

Interactions between Corticosterone Phenotype, Environmental Stressor Pervasiveness and Irruptive Movement-Related Survival

Tim S. Jessop¹, Jonathan Webb², Tim Dempster³, Benjamin Feit^{4,5} and Mike Letnic⁵

¹ Centre for Integrative Ecology, Deakin University, Victoria, 3220, Australia.

² School of the Environment, University of Technology Sydney, NSW 2007, Australia.

³ School of Biosciences, University of Melbourne, Victoria, 3010, Australia.

⁴Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

⁵School of Biological, Earth and Environmental Sciences, University of New South Wales, NSW 2052, Australia.

Corresponding author: Tim Jessop, t.jessop@deakin.edu.au

Summary Statement: Irruptive movement-related survival is influenced by an interaction between corticosterone phenotype and environmental stressor pervasiveness.

Keywords: phenotypic engineering, glucocorticoids, irruptive movement, fitness, stressor magnitude

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 alternate 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 standing, 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 to demonstrate that corticosterone regulated movement performance, nor the interaction with the 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 to 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 among 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.

List of Abbreviations

GC: glucocorticoid

CORT: corticosterone

HPA axis: Hypothalamic-Pituitary-Adrenal Axis

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 induced 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 standing, 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 hormones (GC) 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). GC, 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 performance of 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 CORT levels and the performance of physical or locomotor activity that facilitates animal movement has been extremely well documented in laboratory rodents (Coleman et al., 1998; Girard and Garland Jr, 2002; Malisch et al., 2008; Malisch et al., 2006; Stranahan et al., 2008). Similarly, addition of exogenous CORT has been shown to stimulate activity or increase endurance attributes of movement in mammals (Devenport et al., 1993), birds (Breuner et al., 1998), lizards (Belluore 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 effect 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 GC 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 whilst 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 among an individual's among CORT and movement phenotype, but also 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*) inhabiting semi-arid Northern Australia. Survival and movement of cane toad in this environment are strongly influenced by highly seasonal variation in environmental temperature and water availability (Letnic et al., 2015; Letnic et al., 2014; Webb et al., 2014). In particular, during the hot-dry season (September-November), previous research has indicated that toads must restrict daily movements within a radius of >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. Here 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 it also allowed us to 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 to 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. a 50 m release distance 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 became more pervasive (i.e. 200 and 400m release distances from water), we predicted that selection would favour toads with the high CORT phenotype that would produce] better movement allowing for higher survival. Finally, we predicted that toads exposed to the most intense stressor level (i.e. a 600 m release distance from water), regardless of their CORT phenotype, may achieve poor movement-related survival.

MATERIAL 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; 90% CI] and 38.6°C [35.1-41.9; 90% CI] (sourced from Australian Bureau of Meteorology). Because of these harsh seasonal environmental constraints during the dry season, cane toads are aggregated in close proximity (<100 meters) 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 km²). 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- 19:00, we rapidly hand captured 120 toads/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 1ml syringe and 27 gauge needle 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 intra-peritoneally (IP) with 100 µg/kg metyrapone (Sigma Chemical) suspended in 100 µl of vehicle solution (comprising ethanol: Ringer's saline solution at 1:1 vol/vol). 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 comprising the high CORT phenotype group (40 toads/night) were injected IP with freshly thawed 50 IU/kg 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 more responsive/ larger and hence "high" GC hormone phenotype. Toads that comprised the control CORT phenotype (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 and avoid

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 minutes 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 hr, toads (housed in containers) were moved by vehicle and then as groups of thirty individuals (comprised of 10 each of high, low and control GC hormone phenotypes) were released at one of four distances of 50, 200, 400 and 600 meters 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; Letnic et al., 2014) that allowed us to rapidly inventory all individual that successfully returned from their post-release distance drop off point. Commencing at 8:30 pm 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 hr post release. These intervals reflected that because toads are nocturnally active that they would move at night (hence the intensive nocturnal sampling bouts at 1-7 and 20-24 hrs post release) but during the day they would be inactive(hence the non-intensive diurnal sampling at 13 and 20 hrs post release). The 24 hr post-release duration was decided as the maximum time interval for checking for successful return movement as we recoded no individual beyond the time period within 72 hours of continuous monitoring conducted during the study. 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 hrs 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 hr capture-stress protocol. Here using 90 toads (i.e. 30 toads/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/treatment were immediately blood sampled, whilst the remaining 20 toads/treatment were restrained by placing each individual into a calico bags (10 cm x 10cm) that prevented them from freely moving and escaping. All restrained toads were placed into a large plastic container and held within a vehicle until blood samples were collected from 10 individuals/treatment at 4 and 8 hrs 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 of plasma was sufficient for assay use (Jessop et al., 2013a). Plasma samples were twice extracted in 3 mL of diethyl ether. The efficiency of extraction was measured by adding 20 µl of 3H-CORT (~2000 CPM) (MP Biomedicals, Ohio, USA). To estimate steroid extraction efficiency, 50 µl of each extracted sample was placed into a scintillation vial containing 2 ml of 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). We calculated final steroid concentrations from standard curves and then corrected for individual sample recovery and addition of 3H-CORT. Average extraction efficiency for CORT over the four assays was $88.5 \pm 0.56\%$. 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 EIA 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) was used to analyze 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) a 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 (< 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 * release distance and GC phenotype * time since release) and one three-level interaction (GC phenotype * release distance * time since release) on the categorical dependent variable (i.e. toad survival post release). Toad movement-related survival data was represented by 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 (R Core Team, 2017) and the packages base, nlme (Pinheiro et al., 2014), Car (Fox and Weisberg, 2011) and multcomp (Hothorn et al., 2017).

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 hrs 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 by 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 (mean response: 195.4 ± 12.3 ng/ml) produced significantly more CORT relative to the metyrapone (mean response: 70.87 ± 12.1 ng/ml; $P < 0.001$) and unmanipulated control treated toads (mean response: 111.17 ± 11.2 ng/ml; $P < 0.001$). Metyrapone injected toads produced significantly lower CORT levels compared to 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 hr) on toad movement-related survival under field conditions in semi-arid Australia. All main and interactive model terms had significant effects on toad movement-related survival (Table 1; Table S1; Fig. 2).

The main effect of CORT phenotype treatment induced significant differences in toad movement-related survival (GLMM $\chi^2 = 99.77$; $P < 0.001$). Differences among GC hormone

treatment indicated that high CORT phenotype toads (0.44 ± 0.04 mean survival probability) attained on average better movement-related survival compared to control CORT (0.38 ± 0.04 mean survival probability) and the low CORT phenotype toads (0.33 ± 0.04 mean survival probability) (Fig. 3A). Significant pairwise post-hoc treatment differences were evident between high vs. control ($P < 0.05$), and high vs. low CORT treatments ($P < 0.05$; Fig. 3A, Table S2).

Movement-related survival was significantly influenced by the 2-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 CORT phenotype had significantly increased movement-related survival at the release distances of 50, 200 and 400 m relative to toads with control or low CORT phenotypes. Movement-related survival for toads with the control phenotype was significantly better ($P < 0.05$) than the low CORT at the release distance of 200 m.

Movement-related survival of cane toads was also significantly influenced by the 2-way interaction between CORT phenotype treatment and time post release (GLMM $\chi^2 = 107.66$; $P < 0.001$). Toads with the high CORT phenotype returned faster to the water source resulting in significantly higher survival (post-hoc comparisons with $P < 0.05$; Table S4) being recorded at most time intervals post release compared to toads with control or low CORT phenotypes (Fig. 3C). Movement-related survival for toads with the control phenotype was significantly better than the low CORT phenotype (post-hoc comparisons with $P < 0.05$; Table S4) at most time intervals post release. A significant 3-way interaction among CORT phenotype treatment, release distance and time post release (GLMM $\chi^2 = 64.93$; $P < 0.001$) 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 to control or low CORT phenotypes (Fig. 3A). Similarly, the mean effect of the control CORT phenotype was to obtain better movement-related survival outcomes compared to 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;

Remage-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 that too 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 to 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, compared to the three higher levels of, stressor pervasiveness it also suggests that under weaker selection a broader range of CORT phenotypes can confer more similar fitness outcomes.

At the next two higher levels of environmental stress exposure (i.e. release distances of 200 and 400m), 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 treated with low, control and the 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 (treated 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. As findings from 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 (Bellure 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

No competing interests declared.

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REFERENCES

- Angelier, F. and Wingfield, J. C.** (2013). Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *General and Comparative Endocrinology* **190**, 118-128.
- Anson, J. R., Dickman, C. R., Boonstra, R. and Jessop, T. S.** (2013). Stress triangle: do introduced predators exert indirect costs on native predators and prey? *PLoS One* **8**, e60916.
- Belliure, J., Meylan, S. and Clobert, J.** (2004). Prenatal and postnatal effects of corticosterone on behavior in juveniles of the common lizard, *Lacerta vivipara*. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* **301**, 401-410.
- Belthoff, J. R. and Dufty, A. J.** (1998). Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. *Animal Behaviour* **55**, 405-415.
- Bonier, F., Martin, P. R., Moore, I. T. and Wingfield, J. C.** (2009). Do baseline glucocorticoids predict fitness? *Trends in Ecology & Evolution* **24**, 634-642.
- Bonte, D., Van Dyck, H., Bullock, J. M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K. and Saastamoinen, M.** (2012). Costs of dispersal. *Biological Reviews* **87**, 290-312.
- Breuner, C. W., Greenberg, A. L. and Wingfield, J. C.** (1998). Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). *General and Comparative Endocrinology* **111**, 386-394.
- Breuner, C. W. and Hahn, T. P.** (2003). Integrating stress physiology, environmental change, and behavior in free-living sparrows. *Hormones and behavior* **43**, 115-123.
- Breuner, C. W., Patterson, S. H. and Hahn, T. P.** (2008). In search of relationships between the acute adrenocortical response and fitness. *General and Comparative Endocrinology* **157**, 288-295.
- Breuner, C. W., Sprague, R. S., Patterson, S. H. and Woods, H. A.** (2013). Environment, behavior and physiology: do birds use barometric pressure to predict storms? *Journal of Experimental Biology* **216**, 1982-1990.
- Cash, W. B. and Holberton, R. L.** (1999). Effects of exogenous corticosterone on locomotor activity in the red-eared slider turtle, *Trachemys scripta elegans*. *The Journal of experimental zoology* **284**, 637-644.
- Clobert, J.** (2012). Dispersal ecology and evolution: Oxford University Press.
- Coleman, M. A., Garland Jr, T., Marler, C. A., Newton, S. S., Swallow, J. G. and Carter, P. A.** (1998). Glucocorticoid response to forced exercise in laboratory house mice (*Mus domesticus*). *Physiology & behavior* **63**, 279-285.
- Cote, J., Clobert, J., Meylan, S. and Fitze, P.** (2006). Experimental enhancement of corticosterone levels positively affects subsequent male survival. *Hormones and behavior* **49**, 320-327.
- Crespi, E. J., Williams, T. D., Jessop, T. S. and Delehanty, B.** (2013). Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? *Functional Ecology* **27**, 93-106.
- De Fraipont, M., Clobert, J., John, H., Alder and Meylan, S.** (2000). Increased pre-natal maternal corticosterone promotes philopatry of offspring in common lizards *Lacerta vivipara*. *Journal of Animal Ecology* **69**, 404-413.
- Dickens, M. J., Delehanty, D. J. and Romero, L. M.** (2009). Stress and translocation: alterations in the stress physiology of translocated birds. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb. 2008.1778.
- DuRant, S. E., Romero, L. M., Talent, L. G. and Hopkins, W. A.** (2008). Effect of exogenous corticosterone on respiration in a reptile. *General and Comparative Endocrinology* **156**, 126-133.

- Florance, D., Webb, J. K., Dempster, T., Kearney, M. R., Worthing, A. and Letnic, M.** (2011). Excluding access to invasion hubs can contain the spread of an invasive vertebrate. *Proceedings of the Royal Society of London B: Biological Sciences* **278**, 2900-2908.
- Fox, J. and Weisberg, S.** (2011). An R companion to applied regression: Sage Publications.
- Girard, I. and Garland Jr, T.** (2002). Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. *Journal of applied physiology* **92**, 1553-1561.
- Hardman, B. and Moro, D.** (2006). Optimising reintroduction success by delayed dispersal: is the release protocol important for hare-wallabies? *Biological Conservation* **128**, 403-411.
- Hau, M., Casagrande, S., Ouyang, J. Q. and Baugh, A. T.** (2016). Glucocorticoid-mediated phenotypes in vertebrates: multilevel variation and evolution. *Advances in the Study of Behavior* **48**, 41-115.
- Hayes, T. B. and Wu, T. H.** (1995). Interdependence of corticosterone and thyroid hormones in toad larvae (*Bufo boreas*). II. Regulation of corticosterone and thyroid hormones. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **271**, 103-111.
- Heupel, M., Simpfendorfer, C. and Hueter, R.** (2003). Running before the storm: blacktip sharks respond to falling barometric pressure associated with Tropical Storm Gabrielle. *Journal of fish biology* **63**, 1357-1363.
- Hothorn, T., Bretz, F., Westfall, P., Heiberger, R. M., Schuetzenmeister, A., Scheibe, S. and Hothorn, M. T.** (2017). Package 'multcomp': Obtenido de <http://cran.stat.sfu.ca/web/packages/multcomp/multcomp.pdf>.
- Jessop, T., Sumner, J., Lance, V. and Limpus, C.** (2004). Reproduction in shark-attacked sea turtles is supported by stress-reduction mechanisms. *Proceedings of the Royal Society of London B: Biological Sciences* **271**, S91-S94.
- Jessop, T. S., Letnic, M., Webb, J. K. and Dempster, T.** (2013a). Adrenocortical stress responses influence an invasive vertebrate's fitness in an extreme environment. *Proceedings of the Royal Society of London B: Biological Sciences* **280**, 20131444.
- Jessop, T. S., Woodford, R. and Symonds, M. R.** (2013b). Macrostress: do large-scale ecological patterns exist in the glucocorticoid stress response of vertebrates? *Functional Ecology* **27**, 120-130.
- Ketterson, E. D., Atwell, J. W. and McGlothlin, J. W.** (2009). Phenotypic integration and independence: hormones, performance, and response to environmental change. *Integrative and Comparative Biology* **49**, 365-379.
- Le, P. P., Friedman, J. R., Schug, J., Brestelli, J. E., Parker, J. B., Bochkis, I. M. and Kaestner, K. H.** (2005). Glucocorticoid receptor-dependent gene regulatory networks. *PLoS genetics* **1**, e16.
- Lea, M.-A., Johnson, D., Ream, R., Sterling, J., Melin, S. and Gelatt, T.** (2009). Extreme weather events influence dispersal of naive northern fur seals. *Biology letters* **5**, 252-257.
- Letnic, M., Webb, J. K., Jessop, T. S. and Dempster, T.** (2015). Restricting access to invasion hubs enables sustained control of an invasive vertebrate. *Journal of applied ecology* **52**, 341-347.
- Letnic, M., Webb, J. K., Jessop, T. S., Florance, D. and Dempster, T.** (2014). Artificial water points facilitate the spread of an invasive vertebrate in arid Australia. *Journal of applied ecology* **51**, 795-803.
- Malisch, J. L., Breuner, C. W., Gomes, F. R., Chappell, M. A. and Garland Jr, T.** (2008). Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. *General and Comparative Endocrinology* **156**, 210-217.
- Malisch, J. L., Saltzman, W., Gomes, F. R., Rezende, E. L., Jeske, D. R. and Garland Jr, T.** (2006). Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. *Physiological and Biochemical Zoology* **80**, 146-156.
- Narayan, E. J., Cockrem, J. and Hero, J.-M.** (2013). Changes in serum and urinary corticosterone and testosterone during short-term capture and handling in the cane toad (*Rhinella marina*). *General and Comparative Endocrinology* **191**, 225-230.

- Nathan, R., Getz, W. M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D. and Smouse, P. E.** (2008). A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences* **105**, 19052-19059.
- Piersma, T. and Drent, J.** (2003). Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution* **18**, 228-233.
- Pinheiro, J., Bates, D., DebRoy, S. and Sarkar, D.** (2014). R Core Team (2014) nlme: linear and nonlinear mixed effects models. R package version 3.1-117. Available at <http://CRAN.R-project.org/package=nlme>.
- Preen, A. and Marsh, H.** (1995). Response of dugongs to large-scale loss of seagrass from Hervey Bay, Queensland Australia. *Wildlife Research* **22**, 507-519.
- Remage-Healey, L. and Romero, L. M.** (2001). Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **281**, R994-R1003.
- Romero, L. M.** (2004). Physiological stress in ecology: lessons from biomedical research. *Trends in Ecology and Evolution* **19**, 249-255.
- Romero, L. M. and Wikelski, M.** (2001). Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events. *Proceedings of the National Academy of Sciences* **98**, 7366-7370.
- Scholnick, D. A., Weinstein, R. B. and Gleeson, T. T.** (1997). The Influence of Corticosterone and Glucagon on Metabolic Recovery from Exhaustive Exercise in the Desert Iguana *Dipsosaurus dorsalis*. *General and Comparative Endocrinology* **106**, 147-154.
- Silverin, B.** (1997). The stress response and autumn dispersal behaviour in willow tits. *Animal Behaviour* **53**, 451-459.
- Stranahan, A. M., Lee, K. and Mattson, M. P.** (2008). Central mechanisms of HPA axis regulation by voluntary exercise. *Neuromolecular medicine* **10**, 118-127.
- Streby, H. M., Kramer, G. R., Peterson, S. M., Lehman, J. A., Buehler, D. A. and Andersen, D. E.** (2015). Tornadoic storm avoidance behavior in breeding songbirds. *Current Biology* **25**, 98-102.
- Taff, C. C. and Vitousek, M. N.** (2016). Endocrine flexibility: optimizing phenotypes in a dynamic world? *Trends in Ecology and Evolution* **31**, 476-488.
- Thaker, M., Vanak, A. T., Lima, S. L. and Hews, D. K.** (2009). Stress and aversive learning in a wild vertebrate: the role of corticosterone in mediating escape from a novel stressor. *The American Naturalist* **175**, 50-60.
- Wack, C. L., DuRant, S. E., Hopkins, W. A., Lovern, M. B., Feldhoff, R. C. and Woodley, S. K.** (2012). Elevated plasma corticosterone increases metabolic rate in a terrestrial salamander. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **161**, 153-158.
- Wada, H., Salvante, K. G., Stables, C., Wagner, E., Williams, T. D. and Breuner, C. W.** (2008). Adrenocortical responses in zebra finches (*Taeniopygia guttata*): individual variation, repeatability, and relationship to phenotypic quality. *Hormones and behavior* **53**, 472-480.
- Walls, S. S., Kenward, R. E. and Holloway, G. J.** (2005). Weather to disperse? Evidence that climatic conditions influence vertebrate dispersal. *Journal of Animal Ecology* **74**, 190-197.
- Webb, J. K., Letnic, M., Jessop, T. S. and Dempster, T.** (2014). Behavioural flexibility allows an invasive vertebrate to survive in a semi-arid environment. *Biology letters* **10**, 20131014.
- Wingfield, J. and Ramenofsky, M.** (1997). Corticosterone and facultative dispersal in response to unpredictable events. *Ardea* **85**, 155-166.
- Wingfield, J. C.** (2013). Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. *Functional Ecology* **27**, 37-44.
- Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M. and Richardson, R. D.** (1998). Ecological bases of hormone—behavior interactions: the “emergency life history stage”. *American Zoologist* **38**, 191-206.
- Wingfield, J. C. and Ramenofsky, M.** (2011). Hormone-behavior interrelationships of birds in response to weather. In *Advances in the Study of Behavior*, vol. 43, pp. 93-188: Elsevier.

Table 1. A GLMM model summary table reporting statistical results for the effects of Cort phenotype and its 2-way and 3-way interactions with distance from water and time since release on movement-related survival of cane toads under field conditions in semi-arid Australia.

Term	χ^2	DF	P value
Intercept	15.41	1	< 0.001
CORT Phenotype	99.77	2	< 0.001
CORT Phenotype x distance from water	130.37	3	< 0.001
CORT Phenotype x time since release	107.67	3	< 0.001
CORT Phenotype x distance from water x time since release	64.93	3	< 0.001

Figures

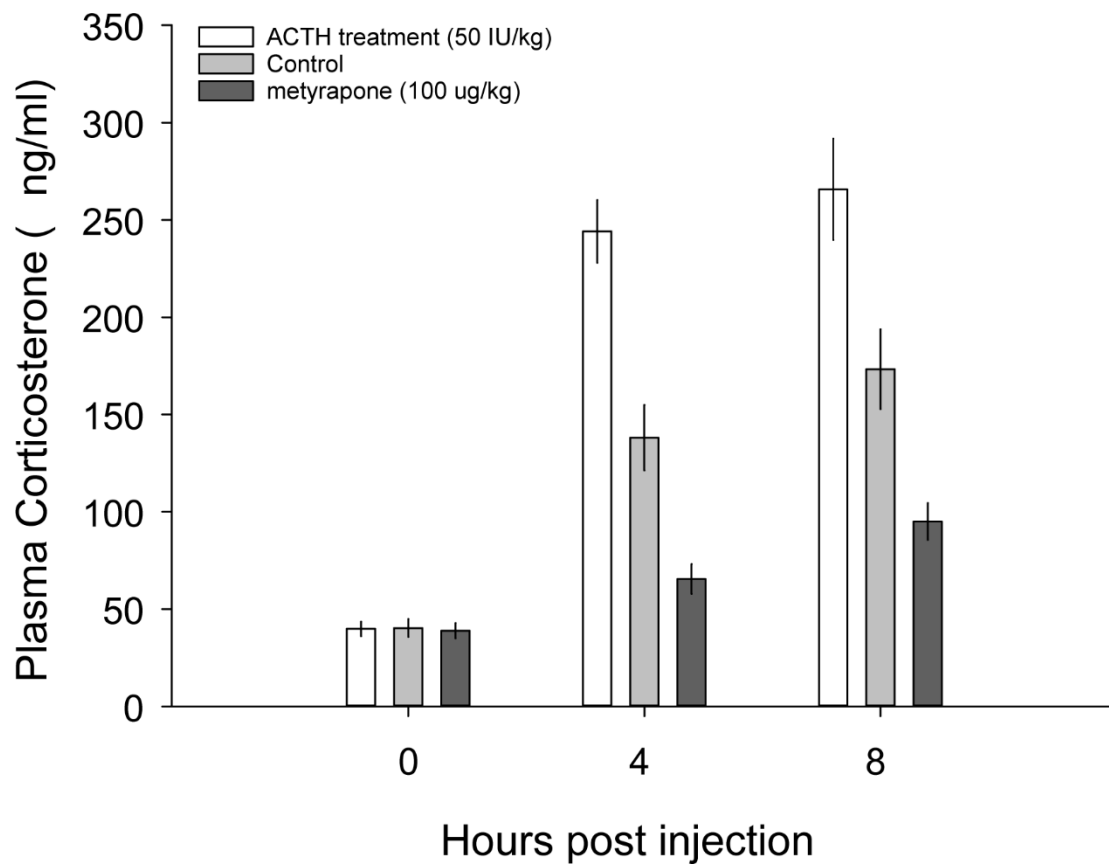


Fig. 1. Effects of ACTH and metyrapone injections, relative to controls, on plasma corticosterone levels of cane toads subjected to a capture stressor under field conditions in semi-arid Australia. The x-axis presents the time post capture at the time of blood sampling. Each bar represents the mean value and standard error for 10 individuals for each treatment sampled at each time period.

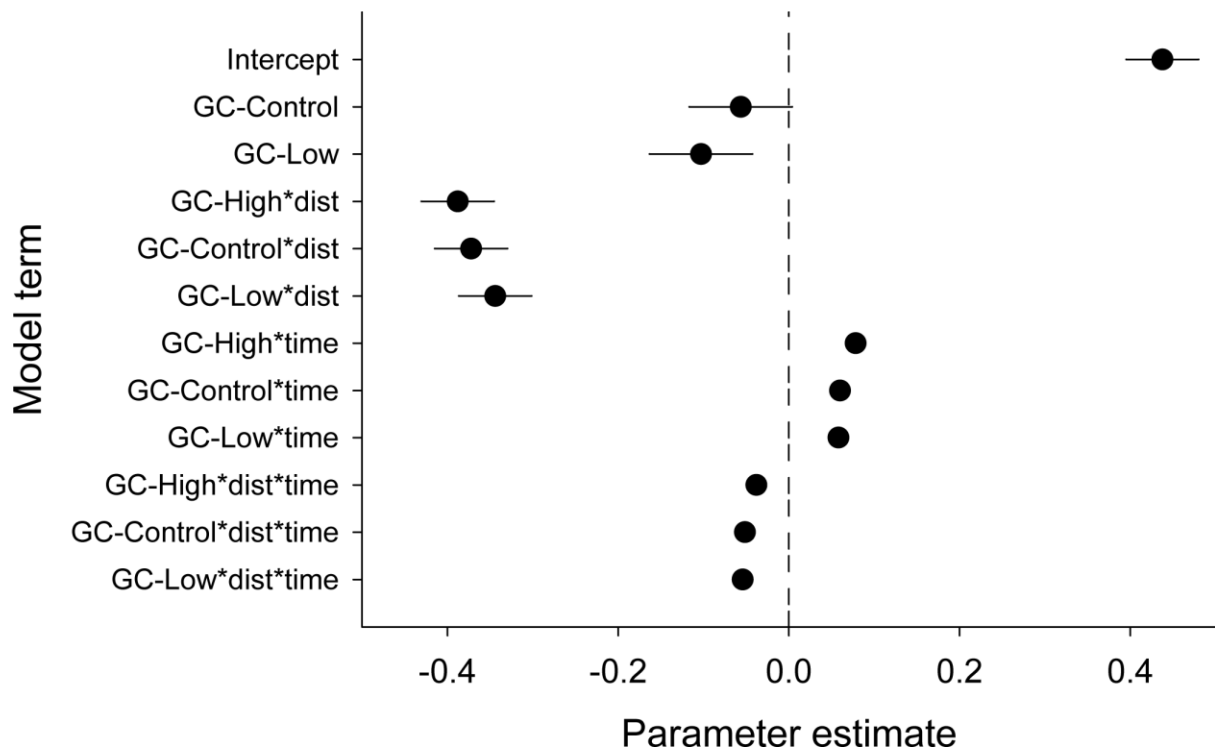


Fig. 2. **Coefficients of measured variables and interactions, and their effects on movement-related survival in cane toads under field conditions in semi-arid Australia.**

Circles indicate parameter estimates and lines represent 95% confidence intervals from a multi-level factorial the effect of CORT phenotype with distance of release and time since release two- and three way- interactions on movement-related survival in cane toads (n = 360).

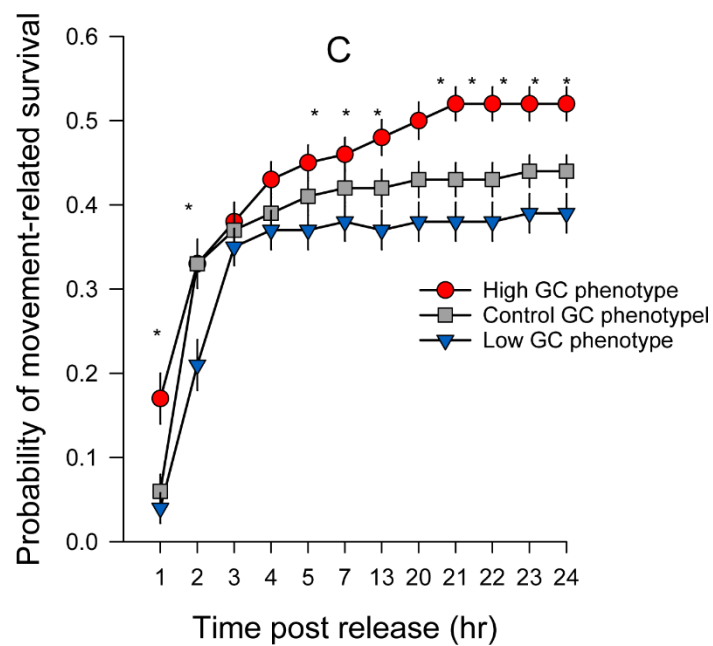
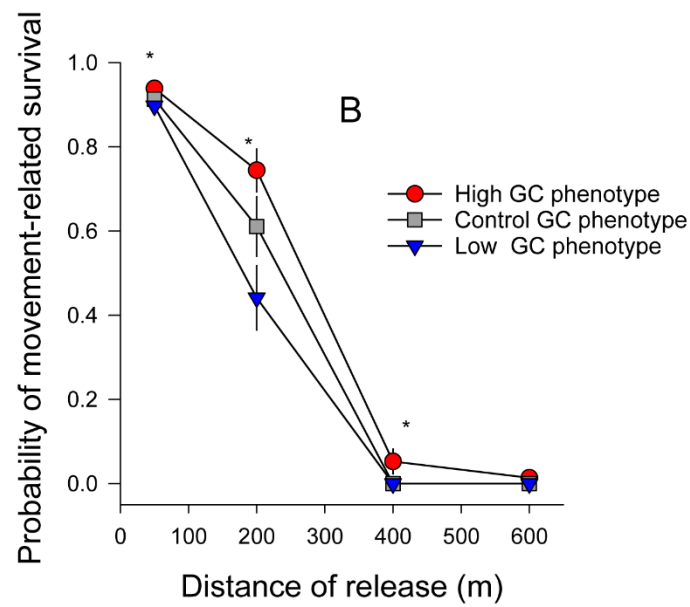
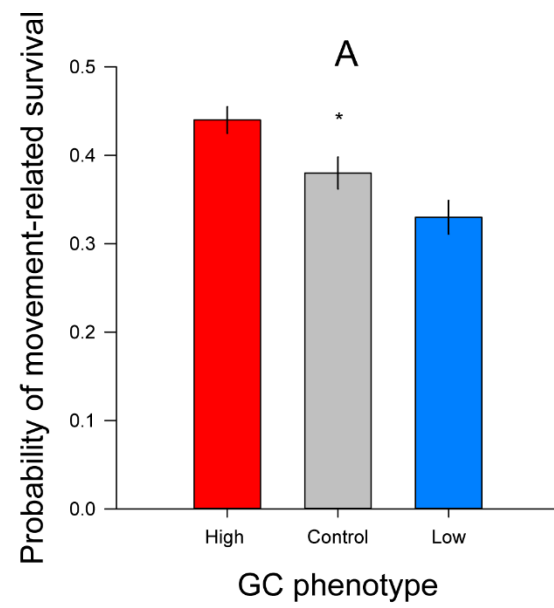


Fig 3. Line and scatter plots demonstrate the effects of different corticosterone phenotypes (A) and interactions with distance from water (B) and hours post release (C) on mean (\pm SEM) movement-related survival of cane toads. The high and low CORT phenotypes refer to toads injected with ACTH and metyrapone, respectively. The control treatment refers to toad with no manipulation to their HPA axis. Each graph represents the survival outcome for 120 individuals sampled within each treatment. The asterix 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.

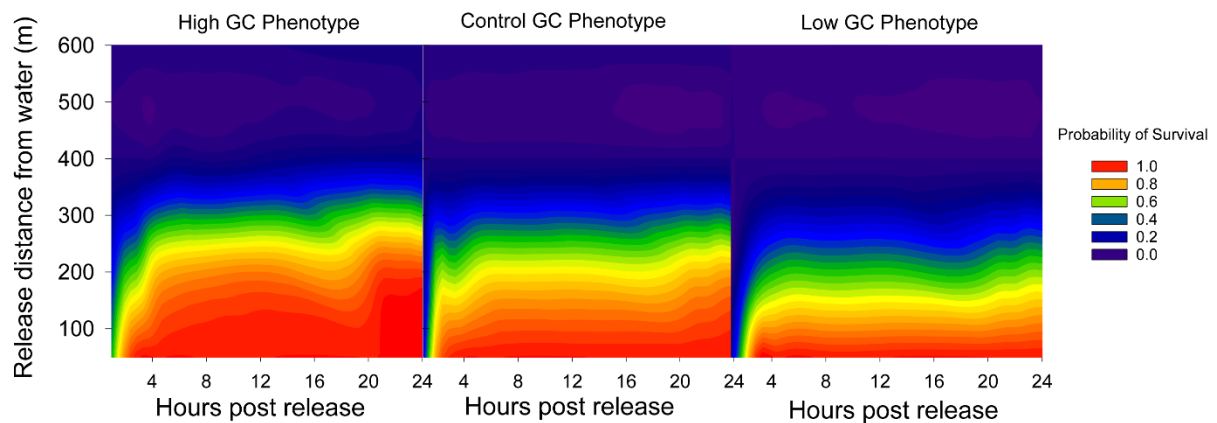


Fig 4. Contour plots demonstrate the 3-way interaction among different corticosterone phenotypes, distance from water and hours post release on movement-related survival of cane toads. The high and low CORT phenotypes refer to toads injected with ACTH and metyrapone, respectively. The control treatment refers to toad with no manipulation to their HPA axis. Each graph represents the survival outcome for 120 individuals sampled within each treatment.

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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