Sex-specific transgenerational plasticity in threespined sticklebacks

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Abstract

1. Transgenerational plasticity (TGP) – when parental environments alter the phenotype of future generations – can influence how organisms cope with environmental change. An intriguing, underexplored possibility is that sex –of both the parent and the offspring – plays an important role in driving the evolution of transgenerational plasticity in both adaptive and nonadaptive ways.
2. Here, we evaluate the potential for sex-specific TGP in a freshwater population of threespined sticklebacks (*Gasterosteus aculeatus*) by independently and jointly manipulating maternal and paternal experiences and separately evaluating their phenotypic effects in sons versus daughters. We tested the adaptive hypothesis that daughters are more responsive to cues from their mother, while sons are more responsive to cues from their father.
3. We exposed mothers, fathers, or both parents to visual cues of predation risk and measured offspring antipredator traits and brain gene expression.
4. Predator-exposed fathers produced sons that were more risk-prone, while predator-exposed mothers produced more anxious sons and daughters. Further, maternal and paternal effects on offspring survival were nonadditive: offspring with a predator-exposed father, but not two predator-exposed parents, had lower survival against live predators. There were also strong sex-specific effects on brain gene expression: exposing mothers versus fathers to predation risk activated different transcriptional profiles in their offspring, and sons and daughters strongly differed in the ways in which their brain gene expression profiles were influenced by parental experience.
5. We found little evidence to support the hypothesis that offspring prioritize their same-sex parent's experience. TGP varied with both the sex of the parent and the offspring in complicated and nonadditive ways. Failing to account for these sex-specific patterns (e.g., by pooling sons and daughters) would have underestimated the magnitude of TGP. Altogether, these results draw attention to the potential for sex to influence patterns of TGP and raise new questions about the interface between transgenerational plasticity and sex-specific selective pressures, sexual conflict, and sexual selection.

43 **Key words:** maternal effect, paternal effect, *Gasterosteus aculeatus*, phenotypic plasticity,

44 intergenerational plasticity, nongenetic inheritance, predation, stress

Introduction

Sex differences in life-histories (e.g. reproductive lifespan, mortality rate) or reproductive strategies can favor different optimal phenotypes in males and females (Andersson 1994). Although a shared genetic basis can constrain phenotypic differences between the sexes (Lande 1980; Reeve & Fairbairn 2001), epigenetic changes can overcome this constraint and allow males and females to respond differently to the same environmental condition (within-generational plasticity). Potentially adaptive sex-specific patterns of within-generational plasticity have been documented in diverse taxa (Stillwell *et al.* 2010; Ceballos & Valenzuela 2011; Xu *et al.* 2014; Meuthen *et al.* 2018); for example, in cichlids, predation risk experienced early in life influenced the development of males, but not females, possibly because males are more vulnerable to predation (Meuthen *et al.* 2018).

While less explored, there also is evidence for sex-specific ***transgenerational*** plasticity (TGP; also referred to as intergenerational plasticity or environmental parental effects); specifically, the sex of the parent and/or the offspring can alter the ways in which environments encountered by recent ancestors affect future generations. Studies and theory to date have primarily focused on the extent to which maternal experiences are integrated into offspring phenotypes; however, the biological reality is that the environment experienced by both mothers and fathers can affect future generations. For example, there is growing evidence for *paternal* effects on ecologically-important traits, which can be transmitted via paternal care as well as epigenetic changes to sperm (reviewed in (Crean & Bonduriansky 2014; Immler 2018)). Because males and females often experience different environments once they reach reproductive age and have different means of transmitting environmental cues to offspring (e.g. eggs versus sperm), the information transmitted by fathers may not match the information encoded by mothers.

Indeed, there is mounting empirical evidence that maternal versus paternal exposure to the same environmental condition can have different effects on offspring (Bonduriansky & Head 2007; Bonduriansky, Runagall-McNaull & Crean 2016; Gilad & Scharf 2019). Further, the influence of maternal environments might depend on paternal environments (or vice versa) (Mashoodh *et al.* 2012; Mashoodh *et al.* 2018; Zirbel & Alto 2018; Gilad & Scharf 2019); for example, a recent study by Lehto and Tinghitella (2020) found that stickleback females preferred duller males when either their mother or father had encountered predation risk, but preferred brighter males when both parents had experienced predation risk. Consequently, careful experimental studies that independently and jointly manipulate maternal and paternal effects are needed to understand the proximate and ultimate causes of similarities and differences between maternal and paternal effects.

Parental effects also often depend on the sex of the offspring. For example, parental environments can have opposing effects on the same trait in sons compared to daughters (Mueller & Bale 2007; Short *et al.* 2016; Braithwaite *et al.* 2017) or can influence different traits in sons versus daughters (Schulz *et al.* 2011; Metzger & Schulte 2016). Because the vast majority of studies that have compared parental effects on sons and daughters have focused on maternal effects, rather than both maternal and paternal effects (but see (He *et al.* 2016; Emborski & Mikheyev 2019)), it is unclear if sex-specific offspring effects are driven by 1) differences in the magnitude of sons' versus daughters' responses to parental environments (e.g., daughters are generally more responsive to parental stress, whether mediated by the mother or the father) or 2) how offspring attend to experiences of their same-sex versus opposite-sex parent. Offspring may attend to experiences of their same-sex parent because sex-specific differences in life history strategies or dispersal mean that daughters are more likely to encounter

the environments experienced by their mothers while sons are likely to encounter environmental pressures similar to their fathers. In order to evaluate the possibility that offspring selectively prioritize experiences of one parent over the other, it is necessary to compare maternal and paternal effects on both sons and daughters.

Sex-specific TGP might have important adaptive implications if it can resolve evolutionary conflicts that occur when selection favors different phenotypes in males and females (Bonduriansky & Day 2008). Mothers and fathers may selectively alter the phenotypes of their sons and daughters in response to the environment with a greater degree of precision than genetic inheritance and in ways that may match the distinct life-history strategies of males and females. Alternatively, sexual conflict could result in complex nonadaptive sex-dependent patterns, especially when sexual selection is strong (Burke, Nakagawa & Bonduriansky 2019). Here, we evaluate the potential for sex-specific TGP in threespined sticklebacks (*Gasterosteus aculeatus*). Male and female sticklebacks are sexually dimorphic in several respects, including in habitat use (Reimchen 1980), diet (Reimchen & Nosil 2001), parasite load (Reimchen & Nosil 2001), and morphology (Reimchen, Steeves & Bergstrom 2016), with these differences beginning to emerge during early adulthood (Reimchen 1980; Reimchen & Nosil 2001). Sexual selection favors a variety of male-specific reproductive traits that can increase male vulnerability to predation risk (Candolin 1998; Johnson & Candolin 2017): male sticklebacks develop bright nuptial coloration, engage in conspicuous territory defense and courtship behavior, and are the sole providers of paternal care that is necessary for offspring survival (Bell & Foster 1994). These sex differences in behavior and life history often expose males and females to different predation regimes (Reimchen & Nosil 2004), likely altering the environment experienced by mothers versus fathers and the optimal phenotype for daughters versus sons in response to

predation risk.

We test the adaptive hypothesis that sex differences in life history strategies cause offspring to attend to cues from their same-sex parent: we predicted that daughters would attend to maternal cues and sons to paternal cues (i.e. a significant interaction between maternal treatment, paternal treatment, and offspring sex). To test this hypothesis, we exposed adult male and female sticklebacks to simulated predation risk prior to fertilization and used a fully factorial design to generate offspring of control (unexposed) parents, offspring of predator-exposed mothers, offspring of predator-exposed fathers, and offspring of predator-exposed mothers and fathers. Because predation risk varies in both space and time, it is likely that there is a mix of reproductively mature males and females who either have or have not recently experienced predation risk within many natural populations. We reared sons and daughters under 'control' conditions (i.e. in the absence of predation risk) and evaluated traits relevant to predator defense. We used brain gene expression data to understand whether experiences of mothers and fathers activate different developmental programs in daughters and sons.

Methods

***Housing conditions*.** Adult, sexually-mature, freshwater threespined sticklebacks were collected from Putah Creek (CA, USA). This population has prickly sculpin (*Cottus asper*), which preys primarily on stickleback eggs, fry, and juveniles. Females were housed in six groups of n=10 fish per tank to mimic shoaling conditions in the wild. To simulate predation risk, we used a clay model sculpin (21cm long) to chase females for 90 seconds each day; unexposed treatment tanks were left undisturbed (similar to Dellinger *et al.* (2018)). Gravid females were removed from tanks and stripped of their eggs for in-vitro fertilization. Mothers were chased between 16-44

137 days; longer exposure increased offspring length at 4.5 months, but the length of exposure did
138 not significantly alter any other measured offspring traits (Supplementary Material).

139 Males were housed singly to build nests. Once their nest was completed, predator-
140 exposed males were chased by a model sculpin for 30 sec every other day for 11 days; control
141 males were left undisturbed. A separate experiment confirmed that the results reported below
142 were not produced when fathers were chased with a net (unpublished data), suggesting that
143 changes in offspring traits are specific to predation risk. The day after the last exposure, males
144 were euthanized to obtain sperm for *in-vitro* fertilization. While female sticklebacks produce
145 eggs throughout the breeding season, stickleback males produce sperm in the beginning of the
146 breeding season (Borg 1982); thus, paternal experiences mediated via sperm in this experiment
147 are likely due to modifications to mature sperm. We used a short stressor because we did not
148 want to potentially reduce sperm quality or fertilization rates by exposing males to a stressor
149 while developing sperm and we wanted to avoid potential habituation to predation risk
150 (Dellinger *et al.* 2018). Further, beginning the treatment when males were transferred to a
151 nesting arena mimics the change in predator regime that males may encounter as they move into
152 a different habitat to nest.

153 F1 offspring were generated using a split clutch design, resulting in: 1) offspring of
154 unexposed fathers and mothers (n=11 half-clutches), 2) offspring of exposed fathers and
155 unexposed mothers (n=11 half-clutches), 3) offspring of unexposed fathers and exposed mothers
156 (n=10 half-clutches), and 4) offspring of exposed fathers and mothers (n=10 half-clutches). By
157 artificially fertilizing the eggs and incubating the embryos using an air bubbler, we controlled for
158 possible pre-fertilization effects mediated by interactions between mothers and fathers
159 (Mashoodh *et al.* 2012; McGhee *et al.* 2015), as well as the post-fertilization effects mediated by

paternal care (Stein & Bell 2014). Separate groups of offspring were used for each assay described below (see Supplementary Material for detailed methods and statistical analysis).

Measuring survival under predation risk and ventilation rate. At 3-5 months of age, groups of n=4 offspring (one from each parental treatment) were exposed to a live sculpin predator. One day prior to the predation assay, fish were weighed, measured, and individually transferred to a 250ml opaque glass beaker containing 100mL of water. We measured opercular beats 30 seconds after transferring to the beaker as a proxy for acute stress (Bell, Henderson & Huntingford 2010) and 30 minutes after transferring to understand response to prolonged stress (n=100 fish per parental treatment group). At the end of thirty minutes, all four fish were moved to the same holding tank until the predation trial the following day. For the predation trial, sticklebacks were simultaneously transferred into the sculpin's tank (n=4 different sculpin, each used once per day); the trial ended two minutes after the first fish was captured by the sculpin. 14/100 trials did not result in any successful captures and were excluded from further analysis of survival data. We euthanized the survivors of the predation assays and used a section of muscle tissue to sex a large portion of the survivors per the methods of Peichel *et al.* (2004). We used generalized linear mixed models (GLMM) with a binomial distribution (R package lme4 (Bates *et al.* 2015)) to analyze differences in survival during the predation assay. Because we found evidence of heteroskedasticity in our opercular beat data, we used MCMC generalized linear mixed models (R package MCMCglmm (Hadfield 2010)) with a weak prior on the variance (V=1, nu=0.002) to analyze stress-induced respiration (breaths/minute). We ran models for 200,000 iterations, with a burn-in of 3000 iterations, thin = 3, and Gaussian distributions (and used these same parameters all MCMC models below).

Measuring risk taking behavior. When offspring were 4.5 months, we measured behavior in an open field before and after a simulated predator attack (as in Bensky *et al.* (2017)). Individuals were placed in an opaque refuge in the center of a circular arena divided into nine sections. After a three minute acclimation period, we removed the plug from the refuge, allowed fish to emerge, and then measured the number of different (exploration) and total (activity) sections visited for three minutes after emergence. We then simulated a sculpin predator attack and measured the latency to resume movement after the simulated attack. Once the individual resumed movement, we again measured the number of different and total sections visited for three minutes. We weighed and measured the fish, euthanized it via decapitation, and preserved the body in ethanol for identification of sex (Peichel *et al.* 2004). We assayed n=118 fish: n=12 females and n=18 males with control parents, n=15 females and n=16 males with predator-exposed fathers, n=13 females and n=14 males with predator-exposed mothers, and n=11 females and n=19 males with two predator-exposed parents.

We used principal components analysis (R package factoextra (Kassambara & Mundt 2017)) to combine exploration and activity (Spearman rank correlation: $\rho=0.92$, $p<0.001$), using data from both before and after the simulated predator attack (two data points per individual). We extracted an eigenvalue of 1.77 that captured 88.4% of the variance in these two behaviors; positive values indicate more active and exploratory individuals. To understand how parental exposure to predation risk altered offspring activity/exploration, length, and body mass, we used MCMC GLMMs with a Gaussian distribution; we used MCMC GLMMs with a Poisson distribution to analyze offspring freezing behavior.

***Measuring anxiety/cautiousness*.** Scototaxis (light/dark preference) protocols have been developed to test anti-anxiety/cautious behavior in fish (Maximino *et al.* 2010). Fish were placed in a clear cylinder in the center of a half-black, half-white tank. After a 5-minute acclimation period, we lifted the cylinder, and fish explored the tank for 15 minutes, during which we measured the latency to enter the white section, total time in the white section, and the number of times the fish moved between the black/white sections. Principal components analysis (R package factoextra) was used to combine these behaviors into one principal component (eigenvalue 2.10, captured 70.1% of the variance in behaviors). We then used this principal component as the dependent variable in the MCMC GLMMs. We interpret greater activity (duration/visits) in the white portion of the tank as anti-anxiety/cautious behavior (Maximino *et al.* 2010). We assayed n=162 fish: n=23 females and n=15 males with control parents, n=22 females and n=17 males with predator-exposed fathers, n=23 females and n=21 males with predator-exposed mothers, and n=24 females and n=17 males with two predator-exposed parents.

Measuring brain gene expression. We dissected whole brains from 4.5 month juvenile offspring (n=5 male and n=5 female offspring per treatment group) and preserved brains in RNAlater. We extracted RNA using Macherey-Nagel NucleoSpin 96 kits and sent n=39 samples to the Genomic Sequencing and Analysis Facility at UT Austin for TagSeq library preparation and sequencing (one sample was of poor quality). To estimate differential expression, pairwise comparisons between the experimental conditions (offspring with only a predator-exposed mother, offspring with only a predator-exposed father, offspring of predator-exposed mothers and fathers) relative to the control condition (offspring of unexposed parents) within each sex were made using edgeR (Robinson, McCarthy & Smyth 2010). To call differential expression,

we used a 'glm' approach and adjusted actual p-values via empirical FDR, where a null distribution of p-values was determined by permuting sample labels for 500 times for each tested contrast and a false discovery rate was estimated (Storey & Tibshirani 2003).

In a separate analysis, WGCNA was used to cluster genes into co-expressed gene modules (Zhang & Horvath 2005; Langfelder & Horvath 2008). To find modules significantly associated with treatment effects, we fitted a linear model (Kuznetsova, Brockhoff & Christensen 2017) which blocked for clutch ID as random factor, along with main and interactive effects of sex, paternal treatment, and maternal treatment on module eigengenes. Eigengenes which were significantly associated ($p < 0.05$) with either the main or interactive effects of sex, paternal treatment, and maternal treatment were retained.

Animal welfare note. All methods were approved by Institutional Animal Care and Use Committee of University of Illinois Urbana-Champaign (protocol ID 15077), including the use of live predators.

Results

Sons, but not daughters, of predator-exposed fathers were more active under risk

In the open field assay, offspring were significantly less active/exploratory after the simulated predator attack compared to before (principal component analysis: higher values indicate more active and explorative individuals; Table 1), confirming that offspring behaviorally responded to the predator attack. There was a significant interaction between paternal treatment and offspring sex on offspring activity/exploration (Table 1; Figure 1A). Specifically, sons of predator-exposed fathers were significantly more active/exploratory compared to sons of control

252 fathers (MCMC GLMM, 95% CI in brackets here and below [-1.30, -0.20], p=0.01), but there
253 was not a detectable effect of paternal treatment on female offspring ([-0.40, 0.81], p=0.49). In
254 other words, paternal effects on activity/exploratory behavior were stronger in sons, which is
255 consistent with our hypothesis. Greater activity in response to exposure to predation risk is
256 consistent with higher risk-taking behavior observed in sticklebacks from high predation
257 populations compared to low predation populations (Bell, Henderson & Huntingford 2010).
258 We did not detect significant maternal or paternal effects on freezing behavior (Table 1).
259 We found no evidence that standard length or body mass at 4.5 months were significantly
260 influenced by maternal (SL [1.14, 1.99], p=0.67; mass [-0.03, 0.02], p=0.91) or paternal (SL [-
261 1.77, 1.38], p=0.78; mass [-0.03, 0.03], p=0.96) exposure to predation risk. Standard length ([-
262 1.24, 0.35], p=0.25), mass ([-0.003, 0.01], p=0.20), and freezing behavior (Table 1) also did not
263 vary between male and female offspring, although larger fish were less active/exploratory (Table
264 1).
265
266 ***Both sons and daughters of predator-exposed mothers, but not fathers, were more cautious***
267 Offspring of predator-exposed mothers were more cautious (principal component
268 analysis: took longer to enter the white area, spent less time in the white area, and switched less
269 between black and white areas) compared to offspring of control mothers (MCMC GLMM: 95%
270 CI [0.06, 1.09], p=0.03; Figure 1B). However, we did not detect an effect of paternal treatment
271 on offspring scototaxis behavior ([-0.79, 0.32], p=0.44). Both female ([-1.27, -0.17], p=0.01)
272 and smaller ([-0.10, -0.006], p=0.03) offspring showed more cautious behavior. We found no
273 evidence of seasonal effects (experimental day [-0.004, 0.01], p=0.33). Consequently, rather than

offspring attending to the experiences of their same-sex parent in the scototaxis assay, we observed that both sons and daughters responded to maternal experiences.

Offspring of predator-exposed fathers were more vulnerable to predation, but not if their mother was also exposed

There was a significant interaction between maternal and paternal treatment on offspring survival in live predation assays (generalized linear mixed effect model: $Z_{335} = -1.98$, 0.047). Specifically, offspring of predator-exposed fathers were more frequently captured by the predator compared to offspring of control parents (Tukey's HSD with parental treatment as a 4-factor variable: Z=2.72, p=0.03), but this was not true for offspring of predator-exposed mothers (Z=0.73, p=0.88) or both a predator-exposed mother and father (Z=-0.80, p=0.85; Figure 1C). This suggests that was a strong fitness cost of having a predator-exposed father, but mothers seemed to mitigate those costs, perhaps by making their offspring more cautious (see above). Survivors of the successful predation trials were heavily female biased (93/148; Chi-squared: χ_2=9.76, p=0.002), suggesting that males are generally more vulnerable to predation risk. The sex-bias was not significantly different across treatment groups (χ_2=3.03, p=0.39); this suggests that paternal exposure influenced sons and daughters equally, although we can cannot conclude this definitively because we do not know the sex of the captured fish. We found no effect of size on how frequently the stickleback were captured by the predator ($Z_{335} = 1.56$, 0.11).

We found no significant difference between stress-induced respiration rates after initial confinement or after 30 minutes of confinement (95% CI [-2.77, 3.16], p=0.90). We did not detect significant maternal ([-4.79, 12.44], p=0.36) or paternal effects ([-16.91, 4.05], p=0.20) on stress-induced respiration, although larger fish tended to have lower stress-induced respiration

297 compared to smaller fish ([-2.24, 0.14], p=0.08). For the portion of offspring where sex was
298 known, we found non-significant interactions between offspring sex and paternal ([-17.67,
299 14.83], p=0.89) or maternal treatment ([-23.89, 8.77], p=0.37), although males tended to have
300 higher opercular beats than females (main effect of sex [-1.48, 25.92], p=0.08). Individuals with
301 lower opercular beat rate at initial confinement (Z_{336}= -1.92, p=0.05) and after 30 minutes of
302 confinement (Z_{336}= -1.75, p=0.08) tended to be more likely to be captured by the predator.
303
304 ***Distinct maternal and paternal effects on offspring brain gene expression***
305 To evaluate whether predation risk experienced by mothers versus fathers has different
306 consequences for offspring development at the molecular level, we compared the baseline brain
307 gene expression profile of offspring of unexposed parents (control) to offspring with a predator-
308 exposed mother, a predator-exposed father, and two predator-exposed parents in male and female
309 offspring (n=39 individuals). In terms of the number of genes, maternal and paternal effects on
310 brain gene expression were approximately equivalent in magnitude, and the genes were largely
311 nonoverlapping (Figure 2A,B): in sons, for example, 1028 genes were differentially expressed in
312 response to maternal experience with risk, 904 genes were differentially expressed in response to
313 paternal experience with risk while only 253 genes were shared between them (daughters show a
314 similar pattern, Figure 2A). This suggests that, in contrast to our prediction, the transcriptomes of
315 sons and daughters are not more responsive to the experiences of their same-sex parent.
316 Interestingly, there was also a large number of genes that were unique to the "both" condition,
317 i.e. between offspring of two predator-exposed parents versus the control; these differentially
318 expressed genes could reflect the ways in which maternal and paternal effects interact at the
319 molecular level.

Of the differentially expressed genes that were shared between the pairwise comparisons, nearly all were concordantly regulated, for both sons and daughters (Figure 2A,B). This suggests that, despite the large-scale differences in brain gene expression between offspring of predator-exposed mothers and fathers, there is a core set of genes that is activated in offspring brains in response to either maternal or paternal exposure to predation risk.

Maternal and paternal exposure to predation risk interacted with offspring sex to influence offspring brain gene expression

The behavioral data suggest that sons and daughters respond to parental experience with predation risk differently, with sons, but not daughters, increasing activity/exploration in response to paternal experience with predation risk. One way that such sex-specific effects could arise is if experiences of one parent (e.g. fathers) activate a particular developmental program in one offspring sex but not the other (e.g. in sons but not daughters).

To bring molecular data to bear on this idea, we used WGCNA to reduce the dimensionality of the transcriptomic dataset, which allowed us to explore the potential for interactive effects of maternal treatment, paternal treatment and offspring sex on modules of genes with correlated expression patterns. WGCNA identified 23 informative clusters ("modules") of genes with coordinated expression patterns in the dataset. The expression of eight of the 23 modules was significantly affected by at least one of the factors in the model: three modules were significantly affected by maternal treatment, two were significantly affected by the two-way interaction between maternal and paternal treatment, and three were significantly affected by the three-way interaction between paternal treatment, maternal treatment and offspring sex (shown in Figure 2C). For example, the module "saddle brown" comprises 48 co-

343 expressed genes (largely enriched for developmental processes) whose expression was
344 influenced by the three way interaction between maternal treatment, paternal treatment and
345 offspring sex. Specifically, daughters of a predator-exposed mother or father showed lower
346 expression of genes in this module compared to daughters of control parents or two predator-
347 exposed parents (Figure 2C). For sons, on the other hand, the expression of genes in this module
348 was more strongly affected by maternal treatment. A similar pattern was observed in the yellow
349 and cyan modules. Overall these results demonstrate that at the molecular level, daughters and
350 sons differ in the extent to which they respond to predation risk that had been experienced by
351 their mother, father or by both parents. However, in contrast to our overall hypothesis, there was
352 no evidence that sons and daughters primarily attend to experiences of their same-sex parent at
353 the molecular level.
354
355 **Discussion**
356 Transgenerational plasticity can allow environmental information to be delivered to
357 offspring earlier and with potentially lower costs to offspring than developmental plasticity,
358 which may allow offspring to develop traits during early development that help them cope with
359 environmental change (Stratmann, Taborsky & Blanckenhorn 2014; Bell & Hellmann 2019).
360 Unlike genetic inheritance, TGP can potentially be fine-tuned to the precise environment that
361 both parents and offspring will encounter (Bonduriansky & Day 2008), potentially including the
362 different environments experienced by males and females because of sex differences in life
363 history and reproductive strategies. However, adaptive sex-specific TGP is unlikely to evolve
364 when sexual conflict and/or sexual selection is strong (Burke et al).

Here, we report the results of a comprehensive comparison of maternal and paternal effects on sons and daughters. Our results illustrate the complexities of sex-specific TGP: offspring phenotypes varied depending on whether predation risk had been experienced by their mother or their father, and a parent's experience with predation risk produced different phenotypes in their sons compared to their daughters. Maternal and paternal effects in response to the same environmental factor (predation risk) were largely distinct: predator-exposed mothers produced more cautious offspring (scototaxis), while predator-exposed fathers produced sons, but not daughters, that were more active under risk (open field assays). There were also non-additive interactions between maternal and paternal effects on some offspring traits. In particular, offspring of predator-exposed fathers had reduced survival against a live predator; however, offspring of two predator-exposed parents did not have reduced survival, suggesting that maternal predation exposure may mitigate the deleterious effects of paternal predation exposure to some degree. This does not seem to arise because offspring of two predator-exposed parents more closely resemble offspring of predator-exposed mothers; instead, our brain gene expression profile results are more consistent with the hypothesis that interactions between the environments experienced by mothers and fathers result in distinct neurogenomic changes in offspring with two predator-exposed parents compared to offspring of either a predator-exposed mother or father.

In addition to interactions between maternal and paternal effects, we found that sons and daughters differ in their response to maternal and paternal exposure to predation risk. These sex-specific patterns emerged in our study well before offspring were reproductively mature, during a period in their life when males and females are shoaling and still occupying similar habitats (Bell & Foster 1994). In contrast to our prediction that offspring would adaptively attend to the

388 cues of their same-sex parent, these sex-specific patterns of transgenerational plasticity did not
389 seem to emerge along a consistent male-female divide (e.g. sons attend to their father and
390 daughters attend to their mother); instead, sons and daughters were both altered by paternal and
391 maternal environments, but in different ways. This is consistent with Emborski and Mikheyev
392 (2019), who found that male offspring were influenced by maternal, but not paternal, diet. These
393 sex-specific effects may be adaptive for offspring, with differences originating in early
394 development to allow offspring to develop phenotypes that are better matched to the different
395 environments they will encounter later in life. For example, it is possible that increased activity
396 under risk for sons, but not daughters, may be adaptive because high variance in male
397 reproductive success favors males that adopt high risk, high reward behaviors to increase growth
398 and access to resources under high predation pressure (Bell, Henderson & Huntingford 2010).
399 Alternatively, these effects may not be adaptive, either resulting from differences in sons and
400 daughters in their susceptibility to parental stress (Bale 2011; Glover & Hill 2012) and/or
401 reflecting sexual conflict and sexually antagonistic selection (Burke, Nakagawa & Bonduriansky
402 2019). Indeed, Burke, Nakagawa and Bonduriansky (2019) suggest adaptive TGP may be
403 unlikely to arise in systems with sex-specific selection because sex-specific ecologies can result
404 in mothers and fathers experiencing different environments and therefore, transmitting
405 conflicting information to their offspring.

406 Our study shows that maternal and paternal predation exposure can have fitness
407 consequences for offspring (i.e., via survival) in the lab; work is needed in a more natural context
408 in the field to assess the fitness consequences of parental effects. For example, there are multiple
409 steps required to avoid predation (Lima & Dill 1990; Guiden *et al.* in press); while our data
410 suggest that offspring with predator-exposed fathers are poor at evading predators once they

411 come into contact with predators, parental experience with predation risk might alter the
412 likelihood that offspring initially avoid coming into contact with predators. Further, offspring of
413 predator-exposed fathers might face a trade-off between survival and reproduction, favoring
414 high-risk, high-reward strategies that reduce survival in high predation environments, but
415 increase reproductive success by ensuring that surviving individuals are in good breeding
416 condition. Indeed, individuals do seem to face a trade-off in surviving predation and gaining the
417 body size necessary for successfully reproducing, with this trade-off being stronger in males
418 compared to females (Bell *et al.* 2011).

419 Whether the fitness interests of mothers, fathers, and offspring align or conflict has
420 important implications for the evolution of sex-specific TGP (Burke, Nakagawa & Bonduriansky
421 2019). When parents' and offspring fitness interests in the face of predation risk are aligned, sex-
422 specific plasticity may arise because mothers and fathers experience their environment in
423 different ways and/or because the same parental environment favors different phenotypes in sons
424 and daughters. However, sex-specific TGP may arise because mothers and fathers favor different
425 optimal offspring phenotypes (Saldivar *et al.* 2017), and/or sons and daughters have different
426 capacities to respond to or ignore information from fathers and mothers. If this is the case, TGP
427 may evolve at the interface between sexual conflict and parent-offspring conflict, with paternal
428 strategies, maternal strategies, and offspring counter-adaptations all ultimately dictating
429 offspring phenotypes. This may result in the evolution of mechanisms that allow mothers to
430 manipulate the ways in which fathers influence offspring (e.g. via cytoplasmic contributions
431 (Crean & Bonduriansky 2014)) or fathers to manipulate the ways in which mothers influence
432 offspring (e.g. via ejaculate composition (Garcia-Gonzalez & Dowling Damian 2015)).

Interactions between maternal effects, paternal effects, and offspring sex could be mediated via a variety of proximate mechanisms. Distinct maternal and paternal effects could reflect different proximate mechanisms that mediate the transmission of cues from mothers versus fathers to offspring (e.g., egg hormones or mRNAs versus sperm small RNAs) as well as the ways in which mothers and fathers were exposed to risk. Both distinct and interactive effects could also be mediated by epigenetic mechanisms such as parent-of-origin effects (Kong *et al.* 2009; Lawson, Cheverud & Wolf 2013) or interactions between maternal and paternal contributions (e.g. egg cytoplasm altering the effect of sperm small RNAs) during early development (Crean & Bonduriansky 2014; Garcia-Gonzalez & Dowling Damian 2015). Differences between sons and daughters in how they respond to parental information could be mediated via trans-acting mechanisms (e.g., regulation of genes on non-sex chromosomes by genes located on the sex chromosome (Metzger & Schulte 2016)), sex-specific differences in epigenetic mechanisms, or genomic imprinting (Bonduriansky & Day 2008; Dunn & Bale 2011). Further, in bulls, Y-bearing and X-bearing spermatozoa have differentially expressed proteins, suggesting a mechanism by which fathers can transmit different information to sons versus daughters (Scott *et al.* 2018). Although mothers in many species can also transmit different information to sons and daughters (e.g., via placental function and gene expression (Bale 2011; Glover & Hill 2012)), it is unclear if mothers can transmit different information to sons and daughters in externally fertilizing species such as sticklebacks, in which mothers do not interact with their offspring post-fertilization. Future work exploring these proximate mechanisms could help explain the extent to which variation in parental effects is due to changes in the information encoded by parents or changes in offspring responsiveness to parental information.

455 Because parents can differentially allocate based on their partner's phenotype or
456 environmental conditions experienced by their partner (Mashoodh *et al.* 2012; McGhee *et al.*
457 2015; Mashoodh *et al.* 2018), in most systems it is difficult to isolate the effects of direct
458 parental exposure to an environmental cue from environmental cues that parents indirectly detect
459 from their mate (e.g. predator-naïve fathers provide less care to offspring of predator-exposed
460 mothers) (Mashoodh *et al.* 2012; McGhee *et al.* 2015; Mashoodh *et al.* 2018). This makes it
461 difficult to understand whether paternal effects can be mediated via sperm alone, or to determine
462 the influence of paternal effects in isolation of maternal effects. In this experiment, we were able
463 to completely isolate paternal effects mediated via sperm because there was no opportunity for
464 parents to interact pre-fertilization or to influence offspring post-fertilization. Although our
465 results suggest that distinct and interactive effects of maternal and paternal effects can be
466 mediated via selective changes to information encoded in eggs and sperm alone, a fascinating
467 direction for future work would be to consider how parental care and mate choice might
468 ameliorate or magnify the sex-specific effects observed here.
469

470 **Conclusions**

471 In conclusion, we show that both the sex of the parent and the sex of the offspring
472 influence the ways in which offspring phenotypes are altered by parental experiences, although
473 we found little evidence for the adaptive hypothesis that offspring would attend primarily to cues
474 from their same-sex parent. We demonstrate that paternal cues mediated via sperm seem to be
475 just as prominent as maternal cues mediated via eggs. However, these sex-specific patterns
476 would have been masked if we had combined cues coming from mothers and fathers (i.e.
477 compared offspring of two predator-exposed parents to a control) or failed to isolate effects

478 emerging in sons versus daughters. Consequently, theoretical and empirical work seeking to
479 understand the evolution of transgenerational plasticity would benefit from considering the
480 conditions which influence *sex-specific* patterns of transgenerational plasticity in both adaptive
481 and nonadaptive ways. Further, given broad interest in understanding the consequences of
482 transgenerational plasticity for future generations and its potential to influence adaptive
483 evolution, future work should consider how sex-specific effects in the first generation may alter
484 the ways in which transgenerational effects persist for multiple generations in lineage-specific
485 and/or sex-specific ways.
486
487

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Author contributions

JKH and AMB designed the study. JKH generated offspring, conducted survival assays, collected opercular beat data, dissected brains and extracted RNA, and oversaw open field assays and offspring sexing. JD conducted scototaxis assays. SAB conducted gene expression analyses. JKH wrote the first draft of the manuscript and JKH/AMB edited the manuscript.

Data accessibility

All datasets (survival, respiration data, scototaxis, behavioral assays, lists of differentially expressed genes, read counts per sample, WGCNA) will be made publicly available on Dryad upon acceptance of this manuscript.

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659

660 **Table 1:** Results of general linear mixed models (MCMCglmm) testing predictors of
661 exploration/activity (higher values indicate more active and exploratory individuals) and freezing
662 behavior. We tested fixed effects of maternal and paternal exposure to predation risk, sex, and
663 standard length, with random effects of maternal and paternal identity. Additionally, we included
664 observation period (before or after simulated predator attack) for activity/exploration, as well as
665 random effects of ID nested within maternal and paternal identity. Non-significant interaction
666 terms were removed.

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669

	Activity and exploration		
	Mean	*95% CI (L, U)*	*P*
Observation period	-0.97	-1.24, -0.70	**<0.001**
Maternal treatment	0.14	-0.27, 0.54	0.48
Paternal treatment	-0.20	-0.81, 0.42	0.52
Offspring sex	-0.25	-0.70, 0.22	0.29
Standard length	-0.12	-0.18, -0.05	**<0.001**
Paternal treatment * sex	0.91	0.25, 1.54	**0.005**
	Freezing behavior		
	Mean	*95% CI (L, U)*	*P*
Maternal treatment	0.31	-0.15, 0.80	0.19
Paternal treatment	-0.17	-0.65, 0.31	0.47
Offspring sex	-0.36	-0.78, 0.07	0.09
Standard length	0.06	-0.02, 0.15	0.14

Figures

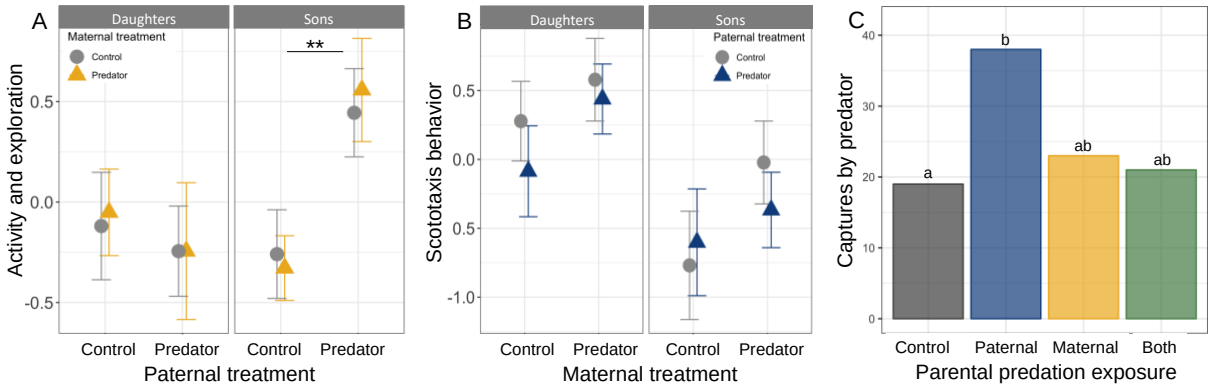
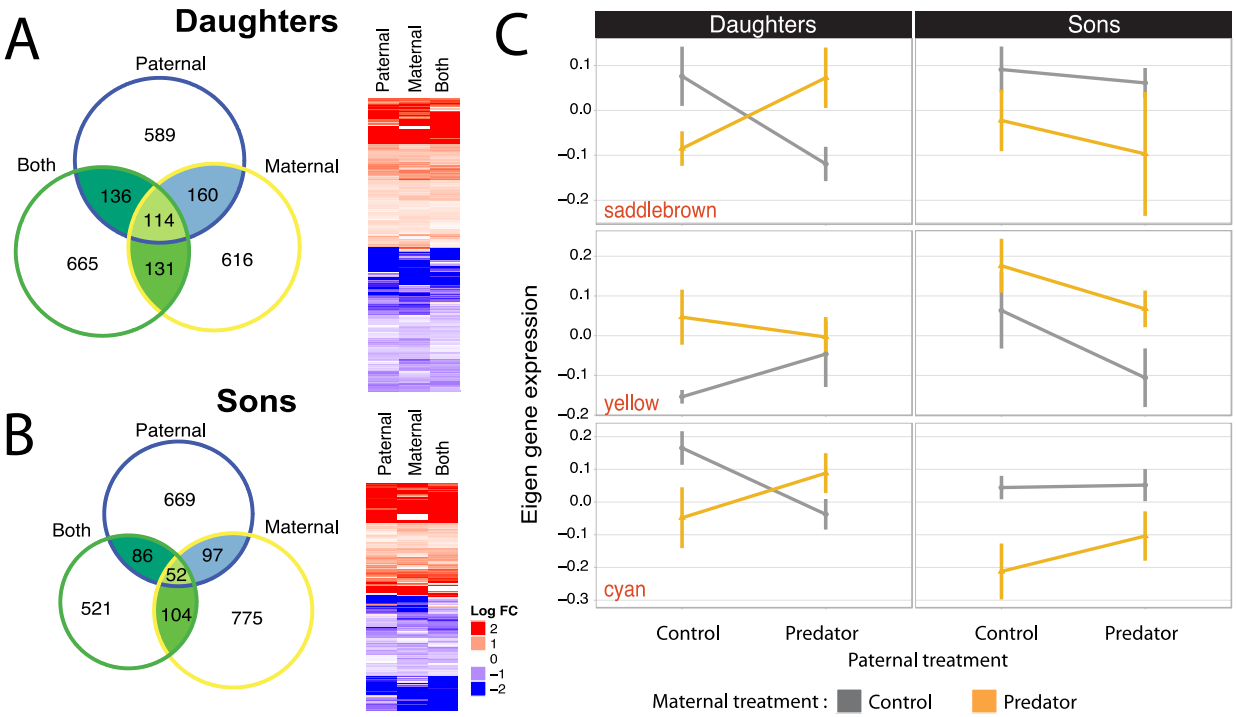


Figure 1: The effects of maternal and paternal treatment on offspring in an open field assay, scototaxis assay, and survival in the face of a live predator. A) Male offspring (right) of predator-exposed fathers were significantly more exploratory and active (PCA: higher values indicate more active and exploratory individuals; mean ± s.e.) compared to male offspring of control fathers; paternal treatment did not affect the exploratory behavior/activity of female offspring (left). The effect of paternal treatment did not depend on maternal treatment (control: grey; predator-exposed: yellow). N= 118 offspring. Stars indicate significant differences across treatment groups. B) Offspring of predator-exposed mothers were more cautious (PCA: high values indicate longer latency to enter the white area and spent less time in the white area; mean ± s.e.) compared to offspring of control mothers. Further, female offspring (left) were more cautious than male offspring (right). The effect of maternal treatment did not depend on paternal treatment (control: grey; predator-exposed: blue). N= 162 offspring. C) In live predation trials, juvenile offspring of predator-exposed fathers, but not two predator-exposed parents, were significantly more likely to be captured and consumed by the sculpin predator relative to offspring of control fathers. Letters indicate significant differences among treatment groups, determined by Tukey's HSD with parental treatment as a 4-level variable. N= 86 trials.



688

689 **Figure 2**: Differential gene and eigen-gene expression analysis. A-B) The three circles in the
690 Venn diagram show the number of genes that were differentially expressed in the brain of
691 offspring of unexposed parents relative to offspring of predator-exposed mothers ("maternal"),
692 predator-exposed fathers ("paternal"), or two predator-exposed parents ("both"), with daughters
693 in (A) and sons in (B). Note that relatively few genes overlap between the different pairwise
694 comparisons. The heatmaps show the direction of gene regulation (blue: downregulated; red:
695 upregulated) of the differentially expressed genes that are shared among the three pairwise
696 comparisons, with daughters and sons shown separately. C) The expression profiles of the four
697 eigen-gene modules which were significantly affected by the three-way interaction among
698 paternal treatment, maternal treatment and offspring sex (mean ± s.e.). N=39 offspring.

699