Comparative Biochemistry and Physiology, Part A xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Plasticity of thermal tolerance and metabolism but not water loss in an invasive reed frog

Sarah J. Davies [a,*], Melodie A. McGeoch [b], Susana Clusella-Trullas [a]

[a] DST-NRF Centre of Excellence for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[b] School of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia

A R T I C L E I N F O

Article history:
Received 10 March 2015
Received in revised form 29 June 2015
Accepted 30 June 2015
Available online xxxx

Keywords:
Biological invasion
Respirometry
Beneficial acclimation
Critical thermal limit
Behavioural inertia
Trade-off

A B S T R A C T

Phenotypic plasticity may buffer the selection pressures on organisms that inhabit novel or rapidly-changing environments. We investigated plasticity of thermal tolerance, energetic and water loss traits and their interaction with behaviour in a small-bodied, arboreal anuran (*Hyperolius marmoratus* Rapp, Hyperoliidae) undergoing rapid range expansion in the winter rainfall region of South Africa. After short-term exposure to three temperatures (acclimation treatments) commonly encountered in their historical and novel ranges, frogs exhibited a broad thermal tolerance range (mean ± s.d.: 42.1 ± 2.9 °C) and higher plasticity in CT_{max} than in CT_{min}. Resting metabolic rate was lowest in cold-acclimated animals, while active metabolic rates were lowest in warm-acclimated frogs, likely reflecting compensation towards energy conservation. Evaporative water loss was not significantly altered by the acclimation treatments in either resting or active animals, indicating limited plasticity in this trait compared to metabolism. Our results suggest that plasticity of temperature limits and metabolism may benefit this species in variable environments such as those encountered in its expanded range. Lack of plasticity in water loss during resting and activity suggests that these frogs rely on their high cutaneous resistance and behavioural means to buffer climate variation. This study highlights the importance of synergistic interactions between physiology and behaviour in determining amphibian responses to temperature variation.

1. Introduction

The capacity of organisms to respond to environmental change through morphological, physiological and behavioural plasticity is likely to contribute to mitigating, at least to a point, the negative effects of habitat loss, climate change, biological invasions and epidemics (e.g. Agrawal, 2001; Cooke et al., 2013; Peacor et al., 2006; Seebacher and Franklin, 2012). Determination of the magnitude and direction of phenotypic plasticity in multiple physiological traits allows the development of mechanistic models for improved prediction of organism responses to environmental change (Basson and Clusella-Trullas, 2015; Buckley et al., 2015; Winwood-Smith et al., 2015). Studies investigating reversible plasticity of multiple thermal traits in anurans are uncommon, and represent only a minority of taxa and functional groups (see Lotshaw, 1977 for *Rana* spp.; Carey 1979 for *Bufo boreas*; Rogers et al., 2007 for *Limnodynastes peronii*; Overgaard et al., 2012 and Seebacher and Franklin, 2011 for *Rhinella marina*). Arboreal frogs face particular thermal and hydric challenges, as they typically travel long distances and spend significant lengths of time away from surface water. Body size influences time to desiccation (Tracy et al., 2010; Tracy et al., 2013), and for small-bodied arboreal frogs, the ability to avoid or delay desiccation is pivotal to survival. Quantifying the level of plasticity in traits of environmental stress resistance for these organisms would therefore be essential for improving predictions of their future distribution and potential vulnerability to environmental change.

The painted reed frog (*Hyperolius marmoratus* Rapp; body mass 1–3 g) has a broad distribution in southern African savannas (Bishop, 2004; Channing, 2001; Fig. A1) and has established breeding populations in the south-western Cape of South Africa since the late 1990s (Bishop, 2004; Davies et al., 2013; Tolley et al., 2008). The novel range is characterised by a more variable, temperate, winter-rainfall Mediterranean climate and dry, hot summers, in contrast with the largely subtropical, summer-rainfall historical range (Tyson, 1986). The breeding phenology (timing of reproduction) of the painted reed frog has remained unchanged in the novel range and the peak activity period coincides with the hottest, driest period of the year (SJD, unpublished data) instead of the warm but humid conditions found in its original geographic range. Correlative models indicate that the cold winters and warm, dry summers typical of the novel range may limit the frogs' ability to occupy further habitat (Davies et al., 2013). However, correlative relationships do not necessarily reveal trait-environment relationships that cause range limits or niche shifts (Buckley et al., 2010; Kearney and Porter, 2004). The magnitude and extent of phenotypic

---

* Corresponding author at: Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa. Tel.: +27 21 808 3922; fax: +27 21 808 2995.
E-mail address: sdavies@sun.ac.za (S.J. Davies).

http://dx.doi.org/10.1016/j.cbpa.2015.06.033
1095-6433/© 2015 Elsevier Inc. All rights reserved.

Please cite this article as: Davies, S.J., et al., Plasticity of thermal tolerance and metabolism but not water loss in an invasive reed frog, Comp. Biochem. Physiol., A (2015), http://dx.doi.org/10.1016/j.cbpa.2015.06.033

plasticity of performance in response to temperature variation of painted reed frog populations in the novel range may help clarify the capacity for this species to persist in these new conditions.

While most anurans lose water at a rate similar to that of a free water surface (Spotila and Berman, 1976), painted reed frogs are highly desiccation-resistant (Geise and Linsenmair, 1988; Withers et al., 1982). The mechanisms of this resistance are unknown, but may involve the structure and composition of the dorsal skin (Kobelt and Linsenmair, 1986; Lillywhite, 2006). In addition, resting frogs adopt a water-conserving posture with limbs tucked under the body and head lowered to be in contact with the substrate. During activity, exposure of the highly permeable ventral and axial skin is associated with high water loss rates (Geise and Linsenmair, 1986; Kobelt and Linsenmair, 1986). Painted reed frogs are prolonged breeders whose period and manner of calling is energetically costly (Bishop, 2004; Wells, 2007). The ability to attend a chorus and call for several consecutive nights increases male mating success, whereas body size and physical condition appear to play secondary roles (Dyson et al., 1998; see also Llusia et al., 2013; Rogers et al., 2007).

This study aimed to assess the thermal acclimation (i.e. phenotypic plasticity) of three key physiological traits: critical thermal limits (measured by CT_{min} and CT_{max}), metabolism (resting and active metabolic rates; RMR and AMR, respectively) and water loss (resting and active water loss rates, WLR). First, we predict that CT_{min} and CT_{max} will respond to acclimation temperature in a way that favours the organism in a variable environment. Second, because metabolic rate represents an energetic cost of living (Hulbert and Else, 2004), we hypothesise that lower RMR is beneficial, with the assumption that additional energetic resources can be allocated to capacity functions such as movement to and from nocturnal choruses, egg production, calling and combat (Boratyński and Koteja, 2010; Burton et al., 2011; Rogers et al., 2007). Therefore, RMR should stay constant or decrease in response to increasing acclimation temperature. Alternatively, RMR may be expected to increase under these conditions if a change in body composition (e.g. muscle mass, organ size) to facilitate movement is associated with increased RMR (reviewed in Piersma and van Gils, 2011). We test this hypothesis by evaluating explicit predictions about the acclimation responses of RMR within a rigorous experimental framework. We distinguish among five competing hypotheses: beneficial acclimation, 'hotter is better', 'colder is better', optimal acclimation temperature and no acclimation response (after Clusella-Trullas et al., 2010; Deere and Chown, 2006; Huey et al., 1999; Kingsolver and Huey, 2008; Marais and Chown, 2008). Predictions of the shape and direction of the expected relationships for metabolic rates are shown in Table 1. Third, we expect AMR to be up-regulated in response to cold acclimation treatments, thereby reflecting the upregulation of biochemical processes for function in the cold, so that animals can undertake the activities required for reproduction, foraging and dispersal during adverse cold conditions. Fourth, due to the known high desiccation resistance of resting frogs and typical water-conserving posture in this species (Geise and Linsenmair, 1988; Withers et al., 1982), we predicted that resting WLR would remain low and constant over a range of acclimation and test temperatures, while active WLR should perhaps demonstrate compensatory responses that minimise excessive water loss during activity (i.e. similar or lower active WLR at high acclimation temperature compared to the acclimation reflecting mean conditions).

Table 1
Hypothesised significance of the polynomial components of treatment effects (acclimation (ACC) and test temperature (TT)) on resting metabolic rate and water loss rate in painted reed frogs, assuming that lower RMR and resting WLR enhance fitness via energy and water savings. Derived from Huey et al. (1999), Deere and Chown (2006), Marais and Chown (2008), Kingsolver and Huey (2008), and Clusella-Trullas et al. (2010).

Hypothesis	ACC	TT	Interaction (ACC × TT)
Beneficial acclimation (BAH)			L+/−
Colder is better (CIB)	L+	L+	ns
Hotter is better (HIB)	L−	L−	ns
Optimal acclimation temperature (OAH)	Q+		
No acclimation response	ns	ns	ns

L denotes a linear and Q a quadratic component; +/− denotes the sign of the component; ns = relationship not significant; blank = no prediction. Because low resting metabolic rate is assumed to be beneficial (see text), CIB is indicated by a significant positive trend, and HIB by a negative trend in ACC and TT; BAH would be supported by a significant interaction between ACC and TT and OAH by a quadratic effect of ACC; non-significant trends throughout indicate no trait plasticity; blank cells indicate no particular hypothesis about the relationship.

2. Materials and methods

2.1. Collections and acclimation treatments

Adult frogs were collected from three artificial water bodies (dams) in the novel range in the Western Cape Province, South Africa (Durbanville: 33.872°S 18.624°E, Stellenbosch: 33.992°S 18.820°E, Strand: 34.098°S 18.821°E) during the peak reproductive activity period (austral summer). These collection sites were selected in order to represent the genetic structure that exists across the novel range populations of this species (Tolley et al., 2008). All collection sites were more than 350 km from the historical range and the inter-site distance averaged 21.4 km. Male and female frogs were captured at night, placed in clear plastic bags and transported to the laboratory where they were transferred to glass aquaria (200 W × 500 L × 350 H mm) within 24 h. Aquaria were assigned to one of three temperature treatments (each treatment contained four aquaria) such that male and female frogs from each collection site were represented equally in each treatment. The temperature treatments are referred to as 'acclimations' hereafter. Aquaria were maintained inside temperature-controlled chambers (LTIE 400 and LTGC 200; LabCon, Maraisburg, South Africa) on a controlled 12 h:12 h L:D cycle for at least 14 days prior to each experiment. We used this intermediate photo-period because the time in captivity was relatively long (mean = 114 d, min. = 105, max. = 131 d) given the multiple trials we conducted; we wished to maintain a constant photo-period throughout the study as the focus was to determine the response to temperature acclimation principally, and not day length or an interaction between the two.

The temperature inside each aquarium was monitored using iButton temperature loggers (Thermochron; Dallas Semiconductor, Sunnyvale, CA, USA; www.maxim-ic.com). Aquaria contained 9–11 frogs of mixed sexes and sites of origin, *c.* 3 l of water, plants for perches (*Polyganum* sp., *Cyperus* sp., *Papyrus* sp.) and a small area of soil, and were sprayed with dechlorinated water several times daily to maintain high humidity levels. Frogs were fed *ad libitum* on captive-bred Mediterranean fruit flies (*Ceratitis capitata*), based on observed feeding rates (5–8 flies per individual per day). Prior to acclimation, frogs were uniquely marked with Visible Implant Alpha tags (1.2 mm × 2.7 mm) inserted under the skin of an upper hind limb (Northwest Marine Technology Inc., Shaw Island, WA, USA) and the incision sealed with tissue glue (Histoacryl; Braun, Melsungen, Germany). Non-gravid females were used as far as gravidity could be ascertained from external examination. During the peak breeding season, a proportion of the females collected (±20%) were unavoidably gravid. These females were not excluded because of the difficulty of finding sufficient females at breeding sites on a given night, and because we wanted to assess the effects of sex on the physiological traits.

Air temperatures were recorded in exposed calling and perching microsites at each of the collection sites for a full year prior to collections. Acclimations (15, 20 and 25 °C) were within the range of conditions encountered at field sites (for details see Table 2). The lowest acclimation treatment of 15 °C did not trigger a dormancy state or torpor and frogs rapidly became active when disturbed (see also Schmuck and Linsenmair, 1997). Fifteen degrees Celsius is a common temperature even in summer in the novel range and painted reed frogs are heard calling at temperatures as low as 12 °C (pers. obs.).

Please cite this article as: Davies, S.J., et al., Plasticity of thermal tolerance and metabolism but not water loss in an invasive reed frog, Comp. Biochem. Physiol., A (2015), http://dx.doi.org/10.1016/j.cbpa.2015.06.033