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Glucocorticoids do not promote prosociality in a wild group-living fish

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ABSTRACT

Individuals often respond to social disturbances by increasing prosociality, which can strengthen social bonds, buffer against stress, and promote overall group cohesion. Given their importance in mediating stress responses, glucocorticoids have received considerable attention as potential proximate regulators of prosocial behaviour during disturbances. However, previous investigations have largely focused on mammals and our understanding of the potential prosocial effects of glucocorticoids across vertebrates more broadly is still lacking. Here, we assessed whether experimentally elevated glucocorticoid levels (simulating endogenous cortisol responses mounted following disturbances) promote prosocial behaviours in wild groups of the cichlid fish, *Neolamprologus pulcher*. Using SCUBA in Lake Tanganyika, we observed how subordinate group members adjusted affiliation, helping, and submission (all forms of prosocial behaviour) following underwater injections of either cortisol or saline. Cortisol treatment reduced affiliative behaviours—but only in females—suggesting that glucocorticoids may reduce overall prosociality. Fish with elevated glucocorticoid levels did not increase performance of submission or helping behaviours. Taken together, our results do not support a role for glucocorticoids in promoting prosocial behaviour in this species and emphasize the complexity of the proximate mechanisms that underlie prosociality.

1. Introduction

Maintaining stability in social groups is a key way to increase the benefits associated with group-living, such as workload sharing (Clutton-Brock et al., 2002; Ulrich et al., 2018), improved foraging (Evans et al., 2016; Ward and Zahavi, 1973), and increased vigilance (Evans et al., 2016; Roberts, 1996). While group stability is often maintained via the formation of social hierarchies, instability within groups can arise from changes in group composition or as a result of fluctuations in resource availability (Creel et al., 2013; Culbert et al., 2018). Following such disturbances, group members will often increase their performance of prosocial behaviours that promote stability within the group. Specifically, the performance of affiliative behaviours—a type of prosocial behaviour that reinforces social bonds—often increases following a disturbance (Anderson et al., 2020; Buchanan and Preston, 2014; Fraser et al., 2008; Radford, 2008). Such responses are thought to play an important role in the persistence and stability of social groups, yet the proximate mechanisms regulating these responses are not well understood. Because glucocorticoids help to coordinate behavioural and physiological responses during periods of stress (Creel et al., 2013; Raulo and Dantzer, 2018; Tort and Teles, 2011), these hormones have been hypothesized to be involved in the regulation of prosocial behaviours during periods of instability.

Glucocorticoid synthesis increases when individuals encounter physical or psychological challenges in their surrounding environment (i.e. a stressor) and elevated glucocorticoid levels help individuals overcome challenges primarily through their effects on metabolism, osmoregulation, and immune function (Mommsen et al., 1999; Sapolsky et al., 2000; Tort and Teles, 2011). Production of glucocorticoids is regulated by the hypothalamic-pituitary-adrenal (HPA) axis in mammals and birds, or by the hypothalamic-pituitary-interrenal (HPI) axis in other vertebrates (Dallman et al., 1994; José et al., 2011; Wendelaar

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Bonga, 1997). Following the perception of a stressor, hypothalamic neurons release corticotropin-releasing factor causing corticotropes located in the anterior pituitary to release adrenocorticotropic hormone into circulation (Aguilera, 1998; Bernier et al., 2009). Adrenocorticotropic hormone then binds melanocortin receptor 2 located on the adrenals or interrenal tissue and stimulates glucocorticoid synthesis from precursory cholesterol (Fridmanis et al., 2017; Payne and Hales, 2004; Tokarz et al., 2015). Glucocorticoid levels can be affected by an individual's social environment (Creel et al., 2013; Gilmour et al., 2005), and the presence of an individual's social partners can even reduce glucocorticoid production following a stressor (Culbert et al., 2019; Smith and Wang, 2014). This 'social buffering' of glucocorticoid synthesis is often associated with increased prosocial behaviours (Kiyokawa and Hennessy, 2018); however, whether glucocorticoids themselves regulate the performance of prosocial behaviours is less clear (Raulo and Dantzer, 2018). While there is evidence that glucocorticoids can promote prosociality in mammals (Dantzer et al., 2017; Santema et al., 2013; Voellmy et al., 2014) and in birds (Thierry et al., 2014), there have been few investigations of the potential prosocial role of glucocorticoids in other vertebrate groups. Thus, to determine whether the prosocial effects of glucocorticoids are broadly evolutionarily conserved across vertebrates, studies in other animals—such as fishes—are critical.

To test the hypothesis that glucocorticoids promote prosocial behaviours in fishes, we assessed how the behaviour of subordinate group members of the cooperatively breeding cichlid—*Neolamprologus pulcher*—changed following treatment with either saline or cortisol (to simulate endogenous cortisol responses mounted following disturbances). These fish live in social groups containing a dominant breeding pair and up to 20 subordinates that help the dominant pair by performing brood care and territory defense (Taborsky and Limberger, 1981; Wong and Balshine, 2011). Previous studies conducted on laboratory populations of *N. pulcher* suggest that glucocorticoids are involved with the development of social competency (Nyman et al., 2018; Reyes-Contreras et al., 2019; Taborsky et al., 2013), and circulating glucocorticoid levels often vary between dominants and subordinates (Culbert et al., 2018; Mileva et al., 2009). However, no study has assessed the potential role of glucocorticoids in regulating social behaviour of *N. pulcher* under ecologically relevant conditions encountered in the field. Therefore, we performed our experiments using wild *N. pulcher* located on the southern tip of Lake Tanganyika in Africa. These fish perform a suite of prosocial behaviours, including affiliation, helping, and submission; however, whether cortisol directly regulates these behaviours has not been examined. Helping and submissive behaviours have been proposed as a means of appeasing dominants, decreasing aggression, and maintaining group stability (Bergmüller et al., 2005b; Bergmüller and Taborsky, 2005; Reddon et al., 2019). Additionally, *N. pulcher* regularly perform affiliative behaviours towards their groupmates which reinforce social relationships and promote cooperation (Anderson et al., 2020; Bruintjes et al., 2016). As such, we predicted that individuals that were treated with cortisol would perform more affiliative, helping, and submissive acts compared to individuals treated with saline.

2. Methods

2.1. Field site and animals

All experiments were conducted off the shore of Mutondwe Island, Lake Tanganyika, Zambia (8°42′45″ S, 31°7′27″ E) in December 2019. Using SCUBA, 29 social groups were located between 6 and 8 m depth and used for this experiment. *N. pulcher* are highly territorial and groups aggressively defend a communal territory containing several rocks which provide shelter for the group (Balshine et al., 2001; Heg et al., 2004). Therefore, groups are readily identifiable by observing which individuals co-inhabit/defend the same territory. Groups contained an average (mean ± SEM) of 7.3 ± 0.1 individuals. All protocols were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol No. 18-04-16) and the Zambian Department of Fisheries and followed the guidelines of the Canadian Council on Animal Care.

2.2. Behavioural analyses

The behaviour of a focal subordinate group member in each social group was observed over two 10 min periods prior to experimentation. We targeted the largest subordinate in each group as our focal fish, except in cases where the largest subordinate spent little time on the territory and may have been in the process of dispersing to join another group (Bergmüller et al., 2005a; Hellmann et al., 2016; Stiver et al., 2007; Zöttl et al., 2013). We focused on subordinates because they routinely perform the entire suite of prosocial behaviours observed in this species. The first observation occurred on the day before treatment (1100–1300 h) and the second observation took place immediately prior to treatment (0900–1100 h). Following a 2 min acclimation period, all affiliative (follows, parallel swims, and soft touches), aggressive (chases, bites, rams, opercular flares, aggressive postures, and lateral displays), helping (moving substrate, brood chamber visits, and aggression towards intruding con- and heterospecifics), and submissive (submissive postures, tail quivers, j-hooks, and flees) behaviours were scored (see Sopinka et al., 2009 for further behavioural details). Following the second behavioural observation period, focal fish were captured using fence and hand nets, measured to the nearest 0.1 cm (average standard length = 4.9 ± 0.1 cm), and sexed underwater via visual examination of the genital papilla. Focal fish each received a unique dorsal fin clip for identification, which does not adversely affect behaviour (Stiver et al., 2004). Fish were then injected underwater (see Section 2.3) with either saline ($N = 15$; 5 females, 10 males) or cortisol ($N = 14$; 6 females, 8 males), and released back onto their territories (an average of 9 ± 1 min elapsed between a diver's first approach to release of the injected fish). Injection solutions (saline vs. cortisol) were haphazardly assigned to fish prior to the first observation period. All social groups resumed normal behaviour within 2–3 min of release (including injected focal fish) and no obvious signs of distress were noted. We returned to observe the behaviour of previously injected focal fish for another 10 min approximately 2 h post-injection (126 ± 3 min post-injection). This timepoint corresponded to when cortisol levels in saline injected fish had returned to baseline and remained elevated in cortisol injected fish (Fig. 1).

2.3. Glucocorticoid manipulation

A pilot study was conducted in October 2019 at McMaster University

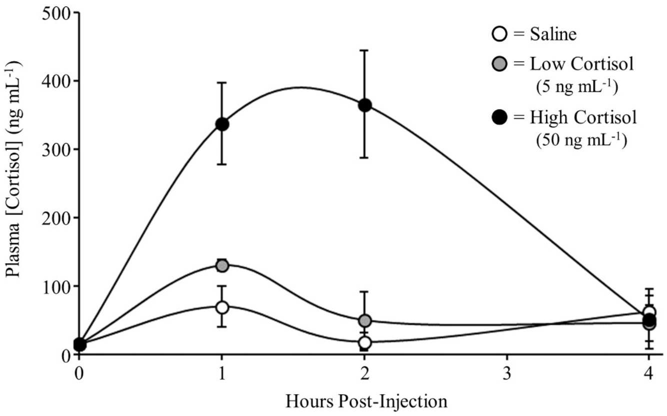

Fig. 1. Time course (0, 1, 2, or 4 hour post-injection) of plasma cortisol levels following an intraperitoneal injection with either saline, low cortisol (5 ng mL^{-1}), or high cortisol (50 ng mL^{-1}). Values are reported as means ± SEM.

in Hamilton, Ontario, Canada to determine the cortisol dosage to be administered in the field. All fish were laboratory-reared descendants of wild-caught *N. pulcher* from Lake Tanganyika. Fish ($N = 21$; average mass of 7.27 ± 0.3 g) were moved from a 527 L stock tank containing ~50 mixed-sex individuals and placed individually into 38 L aquaria that contained a heater (water was held at 27 °C), air stone, mechanical filter, a flower pot half, and 3 cm of coral sand for substrate. The following morning, three fish were immediately captured from their individual aquaria and euthanized via terminal anaesthesia (0.5 g L^{-1} ethyl-*p*-aminobenzoate; Sigma-Aldrich). Blood was collected via caudal severance into heparinized micro-hematocrit capillary tubes (Thermo-Fisher Scientific) within 2 min of approaching each tank and was centrifuged for 3 min (4750*g*). Plasma was then collected, flash frozen in liquid nitrogen, and stored at −20 °C. The remaining 18 fish were weighed and intraperitoneally injected with 10 μL per gram body weight of vehicle (0.9% saline; $N = 6$) or vehicle containing either 5 ng mL^{-1} (Low Dose; $N = 6$) or 50 ng mL^{-1} (High Dose; $N = 6$) of cortisol using a 1 mL syringe and a 26-gauge needle. Two fish in each treatment group

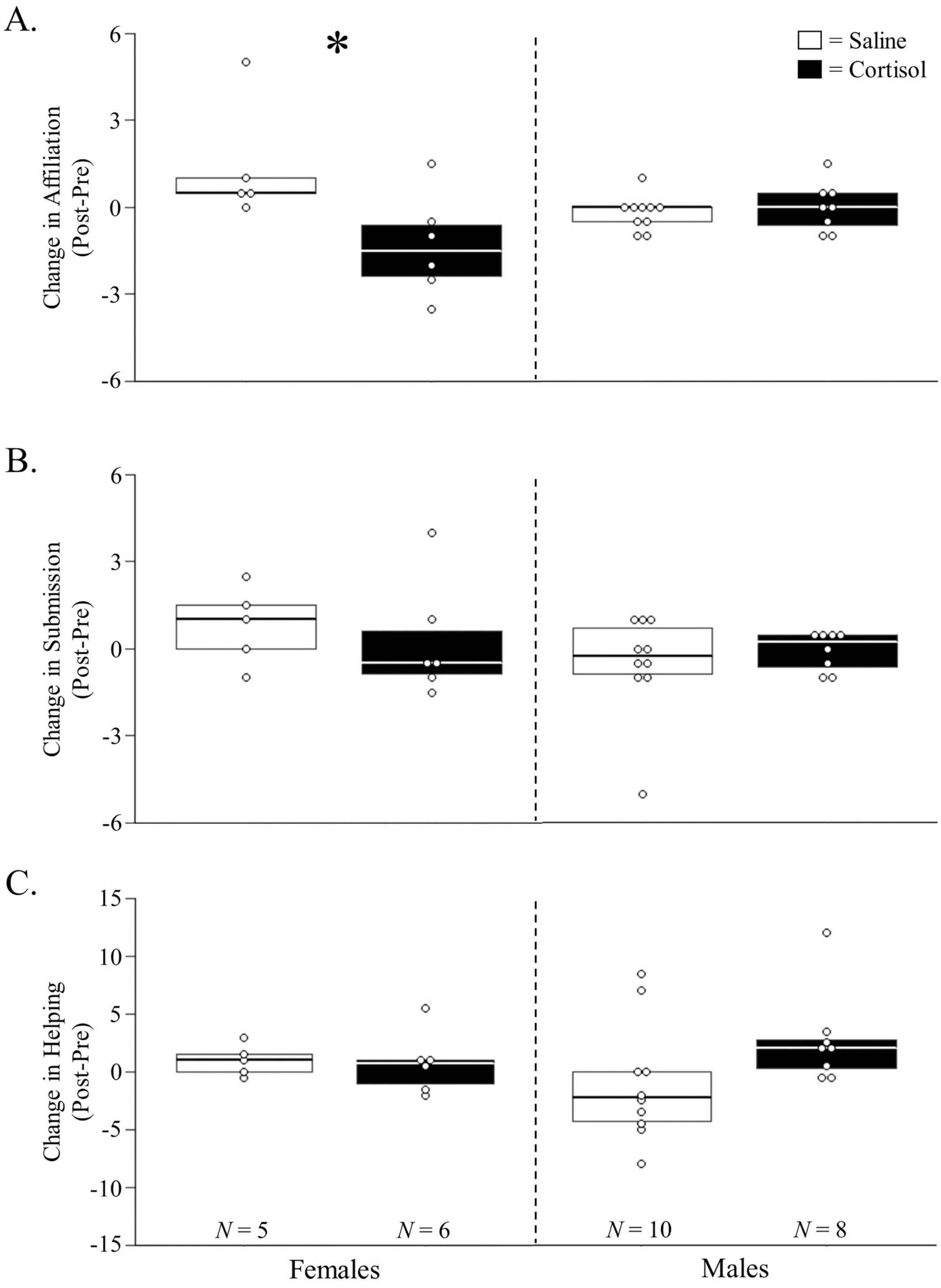

Fig. 2. Changes in the number of affiliative (A), submissive (B) and helping (C) acts performed by focal subordinate *N. pulcher* that were treated with saline (white) or cortisol (black). Values are presented as medians and 1st and 3rd quartiles; points represent individual values. An asterisk indicates a significant difference as a result of cortisol treatment (see text for statistical details).

were sampled at 1, 2 and 4 h post-injection as described above. Plasma cortisol levels were determined using a radioimmunoassay as per Bernier et al. (2008), and showed that the high dosage (50 ng mL^{-1}) elicited physiologically-relevant elevations in circulating cortisol levels (Fig. 1) that were consistent with levels previously observed in *N. pulcher* during periods of acute stress (~100–500 ng mL^{-1}; Culbert et al., 2019; Mileva et al., 2009).

In the field, fish were intraperitoneally injected underwater with 10 μL per gram of body weight of either vehicle (0.9% saline) or vehicle containing 50 ng mL^{-1} of cortisol using a 1 mL syringe and a 26-gauge needle. Because fish could not be weighed underwater, injection volumes were determined using body length measurements since body length and mass are strongly correlated in wild populations of adult *N. pulcher* ($R^2 = 0.94$; $N = 42$, see Hellmann et al., 2015a for collection details). All researchers were blind to the identity of the injection solutions.

2.4. Statistical analysis

Statistical analyses were performed using R (v. 3.6.3; R Core Team, 2020) and a significance level (α) of 0.05 was used for all tests. To assess whether the treatments differentially affected the performance of affiliative, helping, and submissive behaviours, we calculated the difference between each of these behaviours before (mean values of the two observation periods prior to injection) versus after treatment (Diff = Post – (½*(Pre$_1$ + Pre$_2$))). We fit general linear models that included treatment (cortisol or saline), sex (male or female), and their interaction term as fixed factors using the lmer function in the 'lme4' package (Bates et al., 2015). In addition, to determine whether baseline rates of behaviour (performed and received) differed between sexes or treatment groups we fit generalized linear mixed models with a negative binomial distribution (to account for overdispersion of count data) using the 'glmer.nb' function in the 'lme4' package. When overall differences were detected using the Anova function in the 'car' package (Fox and Weisberg, 2011), Tukey's HSD post hoc tests were performed using the emmeans function in the 'emmeans' package (Lenth, 2016). Effect sizes were estimated by calculating eta-squared values (η^2) for general linear models using the 'sjstats' package (Lüdecke, 2020) or semi-partial R^2 values (Nakagawa et al., 2017; Nakagawa and Schielzeth, 2013) for negative binomial models using the 'r2glmm' package (Jaeger et al., 2017).

3. Results

Cortisol treatment had sex-specific effects on the performance of affiliative behaviours (Fig. 2A and Table 1). Females that were treated with cortisol reduced their performance of affiliative acts compared to saline-treated females ($p = 0.008$), while no such differences were detected in males ($p = 0.99$). Additionally, females performed twice as many affiliative acts as males prior to treatment ($p = 0.01$; Table 2). The performance of submissive acts (Fig. 1B and Table 1) or general helping behaviours (Fig. 1C and Table 1) did not differ between treatment groups or sexes. No differences were detected in social behaviours (performed or received) between fish that were treated with saline versus cortisol prior to treatment (Tables 2 & 3) and groupmates did not differentially adjust their performance of social behaviours towards focal fish that were treated with cortisol versus saline (Table 4).

Table 1
Results of linear models assessing changes in the performance of prosocial behaviours by focal subordinate *N. pulcher* that were injected with either saline ($n = 15$) or cortisol ($n = 14$). Significant results ($p < 0.05$) are depicted in **bold** (see text for full description of the statistical analyses).

		F	p	η^2
Affiliation	Treatment	3.74	0.06	0.10
	Sex	0.05	0.82	0.001
	Treatment * Sex	**9.06**	**0.006**	**0.24**
Submission	Treatment	0.01	0.91	0.002
	Sex	1.83	0.19	0.07
	Treatment * Sex	0.70	0.41	0.03
Helping	Treatment	2.70	0.11	0.09
	Sex	0.09	0.76	0.003
	Treatment * Sex	2.15	0.16	0.07

4. Discussion

We found that experimental elevation of glucocorticoid levels did not increase the performance of affiliative, helping, or submissive acts by subordinate group members in a wild group-living fish, *Neolamprologus pulcher*. In fact, subordinate females *decreased* affiliation (e.g., touching and swimming together) following cortisol treatment, suggesting that glucocorticoids may reduce prosocial behaviour in this species. Overall, our findings do not support the hypothesis that elevated glucocorticoids stimulate prosociality in fishes.

Strong affiliative relationships with groupmates can improve an individual's social rank and fitness (Schülke et al., 2010; Silk et al., 2003; Strauss and Holekamp, 2019). In *N. pulcher*, affiliative relationships are likely more important to females because they are less likely to disperse than are males (Hellmann et al., 2016; Stiver et al., 2007) and often ascend to dominant breeder status from within their social group (Dierkes et al., 2005; Stiver et al., 2006). We found that subordinate females performed twice as many affiliative acts as subordinate males, supporting the notion that affiliative relationships are important to female *N. pulcher*; potentially as a mechanism to enhance their social standing within the group. However, females performed fewer affiliative acts when treated with cortisol. Soares et al. (2014) also reported reduced prosociality displayed by cleaner wrasse (*Labroides dimidiatus*) towards their clients following cortisol treatment. These results may reflect glucocorticoids elevating levels of anxiety and vigilance in treated animals (Harris and Carr, 2016), which is thought to be part of an adaptive survival-related response displayed by stressed animals (Crespi et al., 2013). While we did not directly assess anxiety or vigilance in the current study, it is possible that cortisol treated females were temporarily prioritizing awareness and vigilance (related to survival) over affiliation (related to future social success).

Elevated glucocorticoid levels typically induce a number of behavioural and physiological adjustments that function to increase an individual's likelihood of survival (Crespi et al., 2013), but the specific responses that are evoked can vary depending on the cause of the rise in glucocorticoids. For example, exposure to either a predator or novel conspecific resulted in acutely elevated cortisol levels in three-spined sticklebacks (*Gasterosteus aculeatus*), but levels of monoamines in regions of the brain involved in regulating glucocorticoid synthesis (hypothalamus, reticular formation, and telencephalon) showed stressor-specific responses suggesting that different neural regulatory mechanisms are involved (Bell et al., 2007). *N. pulcher* are routinely exposed to a diverse set of challenges that require unique behavioural, physiological, and cognitive responses, including predator attacks (Groenewoud et al., 2016; Heg et al., 2004; Jungwirth et al., 2015), territory disputes with neighbours (Frostman and Sherman, 2004; Saeki et al., 2018), and changes in the composition of their social group (Culbert et al., 2018; Dierkes et al., 2005; Fitzpatrick et al., 2008; Stiver et al., 2006). While each of these challenges is likely to activate the HPI axis and cause elevated glucocorticoid synthesis, the neurohormonal changes that facilitate HPI axis activation may vary owing to the unique responses required to overcome different challenges. Future work should focus on attaining a more thorough understanding of the degree to which the neuroendocrine mechanisms regulating glucocorticoid synthesis are conserved across different ecological challenges.

Following a disturbance, many mammals increase their performance of prosocial and affiliative acts which can help mitigate stress and anxiety via social buffering and consolation (Burkett et al., 2016; Fraser

Table 2
Comparison of pre-treatment performance of social behaviours (over 10 min) by focal subordinate *N. pulcher* that were eventually injected with saline ($n = 15$) or cortisol ($n = 14$). Behaviours are reported as means ± SEM and were recorded over two 10 min periods. Significant differences ($p < 0.05$) are indicated in **bold** (see text for full description of the statistical analyses).

	Females		Males			X^2	p	R^2
	Saline ($N = 5$)	Cortisol (N = 6)	Saline ($N = 10$)	Cortisol ($N = 8$)				
Affiliation	2.4 ± 1.2	2.3 ± 0.9	1.2 ± 0.3	0.9 ± 0.1	Treatment	0.22	0.64	0.001
					Sex	**6.10**	**0.01**	**0.17**
					Treatment * Sex	0.18	0.67	0.01
Aggression	0.6 ± 0.2	1.0 ± 0.5	0.6 ± 0.3	0.4 ± 0.2	Treatment	0.08	0.78	0.005
					Sex	1.01	0.31	0.04
					Treatment * Sex	0.58	0.45	0.02
Helping	2.2 ± 0.3	4.6 ± 1.3	5.4 ± 1.5	5.6 ± 1.6	Treatment	0.83	0.36	0.01
					Sex	2.79	0.09	0.07
					Treatment * Sex	1.37	0.24	0.06
Submission	0.8 ± 0.5	1.1 ± 0.4	1.8 ± 1.1	1.1 ± 0.5	Treatment	0.21	0.64	0.01
					Sex	0.47	0.49	0.02
					Treatment * Sex	0.56	0.46	0.02

Table 3
Comparison of pre-treatment performance of social behaviours by groupmates (over 10 min) towards focal subordinate *N. pulcher* that were eventually injected with saline ($n = 15$) or cortisol ($n = 14$). Behaviours are reported as means ± SEM and were recorded over two 10 min periods (see text for full description of the statistical analyses).

	Females		Males			X^2	p	R^2
	Saline (N = 5)	Cortisol (N = 6)	Saline (N = 10)	Cortisol (N = 8)				
Affiliation	0.4 ± 0.3	0.8 ± 0.2	0.8 ± 0.3	0.9 ± 0.3	Treatment	0.45	0.50	0.01
					Sex	0.78	0.38	0.02
					Treatment * Sex	0.23	0.63	0.01
Aggression	0.4 ± 0.3	0.5 ± 0.1	0.5 ± 0.3	0.3 ± 0.1	Treatment	0.24	0.63	0.007
					Sex	0.10	0.76	0.002
					Treatment * Sex	0.56	0.45	0.02
Submission	0.7 ± 0.6	1.2 ± 0.5	0.6 ± 0.4	0.5 ± 0.4	Treatment	0.10	0.76	0.007
					Sex	0.36	0.34	0.03
					Treatment * Sex	0.26	0.61	0.009

Table 4
Comparison of changes (post-pre) in the performance of social behaviours by groupmates (over 10 min) towards focal subordinate *N. pulcher* that were treated with either saline ($n = 15$) or cortisol ($n = 14$). Behaviours are reported as means ± SEM and were recorded over 10 min periods (see text for full description of the statistical analyses).

	Females		Males			F	p	η^2
	Saline (N = 5)	Cortisol (N = 6)	Saline (N = 10)	Cortisol (N = 8)				
Affiliation	0.4 ± 0.9	0.8 ± 0.7	−0.2 ± 0.3	−0.1 ± 0.6	Treatment	0.01	0.98	0.001
					Sex	0.68	0.42	0.03
					Treatment * Sex	0.31	0.58	0.01
Aggression	0.0 ± 0.5	−0.5 ± 0.1	−0.2 ± 0.3	0.0 ± 0.2	Treatment	0.07	0.80	0.004
					Sex	0.35	0.56	0.01
					Treatment * Sex	1.79	0.19	0.07
Submission	0.3 ± 0.5	0.2 ± 0.7	−0.5 ± 0.4	−0.3 ± 0.1	Treatment	0.03	0.87	0.003
					Sex	1.66	0.21	0.06
					Treatment * Sex	0.14	0.72	0.005

et al., 2008; Kiyokawa and Hennessy, 2018). However, the necessity of affiliative acts during social buffering in other vertebrates is less certain. In fishes, social buffering of stress appears largely dependent on visual stimuli as opposed to physical contact (Faustino et al., 2017; Nadler et al., 2016). While the presence of an individual's social group reduced the cortisol response of subordinate *N. pulcher*, this effect was associated with reduced affiliation (Culbert et al., 2019). These findings are consistent with the results of the current study and highlight a potentially key difference in the apparent interaction between glucocorticoids and prosocial behaviours across vertebrates. Another important distinction between fishes versus mammals and birds is that fish do not typically provision food to juveniles. A common response to elevated glucocorticoids in terrestrial vertebrates is increased food allocation to young (Crossin et al., 2012; Dantzer et al., 2017), which is likely a consequence of the metabolic actions of glucocorticoids. These differences may have ultimately resulted in glucocorticoids fulfilling different behavioural functions in fishes compared to other vertebrates.

The potential role of elevated glucocorticoids as mediators of behavioural responses to social disturbances has received considerable attention (e.g., Dantzer et al., 2017; Santema et al., 2013; Soares et al., 2014; Voellmy et al., 2014); however, many hormones mediate stress responses (Tort and Teles, 2011). It is therefore unlikely that the behavioural responses to stress are mediated by changes in glucocorticoid production alone and responses are more likely the result of interactions between several endocrine systems. In addition to hormones, centrally mediated responses to stress that occur in the brain also regulate social behaviours. Many centrally produced neuropeptides (e.g., corticotropin-releasing factor, arginine vasotocin/vasopressin, and isotocin/oxytocin) and neurotransmitters (e.g., dopamine and serotonin) are widely implicated in the regulation of both social behaviours and stress (Backström and Winberg, 2017; Bernier et al., 2009; Godwin and Thompson, 2012). While we used peripheral injections of cortisol to directly assess the effects of elevated glucocorticoids on prosociality, it is likely that this exogenous source of cortisol influenced the synthesis and release of many centrally produced neurohormones that are involved in glucocorticoid synthesis. For example, peripheral treatment with cortisol causes a reduction in hypothalamic corticotropin-releasing factor synthesis due to negative feedback inhibition (Aguilera, 1998; Bernier et al., 1999). Conversely, elevated endogenous production of cortisol during acute stressors is typically associated with increased synthesis of corticotropin-releasing factor which stimulates cortisol synthesis through the HPI axis (Aguilera, 1998; Bernier et al., 2009). Therefore, we cannot dismiss the possibility that prosocial behaviours in *N. pulcher* are modulated by the HPI axis more broadly, potentially via interactions with neurohormones that promote endogenous cortisol synthesis (as opposed being mediated by cortisol itself). However, few studies have assessed the involvement of these central neurohormone systems in regulating prosociality and promoting stability in social groups (Hellmann et al., 2015b; Madden and Clutton-Brock, 2011; Mooney et al., 2014; Taylor et al., 2017). Additionally, most studies have used peripheral treatments, which may not exert their actions directly in the brain. Unlike steroid hormones (e.g., cortisol), which are lipophilic in their unbound state and can easily diffuse through cell membranes and pass through the blood-brain barrier to reach targets in the brain (Abbott et al., 2010; Kancheva et al., 2011; Marynick et al., 1977), peptide hormones typically exhibit poor penetration of the blood-brain barrier (McKinley and Oldfield, 1998; Meisenberg and Simmons, 1983). As such, peripheral treatments with peptide hormones may elicit different responses versus central manipulations directly in the brain. Future studies should focus on elucidating the role of these central neurohormone systems in regulating social cohesion and prosocial behaviour within social groups.

In conclusion, we did not find evidence that elevated glucocorticoids promote prosocial behaviour in fishes. These findings raise interesting questions about potential differences in the proximate regulation of prosocial behaviours during the evolution of group-living across vertebrates.

Data accessibility

Supporting data can be found in the attached Supplemental file.

CRediT authorship contribution statement

B.M.C., I.Y.L., and M.G.S. conducted the experiment. B.M.C. conducted the pilot study, performed the analyses and wrote the first draft of the manuscript. All authors contributed to the design of the experiment, discussed results, and commented on the manuscript.

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Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2020.104879.

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