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A test of maternal programming of offspring stress response to predation risk in threespine sticklebacks

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HIGHLIGHTS

- Sticklebacks mounted a cortisol response to predation risk.
- Maternal experience with a predator influenced offspring response to a predator.
- The maternal effect on offspring cortisol depended on when cortisol was measured.
- Female sticklebacks had higher cortisol than males.
- There was a buffering effect of the social environment on cortisol.

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ABSTRACT

Non-genetic maternal effects are widespread across taxa and challenge our traditional understanding of inheritance. Maternal experience with predators, for example, can have lifelong consequences for offspring traits, including fitness. Previous work in threespine sticklebacks showed that females exposed to simulated predation risk produced eggs with higher cortisol content and offspring with altered anti-predator behavior. However, it is unknown whether this maternal effect is mediated via the offspring glucocorticoid stress response and if it is retained over the entire lifetime of offspring. Therefore, we tested the hypothesis that maternal exposure to simulated predation risk has long-lasting effects on the cortisol response to simulated predation risk in stickleback offspring. We measured circulating concentrations of cortisol before (baseline), 15 min after, and 60 min after exposure to a simulated predation risk. We compared adult offspring of predator-exposed mothers and control mothers in two different social environments (alone or in a group). Relative to baseline, offspring plasma cortisol was highest 15 min after exposure to simulated predation risk and decreased after 60 min. Offspring of predator-exposed mothers differed in the cortisol response to simulated predation risk compared to offspring of control mothers. In general, females had higher cortisol than males, and fish in a group had lower cortisol than fish that were by themselves. The buffering effect of the social environment did not differ between maternal treatments or between males and females. Altogether the results show that while a mother's experience with simulated predation risk might affect the physiological response of her adult offspring to a predator, sex and social isolation have much larger effects on the stress response to predation risk in sticklebacks.

1. Introduction

Non-genetic maternal effects occur when conditions experienced by mothers influence the phenotype of their offspring [1]. Maternal effects occur in diverse taxa (plants [2]; insects [3]; amphibians [4]; and mammals [5]) and their effect on offspring fitness can be maladaptive [4,6,7] or adaptive [3,8]. There is growing evidence that some maternal effects are mediated by maternal steroid hormones (sex steroids [9], and glucocorticoids [10]). For example high levels of circulating glucocorticoids in stressed mothers are transferred to developing eggs in birds, affecting offspring phenotype [11].

Studies in diverse taxa have shown that maternal stress can have long-lasting effects on offspring traits including survival [5,12–14], growth [5,15,16], morphology [5,12,13], learning [17], and behavior [17–20]. In addition, some studies have shown long-lasting effects of maternal stress on the offspring glucocorticoid response to stressors [21,22], suggesting that maternal steroids have organizational effects [23] on the development of the offspring hypothalamus–pituitary–adrenal (HPA) or, in fishes, hypothalamus–pituitary–interrenal (HPI) axis that persist throughout an offspring's lifetime. Therefore it is possible that mothers might 'program' their offspring for the types of environments they are likely to experience later in life [24,25].

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Predators are one of the most important naturally occurring stressors for animals in natural populations [26,27]. Predator-induced maternal effects have been documented in diverse taxa, with effects on multiple offspring traits [5,8,20,28–30]. A previous study on threespine stickleback fish found that predator-exposed mothers produced offspring that exhibited higher levels of shoaling behavior than offspring of control mothers [31]. Shoaling involves swimming in close proximity to conspecifics, resulting in the formation of groups [32], and is an effective antipredator defense in small fishes [33]. Female sticklebacks exposed to simulated predator attacks also produced eggs with higher concentrations of cortisol [31]. Predator exposure triggers the release of cortisol in sticklebacks [34], therefore a plausible explanation for the maternal effect observed by Giesing et al. [31] is that maternally derived cortisol diffused into eggs and induced an organizational effect on the HPI axis during offspring development. If this is the case, then offspring of predator-exposed mothers might have an altered cortisol response to a stressor compared to offspring of control mothers.

Other studies have shown a buffering effect of the social environment on the cortisol response to stressors (cows [35], pigs [36], guinea pigs [37], and gorillas [38]). Therefore we hypothesized that a maternal effect on the stickleback cortisol response might differ according to the social environment. For example, offspring of predator-exposed stickleback mothers might shoal together in order to cope with simulated predation risk, and might perceive simulated predation risk as more threatening if they do not have the opportunity to shoal.

In this study we tested the maternal programming hypothesis in sticklebacks. Specifically, we examined whether maternal experience with a simulated predation risk by Northern pike (*Esox lucius*), a natural predator of sticklebacks, influences offspring cortisol response to simulated predation risk by pike. We measured circulating plasma cortisol of adult offspring of predator-exposed and unexposed mothers before (baseline), 15 min after and 60 min after exposure to simulated predation risk by pike. We predicted that offspring of predator-exposed mothers have higher baseline and predator-induced cortisol than offspring of control mothers. To test the hypothesis that the presence of a social group during exposure to simulated predation risk buffers the glucocorticoid response of offspring, we compared adult offspring of predator-exposed and unexposed mothers that were either alone or in a group at the time of exposure to simulated predation risk. Finally, hormonally mediated maternal effects are often sex-specific [26,27,39,40]. For example, pregnant mice exposed to stressors during the first week of gestation gave birth to male offspring that as adults exhibited a larger glucocorticoid response than male offspring of control mothers, an effect not seen in female offspring [25]. Therefore we also investigated the influence of sex on the cortisol response to predation risk.

2. Materials and methods

2.1. Maternal predator exposure

Adult threespine sticklebacks were collected from Putah Creek, CA in May 2010 and were acclimated to the laboratory for at least one month before experiments began. Throughout, all fish were maintained in a flow-through system with UV, charcoal, particulate and biological filters that remove olfactory cues and a photoperiod that mimics seasonal changes. Fish were fed *ad libitum* once daily frozen bloodworms, brine shrimp, mysis shrimp, and cyclopeez, and uneaten food removed at the end of the day.

Females were exposed to simulated predation risk by exposing them to a model pike as in McGhee et al. [14]. Briefly, they were housed in 38 L tanks (53 L × 33 W × 24 H cm) with artificial plant refuges, gravel bottom and opaque external shading. Females were randomly assigned to one of six 38 L tanks ('treatment tanks') at a density of five females per tank and either exposed to simulated predation risk ('predator-exposed' mothers) or left undisturbed ('control' mothers) until they became gravid. Predator-exposed females were chased for 30 s/day at a randomly chosen time with a painted clay model of Northern pike (23 cm standard length) to simulate exposure to predation risk [31]. Predator-exposed mothers were chased once daily for 56 ± 21 days (mean ± standard deviation, range 22 to 85 days) before being stripped of eggs. Consistent with a previous study (Giesing et al. 2011), there was no effect of the number of days in the maternal treatment on offspring cortisol (results not shown). Live Northern pike have been shown to elicit a cortisol response in sticklebacks [34] and simulated attack by a model pike has been shown to elicit antipredator behaviors as compared to a non-threatening stimulus [41]. Pike do not inhabit the section of Putah Creek where sticklebacks were caught; therefore, we know that mothers did not have experience with pike before the experiment. When females became gravid, they were stripped of eggs by hand and were replaced by a marked female in order to maintain the same density.

Testes were dissected from wild-caught males from the same population, and macerated to release sperm, and the sperm was used to fertilize eggs. Sperm was used from nine different males: sperm from three males fertilized eggs from control mothers only ($n = 5$ clutches), sperm from three other males fertilized eggs from predator-exposed mothers only ($n = 5$ clutches), and sperm from three additional males fertilized eggs from both control and predator-exposed mothers ($n = 10$ clutches). Altogether, clutches were collected from $n = 10$ predator-exposed mothers and $n = 10$ control mothers. After contributing one clutch of eggs, females were no longer used in the experiment.

2.2. Rearing offspring

Offspring rearing for this experiment was carried out as described in McGhee et al. [14]. Briefly, fertilized eggs were artificially incubated in plastic cups with air bubblers. Juveniles were transferred to 38 L tanks ('tank of origin', 53 L × 33 W × 24 H cm) surrounded by opaque shading and artificial plants for refuge in either single family groups ($n = 11$ families) or mixed family groups ($n = 9$ families) within each maternal treatment. Fry were fed newly hatched brine shrimp nauplii. Offspring were reared in their tank of origin until they entered the simulated predation risk experiment (see below) in August 2011, when they were approximately one year old (standard length 3.83 ± 0.39 cm mean ± standard deviation). On average, the number of offspring surviving to adulthood per mother did not differ between maternal treatments: control mothers had 60.5 ± 15.9 surviving offspring (mean ± standard error) per mother and predator-exposed mothers had 61.2 ± 9.9 surviving offspring per mother.

The number of offspring used in the experiment from each family housed in single family tanks did not differ between maternal treatments. A total of 14.33 ± 5.37 offspring (mean ± standard error) per mother were used from control mothers and 14.83 ± 3.54 offspring per mother used from predator-exposed mothers. Mixed and single family groups of offspring were equally represented across all treatments. Since individuals in mixed family tanks were not identified by parentage, it is possible that some families were overrepresented in the mixed family tanks.

2.3. Simulated predation risk experiment

Twelve 38 L observation tanks (53 L × 33 W × 24 H cm) with artificial plant refugia and surrounded on the sides with opaque material to minimize the effect of external visual stimuli were used for the offspring simulated predation risk experiment. Tanks were assigned to one of three different 'time' treatments for sampling: