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## RESEARCH ARTICLE

# Physiology of invasion: cane toads are constrained by thermal effects on physiological mechanisms that support locomotor performance

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### SUMMARY

**Understanding the mechanisms that constrain the invasiveness of introduced animals is essential for managing invasions and for predicting their limits. In most vertebrate species, the capacity for invasion relies upon the physiological systems that support locomotion, and oxygen transport and metabolism may become limiting as environmental temperatures increase as predicted by the oxygen limitation hypothesis. Here we test the oxygen limitation hypothesis and propose the alternative hypothesis that within-individual plasticity will compensate for thermal variation. We show that during exercise in the invasive cane toad (*Rhinella marina*) oxygen transport by the cardiovascular system was maximised in warm-acclimated toads at high (30°C) temperatures, and that oxygen content of arterial blood was not affected by temperature. Resting oxygen consumption remained stable across a 10°C temperature range (20–30°C) when toads were allowed to acclimate, so that there was no increase in resting oxygen demand that could lead to a decrease in aerobic scope at high temperatures. Additionally, temperature acclimation had no effect on arterial–venous differences in oxygen partial pressures. Toads relied more on glycolytic ATP production at low temperatures to support locomotor activity. Mitochondrial capacities (citrate synthase and cytochrome *c* oxidase activities) were greatest at warmer temperatures. Interestingly, the metabolic cost of exercise increased at low temperatures. In contradiction to predictions by the oxygen limitation hypothesis, aerobic performance was not limited by high temperatures. On the contrary, the relatively slow advance of cane toads to cooler climates can be explained by the constraints of low temperatures on the physiological systems supporting locomotion. It is likely that human-induced global warming will facilitate invasions of environments that are currently too cool to support cane toads.**

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Key words: climate change, oxygen limitation, cane toad, metabolism, body temperature, acclimation, endurance, range expansion.

## INTRODUCTION

Species introduced by human activity are now a major component of many ecosystems, although most exotic species have a limited impact on the endemic ecology. Some introduced species, however, become invasive and rapidly expand their population size and range (Torchin et al., 2003). These invasive species often have a disastrous ecological impact by outcompeting and displacing native species, or by changing the environment in such a way that it becomes unsuitable for native species (Sakai et al., 2001). The reasons why some introduced species become invasive are often unknown (Sexton et al., 2009), but understanding these reasons are fundamental to predicting the occurrence and limitations of invasions, and to managing introduced species before and potentially after they have become invasive.

Many invasive vertebrate species expand their ranges by active movement rather than being passively transported by currents or wind. This active dispersal means that range expansions become a function of the species' locomotor performance, which in turn can be influenced by environmental parameters such as temperature and humidity. Temperature can constrain or enhance dispersal by its effect on physiological functions such as oxygen uptake, oxygen delivery by the cardiovascular system, metabolism and muscle kinetics (Wilson et al., 2000; Johnston and Temple, 2002; Steinhausen et al., 2008).

The aerobic scope hypothesis postulates that an animal's potential for growth and movement is a function of the difference between resting and maximal oxygen use (Fry and Hart, 1948; Farrell et al., 2008). Resting oxygen consumption reflects metabolic maintenance costs necessary to keep cells alive, such as maintaining membrane potentials and protein synthesis (Hulbert and Else, 2000). Maximal oxygen use is constrained by oxygen uptake at the respiratory surface, oxygen delivery to the cells by blood flow and mitochondrial capacities within a cell, specifically by mitochondrial abundance and the activities of cytochrome *c* oxidase and other mitochondrial enzymes. In ectotherms, increases in temperature may cause an increase in resting metabolic rate without, however, changing maximal capacities. Hence, the potential for fitness-related functions such as reproduction and dispersal diminishes along with the metabolic capacity available in excess of that required to maintain cell integrity. The aerobic scope hypothesis has been invoked recently to assess and predict the effect of rising sea temperatures resulting from anthropogenic climate change on marine animals (Pörtner, 2001; Peck et al., 2004). Specifically, the oxygen limitation hypothesis (Pörtner and Knust, 2007) predicts that increasing ocean temperatures cause increases in resting metabolic rates and decreased oxygen transport efficiencies. The resulting decrease in aerobic scope may explain already observed changes in fish distributions

and population densities, and it has been invoked as a possible mechanism responsible for future species extinctions.

Species range expansions and invasions will be particularly affected by oxygen transport and metabolic limitations because their success depends to a large extent on aerobic (oxidative) metabolism. However, it remains unresolved whether limitation of aerobic scope is a general constraint among aquatic animals, and whether it is applicable to terrestrial species. In particular, many ectotherms have the capacity to compensate for environmental changes by remodelling physiological responses (reversible phenotypic plasticity, acclimation and acclimatisation) (Wilson and Franklin, 2002; Piersma and Drent, 2003). This capacity can counteract aerobic scope limitations at high temperatures by either curtailing the increase in resting metabolic rate or increasing oxygen transport efficiency and metabolic capacity, thereby maintaining aerobic scope (Franklin et al., 2007). Animals that have the capacity for such high temperature compensation would be relatively resilient against global warming (Seebacher et al., 2005). Similarly, invasive species could be successful in colonising novel habitats and climates because they possess plastic phenotypes that allow compensation for environmental changes (Dukes and Mooney, 1999; Schnell and Seebacher, 2008). If that were the case, global warming would facilitate invasions. Hence, our aim was to test the applicability of the oxygen limitation hypothesis in an invasive terrestrial ectotherm, the cane toad *Rhinella marina* (formerly *Bufo marinus*). Our alternative hypothesis was that reversible phenotypic plasticity of traits related to metabolism and exercise counteracts temperature limitations of aerobic scope in this invasive species. Cane toads are native to Central America and were introduced throughout the Pacific, including Australia, to control sugar cane beetles. In Australia, cane toads became invasive and have spread many thousands of kilometres in the tropics and subtropics and, to a lesser degree, to temperate regions (Kearney et al., 2008).

Our approach was to measure several physiological traits in the integrated oxygen transport–metabolism system during rest and exercise and at different combinations of acute and chronic temperature treatments. Hence, we measured cardiovascular performance (heart rate and blood pressure), oxygen transport (venous and arterial oxygen content), metabolic rates (oxygen consumption and lactate production) and metabolic capacity (enzyme activities) to test the hypothesis that toads compensate for temperature change and are not limited by increasing ambient temperatures.

## MATERIALS AND METHODS

### Study animals and experimental treatments

Cane toads [*Rhinella marina* (Linnaeus 1758); total $N$=50] were collected from the wild near Brisbane, QLD, Australia (27°28′S, 153°21′E). Toads were kept in large plastic tanks (640×430×250 mm) containing a substrate of moist bark chips deep enough to allow burrowing, and containers of open water. Toads were divided between two experimental acclimation treatments and kept in constant temperature rooms at body temperatures of either 20°C (cold acclimation) or 30°C (warm acclimation) for 4 weeks before experimentation. Acclimation temperatures were chosen to represent temperatures at the toads' southernmost and northernmost limits of distribution, respectively (Australian Bureau of Meteorology, http://www.bom.gov.au/climate) (Fig. 6). Upon completion of experiments, toads were killed by double pithing. All procedures were approved by The University of Sydney Animal Ethics Committee (approval no. L04-5-2009-2-5047).

### Cardiovascular and blood chemistry

We cannulated the femoral arteries and veins of toads to measure arterial and venous blood chemistry before and after exercise at different test temperatures. Toads were anaesthetised in a solution of buffered MS 222 (250 mg $l^{-1}$; Sigma, Sydney, Australia). We made a small incision in the skin of the upper leg and teased the underlying triceps, sartorius and gracilis muscles apart to expose the femoral artery and vein. Saline-filled and heparinised (100 IU $ml^{-1}$ sodium heparinate; Sigma) PE50 cannulae with PE10 tips were inserted into the vein and artery and sutured in place. The incision was closed with silk sutures and sealed with tissue glue, and toads were allowed to recover overnight. The cannulae had the dual role of recording heart rate and blood pressure, and providing the means of withdrawing blood without disturbing the toad at rest or during exercise. Cannulae were connected to a calibrated pressure transducer (MLT844, AD Instruments, Sydney, Australia), which was connected to a recording system (PowerLab) *via* a bridge amplifier (both AD Instruments). Heart rates were determined from blood pressure traces. Toads were allowed to recover at their acclimation temperature for 24 h.

Before recording, toads were placed in a constant temperature room set at the correct test temperature and allowed to recover for at least 1 h or until recovered from handling stress, i.e. when heart rates had stabilised. At this point we measured resting heart rates and blood pressure and withdrew an arterial and a venous blood sample. All sampling was done remotely without handling the toads. For the exercise measurements, we transferred toads into a water bath and induced vigorous exercise by prodding the toads with a blunt wooden stick, thereby causing toads to swim continuously for 10 min. At the end of the exercise period we withdrew venous and arterial blood samples and recorded heart rates and blood pressure. All toads showed reduced activity, indicative of fatigue, at the end of the exercise period. Blood lactate, pH and partial pressures of $CO_2$ ($P_{CO_2}$) and $O_2$ ($P_{O_2}$) were determined with an iStat 1 Analyser (Abbott Laboratories, Sydney, Australia). We measured resting and exercise-induced responses at two test temperatures (20 and 30°C) for each toad in a repeated-measures design, with at least 24 h between experimental trials.

Sample sizes vary because of the difficulty in keeping both cannulae patent and in place during the experiments. For blood chemistry measurements, we obtained data from six animals for each treatment (before and after excercise at 20 and 30°C; $N$=24 toads). We obtained cardiovascular data from an additional six animals (to give six to 12 animals per treatment) in which cannulae were dislodged before we were able to obtain blood samples.

### Oxygen consumption

We measured resting oxygen consumption rates by closed-system respirometry in 10 toads from each acclimation experiment at 20 and 30°C test temperatures (total $N$=20 toads; note that eight toads were used for the cold-acclimated experiment at 30°C); these toads were different from those used for cardiovascular and blood chemistry measurements. Toads were placed in respiration chambers (130×90×95 mm) that were fitted with a sealed port, which allowed withdrawal of air samples without disturbing the toads. Toads were left undisturbed in the unsealed chambers at the correct test temperature for 2 h before we sealed the chamber and obtained an initial air sample. We obtained air samples after 30 and 60 min to calculate repeated rates of oxygen consumption, and used the lowest rate in the analysis. Fractional concentrations of $O_2$ and $CO_2$ in the samples were analysed by passing the sample through a drying column (Drierite; Sigma, Sydney, Australia) into a gas analyser (AD

Instruments) connected to a Power Lab (AD Instruments). Oxygen consumption and $CO_2$ production were determined using equations in Vleck (Vleck, 1987) as previously described (Kayes et al., 2009).

### Enzyme assays

Maximal activity of regulatory enzymes in metabolic pathways determine the maximum flux through that pathway, and may thereby limit ATP production and locomotion. Hence, enzyme activities are a useful measure to assess whether metabolic capacities are limiting and whether animals compensate their metabolism for different thermal conditions (Seebacher et al., 2003). We measured the activities of lactate dehydrogenase, citrate synthase and cytochrome *c* oxidase to assess the capacities of glycolytic ATP production, the mitochondrial Krebs cycle and the mitochondrial electron transport chain, respectively. Skeletal muscle (vastis lateralis) and liver tissue samples were collected at the time of death from nine cold-acclimated and seven warm-acclimated toads, which were previously used for resting oxygen consumption measurements; tissues were transferred into liquid nitrogen immediately after collection and stored at –80°C for later analysis. Enzyme activities were determined according to published protocols (Seebacher et al., 2003).

### Statistical analysis

We analysed heart rates and blood pressures at rest and exercise separately using ANOVAs with acclimation treatment as a fixed factor and test temperature as a repeated measure. We compared heart rates of resting and exercising toads by calculating cardiac factorial scope (exercise/rest), which we analysed with an ANOVA using acclimation treatment as a fixed factor and test temperature as a repeated measure. Similarly, we analysed $P_{O_2}$ and $P_{CO_2}$ separately for arterial and venous blood during rest and exercise with an ANOVA using acclimation treatment as fixed factor and test temperature as repeated measure. We analysed oxygen uptake at the tissue level by calculating the arterial–venous differences in $P_{O_2}$, which we analysed with an ANOVA with acclimation treatment and test temperature as fixed factors and activity (rest and exercise) as a repeated measure.

Resting oxygen consumption and carbon dioxide production of whole animals were analysed using ANOVA with acclimation treatment as a fixed factor and test temperature as a repeated measure. Blood lactate concentrations and pH were analysed by ANOVA with acclimation treatment as a fixed factor and test temperature as a repeated measure. Additionally, we compared resting and exercise-induced concentrations of lactate and pH in venous blood at each test temperature separately by paired *t*-tests, by pooling data from the two acclimation groups, which had similar responses (see Results).

We used the truncated-product method (Zaykin et al., 2002) to assess the effect of multiple comparisons on the validity of *P*-values. Briefly, the truncated-product method considers the distribution of all *P*-values from multiple hypothesis tests to test for bias in their distribution. Multiple comparisons did not bias the statistical results presented here ($P<0.001$).

## RESULTS

### Cardiovascular system

Increasing the test temperature from 20 to 30°C caused an increase in both resting and exercise-induced heart rates (both $F_{1,12}>40.0$, $P<0.0001$; Fig. 1A). Resting heart rates were higher in cold-acclimated toads at both test temperatures (main effect $F_{1,12}=5.19$, $P<0.05$). Heart rates during exercise were lower in warm-acclimated toads at 20°C compared with cold-acclimated toads (main effect $F_{1,12}=5.76$, $P<0.04$). The factorial scope of heart rate (resting/maximal heart rate), which provides a measure of the capacity of the cardiovascular system to support exercise, was greatest in warm-acclimated toads at 30°C (interaction $F_{1,12}=9.30$, $P<0.01$; Fig. 1B). Blood pressure increased with increasing test temperature, but there was no effect of acclimation (see Appendix).

### Oxygen transport

$P_{O_2}$ values did not change with either acclimation treatment or test temperature, except that after exercise $P_{O_2}$ was higher in venous blood at 30°C compared with 20°C (Table 1, supplementary material Fig. S1). Oxygen uptake by cells may be expressed as the difference in $P_{O_2}$ between arterial and venous blood, assuming that blood flow through the tissue is constant. As expected, this arterial–venous difference was greater during exercise than at rest ($F_{1,20}=18.39$, $P<0.0001$; Fig. 2), but during exercise it was lower at 30°C than at 20°C (interaction $F_{1,20}=4.69$, $P<0.05$), which indicates that toads used less oxygen at 30°C despite similar arterial $P_{O_2}$ at all acclimation treatment–test temperature combinations. There was no effect of acclimation on arterial–venous differences in $P_{O_2}$ ($F_{1,20}=0.91$, $P=0.35$).

Blood $P_{CO_2}$ values, reflecting activity of the Krebs cycle, were more thermally sensitive than $P_{O_2}$ values, and $P_{CO_2}$ increased with increasing temperature except for arterial blood during exercise (Table 1, supplementary material Fig. S1).

### Metabolism

Whole animal resting oxygen consumption was significantly higher at 30°C than at 20°C ($F_{1,18}=69.67$, $P<0.0001$). However, there was

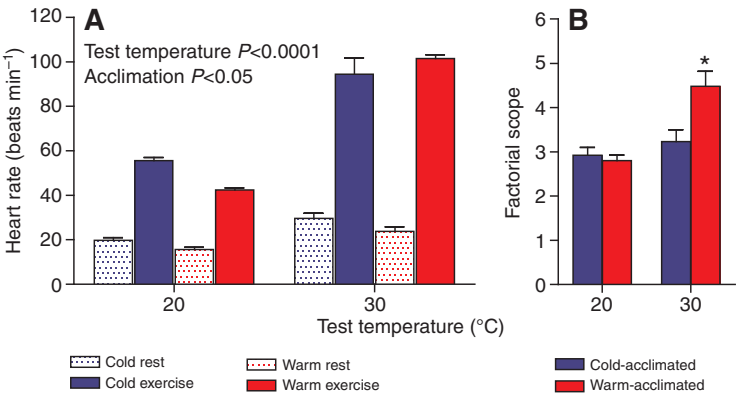


Fig. 1. (A) Heart rates of toads at rest and during exercise increased significantly with increasing test temperature. There was an interaction between acclimation treatment and test temperature, and resting heart rates were significantly lower in warm-acclimated toads at both test temperatures. (B) Factorial scope for heart rate which is a measure of the capacity of the cardiovascular system to support activity, was greatest in warm-acclimated toads at 30°C (asterisk).

Table 1. Results of statistical analyses of the partial pressures of $O_2$ ($P_{O_2}$) and $CO_2$ ($P_{CO_2}$) in arterial and venous blood in cane toads at rest and during exercise

| Blood | Activity | Factor | Variable | $F_{1,10}$ | $P$ |
|---|---|---|---|---|---|
| Arterial | Rest | Acclimation | $P_{O_2}$ | 2.51 | 0.14 |
| | | Test temperature | | 0.002 | 0.96 |
| | | Acclimation × test temperature | | 4.67 | 0.056 |
| | | Acclimation | $P_{CO_2}$ | 10.33 | <0.01 |
| | | Test temperature | | 58.91 | <0.0001 |
| | | Acclimation × test temperature | | 0.17 | 0.69 |
| | Exercise | Acclimation | $P_{O_2}$ | 0.45 | 0.52 |
| | | Test temperature | | 2.99 | 0.11 |
| | | Acclimation × test temperature | | 1.19 | 0.30 |
| | | Acclimation | $P_{CO_2}$ | 0.15 | 0.71 |
| | | Test temperature | | 0.92 | 0.22 |
| | | Acclimation × test temperature | | 0.28 | 0.49 |
| Venous | Rest | Acclimation | $P_{O_2}$ | 1.34 | 0.27 |
| | | Test temperature | | 0.77 | 0.40 |
| | | Acclimation × test temperature | | 2.20 | 0.17 |
| | | Acclimation | $P_{CO_2}$ | 0.62 | 0.45 |
| | | Test temperature | | 23.87 | <0.001 |
| | | Acclimation × test temperature | | 3.99 | 0.074 |
| | Exercise | Acclimation | $P_{O_2}$ | 0.17 | 0.69 |
| | | Test temperature | | 10.25 | <0.01 |
| | | Acclimation × test temperature | | 0.38 | 0.55 |
| | | Acclimation | $P_{CO_2}$ | 0.74 | 0.41 |
| | | Test temperature | | 5.15 | 0.047 |
| | | Acclimation × test temperature | | 0.078 | 0.79 |

a significant effect of acclimation ($F_{1,18}$=67.35, $P$<0.0001) so that oxygen consumption did not differ between acclimation groups when measured at their respective acclimation temperatures, despite the 10°C difference in test temperature (Fig. 3).

Arterial and venous blood lactate levels, indicating anaerobic ATP production *via* lactate dehydrogenase activity, were significantly higher during exercise than at rest at both test temperatures (both $t_{11}$>10.0, $P$<0.0001; Fig. 4, supplementary material Fig. S2). However, during exercise, lactate concentrations were significantly lower at 30°C than at 20°C ($F_{1,10}$=5.3, $P$<0.05; Fig. 4), indicating that toads rely less on anaerobic metabolism at high temperatures. Additionally, warm-acclimated toads at rest produced less lactate at 20°C than cold-acclimated toads (interaction $F_{1,10}$=5.12, $P$<0.05). Patterns of blood pH mirrored those of lactate, and pH was significantly lower during exercise than at rest (see Appendix).

All enzyme activities, reflecting maximal capacity of their respective pathways, increased with increasing test temperature (all $F_{1,14}$>100.0, $P$<0.0001; Fig. 5). Warm-acclimated toads had significantly greater activity of citrate synthase and lactate dehydrogenase in liver at both test temperatures ($F_{1,14}$=5.28, $P$<0.05 and $F_{1,14}$=4.69, $P$<0.05, respectively). Conversely, lactate dehydrogenase activity was significantly greater in muscle of cold-

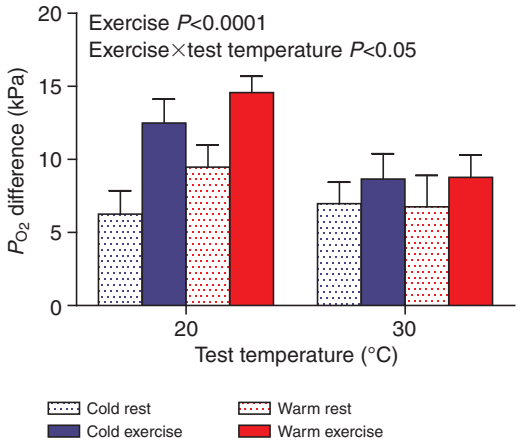


Fig. 2. Difference in $P_{O_2}$ between arterial and venous blood, which is an indicator of muscle oxygen use, at rest and during exercise in cold- (blue bars) and warm-acclimated (red bars) toads at 20 and 30°C test temperatures. The arterial–venous difference in $P_{O_2}$ was less during exercise at 30°C than at 20°C, indicating metabolically more efficient muscle function.

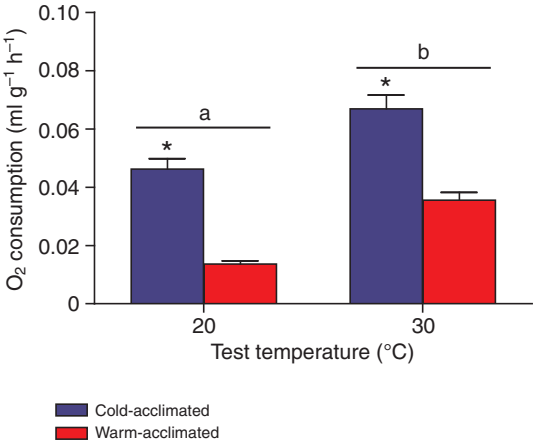


Fig. 3. Resting oxygen consumption in cold- (blue bars) and warm-acclimated (red bars) toads at 20 and 30°C test temperatures. Oxygen consumption was higher at 30°C than at 20°C (indicated by different letters), but there was a significant effect of acclimation (asterisks) so that oxygen consumption of the warm-acclimated group at 30°C did not differ from that of the cold-acclimated group at 20°C.

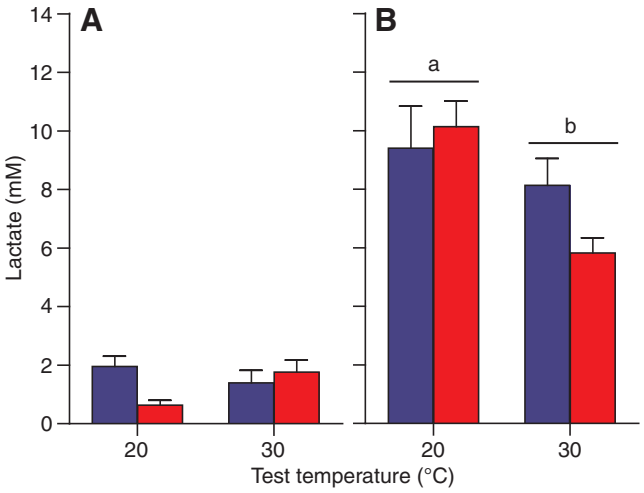


Fig. 4. Venous blood lactate concentrations (A) at rest and (B) during exercise in cold- (blue bars) and warm-acclimated (red bars) toads at 20 and 30°C test temperatures. Lactate concentrations were significantly higher during exercise than at rest. During exercise, lactate levels were significantly lower at 30°C than at 20°C (indicated by different letters), indicating a reduced reliance on anaerobic metabolism at high temperatures. At 20°C, warm-acclimated toads had significantly lower venous blood lactate levels at rest than cold-acclimated toads.

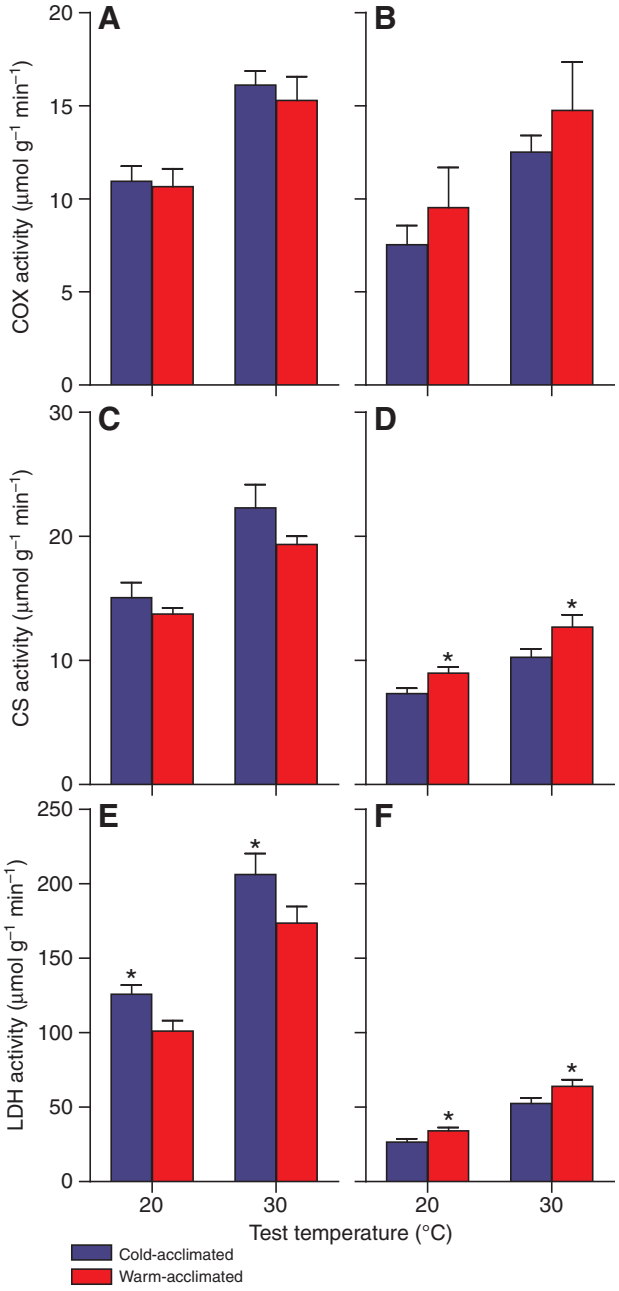


Fig. 5. Activities of metabolic enzymes in cold- (blue bars) and warm-acclimated (red bars) toads in skeletal muscle (left column) and liver (right column) at at 20 and 30°C test temperatures. Test temperature had a significant effect on all activities. Asterisks indicate significant effects of acclimation. COX, cytochrome *c* oxidase; CS, citrate synthase; LDH, lactate dehydrogenase.

acclimated toads ($F_{1,14}$=4.55, $P$<0.05). Acclimation treatment did not have an effect on cytochrome *c* oxidase activities in either tissue, or on citrate synthase activity in muscle.

## DISCUSSION

Elevated temperatures did not limit oxygen transport or metabolism in cane toads, and metabolic performance supporting locomotion was maintained at higher temperatures. Furthermore, the physiological systems that underlie exercise performance functioned better at higher temperatures, which could explain the slow spread of cane toads at their southern limit. A corollary to this is that these toads would benefit from climate warming in that they would be able to disperse into the cooler southern areas of Australia, particularly because, as seen in the present study, they have a limited capacity for cold acclimation.

Movement of toads and other amphibians depends strongly on water availability as well as temperature (Schwarzkopf and Alford, 1996; Rowley and Alford, 2007). For example, movement of toads near their original release site in north Queensland, Australia, decreased rapidly at soil moisture levels of less than 0.3 ml $g^{-1}$ dry mass (Seebacher and Alford, 1999), which corresponds to rainfall of less than approximately 60–70 mm per month (Australian Bureau of Meteorology, http://www.bom.gov.au/climate). The seasonality of rainfall decreases with increasing latitude; thus, according to the above estimates, toad movement would be restricted by low rainfall in winter in northern monsoonal climates but not at all in more southern temperate climates (Fig. 6). Hence, the major limitation to toad movement at the southern extreme of their distribution is likely to be lower temperatures, and here we show that the thermal sensitivity of exercise physiology can provide the proximate mechanisms explaining this limitation.

Toads have a limited capacity to acclimate to cold temperatures. There was temperature compensation in the capacity of cane toads in the present study for anaerobic energy (ATP) production by lactate dehydrogenase. Acclimation of muscle lactate dehydrogenase in addition to the increased accumulation of blood lactate levels during exercise at low temperatures indicates that toads become more anaerobically poised as temperatures decrease (Petersen and Gleeson, 2009). Hence, endurance performance and the capacity for extended movement decrease as temperatures decrease. Conversely, mitochondrial ATP production capacity and cardiovascular performance increase at higher temperatures, thereby facilitating oxygen transport and aerobically fuelled movement and dispersal. The greater increase in activity of mitochondrial enzymes in liver *versus* muscle with warm acclimation may reflect a greater

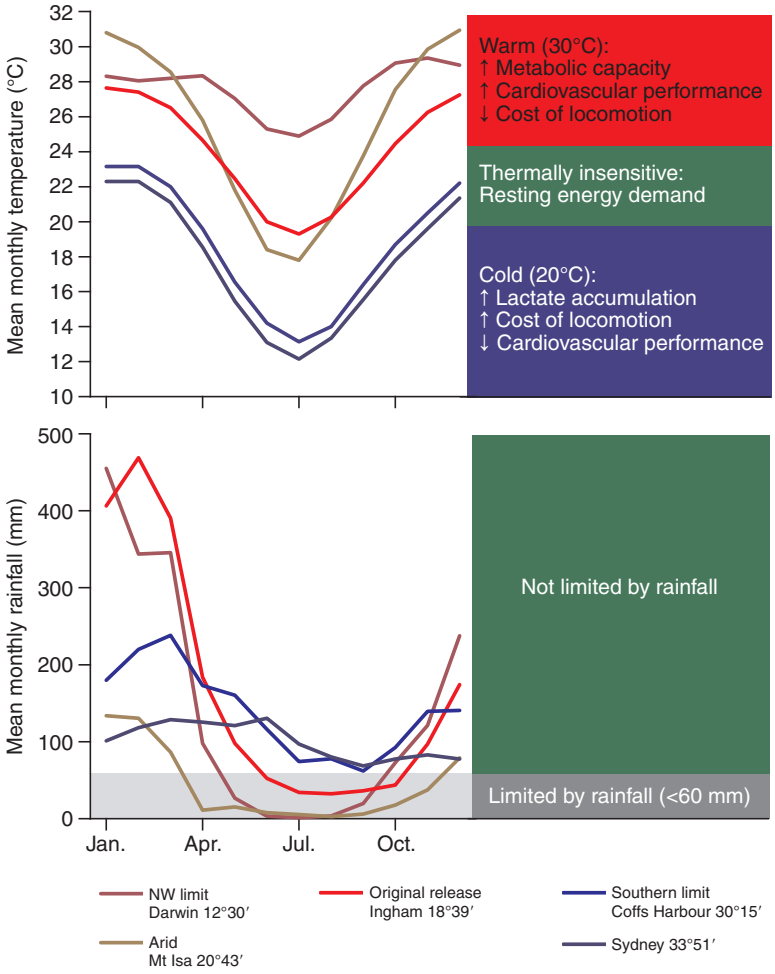


Fig. 6. Summary of thermal sensitivities of exercise-related traits relative to (A) mean monthly temperatures and (B) rainfall throughout the distributional range of cane toads. Toads were introduced near Ingham in tropical north Queensland and spread across arid north Queensland (e.g. Mt Isa), where movement would have been limited by low rainfall most of the year and by low temperature in mid-winter. Toads colonised the Northern Territory (e.g. Darwin) very rapidly, where movement would be limited only by low rainfall in winter. At the southern extent of their range (Coffs Harbour and, by extension, Sydney), movement would be limited by low temperature for most of the year, but not by rainfall.

capacity for gluconeogenesis and substrate cycling at higher temperatures (Fernandez and Des Rosiers, 1995; Seebacher et al., 2009).

The conclusion that movement is physiologically limited by cold is further supported by our finding that toads used more oxygen during exercise at low temperatures than at high temperatures. The lower oxygen use at higher temperature is not the result of limited oxygen availability, because partial pressures in arterial blood were constant across all temperature treatments, indicating that there is no thermal limitation of oxygen uptake. Also, the thermal sensitivity of myoglobin-facilitated oxygen transport is very low (Dowd et al., 1991) so oxygen transport at the tissue level is unlikely to be a limiting factor. In other words, locomotion is metabolically more costly at low temperatures, if it can be assumed that blood flow to the muscle is constant at different temperatures. Indeed, this is the case in resting crocodiles, where heating from 20 to 27°C caused a significant increase in heart rate and blood flow to the periphery of the animal and, conversely, cooling decreased heart rate and caused blood flow from the periphery to the duodenum (Seebacher and Franklin, 2007). However, flow to the locomotory muscles in the leg and tail remained constant during the heating and cooling cycle. The effect of exercise on blood flow overrides any temperature effect in human skeletal muscle, and muscle blood flow increases with exercise but does not differ between muscles at 33–34 and 37–38°C (Ferguson et al., 2006; Cook and Ray, 2009). In cane toads, total blood flow in the systemic artery during exercise is similar at 20 and 30°C (Hedrick et al., 1999), so that there is no temperature-induced central limitation to tissue blood flow over that temperature range. Additionally, the efficiency of muscle to convert chemical energy into mechanical energy is independent from temperature (Barclay et al., 2010), so that our results cannot be explained by a loss in efficiency at lower temperatures. A possible explanation is that there may be greater resistance in the contracting muscle, maybe resulting from greater viscosity of membranes and cytoplasm (Mutungi and Ranatunga, 1998), which increases the amount of ATP necessary to perform an equal amount of work at low temperatures.

Together, these data show that toads have greater endurance capacity and can disperse further in warmer seasonal and geographical conditions. Temperatures during winter at their original release site in coastal north Queensland as well as in central north Queensland are low enough to curtail dispersal. However, thermal conditions at their current northwestern invasion front in the Northern Territory are rarely limiting (Fig. 6). Hence, our data provide a mechanistic explanation for the greater endurance performance and rapid expansion of toads at their tropical invasion front (Llewelyn et al., 2010).

Importantly, resting oxygen use remained stable when the toads were allowed to acclimate, despite a 10°C increase in temperature. Hence, aerobic scope would not diminish at higher temperatures as a result of increased resting oxygen demand; this is in contrast to predictions that a temperature increase of as little as 2–3°C can cause an increase in resting oxygen consumption, leading to diminished

aerobic capacity (Pörtner and Knust, 2007). Similarly, cardiovascular performance in toads was greatest at the higher test temperature, so increasing body temperature to 30°C does not constrain oxygen transport. The ecological consequences of oxygen limitation and decreasing aerobic scope would be curtailed movement, behaviour and reproduction, leading to a decrease in fitness. This is clearly not the case for cane toads, and our data suggest that the oxygen limitation hypothesis is not a general principle for terrestrial animals (Franklin and Seebacher, 2009). In particular, physiologically plastic phenotypes would be able to offset the effects of environmental temperature variation, as we show for resting oxygen consumption in toads. Acclimation of cardiovascular function, metabolic capacity and locomotor performance is well known in terrestrial and aquatic species (St Pierre et al., 1998; Hammill et al., 2004; Franklin et al., 2007), and its importance for metabolic scope must be incorporated in predictions of animal performance in variable climates (Seebacher et al., 2010).

The success of invasive species may lie in plastic phenotypes that can compensate for the rapid changes experienced during dispersal into novel environments (Chown et al., 2007; Schnell and Seebacher, 2008). Alternatively, range-expanding phenotypes may predispose some species to colonise novel environments at a greater rate. This has been suggested for toads, where traits such as a terrestrial adult niche, large body size, energy reserves and toxicity may promote range expansion (Van Bocxlaer et al., 2010). We suggest that physiological variables such as acclimation and/or acclimatisation of resting energy demand and exercise physiology should also be considered in the context of predisposing animals to range and niche expansions (Kearney and Porter, 2004). Lastly, dispersal into novel environments and invasion success may be facilitated by rapid evolution, i.e. relatively strong selection pressures at the invasion front lead to a shift in allele frequencies, which heritably affect phenotypic traits that benefit range expansion and invasion (Lee, 2002; Hassel et al., 2005). However, the genetic basis for rapid adaptive processes are difficult to detect, because in all likelihood these occur by small changes in many genes (polygenic adaptation) rather than by substitution of a few (Pritchard and DiRienzo, 2010). At a temporal level, it is likely that phenotypic plasticity in the form of acclimation or acclimatisation, or developmental processes (Wilson and Franklin, 2002), is most effective in the initial dispersal of species at invasion fronts.

## APPENDIX

### Cardiovascular system

Increasing test temperatures from 20 to 30°C caused a significant increase in blood pressure of toads at rest and during exercise (both $F_{1,12}$>11.0, $P$<0.001), but there was no effect of acclimation on blood pressure (all $F_{1,12}$<1.0, $P$>0.70; supplementary material Fig. S3).

### Metabolism

Arterial blood lactate concentrations did not change with either acclimation or test temperature at rest or during exercise (all $F_{1,10}$<4.0, $P$>0.05; supplementary material Fig. S2). However, the patterns of lactate production parallel those of venous lactate levels.

Arterial and venous blood pH was significantly lower during exercise than at rest at both test temperatures (both $t_{11}$>8.0, $P$<0.0001). However, blood pH did not change significantly with either acclimation or test temperature at rest or during exercise (all $F_{1,10}$<2.5, $P$>0.15; supplementary material Fig. S4), except that warm-acclimated toads at rest produced less lactate at 20°C than cold-acclimated toads (interaction $F_{1,10}$=5.12, $P$<0.05).

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