**Effects of mothers' and fathers' experience with predation risk on the behavioral
development of their offspring in threespined sticklebacks**

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18 *Abstract*

19 Stressors experienced by parents can influence the behavioral development of their
20 offspring. Here, we review recent studies in threespined sticklebacks (a species in which males
21 are the sole providers of parental care) showing that when parents are exposed to an ecologically
22 relevant stressor (predation risk), there are consequences for offspring. For example, female
23 sticklebacks exposed to predation risk produce eggs with higher concentrations of cortisol, a
24 stress hormone, and offspring with altered behavior and physiology. Male sticklebacks exposed
25 to predation risk produce offspring that are less active, smaller, and in poorer condition. The
26 precise mechanisms by which maternal and paternal experiences with predators affect offspring
27 phenotypes are under investigation, and could include steroid hormones, olfactory cues and/or
28 parental behavior. As in other species, some of the consequences of parental exposure to
29 predation risk for offspring in sticklebacks might be adaptive, but depend on the stressor, the
30 reliability of the parental and offspring environments and the evolutionary history of the
31 population.

32

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Introduction

There is growing evidence that parents' experiences can influence the next generation. For example, female crickets exposed to cues of predatory spiders produced offspring with heightened antipredator behavior that were more likely to survive encounters with predators [1]. Indeed, offspring of stressed parents differ from offspring of unstressed parents in many respects, including morphology [2], physiology [3], and behavior [1,4]. Because paternal care is relatively rare among taxa, and because mothers often have intimate contact with their developing embryos and offspring, most studies to date have focused on the consequences of maternal stress for offspring. However, there is growing appreciation that stressors experienced by fathers can also affect the next generation [5-7]. For example, stressors experienced by fathers prior to mating influence offspring via effects on seminal fluid [8] and sperm [7,9]. There is also evidence that stressors experienced by fathers *post*-fertilization can affect offspring—the mechanisms underlying post-fertilization paternal effects are less understood, but could be mediated indirectly through changes in maternal care [10] or could reflect direct effects of fathers on offspring via, for example, changes in paternal provisioning behavior.

Recent work in our lab has investigated the effects of maternal and paternal stress on offspring phenotypes in threespined sticklebacks (*Gasterosteus aculeatus*). Sticklebacks are a small fish distributed throughout the northern hemisphere that are renowned for their geographic variation in behavior [11]. For example, sticklebacks from populations that are subject to strong predation have morphological, behavioral and physiological adaptations for coping with predators [12]. Another important feature of the stickleback system is that like many other fish species, sticklebacks exhibit paternal care, where the father is solely responsible for providing care that is necessary for offspring survival. In this paper, we summarize the effects of maternal

and paternal exposure to predation risk on offspring in sticklebacks, discuss their potential adaptive significance and mechanisms, and outline several key areas for future work. While we focus on sticklebacks, it is worth pointing out that many of our findings are similar to studies on mammals and other taxa, which suggests that the way parental stress can affect offspring extends across systems that differ dramatically in parental investment (placentas vs eggs, maternal vs paternal care, etc.).

Effects of maternal stress on offspring outcomes in sticklebacks

In a wide range of organisms, mothers that experience predation risk improve the antipredator defenses of their offspring [1-4]. These studies suggest that when females can detect reliable environmental cues of predation risk, natural selection favors adaptive maternal 'programming' of offspring behavior to match the anticipated environment [13]. However, a large literature in diverse vertebrate species has shown that maternal stress can have deleterious consequences for offspring, often mediated by glucocorticoid stress hormones (reviewed in [14-16]. We hypothesize that adaptive maternal effects are more likely to be observed when natural selection has had an opportunity to produce them, therefore we are more likely to detect adaptive maternal effects in response to an ecologically relevant stressor as opposed to a stressor that animals in natural populations are unlikely to encounter.

We examined the consequences of maternal exposure to predation risk in a series of studies in sticklebacks. The experimental protocol involves exposing female sticklebacks to predation risk during the time when they are yolking eggs (pre-fertilization), and comparing their offspring to offspring of females that were not exposed to predation risk (control females).

Females are chased by a model of a predator at an unpredictable time of day once a day for 30-40 seconds per day. This regime is meant to simulate experience in a high-predation locality.

Being chased by predators alters the composition of a female's eggs, and thus, the future developmental environment her offspring experience. Eggs of females exposed to predation risk are larger and have higher cortisol content than the eggs of control females [17], and an RNASeq study showed that maternal predator exposure influences the expression of hundreds of genes in embryos, including noncoding RNAs, genes involved in epigenetic modifications and genes involved in neural growth [18]. Moreover, there are long-lasting consequences of maternal stress for stickleback offspring. For example, maternal predator exposure influences the development of the offspring stress axis [19]. In addition, having a predator-exposed mother affects how offspring react to a predator later in life. Offspring of predator-exposed mothers are less likely to orient to a live predatory Northern pike (*Esox lucius*) than offspring of unexposed mothers [20]. This seemingly slight behavioral difference has serious survival consequences: offspring of predator-exposed mothers are captured more quickly by the predator compared to offspring of unexposed mothers [21]. Additionally, offspring of predator-exposed mothers show deficits in their ability to learn a color-reward association compared to offspring of unexposed mothers [21]. However, some of these learning deficits might be overcome by the increased tendency of offspring of predator-exposed mothers to use social information from shoalmates [22] and shoal more closely with one another than offspring of control mothers [17].

We originally hypothesized that the effects of maternal stress on offspring were mediated by glucocorticoids, specifically cortisol. Glucocorticoids have been implicated with the maladaptive consequences of maternal stress for offspring in many taxa [14-16,23]. Consistent with this literature, female sticklebacks mount a cortisol response to predation risk [24] and this

results in eggs with a higher cortisol content [25]. However, subsequent studies found that egg cortisol content drops quickly after fertilization (within 72 hours [25]), and experimental application of exogenous cortisol to developing eggs fails to affect offspring development (Paitz et al, in prep). These results suggest that predator-induced maternal effects on offspring in sticklebacks are not directly caused by cortisol, a result consistent with the emerging literature in other organisms showing that the mechanisms underlying the effects of maternal stress on offspring are complex and often include more than just the HPA axis [23].

Effects of paternal stress on offspring outcomes in sticklebacks

While maternal effects are well-documented in a diverse array of species (reviewed in [14-16]), paternal effects are less studied, even though it has been suggested that paternal effects may be similar in magnitude to maternal effects [26]. The stickleback system provides an excellent opportunity to examine post-fertilization paternal effects, as fathers are the sole providers of parental care that is necessary for offspring survival, and early studies suggested that paternal behavior shapes offspring behavior in this species [27]. During the breeding season, males defend their breeding territory from intruders and predators and attract females to spawn in their nests. After spawning, the female leaves the male's territory and the male is solely responsible for the care of the eggs. During the ~6-day incubation period, the male 'fans' (oxygenates) the eggs, removes rotten eggs and debris, and defends the territory. After embryos hatch, father sticklebacks continue to tend their newly-hatched offspring for ~7 days, chasing and retrieving fry that stray from the nest and spitting them back into the nest.

Stickleback nests are highly susceptible to predation, and fathers are constantly vigilant toward intruders. We hypothesized that fathers adjust their behavior in response to predation

125 risk, and that such adjustments might have consequences for offspring. To test this hypothesis,
126 male sticklebacks were either briefly exposed to predation risk by a model of a common nest
127 predator (sculpin, *Cottus asper*) or were unexposed to predation risk while they were caring for
128 embryos in their nest. Males were presented with a model sculpin for three minutes on the third
129 day after fertilization. This treatment was designed to simulate the types of conditions males in
130 high-predation environments might encounter – brief but dangerous encounters with local
131 predators that regularly predate nests. Adult offspring of predator-exposed fathers were smaller,
132 in poorer condition and less active than offspring of unexposed fathers [28]. Interestingly,
133 sticklebacks that were reared with predation risk [29], or have evolved with predation risk [12],
134 also have these characteristics. These data are consistent with the hypothesis that fathers alter
135 their paternal behavior in response to predation risk, and that fathers can ‘program’ their
136 offspring for living in a high-predation environment. These findings are important because while
137 we know that the care provided by mothers early in life can have a long-lasting influence on
138 offspring [30], these are some of the first data to suggest that fathers can do the same. While
139 recent studies are documenting the important consequences of paternal stress prior to conception
140 on offspring brain development [31] and HPA axis regulation [32,33] potentially via changes in
141 DNA methylation in sperm [34], these are some of the first data to show that fathers, like
142 mothers, can influence their offspring *post-fertilization*. Furthermore, we have found that father
143 care alters expression of a gene responsible for *de novo* methylation (DNMT3a) in offspring
144 brains [35], suggesting that paternal care might have long-lasting effects on offspring behavior
145 via DNA methylation.

146
147 *Conclusions and future directions*

148

149 *1. Are there interactions between maternal and paternal effects?*

150 Maternal and paternal effects on offspring are often studied in isolation of each other,
151 even though it is likely that they frequently co-occur and interact with one another [36]. For
152 example, in sticklebacks, the previous predator experiences of a mother affects the amount of
153 paternal care males provide to her offspring, with fathers providing less care, both pre- and post-
154 hatching, to offspring of predator-exposed mothers [37]. Such examples of differential allocation
155 are common [5,6]. For example, a parent might compensate or differentially allocate care based
156 on the behavior of its partner. Teasing apart the influence of fathers from the influence of
157 mothers can be challenging, but can be facilitated by studying a diverse array of species that
158 exhibit different forms of parental care (exclusive maternal care, exclusive paternal care or
159 biparental care). For example, in species such as sticklebacks with exclusive paternal care, we
160 can ask if attentive paternal care can compensate for the effects of maternal stress on offspring.
161 Moreover, it is likely that both mothers and fathers in natural populations experience stressors
162 such as predation risk, therefore examining the consequences for offspring of simultaneously
163 exposing both mothers and fathers to stressors will move us toward a more complete
164 understanding of the role parental effects play in nature (Figure 1).

165

166 *2. Are predator-induced parental effects adaptive?*

167 Determining whether parental effects are adaptive can be challenging. A recent meta-
168 analysis by Uller and colleagues [38] argued that the most convincing test involves comparing
169 the fitness of parental effects in two environments: one where offspring fitness is measured in the
170 type of environment that resembles their parent ('matched'), the other where offspring fitness is

measured in an environment different from their parents' ('mismatched'). Strong evidence for an adaptive parental effect would occur if offspring fitness (survival and reproductive success) is highest in the 'matched' environment [13,38]. Other considerations when examining the adaptive significance of predator-induced parental effects are the reliability of the environment within and across generations [13,38,39] and the evolutionary history of the population [27,40]. For example, if the environment is so variable that the likelihood that the parental and offspring environments will match is low, then selection should not favor parental effects. Instead, selection might favor adaptive parental effects if offspring are likely to encounter the same environment as their parents. However, if the environment is highly predictable across generations, we may instead predict selection to favor genetic adaptation over parental effects. In this way, genetic adaptation may allow offspring to be "buffered" from the noise experienced by their parents.

3. Can parental effects facilitate evolution?

If parental effects can be adaptive, that opens the possibility that they might evolve and even facilitate adaptation [41,42]. Parental effects might play an important role in adaptive radiations, if, for example, parents can prepare their offspring for the type of environment they are likely to encounter as populations move into new habitats or niches. Such transgenerational plasticity could allow an initial colonizing population to persist in a novel environment, and allow it to persist until genetic adaptation can occur [43-45]. Determining whether predator-induced parental effects might allow populations to cope with novel predator regimes would help us understand how particular species might become successful invaders or cope with changing environmental conditions.

194

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Figure 1. Model conceptualizing the direct and indirect effects of predation risk to mothers and fathers on offspring.