Parental predator exposure affects offspring boldness and laterality in the stickleback

Berlinghieri, Flavia; Vittorietti, Martina; Savoca, Dario; Pace, Andrea; Nicolaus, Marion; Riedstra, Bernd; Brown, Culum; Groothuis, Ton G.G.

DOI
10.1016/j.anbehav.2025.123154

Publication date
2025

Document Version
Final published version

Published in
Animal Behaviour

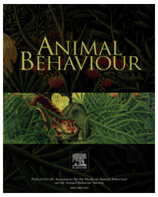
Citation (APA)
Berlinghieri, F., Vittorietti, M., Savoca, D., Pace, A., Nicolaus, M., Riedstra, B., Brown, C., & Groothuis, T. G. G. (2025). Parental predator exposure affects offspring boldness and laterality in the stickleback. *Animal Behaviour*, *223*, Article 123154. https://doi.org/10.1016/j.anbehav.2025.123154

Animal Behaviour 223 (2025) 123154

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



Parental predator exposure affects offspring boldness and laterality in the stickleback

Flavia Berlinghieri [a, b, *], Martina Vittorietti [c], Dario Savoca [d, e], Andrea Pace [d], Marion Nicolaus [a], Bernd Riedstra [a], Culum Brown [b], Ton G. G Groothuis [a]

[a] Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, the Netherlands
[b] School of Natural Sciences, Macquarie University, Sydney, Australia
[c] Department of Applied Mathematics, Delft University of Technology, Delft, the Netherlands
[d] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy
[e] NBFC, National Biodiversity Future Center, Palermo, Italy

ARTICLE INFO

Article history:
Received 11 March 2024
Initial acceptance 6 May 2024
Final acceptance 10 January 2025
Available online xxx
MS. number: 24-00148R

Keywords:
cortisol
laterality
LC-MS/MS
parental effect
personality
predation
stickleback

Parental influences on offspring phenotype occurring through pathways other than via inherited DNA sequences are known as parental effects. Parental effects profoundly influence offspring behaviour, including behaviour laterality and personality, two traits that are widespread and of fundamental importance in the animal kingdom with clear fitness consequences. However, the impact of parental effects on the interaction between behavioural laterality and personality within the same species has not been previously explored. If such a link exists, it would deepen our understanding of personality traits, extending them to brain laterality and its underlying neurobiology. In addition, if both traits are causally linked, it may constrain evolution as changing one of these traits would affect the other. The aim of this study was to examine whether offspring personality traits and behavioural laterality are related and can simultaneously be influenced by parental effects, suggesting a common underlying mechanism. Here we exposed parents of an egg-laying species, the stickleback *Gasterosteus aculeatus*, to a predator cue or not and examined the impact of this exposure on two behavioural traits of their offspring. Shortly after laying, clutches were split: half were reared without predator cues for 12 weeks before behavioural testing, and the other half were used for cortisol analysis. We found that both parents and offspring from predator-exposed parents were bolder, with the offspring more likely to show lateralized behaviour, and were smaller than offspring from parents that were not exposed to predation. The egg cortisol levels were too low to be detected by LC-MS/MS. To our knowledge, these results are the first to indicate that parental effects under varying predation conditions can influence laterality, personality and growth of offspring within the same individuals, although further evaluation and experiments are needed to determine the role of maternal cortisol.

Parents can modify the phenotype of their offspring outside the bounds of inherited genetic material, a process called parental effects (Mousseau & Fox, 1998). Such modifications have been found to affect a wide array of offspring traits (e.g. behaviour, morphology, physiology), often simultaneously (Burgess & Marshall, 2014; Lind et al., 2020). It is thought that through these modifications, parents prepare their offspring for the environment that they are likely to encounter in the future, although a demonstration in the literature of adaptive and predictive parental effects is inconsistent.

The pathways inducing parental effects are highly diverse, ranging from habitat choice and parental rearing behaviour to transfer of food and exposure to immune factors and hormones. These effects can be prenatally induced through modifications to the eggs or sperm/seminal fluid (Bell et al., 2011; Giesing et al., 2011; Hellmann et al., 2021; Lelono et al., 2019; McGhee et al., 2020; Moisiadis & Matthews, 2014; Rakers et al., 2020; Rivera et al., 2021; Rogers & Deng, 2005; von Engelhardt & Groothuis, 2011). For example, stressed human mothers who have higher levels of circulating cortisol can pass this on to their young in utero and thereby influence the developing embryos (Moisiadis & Matthews, 2014; Rakers et al., 2020). In this way, the parental

---

* Corresponding author.
E-mail address: flavia.berlinghieri93@gmail.com (F. Berlinghieri).

https://doi.org/10.1016/j.anbehav.2025.123154

environment causes changes in the offspring's morphology, physiology and behaviour. Many maternal effects are claimed to be mediated by prenatal exposure to glucocorticoids (birds: Hayward & Wingfield, 2004; Saino et al., 2005; fish: Gagliano & McCormick, 2009; reptiles: Meylan & Clobert, 2005; mammals: Hayward & Wingfield, 2004). However, these results are controversial because they relied on ELISA (enzyme-linked immunosorbent assay) or RIA (radioimmunoassay), which are not appropriate for glucocorticoid determination in eggs samples (Henriksen et al., 2011; Rettenbacher et al., 2013).

One well-studied context in which parental effects shape offspring traits is in response to environmental pressures such as predation risk. The effect of predation pressure on parents and the subsequent influence on offspring behaviour is one of the most well-studied contexts of parental effects. In several species, mothers exposed to predators generate offspring with enhanced antipredator behaviour (invertebrates: Storm & Lima, 2010; reptiles: Bestion et al., 2014; Shine & Downes, 1999; fish: Giesing et al., 2011; birds; Coslovsky et al., 2012; Morales et al., 2018; mammals: St-Cyr & McGowan, 2015; metanalysis studies: MacLeod et al., 2022; Tariel et al., 2020b). For example, in the common lizard, *Zootoca vivipara,* gravid females exposed to predator cues generated offspring with more successful antipredator tactics, longer tails and higher dispersal tendencies than offspring from unexposed females (Bestion et al., 2014). In mice, parental exposure to predator odour increased the antipredator behaviour of their offspring (St-Cyr & McGowan, 2015), while in fish, female sticklebacks, *Gasterosteus aculeatus*, exposed to predation risk produced eggs with higher concentrations of cortisol and their offspring shoaled more (Giesing et al., 2011; but see Henriksen et al., 2011; Rettenbacher et al., 2013 for methodological issues), which is an effective antipredator behaviour in fishes (Queiroz & Magurran, 2005).

In addition to these maternal effects, fathers may also affect offspring development. Indeed, Stein and Bell (2014) showed that the brood-nursing stickleback father produced offspring that tended to be smaller in size and had reduced body condition but had a stronger antipredator response and lower general activity under predation pressure. Much of this is mediated by the paternal care, which involves chasing and collecting the fry to return them to the nest (Tulley & Huntingford, 1987, 1988).

Egg laying species are good study species for studying such prenatal effects, as egg components can be analysed and manipulated to identify potential underlying mechanisms without interfering with the mother. The prenatal stage is an important developmental phase for parental effects because offspring have relatively low costs for plasticity as most of the tissue has still to be formed and the embryo relies almost entirely on the parents for environmental information (T. G. Groothuis & Taborsky, 2015). Research has demonstrated that hormone-mediated maternal effects influence not only egg size and egg components but also various offspring traits including growth, physiology, behaviour and survival (Eriksen et al., 2006; Giesing et al., 2011; Henriksen et al., 2011; von Engelhardt & Groothuis, 2011).

These prenatal influences can also interact with the development of personality. Personality is described as consistent individual differences in behaviour across time and contexts (Stamps & Groothuis, 2010) and has wide-ranging ramifications for individual fitness, with potential consequences at the level of the ecosystem (Mittelbach et al., 2014; Smith & Blumstein, 2008). Personality has been identified in a wide range of animals (Carere & Maestripieri, 2013) including invertebrates (Tariel et al., 2020a), fish (Byrnes & Brown, 2016; Colléter & Brown, 2011), lizards (Barrett et al., 2022), birds (T. G. G. Groothuis & Carere, 2005) and mammals (Mazza et al., 2019). Boldness, which is the propensity to take risks, is one of the most studied personality traits and has repeatedly been shown to vary with exposure to predation. In the tropical poeciliid *Brachyrhaphis episcopi*, fish from high-predation areas were bolder compared to those from low-predation areas (Brown et al., 2005). Indeed, fish tend to be bolder in high-predation areas because they must continue to live their lives conducting important activities like foraging despite the greater threat (Brown et al., 2005).

Exposing sticklebacks to predation pressure generated a boldness—aggressiveness behavioural correlation that did not emerge in nonexposed fish (Bell & Sih, 2007). Also, in sticklebacks, simulated predation risk generated repeatable behaviour for boldness and aggression (Edenbrow & Croft, 2012; Stein & Bell, 2014). Collectively these studies indicate that personality traits can be affected by predation pressure.

Behavioural lateralization results from an asymmetrical organization of the brain with respect to behavioural domains and functions, where one hemisphere is functionally different from the other (Bisazza et al., 1998). Laterality has been suggested to provide fitness benefits across multiple domains (Bibost & Brown, 2013; Dadda et al., 2015; Magat & Brown, 2009). Lateralization is often manifested as behavioural side biases such as turning or hand preferences. For example, in animal species where the visual fields do not overlap much, an individual can prefer to use one eye for predator observation and the other for foraging (Lucon-Xiccato & Dadda, 2017). Similarly, toads are more likely to strike a prey moving in their right lateral field than in the left (Bisazza et al., 1998). As with personality, predation pressure plays a crucial role in the development of lateralized behaviour in fish (C. Brown & Braithwaite, 2004; Brown, Burgess, & Braithwaite, 2007; Brown, Western, & Braithwaite, 2007; Dadda et al., 2020). Brown et al. (2004), for example, found that fish from high-predation areas were more lateralized compared to those from low-predation areas (Brown et al., 2004). Moreover, laterality has a heritable and an experiential component (Bisazza et al., 2000; Brown, Burgess, & Braithwaite, 2007; Paracchini, 2021), and there is some evidence that parental effects play a role in shaping laterality. Dadda et al. (2020), exposed topminnows to simulated predation threats during pregnancy and found that predator exposure increased both visual and motor lateralization in their offspring (Dadda et al., 2020).

However, since laterality is not entirely under genetic control (Bisazza et al., 2000) and some lateralized functions cannot be artificially selected (Bisazza et al., 2005), environmental factors may play a significant role. Relevant stimuli, such as exposure to predators are well known to influence both the development and evolution of laterality (Dadda et al., 2009; Lucon-Xiccato et al., 2016; Rogers, 2002; Vallortigara & Rogers, 2005). This idea is largely based on the concept that increased neural capacity, dominance of one brain hemisphere and multitasking capability can help prey more effectively avoid predators, thereby improving their chances of survival in environments with high predation threats (Hulthén et al., 2021). Indeed, some studies have shown that more strongly lateralized individuals exhibit increased performance in antipredator behaviours, such as escape behaviour (Dadda et al., 2010) and predator recognition (Ferrari et al., 2017; Lucon-Xiccato et al., 2016).

Since personality and lateralization encompass a wide array of partly overlapping behavioural and cognitive domains, they may be causally related. Specific emotions that can affect some personality traits are mainly processed by a specific hemisphere and are therefore lateralized (Rogers, 2010). Thus, it can be expected that some personality traits, such as boldness and aggression, may be expressed behaviourally in a lateralized fashion (C. Brown & Bibost, 2014; Byrnes et al., 2016; Irving & Brown, 2013; Reddon & Hurd, 2008, 2009). For example, emotions that give rise to aggression or fear (e.g. of novel objects) are strongly lateralized and are well-known personality traits (Leliveld et al., 2013). Aggression is often processed in the right hemisphere in a wide range of animals, ranging from invertebrates (Rogers et al., 2016) to reptiles (Bisazza et al., 1998; Deckel, 1995), fish (Ariyomo & Watt, 2013; Reddon & Hurd, 2008), birds (Lemaire et al., 2019) and mammals (Austin & Rogers, 2012, 2014). In addition, both can be affected by early experience, including prenatal hormone exposure, of which cortisol may be one possible candidate (Deckel, 1998; Jutfelt et al., 2013; Ocklenburg et al., 2016; Raoult et al., 2012, 2017). Therefore, both may be correlated perhaps via a common underlying mechanism. However, both traits are mostly studied separately and only more recently has their relationship received attention. A link between laterality and personality traits has been studied in a few animal species, including teleost fish (C. Brown & Bibost, 2014), sharks (Byrnes et al., 2016) and mammals (Barnard, Wells, Hepper, & Milligan, 2017; Goursot et al., 2019). For example, in rainbowfish, nonlateralized fish were bolder than strongly lateralized fish (C. Brown & Bibost, 2014). In cichlids, the strength of laterality was linked to stress and aggressiveness (Reddon & Hurd, 2008). That such a correlation may be causal is suggested by several studies finding a link between individual stress levels, laterality and personality traits in nonhuman primates (e.g. Fernández-Lázaro et al., 2019). It is possible that a common causal factor connects laterality and personality. In such a scenario, the manipulation or evolution of laterality might also influence personality, and vice versa. To date, however, there are no studies that have determined if the link between laterality and personality traits can both be influenced by the same parental effects in a single study system and may therefore be causally linked.

Here we used three-spined sticklebacks, *G. aculeatus*, as our model to determine if parental exposure to simulated predation influenced the development of both laterality and personality in their offspring, suggestive of a causal link between the two. Sticklebacks are a key species for the study of behavioural neurosciences and genetics, and for ecoevolutionary studies, including maternal effects and personality (Aguirre et al., 2022; Arai et al., 2020; McGhee et al., 2012, 2020; Minter et al., 2017; Ramesh et al., 2021; Stein & Bell, 2014). Sticklebacks are an egg laying species, which facilitates the study of prenatal parental effects. Here we exposed parents to simulated predation threat or not and examined the behaviour of the parents and of their offspring, which were reared without parents in a predator-free environment (to exclude any further parental effects). We hypothesized that offspring from parents exposed to simulated predation would be consistently more strongly lateralized and bolder relative to offspring from nonexposed individuals and that parental exposure to predation would induce a stronger correlation between both traits. Additionally, concerning shoaling behaviour, fishes exposed to predation threats should display tighter shoal cohesion. Using liquid chromatography tandem mass spectrometry (LC-MS/MS) we also checked whether mothers exposed to predation pressure deposited more cortisol in their eggs and, if so, whether this would correlate with the shifts in offspring behaviour.

METHODS

Study System

The three-spined stickleback is a widespread anadromous fish species found in the northern hemisphere (Fang et al., 2018; Giles, 1987). Some populations inhabit freshwater environments all year round, while others migrate from a marine environment to freshwater for reproduction (Clavero et al., 2009). Sticklebacks are gregarious fish forming schools outside the reproductive season and are sexually dimorphic, which is most pronounced in the breeding period from spring until summer. The male crafts a nest using plant materials, and females lay eggs inside it, which are subsequently fertilized by the male. The male guards and ventilates the eggs and young (Scott, 2004; Wootton, 1973). In the present study we used adult fish derived from a wild anadromous population and their offspring bred at our facility at the University of Groningen, The Netherlands.

Housing and Experimental Design

Adult fish used as parents in this experiment were born between May and June 2020 from wild sticklebacks caught in April 2020 at the Ems-Dollard Estuary, on the northern Dutch−German border, and were reared in a seminatural environment (Ramesh et al., 2021).

In March 2021, fish were housed in a seminatural system that comprised five interconnected ponds (Fig. 1), each with a diameter 1.6 m, connected by four pipes of length 1.5 m and diameter 11 cm (ca. 600-litre tanks filled with freshwater from the tap) under natural temperature and light conditions (described by Ramesh et al., 2023). The floor of each pond was covered with a thin layer of sand and plastic plants. Green cotton threads were provided to facilitate nest building. Fish were fed a mixture of artemia and bloodworms once a day, ad libitum. We had two systems; each system contained 50 fish.

Both pond systems were located in the same aviary (to protect against predation), exposed to the same environmental conditions, fed with water from the same source and treated (apart from the experimental manipulation) exactly the same way. Therefore, the likelihood that differences between the pond systems would have overruled the effect of manipulation is unlikely. Additionally, after the collection of the egg clutches, they were each housed in their own tank (35 × 45 cm).

Fish started to breed in April 2021 at which time we started the predation treatment (see below). Fish from one system were designated as ‘treatment’ and from the other system ‘control’. The fish in each pond in the treatment system were exposed to 50 ml of conspecific alarm cues, 50 ml of predator alarm cues (perch, *Perca fluviatilis*) and chased by a model perch-like ‘predator’ for 5 min. Perch chasing occurred five times a week, while conspecific and

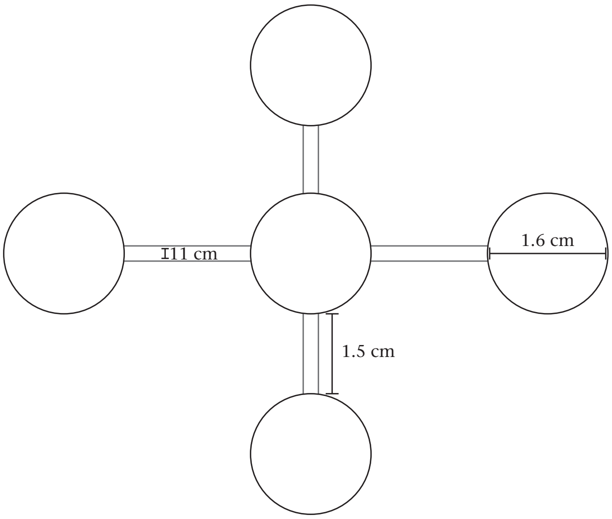


Figure 1. Arrangement of the ponds for the treatment and control groups.

predator alarm cues were presented three times a week during the five instances of predator chasing, on random days each week.

The treatment simulated a real predation risk that sticklebacks experience in the natural environment. Conspecific alarm cues were created from 200 g of frozen sticklebacks with their head and organs removed. The tissue was homogenized with 600 ml of tap water, passed through a sieve (Cattelan et al., 2020; Evans et al., 2007; Speedie & Gerlai, 2008) and then stored in 10 ml aliquots at −20 °C until the day of use. Each pond received a 10 ml dose of alarm cue diluted with an additional 40 ml of pond water (250 ml total volume for the entire system). Predator odour was collected from a separate pond (ca. 600 litres) housing three perch, one of the primary piscivorous predators of sticklebacks in The Netherlands (Jacobson et al., 2019). The parental predation treatment lasted from March 2021 to July 2021. We started collecting eggs to be analysed later for hormonal analysis and to obtain offspring three weeks after the treatment began.

We removed the eggs from the males' nests every 2 days, thereby limiting the exposure of eggs themselves to predation cues and paternal care. It is improbable that the eggs were sufficiently developed at 2 days postfertilization to perceive alarm cues (Swarup, 1958). Clutches were divided in half; one half was placed in smaller (ca. 20 litre) ponds to let them hatch (see below) and the other half was frozen at −80 °C awaiting hormonal analysis by extraction and LC-MS/MS (see Appendix). LC-MS/MS is the technique that can reliably detect concentrations of the target hormones with very high specificity. Mass spectrometry (MS) associated with liquid chromatography (LC-MS) is the principal antibody-free method used for the detection of cortisol. Cortisol, extracted from the sample is isolated by liquid chromatography (LC). The stationary and adsorbent octadecylsilane-bonded phase (C18) is often used at high pressure (HPLC) to separate chemical species in the sample. MS then ionizes isolated components, measures the separation of the molecules according to their mass-to-charge ratio (M/z) and determines the relative abundance of each ion produced. This technique is much more reliable and specific for determining hormone concentrations than techniques using antibodies, such as the traditional and commonly used RIA or ELISA, as their antibodies cross-react with hormones other than the target hormones. This is especially relevant for eggs that contain many hormones, both their precursors and metabolites (Henriksen et al., 2011; Rettenbacher et al., 2009, 2013).

We used offspring that were sexually immature; therefore, we could not discriminate their sex throughout the experiments.

Parental Boldness

To confirm that prolonged exposure to simulated predation influenced the parents, we observed their responses to a predator model under controlled lab conditions. We tested 26 fish from the predation treatment and 24 from the control. These fish were moved from their home pond a day before the test and placed into one of four aquaria (80 × 35 cm and 40 cm high) to allow them to acclimatize.

A focal fish was selected from the housing aquarium and gently placed in a novel experimental tank (40 × 30 cm and 40 cm high). The experimental tank was furnished with a tube as shelter (5 × 10 cm), gravel on the floor and a tethered perch model, the movement of which was remotely controlled by hand (Fig. 2). To further simulate the presence of predators in the environment, we added perch odour (5 ml) to the test aquarium. The experimental tank was positioned in an illuminated wooden box to avoid external disturbance. The duration of the experiment was 10 min and the behaviour of the subjects was recorded using an overhead camera (Raspberry Pi NoIR Camera Board V2 8MP, Raspberry Pi Foundation, U.K., https://www.raspberrypi.org). We replaced the water in the experimental tank with freshwater and new predator odour each time after four fish were tested.

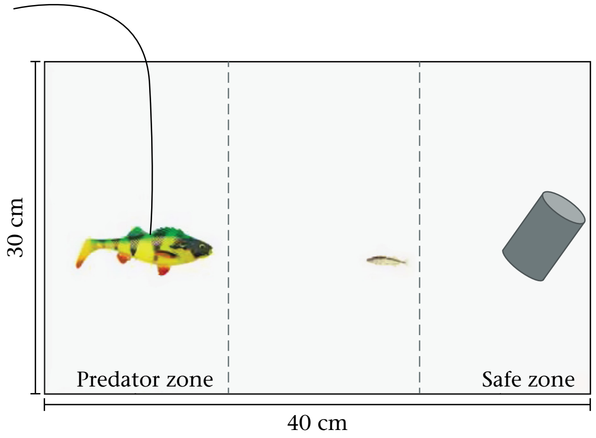


Figure 2. Test aquarium set-up for the boldness test. Predator zone is where the perch model was positioned and the safe zone was supplied with a tube as shelter.

Behaviour was scored using the software BORIS 7.10.2 (Friard & Gamba, 2016). We recorded the time spent in the safe zone (the third of the arena nearest to the shelter) and the predator zone (the third of the tank nearest the predator model; Fig. 2).

Offspring Housing Conditions

Half of each clutch collected was placed in small ponds (ca. 20 litres) and allowed to hatch in the absence of parental care to avoid potential posthatch paternal effects on juvenile behaviour. The larvae hatched 5—7 days after fertilization and started to independently forage around 1 week after hatching. Larvae were then fed daily with frozen cyclops and freshly hatched Artemia. Larvae remained in the small ponds for 3 weeks after which they were moved into submerged cages (40 × 40 cm and 60 cm high) inside larger ponds (ca. 600 litres). We placed 40 larvae per cage, twelve cages in total (six cages per pond per treatment and six cages per pond per control). The larvae remained in this semi-natural set-up for 4 weeks, after which the fish were pooled by treatment, divided into groups of eight and moved to a standardized environment in a climate room. Subsequently, fish were placed in rectangular tanks (30 × 16 cm and 18 cm high) connected to a recirculating water system at 17(±1) °C. The photoperiod was set at 14:10 h light:dark. Each tank had a plastic plant, PVC tube hides and a gravel substrate. Three months after hatching fish were lightly anaesthetized using a buffered MS-222 solution (see Ethical Note) and tagged with a visible implant elastomer (VIE; NorthWest Marine Technology, https://www.nmt.us) for individual identification. VIE was implanted underneath the dorsal fin at the subcutaneous level using an insulin syringe (29G). The whole tagging procedure lasted about 20 s per individual. The fish were then allowed to recover in tanks with freshwater oxygenated with air stones. We tagged 100 juveniles from the treatment group and 100 from the control group.

Fish were exposed to a series of behavioural assays and tested in the following order: predator inspection test, shoaling test, mirror test and, after 2 weeks, the second predator inspection test. All four tests for each fish occurred on 2 subsequent days, so on the first day they were tested with predator inspection test and shoaling test, and the following day with a mirror test. The second predator test took place 2 weeks after the first predator inspection test.

Offspring Boldness

Offspring were tested at 4 months old with a predator inspection test twice, 3 weeks apart, to assess consistency in boldness (following Ramesh et al., 2023). The sample size tested was 100 fish from the control group and 94 from the treatment group. Two tanks were placed side-by-side; one (40 × 20 cm and 30 cm high) housed a live perch, *P. fluviatilis*, and the other (25 × 20 cm and 20 cm high) housed the focal fish and a shelter (Fig. 3). The tanks were positioned in an illuminated wooden box to avoid external disturbance. The duration of the experiment was 10 min. We scored the time spent inside the shelter (B) and outside the shelter (A) as proxies for shyness and boldness respectively.

Offspring Shoaling (Antipredator Behaviour)

To measure shoaling behaviour, five individuals from the same home tank were placed in a novel arena (33 × 18 cm and 33 cm high), which was surrounded with black plastic. The test lasted 10 min. Every 10 s a snapshot of the video was taken and the *x,y* coordinates of each fish's head were identified. The area of the polygon generated by the coordinates (Fig. 4) was calculated using ImageJ 13.1 (Schneider et al., 2012) as a measure of shoal cohesion. We averaged the area generated by the shoal at each snapshot over the total observation time (Bibost & Brown, 2013; Bisazza & Dadda, 2005).

Offspring Laterality

We employed a rotational mirror test to score laterality in a social context (sensu Sovrano et al., 1999). Fish were placed in a circular glass tank (diameter 19 cm, water depth 4 cm) where the wall was covered with mirror foil to create a continuous mirror for the subject to swim alongside their reflection (Fig. 5). Subjects were gently placed in the arena, and we recorded which eye the individual used to observe their mirror image every 5 s for 10 min. When the fish was perpendicular to the mirror it was scored as nonlateralized. If the fish was not looking at the mirror, as indicated with B in Fig. 5, it was not included in the lateralized formula. When the fish was looking at the mirror with one eye (<90° to the mirror) we noted if it was using the right or left eye, and if the fish was at >90° to the mirror it was excluded from the data set (Sovrano et al., 1999). We then calculated the total number of times each fish watched the mirror with its left, right or both eyes. The sample size tested was 100 fish from the control group and 94 from the treatment group.

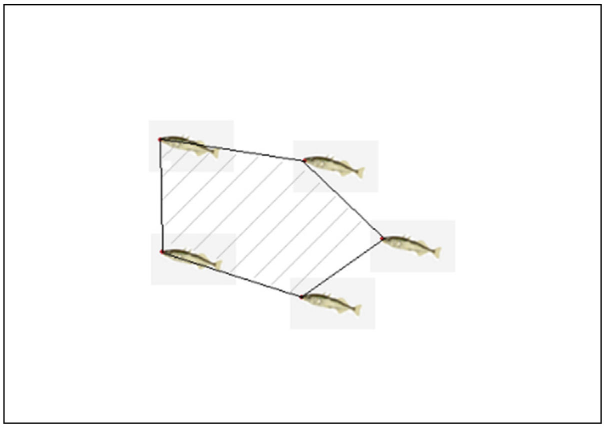


Figure 4. Shoaling test. The shoal area was determined by the size of the area, in cm^2, enclosed by connecting the fish's heads with hypothetical straight lines (shaded area).

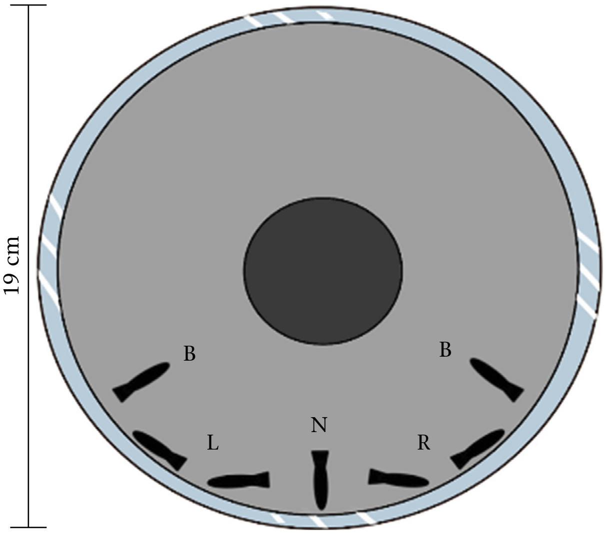


Figure 5. Rotational mirror test. R indicates when the fish was looking at the mirror with the right eye, L with the left eye, N with both eyes (nonlateralized) and B if the fish was not oriented towards the mirror.

Egg Cortisol Analysis

Eggs sampled during the treatment period were preserved in a freezer −80 °C and later analysed for cortisol concentrations using LC-MS/MS. Due to the required sample volume, we pooled eggs

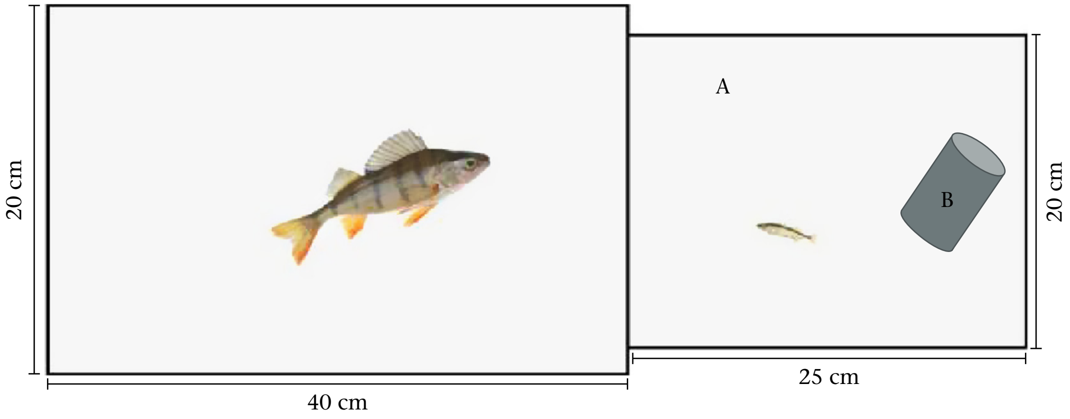


Figure 3. Boldness test in offspring. The tank on the left housed a live perch. In the tank on the right, Zone A is the predator zone and zone B (the shelter) is the safe zone.

from the same clutch, resulting in a sample size of 10 clutches of the treatment and 10 clutches of controls. Two procedures were used for cortisol extraction: SPE and liquid extraction (LLE; see Appendix). The limit of detection (LOD) was 0.5 ng/ml and limit of quantification (LOQ) was 2.5 ng/ml, as determined by the IUPAC method.

Ethical Note

The parents were descended from wild-caught fish in 2020 (Ramesh et al., 2021). Adult three-spined sticklebacks were housed in different ponds as previously described (100 individuals, 50 in the treatment group and 50 in the control group). The adults bred during this period, and we simulated predation risk. Adult fish had sufficient space and physical structure to avoid the simulated predator. We then tested 26 adult fish from the predation treatment group and 24 from the control group to generate sufficient power to test our hypotheses. Since we moved them from their home ponds to the aquarium, we allowed them to acclimatize before testing, for 1 day.

We tagged the fish using Visible Implant Elastomer (VIE). The tagging procedure was as follows: the fish were first placed in a pretagging and recovery tank, which was the same size as their home tank. After approximately 20 min, the fish were transferred to a container with buffered MS-222 (tricaine methane sulfonate; 0.3 g/litre; pH ca. 7.0) anaesthetic. The fish remained in the anaesthetic solution for about 1 min, until they lost equilibrium.

Once the fish were anesthetized, they were removed from the solution and placed on a wet sponge with a groove to facilitate tagging. Prefilled microfine 0.5 ml insulin syringes containing the VIE elastomer were prepared for use. Each fish was gently held in the wet sponge, and the needle was inserted at a shallow angle beneath the first layer of skin on the dorsal side.

After tagging, the fish were returned to the pretagging and recovery tank, which was aerated to aid in their recovery. The entire tagging process took approximately 2 min per fish. Following the procedure, the fish quickly resumed their typical swimming and exploratory behaviours. We observed a 100% recovery rate after the tagging process.

Eggs from the adults were collected from nests in each treatment. Male parental care primarily protects the eggs from predation and increases water flow, so removing them and rearing them to hatch in the laboratory has limited welfare implications. We tested 100 offspring from the control group and 94 offspring from the treatment group. Six fish from the treatment group were not tested because they appeared stressed prior to experimentation (their behaviour and eating habits were abnormal).

We always minimized the stress of handling and moving the fish, allowing them to acclimatize when necessary.

Fish were kept in the animal facility at the University of Groningen for future experiments. Indeed, after 6 months, 62 fish were used in another study (Berlinghieri et al., 2024). Additionally, we donated around 40 fish to another research group at the University of Leiden in The Netherlands, while the remaining fish were released into the Reitdiep canal near the Zernike Campus of the University of Groningen.

Housing and testing of behaviours were in adherence to the project permit from the CCD (Centrale Commissie Dierproeven, The Netherlands) under licence number AVD1050020174084 and the IVD (Instantie voor Dierenwelzijn) under the licence number 174084-01-006 (University of Groningen, The Netherlands). Further, the research conducted here adheres to the ASAB Ethical Committee & ABS Animal Care Committee guidelines for the ethical treatment of nonhuman animals.

Data Analyses

We tested the personality and laterality data for normality using the Shapiro–Wilk test and because the data were not normally distributed, we analysed the data using nonparametric procedures. We used the Mann–Whitney *U* test (MWU) to check for differences in personality traits (shyness: time spent in the safe zone) between the treatments in both the parental and the offspring generations and length in the latter. To check for a relationship between size (length) and laterality (strength and direction, see below) or personality traits we used Spearman rank correlations. Nonparametric tests were performed using SPSS 26.0.

We also used Spearman correlation to assess the repeatability of the personality trait during the predator inspection test (Mazué et al., 2015; Svartberg et al., 2005).

For the laterality test, we calculated, the laterality index and absolute laterality using the formulas:

$$\text{Laterality index} = \frac{\text{Left eye} - \text{Right eye}}{\text{Left eye} + \text{Right eye}}$$

$$\text{Absolute laterality} = \left|\frac{\text{Left eye} - \text{Right eye}}{\text{Left eye} + \text{Right eye}}\right|$$

To analyse the data, we followed the methods used by Dadda et al. (2015) and categorized right and left lateralized respectively when the index was $\geq$0.75 (right) or $\leq$0.75 (left). Otherwise, the fish were classified as nonlateralized (NL). Because of this categorization, we conducted a two-sample Z proportion test to compare the number of lateralized individuals between control and treatment groups.

Because the data were highly skewed, we analysed the relationship between personality (boldness) and laterality (categorized in three levels: right lateralized, left lateralized and nonlateralized) with a multinomial logistic regression (MLR). For the estimation, we used the R package VGAM (Yee, 2010). Offspring shoaling test results were also analysed with a nonparametric analysis, Mann–Whitney *U* test (MWU), comparing the difference between the treatment and the control group.

RESULTS

Parents Boldness Test

Adult fish exposed to simulated predation spent less time in the safe zone compared to the control fish (MWU: $U = 207.000$, $P = 0.017$; Fig. 6) confirming that the predator treatment was effective in changing the behaviour of the adults.

Offspring Standard Length

Offspring ($N = 93$ treatment, $N = 132$ control) of predator-exposed parents (SD = 28.63; 95% confidence interval, CI = 18.544–19.717; median = 18.700; interquartile range = 3.500) were smaller than offspring from the control parents (SD = 3.885; CI = 21.063–22.391; median = 21.600; interquartile range = 5.5750; MWU: $U = 3638.000$, $P < 0.001$). These results confirm that exposing parents to predation had an effect on the growth of their offspring.

Offspring Boldness Test

Similar to the parents, offspring from control parents spent more time in the safe zone than offspring from the treatment group (*N* control = 83, treatment = 77; MWU: $U = 2581.000$, $P = 0.034$; Fig. 6). The repeatability of the personality trait (shyness) during

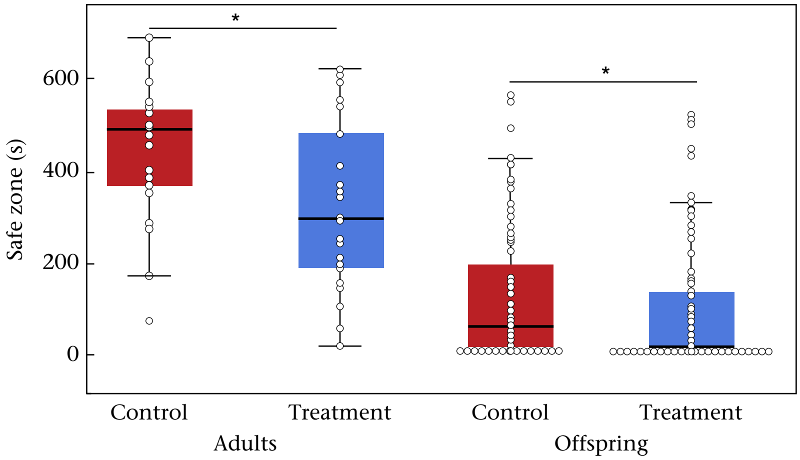


Figure 6. Box plots with jittered raw data showing the time spent in the safe zone (s) by adult parents and offspring in the control and treatment groups. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range. *P<0.05.

the predator inspection assay was significant only in the treatment group (treatment: Spearman rho = 240, P = 0.036; control: Spearman rho = 179, P = 0.106). Furthermore, the time spent in the shelter was weakly positively correlated with body length in the control group (Spearman rho = 239, P = 0.049) but not in the treatment group (Spearman rho = 091, P = 0.534).

Offspring Shoaling Behaviour

Shoaling behaviour of the control (median = 2259; CI = 2001−2848) and predator-exposed offspring (median = 2731; CI = 2168.096−3248) did not differ (MWU: P = 0.55, control N = 16 versus treatment N = 15; Fig. 7).

Laterality and the Association with Boldness

The proportion of lateralized fish in the rotational mirror test was significantly higher in the treatment group (43%) than in the control group (24%; treatment N = 76 versus control N = 90; two-sample Z proportion test: P = 0.015).

The multinomial logistic regression indicated that the parameters associated with boldness and its interaction with treatment were not significant, suggesting the two traits were not correlated (Table 1). However, the parameters associated with treatment were significant (β_{2R}) or approached significance (β_{2L}). This indicates that there is a trend of being more lateralized in the treatment group, especially towards the right (β_{2R}), than fish in the control group (Table 1, Fig. 8).

Cortisol

In all egg samples, cortisol concentrations were below the level of detection limit. The limit of detection was 0.5 ng/ml and the limit of quantification was 2.5 ng/ml, determined according to the IUPAC method.

DISCUSSION

The aim of the study was to test whether offspring personality and laterality are related and can simultaneously be influenced by parental effects, suggesting a common underlying mechanism. We found that our treatment simulating a high predation risk environment for the parents was effective in two ways. First, parents exposed to predators spent less time in the safe zone (i.e. they were

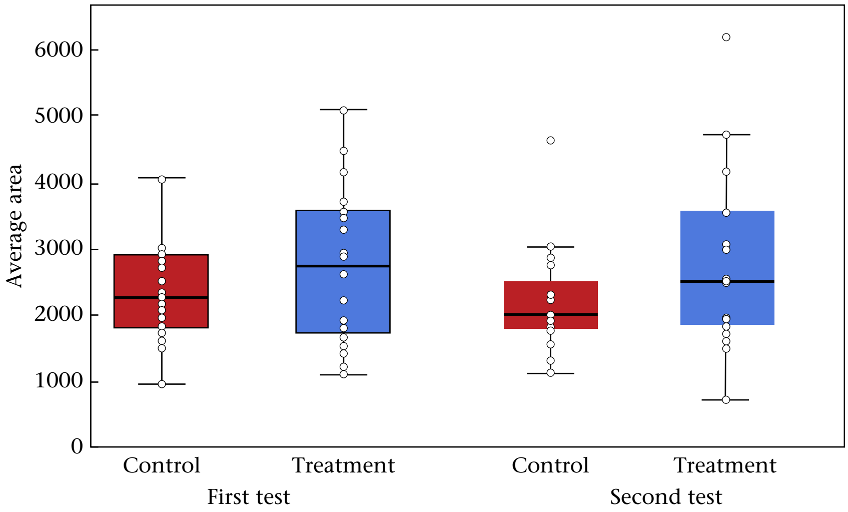


Figure 7. Shoaling behaviour. Average area of the polygon generated by coordinates every 10 s from a 10 min video, representing shoal cohesion for the control and treatment groups in the first and second tests. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range.

Table 1
Strength of laterality as dependent variable

Parameters	Estimate	SE	*P*
β_{0L}	−2.178	0.489	8.40×10^{6} ***
β_{0R}	−2.188	0.507	1.59×10^{5} ***
β_{1L}	0.003	0.002	0.1355
β_{1R}	0.002	0.003	0.4577
β_{2L}	0.966	0.561	0.0849
β_{2R}	1.136	0.570	0.0464*
β_{3L}	−0.003	0.003	0.2096
β_{3R}	−0.003	0.003	0.3188

Estimates of the parameters of the MLR with their standard error and *P* value. The dependent variable is the categorized laterality score (right and left lateralized respectively when the index was $\geq$0.75 or $\leq$0.75) . The subscript letters R and L indicates laterality, specifically right and left. β_{0R} and β_{0L} are the two intercepts, β_{1R} and β_{1L} are the regression parameters associated with shyness, β_{2R} and β_{2L} are the regression parameters associated with treatment and β_{3R} and β_{3L} are the regression parameters associated with the interaction between shyness and the treatment.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

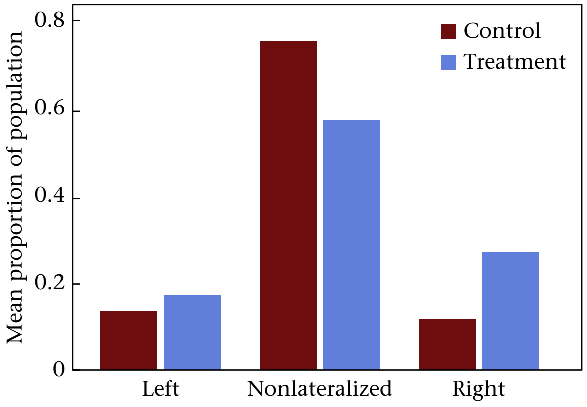


Figure 8. The mean proportion of the population that was either left, right or non-lateralized for the control and treatment groups.

bolder) compared to parents in the control group, and second the parental environment affected size, laterality and personality traits in the offspring even though the offspring themselves had not experienced the treatment. In particular, relative to control offspring, offspring from predator-exposed parents (1) were smaller in size; (2) were bolder as exemplified by the increased time spent close to the predator, mirroring the behaviour of their parents; (3) showed repeatability in boldness; (4) were more lateralized. However, we found no differences in shoaling cohesion.

Contrary to our hypothesis, however, laterality and boldness were not linked; rather they were affected by the treatment independently. We also assumed that the underlying causal mechanism would involve cortisol, but our analysis indicated that cortisol levels in the eggs from both treatments were extremely low (below threshold detection levels).

Many fish alter their behaviour long after being exposed to stressful cues such as alarm signals and predator exposure (C. Brown, 2003; G. E. Brown, 2003; Crane et al., 2022). Consistent with previous studies (e.g. Brown et al., 2004), we found that parents exposed to such cues spent more time close to a predator (i.e. bolder) compared to the control group, indicating that exposure to simulated predation threat altered the behaviour of the parents. However, it is important to note that we did not test the parents before starting the treatment, so there is a possibility that their behaviour differed initially. This seems highly unlikely, since we randomly chose the fish for the treatment groups. We also found that offspring from predator-exposed parents spent more time close to the predator than the control offspring, providing evidence for a parental effect on offspring behaviour. Intriguingly, boldness was repeatable for offspring from predator-exposed parents, but not in those from control parents, suggesting that exposure to predation generates personality in their offspring. This finding is consistent with previous research showing that exposure to predation in adult fish leads to behavioural repeatability (i.e. personality traits) in sticklebacks (Bell & Sih, 2007). As far as we are aware, this is the first demonstration of parental exposure to predators inducing repeatability in a behaviour, generating a personality trait in offspring at the juvenile stage.

As predicted, we also found evidence for parental effects on laterality. We discovered that predator-exposed parents were more likely to produce strongly lateralized offspring than those of the control group, as tested in the mirror assay. This result is consistent with the theory that laterality probably provides a fitness benefit in the context of antipredator behaviour since strongly lateralized fish can perform two tasks simultaneously, such as looking out for a predator with one hemisphere while shoaling with shoal mates using the other hemisphere (Bisazza & Brown, 2011). This result mirrors that found in poeciliids, where fish from high-predation areas are more strongly lateralized than those from low-predation areas (Brown, Burgess, & Braithwaite, 2007). Research in the guppy, *Poecilia reticulata*, found that laterality appeared only in fish that had received previous experience with predators (De Santi et al., 2000) and exposure of pregnant goldbelly topminnow mothers to a visual predator increased offspring lateralization. (Dadda et al., 2020). Importantly, here we removed the eggs after a maximum of 2 days of oviposition, which probably prevented embryos from being influenced by the predator and conspecific odour (Horn & Chivers, 2021; Swarup, 1958). Our results add to the growing literature suggesting that the laterality of young animals can be heavily influenced by maternal effects and that laterality is highly plastic very early in development.

Surprisingly we found no difference in shoal cohesion between offspring from the treatment groups, which is often one of the first traits to respond to increased predation threat. Although the tank used was relatively small and rectangular, it was the same dimensions as their home tank. Given that the fish were only 4 months old, their small body size allowed them to move freely within the tank. Thus, we think this null result is real, but it could be revisited in future studies using larger circular arenas.

If we had found evidence for maternal effects affecting both laterality and personality, this would still not necessarily present evidence for a causal link between the two traits, as the physiological mechanisms by which maternal effects influence these traits may differ. We initially hypothesized that laterality and personality would have similar underlying mechanisms, such as circulating hormones, specifically maternally derived cortisol (Eriksen et al., 2011; Giesing et al., 2011). Such evidence would only be correlative, warranting experimental manipulation of the hormone to conclude on a potential causal mechanism. Instead, our results suggest that these traits developed independently in offspring whose parents were exposed to predation. Previous authors have found that cortisol can impact both laterality and personality in a range of animals (Schaafsma & Groothuis, 2011, 2012). While we did find that predator-exposed parents delivered smaller offspring, a common finding after maternal treatment with corticosterone or cortisol (von Engelhardt et al., 2009), contrary to expectation, cortisol levels in the eggs were below detection limits in both treatment groups. This indicates that prenatal exposure to cortisol is probably not underlying any differences found in laterality or personality, but there may be other hormones or derivatives responsible.

The failure to demonstrate the presence of cortisol in the eggs may be due to the following reasons. First, the sensitivity of the LC-MS/MS could have been insufficient. However, a study by Reyes-Contreras et al. (2023) did not detect cortisol in unfertilized and fertilized eggs of a cichlid species with a 20-fold increase in sensitivity by LC-MS/MS, whereas they detected several of its metabolites that are thought to be mostly biologically inactive (Reyes-Contreras et al., 2023). Also, egg cortisol levels have been reported to be much higher than our threshold. Giesing et al. (2011) did report cortisol concentrations in stickleback eggs, but this was determined by ELISA, a much less reliable technique due to its cross-reactivity with the many gestagens in the egg (Rettenbacher et al., 2013). Second, it may have been that cortisol was initially present but very rapidly converted to its metabolites, such as 11-deoxo forms and cortison, before eggs were collected, as early conversion of steroids has been demonstrated in avian eggs (e.g. Wang et al., 2023). However, this conversion mostly results in biologically inactive hormones. Moreover, this conversion takes place by the embryo and, in the abovementioned study of eggs of cichlid fish, authors did not detect corticosterone in unfertilized eggs where such conversion would not have taken place (Reyes-Contreras et al., 2023). Nevertheless, as our eggs were collected 1–2 days after fertilization, this may be a possible reason for not detecting cortisol. Third, the hormone may have lowered in concentration between oviposition and sampling because the hormone migrated to the water. We did not expect this, as eggs are lipophilic in contrast to water and thus the steroids will have difficulty leaving the egg. However, Paitz et al. (2016) demonstrated a very rapid embryo-mediated efflux of cortisol from the egg to the water by using radioactively labelled cortisol. Such a defence mechanism against maternal cortisol, in addition to rapid conversion to other metabolites from cortisol, is known in placental animals. Although the authors used pharmacological doses, the current stage of the field suggests that embryos are well protected against maternal stress hormones. Finally, McGhee et al. (2020) did not detect an elevation in cortisol concentrations in adult sticklebacks exposed to predator cues relative to controls, making differences in egg cortisol levels unlikely. So, although we cannot exclude the possibility that cortisol was present in very low concentrations in the eggs and still biologically relevant, the current state of knowledge suggests that the hormone is not mediating maternal effects in this (and several other) species. Although we clearly found evidence of multiple parental effects, we failed to identify the underlying mechanism, but cannot exclude a role for maternal hormones. For example, glucocorticoids other than cortisol or those of the androgenic pathway (Schaafsma & Groothuis, 2012) could have been responsible for the changes in offspring behaviour.

Lastly, predation is a well-known selective agent for life history traits. Here we found that offspring from parents that had been exposed to predation threat were significantly smaller than those whose parents had not been exposed to predation. The fact that mothers that are placed under predation threat or are stressed tend to produce smaller offspring is well established in the literature. For example, great tits, *Parus major*, exposed to a model predator produced smaller nestlings with higher wing growth rates (Coslovsky & Richner, 2011). Similarly in fishes, females exposed to predation tend to produce smaller offspring (Johnson & Belk, 2001; Reznick & Endler, 1982).

In conclusion, our results support the hypothesis that predation pressure on parents enhances lateralization and boldness in the offspring via parental effects. Although both traits were affected by the treatment, we found no evidence that they are correlated, suggesting that they are not expressions of the same proximate mechanism. Whether the effects are transferred through the eggs by maternal hormones other than cortisol remains an open question. Offspring from predator-exposed parents displayed personality (boldness) that might suggest that consistent behaviour (predictability) could be beneficial in high-threat social contexts. Future research should examine the possibility that other hormones or their by-products are responsible for maternal effects and thereby shape the behavioural trajectories of their offspring.

Author Contributions

Flavia Berlinghieri: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Culum Brown:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ton G.G. Groothuis:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marion Nicolaus:** Writing – review & editing, Supervision, Project administration. **Andrea Pace:** Writing – review & editing, Methodology. **Bernd Riedstra:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dario Savoca:** Writing – review & editing, Methodology, Formal analysis. **Martina Vittorietti:** Writing – review & editing, Formal analysis, Data curation.

Data Availability

The raw data are available at the data share repository Dryad at https://doi.org/10.5061/dryad.ncjsxkt5x.

Declaration of Interest

The authors declare there are no conflicts of interest.

Acknowledgments

We thank Miriam van der Meulen-Frank and Martijn Salomons from the University of Groningen for their support and assistance. We are also grateful to Willem Diderich for his daily help with the animals and experimental set-up, and to the students Tessa Mulder, Jolene Schotanus and Gijs Ernst for their contributions to data collection. We thank Jakob Gismann and Paolo Panizzon for their help in rearing and housing the sticklebacks, as well as for their assistance with setting up the experiment. Additionally, we thank the Groothuis group at the University of Groningen for the helpful discussions regarding the manuscript. The comments from three anonymous referees and the Editor also greatly improved the manuscript.

References

Aguirre, W. E., Reid, K., Rivera, J., Heins, D. C., Veeramah, K. R., & Bell, M. A. (2022). Freshwater colonization, adaptation, and genomic divergence in threespine stickleback. *Integrative and Comparative Biology, 62*(2), 388–405. https://doi.org/10.1093/ICB/ICAC071

Arai, T., Ueno, D., Kitamura, T., & Goto, A. (2020). Habitat preference and diverse migration in threespine sticklebacks, *Gasterosteus aculeatus* and *G. nipponicus*. *Scientific Reports, 10*(1), Article 14311. https://doi.org/10.1038/s41598-020-71400-4

Ariyomo, T. O., & Watt, P. J. (2013). Aggression and sex differences in lateralization in the zebrafish. *Animal Behaviour, 86*(3), 617–622. https://doi.org/10.1016/j.anbehav.2013.06.019

Austin, N. P., & Rogers, L. J. (2012). Limb preferences and lateralization of aggression, reactivity and vigilance in feral horses, *Equus caballus*. *Animal Behaviour, 83*(1), 239–247. https://doi.org/10.1016/j.anbehav.2011.10.033

Austin, N. P., & Rogers, L. J. (2014). Lateralization of agonistic and vigilance responses in Przewalski horses (*Equus przewalskii*). *Applied Animal Behaviour Science, 151*, 43–50. https://doi.org/10.1016/j.applanim.2013.11.011

Barnard, S., Wells, D. L., Hepper, P. G., & Milligan, A. D. (2017). Association between lateral bias and personality traits in the domestic dog (*Canis familiaris*). *Journal of Comparative Psychology, 131*(3), 246. https://doi.org/10.1037/com0000074

Barrett, L. P., Anthony, K. L., Eliades, S. J., Siler, C. D., Lock, B., & Snyder, R. J. (2022). Personality assessment of headstart Texas horned lizards *(Phrynosoma cornutum)* in human care prior to release. *Applied Animal Behaviour Science, 254*, Article 105690. https://doi.org/10.1016/j.applanim.2022.105690

Bell, A. M., Dingemanse, N. J., Hankison, S. J., Langenhof, M. B. W., & Rollins, K. (2011). Early exposure to nonlethal predation risk by size-selective predators increases somatic growth and decreases size at adulthood in threespined sticklebacks. *Journal of Evolutionary Biology, 24*(5), 943−953. https://doi.org/10.1111/j.1420-9101.2011.02247.x

Bell, A. M., & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecology Letters, 10*(9), 828−834. https://doi.org/10.1111/j.1461-0248.2007.01081.x

Berlinghieri, F., Jansen, N., Riedstra, B., Brown, C., & Groothuis, T. G. (2024). The effect of light during embryonic development on laterality and exploration in Western Rainbowfish. *Laterality, 29*(1), 1−18. https://doi.org/10.1080/1357650X.2023.2252567

Bestion, E., Teyssier, A., Aubret, F., Clobert, J., & Cote, J. (2014). Maternal exposure to predator scents: Offspring phenotypic adjustment and dispersal. *Proceedings of the Royal Society B: Biological Sciences, 281*(1792). https://doi.org/10.1098/rspb.2014.0701

Bibost, A. L., & Brown, C. (2013). Laterality influences schooling position in rainbowfish, Melanotaenia spp. *PLoS One, 8*(11), Article e80907. https://doi.org/10.1371/journal.pone.0080907

Bisazza, A., & Brown, C. (2011). Lateralization of cognitive functions in fish. In C. Brown, K. Laland, & J. Krause (Eds.), *Fish cognition and behavior* (pp. 298−324). https://doi.org/10.1002/9781444342536.CH14

Bisazza, A., & Dadda, M. (2005). Enhanced schooling performance in lateralized fishes. *Proceedings of the Royal Society B: Biological Sciences, 272*(1573), 1677−1681. https://doi.org/10.1098/RSPB.2005.3145

Bisazza, A., Dadda, M., & Cantalupo, C. (2005). Further evidence for mirror-reversed laterality in lines of fish selected for leftward or rightward turning when facing a predator model. *Behavioural Brain Research, 156*(2), 165−171. https://doi.org/10.1016/j.bbr.2004.05.022

Bisazza, A., Facchin, L., & Vallortigara, G. (2000). Heritability of lateralization in fish: Concordance of right−left asymmetry between parents and offspring. *Neuropsychologia, 38*(7), 907−912. https://doi.org/10.1016/S0028-3932(00)00018-X

Bisazza, A., Rogers, L. J., & Vallortigara, G. (1998). The origins of cerebral asymmetry: A review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neuroscience & Biobehavioral Reviews, 22*(3), 411−426. https://doi.org/10.1016/S0149-7634(97)00050-X

Brown, C. (2003). Habitat−predator association and avoidance in rainbowfish (*Melanotaenia* spp.). *Ecology of Freshwater Fish, 12*(2), 118−126. https://doi.org/10.1034/j.1600-0633.2003.00007.x

Brown, G. E. (2003). Learning about danger: Chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries, 4*(3), 227−234. https://doi.org/10.1046/J.1467-2979.2003.00132.X

Brown, C., & Bibost, A. L. (2014). Laterality is linked to personality in the black-lined rainbowfish, *Melanotaenia nigrans*. *Behavioral Ecology and Sociobiology, 68*(6), 999−1005. https://link.springer.com/article/10.1007/s00265-014-1712-0.

Brown, C., & Braithwaite, V. A. (2004). Size matters: A test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour, 68*(6), 1325−1329. https://doi.org/10.1016/J.anbehav.2004.04.004

Brown, C., Burgess, F., & Braithwaite, V. A. (2007). Heritable and experiential effects on boldness in a tropical opulati. *Behavioral Ecology and Sociobiology, 62*(2), 237−243. https://doi.org/10.1007/s00265-007-0458-3

Brown, C., Gardner, C., & Braithwaite, V. A. (2004). Population variation in lateralized eye use in the poeciliid Brachyraphis episcopi. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 271*(suppl_6), S455−S457. https://doi.org/10.1098/rsbl.2004.0222

Brown, C., Jones, F., & Braithwaite, V. (2005). In situ examination of boldness−shyness traits in the tropical opulati, *Brachyraphis episcopi*. *Animal Behaviour, 70*(5), 1003−1009. https://doi.org/10.1016/j.anbehav.2004.12.022

Brown, C., Western, J., & Braithwaite, V. A. (2007). The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour, 74*(2), 231−238. https://doi.org/10.1016/j.anbehav.2006.08.014

Burgess, S. C., & Marshall, D. J. (2014). Adaptive parental effects: The importance of estimating environmental predictability and offspring fitness appropriately. *Oikos, 123*(7), 769−776. https://doi.org/10.1111/oik.01235

Bussy, U., Wassink, L., Scribner, K. T., & Li, W. (2017). Determination of cortisol in lake sturgeon (*Acipenser fulvescens*) eggs by liquid chromatography tandem mass spectrometry. *Journal of Chromatography B, 1040*, 162−168. https://doi.org/10.1016/j.jchromb.2016.11.028

Byrnes, E. E., & Brown, C. (2016). Individual personality differences in Port Jackson sharks *Heterodontus portusjacksoni*. *Journal of Fish Biology, 89*(2), 1142−1157. https://doi.org/10.1111/JFB.12993

Byrnes, E. E., Pouca, C. V., & Brown, C. (2016). Laterality strength is linked to stress reactivity in Port Jackson sharks (Heterodontus portusjacksoni). *Behavioural brain research, 305*, 239−246.

Carere, C., & Maestripieri, D. (Eds.). (2013). *Animal personalities: Behavior, physiology, and evolution*. University of Chicago Press.

Cattelan, S., Herbert-Read, J., Panizzon, P., Devigili, A., Griggio, M., Pilastro, A., & Morosinotto, C. (2020). Maternal predation risk increases offspring's exploration but does not affect schooling behavior. *Behavioral Ecology, 31*(5), 1207−1217. https://doi.org/10.1093/beheco/araa071

Clavero, M., Pou-Rovira, Q., & Zamora, L. (2009). Biology and habitat use of three-spined stickleback (*Gasterosteus aculeatus*) in intermittent Mediterranean streams. *Ecology of Freshwater Fish, 18*(4), 550−559. https://doi.org/10.1111/J.1600-0633.2009.00369.x

Colléter, M., & Brown, C. (2011). Personality traits predict hierarchy rank in male rainbowfish social groups. *Animal Behaviour, 81*(6), 1231−1237. https://doi.org/10.1016/j.anbehav.2011.03.011

Coslovsky, M., Groothuis, T., de Vries, B., & Richner, H. (2012). Maternal steroids in egg yolk as a pathway to translate predation risk to offspring: Experiments with great tits. *General and Comparative Endocrinology, 176*(2), 211−214. https://doi.org/10.1016/j.ygcen.2012.01.013

Coslovsky, M., & Richner, H. (2011). Predation risk affects offspring growth via maternal effects. *Functional Ecology, 25*(4), 878−888. https://doi.org/10.1111/j.1365-2435.2011.01834.x

Crane, A. L., Bairos-Novak, K. R., Goldman, J. A., & Brown, G. E. (2022). Chemical disturbance cues in aquatic systems: A review and prospectus. *Ecological Monographs, 92*(1), Article e01487. https://doi.org/10.1002/ecm.1487

Dadda, M., Agrillo, C., Bisazza, A., & Brown, C. (2015). Laterality enhances numerical skills in the guppy, *Poecilia reticulata*. *Frontiers in Behavioral Neuroscience, 9*, 285. https://doi.org/10.3389/fnbeh.2015.00285

Dadda, M., Koolhaas, W. H., & Domenici, P. (2010). Behavioural asymmetry affects escape performance in a teleost fish. *Biology Letters, 6*(3), 414−417.

Dadda, M., Vendramin, V., & Agrillo, C. (2020). Prenatal visual exposure to a predator influences lateralization in goldbelly topminnows. *Symmetry, 12*(8), 1257. https://doi.org/10.3390/SYM12081257

Dadda, M., Zandona, E., Agrillo, C., & Bisazza, A. (2009). The costs of hemispheric specialization in a fish. *Proceedings of the Royal Society B: Biological Sciences, 276*(1677), 4399−4407. https://doi.org/10.1098/rspb.2009.1406

De Santi, A., Bisazza, A., Cappelletti, M., & Vallortigara, G. (2000). Prior exposure to a predator influences lateralization of cooperative predator inspection in the guppy, *Poecilia reticulata*. *Italian Journal of Zoology, 67*(2), 175−178. https://doi.org/10.1080/11250000009356312

Deckel, A. W. (1995). Laterality of aggressive responses in *Anolis*. *Journal of Experimental Zoology, 272*(3), 194−200. https://doi.org/10.1002/JEZ.1402720304

Deckel, A. W. (1998). Hemispheric control of territorial aggression in *Anolis carolinensis*: Effects of mild stress. *Brain, Behavior and Evolution, 51*(1), 33−39. https://doi.org/10.1159/000006527

Edenbrow, M., & Croft, D. P. (2012). Sequential hermaphroditism and personality in a clonal vertebrate: The mangrove killifish. *Behavioural Processes, 90*(2), 229−237. https://doi.org/10.1016/j.beproc.2012.02.001

Eriksen, M. S., Bakken, M., Espmark, Å., Braastad, B. O., & Salte, R. (2006). Prespawning stress in farmed Atlantic salmon *Salmo salar*: Maternal cortisol exposure and hyperthermia during embryonic development affect offspring survival, growth and incidence of malformations. *Journal of Fish Biology, 69*(1), 114−129. https://doi.org/10.1111/j.1095-8649.2006.01071.x

Eriksen, M. S., Faerevik, G., Kittilsen, S., McCormick, M. I., Damsgård, B., Braithwaite, V. A., Braastad, B. O., & Bakken, M. (2011). Stressed mothers − troubled offspring: A study of behavioural maternal effects in farmed *Salmo salar*. *Journal of Fish Biology, 79*(3), 575−586. https://doi.org/10.1111/J.1095-8649.2011.03036.x

Evans, J. P., Gasparini, C., & Pilastro, A. (2007). Female guppies shorten brood retention in response to predator cues. *Behavioral Ecology and Sociobiology, 61*(5), 719−727. https://link.springer.com/article/10.1007/s00265-006-0302-1.

Fang, B., Merilä, J., Ribeiro, F., Alexandre, C. M., & Momigliano, P. (2018). Worldwide phylogeny of three-spined sticklebacks. *Molecular Phylogenetics and Evolution, 127*, 613−625. https://doi.org/10.1016/J.YMPEV.2018.06.008

Fernández-Lázaro, G., Latorre, R., Alonso-García, E., & Barja Núñez, I. (2019). Nonhuman primate welfare: Can there be a relationship between personality, lateralization and physiological indicators? *Behavioural Processes, 166*, Article 103897. https://doi.org/10.1016/j.beproc.2019.103897

Ferrari, M. C., McCormick, M. I., Mitchell, M. D., Allan, B. J., Gonçalves, E. J., & Chivers, D. P. (2017). Daily variation in behavioural lateralization is linked to predation stress in a coral reef fish. *Animal Behaviour, 133*, 189−193. https://doi.org/10.1016/j.anbehav.2017.09.020.

Friard, O., & Gamba, M. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution, 7*(11), 1325−1330. https://doi.org/10.1111/2041-210X.12584

Gagliano, M., & McCormick, M. I. (2009). Hormonally mediated maternal effects shape offspring survival potential in stressful environments. *Oecologia, 160*(4), 657−665. https://doi.org/10.1007/s00442-009-1335-8

Giesing, E. R., Suski, C. D., Warner, R. E., & Bell, A. M. (2011). Female sticklebacks transfer information via eggs: Effects of maternal experience with predators on offspring. *Proceedings of the Royal Society B: Biological Sciences, 278*(1712), 1753−1759. https://doi.org/10.1098/rspb.2010.1819

Giles, N. (1987). Population biology of three-spined sticklebacks, *Gasterosteus aculeatus*, in Scotland. *Journal of Zoology, 212*(2), 255−265. https://doi.org/10.1111/J.1469-7998.1987.TB05988.X

Goursot, C., Düpjan, S., Kanitz, E., Tuchscherer, A., Puppe, B., & Leliveld, L. M. (2019). Assessing animal individuality: Links between personality and laterality in pigs. *Current Zoology, 65*(5), 541−551. https://doi.org/10.1093/cz/zoy071

Groothuis, T. G. G., & Carere, C. (2005). Avian personalities: Characterization and epigenesis. *Neuroscience & Biobehavioral Reviews, 29*(1), 137−150. https://doi.org/10.1016/j.neubiorev.2004.06.010

Groothuis, T. G., & Taborsky, B. (2015). Introducing biological realism into the study of developmental plasticity in behaviour. *Frontiers in Zoology, 12*(Suppl 1), S6. https://doi.org/10.1186/1742-9994-12-S1-S6

Hayward, L. S., & Wingfield, J. C. (2004). Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *General and Comparative Endocrinology, 135*(3), 365–371. https://doi.org/10.1016/j.ygcen.2003.11.002

Hellmann, J. K., Carlson, E. R., & Bell, A. M. (2021). The interplay between sperm-mediated and care-mediated paternal effects in threespine sticklebacks. *Animal Behaviour, 179*, 267–277. https://doi.org/10.1016/j.anbehav.2021.07.005

Henriksen, R., Rettenbacher, S., & Groothuis, T. G. G. (2011). Prenatal stress in birds: Pathways, effects, function and perspectives. *Neuroscience & Biobehavioral Reviews, 35*(7), 1484–1501. https://doi.org/10.1016/j.neubiorev.2011.04.010

Horn, M. E., & Chivers, D. P. (2021). Embryonic exposure to predation risk and hatch time variation in fathead minnows. *PLoS One, 16*(8), Article e0255961. https://doi.org/10.1371/journal.pone.0255961

Hulthén, K., Heinen-Kay, J. L., Schmidt, D. A., & Langerhans, R. B. (2021). Predation shapes behavioral lateralization: Insights from an adaptive radiation of live-bearing fish. *Behavioral Ecology, 32*(6), 1321–1329. https://doi.org/10.1093/beheco/arab098

Irving, E., & Brown, C. (2013). Examining the link between personality and laterality in a feral guppy *Poecilia reticulata* population. *Journal of Fish Biology, 83*(2), 311–325. https://doi.org/10.1111/JFB.12165

Jacobson, P., Bergstrom, U., & Eklof, J. (2019). Size-dependent diet composition and feeding of Eurasian perch (*Perca fluviatilis*) and northern pike (*Esox lucius*) in the Baltic Sea. *Boreal Environment Research, 24*. https://res.slu.se/id/publ/102238.

Johnson, J. B., & Belk, M. C. (2001). Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. *Oecologia, 126*, 142–149. https://doi.org/10.1007/s004420000504

Jutfelt, F., Bresolin de Souza, K., Vuylsteke, A., & Sturve, J. (2013). Behavioural disturbances in a temperate fish exposed to sustained high-CO_2 levels. *PLoS One, 8*(6), Article e65825. https://doi.org/10.1371/journal.pone.0065825

Leliveld, L. M. C., Langbein, J., & Puppe, B. (2013). The emergence of emotional lateralization: Evidence in non-human vertebrates and implications for farm animals. *Applied Animal Behaviour Science, 145*(1–2), 1–14. https://doi.org/10.1016/j.applanim.2013.02.002

Lelono, A., Riedstra, B., & Groothuis, T. (2019). Ejaculate testosterone levels affect maternal investment in red junglefowl (*Gallus gallus gallus*). *Scientific Reports, 9*, Article 12126. https://doi.org/10.1038/s41598-019-48563-w

Lemaire, B. S., Viblanc, V. A., & Jozet-Alves, C. (2019). Sex-specific lateralization during aggressive interactions in breeding king penguins. *Ethology, 125*(7), 439–449. https://doi.org/10.1111/eth.12868

Lind, M. I., Zwoinska, M. K., Andersson, J., Carlsson, H., Krieg, T., Larva, T., & Maklakov, A. A. (2020). Environmental variation mediates the evolution of anticipatory parental effects. *Evolution Letters, 4*(4), 371–381. https://doi.org/10.1002/evl3.177

Lucon-Xiccato, T., Chivers, D. P., Mitchell, M. D., & Ferrari, M. C. (2016). Prenatal exposure to predation affects predator recognition learning via lateralization plasticity. *Behavioral Ecology, 20*(1), 253–259. https://doi.org/10.1093/beheco/arw155

Lucon-Xiccato, T., & Dadda, M. (2017). Individual guppies differ in quantity discrimination performance across antipredator and foraging contexts. *Behavioral Ecology and Sociobiology, 71*(1), 1–9. https://doi.org/10.1007/s00265-016-2231-y

MacLeod, K. J., Monestier, C., Ferrari, M. C., McGhee, K. E., Sheriff, M. J., & Bell, A. M. (2022). Predator-induced transgenerational plasticity in animals: a meta-analysis. *Oecologia, 200*(3), 371–383. https://doi.org/10.1007/s00442-022-05274-w.

Magat, M., & Brown, C. (2009). Laterality enhances cognition in Australian parrots. *Proceedings of the Royal Society B: Biological Sciences, 276*(1676), 4155–4162. https://doi.org/10.1098/rspb.2009.1397

Mazué, G. P., Dechaume-Moncharmont, F. X., & Godin, J. G. J. (2015). Boldness–exploration behavioral syndrome: Interfamily variability and repeatability of personality traits in the young of the convict cichlid (*Amatitlania siquia*). *Behavioral Ecology, 26*(3), 900–908. https://doi.org/10.1093/beheco/arv030

Mazza, V., Jacob, J., Dammhahn, M., Zaccaroni, M., & Eccard, J. A. (2019). Individual variation in cognitive style reflects foraging and anti-predator strategies in a small mammal. *Scientific Reports, 9*(1), Article 10157. https://doi.org/10.1038/s41598-019-46582-1

McGhee, K. E., Paitz, R. T., Baker, J. A., Foster, S. A., & Bell, A. M. (2020). Effects of predation risk on egg steroid profiles across multiple populations of threespine stickleback. *Scientific Reports, 10*, 5239. https://doi.org/10.1038/s41598-020-61412-5

McGhee, K. E., Pintor, L. M., Suhr, E. L., & Bell, A. M. (2012). Maternal exposure to predation risk decreases offspring antipredator behaviour and survival in threespined stickleback. *Functional Ecology, 26*(4), 932–940. https://doi.org/10.1111/J.1365-2435.2012.02008.X

Meylan, S., & Clobert, J. (2005). Is corticosterone-mediated phenotype development adaptive? Maternal corticosterone treatment enhances survival in male lizards. *Hormones and Behavior, 48*(1), 44–52. https://doi.org/10.1016/j.yhbeh.2004.11.022

Minter, R., Keagy, J., & Tinghitella, R. M. (2017). The relationship between male sexual signals, cognitive performance, and mating success in stickleback fish. *Ecology and Evolution, 7*(15), 5621–5631. https://doi.org/10.1002/ece3.3091

Mittelbach, G. G., Ballew, N. G., & Kjelvik, M. K. (2014). Fish behavioral types and their ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences, 71*(6), 927–944. https://doi.org/10.1139/cjfas-2013-0558

Moisiadis, V. G., & Matthews, S. G. (2014). Glucocorticoids and fetal programming part 1: Outcomes. *Nature Reviews Endocrinology, 10*(7), 391–402. https://doi.org/10.1038/nrendo.2014.73

Morales, J., Lucas, A., Velando, A., Margalef, R., San, C., & Del Raspeig, V. (2018). Maternal programming of offspring antipredator behavior in a seabird. *Behavioral Ecology, 29*(2), 479–485. https://doi.org/10.1093/beheco/arx197

Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution, 13*(10), 403–407. https://doi.org/10.1016/S0169-5347(98)01472-4

Ocklenburg, S., Ströckens, F., Bless, J. J., Hugdahl, K., Westerhausen, R., & Manns, M. (2016). Investigating heritability of laterality and cognitive control in speech perception. *Brain and Cognition, 109*, 34–39. https://doi.org/10.1016/J.BANDC.2016.09.003

Paitz, R. T., Bukhari, S. A., & Bell, A. M. (2016). Stickleback embryos use ATP-binding cassette transporters as a buffer against exposure to maternally derived cortisol. *Proceedings of the Royal Society B: Biological Sciences, 283*(1826), Article 20152838. https://doi.org/10.1098/rspb.2015.2838

Paracchini, S. (2021). Recent advances in handedness genetics. *Symmetry, 13*(10), 1792. https://doi.org/10.3390/SYM13101792

Queiroz, H., & Magurran, A. E. (2005). Safety in numbers? Shoaling behaviour of the Amazonian red-bellied piranha. *Biology Letters, 1*(2), 155–157. https://doi.org/10.1098/RSBL.2004.0267

Rakers, F., Rupprecht, S., Dreiling, M., Bergmeier, C., Witte, O. W., & Schwab, M. (2020). Transfer of maternal psychosocial stress to the fetus. *Neuroscience & Biobehavioral Reviews, 117*, 185–197. https://doi.org/10.1016/j.neubiorev.2017.02.019

Ramesh, A., Domingues, M. M., Stamhuis, E. J., Groothuis, T. G. G., Weissing, F. J., & Nicolaus, M. (2021). Does genetic differentiation underlie behavioral divergence in response to migration barriers in sticklebacks? A common garden experiment. *Behavioral Ecology and Sociobiology, 75*(12), 1–12. https://link.springer.com/article/10.1007/s00265-021-03097-y.

Ramesh, A., Gismann, J., Groothuis, T. G. G., Weissing, F. J., & Nicolaus, M. (2023). Mesocosm experiment reveals scale dependence of movement tendencies in sticklebacks. *Biology Letters, 19*(4). https://doi.org/10.1098/rsbl.2022.0602

Raoult, V., Brown, C., Zuberi, A., & Williamson, J. E. (2012). Blood cortisol concentrations predict boldness in juvenile mulloway (*Argyosomus japonicus*). *Journal of Ethology, 30*(2), 225–232. https://doi.org/10.1007/s10164-011-0314-9

Raoult, V., Trompf, L., Williamson, J. E., & Brown, C. (2017). Stress profile influences learning approach in a marine fish. *PeerJ, 5*, Article e3445. https://doi.org/10.7717/peerj.3445

Reddon, A. R., & Hurd, P. L. (2008). Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology Letters, 4*(4), 338–340. https://doi.org/10.1098/rsbl.2008.0206

Reddon, A. R., & Hurd, P. L. (2009). Individual differences in cerebral lateralization are associated with shy–bold variation in the convict cichlid. *Animal Behaviour, 77*(1), 189–193. https://doi.org/10.1016/j.anbehav.2008.09.026

Rettenbacher, S., Groothuis, T. G., Henriksen, R., & Möstl, E. (2013). Corticosterone in bird eggs: The importance of analytical validation. *WTM Wiener Tierärztliche Monatsschrift, 100*(9–10), 283–290.

Rettenbacher, S., Möstl, E., & Groothuis, T. G. G. (2009). Gestagens and glucocorticoids in chicken eggs. *General and Comparative Endocrinology, 164*(2–3), 125–129. https://doi.org/10.1016/j.ygcen.2009.05.019

Reyes-Contreras, M., de Vries, B., van der Molen, J. C., Groothuis, T. G. G., & Taborsky, B. (2023). Egg-mediated maternal effects in a cooperatively breeding cichlid fish. *Scientific Reports, 13*(1), 9759. https://doi.org/10.1038/s41598-023-35550-5

Reznick, D., & Endler, J. A. (1982). The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution, 36*(1), 160–177. https://doi.org/10.2307/2407978

Rivera, H. E., Chen, C. Y., Gibson, M. C., & Tarrant, A. M. (2021). Plasticity in parental effects confers rapid larval thermal tolerance in the estuarine anemone *Nematostella vectensis*. *Journal of Experimental Biology, 224*(5), Article jeb236745. https://doi.org/10.1242/jeb.236745

Rogers, L. J. (2002). Advantages and disadvantages of lateralization. In L. J. Rogers, & R. J. Andrew (Eds.), *Comparative vertebrate lateralization* (pp. 126–154). Cambridge University Press. https://doi.org/10.1017/CBO9780511546372.006.

Rogers, L. J. (2010). Relevance of brain and behavioural lateralization to animal welfare. *Applied Animal Behaviour Science, 127*(1–2), 1–11. https://doi.org/10.1016/j.applanim.2010.06.008

Rogers, L. J., & Deng, C. (2005). Corticosterone treatment of the chick embryo affects light-stimulated development of the thalamofugal visual pathway. *Behavioural Brain Research, 159*(1), 63–71. https://doi.org/10.1016/J.BBR.2004.10.003

Rogers, L. J., Frasnelli, E., & Versace, E. (2016). Lateralized antennal control of aggression and sex differences in red mason bees, *Osmia bicornis*. *Scientific Reports, 6*, Article 29411. https://doi.org/10.1038/srep29411

Saino, N., Romano, M., Ferrari, R. P., Martinelli, R., & Møller, A. P. (2005). Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *Journal of Experimental Zoology Part A: Comparative Experimental Biology, 303A*(11), 998–1006. https://doi.org/10.1002/JEZ.A.224

Schaafsma, S. M., & Groothuis, T. G. (2011). Sex-specific effects of postnatal testosterone on lateralization in cichlid fish. *Animal Behaviour, 81*(1), 283–288. https://doi.org/10.1016/j.anbehav.2010.10.019

Schaafsma, S. M., & Groothuis, T. G. G. (2012). Sex-specific effects of maternal testosterone on lateralization in a cichlid fish. *Animal Behaviour, 83*(2), 437–443. https://doi.org/10.1016/j.anbehav.2011.11.015

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Scott, R. J. (2004). Assortative mating between adjacent populations of threespine stickleback (*Gasterosteus aculeatus*). *Ecology of Freshwater Fish, 13*(1), 1–7. https://doi.org/10.1111/J.0906-6691.2004.00037.X

Shine, R., & Downes, S. J. (1999). Can pregnant lizards adjust their offspring phenotypes to environmental conditions? *Oecologia, 119*, 1–8. https://doi.org/10.1007/S004420050754

Silva, P. I., Martins, C. I., Khan, U. W., Gjøen, H. M., Øverli, Ø., & Höglund, E. (2015). Stress and fear responses in the teleost pallium. *Physiology & Behavior, 141*, 17–22. https://doi.org/10.1016/j.physbeh.2014.12.020

Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: A meta-analysis. *Behavioral Ecology, 19*(2), 448–455. https://doi.org/10.1093/beheco/arm144

Sovrano, V. A., Rainoldi, C., Bisazza, A., & Vallortigara, G. (1999). Roots of brain specializations: Preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behavioural Brain Research, 106*(1–2), 175–180. https://doi.org/10.1016/S0166-4328(99)00105-9

Speedie, N., & Gerlai, R. (2008). Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). *Behavioural Brain Research, 188*(1), 168–177. https://doi.org/10.1016/J.BBR.2007.10.031

St-Cyr, S., & McGowan, P. O. (2015). Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. *Frontiers in Behavioral Neuroscience, 9*, 145. https://doi.org/10.3389/fnbeh.2015.00145

Stamps, J., & Groothuis, T. G. G. (2010). The development of animal personality: Relevance, concepts and perspectives. *Biological Reviews, 85*(2), 301–325. https://doi.org/10.1111/j.1469-185X.2009.00103.x

Stein, L. R., & Bell, A. M. (2014). Paternal programming in sticklebacks. *Animal Behaviour, 95*, 165–171. https://doi.org/10.1016/j.anbehav.2014.07.010

Storm, J. J., & Lima, S. L. (2010). Mothers forewarn offspring about predators: A transgenerational maternal effect on behavior. *American Naturalist, 175*(3), 382–390. https://doi.org/10.1086/650443

Svartberg, K., Tapper, I., Temrin, H., Radesäter, T., & Thorman, S. (2005). Consistency of personality traits in dogs. *Animal Behaviour, 69*(2), 283–291. https://doi.org/10.1016/j.anbehav.2004.04.011

Swarup, H. (1958). Stages in the development of the stickleback *Gasterosteus aculeatus* (L.). *Development, 6*(3), 373–383. https://doi.org/10.1242/dev.6.3.373

Tariel, J., Plénet, S., & Luquet, E. (2020a). How do developmental and parental exposures to predation affect personality and immediate behavioural plasticity in the snail *Physa acuta*? Parental environment and personality. *Proceedings of the Royal Society B: Biological Sciences*, (1941), 287. https://doi.org/10.1098/rspb.2020.1761

Tariel, J., Plénet, S., & Luquet, É. (2020b). Transgenerational plasticity in the context of predator-prey interactions. *Frontiers in Ecology and Evolution, 8*. https://doi.org/10.3389/fevo.2020.548660

Tulley, J. J., & Huntingford, F. A. (1987). Age, experience and the development of adaptive variation in anti-predator responses in three-spined sticklebacks (*Gasterosteus aculeatus*). *Ethology, 75*(4), 285–290. https://doi.org/10.1111/j.1439-0310.1987.tb00660.x

Tulley, J. J., & Huntingford, F. A. (1988). Additional information on the relationship between intra-specific aggression and anti-predator behaviour in the three-spined stickleback, *Gasterosteus aculeatus*. *Ethology, 78*(3), 219–222. https://doi.org/10.1111/j.1439-0310.1988.tb00232.x

Vallortigara, G., & Rogers, L. J. (2005). Survival with an asymmetrical brain: Advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences, 28*(4), 575–589. https://doi.org/10.1017/S0140525X05000105

von Engelhardt, N., & Groothuis, T. G. G. (2011). Maternal hormones in avian eggs. In D. O. Norris, & K. H. Lopez (Eds.), *Hormones and reproduction of vertebrates* (Vol. 4, pp. 91–127). Academic Press. https://doi.org/10.1016/B978-0-12-374929-1.10004-6.

von Engelhardt, N., Henriksen, R., & Groothuis, T. G. G. (2009). Steroids in chicken egg yolk: Metabolism and uptake during early embryonic development. *General and Comparative Endocrinology, 163*(1–2), 175–183. https://doi.org/10.1016/j.ygcen.2009.04.004

Wang, Y., Riedstra, B., van Faassen, M., Pranger, A., Kema, I., & Groothuis, T. G. (2023). Dynamics of maternal androgens and its metabolites during early embryonic development: Embryonic modification of a maternal effect. *Journal of Endocrinology, 258*(2). https://doi.org/10.1530/joe-22-0299

Wootton, R. J. (1973). The effect of size of food ration on egg production in the female three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology, 5*(1), 89–96. https://doi.org/10.1111/J.1095-8649.1973.tb04433.x

Yee, T. W. (2010). The VGAM package for categorical data analysis. *Journal of Statistical Software, 32*(10), 1–34. https://doi.org/10.18637/JSS.V032.I10

Appendix

Chemicals and Reagents

For extraction and analyses we used hydrocortisone (HO888, 1G Sigma), water (LC-MS grade water from PanReac Applichem, https://www.itwreagents.com), methanol (LC-MS grade methanol from Honeywell, https://www.researchchemicalsconnect.com), ethyl acetate (ACS grade from Sigma-Aldrich, https://www.sigmaaldrich.com), formic acid (eluent additive for LC-MS from Honeywell), acetonitrile (LC-MS grade from Honeywell) and C18 SPE (500 mg/3 ml from Chromabond).

Egg Cortisol Analysis

Hydrocortisone was used both as a reference for calibration in the range 1–100 ng/ml before each round of analyses with linear response R^2 >0.999 and as an internal standard added in spiked samples before extraction. Spiked samples underwent the same procedure as the corresponding unspiked samples.

For sample preparation 2 g of eggs were divided in four aliquots in order to perform cortisol extraction in duplicate with 10 ng of standard cortisol and without the addition of standards. Each subsample was homogenized with 0.5 ml of PBS1x (phosphate buffered saline). In the specific case of solid phase extraction (SPE) at each aliquot, 4.5 ml of water was added and vortexed, centrifuged (4000 rpm, 10 min, 4 °C) before loading into the cartridge. Two procedures were used for cortisol extraction SPE and liquid extraction (LLE).

SPE extraction was performed according to the protocol of McGhee et al. (2020) with some modifications using cartridges C18 SPE. These were conditioned with 5 ml methanol and equilibrated with 5 ml water, then the sample was loaded and washed with 5 ml water. After drying the cartridges, elution was performed with 2 ml of methanol. The eluate was lyophilized and reconstituted in 200 μl methanol, centrifuged (4000 rpm, 10 min, 4 °C), transferred to an autosampler vial and stored at −18 °C until LC-MS/MS analysis.

The procedure for LLE extraction was adapted from Silva et al. (2015) and Bussy et al. (2017). Each sample was vigorously mixed with 5 ml of ethyl acetate and stored at −18 °C overnight. Once the aqueous and fat layers were frozen, the sample was centrifuged (4000 rpm, 10 min, 4 °C) and the organic layer was withdrawn, transferred to a new tube, stored at −18 °C and the procedure was repeated. The 10 ml of ethyl acetate per sample was dried under gentle flow of nitrogen (N_2) and the residues were reconstituted in 200 μl of methanol, centrifuged, transferred to an autosampler vial and stored at −18 °C until LC–MS/MS analysis.

The extraction procedure was carried out in duplicates while LC-MS/MS analyses were carried out in triplicates. Analyses were performed on an HPLC-ESI-QTOF Agilent 6540 (https://www.agilent.com), operating in the positive ion monitoring mode and injecting 20 μl of a sample in a Luna Omega 5 μm Polar C18 LC Column 150 × 2.1 mm using a mixture of water (with 0.1% formic acid) and acetonitrile (with 0.1% formic acid) as eluents with a fixed flow of 0.8 ml/min with the following gradient: 0 min (5% B), 10 min (95% B), 12 min (5% B) maintaining this gradient for a further 3 min.

The analyses were conducted in multiple reaction monitoring (MRM) with transitions m/z selected of 363.21 → 121.06 and retention time recorded was 5.52 min.

In order to avoid any cross-contamination during the HPLC-MS analysis of subsequent samples, blank solvent (methanol) and no injection runs were performed in between one sample analysis. Quality checks were performed by analysing a 10 ng/ml (10 ppb) of a standard solution every four samples, followed by a blank solvent injection and a no injection run. No traces of cortisol were detected in blank analyses. The relative standard deviations (RSDs) on four replicates of quality checks were less than 10% and the analyses were corrected accordingly. Limit of detection (LOD) of 0.5 ng/ml and limit of quantification (LOQ) of 2.5 ng/ml were determined according to the IUPAC method.

The percentage of recovery R was calculated according to equation (1), where C_{spike} is the known concentration (ng/g) of spiked internal standard, D_{spiked} is the instrumental (LC-MS) analytical response of the spiked sample (i.e. the 'detected' concentration) and $D_{unspiked}$ is the analytical response of the unspiked sample. R was then used in equation (2) to calculate the actual values of each PAEs concentration in unspiked analysed samples.

$$R(\%) = 100 \times \left(D_{spiked} - D_{unspiked}\right) \Big/ C_{spike} \quad (1)$$

$$[\text{Cortisol}](\text{ng} / \text{g}) = 100 \times D_{unspiked} \Big/ R \quad (2)$$

The best average of recovery was obtained from LLE extraction of 80% while from SPE extraction was of 60%.