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RESEARCH ARTICLE

Rapid adaptive response to a Mediterranean environment reduces phenotypic mismatch in a recent amphibian invader

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ABSTRACT

Invasive species frequently cope with ecological conditions that are different from those to which they adapted, presenting an opportunity to investigate how phenotypes change across short time scales. In 2000, the guttural toad *Sclerophrys gutturalis* was first detected in a peri-urban area of Cape Town, where it is now invasive. The ability of the species to invade Cape Town is surprising as the area is characterized by a Mediterranean climate significantly drier and colder than that of the native source area. We measured field hydration state of guttural toads from the invasive Cape Town population and a native source population from Durban. We also obtained from laboratory trials: rates of evaporative water loss and water uptake, sensitivity of locomotor endurance to hydration state, critical thermal minimum (CT_{min}) and sensitivity of CT_{min} to hydration state. Field hydration state of invasive toads was significantly lower than that of native toads. Although the two populations had similar rates of water loss and uptake, invasive toads were more efficient in minimizing water loss through postural adjustments. In locomotor trials, invasive individuals noticeably outperformed native individuals when dehydrated but not when fully hydrated. CT_{min} was lower in invasive individuals than in native individuals, independent of hydration state. Our results indicate that an invasive population that is only 20 years old shows adaptive responses that reduce phenotypic mismatch with the novel environment. The invasion potential of the species in Cape Town is higher than we could infer from its characteristics in the native source population.

KEY WORDS: Anuran, Environmental novelty, Invasive species, Locomotor performance, Thermal tolerance, Water exchange

INTRODUCTION

The mean phenotype of a population should maximize the fitness of its individuals in the given environment (Lande, 1976). Such maximization emerges from the interplay between environmental pressures and mechanisms of genetic and non-genetic inheritance occurring on evolutionary time scales (Lande, 1976; Laland et al., 2015). Invasive populations at the incursion stage (Van Wilgen et al., 2014) may, however, be an exception to this generalization. At the onset of an invasion process, ecological conditions are often dissimilar from those to which the invaders adapted (Novak, 2007); therefore, a mismatch between the current available phenotypes and the phenotype maximizing fitness in the invaded environment occurs (Prentis et al., 2008; Hendry et al., 2011). This may lead the invasive population to respond adaptively to the novel environmental conditions to reduce phenotypic mismatch (Lee, 2002; Whitney and Gabler, 2008).

Adaptive responses may be promoted by various rapid evolutionary and non-evolutionary mechanisms such as environmentally induced plasticity (Strauss et al., 2006; Ghalambor et al., 2007; Liao et al., 2016), maternal effects (Monty et al., 2013) and epigenetic changes (Moran and Alexander, 2014). As these mechanisms can act on much shorter time scales than those required for adaptive genetic changes (hereafter 'adaptation', as used in the strict sense by Hendry et al., 2011), their key role in reducing phenotypic mismatch at the onset of an invasion has often been invoked (Ghalambor et al., 2007; Davidson et al., 2011; Liao et al., 2016). However, the occurrence of contemporary adaptation (Carroll et al., 2007) early in an invasion process cannot be neglected; recent studies have shown cases of rapid adaptive genetic changes to human-induced environmental modifications within one or a few years (Sultan et al., 2012; Stuart et al., 2014; de Amorim et al. 2017; Campbell-Staton et al., 2017).

Differentiating evolutionary and non-evolutionary mechanisms that promote adaptive responses in invasive populations usually requires long and/or laborious studies (Colautti and Lau, 2015; Rollins et al., 2015). However, prompt exploratory efforts to find mismatch reductions in the invader's phenotype are often necessary and recognizing the occurrence of adaptive responses at the onset of the invasion has practical importance. Invasive populations may have considerable impacts on native species and communities (Simberloff et al., 2013), and efforts should thus be directed to understanding the mechanisms of invasion, predicting future invasions and obtaining insights for prioritization and control. Ignoring the occurrence of contemporary adaptive responses hampers our ability to forecast invasion potential (Broennimann et al., 2007). For example, models that predict the invasion potential of a species from the niche space estimated in the native distribution may fail to estimate robustly the invaded range because they do not consider adaptive responses that minimize phenotypic mismatches during invasion (Urban et al., 2007).

If we aim to investigate the occurrence of phenotypic responses in an invasive population, a sensible first step is to compare species phenotype between native and invaded populations, defined by Hierro et al. (2005) as 'biogeographical intraspecific comparison'. The next step is to compare a possible phenotypic response with other similar adaptive responses detected in closely related populations or species. Although this approach does not allow us to make a conclusive distinction between adaptive phenotypic plasticity and local adaptation (Strauss et al., 2006; Van Kleunen et al., 2010), it helps us to explore the extent to which invaders express novel characteristics that confer higher fitness in the novel environment.

Toads (Amphibia, Anura, Bufonidae) offer excellent prospects for finding rapid phenotypic responses to new environmental contexts. Evolutionary accumulation of adaptive traits promoting range expansion has allowed this group to attain a cosmopolitan distribution and invade disparate environments over relatively short time scales (Van Bocxlaer et al., 2010). Furthermore, the deliberate introduction of and subsequent invasion by the cane toad *Rhinella marina* in more than 40 countries across the globe has yielded an extensive literature on the physiological and behavioural response of these toads to novel environmental challenges (Lever, 2001; Rollins et al., 2015, in Australia; Kosmala et al., 2017). Also, toad invasions are known to have disproportionate environmental and economic impacts when compared with those of other amphibians (Shine, 2010; Measey et al., 2016). Recent observations of bufonids accidentally moved outside their native range (Kolby, 2014; Measey et al., 2017; Reilly et al., 2017; Tingley et al., 2017) highlight the need to dedicate more study to their capacity to adapt rapidly to novel environments.

Amphibians are particularly vulnerable to dehydration. As many important activities such as locomotion are strongly affected by an individual's hydration state (Preest and Pough, 1989), they have evolved several adaptations to regulate water balance (McClanahan and Baldwin, 1969; Prates and Navas, 2009). However, the sensitivity of physiology to hydration state differs among species and populations, with amphibians that evolved in drier environments generally outperforming those from wetter environments when dehydrated (Beuchat et al., 1984). Although the capacity to adapt to the challenges imposed by drier environmental conditions may emerge on evolutionary time scales (Titon et al., 2010), it is not clear to what extent adaptive responses can rapidly reduce a phenotypic mismatch caused by the introduction of a sub-optimal genotype into an unfamiliar environment. To address this important question, we studied an invasive population of guttural toads *Sclerophrys gutturalis* recently established (in 2000) in Cape Town, South Africa, in comparison to a native population of the same species from Durban, South Africa. We selected Durban to represent the native population as it is hypothesized that this was the source of founder individuals by genetic analyses (Telford, 2015).

The guttural toad naturally inhabits areas of tropical and subtropical southern Africa characterized by summer rainfall, where the species adaptively synchronizes reproduction with rainfall to exploit favourable conditions of higher temperatures and water availability (du Preez et al., 2004). Invasive guttural toads in Cape Town still breed in the warmer months, despite the winter rainfall regime of the area resulting in summers that are notably drier in terms of precipitation and general humidity than those characterizing Durban (Fig. 1). The invasion has been facilitated by the occurrence in the invaded area of numerous artificial ponds (Vimercati et al., 2017a) and the synanthropic behaviour of the species (Measey et al., 2017). However, it is not clear whether and how the phenotype of the guttural toad has rapidly responded to an environment that is climatically drier than the native range.

Invasive toads in Cape Town are actively invading an area that is characterized by a climate significantly drier and colder than that of the native source area of Durban. Thus, we hypothesize that the invasive population has undergone a rapid adaptive response to reduce its phenotypic mismatch with the novel environment with regard to hydric balance and thermal tolerance. To investigate this hypothesis, we conducted two separate sets of experiments on toads from Cape Town and Durban. (1) In the field, we checked the extent to which individuals from the invasive population are exposed to more severe desiccating conditions than native conspecifics by measuring each toad's hydration state during the reproductive period. (2) In the laboratory, we estimated the extent to which the physiological phenotype of the guttural toad differs between invasive and native individuals by measuring: (i) the rate of evaporative water loss (EWL) and rate of water uptake (WU), (ii) the sensitivity of locomotor endurance to hydration state, (iii) the critical thermal minimum (CT_{min}) and (iv) the sensitivity of CT_{min} to hydration state. These traits are commonly measured in amphibians to assess phenotypic response to local conditions of temperature and water availability across populations (Tingley et al., 2012; McCann et al., 2014), and to forecast anuran invasion potential by defining terrestrial niche space (Kolbe et al., 2010; Tingley et al., 2012).

MATERIALS AND METHODS
Study species and locations
The guttural toad *Sclerophrys gutturalis* (Power 1927) is a widely distributed African bufonid native to central and southern Africa at a range of altitudes (from sea level to about 1800 m a.s.l.) and latitudes (from the equator to 30°S) (du Preez et al., 2004). The species inhabits disparate vegetation types like savanna, grassland and thicket biomes and frequently occupies peri-urban areas as a
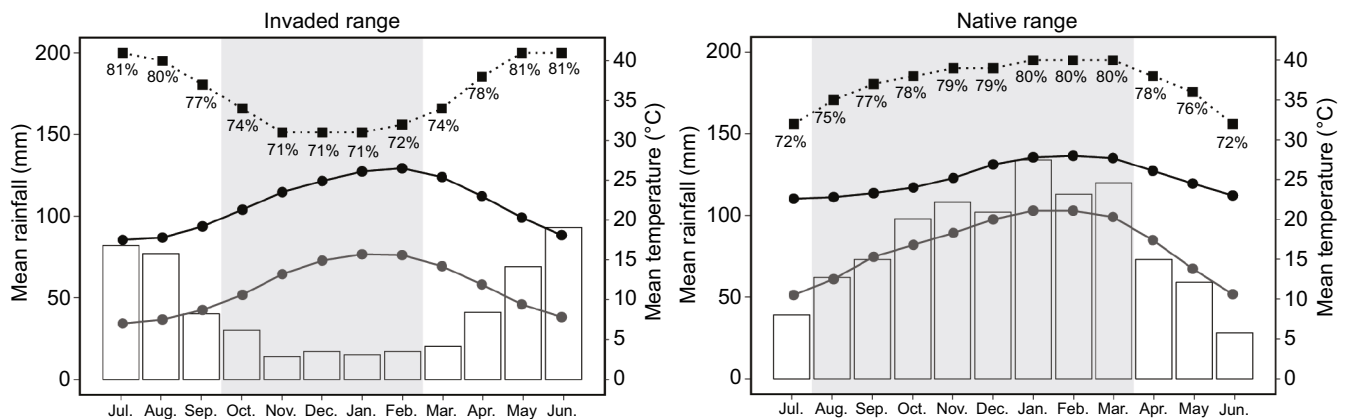

Fig. 1. Climate data from the invaded range of Cape Town and the native range of Durban. Mean monthly values for rainfall (bars), maximum temperature (black circles and solid line), minimum temperature (grey circles and solid line) and relative humidity (black squares and dotted line) are reported. The shaded area represents the breeding season of guttural toads, *Sclerophrys gutturalis*, in each sampling location. Climate data sourced from the World Meteorological Organization, http://public.wmo.int/.

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result of highly synanthropic behaviour (du Preez et al., 2004). The guttural toad is a domestic exotic in South Africa (Measey et al., 2017), being native in most of the country but not in the Western Cape, where an invasive population was first identified in Cape Town in 2000 (De Villiers, 2006).

Adult toads were collected within an area of 10 km^2 both in Cape Town (87 m a.s.l., 34°01′S, 18°25′E), where the population is still expanding every year (Measey et al., 2017), and in Durban (75 m a.s.l., 29°47′S, 31°01′E), where the species is native. The two populations share similar altitudinal ranges and very similar peri-urban landscapes characterized by artificial breeding ponds (Vimercati et al., 2017a).

Ethics clearance for research on captive toads was obtained from Stellenbosch University Animal Ethics Committee (protocol number U-ACUD14-00112); collections in the native area (Durban) and in the invasive area (Cape Town) occurred under permission from KZN Wildlife (permit number OP553/2015) and Cape Nature (permit number 0056-AAA041-00088), respectively.

Hydration state in the field

In January and February 2016, adult toads were captured in Cape Town (sample size: n=35, n=17 males, n=18 females; snout–vent length (SVL): mean±s.d. 75.2±8.7 mm, range 60.0–99.0 mm; body mass: mean±s.d. 43.5±19.0 g, range 20.7–114.5 g) and Durban (sample size: n=43, n=20 males, n=23 females; SVL: mean±s.d. 79.2±7.5 mm, range 66.4–96.0 mm; body mass: mean±s.d. 51.3± 18.5 g, range 27.3–103.0 g) every third night for a total of 2 weeks. At capture, we recorded air temperature and relative humidity (using a pocket weather meter, AZ-8910 5 in 1, AZ Instrument Corp., Taichung, Taiwan) and cloacal body temperature (using a thermocouple connected to a digital thermometer, CHY 507, CHY Firemate, Tainan City, Taiwan). Immediately after capture, each toad was first blotted with a paper towel, its bladder emptied by gently pressing the abdomen, and its body mass measured with a portable balance (±0.01 g, WTB 2000, Radwag, Radom, Poland). Then, the toad was placed in a plastic container filled to a depth of 20 mm with water to promote hydration and its body mass was measured every 15 min until it did not change more than 0.01 g between two consecutive measurements. As hydration state is a measure of the tissue hydration that does not include bladder water (Tracy et al., 2014), the toad's bladder was emptied before each body mass measurement. The final measurement was considered the fully hydrated body mass of the individual (hydration state of 100%; Tracy et al., 2014). Hydration state was calculated as initial body mass as a percentage of fully hydrated body mass. Toads that defecated during the experiment were removed from the analysis.

To investigate differences in field hydration state between the two populations, we performed a Kruskal–Wallis test. A Spearman correlation test was also performed between the hydration state and air temperature, body temperature and relative humidity to investigate their effect on individual hydration state.

Laboratory housing

To test toad traits in the laboratory, adult toads were captured between December 2015 and February 2016 and housed in tanks with water and shelter *ad libitum* on a natural photoperiod, and fed mealworms (mealworm beetle *Tenebrio molitor* larvae) twice a week. The collection of preliminary data in the field led us to set the temperature and relative humidity of the room at a constant 23±2°C and 70±5%, respectively. Animals were not fed for 3 days before any experiment to avoid the effects of defecation and specific dynamic action (i.e. the increase in metabolic rate elicited by

feeding) on hydration and body mass (Secor and Faulkner, 2002). Individuals tested in the laboratory were different from those used to estimate hydration state in the field and each toad was utilized in only one of the physiology experiments within 2 weeks from the day of capture.

EWL and WU

To estimate EWL and WU, the toads from Cape Town (sample size: n=22, n=11 males, n=11 females; SVL: mean±s.d. 72.5±5.9 mm, range 62.8–83.0 mm; body mass: mean±s.d. 36.7±9.4 g, range 23.4–57.3 g) and Durban (sample size: n=20, n=10 males, n=10 females; SVL: mean±s.d. 74.2±4.7 mm, range 65.3–83.8 mm; body mass: mean±s.d. 39.1±8.6 g, range 26.0–59.7 g) were placed in individual plastic containers filled with water to a depth of 20 mm for 1 h and left in a climate-controlled room (23°C and relative humidity 65%), to ensure that they were fully hydrated before the tests. Then, the fully hydrated body mass of each individual (hydration state of 100%) was measured following the protocol described above to estimate hydration state in the field.

Each individual was subsequently placed inside a plastic wind tunnel (diameter 0.25 m, length 1 m) equipped at one end with a small electric fan that created an air flow of 0.1 m s^{-1}, and weighed (±0.01 g) at intervals of 15 min. Following Titon et al. (2010), the toad was restricted to the tunnel end opposite the fan using a mesh barrier. The EWL trial stopped when the toad reached 80% of its fully hydrated body mass. We derived total surface area from fully hydrated body mass through the equation empirically derived by McClanahan and Baldwin (1969), and calculated the effective surface area assuming the water-conserving posture exposes two-thirds of the total area of the toad's body to the air (Withers et al., 1984; Titon et al., 2010). The rate of EWL was calculated by dividing the regression of body mass (mg) against time (min) by the effective surface area (cm^2) from each individual. As EWL may co-vary with the proportion of time spent in the water-conserving posture, we inspected toad posture in the plastic tube every 15 min following Tingley et al. (2012).

After the EWL test, the dehydrated toads (hydration state of 80%) were placed individually in a plastic container filled with water to a depth of 10 mm, blotted with a paper towel and weighed (±0.01 g) every 5 min. The experiment was stopped when body mass did not change between two consecutive weighings. Rate of WU was calculated from the regression of body mass (g) against time (min) following Titon et al. (2010).

As water loss can be affected by the water-conserving posture, differences in EWL between the two populations were explored using ANCOVA with the proportion of time spent in a water-conserving posture used as a covariate, following Tingley et al. (2012). Differences in terms of WU between the two populations were evaluated by a *t*-test. To explore the occurrence of a correlation between EWL and WU and population of origin, we performed an ANCOVA with WU as the response variable and EWL as the covariate.

Sensitivity of locomotor endurance to hydration state

The effect of hydration on performance was tested in 30 toads from Cape Town (n=15 males, n=15 females; SVL: mean±s.d. 74.2± 8.6 mm, range 62.2–94.3 mm; body mass: mean±s.d. 41.5±16.1 g, range 22.9–85.4 g) and 24 toads from Durban (n=12 males, n=12 females; SVL: mean±s.d. 73.7±6.5 mm, range 63.7–89.0 mm; body mass: mean±s.d. 42.2±12.9 g, range 27.5–77.7 g). To determine whether hydration status influenced locomotor endurance, toads at three hydration states (100%, 90% and 80% of fully hydrated mass;

10 toads from Cape Town and 8 toads from Durban for each treatment) were tested on an indoor circular racetrack (4.1 m) using a rubber grip mat as a substrate (Tingley et al., 2012) at constant temperature and humidity (23°C and 70%, respectively). Different hydration states were obtained using the same protocol described in the previous sections. As toads were active after sunset at both sampling locations, we performed performance trials at night. Each toad was individually placed on the racetrack and stimulated to hop by gently tapping it on the urostyle with a stick. To regularize tapping time among individuals and between populations, the toads were tapped by G.V. at intervals of 1 s after each hop. For each toad, we counted the number of laps it performed until it did not voluntarily hop for 60 s (i.e. exhaustion). For each lap around the racetrack, we also recorded the time taken and the number of hops until exhaustion.

As two 80% hydrated individuals from Cape Town showed anomalous behaviour when placed on the racetrack (i.e. they did not hop for the first 45 s of the experiment and refused to hop again after about 30 s), we removed them from the analysis. Each individual was killed straight after the experiment by immersion in 1 g l^{-1} tricaine methanesulfonate (MS 222) for 20 min; SVL and tibia length were measured using a digital calliper in order to explore a possible role of morphology on hopping ability (Phillips and Shine, 2006).

We used ANOVA to investigate effects of population and hydration state on endurance (i.e. the total distance travelled expressed in m), speed (m s^{-1}), distance covered in the first 10 min (m) and number of hops per metre. Also, we performed ANOVA to study the effect of independent variables on endurance, speed and distance covered in the first 10 min, all expressed in multiples of SVL (body lengths).

CT_{min} and its sensitivity to hydration state

We used 30 toads from Cape Town (n=15 males, n=15 females; SVL: mean±s.d. 70.6±6.5 mm, range 52.0–80.8 mm; body mass: mean±s.d. 34.0±9.3 g, range 14.0–51.5 g) and 20 toads from Durban (n=10 males, n=10 females; SVL: mean±s.d. 68.8±5.6 mm, range 59.9–76.9 mm; body mass: mean±s.d. 31.1±7.3 g, range 20.6–42.8 g) to estimate CT_{min}. One hour before the experiment, individuals were placed in individual plastic containers filled with water to a depth of 20 mm, and kept inside a climate-controlled room (23°C and relative humidity 65%), to ensure that they were fully hydrated before the tests. Then each toad was weighed and individually placed in a metal chamber (80 mm L×100 mm W×150 mm H) submerged in a fluid-filled Perspex jacket and connected to a water bath (Grant Gr150, Grant Instruments, Shepreth, UK) containing a 1:1 water:glycol mixture at 0°C. The aperture of the chamber was closed with acetate film to prevent the toad escaping and to maintain the targeted temperature and high humidity (~100% relative humidity) within the chamber. Every 2 min, the toad was turned on its back in order to test the righting reflex (Spellerberg, 1972). In addition, body temperature was collected every 2 min with a thermocouple inserted into the cloaca to estimate cooling rate as the regression of temperature over time. The experiment was repeated until the toad was unable to right itself for 15 s (Kolbe et al., 2010); the CT_{min} was considered the highest body temperature at which the toad first lost its righting response. This method closely follows the protocol adopted by Kolbe et al. (2010) and McCann et al. (2014) to estimate CT_{min} in cane toads, but we utilized a water bath, instead of a cooler box with ice, to standardize the chamber temperature and reduce the variation in cooling rate among individuals.

In order to test how the hydration state affects CT_{min}, the same toads previously tested for CT_{min} were placed 1 week later into the same plastic wind tunnel previously used to estimate EWL, and dehydrated until they reached 80% of their fully hydrated body mass. After that, we repeated the CT_{min} experiment as described above.

To explore differences between the two populations for CT_{min} in fully hydrated toads (100% hydration state), we used ANCOVA with body mass and cooling rate as covariates. Although body mass significantly affected cooling rate (P=0.0009), body mass was not significant as a covariate and did not significantly differ between the two populations. Therefore, it was removed from the successive analyses (Kolbe et al., 2010; McCann et al., 2014). As each individual was tested for CT_{min} at the two different hydration states, we used repeated measures MANCOVA with population and hydration state as factors and cooling rate as covariate in order to test the effect of hydration state on CT_{min}. All analyses were conducted using R version 3.3.0 (https://www.r-project.org).

RESULTS
Field hydration state
On capture from the field, invasive toads were significantly less hydrated than were native toads (Cape Town 89.9±5.0%, Durban 96.2±4.3%, Kruskal–Wallis χ^2=27.75, P<0.0001; Fig. 2). Although 10 out of 43 individuals collected in Durban were fully hydrated (hydration state of 100%) when collected as a result of being captured on a single rainy night (Dataset 1), their removal from the analysis did not significantly change the mean field hydration state of the population (95.1±4.3%) so we retained them in the analysis. Hydration state was positively correlated with relative humidity (Spearman's ρ=0.67, P<0.0001) but not correlated with air temperature (ρ=0.09, P=0.41) or body temperature (ρ=0.18, P=0.11).

EWL and WU
The two populations did not differ in EWL once corrected for the time spent in water-conserving posture (Cape Town 0.93±0.22 mg cm^{-2} min^{-1}, Durban 0.89±0.18 mg cm^{-2} min^{-1}; $F_{1,38}$=0.35, P=0.558); notably, the time spent in this posture did
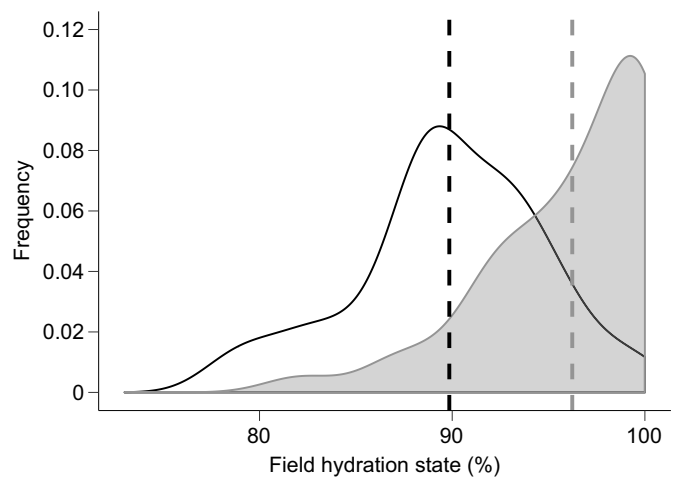

Fig. 2. Smoothed frequency distributions of field hydration states in invasive and native toads. The field hydration state is expressed as a percentage of the empty bladder body mass against the fully hydrated body mass, both measured in the field. Vertical lines represent means. Guttural toads, *S. gutturalis*, were collected from the invasive population of Cape Town (black curve, white shading, n=35) and the native population of Durban (grey curve and shading, n=43).

not differ between the two populations either. However, an interaction between time spent in water-conserving posture and population was detected ($F_{1,38}$=4.62, P=0.038); toads from Cape Town were more efficient than those from Durban in minimizing water loss through postural adjustments (Fig. 3; Cape Town, r= −0.84, P<0.0001; Durban, r=−0.45, P=0.044).

The two populations did not differ in terms of WU (Cape Town 24.41±11.21 g 100 g⁻¹ h⁻¹, Durban 23.38±6.65 g 100 g⁻¹ h⁻¹; $t_{34.7}$=0.37, P=0.72). Globally (among all individuals from both populations), EWL was positively correlated with WU rate ($F_{1,38}$=16.55, P=0.0002); toads that lost water faster also gained water more rapidly. However, this relationship showed a population effect ($F_{1,38}$=5.53, P=0.024); EWL was positively correlated with WU in the invasive population (r=0.69, P=0.0003) but not correlated with WU in the native population (r=0.11, P=0.623).

Sensitivity of locomotor endurance to hydration state
Although endurance in invasive toads was about twice that of native toads for both dehydration treatments (Fig. 4A; Dataset 1), the effect of desiccation on locomotion did not differ significantly between the two populations (Table 1). More generally, invasive toads seemed to outperform native toads when 90% and 80% hydrated but not when they were fully hydrated (Fig. 4). This may suggest that invasive toads were less sensitive to desiccation than native toads but that the small sample size did not allow detection of a significant difference between the two populations. Globally, hydration state affected locomotor endurance, with fully hydrated toads (100%) outperforming toads that were dehydrated (90% and 80%; Table 1). A population effect was detected for endurance, with invasive individuals able to cover longer distances than individuals from the native range (Table 1). All the results reported above were analogous when locomotor endurance was expressed in SVL (body lengths) instead of distance (data not shown), suggesting that body size does not significantly affect endurance in this species. Also, neither SVL nor tibia length differed between populations or among treatments. This suggests that these morphological traits did not explain the variations observed in our groups (Tingley et al., 2012; McCann et al., 2014).

CT$_{min}$ and its sensitivity to hydration state
When fully hydrated, invasive toads showed a lower CT$_{min}$ than native conspecifics (Cape Town 7.1±1.3°C, Durban 8.2±1.9°C; $F_{1,46}$=3.95, P=0.048). Cooling rate significantly affected CT$_{min}$ ($F_{1,46}$=10.93, P=0.0018) while an interaction effect between cooling rate and population was also observed ($F_{1,46}$=9.51, P=0.0034).

The two populations did not differ from each other in CT$_{min}$ when tested across the two hydration states ($F_{1,46}$=1.66, P=0.10). However, hydration state did not affect the CT$_{min}$ ($F_{1,46}$=0.33
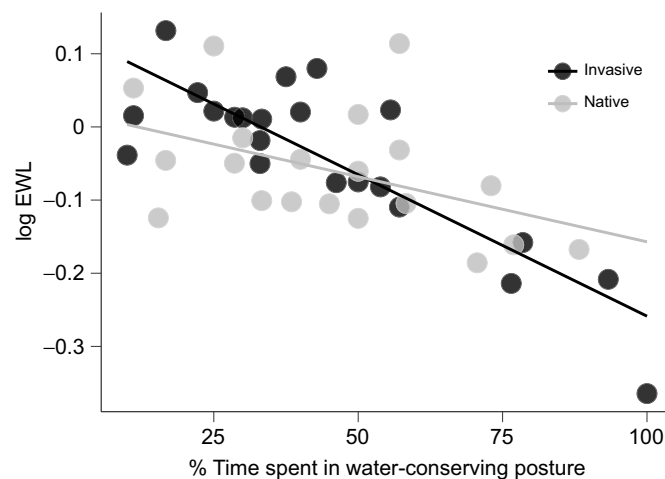

Fig. 3. Linear regression of evaporative water loss on percentage of time spent in water-conserving posture in invasive and native toads. Values of evaporative water loss (EWL, expressed in mg cm⁻² min⁻¹) from the y-axis are log-transformed. Guttural toads, *S. gutturalis*, were collected from the invasive population of Cape Town (r=−0.84, P<0.0001, n=22) and the native population of Durban (r=−0.45, P=0.044, n=20).
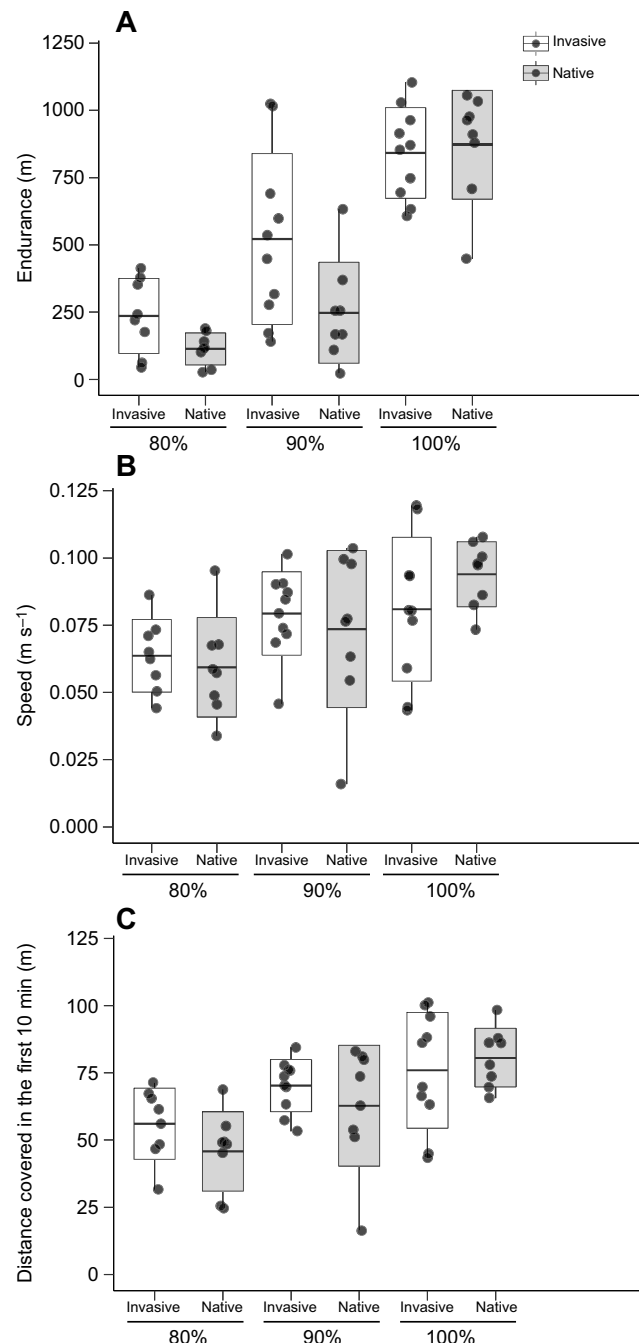

Fig. 4. Effect of hydration state on locomotor endurance in invasive and native toads. (A) Endurance, (B) speed and (C) distance covered in the first 10 min. Boxes represent means±s.d.; whiskers extend to maxima and minima. Guttural toads, *S. gutturalis*, were collected from the invasive population of Cape Town (n=28) and the native population of Durban (n=24).

Table 1. Effects of population and hydration state on locomotor endurance in guttural toads, *Sclerophrys gutturalis*, from invasive Cape Town and native Durban populations

Variable	Statistical test	P
Endurance (m)		
Population	$F_{1,46}=9.16$	**P=0.004**
Hydration state	$F_{2,46}=31.19$	**P<0.0001**
Population:hydration state	$F_{2,46}=2.18$	P=0.124
Speed (m s⁻¹)		
Population	$F_{1,46}=0.723$	P=0.127
Hydration state	$F_{2,46}=4.15$	**P=0.022**
Population:hydration state	$F_{2,46}=1.44$	P=0.25
Distance covered in the first 10 min (m)		
Population	$F_{1,46}=1.95$	P=0.169
Hydration state	$F_{2,46}=8.17$	**P=0.0009**
Population:hydration state	$F_{2,46}=1.36$	P=0.27
Number of hops per metre		
Population	$F_{1,46}=0.24$	P=0.627
Hydration state	$F_{2,46}=1.10$	P=0.340
Population:hydration state	$F_{2,46}=0.32$	P=0.730

Significant differences (P<0.05) are highlighted in bold.

$P=0.75$), while cooling rate had a significant effect ($F_{1,46}=2.66$, $P=0.0106$).

DISCUSSION

We found that invasive guttural toads exposed to a novel environment for <2 decades show an adaptive response that reduces phenotypic mismatch in the invaded area. Consistent with the drier, colder environment of Cape Town, the invasive population has responded physiologically and behaviourally to reduce its sensitivity and exposure to potential stressors. Such a response indicates that invasive guttural toads have a higher potential to invade Cape Town, and possibly other drier environments, than we could infer from studies of the source population alone.

Invasive guttural toads exhibited lower field hydration states than native toads during the breeding season (Fig. 2), suggesting that the lower precipitation and relative humidity that characterize the invaded Mediterranean range in summer expose the toads to more desiccating conditions than those experienced by native conspecifics (Fig. 1). Both the sampling sites (Cape Town and Durban) are characterized by a peri-urban landscape where residents maintain artificial ponds and irrigate their gardens. Thus, the climatic difference between invaded and native ranges is still evident despite the landscape homogenization caused by urbanization, and may have relevant repercussions for the fitness of the invaders, especially during the reproductive season.

Amphibian physiological performance is negatively affected by a decrease in hydration state (Preest and Pough, 1989; Titon et al., 2010). As a consequence, we predicted that populations invading environments characterized by more desiccating conditions should respond by regulating water exchange more effectively and/or developing lower physiological sensitivity to dehydration. Our findings confirm this prediction and show that adaptive responses reducing phenotypic mismatch with the invaded environment may occur on a relatively short time scale (<2 decades). Although the invasive and native populations did not exhibit markedly different rates of EWL or WU, individuals from Cape Town minimized water loss more effectively through a more efficient use of a water-conserving posture (Fig. 3). Therefore, in addition to an adaptive shift, plastic behavioural mechanisms could partially compensate for the low plasticity of physiological traits such as skin permeability (Snell-Rood, 2013; Davies et al., 2015).

Changes in behaviour have been detected in invasive species exposed to environmental conditions different from those to which they had adapted, both across (Pizzatto and Shine, 2008; Liebl and Martin, 2012) and within generations, through behavioural plasticity (Terkel, 1995, in mammals; Price et al., 2008, in birds; Pettit et al., 2016, in frogs). Behavioural plasticity is also a predictor of invasion success across many taxa (Wright et al., 2010; Amiel et al., 2011).

Intriguingly, EWL and WU were positively correlated in the invasive toad population (Cape Town), while no such correlation was detected in toads from the native area (Durban). This suggests that only toads inhabiting the drier environment counterbalance a faster water loss through fast WU. This is similar to observations by Tingley et al. (2012) in invasive cane toads, *Rhinella marina*, from a semi-arid and a mesic environment. We used the same dessication protocol as Tingley et al. (2012), i.e. air flow in a wind tunnel, to induce a relatively rapid dehydration rate, which may have imposed stress on the animals and impeded a prompt effective physiological response such as water absorption through the pelvic patch in native, but not in invasive, toads (McClanahan and Baldwin, 1969; Titon et al., 2010). Testing the correlation between EWL and WU across populations and species exposed to differential hydric regimes seems ripe for further investigation. However, we suggest inducing desiccation through more natural experimental procedures, as recently done by Kosmala et al. (2017).

As reported in other species of amphibians (Titon et al., 2010; Tingley et al., 2012; Kosmala et al., 2017), guttural toad locomotor performance is affected by hydration state (Table 1); this implies that in the invaded range, the capacity of the species to disperse and migrate during the breeding season is constrained by the hot, dry Mediterranean summers. Invasive toads are exposed to desiccating conditions that make the phenotype of the species sub-optimal in the invaded area, thus causing a phenotypic mismatch. We show that invasive toads may be responding to these constraints (1) through behavioural means (adopting a more effective water-conserving posture) and (2) by balancing high rates of EWL with fast WU. However, our study also shows that individuals from the invaded range notably, but not significantly, outperformed native conspecifics when dehydrated; for example, endurance of invasive toads was about twice that of native toads in both dehydration treatments (Fig. 4A; Dataset 1). Conversely, native toads had slightly higher endurance than invasive toads when fully hydrated (Fig. 4). This contrast could suggest a trade-off in performance, such that enhanced performance when dehydrated has come at the cost to performance when hydrated (Kosmala et al., 2017). Given the striking difference in sensitivity of locomotion to hydration state between the two populations, the lack of statistical power in our analysis (Table 1) is probably due to sample size constraints and wide inter-individual variation in locomotor ability (Fig. 4); although logistic constraints did not allow us to test endurance in a larger number of individuals, increasing the sample size in future studies may reveal significant differences between toads in native and invaded ranges as recently observed in the cane toad (Kosmala et al., 2017).

Although at this stage we cannot conclusively identify the mechanism responsible for reducing the phenotypic mismatch (e.g. local adaptation versus phenotypic plasticity; see Rollins et al., 2015), our findings have both evolutionary and conservation implications. Firstly, reduction of phenotypic mismatch could suggest the onset of a genetic change (Lee, 2002; Strauss et al., 2006; Whitney and Gabler, 2008) as a result of the classic mechanism of natural selection (Lande, 1976); for example, if individuals less sensitive to dehydration have higher fitness than

conspecifics as a consequence of reaching breeding sites faster (i.e. local adaptation). A genetic change may also be due to spatial disequilibrium dynamics such as spatial selection (Shine et al., 2011); for example, if individuals less sensitive to dehydration disperse further than conspecifics and exploit low-density areas beyond the invasion front. Alternatively, the mismatch reduction may be promoted by environmentally induced plasticity, where prolonged exposure to dry conditions causes changes in individuals, permitting a better performance when dehydrated but at a cost to performance when hydrated (DeWitt et al., 1998; Relyea, 2002). As the exact translocation pathway of guttural toads to Cape Town is unknown (Measey et al., 2017), we also cannot exclude the possibility that severe conditions of temperature and desiccation during transport may have selected only the most tolerant individuals, thus filtering the founders' phenotype (Tingley et al., 2010). Lastly, neutral mechanisms, such as genetic drift or random founder effect, could also have reduced phenotypic mismatch, although this seems improbable considering the clear adaptive significance of the reduction. Controlled translocation or common-garden experiments may help to investigate whether this rapid response has an evolutionary component or derives from phenotypic plasticity (Moloney et al., 2009; Pettit et al., 2016; Gruber et al., 2017; Ramenofsky et al., 2017). As local adaptation may reduce physiological sensitivity to dehydration over time and accelerate the invasion spread, this investigation could have additional management implications. More generally our study suggests that models incorporating locomotion ability to forecast invasion potential should consider the lower sensitivity of Cape Town toads to dehydration; ignoring this distinction could significantly underestimate invasion potential (Urban et al., 2007; Whitney and Gabler, 2008).

The invasive population of guttural toads is currently spreading, and toads colonize new ponds every year through leading edge dispersal (Measey et al., 2017). Since they were first detected, guttural toads have invaded an area of 10 km^2; in the last 5 years, the invasion front has advanced about 2 km (Vimercati et al., 2017a). However, this spread rate is low when compared with that of other invasive toad populations (Urban et al., 2007; Moore et al., 2015). Many factors such as the constant removal of guttural toads by an eradication programme or the high density of available breeding sites in Cape Town (Vimercati et al., 2017b) may contribute to this rate of spread. We suggest that mark–recapture and radio-tracking studies performed in both native and invasive populations (Pizzatto et al., 2017) could link physiological and behavioural constraints to the actual dispersal of the species in the field.

Cape Town is generally colder than Durban (Fig. 1); furthermore, the mean July minimum temperature, known to be a reliable predictor of CT_{min} for toads in the southern hemisphere (Kolbe et al., 2010), is notably lower in Cape Town than in Durban (7°C and 10.5°C, respectively, World Meteorological Organization, http://public.wmo.int/). As CT_{min} and thermal tolerance are highly plastic in some amphibian species (Kolbe et al., 2010; McCann et al., 2014, 2018), the difference between the two populations may be due to thermal acclimation (i.e. phenotypic plasticity) on a short time scale. This rapid phenotypic response may be adaptive in the invaded range, prolonging the activity of invasive toads in the coldest months of the year (Seebacher and Franklin, 2011; McCann et al., 2014). Interestingly, CT_{min} recorded in guttural toads and its relationship with the mean July minimum temperature of Cape Town and Durban are analogous to the values detected in cane toads, *Rhinella marina*, invading areas of Australia across a broad latitudinal and longitudinal range (see table 1 in Kolbe et al., 2010). As the guttural toad inhabits areas characterized by disparate

latitudes and elevations across central and southern Africa, more studies should be conducted on this species to investigate to what extent its thermal tolerance is fine-tuned by environmental conditions (Seebacher and Franklin, 2011; McCann et al., 2018).

In summary, we have shown that invasive guttural toads have undergone an adaptive response that reduces the difference (i.e. mismatch) between their actual phenotypes and the phenotypes that 'would be best suited in the invaded environment' (Hendry et al., 2011). We have also shown that this response does not necessarily require long time scales to occur and instead can be detected during the initial phase of an invasion. Although more studies are needed to distinguish among rapid genetic–epigenetic adaptation, phenotypic plasticity and founder effects/genetic drift, the consequences of this response should not be underestimated (Carroll, 2008; Whitney and Gabler, 2008). It may allow guttural toads, like other toad species (Urban et al., 2007; Van Bocxlaer et al., 2010; Kosmala et al., 2017), to survive, breed and expand into environments that are theoretically inhospitable, and hamper our capacity to predict invasion potential and/or adopt management countermeasures.

Author contributions
Conceptualization: G.V., S.J.D., J.M.; Methodology: G.V., S.J.D., J.M.; Software: G.V.; Validation: G.V.; Formal analysis: G.V.; Investigation: G.V.; Resources: G.V., S.J.D., J.M.; Data curation: G.V.; Writing - original draft: G.V.; Writing - review & editing: G.V., S.J.D., J.M.; Visualization: G.V.; Supervision: S.J.D., J.M.; Project administration: S.J.D., J.M.; Funding acquisition: S.J.D., J.M.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.174797.supplemental.

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