

# Sperm-mediated effects of predation risk on reproduction in male threespined sticklebacks

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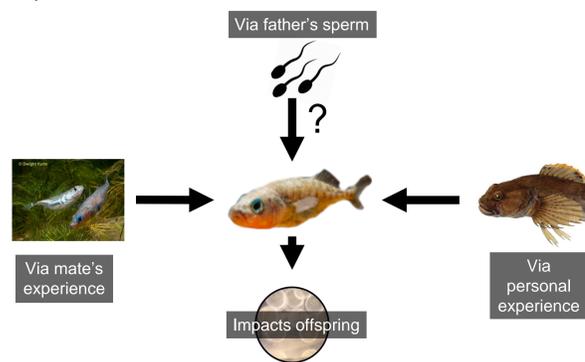
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**PRECS** Phenotypic Plasticity Research Experience for Community College Students

## Introduction

- Threespined sticklebacks, *Gasterosteus aculeatus*, exhibit **male-only parental care**.
- Male sticklebacks **decrease paternal care** if they are exposed to predators while giving care<sup>1</sup> or if their mate has prior experience with predation<sup>2</sup>. This has lasting effects on offspring behavior (behavior-mediated transgenerational plasticity)<sup>3</sup>.
- Recent studies in other animals have concluded that male exposure to predation risk **prior** to fertilization impacts offspring behavior (sperm-mediated transgenerational plasticity)<sup>4,5</sup>.
- Whether **sperm-mediated effects** exist in sticklebacks, and for how many generations the effects persist is **unknown**.



## Questions

- How does a father's experience with predation risk prior to mating influence the parenting behavior of his sons?
- Does sperm mediated plasticity persist over multiple generations (i.e. are grandsons influenced by their grandfather's experience with predation risk)?

## Hypotheses

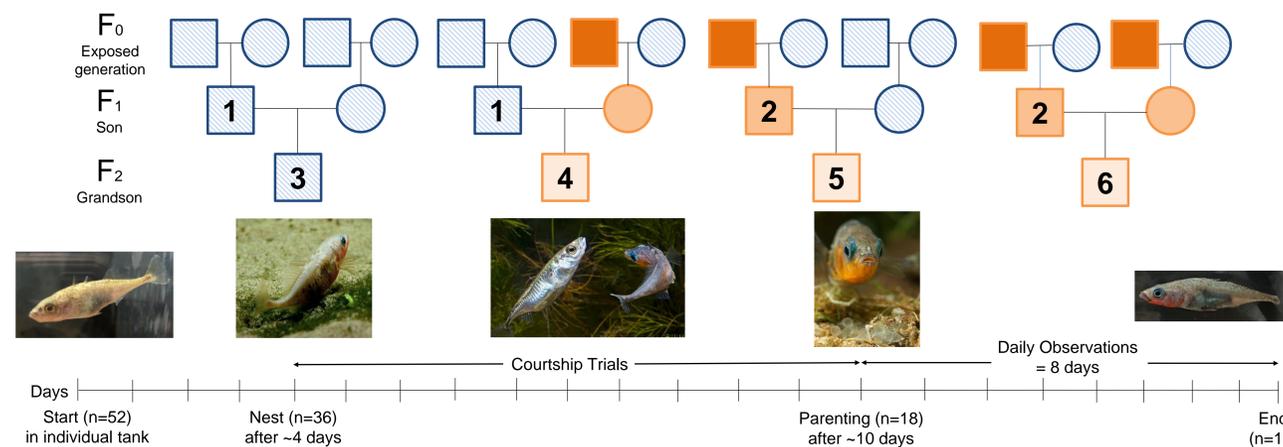
- Males with predator-exposed fathers are expected to have traits associated with high predation risk, such as reduced nuptial color and reduced fanning of nests<sup>1</sup>.
- If sperm-mediated plasticity persists over multiple generations, then males with predator-exposed grandfathers will also have traits associated with high predation risk.

## Methods

- Two treatment groups for sons: **1)** Males whose fathers had not been exposed to predators or **2)** Males whose fathers had been exposed to predators prior to fertilization (Fig. 1).
- Four treatment groups for grandsons: **3)** Neither grandfather exposed, **4)** Exposed maternal grandfather, **5)** Exposed paternal grandfather, **6)** Both grandfathers exposed (Fig. 1).
- We conducted courtship trials pairing predator unexposed females with males from each treatment group. We observed parenting behavior of males for five minutes daily for eight days post-fertilization via JWatcher (Fig. 2). Before parenting, we scored each male for nuptial colors, including throat, eye, and body coloration<sup>6</sup>. After parenting, we weighed and measured the length of each male.



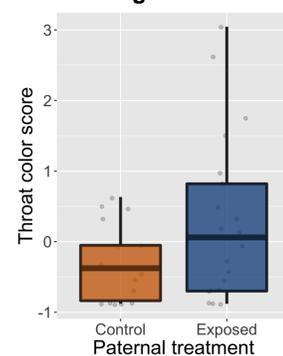
**Figure 2:** (Left) Female stickleback lays eggs in a nest built by the male. (Right) Shayne Kempfer performs JWatcher observation on parenting male post-fertilization.



**Figure 1:** (Top) The four pedigrees track ancestral predation experience of all treatment groups (1-6). Shading represents generational time since predation took place i.e. dark for individual predation experience and light for grandfather experience. Blue signifies control and orange signifies predation exposure. (Bottom) Experimental timeline with average number of days per stage and sample size for each stage. Photo credit: Shayne Kempfer 2018 (1,5) and [www.arkive.org](http://www.arkive.org) (2-4).

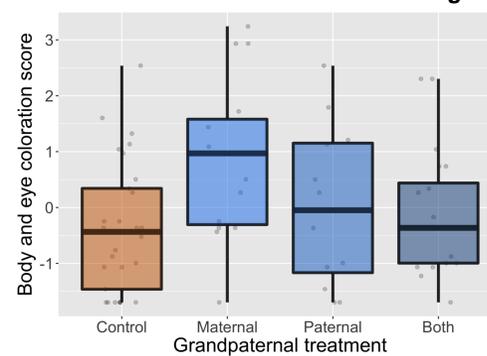
## Results

**F1s with a predator exposed father tend to have brighter throats.**

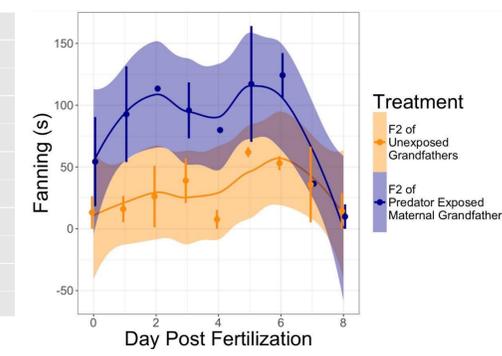


**Figure 3:** There is a borderline significant difference between the control and predator exposed paternal treatment on throat coloration (linear mixed effect model:  $F_{1,7.76}=4.04$ ,  $p=0.08$ ).

**F2s with a predator exposed maternal grandfather are significantly brighter and fan more compared to F2s with a control grandfather.**



**Figure 4:** There is a significant difference between the control and maternal grandfather treatment on male body hue and eye color (linear mixed effect model with Tukey HSD:  $Z_{13.89}=2.65$ ,  $p=0.04$ ).



**Figure 5:** Males whose maternal grandfather encountered predation fanned more than the control group (repeated measures quadratic model, fanning = treatment + day + day<sup>2</sup> with each male modeled with his own fanning curve shape):  $F_{1,3.85}=23.65$ ,  $p=0.009$ .

## Conclusions

- Preliminary F1 and F2 nuptial color data, as well as F2 fanning data, are in contrast to our initial predictions that predation exposure would reduce coloration and paternal care.
- Although parenting is a male-only trait, the significant impact of maternal grandfather predation exposure suggests that daughters of predator-exposed fathers are passing down cues to their sons.

## Future Work

- Expand the sample size in order to analyze differences in paternal care between the two F1 treatment groups as well as among the four F2 treatment groups.
- Understand how transgenerational plasticity is mediated via molecular changes to sperm.
- Investigate how paternal exposure to predation risk impacts female mate choice in the F1 and F2 generations.

## Acknowledgements

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