



Research article

Ecologically relevant arsenic exposure alters female mate preference and anxiety-like behavior in *Betta splendens*



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ABSTRACT

Arsenic is a metalloid pollutant that is commonly found in surface and groundwater worldwide. Toxicological effects of arsenic are relatively well-known, but much less studied are its effects on behavioral endpoints, which may have considerable evolutionary and population-level consequences. Here we investigated the effects of exposure to environmentally relevant concentrations of arsenic (0, 10 and 100 µg/L) for 96-hours on female preference for male color (i.e. red versus blue) in *Betta splendens*, an increasingly popular fish model for contaminant-induced behavioral dysfunction. Further, we examined whether arsenic exposure altered anxiety-like behaviors using a standard scototaxis test (preference for light or dark), as well as measured tissue cortisol concentrations to increase our understanding of possible mechanisms driving behavioral responses. We found exposure to 100 µg/L arsenic results in a loss of female preference for red males, and arsenic exposed females showed increased anxiety-like behavior. The loss in preference for male coloration may have been driven by anxiety, as preference for red was negatively correlated with anxiety-like behavior for all fish. Interestingly, increase in anxiety-like behavior occurred without a parallel increase in cortisol. Female preference for red colored males may confer fitness benefits, and this study highlights important arsenic-induced behavioral changes that could have population level consequences.

1. Introduction

Arsenic is a ubiquitous metalloid found in surface and groundwater worldwide, including both fresh and ocean waters [1, 2]. While arsenic occurs naturally, coal combustion, mining activities, industrial processes, and the application of arsenic-containing pesticides contribute substantially to environmental burdens [3, 4]. The drinking water standard for arsenic set by the United States (U.S.) Environmental Protection Agency and the World Health Organization is 10 µg/L, although some states in the U.S. have lowered acceptable drinking limits to 5 µg/L. Environmental concentrations in both fresh and ocean water are typically below 2 µg/L [2, 4], although water bodies in many regions of the world with anthropogenic activity or hydrogeologic conditions conducive to elevated arsenic, may have concentrations well above safe drinking limits, and concentrations of more than 21,000 µg/L have been reported [4].

The toxicity of arsenic to fish is dependent upon its form (e.g. organic

versus inorganic), and by a variety of aquatic variables including temperature, pH, organic content, the presence of other contaminants, and the duration of exposure [5]. Acute toxicity studies in fish show that 96-h LC₅₀ concentrations for arsenic tend to range between 10-30 mg/L, with variation driven by both fish and arsenic species [5]. Fish may be especially vulnerable to arsenic, as it is readily absorbed across the gills, accumulates further via ingested sources, and has been shown to affect a variety of physiological processes including growth, reproduction, osmoregulation, and immune function [6, 7, 8]. Much less studied, however, are sublethal effects of arsenic at ecologically relevant concentrations, especially related to behaviors that may alter reproductive outcomes. This is a critical gap in our knowledge, given the pervasiveness of arsenic contamination and the potential for behavioral endpoints to have population level consequences. Behavioral changes represent an important endpoint for environmental exposures, as they are the result of complex physiological processes across numerous biological systems, and demonstrate the downstream consequences of contaminant exposure.

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Moreover, because behavior is the culmination of numerous physiological inputs, effects may be observed at lower concentrations than for other established biological endpoints. Reproductive behaviors such as female mate preference can have considerable ecological and evolutionary consequences, since the offspring of females that are able to mate with preferred males have heritable increases in growth rate and body size [9], and there is greater maternal investment in the offspring [10]. Further, studies suggest that when females are unable to mate with preferred males, there may be a decrease in population viability due to the persistence of deleterious genes [11]. Notwithstanding, the role of contaminants in sexual selection and population resilience is poorly understood.

The mechanisms by which arsenic could alter behavior are unclear, but both direct and indirect actions are possible. For example, arsenic-induced behavioral changes could be via direct action on neural function [12, 13], or changes could be induced by influencing other behaviors that result in a reduced expression of preference. For example, arsenic has been shown to increase anxiety-like behavior in mice as measured by open-field and elevated-maze tests [14], and anxiety has been associated with altered behavior in a variety of contexts, including reproduction [15]. Scototaxis, the preference for light vs. dark, has been shown to be an effective metric of anxiety in fish, and has been used to examine the behavioral effects of environmental contaminants [16, 17]. This test creates a trade-off between risk-aversion behavior (i.e. spending more time in the dark section) and exploratory behavior (i.e. spending time in both light and dark sections). Scototaxis as a method of measuring anxiety in fish has been validated in zebrafish using pharmaceuticals known to alter anxiety in humans [18], with more anxious individuals spending more time in the dark portions of the testing environment.

Contaminant-induced behavioral changes with implications for population-level consequences have been shown for other pollutants at environmentally relevant concentrations [15, 17, 19, 20, 21, 22, 23, 24, 25, 26]. 17 β -trenbelone, an androgenic steroid associated with the production of livestock, altered male courtship and the receptivity of females toward exposed males in guppies (*Poecilia reticulata*) [21]. Further, 17 β -trenbelone was shown to reduce the amount of time females associated with males, and unexposed females showed a higher degree of discretion in choosing males that had not been previously exposed to the contaminant [27]. European perch (*Perca fluviatilis*) exposed to the psychoactive pharmaceutical oxazepam showed increased boldness as well as context-specific anxiety-like behaviors [17].

This study seeks to explore the effects of arsenic exposure on female mate preference and anxiety using *Betta splendens*. This is an excellent behavioral model for contaminant exposure given that these fish are easily reared in the laboratory, many of their behaviors are well-described [28, 29, 30], and it has shown itself to be a valuable model for studies of contaminant-induced behavioral changes [28, 29, 31, 32, 33, 34]. Further, studies have determined that female bettas prefer red males, permitting the exploration of how contaminants may perturb these previously established norms [35].

We examined the effects of an acute (96-hour) arsenic exposure on female preference for male color, as well as female anxiety measured via scototactic response. We also examined plasma cortisol concentrations to increase our understanding of possible mechanisms driving behavioral responses. We hypothesized that exposure to ecologically relevant concentrations of arsenic would reduce female mate preference via increases in anxiety-like behavior.

2. Methods

2.1. Study species and housing

Betta splendens (Siamese fighting fish, or bettas) were obtained from a commercial fish supplier (Segrest Farms, Gibsonton, FL). All fish were fed daily to satiation with a commercial pellet diet (Aqueon betta pellets) and maintained on a 12:12 h light:dark photoperiod at an average

temperature of 28.1 °C \pm 0.23 (SE). Since arsenic can occur naturally in well water, we used reverse osmosis purified water supplemented with a commercial salt/mineral mix (2 ppt; Kent R/O right) to ensure fish were not exposed to extraneous arsenic. All males were individually housed in 4-liter tanks separated by opaque partitions to prevent male-male interactions prior to the experiment. All females were housed in one-liter aquaria and had visual contact with other females, as extended social isolation has been suggested to negatively affect females [36]. All females used in preference tests exhibited a white ovipositor, which indicates reproductive maturity and gravidity [37]. All fish were acclimated in these aquaria for at least seven days prior to the initiation of experiments. This project was approved by and followed the guidelines of the University of Maine's Animal Care and Use Committee (protocol number A2018-05-03).

2.2. Arsenic exposure

A solution of sodium arsenite (Sigma-Aldrich) and ultra-pure water was used to create arsenic treatments for focal females. Arsenite is the most commonly found form of arsenic in aquatic environments [2]. In addition to a control group (0 μ g/L arsenic), two arsenic treatment groups were created: 10 μ g/L and 100 μ g/L. These concentrations were chosen as 10 μ g/L is currently the safe drinking water limit set by the US Environmental Protection Agency (EPA), and 100 μ g/L is an elevated, but still ecologically relevant concentration, and is approximately 1000 times less than the LC₅₀ for most fish [5].

Females were exposed for 96-hrs, which is an arsenic exposure period that has been shown to alter behavior in other fish models [38]. Further, acute exposures to arsenic are common due to agricultural runoff or rain events causing overflow from coal ash retention ponds. Complete water changes (100%) were conducted daily to reduce the potential for concentration variation in arsenic exposure during the course of the study. At the end of the 96-h exposure period, a sub-set of females were placed into the behavioral observation tanks that contained arsenic-free water and underwent female preference testing, followed immediately by scototaxis behavioral assays (n = 18, 20 and 21 for the control, 10 μ g/L and 100 μ g/L treatments respectively). Another sub-set of females (n = 26 females/treatment) were euthanized with buffered MS222 and the tissues sampled for cortisol concentration. Sampling for cortisol occurred between 10:00am and 1:00pm, and samples were collected randomly from each treatment to ensure sampling was evenly spread out among each treatment throughout that time period. Males were not exposed to arsenic treatments.

2.3. Preference tests

Female preference for male color (red versus blue) was assessed by recording association time using a dichotomous preference test. Association time is a good predictor of the mating patterns in fish [39] and has been used in *B. splendens* to assess female mate preference for male color [35]. Only red females were used to limit the potential of assortative mating. Male pairs consisted of one red male and one blue male that were uniform in their respective color. We used 13 unique male pairs that were size matched for each treatment. Individual females from all three treatments (0, 10, 100 μ g/L sodium arsenite) were randomly assigned to a male pair; male pairs were used no more than four times within a treatment group. The mean standard length \pm SEM of the red males was 35.9 \pm 0.42 mm (range 33.1–39.1 mm) and that of the blue males was 35.9 \pm 0.43 mm (range 33.9–39.0 mm). The mean absolute size difference \pm SE between males within a pair was 0.39 \pm 0.12 mm (range 0.1–1.3 mm). Females were measured for standard length after behavioral testing to reduce pre-trial stress. There was no difference in size of the females measured among treatment groups (mean size \pm SE: 0 μ g/L = 30.1 \pm 1.03mm, range 24.0–33.2mm; 10 μ g/L = 30.3 \pm 0.67mm, range 24.7–33.2mm; 100 μ g/L = 30.7 \pm 0.86 mm, range 23.6–35.0 mm; ANOVA: F_{2,42} = 1.286, p = 0.881). Males and females were measured

without anaesthetization by immobilizing them gently against the side of the holding tanks.

Preference tests were carried out in a 75.7-liter aquarium ($76.2 \times 30.5 \times 30.5$ cm, L \times W \times H) divided into five equal sections (15.2 cm wide each), with the two outermost sections separated from the inner three sections by plexiglas partitions (Fig. 1). The outer two sections were sealed with silicone to prevent olfactory cues from passing into the inner most sections. Female *B. splendens* have been shown to exhibit a preference for red males in the absence of olfactory cues [35]. Lines drawn on the outside of the aquarium designated the inner three sections of the preference aquarium. For each test, a single male was placed in each of the two outer sections of the tank, and the placement of the red and blue males was randomized among tests. The female was placed in the middle section, which was considered the neutral zone, inside a clear Plexiglas tube for a 10-minute acclimation. After the acclimation, the clear plexiglas tube was removed and the female was allowed to swim freely among the three middle sections. The time the female spent in the sections adjacent to a male (association zones; within ~ 15 cm of each male) was recorded for 10 minutes (trial one). The female was placed back into the tube and the placement of the males was switched. A second trial was conducted starting with the 10-minute acclimation period. Two trials were run with different placement of the same males to control for any side biases females may exhibit. Observations in which the female did not spend any time in one of the association zones across (not within) the two trials were considered to be side-biased, however, no females needed to be excluded from the analyses due to side-bias.

2.4. Scototaxis test

We assessed female scototaxis directly after preference tests for male color. Scototaxis tests were conducted in a 37.9-liter tank ($50.8 \times 25.4 \times 30.5$ cm, L \times W \times H) divided in half along the length of the tank with black and white felt to create one black section and one white section (Fig. 2). Felt was adhered to the inside of the tank creating a matte, non-reflective surface. Although it has been suggested that non-reflective surfaces are important for all fish when using this test [40], it may be especially important for bettas that exhibit exaggerated and agonistic responses to their own reflections. Fluorescent lighting was located directly above the testing tank to ensure uniform lighting. Females were placed into a clear tube in the middle of the tank and allowed to acclimate for 10 minutes. The tube was then removed from the tank, and the amount of time the females spent in the black section was recorded for 10 minutes (600 seconds). The transition from one section to another was defined as the point at which the back of the female's eye went beyond the section demarcation. Females were scored as being in the light or the dark section throughout the observation period, as there was no neutral section.

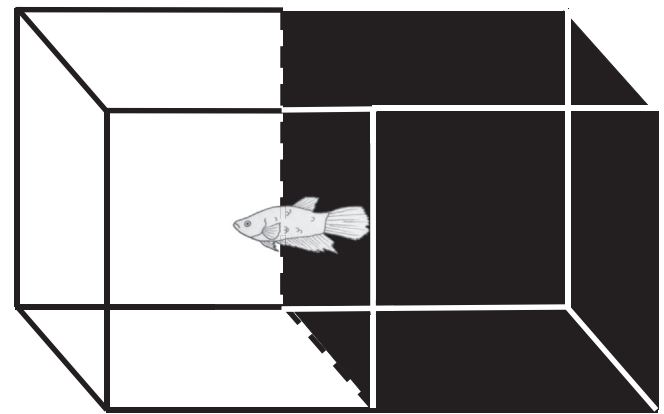


Fig. 2. Schematic of the tank set-up for the scototaxis tests. A 37.9-liter tank ($50.8 \times 25.4 \times 30.5$ cm, L \times W \times H) was divided in half along the length of the tank with black and white felt to create one black and one white section (25.4×30.5 cm, L \times W \times H). *Betta* illustration by: Torey J. Bowser.

2.5. Cortisol assays

Cortisol was measured from double-ether extracted betta tissue according to modified protocols described in Edwards et al. (2006). Following euthanization with buffered MS222, the viscera, fins, and head was removed just past the operculum and discarded. The remaining body tissue was placed in a tube and stored at -80°C until assayed. Tissues were weighed, thawed on ice, and homogenized in 2 mls 65 mM borate buffer (pH 8.0) for 1-minute. For extraction, 5 mls diethyl ether was added to the borate buffer solution and mixed for 5 minutes on a multi-tube vortex mixer. After being allowed to settle for 3 minutes, the tubes were snap-frozen in a methanol bath chilled to $< -20^{\circ}\text{C}$ with dry ice, and the aqueous (lipophilic) portion was poured into a new tube. This process was repeated on the tissue portion, except in this second extraction, following the 5 minutes of mixing with ether, the tubes were centrifuged for 2 minutes to facilitate optimal phase separation. Following snap-freezing, the lipophilic portion was then combined with the first tube. The ether portion containing extracted hormones was dried down under forced air, and the dry extract was reconstituted in 1 ml ELISA buffer before being assayed according to directions supplied with a commercial cortisol ELISA kit (Cayman Chemical, Ann Arbor, Michigan, USA, item #500360) using an 18-hr refrigerated incubation to increase sensitivity. This kit was previously validated by our lab for this use by ensuring that serial dilutions of extracted *B. splendens* tissue samples were parallel to a standard curve.

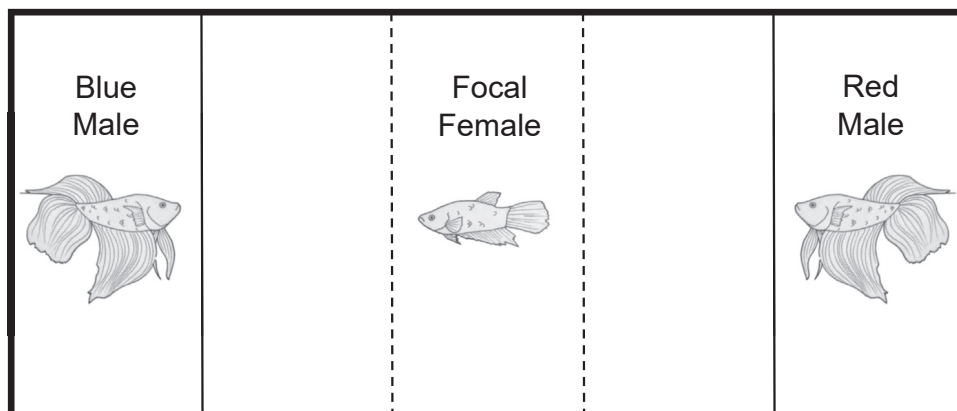


Fig. 1. Schematic of the tank set-up used for the female mate preference tests. A 75.7L aquarium (Fig. 1; $76.2 \times 30.5 \times 30.5$ cm, L \times W \times H) was divided into five equal sections ($15.2 \times 30.5 \times 30.5$ cm, L \times W \times H). The two outermost sections were divided with clear partitions and sealed with silicone. Lines drawn on the outside of the aquarium designated the inner three sections of the preference aquarium (dotted lines). The two inner sections adjacent to the male sections are the association section. *Betta* illustration by: Torey J. Bowser.

2.6. Statistical analyses

Strength of preference for red males was defined for each female as the time spent with the red male minus the amount of time the female spent with the blue male; thus positive values indicate a preference for red males, while a negative value indicates a preference for blue males [42]. We determined whether the strength of preference by a female was repeatable across trials one and two by assessing the correlation between the strengths using a Pearson's correlation. For all treatment groups combined, females associated with the same male across trials one and two ($r = 0.269$; $n = 59$; $p = 0.04$), thus we used the strength of preference calculated from the combination of trial 1 and 2 observations. To describe the baseline patterns of behavior in the females, we examined both female preference and scototaxis within the control female group. We evaluated whether females within a treatment exhibited a mate preference for red males by comparing the total time (summed across the two tests) they spent with the red male to the total time they spent with the blue male using a paired t test. We evaluated whether control females spent significantly more time in the dark section than expected at random (*i.e.*, differing from 300 seconds which is half of the total observation) by comparing the time females spent in the dark section to a null hypothesis of 50% of the observation time using a one-sample t -test. The time spent in the dark section among all treatment groups was compared using t -test comparing the arsenic treatments to the control. A general linear model was used to assess the effects of scototaxis, treatment, and the interaction between scototaxis and treatment on strength of preference for red males. Finally, a one-way ANOVA was used to determine differences in tissue cortisol concentrations between arsenic treatments. All analyses were conducted in either SPSS version 25, or JMP version 13.0.0.

3. Results

3.1. Baseline behavioral data

Control females (0 $\mu\text{g/L}$ arsenic treatment) spent significantly more time associating with red males than blue males (mean association time \pm SE: red male = $621.7 \pm 47.2\text{s}$; blue male = $364.7 \pm 43.8\text{s}$; paired t test: $t_{17} = 2.962$; $p = 0.009$; Fig. 3). Control females did not spend significantly more time in the dark sections than expected at random (mean time in the dark section \pm SE: $324.4 \pm 25.8\text{s}$; one-sample t test: $t_{17} = 0.357$; $p = 0.357$; Fig. 4).

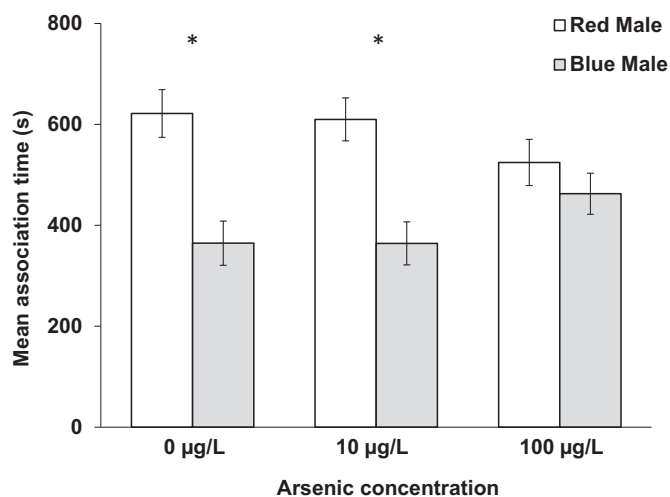


Fig. 3. Comparison of the mean association time (\pm SE) females spent associating with red or blue males exposed to either 0, 10, or 100 $\mu\text{g/L}$ arsenic for 96-h. Asterisks indicate $P < 0.05$.

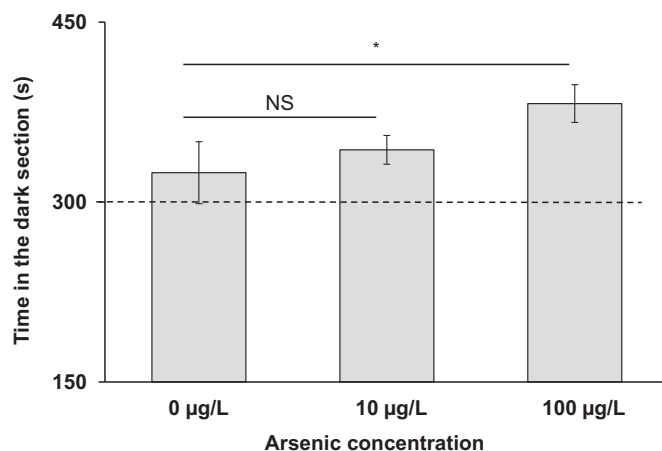


Fig. 4. Mean proportion of time (\pm SE) females spent in the dark section of the tank following exposure to either 0, 10, or 100 $\mu\text{g/L}$ arsenic for 96-h. An asterisk indicates a significant difference between the control group (0 $\mu\text{g/L}$) and the 100 $\mu\text{g/L}$ arsenic exposure treatment group; NS = non-significant. Dotted line indicates half the total assessment time of 600 s, and reflects the expectation due to random movement alone.

3.2. Effects of arsenic exposure on behavior

There was no significant difference in the total amount of time females spent with males among treatment groups (mean association time with both males \pm SE: 0 $\mu\text{g/L}$ = $986.4 \pm 27.7\text{s}$; 10 $\mu\text{g/L}$ = $974.0 \pm 22.0\text{s}$; 100 $\mu\text{g/L}$ = $987.3 \pm 21.1\text{s}$; ANOVA: $F_{2,58} = 0.102$, $p = 0.903$). Similar to control females, females exposed to 10 $\mu\text{g/L}$ arsenic showed a preference for red males and spent significantly more time in their association zone (mean association time \pm SE: red male = $609.9 \pm 42.6\text{s}$; blue male = $364.1 \pm 42.6\text{s}$; paired t test: $t_{19} = 2.987$; $p = 0.008$; Fig. 3). Conversely, females exposed to 100 $\mu\text{g/L}$ arsenic showed no preference for male color (mean association time \pm SE: red male = $524.6 \pm 45.9\text{s}$; blue male = $462.7 \pm 40.7\text{s}$; paired t test: $t_{20} = 0.736$; $p = 0.471$; Fig. 3). There was a significant effect of scototaxis on the strength of preference for red males (GLM: Scototaxis: $df = 1$, $F = 10.332$, $p = 0.002$; Fig. 5) and the relationship between scototaxis and strength of preference was similar

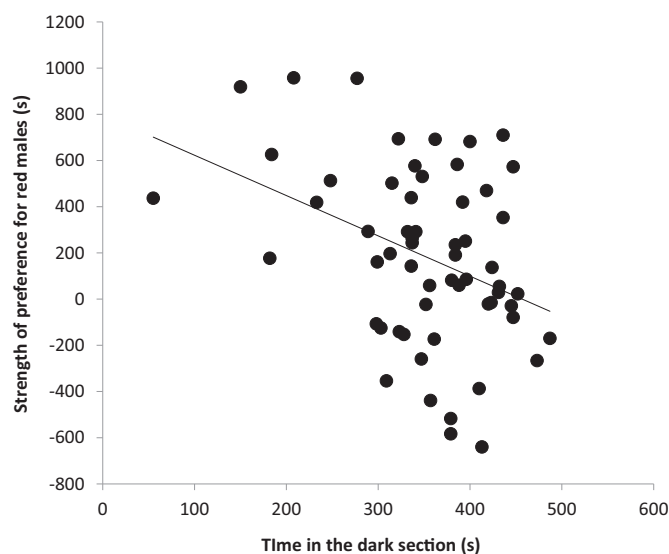


Fig. 5. The relationships between female strength of preference for red males and the time spent in the dark section. Positive strength of preference scores indicate more time females spent with the red males; negative strength of preference scores indicate more time females spent with blue males.

among treatments (GLM: Scototaxis*Treatment: $df = 2$, $F = 1.646$, $p = 0.202$; Fig. 5).

Comparisons of each arsenic dosage group (*i.e.*, 10 $\mu\text{g/L}$ and 100 $\mu\text{g/L}$) to the control showed females exposed to 100 $\mu\text{g/L}$ arsenic spent significantly more time in the dark section than control females, where the females exposed to 10 $\mu\text{g/L}$ did not differ from control females (*t*-test; 10 $\mu\text{g/L}$: $p = 0.246$; 100 $\mu\text{g/L}$: $p = 0.028$; Fig. 4).

3.3. Cortisol

Tissue cortisol concentrations were not significantly different among treatments (ANOVA $p = 0.52$), and the mean (\pm S.E.) was 1448 ± 443 , 1764 ± 418 , and 1143 ± 256 pg/g for the 0, 10 and 100 $\mu\text{g/L}$ arsenic treatments, respectively. Inter-assay variance between plates was $<15\%$ and intra-assay variance was $<7\%$.

4. Discussion

Exposure to arsenic resulted in a loss of female preference for male coloration, a novel finding in fish exposed to a metalloid contaminant. Arsenic-exposed fish also showed increased anxiety-like behavior, which may have been a key driver for the preference loss, since female preference for red males was negatively correlated with anxiety-like behavior for all fish.

Loss of female mate preference may have significant evolutionary consequences, as females across a variety of taxa have been shown to choose male traits that confer fitness benefits [43, 44, 45, 46]. The consequence of the loss in female preference for red color observed in this study is uncertain, however, there is evidence from other studies that the preference for red coloration may provide important benefits. Carotenoid pigments, such as red, have been shown to be attractive to prospective mates in a variety of taxa, possibly because these pigments must be derived from dietary sources and could serve as an honest signal of foraging ability. Carotenoids also play an important role in immune function, leading to a potential trade-off between immunity and pigmentation. Individuals that display red could be viewed as having sufficient carotenoids to satisfy both immunity and coloration, and may be more desirable than those with more drab coloration or those in which diet has less influence on pigmentation. Coloration in bettas is genetically determined, but can be enhanced by diet, and genetically red bettas without dietary carotenoid supplementation would appear drab and brownish. Studies have shown that dietary carotenoid supplementation leads to enhanced coloration in red male bettas, but not blue [35]. Unlike red males, blue betta males shunt excess carotenoids to immune function only, suggesting females may be able to gather less information about the health status of blue colored males, which could underlie female preference for red.

Studies have shown that exposure to some environmental contaminants, such as 17 β -trenbolone, causes females to spend less time associating with males, suggesting a decreased interest in mating [27]. Females in our study spent an equal amount of time associating with males, and association time is an established measure of a female's intention to mate in fish. This suggests that females in our study were still interested in mating, but showed no discretion for color.

The physiological mechanisms for the loss in female preference for red colored males following exposure to arsenic remain obscure. While arsenic has been shown to impact neurological function in a number of animal models [5] there is little evidence to support neurological toxicity at the concentrations tested in this study. Our study shows a contaminant-induced reduction in mate preference for a visual trait, and other studies have shown some heavy metals to negatively affect visual acuity [47]. Zebrafish exposed to arsenic throughout embryonic development showed significant increases in eye diameter by 14 days post fertilization in as low as 10 $\mu\text{g/L}$ arsenic [48]. However, these changes were accompanied by altered expression of key genes associated with eye development and formation, and it is unclear if these changes were

accompanied by altered vision. Arsenic has been shown to accumulate in eye tissue in a number of vertebrate models, including fish [49, 50], so more work is needed to understand the effects of arsenic on visual acuity in adults.

Interestingly, female strength of preference for red males negatively correlated with the amount of time they spent in the dark portion of the testing chamber for all females, regardless of treatment. This implies that anxiety may have been an important driver for the loss of preference.

Scototaxis, or the preference for light or dark, is a well-established metric of anxiety in fish, and has been validated for a variety of fish models [16]. This test puts the fish at odds with remaining "safe" in the dark section of the tank, versus exploring a novel environment in which the fish is more exposed. Fish exposed to 100 $\mu\text{g/L}$ arsenic spent significantly more time in the dark portion of the scototaxis arena, suggesting increases in anxiety. Though boldness (*e.g.* spending time in the light portion) may have negative consequences for the risk of predation, it has the benefit of increasing the chances of finding resources such as food or potential mates. If fish exposed to arsenic show decreased boldness, then this could have negative consequences for resource allocation and mate sampling. The consequences of anxiety and reduced boldness would depend on the ecological makeup of the environment, however, it may lead to slower growth and sub-optimal mate selection, and thereby have population-level consequences.

While anxiety-like behavior can be viewed as a form of stress, it may be difficult to uncouple from a generalized stress response. The hypothalamic-pituitary-adrenal (HPA) axis regulates many responses to environmental stressors. Activation of the HPA axis results in rapid increases in glucocorticoids such as cortisol, which in turn mediates numerous physiological responses to deal with the stressor. Therefore, it is not surprising that a variety of environmental pollutants, including arsenic, are capable of activating the HPA axis in fish and eliciting an increase in circulating stress hormones [51, 52]. Cortisol is an acute stress hormone that increases rapidly in response to stressful stimuli. Rockfish (*Sebastes schlegelii*) exposed to 100 $\mu\text{g/L}$ arsenic for 20 days showed significantly increased concentrations in plasma cortisol [53]. *Oreochromis* sp. also showed significant increases in plasma cortisol concentrations during a 12-day exposure, but by day 20, cortisol concentrations were significantly lower than control fish [54]. Dipp et al. (2018) report that zebrafish exposed to 500 $\mu\text{g/L}$ arsenic showed anxiety-like behavior without changes in whole-body cortisol, which they report as an unexpected finding given that the few studies available support arsenic-induced elevations in cortisol [55]. Our results with bettas align with that of Dipp et al. (2018) in zebrafish, showing that acute exposure to arsenic induces anxiety-like behavior without a parallel increase in tissue cortisol [55]. These equivocal findings among studies support that responses to arsenic are species and/or context dependent, and more work is needed to determine the underlying mechanisms involved.

Here we report that female bettas exposed to elevated concentrations of arsenic show a loss of preference for male coloration, and show increases in anxiety-like behavior. Preference for male coloration was negatively correlated with anxiety-like behavior, occurring without an increase in tissue cortisol, indicating anxiety may have been a key driver for the loss of preference. Fish behavior is the downstream culmination of numerous physiological inputs, and data suggest it is sensitive to environmental chemicals. Altered behavior may influence ecologically important effects, and have population-level consequences. Contaminants like arsenic do not occur in isolation, and future studies should seek to understand the effects of contaminants that commonly co-occur with arsenic in surface waters on ecologically important behaviors.

Declarations

Author contribution statement

M. Scarlett Tudor: Conceived and designed the experiments;

Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Rebecca N. Lopez-Anido, Charly A. Yocius, Sarah M. Conlin: Performed the experiments.

Heather J. Hamlin: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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