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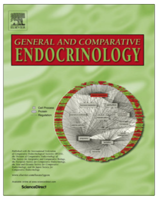

Does corticosterone mediate predator-induced responses of larval *Hylarana indica*?

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ABSTRACT

Prey-predator interactions have been studied extensively in terms of morphological and behavioural responses of prey to predation risk using diverse model systems. However, the underlying physiological changes associated with morphological, behavioural or life historical responses have been rarely investigated. Herein, we studied the effect of chronic predation risk on larval growth and metamorphosis of *Hylarana indica* and the underlying physiological changes in prey tadpoles. In the first experiment, tadpoles were exposed to a caged predator from Gosner stage 25–42 to record growth and metamorphosis. Further, whole body corticosterone (CORT) was measured to determine the physiological changes underlying morphological and life historical responses of these prey tadpoles. Surprisingly, tadpoles experiencing continuous predation risk grew and developed faster and metamorphosed at a larger size. Interestingly, these tadpoles had significantly lower CORT levels. In the second experiment, tadpoles were exposed to predation risk (PR) or PR + CORT from stage 25–42 to determine the role of CORT in mediating predator-induced responses of *H. indica*. Tadpoles facing continuous predation risk grew and developed faster and metamorphosed at a larger size reinforcing the results of the first experiment. However, when CORT was administered along with predation risk, tadpoles grew and developed slowly leading to delayed metamorphosis. Interestingly, growth and metamorphic traits of tadpoles exposed to PR + CORT were comparable to those of the control group indicating that exogenous CORT nullified the positive effect of predation risk. Apparently, CORT mediates predator-induced morphological responses of *H. indica* tadpoles by regulating their physiology.

1. Introduction

Though phenotypic traits are controlled by genes, environment plays a crucial role in shaping these traits by interacting with genes thus, affecting their expression leading to a particular phenotypic trait (Miner et al., 2005). In many animal taxa, organisms have an inherent capacity of producing a range of phenotypes depending on the environmental conditions experienced during early development. This flexibility of producing alternative phenotypes referred to as phenotypic plasticity, involves changes in behaviour, morphology and/or physiology in response to changing environment (Pigliucci, 2001). Phenotypic responses of organisms to environmental alterations are the consequences of modifications in developmental programs hence, also referred to as developmental plasticity as a substitute for phenotypic plasticity (Gilbert, 2001). Although phenotypic or developmental plasticity helps in coping with the changing environment and survival during critical periods, phenotypic adaptations are costly to produce and maintain, thus bear additional costs (Denver, 2009). Hence, these adaptations carry fitness costs leading to a trade-off between fitness and survival (Denver et al., 1998). Recent studies have shown that glucocorticoids are important in mediating the environmental effects on phenotypic traits (Denver, 2009). In vertebrates, the activation of neuroendocrine stress axis (hypothalamus–pituitary adrenal axis, HPA axis) in response to environmental stressors is essential for survival during critical periods (Romero, 2002). Environmental changes acting as stressors stimulate higher brain centres, which in turn, activate endocrine hypothalamus to secrete corticotropin releasing factor (CRF). The CRF stimulates anterior pituitary leading to the release of corticotropin (adrenocorticotrophic hormone, ACTH). Eventually, corticotropin released into the peripheral blood reaches adrenal cortex and stimulates it to secrete corticosteroids (glucocorticoids and mineralocorticoids). Glucocorticoids thus released in response to acute stress are critical for coordinating many physiological processes in addition to mobilising energy

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reserves helping the animals to overcome the stress situation (Maher et al., 2013; Romero, 2002; Wingfield and Kitaysky, 2002).

Prey-predator interaction plays a crucial role in shaping ecological communities. Predation acts as a major selective force in determining the distribution and abundance of prey populations in any ecosystem (Relyea, 2000). Moreover, predators mould life histories of prey populations by indirectly affecting different aspects of their life such as morphology, physiology, behaviour and life history (Alford, 1999; Kats and Dill, 1998; Orizaola and Braña, 2005). Consequently, prey animals are always under intense pressure to evolve novel strategies that would help them in escaping/reducing predation risk. Predators can affect their prey either directly by killing and thus reducing the prey density or indirectly by altering prey behaviour, morphology and physiology leading to a compromised phenotype (Lima, 1998). The majority of studies have focussed on the indirect (non-lethal) effects of predation on morphology and behaviour of diverse vertebrate prey (Lima, 1998). However, very few studies have tried to link the physiological changes underlying phenotypic responses of prey to predation risk (Hossie et al., 2010). The activation of HPA axis leading to secretion of glucocorticoids works in many kinds of stress situations and thus, appears to be the ideal pathway for the manifestation of predator-induced responses (Hossie et al., 2010). Hence, anti-predator responses of prey animals might be mediated through glucocorticoids, known as stress response hormones in animals (Hossie et al., 2010).

Anurans are excellent model systems to understand prey-predator interactions due to their complex life cycles and encounter diverse native as well as non-native predators throughout their larval development. Moreover, predator-induced changes in behaviour and morphology that together cause slower growth and development have been established very well inanuran tadpoles (Relyea, 2002; Van Buskirk and Yurewicz, 1998). Recent studies have implicated corticosterone (CORT), the principal physiological glucocorticoid in amphibians, in mediating predator-induced responses of amphibians (Hossie et al., 2010; Maher et al., 2013). Further, CORT is known to inhibit tadpole growth and development thus delaying metamorphosis (Glennemeier and Denver, 2002). Furthermore, CORT is known to alter many physiological processes leading to changes in tadpole morphology such as tail length and tail depth that serve as an adaptation in escaping from predators (Hossie et al., 2010; Maher et al., 2013). Increased CORT levels are known to increase activity and stimulate foraging behaviour (Fraker et al., 2009; Maher et al., 2013).

Effects of predation may not be restricted only to the period of exposure, but might have latent effects that are visible at or even after metamorphosis (Pechenik, 2006). Experience with predators can delay metamorphosis of amphibian larvae leading to the emergence of smaller froglets, which in turn, can affect post-metamorphic survival and growth rate (Cabrera-Guzman et al., 2013). Experiences during larval development might alter adult traits as the latter are dependent on the former (Pechenik et al., 1998). Therefore, it is important to study the association between early larval traits and metamorphic traits. It is suggested that CORT in combination with thyroid hormones regulates metamorphosis in amphibians (Hayes, 1997). Interestingly, CORT may work either synergistically with or antagonistically to thyroid hormones depending upon the developmental stage of exposure and energy levels (Hayes, 1997). Moreover, CORT is known to affect size at metamorphosis by regulating food intake during larval development (Denver, 2009). Thus, CORT seems to be important in determining predator-induced changes in larval life-history traits of amphibian tadpoles.

Tadpoles of the Indian Golden-backed frogs (*Hylarana indica*) inhabit gently flowing rivers, streams and isolated pockets of water and require 3–4 months to complete metamorphosis (Mogali et al., 2016). These perennial sources of water are home to a diverse community of predators including the nymphs of dragonflies that are known to feed voraciously on different stages of anuran larvae (Batabyal et al., 2014). Surprisingly, tadpoles of *H. indica* respond to chronic predation risk by metamorphosing early but, at a larger size (Mogali et al., 2016). Moreover, they do not alter their morphology in response to chronic predation risk. Since stress hormones (glucocorticoids) are known to mediate the effects of stress in many animals including amphibians, we hypothesised that the antipredator responses of larval *H. indica* could be the result of alteration in the levels of corticosterone. Hence, we used larval *H. indica* to determine the role of corticosterone in mediating the predator-induced changes in growth, morphology and metamorphic traits.

2. Materials and methods

2.1. Study animal and rearing

Two freshly deposited egg masses of *H. indica* were collected from a gently flowing stream in the Western Ghats near Anmod, Karnataka, India (15°4′*N*, 74°33′*E*) in the morning hours of December 8, 2013, and December 9, 2014. At collection, the embryos were undergoing gastrulation (stage 10–11) according to Gosner's staging table (1960). The egg masses were quickly transported to the laboratory, where they were maintained in plastic tubs (33 cm diameter) at room temperature until hatching. The eggs hatched 7–8 days after collection, following which the hatchlings/tadpoles were maintained in glass aquaria (60 cm × 45 cm × 15 cm) until used for experimentation.

Nymphs of the dragonfly (*Bradinopyga geminata*) collected from a semi-permanent pond located on the S. P. Pune University campus were used as predators. The nymphs ($M = 22.7$ mm ± 0.61) were maintained individually in perforated plastic cups until used for experimentation and fed with conspecific tadpoles. The study was carried out following the guidelines of departmental committee for animal ethics (In India, animals other than mammals do not come under the purview of institutional committee for animal ethics, No. 538/CPCSEA).

Two experiments were conducted; experiment I involving assessment of the effect of chronic predation risk on larval growth, morphology, physiology and metamorphic traits of *H. indica* and Experiment II, to determine the effects of predation risk in combination with CORT (10 and 20 μg/L) on growth, morphology and metamorphic traits. The experiments were conducted during December 2013–March 2014 and December 2014–March 2015 respectively.

2.2. Experimental design

To minimise the effect of heterogeneity in anti-predator responses, tadpoles from a single clutch were used for experimentation. The experiments were initiated when the tadpoles reached stage 25 (feeding stage). Tadpoles were reared in glass aquaria equipped with a transparent, perforated plastic cup (8 cm diameter) that served as a predator cage. Use of a transparent and perforated cup as a predator cage enabled the transmission of both visual as well as chemical cues (kairomones, alarm cues and dietary cues; Peacor, 2002; Van Buskirk and Saxer, 2001). The experimental setups were as follows:

2.3. Experimental setup

Effects of predation and predation + CORT on growth, larval morphology, physiology and life-history traits were studied using

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