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

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

3

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11 **Abstract**

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

14 2. We used a combination of field and laboratory based experiments to assess physiological
15 tolerance to limited water access at four sites distributed across the historical invasion path of
16 cane toads (*Rhinella marina*) in Australia that, from east to west, alternated between mesic and
17 seasonally xeric habitats.

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

22 4. In contrast, bactericidal ability was not sensitive to hydric state and was greater in toads from
23 eastern (long-colonized) areas. Similar patterns in lytic ability in hydrated toads and
24 agglutination ability in wild toads suggest that toads along the invasion front face a tradeoff
25 between enhanced dispersal ability and physiological responses to dehydration.

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

28 **Keywords**

29 *Bufo marinus*, hydration, hydroregulation, innate immunity, invasive species, osmolality

30 **Introduction**

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

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

49 Within Australia, cane toads and native species with which they interact are undergoing
50 rapid evolutionary change (Brown et al., 2014; Pizzatto et al., 2013; Shine, 2012; Tingley &
51 Shine, 2011), generating substantial differences between individuals at the invasion front
52 compared to individuals at the range core (Hudson et al., 2016; Lindstrom et al., 2013). Cane

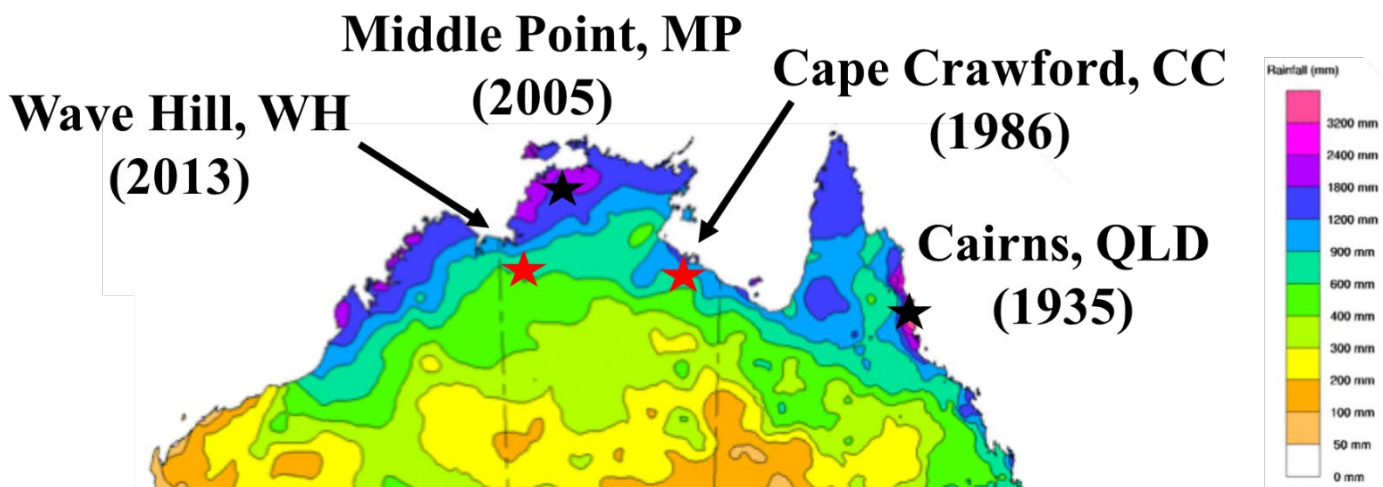
53 toads at the invasion front have been shown to rapidly acclimatize to temperature regimes
54 previously thought to be too cold (McCann et al., 2014; 2018). Those inhabiting drier regions of
55 Australia exhibit dramatic fluctuations in plasma osmolality (250-370 mOsm kg⁻¹, Reynolds &
56 Christian, 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

76 terrestrial amphibians particularly interesting for studying the relationship between hydration
77 state and immune function.

78 We used four Australian sites distributed across the historical invasion path of cane toads
79 that, from east to west, alternated between mesic and seasonally xeric habitats (Fig. 1). We
80 evaluated indicators of physiological tolerance to limited water access and used these results to
81 ascertain whether inter-populational differences are best explained by rapid evolution or
82 plasticity. We hypothesized that cane toads have rapidly evolved a physiological tolerance to
83 limited water access. Accordingly, we predicted that tolerance to water limitations and
84 modification of dehydration sensitivity of immune function progressively changes from east to
85 west. Alternatively, cane toad dehydration tolerance and effects on immune function is a result of
86 plasticity, which would be indicated by performances reflecting the hydric environment in which
87 the toads live (mesic vs. xeric) rather than the historical progression of the invasion across the
88 sites.



89
90 Figure 1: Four study locations across the historical invasion path (year) of cane toads in Australia
91 that alternated between mesic (black star) and seasonally xeric (red star). Annual rainfall map for
92 2016 modified from the Australian Bureau of Meteorology (<http://www.bom.gov.au/climate>).

93

94 **Methods**

95 All procedures were approved by the Arizona State University Institutional Animal Care and Use
96 Committee (protocol #16-1495R), the University of Sydney Animal Ethics Committee (protocol
97 #2016/997), and the Charles Darwin University Animal Ethics Committee (protocol #A16010).

98

99 *Study species and sites*

100 Cane toads are large (to > 1 kg) toxic bufonid anurans that are native to tropical and subtropical
101 areas of the Americas. After being introduced to northeastern Australia in 1935, cane toads
102 spread across almost one-quarter of the Australian continent (Kearney et al., 2008; Tingley et al.,
103 2016; Urban et al., 2008). We collected toads during June and July of 2016 in the midst of the
104 tropical ‘dry-season’ (May-October) when rainfall is scarce or absent (Shine & Brown, 2008).
105 Toads were collected from four locations: 1) Cairns, Queensland (QLD), the mesic environment
106 (average rainfall & mean daily maxima during June-July = 38.9 mm / 26°C [Australian Bureau
107 of Meteorology, <http://www.bom.gov.au/climate/>]) where the species was first introduced to
108 Australia. 2) Cape Crawford, Northern Territory (CC), an arid area (1.2 mm / 30°C) along the
109 southern edge of the Gulf of Carpentaria where the cane toad invasion slowed in the 1980s
110 apparently as a result of arid conditions (Tingley et al., 2012). 3) Middle Point, Northern
111 Territory (MP), a wet-dry tropical site along the Adelaide River that receives high annual
112 precipitation (~1400 mm) that provides constant access to water for toads (Warfe et al., 2011)
113 even during June and July when little precipitation occurs (0.5 mm / 32°C). 4) Wave Hill,
114 Northern Territory (WH), the arid habitat (0.2 mm / 29°C) near the leading edge of the species’
115 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.

162 To evaluate the involvement of complement (C') and natural antibodies (NAbs) in
163 reacting to a novel, eukaryotic antigen, we used sheep red blood cells ([SRBC]; SB050, Thermo
164 Fisher Scientific, Scoresby, Victoria, Australia) to quantify agglutination and lysis, which are
165 standard measures of soluble constitutive immunity (Matson et al., 2005). Briefly, 20 μ l of
166 plasma were serially diluted with phosphate buffered saline (PBS) along a row of a 96-well plate,
167 after which twenty μ l of 1% SRBC were added to each well. Plates were incubated at 37°C for
168 90 minutes and then placed at room temperature (\sim 25°C) for 20 minutes after which point they
169 were scanned at 600 dots per inch (Hewlett-Packard Co., ScanJet 3670) for agglutination images.
170 After an additional 70 minutes, plates were centrifuged for 5 min (500 rpm, Sorvall, Newtown,
171 CT, USA) and the supernatant was dispensed into a clean 96-well plate. Absorbance values were
172 measured (405 nm, Bio-Rad, Hercules, CA, USA) to calculate lysis scores. Hemolytic-
173 complement activity was expressed in CH_{50} units ml plasma⁻¹, where 1 CH_{50} unit equals the
174 reciprocal of the dilution of plasma required to lyse 50% of the SRBC.

175 Bactericidal activity was also assessed to determine the ability of cane toad plasma to kill
176 a prokaryotic microorganism (French & Neuman-Lee, 2012). For this assay, *Escherichia coli*, a
177 gram-negative bacteria that has been previously reported in wild populations of cane toads
178 (Shilton et al., 2008), was used to provide ecological relevance. In brief, 1:4 plasma dilution with
179 CO₂-independent media plus 4 mM L-glutamine, 10⁵ colony-producing units of *E. coli* (Lot#483-
180 306-1, ATCC 8739, MicroBioLogics, St. Cloud, MN, USA), and agar broth were combined on a
181 96-well microplate. Absorbance values were measured (300 nm, Bio-Rad, Hercules, CA, USA)
182 immediately and again after 12 hours of incubation at 37°C. Bactericidal ability percentages were
183 calculated as the mean number of colonies for each sample, run in triplicate, divided by the mean
184 number of colonies for the positive control, and then multiplied by 100. Together, these three

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

188

189 *Statistical Analyses*

190 To explore physiological responses to water deprivation, we used linear mixed-effect models to
191 compare total water loss (i.e., initial mass – final mass) of the captive toads from all four
192 populations. Location was used as a fixed effect and SVL as a random effect (to remove any
193 potential confounding influence from surface-area-to-volume ratios). Initially, analyses were
194 carried out separately using size based on either body mass or SVL. However, these analyses
195 yielded qualitatively identical results and therefore the reported analyses are based on SVL only.
196 The data were checked to ensure they met the assumptions for parametric testing,
197 transformations were used where necessary, and the *agricolae* package (De Mendiburu, 2014)
198 was used for post-hoc tests.

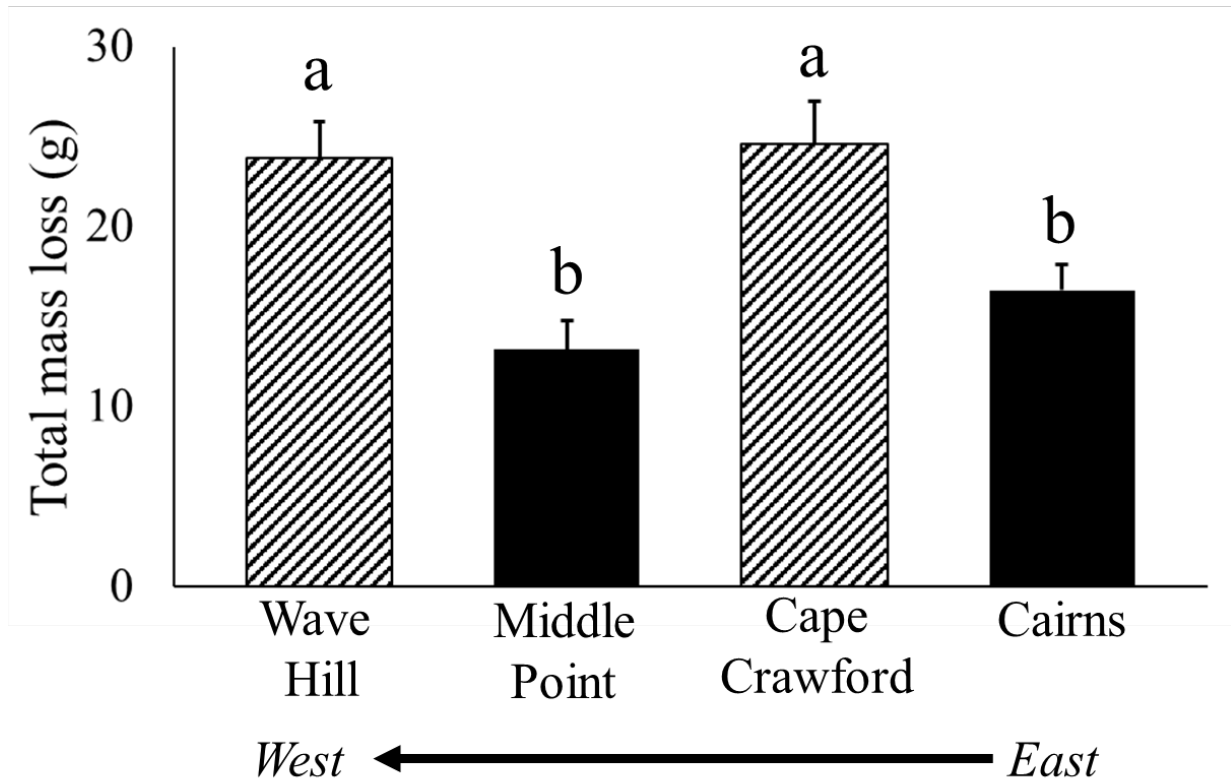
199 To quantify sources of variation in osmolality values and immune scores, we used
200 multimodel inference to estimate the most likely values of means and standard deviations for
201 each dependent variable using the full-average method (Burnham & Anderson, 2002). Size, sex,
202 treatment, and location were used as categorical fixed factors. Using the *nlme* (Pinheiro et al.,
203 2016) and *MuMIn* libraries (Barton, 2015) the R Statistical Software (version 3.3.2; R
204 Development Core Team 2016) was used to fit all possible models, including potential possible
205 interactions between fixed factors. The Akaike information criterion (AIC_c) and Akaike weight
206 (w_i) of each model (see Tables S1-S4) were calculated to determine the best model. Finally, we
207 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

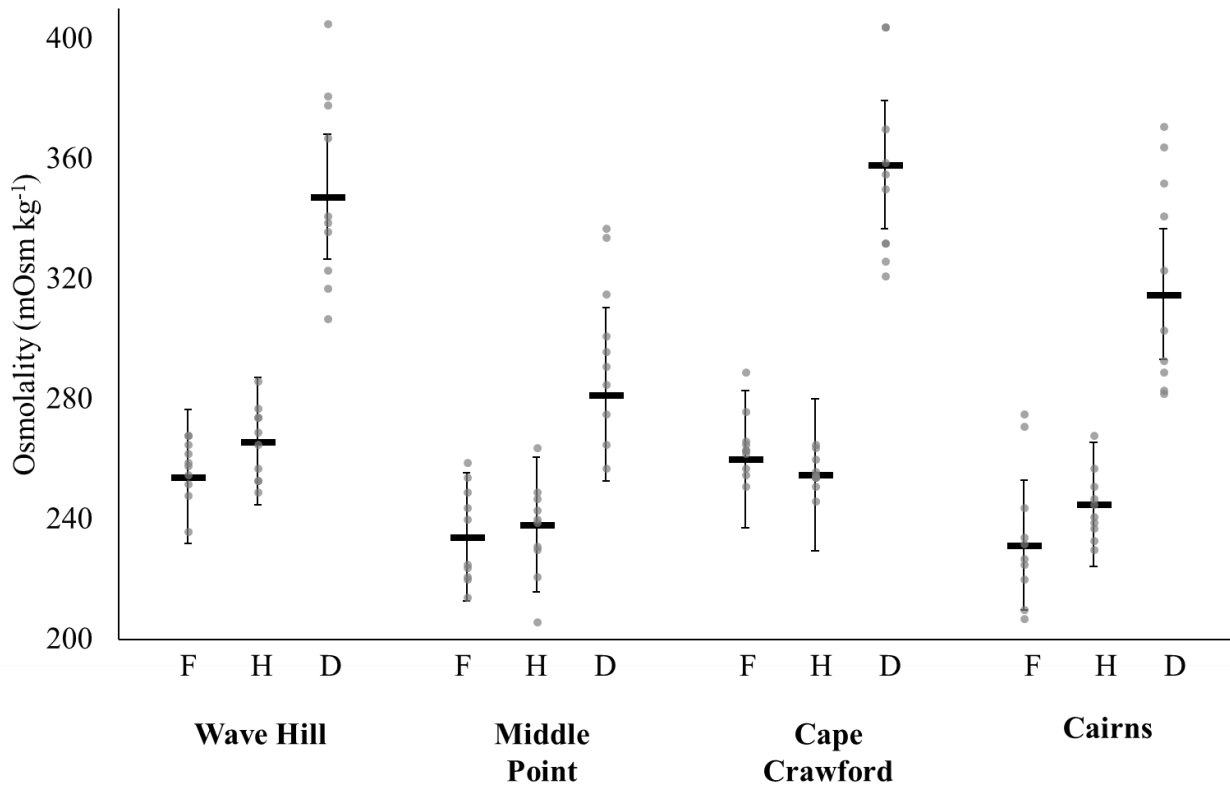
211 **Results**

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 \pm 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.



235

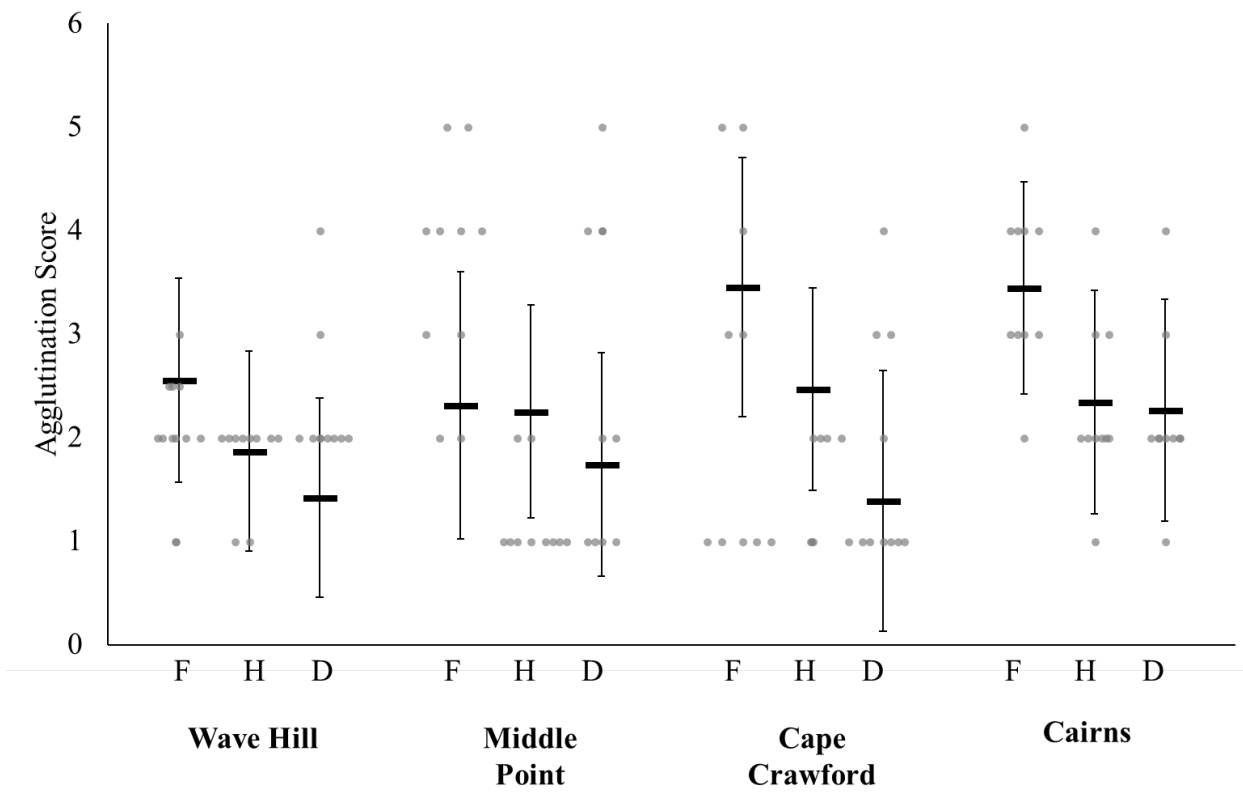
236 Figure 3: Plasma osmolality (mOsm kg^{-1}) of cane toads increased after the dehydration
 237 treatment, but was similar for toads in the field and toads after hydration treatment. Toads from
 238 xeric populations (Wave Hill and Crape Crawford) had larger increases in osmolality as a result
 239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

243

244 Agglutination scores were higher for toads in the field than for toads after experimental
 245 hydration, which were higher in turn than were scores for toads after dehydration (except for the
 246 MP population where agglutination was relatively similar among the three groups; Fig. 4).

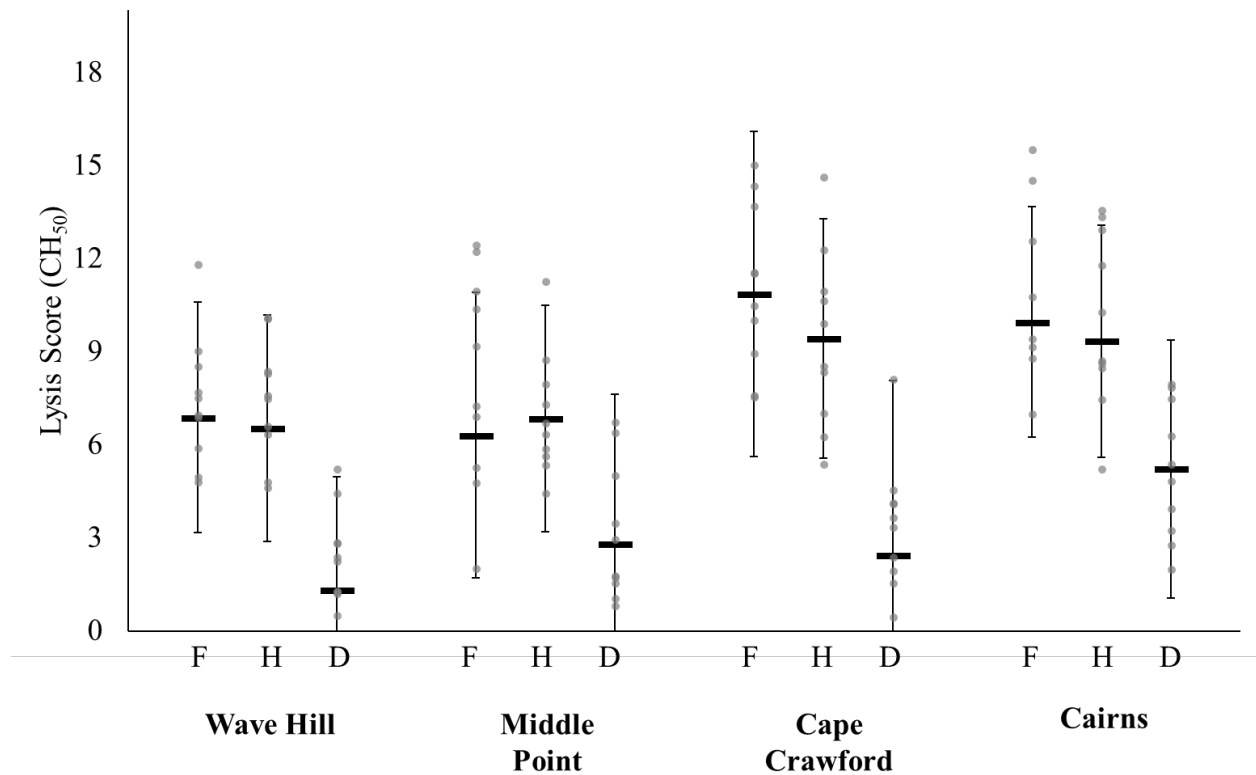
247 Because agglutination scores varied within populations and within treatment groups, our results

248 should be interpreted cautiously. Toads from all locations had decreased lysis scores after
 249 dehydration. However, lysis scores decreased, on average, 25% more in toads from WH ($5.2 \pm$
 250 3.6 CH_{50}) and 75% more in toads from CC ($7.0 \pm 3.8 \text{ CH}_{50}$) compared to toads from MP ($4.0 \pm$
 251 3.4 CH_{50}) and QLD ($4.1 \pm 3.7 \text{ CH}_{50}$) (Fig. 5). In contrast, bactericidal ability showed little
 252 change among treatment groups. There was a clear effect of location, although unlike the pattern
 253 in osmolality and lysis values, bactericidal ability averaged 125% and 150% higher in the QLD
 254 ($39.5 \pm 10.5 \%$ bacteria killed) populations compared to the MP ($17.5 \pm 10.3 \%$ killed) and WH
 255 ($14.6 \pm 10.4 \%$ killed) populations, respectively. Similarly, toads from CC ($36.7 \pm 10.3 \%$ killed)
 256 had a 110% and 151% higher average bactericidal ability compared to MP and WH, respectively
 257 (Fig. 6).



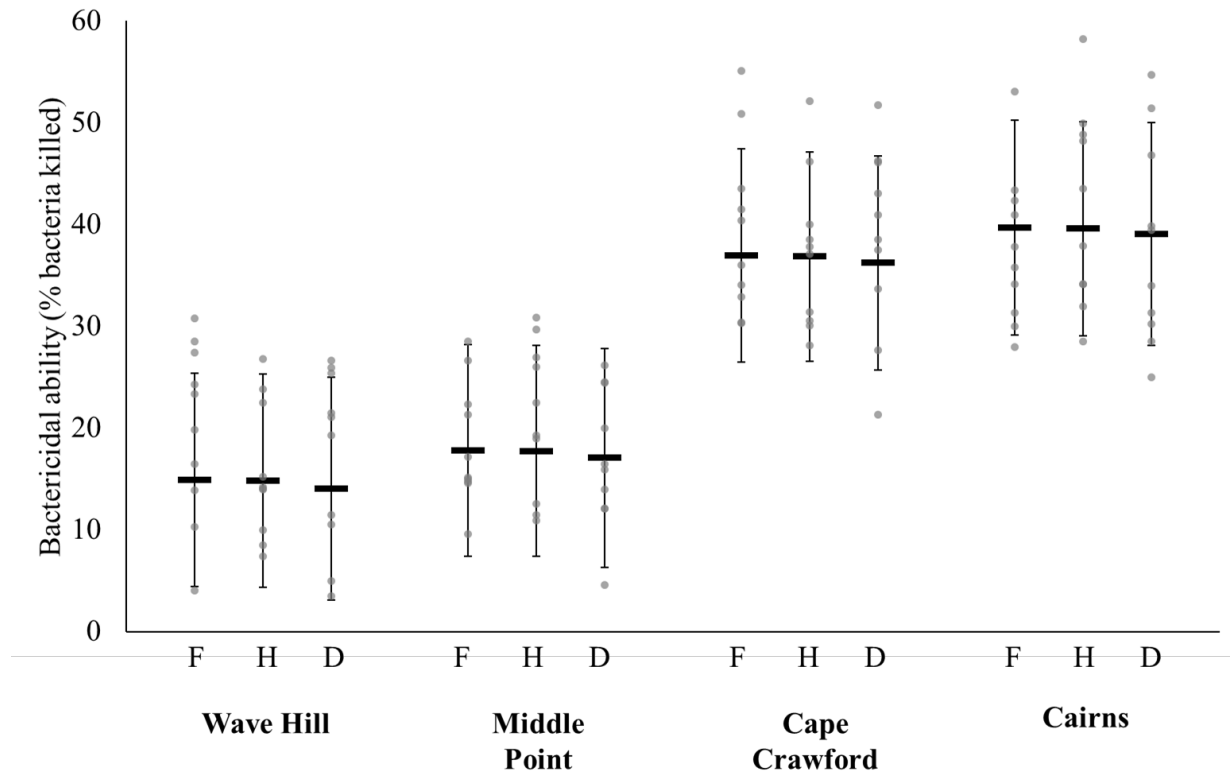
258
 259 Figure 4: Agglutination scores of cane toads showed a stepdown pattern, where toads in the field
 260 had higher scores than experimentally hydrated toads, and both had higher scores than

261 experimentally dehydrated toads. Grey circles represent agglutination scores of individual toads
262 in a treatment (F = field; H = experimentally hydrated; D = experimentally dehydrated). Black
263 horizontal lines denote means and error bars denote standard deviations estimated from
264 multimodel averaging.



265
266 Figure 5: Lysis scores (CH₅₀) of cane toads were similar for toads in the field and toads after the
267 hydration treatment, but decreased after the dehydration treatment. Toads from xeric populations
268 (Wave Hill and Crape Crawford) had larger decreases after the dehydration treatment compared
269 to toads from mesic populations (Middle Point and Cairns), and toads closer to the invasion
270 origin (Cairns and Cape Crawford) had higher scores, in general, than those closer to the
271 invasion front (Middle Point and Wave Hill). Grey circles represent lysis scores of individual
272 toads in a treatment (F = field; H = experimentally hydrated; D = experimentally dehydrated).

273 Black horizontal lines denote means and error bars denote standard deviations estimated from
 274 multimodel averaging.



275
 276 Figure 6: Bactericidal ability (% bacteria killed) of cane toads were similar for toads in the field,
 277 after hydration, and after dehydration. Toads closer to the invasion origin (Cairns and Cape
 278 Crawford) had higher scores than those closer to the invasion front (Middle Point and Wave
 279 Hill). Grey circles represent bactericidal ability values of individual toads in a treatment (F =
 280 field; H = experimentally hydrated; D = experimentally dehydrated). Black horizontal lines
 281 denote means and error bars denote standard deviations estimated from multimodel averaging.

282

283 **Discussion**

284 Cane toads from seasonally xeric populations had larger increases in plasma osmolality after five
 285 days without water, which, coupled with larger decreases in mass (see Figs. 2-3), suggests that

286 toads from these populations are more prone to evaporative water loss (EWL). This pattern runs
287 counter to the typical situation whereby animals adapted to living in xeric environments have
288 reduced rates of EWL (reptiles - Cox & Cox, 2015; Dmi'el, 2001; bats - Muñoz-Garcia et al.,
289 2016), thereby conserving water in drier climates. However, our results are consistent with a
290 previous study (Tingley et al., 2012) that found the opposite to be true in cane toads; animals
291 from xeric environments had increased desiccation rates due to higher EWL compared to toads
292 from mesic environments. This counter-intuitive, result may reflect a reliance on access to
293 standing water by toads from xeric populations, which places a selective premium on the ability
294 to rapidly absorb water if it becomes available (Tingley et al., 2012). Differences in resistance to
295 EWL between mesic and xeric populations have also been documented in other neotropical toads
296 (Anderson et al., 2017; Prates & Navas, 2009; Prates et al., 2013), suggesting that dehydration
297 tolerance is a relatively plastic trait.

298 Plasma osmolality was similar for toads from the wild (regardless of location) and after
299 experimental hydration. Thus, despite drastically different temperature and rainfall patterns
300 among the sites, cane toads in the wild are staying hydrated. Although we avoided collecting
301 toads that had recently emerged from water or wet substrate, collecting animals at the beginning
302 of their nocturnally active period may have inadvertently sampled animals when they were at an
303 optimal hydric state, after emerging from moist refugia (Schwarzkopf & Alford, 1996).
304 However, soil moisture levels during the Austral dry season would presumably be low enough to
305 garner no net hydric benefit to the cane toads (Seebacher & Alford, 1999). In arid parts of
306 Australia, cane toads remain active when there is no rainfall, although they limit their activity to
307 areas with permanent water sources (Brown et al., 2011). Moreover, toads can switch from
308 nocturnal to diurnal activity to stay hydrated via the aforementioned permanent water sources

309 (Webb et al., 2014). Behavioral flexibility to remain hydrated might explain our results, and the
310 existence of toads in xeric environments likely relies on them finding sporadically located water
311 resources.

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

323 Bactericidal ability was not sensitive to hydration treatment (i.e., at each of the four sites,
324 hydrated and dehydrated toads had similar killing ability). However, bacterial killing was greater
325 in the two eastern population than the western populations. These bactericidal results are in
326 contrast with previous research that found toads from the western edge of their expansion had
327 higher bactericidal ability compared to toads from the range core in Queensland (Brown et al.,
328 2015); however, these results were from captive-raised toads which had not been exposed to
329 ecologically relevant influences on immune performance in cane toads such as pathogen pressure
330 (Brown et al., 2015) and trade-offs with activity (Brown & Shine, 2014). For example, higher
331 levels of activity among wild cane toads (typical of invasive populations) are associated with

332 reduced ability of kill *E. coli* (Brown & Shine 2014). The interpopulational pattern in our
333 bacterial killing results is also seen with the lysis scores of hydrated toads (whether naturally or
334 experimentally hydrated) and agglutination of field-sampled toads (Figs. 3 and 4). Additionally,
335 decreased immune performance correlates with higher disease prevalence in toads at the xeric
336 expansion front (Brown et al., 2007; Shilton et al., 2008) and similar immunosuppressive effects
337 of range expansion have been documented in other invasive species (Martin et al., 2017; Riddick
338 et al., 2017; Silva-Rocha et al., 2015). Stressful conditions or activities near the expanding range
339 edge might increase levels of glucocorticoids (e.g. corticosterone) which could suppress immune
340 performance in invasion-front populations (Goetz et al., 2017).

341 Reduced immunocompetence in individuals at the invasion front may reflect a tradeoff
342 with morphological changes that enhance dispersal ability (Hudson et al., 2016; Phillips et al.,
343 2006). Individuals along an invasion front disperse the greatest distances (Alford et al., 2009)
344 and this might reduce immune capacities due to physically demanding movement (Brown &
345 Shine, 2014; Freidenreich & Volek, 2013). Alternately, species at the invasion front may face
346 fewer potential pathogens (Perkins et al., 2013), dampening immune responses (Devalapalli et
347 al., 2006), a phenomenon that can be rapidly reversed within an individual's lifetime
348 (Montecino-Rodriguez et al., 2013). Further work is needed to better understand the relationship
349 between dispersal and immunocompetence.

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

355 al., 2010) as have adaptive shifts by invasive species (Gruber et al., 2017; Myles-Gonzalez et al.,
356 2015). However, these mechanisms are not mutually exclusive (Mery & Burns, 2010; Rollins et
357 al., 2015) and similar synergistic changes occur in other invasive species (Colautti & Lau, 2015;
358 Kilvitis et al., 2017; Reisinger et al., 2017). Invasiveness appears to be based on a complex
359 interaction of ecology, evolution, and physiology, and future work should incorporate a broad
360 range of organismal traits when trying to decipher the invasion potential of a species.

361

362 **Data Accessibility**

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

364

365 **Authors' contributions**

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

369

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379

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