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Growing up in a new world: trait divergence between rural, urban, and invasive populations of an amphibian urban invader

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Abstract

Cities are focal points of introduction for invasive species. Urban evolution might facilitate the success of invasive species in recipient urban habitats. Here we test this hypothesis by rearing tadpoles of a successful amphibian urban coloniser and invader in a common garden environment. We compared growth rate, morphological traits, swimming performance, and developmental rate of guttural toad tadpoles (*Sclerophrys gutturalis*) from native rural, native urban, and non-native urban habitats. By measuring these traits across ontogeny, we were also able to compare divergence across different origins as the tadpoles develop. The tadpoles of non-native urban origin showed significantly slower developmental rate (e.g., the proportion of tadpoles reaching Gosner stage 31 or higher was lower at age 40 days) than tadpoles of native urban origin. Yet, tadpoles did not differ in growth rate or any morphological or performance trait examined, and none of these traits showed divergent ontogenetic changes between tadpoles of different origin. These findings suggest that prior adaptation to urban habitats in larval traits likely does not play an important role in facilitating the invasion success of guttural toads into other urban habitats. Instead, we suggest that evolutionary changes in larval traits after colonization (e.g., developmental rate), together with decoupling of other traits and phenotypic plasticity might explain how this species succeeded in colonising extra-limital urban habitats.

Keywords
AIAI hypothesis, development, growth rate, invasion biology, morphology, performance, tadpole, urban evolution

Introduction

Invasive species pose a major threat to global biodiversity, human wellbeing, and the economy (Pejchar and Mooney 2009; Gallardo et al. 2016; Mollot et al. 2017; Hanley and Roberts 2019; Diagne et al. 2021). The introduction of invasive populations is fundamentally linked to human activities such as global transport and habitat alteration (Pyšek et al. 2010; Blackburn et al. 2011; Hill et al. 2017b; McLean et al. 2017). Heavily-modified landscapes, like cities, harbour a significant proportion of invasive species (Cadotte et al. 2017; Gaertner et al. 2017), moreover, urban land cover is predicted to expand rapidly with rising human populations - especially in countries within biodiversity hotspots (Seto et al. 2012). Urban areas are focal points of introduction for alien biota due to global trade and transportation (Padayachee et al. 2017; Bullock et al. 2018; Gippet et al. 2019; Rivkin et al. 2019), and they show distinct habitat characteristics that make cities more similar to each other than when comparing each city to its surrounding less-disturbed environment (McKinney 2006; Grimm et al. 2008). Those habitat characteristics (e.g., reduced native biodiversity and predator species richness, higher rates of impervious surface area, and the urban heat-island effect; McKinney 2006; Grimm et al. 2008; Ramamurthy and Bou-Zeid 2017) can also facilitate, if not promote, the establishment of invasive populations (McKinney 2008; Santangelo et al. 2018; Des Roches et al. 2020; Reed et al. 2020). Thus, if an alien population has established in one city, there may be an increased likelihood that propagules from that population could disperse to other cities where they encounter similar environmental conditions due to the homogenisation of urban landscapes, making their establishment and spread more likely (McKinney 2006; Rivkin et al. 2019; Reed et al. 2020).

Recently, evolutionary biologists have begun studying the adaptive divergence of traits in urban populations compared to populations from rural habitats (Johnson and Munshi-South 2017; Santangelo et al. 2018). Research has shown that urban populations can evolve to cope with novel challenges that cities pose (e.g., Brans et al. 2017a, b; Tüzün et al. 2017; Putman et al. 2019; Corsini et al. 2021). However, whether and how adaptation to urban habitats facilitates the establishment success of populations transported to cities outside their natural ranges remains largely unknown (Reed et al. 2020; Borden and Flory 2021). The ”Anthropogenically Induced Adaptation to Invade” (AIAI) hypothesis (Hufbauer et al. 2012) postulates that anthropogenically modified habitats force adaptations in native populations to the anthropogenic selection regime, and given that altered landscapes are well connected due to global trade, the likelihood of prior adapted populations being transported to another anthropogenically modified habitat is high (e.g., Brady and Hay 2020). As human landscape

alterations lead to homogenisation of habitats (McKinney 2006), a prior adapted population will display a phenotype that is less likely to be mismatched in the recipient altered habitat compared to when being introduced to an unaltered habitat (Hufbauer et al. 2012). Thus, cities – as highly modified and interconnected habitats - pose an excellent opportunity to test the AIAI hypothesis.

To date, few studies have investigated whether prior adaptation to urban habitats facilitates invasion success in introduced habitats (Borden and Flory 2021). One such prior adaptation shown to provide invasive populations an advantage in urban landscapes are adaptive shifts in thermotolerance which occur in human-modified habitats before colonisation of novel ranges of the little fire ant (*Wasmannia auropunctata*; Foucaud et al. 2013) and several species of parrot (Jackson et al. 2015; Strubbe et al. 2015). Furthermore, several studies have shown that prior adaptation to the selective regime of the introduced range enhances invader fitness (Rey et al. 2012; Vahsen et al. 2018; Saarinen et al. 2019; Sherpa et al. 2019; Alzate et al. 2020). To study if and how urban evolution leads to prior adaptation in invasive populations some key attributes are required of the model system. First, there needs to be a population in an urban and in a rural habitat (preferably in close proximity) in the native range where the urban population is confronted with novel environmental challenges compared to the ancestral rural environment (Hufbauer et al. 2012; Borden and Flory 2021). Second, an invasive population must have been established in an urban area and this population must have originated from the native urban population (Hufbauer et al. 2012; Borden and Flory 2021). Third, to study evolutionary changes, the traits examined must be shown to be genetically fixed (e.g., by using a common garden experiment; Hufbauer et al. 2012; Lambert et al. 2020; Borden and Flory 2021).

Amphibians provide an excellent model system for examining the relationship between urban adaptations and invasions. Currently, there are more than 120 amphibian species with recognised invasive populations globally (Measey et al. 2020a), many of which were established through urban/suburban landscapes (e.g., Rebelo et al. 2010; Moore et al. 2015; Tingley et al. 2015) or other anthropogenically-altered habitats (e.g., aquacultural areas; Wang et al. 2019). Furthermore, a number of traits (e.g., growth rate, body shape, and developmental rate) have been demonstrated to diverge between rural/urban, and native/invasive amphibian populations (Iglesias-Carrasco et al. 2017; Iglesias-Carrasco et al. 2018; Eakin et al. 2019) and amphibians are well-known to show rapid evolution particularly in larval traits (e.g., Skelly and Freidenburg 2008; Nunes et al. 2014; Melotto et al. 2020). For example, comparisons of rural and urban populations have shown that wood frog tadpoles (*Lithobates sylvaticus*) in suburban pools were larger and developed earlier compared to tadpoles from rural pools (Eakin et al. 2019), also urban adult males tended to be larger and have a better body condition in several amphibian species compared to rural adult males (Iglesias-Carrasco et al. 2017). Thus, urban amphibian populations, especially of urban exploitative species, benefit from altered biotic and abiotic factors in urban wetlands, such as reduced interspecific competition and longer hydroperiods (i.e., wetland permanency) (Rubbo and Kiesecker 2005; Hassall 2014; Hill et al. 2017a). This trend is continued for invasive

amphibian populations that benefit from reduced competition by growing and developing faster, presumably to begin reproducing sooner (Phillips 2009; Pujol-Buxó et al. 2020). There has not been much work done on how tadpole swimming performance and the underlying morphological traits (i.e., body shape and tail morphology; Van Buskirk et al. 1997; Dayton et al. 2005; Teplitsky et al. 2005; Arendt 2010) diverge between rural and urban or between native and invasive populations. These traits, however, are known to change in response to novel selection regimes such as reduced predation and/or competition (i.e., larger bodies and smaller tail fins; Smith and Van Buskirk 1995; Relyea 2002; Relyea 2004; Dayton et al. 2005). Since urban bodies of water often represent habitats with reduced predation and interspecific competition for aquatic organisms (Rubbo and Kiesecker 2005; Hassall 2014), selection on morphological and performance traits may differ between rural and urban populations.

Here, we will examine the trait divergence in tadpoles of the guttural toad (*Sclerophrys gutturalis*) of three different origins in South Africa: native rural (Durban Rural), native urban (Durban Urban), and non-native urban (Cape Town, an invasive population that originated from Durban; Telford et al. 2019), within a common garden experiment. We focus on a suite of traits across development including tadpole body shape, tail morphology, and swimming performance. Furthermore, we examine whether these traits diverge across larval ontogeny between tadpoles of different origin. In doing so, we aim to test three hypotheses relating to the tadpoles' development, morphology, and performance capacity. Firstly, we predict that native rural tadpoles grow and develop slower than native urban tadpoles and urban invasive tadpoles grow and develop the fastest (i.e., relating to evolved increases in growth and developmental rate in urban/invasive populations; Sargent and Lodge 2014; Brans and De Meester 2018). Secondly, we expect that native rural tadpoles will have more slender bodies, but larger tail fins relative to body size, compared to native urban tadpoles and that invasive urban tadpoles have the bulkiest bodies with the smallest tail fins relative to body size (i.e., relating to reduced predation and competition in urban/invasive habitats; Rubbo and Kiesecker 2005; Hassall 2014; Hill et al. 2017a and following the findings of previous work on tadpole morphology; Smith and Van Buskirk 1995; Relyea 2002; Relyea 2004; Dayton et al. 2005). With these differences in growth, development and shape established, we then predict that native rural tadpoles will exhibit the fastest swimming speeds with native urban tadpoles being intermediary and invasive urban tadpoles to exhibit the slowest swimming speeds, owing to the expected differences in morphology and following the known effect of body and tail shape on performance (Van Buskirk et al. 1997; Dayton et al. 2005; Teplitsky et al. 2005; Arendt 2010).

Materials and methods

Study species

The guttural toad is a large bufonid (maximum snout-vent length (SVL)) of 140mm; du Preez et al. 2004), which is sexually dimorphic in body size (Baxter-Gilbert et al.

2020; Suppl. material 1: Fig. S1) and has a wide distribution in sub-Saharan Africa (Fig. 1A, B; du Preez et al. 2004; Telford et al. 2019). This habitat generalist can be found in a variety of environments including forests, grasslands, agricultural, and urban areas (du Preez et al. 2004; Channing et al. 2012; Baxter-Gilbert et al. 2020). Guttural toads breed in natural water bodies, such as shallow pools in rivers, and anthropogenic bodies of water, such as garden ponds and ditches. A single clutch can contain up to 25,000 eggs, laid in gelatinous strings (du Preez et al. 2004). Tadpoles usually develop over the course of five to six weeks with toadlets leaving the water as soon as the front legs have fully developed (du Preez et al. 2004; Suppl. material 1: Fig. S2).

The species has successfully established invasive populations in Mauritius, Réunion, and near Cape Town (Constantia, South Africa) (Measey et al. 2017; Telford et al. 2019; Measey et al. 2020b). Recent genetic analyses have confirmed that all three invasive populations originated from a clade located in the area around the port city of Durban in eastern South Africa (Telford et al. 2019). In the case of the invasive Cape Town population, guttural toads were most likely introduced as eggs or tadpoles within a shipment of aquatic plants at the end of the 1990s originating from a residential area of Durban (De Villiers 2006; Measey et al. 2017).

Sampling sites, animal collection, husbandry, and breeding

Breeding-sized adults (Suppl. material 1: Fig. S1) were collected from the end of November 2019 to the beginning of February 2020. We collected toads from two sites in the rural area surrounding Durban (hereafter referred to as "Durban Rural": 29°51'31"S, 30°43'18"E and 29°28'17"S, 31°13'25"E with 302 m and 54 m elevation above sea-level [a. s. l.], respectively), from two urban sites in and near Durban (hereafter referred to as "Durban Urban": 29°50'55"S, 31°00'30"E and 29°49'08"S, 30°56'37"E with 24 m and 194 m elevation a. s. l., respectively). Toads from the extra-limital range in Constantia near Cape Town (hereafter referred to as "Cape Town": 34°01'29"S, 18°26'03"E with 50–100 m elevation a. s. l.) were collected as part of an invasive species control operation (Davies et al. 2020a, 2020b). We chose the Durban Urban sites because they show similar habitat characteristics as the Cape Town sites, like high relative proportion of impervious surface area, well-established human populations, high numbers of buildings and roads, and the bodies of water within those urban sampling sites were either anthropogenically altered or created (e.g., fish ponds, fountains, or ditches) with heavily modified shorelines (Fig. 1D, E) and frequently contained ornamental fish species. All these characteristics are typical of urban environments (Rubbo and Kiesecker 2005; du Toit and Cilliers 2011; Moll et al. 2019) and have frequently been shown to drive adaptation in urban populations (e.g., Winchell et al. 2016; Corsini et al. 2021). Conversely, the Durban Rural sites have very low relative proportions of impervious surface area, few human settlements nearby with low human population densities and little human alteration. The bodies of water within our rural sites (i.e., a large lake and a series of ponds) are characterised by only very few anthropogenic modifications of the shoreline with riparian

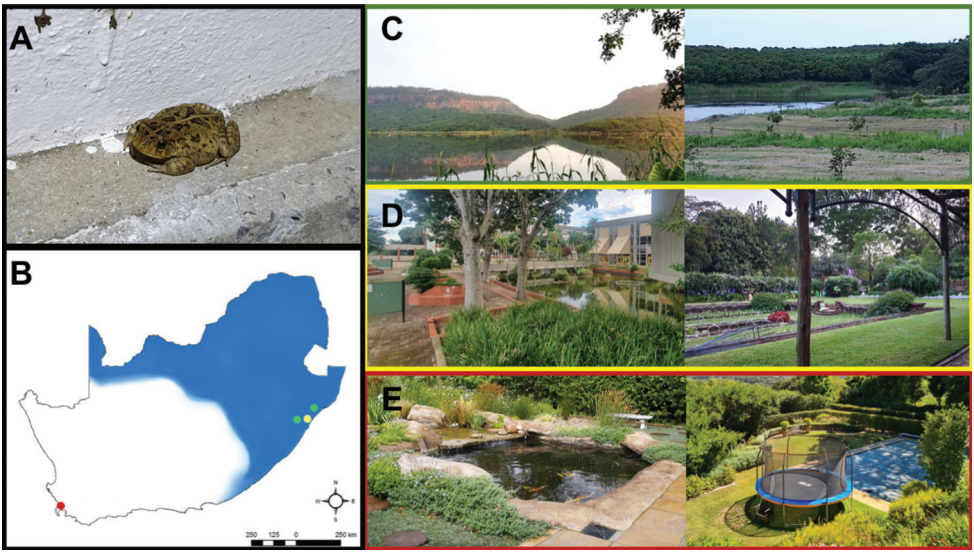

Figure 1. Overview of the study system **A** a guttural toad (*Sclerophrys gutturalis*): this female was photographed in Cape Town **B** the species' natural and non-native distribution in South Africa. The approximate locations of sampling sites are demarcated for Durban Rural (green), Durban Urban (yellow), and Cape Town (red). Further we show the general appearance of **C** the Durban Rural **D** durban Urban, and **E** Cape Town sampling sites.

grasslands consisting of mainly native plants and the presence of native fish species (Fig. 1C).

Shortly after collection, toads were transported to an experimental facility located at the University of KwaZulu-Natal (Westville Campus) situated at one of our sampling locations for Durban Urban. In the facility, toads were housed by sex and collection site in large plastic mesocosms (110 cm L × 130 cm W × 50 cm H) until they were used for breeding. Each mesocosm contained at least two water bowls (~ 15 cm L × 10 cm W × 5 cm H) on a 10 cm layer of soil mixed with leaf litter collected outside of the greenhouse. Crickets (*Acheta domesticus*) were fed to adults *ad libitum* every other day.

To initiate breeding, we injected adults with a synthetic gonadotrophin, leuroprorelin acetate (Lucrin Depot, Abbott), diluted 1:20 with Ringer's solution using 0.666 ml of that dilution for females and 0.333 ml for males (Hamilton et al. 2005; Hudson et al. 2015). Breeding was induced from December 2019 to the beginning of March 2020 in batches of breeding pairs due to logistical reasons. This means that clutches were not all laid at the same time but rather breeding was carried out over the whole period described. However, we spread breeding for the different toad origins over this entire period. Injections took place at ~17:00 h. After a breeding pair was injected, pairs of male and female toads were placed into plastic containers (26 × 41 cm) filled to a height of 4 cm with aged tap water and left overnight. Usually, males would initiate amplexus shortly after being introduced to the female. The next morning at ~09:00 h containers were checked for fertilised clutches and adults were removed from the containers. All adults were used for

breeding only once. New clutches were cleaned from any faecal material and water was replaced with aged tap water and filled to a height of 15 cm in the plastic containers. We replaced water regularly with aged tap water and made sure tanks were well aerated and clean. Embryonal development was rapid and, after one day, most embryos had hatched. For our mesocosm experiment, this was considered as "age 0" day for tadpoles.

Mesocosm experiment

Prior to the experiment, large plastic mesocosms (110 cm L × 130 cm W × 50 cm H; n = 33) located in the experimental facility were filled with 600 L of tap water and left to age for a week. Subsequently, the water was inoculated with water from a standing water tank to induce establishment of phyto- and zooplankton communities within the mesocosms. This water tank was located in the greenhouse (i.e., preventing access from toads and fish) and had live aquatic vegetation and algae growing within it. After another week, 50 g of rabbit chow (Rabbit Pellets, Westerman's Premium; 9% protein, 1.25% fat, 0.75% calcium by weight) was added for additional nutrients (Semlitsch and Boone 2009). Mesocosms were placed under work benches so that half of the water surface was shaded and the other half received sunlight through the roof panels of the facility. After approximately one month, all mesocosms contained a visible phyto- and zooplanktonic community and were subsequently deemed ready for use. At the clutch age of three days, 1000 tadpoles from a single clutch (i.e., full siblings) were enumerated and introduced to a readied mesocosm where they would be housed until the end of the experiment. For a summary of the average daily maximum and minimum temperatures inside the mesocosms over the course of the experiment see Suppl. material 1: Fig. S3.

Sample sizes and measurements across ontogeny

At clutch age of three days, 20 tadpoles from one clutch were randomly selected for measurements (see below) and were returned to their respective mesocosms afterwards. At the age of ten days, and subsequently every ten days (i.e., age 20, 30, 40, 50 and 60 days), 20 tadpoles were randomly selected from each mesocosm, and measurement procedures were repeated (for sample sizes see Table 1). Note that for some clutches we were not able to conduct measurements on the scheduled day and instead conducted measurements on the next possible day (Suppl. material 1: Table S1). For all trait measurements, we made sure to only include tadpoles that did not show development past Gosner stage 41 (i.e., hindlimbs fully developed but tail not resorbed and front limbs did not emerge; Gosner 1960) as metamorphosis strongly affects the expression of the traits we examined in subsequent stages (Watanabe and Sasaki 1974; Gilbert 2000; Vitt and Caldwell 2013). Notes were made on any individuals that reached metamorphosis as they climbed on to floating platforms placed inside the mesocosms.
After the measurements (see below), tadpoles were returned to their respective mesocosms. We acknowledge that our replicates cannot be assumed to be fully independent

Table 1. Sample sizes for guttural toad (*Sclerophrys gutturalis*) tadpoles across age: numbers below the specific traits correspond to the total number of tadpoles measured at the specific age. The total number of clutches used in this experiment was 10 (3/7), 14 (8/6), and 9 for Durban Rural, Durban Urban and Cape Town, respectively. The numbers in brackets correspond with the specific number of clutches derived from adults collected in either the first or seco nd sampling location for Durban Rural or Durban Urban, respectively (see Methods). For a more detailed report of the sample sizes in this experiment see Suppl. material 1: Table S1.

Traits / Age (Days)	Durban Rural			Durban Urban			Cape Town		
	SVL, body width, tail length	Body height, tail fin height, tail muscle height	Maximum velocity, maximum acceleration	SVL, body width, tail length	Body height, tail fin height, tail muscle height	Maximum velocity, maximum acceleration	SVL, body width, tail length	Body height, tail fin height, tail muscle height	Maximum velocity, maximum acceleration
2–4	199	199	199	272	271	272	180	180	179
10–12	200	199	200	240	235	240	160	160	160
20	200	200	200	180	179	180	160	160	160
30	200	199	200	180	180	180	80	80	80
40–41	160	160	160	180	180	180	80	80	80
50	60	60	60	180	180	180	60	60	60
60	60	60	60	100	100	100	40	40	40

(e.g., a tadpole that was randomly picked for measurements at an age of three days might have been picked for another subsequent measurement at a higher age). However, since each mesocosm housed 1000 tadpoles at the start of the experiment, it is unlikely that a single tadpole was recurrently picked for measurements. As such our statistical analyses use the assumption that repeated measurements of an individual tadpole did not occur.

Morphological measurements and developmental rate

We measured standard morphological variables of tadpoles: SVL, tail length, body width, body height, tail muscle height, and tail fin height to 0.001 mm (Altig 2007; Suppl. material 1: Fig. S4). Images were scaled using millimeter paper in the background of tadpole pictures. Tadpole morphology was measured using the measurement function of the image and video analysis software Tracker (Open Source Physics, USA) (Brown and Cox 2009). To observe differences in body shape, we also calculated body volume (mm^3) using the formula ¾ × π x.*SVI*. × hady width. × height ¾ × π x.*SVI*. × hady width. × height (e.g., tadpoles with bulkier bodies have higher values than tadpoles with more slender bodies). At the age of 40/41 days (see Table 1 for sample sizes, Suppl. material 1: Table S1), we also carefully examined tadpole images to see whether tadpoles had developed to, or past, Gosner stage 31 (i.e., well-developed hindlimbs; Gosner 1960) to study if developmental rate diverges between tadpoles of different origin. The purpose of using Gosner stage 31 as a threshold was to have a clear morphological marker (i.e., hindlimb; Gosner 1960) that is unambiguously identifiable from images of the tadpoles.

Performance measurements and video analysis

All tadpoles that underwent morphological measurements were also tested for swimming performance on the same day. Performance trials were carried out in a clear plexiglass tank (30 × 5 cm) filled with 3 cm of aged tap water (Wagener et al. 2021). A mirror was attached to the tank at an angle of 45° to enable us to record the movement of tadpoles using a dorsal and lateral point of view. At the start of the trial, an individual tadpole was gently placed inside the arena and left to acclimate for ~5 s. Water temperature was recorded using a standard digital thermometer. Tadpoles were tapped at the tail fin with a fine metal wire to initiate burst escape swimming for ~30 s. Videos of tadpoles swimming were recorded using a camera (Canon PowerShot G16) at 120 frames per second.

High-speed videos were recorded from a dorsal and lateral perspective to the tadpole, using an angled mirror attached to the tank. As a swimming bout we defined the movement of a tadpole, initiated by rapid lashes of the tail fin in response to an approach or a touch by the metal wire, from the beginning to the end of the displacement. Only swimming bouts that were carried out on a horizontal plane with a displacement at least 2 cm away from its initial position were selected. For each tadpole, we analysed three swimming bouts that were judged to yield the highest values for velocity and acceleration. Using the image and video analysis software Tracker (Brown and Cox 2009), we extracted the x- and y-coordinates from the videos and the displacement (mm) of the tadpole per frame was calculated. Videos were scaled using millimeter paper in the background of videos. We filtered the displacement data using a fourth order zero-phase shift low-pass Butterworth filter (Christodoulakis et al. 2010) and used a 12 Hz cut-off frequency which was determined as a 10^{th} of the video recording frame rate. The Butterworth fourth order zero-phase shift low-pass filter is used to reduce noise in a data set by a given cut-off frequency (i.e., reducing the number of data points to filter noise in a data set) with the possibility to differentiate the filtered data into velocity (first derivate against time) and acceleration (second derivate against time) (Winter 2004; Erer 2007). From the filtered data we extracted maximum swimming velocity ($mm \times s^{-1}$) and maximum swimming acceleration ($mm \times s^{-2}$) for each tadpole.

Statistical analysis

All statistical analyses were conducted using R version 4.0.1 (R Core Team 2020). Before analysis, we explored our data following Zuur et al. (2010). We did not find any unexplainable outliers and ensured the models did not contain strongly confounded predictor variables. All figures presented in the results section were created using the R package "ggplot2" (Hadley 2016). All morphological and performance traits were log-transformed prior to analysis. Following model analysis (see details below), we verified assumptions of normality and homoscedasticity of outliers prior to interpretation. Also, we conducted post-hoc multiple comparison tests between all origins (Durban

Rural, Durban Urban, Cape Town) using the function "emmeans" from the R package "emmeans" (Lenth et al. 2018). All *p*-values generated for these comparisons were Tukey-adjusted (Lenth et al. 2018).

Ontogenetic differentiation of morphological and performance traits

To examine differences in growth rate and body volume, we fitted linear mixed effect models (LMM) using the R package "lme4" (Bates et al. 2015). As response variables, we used SVL and body volume, while the fixed effects we used were origin (categorical variable with three levels: Durban Rural, Durban Urban, Cape Town), age (days; continuous), and their interaction term. If the interaction term was non-significant, it was removed and the models re-run. The models also included a random intercept and slope of mesocosm ID to control dependencies in our data due to clutch effects and effects of being reared in the same mesocosm, as well as a random intercept of parentage site to accommodate dependency among observations of tadpoles with parentage from the same sampling site. To examine differences in the other variables of interest (tail length, tail muscle height, tail fin height, maximum swimming velocity, and maximum swimming acceleration), we fitted separate LMMs with the variables each using the same fixed and random effects as detailed for the growth rate and body volume models, but these models also included SVL as a fixed effect to standardise the variables with respect to body length. We did not include water temperature in any of our models examining differences in performance traits, because temperature and tadpole age are confounded. Specifically, water temperatures were lower at a higher age (β = –0.033, t = -15.76, p < .001; results from a linear model performed using the "lm" function in the R package "stats" with age as fixed effect; R Core Team 2020).

Trait differences at 40 days

We also examined trait differences at a specific point in development. We chose age 40/41 days because previous literature has shown that at this age individual traits can diverge between populations (e.g., in European common frogs, *Rana temporaria*; Van Buskirk and Arioli 2005), and also, because at this age our sample size was still relatively high (Table 1). Furthermore, metamorphosis in this species has been previously reported to commence at around an age of 40 days (du Preez et al. 2004), which also corresponded to observations made in this experiment (Suppl. material 1: Fig. S2). Once again, we fitted separate LMMs using the R package "lme4" (Bates et al. 2015) with SVL, body volume, tail length, tail muscle height, tail fin height, maximum swimming velocity, and maximum swimming acceleration as response variables. For all models, apart from the models with SVL or body volume as response variables, we included SVL as a fixed effect. For all models, we also included origin as a fixed effect and included the random intercepts of mesocosm ID and parentage site. Again, we did not include water temperature in our models examining differences in performance variables (see above) to ensure that model results were comparable.

Developmental rate

To examine differences in developmental rate between tadpoles from different origin populations, we fitted a generalised linear mixed effects model (GLMM) with logit link function using the R package "lme4" (Bates et al. 2015). The binomial response variable described whether tadpoles had developed to or past Gosner stage 31 (Gosner 1960) at an age of 40/41 days or not. We included origin as a fixed effect (categorical variable with three levels: Durban Rural, Durban Urban, Cape Town) and the random intercepts of mesocosm ID and parentage site.

Results

Ontogenetic differentiation of morphological and performance traits

None of the observed morphological or performance traits showed significant differences between tadpoles of different origin (Table 2, Suppl. material 1: Table S2, Fig. 2). Snout-vent length (SVL) and body volume increased with age (Table 2 and Fig. 2A, B). Tail length, tail muscle height, tail fin height, maximum swimming velocity, and maximum swimming acceleration were positively correlated with SVL (Table 2). Only tail length, relative to SVL, increased with age, whereas in relation to SVL, tail muscle height, maximum swimming velocity, and maximum swimming acceleration decreased with age (Table 2). Tail fin height, in relation to SVL, did not change with age (Table 2).

Morphological and performance trait differences at the age of 40 days

We did not find significant effects of tadpole origin on any morphological or performance trait at the age of 40/41 days (Table 3, Suppl. material 1: Table S3, Suppl. material 1: Fig. S5). Tail muscle height, tail fin height, tail length, maximum swimming velocity, and maximum swimming acceleration were all positively correlated with SVL (Table 3).

Developmental rate

The proportion of tadpoles having developed to or past Gosner stage 31 (Gosner 1960) was highest for Durban Urban (0.189), followed by Durban Rural (0.179) and was lowest for Cape Town tadpoles (0.05) as calculated from the raw data. Based on our statistical analyses, the proportion of tadpoles developing to or past Gosner stage 31 in Cape Town was only significantly lower in comparison with Durban Urban and no other significant differences were found (Table 4 and Fig. 3). This general pattern also corresponds with our data indicating that time to metamorphosis tended to be longer in Cape Town clutches than in clutches from the other habitats, although we were not able to run a statistical test on this data set due to a limited sample size (Suppl. material 1: Fig. S2).

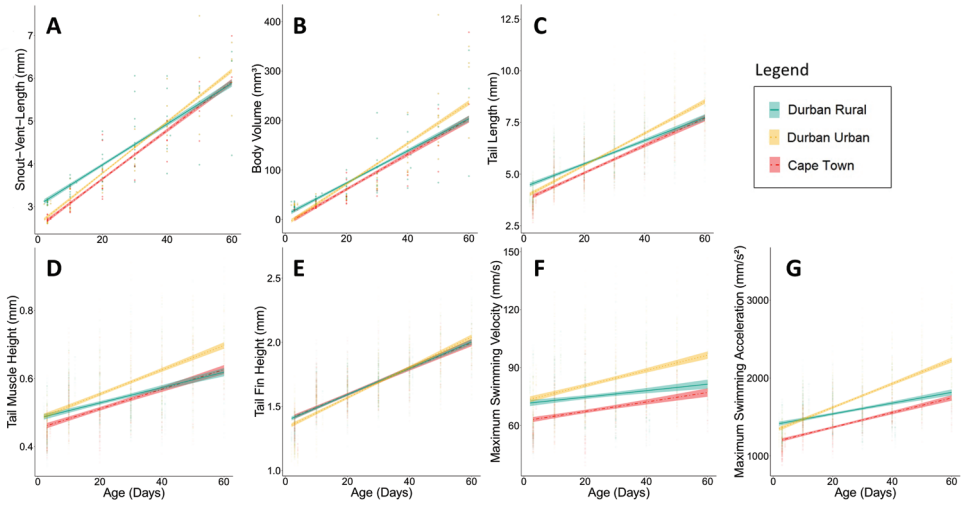

Figure 2. Trait changes across 60 days post-hatching in tadpoles: none of the observed traits were significantly different between origins (native rural – Durban Rural, native urban – Durban Urban, non-native urban – Cape Town). Presented are **A** snout-vent length (growth rate) **B** body volume **C** tail length **D** tail muscle height **E** tail fin height **F** maximum swimming velocity, and **G** maximum swimming acceleration. All morphological and performance variables were log-transformed prior to analysis and predicted data was back-transformed before plotting. Circles represent predictions from linear mixed effect models and the lines represent predicted linear regressions with 95% confidence intervals.

Discussion

Here we show, in a common garden experiment, that the invasive urban Cape Town population of the guttural toad has a slower larval development (i.e., the proportion of tadpoles having developed to or past Gosner stage 31 at the age of 40/41 days) compared to the native urban population from Durban. Contrary to our predictions, tadpoles of urban/rural or native/invasive origin do not differ in growth rate, or any of the other morphological or performance traits we examined. From this, we suggest that prior adaptation to urban habitats (AIAI hypothesis *sensu* Hufbauer et al. 2012) in larval morphological, performance, and developmental traits of the guttural toad did not enhance its invasion success in Cape Town. Rather, the slower larval development in Cape Town toads arose within the short period (~ 20 years or 10 generations) since introduction, indicating bridgehead effects (e.g., Bertelsmeier and Keller 2018). Had our experiment been able to continue to metamorphosis, we would predict larger metamorphs from Cape Town given that growth rates did not differ, and which is also consistent with previous literature on the topic (e.g., Harkey and Semlitsch 1988; Newman 1989; Touchon et al. 2013; Tarvin et al. 2015). Owing to the study design used (i.e., a common garden experiment), we cannot rule out potential different habitat- or site-specific factors that may result in situational or plastic response in wild populations, however from an innate trait standpoint, we assert that the morphological

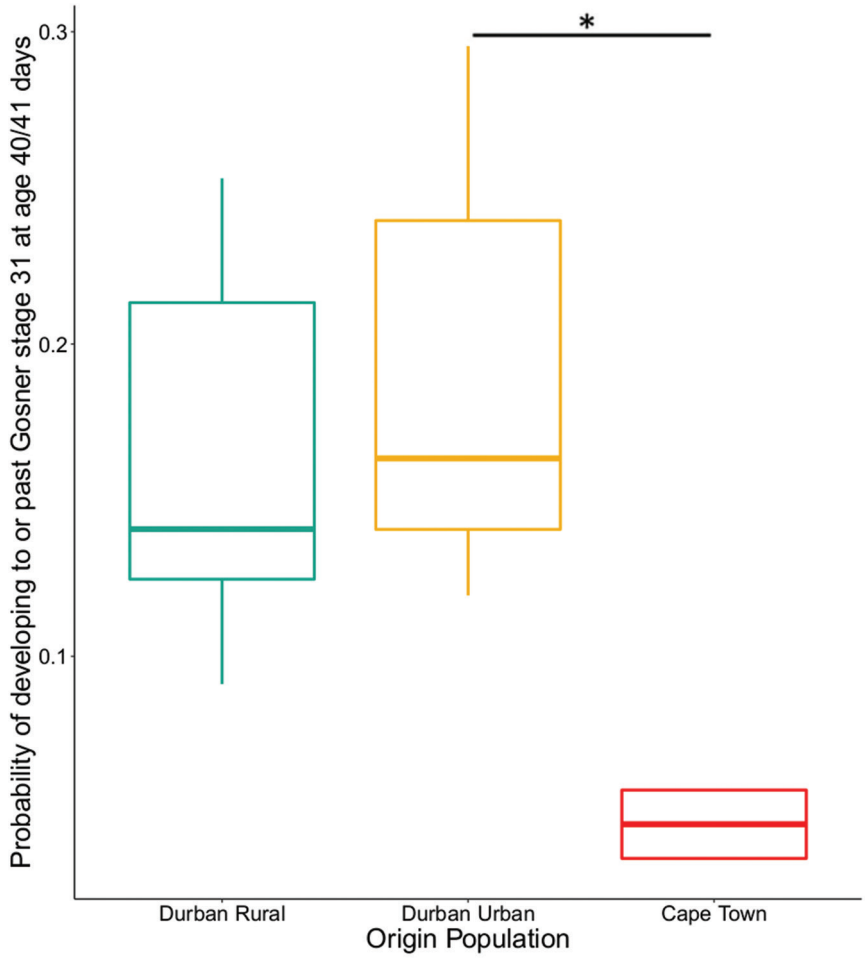

Figure 3. Probability of reaching Gosner stage 31 or higher (Gosner 1960) for tadpoles of native rural (Durban Rural), native urban (Durban Urban), and non-native urban (Cape Town) origin at an age of 40/41 days as predicted from our generalised linear mixed effect model. Presented are boxplots. The boxes denote the interquartile ranges (IQR), the bars inside the boxes correspond with the predicted medians from the models. The whiskers extend to 1.5 × IQR. Asterisks denote significant differences as determined by post-hoc multiple comparisons.

or performance traits we examined are not diverging within the larval stage between urban/rural or native/invasive origin populations.

One of the most prominent differences between the areas of Durban and Cape Town is the Mediterranean climate in Cape Town compared to subtropical Durban. The invasive toad population in Cape Town is confronted with a colder, drier, and more seasonal climate compared to the climate of the source habitat (Vimercati et al. 2019). Previous studies have already shown that this novel climatic regime likely led to both physiological as well as behavioural changes in adult toads (Vimercati et al.

Table 2. Outcomes of linear mixed effect models testing for differences in morphological and performance traits between guttural toad tadpoles of native rural origin (Durban Rural), native urban origin (Durban Urban), and invasive urban origin (Cape Town) raised in a common garden environment. All variables were log-transformed prior to analysis. Presented are model coefficient estimates (β) with their corresponding standard errors (*SE*) for fixed effects and variance estimates (σ^2) for random effects and residuals. Test statistics (*t*) are given, and all significant values ($p < .05$) are presented in bold. For categorical variables, reference levels are presented in brackets behind the variable name.

Model	Variable Names	Model Output			
Snout-Vent Length	Fixed Effects	*β*	*SE*	*t*	*p*
	Intercept (Cape Town)	**0.412**	**0.042**	**9.740**	**.011**
	Origin (Durban Rural)	0.064	0.052	1.239	.341
	Origin (Durban Urban)	0.025	0.052	0.492	.672
	Age	**0.006**	**< 0.001**	**11.970**	**< .001**
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	0.002			
	Residuals	0.008			
Body Volume	Fixed Effects	*β*	*SE*	*t*	*p*
	Intercept (Cape Town)	**1.208**	**0.151**	**7.983**	**.016**
	Origin (Durban Rural)	0.220	0.186	1.187	.358
	Origin (Durban Urban)	0.114	0.185	0.614	.602
	Age	**0.020**	**0.002**	**12.606**	**< .001**
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	0.026			
	Residuals	0.085			
Tail Muscle Height	Fixed Effects	*β*	*SE*	*t*	*p*
	Intercept (Cape Town)	**-0.544**	**0.035**	**-5.690**	**.003**
	Snout-Vent Length	**0.542**	**0.017**	**31.681**	**< .001**
	Origin (Durban Rural)	-0.013	0.042	-0.306	.789
	Origin (Durban Urban)	0.002	0.042	0.041	.971
	Age	**-0.003**	**< 0.001**	**-5.578**	**< .001**
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	0.001			
	Residuals	0.008			
Tail Fin Height	Fixed Effects	*β*	*SE*	*t*	*p*
	Intercept (Cape Town)	-0.009	0.064	-0.139	.902
	Snout-Vent Length	**0.393**	**0.012**	**31.917**	**< .001**
	Origin (Durban Rural)	-0.049	0.078	-0.577	.623
	Origin (Durban Urban)	-0.040	0.078	-0.517	.657
	Age	-0.004	0.009	-0.434	.670
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	0.004			
	Residuals	0.004			
Tail Length	Fixed Effects	*β*	*SE*	*t*	*p*
	Intercept (Cape Town)	**0.265**	**0.009**	**29.937**	**< .001**
	Snout-Vent Length	**0.729**	**0.011**	**68.726**	**< .001**
	Origin (Durban Rural)	0.009	0.010	0.992	.437
	Origin (Durban Urban)	0.014	0.009	1.522	.290
	Age	**0.001**	**< 0.001**	**6.995**	**< .001**
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	< 0.001			

Model	Variable Names	Model Output			
	Residuals	0.003			
Maximum Swimming Velocity	Fixed Effects	*β*	*SE*	*t*	*p*
	Intercept (Cape Town)	**1.529**	**0.090**	**17.066**	**.003**
	Snout-Vent Length	**0.667**	**0.026**	**25.656**	**< .001**
	Origin (Durban Rural)	0.030	0.109	0.277	.808
	Origin (Durban Urban)	0.046	0.109	0.423	.714
	Age	**-0.004**	**0.001**	**-3.348**	**.004**
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	0.008			
	Residuals	0.018			
Maximum Swimming Acceleration	Fixed Effects	*β*	*SE*	*t*	*p*
	Intercept (Cape Town)	**2.819**	**0.042**	**66.838**	**< .001**
	Snout-Vent Length	**0.592**	**0.032**	**18.303**	**< .001**
	Origin (Durban Rural)	0.056	0.049	1.142	.374
	Origin (Durban Urban)	0.051	0.049	1.036	.414
	Age	-0.001	< 0.001	-2.505	.017
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	0.002			
	Residuals	0.028			

2018; Vimercati et al. 2019; Madelaire et al. 2020; Barsotti et al. 2021). For example, Madelaire et al. (2020) demonstrated that Cape Town adults show a more efficient water-conservation behaviour than toads from Durban. From extensive research on the effects of toad metamorph size, we know that larger metamorphs desiccate less rapidly and, are able to disperse further, potentially increasing chances of reaching new bodies of water (Cohen and Alford 1993; Goater et al. 1993; Beck and Congdon 2000; Chelgren et al. 2006; Child et al. 2008; Cabrera-Guzmán et al. 2013). Thus, the drier summers in Cape Town, during which guttural toads metamorphose, may lead to strong selection for larger metamorph body sizes (as we hypothesize will arise via the longer larval developmental time found in this study) in the invasive population.

Given the substantial literature reporting differences in tadpole growth rates due to ecological or evolutionary factors, we were surprised that we did not find any differences in growth rate among the three location types. Several studies on body size differences among rural and urban, as well as among native and invasive populations, across a wide range of taxa report higher growth rates and larger adult body sizes for urban and/or invasive populations (Phillips 2009; y Gomez and Van Dyck 2012; Sargent and Lodge 2014; Hall and Warner 2017; Iglesias-Carrasco et al. 2017; Pujol-Buxó et al. 2020; Putman and Tippie 2020), which is widely attributed to lower interspecific competition, higher food abundance, and novel ecological opportunities in urban and/or invasive ranges. The opposite trend is, however, also possible. For example, studies of cladocerans, several orders of insects, and on passerine birds (Brans et al. 2017a, b; Gianuca et al. 2018; Merckx et al. 2018; Corsini et al. 2021) have found negative effects of urban environments on body size. In the case of our study species, the guttural toad, Baxter-Gilbert et al. (2020) showed that invasive populations of guttural toads

Table 3. Model output of linear mixed effect models examining differences in guttural toad tadpoles (*Sclerophrys gutturalis*) at the age of 40/41 days between guttural toad tadpoles of native rural origin (Durban Rural), native urban origin (Durban Urban), and invasive urban origin (Cape Town) raised in a common garden environment. All morphological and performance variables were log-transformed prior to analysis. Given are model coefficient estimates (β) with their corresponding standard errors (*SE*) for fixed effects and variance estimates (σ^2) for random effects and residuals. Test statistics (*t*) are presented, and all significant values ($p < .05$) are presented in bold. For categorical variables, reference levels are presented in brackets behind the variable name.

Model	Variable Names	Model Output			
Snout-Vent Length	Fixed Effects	β	*SE*	*t*	*p*
	Intercept (Cape Town)	**0.659**	**0.026**	**25.291**	**< .001**
	Origin (Durban Rural)	0.025	0.032	0.796	.437
	Origin (Durban Urban)	0.043	0.031	1.362	.190
	Random Effects	σ^2			
	Mesocosm ID	0.002			
	Parentage Site	0.000			
	Residuals	0.006			
Body Volume	Fixed Effects	β	*SE*	*t*	*p*
	Intercept (Cape Town)	**1.993**	**0.075**	**26.487**	**< .001**
	Origin (Durban Rural)	0.070	0.092	0.757	.459
	Origin (Durban Urban)	0.141	0.090	1.564	.135
	Random Effects	σ^2			
	Mesocosm ID	0.020			
	Parentage Site	0.000			
	Residuals	0.051			
Tail Muscle Height	Fixed Effects	β	*SE*	*t*	*p*
	Intercept (Cape Town)	**-0.684**	**0.080**	**-8.572**	**.004**
	Snout-Vent Length	**0.659**	**0.050**	**13.212**	**< .001**
	Origin (Durban Rural)	-0.010	0.089	-0.113	.921
	Origin (Durban Urban)	0.029	0.089	0.327	.775
	Mesocosm ID	0.001			
	Parentage Site	0.005			
	Residuals	0.007			
Tail Fin Height	Fixed Effects	β	*SE*	*t*	*p*
	Intercept (Cape Town)	-0.038	0.025	-1.525	.147
	Snout-Vent Length	**0.456**	**0.031**	**14.914**	**< .001**
	Origin (Durban Rural)	-0.024	0.018	-1.363	.315
	Origin (Durban Urban)	-0.018	0.018	-1.032	.424
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	< 0.001			
	Residuals	0.002			
Tail Length	Fixed Effects	β	*SE*	*t*	*p*
	Intercept (Cape Town)	**0.312**	**0.023**	**13.772**	**< .001**
	Snout-Vent Length	**0.734**	**0.031**	**23.409**	**< .001**
	Origin (Durban Rural)	0.014	0.011	1.220	.238
	Origin (Durban Urban)	0.017	0.011	1.534	.142
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	0.000			
	Residuals	0.003			
Maximum Swimming Velocity	Fixed Effects	β	*SE*	*t*	*p*
	Intercept (Cape Town)	**1.329**	**0.089**	**14.999**	**< .001**
	Snout-Vent Length	**0.754**	**0.075**	**10.012**	**< .001**
	Origin (Durban Rural)	-0.007	0.090	-0.083	.942
	Origin (Durban Urban)	0.078	0.090	0.865	.479

Model	Variable Names	Model Output			
	Random Effects	σ^2			
	Mesocosm ID	< 0.001			
	Parentage Site	0.005			
	Residuals	0.018			
Maximum Swimming Acceleration	Fixed Effects	β	*SE*	*t*	*p*
	Intercept (Cape Town)	**2.675**	**0.110**	**24.272**	**< .001**
	Snout-Vent Length	**0.737**	**0.099**	**7.421**	**< .001**
	Origin (Durban Rural)	0.008	0.109	0.077	.945
	Origin (Durban Urban)	0.112	0.109	1.028	.413
	Random Effects	σ^2			
	Mesocosm ID	0.001			
	Parentage Site	0.007			
	Residuals	0.029			

on the islands of Mauritius and Réunion have smaller adult body sizes compared to native populations. Observations made in this study (see Suppl. material 1: Fig. S1) indicate that adult body sizes might diverge between rural, urban, and invasive populations, as well. Yet, our study suggests that any adult body size differences are not driven by innate differences in tadpole growth rate, and it is unclear how they arose in adults (e.g., due to environmental factors such as food, due to phenotypic plasticity or due to directional selection for smaller or larger adult body sizes). In another successful amphibian invader, the African clawed frog (*Xenopus laevis*), Kruger et al. (2021) showed that despite differences in adult body sizes between the core and periphery of the species' invasive distribution in France, the tadpoles did not show significant differences in growth rates when raised in a common garden experiment, indicating the decoupling of traits between life stages (but see Phillips 2009 for coupling of growth rate across larval and juvenile stages in cane toads, *Rhinella marina*). The decoupling of traits might also be a potential driver for the apparent similarities in growth rates of guttural toad tadpoles. Furthermore, the guttural toad is an infamous urban exploiter with synanthropic behaviour, frequently making use of artificial light sources for foraging and artificial bodies of water for hydration and reproduction (du Preez et al. 2004; Measey et al. 2017; Vimercati et al. 2017; Baxter-Gilbert et al. 2020; Measey et al. 2020b). Thus, the terrestrial stage of the species might benefit from ecological opportunities in urban environments that are not available for the aquatic stage and growth rate might be decoupled between the tadpole and adult stage.

Urban bodies of water have been reported to frequently show high levels of modification (especially of the riparian zone) and, partly as a consequence, show reduced native biodiversity and high abundance of invasive species (reviewed in Hassall 2014), but can also act as biodiversity hotspots in a city (Hassall 2014; Hill et al. 2017a). Given the potential reduction in predation and competition for Durban Urban and Cape Town tadpoles, we expected that Durban Rural tadpoles would show the most conducive phenotype for predator escape and overall better performance under high competition scenarios (i.e., high burst escape swimming speed, slim bodies and large tail fins; Smith and Van Buskirk 1995; Relyea 2002; Relyea 2004; Dayton et al. 2005), which is in line with the enemy release hypothesis (Colautti et al. 2004; Liu and Stiling

Table 4. **(A)** Model output of a generalised linear mixed effects model used to examine differences in guttural toad (*Sclerophrys gutturalis*) tadpole developmental rates across origins. We present model coefficient estimates (β) and the corresponding standard errors (*SE*) for fixed effects, as well as variance estimates (σ^2) for random effects. Test statistics (z) are presented and all significant values ($p < .05$) are presented in bold. For the categorical variables, reference levels are presented in brackets behind the variable name. **(B)** Results of post-hoc multiple comparisons testing for differences in developmental rate among guttural toad (*Sclerophrys gutturalis*) tadpole origins. Presented are conditional odds ratios with their corresponding standard errors (*SE*). Test statistics (z) and p-values (p_{corr}) corrected using a "tukey" adjustment (Lenth et al. 2018) are given. Significant values are bolded.

(A) Output from the generalised linear mixed effect model				
Variable Names				
Fixed Effects	β	*SE*	z	p
Intercept (Cape Town)	**-3.08**	**0.599**	**-5.142**	**< .001**
Origin (Durban Rural)	**1.37**	**0.663**	**2.066**	**.039**
Origin (Durban Urban)	**1.534**	**0.654**	**2.347**	**.019**
Random Effects	σ^2			
Mesocosm ID	0.324			
Parentage Site	< 0.001			
(B) Multiple Comparisons between Origins				
Origin Comparison	Conditional Odds Ratio	*SE*	z	p_{corr}
Cape Town – Durban Rural	0.254	0.169	-2.066	0.097
Cape Town – Durban Urban	**0.216**	**0.141**	**-2.347**	**0.049**
Durban Rural – Durban Urban	0.849	0.344	-0.404	0.914

2006). However, similar to growth rate, swimming performance and the underlying morphological variables did not diverge between tadpoles of different origin. The urban (Durban Urban) and non-native urban (Cape Town) sampling sites frequently had artificial ponds that were used for breeding by guttural toads (Vimercati et al. 2017) and, also frequently contained non-native ornamental fish species such as goldfish (*Carassius auratus*) and koi (*Cyprinus rubrofuscus*) (MM, JB-G & JM pers. obs.). Similarly, the water bodies in our rural sampling sites also contained fish, but of native fish species (MM, JB-G & BM pers. obs.) Ornamental fish species as well as many fish species, native to sub-tropical South Africa, are well-known to reduce invertebrate densities such as dragonfly larvae (reviewed in Wellborn et al. 1996; Brown et al. 2012; see also Richardson et al. 1995; Preston et al. 2017) but may not predate on toad tadpoles due to unpalatability (see Brown et al. 2012 and citations within; Manteifel and Reshetnikov 2002; Üveges et al. 2019). Invertebrate predators, on the other hand, have been reported to reduce densities of the larvae of some invasive amphibian species, like cane toads (Crossland and Alford 1998; Cabrera-Guzmán et al. 2012) and African clawed frogs (Warren et al. 2021). Thus, ornamental fish species may carry out a similar ecological function as native fish species and therefore, a similar selection regime is present in both rural and urban ponds from the perspective of guttural toads. More research is needed, however, to understand the facilitating role ornamental and/or invasive fish species might play in urban bodies with respect to the invasion success of pond-breeding amphibians (e.g., Adams et al. 2007).

In this common garden experiment, we raised the F1 progeny from toads collected in the wild. Thus, we cannot rule out maternal/paternal effects on differences or simi-

larities between tadpoles (e.g., Kawecki and Ebert 2004), and the effect of phenotypic plasticity induced by environmental cues in the tadpole's respective habitats that might enhance fitness under the current selective regime. Anuran tadpoles are well-known to show high levels of phenotypic plasticity in swimming performance and underlying morphological traits in response to a variety of environmental factors (e.g., Loman and Claesson 2003; Relyea 2004; Hoverman et al. 2005; Castaneda et al. 2006; Whiles et al. 2010; Gomez-Mestre et al. 2013). Interestingly, Bókony et al. (2021) demonstrated that urban tadpoles of common toads (*Bufo bufo*) showed higher baseline stress and a more efficient recovery to baseline levels compared to tadpoles from natural habitats, partly as a response to water pollutants. These differences disappeared, however, when individuals were raised in a common garden environment, suggesting that divergence in the efficacy of negative feedback is driven by phenotypic plasticity rather than microevolutionary change (Bókony et al. 2021). Urban-exploitative and invasive species often express highly flexible generalist phenotypes that enable them to colonise new habitats such as cities (Rodewald and Gehrt 2014; Ducatez et al. 2018; Franzén et al. 2020; Palacio 2020) and phenotypic plasticity plays an important role in facilitating the success of a small number of highly abundant species in cities (reviewed in Lowry et al. 2013; Rodewald and Gehrt 2014). Therefore, phenotypic flexibility might also play an important role in the invasion success of guttural toads and we suggest future research to investigate if and how phenotypic plasticity might drive invasion success in this and other urban adapted species.

We show here that prior adaptation in larval growth rate as well as morphological and performance traits is unlikely to have facilitated the invasion success of guttural toads in Cape Town. Furthermore, the reduction in developmental rate likely arose after the introduction to Cape Town. Thus, bridgehead effects and decoupled evolution of traits are more likely to drive successful colonisation of new habitats in this species. Our findings suggest several promising avenues of future research. For example, we suggest investigations examine divergent selection for aquatic and terrestrial life stages in amphibian invaders, and how this might lead to coupling or decoupling of traits across life stages. We also know little about how certain habitat characteristics in urban environments, such as altered species composition or anthropogenic structures, might facilitate colonisation of invasive populations or how possible plastic traits can enhance invasion success across different habitat types - which are both important knowledge gaps to address.

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Supplementary material 1

Supporting information

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