The transgenerational consequences of paternal social isolation and predation exposure in threespined sticklebacks

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Abstract

1. Although parents routinely encounter stress in the ecological environment that can affect offspring development (transgenerational plasticity: TGP), nearly all organisms interact with conspecifics that can either independently induce parental stress or can alter how parents respond to ecological stressors.
2. During social buffering, the presence of conspecifics can reduce the response to or increase the speed of recovery from a stressor. This may have cascading effects on offspring if the presence of conspecifics can mitigate parental responses to ecological stress in ways that blunt transmission of stress-induced transgenerational effects.
3. Here, we simultaneously manipulated both paternal social isolation and experience with predation risk prior to fertilization in threespined stickleback (*Gasterosteus aculeatus*). If social buffering mitigates TGP induced by paternal exposure to predation risk, then we expected fewer phenotypic effects in offspring when fathers are exposed to predation risk with a conspecific with present compared to fathers exposed to predation risk while isolated.
4. Offspring of predator-exposed fathers showed reduced anxiety-like behavior. Fathers who were socially isolated had offspring that were captured faster by a live predator, suggesting that social isolation may be a stressor that has maladaptive consequences for offspring. Further, we also found evidence that the presence of a conspecific buffered fathers against the effects of predation risk: offspring of predator-exposed fathers tended to be captured faster by the predator, especially when fathers were isolated compared to when they had neighbors.

5. Our results suggest that socially-induced stress is an important, yet underappreciated, mediator of TGP and can elicit transgenerational effects even in species that do not form permanent social groups. Future studies should therefore consider the broader social environment when predicting both within and trans-generational responses to ecological change.

Key words: *Gasterosteus aculeatus*, paternal effects, phenotypic plasticity, predation risk, social buffering

Introduction

Organisms encounter a wide variety of threats and stressors in their environment that can push individuals beyond their homeostatic limits and induce physiological and behavioral changes that allow them to cope with those stressors (Romero, Dickens & Cyr 2009). It is well known that individuals differ drastically in how they respond to these environmental stressors: the same external stressor can induce strong responses in some individuals and weak response in others (Koolhaas *et al.* 1999; Cockrem 2013). Although some of this variation can be attributed to traits of the organism itself (e.g., personality (Cockrem 2013), sex (DeVries, Glasper & Detillion 2003)), some of this variation is environmentally dependent. In particular, there is ample evidence that the social environment alters how animals cope with ecological stress, influencing both the perception of and response to ecological cues (DeVries, Glasper & Detillion 2003; Brandl, Pruessner & Farine 2022). In many cases, the presence of conspecifics can reduce physiological and behavioral responses to stress via social buffering, which occurs when the presence of a conspecific reduces the response to or increases the speed of recovery from an external stressor (Kikusui, Winslow & Mori 2006; Gilmour & Bard 2022). For example, socially isolated mice showed greater immune impairment and a higher basal corticosterone increase in response to a mild stress compared to group housed mice (Bartolomucci *et al.* 2003). Similarly, in humans, anxiety and negative emotions induced by a social stress test were attenuated when women had social support (McQuaid *et al.* 2016). This social buffering may be an adaptive response to minimize the transmission of stress among group members (Gilmour & Bard 2022), which can occur when a stress response system (e.g., glucocorticoids) in one individual is activated after interacting with another stressed individual (Brandl, Pruessner & Farine 2022). Further, social buffering can even increase the limits of the physical conditions organisms are

willing to tolerate (Gilmour & Bard 2022), suggesting that sociality can mediate response to ecological stressors.

While social buffering has been well studied in highly social mammals, there is mounting evidence that social buffering of stress also occurs in less social species, including solitary species and species with aggregations or schooling behavior (Gilmour & Bard 2022). For example, in lake sturgeon, group-housed fish had significantly reduced endocrine and cellular stress markers after exposure to heat stress compared to isolated individuals (Yusishen *et al.* 2020). This social buffering effect can even occur in small groups of two individuals (Faustino, Tacão-Monteiro & Oliveira 2017; Schumann *et al.* 2023) or in response to mere visual cues of a conspecific (da Costa *et al.* 2004; Faustino, Tacão-Monteiro & Oliveira 2017), suggesting that these social buffering effects are robust and occur in a wide range of taxonomic groups with differing levels of sociality.

While social buffering effects can have consequences for the individuals that directly experience the stressor, social buffering in parents may also have consequences for offspring. Transgenerational plasticity (TGP) occurs when the experiences of a previous generation alter the phenotypes of future generations (Bell & Hellmann 2019). TGP can occur in response to a variety of ecological stressors, including predation risk (Tariel, Plénet & Luquet 2020; MacLeod *et al.* 2022), heat exposure (Donelson *et al.* 2016; Pessato, McKechnie & Mariette 2022), immune challenges (Beemelmanns & Roth 2016; Moore, Kaletsky & Murphy 2019), and food limitation (Bygren *et al.* 2014; Hagmayer *et al.* 2022). These ecological stressors induce changes in parental state, including the activation of the hypothalamic-pituitary-adrenal axis (HPA axis), that influence offspring development, such as stress responsiveness, growth, and behavior (Bell & Hellmann 2019; Potticary & Duckworth 2020). Given that the magnitude of the stress

response in parents can be linked to the magnitude of the phenotypic changes in offspring (Sheriff, Krebs & Boonstra 2010), social buffering may affect both the strength and frequency with which parental experiences affect offspring traits. This is supported by a recent study in humans which found that the adverse health outcomes differentiating offspring of healthy and psychologically stressed mothers could be specifically linked to a lack of social support in psychologically stressed mothers (Walsh *et al.* 2019). Similarly, in rats, offspring of socially-isolated mothers showed cognitive impairments and increased anxiety-like behaviors (McDonald *et al.* 2023). These effects may arise because conspecifics provide a buffering effect that abates maternal response to external stress (e.g., brings glucocorticoids back to baseline quickly (Kikusui, Winslow & Mori 2006)), thereby mitigating adverse outcomes for offspring (Séguin *et al.* 1995; Nylen, O'Hara & Engeldinger 2013).

Although this previous work suggests the importance of the social environment in mediating transgenerational effects for mothers, we know nothing about the transgenerational effects of social isolation for fathers. This is problematic, as paternal experiences can affect offspring phenotypes in magnitudes that are similar to maternal experiences (Bonduriansky & Head 2007; Beemelmanns & Roth 2016; Gilad & Scharf 2019; Hellmann *et al.* 2020). Further, because males and females differ in their susceptibility to stress (DeVries, Glasper & Detillion 2003) and the effects of paternal experiences often have different effects on offspring than maternal experiences (Bonduriansky & Head 2007; Bonduriansky, Runagall-McNaull & Crean 2016; Bell & Hellmann 2019; Gilad & Scharf 2019; Hellmann *et al.* 2020), it is likely that the transgenerational effects of paternal isolation may be different from maternal isolation. Thus, understanding how the social environment mediates paternal effects can help us better predict the extent to which paternal experiences influence offspring phenotypes and understand how we

112 might predict the effects of paternal stress if, for instance, social buffering during or after a
113 stressor mitigates or reverses changes in parental state that induce transgenerational effects.
114 We sought to understand the ways that the social environment alters transgenerational
115 consequences of paternal stress in threespined stickleback *(Gasterosteus aculeatus).* There is
116 abundant evidence for predator-induced paternal effects in stickleback, which alter offspring
117 behavior, growth, gene expression, survival, and stress responsiveness (Hellmann *et al.* 2020;
118 Hellmann, Carlson & Bell 2020; Chen *et al.* 2021; Hellmann, Carlson & Bell 2021; Afseth *et al.*
119 2022). There is also previous evidence of social buffering effects in stickleback: fish that are
120 exposed to simulated predation risk while isolated had higher circulating levels of cortisol
121 compared to fish in a group (Mommer & Bell 2013) and pairs of sticklebacks co-regulate their
122 cortisol responses in stressful environments (Fürtbauer & Heistermann 2016). However, we have
123 very little understanding as to how the social environment might modulate parental responses to
124 ecological stress in ways that might have cascading effects on the adaptive nature and frequency
125 of transgenerational plasticity.
126 We used a full factorial design to compare the transgenerational consequences of
127 exposing fathers to predation risk while fathers were isolated or with another conspecific.
128 Offspring were generated via in-vitro fertilization to isolate paternal effects mediated via sperm
129 and/or seminal fluid. We then measured offspring behavioral traits relevant to predation defense,
130 including anti-anxiety behavior, shoaling behavior, and survival against a live predator. If social
131 buffering mitigates TGP induced by paternal exposure to predation risk, then we expect to see
132 fewer phenotypic effects in offspring when fathers are exposed to predation risk with a
133 conspecific with present compared to fathers exposed to predation risk while isolated.
134

Methods

Housing information and F0 exposures. In June 2021, we collected adult three-spined stickleback *(Gasterosteus aculeatus)* from Putah Creek (CA, USA), a freshwater stream in northern California, and shipped them to University of Dayton. We maintained the F0 generation on a summer photoperiod schedule (16 L : 8 D) at 20° ± 1°C and fed them ad libitum daily with a mix of frozen bloodworms (*Chironomus* spp.), brine shrimp (Artemia spp.) Mysis shrimp, and Cyclop-eeze. We obtained 10-15 cm long rainbow trout *(Oncorhynchus mykiss)* from Freshwater Farms of Ohio in June 2021. Putah Creek has piscivorous predators, including the prickly sculpin (*Cottus asper*); while rainbow trout are present in Putah Creek, they are not present in the isolated section of Putah Creek where we collected stickleback (Beaver Pond), suggesting that rainbow trout is an environmentally relevant, but novel predator.

In August 2021, we moved males into experimental 37.9 L (53L x 33W x 25H cm) tanks divided into 3 equal sections using removable barriers. Males in the neighbor treatment were separated with clear barriers with 9, 3mm holes to allow for water flow between the sections. Males in the isolated treatment were separated with permanent opaque barriers that did not allow for water flow between sections. In both treatments, we placed one male stickleback in each of the outer sections and left the middle section empty. Each male section contained gravel, one fake plant, and nesting materials: a container of sand, a terra cotta pot for cover, and algae. The middle section contained only gravel. Next to each nesting male tank, we placed an undivided 37.9L tank containing gravel and 2 sponge filters, which either contained one live rainbow trout or was left empty depending on the predator treatment. All experimental tanks were separated visually from each other with a removable opaque divider.

157 We exposed male stickleback to predator cues once a day for 2 hours, for 11-14
158 consecutive days depending on the availability of gravid females. We used a short stressor to
159 avoid potential habituation to predation risk (Dellinger *et al.* 2018). Further, by beginning the
160 treatment when males were transferred to a nesting arena, we sought to mimic the change in
161 predator regime that males may encounter as they move into a different habitat to nest. At a
162 random time of day between 0800 hrs and 2000 hrs, we turned off the bubblers in the tanks and
163 removed the opaque divider to reveal the adjacent tank (which was empty or contained a live
164 trout). At the same time, we added either 100mL of control RO water or the prepared predator
165 cue to each end compartment containing one male stickleback. We prepared predator cues using
166 methods consistent with Crane and Ferrari (2015). Briefly, we placed a trout in a 37.9 L tank
167 with clean, conditioned tap water proportional to the size of the trout (500mL per gram of fish).
168 We fed the trout one juvenile stickleback per day for four days and after 96hrs, we froze the tank
169 water into 200 mL aliquots.
170 In the control treatment, males saw an empty tank and received 100 mL of RO water. In
171 the predator treatment, males saw a live trout and received 100 mL of the prepared predator cue.
172 Every other day, we fed the trout one juvenile stickleback during the two hour exposure to
173 reinforce the visual cue. Importantly, despite holes between the sections, we found very limited
174 diffusion of water between the sections (tested using dyed water) when bubblers were turned off,
175 suggesting that these olfactory cues did not disperse throughout the tank during the predation
176 exposure and therefore, the neighbor treatment did not receive weaker olfactory cues or
177 predation risk than the isolated group. After two hours, we replaced the dividers and completed a
178 50% water change on all treatments. We removed males after the last day of exposure.
179

180 ***Generating Offspring.*** The day after the last exposure, we generated offspring by manually
181 squeezing the eggs out of a gravid, unexposed female and fertilizing them with sperm from the
182 testes of the dissected experimental male. We checked clutches for successful fertilization via
183 microscope and then placed into a small cup with a mesh bottom above an air bubbler in an
184 18.9L (38L x 25W x 21H) tank containing gravel and two fake plants, with each clutch housed
185 individually on a recirculating flow-through system. Once hatched, we fed offspring newly
186 hatched brine shrimp for two months and then gradually transitioned to frozen food (as above).
187 We switched offspring to a winter light schedule (8 L: 16 D) prior to starting behavioral assays.
188 We generated a total of n = 35 clutches: n = 9 of control fathers with a neighbor, n = 9 of
189 control, isolated fathers, n = 8 of predator-exposed fathers with a neighbor, and n = 9 of predator-
190 exposed, isolated fathers. By artificially fertilizing the eggs and incubating the embryos using an
191 air bubbler, we controlled for the possibility that predator-exposed or isolated fathers might be
192 less likely to successfully mate with females or parent offspring.
193
194 ***Offspring assays.***
195 ***Shoaling assays.*** We used experimental 37.9L (53 x 33 x 24 cm high) tanks lined with gravel.
196 On one end of the tank, we positioned three small plastic plants; on the other end of the tank, we
197 created a small compartment for shoaling fish by inserting a clear plastic barrier (10 cm from the
198 edge) perforated with small holes to allow for flow of water and olfactory cues. The evening
199 before a day of trials, we placed five size-matched juveniles into the shoaling side compartment
200 of each tank to habituate overnight. These fish were unrelated and unfamiliar to the focal fish.
201 At the start of the shoaling trial, we placed an experimental offspring (focal fish) into the
202 open area in the middle of the tank and allowed the focal fish to habituate for 10 minutes. After

203 the habitation, we used BORIS to record total time spent shoaling, total time spent oriented to the
204 shoal while shoaling (the focal fish's head had to be pointing in the direction of at least one
205 member of the shoal), total time spent hiding in plants, and time spent in the gravel in the center
206 of the tank over a 10 minute period. We defined a focal fish to be shoaling or hiding in the plant
207 if it was within one body length of the shoal barrier or plants, respectively. Upon completion of
208 the assay, we gently removed the focal fish from the tank with a cup, weighed and measured
209 them, and then placed them into an individual holding tanks. We assayed and measured n = 242
210 fish: n = 61 of control fathers with a neighbor, n = 57 of control, isolated fathers, n = 66 of
211 predator-exposed fathers with a neighbor, and n = 58 of predator-exposed, isolated fathers.
212
213 ***Measuring anxiety/cautiousness.*** Scototaxis (light/dark preference) protocols have been
214 developed to test anti-anxiety/cautious behavior in fish (Maximino *et al.* 2010). On the day after
215 the shoaling assay, offspring were gently caught with a cup from their holding tank and placed in
216 a clear cylinder (10.5cm diameter) in the center of a half-black, half-white tank (51L x 28W x
217 19H cm). After a 5-minute acclimation period, we lifted the cylinder, and fish explored the tank
218 for 15 minutes, during which we measured the latency to enter the white section, total time in the
219 white section, and thigmotaxis behavior (time spent within a body length of the wall) within the
220 white section. After the 15-minute testing period, we removed the fish from the tank and placed
221 them back in their holding tank. We assayed n = 234 fish: n = 58 of control fathers with a
222 neighbor, n = 55 of control, isolated fathers, n = 64 of predator-exposed fathers with a neighbor,
223 and n = 57 of predator-exposed, isolated fathers.
224

225 ***Survival assay***. 48-72 hours after the scototaxis assay, we conducted survival assays in groups of
226 two siblings (same paternal treatment and genetic family). We filled circular portable pools with
227 conditioned tap water (1m diameter, 13 cm water depth) and evenly placed 6 plants around each
228 arena. We placed an individually marked rainbow trout in each pool 24-hours before the first day
229 of assays. We fasted the trout for 48 hours prior to being placed in the pools and we fed them one
230 non-experimental juvenile stickleback after being placed in the pool to keep hunger levels
231 standardized across days. On each assay day, we used each trout for two assays, once in the
232 morning and once in the afternoon. For each assay, we placed two siblings in a clear cylinder
233 (20.3 cm diameter) in the middle of the arena. After acclimating for 20 minutes, we lifted the
234 cylinder and used a stopwatch to time how long it took the trout to capture one of the fish
235 (survival time). We had a total of n=91 trials: n = 29 of control fathers with a neighbor, n = 15 of
236 control, isolated fathers, n = 25 of predator-exposed fathers with a neighbor, and n = 22 of
237 predator-exposed, isolated fathers. We analyzed all trials where one of the fish was captured
238 within one hour (n=88 trials).
239
240 ***Statistical methods***. To get an estimate of shoaling behavior, we used principal components
241 analysis (R package factoextra (Kassambara & Mundt 2017)) to combine several behaviors: time
242 spent hidden in the plant, time spent shoaling, time spent oriented to the shoal, and time spent in
243 the gravel in the center of the tank. We extracted an eigenvalue of 2.74 that captured 68.5% of
244 the variance in these behaviors; positive values indicate more social individuals who spent more
245 time shoaling (contribution: 33.4%) and oriented to the shoal (31.3%), and spent less time hiding
246 in plants (25.1%) and gravel (10.2%). This PC was used as the dependent variable in the model
247 described below (Gaussian distribution). For the scototaxis assay, latency to enter the white arena

was negatively correlated with time spent in the white section (Spearman rank correlation: $\rho = -0.42$, $p<0.001$), so we analyzed variation in time spent in the white section of the tank, as well as time spent performing thigmotaxis in the white section of the tank (both Poisson distribution).

We used the package MCMCglmm (Hadfield 2010) to analyze offspring traits. We ran models with a weak prior on the variance (V=1, nu=0.002). We ran models for 200,000 iterations, with a burn-in of 3000 iterations and thin = 3. Models testing variation in shoaling and scototaxis behavior included fixed effects of paternal predation exposure, paternal isolation, and offspring standard length and random effects of clutch identity and observer identity. For the thigmotaxis model, we also included a fixed effect of total time in the white section of the tank. The survival model (time to capture: Poisson distribution) included fixed effects of paternal predation exposure and paternal isolation, and random effects of clutch identity, observer identity, and trout identity. The length model (Gaussian distribution) included fixed effects of paternal predation exposure, paternal isolation, and offspring age, while the mass model (Gaussian distribution) included fixed effects of paternal predation exposure, paternal isolation, and offspring length; both models included random effects of clutch identity and observer identity. For all models, we tested for interactions between paternal predation exposure and paternal isolation and removed all non-significant interactions.

Animal welfare note. All methods were approved by Institutional Animal Care and Use Committee of University of Dayton (protocol ID 021-05), including the use of live predators.

Results

We examined how paternal exposure to predation risk and paternal social isolation altered offspring behavior, size, and survival against live predators.

Anti-anxiety behavior. We found that offspring of predator-exposed fathers spent more time in the white section of the tank compared to offspring of control fathers (MCMC GLMM, 95% CI [0.04, 0.29], p = 0.01; Figure 1). After controlling for the total time in the white area, we found that offspring of predator-exposed fathers tended to do more thigmotaxis in the white section compared to offspring of control fathers ([-0.49, 42.5], p = 0.06). We found no effect of paternal isolation on time spent in the white section ([-0.20, 0.06], p = 0.30) or on thigmotaxis behavior ([-24.5, 18.5], p = 0.77).

Shoaling behavior. We found no change in offspring shoaling behavior based on either paternal isolation (MCMC GLMM: 95% CI [-0.54, 0.40], p = 0.77) or paternal predation exposure ([-0.60, 0.33], p = 0.59), although longer offspring spent more time shoaling than smaller offspring ([0.06, 0.24], p <0.001).

Offspring size. We also found no differences in length or mass based on either paternal isolation (length: 95% CI [-2.32, 1.21], p = 0.49; mass: [-0.01, 0.01], p = 0.90) or paternal predation exposure (length: [-1.15, 2.39], p = 0.49; mass: [-0.01, 0.01], p = 0.71).

Survival. We found that offspring of fathers who were isolated were captured significantly faster by the predator compared to offspring of fathers with neighbors (95% CI [-1.55, -0.09], p = 0.03;

Figure 2). Similarly, offspring of fathers who were predator-exposed tended to be captured significantly faster by the predator compared to offspring of control fathers (95% CI [-1.38, 0.07], p = 0.07; Figure 2). We found some evidence for social buffering: considering paternal treatment as a 4-level factor, we found a borderline significant reduction in survival time between offspring of predator-exposed fathers who had a neighbor and offspring of control fathers with a neighbor (95% CI [-1.83, 0.04], p = 0.052), with a strong difference between offspring of isolated, predator-exposed fathers relative to offspring of control fathers with a neighbor (95% CI [-2.40, -0.43], p = 0.006).

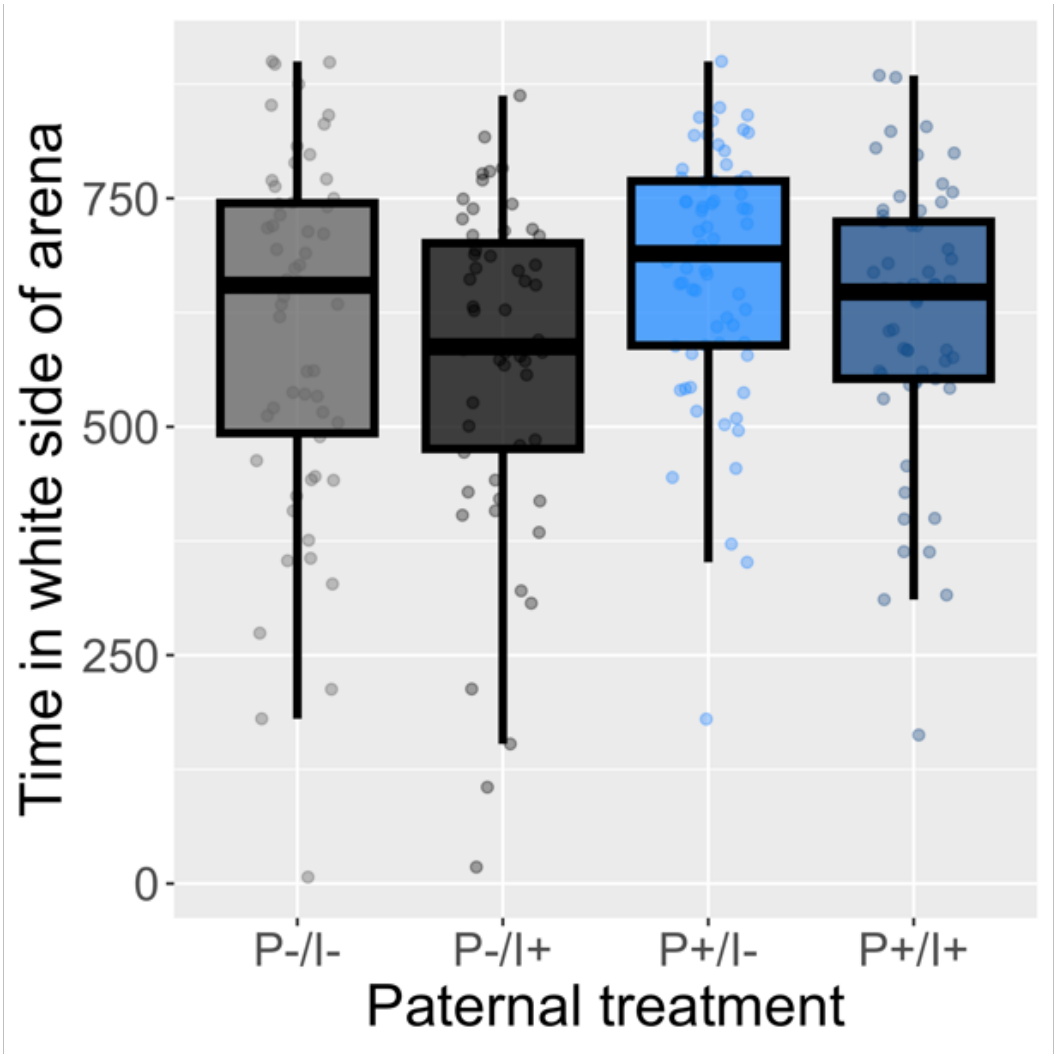

Figure 1: Offspring of predator-exposed fathers (blue) spent more time in the white section of the tank compared to offspring of control fathers (grey), suggesting an anti-anxiety phenotype (median with interquartile range, dots correspond to individuals). P- indicates control, unexposed fathers while P+ indicates predator-exposed fathers; I- indicates fathers who had a male neighbor, while I+ indicates isolated fathers.

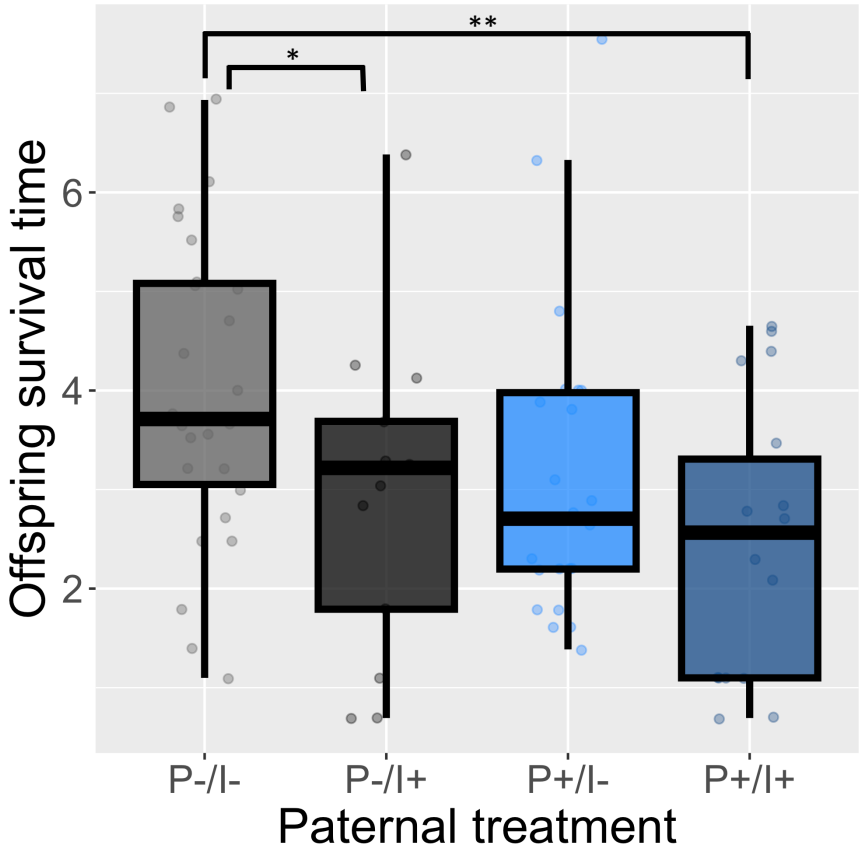

Figure 2. Offspring of socially isolated fathers (darker shade), regardless of paternal predation exposure, were captured more quickly by the predator compared to offspring of a father with a male neighbor (lighter color). Data are median with interquartile range, dots correspond to individuals. Although raw integer data were used for the statistical analysis, we log-transformed the data here for better visibility of results.

Discussion

Although parents routinely encounter stress in the ecological environment that can affect offspring development, the biological reality is that nearly all organisms are embedded within a social environment that can either independently induce parental stress or can alter how parents respond to ecological stressors. Here, we sought to understand how paternal social buffering (via the presence or absence of conspecific neighbors) altered the transgenerational consequences of paternal predation exposure. Offspring of predator-exposed fathers spent more time in the white section of the tank, consistent with an anti-anxiety phenotype. We found that fathers who were socially isolated had offspring who were captured faster by a live predator, suggesting that social isolation in parents may be a stressor that leads to maladaptive effects in offspring. Further, we found some evidence for social buffering: offspring of fathers who were exposed to predation risk were more likely to get eaten by the predator, particularly when the father was isolated compared to when he had a neighbor. This suggests that the social environment can mediate transgenerational effects of paternal predation risk, with the presence of a conspecific buffering fathers from potentially stressful environmental cues.

Previous studies have found that social isolation itself can have a detrimental effect on those directly experiencing it, including an immunosuppressive effect in greylag geese (Ludwig *et al.* 2017), oxidative stress in fish (Schumann *et al.* 2023), and elevated adrenocorticotropic hormone and corticosterone in mice (Dronjak *et al.* 2004). This likely occurs because social contact improves homeostatic control over the HPA axis and reduces exposure to glucocorticoids (DeVries, Glasper & Detillion 2003). Here, we demonstrate that social isolation in adult males does not just affect individuals within a lifetime, but also has detrimental consequences for offspring, leading to lower survival against a predator. This may arise because ancestral stress

340 can make offspring more vulnerable to stress by reprograming the HPA axis, hampering
341 offspring ability to respond appropriately to stress encountered within their own lifetime (Yehuda
342 *et al.* 2008; Rodgers *et al.* 2013; Faraji *et al.* 2017; McDonald *et al.* 2023). Although no studies
343 (to our knowledge) have looked at the transgenerational consequences of paternal isolation prior
344 to fertilization, our results are consistent with previous studies demonstrating that other forms of
345 paternal social stress, including maternal separation early in life (Gapp *et al.* 2014), social defeat
346 (Dietz *et al.* 2011), and social instability (Kong *et al.* 2021), alter offspring anxiety and
347 corticosterone levels. Consequently, although nearly all TGP studies focus on the effects of
348 parental exposure to ecological stress on offspring, here we demonstrate the importance of
349 considering how social stress can also have transgenerational consequences for offspring.
350 The mechanism by which paternal social isolation affects offspring is unknown, but is
351 clearly different than mechanisms of maternal social isolation, largely because those maternal
352 social effects seem to be passed down via glucocorticoids in eggs or during gestation (Walsh *et*
353 *al.* 2019; MacLeod *et al.* 2023; McDonald *et al.* 2023). Dietz *et al.* (2011) found that paternal
354 social defeat in mice increased offspring susceptibility to depression and anxiety-like behaviors,
355 but that this effect was largely not present when offspring were generated via IVF, suggesting
356 that these transgenerational effects were mediated by changes in maternal investment in her
357 offspring in response to perceived paternal quality (acquired during behavioral interactions
358 between the mother and father). Here, we did not allow for the effects of maternal compensation
359 on paternal phenotypes, as offspring were generated via in-vitro fertilization without behavioral
360 interactions between the mother and father. Therefore, our study demonstrates that these paternal
361 effects can be mediated by fathers alone; as our fathers also did not interact behaviorally with
362 their offspring (no paternal care), these effects are presumably mediated via epigenetic changes

363 to sperm and/or seminal fluid. Future studies linking the precise mechanism (e.g., small RNAs or
364 methylation in sperm (Immler 2018)) to phenotypic effects in offspring would be useful for
365 predicting when and how paternal social experiences affect offspring.

366 We found that offspring of predator-exposed fathers spent more time in the white side of
367 the scototaxis arena, suggesting that they were less anxious (Maximino *et al.* 2010). This is
368 consistent with previous studies demonstrating that paternal stress results in offspring with
369 reduced anxiety and HPA responsiveness (Rodgers *et al.* 2013; Gapp *et al.* 2014; He *et al.* 2016;
370 Brass *et al.* 2020). Further, offspring of predator-exposed fathers tended to be consumed faster by
371 the predator, which is consistent with previous work in sticklebacks that found that paternal
372 exposure to predation risk lowered offspring survival against a predator (Hellmann *et al.* 2020).
373 We found some evidence that the presence of a conspecific buffered fathers against the effects of
374 predation risk: there was a stronger reduction in survival time in offspring of isolated, predator-
375 exposed fathers compared to offspring of predator-exposed fathers with a neighbor. This is
376 consistent with previous work demonstrating that isolated individuals show high levels of stress
377 in response to environmental stimuli, while individuals in social groups recover more quickly
378 from or have a reduced response to stressful experiences (Dronjak *et al.* 2004; Kikusui, Winslow
379 & Mori 2006; Ozbay *et al.* 2007; Culbert, Gilmour & Balshine 2019). Interestingly, we show that
380 social buffering can occur even under circumstances where we wouldn't necessarily predict
381 strong effects of social buffering: we used an experimental design where we paired two
382 unfamiliar males together, both of whom were exposed to the predator. This would typically lead
383 to weak effects of social buffering, as previous studies have found stronger effects of social
384 buffering when individuals are paired with a known conspecific compared to an unknown
385 conspecific (Hennessy, Kaiser & Sachser 2009), when individuals are in groups compared to

386 pairs (Stanton, Patterson & Levine 1985; Kikusui, Winslow & Mori 2006), and when the partner
387 is unstressed compared to stressed (Kiyokawa *et al.* 2004; Kikusui, Winslow & Mori 2006).
388 Consequently, future studies manipulating the quality of the social environment could help us
389 better understand how transgenerational effects of social buffering may vary with the number,
390 familiarity, and state of the other conspecifics.
391
392 **Conclusions**
393 We have long known that the social environment is an important source of selection, and
394 that any given trait (e.g., body condition) is not necessarily the property of a single individual
395 because social interactions will influence the traits and fitness of other individuals, even if those
396 individuals have never physically met (Fisher & McAdam 2017; Fisher *et al.* 2019). Currently,
397 we view transgenerational plasticity as a linear and relatively isolated relationship between
398 parent and offspring. Here, we demonstrate that the broader social environment can induce
399 transgenerational effects even in relatively asocial species such as stickleback, which do not form
400 permanent social groups. Previous work has shown that the social environment can also mediate
401 parental response to ecological cues, providing an opportunity for parents to learn about
402 ecological change in their environment in ways that have cascading effects on offspring (Afseth
403 *et al.* 2022). Consequently, the social environment can provide information about ecological cues
404 (Silva, Garcia de Leaniz & Luchiari 2019), act as a buffer against ecological stress (Kikusui,
405 Winslow & Mori 2006; Gilmour & Bard 2022), and induce stress (via both stress transmission
406 (Sterley *et al.* 2018), as well as via aggression, social instability, social isolation (Brandl,
407 Pruessner & Farine 2022)). Future studies exploring how these socially-induced effects alter both

408 within and trans-generational responses to ecological change can help us better predict when and
409 to what extent individuals will be able to adaptively response to rapidly changing environments.

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