Associations Among Brain Size and Social-Modulating Hormones with Aggression and Cognitive Performance in a Monogamous Cichlid Fish

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Research Article

Keywords:

Posted Date: February 24th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1349913/v1>

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Abstract

Social behavior is shaped by neural and endocrine mechanisms. In this framework, the social brain hypothesis assumes that bigger brains are selected to cope with complex social environments. On the other hand, hormones can act through organizational and activational effects, modulating brain function during ontogeny and in a fast-changing environment. Here, we test correlations among social-modulating hormones – cortisol, testosterone (T), and 11-ketotestosterone (11KT) – and the volume of specific brain areas with individuals' social aggression and cognitive abilities in both males and females of the pearl cichlid *Geophagus brasiliensis*. We observed that, although both parents present similar roles during parental care – biparental brood care – reflecting in similar brain volumes, besides social and cognitive behaviors, the correlation between these factors and hormone levels are different comparing sexes. Females are associated with cortisol, which possibly enhances their cognitive performance, and is linked to bigger telencephalons and diencephalons. Males are influenced by testosterone and its conversion into 11KT, which seems to modulate their behavior. Thus, males' and females' social behaviors seem to be evolutionarily driven by similar brains, yet different hormonal activation for coping with the social environment.

Introduction

Social behavior encompasses any kind of interaction between conspecific or heterospecific individuals which influences their immediate or future behavior and fitness ¹. These interactions are a constant in group-living animals: some can be beneficial whereas others, detrimental. In this conceptual framework, evolution will drive the selection of group-living animals whether an individual's gain of living in a social system surpasses the gains of living alone ². Nonetheless, living in groups creates several challenges to the individuals of a same species, due to the variety of age, sex, and personalities; therefore, some skills are necessary for them to cope with such an unpredictable social environment ². Hence, living in social groups demands a certain degree of behavioral flexibility, which is mediated by a higher brain complexity, as postulated by the social brain hypothesis ^{2,3}.

Social behavior is also influenced by hormones that control or modulate behaviors through organizational and activational effects on the brain ⁴. The organizational effect is considered to shape ontogenetic differences in male and female phenotypes, including their nervous system ⁴. On the other hand, fast environmental changes (e.g., social environment) may trigger endocrine changes which will shape individual behavior through a hormonal activational effect ⁵. According to the challenge hypothesis, for instance, social interactions, namely competition among males and paternal care style, modulate individual androgen responses ^{6,7}. Hormones, in turn, act as input-modulators of the social brain ⁸, probably improving social cognitive demands, such as individual discrimination, learning and attention ⁹. Hence, an integrative approach regarding ultimate (brain evolution) and proximate causes (activational effect of hormones) can provide a better understanding about the regulation of social behavior.

Reproduction is also intrinsically related to brain evolution due to its social context^{10–12}. Monogamous species, for example, require a large amount of energy investment and a refined social synchronization between parents during brood care to succeed². In some biparental fish species, both parents must defend their youngsters from predators and environmental fluctuations while feeding and resting themselves^{2,13}. The lack of synchronicity during this care may result in brood loss and reproductive failure. Therefore, the mating system and parental care are related to the brain architecture, with monogamy being associated with bigger relative brain areas in different groups of animals^{2,14–17}.

Reproduction can also encompass aggression in many species, which is strongly related to hormones, such as sex steroids and glucocorticoids^{12,18,19}. In fish, for example, cortisol is the most important glucocorticoid, and its levels can change according to individuals' hierarchy and reproductive state¹⁹. Moreover, cortisol affects cognitive processes, such as learning and memory²⁰, and can be detrimental to the individual's cognitive performance²¹ as well as to brain cells' composition²². Thus, besides its metabolic action, cortisol can also affect social behavior through effects on the cognitive flexibility of fish.

Aggression during the reproductive season is also associated with androgen levels which usually increase in winners, particularly in polygynous species^{6,7,23}, or decrease in species whose males engage in brood care⁷. Although males and females produce and present the same androgens, their function and target tissues can differ²⁴. Concerning fish, the two most important androgens are testosterone (T) and 11-ketotestosterone (11KT), the latter being considered more potent than the former^{5,25}.

Regarding connections between brain and steroid hormones, we tested whether androgen (i.e., testosterone (T) and 11-ketotestosterone (11KT)) and cortisol levels would be associated with the volumes of specific brain structures (i.e., telencephalon, diencephalon, optic tectum, brain stem, and cerebellum), and with individual social and cognitive performances. Our target species was a Neotropical fish, the pearl cichlid *Geophagus brasiliensis*, which is a monogamous substrate-brooder with biparental care of the fry. As other Neotropical cichlids, *G. brasiliensis* defends its territory making use of aggressive interactions²⁶ and provides biparental brood care. In this context, we aimed to test whether a species considered to present highly demanding cognitive synchronization during parental care would present associations between aggressive interactions (mirror test), cognition (inhibitory control test), hormone levels (cortisol, T, and 11KT), and volume of brain areas (Fig. 1). We found that although males and females have similar social and cognitive performances (which is in line with their very similar roles during parental care), both sexes differ in the patterns of how brain and hormonal factors correlate with their behavior, being associated to T in males and, surprisingly, to cortisol in females. Therefore, social behavior of males and females seems to be evolutionarily driven by similar brains, but different hormonal activation for coping with the social environment.

Results

Female and male pear cichlids have different levels of 11KT, whereas cortisol and testosterone levels are similar

Behavioral variables and relative brain volumes did not show any difference between males and females (Table 1). Cortisol ($F_{(1,34)} = 0.59$; $p = 0.44$) and testosterone ($F_{(1,34)} = 2.05$; $p = 0.16$) levels were also similar between sexes (Fig. 2a, b). Significant differences between males and females were observed only in 11KT ($F_{(1,33)} = 18.74$; $p = 0.0001$) and the 11KT/T ratio of conversion ($F_{(1,33)} = 36.18$; $p < 0.0001$), with females showing the lowest levels for both variables (Fig. 2c, d).

Table 1

Linear Models regressions with sex as a predictor variable; biological measures, such as behavior and brain volume, as response variables. Data are shown as mean $\pm$ SE.

Biological measures	Response variables	Replicates	Mean $\pm$ SE	F	P
Aggressiveness (Mirror-elicited fights) 30 min total	Attacks (frequency)	n = 16 females	30.69 $\pm$ 12.17	$F_{(1,27)} = 0.02$	0.89
		n = 13 males	27.99 $\pm$ 10.01		
	Displays (frequency)	n = 16 females	4.14 $\pm$ 0.68	$F_{(1,27)} = 1.62$	0.21
		n = 13 males	7.24 $\pm$ 2.58		
	Latency to attack (s)	n = 16 females	201.26 $\pm$ 33.24	$F_{(1,27)} = 0.56$	0.46
		n = 13 males	244.32 $\pm$ 48.73		
Inhibitory control (Detour-reaching task) 10 min each trial	# trials to learn	n = 19 females	9.37 $\pm$ 1.27	$F_{(1,34)} = 0.02$	0.89
		n = 17 males	9.18 $\pm$ 0.67		
	Time on glass (s)	n = 19 females	11.77 $\pm$ 1.34	$F_{(1,34)} = 0.64$	0.43
		n = 17 males	10.21 $\pm$ 1.41		
	Latency to detour (s)	n = 19 females	40.07 $\pm$ 7.29	$F_{(1,34)} = 0.65$	0.42
		n = 17 males	51.78 $\pm$ 12.97		
	% correct responses	n = 19 females	31.58 $\pm$ 3.79	$F_{(1,34)} = 2.68$	0.11
		n = 17 males	41.76 $\pm$ 5.04		
Brain structure volumes (μm^3)	Telencephalon	n = 19 females	2.09 $\pm$ 0.09	$F_{(1,36)} = 2.76$	0.1
		n = 19 males	1.88 $\pm$ 0.09		
	Diencephalon	n = 17 females	1.16 $\pm$ 0.08	$F_{(1,31)} = 1.89$	0.18
		n = 16 males	1.32 $\pm$ 0.07		
	Optic tectum	n = 19 females	4.35 $\pm$ 0.19	$F_{(1,36)} = 0.08$	0.77
		n = 19 males	4.24 $\pm$ 0.31		

Biological measures	Response variables	Replicates	Mean $\pm$ SE	F	P
	Brain stem	n = 17 females	0.45 $\pm$ 0.04	$F_{(1,28)} = 0.13$	0.72
		n = 13 males	0.48 $\pm$ 0.04		
	Cerebellum	n = 19 females	1.2 $\pm$ 0.07	$F_{(1,36)} = 1.34$	0.25
		n = 19 males	1.31 $\pm$ 0.06		

Hormone levels are distinctly correlated with behavior and brain volume in each sex

Although no difference between sexes was observed in behavior and brain volume, we found several correlations between these two variables and hormone levels in females (Table 2) and males (Table 3).

Table 2

Spearman's correlations among quantified hormones, behavioral responses, and brain volumes in female pearl cichlids.

	Cortisol (ng/ml)	T (ng/ml)	11KT (ng/ml)	11KT/T
T (ng/ml)	$r = 0.07$			
	$p = 0.79$			
11KT (ng/ml)	$r = -0.12$	$r = 0.40$		
	$p = 0.67$	$p = 0.14$		
11KT/T	$r = -0.41$	$r = -0.05$	$r = 0.84$	
	$p = 0.12$	$p = 0.85$	$p < 0.001$	
Attacks (frequency)	$r = 0.28$	$r = -0.32$	$r = -0.09$	$r = -0.002$
	$p = 0.30$	$p = 0.24$	$p = 0.75$	$p = 0.99$
Displays (frequency)	$r = 0.17$	$r = -0.03$	$r = 0.16$	$r = 0.22$
	$p = 0.54$	$p = 0.90$	$p = 0.31$	$p = 0.42$
Latency to attack (s)	$r = -0.26$	$r = -0.07$	$r = 0.39$	$r = 0.54$
	$p = 0.34$	$p = 0.81$	$p = 0.14$	$p = 0.03$
# trials to learn	$r = -0.55$	$r = -0.07$	$r = 0.02$	$r = 0.24$
	$p = 0.03$	$p = 0.79$	$p = 0.93$	$p = 0.40$
Time on glass (s)	$r = -0.56$	$r = -0.03$	$r = 0.006$	$r = 0.09$
	$p = 0.03$	$p = 0.89$	$p = 0.98$	$p = 0.74$
Latency to detour (s)	$r = -0.47$	$r = 0.23$	$r = 0.15$	$r = 0.10$
	$p = 0.10$	$p = 0.41$	$p = 0.59$	$p = 0.72$
% correct responses	$r = 0.06$	$r = 0.27$	$r = 0.19$	$r = 0.10$
	$p = 0.82$	$p = 0.33$	$p = 0.48$	$p = 0.72$
Volume tl (μm^3)	$r = 0.60$	$r = -0.18$	$r = -0.33$	$r = -0.42$
	$p = 0.01$	$p = 0.50$	$p = 0.22$	$p = 0.12$
Volume di (μm^3)	$r = 0.49$	$r = 0.14$	$r = -0.05$	$r = -0.28$
	$p = 0.06$	$p = 0.62$	$p = 0.86$	$p = 0.31$

Significance was settled at $p \leq 0.05$. Significant correlations are highlighted in bold and gray. Trends ($p < 0.10$) are highlighted in bold and italic.

	Cortisol (ng/ml)	T (ng/ml)	11KT (ng/ml)	11KT/T
Volume ot (μm^3)	$r = 0.28$	$r = -0.34$	$r = -0.16$	$r = -0.09$
	$p = 0.30$	$p = 0.21$	$p = 0.56$	$p = 0.74$
Volume bs (μm^3)	$r = 0.11$	$r = 0.06$	$r = -0.48$	$r = -0.59$
	$p = 0.70$	$p = 0.82$	$p = 0.06$	$p = 0.02$
Volume cb (μm^3)	$r = -0.38$	$r = -0.22$	$r = -0.36$	$r = -0.14$
	$p = 0.15$	$p = 0.43$	$p = 0.18$	$p = 0.61$
Significance was settled at $p \leq 0.05$. Significant correlations are highlighted in bold and gray. Trends ($p < 0.10$) are highlighted in bold and italic.				

Table 3
Spearman's correlations among quantified hormones, behavioral responses, and brain volumes in males
pearl cichlid.

	Cortisol (ng/ml)	T (ng/ml)	11KT (ng/ml)	11KT/T
T (ng/ml)	r = -0.12 p = 0.74			
11KT (ng/ml)	r = -0.90 p = 0.003	r = 0.31 p = 0.38		
11KT/T	r = -0.28 p = 0.42	r = -0.76 p = 0.01	r = 0.19 p = 0.60	
Attacks (frequency)	r = -0.16 p = 0.64	r = -0.86 p = 0.001	r = -0.14 p = 0.69	r = 0.62 p = 0.05
Displays (frequency)	r = 0.12 p = 0.74	r = -0.74 p = 0.01	r = -0.36 p = 0.31	r = 0.31 p = 0.38
Latency to attack (s)	r = -0.16 p = 0.64	r = 0.33 p = 0.34	r = 0.40 p = 0.24	r = 0.07 p = 0.84
# trials to learn	r = -0.19 p = 0.58	r = 0.13 p = 0.71	r = 0.27 p = 0.45	r = -0.26 p = 0.47
Time on glass (s)	r = -0.47 p = 0.16	r = -0.47 p = 0.16	r = 0.47 p = 0.16	r = 0.83 p = 0.003
Latency to detour (s)	r = -0.33 p = 0.34	r = 0.71 p = 0.02	r = 0.47 p = 0.16	r = -0.28 p = 0.42
% correct responses	r = 0.42 p = 0.23	r = 0.57 p = 0.08	r = -0.26 p = 0.47	r = -0.84 p = 0.002
Volume tl (μm^3)	r = 0.40 p = 0.24	r = -0.69 p = 0.03	r = -0.47 p = 0.16	r = 0.33 p = 0.34
Volume di (μm^3)	r = 0.24 p = 0.51	r = -0.24 p = 0.51	r = -0.09 p = 0.79	r = 0.09 p = 0.79

Significance was settled at $p \leq 0.05$. Significant correlations are highlighted in bold and gray. Trends ($p < 0.10$) are highlighted in bold and italic.

	Cortisol (ng/ml)	T (ng/ml)	11KT (ng/ml)	11KT/T
Volume ot (μm^3)	<i>r = 0.14</i>	<i>r = 0.05</i>	<i>r = 0.09</i>	<i>r = 0.40</i>
	<i>p = 0.69</i>	<i>p = 0.89</i>	<i>p = 0.79</i>	<i>p = 0.24</i>
Volume bs (μm^3)	<i>r = 0.14</i>	<i>r = 0.02</i>	<i>r = -0.16</i>	<i>r = -0.05</i>
	<i>p = 0.69</i>	<i>p = 0.95</i>	<i>p = 0.64</i>	<i>p = 0.89</i>
Volume cb (μm^3)	<i>r = 0.33</i>	<i>r = -0.43</i>	<i>r = -0.24</i>	<i>r = 0.31</i>
	<i>p = 0.34</i>	<i>p = 0.21</i>	<i>p = 0.51</i>	<i>p = 0.38</i>
Significance was settled at $p \leq 0.05$. Significant correlations are highlighted in bold and gray. Trends ($p < 0.10$) are highlighted in bold and italic.				

Females showed several correlations regarding cortisol, assessed behaviors and brain volumes. During the inhibitory control test, the number of trials to learn the task (training phase) and the time spent trying to cross the glass (test phase) were negatively correlated to cortisol levels. Higher levels of cortisol were positively related to females with bigger telencephalons and a positive trend correlation also appeared in respect to the diencephalon. Therefore, the higher the cortisol levels, the better the cognitive performance of females. On the other hand, females that presented higher levels of 11KT showed a tendency to have a smaller brain stem. The levels of 11KT were positively correlated to the 11KT/T ratio of conversion, which was significantly related to smaller brain stems, whilst positively associated with females that were faster to start aggressive encounters, thus indicating that a balance between androgens is associated with aggressive motivation in females.

Males had several different correlations from those observed for females. Cortisol, for instance, was negatively (and strongly) correlated with 11KT levels (Table 3), yet it was not correlated to any other behavioral or brain data. Regarding testosterone, this hormone presented stronger correlations with 11KT/T ratio, attacks and displays, and telencephalon volume, all of them negative correlations. During the inhibitory control test, males that presented higher testosterone levels were faster in detouring the transparent barrier and achieving the food reward. A correlation between testosterone and behavioral data was also observed as a trend, with a high positive R value concerning the percentage of correct responses during the test phase of the inhibitory control test. Ratio of conversion from 11KT to T showed significant correlations among attacks, time fish spent trying to cross the glass barrier (both positive correlations), and percentage of correct responses (negatively correlated). The levels of 11KT were correlated neither with behavior nor with brain volumes.

Discussion

This study showed that monogamous male and female pearl cichlids have similar brain volumes as well as aggressive and cognitive performances, probably due to their similar roles in parental care. Hormone

levels were expected to be different, at least to what androgens were concerned. This difference was observed for 11KT and for the ratio of conversion 11KT/T, which were lower in females compared to males, yet not for cortisol and T levels. Even though males and females were similar in most of the variables tested herein, we found distinct correlations between sexes that deserve attention. While cognitive performance and brain size are highly correlated to cortisol levels in females, males' social and cognitive behaviors, as well as brain sizes, are strongly related to T. These results suggest that, besides similar pressures provided by monogamy and biparental care, male and female pearl cichlids still have behavioral and physiological particularities coordinated by different hormonal patterns and brain areas.

As a monogamous species, female and male pearl cichlids have similar social and cognitive abilities

Males and females of *G. brasiliensis* showed similar aggressiveness and inhibitory control. These results corroborate our predictions, since both parents must coordinate their behavior to defend and assist the offspring while chasing away predators²⁶. This coordination is a highly demanding cognitive exercise for social animals, which is hypothesized to have shaped the brain in monogamous species¹⁶. Moreover, both parents seem to receive similar cognitive demands during parental care synchronization, as expected, since males and females performed very much alike in a test considered to be highly cognitively demanding, such as the inhibitory control task, which is the opposite scenario observed for other species that do not perform biparental brood care (e.g., Nile tilapia with female-only care: ²⁷) or perform no brood care at all (e.g., guppies: ^{28,29}). There were no differences regarding specific brain volumes. Therefore, both sexes from this species seem to be under a similar evolutionary pressure, which concerns sociability, cognition, and brain size.

Cortisol and testosterone are similar in both males and females, although males present higher plasma levels of 11KT

Males showed similar T levels compared to females, and higher 11KT levels and 11KT/T rates, thus in accordance with a teleost fish pattern, in which 11KT is considered the main androgen associated to reproductive behavior in males^{5,25}. This second androgen is usually found in very low levels or is sometimes absent in females⁵. The effect of sexual steroids on the social and reproductive behavior, however, is highly variable among species, since castration can either impair, increase, or not affect at all aggressiveness, depending on the species^{12,30}. Sapolsky (2017) states that T has a very flexible utility in vertebrates, and could be more related to the maintenance of an individual's status in a challenging scenario than to the aggressive behavior *per se*. As proposed by the challenge hypothesis, males' androgens levels are strongly influenced by the presence of male competitors and females^{6,7}.

In teleost fishes, females tend to prefer males that have nesting-care habits³²; therefore, androgens in males will be coordinated by the presence of females rather than by competitors' threats⁷. Nevertheless, in our study, males were always allocated into individual aquaria, facing another same-sex neighbor in

order to avoid social isolation stress (the same was done for females). Thus, a possible hierarchy could have been achieved through aquaria facing walls during the days of the experiment. However, given that there were no females at sight and males' (and females') territories were never invaded or transposed by a competitor, this probably provided the necessary stability for the lack of abrupt changes in T levels observed in our study.

The second androgen tested herein was the one that was differently expressed between sexes: 11KT. Males presented higher plasma levels of 11KT, which was reflected in the higher 11KT/T ratio also observed for males. The 11KT is the most potent androgen in teleost fishes; it is behind secondary sexual traits for males and females and is related to neurogenesis (see ³³, a study with Mozambique tilapia, *Oreochromis mossambicus*), a process directly related to cognition ³⁴. Despite that, there was no difference in cognitive and social performances between sexes, and males and females performed equally in both behavioral tests.

The pearl cichlid is a monogamous species in which androgen levels are usually similar between sexes, particularly when males are not defending territories (male-male challenges) and searching reproductive partners (male-female interactions), as during the breeding season ^{6,7}. Goymann et al. (2019) state that males providing nest-tending are more attractive to females and, therefore, there will be no conflict between mating and parenting that causes increases in T levels, since males can attract sexual partners and care for the youngsters at the same time ⁷. Interestingly, Scaia et al. (2018) observed in their study that aggression in females of the Neotropical cichlid fish *Cichlasoma dimerus*, rather than being modulated by androgens, were determined by initial levels of estrogens, with female winners showing higher levels of E₂ than losers ³⁵. Therefore, these results show that other sex steroids, not measured in our study, may modulate female social behaviors.

Glucocorticoids, although primarily linked to stress responses, modulate social behavior and cognitive performance, such as learning and memory ^{21,36}. For instance, social animals, such as the pearl cichlid, present high levels of cortisol when isolated, which will help the individual to cope with such a stressful situation (e.g., as an energy provisioner, glucocorticoids may allow the social animal to look for conspecifics). In our study, males and females showed no differences in cortisol levels, evidencing the same predisposition to do the tests, without any perceived stress biasing our results. Moreover, we cannot consider that the cortisol levels presented by our subjects were detrimental, since subjects did not present significant weight loss throughout the days of experimentation and were prone to do all the behavioral tests we proposed by swimming, exploring, and feeding in an apparently healthy way.

Females have brain and behavioral factors mainly linked to cortisol, whereas males present several associations with testosterone

Although they presented no differences when compared to each other, female and male pearl cichlids showed remarkable correlations regarding hormone levels, behavioral performance, and brain volumes. Cortisol levels were interestingly correlated to cognitive function and to telencephalon size in females, yet

not in males. During the inhibitory control test, higher levels of cortisol were related to females that took fewer trials to learn the detour-reaching task in the training phase. These females also spent less time trying to cross the glass of the transparent barrier in the test phase (i.e., better memory recall and inhibitory control), showing that higher cortisol levels seem to facilitate cognitive performance. As mentioned, higher cortisol concentrations were also related to bigger telencephalons, an important brain area related to social behavior and spatial learning³⁷. Cortisol levels also had a tendency of positive correlation with bigger diencephalons. The diencephalon controls several pathways linked to social organization³⁸, such as gonadotropin and corticotropin-releasing factors; it is, therefore, logical that bigger diencephalons might be related to higher levels of cortisol.

Long exposure to higher levels of cortisol is known to cause damage in brain cells^{20,22}, reflecting in lower cognitive performances²¹. Nevertheless, glucocorticoids can be related to an improvement in environmental perception and attention as well, which increases memorization and learning^{39,40} — in our study, spatial learning demanded by the detour-reaching paradigm. Therefore, even though both sexes did not present different levels of cortisol when compared to each other, female pearl cichlids seem to be positively responsive to cortisol. Males, on the other hand, did not show any relation between cortisol and the other tested variables, revealing that this sex is somehow less susceptible to cortisol effects on aggression and cognitive performance, as well as on brain size. Such lower susceptibility may be associated to the cichlid's role in natural environments, in which males are more territorial and aggressive than females before and during brood care⁴¹.

Pearl cichlid females also showed that the more T was metabolized into 11KT, the less prone they were to attack their mirror image; in other words, the 11KT/T ratio of conversion was negatively correlated to aggressiveness in females. However, males presenting a higher 11KT/T ratio of conversion showed a positive correlation with higher levels of aggression, which is the opposite from what we observed for females. Brain structure volumes also presented different correlations in each sex, revealing, again, the evolutive organizational particularities driving different evolutionary pressures on males' and females' reproductive roles.

In females, 11KT and 11KT/T were negatively associated with the volume of the brain stem. This brain area is known to be linked to motor coordination and autonomic activity in vertebrates, such as cardiovascular, respiratory, and sexual function⁴², as well as to the lateral line and to auditory information⁴³. The correlation observed herein could mean that this area of the brain helps fish to better interpret its environment. The brain stem has a lot of T neurons⁴²; it may also be the case for 11KT, although it has not been investigated yet.

As observed in tetrapods, the brain stem of teleost fish is also related to the auditory-vocal integration and presents the same auditory pathways⁴⁴. Teleosts also use acoustic information for sexual selection^{45,46}. In cichlids, females show higher levels of T and 11KT when they are close to spawn^{47,48}. In *G. brasiliensis*, a lower ratio of conversion of T into 11KT linked to a bigger brain stem and followed by the

same tendency regarding 11KT concentrations lead us to speculate that evolutionary pressure would select females with bigger brain stems which would balance the lower 11KT production. This could be the reason why a correlation between this androgen level with the brain stem size is observed in females, whereas in males it does not happen.

During the aggressiveness test, females showed a tendency to be less prone to fight when there was a higher 11KT/T ratio of conversion. Herein, we assessed aggression by measuring mirror-elicited fights. This methodology is pervaded by some concerns, and some researchers state that brain measures and hormonal responses are different when individuals fight with real vs virtual opponents ⁴⁹. Nevertheless, it is still an efficient method to measure aggression and avoid physical injuries in cichlids, thus improving animals' welfare and survival ^{50–52}.

In this study, aggressiveness was inversely correlated with T and positively correlated with 11KT/T, meaning that individuals with higher T were less aggressive. On the other hand, 11KT seems to increase aggression. T levels in males, for instance, were related to decreased aggression (in both attacks and displays), a correlation not observed for females. We did not measure hierarchical status; thus, we cannot speculate about dominance or subordination driving changes in androgen levels. However, T is an androgen commonly related to high rates of aggression ^{6,12,25} yet we observed the contrary in this study — a negative correlation. Nevertheless, as mentioned, T seems to be more related to the maintenance of an individual's status ³¹, and the lack of an intruder throughout the experimentation combined with a stable pairing to a same neighbor conspecific might be pointing to this status stability, given that blood sampling for hormonal analysis happened only on the last day.

In males, androgens also seem to be detrimental to cognitive performance, as individuals with higher levels of androgens took more time to conclude the detour-reaching task, with the trend of less correct responses as well. In the polygamous African cichlid Nile tilapia, males and females play very distinct roles during the reproductive season, with males being more aggressive and territorial, and leaving after reproducing with the females, which will care alone for the offspring inside their mouth ⁵³. Using a similar approach, Brandão et al. (2019) observed that these different roles in the reproductive repertoire of Nile tilapia seem to lead to different cognitive demands in inhibitory control. In that study, females were quicker to learn the detour-reaching task than males ²⁷, differently from the monogamous pearl cichlid, whose males and females obtained similar results in the same task, in spite of the apparent different action of T levels on cognitive performance. Moreover, the only relation regarding the brain volume in males was between the telencephalon and T levels, showing that the bigger that brain area, the lower the concentration of T in the plasma. Besides its role in spatial learning, the telencephalon is also related to the coordination of social abilities ³⁸, and a negative correlation to a hormone linked to aggression corroborates this idea.

In male pearl cichlids, the higher the cortisol, the lower the 11KT levels. This is in accordance with other studies showing this negative correlation between cortisol and androgen levels ^{54,55}. In teleost fish, this effect can be seen in the direct effect of cortisol on the production of 11KT, by affecting the producing

capacity of the testis, and consequently, decreasing 11KT levels in the plasma, since cortisol is converted in the testis into 11-keto-derivatives⁵⁶. It is important to point that we only observed this negative correlation between cortisol and 11KT (and not with T) for males. We also collected the hormones only at the end of the experiment and we did not observe if there was a synchronized increase or decrease in cortisol/11KT during the experiment. Nevertheless, the correlation between these two steroids, already observed in other teleost fish, seems to exist in the pearl cichlid as well.

Surprisingly, the cerebellum and the diencephalon were not correlated to any hormone we tested herein, either in males or females. Both areas are known to be involved in several spatial-learning processes and coordination of social behaviors, respectively⁵⁷. This lack of correlation shows us that, although extensively found in the literature, the volume of brain areas might not answer all questions regarding correlations between brain and other physiological measures, as is the case of the hormones tested herein. Thus, other comparative methods (e.g., molecular analysis or quantification of the types of brain cells composing specific brain areas) could be more efficient to detect the associations among hormones, behavior, and brain in teleost fish.

In summary, our correlational study aggregated, for the first time, the two most important units of individual's fitness: animals' behavior (measured by aggressiveness and cognitive tests) and the physiology coordinating it (measured by hormone levels and brain size). Males and females of *G. brasiliensis*, although showing very similar roles during parental care, evidenced in similar aggressiveness and inhibitory control, show different patterns of brain and hormonal correlations. Nevertheless, further studies concerning more specific anatomical components – quantifying the number of different types of brain cells, for instance – are necessary in order to elucidate how brain and behavior work together in such socially complex individuals.

Methods

Fish housing

We used 19 adult female and 19 adult male pearl cichlids from the *Geophagus brasiliensis* species (Quoy & Gaimard, 1824), which were collected in the Rio Grande river, in the state of Minas Gerais, southern Brazil. Specimens were transported and kept in the laboratory in polyethylene tanks (ca. 500 L, 1 fish/10 L) and acclimated for 30 days. The water temperature was controlled at 27°C with heaters and thermostats. The light regime was set from 7AM to 7PM. Water quality was maintained with biological filters (400 L/h, with constant aeration) and 25% of water renewal every week to clean accumulated organic residue without disturbing the group's social stability⁵⁸. Fish were fed once a day to apparent satiation with commercial food for tropical fish (28% CP).

Experimental design

Fish were anesthetized (tricaine methanesulfonate, MS222, 150 mg.L⁻¹), then measured, weighed, and allocated individually in a home aquarium (40 × 30 × 40 cm, ca. 48 L) for 24 hours previous to behavioral

tests. Although we allocated fish individually, aquaria were always paired along in a way that two fish could always see each other through a lateral transparent wall of the tank. These transparent walls were, however, covered with an opaque partition during behavioral tests. We used this design aiming to avoid the detrimental effects from social isolation on fish learning and welfare⁵⁹. Moreover, we noticed that fish were more active under this protocol than when completely isolated. *G. brasiliensis* is a monomorphic species, i.e., it does not present evident morphological differences between males and females, which is why several studies using this species will opt for using juvenile individuals instead of adults²⁶. In addition, we noticed that even during the reproductive season, not all males developed a nuchal hump at their head (personal observation), a major assertion used to distinguish sexes in this species. In our study, we could differentiate males from females by observing their genital papillae. As it occurs for other cichlid species^{19,60}, male pearl cichlids present a more cuneiform genital with a small pointed ending, whilst females present a rounder genital with their ovipositor duct being slit-shaped. By using this method, sex was correctly confirmed for 37 (out of the 38 subjects used in this study) after euthanasia.

The subjects were assigned to behavioral tests in a sequence of days (Fig. 1a), focused on social behavior (aggressiveness test) and cognitive performance (inhibitory control test). These two tasks were carried out in four consecutive days as detailed below. After the tests, individuals had their blood sampled and were killed with an anesthetic overdose of benzocaine at 180 mg.L^{-1} for sex confirmation and brain collection (Fig. 1a). During data gathering, some samples had insufficient plasma volume for hormonal analysis, or the subject did not fight during the aggressiveness test, which were reflected in the unequal number of replicates seen in Table 1.

There were no sex differences concerning weight and length at the beginning and end of the experiments (non-parametrical test of Mann-Whitney), neither when comparing initial and final measures between males and females, nor when comparing initial and final measures within the same sex. Female subjects used in our study presented an initial weight and length (mean $\pm$ SE for all the measures) of 30.54 ± 2.31 g and 9.54 ± 0.2 cm, respectively, and a final weight and length of 29 ± 2.4 g and 9.32 ± 0.2 cm. Males presented an initial weight and length of 26.76 ± 1.46 g and 9.03 ± 0.16 cm, and a final weight and length of 25.68 ± 1.35 g and 8.94 ± 0.14 cm, respectively.

Aggressiveness test

Agonistic interactions are part of the repertoire of a social species; they are hierarchy modulators in the social group, quite often, for both males and females⁵³. Hierarchical ranks in social species are very important to establish priority in accessing resources, such as food, territory, or reproductive partners⁵³. Thus, tests that measure aggressiveness are a good way of assessing social complexity. Therefore, we quantified the frequency of aggressive behaviors and the latency for the first attack during a 30-min video recording by using a mirror-elicited fight paradigm, which allows us to quantify aggressive behavior without physically injuring subjects and the opponent sex's bias (Fig. 1b).

Aggressive behaviors were grouped in displays (exhibitions without “physical contact”, requiring lower energy expenditure) and attacks (physical encounters, higher energy expenditure). The behaviors were quantified using an adaptation of the ethogram already developed for the pearl cichlid’s interaction with a real opponent (e.g., Kadry and Barreto 2010) and adjusted for mirror-elicited fights. We categorized undulation and butting as attacks, and frontal and lateral exhibitions as displays.

Inhibitory control test

The inhibitory control test is considered a robust method to measure cognitive flexibility in problem resolution²⁷. Fish were supposed to learn how to detour around an opaque U-shaped barrier to reach a food reward; then, the opaque barrier was replaced by a transparent one, which fish should detour in order to reach the food, thus showing inhibitory control by recovering the learned response from the previous tests (Fig. 1c). The test had two phases: a training phase, which was set to establish the learning criterion; and a test phase, which encompassed the inhibitory control *per se*. In the training phase, the U-shaped barrier was opaque. The subject was tested in four blocks of 5 trials (20 trials of 10 min each). Fish had to detour the opaque barrier and eat the food without entering the “incorrect zone”, represented by the barriers’ wings (Fig. 1c), in 4 out of 5 trials, at least in one of these four blocks (i.e., 80% correct responses). Only individuals that achieved the learning criterion in the training phase moved onto the next phase.

In the test phase, the opaque U-shaped barrier was replaced by a transparent one. There were 20 trials in this phase as well, yet now they were carried out on two different days not to overwhelm the subjects due to the highly demanding cognitive task (Fig. 1a, for timeline). We quantified the time fish spent trying to achieve the food through the transparent U-shaped barrier (i.e., latency to detour the barrier and time swimming against the glass wall), as well as the percentage of correct responses (fish detouring the transparent barrier to reach the reward without entering the “incorrect zone”).

Hormone assay

At the end of the behavioral experiments, we sampled blood from the subjects’ caudal vein, after they had been anesthetized by immersion in benzocaine at 90 mg.L⁻¹. We used hypodermic needles and heparinized syringes. The blood sampling lasted less than 2 minutes to avoid stress and interference of the manipulation in the results. Blood was centrifuged at 3,000 rpm for 10 min and the plasma was frozen at -20°C for further hormone assays — i.e., cortisol, testosterone, and 11-ketotestosterone (11KT). The hormones were measured through ELISA (Enzyme Linked Immunosorbent Assay), using commercial kits (IBL – Immuno Biological Laboratories, Hamburg, Germany, for cortisol assays; and Cayman Chemical Company, MI, USA, for testosterone and 11KT). Working plasma dilutions were 1:2 for cortisol assays, and 1:4 for T and 11KT, based on previous hormone analysis ran in our lab; samples were analyzed in duplicates. For cortisol, the intra-assay CV was 6.86% and 8.69% in each plate, and the inter-assay was CV = 3.94%. For T, the intra-assay was CV = 15.75%, 16.4%, and 12.02%, whereas the inter-assay CV = 9.37%. For last, the intra-assay of 11KT was CV = 10.76%, 16.46%, and 9.57% in each plate;

the intra-assay, CV = 7.97%. Some samples had insufficient plasma volume for the hormone analysis to be conducted, which reflected in the unequal number of replicates seen in Table 1.

Measurements of brain area volume

After blood sampling, fish were euthanized with a benzocaine overdose at 180 mg.L⁻¹ and had their skull removed for brain collection. The brain was placed in a Petri dish and photographed by the dorsal, lateral (left) and ventral sides. Afterward, the volume of the specific brain areas (i.e., telencephalon, diencephalon, optic tectum, brain stem, and cerebellum) were obtained by using the ellipsoid model, already described and used by Pollen et al. (2007) and Gonzalez-Voyer & Kolm (2010) for cichlids. Each value was divided by the standard length (in cm) of its corresponding subject, in order to standardize the data.

Statistical analysis

Data analysis was carried out using the free software R, version 4.0.2 (<http://www.r-project.org>). We checked our data using Kolmogorov-Smirnov test for normality and Fmax test for homoscedasticity. Data which were not normally distributed (testosterone and 11KT/T ratio) were log-transformed to fit parametric assumptions. Then, we compared hormone levels, behaviors, and brain volumes in males and females using Linear Models (LM), with hormones, behaviors, and brain volumes as response variables and sex as predictors. In order to avoid missing correlations in non-linear data, we used Spearman's correlation test corrected by Bonferroni's ("corr.test" function of the "psych" package⁶¹), in order to evaluate interactions between variables. Statistical significance was set at $p \leq 0.05$. However, in the correlations, we considered a marginal trend towards significance when $p < 0.10$, based on Pollen et al. (2007).

Declarations

Ethical statement

This project was registered in the Authorization and Information System in Biodiversity (SISBIO-ICMBio, Brazil, #54287-1), which allows the collection of biological material in natural environments for research purposes. It was also conducted according to the ethical principles on animal experimentation adopted by the National Council for the Control of Animal Experimentation (CONCEA – Brazil) and approved by the Committee on Ethics in Animal Use, UNESP, São José do Rio Preto, SP, permit number 156/2016.

These ethical procedures are also following the ARRIVE's guidelines, being fish allocated in aquaria with enough space, with small gravel substrate, walls covered with blue color plastic adhesive to prevent stress, specific feeding for cichlids and maintenance of water quality. As a social fish, we reduced the impact of isolation by allowing individuals to visualize their neighbors outside of behavior testing time. Handling for biometry and blood collection was always gentle and preceded by anesthesia. At the end of the experiment, they were euthanized by deepening anesthesia. The number of replicates was adequate

to answer our questions. Altogether, we avoid suffering and pain while valuing the life of fish, as well as the quality of our study.

ACKNOWLEDGEMENTS

We thank Ana Carolina dos Santos Gauy and Marcela Cesar Bolognesi for their assistance during data collection. This research project was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant #2016/26160-2 to EGF. MLB was supported by CAPES (PhD scholarship DS #001) and by FAPESP (Technical Training 3 #2019/10808-1). SPL was supported by CNPQ (PhD scholarship #141371/2019–6). TBC was supported by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) #06200679/2015.

AUTHOR CONTRIBUTIONS

M.L.B., E.G.F. and T.B.C. conceived the idea of the study. M.L.B. and S.P.L. performed the experiments and collected the data. M.L.B. analyzed the data and provided the figures. M.L.B. and E.G.F. wrote the manuscript. All authors discussed the results and agreed with data interpretation and conclusions. All authors reviewed the manuscript.

DATA AVAILABILITY

All data generated or analyzed during this study are included in the Supplementary Information file.

DECLARATION OF INTERESTS

The authors declare no competing interests.

References

1. Oliveira, R. F. & Bshary, R. Expanding the concept of social behavior to interspecific interactions. *Ethology* **127**, 758–773 (2021).
2. Dunbar, R. I. M. & Shultz, S. Evolution in the Social Brain. *Science* (80-.). **317**, 1344–1347 (2007).
3. Wascher, C. A. F., Kulahci, I. G., Langley, E. J. G. & Shaw, R. C. How does cognition shape social relationships? *Philos. Trans. R. Soc. B Biol. Sci.* **373**, 20170293 (2018).
4. Rosenfeld, C. S., Denslow, N. D., Orlando, E. F., Gutierrez-Villagomez, J. M. & Trudeau, V. L. Neuroendocrine disruption of organizational and activational hormone programming in poikilothermic vertebrates. *J. Toxicol. Environ. Heal. Part B* **20**, 276–304 (2017).
5. Oliveira, R. F. & Gonçalves, D. M. Hormones and Social Behaviour of Teleost Fish. in *Fish Behaviour* (eds. Magnhagen, C., Braithwaite, V. A., Forsgren, E. & Kapoor, B. G.) 61–150 (Science Publishers, 2008). doi:10.1201/b10757-5.
6. Wingfield, J. C., Hegner, R. E., Dufty, A. M. & Ball, G. F. The ‘challenge hypothesis’: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies’. *Am.*

- Nat. **136**, 829–846 (1990).
7. Goymann, W., Moore, I. T. & Oliveira, R. F. Challenge Hypothesis 2.0: A Fresh Look at an Established Idea. *Bioscience* **69**, 432–442 (2019).
 8. Soares, M. C. *et al.* Hormonal mechanisms of cooperative behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* **365**, 2737–2750 (2010).
 9. Cynx, J. & Nottebohm, F. Testosterone facilitates some conspecific song discriminations in castrated zebra finches (*Taeniopygia guttata*). *Proc. Natl. Acad. Sci.* **89**, 1376–1378 (1992).
 10. Cummings, M. E. The mate choice mind: studying mate preference, aversion and social cognition in the female poeciliid brain. *Anim. Behav.* **103**, 249–258 (2015).
 11. Boucherie, P. H., Loretto, M.-C., Massen, J. J. M. & Bugnyar, T. What constitutes “social complexity” and “social intelligence” in birds? Lessons from ravens. *Behav. Ecol. Sociobiol.* **73**, 12 (2019).
 12. Maruska, K. P. *et al.* Endocrine and neuroendocrine regulation of social status in cichlid fishes. *Horm. Behav.* **139**, 105110 (2022).
 13. Emlen, S. T. & Oring, L. W. Ecology, Sexual Selection, and the Evolution of Mating Systems. *Science* (80-). **197**, 215–223 (1977).
 14. Pollen, A. A. *et al.* Environmental complexity and social organization sculpt the brain in Lake Tanganyikan cichlid fish. *Brain. Behav. Evol.* **70**, 21–39 (2007).
 15. Zeng, Y., Lou, S. L., Liao, W. B., Jehle, R. & Kotrschal, A. Sexual selection impacts brain anatomy in frogs and toads. *Ecol. Evol.* **6**, 7070–7079 (2016).
 16. West, R. J. D. The evolution of large brain size in birds is related to social, not genetic, monogamy. *Biol. J. Linn. Soc.* **111**, 668–678 (2014).
 17. Gonzalez-Voyer, A. & Kolm, N. Sex, Ecology and the Brain: Evolutionary Correlates of Brain Structure Volumes in Tanganyikan Cichlids. *PLoS One* **5**, e14355 (2010).
 18. Dunlap, K. D., Castellano, J. F. & Prendaj, E. Social interaction and cortisol treatment increase cell addition and radial glia fiber density in the diencephalic periventricular zone of adult electric fish, *Apteronotus leptorhynchus*. *Horm. Behav.* **50**, 10–17 (2006).
 19. Alonso, F., Cánepa, M., Moreira, R. G. & Pandolfi, M. Social and reproductive physiology and behavior of the Neotropical cichlid fish *Cichlasoma dimerus* under laboratory conditions. *Neotrop. Ichthyol.* **9**, 559–570 (2011).
 20. McEwen, B. S. & Sapolsky, R. M. Stress and cognitive function. *Curr Opin Neurobiol.* **5**, 205–16 (1995).
 21. Wood, L. S., Desjardins, J. K. & Fernald, R. D. Effects of stress and motivation on performing a spatial task. *Neurobiol. Learn. Mem.* **95**, 277–285 (2011).
 22. Sørensen, C., Bohlin, L. C., Øverli, Ø. & Nilsson, G. E. Cortisol reduces cell proliferation in the telencephalon of rainbow trout (*Oncorhynchus mykiss*). *Physiol. Behav.* **102**, 518–523 (2011).
 23. Soma, K. K. Testosterone and Aggression: Berthold, Birds and Beyond. *J. Neuroendocrinol.* **18**, 543–551 (2006).

24. Koren, L. *et al.* Sexually opposite effects of testosterone on mating success in wild rock hyrax. *Behav. Ecol.* **30**, 1611–1617 (2019).
25. Borg, B. Androgens in teleost fishes. *Comp. Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrinol.* **109**, 219–245 (1994).
26. Kadry, V. O. & Barreto, R. E. Environmental enrichment reduces aggression of pearl cichlid, *Geophagus brasiliensis*, during resident-intruder interactions. *Neotrop. Ichthyol.* **8**, 329–332 (2010).
27. Brandão, M. L., Fernandes, A. M. T. de A. & Gonçalves-de-Freitas, E. Male and female cichlid fish show cognitive inhibitory control ability. *Sci. Rep.* **9**, 15795 (2019).
28. Lucon-Xiccato, T., Gatto, E. & Bisazza, A. Fish perform like mammals and birds in inhibitory motor control tasks. *Sci. Rep.* **7**, 13144 (2017).
29. Lucon-Xiccato, T., Gatto, E. & Bisazza, A. Male and female guppies differ in problem-solving abilities. *Curr. Zool.* **66**, 83–90 (2020).
30. Gonçalves, D. M. & Oliveira, R. F. Hormones and Sexual Behavior of Teleost Fishes. in *Hormones and Reproduction of Vertebrates* vol. 1 119–147 (Elsevier, 2011).
31. Sapolsky, R. M. *Behave: The Biology of Humans at Our Best and Worst.* (Penguin Books, 2017).
32. Hale, R. E. & St Mary, C. M. Nest tending increases reproductive success, sometimes: environmental effects on paternal care and mate choice in flagfish. *Anim. Behav.* **74**, 577–588 (2007).
33. Narita, Y. *et al.* Androgen induced cellular proliferation, neurogenesis, and generation of GnRH3 neurons in the brain of mature female Mozambique tilapia. *Sci. Rep.* **8**, 16855 (2018).
34. Salvanes, A. G. V. A. *et al.* Environmental enrichment promotes neural plasticity and cognitive ability in fish. *Proc. R. Soc. B Biol. Sci.* **280**, 20131331–20131331 (2013).
35. Scaia, M. F. *et al.* Can estrogens be considered as key elements of the challenge hypothesis? The case of intrasexual aggression in a cichlid fish. *Physiol. Behav.* **194**, 481–490 (2018).
36. Woolley, C. S., Gould, E. & McEwen, B. S. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res.* **531**, 225–231 (1990).
37. Kotrschal, K., Van Staaden, M. J. & Huber, R. Fish brains: evolution and environmental relationships. *Rev. Fish Biol. Fish.* **8**, 373–408 (1998).
38. Shumway, C. a. The evolution of complex brains and behaviors in African cichlid fishes. *Curr. Zool.* **56**, 144–156 (2010).
39. Ackermann, S., Hartmann, F., Papassotiropoulos, A., de Quervain, D. J.-F. & Rasch, B. Associations between Basal Cortisol Levels and Memory Retrieval in Healthy Young Individuals. *J. Cogn. Neurosci.* **25**, 1896–1907 (2013).
40. Luine, V., Martinez, C., Villegas, M., Magariños, A. M. & McEwen, B. S. Restraint stress reversibly enhances spatial memory performance. *Physiol. Behav.* **59**, 27–32 (1996).
41. Arnott, G. & Elwood, R. W. Gender differences in aggressive behaviour in convict cichlids. *Anim. Behav.* **78**, 1221–1227 (2009).

42. Coolen, R. L., Cambier, J. C., Spantidea, P. I., Asselt, E. & Blok, B. F. M. Androgen receptors in areas of the spinal cord and brainstem: A study in adult male cats. *J. Anat.* **134**, 7 (2021). doi:10.1111/joa.13407.
43. Braithwaite, V. A. Cognitive Ability in Fish. in *Behaviour and Physiology of Fish* (eds. Sloman, K., Balshine, S. & Wilson, R.) 1–37 (Elsevier Academic Press, 2006).
44. Bass, A. H., Rose, G. J. & Pritz, M. B. Auditory Midbrain of Fish, Amphibians, and Reptiles: Model Systems for Understanding Auditory Function. in *The Inferior Colliculus* (eds. Winer, J. A. & Schreiner, C. E.) 459–492 (Springer-Verlag, 2005). doi:10.1007/0-387-27083-3_16.
45. Amorim, M. C. P., Fonseca, P. J. & Almada, V. C. Sound production during courtship and spawning of *Oreochromis mossambicus*: Male-female and male-male interactions. *J. Fish Biol.* **62**, 658–672 (2003).
46. Vasconcelos, R. O. *et al.* Vocal behavior predicts reproductive success in a teleost fish. *Behav. Ecol.* **23**, 375–383 (2012).
47. Maruska, K. P. & Sisneros, J. A. Sex Steroid-Dependent Modulation of Acoustic Communication Systems in Fishes. in *Sound Communication in Fishes* (ed. Ladich, F.) 207–233 (Springer, 2015). doi:10.1007/978-3-7091-1846-7_7.
48. Maruska, K. P. & Fernald, R. D. Steroid receptor expression in the fish inner ear varies with sex, social status, and reproductive state. *BMC Neurosci.* **11**, (2010).
49. Li, C.-Y., Hofmann, H. A., Harris, M. L. & Earley, R. L. Real or fake? Natural and artificial social stimuli elicit divergent behavioural and neural responses in mangrove rivulus, *Kryptolebias marmoratus*. *Proc. R. Soc. B Biol. Sci.* **285**, 20181610 (2018).
50. Reddon, A. R., O'Connor, C. M., Marsh-Rollo, S. E. & Balshine, S. Effects of isotocin on social responses in a cooperatively breeding fish. *Anim. Behav.* **84**, 753–760 (2012).
51. Scherer, U., Buck, M. & Schuett, W. Lateralisation in agonistic encounters: do mirror tests reflect aggressive behaviour? A study on a West African cichlid. *J. Fish Biol.* **89**, 1866–1872 (2016).
52. Falsarella, L. do N., Brandão, M. L. & Gonçalves-de-Freitas, E. Fish adjust aggressive behavior to audience size with limited information on bystanders' fighting ability. *Behav. Processes* **142**, 116–118 (2017).
53. Gonçalves-de-Freitas, E. *et al.* Social Behavior and Welfare in Nile Tilapia. *Fishes* **4**, 23 (2019).
54. Pickering, A. D., Pottinger, T. G., Carragher, J. & Sumpter, J. P. The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* **68**, 249–259 (1987).
55. Welsh, T. H., Bambino, T. H. & Hsueh, A. J. W. Mechanism of Glucocorticoid-Induced Suppression of Testicular Androgen Biosynthesis In Vitro¹. *Biol. Reprod.* **27**, 1138–1146 (1982).
56. Consten, D., Keuning, E. D., Terlouw, M., Lambert, J. G. D. & Goos, H. J. T. Cortisol effects on the testicular androgen synthesizing capacity in common carp, *Cyprinus carpio* L. *Fish Physiol. Biochem.* **25**, 91–98 (2001).

57. Rodríguez, F. *et al.* Cognitive and emotional functions of the teleost fish cerebellum. *Brain Res. Bull.* **66**, 365–370 (2005).
58. Gauy, A. C. dos S., Boscolo, C. N. P. & Gonçalves-de-Freitas, E. Less water renewal reduces effects on social aggression of the cichlid *Pterophyllum scalare*. *Appl. Anim. Behav. Sci.* **198**, 121–126 (2018).
59. Brandão, M. L., Braithwaite, V. A. & Gonçalves-de-Freitas, E. Isolation impairs cognition in a social fish. *Appl. Anim. Behav. Sci.* **171**, 204–210 (2015).
60. Stiver, K. A. *et al.* Relatedness and helping in fish: examining the theoretical predictions. *Proc. R. Soc. B Biol. Sci.* **272**, 1593–1599 (2005).
61. Revelle, W. *psych: Procedures for Psychological, Psychometric, and Personality Research*. <https://cran.r-project.org/package=psych> (2018).

Figures

Figure 1

Schematic view of the experimental design and set-ups of behavioral tests. **a.** Timeline of events showing the beginning of experiment, when fish were anesthetized, measured, weighed, and allocated in a home aquarium where behavioral tests were carried out. **b.** Aggressiveness test consisting of fish interacting

with their mirror image (30 min). **c.** Inhibitory control test set-up (seen from above), showing the plastic contingency barrier that released the fish at the beginning of the task, as well as the U-shaped barrier that fish had to detour in order to reach the food reward. In this last task, fish were assigned to a training phase, in which they were tested with an opaque U-shaped barrier for meeting a learning criterion. Fish that met the criterion moved onto the test phase, wherein the opaque U-shaped barrier was replaced by a transparent one; fish had to detour it in order to reach the food instead of trying to go through it, thus showing inhibitory control by recovering the learned response from the training phase. Subjects were killed by an anesthetic overdose on day 5; brain and blood were then collected for further analyses.

Figure 2

Hormone levels of pear cichlid females and males. **a.** cortisol; **b.** testosterone; **c.** 11-ketotestosterone (11KT); and **d.** 11-ketotestosterone to testosterone ratio of conversion. Variables were compared through Linear Models. Hormone levels are plotted in different graphic scales. Data are represented as mean $\pm$ SE.

Supplementary Files

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