

## RESEARCH ARTICLE

# Interactions between corticosterone phenotype, environmental stressor pervasiveness and irruptive movement-related survival in the cane toad

Tim S. Jessop<sup>1,\*</sup>, Jonathan Webb<sup>2</sup>, Tim Dempster<sup>3</sup>, Benjamin Feit<sup>4,5</sup> and Mike Letnic<sup>5</sup>

**ABSTRACT**

Animals use irruptive movement to avoid exposure to stochastic and pervasive environmental stressors that impact fitness. Beneficial irruptive movements transfer individuals from high-stress areas (conferring low fitness) to alternative localities that may improve survival or reproduction. However, being stochastic, environmental stressors can limit an animal's preparatory capacity to enhance irruptive movement performance. Thus individuals must rely on pre-existing, or rapidly induced, physiological and behavioural responses. Rapid elevation of glucocorticoid hormones in response to environmental stressors are widely implicated in adjusting physiological and behaviour processes that could influence irruptive movement capacity. However, there remains little direct evidence demonstrating that corticosterone-regulated movement performance or interaction with pervasiveness of environmental stress, confers adaptive movement outcomes. Here, we compared how movement-related survival of cane toads (*Rhinella marina*) varied with three different experimental corticosterone phenotypes across four increments of increasing environmental stressor pervasiveness (i.e. distance from water in a semi-arid landscape). Our results indicated that toads with phenotypically increased corticosterone levels attained higher movement-related survival compared with individuals with control or lowered corticosterone phenotypes. However, the effects of corticosterone phenotypes on movement-related survival to some extent co-varied with stressor pervasiveness. Thus, our study demonstrates how the interplay between an individual's corticosterone phenotype and movement capacity alongside the arising costs of movement and the pervasiveness of the environmental stressor can affect survival outcomes.

**KEY WORDS:** Phenotypic engineering, Glucocorticoids, Movement behaviour, Fitness, Stressor magnitude

**INTRODUCTION**

Animal movements, including dispersal and migration, are used by individuals to travel beyond their current home range to influence individual fitness and population persistence (Clobert, 2012; Nathan et al., 2008). Such movements arise in response to both

predictable (e.g. natal dispersal or seasonal migration) and unpredictable (i.e. stochastic) events (Clobert, 2012). Animal movements triggered by stochastic environmental or ecological events, are often referred to as irruptive movements or facultative (or conditional) dispersal, and represent a key strategy through which individuals attempt to avoid the fitness consequences of exposure to potentially pervasive local events (i.e. stressors) (Walls et al., 2005; Wingfield and Ramenofsky, 1997). Irruptive type movements vary considerably in their scale and duration but are typically triggered by the approach of, or exposure to, pervasive environmental stressors. For example, animals use irruptive movements in response to approaching or exposure to storms, floods, heat waves, wild fires and anthropogenic disturbance events (Heupel et al., 2003; Preen and Marsh, 1995; Streby et al., 2015). The fitness implications of these movements can be extremely valuable if they afford individuals higher survival, or allow for reproduction, through the avoidance of exposure to environmental stressors and any arising costs of movement (Clobert, 2012; Hardman and Moro, 2006; Lea et al., 2009; Wingfield and Ramenofsky, 1997).

Movement performance in animals is influenced by many extrinsic and intrinsic factors (Clobert, 2012; Nathan et al., 2008). Intrinsic factors include the physiological, morphological and behavioural attributes that affect how animals navigate and physically move in their environment (Nathan et al., 2008). However, an obvious constraint for animals that use irruptive movements to avoid stochastic and pervasive environmental phenomena, is the lack of preparatory time (e.g. unlike annual migration), to make the often extensive and complex phenotypic adjustments to enhance movement capacity (Breuner et al., 2013; Piersma and Drent, 2003; Wingfield and Ramenofsky, 1997). Consequently, irruptive movement performance is likely to be strongly influenced by the ability of individuals to use pre-existing, or rapidly induced, phenotypic responses to mitigate and reduce consequences of exposure to environmental stressors (Wingfield and Ramenofsky, 1997; Wingfield et al., 1998).

In vertebrates, the hypothalamic–pituitary–adrenal axis (HPA axis) produces the glucocorticoid (GC) hormones corticosterone and cortisol, which regulate behavioural and physiological traits that influence the ability of an individual to respond to unpredictable and extreme environmental events (Breuner et al., 2008; Crespi et al., 2013; Jessop et al., 2013a; Romero, 2004; Wingfield, 2013). GCs, through broad-scale effects on gene transcription, allow for diverse and complex control over behaviour, metabolism, reproduction, growth and immune functions (Le et al., 2005; Romero, 2004). Thus many GC-regulated processes are likely to influence multiple traits that affect movement performance and subsequent fitness outcomes for animals exposed to environmental stressors (Breuner and Hahn, 2003; Breuner et al., 2008; Wingfield et al., 1998).

Positive relationships between corticosteroid (CORT) levels and the performance of physical or locomotor activity that facilitates

<sup>1</sup>Centre for Integrative Ecology, Deakin University, Victoria, 3220, Australia. <sup>2</sup>School of the Environment, University of Technology Sydney, NSW 2007, Australia. <sup>3</sup>School of Biosciences, University of Melbourne, Victoria, 3010, Australia. <sup>4</sup>Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden. <sup>5</sup>School of Biological, Earth and Environmental Sciences, University of New South Wales, NSW 2052, Australia.

\*Author for correspondence (t.jessop@deakin.edu.au)

 T.S.J., 0000-0002-7712-4373

animal movement has been extremely well documented in laboratory rodents (Coleman et al., 1998; Girard and Garland, 2002; Malisch et al., 2008, 2006; Stranahan et al., 2008). Similarly, addition of exogenous CORT has been shown to stimulate activity or increase endurance attributes of movement in mammals, birds (Breuner et al., 1998), lizards (Belluire et al., 2004 and Cote et al., 2006) and turtles (Cash and Holberton, 1999). Coupled with this physiological enhancement of movement, increased GC levels can affect behavioural attributes that promote motivation, causing inducement of irruptive or escape-type movements to avoid stressors (Breuner et al., 1998; Dickens et al., 2009; Thaker et al., 2009).

However, despite clear evidence for the positive effects of GCs on physical and behavioural attributes of movement performance, there is less evidence for how CORT might influence adaptive movement outcomes in response to exposure to environmental stressors in free-living populations (Breuner and Hahn, 2003). This is because while movement allows individuals to avoid exposure to the environmental stressors, the act of movement can also present direct (e.g. energetic), or deferred, costs that can be paid by individuals during departure, transit and settlement phases of movement (Bonte et al., 2012). Furthermore, activation of the HPA axis in response to environmental stressors and through its capacity to influence many other attributes of phenotypic performance could cause performance trade-offs that produce fitness costs in individuals that utilise irruptive movement (Ketterson et al., 2009). Thus, interplay not only between an individual's CORT and movement phenotype, but also between other external and internal environmental attributes, could lead to complex fitness outcomes (Bonier et al., 2009; Breuner et al., 2008; Jessop et al., 2013b).

In this field study, we investigated how variation in CORT hormone phenotypes and different severities of environmental stressor pervasiveness affected movement-related survival in an introduced amphibian – the cane toad [*Rhinella marina* (Linnaeus 1758)] – inhabiting semi-arid northern Australia. Survival and movement of cane toads in this environment are strongly influenced by highly seasonal variation in environmental temperature and water availability (Letnic et al., 2015, 2014; Webb et al., 2014). In particular, during the hot-dry season (September–November), previous research has indicated that toads must restrict daily movements when they are more than 400 m from bodies of permanent natural or artificial water bodies that are bordered by highly unfavourable (i.e. extremely stressful to amphibians) semi-arid habitat (Florance et al., 2011; Jessop et al., 2013a; Webb et al., 2014). Simply, as distance from the water increases, a rapid transition from mesic to semi-arid habitat presents toads with an acute spatial gradient of environmental stress that increasingly exposes toads to unfavourable and potentially lethal abiotic conditions alongside increased costs of movement needed to access water (Florance et al., 2011; Jessop et al., 2013a).

Our study used two experimental approaches to concurrently evaluate how the CORT phenotype and the severity of stressor pervasiveness influenced cane toad irruptive movement-related survival in semi-arid Australia. We used physiological engineering to administer CORT agonists/antagonists to increase (i.e. high CORT phenotype) and reduce (i.e. low CORT phenotype) endogenous CORT levels relative to naturally occurring individuals (i.e. control CORT phenotype). In effect, physiological engineering allowed us to consider how three experimentally distinct CORT phenotypes could influence movement-related survival in toads. In addition, we also manipulated the level of stressor pervasiveness by releasing toads at four increasing distances from water. With each increase in release distance, we expected toads to face a higher risk/duration of exposure

to harsh environmental conditions and incur greater costs of movement. By coupling these two experimental approaches we could measure the interaction between the different CORT phenotypes and the different levels of stressor pervasiveness on movement-related survival in toads.

We predicted that different GC hormone phenotypes could lead to variation in toad movement capabilities that could result in different survival outcomes. In particular, and if consistent with expectations of increased locomotor performance, then toads with high CORT phenotypes could, on average, attain better movement-related survival compared with control or low CORT phenotypes across all levels of stressor exposure. However, we also predicted that differences in CORT-mediated effects on movement-related survival outcomes would also arise from an interaction with the pervasiveness of the stressor. Within the context of our experimental design, we predicted that those toads exposed to the most benign stressor (i.e. release 50 m from water), irrespective of CORT phenotypes differences, could achieve similar movement-related survival outcomes: a finding that would support the idea that under benign conditions a broad range of CORT phenotypes can achieve similar fitness outcomes. However, as exposure to stressors becomes more pervasive (i.e. release 200 and 400 m from water), we predicted that selection would favour toads with the high CORT phenotype that would produce better movement, allowing for higher levels of survival. Finally, we predicted that toads exposed to the most intense stressor level (i.e. release 600 m from water), regardless of their CORT phenotype, may achieve poor movement-related survival.

## MATERIALS AND METHODS

### Study area

Research was conducted at Camfield Station (17°04'S, 131°39'E) on the northern edge of the Tanami Desert in Australia's Northern Territory during late September (seasonally hot and dry). This semi-arid region experiences a bi-phasic wet-dry season with most rainfall occurring from December to April. The mean annual rainfall at the nearest weather station to the study site (Wave Hill) is 580 mm. Monthly peak air temperatures during the mid-late dry season (September–November) are 35.1°C (30.8–38.5; 90% CI), 37.9°C (34.3–40.7) and 38.6°C (35.1–41.9) (sourced from Australian Bureau of Meteorology). Because of these harsh seasonal environmental constraints during the dry season, cane toads aggregate in close proximity (<100 m) to permanent water bodies. In our study area, permanent water is only found at a single river or at small man-made dams (1 per 100 km<sup>2</sup>). We used a man-made dam with permanent water that harboured a large resident population of toads to evaluate the effects of different GC hormone phenotypes on movement-related survival within the context of a semi-arid range front environment.

### Protocols for manipulating toad CORT phenotypes

Over the course of three nights between 18:30 h and 19:00 h, we rapidly hand captured 120 toads per night ( $N=360$  adult individuals of mixed sex for entire experiment) from the dam, with individuals allocated to one of three GC hormone treatments. To manipulate endogenous CORT levels of toads, we administered via injection using a 1 ml syringe and 27 gauge needle, a mass-calibrated dose of either metyrapone, adrenocorticotrophic hormone (ACTH) or vehicle only to produce toads with low, high and control CORT phenotypes, respectively. Low CORT phenotype toads (40 toads/night) were injected intraperitoneally (IP) with 100 µg kg<sup>-1</sup> metyrapone (Sigma) suspended in 100 µl vehicle solution (comprising ethanol:Ringer's saline solution at 1:1 v/v). Metyrapone is a potent glucocorticoid

inhibitor that causes rapid and sustained suppression of endogenous CORT and was used to produce a cohort of 'low' GC hormone phenotypes in toads (Hayes and Wu, 1995; Thaker et al., 2009). Toads constituting the high CORT phenotype group (40 toads/night) were injected IP with freshly thawed 50 IU kg<sup>-1</sup> porcine ACTH (Sigma Chemical) suspended in 100 µl of vehicle solution. ACTH increases synthesis of CORT and causes faster rates of GC receptor activation and was used to produce a cohort of toads with a greater magnitude and hence 'high' GC hormone phenotype. The control CORT phenotype toads (40 toads/night) were injected IP with 100 µl of vehicle solution. On each night, all individuals were injected within 10 min of each other to produce similar exposure to agents manipulating endogenous CORT levels. Metyrapone and ACTH dosage protocols were based on other studies that investigated their effects on the acute CORT stress response in ectothermic vertebrates (Narayan et al., 2013; Scholnick et al., 1997; Thaker et al., 2009). Immediately following injection, individual toads were marked with a uniquely coloured and labelled cable tie secured to their right hind leg (sufficiently secure to prevent loss but avoiding any impediment to movement). Labelled cable ties thus provided a means to identify individual animals to the day and distance of release alongside their GC treatment phenotype. Thirty toads, comprising 10 individuals from each GC treatment were then placed into one of four 60 l partially water-filled containers and held for 30 min prior to release. This holding time was to ensure that all individuals had adequate time to reach similar states of hydration prior to release. During the holding period, toads were free to move about in their container to limit the effects of restraint stress.

#### Measurement of movement-related survival

To measure the effect of different CORT phenotypes on movement-related survival over a gradient of environmental stress, we used a translocation approach. Across each of the three nights at 20:00 h, toads (housed in containers) were moved by vehicle and then as groups of 30 individuals (comprising 10 each of high, low and control GC hormone phenotypes, respectively) were released at one of four distances of 50, 200, 400 and 600 m from the dam (i.e. place of residence and access to water). We randomised the bearing of release for each group at each distance. The purpose of increasing the release distance was to incrementally increase the level of stressor pervasiveness. This simple experimental manipulation allowed us to increase durations of exposure to harsh daily abiotic conditions and the arising costs (e.g. energy) of movement of toads as they attempted to successfully return to the safety of water. To increase detection of marked toads that successfully returned to the dam post-release we constructed a shade cloth fence (standing 50 cm) that surrounded the dam which forced toads to aggregate along its perimeter (Letnic et al., 2015, 2014) that allowed us to rapidly inventory all individual that successfully returned from their post-release distance drop-off point. Commencing at 20:30 h we began walking the fence perimeter to hand capture any marked individuals at 1, 2, 3, 4, 5, 7, 13, 20, 21, 22, 23 and 24 h post-release. These intervals reflected the fact that because toads are nocturnally active, they would move at night (hence the intensive nocturnal sampling bouts at 1–7 and 20–24 h post-release) but during the day they would be inactive (hence the non-intensive diurnal sampling at 13 and 20 h post-release). The 24 h post-release duration was decided as the maximum time interval for checking for successful return movement as we recorded no individual returning beyond this time period after 72 h of continuous monitoring. Given the absence of alternative water bodies at the study site, any toad that failed to move back to permanent water (i.e. the dam) to allow rehydration

within ~24 h was assumed to have succumbed to subsequent dehydration or thermal stress (Florance et al., 2011; Jessop et al., 2013a). Thus, at every sampling event, each marked individual was recorded present (successful dispersal/homing event) or absent (unsuccessful dispersal/homing event).

#### Validation of CORT phenotypes

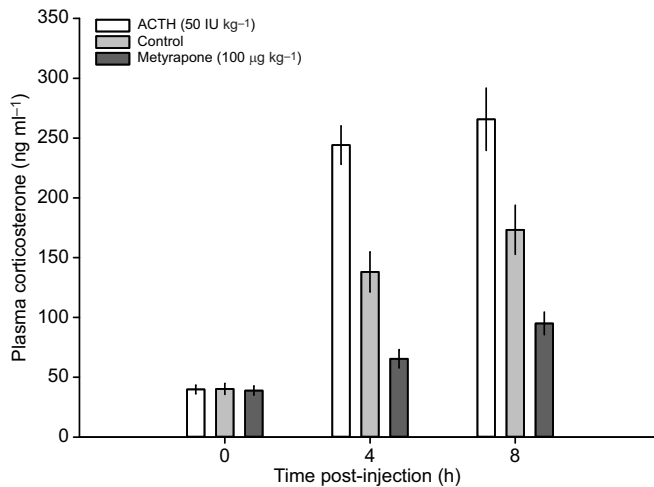
To ensure that hormone manipulations produced different CORT phenotypes, we monitored changes in CORT levels in toads post-injection with ACTH, metyrapone and vehicle solution (i.e. controls) subjected to an 8 h capture-stress protocol. Here, using 90 toads (i.e. 30 toads per treatment), independent of those used in the main experiment and also captured from the dam at night, we used identical injection protocols to manipulate CORT phenotypes as described above. Once injected, 10 toads per treatment were immediately blood sampled, whilst the remaining 20 toads per treatment were restrained by placing each individual into a calico bags (10 cm×10 cm) that prevented them from freely moving and escaping. All restrained toads were placed in a large plastic container and held within a vehicle until blood samples were collected from 10 individuals per treatment at 4 and 8 h post injection, respectively. Each toad was thus blood sampled once at a designated time period. Here, 2 ml of blood was collected in heparinised Eppendorf containers following rapid euthanasia of individuals and then stored briefly on ice prior to centrifugation (5 min at 2500 rpm) to harvest plasma that was stored frozen at -20°C until assay.

#### CORT assay

Total plasma concentrations of CORT were measured using a commercially available ELISA kit (Cayman Chemical, Michigan, USA). Previous studies have determined that 20 µl plasma was sufficient for assay use (Jessop et al., 2013a). Plasma samples were twice extracted in 3 ml diethyl ether. The efficiency of extraction was measured by adding 20 µl [<sup>3</sup>H]CORT (~2000 cpm) (MP Biomedicals). To estimate steroid extraction efficiency, 50 µl of each extracted sample was placed in a scintillation vial containing 2 ml scintillation fluid (Ultima Gold). Sample radioactivity was estimated using a Beckman 2100R Liquid Scintillation Counter. We followed the Cayman Chemical CORT EIA assay procedures without modification to measure plasma CORT concentrations. During each assay, samples were run in duplicate alongside a standard curve of eight known concentrations of corticosterone (5000, 2000, 800, 320, 128, 51.2, 20.5, 8.2 pg ml<sup>-1</sup>). We calculated final steroid concentrations from standard curves and then corrected for individual sample recovery and addition of [<sup>3</sup>H]CORT. Average extraction efficiency for CORT over the four assays was 88.5±0.56% (mean±s.e.m.). For CORT assays, intra-assay coefficient of variance and inter-assay coefficient of variance were estimated at 3.7% and 8.0%, respectively. To validate the use of the CORT enzyme immunoassay kit with toad plasma, we established parallelism between the standard curve and serial dilutions of pooled plasma samples.

#### Data analysis

A linear model (LM) and generalized linear mixed effect model (GLMM) were used to analyse plasma CORT concentration and movement-related survival data of toads, respectively. To evaluate the effects of different hormone injection protocols on toad plasma CORT levels (i.e. to produce different stress phenotypes) an LM fitted with a Gaussian distribution and identity canonical link was used. This model tested for the effect of hormone treatment, restraint time and their interaction on toad plasma CORT levels. *P*-values



**Fig. 1. Effects of ACTH and metyrapone on plasma corticosterone levels of cane toads subjected to a capture stressor under field conditions in semi-arid Australia.** Bars represent the mean  $\pm$  s.e.m. for 10 individuals for each treatment sampled at each time period. ACTH, adrenocorticotropic hormone: high GC samples; Control: control GC samples; Metyrapone: low GC samples.

(<0.05) and Tukey's HSD were used to evaluate significant inference among and within main and interaction model terms.

A GLMM model was specified to include the fixed main effect of GC hormone phenotype, two two-level interactions (GC phenotype  $\times$  release distance and GC phenotype  $\times$  time since release) and one three-level interaction (GC phenotype  $\times$  release distance  $\times$  time since release) on the category-dependent variable (i.e. toad survival post-release). Toad movement-related survival data were represented by a binomial score of 1 that represented successful movement-related survival or 0 for an unsuccessful movement-related survival outcome recorded for each individual at each time interval. Individual toad identity was included as a random effect in the model. *P*-values (<0.05), parameter estimates and associated 95% confidence intervals and Tukey's HSD were used to evaluate significant inference among and within main and interaction model terms. Analyses were conducted using program R (2017) and the packages base, nlme (<http://CRAN.R-project.org/package=nlme>), Car (Fox and Weisberg, 2011) and multcomp (<http://cran.stat.sfu.ca/web/packages/multcomp/multcomp.pdf>).

**Table 1. GLMM model results for the effects of GC phenotype and its two- and three-way interactions with distance from water and time since release on movement-related survival of cane toads**

Term	$\chi^2$	d.f.	<i>P</i> -value
Intercept	15.41	1	<0.001
GC phenotype	99.77	2	<0.001
GC phenotype $\times$ distance from water	130.37	3	<0.001
GC phenotype $\times$ time since release	107.67	3	<0.001
GC phenotype $\times$ distance from water $\times$ time since release	64.93	3	<0.001

### Ethics statement

All research-related procedures were conducted under the approval of the University of Melbourne Animal Ethics committee (permit ID number 1313024.1).

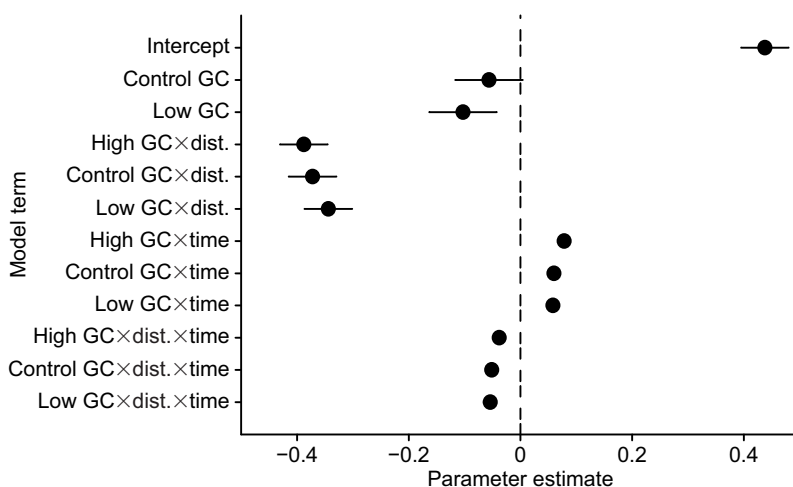
## RESULTS

### Validation of effects of ACTH and metyrapone on toad plasma CORT profiles

Experimental protocols produced successfully manipulated CORT phenotypes for at least 8 h under field conditions (Fig. 1). As toads injected with ACTH, metyrapone or vehicle solution (i.e. controls) and then subjected to a restraint capture protocol indicated significant hormone treatment (LM,  $F=46.90$ ;  $P<0.001$ ), time post-restraint (LM,  $F=65.96$ ;  $P<0.001$ ) and hormone treatment  $\times$  time post-restraint interaction effects (LM,  $F=12.55$ ;  $P<0.001$ ) on plasma CORT levels (Fig. 1). For the hormone treatment effect, *post hoc* analyses indicated that ACTH-injected toads ( $195.4 \pm 12.3$  ng ml<sup>-1</sup>; mean  $\pm$  s.e.m.) produced significantly more CORT relative to the metyrapone ( $70.87 \pm 12.1$  ng ml<sup>-1</sup>;  $P<0.001$ ) and control-treated toads ( $111.17 \pm 11.2$  ng ml<sup>-1</sup>;  $P<0.001$ ). Metyrapone-injected toads produced significantly lower CORT levels compared with unmanipulated control toads ( $P<0.05$ ).

### Effects of GC phenotype and interactions with distance and time on movement-related survival

Using a GLMM, we evaluated the effects of three GC treatments (high, control and low) and their interactions with four release distances from water (50, 200, 400, 600 m) at 12 post-release time intervals (1, 2, 3, 4, 5, 7, 13, 20, 21, 22, 23 and 24 h) on toad movement-related survival under field conditions in semi-arid Australia. All main and interactive model terms had significant



**Fig. 2. Coefficients of measured variables and interactions, and their effects on movement-related survival in cane toads.** Circles indicate mean parameter effects and associated 95% confidence intervals estimated from a GLMM. Here, the effects of GC phenotype, distance of release and time since release and their two- and three-way interactions on cane toad ( $n=360$ ) movement-related survival are presented.

effects on toad movement-related survival (Table 1, Fig. 2; Table S1).

CORT phenotype treatment induced significant differences in toad movement-related survival (GLMM  $\chi^2=99.77$ ;  $P<0.001$ ). Differences among GC hormone treatments indicated that high GC phenotype toads ( $0.44\pm 0.04$ ; mean $\pm$ s.d. survival probability) attained on average better movement-related survival compared with control GC ( $0.38\pm 0.04$ ) and the low GC phenotype toads ( $0.33\pm 0.04$ ) (Fig. 3A). Significant pairwise *post hoc* treatment differences were evident between high versus control ( $P<0.05$ ), and high versus low GC treatments ( $P<0.05$ ; Fig. 3A, Table S2).

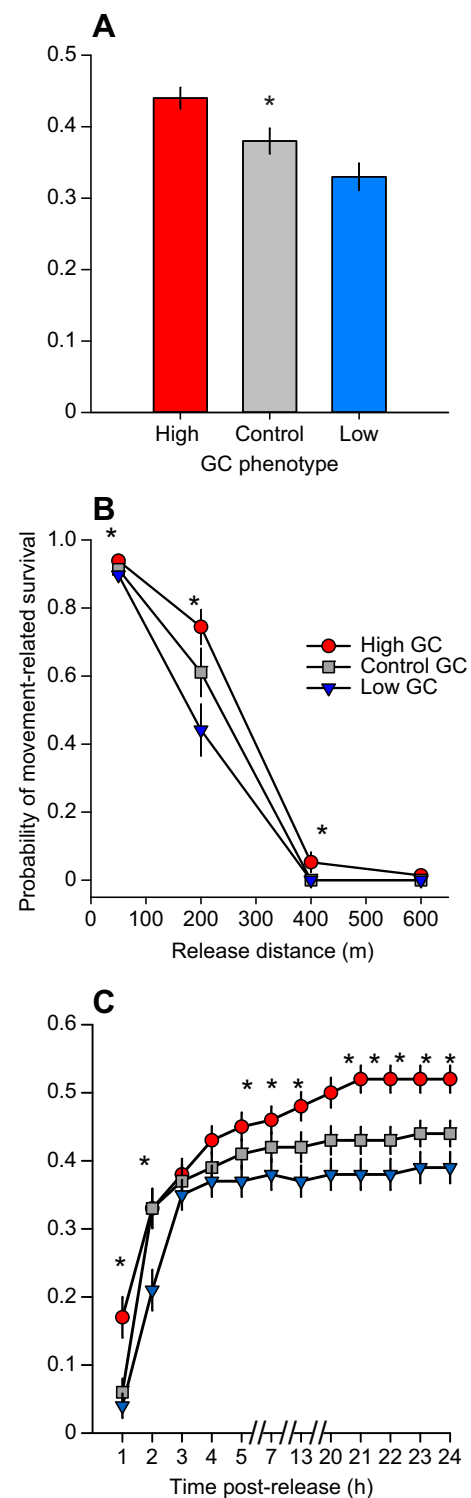
Movement-related survival was significantly influenced by the two-way interaction between CORT phenotype treatment and release distance from water (GLMM  $\chi^2=130.37$ ;  $P<0.001$ ). Significant *post hoc* comparisons ( $P<0.05$ ) indicated that this effect was evident at 50, 200 and 400 m release distances; but not at 600 m where survival was extremely low for all GC phenotypes (Fig. 3B, Table S3). Toads with the high GC phenotype had significantly increased movement-related survival at the release distances of 50, 200 and 400 m relative to toads with control or low GC phenotypes. Movement-related survival for toads with the control phenotype was significantly better ( $P<0.05$ ) than the low GC at the release distance of 200 m.

Movement-related survival of cane toads was also significantly influenced by the two-way interaction between GC phenotype treatment and time post-release (GLMM  $\chi^2=107.66$ ;  $P<0.001$ ). Toads with the high GC phenotype returned to the water source more quickly, resulting in significantly higher levels of survival (*post hoc* comparisons with  $P<0.05$ ; Table S4) at most time intervals post-release compared with levels in toads with control or low GC phenotypes (Fig. 3C). Movement-related survival for toads with the control phenotype was significantly better than with the low GC phenotype (*post hoc* comparisons with  $P<0.05$ ; Table S4) at most time intervals post-release. A significant three-way interaction among GC phenotype treatment, release distance and time post-release (GLMM  $\chi^2=64.93$ ;  $P<0.001$ ; *post hoc* comparisons with  $P<0.05$ ; Table S5) also indicated how complex dynamics affected toad movement-related survival (Fig. 4).

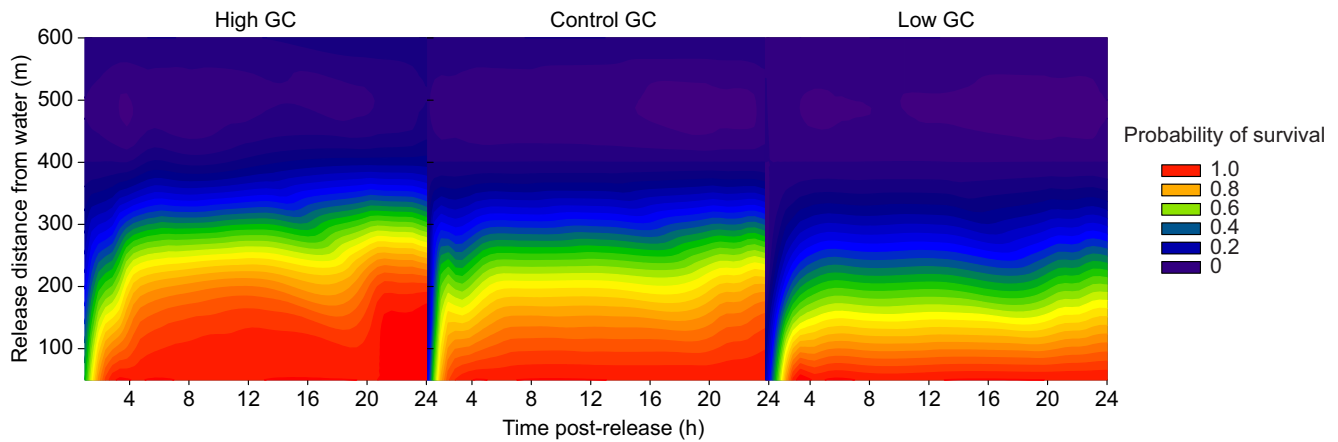
## DISCUSSION

Animals use irruptive movement triggered by pervasive environmental phenomena to avoid fitness costs arising from stressor exposure and subsequent disturbance to their local environment (Walls et al., 2005; Wingfield and Ramenofsky, 2011). However, with little warning to prepare phenotypic responses to pervasive and stochastic environmental stressors, individuals must rely on standing, or rapidly induced, physiology and behaviour (Breuner and Hahn, 2003; Wingfield and Ramenofsky, 1997). Intuitively, in such circumstances, an individual's GC phenotype could regulate many behavioural and physiological traits, that affect irruptive movement capacity (Breuner and Hahn, 2003; Crespi et al., 2013; Wingfield and Ramenofsky, 1997).

This study investigated the interplay between three different CORT phenotypes and four different levels of environmental stressor pervasiveness on movement-related survival in the invasive cane toad under natural conditions. Our study suggested an important role for the magnitude of the CORT-mediated stress response in regulating irruptive movement performance to facilitate the survival of individual cane toads under extreme environmental condition faced at their arid range front. On average, toads engineered to produce a high CORT phenotype obtained better movement-related survival outcomes compared with control or low CORT phenotypes (Fig. 3A). Similarly, the mean effect of the



**Fig. 3. Effect of different corticosterone phenotypes and interactions with distance from water and time post-release on movement-related survival of cane toads.** (A) Survival in toads with different corticosterone phenotypes. (B,C) Interactions with (B) distance from water and (C) time post-release on movement-related survival of cane toads. The high and low GC phenotypes refer to toads injected with ACTH and metyrapone, respectively. The control treatment refers to toads with no manipulation to their HPA axis. Each graph represents the survival outcome for 120 individuals sampled within each treatment. The asterisk denotes at least one significant ( $*P<0.05$ ) *post hoc* comparison among treatments or within each distance or time sampling interval. Each graph represents the survival outcome for 120 individuals sampled within each treatment. All values are means $\pm$ s.e.m.



**Fig. 4. Contour plots demonstrating the three-way interaction among different corticosterone phenotypes, distance from water and time post-release on movement-related survival of cane toads.** The high and low CORT phenotypes refer to toads injected with ACTH and metyrapone, respectively. The control treatment refers to toads with no manipulation to their HPA axis. Each graph represents the survival outcome for 120 individuals sampled within each treatment.

control CORT phenotype was to obtain better movement-related survival outcomes compared with low CORT phenotypes. This suggests that higher CORT phenotypes can affect regulation of many traits to enhance physiological and behavioural attributes of movement to increase survival. For example, elevated CORT is well demonstrated to trigger intracellular GC receptors that lead to upregulation of intermediary (e.g. glucose) and organismal metabolism. Increased intermediary metabolism could promote improved locomotor activity and endurance capacity, facilitating movement in vertebrates (Cash and Holberton, 1999; DuRant et al., 2008; Ramage-Healey and Romero, 2001; Wack et al., 2012). High CORT phenotypes could also influence behavioural attributes that facilitate movement-related survival in toads by affecting motivation and fearfulness, which also affect physical performance (Anson et al., 2013; Belliure et al., 2004; Cote et al., 2006; Thaker et al., 2009; Wingfield and Ramenofsky, 2011).

More importantly, our results clearly demonstrated the contextual nature of CORT-mediated effects on movement-related survival outcomes to environmental stress. This result was evident because unlike most studies that only manipulate an animal's CORT phenotype, we also experimentally regulated the level of environmental stressor pervasiveness (i.e. release distance from water). This additional manipulation allowed us to subject toads to both different exposure durations to harsh daily abiotic conditions and the associated costs of movement needed by individuals to successfully return to the safety of water. As a consequence, we were able to observe a significant interaction between CORT phenotype and stressor pervasiveness to mediate different movement-related survival outcomes. At the most benign stressor exposure (i.e. a release distance from water of 50 m), toads with the high CORT phenotype attained significantly better movement-related survival compared with survival in toads with control and low CORT phenotypes. We attribute this difference to the high CORT phenotype causing greater upregulation of physiological and behavioural traits that allowed toads to simply move faster. However, given the significant overall increase in movement-related survival at this low level, compared with the three higher levels of stressor pervasiveness, it also suggests that under weaker selection a broader range of CORT phenotypes can confer a greater number of similar fitness outcomes.

At the next two higher levels of environmental stress exposure (i.e. release distances of 200 and 400 m), it was more evident that the

experimental CORT phenotypes could produce greater variation the movement-related survival among individuals. Here, movement-related survival increased across individuals with low, control and high CORT phenotypes, respectively. For toads to successfully return to water at these release distances they presumably need increased time and greater physical effort to survive. Thus increased physiological demands arising from exposure to hot diurnal temperatures and higher costs of movement could then explain how different CORT phenotypes, via effects on physiological and behaviour attributes, produce significant differences in toad movement capacity and ensuing survival.

However, for the longest and most pervasive release distance (600 m), the three CORT phenotypes did not differ in their influence on movement-related survival. Indeed, with the exception of a single individual (with the high CORT phenotype) that successfully returned to water, toad survival was otherwise negligible for all CORT phenotypes. We attribute this result to the fact that this longest release distance caused toads to face extended exposures to lethally hot diurnal temperatures and higher energetic costs that effectively caused homeostatic overload and high mortality (Romero, 2004; Wingfield, 2013). This result is important as it clearly demonstrates that pending a stressor's pervasiveness, variation in an individual's CORT phenotype, although affecting irruptive movement, can confer no fitness advantage.

Given toads with the control CORT phenotype had on average intermediate movement-related survival capacity compared to individuals with the experimentally increased and decreased CORT phenotypes, what might be the relevance of our results? First, we acknowledge that by using ACTH and metyrapone to physiologically manipulate the toad HPA axis we have produced individuals within each treatment that exhibit a distribution of CORT phenotypes that are significantly different in their mean (i.e. Fig. 1), and possibly variances, relative to controls (i.e. natural phenotypic variation). The second consequence of experimental manipulations is to then produce individuals with movement-related survival outcomes that exist beyond the normal range of phenotypic abilities observed for this toad population. Thus additional study is now necessary to evaluate to what extent natural phenotypic variation in CORT might influence movement-related survival in cane toads. This study would require experiments that first involve 'CORT phenotyping' of individuals to demonstrate the repeatability of CORT measures within, and among,

individuals. This is a clear requirement to demonstrate that individuals indeed possess distinct CORT phenotypes (Wada et al., 2008). Performing such phenotyping experiments, via repeated blood-sampling and timely hormone analysis (under field conditions), prior to conducting movement-related survival experiments would clearly add considerable logistical complexity to a study such as ours. Nevertheless, it is plausible to suggest that natural phenotypic variation in CORT is correlated with variation in toad movement-related survival. Other studies have reported significant relationships between natural variation in an individual's CORT phenotype and its fitness (Breuner et al., 2008; Romero and Wikelski, 2001).

In conclusion, variation in GC phenotypes and subsequent regulatory consequences for phenotypic performance are thought to be instrumental in how animals mediate fitness outcomes to ecological or environmental stressors (Angelier and Wingfield, 2013; Bonier et al., 2009; Jessop et al., 2004; Wingfield, 2013). Here, we focused on how experimental differences in the CORT phenotype influenced irruptive movement, a key strategy by which animals avoid fitness consequences during exposure to unpredictable environmental stressors. This study contributes to the role the GC hormones can have in explaining fitness outcomes arising from animal movements undertaken during exposure to environmental stressors or during life-history related dispersal events (Belliere et al., 2004; Belthoff and Dufty, 1998; Clobert, 2012; De Fraipont et al., 2000; Silverin, 1997; Wingfield and Ramenofsky, 1997). However, as suggested here, generalizations for how variation in CORT phenotypes affects movement performance and arising fitness implications in animals could be complex and contextual (Angelier and Wingfield, 2013; Hau et al., 2016; Taff and Vitousek, 2016). Thus, to better understand how phenotypic variation in CORT could affect movement-related fitness in animals clearly necessitates detailed consideration of multiple attributes relating to the environmental stressor (e.g. pervasiveness, scale and duration), costs of movement (e.g. energetic, time or opportunity costs) and the inherent movement capacity of the organism (Bonte et al., 2012; Clobert, 2012; Ketterson et al., 2009). We suggest that future studies could attempt to explicitly measure these different attributes, and their interactions, with variation in CORT phenotypes that affect an individual's movement, or other components of organismal performance, to better understand the arising animal fitness outcomes to stressors.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: T.J., J.W., T.D., M.L., B.F.; Methodology: T.J., J.W., T.D., M.L., B.F.; Software: T.J.; Validation: T.J.; Formal analysis: T.J.; Investigation: T.J., J.W.; Resources: T.J., J.W.; Data curation: T.J.; Writing - original draft: T.J., B.F.; Writing - review & editing: T.J., M.L., B.F.; Visualization: T.J.; Supervision: T.J.; Project administration: T.J., T.D., M.L.; Funding acquisition: T.J.

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#### Supplementary information

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**Table S1.** GLMM results evaluating the effects of GC treatment and interactions with release distance from water and time since release on cane toad movement-related survival

**Table S2.** Pairwise comparison results for post-hoc analyses for GC treatment on toad movement-related survival.

**Table S3.** Pairwise comparison results for post-hoc analyses for GC treatment by release distance interval on toad movement-related survival

**Table S4.** Pairwise comparison results for post-hoc analyses for GC treatment by time since release on toad movement-related survival.

**Table S5.** Pairwise comparison results for post-hoc analyses for GC treatment by distance from water by time since release on toad movement-related survival.

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