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Research paper

Individual variation in ACTH-induced cortisol levels in females of a livebearing fish at different gestational stages

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ABSTRACT

Individuals vary in their baseline levels of stress hormones (predictive homeostasis) and in their stress responses (reactive homeostasis). Variation in normal reactive scope, both predictive and reactive homeostasis, may be important for understanding how endocrine traits respond to selection. Reactive homeostasis is the increase in glucocorticoid (GCs) hormones above baseline. Individuals at different life history stages, such as gestation in females, may show variation in normal reactive scope. We performed an adrenocorticotropic hormone (ACTH) challenge and measured changes in circulating GCs to estimate the reactive scope of female sailfin mollies (*Poecilia latipinna*) at different gestational states. We measured cortisol, primary GC in teleost fishes, to obtain baseline release rates prior to injection with either ACTH or saline control. Using water-borne hormones, we measured cortisol release rates at four time intervals post-injection. Females were then sacrificed to determine the developmental stage of embryos, if present, and the number of developing embryos or mature ova. We found that ACTH-injected females had significant increases in cortisol releases rates, whereas cortisol release rates of control females did not change during the 4 h post-injection period. We found high repeatability in predictive homeostasis of cortisol and moderate repeatability in reactive homeostasis and a phenotypic correlation between predictive and reactive homeostasis. Gestational state did not affect female predictive or reactive homeostasis. We applied the reactive scope model to *P. latipinna* and gained a further understanding of how among- and within-individual variation in both predictive and reactive homeostasis are partitioned and how these traits vary under certain life-history conditions.

1. Introduction

Endocrine systems are highly variable within populations and across contexts (e.g. circadian and seasonal differences, age-dependence, life-history stages), and we are beginning to understand the extent to which endocrine traits vary among individuals (Biro and Stamps, 2015; Cox et al., 2016; Williams, 2008). Hormones are important for coordinating multiple facets of the phenotype including physiology, behavior, life history, and morphology (Taff and Vitousek, 2016). Therefore, understanding individual differences in hormone responses can be informative about adaptation and the evolution of complex traits, such as the stress response (Dufty et al., 2002; Hau et al., 2016; Ketterson and Nolan, 1999; Zera et al., 2007). The stress response promotes immediate survival through mobilizing energy stores, often at the expense of other life history traits such as reproduction (Sapolsky et al., 2000; Wingfield and Sapolsky, 2003). The stress response may also show some degree of heritability (Cox et al., 2016). As a trait that shows variation among individuals and is heritable, the stress response can respond to selection in ways that maximize fitness benefits.

The stress response is a complex physiological mechanism that regulates an organism's response to perturbations and typically is measured through changes in glucocorticoid (GC) hormones before and after exposure to an aversive stimulus. The GC stress response is mediated through the HPA/I-axis (hypothalamus–pituitary–adrenal/interrenal) and GCs are the signaling hormones for target tissues in this physiological pathway (Wendelaar Bonga, 1997). Exposure to stressors initiate the release of CRH (corticotropin-releasing hormone) from the hypothalamus, which in turn induces the release of ACTH (adrenocorticotropic hormone) from the anterior pituitary. ACTH activates glucocorticoid synthesis, which then produces several physiological responses to cope with stressors. Romero et al. (2009) proposed the reactive scope model as an explanatory, graphical model that integrates homeostasis and allostasis to describe the stress response. According to the reactive scope model, individuals

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vary in some physiological mediator, such as GC levels (we use cortisol for this study), at both baseline levels (predictive homeostasis) and at increases above baseline levels in response to unpredictable events (reactive homeostasis). Predictive homeostasis varies according to life-history demands and therefore encompass circadian variation for seasonal and non-seasonal species. Species may show seasonal variation that corresponds to breeding or gestation events or species may show non-seasonal patterns (little to no variation across seasons) in predictive homeostasis (Romero, 2002). Any exposure to a stressor will drive increases in cortisol into the reactive range, but in healthy individuals, levels should rapidly return to baseline after the stressor has ended to maintain homeostasis. Combined, both predictive and reactive homeostasis constitute the normal reactive scope. Below the normal reactive scope is homeostatic failure, in which levels of cortisol are too low to maintain homeostasis. Any further increases in cortisol beyond the normal reactive scope is homeostatic overload, which can result in reduced immune function, suppressed reproduction, and decreased growth (DuRant et al., 2016; McEwen and Wingfield, 2003; Romero, 2004). The threshold for homeostatic overload presumably does not vary with circadian or circannual rhythms but may be reduced when an individual experiences frequent, chronic exposure to stressful events (e.g., Narayan et al., 2015).

The reactive scope model accounts for some among-individual variation in predictive homeostasis but the extent to which among- and within-individual differences explain variation in the normal reactive scope is not consistent across taxa, nor across study conditions (Hau et al., 2016; Taff and Vitousek, 2016). Calculating repeatability of traits is a useful estimate to understand how among- and within-individual phenotypic variance is partitioned. However, studies on the repeatability of stress hormone titers do not show consistent patterns with respect to either the predictive or reactive ranges. Greater within-individual variation than among-individual variation (low repeatability) in predictive ranges has been shown in birds (e.g. great tits, *Parus major*, Baugh et al., 2014; house sparrows, *Passer domesticus*, Romero and Reed, 2008), and fish (e.g. largemouth bass, *Micropterus salmoides*, Cook et al., 2011). Conversely, high repeatability in predictive ranges has also been shown in other bird species (e.g. Florida scrub-jay, *Aphelcoma coerulescens*, Rensel and Schoech, 2011) and amphibians (e.g. Fijian ground frog, *Platymantis vitiana*, Narayan et al., 2013; Narayan and Hero, 2013). In contrast to the mixed results of predictive ranges, reactive ranges shows more consistent patterns of high repeatability (largemouth bass, *Micropterus salmoides*: Cook et al., 2011; Fijian ground frog, *Platymantis vitiana*: Narayan et al., 2013; Narayan and Hero, 2013; Florida scrub-jay, *Aphelcoma coerulescens*: Rensel and Schoech, 2011), but see Baugh et al. (2014) for an example of no repeatability of reactive homeostasis.

Additionally, there may be some correlation between predictive and reactive ranges within individuals. Predictive and reactive ranges can be positively correlated (e.g., individuals with high predictive values have high reactive values) as was found in great tits, *Parus major*, exposed to acute handling stress (Baugh et al., 2014), and predictive and reactive ranges can also be negatively correlated (e.g., individuals with higher predictive values have a constrained reactive value) as was found in Fijian ground frogs, *Platymantis vitiana* (Narayan et al., 2013). The direction of the correlation between predictive and reactive ranges may provide insight into the flexibility of the homeostatic overload threshold. If the homeostatic overload threshold is fixed, then individuals with high predictive values may show reduced or constrained reactive values. Conversely, a flexible homeostatic overload threshold may allow both predictive and reactive values to show correlated increases in the presence of stressors. Measuring repeatability of both the predictive and reactive ranges provides the upper limit to the heritability of these endocrine traits and therefore provides information about the extent to which the stress response can evolve (Bonier and Martin, 2016; Cox et al., 2016; Hau et al., 2016).

Gestation is likely to be a major source of circannual variation observed in the predictive range of cortisol in seasonal species (Romero, 2002; Wingfield and Sapolsky, 2003). Reproduction requires considerable energetic investment (Stearns, 1992) and increases in stress hormones during this period may aid females by facilitating access to energy stores such as increasing blood glucose levels, breakdown of lipids, and inhibition of protein synthesis (Sapolsky et al., 2000). Therefore, gestating females may have greater predictive values of cortisol than non-gestating females (Romero, 2002). For example in female Fijian ground frogs, *Platymantis vitiana*, both baseline and stress response corticosterone (primary GC in amphibians and reptiles) values were higher in vitellogenic females than in non-vitellogenic females (Narayan and Hero, 2013). However, gravid female tuatara, *Sphenodon punctatus*, had greater baseline corticosterone and a dampened corticosterone response compared to non-gravid females (Anderson et al., 2014). Gestational stage of developing embryos may also affect circulating stress hormones of females, particularly at later stages of development. In some mammals, females show increases in cortisol just prior to parturition (Cavigelli, 1999; Pavitt et al., 2016). In addition, females with larger brood sizes may have greater reproductive effort, hence greater energetic investment, and may also show greater levels of stress hormones (Algera et al., 2017). Therefore, understanding how predictive and reactive ranges of cortisol differ within a species based on breeding phenology can identify different sources of variation in the normal reactive scope.

In this study, we estimate the parameters of the reactive scope model as proposed by Romero et al. (2009), using changes in cortisol as the physiological mediator. First, we test the hypothesis that there is a correlation between predictive (nominal baseline) and reactive (stress response) homeostasis of cortisol, regardless of within- and among-individual variation in these ranges. We also test the hypothesis that baseline and stress response ranges of cortisol in individual females will correlate with reproductive status. We predict that as females progress in their gestational state, baseline should increase. Similarly, we predict that female baseline should increase as a function of increasing brood size. Additionally, stress response also may vary with gestation and brood size.

We tested the reactive scope model using female sailfin mollies, *Poecilia latipinna*. This species of livebearing fish typically carry broods for ~30 days and are mainly lecithotrophic, where embryos rely on yolk for nutrition rather than through maternal provisioning (i.e. placental nutrition, Pollux et al., 2014). *Poecilia latipinna* have a long breeding season, especially in constant temperature springs in southern temperate North America (Robinson et al., 2011) and females have multiple broods per year. Female and male *P. latipinna* form loose aggregations (shoals) of conspecific and heterospecific individuals (Schlupp and Ryan, 1996). There is no social structure or dominance hierarchies among females, but males exhibit alternative mating phenotypes based on male size (Ptacek and Travis, 1996; Snelson, 1985). To test our hypotheses, we performed an ACTH challenge, which should provide upper range estimates for the reactive homeostasis, on female *P. latipinna* at different stages of gestation and measured changes in cortisol, the primary GC of teleosts (Arterbery et al., 2010; Mommsen et al., 1999; Wendelaar Bonga, 1997).

2. Materials and methods

2.1. Animal collection and housing

We collected *P. latipinna* (n ≈ 120) from Spring Lake, Hays County, Texas (29.89°N, 97.82°W) in January 2015 and brought