

**Effects of Elevated Predation Risk on Female Mate Selection and Maternal Effects in
Trinidadian guppies**

Arun Dayanandan

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By: Arun Dayanandan

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Signed by the final Examining Committee:

Dr. Selvadurai Dayanandan

Chair

Dr. Emma Despland

Examiner

Dr. Robert Weladji

Examiner

Dr. Ian Ferguson

Examiner

Dr. Grant E. Brown

Supervisor

Approved by _____
Dr. Robert Weladji, Graduate Program Director

2022

Dr. Pascale Sicotte, Dean of Arts & Science

ABSTRACT

Effects of Elevated Predation Risk on Female Mate Selection and Maternal Effects in Trinidadian guppies

Arun Dayanandan

Predation carries non-consumptive behavioural effects over multiple time scales through which population-level changes in innate antipredator behaviour may occur by (1) shaping female mate selection decision-making behaviours to reduce the risk of a predation event, and (2) improving offspring fitness through anticipatory maternal effects on offspring antipredator behaviour.

While acute and long-term risk are better understood, studies on populations reared under short-term elevated background predation risk are lacking. I looked at the impact of elevated predation risk over a period of several days on the reproductive behaviours of female Trinidadian guppies (*Poecilia reticulata*) and their offspring. I first exposed adult female guppies to intermediate and elevated ambient short-term predation risk and predicted that female guppies would reduce their preference for brightly coloured males and increase antipredator behaviours. I measured sexual activity and movement and found no evidence of intermediate and elevated ambient short-term predation risk affecting female mate antipredator behaviours. I then examined the impact of elevated maternal predation risk on antipredator behaviour of guppy offspring. I exposed pregnant female guppies to predation risk and measured offspring antipredator behaviour, predicting that offspring of high risk female guppies would exhibit increased dispersal, exploration, and neophobia. I measured offspring response to a novel odour and movement. I

found no evidence of elevated predation risk affecting offspring antipredator responses nor inducing neophobia. My results did not provide evidence for elevated predation risk over short-term time frames altering reproductive behaviour in Trinidadian guppies which stands in contrast to similar research on wild fish populations.

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*For fish, featherless bipeds
and the follies of certainty*

Contribution of Authors

AD was responsible for Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Software, Validation, Visualization, Writing – Original Draft, and Writing – Review & Editing as per CRediT Contributor Roles Taxonomy. **GEB** was responsible for Conceptualization, Funding Acquisition, Project Administration, Resources, Supervision, Validation, and Writing – Review & Editing as per CRediT Contributor Roles Taxonomy.

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Chapter 1

Predation exerts non-consumptive effects on prey populations. Defined as predator-induced changes in prey traits such as life history, morphology, and behaviour over ecological time scales (Peckarsky et al., 2008), this includes time and energy trade-offs between resource acquisition and antipredator behaviour (Lima and Dill, 1990; Sih et al., 2000; Welton et al., 2003). These trade-offs affect development, morphology, physiology, and behavioural traits (Werner and Peacor, 2003) such as ontogeny and growth rates (Davenport et al., 2020), habitat use (Gotceitas and Brown, 1993), foraging behaviours (Godin and Smith, 1988; Sih, 1992), and reproduction (Brown et al., 2009; Lima and Steury, 2005; Pilakouta and Alonzo, 2014). Thus, it is important for prey to adequately assess predation risk and respond appropriately in order to minimize the negative impacts of predation.

Predation risk assessment influences fish behavioural decision-making over multiple time scales of exposure to risk: acute (i.e. immediate risk) (Ferrari and Chivers, 2006), short-term (i.e. over several days) (Brown et al., 2006a; Chivers and Smith, 1998; Foam et al., 2005), and long-term (i.e. over an individual's lifetime) (Kelley and Magurran, 2003a). Variability of predation threats over these multiple time scales interact to shape both the overall intensity and pattern of antipredator behaviour (Brown et al., 2006a, 2006b; Lima and Bednekoff, 1999). For example, short-term behavioural changes may persist long after a threat is gone in environments absent of acute predation risk (Crane et al., 2021; Mettke-Hofmann, 2017; Rogan et al., 1997; Zanette et al., 2019).

The impact of long-term predation pressure on the mating tactics of Trinidadian guppies is well-studied (Kniel et al., 2020; Magurran and Seghers, 1994). Female mate choice under elevated predation conditions is a costly strategy when compared to random mating

(Pomiankowski, 1987), with costs incurred during search, assessment, and selection of males. Such costs include increased vulnerability to predation during elaborate and colourful courtship displays (Godin, 2003; Lima and Dill, 1990; Sih, 1994), expenditure of time and energy, injury from conspecifics and heterospecifics, increased disease risk, lost mating opportunities with potentially more fit males, and a reduction in future parental care provisioning (Lima and Dill, 1990; Pomiankowski, 1987; Sih, 1994). These costs influence the survival and fecundity of females, and constrain selection on male traits by influencing female trait preferences (Houde, 1993; Kirkpatrick and Ryan, 1991; Pomiankowski, 1987). For example, Stoner and Breden (1988) showed that wild female guppies from high-predation populations prefer duller males, while females from low-predation populations prefer brighter males. These preferences evolved through predation on brightly coloured and conspicuous males and their male offspring, in addition to direct selection on female preferences through predation on females associated with bright males, leading to changes in male traits through sexual selection (Endler and Houde, 1995; Houde and Endler, 1990). Thus, for the first question of my thesis, I asked if female guppies reared under short-term elevated predation risk would reduce their preference for brightly coloured males and exhibit increased antipredator behaviours.

Differences in antipredator behaviour persist between generations of laboratory-reared fish indicating that Trinidadian guppies have an innate ability to recognize predatory fish species (Kelley and Magurran, 2003b). Anticipatory maternal effects, defined as when environmental stressors such as elevated predation risk increase maternal glucocorticoid levels and influence offspring phenotype (Love et al., 2013; Sheriff and Love, 2013, Sheriff et al., 2017) have been found to both positively and negatively affect offspring fitness in high-predation environments (McGhee et al., 2021; Storm and Lima, 2010).

Behavioural changes of Trinidadian guppy offspring responding to elevated predation risk include increased exploration in novel or stressful environments (Cattelan et al., 2020; Zimmer et al., 2013, 2017), which are correlated with increased dispersal away from stressful conditions similar to those experienced by their mother (Cote et al., 2010; Hoset et al., 2011; Korsten et al., 2013). Recent work by McGhee et al. (2021) has shown that brief stressors during a female's pregnancy may also interact with offspring learned experiences of predation risk to influence offspring behaviour. These two findings suggest the existence of intra- and inter-population differences in antipredator behaviour under different levels of predation risk. Thus, for my second question of my thesis, I asked if offspring of female guppies reared under elevated predation risk would exhibit increased dispersal, exploratory behaviour, and neophobic predator-avoidance behaviour under the same predation risk environment as that exposed to their mother.

While it is known that periods of high risk acute threats induce antipredator behavioural changes in the short-term and over multiple generations, my study is novel in that it examines the impact of short-term elevated background predation risk levels on mate choice and offspring antipredator behaviour.

Research Objectives

In my thesis, I looked at the impact of short-term elevated background predation risk on the reproductive behavioural decision-making of female Trinidadian guppies. In Chapter 2, I exposed adult female guppies to intermediate and elevated ambient short-term predation risk, predicting that female guppies would reduce their preference for brightly coloured males and exhibit increased antipredator behaviours. In Chapter 3, I examined the impact of elevated maternal predation risk on the antipredator behaviour of Trinidadian guppy offspring. I did this

by exposing pregnant female guppies to elevated predation risk and measuring their offspring's antipredator behaviour, predicting they would exhibit increased dispersal, exploratory behaviour, and neophobic predator-avoidance behaviour under conditions of elevated background predation risk.

Chapter 2

Intermediate and Elevated Background Predation Risk and Female Mate Choice in the Trinidadian guppy

Introduction

Conspicuous secondary sexual characteristics in males, such as body colour and mating displays, have arisen through female mate preferences that are assumed to be static (Kirkpatrick and Ryan, 1991; Møller, 1994; Pomiankowski, 1988). These characteristics are driven by strong directional selection (Chaine and Lyone, 2008) and favour extreme expression of male traits that increase male mating opportunities in addition to conferring direct and indirect genetic benefits to females (Pilakouta and Alonzo, 2014). However, there is evidence for within-individual and among- and within-population variation in these female preferences (Brooks and Endler, 2001; Chaine and Lyone, 2008; Endler and Houde, 1995). These variations in female preference can slow the exaggeration of male traits and may explain the maintenance of population-level genetic variation (Brooks, 2002). As background predation risk affects the mate choice decision-making behaviour of females, natural selection is expected to favour individuals that can assess changes in predation risk and modify their reproductive behaviours accordingly (Godin and Briggs, 1996; Lima and Dill, 1990; Magnhagen, 1991). Recent studies have also demonstrated that exposure to elevated predation risk over the short term (i.e. over 3-4 days) is sufficient to induce phenotypically plastic neophobia in guppies (Crane et al., 2020). These short periods of risk are representative of the naturally occurring isolated pools which emerge due to low water levels during the Trinidadian dry season in which guppies are restricted to small bodies of water (approx 3 m x 10 m) with fluctuating levels of predation risk and are unable to escape (Brown,

G. E., Personal Observations). However, it remains unknown if this level of elevated risk is sufficient to induce changes in mating tactics. Given the effects of acute and long-term elevated predation risk on mate choice (see ‘Introduction’), I chose to examine if short-term elevated background predation risk (i.e. over a period of a few days) has an effect on female mate selection behaviours.

Female Trinidadian guppies mate with multiple males during their lifetime, with adult males pursuing and courting females through normal and sneaky copulatory behaviour (Farr, 1989; Magurran and Seghers, 1990, 1994). While females choose males based on social courtship cues, the main driver of female mate selection in this species is body colour pattern of males (Endler and Houde, 1995; Godin and Dugatkin, 1995; Houde and Endler, 1990) consisting of orange-red spots, black-brown spots or bars, and iridescent blue-greens. These colour patterns are population-specific and genetically variable, corresponding to variation in the number and species composition of predator communities (Kemp et al., 2008).

Thus, I examined if female Trinidadian guppies exposed to intermediate and elevated ambient short-term predation risk levels reduced their preference for brighter coloured males by selecting mates with muted secondary sexual characteristics. I also examined if female guppies displayed altered mating behaviour when compared to females exposed to a short-term, non-predator prone, environment. I exposed adult female guppies to publicly-available risk assessment cues representing intermediate and elevated ambient short-term predation risk and predicted that female guppies exposed to a short-term high-predation environment would minimize the reproductive risks they incurred.

Methods

Study System

I used a total of 101 female (Alarm Cue Treatment $n = 23$, Alarm Cue Control $n = 22$; Disturbance Cue Treatment $n = 29$, Disturbance Cue Control $n = 27$) and 14 male lab-born ten-generation descendants of wild Trinidadian guppies collected from the Quare River, Trinidad, for the duration of this experiment (Fig. 1). Prior to experimentation, fish were housed in 110 L glass aquaria kept at 25°C on a 12:12 L:D cycle. Fish were fed twice a day with Nutrafin commercial flake food.

Chemical Cues

I used disturbance cue and damage-released alarm cue for this experiment as both have been experimentally demonstrated to influence guppy antipredator behaviour (Elvidge et al., 2016; Goldman et al., 2021). Aquatic prey species in both field and lab settings have been shown to rely on conspecific and heterospecific chemosensory cues as reliable sources of publicly available information to assess the level of local predation risk (Chivers and Smith, 1998; Elvidge et al., 2016; Wisenden and Chivers, 2006). Damage-released alarm cues are a reliable indicator of high risk environments (Brown and Godin, 1999; Ferrari et al., 2010; Wisenden and Chivers, 2006). Disturbance cues, released prior to an attack by individuals when disturbed but uninjured (Crane et al., 2022), are a reliable indicator of intermediate risk environments (Brown et al., 2012; Bryer et al., 2001, Crane et al., 2021; Vavrek et al., 2008) and can elicit predator-avoidance phenotypic changes. Disturbance cues were used to reduce the number of guppies required for the duration of the experiment as per animal care protocol. These chemical cues allow prey to respond with context-appropriate behavioural, morphological, and life history defences (Chivers et al., 2012).

I collected disturbance cues from a shoal of 30 mixed-sex guppies in a 9.46 L aquarium by waving a pike cichlid model made from a 12.7 cm painted fishing lure back-and-forth underwater 10 times. I collected water from the same aquarium prior to disturbance with the pike model as the control cue for the disturbance cue experiment (Crane et al. 2022). I collected damage-released alarm cue from 17 male guppies (mean standard length = 14.01 ± 2.40 mm) by euthanizing donors by cervical dislocation (Concordia University Animal Research Ethics protocol 30000255) and using whole body extracts after removal of the head and tail. I placed all tissues in 100 mL of dechlorinated water, homogenized the tissues, filtered the mix through polyester floss, and then diluted the solution in dechlorinated water to the desired final volume (2000 mL) and concentration ($0.1 \text{ cm}^2\text{mL}^{-1}$). I collected a total of 23.67 cm^2 of Trinidadian guppy skin extract which was subsequently frozen in 20 mL aliquots at -20°C . I used dechlorinated water as the control cue where alarm cue treatments were administered.

Background risk

To manipulate background predation risk, I installed six 75.71 L aquaria with seven females guppies each and independent filtration, heating, and aeration (Fig. 2). After one day of acclimation, I separated the aquaria into two experiments: high risk treatment groups (ie. alarm cue and distilled water control) and intermediate risk treatment groups (i.e. disturbance cue and aquarium water control).

For the high risk experiment, I administered a 20 mL dose of alarm cue with a lure waved ten times back-and-forth in all high risk treatment aquaria two times a day for three days. I used the lure as previous research shows that prey fishes display increased threat-sensitive antipredator responses to both visual (Bishop and Brown, 1992; Chivers et al., 2001) and

chemosensory cues (Brown et al., 2006b; Lawrence and Smith, 1989; Vavrek and Brown, 2009). I administered a 20 mL dose of distilled water along with a hand waved above the aquarium ten times back-and-forth to the high risk control aquaria two times a day for three days. For the intermediate risk experiment I administered a 20 mL doses of disturbance cue with a lure waved ten times back-and-forth to all intermediate risk treatment aquaria two times a day for three days. I administered a 20 mL dose of aquarium water control with a hand waved above the aquarium ten times back-and-forth to intermediate risk control aquaria two times a day for three days. I fed all fish once daily with commercial flake food for the duration of the experiment. Following the third day of exposure to risk, I moved individual females to a plastic cylinder in the central aquarium of the test arena.

Behavioural Observations

I installed a test arena consisting of a central 9.46 L aquarium with two 9.46 L aquaria positioned 90° from the central aquarium centered to both short sides (Fig. 3). I placed an opaque plastic divider between each aquarium, as well as an opaque tarpaulin covering all external sides except for the top and bottom. I used two GoPro Hero 3+ Silver cameras, one located at the far left and one on top of the aquarium 15 cm from the surface to record movement within the central aquarium and both side aquaria. I drew black lines 8 cm inward from either side of the central aquarium to demarcate two male inspection zones from the larger central zone. I placed a clear plastic cylinder inside the central aquarium. To avoid differences in lighting between aquaria, I lit all aquaria evenly using a white overhead diffuser.

I placed one male with visibly blue colouration into one perpendicular side aquarium and one male without visibly blue colouration into the second perpendicular side aquarium as per

standard behavioural assay (Auld et al., 2016). I reused males from two populations of 14 males, with one population of males displaying blue body colouration and the other without blue body colouration. After 5 minutes inside the cylinder, I removed the two opaque dividers, allowing the female fish to see the males but not approach them. After another 5 minutes elapsed, I removed the plastic cylinder and collected video for 10 minutes at a resolution of 720 p, framerate of 30 fps, and a wide angle lens camera setting. After 10 minutes of recording, I replaced the opaque dividers and placed the female fish back inside the plastic cylinder. I swapped the male aquarium positions and repeated the test. After each pair of recordings, I returned males to their respective holding aquaria, removed the female fish, and replaced aquarium water in the test set-up with dechlorinated water. I photographed all fish prior to finishing the experiment by setting each fish against an evenly lit neutral grid background and using a coloured tape for colour standardization. I repeated this protocol until all 101 female fish were tested. I colour-corrected photographs in Adobe Photoshop CS6 version 13.0.6 and measured colouration and standard length in ImageJ version 1.52n.

Behavioural Assay

I analyzed all recordings using BORIS version 7.0.13 (Friard and Gamba, 2016) for female latency to enter either inspection zone, amount of time the female spent in each demarcated section, and the amount of overall movement between demarcated sections indicated by the number of black lines crossed.

From these initial measures, I quantified choice and movement: (1) ‘latency to enter’ as the time until the female entered either of the two inspection zones, (2) ‘sexual activity’ as the time the female spent within either inspection zone, (3) ‘predicted choice’ as the time the female

spent with the blue male, (4) ‘sampling frequency’ as the number of times a female moved from one of the inspection zones to the centre, and (5) ‘predicted choice while controlling for overall sexual activity’ as the amount of time the female spent in the inspection zone closest to the blue male while controlling for the time the female was with either male. I was kept double-blind as to the treatment identity of the fish being analyzed.

Statistical Analysis

In order to test for the effect of short-term exposure to a high risk (alarm cue) or intermediate risk (disturbance cue) environment on the mate selection behaviour of female Trinidadian guppies, I conducted a mixed model analysis of variance. In order to test for the effect of side bias in male placement, I performed a repeated measures analysis of variance on females exposed to high risk and intermediate risk environments. After selecting for fish with a standard length within one standard deviation of the mean (Alarm Cue Treatment $n = 18$, Alarm Cue Control $n = 12$; Disturbance Cue Treatment $n = 18$, Disturbance Cue Control $n = 20$), I analyzed the data in Jamovi version 1.2.27.0 (The Jamovi Project, 2020), a graphical user interface for the R programming language (version 3.6, R Core Team, 2019), using the packages GAMLj (Gallucci, 2019), afex (Singmann, 2018), and emmeans (Lenth, 2020). Latency to enter and sampling frequency were log-transformed in order to reduce the skew of the data.

Results

To test if alarm cue treated females showed differences in mate selection behaviour, I conducted a mixed model analysis of variance and found that log transformed latency to enter was not significant ($F_{1,28} = 2.00$, $p = 0.17$). Additionally, sexual activity ($F_{1,28} = 2.07$, $p =$

0.16), predicted choice ($F_{1,58} = 0.29$, $p = 0.59$), log transformed sampling frequency ($F_{1,58} = 1.21$, $p = 0.28$), and predicted choice while controlling for overall sexual activity ($F_{1,58} = 0.16$, $p = 0.69$) were also found to be not significant. Overall, I found that females treated with alarm cue i.e. high background risk for three days prior to testing did not show any differences in mate selection behaviour when compared to females treated with a distilled water control (Fig. 4).

To test for the presence of confounding side bias due to male side placement on female behaviour, I performed a repeated measures analysis of variance on females treated with alarm cue with their associated distilled water control group and showed a significant effect of male side placement on female time spent with the blue male during both trials ($F_{1,58} = 134.5$, $p < 0.05$). My result suggests that the side aquarium the male was placed in affected the mate selection behaviour of alarm cue treated females (Fig. 6).

To test if disturbance cue treated females showed differences in mate selection behaviour, I performed a mixed model analysis and found that log transformed latency to enter was not significant ($F_{1,36.3} = 1.22$, $p = 0.28$). Additionally, sexual activity ($F_{1,36} = 2.29$, $p = 0.14$), predicted choice ($F_{1,74} = 2.39$, $p = 0.13$), log transformed sampling frequency ($F_{1,36} = 2.32$, $p = 0.14$), and predicted choice while controlling for overall sexual activity ($F_{1,74} = 1.39$, $p = 0.24$) were also found to be not significant. Overall, I found that females treated with disturbance cue for three days prior to testing did not show any differences in mate selection behaviour when compared to females treated with an undisturbed aquarium water control (Fig. 5).

To test for the presence of confounding side bias of male side placement on female behaviour, I performed a repeated measures analysis of variance on females treated with disturbance cue and their associated control group of undisturbed water treated females and showed a significant effect of male side placement on the amount of time females spent with the

blue male during both trials ($F_{1,74} = 32.2, p < 0.05$). My result suggests that the side aquarium the male was placed in determined the mate selection behaviour of disturbance cue treated females independent of any background treatment or stimulus treatment (Fig. 7).

Discussion

Despite the well established effects of long-term elevated predation risk on mate choice (see ‘Introduction’), my results suggest that periods of short-term elevated background predation risk is insufficient to alter the mate selection behaviours of female Trinidadian guppies under both elevated and intermediate predation risk scenarios. In both high risk and intermediate risk scenarios, female fish did not show significant differences in antipredator behaviours when compared to control groups in female latency to enter, sexual activity, predicted choice, sampling frequency, and predicted choice while controlling for overall female sexual activity. These results suggest that short-term risk is not sufficient to alter female mate choice patterns in Trinidadian guppies.

While prior research in guppies have shown compensatory behavioural changes in response to acute predation threats (Elvidge et al. 2014), there exists a number of reasons as to why I did not observe behavioural changes in response to short-term risk. First, the guppies in my experiment may have exhibited covert behavioural changes as described by Brown et al. (2001), who showed that prey fish species from high predation populations exhibit covert antipredator behaviours at lower concentrations of chemical cues than normally required for overt behavioural responses. Second, the guppies in our experiment behaved as would be expected in a low-predation environment where the short-term risk of predation did not reach the threshold required to elicit a response. Brown et. al (2009) found that Trinidadian guppies from

high predation environments exhibit graded, threat-sensitive, antipredator responses in proportion to the concentration of chemical cues, while guppies from low predation environments react in a hypersensitive, all-or-nothing, manner and thus require a higher threshold of stimulus to show a behavioural change. In another species, *Amatitlania nigrofasciata*, Brown et al. (2006b) showed that short-term variability in predation risk did not affect these graded versus hypersensitive response patterns, indicating that the intensity and patterns of antipredator behaviour are influenced more strongly by recent experiences as compared to prior experiences (Ferrari and Chivers, 2006; Vainikka et al., 2005). Third, changes in female mating behaviour under elevated predation risk may not be present as prior research has shown that when only female guppies are subjected to elevated predation risk, they are less receptive to male courtship (Dill, 1999). Lastly, it is possible that mate choice behavioural decision-making in Trinidadian guppies is not plastic but are instead driven by long-term changes.

While the generalizability of my study is limited to laboratory-reared populations, the results of this study points toward potential future work comparing laboratory-reared and wild populations as laboratory-reared populations lack experience in foraging, territorial defense, and courting under predation risk and have been shown to differ in their antipredator responses when compared to wild populations (Magurran et al., 1996). Further, these same behaviours are also dependent upon factors such as body size (Cooke et al., 2003), foraging tactic, prey group size (Brown et al., 2006b; Helfman and Winkelman, 1997), and the influence of ecological factors (Botero and Rubenstein, 2012; Walling et al., 2008).

Chapter 3

Elevated Maternal Predation Risk and Antipredator Responses in Juvenile Trinidadian Guppies

Introduction

Behaviourally plastic responses to local ambient predation risk occurs over a single generation through the induction of phenotypically plastic responses (Brown et al., 2009; Brown, et al., 2013; Magurran, 2005), or over several generations through natural selection (O'Steen et al., 2002). Under conditions of intense predation pressure, or in the presence of a small and stable predator guild, selection should favour an experience-independent response to novel predators (Breden et al., 1987; Riechert, 2005; Vilhunen and Hirvonen, 2003). Exposure to novel situations, including a novel predator, may elicit a strong predator avoidance response as the cost of not responding would be higher than the cost associated with acting in the absence of a predation threat (Hirvonen et al., 2000). Such 'experience-independent' responses include phenotypically plastic responses such as neophobia (Brown et al. 2013), and is beneficial for species lacking sufficient behavioural plasticity in early life history phases as it allows for the development of context-appropriate threat-sensitive responses (Killen and Brown, 2006).

Consensus on the differences in antipredator behaviour between generations of fish is mixed. McGhee et al. (2012) found that offspring of predator-exposed *Gasterosteus aculeatus* did not exhibit antipredator behaviour in the form of orienting toward a predator. The authors suggest this is due to the mothers' immediate increase in endogenous glucocorticoid levels providing a survival benefit for the mother at the expense of future offspring survival (Gluckman et al., 2005). Indeed, the eggs of predator-exposed mothers have higher concentrations of

glucocorticoid (Giesing et al., 2011), with these same hormones found to be responsible for the deleterious effects of maternal stressors on offspring in other vertebrate species (Henriksen et al., 2011; Schoech et al., 2011; Weinstock, 2008).

On the other hand, population differences in antipredator behaviour in Trinidadian guppies persist between generations of laboratory-reared fish, indicating that this species has an innate ability to recognize predatory fish (Kelley and Magurran, 2003b). Observed behavioural changes include increased exploration (Crane et al., In Press) in novel or stressful environments (Cattelan et al., 2020; Zimmer et al., 2013, 2017) for increased information gathering, which are correlated with increased dispersal away from stressful conditions similar to those experienced by their mother (Cote et al., 2010; Hoset et al., 2011; Korsten et al., 2013). These anticipatory maternal effects, defined as when an environmental stressor such as elevated predation risk increases maternal glucocorticoid levels which then influences offspring phenotype (Love et al., 2013; Sheriff and Love, 2013, Sheriff et al., 2017), affect offspring fitness in high-predation environments (McGhee et al., 2021; Storm and Lima, 2010) by creating changes in physiological (Love and Williams, 2008b), morphological, and behavioural defenses (Agrawal et al., 1999; Coslovsky and Richner, 2011; Storm and Lima, 2010).

To add an additional layer of complexity, recent work by McGhee et al. (2021) in *Gambusia affinis* showed that brief stressors during a female's pregnancy interacts with offspring sex to influence offspring behaviour, potentially leading to both intra- and inter-population differences in antipredator behaviour under varying levels of predation risk and over time.

Thus, I examined if the offspring of Trinidadian guppies exposed to elevated predation risk increased dispersal and exploratory behaviour such that it would confer a potential survival benefit in high-predation environments. I did this by exposing pregnant female guppies to short-

term elevated predation risk and measuring antipredator behaviours in their offspring. I predicted that offspring of female guppies reared under elevated predation risk would exhibit higher dispersal, exploratory behaviour, and neophobia when exposed to a novel cue when compared to females that were not exposed to elevated predation risk.

Methods

Study System

I used a total of 113 newborn juvenile fry offspring of 24 lab-born descendants of wild Trinidadian guppies collected from the Quare River, Trinidad, for the duration of this experiment (Fig. 8). Prior to experimentation, fish were housed in 110 L glass aquaria kept at 25°C on a 12:12 L:D cycle. Fish were fed twice a day with Nutrafin commercial flake food.

Behavioural Recordings

I placed one male and four visibly pregnant i.e. gravid spot-displaying female lab-born descendants of wild Trinidadian guppies of equal size in one of six aquaria for three days. I removed the male at the end of the third day (Fig. 9). By including visibly pregnant females and housing the female fish with a male, I ensured all females used for the experiment were pregnant and would produce offspring for the duration of the study. I collected water from a 9.46 L aquarium with 30 mixed-sex guppies for control cue. I collected disturbance cue from the same aquarium after using a painted lure waved back-and-forth underwater ten times as described in Chapter 2. After one day, I divided the aquaria into two groups of three aquaria, with one group receiving 5 mL of disturbance cue and ten underwater back-and-forth lure movements twice-a-day, and the other group receiving 5 mL of aquarium water control and ten times overhead hand

movement twice-a-day. Each morning, I removed newborn juvenile (less than 24-hour old) fry (mean length = 9.45 mm \pm 1.15 mm) and individually tested each offspring in a 100 mm petri dish filled with dechlorinated water positioned on a diffused light box illuminated from below (Fig. 10). I tested individual offspring in order to control for the effects of shoaling, as well as to focus on individual antipredator behaviours (McGhee et al., 2012). After 5 minutes of acclimation, I recorded the fish for 5 minutes using a GoPro Hero 3+ Silver camera placed directly above the installation at a resolution of 720 p, framerate of 30 fps, and a wide angle lens camera setting. Following this first recording, I delivered 1 mL of either a novel odour of Clubhouse™ Lemon extract diluted to 1/100 concentration in distilled water or a distilled water control, after which I recorded the fish for 5 minutes. I delivered cue and lure movements to the mother fish every day until I collected all offspring needed for the experiment. I analyzed offspring movement using Open Source Physics Tracker version 5.0.6 (Brown, 2018).

Behavioural Assay

After binning movement coordinates into time intervals of 0.5 s, I used Microsoft Excel for Mac version 16.46 to calculate total distance travelled (m), mean velocity (m/s), number of darting movements defined as the number of times where the fish travelled at a velocity greater than the upper confidence limit of the mean velocity, average acceleration of darts (m/s²), and the time the fish spent paused (s).

Statistical Analysis

As smaller individuals are often at a disproportionately higher risk than larger individuals due to gape-limitation of predators, their weaker swimming abilities (Lundvall et al., 1999), and

based on previous experiments related to maternal effects (McGhee et al., 2012), I chose to analyze fish with a body size within one standard deviation of the mean. After selecting for offspring of appropriate body size, I performed a two-way analysis of variance in Jamovi version 1.2.27.0 (The Jamovi Project, 2020) to test for differences in the aforementioned behaviours in the offspring due to the maternal treatment and the offspring novel odour exposure. I also performed an independent samples t-test to test for differences in offspring behaviour due to maternal treatment.

Results

To test for the effect of maternal treatment and offspring treatment, I conducted a two-way analysis of variance on total distance travelled and found no statistically significant interaction between the effects of maternal treatment and offspring treatment ($F_{1,74} = 0.11, p = 0.74$). Likewise, I found no statistically significant interaction between the effects of maternal treatment and offspring treatment on mean velocity ($F_{1,74} = 0.11, p = 0.74$), number of darts ($F_{1,74} = 0.48, p = 0.49$), average acceleration of darts ($F_{1,74} = 0.93, p = 0.34$), and time spent paused ($F_{1,74} = 1.66, p = 0.20$) (Fig. 11).

To test for the effects of maternal treatment, I conducted an independent samples t-test on total distance travelled by offspring and found no statistically significant effect ($t_{74} = -0.58, p = 0.56$). Likewise, I found no statistically significant effect of maternal treatment on mean velocity of offspring ($t_{74} = -0.58, p = 0.56$), number of darts by offspring ($t_{74} = 0.73, p = 0.47$), average acceleration of darts by offspring ($t_{74} = 1.49, p = 0.14$), and time spent paused by the offspring ($t_{74} = -1.97, p = 0.052$) using the same statistical analysis.

Discussion

The results of my experiment suggest there is no significant effect of short-term intermediate maternal predation risk on offspring antipredator behaviour contrasting previous research showing that mothers prepare their future offspring for the environment they will encounter (Gluckman et al., 2005; Uller, 2008; Weaver et al., 2004) by providing them with survival benefits over offspring of unexposed mothers (Giesing et al., 2011; Shine and Downes, 1999; Storm and Lima, 2010). It is important to note that within my experiment there was near-significance ($p = 0.052$) in the effect of maternal treatment on offspring time spent paused. The offspring of predator-exposed females spent less time paused than the offspring of unexposed females, indicating there may be minor increases in offspring exploratory and dispersal behaviour. These changes, however minor, may be in line with the findings of Giesing et al. (2011) who demonstrated that offspring of predator-exposed female sticklebacks displayed antipredator defensive behaviours, but that these behaviours may be mediated by exposure to maternally-derived cortisol.

The generalizability of my study is limited by a number of factors. While McGhee et al. (2012) suggested that prenatal exposure to glucocorticoids may be responsible for the lack of antipredator response observed in their experiment, other research has shown that these same hormones may also have positive effects on other components of an offspring's phenotype (Chin et al., 2009; Coslovsky and Richner, 2011; Gagliano and McCormick, 2009), potentially negating any expected behavioural changes. Further, the environment offspring perceive may not be the same as the perceived maternal environment (McGhee et al., 2012), where instances of predator-induced maternal effects are adaptive under some circumstances, but deleterious in others (Matthews and Phillips, 2010; Schoech et al., 2011; Weinstock, 2008). This could

potentially lead to a mismatch in predictively adaptive behaviours as suggested in Gluckman et al. (2005). Additionally, due to the rapid growth of offspring during early developmental stages, my results are only indicative of antipredator behaviour at a specific developmental stage and may differ from responses seen earlier or later in development as innate behaviours are replaced by learned behaviours (Brown and Chivers, 2006b; Brown et al., 2011). Younger offspring may display increased antipredator behaviours while offspring in later developmental stages may not show any responses as there may be no immediate risk of predation. Stressed females may also compensate for stressful environments by shifting their reproductive investment toward smaller offspring as opposed to our study's expected modulation of offspring behaviour (Love et al., 2005; Love and Williams, 2008b; Monclus et al., 2011). These offspring may not behave differently than others reared in low predation environments except that they may be developmentally stunted, effects which may continue passed the juvenile stage (Gluckman et al., 2005). Lastly, other factors which may play a role in the applicability of my findings are the type, magnitude, predictability, and timing of the stressor (Sheriff et al., 2009, 2010; Storm and Lima, 2010), all of which increase the complexity of possible antipredator interactions.

Chapter 4

General Discussion

In interpreting both the mate selection and anticipatory maternal effects experiments together, my results suggest that short-term elevated predation risk does not alter reproductive antipredator behaviour in Trinidadian guppies. In comparing with the literature, my results suggest that while long term predator exposure shapes behavioural responses and acute exposure affects trade-offs, short-term exposures may not be enough to elicit changes. Populations of Trinidadian guppies exposed to predation pressures over long periods of time have been demonstrated to be bolder and display non-graded anti-predator responses when exposed to acute predation risk. Further, these populations exhibit compensatory behaviours following a predation threat whereby guppies from high risk populations resume pre-exposure behaviours, such as foraging, at a higher rate than prior to predator exposure when compared to guppies from low risk populations (Elvidge 2014). In addition, under conditions of acute predation risk, female guppies have been shown to prefer bolder males irrespective of prior background risk (Godin and Dugatkin, 1996). Taken together, these findings suggest that predator avoidance is shaped by acute risk in combination with past experiences and individual behaviours (Brown et al., 2018).

A number of interesting future research goals are brought to light in the context of my findings. First, one continued approach is to test for cross-species responses to chemical cues as these interactions have been noted in a variety of other taxa in the absence of individual prior experience (Ferrari et al., 2010; Pollock et al., 2003). This is especially interesting in species with differing reproductive strategies, such as the asexually-reproducing Amazon molly (*Poecilia formosa*) which requires sperm from a male of a different species for egg activation and is reliant upon the female mating behaviour of a different species facing a potentially

dissimilar predation-risk scenario. Second, this research may be implemented in a model fish species that matures rapidly, such as the Turquoise killifish (*Nothobranchius furzeri*) which reach sexual maturity 14 days after hatching and must make faster reproductive decisions than the model studied here. Third, the current work may continue on the sex of the offspring studied, as previous research shows maternal carryover effects are sex-dependent (Love and Williams, 2008a, 2008b; Monclus et al., 2011). These sex-dependent maternal effects may also impact differences in mate selection behaviour, with additional focus required on both male and female mate choice behaviours.

My results have a number of important conservation and industry applications. Predator training in fisheries, where hatchery-reared fish are trained to recognize and respond to potential predators before being released into the wild (Bischof and Zedrosser, 2009; Brown and Day, 2002; Brown and Laland, 2003), is important as fish often succumb to predation pressure at early life stages (Henderson and Letcher, 2003; Shively et al., 1996). As wild and captive-bred fish populations show different responses to predation (Lepage et al., 2000), training fish to respond to predators at these early life stages would be beneficial to ensure survival of captive-reared species (Beck et al., 1994; Fleming, 1994; Snyder et al., 1996) over both ontogenetic and evolutionary timeframes (Carthey and Blumstein, 2018; McLean, 1998; Wallace, 2000).

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Fig. 1: Experimental overview where female Trinidadian guppies were grouped into high and intermediate risk treatments and administered alarm cue or disturbance cue and their appropriate controls. After a three day acclimation period, fish were grouped into high and intermediate risk treatments and administered cue twice a day over a three day period after which they were tested for mate selection behaviours.

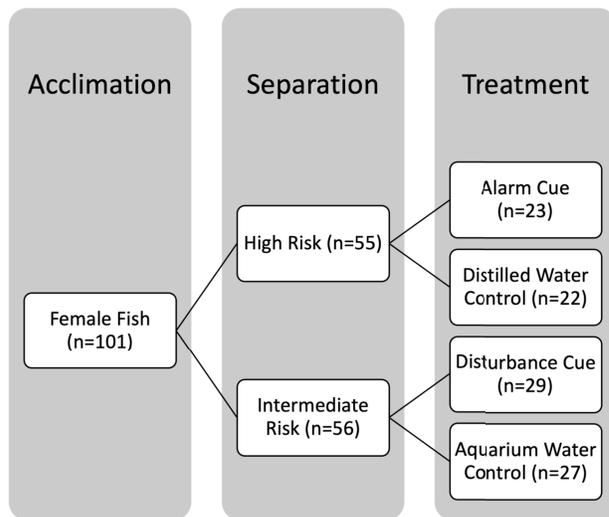


Fig. 2: Experimental setup of treatment aquaria where female Trinidadian guppies were administered alarm cue, disturbance cue, distilled water control, or aquarium water control twice a day for three days before testing. Treatment aquaria of 37.85 L had a box filter setup with air (A), were lined with gravel substrate (B), had a central plastic plant (C), and were lined externally on two sides with tarpaulin to prevent fish from different aquaria interacting with each other (D).

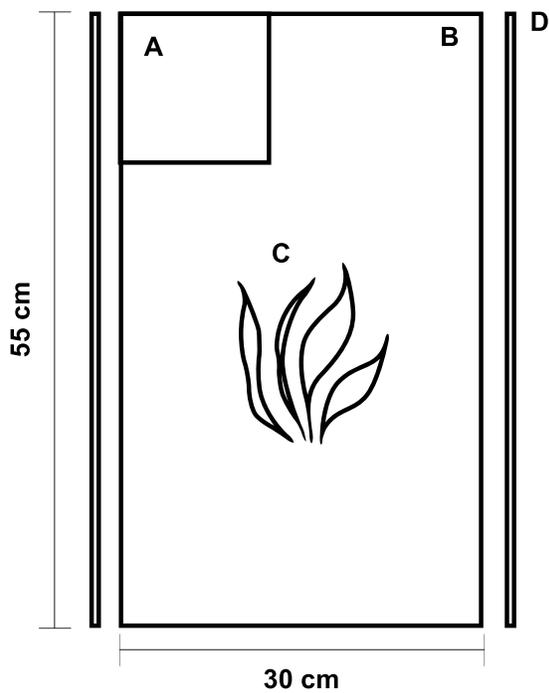


Fig. 3: Experimental setup of test aquaria where female Trinidadian guppies were tested for mate selection behaviours. All test aquaria were 9.46 L. Male aquaria (A) contained either a blue or a dull male and were separated externally by an opaque divider (B) from the female holding aquarium (C) that was sub-divided with two inspection zones on either side. The whole setup was covered by opaque tarpaulin (E) in order to prevent external movement or lighting from interfering with the experiment. Cameras were placed overhead (F) as well as within an enclosed pocket (G) to record behaviours.

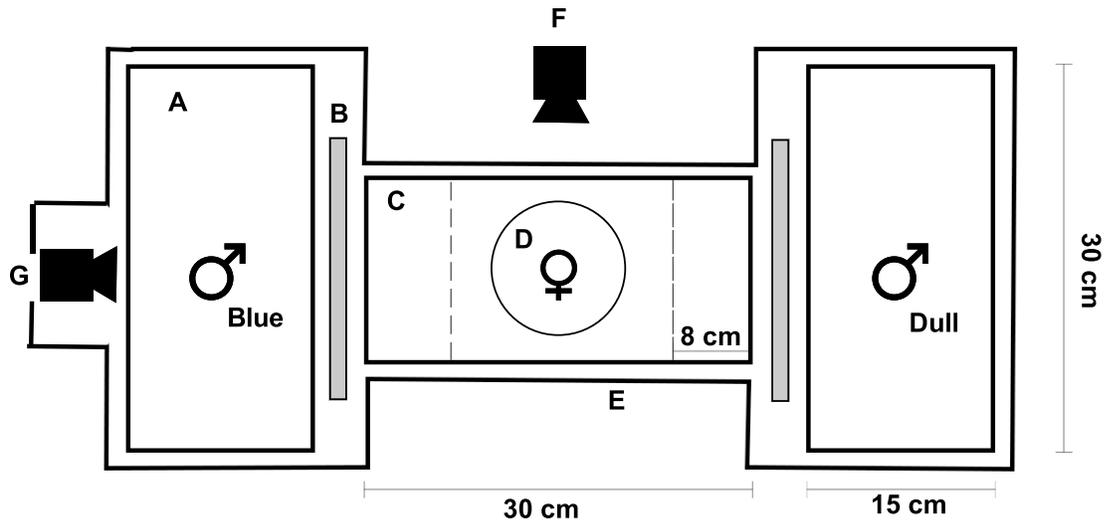


Fig. 4: A mixed model analysis of variance of female mate selection behaviours under high risk conditions. Log transformed latency to enter was not significant (D), nor were the time the female spent with both males (C), the time the female spent on the side with the blue male (B), the log transformed total number of line crosses, and time the female spent on the side with the blue male after controlling for the time the female spent with both males (A).

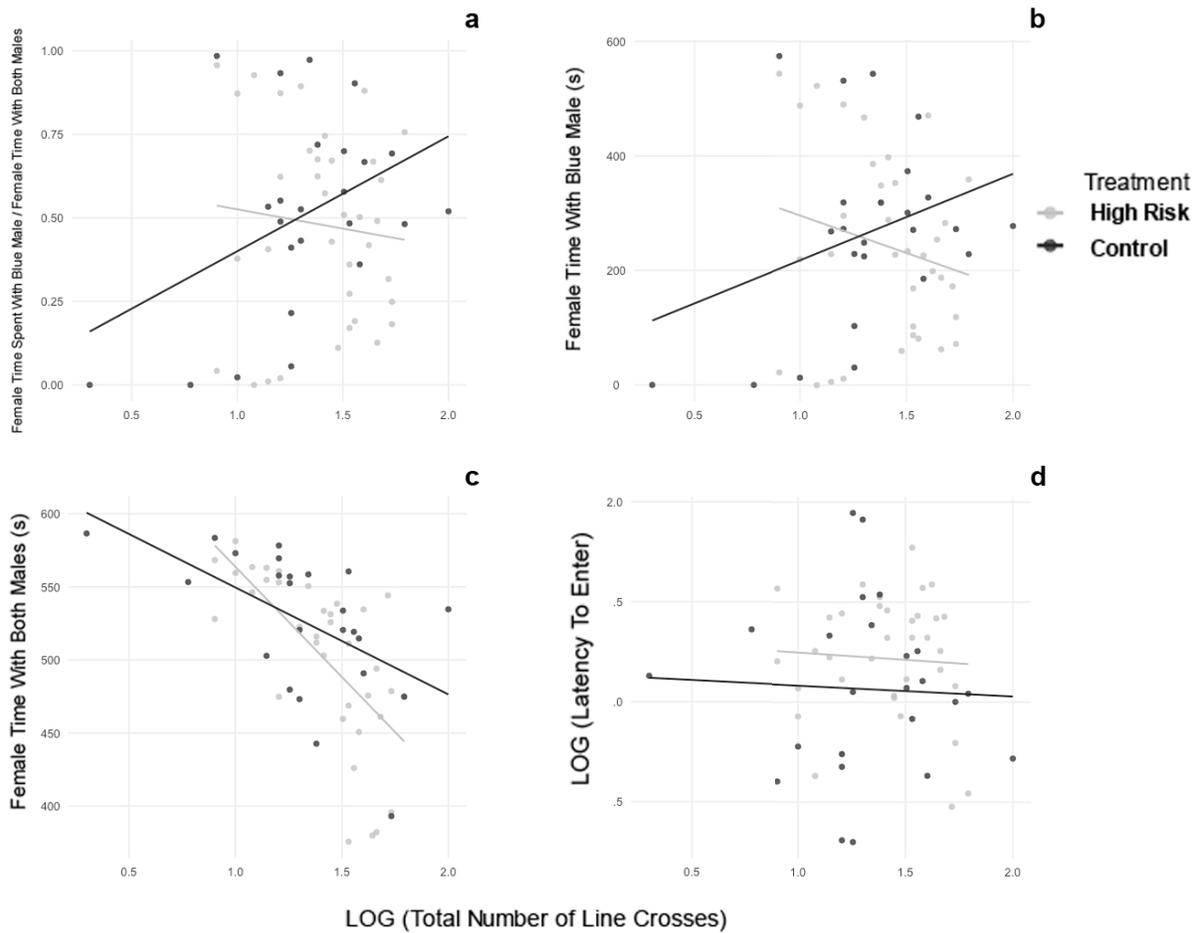


Fig. 5: A mixed model analysis of variance of female mate selection behaviours under intermediate risk conditions. Log transformed latency to enter was not significant (D), nor were the time the female spent with both males (C), the time the female spent on the side with the blue male (B), the log transformed total number of line crosses, and time the female spent on the side with the blue male after controlling for the time the female spent with both males (A).

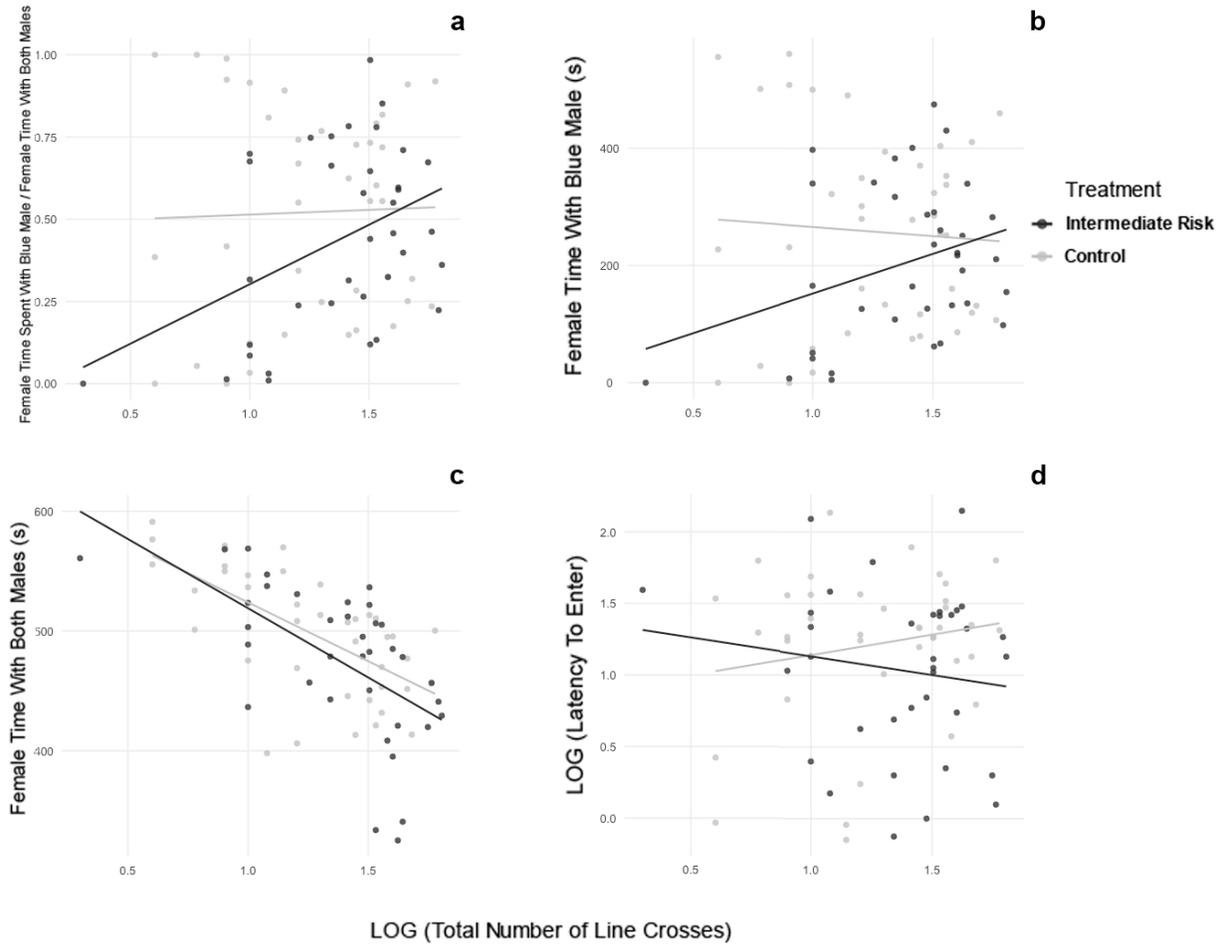


Fig. 6: A repeated measures analysis of variance on females from the high risk experiment along with their associated distilled water control group. There was a significant effect of the side of the aquarium the male was placed on female time spent with the blue male during both trials.



Fig. 7: A repeated measures analysis of variance on females from the intermediate risk experiment along with their associated distilled water control group. There was a significant effect of the side of the aquarium the male was placed on female time spent with the blue male during both trials.

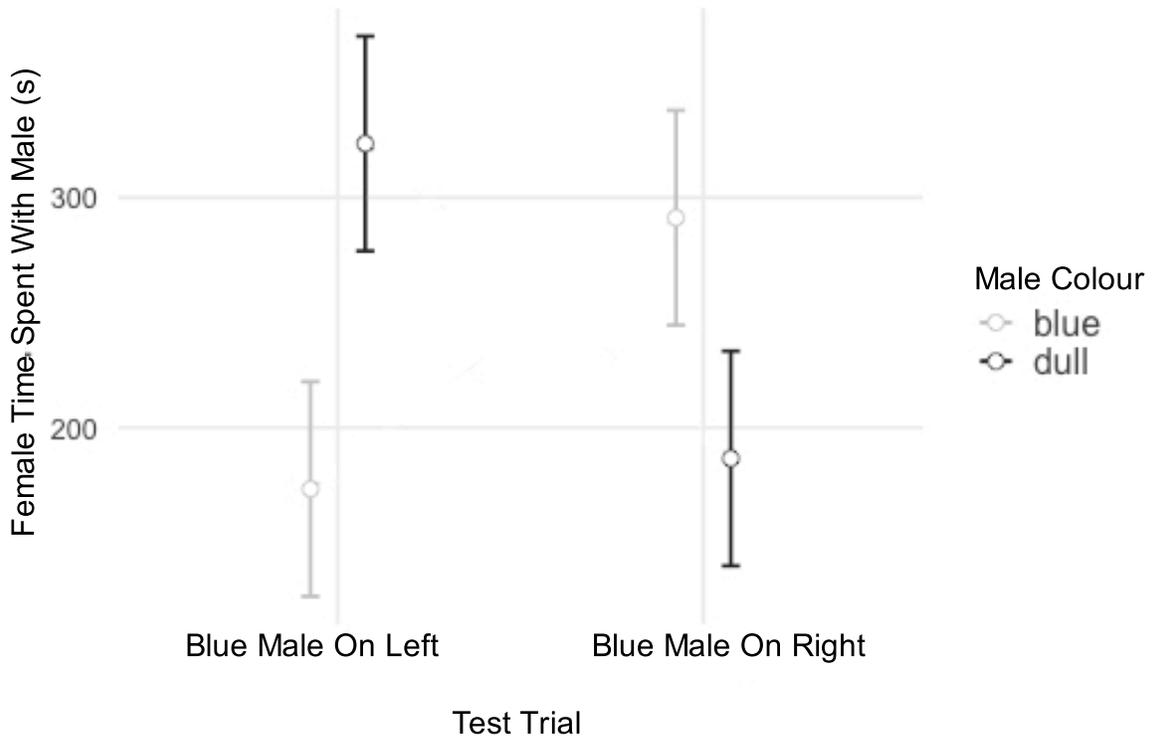


Fig. 8: Experimental overview where female Trinidadian guppies were grouped into disturbance cue and aquarium water control treatments and administered. After a three day acclimation period in which females were placed in groups of three females with one male, males were removed and females were administered cue twice a day over the course of the experiment. Offspring were tested on the same day they were collected for antipredator behaviours, including neophobic predator avoidance with a novel odour.

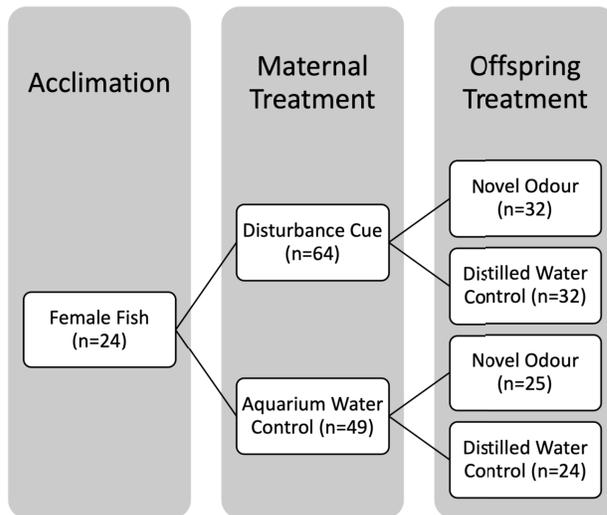


Fig. 9: Experimental setup of treatment aquaria for the maternal carryover effect experiment where female Trinidadian guppies were administered either disturbance cue or aquarium water control. All test aquaria were 9.46 L and contained a sponge filter (A), plastic plants and floating plants (B), and a Indian almond (*Terminalia catappa*) leaf to prevent fungal infections (C).

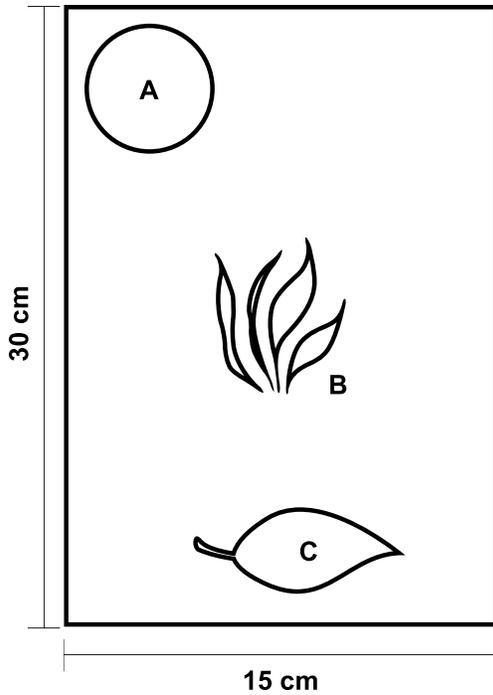


Fig. 10: Experimental setup for the maternal carryover effect experiment where offspring of Trinidadian guppies were recorded before and after administering either a lemon extract novel odour or distilled water control. Newly born offspring are placed in a 10 cm petri dish (A) on top of a light box (B) and recorded with an overhead camera (C).

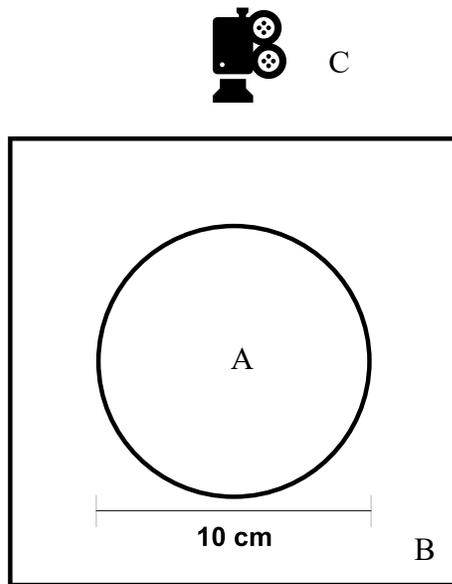


Fig. 11: A two-way analysis of variance to examine the effect of maternal treatment and offspring treatment before and after administering novel odour. The results show that there was no statistically significant interaction between the effects of maternal treatment and offspring treatment on proportional total distance travelled (D), number of darts (C), average acceleration of darts (B), and time spent paused (C).

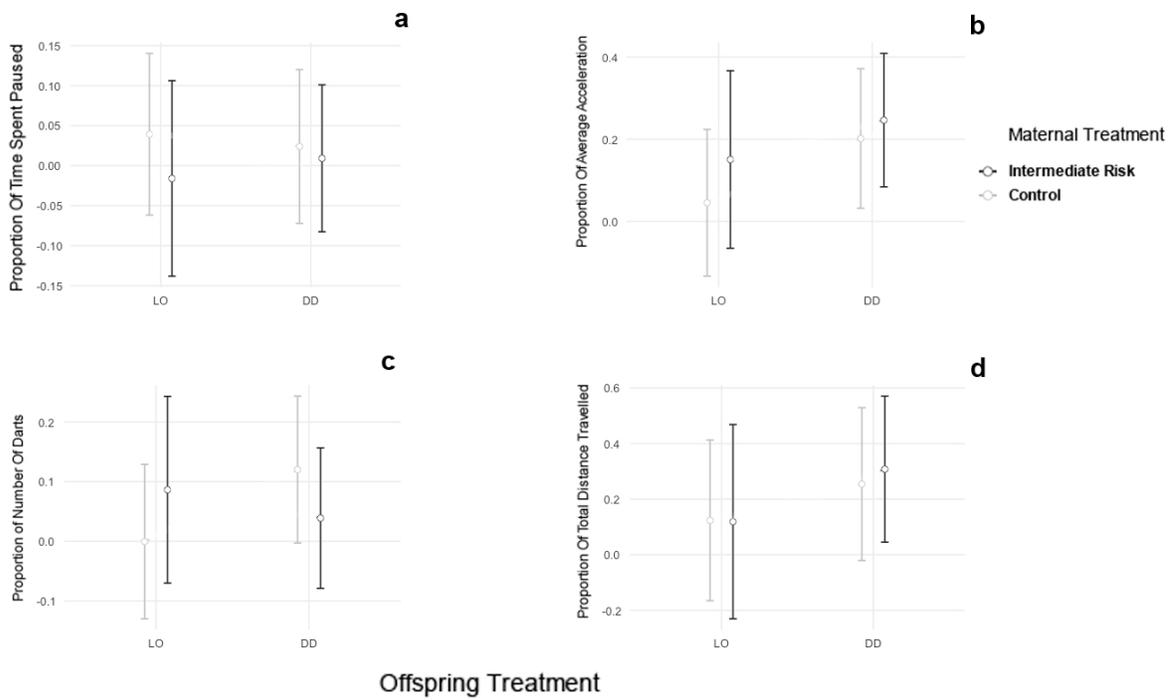


Table 1: Mixed model analysis of variance of female mate selection behaviours under high risk conditions.

	<i>df</i>	<i>t</i>	<i>p</i>
<i>LOGLatencyToEnter</i>	28	-1.41	0.169
<i>TimeFemaleNotInCenter</i>	28	1.44	0.161
<i>TimeWithPreferredMale</i>	58	0.537	0.594
<i>LOGTotalLineCross</i>	58	-1.1	0.275
<i>TimeWithPreferredMale/TimeFemaleNotInCenter</i>	58	0.397	0.693

Table 2: Repeated measures analysis of variance on females from the high risk experiment along with their associated distilled water control group.

	<i>df</i>	<i>F</i>	<i>p</i>
<i>TimeFemaleSpentWithMales</i>	1	0.0249	0.875
<i>TimeFemaleSpentWithMales*MaleColour</i>	1	134.5061	<0.001
<i>Residual</i>	58		

Table 3: Mixed model analysis of variance of female mate selection behaviours under intermediate risk conditions.

	<i>df</i>	<i>t</i>	<i>p</i>
<i>LOGLatencyToEnter</i>	36.3	-1.1	0.277
<i>TimeFemaleNotInCenter</i>	36	-1.51	0.139
<i>TimeWithPreferredMale</i>	74	-1.54	0.127
<i>LOGTotalLineCross</i>	36	1.52	0.136
<i>TimeWithPreferredMale/TimeFemaleNotInCenter</i>	74	-1.18	0.243

Table 4: Repeated measures analysis of variance on females from the intermediate risk experiment along with their associated aquarium water control group.

	<i>df</i>	<i>F</i>	<i>p</i>
<i>TimeFemaleSpentWithMales</i>	1	0.178	0.674
<i>TimeFemaleSpentWithMales*MaleColour</i>	1	32.207	<0.001
<i>Residual</i>	74		

Table 5: A two-way analysis of variance to examine the effect of maternal treatment and offspring treatment before and after administering novel odour.

	<i>df</i>	<i>F</i>	<i>p</i>
<i>PROP_TotalDistance</i>	1	0.0389	0.844
<i>PROP_MeanVelocity</i>	1	0.0389	0.844
<i>PROP_NumberOfDarts</i>	1	1.5997	0.21
<i>PROP_AverageAcceleration</i>	1	0.109	0.742
<i>PROP_TimePaused</i>	1	0.152	0.698