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Does experimental cortisol elevation mediate risk-taking and antipredator behaviour in a wild teleost fish?

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ABSTRACT

The hypothalamic-pituitary-interrenal (HPI) axis is centrally implicated in stressor mitigation in teleost fishes. Sustained HPI axis activation can be detrimental to the physiological functioning of an organism and can result in fitness-related trade-offs. Predator-induced mortality is known to be higher in stressed fish than in unstressed conspecifics, suggesting a role for the HPI axis in mediating fish behaviour. However, the underlying specific mechanism(s) for this phenomenon is(are) unknown. The purpose of the current study was to address how the HPI axis influences risk-taking, and antipredator behaviours in a wild teleost, the pumpkinseed sunfish (*Lepomis gibbosus*). Here, individual juvenile pumpkinseed were implanted either with cocoa butter as a sham control or with a biologically-relevant concentration of cortisol. Forty-eight hours post-implantation, fish were assessed for behavioural metrics associated with boldness and risk taking in three sequential behavioural tests: (i) a predation-risk test, (ii) an exploration tendency test, and (iii) a shoaling tendency test, with test order randomized among different trials. Cortisol treatment had no influence on antipredator, exploratory, or shoaling behaviours. However, post-attack swimming duration (in predation-risk test) and exploratory activity (in Z-maze exploration test) were significantly affected by body mass. Collectively, our results indicate that cortisol may not have a role in mediating sociability, boldness, and risk-taking behaviours in pumpkinseed sunfish, at least under the current laboratory conditions. However, cortisol may nonetheless play a role in mediating predator-prey interactions in fishes in more natural environmental settings that were not considered here.

1. Introduction

In teleost fishes, the hypothalamic-pituitary-interrenal (HPI) axis represents one of primary axes involved in the stress response. Briefly, under hypothalamic coordination, the HPI axis regulates the biosynthesis of cortisol, the primary glucocorticoid hormone in teleosts, which is upregulated in response to stressors (reviewed in Barton and Iwama, 1991; Mommsen et al., 1999; Barton, 2002). Stressor mitigation is typically considered to be an energetically expensive process (Davis and Schreck, 1997; Schreck, 2010; Schreck and Tort, 2016). As such, cortisol's functional role facilitates the prioritization of metabolic energy towards homeostatic readjustment while simultaneously increasing energy substrate mobilization via gluconeogenesis (reviewed in Mommsen et al., 1999; Schreck and Tort, 2016). In this manner, cortisol's actions enable the animal to cope with the stressor-induced physiological challenge, ensuring continued survival during stressor exposure (Romero et al., 2009).

Chronic HPI axis stimulation can be detrimental to optimal physiological performance. Under sustained cortisol elevation, such as in a chronically-stressed state, metabolic power is diverted away from non-essential activities resulting in divestment from fitness-enhancing processes (reviewed in Pankhurst, 2016, Sadoul and Vijayan, 2016; Yada and Tort, 2016). Furthermore, basal metabolic expenditures are typically elevated under chronic elevations of cortisol (De Boeck et al., 2001; O'Connor et al., 2011), which may constrain available energy budgeting (Sokolova, 2013). Sustained cortisol elevation is also associated with glucocorticoid receptor downregulation (Sathiyaa and Vijayan, 2003; Aluru and Vijayan, 2007), which could conceivably impair the animal's response to subsequent stressors resulting in a decreased ability to cope with environmental challenges (Sneddon et al., 2016). Thus, there is considerable evidence suggesting that continued HPI axis stimulation can be costly to an animal in certain contexts, especially during chronic stimulation (i.e. homeostatic overload; Romero et al., 2009).

Currently, our understanding of how a homeostatic overload, specifically cortisol, influences organismal performance/fitness and its

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effects on higher orders of biological scale (e.g. behaviour and population dynamics) in wild teleosts is relatively poor. This is especially true for how the HPI axis regulates predator-prey interactions in nature, wherein stressed teleosts tend to exhibit riskier behaviour (Järvi, 1989; Brown et al., 1985; Handeland et al., 1996; Piato et al., 2011) and suffer higher rates of predation (reviewed in Mesa et al., 1994; Raby et al., 2014), relative to unstressed conspecifics. However, no apparent mechanism(s) underlying these observations has been characterized (Schreck et al., 1997). Behavioural decision making, in the context of predator-prey interactions, in prey fish is considered state dependent and reflects a trade-off between individual risk of mortality to predation and fitness-enhancing activities such as foraging and reproduction (reviewed in Lima and Dill, 1990; Lima, 1998). Indeed, fish experiencing energetic distress, such as starvation or parasitism, are more likely to accept a greater degree of predation risk (i.e. the vulnerability to a predation event; Lima and Dill, 1990) as exemplified in reduced post-attack behavioural latencies (Giles, 1983; Godin and Sproul, 1988; Gotceitas and Godin, 1991), a greater proportion of their time foraging in open environments (Magnhagen, 1988; Godin and Smith, 1988) and reduced refuge use (Skajaa et al., 2003; Vehanen, 2003; Petrie and Ryer, 2006; Killen et al., 2011). Additionally, the ability to sustain vigilance behaviours is believed to be directly tied to a fish's available metabolic scope (Millidine et al., 2006; Killen et al., 2015), with metabolism playing a role in mediating risk-taking behavioural phenotypes (i.e. represented as a suite of consistent behavioural metrics) (reviewed in Conrad et al., 2011; Godin and Sproul, 1988; Krause et al., 1998; Killen et al., 2011). Collectively, these findings suggest that the metabolic changes associated with chronic cortisol elevations may be an important mediator of predator-prey interactions in wild fish; an effect tested only in a limited number of settings to date (Cull et al., 2015; Pleizier et al., 2015; Lawrence et al., 2017, 2018).

The objective of the current study was therefore to further our understanding of the role of the HPI axis, specifically cortisol, in mediating risk-taking and antipredator behaviours in a wild teleost fish, the pumpkinseed sunfish (*Lepomis gibbosus*, Linnaeus 1758). Previous work has shown that externally-administered cortisol in this species elicits an increased standard metabolic rate (Lawrence et al., unpubl. data). As such, we hypothesized that animals subject to sustained cortisol elevations should exhibit riskier behavioural phenotypes as a product of elevated metabolic expenditures. To test this hypothesis, wild-caught sunfish were treated with either a sham- or cortisol-containing implant and assessed 48 h post-implantation for a variety of behavioural indices of risk-taking and antipredator activities (see Chapman et al., 2010).

2. Methods

2.1. Fish collection and holding conditions

Juvenile pumpkinseed sunfish (mass = 8.4 ± 0.2 g; total length = 81.4 ± 7.5 mm) were haphazardly collected using a hand seine in the shallow nearshore reaches of Lake Opinicon, Ontario, Canada (44.5590° N, 76.3280° W) during June and July 2017 (under Ontario Ministry of Natural Resources permit #1086180). Capture sites were always of the same habitat type, which consisted of a muddy bottom with short vegetation interspersed with woody debris. This was done to avoid potential confounds with specific behavioural phenotypes being associated with habitat type (e.g. Kobler et al., 2011; Wolf and Weissing, 2012). Seine netting was the preferred capture method to avoid any potential biases in the selection of specific personality types (i.e. angling; see Wilson et al., 2011; Gutowsky et al., 2017). Collected fish were transferred to an indoor holding tank (~212 l) at the nearby Queen's University Biological Station (Chaffey's Lock, ON, Canada) and held overnight prior to receiving a cocoa butter implant. Here, fish were maintained on a flow-through circulation (23.82 ± 0.3 °C; $O_2 > 90\%$ saturation) with independent aeration under a seasonally-appropriate illumination cycle (15 h L: 9 h D). A subset of the captured fish were retained in a large, free-floating net pen (1.3 × 1.3 × 1.1 m) situated in the lake. These fish were used solely as stimulus conspecifics in the shoaling tendency test (see below). All experimental procedures received prior approval of the Carleton University Animal Care Committee (AUP's #104262 & #104281) and therefore are consistent with the guidelines for the care and use of research animals of the Canadian Council on Animal Care and the laws of Canada.

2.2. Experimental treatments

Hunger state commonly influences foraging and risk-taking decisions in teleost fishes (e.g. Godin and Smith, 1988; Gotceitas and Godin, 1991; Godin and Crossman, 1994). Therefore, fish were not fed during the holding period and experimental trials. Test fish were subjected to the implantation of either cocoa butter (5 ml kg^{-1} body weight [BW]) containing suspended cortisol (hydrocortisone 21-hemisuccinate; 25 mg kg^{-1} BW) or a sham implant (i.e. no cortisol). Cocoa butter-containing implants are a common and validated means by which cortisol can be elevated in the circulatory system of teleost fishes over semi-chronic durations (Gamperl et al., 1994; Sopinka et al., 2016). We selected the aforementioned dosage based on previous validation work with this species (Lawrence et al., unpubl. data). Here, cortisol levels in experimental fish were on average higher over the first 48 h following implantation (~67 ng ml^{-1} and 19 ng ml^{-1} for 24 h and 48 h cortisol-treated fish, respectively) than in sham-control fish (~14 ng ml^{-1} and 8 ng ml^{-1}, respectively). Preparation of the cortisol-treated cocoa butter followed the methods of Hoogenboom et al. (2011). Fish were selected haphazardly from a pool of available fish and assigned to a treatment group. The order of which fish were implanted with the cortisol or sham treatment was alternated on a daily basis to avoid possible biases in fish selection. Cocoa butter implants were injected intraperitoneally in the fish's abdomen, at a site just posterior to the pelvic fins, using a 1 ml syringe tipped with a 16 G needle. Following implantation, individual fish were immediately transferred to a blacked-out holding chamber (~2.6 l) that was maintained on a flow-through of fresh, aerated lake water (McConnachie et al., 2012). Animals were held in these individual blacked-out (darkened) chambers for 48 h prior to behavioural testing to allow the administered cortisol to reach biologically active concentrations in their blood (McConnachie et al., 2012). Sham and cortisol-treated fish had comparable mean body masses (sham = 8.8 ± 0.3 g, cortisol = 8.1 ± 0.3; *t* = 1.608, DF = 50, *P* = 0.114) and total lengths (sham = 82.2 ± 9.9 mm, cortisol = 86.7 ± 11.0 mm; *t* = 1.029, DF = 50, *P* = 0.308). Water conditions in the experimental arenas were maintained at > 90% O_2 saturation and 23.59 ± 0.1 °C.

2.3. Behavioural trials

Individual implanted pumpkinseed (*n* = 28 sham-treated fish, *n* = 29 cortisol-treated fish) were subjected to three sequential behavioural tests: (i) a predation-fright test, (ii) an exploration tendency test, and (iii) a shoaling tendency test, with 2–3.7 h elapsed between consecutive tests. Testing occurred on fish that had been held for ~72 h post-capture. The order of the tests was randomized for each individual fish using a random number generator to minimize any potential effects of trial time and handling stress on their behaviour in the three tests. A maximum of 10 fish (5 from each treatment group) were run through the experimental series on any given day. Following a behavioural trial, the test fish was removed from the experimental arena and returned to its original holding chamber, where it was allowed to recover for at least 2 h before being used in the next test in sequence. Systematic randomization was used to determine the order of the treatment groups (i.e. cortisol, sham, cortisol, sham, etc.) and was alternated on a daily basis. Fish behaviour in each of the tests was recorded using an overhead Go Pro camera (Go Pro Hero 3; Struthers et al., 2015) and water temperature was recorded at the end of each trial. Behavioural data

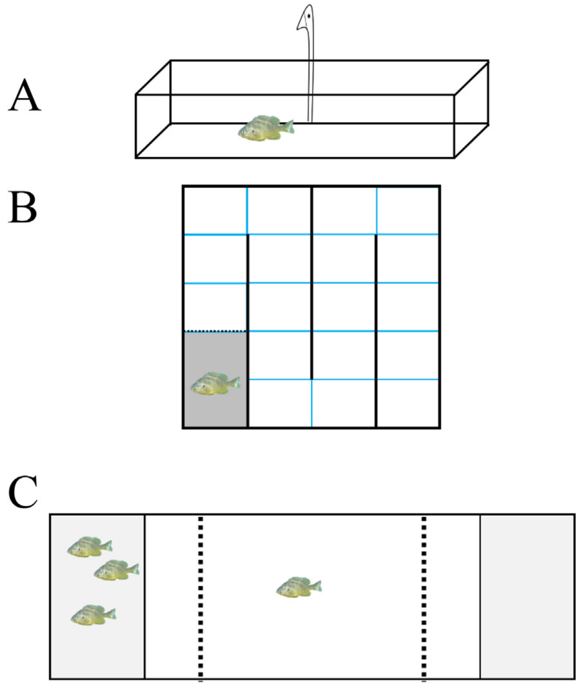

Fig. 1. Schematic representation of the behavioural trials used in this experiment including the predation fright trial (A), the Z-maze (B), and the shoaling trial (C). In the *predation fright trial*, a model of a great blue heron (*Ardea herodias*) was centred over the side of the raceway tank. On the attack, the heron's beak penetrated 10 cm below the water surface. In the *Z-maze trial*, the grid pattern consists of 18, 10 cm × 10 cm squares (blue lines) arranged in a Z pattern. The grey rectangular box represents the refuge area where the fish was acclimated in. This was gated (dash line) until the experiment commenced. In the *shoaling trial*, shaded areas represent the two choice compartments, containing 3 conspecifics or nothing (control), which were separated by means of perforated Plexiglas. Dashed lines represent the 20 cm association zone with each choice compartment. The empty region in the centre of the tank, in between the association zones, represents the "no-man's land" region of the experiment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Photo credit: Michael Lawrence, 2017.

were subsequently extracted from the video films. Fish wet body mass was recorded to the nearest half gram using a Valor 2000W balance (Ohaus, Parsippany, NJ, USA) following the end of the experimental series.

2.4. *Predation-fright test*

The experimental arena (Fig. 1A) consisted of a fibreglass raceway style tank (156 cm × 27.7 cm, L × W), with a water depth of 24.8 cm and devoid of any sort of cover or substrate. A realistically painted model of the head and neck of a great blue heron (*Ardea herodias*, Linnaeus 1758), designed and constructed by Godin and Sproul (1988), was placed near the tank's rim in the horizontal centre of the tank. In the wild, great blue herons are natural predators of sunfish and are perceived as a significant predation threat to them (Forbes, 1987; Coleman and Wilson, 1996). As described in Godin and Sproul (1988), the heron model was hinged on a frame external to the experimental tank, allowing it to fall forward (when triggered) and its bill to penetrate the water's surface (to ~10 cm depth) thereby simulating an overhead strike event by the bird. Post-strike, the model was immediately returned to its previous upright position by an overhead spring and braided fishing line suspension system anchored to the external frame. The model was present above the water surface near the rim of the experimental arena, presumably within the test fish's visual field, throughout the experimental test including during the acclimatization phase.

For each experimental trial, a focal test fish was transferred from its holding cell to the centre of the aforementioned test arena and allowed to swim freely. Care was taken to minimize handling and air exposure times during the transfer. The fish was then left undisturbed for a 5-min acclimatization period. Following this period, an avian predator attack was simulated by gently tipping the heron model forward to strike the water surface near the fish. The test fish's behavioural response to the simulated attack and thereafter was recorded over 5 min. Behavioural variables were the type of immediate antipredator response (i.e. immobility/freezing vs. escape/flight) and the time spent swimming or total time spent immobile following the attack. Time spent swimming constituted the time from when the fish started swimming following the attack until it ceased activity for > 5 s. At the end of the trial, the test fish was returned to its holding cell until the onset of the next test, as described above.

2.5. *Exploration tendency test*

To assess the potential influence of cortisol on exploratory activity, individual focal fish were introduced into a novel environment that constituted a Z maze (Fig. 1B), following the methodology of Chapman et al. (2010). The maze comprised an arena (40 cm × 50 cm) that contained a shaded and gated refuge (10 cm × 20.3 cm) in one corner and three staggered opaque partitions arranged so as to form a Z-pattern. Black plastic marker lines on the bottom of the arena delineated eighteen equal squares (10 × 10 cm), used to record fish location and activity. Water depth was 6.6 cm. Prior to the onset of a trial, a test fish was introduced into the refuge (with gate down) and allowed to acclimatize undisturbed for 5 min. Following this period, the refuge gate was remotely raised using a pulley system and the fish allowed to explore the maze for 10 min. The experiment was filmed from above using a Go Pro Hero 3 camera. We recorded (i) latency time to exit the refuge, (ii) the number of lines crossed (='exploration' of the novel environment), (iii) total time spent inside the refuge and (iv) the square in the maze (out of 18) furthest from the refuge entered by the fish. At the end of the trial, the test fish was returned to its holding cell until the onset of the next test, as described above, and the maze was completely drained and re-filled with fresh lake water in preparation for the next test fish.

2.6. *Shoaling tendency*

To assess the potential influence of cortisol on sociability, we quantified the tendency of individual test fish to socially associate (i.e. 'shoal') with a stimulus group of conspecifics in choice apparatus (Fig. 1C). Shoaling is a common response to perceived predation threats, and as such reduces individual risk of mortality to predation, in teleost fishes (reviewed in Godin, 1986). Following Chapman et al. (2010), we used a raceway style tank arranged into three compartments separated by clear Plexiglas partitions that were perforated with small holes to permit water flow between the compartments (Fig. 1C). Two smaller compartments (20.0 × 27.7 cm; either of which would contain a conspecific stimulus shoal) flanked a large central experimental arena (112 × 27.7 cm), wherein the test fish could freely swim. Associated with each end compartment was a 20-cm wide social association zone used to assess the test fish's preference for either end compartment. Water depth was maintained at 26.8 cm. Consistent with Chapman et al. (2010), we used a stimulus shoal of three pumpkinseed sunfish of similar body size to the test fish. The stimulus fish were not implanted with cocoa butter and were taken from the floating net pen in the lake, previously described above. As such, they were presumably socially unfamiliar with the test fish. Individual stimulus fish were only used once per day but were randomly reused on subsequent test days throughout the experiment. The lake net-pen shoal contained approximately 40 individuals at any given time, with any mortalities being compensated for with the addition of new fish.

Prior to the onset of a behavioural trial, the stimulus shoal was placed in one of the two end compartments, determined pseudo-randomly (with a coin toss). The other end compartment remained empty. The test fish was then introduced into the central arena and allowed to swim freely. Both the test fish and the stimulus shoal were left undisturbed to acclimatize to the experimental tank for 5 min. Following this period, we observed the behaviour of the test fish using a Go Pro camera mounted above the experimental arena and we recorded the cumulative time that it spent near either of the end compartments over a 5-min trial. We quantified the test fish's shoaling tendency as a difference score (S_{DS}), calculated as the amount of time spent in the association zone near the stimulus shoal (t_c) minus the time it spent in the association zone near the empty chamber (t_e), such that $S_{DS} = (t_c - t_e)$.

2.7. Behavioural metrics and data analyses

All statistical analyses were conducted in R Studio (Version 1.1.423; RStudio Team, 2015). Statistical significance was accepted at $\alpha = 0.05$ and, unless otherwise noted, data are presented as means ± SE. Our statistical models included the main effect of treatment (i.e. cortisol vs. sham) and three covariables (mass of test fish, trial time of day, test order [the number of trials prior to the current assessment]). For shoaling tendency only, the statistical model included the location (left or right) of the particular end compartment containing the stimulus shoal as an additional covariable. All models were subjected to model simplification using AICc methodology (Hurvich and Tsai, 1989; Burnham and Anderson, 2002). Data on the type of antipredator response (Predation-fright test) were fitted to a generalized linear model (GLM) with a binomial distribution specified. Data on time spent swimming were fitted to a GLM with a Gaussian distribution. Data on time spent swimming were normalized using a logarithmic transformation. Latency time to emerge from refuge, total refuging time, and furthest square reached (Exploration tendency test) were converted to proportional data (out of total trial duration/maximum count) and analyzed using a beta regression model (package: 'betareg', V3.1-0; Cribari-Neto and Zeileis, 2009, 2010). Data on the number of lines crossed were fitted to a GLM with a Poisson distribution. The relationship between lines crossed and emergence latency time was characterized using a linear regression. Shoaling tendency data was transformed to a proportion of the amount of time that the animal spent with the shoal out of the total time spent in both association end zones and was assessed using a beta regression model, as described above.

3. Results

3.1. Antipredator behaviour

Pumpkinseed sunfish responded to a simulated heron attack by either immediately fleeing (i.e. rapidly swimming away from the threat) or becoming immobile ('freezing'). Most (85.4%) fish exhibited a fleeing (escape) response to the perceived predation threat. However, neither the cortisol treatment ($t = -0.189$, $P = 0.850$), fish body mass ($t = -1.498$, $P = 0.134$), trial order ($t = 0.336$, $P = 0.737$) nor time of day ($t = -0.438$, $P = 0.661$) influenced the particular antipredator response tactic adopted by the test fish (Table 1). Post-attack swimming duration was also not affected by cortisol treatment ($t = -1.122$, $P = 0.270$, Fig. 2A, Table 1), trial order or time of day, but interestingly was influenced negatively by individual body mass (Table 1). The number of animals that froze in response ($n = 7$) was too small to conduct statistical analyses on, with respect to treatment effects, and has thus been omitted from the results.

3.2. Exploratory behaviour

In the exploration tendency test, neither latency to emerge from the refuge ($z = -0.543$, $P = 0.587$; Fig. 3A), exploratory activity, measured as number of lines crossed ($z = 0.426$, $P = 0.670$, Fig. 3B), total time spent in the refuge ($z = -0.353$, $P = 0.724$, Fig. 3C) nor the furthest square reached ($z = 0.441$, $P = 0.659$, Fig. 3D) was affected by the cortisol treatment (Table 1). None of the covariates influenced refuge emergence time, total time spent in the refuge and furthest square reached (Table 1), but all covariates significantly affected exploratory activity (Table 1), with fish body mass negatively influencing exploratory activity ($z = -5.176$, $P < 0.001$). A strong negative relationship was observed between refuge emergence time and exploratory activity, with individual fish that exited the refuge sooner exhibiting greater exploratory activity in the maze ($r^2 = 0.923$, $F = 550.773$, $P < 0.001$; Fig. 4).

Table 1

Summary statistics for all behavioural variables measured in the behavioural trials here relating the main effect of cortisol-treatment alongside a number of covariate parameters (body mass, trial order, time of day, side). Bolded values indicated statistically significant results ($\alpha = 0.05$). Test parameters are specific to the statistical model used (see Methods 2.7), The constant represents the Y intercept of the model.

Trial	Behavioural metric		Test statistic	P value
Predation fright	*Fright response*		*t-Value*	
		Constant	0.877	0.380
		Treatment group	−0.189	0.850
		Body mass	−1.498	0.134
		Trial order	0.336	0.737
		Time of day	−0.438	0.661
	Post-attack swimming duration		*t-Value*	
		Constant	3.320	**0.002**
		Treatment group	−1.122	0.270
		Body mass	−2.235	**0.032**
		Trial order	−1.548	0.131
		Time of day	1.060	0.296
Z-maze trial Trial	*Emergence time*		*z-Value*	
		Constant	1.323	0.186
		Treatment group	−0.543	0.587
		Body mass	0.306	0.760
		Trial order	1.175	0.240
		Time of day	−0.376	0.707
	Exploratory activity		*z-Value*	
		Constant	0.097	0.923
		Treatment group	0.426	0.670
		Body mass	−5.176	< **0.001**
		Trial order	14.611	< **0.001**
		Time of day	11.187	< **0.001**
	Total refuge time		*z-Value*	
		Constant	1.870	0.062
		Treatment group	−0.353	0.724
		Body mass	0.347	0.728
		Trial order	1.145	0.252
		Time of day	−0.472	0.637
	Furthest square		*z-Value*	
		Constant	−0.902	0.367
		Treatment group	0.441	0.659
		Body mass	−0.113	0.910
		Trial order	−1.385	0.166
		Time of day	0.753	0.452
Shoaling tendency trial	*Difference score*		*z-Value*	
		Constant	−1.196	0.233
		Treatment group	−0.455	0.649
		Body mass	0.927	0.354
		Trial order	0.498	0.619
		Time of day	0.778	0.437
		Side	−0.125	0.901

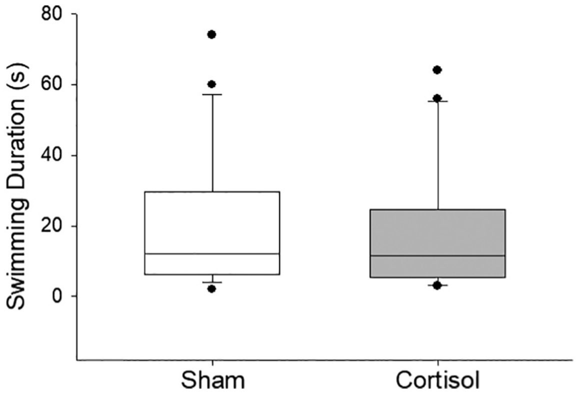

Fig. 2. Swim duration of pumpkinseed following a mock predator attack for 48-h post-implant sham- (white bars; 5 ml kg^{-1} body weight; n = 20) and cortisol-treated (grey bars; 25 mg kg^{-1} body weight; n = 21) fish. No significant effects of cortisol, body mass, trial order or time of day were found. Data are presented as a box plot containing the median value delineated by the interquartile range (1st to 3rd quantile) and an accompanying whisker that represents 1.5 × beyond this range. Suspected statistical outliers are presented as black circles outside of the interquartile range. Statistical significance was accepted at α = 0.05.

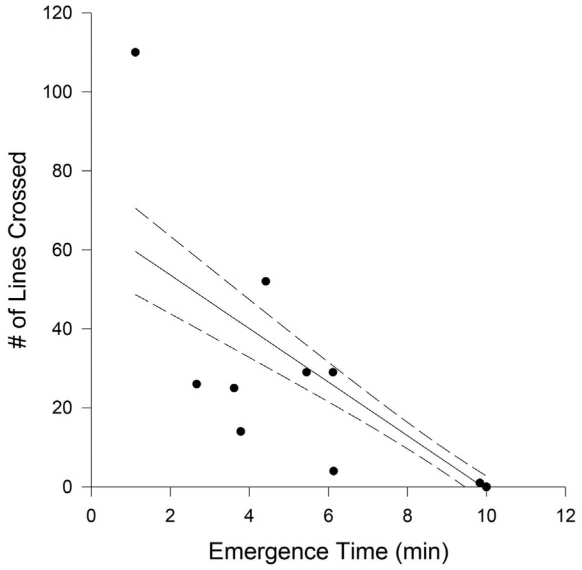

Fig. 4. Relationship between the emergence time from a refuge environment and the activity, presented as the number of lines crossed, for individual pumpkinseed in the Z maze trial. A significant relationship was detected between the two variables (F = 550.773; $P < 0.001$; r^2 = 0.923; n = 47).

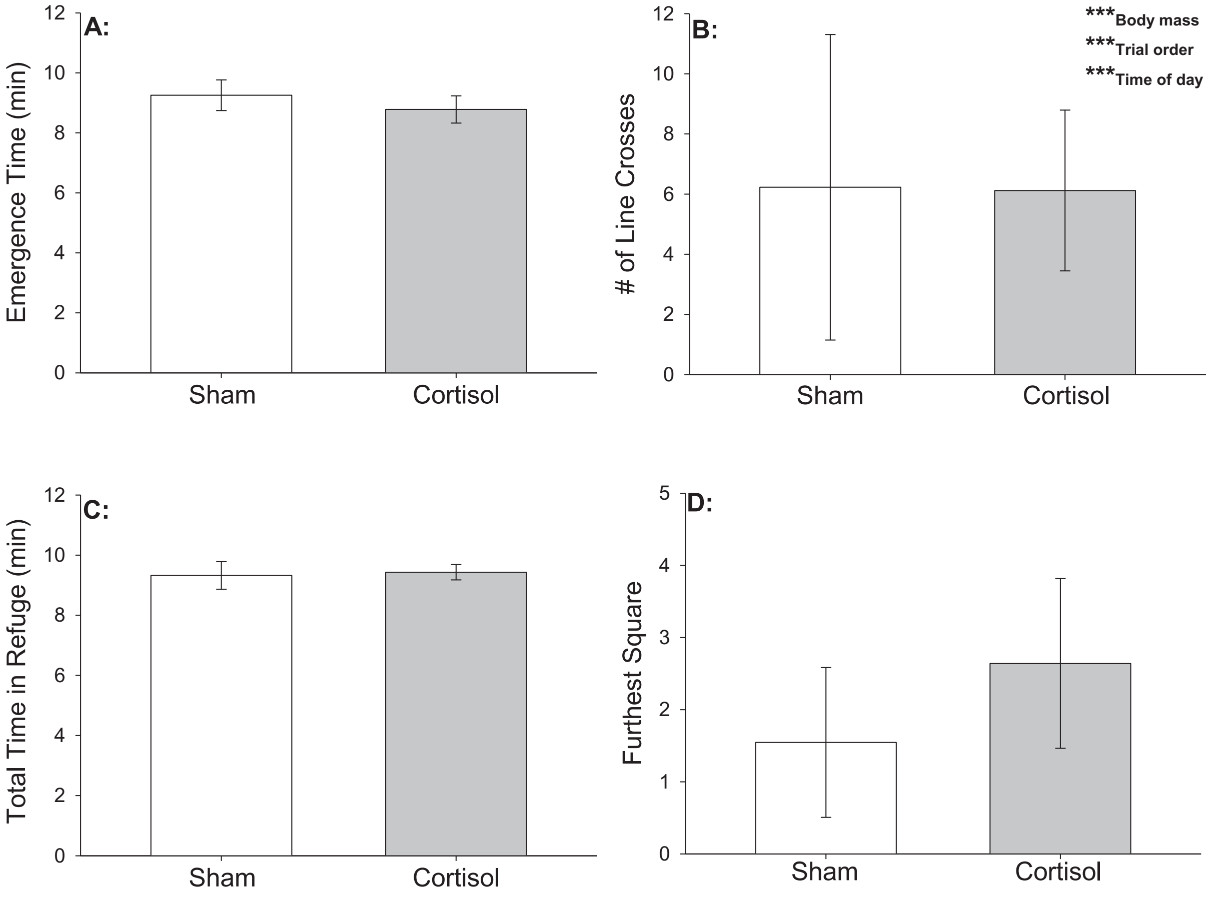

Fig. 3. Metrics for sham- (white bars; 5 ml kg^{-1} body weight; n = 22) and cortisol-treated (grey bars; 25 mg kg^{-1} body weight; n = 25) pumpkinseed in the Z maze trial displaying refuge emergence time (A), the number of lines crossed in the maze (B), the total amount of time spent in the refuge area (C), and the furthest square reached (D). For the number of lines crossed, a statistically significant effect of body mass (z = −5.176; $P < 0.001$), trial order (z = 14.611; $P < 0.001$) and time of day (z = 11.187; $P < 0.001$) was noted. Values are shown as mean ± 1 SE. Statistical significance was accepted at α = 0.05 with differences between treatment groups represented by an asterisk (***$P < 0.001$).

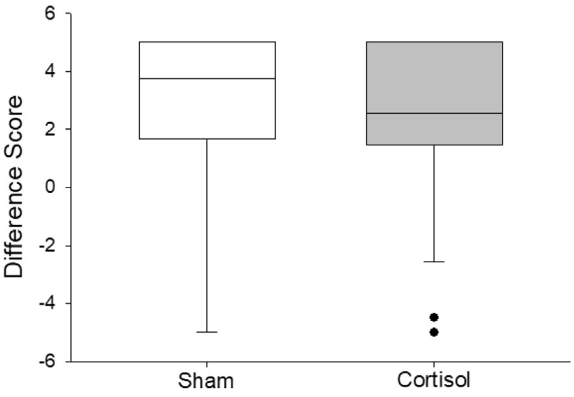

Fig. 5. Box plot depicting a difference score for the strength of shoaling association for sham- (white bars; $N = 22$) and cortisol-treated (grey bars; $N = 23$) fish in the shoaling tendency trial. Positive values indicate the animal is spending its time associating with the conspecific choice-compartment, a negative value represents the focal fish associating with the empty choice-compartment and a value of zero represents no preference with either compartment. No significant effects of cortisol, body mass, trial order or time of day were found. Data are presented as a box plot containing the median value delineated by the interquartile range (1st to 3rd quantile) and an accompanying whisker that represents 1.5× beyond this range. Suspected statistical outliers are presented as black circles outside of the interquartile range. Statistical significance was accepted at $\alpha = 0.05$.

3.3. Shoaling behaviour

Pumpkinseed sunfish exhibited a strong preference to socially associate with a conspecific stimulus shoal over an empty end stimulus compartment, which resulted in positive difference scores for both cortisol- and sham-treatment groups (Fig. 5). However, fish in the two treatment groups did not differ in their shoaling tendency ($z = -0.455$, $P = 0.649$, Fig. 5, Table 1). Similarly, individual body mass, trial order, time of day, and the position (left or right) of the stimulus shoal did not significantly influence the shoaling tendency of test fish (Table 1).

4. Discussion

4.1. Behavioural responses to a predation threat

In the simulated predation threat test, most test fish chose to engage in a rapid burst fleeing response rather than remain immobile post-attack, which may have reflected a lower cost of fleeing and a relatively high cost of remaining in terms of perceived risk of predation from a sit-and-wait predator such as a great-blue heron (Ydenberg and Dill, 1986; Godin, 1997). This response is a common strategy among teleosts in general (e.g. Faber et al., 1989; Domenici and Blake, 1991; Marras et al., 2011; reviewed in Godin, 1997; Domenici, 2010) and in centrarchid fishes in particular (Moody et al., 1983; Webb, 1986; Tytell and Lauder, 2008; Chadwell et al., 2012). In contrast to our predictions, cortisol had no influence on the predator avoidance tactic used or on post-attack swimming duration of pumpkinseed. This may reflect a lack of fitness incentive for fish to remain in the area (i.e. foraging opportunities; Godin and Sproul, 1988; Ydenberg and Dill, 1986; Godin, 1997). While data on cortisol-predation responses is lacking, cortisol treatment in checkered pufferfish (*Sphoeroides testudineus*) resulted in post-startle durations comparable to sham controls (Pleizier et al., 2015), which supports our current findings. As it stands, our data suggest that cortisol has little role in mediating antipredator behaviours in juvenile sunfish in this laboratory context.

4.2. Boldness and exploration activity

Contrary to our a priori prediction, cortisol did not influence refuge emergence time, exploratory activity, or the total time spent refuging. These negative results likely stem from the context-dependent nature of metabolism-behaviour interactions (Dowling and Godin, 2002; Killen et al., 2011, 2012, 2013; Metcalfe et al., 2016). Specifically, metabolism-boldness relationships are usually only apparent under additional stressors such as food deprivation (Killen et al., 2011) and hypoxia (Killen et al., 2012). In other contexts, no such metabolism-boldness relationship exists (Farwell and McLaughlin, 2009; Polverino et al., 2016) or the behavioural outcome is highly variable (Biro et al., 2010). As such, perhaps cortisol alone is not sufficient to alter pumpkinseed behavioural phenotypes in this context (reviewed in Schreck et al., 1997, Sopinka et al., 2015, Crossin et al., 2016). Interestingly, we observed a significant relationship between refuge emergence time and lines crosses in the maze, suggesting that individual traits may be important in mediating behavioural phenotypes (Wilson and Godin, 2009a, 2009b; Wilson et al., 2011).

4.3. Shoaling behaviour

We found that juvenile pumpkinseed sunfish associated strongly with conspecifics (i.e. shoaled) under laboratory experimental conditions, which they also do in their natural habitats (Miller, 1963; Brown and Colgan, 1982; Golub et al., 2005) as part of an anti-predator defense strategy (Godin, 1986; Pitcher and Parrish, 1993; McCartt et al., 1997; Marcus and Brown, 2003). However, we observed no effect of cortisol treatment on shoaling tendency in pumpkinseed sunfish in the current study, despite previous works showing HPI axis involvement in mediating shoal cohesion (Piato et al., 2011; Pavlidis et al., 2015). The chronic period of HPI axis stimulation in the latter studies (i.e. 12–14 days) was much longer than in our current study (48 h). This suggests that our fish likely had sufficient capacity to maintain ‘normal’ shoaling behaviour in the face of relatively short-term physiological dysregulation (Romero et al., 2009; Piato et al., 2011; Sopinka et al., 2015; Lawrence et al., 2017).

4.4. Body mass and pumpkinseed behaviour

Body mass influenced post-attack swimming duration in juvenile pumpkinseed sunfish in the current study. In wild bluegill sunfish (*Lepomis macrochirus*), predator-induced mortality is inversely correlated with body size as a result of limitations in predator gape (Werner and Hall, 1988; Santucci and Wahl, 2003; Hill et al., 2004). Smaller fish in the current study were likely more vulnerable to gape-limited predators, and thus exhibited behavioural traits that were risk adverse, compared with larger conspecifics (Sogard, 1997; Metcalfe et al., 1998; Dowling and Godin, 2002; Brown and Braithwaite, 2004; Ioannou et al., 2008; Polverino et al., 2016).

5. Conclusion

Here, we investigated the role of the HPI axis, specifically cortisol, in mediating predator-prey interactions in wild-caught juvenile pumpkinseed sunfish, as previous works indicate a role for an individual's stress state in determining its predator susceptibility (reviewed in Mesa et al., 1994; Raby et al., 2014). Contrary to our predictions, cortisol treatment did not alter risk taking or boldness in this species, suggesting that cortisol has no influence over predator-prey dynamics in agreement with previous research (Cull et al., 2015; Pleizier et al., 2015; Lawrence et al., 2017, 2018). However, we cannot completely discount a role for cortisol in mediating predator-prey interactions, as this hormone can increase resting/routine metabolic rate (De Boeck et al., 2001; O'Connor et al., 2011) and, through this effect, can sustain antipredator and foraging activities (Millidine et al., 2006; Killen et al., 2007, 2015), both important behaviours governing predator-prey interactions (reviewed in Lima and Dill, 1990; Milinski, 1993; Godin, 1997). Moreover, because of the context-dependent nature of cortisol-behavioural interactions (Crossin et al., 2016; Sopinka et al., 2015), it is possible that cortisol treatment may nonetheless mediate behavioural costs or trade-offs with respect to predator avoidance in other contexts beyond the scope of our current study. As well, we are limited in some of our interpretations because we did not include a pure control group (i.e. no implant treatment) in our experimental design. Consequently, we do not know whether and to what degree the stress related to implantation per se (for both sham- and cortisol-treated fish) may have masked the effects of cortisol on behaviour. Future research should address cortisol-behavioural dynamics in more ecologically-relevant settings that includes access to fitness gaining opportunities (e.g. foraging patches) to fully appreciate the potential role of cortisol in mediating predator-prey interactions.

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Author contributions

All authors contributed to the design of the experiment. The experimental trials were conducted by M.J.L. Data analyses were performed by M.J.L. with help from J.-G.J.G. The manuscript was written by M.J.L., with all authors contributing to revisions.

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