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Cane toads (*Rhinella marina*) rely on water access, not drought tolerance, to invade xeric Australian environments

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Abstract

The invasion of habitats with novel environmental challenges may require physiological tolerances not seen in conspecifics from the native range. We used a combination of field and laboratory-based experiments to assess physiological tolerance to limited water access at four sites distributed across the historical invasion path of cane toads (*Rhinella marina*) in Australia that, from east to west, alternated between mesic and seasonally xeric habitats. Toads from all locations were well hydrated at the time of capture. However, experimental dehydration caused greater mass loss, higher plasma osmolality, and inhibition of lytic ability in toads from xeric compared to mesic locations. These results suggest somewhat surprisingly that toads from xeric environments are physiologically more vulnerable to water loss. In contrast, bactericidal ability was not sensitive to hydric state and was greater in toads from eastern (long-colonized) areas. Similar patterns in lytic ability in hydrated toads and agglutination ability in wild toads suggest that toads along the invasion front face a tradeoff between enhanced dispersal ability and physiological responses to dehydration. The ability of this invasive species to spread into drier environments may be underpinned by a combination of phenotypic plasticity and evolved (heritable) traits.

Keywords *Bufo marinus* · Hydroregulation · Innate immunity · Invasive species · Osmolality

Introduction

Invasive species can perturb biotic communities, especially in areas where the invader and the native taxa have substantially different eco-evolutionary histories (Donohue et al. 2013; Simberloff et al. 2013; Dick et al. 2017). Despite the impact of invasive species, physiological mechanisms that underpin extensive range expansion are little understood, especially when the invader moves into areas that expose it to conditions more extreme than in its natural habitat (Sexton et al. 2009). Cane toads (*Rhinella marina*) have successfully invaded over 150 countries, and are among the most intensively studied colonizing species (Shine 2010; Pizzatto et al. 2014). Nonetheless, there remain substantial gaps in our understanding of physiological mechanisms which have allowed toads from stable warm, moist climates in Latin America to successfully invade severely xeric areas of Australia (Tingley et al. 2014; Kosmala et al. 2018).

Cane toads were introduced into northeastern Australia in 1935 as a potential pest control agent (Lever 2001). Based on environmental characteristics of their native habitats, it was assumed that spread of these toads in Australia would be restricted by limited water availability and high temperatures (Sutherst et al. 1996). However, the toads' range in Australia has expanded considerably, and they have moved from relatively seasonal east-coast environments into the wet–dry tropics of the Northern Territory and Western Australia where extended seasonal drought occurs (Phillips et al. 2007). Models based on these advances project an almost

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tripling of the toads' range in the near future (Urban et al. 2007).

Within Australia, cane toads and native species with which they interact are undergoing rapid evolutionary change (Tingley and Shine 2011; Shine 2012; Pizzatto et al. 2013; Brown et al. 2014), generating substantial differences between individuals at the invasion front compared to individuals at the range core (Lindstrom et al. 2013; Hudson et al. 2016). Cane toads at the invasion front have been shown to rapidly acclimatize to temperature regimes previously thought to be too cold (McCann et al. 2014, 2018). Those inhabiting drier regions of Australia exhibit dramatic fluctuations in plasma osmolality (250–370 mOsm kg^{-1}, Reynolds and Christian 2009). Desiccation risk reduces dispersal of juvenile cane toads (Child et al. 2009) but, despite presumed constraints due to lack of water, cane toads are physiologically acclimating to invade semi-arid regions of Australia (Tingley and Shine 2011; Tingley et al. 2012; Kosmala et al. 2018). Indeed, the cane toad invasion is expanding more rapidly as it moves through drier habitats (Phillips et al. 2006).

Exploring physiological traits that enable toads to expand their range into xeric areas may help us to predict the extent of the species' eventual distribution. Although a few studies have examined the role of immune function in the invasiveness of cane toads (Llewellyn et al. 2011; Brown and Shine 2014; Brown et al. 2015a, b), the interaction between immune function and hydric state has remained unstudied. Water is a fundamental, non-energetic resource that can modulate immune function. Enhanced immune function in response to dehydration has been documented in an invertebrate (Hoang 2007) and multiple squamates (Moeller et al. 2013; Brusch et al. 2017; Brusch and DeNardo 2017).

Many animals maintain a plasma osmolality of approximately 300 mOsm kg^{-1} (Stockham and Scott 2013) even during periods when they do not drink (Ramsay and Thrasher 1984). Juxtaposed against these norms are terrestrial amphibians, which typically have a low resistance to transcutaneous water loss (Young et al. 2005). As a result, many species in dry environments are constantly at risk of desiccation due to the inability to balance water influx with water efflux (Hillman 1980). Accordingly, many terrestrial amphibians tolerate high osmolality values that are indicative of dehydration (Zug and Zug 1979; Reynolds and Christian 2009). This makes terrestrial amphibians particularly interesting for studying the relationship between hydration state and immune function.

We used four Australian sites distributed across the historical invasion path of cane toads that, from east to west, alternated between mesic and seasonally xeric habitats (Fig. 1). We evaluated indicators of physiological tolerance to limited water access and used these results to explore inter-populational differences. We hypothesized that cane toads invading xeric habitats have a greater physiological tolerance to limited water access. Accordingly, we predicted that tolerance to water limitations and modification of dehydration sensitivity of immune function progressively changes from east to west. Alternatively, cane toad dehydration tolerance and effects on immune function is a result of plasticity, which would be indicated by performances reflecting the hydric environment in which the toads live (mesic vs. xeric) rather than the historical progression of the invasion across the sites.

Methods

All procedures were approved by the Arizona State University Institutional Animal Care and Use Committee (protocol #16-1495R), the University of Sydney Animal Ethics Committee (protocol #2016/997), and the Charles Darwin University Animal Ethics Committee (protocol #A16010).

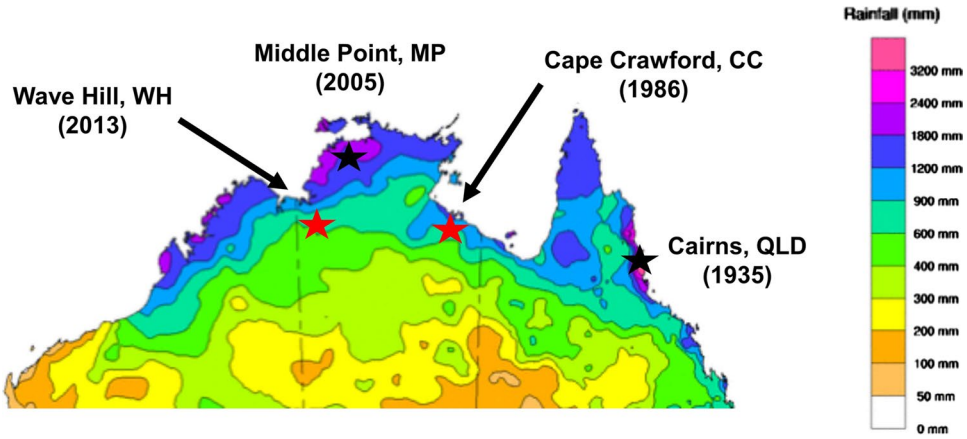

Fig. 1 Four study locations across the historical invasion path (year of arrival) of cane toads in Australia that alternated between mesic (black star) and seasonally xeric (red star). Annual rainfall map for 2016 modified from the Australian Bureau of Meteorology (http://www.bom.gov.au/climate)

Study species and sites

Cane toads are large (to > 1 kg) toxic bufonid anurans that are native to tropical and subtropical areas of the Americas. After being introduced to northeastern Australia in 1935, cane toads spread across almost one-quarter of the Australian continent (Kearney et al. 2008; Urban et al. 2008; Tingley et al. 2016). We collected toads during June and July of 2016 in the midst of the tropical 'dry-season' (May–October) when rainfall is scarce or absent (Shine and Brown 2008). Toads were collected from four locations: (1) Cairns, Queensland [QLD; average rainfall and mean daily maxima during June–July = 38.9 mm/26 °C (Australian Bureau of Meteorology, http://www.bom.gov.au/climate)] the mesic environment where the species was first introduced to Australia. (2) Cape Crawford, Northern Territory (CC 1.2 mm/30 °C), an arid area along the southern edge of the Gulf of Carpentaria where the cane toad invasion slowed in the 1980s apparently as a result of arid conditions (Tingley et al. 2012). (3) Middle Point, Northern Territory (MP 0.5 mm/32 °C), a wet–dry tropical site along the Adelaide River that receives high annual precipitation (~ 1400 mm) that provides constant access to water for toads (Warfe et al. 2011) even during June and July when little precipitation occurs. (4) Wave Hill, Northern Territory (WH 0.2 mm/29 °C), the arid habitat near the leading edge of the species' current range (Phillips et al. 2006). Over the last 20 years, cane toads have rapidly expanded their range despite the aridity of the area (González-Bernal et al. 2012).

Field-based experiment

To evaluate variation in plasma osmolality and innate immune function of cane toads across their Australian range, blood samples (see details below) were collected from ten adult toads at each of the four sites during the dry season (June and July). Toads were captured by hand between 1800 and 2100 hours when toads typically emerge from their daytime refugia to begin nocturnal activity. For consistency, all toads were captured > 5 m from any visible water source and toads were not selected if they had moist skin (suggesting they had just exited the water). Upon capture, we determined mass, sex, and snout–vent length (SVL), and a blood sample was collected (see details below). For all samples, plasma osmolality was determined using a vapor pressure osmometer (± 3 mOsm kg^{-1}; model 5100C; Wescor Inc., Logan, UT, USA). Samples were run in triplicate as described in Davis and DeNardo (2009). Additionally, a suite of plasma-based immune function assays was performed on samples (see details below).

Laboratory-based experiment

To determine whether there is a causal effect of osmolality on innate immunity, 20 toads from each site (total 80) were captured by hand and temporarily held in wet cloth bags. Mass, sex, and snout-vent length were recorded before toads were housed individually in 30 × 20 × 20-cm plastic containers filled with ~ 4 cm of water to enable them to hydrate overnight (Hillyard et al. 1998). After 12–14 h, blood samples were collected from ten of the hydrated toads from each site (total 40). The remaining ten toads from each site (total 40) were moved to individual plastic containers without access to food or water and allowed to dehydrate for 120 h (5 days) to reach an ecologically relevant level of dehydration based on previously published work (360 mOsm kg^{-1}; Reynolds and Christian 2009). Containers were held at ambient temperature with natural light from rooftop windows. All toads were weighed every 24 h to evaluate rate of dehydration as change in mass is a good proxy for water loss in amphibians because of their very low metabolic rate. No toads showed clinical signs of dehydration (lethargy, slow righting reflex) or lost more than 35% of their body mass. After the 5-day dehydration period, toads were weighed and a blood sample was collected for determining osmolality and performing immune function assays.

Blood sample collection

Prior to blood sample collection, all toads were euthanized with an overdose of sodium pentobarbital (50% Lethabarb diluted in water: Pizzatto et al. 2013). Blood samples (0.8 ml) were collected via cardiocentesis using heparinized 1-ml syringes with a 25-gauge × 1.6-cm (5/8 in.) needle. Total time for capture, restraint, and blood collection was typically less than 5 min and did not exceed 8 min in either lab or field procedures. Blood samples were immediately centrifuged at 3000 rpm for 3 min to separate plasma from blood cells. Plasma samples were aliquoted (~ 50 µl) into separate vials and frozen at − 80 °C until they were used (within 21 days) to measure plasma osmolality and evaluate immune function.

Immune function assays

To examine immunocompetence, we performed several plasma-based innate immune function assays. Although amphibians possess both innate and adaptive immunities (Chen and Robert 2011), we focused on innate immune components for logistical reasons.

To evaluate the involvement of complement (C′) and natural antibodies (NAbs) in reacting to a novel, eukaryotic antigen, we used sheep red blood cells [(SRBC); SB050, Thermo Fisher Scientific, Scoresby, VIC, Australia] to

quantify agglutination and lysis, which are standard measures of soluble constitutive immunity (Matson et al. 2005). Briefly, 20 µl of plasma was serially diluted with phosphate-buffered saline (PBS) along a row of a 96-well plate, after which 20 µl of 1% SRBC was added to each well. Plates were incubated at 37 °C for 90 min and then placed at room temperature (~ 25 °C) for 20 min after which point they were scanned at 600 dots per inch (Hewlett-Packard Co., ScanJet 3670) for agglutination images. After an additional 70 min, plates were centrifuged for 5 min (500 rpm, Sorvall, Newtown, CT, USA) and the supernatant was dispensed into a clean 96-well plate. Absorbance values were measured (405 nm, Bio-Rad, Hercules, CA, USA) to calculate lysis scores. Hemolytic-complement activity was expressed in CH_{50} units ml plasma^{-1}, where 1 CH_{50} unit equals the reciprocal of the dilution of plasma required to lyse 50% of the SRBC.

Bactericidal activity was also assessed to determine the ability of cane toad plasma to kill a prokaryotic microorganism (French and Neuman-Lee 2012). For this assay, *Escherichia coli,* a Gram-negative bacteria that has been previously reported in wild populations of cane toads (Shilton et al. 2008) was used to provide ecological relevance. In brief, 1:4 plasma dilution with CO_2-independent media plus 4 nm L-glutamine, 10^5 colony-producing units of *E. coli* (Lot #483-306-1, ATCC 8739, MicroBioLogics, St. Cloud, MN, USA), and agar broth were combined on a 96-well microplate. Absorbance values were measured (300 nm, Bio-Rad, Hercules, CA, USA) immediately and again after 12 h of incubation at 37 °C. Bactericidal ability percentages were calculated as the mean number of colonies for each sample, run in triplicate, divided by the mean number of colonies for the positive control, and then multiplied by 100. Together, these three assays provided a detailed assessment of innate immune function that could be used to compare populations in terms of immunocompetence and the sensitivity of immune function to hydration state.

Statistical analyses

To explore physiological responses to water deprivation, we used linear mixed-effect models to compare total water loss (i.e., initial mass − final mass) of the captive toads from all four populations. Location was used as a fixed effect and SVL as a random effect (to remove any potential confounding influence from surface-area-to-volume ratios). Initially, analyses were carried out separately based on size on either body mass or SVL. However, these analyses yielded qualitatively identical results and, therefore, the reported analyses are based on SVL only. The data were checked to ensure they met the assumptions for parametric testing, transformations were used where necessary, and the *agricolae* package (de Mendiburu 2017) was used for post hoc tests.

To quantify sources of variation in osmolality values and immune scores, we used multimodel inference to estimate the most likely values of means and standard deviations for each dependent variable using the full-average method (Burnham and Anderson 2002). Size, sex, treatment, and location were used as categorical fixed factors. Using the *nlme* (Pinheiro et al. 2018) and *MuMIn* libraries (Bartoń 2015), the R Statistical Software (version 3.3.2; R Development Core Team 2016) was used to fit all possible models, including potential possible interactions between fixed factors. The Akaike information criterion (AIC_c) and Akaike weight (w_i) of each model (see Online Resource 1–4) were calculated to determine the best model. Finally, we used multimodel averaging following Burnham and Anderson (2002) to calculate the weighted average of each parameter using estimates from all models. These resulting values were used to calculate the most likely mean and standard deviations for each parameter.

Results

Location had a significant effect on physiological responses to water deprivation ($F_{3,3} = 10.81$, $P = 0.04$), whereby toads from WH and CC had the highest average water losses over the 5-day dehydration treatment (Fig. 2). Treatment and location had the greatest effects on plasma osmolality, lysis, agglutination, and bactericidal ability. Although toads from all locations had higher plasma osmolality after dehydration, the difference in osmolality between hydrated versus dehydrated treatments on average were 88% and 16% greater in toads from WH (mean ± SD = 81 ± 20 mOsm kg^{-1})

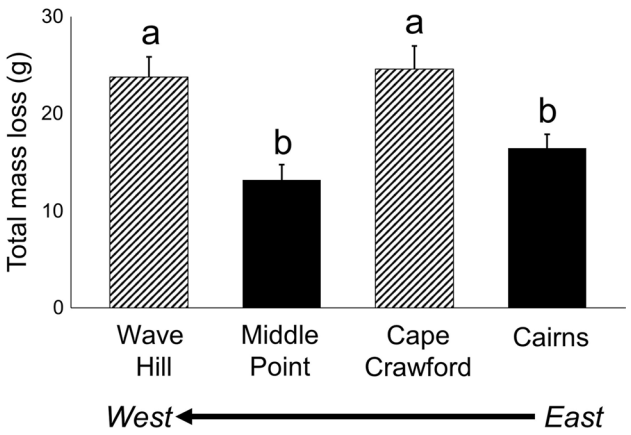

Fig. 2 Average total loss in mass (g) after 5 days without food and water measured in cane toads from mesic (solid) and xeric (striped) populations distributed across the historical invasion from east (Cairns, Queensland) to west (Wave Hill, Northern Territory) in Australia. Groups that share the same letter did not have statistically significant differences in means. Error bars represent ± 1 SD

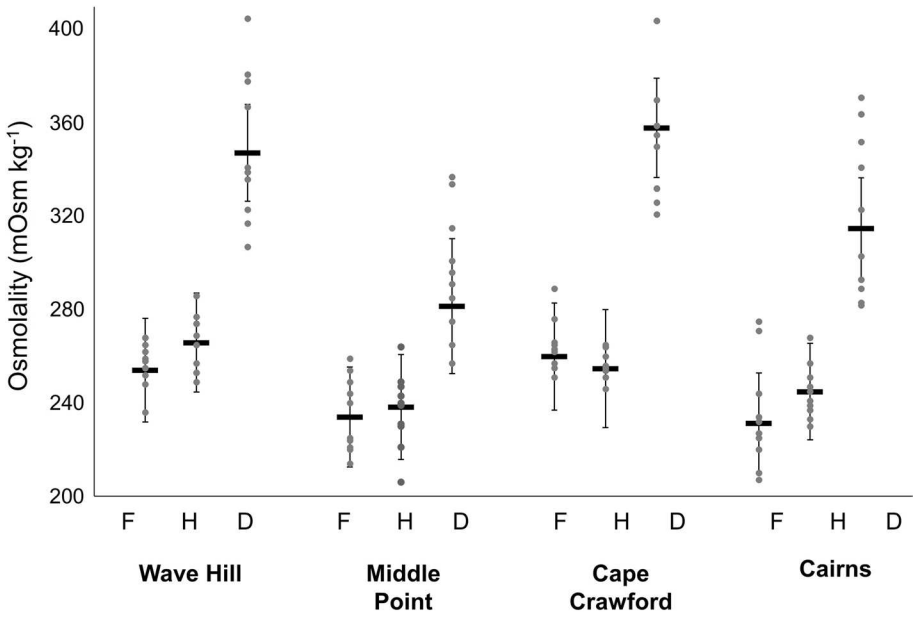

Fig. 3 Plasma osmolality (mOsm kg^{-1}) of cane toads increased after the dehydration treatment, but was similar for toads in the field and toads after hydration treatment. Toads from xeric populations (Wave Hill and Crape Crawford) had larger increases in osmolality as a result of dehydration than did toads from mesic populations (Middle Point and Cairns). Gray circles represent plasma osmolality of individual toads by treatment (F = field; H = experimentally hydrated; D = experimentally dehydrated). Black horizontal lines denote means and error bars denote standard deviations estimated from multimodel averaging

compared to MP (43 ± 21 mOsm kg^{-1}), and QLD (70 ± 19 mOsm kg^{-1}), respectively (Fig. 3). Although there was an expected relationship between size and plasma osmolality at each site during the dehydration treatment (Online Resource 5), likely reflecting a surface-area-to-volume ratio effect, there was no detectable influence of body size on osmolality in the field or after the hydration treatment. There was also no significant relationship when dehydration treatment data were pooled from all locations (Online Resource 6), further emphasizing the importance of location on plasma osmolality post-desiccation. Similarly, the difference in plasma osmolality of toads in the hydration versus dehydration treatments from CC (103 ± 20 mOsm kg^{-1}) were 47% and 139% greater than in toads from QLD and MP, respectively (Fig. 3). Plasma osmolality of toads in the field and toads after the hydration treatment was nearly identical for all locations.

Agglutination scores were higher for toads in the field than for toads after experimental hydration, which were higher in turn than were scores for toads after dehydration (except for the MP population where agglutination was relatively similar among the three groups; Fig. 4). Because agglutination scores varied within populations and within treatment groups, our results should be interpreted cautiously. Toads from all locations had decreased lysis scores after dehydration. However, lysis

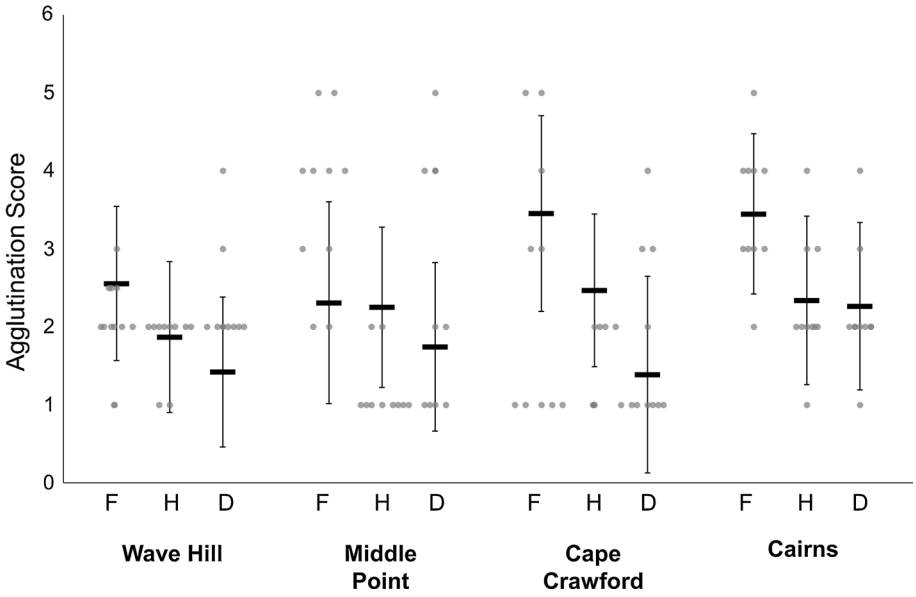

Fig. 4 Agglutination scores of cane toads showed a stepdown pattern, where toads in the field had higher scores than experimentally hydrated toads, and both had higher scores than experimentally dehydrated toads. Gray circles represent agglutination scores of individual toads in a treatment (F = field; H = experimentally hydrated; D = experimentally dehydrated). Black horizontal lines denote means and error bars denote standard deviations estimated from multimodel averaging

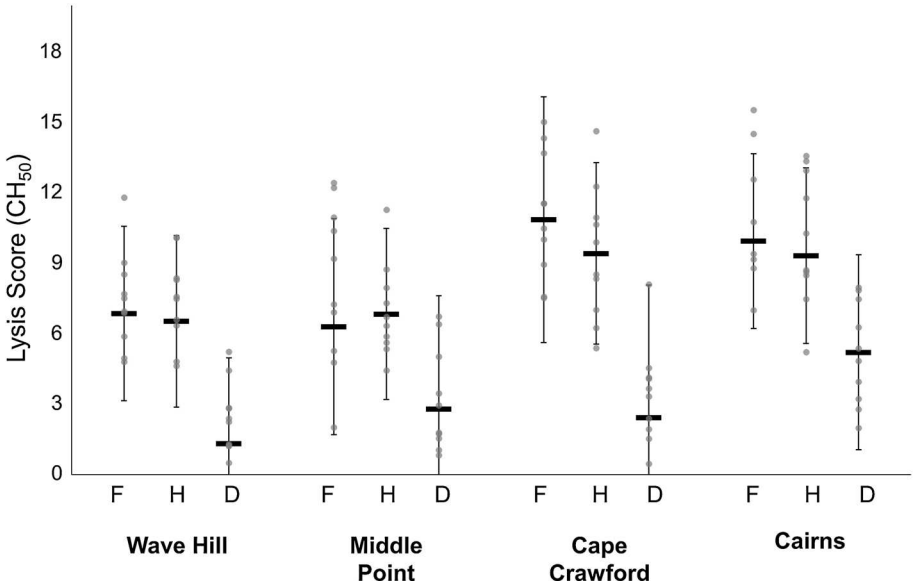

Fig. 5 Lysis scores (CH_{50}) of cane toads were similar for toads in the field and toads after the hydration treatment, but decreased after the dehydration treatment. Toads from xeric populations (Wave Hill and Crape Crawford) had larger decreases after the dehydration treatment compared to toads from mesic populations (Middle Point and Cairns), and toads closer to the invasion origin (Cairns and Cape Crawford) had higher scores, in general, than those closer to the invasion front (Middle Point and Wave Hill). Gray circles represent lysis scores of individual toads in a treatment (F = field; H = experimentally hydrated; D = experimentally dehydrated). Black horizontal lines denote means and error bars denote standard deviations estimated from multimodel averaging

scores decreased, on average, 25% more in toads from WH (5.2 ± 3.6 CH_{50}) and 75% more in toads from CC (7.0 ± 3.8 CH_{50}) compared to toads from MP (4.0 ± 3.4 CH_{50}) and QLD (4.1 ± 3.7 CH_{50}) (Fig. 5). In contrast, bactericidal ability showed little change among treatment groups. There was a clear effect of location, although unlike the pattern in osmolality and lysis values, bactericidal ability averaged 125% and 150% higher in the QLD (39.5 ± 10.5% bacteria killed) populations compared to the MP (17.5 ± 10.3% killed) and WH (14.6 ± 10.4% killed) populations, respectively. Similarly, toads from CC (36.7 ± 10.3% killed) had a 110% and 151% higher average bactericidal ability compared to MP and WH, respectively (Fig. 6).

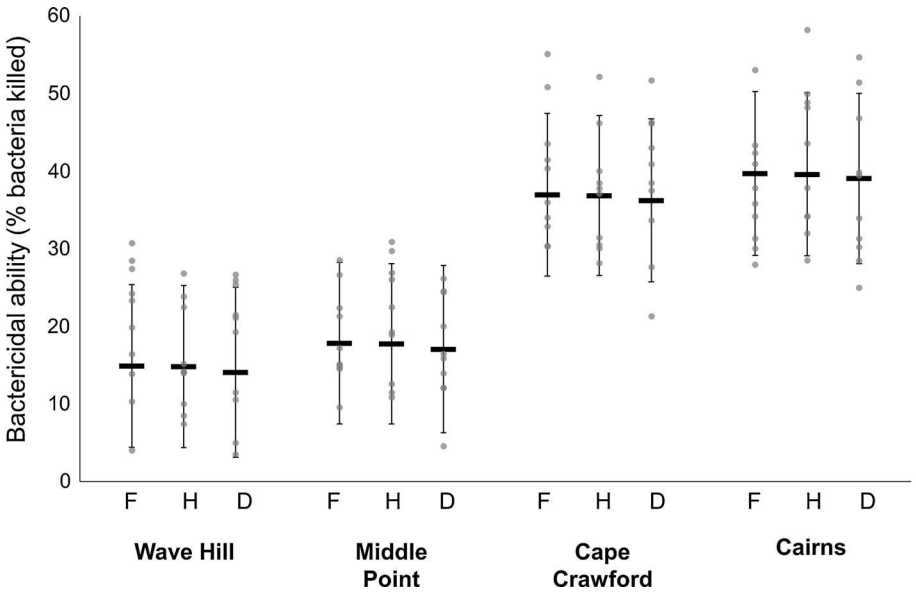

Fig. 6 Bactericidal ability (% bacteria killed) of cane toads were similar for toads in the field, after hydration, and after dehydration. Toads closer to the invasion origin (Cairns and Cape Crawford) had higher scores than those closer to the invasion front (Middle Point and Wave Hill). Gray circles represent bactericidal ability values of individual toads in a treatment (F = field; H = experimentally hydrated; D = experimentally dehydrated). Black horizontal lines denote means and error bars denote standard deviations estimated from multimodel averaging

Discussion

Cane toads from seasonally xeric populations had larger increases in plasma osmolality after 5 days without water, which, coupled with larger decreases in mass (see Figs. 2, 3), suggests that toads from these populations are more prone to evaporative water loss (EWL). This pattern runs counter to the typical situation whereby animals adapted to living in xeric environments have reduced rates of EWL (reptiles—Dmi'el 2001; Cox and Cox 2015; bats—Muñoz-Garcia et al. 2016), thereby conserving water in drier climates. However, our results are consistent with a previous study (Tingley et al. 2012) that found the opposite to be true in cane toads; animals from xeric environments had increased desiccation rates due to higher EWL compared to toads from mesic environments. This counter-intuitive result may reflect a reliance on access to standing water by toads from xeric populations, which places a selective premium on the ability to rapidly absorb water if it becomes available (Tingley et al. 2012). Differences in resistance to EWL between mesic and xeric populations have also been documented in other neotropical toads (Prates and Navas 2009; Prates et al. 2013; Anderson et al. 2017), suggesting that dehydration rate is a relatively plastic trait.

Plasma osmolality was similar for toads from the wild (regardless of location) and after experimental hydration. Thus, despite drastically different temperature and rainfall patterns among the sites, cane toads in the wild tend to remain hydrated. Although we avoided collecting toads that had recently emerged from water or wet substrate, collecting animals at the beginning of their nocturnally active period may have inadvertently sampled animals when they were at an optimal hydric state, after emerging from moist refugia (Schwarzkopf and Alford 1996). However, soil moisture levels during the Austral dry season would presumably be low enough to garner no net hydric benefit to the cane toads (Seebacher and Alford 1999). In arid parts of Australia, cane toads remain active when there is no rainfall, although they limit their activity to areas with permanent water sources (Brown et al. 2011). While toads in their native habitat come out only at night to forage and rehydrate, invasive toads in arid regions come out throughout the day to soak up water, and go back underground until coming out to forage at night (Webb et al. 2014). Behavioral flexibility to remain hydrated might explain our results, and the existence of toads in xeric environments likely relies on them finding sporadically located water resources.

Previous research has shown a positive relationship between dehydration and immune performance in a variety of taxa (snakes—Brusch and DeNardo 2017; flies—Hoang 2007; lizards—Moeller et al. 2013). In contrast to those results, toads from all four of our populations had decreased lytic abilities when dehydrated. Similar to the greater lab-based water loss rates observed in toads from xeric habitats, dehydration caused a greater inhibition of lysis (i.e., hydrated lysis score – dehydrated score) in toads from xeric populations. These two results surprisingly suggest that toads from more xeric environments are physiologically more vulnerable to water loss. This possibility is consistent with previous work that has shown that amphibians are more susceptible to disease during droughts (Kiesecker and Skelly 2001; Adams et al. 2017) and parasitic infections are highest in xeric habitats, especially during dry seasons (Pizzatto et al. 2013; Laverty et al. 2017).

Bactericidal ability was not sensitive to hydration treatment (i.e., at each of the four sites, hydrated and dehydrated toads had similar killing ability). However, bacterial killing was greater in the two eastern populations compared to the western populations. These bactericidal results are in contrast with previous research that found toads from the western edge of their expansion had higher bactericidal ability compared to toads from the range core in Queensland (Brown et al. 2015a, b); however, these previous results were from captive-raised toads which had not been exposed to ecologically relevant influences on immune performance in cane toads such as pathogen pressure (Brown et al. 2015a, b) and trade-offs with activity (Brown and Shine 2014). For example, higher levels of activity among wild cane toads (typical of invasive populations) are associated with reduced ability to kill *E. coli* (Brown and Shine 2014). The inter-populational pattern in our bacterial killing results is also seen with the lysis scores of hydrated toads (whether naturally or experimentally hydrated) and agglutination of field-sampled toads (Figs. 3, 4). Additionally, decreased immune performance correlates with higher disease prevalence in toads at the xeric expansion front (Brown et al. 2007; Shilton et al. 2008), and similar immunosuppressive effects of range expansion have been documented in other invasive species (Silva-Rocha et al. 2015; Martin et al. 2017; Riddick et al. 2017). Stressful conditions or activities near the expanding range edge might increase levels of glucocorticoids (e.g., corticosterone) which could suppress immune performance in invasion front populations (Goetz et al. 2017).

Reduced immunocompetence in individuals at the invasion front may reflect a tradeoff with morphological changes that enhance dispersal ability (Phillips et al. 2006; Hudson et al. 2016). Individuals along an invasion front disperse the greatest distances (Alford et al. 2009) and this might reduce immune capacities due to physically demanding movement (Freidenreich and Volek 2013; Brown and Shine 2014). Alternately, individuals at the invasion front may face fewer potential pathogens (Perkins et al. 2013), dampening immune responses (Devalapalli et al. 2006), a phenomenon that can be rapidly reversed within an individual's lifetime (Montecino-Rodriguez et al. 2013). Further work is needed

to better understand the relationship between dispersal and immunocompetence.

The ability of invaders to adjust phenotypic characteristics via plasticity has been well studied (Wright et al. 2010; Hendry 2015; Peneaux et al. 2017) as have adaptive shifts by invasive species (Myles-Gonzalez et al. 2015; Gruber et al. 2017). However, these mechanisms are not mutually exclusive (Mery and Burns, 2010; Rollins et al. 2015), and synergistic changes have been suggested in other invasive species (Colautti and Lau 2015; Kilvitis et al. 2017; Reisinger et al. 2017). While our experimental design cannot distinguish whether our results are a result of plasticity or adaptation, there are some indications that both are involved. This is consistent with previous studies that suggest physiological adjustments by individuals (i.e., phenotypic plasticity; McCann et al. 2018) and rapid evolutionary changes (Brown et al. 2014, 2015a, b) are likely both involved in the spread of the cane toad. Invasiveness appears to be based on a complex interaction of ecology, evolution, and physiology, and future work should incorporate a broad range of organismal traits when trying to decipher the invasion potential of a species.

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Author contribution statement GABIV, KC, GPB, RS and DD designed the study. GABIV and KC conducted the field work. GABIV conducted all assays, performed the statistical analyses, and led the writing of the manuscript. DD, KC, GPB, and RS contributed to revisions and gave final approval for publication.

Compliance with ethical standards

Data accessibility The datasets supporting this article can be accessed at https://doi.org/10.6084/m9.figshare.6431108.

Conflict of interest The authors declare no conflicts of interest.

References

Adams AJ, Kupferberg SJ, Wilber MQ, Pessier AP, Grefsrud M, Bobzien S, Vredenburg VT, Briggs CJ (2017) Extreme drought, host density, sex, and bullfrogs influence fungal pathogen infection in a declining lotic amphibian. Ecosphere 8:e01740. https://doi.org/10.1002/ecs2.1740

Alford RA, Brown GP, Schwarzkopf L, Phillips BL, Shine R (2009) Comparisons through time and space suggest rapid evolution of dispersal behaviour in an invasive species. Wildl Res 36:23. https://doi.org/10.1071/WR08021

Anderson RCO, Bovo RP, Eismann CE, Menegario AA, Andrade DV (2017) Not good, but not all bad: dehydration effects on body fluids, organ masses, and water flux through the skin of *Rhinella schneideri* (Amphibia, Bufonidae). Physiol Biochem Zool 90:313–320. https://doi.org/10.1086/690189

Bartoń K (2015) MuMIn: multi-model inference, R package version 1.15.1. https://CRAN.R-project.org/package=MuMIn. Accessed 1 May 2018

Brown G, Shine R (2014) Immune response varies with rate of dispersal in invasive cane toads (*Rhinella marina*). PLoS One 9:e99734. https://doi.org/10.1371/journal.pone.0099734

Brown GP, Shilton C, Phillips BL, Shine R (2007) Invasion, stress, and spinal arthritis in cane toads. Proc Natl Acad Sci 104:17698–17700. https://doi.org/10.1073/pnas.0705057104

Brown GP, Kelehear C, Shine R (2011) Effects of seasonal aridity on the ecology and behaviour of invasive cane toads in the Australian wet-dry tropics. Funct Ecol 25:1339–1347. https://doi.org/10.1111/j.1365-2435.2011.01888.x

Brown G, Phillis BL, Shine R (2014) The straight and narrow path: the evolution of straight-line dispersal at a cane toad invasion front. Proc R Soc Lond B Biol Sci 281:20141385. https://doi.org/10.1098/rspb.2014.1385

Brown GP, Kelehear C, Shilton CM, Phillips BL, Shine R (2015a) Stress and immunity at the invasion front: a comparison across cane toad (*Rhinella marina*) populations. Biol J Linn Soc 116:748–760. https://doi.org/10.1111/bij.12623

Brown G, Phillips B, Dubey S, Shine R (2015b) Invader immunology: invasion history alters immune system function in cane toads (*Rhinella marina*) in tropical Australia. Ecol Lett 18:57–65. https://doi.org/10.1111/ele.12390

Brusch GA, DeNardo DF (2017) When less means more: dehydration improves innate immunity in rattlesnakes. J Exp Biol 220:2287–2295. https://doi.org/10.1242/jeb.155028

Brusch GA, Billy G, Blattman JN, DeNardo DF (2017) Reproduction alters hydration state but does not impact the positive effects of dehydration on innate immune function in Children's pythons (*Antaresia childreni*). Physiol Biochem Zool 90:646–654. https://doi.org/10.1086/694834

Burnham KP, Anderson DR (2002) Model selection and multimodel inference a practical information-theoretic approach, 1st edn. Springer Science & Business Media, New York

Chen G, Robert J (2011) Antiviral immunity in amphibians. Viruses 3:2065–2086. https://doi.org/10.3390/v3112065

Child T, Phillips BL, Shine R (2009) Does desiccation risk drive the distribution of juvenile cane toads (*Bufo marinus*) in tropical Australia? J Trop Ecol 25:193–200. https://doi.org/10.1017/S0266467408005695

Colautti RI, Lau JA (2015) Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. Mol Ecol 24:1999–2017. https://doi.org/10.1111/mec.13162

Cox CL, Cox RM (2015) Evolutionary shifts in habitat aridity predict evaporative water loss across squamate reptiles. Evolution 69:2507–2516. https://doi.org/10.1111/evo.12742

Davis JR, DeNardo DF (2009) Water supplementation affects the behavioral and physiological ecology of Gila monsters (*Heloderma suspectum*) in the Sonoran Desert. Physiol Biochem Zool 82:39–748. https://doi.org/10.1086/605933

de Mendiburu F (2017) Agricolae: statistical procedures for agricultural research, R Package Version, 1.2. https://CRAN.R-project.org/package=agricolae. Accessed 1 May 2018

Devalapalli AP, Lesher A, Shieh K, Solow JS, Everett ML, Edala AS, Whitt P, Long RR, Newton N, Parker W (2006) Increased levels of IgE and autoreactive, polyreactive IgG in wild rodents: implications for the hygiene hypothesis. Scand J Immunol 64:125–136. https://doi.org/10.1111/j.1365-3083.2006.01785.x

Dick JTA, Laverty C, Lennon JJ, Barrios-O'Neill D, Mensink PJ, Britton RJ, Medoc V, Boets P, Alexander ME, Taylor NG, Dunn AM, Hatcher MJ, Rosewarne PJ, Crookes S, Maclsaac HJ, Xu M, Ricciardi A, Wasserman RJ, Ellender BR, Weyl OLF, Lucy FE, Banks PB, Dodd JA, MacNeil C, Penk MR, Aldridge DC, Caffrey JM (2017) Invader relative impact potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. J Appl Ecol 54:1259–1267. https://doi.org/10.1111/1365-2664.12849

Dmi'el R (2001) Skin resistance to evaporative water loss in reptiles: a physiological adaptive mechanism to environmental stress or a phyletically dictated trait? Isr J Zool 47:55–67. https://doi.org/10.1560/ENQ9-KD7R-WFGW-KUQW

Donohue I, Petchey OL, Montoya JM, Jackson AL, Mcnally L, Viana M, Healy K, Lurgi M, O'Connor NE, Emmerson MC (2013) On the dimensionality of ecological stability. Ecol Lett 16:421–429. https://doi.org/10.1111/ele.12086

Freidenreich DJ, Volek JS (2013) The immune response to exercise: effects on cellular mobilization, immune function and muscle regeneration. In: Bagchi D, Nair S, Sen CK (eds) Nutrition and enhanced sports performance: muscle building, endurance, and strength. Academic Press, Cambridge, pp 95–101

French SS, Neuman-Lee LA (2012) Improved ex vivo method for microbiocidal activity across vertebrate species. Biol Open 1:482–487. https://doi.org/10.1242/bio.2012919

Goetz SM, Romagosa CM, Appel AG, Guyer C, Mendonça MT (2017) Reduced innate immunity of Cuban tree frogs at leading edge of range expansion. J Exp Zool A Ecol Integr Physiol 327:592–599. https://doi.org/10.1002/jez.2146

González-Bernal E, Greenlees M, Brown GP, Shine R (2012) Cane toads on cowpats: commercial livestock production facilitates toad invasion in tropical Australia. PLoS One 7:e49351. https://doi.org/10.1371/journal.pone.0049351

Gruber J, BrownG Whiting MJ, Shine R (2017) Is the behavioural divergence between range-core and range-edge populations of cane toads (*Rhinella marina*) due to evolutionary change or developmental plasticity? R Soc Open Sci 4:170789. https://doi.org/10.1098/rsos.170789

Hendry AP (2015) Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. J Hered 107:25–41. https://doi.org/10.1093/jhered/esv060

Hillman SS (1980) Physiological correlates of differential dehydration tolerance in anuran amphibians. Copeia 1980:125–129

Hillyard SD, Hoff KS, Propper C (1998) The water absorption response: a behavioral assay for physiological processes in terrestrial amphibians. Physiol Zool 71:127–138. https://doi.org/10.1086/515900

Hoang A (2007) Immune response to parasitism reduces resistance of *Drosophila melanogaster* to desiccation and starvation. Evolution 55:2353–2358. https://doi.org/10.1111/j.0014-3820.2001.tb00748.x

Hudson CM, McCurry MR, Lundgren P, McHenry CR, Shine R (2016) Constructing an invasion machine: the rapid evolution of a dispersal-enhancing phenotype during the cane toad invasion of Australia. PLoS One 11:e0156950. https://doi.org/10.1371/journal.pone.0156950

Kearney M, Phillips BL, Tracy CR, Christian KA, Betts G, Porter WP (2008) Modelling species distributions without using species distributions: the cane toad in Australia under current and future climates. Ecography 31:423–434. https://doi.org/10.1111/j.2008.0906-7590-05457.x

Kiesecker JM, Skelly DK (2001) Effects of disease and pond drying on gray tree frog growth, development, and survival. Ecology 82:1956–1963. https://doi.org/10.1890/0012-9658(2001)082%5b1956:EODAPD%5d2.0.CO;2

Kilvitis HJ, Hanson H, Schrey AW, Martin LB (2017) Epigenetic potential as a mechanism of phenotypic plasticity in vertebrate range expansions. Integr Comp Biol 57:385–395. https://doi.org/10.1093/icb/icx082

Kosmala GK, Brown GP, Christian KA, Hudson CM, Shine R (2018) The thermal dependency of locomotor performance evolves rapidly within an invasive species. Ecol Evol 8:4403–4408. https://doi.org/10.1002/ece3.3996

Laverty C, Brenner D, McIlwaine C, Lennon JJ, Dick JTA, Lucy FE, Christian KA (2017) Temperature rise and parasitic infection interact to increase the impact of an invasive species. Int J Parasitol 47:291–296. https://doi.org/10.1016/j.ijpara.2016.12.004

Lever C (2001) The cane toad: the history and ecology of a successful colonist, 1st edn. Westbury Academic & Scientific Publishing, Otley

Lindstrom T, Brown GP, Sisson SA, Phillips BL, Shine R (2013) Rapid shifts in dispersal behavior on an expanding range edge. Proc Natl Acad Sci 110:13452–13456. https://doi.org/10.1073/pnas.1303157110

Llewellyn D, Brown GP, Thompson MB, Shine R (2011) Behavioral responses to immune-system activation in an anuran (the cane toad, *Bufo marinus*): field and laboratory studies. Physiol Biochem Zool 84:77–86. https://doi.org/10.1086/657609

Martin LB, Kilvitis HJ, Brace AJ, Cooper L, Haussmann MF, Mutati A, Fasanello V, O'Brien S, Ardia DR (2017) Costs of immunity and their role in the range expansion of the house sparrow in Kenya. J Exp Biol 220:2228–2235. https://doi.org/10.1242/jeb.154716

Matson KD, Ricklefs RE, Klasing KC (2005) A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. Dev Comp Immunol 29:275–286. https://doi.org/10.1016/j.dci.2004.07.006

McCann S, Greenlees MJ, Newell D, Shine R (2014) Rapid acclimation to cold allows the cane toad to invade montane areas within its Australian range. Funct Ecol 28:1166–1174. https://doi.org/10.1111/1365-2435.12255

McCann SM, Kosmala GK, Greenlees MJ, Shine R (2018) Physiological plasticity in a successful invader: rapid acclimation to cold occurs only in cool-climate populations of cane toads (*Rhinella marina*). Cons Physiol 6:cox072. https://doi.org/10.1093/conphys/cox072

Mery F, Burns JG (2010) Behavioural plasticity: an interaction between evolution and experience. Evol Ecol 24:571–583. https://doi.org/10.1007/s10682-009-9336-y

Moeller KT, Butler MW, DeNardo DF (2013) The effect of hydration state and energy balance on innate immunity of a desert reptile. Front Zool 10:23. https://doi.org/10.1186/1742-9994-10-23

Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K (2013) Causes, consequences, and reversal of immune system aging. J Clin Investig 123:958–965. https://doi.org/10.1172/JCI64096

Muñoz-Garcia A, Larraín P, Ben-Hamo M, Cruz-Neto A, Williams JB, Pinshow B, Korine C (2016) Metabolic rate, evaporative water loss and thermoregulatory state in four species of bats in the Negev desert. Comp Biochem Physiol A Mol Integr Physiol 191:156–165. https://doi.org/10.1016/j.cbpa.2015.10.010

Myles-Gonzalez E, Burness G, Yavno S, Rooke A, Fox MG (2015) To boldly go where no goby has gone before: boldness, dispersal tendency, and metabolism at the invasion front. Behav Ecol 26:1083–1090. https://doi.org/10.1093/beheco/arv050

Peneaux C, Machovsky-Capuska GE, Raubenheimer D, Lermite F, Rousseau C, Ruhan T, Rodger JC, Griffin AS (2017) Tasting novel foods and selecting nutrient content in a highly successful

ecological invader, the common myna. J Avian Biol 48:1432–1440. https://doi.org/10.1111/jav.01456
Perkins TA, Phillips BL, Baskett ML, Hastings A (2013) Evolution of dispersal and life history interact to drive accelerating spread of an invasive species. Ecol Lett 16:1079–1087. https://doi.org/10.1111/ele.12136
Phillips BL, Brown GP, Webb JK, Shine R (2006) Invasion and the evolution of speed in toads. Nature 439:803. https://doi.org/10.1038/439803a
Phillips BL, Brown GP, Greenlees M, Webb JK, Shine R (2007) Rapid expansion of the cane toad (*Bufo marinus*) invasion front in tropical Australia. Austral Ecol 32:169–176. https://doi.org/10.1111/j.1442-9993.2007.01664.x
Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2018) nlme: linear and nonlinear mixed effects models. R package version 3.1-137. https://CRAN.R-project.org/package=nlme
Pizzatto L, Kelehear C, Shine R (2013) Seasonal dynamics of the lungworm, *Rhabdias pseudosphaerocephala*, in recently colonised cane toad (*Rhinella marina*) populations in tropical Australia. Int J Parasitol 43:753–761. https://doi.org/10.1016/j.ijpara.2013.05.002
Pizzatto L, Both C, Shine R (2014) Quantifying anuran microhabitat use to infer the potential for parasite transmission between invasive cane toads and two species of Australian native frogs. PLoS One 9:e106996. https://doi.org/10.1371/journal.pone.0106996
Prates I, Navas CA (2009) Cutaneous resistance to evaporative water loss in Brazilian *Rhinella* (Anura: Bufonidae) from contrasting environments. Copeia 2009:618–622. https://doi.org/10.1643/CP-08-128
Prates I, Angilleta MJ, Wilson RS, Niehaus AC, Navas CA (2013) Dehydration hardly slows hopping toads (*Rhinella granulosa*) from xeric and mesic environments. Physiol Biochem Zool 86:451–457. https://doi.org/10.1086/671191
Ramsay DJ, Thrasher TN (1984) The defence of plasma osmolality. J Physiol (Paris) 79:416–420
Reisinger LS, Elgin AK, Towle KM, Chan DJ, Lodge DM (2017) The influence of evolution and plasticity on the behavior of an invasive crayfish. Biol Invasions 19:815–830. https://doi.org/10.1007/s10530-016-1346-4
Reynolds SJ, Christian KA (2009) Environmental moisture availability and body fluid osmolality in introduced toads, *Rhinella marina*, in monsoonal northern Australia. J Herpetol 43:326–331. https://doi.org/10.1670/08-062R2.1
Rollins LA, Richardson MF, Shine R (2015) A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). Mol Ecol 24:2264–2276. https://doi.org/10.1111/mec.13184
Schwarzkopf L, Alford RA (1996) Desiccation and shelter-site use in a tropical amphibian: comparing toads with physical models. Funct Ecol 10:193–200. https://doi.org/10.2307/2389843
Seebacher F, Alford RA (1999) Movement and microhabitat use of a terrestrial amphibian (*Bufo marinus*) on a tropical island: seasonal variation and environmental correlates. J Herpetol 33:208–214. https://doi.org/10.2307/1565716
Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009) Evolution and ecology of species range limits. Annu Rev Ecol Evol Syst 40:415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317
Shilton CM, Brown GP, Benedict S, Shine R (2008) Spinal arthropathy associated with *Ochrobactrum anthropi* in free-ranging cane toads (*Chaunus* [*Bufo*] *marinus*) in Australia. Vet Pathol 45:85–94. https://doi.org/10.1354/vp.45-1-85
Shine R (2010) The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. Q Rev Biol 85:253–291. https://doi.org/10.1086/655116
Shine R (2012) Invasive species as drivers of evolutionary change: cane toads in tropical Australia. Evol Appl 5:107–116. https://doi.org/10.1111/j.1752-4571.2011.00201.x
Shine R, Brown GP (2008) Adapting to the unpredictable: reproductive biology of vertebrates in the Australian wet-dry tropics. Philos Trans R Soc B Biol Sci 363:363–373. https://doi.org/10.1098/rstb.2007.2144
Silva-Rocha I, Salvi D, Sillero N, Mateo JA, Carretero MA (2015) Snakes on the balearic islands: an invasion tale with implications for native biodiversity conservation. PLoS One 10:e0121026. https://doi.org/10.1371/journal.pone.0121026
Simberloff D, Martin JL, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, Garcia-Berthou E, Pascal M, Pysek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's what and the way forward. Trends Ecol Evol 28:58–66. https://doi.org/10.1016/j.tree.2012.07.013
Stockham S, Scott M (2013) Fundamentals of veterinary clinical pathology, 1st edn. Wiley, Hoboken
Sutherst RW, Floyd RB, Maywald GF (1996) The potential geographical distribution of the cane toad, *Bufo marinus* L. in Australia. Cons Biol 1:294–299. https://doi.org/10.1046/j.1523-1739.1996.10010294.x
Tingley R, Shine R (2011) Desiccation risk drives the spatial ecology of an invasive anuran (*Rhinella marina*) in the Australian semi-desert. PLoS One 6:e25979. https://doi.org/10.1371/journal.pone.0025979
Tingley R, Greenlees MJ, Shine R (2012) Hydric balance and locomotor performance of an anuran (*Rhinella marina*) invading the Australian arid zone. Oikos 121:1959–1965. https://doi.org/10.1111/j.1600-0706.2012.20422.x
Tingley R, Vallinoto M, Sequeira F, Kearney MR (2014) Realized niche shift during a global biological invasion. Proc Natl Acad Sci 111:10233–10238. https://doi.org/10.1073/pnas.1405766111
Urban MC, Phillips BL, Skelly DK, Shine R (2007) The cane toad's (*Chaunus* [*Bufo*] *marinus*) increasing ability to invade Australia is revealed by a dynamically updated range model. Proc R Soc B Biol Sci 274:1413–1419. https://doi.org/10.1098/rspb.2007.0114
Urban MC, Phillips BL, Skelly DK, Shine R (2008) A toad more traveled: the heterogeneous invasion dynamics of cane toads in Australia. Am Nat 171:E134–E148. https://doi.org/10.1086/527494
Warfe DM, Pettit NE, Davies PM, Pusey BJ, Hamilton SK, Kennard MJ, Townsend SA, Bayliss P, Ward DP, Douglas MM, Burford MA, Finn M, Bunn SE, Halliday IA (2011) The "wet-dry" in the wet-dry tropics drives river ecosystem structure and processes in northern Australia. Freshw Biol 56:2169–2195. https://doi.org/10.1111/j.1365-2427.2011.02660.x
Webb JK, Letnic M, Jessop TS, Dempster T (2014) Behavioural flexibility allows an invasive vertebrate to survive in a semi-arid environment. Biol Lett 10:20131014. https://doi.org/10.1098/rsbl.2013.1014
Wright TF, Eberhard JR, Hobson EA, Avery ML, Russello MA (2010) Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. Ethol Ecol Evol 22:393–404. https://doi.org/10.1080/03949370.2010.505580
Young JE, Christian KA, Donnellan S, Tracy CR, Parry D (2005) Comparative analysis of cutaneous evaporative water loss in frogs demonstrates correlation with ecological habits. Physiol Biochem Zool 78:847–856. https://doi.org/10.1086/432152
Zug GR, Zug PB (1979) The marine toad, *Bufo marinus*: a natural history resumé of native populations. Smithson Contrib Zool 1979:1–58. https://doi.org/10.5479/si.00810282.284