoles and size. We decided however, to decrease the tadpole density to as low as one tadpole per litre to exclude high density effects**.** Every week, five tadpoles from each mesocosm were captured at random with a dipnet, euthanised by overdosing with 300–500 mg/L of tricaine methanesulfonate (ms-222) buffered with $NaHCO_3$, and preserved in 70% ethanol (EtOH). We measured total length (snout-to-tail), snout-to-vent length (SVL), body depth, tail depth, head width of larvae and climax individuals, and femur length of climax individuals (Fig. S1, Kruger

Table 1 Maximum, mean and minimum daily temperature (°C ± SD) and coefficient of variation in the summer and winter rainfall region during the 10-week experimental period

Region	Maximum daily temperature (°C ± SD)	Mean daily temperature	Minimum daily temperature (°C ± SD)	Coefficient of variation (%)
Summer rainfall region	25.82 ± 2.28	22.37 ± 1.69	19.84 °C ± 1.26	8.95
Winter rainfall region	21.42 ± 2.05	20.50 ± 2.78	19.48 ± 3.60	3.16

et al. 2020). Additionally, we staged individuals according to Nieuwkoop and Faber (1994) and placed staged animals in stage categories according to Segerdell et al. (2008).

Statistical analyses

Morphological variation

We measured the morphology of larval individuals at NF stage 45–57. The sample size for climax individuals (NF stage 58–65) was too small to be included in statistical treatment after the 10 week experimental period. To assess the effect of experimental venue (summer or winter rainfall region) and population locality (summer-or winter rainfall) on larval morphology we performed a PCA using linear measurements (Fig. S2). The first principal component accounted for 94% of variation (± 2.16 SD) (Table S1). Loadings were positive for head width (0.442), SVL (0.450), maximum body depth (0.453), tail depth (0.448) and tail length (0.443). Loadings were similar if we ran this for the individual populations. We retained only the first PCA axis in our analysis as it represents a global measurement of body size.

Using the *lme4 R* package (Bates et al. 2015), linear mixed models were utilized with body size (PC1) as the response variable, and experimental venue, population locality, their interaction and NF stage (discrete variable) and its interaction with experimental venue and population locality as fixed effects. Clutch, nested within collection site, was considered as a random effect. All variables (measurements for PC1) were tested for normality and normalised with the *bestNormalize R* package (Peterson and Cavanaugh 2019). The best transformation informed by *bestNormalize* was Ordered Quantile transformation. PC1 was plotted using *ggplot2 R* package (Wickham 2009).

Time to metamorphosis

To determine the time to metamorphosis we counted the number of collected individuals that underwent a transition event from larvae (NF stage 45–57) to metamorphic climax (NF stage 58–65). We conducted a Cox proportional hazards analysis on transition between larvae and metamorphic climax stages using the *coxme* (Therneau 2020a) and *survival* (Therneau 2020b) *R* packages. The week when a transition occurred between larvae and climax was considered as the response variable, and experimental venue and population locality as fixed effects and clutch nested within collection site as random effects.

Variation in survival

We removed a total of 50 individuals from the mesocosms owing to our weekly sampling for the morphological study. Thus, the number of surviving individuals at the end of the experiment was tallied out of 150. We modelled the probability of surviving until the end of the experiment in mesocosms using generalised binomial mixed models with fate ($1 = \text{survived}, 0 = \text{died}$) as the response variable, experimental venue and population locality as fixed effects, and clutch nested within collection site as a random effect.

For all analyses, we selected the best fitting model using corrected Akaike information criterion (AICc) according to parsimony (Burnham and Anderson 2002). Multi-model inference (model averaging) was carried out to account for model selection uncertainty between the top models ($\Delta\text{AICc} < 2$) using the *MuMIn* package (Barton 2020). Model coefficients were subsequently averaged across the set of top models and the resulting averaged coefficients were used for predictions. If the top model was equal ($\Delta\text{AICc} < 2$) to the null model, the null model was not rejected. The chosen model was then used to calculate the least square means of interactions using the *lsmeans R* package (Lenth 2016). Model diagnostics were carried out using the DHARMa *R* package (Hartig 2019). All analyses were carried out using the statistical software R 3.4.1 (R Core Team 2018).

Results

Variation in morphology

Experimental venue, the interaction between experimental venue and stage, and the interaction between population locality and stage were retained in the top model explaining body size variation (PC1) (Fig. 2, Table 2 and Table S2, GLMM, $\Delta\text{AICc} = 2.58$, $R^2 = 0.906$). Post hoc tests for the two interactions revealed that the significant differences between experimental venue and population locality were stage-specific. All tadpoles (SR_{Home} and WR_{Away}) reared in the summer rainfall region, regardless of origin, were significantly smaller than tadpoles reared in the winter rainfall region (WR_{Home} and SR_{Away}) at NF stages 45 ($P = 0.001$) and 46 ($P < 0.001$) and significantly larger at NF stages 49 ($P = 0.001$); 51 ($P = 0.026$); 52 ($P = 0.0001$); 53 ($P = 0.002$), 54 ($P = 0.0001$); 55 ($P < 0.001$); 56 ($P < 0.001$); and 57 ($P = 0.015$). However, all tadpoles from the summer rainfall population (SR_{Home} and SR_{Away}) were significantly smaller than winter rainfall animals (WR_{Home} and WR_{Away}) at NF stage 46 ($P = 0.01$) but summer rainfall animals were significantly larger (SR_{Home} and SR_{Away}) at NF stages 55 ($P = 0.005$) and 56 ($P = 0.0002$) (Fig. 2; Table S3).

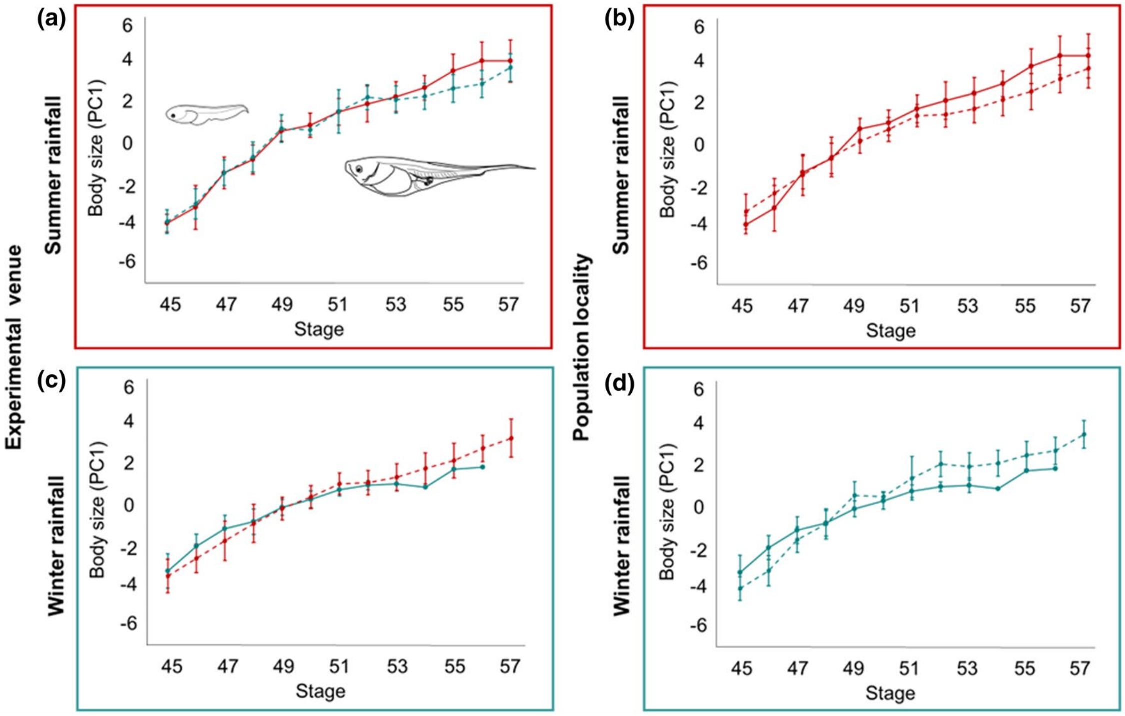

Fig. 2 Body size (PC1) variation and standard deviation of *Xenopus laevis* during the larval development. **a** Pairwise comparison of the summer rainfall home individuals ($N=189$; $\mathrm{SR_{Home}}$: *solid red line*) and the winter rainfall away individuals ($N=157$; $\mathrm{WR_{Away}}$: *dashed blue line*) reared in the summer rainfall region. **b** Pairwise comparison of $\mathrm{SR_{Home}}$ and $\mathrm{SR_{Away}}$ originating from the summer rainfall region. **c** Pairwise comparison of winter rainfall home individuals ($N=254$; $\mathrm{WR_{Home}}$: *solid blue line*) and summer rainfall away individuals ($N=207$; $\mathrm{SR_{Away}}$: *dashed red line*) reared in the winter rainfall region. **d** Pairwise comparison of $\mathrm{WR_{Home}}$ and $\mathrm{WR_{Away}}$ originating from the winter rainfall region

Table 2 Model selection of top models (ΔAICc < 2) for each response variable: larval body size (PC1), time to metamorphosis and survival of *Xenopus laevis* larvae

Response variable	Explanatory variables		LogLik	*k*	AICc	ΔAICc	Weight
	Fixed	Random					
Body size (PC1)	Experimental venue + Population locality + Stage + Experimental venue × Stage + Population locality × Stage	Site|Clutch	− 850.704	42	1790.1	0.00	0.784
Time to metamorphosis	Experimental venue + Population locality	Site|Clutch	− 158.88	12	343.7	0.00	0.38
	Experimental venue	Site|Clutch	− 158.97	12	344.0	0.28	0.33
	Experimental venue × Population locality	Site|Clutch	− 159.18	12	344.3	0.61	0.28
Survival	Experimental venue × Population locality	Site|Clutch	− 1296.14	6	2604.3	0.00	1

Predictors in the models were NF stages within stage category (Stage), Population locality and Experimental venue. Models are ranked by AICc weight, where higher weighted models have more support. *K* indicates the number of model parameters and logLik the log-likelihood of the model

Variation in time to metamorphosis

From the full averaged model, experimental venue ($P=0.0004$) was retained as the most important predictor for time to metamorphosis among all competing models (Table 2, Table S3). Other predictors such as population locality ($P=0.536$) and the interaction between population locality and experimental venue ($P=0.128$) were retained in the averaged model but had minor effects (Table S5). The post hoc test found that $\mathrm{SR_{Home}}$ and $\mathrm{WR_{Away}}$ at the summer rainfall experimental venue had a significantly higher number of individuals transitioning from larvae to climax in ten weeks than $\mathrm{SR_{Away}}$ and $\mathrm{WR_{Home}}$ at the winter rainfall experimental venue (Fig. 3). $\mathrm{SR_{Away}}$ individuals had the fastest transitioning with the first climax

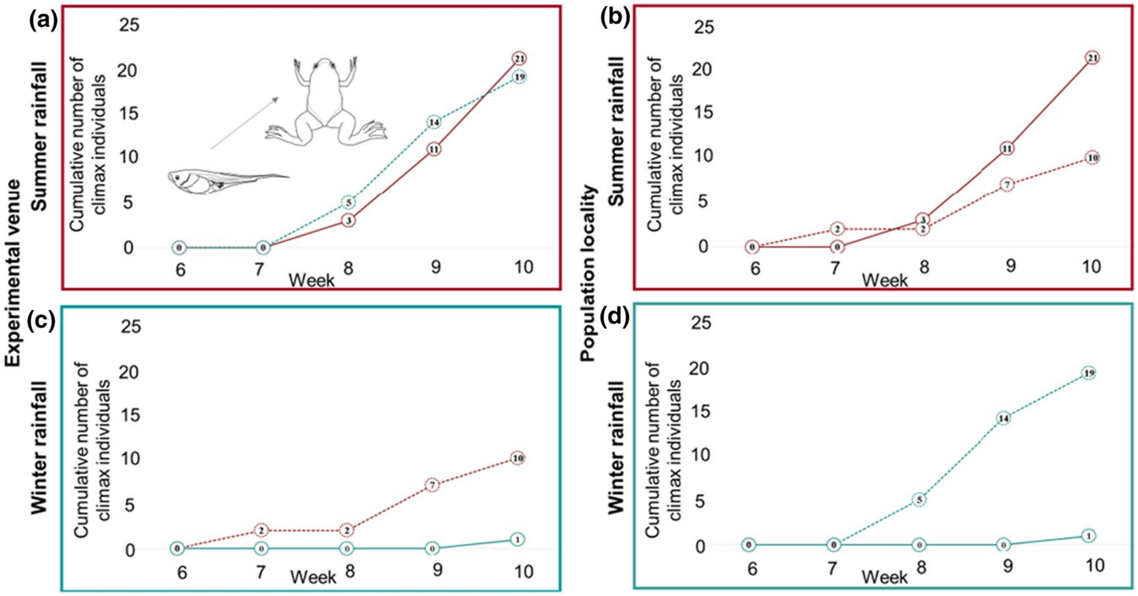

Fig. 3 Cumulative number of *Xenopus laevis* individuals that transitioned between larvae and climax per week in all mesocosms. **a** Pairwise comparison of summer rainfall home individuals (SR_{Home}: *solid red line*) and the winter rainfall away individuals (WR_{Away}: *dashed blue line*) reared in the summer rainfall region. **b** Pairwise comparison of SR_{Home} and SR_{Away} tadpoles originating from the summer rainfall region. **c** Pairwise comparison of winter rainfall home individuals (WR_{Home}: *solid blue line*) and the summer rainfall away individuals (SR_{Away}: *dashed red line*) in the winter rainfall region. **d** Pairwise comparison of WR_{Home} and WR_{Away} tadpoles originating from the winter rainfall region

individuals ($n = 2$) emerging in week 7. WR_{Home} had the slowest transition with the first climax individual emerging in week 10.

Variation in survival

The top model included the interaction between population, locality and experimental venue ($P < 2e{-}16$) for larval survival (Fig. 4, Table 2 and S4, ΔAICc = 146.35). According to the post hoc test the survival between SR_{Home} (26/150 tadpoles ± 15.24 SD) and WR_{Away} (29/150 tadpoles, ± 8.41 SD) tadpoles reared at the summer rainfall experimental venue did not significantly differ (Fig. 4). The survival of SR_{Away} (9/150 tadpoles, ± 11.72 SD) was significantly lower than the survival of WR_{Home} (64/150 tadpoles ± 40.03 SD) in the winter rainfall region.

Discussion

In agreement with our predictions for larval body size and time to metamorphosis, the results suggest that phenotypic plasticity and local adaptation both contribute to the phenotypic variation of *Xenopus laevis* tadpoles from two contrasting rainfall regimes; which agrees with previous studies (Orizaola et al. 2010; Amburgey et al. 2012). The contribution of each mechanism seems to depend on the trait. For instance, our findings on morphological variation are consistent with those of previous studies that showed that phenotypic variation results from the combined effects of selection and plasticity over divergent environmental conditions (e.g., Van Buskirk and McCollum 1999; Lind and Johansson 2007). However, the joint effect of plasticity and local adaptation on morphological variation was only detected within the last developmental stages before metamorphosis. This emphasises the importance of standardising the stage of amphibian larvae when studying the mechanisms underlying phenotypic variation as variation among stages can be high. Regarding the differences in body size between the two contrasting climatic regions, the larger body size in the summer rainfall region may be due to the higher daily temperature variation and higher maximum temperature (Niehaus et al. 2006).

For time to metamorphosis the interaction between experimental venue and population locality was retained in the averaged model but the coefficients were not significant. Experimental venue was retained as the most important predictor, thus, the expression of this trait in *X. laevis* tadpoles may be more dependent on the immediate local environment rather than the evolutionary history of the population (as seen in Walsh et al. 2008). This result is also consistent with previous findings highlighting the importance of plasticity over local adaptation for developmental phenology (Smith-Gill and Berven 1979; Newman

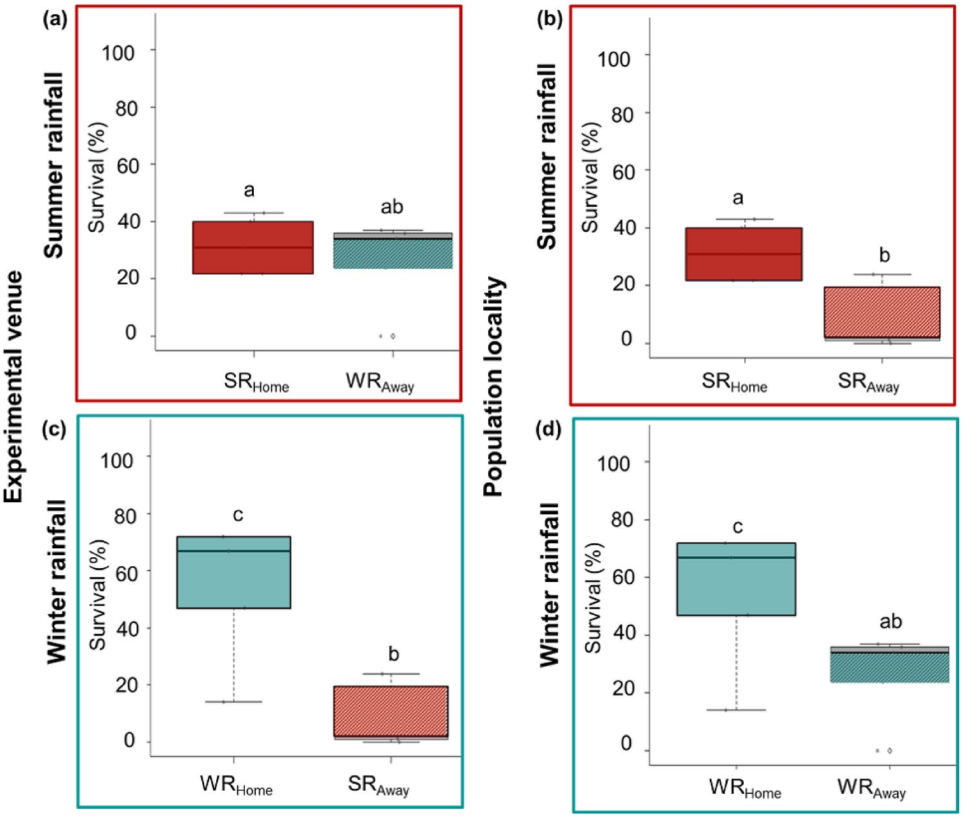

Fig. 4 Survival of *Xenopus laevis* tadpoles after ten weeks. **a** Pairwise comparison of summer rainfall home individuals (SR$_{Home}$: *solid red fill*) and the winter rainfall away individuals (WR$_{Away}$: *dashed blue fill*) reared in the summer rainfall region. **b** Pairwise comparison of SR$_{Home}$ and SR$_{Away}$ tadpoles originating from the summer rainfall region. **c** Pairwise comparison of winter rainfall home individuals (WR$_{Home}$: *solid red fill*) and the summer rainfall away individuals (SR$_{Away}$: *dashed red fill*) in the winter rainfall region. **d** Pairwise comparison of WR$_{Home}$ and WR$_{Away}$ tadpoles originating from the winter rainfall region. Survival significantly decreased between individuals of the same locality. In the boxplot, the lowest boundary indicates the 25th percentile, a black line within the box indicates the median, and the highest boundary indicates the 75th percentile. Whiskers above and below the box indicates the 10th and 90th percentile. Points above and below and the whiskers represent outliers above and below the 10th and 90th percentile. Boxplots sharing a letter are not significantly different ($p > 0.05$) according to post hoc pairwise comparisons

1989; Lind and Johansson 2007). However, plasticity alone could not explain our results of time to metamorphosis, which is also congruent with studies showing a contribution of local adaptation to this trait (Ficetola and Bernardi 2005; Orizaola et al. 2010; Amburgey et al. 2012; Mühlenhaupt et al. 2021).

The time to metamorphosis of *X. laevis* tadpoles significantly differed between the two contrasting climatic regions, where all tadpoles reared in the summer rainfall experimental venue started metamorphosing earlier (and in larger numbers) than the tadpoles reared in the winter rainfall experimental venue. This result is congruent with the findings from a recent study by Mühlenhaupt et al. (2021) where guttural toads (*Sclerophrys gutturalis*) from the summer rainfall region (Durban, KwaZulu-Natal) were found to have a faster larval development than those from the winter rainfall region. The decreased duration to metamorphosis may be due to the increase in temperature detected in the summer rainfall region as an increase in temperature can decrease larval period (Loman 2001). One possible explanation for the fact that this trait is not entirely plastic could be due to the influence of the performance of the larval stage on adult survival and fitness (Tejedo 1992; Altwegg and Reyer 2003; Cabrera-Guzman et al. 2013). Such carry over effects have been observed in other groups with complex life cycles such as odonates where faster larval development results in stronger immune system in adults (Therry et al. 2014). Additionally, there is a trade-off between time to metamorphosis and adult fecundity in insects (Nylin and Gotthard 1998). Genotypes expressing a fully plastic trait that closely tracks environmental conditions could complete metamorphosis early and result in reduced juvenile survival or adults producing less offspring than individuals with less plastic phenotypes.

We found that *X. laevis* tadpoles from different rainfall regimes display different reaction norms when translocated.

For the reaction norm to evolve, there must be enough genetic variation (Via and Lande 1985; Newman 1989). The evolution of reaction norms between populations may be because of selection on the maintenance of genetic variation in heterogeneous environments (Stearns 1982). This suggests that there is an advantage to maintain developmental plasticity in each region. Expressing the full range of phenotypic variation requires maintenance of the physiological machinery to meet the whole range of environmental variation, some of which may not be necessary in areas experiencing only a part of the climatic gradient. For this reason, theory predicts a non- or less plastic phenotype to incur lower costs than fully plastic phenotypes, and thus an advantage of specialist (non-plastic) over generalist (plastic phenotypes) in stable/predictable environments. This physiological constraint could drive the evolution of the reaction norm (van Tienderen 1991; DeWitt et al. 1998).

One result from this study displaying differing reaction norms is the change in time to metamorphosis when translocated. When translocated, *X. laevis* tadpoles from the winter rainfall region aligned with the reaction norm of local tadpoles. In other words, the direction of change in the reaction norm is toward the local phenotype (Radersma et al. 2020). Regarding thermal tolerance, the environmentally induced phenotype expresses characteristics that would allow survival in the novel environment, without considering other factors (e.g., predation regime). This suggests that plasticity contributes to local adaptation of *X. laevis* in contrasting environments (Kovaka 2019; Uller et al. 2019; Parsons et al. 2020; Radersma et al. 2020). The phenotypic response of translocated summer rainfall tadpoles compared to the local winter rainfall tadpoles may display what is described as an “undershoot”. An “undershoot” corresponds to the under expression of the phenotype in translocated individuals compared to the optimal phenotype expressed by local individuals (Radersma et al. 2020). Summer rainfall tadpoles matched the body size but “undershot” the time to metamorphosis of local tadpoles in the winter rainfall experimental venue. Due to the apparent “undershoot” the plasticity first evolution hypothesis might be a plausible scenario for this population (Radersma et al. 2020). The translocated summer rainfall tadpoles appeared to incur developmental and genetic constraints to shorten time to metamorphosis to such an extent that their survival was reduced. Nevertheless, translocated summer rainfall tadpoles decreased their development enough to significantly differ from local summer and local winter rainfall tadpoles.

Supporting differing reaction norms, we found that both summer and winter rainfall tadpoles incurred an asymmetric decrease in survival when translocated. Our results are congruent with the findings of another study that observed that the benefits of phenotypic plasticity of life-history traits also lower tadpole survival (Amburgey et al. 2016). Survival of *X. laevis* from the winter rainfall region decreased when translocated. However, survival of translocated winter rainfall tadpoles did not differ from the local tadpoles from the summer rainfall region. In contrast, the survival of summer rainfall tadpoles was significantly lower when translocated compared to the survival of local tadpoles from the winter rainfall region. Therefore, the apparent constraint on time to metamorphosis and low survival of *X. laevis* tadpoles from the summer rainfall region when translocated may indicate that the benefits of matching the local phenotype was the lowest for this group. Our result of evolved reaction norms in relation to spatial heterogeneity is in agreement with those of previous studies (Via and Lande 1985; Gomulkiewicz and Kirkpatric 1992; Moran 1992; Van Tienderen 1997; Sultan and Spencer 2002; Ernande and Dieckmann 2004).

Differential survival in mesocosms changes larval density, which in turn alters development in amphibians (Dash and Hota 1980; Wilbur 1977). Density variation is inherent to mesocosm studies, but we do not believe this was a major issue in our experiment. We made sure food intake was not limiting throughout the experiment. Additionally, larger body size was expected in mesocosms with lower densities according to the literature (Dash and Hota 1980; Newman 1994; Wilbur 1977). However, in our setup a lower density at the end of the experiment was associated with a lower survival and smaller body size, in surviving tadpoles. Summer rainfall tadpoles reared in the winter region had lower survival and a smaller body size than those reared at home, which suggests that the changes in density experienced during the experiment did not drastically affect the outcome of our experiment.

Winter rainfall tadpoles are able to express the full phenotypic reaction norm when translocated. This has implications for the dynamics of translocated *X. laevis*. Evidence of admixture and subsequently gene flow between *X. laevis* from the southwestern Western Cape clade in the winter rainfall region and other clades of *X. laevis* has been found (Measey and Channing 2003; Furman et al. 2015). Gene flow between populations can maintain plasticity of *X. laevis* in the winter rainfall region (see Richardson et al. 2014). In contrast, *X. laevis* tadpoles from the summer rainfall region display limited ability to express the full phenotype when translocated.

Although this study examined the phenotypic responses of native populations, *X. laevis* is also invasive on four continents (Measey et al. 2012). The ability to rapidly undergo adaptive change and plasticity are important characteristics of successful invasive species (Bomford et al. 2009; Tingley et al. 2012; Allen et al. 2017). The plasticity of this species at the larval stage can explain its capacity to establish in new areas with novel environmental conditions (Rödder et al. 2017). These traits can assist introduced populations to become invasive outside of their typical and natural niche

(Li et al. 2013). The ability of *X. laevis* to cope with the novel environment through plasticity could contribute to explaining the invasion success of introduced populations on several continents, and maybe an important invasion syndrome (Novoa et al. 2020). When introduced to a new area, intense selection will keep individuals with higher plasticity due to their higher survival rate. The mitochondrial DNA of all the invasive populations of *X. laevis* globally correspond to the phylogeographic lineage found in the winter rainfall region (Wang et al. 2019), with the exception of the invasive population in western France, where haplotypes from both winter and summer rainfall lineages have been detected (De Busschere et al. 2016). Our findings suggest that tadpoles from the winter rainfall region may be better invaders due to their plastic and adaptive responses.

Conclusion

Our study suggests that *Xenopus laevis* tadpoles, from two contrasting climatic regimes, are both locally adapted and have an asymmetrical capacity to respond to translocation in a novel environment. Specifically, responses of tadpoles' life-history traits were plastic but differed depending on their climatic origin. Such an asymmetric response to transplantation has been observed elsewhere and in different taxa with complex life histories (e.g., Amarillo-Suárez and Fox, 2006). These contrasting responses may arise from the various effects of phenotypic plasticity. Plasticity allows organisms to withstand novel conditions (Via and Lande, 1985; Price et al. 2003). Phenotypic plasticity alleviates maladaptation caused by incoming gene flow in heterogenous environments (Chevin and Lande 2011) while it reduces the rate of local adaptation and the development of an optimum or single phenotype in more stable environments (de Jong 1999). Therefore, the relative weight of the mechanisms that drive phenotypic variation between populations of a widely distributed species might differ across its range and account for asymmetric capacity to overcome novel conditions by phenotypic plasticity alone. These findings can assist future studies in predicting species' response to environmental change by understanding the current diversity of phenotypes and the mechanisms that drive them. Additionally, we emphasise the potential of *X. laevis* from the winter rainfall region of South Africa as an important source of potential invasions in novel environments.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-022-05240-6.

Acknowledgements The authors would like to thank the Editor and anonymous reviewers for their contribution to improve the manuscript. NK and JM would like to thank the DSI-NRF Centre of excellence for Invasion Biology (South Africa). NK would like to acknowledge the Ambassade de France en Afrique du Sud (France). We would like to acknowledge Hannes van Wyk, Erika Nortje, Henk Stander, Anvor Adams, and Carla Wagener for their field and laboratory assistance. We declare that we know of no conflicts of interest associated with this publication.

Author contribution statement NK, JM, JS conceived and designed the study, NK performed the experiments and data acquisition, NK and JS contributed to the data analysis. NK, JM, LDP, AH and JS were involved with the interpretation of data. NK drafted the manuscript and JM, AH, LDP and JS revised the manuscript and approved the final version.

Funding DSI-NRF Centre of Excellence for Invasion Biology; Ambassade de France en Afrique du Sud.

Data availability The datasets generated during and/or analysed during the current study are available in the Zenodo repository, [https://doi.org/10.5281/zenodo.4721370].

Declarations

Conflict of interest We declare that we know of no conflicts of interest associated with this publication.

Ethical statement The utilisation of the frogs and the research protocols were approved by Stellenbosch University Research Ethics Committee: Animal Care and Use (ethics number: 1535) as well as the Anim-Care Ethics committee of North-West University (NWU-0061-19-A5). Collections in the Western Cape province occurred with permission from CapeNature (collection permit: CN44-31-3376; transport and import permit: CN28-31-3722; export and transport permit: CN13-31-3740). Collections in the North-West province occurred with permission from NW READ (collection, import, export and transport permit: 5528).

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