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

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Title: Invasion of cane toads (*Rhinella marina*) into xeric Australian environments is a product of physiological adaptation and phenotypic plasticity.

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Abstract

1. The invasion of habitats with novel environmental challenges may require physiological tolerances not seen in conspecifics from the native range.

2. 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.

3. Toads from all locations were well-hydrated at the time of capture. However, experimental dehydration caused greater mass loss, plasma osmolality and inhibition of lytic ability in toads from xeric rather than mesic locations. These results suggest that toads from xeric environments are physiologically more vulnerable to water loss.

4. 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.

5. The ability of this invasive species to spread into drier environments is underpinned by a combination of phenotypic plasticity and evolved (heritable) traits.

Keywords

Bufo marinus, hydration, 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 (Dick et al., 2017; Donohue et al., 2013; Simberloff et al., 2013). 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 (Pizzatto et al., 2014; Shine, 2010). Nonetheless, there remain substantial gaps in our understanding of physiological mechanisms which have allowed toads to effectively spread from equable climates in Latin America into severely xeric areas of Australia (Kosmala et al., 2018; Tingley et al., 2014).

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 aseasonal 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 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 (Brown et al., 2014; Pizzatto et al., 2013; Shine, 2012; Tingley & Shine, 2011), generating substantial differences between individuals at the invasion front compared to individuals at the range core (Hudson et al., 2016; Lindstrom et al., 2013). Cane

53 toads at the invasion front have been shown to rapidly acclimatize to temperature regimes
54 previously thought to be too cold (McCann et al., 2014; 2018). Those inhabiting drier regions of
55 Australia exhibit dramatic fluctuations in plasma osmolality (250-370 mOsm kg^{-1}, Reynolds &
56 Christian, 2009). Desiccation risk reduces dispersal of juvenile cane toads (Child et al., 2009)
57 but, despite presumed constraints due to lack of water, cane toads are physiologically
58 acclimating to invade semi-arid regions of Australia (Kosmala et al., 2018; Tingley et al., 2012;
59 Tingley & Shine, 2011). Indeed, the cane toad invasion is expanding more rapidly as it moves
60 through drier habitats (Phillips et al., 2006).
61 Exploring physiological traits that enable toads to expand their range into xeric areas may
62 help us to predict the extent of the species' eventual distribution. Although a few studies have
63 examined the role of immune function in the invasiveness of cane toads (Brown et al., 2015;
64 Brown & Shine, 2014; Llewellyn et al., 2011), the interaction between immune function and
65 hydric state has remained unstudied. Water is a fundamental, non-energetic resource that can
66 modulate immune function. Enhanced immune function in response to dehydration has been
67 documented in an invertebrate (Hoang, 2007) and multiple squamates (Brusch et al., 2017;
68 Brusch & DeNardo, 2017; Moeller et al., 2013).
69 Many animals maintain a plasma osmolality of approximately 300 mOsm kg^{-1} (Stockham
70 & Scott, 2013) even during periods when they do not drink (Ramsay & Thrasher, 1984).
71 Juxtaposed against these norms are terrestrial amphibians, which typically have a low resistance
72 to transcutaneous water loss (Young et al., 2005). As a result, many species in dry environments
73 are constantly at risk of desiccation due to the inability to balance water influx with water efflux
74 (Hillman, 1980). Accordingly, many terrestrial amphibians tolerate high osmolality values that
75 are indicative of dehydration (Reynolds & Christian, 2009; Zug & Zug, 1979). 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 ascertain whether inter-populational differences are best explained by rapid evolution or plasticity. We hypothesized that cane toads have rapidly evolved a 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.

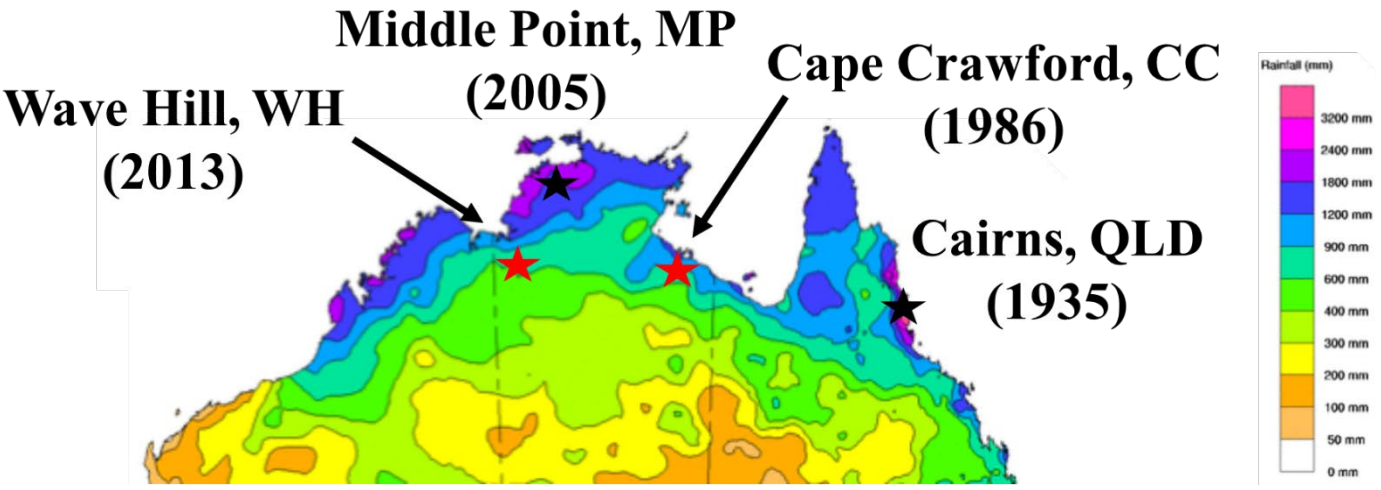


Figure 1: Four study locations across the historical invasion path (year) of cane toads in Australia that alternated between mesic (black star) and seasonally xeric (red star). Annual rainfall map for 2016 modified from the Australian Bureau of Meteorology (http://www.bom.gov.au/climate).

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).

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; Tingley et al., 2016; Urban et al., 2008). We collected toads during June and July of 2016 in the midst of the tropical 'dry-season' (May-October) when rainfall is scare or absent (Shine & Brown, 2008). Toads were collected from four locations: 1) Cairns, Queensland (QLD), the mesic environment (average rainfall & mean daily maxima during June-July = 38.9 mm / 26°C [Australian Bureau of Meteorology, http://www.bom.gov.au/climate]) where the species was first introduced to Australia. 2) Cape Crawford, Northern Territory (CC), an arid area (1.2 mm / 30°C) 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), 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 (0.5 mm / 32°C). 4) Wave Hill, Northern Territory (WH), the arid habitat (0.2 mm / 29°C) near the leading edge of the species' current range (Phillips et al., 2006). Over the last 20 years, cane toads have rapidly expanded

116 their range despite the aridity of the area (González-Bernal et al., 2012).

117

118 *Field-based Experiment*

119 To evaluate variation in plasma osmolality and innate immune function of cane toads across their
120 Australian range, blood samples (see details below) were collected from 10 adult toads at each of
121 the four sites during the dry season (June and July). Toads were captured by hand between 1800
122 and 2100 h when toads typically emerge from their daytime refugia to begin nocturnal activity.
123 For consistency, all toads were captured > 5 m from any visible water source and toads were not
124 selected if they had moist skin (suggesting they had just exited the water). Upon capture, we
125 determined mass, sex, and snout-vent length (SVL), and a blood sample was collected (see
126 details below). For all samples, plasma osmolality was determined using a vapor pressure
127 osmometer (±3 mOsm kg^{-1}; model 5100C; Wescor Inc., Logan, Utah, USA). Samples were run
128 in triplicate as described in Davis and DeNardo (2009). Additionally, a suite of plasma-based
129 immune function assays were performed on samples (see details below).

130

131 *Laboratory-based Experiment*

132 To determine whether there is a causal effect of osmolality on innate immunity, 20 toads from
133 each site (total = 80) were captured by hand and temporarily held in wet cloth bags. Mass, sex,
134 and snout-vent length were recorded before toads were housed individually in 30 x 20 x 20 cm
135 plastic containers filled with ~4 cm of water to enable them to hydrate overnight (Hillyard et al.,
136 1998). After 12-14 h, blood samples were collected from ten of the hydrated toads from each site
137 (total = 40). The remaining ten toads from each site (total = 40) were moved to individual plastic
138 containers without access to food or water and allowed to dehydrate for 120 h (5 days) to reach

139 an ecologically relevant level of dehydration based on previously published work (360 mOsm
140 kg^{-1}; Reynolds & Christian, 2009). Containers were held at ambient temperature with natural
141 light from rooftop windows. All toads were weighed every 24 h to evaluate rate of dehydration
142 as change in mass is a good proxy for water loss in amphibians because of their very low
143 metabolic rate. No toads showed clinical signs of dehydration (lethargy, slow righting reflex) or
144 lost more than 35% of their body mass. After the five-day dehydration period, toads were
145 weighed and a blood sample was collected for determining osmolality and performing immune
146 function assays.
147
148 *Blood Sample Collection*
149 Prior to blood sample collection, all toads were euthanized with an overdose
150 of sodium pentobarbital (50% Lethabarb diluted in water: Pizzatto et al., 2013). Blood samples
151 (0.8 mL) were collected via cardiocentesis using heparinized 1 mL syringes with a 25-gauge X
152 1.6 cm (5/8 in) needle. Total time for capture, restraint, and blood collection was typically less
153 than five minutes and did not exceed eight minutes in either lab or field procedures. Blood
154 samples were immediately centrifuged at 3000 rpm for three minutes to separate plasma from
155 blood cells. Plasma samples were aliquoted (~50 µL) into separate vials and frozen at -80°C until
156 they were used (within 21 days) to measure plasma osmolality and evaluate immune function.
157
158 *Immune Function Assays*
159 To examine immunocompetence, we performed several plasma-based innate immune function
160 assays. Although amphibians possess both innate and adaptive immunity (Chen & Robert, 2011),
161 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, Victoria, Australia) to quantify agglutination and lysis, which are standard measures of soluble constitutive immunity (Matson et al., 2005). Briefly, 20 µl of plasma were serially diluted with phosphate buffered saline (PBS) along a row of a 96-well plate, after which twenty µl of 1% SRBC were added to each well. Plates were incubated at 37ºC for 90 minutes and then placed at room temperature (~25°C) for 20 minutes after which point they were scanned at 600 dots per inch (Hewlett-Packard Co., ScanJet 3670) for agglutination images. After an additional 70 minutes, 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 & 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 hours of incubation at 37ºC. 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

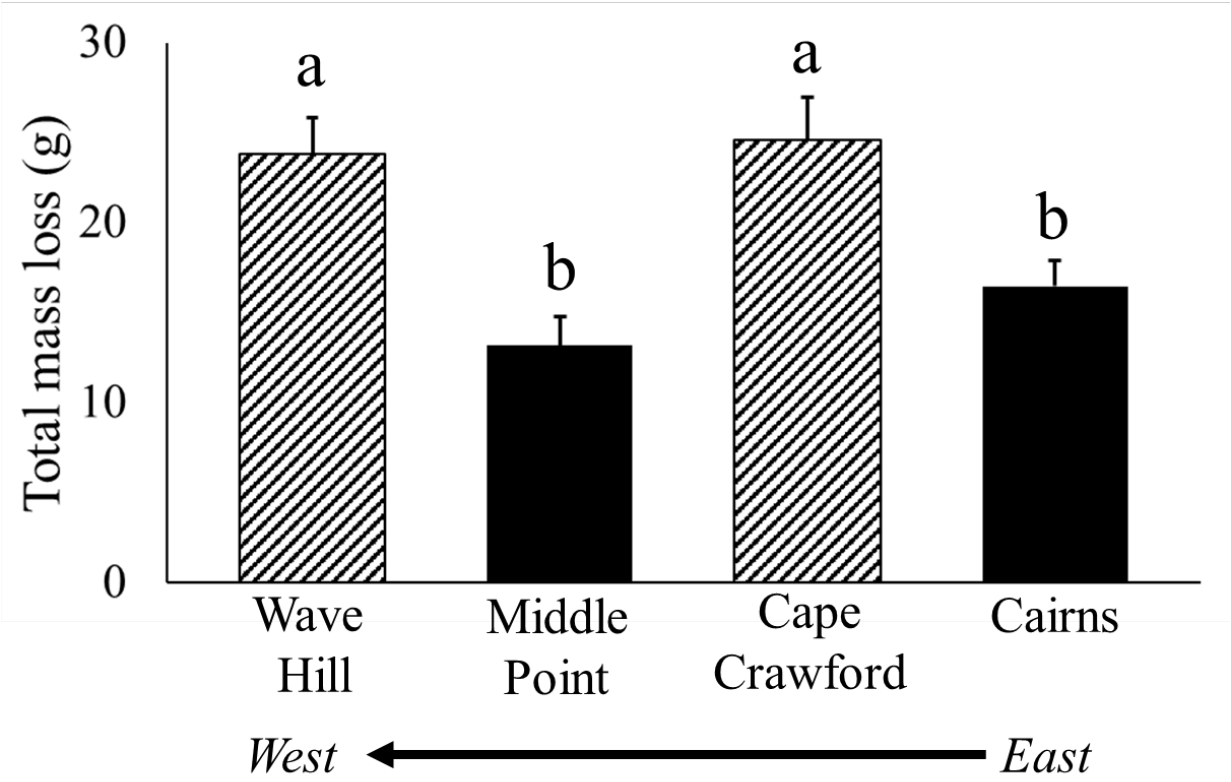
assays provided a detailed assessment of innate immune function that could be used to compare populations in terms of immunocompetence and the sensitivity of immune function to hydration state.

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 using size based 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, 2014) 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 & Anderson, 2002). Size, sex, treatment, and location were used as categorical fixed factors. Using the *nlme* (Pinheiro et al., 2016) and *MuMln* libraries (Barton, 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 Tables S1-S4) were calculated to determine the best model. Finally, we used multimodel averaging following Burnham and Anderson (2002) to calculate the weighted

208 average of each parameter using estimates from all models. These resulting values were used to
209 calculate the most likely mean and standard deviations for each parameter.
210
211 **Results**
212 Location had a significant effect on physiological responses to water deprivation ($F_{3,3} = 10.81$, P
213 = 0.04), whereby toads from WH and CC had the highest average water losses over the five-day
214 dehydration treatment (Fig. 2). Treatment and location had the greatest effects on plasma
215 osmolality, lysis, agglutination, and bactericidal ability. Although toads from all locations had
216 higher plasma osmolality after dehydration, the difference in osmolality between hydrated versus
217 dehydrated treatments on average were 88% and 16% greater in toads from WH (mean ± SD =
218 81 ± 20 mOsm kg^{-1}) compared to MP (43 ± 21 mOsm kg^{-1}), and QLD (70 ± 19 mOsm kg^{-1}),
219 respectively (Fig. 3). Although there was an expected relationship between size and plasma
220 osmolality at each site during the dehydration treatment (Fig. S1), likely reflecting a surface-
221 area-to-volume ratio effect, there was no detectable influence of body size on osmolality in the
222 field or after the hydration treatment. There was also no significant relationship when
223 dehydration treatment data were pooled from all locations (Fig. S2), further emphasizing the
224 importance of location on plasma osmolality post-desiccation. Similarly, the difference in plasma
225 osmolality of toads in the hydration versus dehydration treatments from CC (103 ± 20 mOsm kg^{-1})
226 were 47% and 139% greater than in toads from QLD and MP, respectively (Fig. 3). Plasma
227 osmolality of toads in the field and toads after the hydration treatment were nearly identical for
228 all locations.



229

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

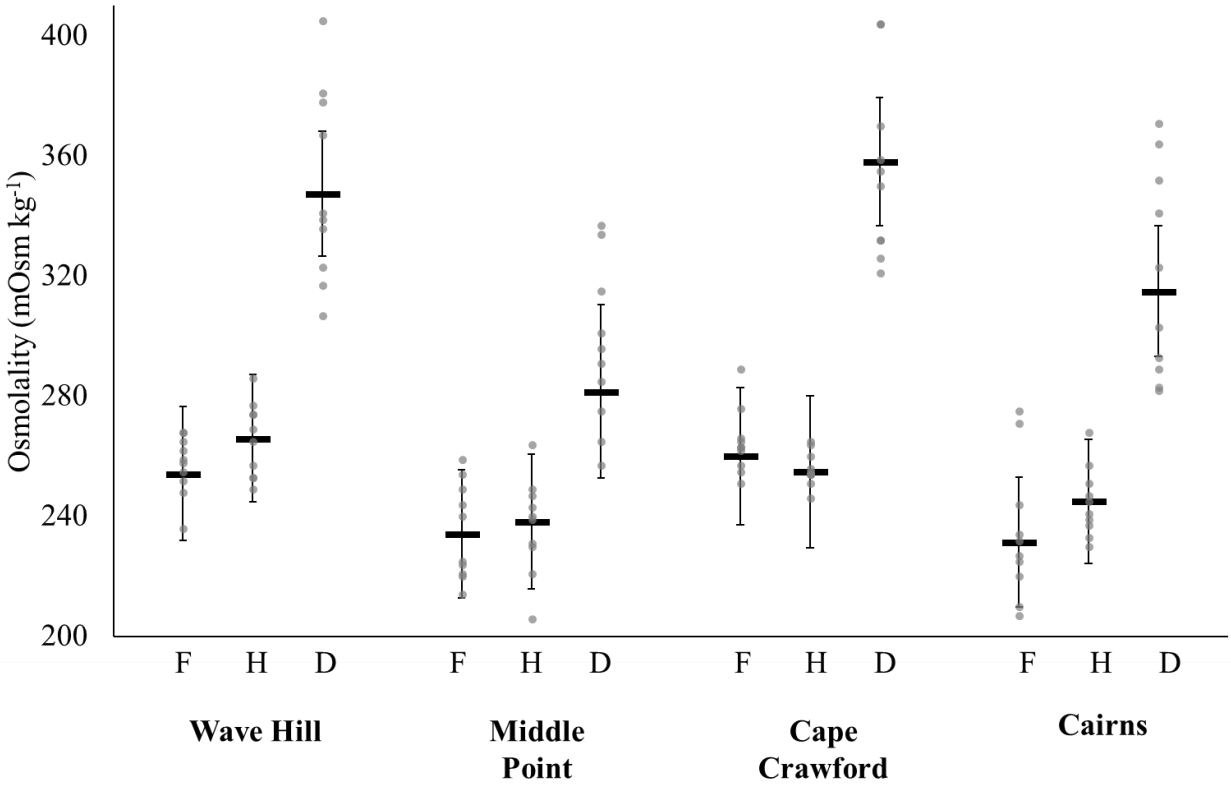


Figure 3: Plasma osmolality (mOsm kg^{-1}) of cane toads increased after the dehydration treatment, but was similar for toads in the field and toads after hydration treatment. Toads from xeric populations (Wave Hill and Crape Crawford) had larger increases in osmolality as a result of dehydration than did toads from mesic populations (Middle Point and Cairns). Grey 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.

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 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).

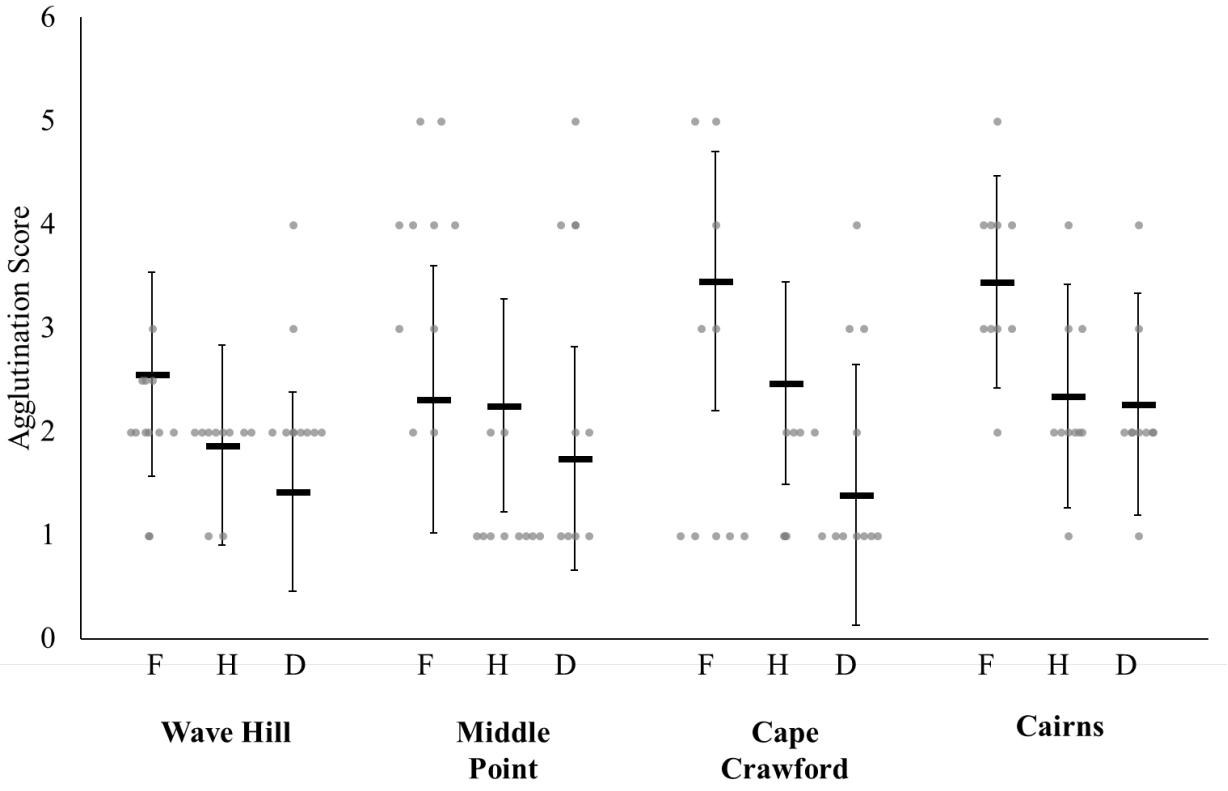


Figure 4: Agglutination scores of cane toads showed a stepdown pattern, where toads in the field had higher scores than experimentally hydrated toads, and both had higher scores than

experimentally dehydrated toads. Grey 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.

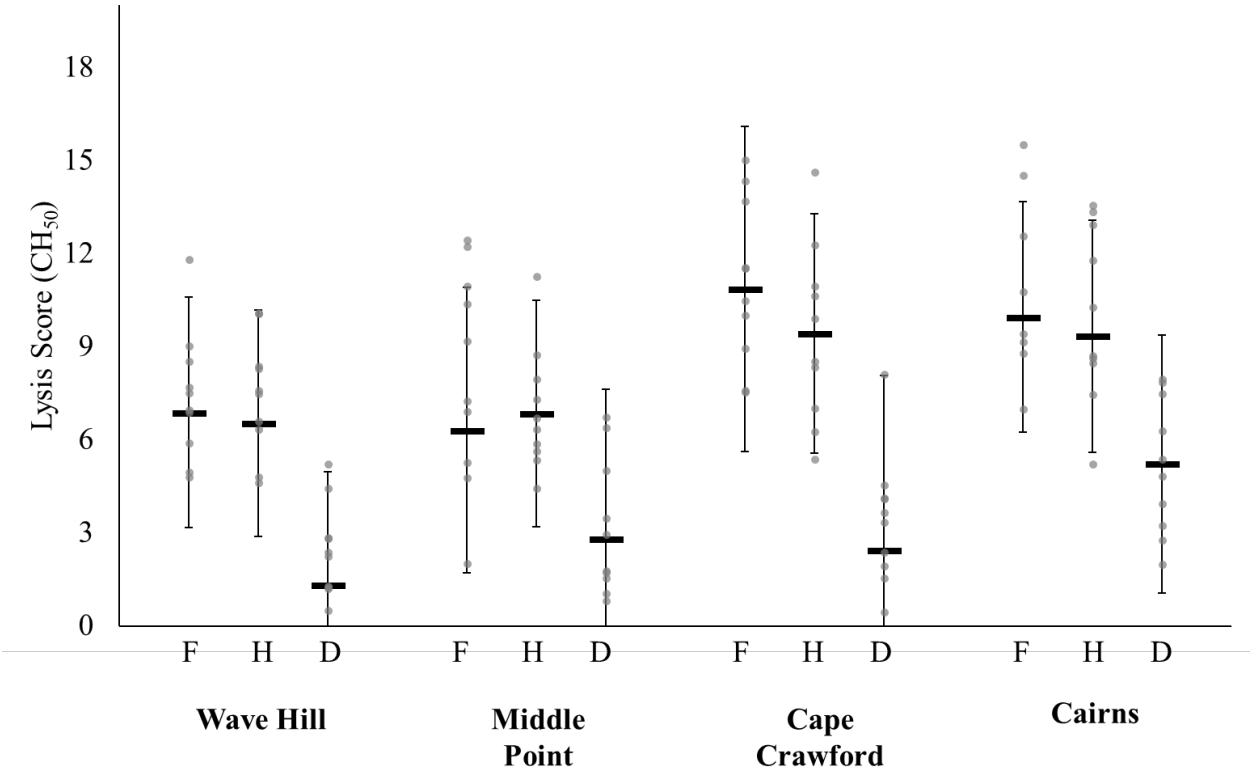


Figure 5: Lysis scores (CH_{50}) of cane toads were similar for toads in the field and toads after the hydration treatment, but decreased after the dehydration treatment. Toads from xeric populations (Wave Hill and Crape Crawford) had larger decreases after the dehydration treatment compared to toads 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). Grey 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.

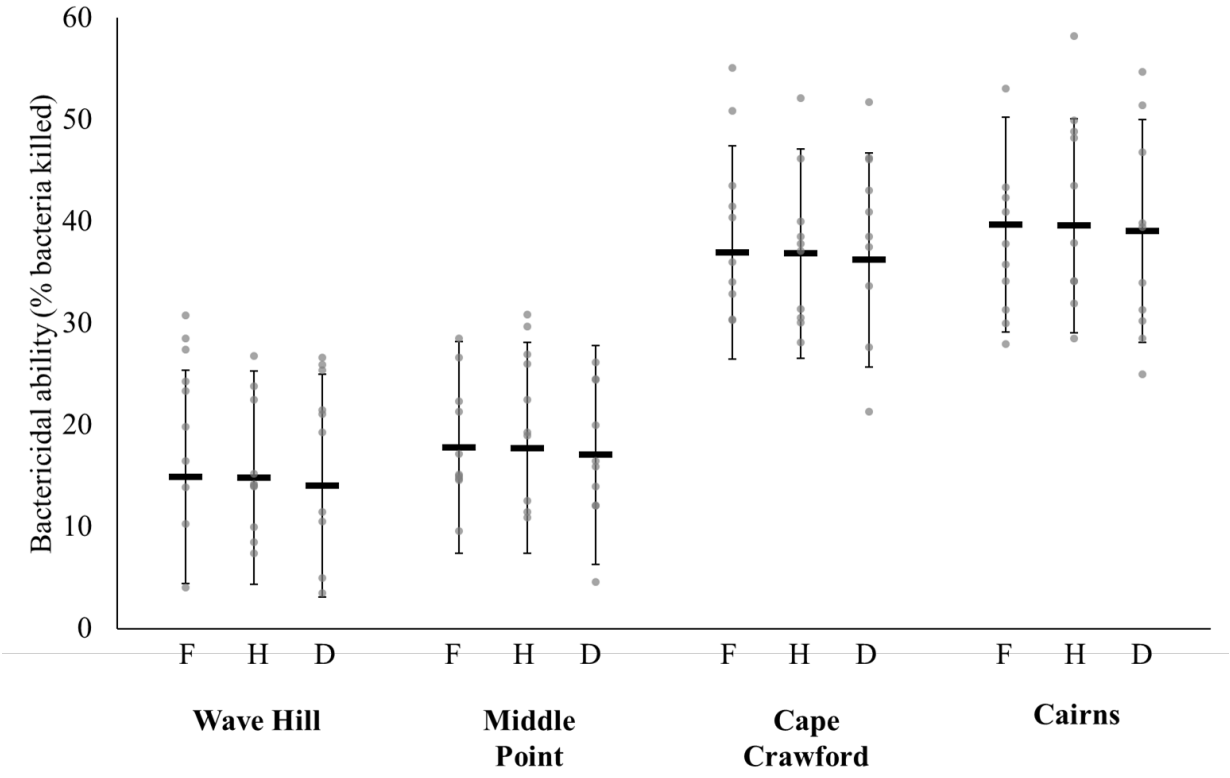


Figure 6: Bactericidal ability (% bacteria killed) of cane toads were similar for toads in the field, after hydration, and after dehydration. Toads closer to the invasion origin (Cairns and Cape Crawford) had higher scores than those closer to the invasion front (Middle Point and Wave Hill). Grey circles 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 five 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 - Cox & Cox, 2015; Dmi'el, 2001; 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 (Anderson et al., 2017; Prates & Navas, 2009; Prates et al., 2013), suggesting that dehydration tolerance 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 are staying 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 & 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 & 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). Moreover, toads can switch from nocturnal to diurnal activity to stay hydrated via the aforementioned permanent water sources

(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 & 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 (Adams et al., 2017; Kiesecker & Skelly, 2001) and parasitic infections are highest in xeric habitats, especially during dry seasons (Laverty et al., 2017; Pizzatto et al., 2013).

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 population than 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., 2015); however, these 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., 2015) and trade-offs with activity (Brown & Shine, 2014). For example, higher levels of activity among wild cane toads (typical of invasive populations) are associated with

reduced ability of kill *E. coli* (Brown & Shine 2014). The interpopulational 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 and 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 (Martin et al., 2017; Riddick et al., 2017; Silva-Rocha et al., 2015). 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 (Hudson et al., 2016; Phillips et al., 2006). Individuals along an invasion front disperse the greatest distances (Alford et al., 2009) and this might reduce immune capacities due to physically demanding movement (Brown & Shine, 2014; Freidenreich & Volek, 2013). Alternately, species 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.

Overall, our results include some metrics that suggest plasticity and others that support adaptation. Therefore, physiological adjustments by individuals (i.e., phenotypic plasticity; McCann et al., 2018) and rapid evolutionary changes (Brown et al. 2014; 2015) are likely both involved in the spread of the cane toad. The ability of invaders to adjust phenotypic characteristics via plasticity has been well studied (Hendry, 2016; Peneaux et al., 2017; Wright et

al., 2010) as have adaptive shifts by invasive species (Gruber et al., 2017; Myles-Gonzalez et al., 2015). However, these mechanisms are not mutually exclusive (Mery & Burns, 2010; Rollins et al., 2015) and similar synergistic changes occur in other invasive species (Colautti & Lau, 2015; Kilvitis et al., 2017; Reisinger et al., 2017). 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.

Data Accessibility

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

Authors' contributions

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

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