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Social environment affects inhibitory control via developmental plasticity in a fish

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Abstract

Living in a social group may impose cognitive demands, for example individual recognition, social memory, and the inhibition of behaviour when it is not adaptive. As the neural substrates for these cognitive skills are metabolically expensive, cognitive abilities may be positively related to the complexity of the social system. Where there is large spatio-temporal variation in the ecological conditions experienced and hence in the social system exhibited by species, selection may favour adaptive phenotypic plasticity of cognitive abilities involved in social tasks rather than evolved differences across populations. Here, we tested this hypothesis in a social living teleost fish, the guppy, *Poecilia reticulata*. We exposed new-born guppies to treatments that altered two parameters of social environmental complexity: group size (experiment 1) and group stability (experiment 2). Then, we assessed guppies' inhibitory control, the ability to withhold responding to a stimulus, a cognitive function that is critically involved in social interactions. In experiment 1, guppies reared alone showed higher levels of behavioural inhibition in a foraging task compared to guppies reared in pairs or in groups of 6 individuals. In addition, we found that individuals' variance in performance was smaller for fish raised as singletons. In experiment 2, guppies reared in a stable social group showed greater inhibition compared to guppies from groups subjected to frequent fission-fusion events. These results reveal phenotypic plasticity of inhibitory control in guppies, however, contrary to prediction, indicate greater inhibitory abilities developing in individuals exposed to 'simpler' social environments.

Keywords: executive functions; cognition; individual differences; *Poecilia reticulata*; social brain.

Introduction

The survival and reproduction of a social animal strongly depends on its ability to succeed in social interactions, which necessitates advanced cognitive skills to generate behavioural responses that are adapted to the social context (Fernald, 2017). Inhibitory control is one of the skills considered critically important to optimise social interactions (Amici et al., 2008; Santos et al., 1999), allowing the animal to withhold a behaviour when it is not adapted to the context (Diamond, 2013). For example, in several social species, subordinate individuals inhibit feeding or mating in presence of dominant individuals to avoid aggression (Byrne & Whiten, 1992; Estep et al., 1988; Lindsay et al., 1976).

As developing the neural substrates for cognition is energetically demanding (Kool & Botvinick, 2013; Kotrschal et al., 2013), the evolution of cognitive skills involved in social tasks is expected to be strongly tied to levels of social complexity (the social brain hypothesis; Dunbar et al., 1998). According to this hypothesis, inhibitory control should be enhanced in those species in which social relationships are, on average, more complex (Byrne & Bates, 2007; Bond et al., 2003; Dumbar & Shultz, 2007; Kamil, 2004; Jolly, 1966). A comparative study on seven primates supported this prediction, finding that inhibitory control is enhanced in species with higher levels of fission-fusion dynamics (Amici et al., 2008). Conversely, a subsequent study reported no effect of group size on inhibitory control in 23 primate species (MacLean et al., 2014).

However, the social system experienced by members of the same species often varies widely as a result of spatio-temporal fluctuations in environmental conditions (Creel & Winnie, 2005; Crockett & Eisenberg, 1987; Edenbrow et al., 2011; Foster et al., 2012; Jo Safran et al., 2004; Rushmore et al., 2013; Seghers & Magurran, 1994). In addition, a growing literature suggests the presence of adaptive phenotypic plasticity in cognitive abilities in response to individuals’ experiences during early life (Chivers et al., 2016; Fond et al., 2019; Kotrschal & Taborsky, 2010; Lucon-Xiccato et al., 2016; Lucon-Xiccato et al., 2020a; Salvanes et al., 2013). An individuals' inhibitory control may therefore vary depending on the social environment experienced by an individual, perhaps especially early in life. Such adaptive phenotypic plasticity of inhibitory control would allow matching of the costs of the required neural substrates with the cognitive demands of the specific social environmental experienced by an individual when populations are exposed to changing socio-ecological conditions across generations. A study on a mammal, the spotted hyaenas *Crocuta Crocuta* (Johnson-Ulrich, et al., 2020) and a study on a bird, the Australian magpie *Gymnorhina tibicen* (Asthon et al., 2018) have provided some experimental support to the link between group size and inhibitory control at the intraspecific level. However, there has not yet been any investigation of the link between social complexity and inhibitory control within species in poikilothermic vertebrate taxa.

We tested the presence of a socially-mediated plasticity in inhibitory control in the guppy fish, *Poecilia reticulata*. In this species, several studies have reported refined inhibitory abilities (Lucon-Xiccato et al., 2017; Santacà et al., 2019) and remarkable intraspecific variability in cognition (reviewed in Lucon-Xiccato & Bisazza, 2017), including in inhibitory control (Macario et al., 2021; Savaşçı et al., 2021). Furthermore, variation in social environment has been consistently reported in natural guppy populations (Darden et al., 2020; Edenbrow et al., 2011; Magurran & Seghers, 1990, Seghers & Magurran, 1991; Seghers & Magurran, 1991). We analysed two parameters that are important determinants of social environment complexity, group size and group stability (Amici et al., 2008; MacLean et al., 2014). In our first experiment, we manipulated the group size experienced by guppies by maintaining subjects either alone, in pairs, or in groups of 6 individuals from their first day of life. In our second experiment, we manipulated group stability. We simulated fission-fusion events experienced by guppies in the wild (Croft et al., 2003; Wilson et al., 2014) and manipulated the exposure to these events by either keeping constant social groups or by regularly altering group membership. Following these social treatments, we measured subjects' inhibitory control. We expected that individuals' inhibitory control will be enhanced in experimental populations with larger social groups and the occurrence of frequent fission-fusion events (Amici et al., 2008; MacLean et al., 2014). Larger groups involve a greater number and diversity of social relationships, and hence maintaining a position in the hierarchy should require greater ability to inhibit behavioural responses. Populations with many fission-fusion events should be characterised by continuous changes in social networks and dominance hierarchies, requiring greater inhibition by the individuals to cope with an uncertain social environment.

Materials and methods

Subjects

We used guppies from a population of the snake cobra green strain, maintained in our laboratory since 2012. These guppies were chosen because they adapt quickly to training procedures involving interactions with human experimenters (Mair et al., 2021; Montalbano et al., 2020). The population's minimum size was approximately 1000 individuals. To reduce the chances of inbreeding, we moved guppies between multiple maintenance aquaria and we added new guppies once or twice per year. Mixed-sex groups of guppies were maintained in 200 L plastic aquaria, enriched with gravel and natural plants to simulate natural habitat. Aquaria were provided with air pumps, filters, and heaters (27°C ± 1°C) to ensure stable water conditions. Fluorescent lamps provided illumination with a light/dark cycle of 12h:12h. We fed the guppies twice per day with live *Artemia salina* and crumbled commercial flake food. In the current study, we used 144 guppies (72 in each experiment). They were collected in their first day of life as juvenile guppies begin to engage in social interactions approximately 72h after birth (Gorlick, 1976). This was done by isolating pregnant females and inspecting for the presence of offspring daily.

Social environment treatments

In experiment 1, we manipulated the size of the social group (1, 2, or 6 fish; figure 1a). Social group sizes used in the treatment have been reported in guppies' natural populations (Croft et al., 2003). We randomly allocated 72 guppies to the 3 treatments: 6 groups of 6 subjects were maintained in 6-L aquaria; 12 groups of 2 subjects in 2-L aquaria; and 12 individual guppies in 1-L aquaria so that the density of individuals per water volume was equal in the 3 treatments. The treatment aquaria were enriched with gravel, natural and artificial plants, and an aerator. The treatments were maintained for 6 weeks and we changed 50% of the water from each aquarium with clean, conditioned and temperature matched water three times per week.

In experiment 2, we manipulated the stability of the social group (stable and unstable; figure 1b) using a resampling procedure to mimic a fission-fusion society, which is commonly reported in wild guppies (Auge et al., 2016; Croft et al., 2003; Wilson et al., 2014). We randomly allocated 72 guppies to the 2 treatments. For the variable social group treatment, 6 groups of 6 subjects were maintained in 6 L aquaria as described for experiment 1. Three times per week, these 36 guppies were moved into a 20 L aquarium and immediately resampled to form 6 novel groups. For the stable social group treatment, 6 groups of 6 subjects were maintained in 6 L aquaria. Three times per week, each of these 6 groups was moved one at a time into a 20 L aquarium for 5 minutes and successively placed back into a new 6 L aquarium. This pseudo-resampling treatment controlled for the disruption of moving between aquaria, but maintained the membership of the groups throughout the treatment phase. The treatment phase lasted for 6 weeks and hence each group underwent 18 resampling/pseudo-resampling events. During the resampling/pseudo-resampling procedure, we performed a 50% water change in each aquarium.

Foraging inhibition task

After six weeks in the social treatments, each guppy was tested individually in a foraging inhibition task (figure 1c). Individual testing was necessary to assess individual differences in cognition. Guppies cannot be reliably sexed at this age and therefore our sample was comprised of mixed sex subadults. In the foraging inhibition task, we followed the paradigm developed for this species and other small teleost fish (Lucon-Xiccato et al., 2020b; Lucon-Xiccato & Bertolucci, 2020). It relies on measuring the ability to inhibit foraging behaviour towards a visible prey that cannot be reached because of a transparent barrier. Individuals with higher inhibitory abilities are expected to attempt to attack the prey less often, after experiencing the transparent barrier. This paradigm has been shown to be successful in detecting significant individual differences and it correlates with other measures of inhibitory control (Lucon-Xiccato et al., 2020b; Lucon-Xiccato et al., 2020c; Montalbano et al., 2020).

Initially, we moved each individual into an experimental aquarium filled with 4 L of water. By using multiple aquaria, we tested all guppies from an experiment at the same time. The experimental aquaria had green plastic walls and a transparent lid. Near the front of the lid (figure 1c), there was a circular opening (1.2 cm ∅) that allowed for feeding the subject and performing the experimental procedure. For 3 days (training phase), we fed the guppies with crumbled food mixed with water by inserting a Pasteur pipette through the opening. We took care to release the food only when the guppies showed interest toward the pipette. This habituated the guppies to receiving food in association with the opening in the lid. We performed this feeding procedure 2, 4, and 6 times on days 1, 2, and 3 of the training phase, respectively. All the fish promptly learned this feeding procedure.

On the day following the third day of training, we performed the foraging inhibition test. We inserted a transparent glass tube (length: 10 cm; Ø: 1.2 cm) into the experimental aquaria, through the opening in the lid. The tube contained 4 mL of a solution with live prey, *A. salina* (*N* = approximately 500). We video recorded the response of the subject for 10 minutes using a webcam (ELP 5-50 mm 8MP) placed 1 m above the experimental aquaria. From the video recordings played back on a computer monitor, we recorded the minute from the beginning of the experiment in which each subject attempted to attack the prey for the first time (latency). This variable was considered as a proxy of feeding/foraging motivation in the analyses assuming that more motivated individuals would approach the prey faster. We then scored the number of attacks performed by each subject for 10 consecutive minutes after the first approach to the live prey. This analysis allowed us to standardise the data for differences in motivation or other factors that could cause the subjects to initially approach the prey with different latency. In addition, the fact that the trial started from the first attack for each subject was useful for examining differences in inhibition rate between subjects and treatments: two subjects displaying a different number of attacks in a given minute implies that one of them was faster at inhibiting predatory behaviour after discovering that the prey was inaccessible. Attacks were counted when a guppy touched the glass tube with its snout. Because the fish could not capture the prey through the tube, they were expected to inhibit the attack behaviour. Individuals with higher inhibitory control were expected to perform fewer attacks. Prior experiments have highlighted that non-cognitive factors, such as neophilia/exploration and motivation did not affect this measure of performance (Lucon-Xiccato & Bertolucci, 2019). Fish that did not perform any attacks within 10 minutes were excluded from further analysis. The final sample size was as follows: experiment 1: 1 fish treatment $N = 11$, 2 fish treatment $N = 16$, 6 fish treatment $N = 27$; experiment 2: variable social group treatment $N = 20$, stable social group $N = 22$.

Statistical analysis

We used R Statistical software version 4.0.1 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). All statistical tests were two-tailed and significance was set at $\alpha = 0.05$ unless stated otherwise. First, we compared the measure of motivation (latency with which the fish firstly attempted to reach the prey) across the treatments of the two experiments. Given a non-normal distribution, we used non parametric tests (Wilcoxon test for experiment 1 and Kruskal-Wallis test for experiment 2). Then, we analysed the main dependent variable obtained for each fish, i.e., the number of attacks towards the stimulus prey in 10 1-min time bins. The use of time bins allows to compare inhibition rate across treatments (Lucon-Xiccato & Bertolucci, 2019; Lucon-Xiccato & Bertolucci, 2020). To account for the Poisson distribution and the repeated measures structure of this depended variable, we analysed it with generalised linear mixed-effects models with Poisson error distribution (GLMMs) fitted with the *glmer* function of the *lme4* R package (Bates et al., 2007). We included social environment treatment and time (minutes from 1 to 10) as fixed effects, and subject ID as random effect in the models. The effect of time is difficult to model because its residual plotting suggested a non-linear effect on the dependent variable. This makes the data partially violating assumptions for covariance analysis (Ernst & Albers, 2017; Nimon, 2012). In addition, the experimental design based on recording the dependent variable in 1-minute bins makes the time variable *de facto* a non-continuous variable, which is also not ideal for covariance analysis. An alternative analysis approach would be fitting time as categorical factor; this approach is expected to achieve increased power in the comparison between the experimental groups in a specific experimental period but might suffer limits in considering the order of the time series. As each of the two aforementioned approaches has inferential advantages and disadvantages, we ran each model twice, first fitting time in 1-min bins as a categorical factor and then again fitting time as a continuous covariate. In experiment 1, the social environment treatment was treated as a fixed factor with 3 levels (social group size: 1 fish, 2 fish, or 6 fish). In experiment 2, the social environment treatment was treated as a fixed factor with 2 levels (social group stability: variable or stable). When significant interactions between time and social treatment were identified, we fit post-hoc models on each minute of the experiment separately using generalised linear models (GLMs) with Poisson error distribution and an α level corrected with the Bonferroni method. The post-hoc models were critical to assess differences in inhibition between the experimental groups. The number of attacks per minute was counted from the first attempt to attack the prey; therefore, differences in the number of attacks in a minute indicated that one group of subjects have a faster decrease in attack rate, which is indicative of greater inhibitory abilities. In experiment 1, to further investigate the differences between the 3 levels of the social environment treatment, we used Tukey post-hoc tests. Last, we compared variance of the overall inhibitory performance (sum of the 10 min bins) across the experimental groups of the two experiments using Bartlett test. We log transformed the data to account for the Poisson distribution. For Poisson data (range > 0) variance is expected to increase with sample size. In experiment 1, the sample size of the three experimental groups varied (range 11-27), which could artifactually lead to differences in variance. We therefore ran a simulation analysis that compared same-sized groups ($N = 11$) obtained from 10000 iterations of a resampling procedure for each experimental treatment group in experiment 1. For each simulated dataset, we ran a Bartlett test to compare the variances of the simulated groups and calculated an overall P-value supporting the presence of differences in group variances that are independent of sample size (P_{sim} = N non-significant P-values obtained in the 10000 simulations / 10000).

Animal welfare

Experiments were conducted in accordance with the ABS/ASAB 'Guidelines for the treatment of animals in behavioural research and teaching' (doi: 10.1016/j.anbehav.2019.11.002) and the law of the country in which they were performed (Italy, D.L. 4 Marzo 2014, n. 26). The Ethical Committee of University of Ferrara reviewed and approved the experimental procedures (protocol n. TLX 2-2018-PR).

Results

Experiment 1 – Group size

Analysis on the latency to attack the prey did not reveal differences between the two experimental treatments (*Kruskal-Wallis* $X^2_2 = 0.383$, $P = 0.826$; single fish treatment, mean ± standard deviation: 1.36 ± 1.21; 2 fish treatment: 1.38 ± 1.02; 6 fish treatment: 1.89 ± 2.49).

The GLMM on the number of attacks revealed a significant interaction between group size treatment and time ($X^2_{18} = 212.756$, $P < 0.0001$; figure 2). The interaction was significant also in the model fitted with time as continuous covariate ($X^2_2 = 249.796$, $P < 0.0001$). The main effect of time was also significant ($X^2_9 = 1979.135$, $P < 0.0001$) but the main effect of group size treatment was not ($X^2_2 = 2.121$, $P = 0.346$). The significant interaction was further investigated by testing for the effect of treatment in each minute of the experiment separately with GLMs. The GLMs showed significant effect of group size treatment in minutes 1, 2, 3, 6, 7, 8, 9, and 10, but not in minutes 4 and 5 (table 1). Tukey's post-hoc tests on the GLMs with a significant effect of group size treatment indicated that the subjects from the single fish treatment exhibited lower number of attacks especially in the second half of the experiment (figure 2; table 1).

Analysis of individual variance in the number of attacks revealed a significant difference between group size treatments (*Bartlett's* $K^2_2 = 9.647$, $P = 0.008$; figure 3), mostly due to the single fish treatment exhibiting lower performance variance (0.35) compared to the 2-fish treatment (2.3) and the 6-fish treatment (2.52). The simulation analysis suggested that this effect was not due to the difference in sample size between groups ($P_{sim} = 0.012$).

Experiment 2 – Group stability

Analysis on the latency to attack the prey did not find differences between the two experimental treatments (*Wilcoxon* $W = 198$, $P = 0.535$; stable social group, mean ± standard deviation: 3.05 ± 2.95; unstable social group: 2.35 ± 2.45).

The GLMM on the number of attacks revealed a significant interaction between group stability treatment and time ($X^2_9 = 19.7499$, $P = 0.020$; figure 4). The interaction was marginally significant in the model fitted with time as covariate ($X^2_1 = 3.129$, $P = 0.077$). The main effect of time was also significant ($X^2_9 = 408.919$, $P < 0.0001$) but the main effect of group stability treatment was not ($X^2_1 = 1.945$, $P = 0.163$). The significant interaction was further investigated by testing for the effect of treatment in each minute of the experiment separately with GLMs. The GLMs showed that the subjects from the stable social group treatment performed a lower number of attacks in minutes 1 to 8 (table 1). This difference was not present in the last minutes of the task (9 and 10; table 1), suggesting an effect of habituation and/or learning.

Analysis of individuals' variance in the number of attacks indicated no significant difference between group stability treatments (*Bartlett's* $K^2_1 = 0.039$, $P = 0.843$; stable social group variance = 1.95; stable social group variance = 1.78).

Discussion

The requirements for metabolically expensive cognitive functions due to group living (Kool & Botvinick, 2013; Kotrschal et al., 2013) and the high levels of spatio-temporal variation in the social system experienced by many species (Creel & Winnie, 2005; Foster et al., 2012; Rushmore et al., 2013; Seghers & Magurran, 1994) suggest that social animals might exhibit adaptive phenotypic plasticity of cognitive abilities involved in social tasks (Asthon et al., 2018; Johnson-Ulrich, et al., 2020). Here, we reported evidence of such phenotypic plasticity for inhibitory control in a teleost fish, the guppy, *P. reticulata*. For half of the testing phase in experiment 1, the guppies reared alone exhibited a lower number of attacks towards the inaccessible prey. This effect indicates that guppies reared alone decreased the number of attacks after experiencing that the prey could not be reached more rapidly than did guppies reared in groups. Therefore, is possible to conclude that guppies reared alone exerted greater levels of behavioural inhibition. In experiment 2, the guppies reared in a stable social group consistently showed a reduced number of predation attempts, and therefore greater inhibitory control, compared to the guppies reared in groups with changing composition simulating a high turnover fission-fusion society, commonly observed in guppies from some natural populations (Croft et al., 2003; Wilson et al., 2014). Overall, these results suggest that simpler social environments characterised by small and stable social groups may favour the development of greater inhibitory control in guppies.

Interestingly, our results on the plastic response of the guppy to social experiences early in life do not fit with the logic of the social brain hypothesis which predicts that more complex social environments should be linked to greater cognitive abilities on evolutionary time scales (Dunbar, 1998). Empirical support for the social brain hypothesis has been mostly drawn from primates (e.g., Amici et al., 2008; MacLean et al., 2014), but it is also provided by studies in other mammals such as ungulates and carnivores (Dunbar & Shultz, 2007) and in some bird taxa (Ashton et al., 2018; Emery et al., 2007). However, evidence also indicates that this evolutionary hypothesis might not consistently apply across all taxa (e.g., Shultz & Dunbar, 2007). The specific ecological, life history, and social characteristics of a given species or taxon may affect the relationship between cognition and social system (Thornton & McAuliffe, 2015). Guppies show remarkable social sophistication, including tit-for-tat cooperation strategies, complex social networks and durable social relationships (Cattelan et al., 2019; Croft et al., 2006; Dugatkin, 1988), however, it is undeniable that the breadth and depth of their social behaviour repertoire is limited compared to that of higher vertebrates. For instance, highly structured primate societies with strong hierarchies and long-term relationships likely create a situation wherein an individuals' behaviours have long term consequences, providing selective pressures for heightened inhibitory control. Conversely, in guppy societies with high levels of fission-fusion events (Croft et al., 2003; Wilson et al., 2014), these consequences might be less relevant. High competitiveness during foraging (Bashey, 2008; Chuard et al., 2018) allows a guppy to obtain more food leading to fitness benefits with limited socially mediated costs when other group members are absent or often changing. This might explain the finding that guppies raised in stable social groups developed higher inhibitory control compared to guppies raised in unstable social groups (experiment 2). Considering that aggression and competition increase with group size in guppies (Magurran & Seghers, 1991), it also seems reasonable that guppies from the more complex social treatments may have developed reduced inhibitory control compared to guppies raised alone (experiment 1). However, this interpretation is difficult to reconcile with a recent report showing that in some circumstances foraging efficiency is improved when guppies are in larger social groups (Snijders et al., 2021).

Before accepting an interpretation based on guppies' social ecology, two other explanations should be considered in future studies. First, the contradictory effects of social complexity on inhibitory control between guppies and primates may be due to differential effects of the social environment on developmental plasticity within species compared to interspecific variation on evolutionary timescales (but see Ashton et al., 2018). Second, we cannot exclude that the domesticated nature of the guppies used in this study had a role in their response to the developmental social environment. For example, domesticated zebrafish, *Danio rerio*, rely less on social information than their wild counterparts (Zala et al., 2012), and the fact that domesticated guppies shoal less than feral guppies (Swaney et al., 2015) suggest differences in sociability might exist also between domesticated and wild guppies. To disentangle these possibilities, it will be necessary to gather more data on the social brain hypothesis in teleost fishes, as well as in other vertebrates. Further comparisons of wild and domestic guppy strains would also be informative.

Given the broad effects of social environment on the endocrine system (Hellmann et al., 2015; Reddon et al., 2015) and the effects of hormones on cognition (Eaton et al., 2015), it is also worth considering hormonal underpinnings of the plasticity that we detected. In humans, substantial evidence indicates that stress hormones increase inhibitory control (reviewed in Shields et al., 2016). Stress due to the lack of social companions might have caused the higher inhibition in the individuals raised alone in experiment 1. However, guppies are often found in small social groups (< 6 individuals) or alone in the wild (Croft et al., 2006), suggesting that at least short-term isolation from social fellows is ecologically realistic, and unlikely to be highly stressful. Chouinard-Thuly et al. (2018) did not detect any effect of acute social isolation on the stress response in guppies. The stress mediated explanation does not seem to fit with the effects of group stability in experiment 2 because social fish prefer familiar conspecifics (Griffith & Magurran, 1999; Magurran et al., 1994), and social disruption is likely to be more stressful than consistent group membership (Bhat & Magurran, 2006; Chivers et al., 1995; Lucon-Xiccato & Griggio, 2017), yet the fish from the socially unstable treatment showed lower inhibitory control. It should be also noted that a recent study has suggested that early social experience has no effect on stress-induced cortisol levels in another species of fish (Antunes et al., 2020). Collectively, the interpretation based on developmental organisation of the stress response seems an unlikely explanation for the results of this study, however, confirmation by examining the stress response in guppies following similar social manipulations would be worthwhile. Future studies should also investigate the effects on inhibitory control of other hormones. For example, manipulation of isotocin (the teleost fish homologue of the mammalian nonapeptide oxytocin) has been shown to alter the response to social disruption in the daffodil cichlid, *Neolamprologus pulcher* (Hellmann et al., 2015). The relationship between isotocin and inhibitory control is so far unknown, yet oxytocin has been related to inhibitory processes of hunger in rats (Stricker & Verbalis, 1987).

One or more non-hormonal mechanisms may be also involved in guppies' inhibitory control plasticity. For example, in fishes, the social environment can affect cerebral lateralisation (Biost et al., 2013; Schaafsma & Groothuis, 2011), namely how the brain splits information processing between the two hemispheres. The degree of cerebral lateralisation affects inhibitory control performance in the zebrafish, *Danio rerio* (Lucon-Xiccato et al., 2020c). Furthermore, both personality and brain size, which in fish, are affected by both group size (Castanheira et al., 2019; Gonda et al., 2009) and familiarity with group members (Galhardo et al., 2012), have been related to inhibitory control (Buechel et al., 2018; Lucon-Xiccato et al., 2020b). These factors should be investigated along with physiological mechanisms to explain the effects observed in the present study.

Socially-driven plasticity of inhibitory control might have consequences for individual guppies and their fitness that go beyond social interactions. Core executive functions, to which inhibitory control belongs, are simple cognitive modules that are activated when an animal performs many activities (Diamond, 2013). For example, inhibition of a behaviour is expected to be advantageous anytime the animal must adapt to a changing situation (Chow et al., 2020). Inhibitory control can help animals to deal with changes in levels of competition, including in the case of the invasion of an alien species (Hoare et al., 2007). Literature also suggests that low inhibition and high persistency might help individuals to find the solution for a problem during foraging (Chow et al., 2016). Other effects of inhibitory control can be visible during reproductive processes, such as mate choice, as suggested by two recent studies on sticklebacks, *Gasterosteus aculeatus* (Keagy et al., 2019; Minter et al., 2017). To understand these and other indirect consequences of socially-driven changes in executive functions it will be important to fully characterise their relationship with fitness.

Notably, our analysis indicated that the group size manipulation of experiment 1 not only affected average inhibitory control performance of guppies, but also its variability. Guppies reared alone displayed lower interindividual variance in performance compared to the other groups. Within-group variability was not affected by the manipulation of group stability performed in experiment 2. Changes in cognitive variance have recently been described in response to predation risk (Lucon-Xiccato et al., 2020d). However, their causes and consequences are currently unclear. Undeniably, life in a group allows individuals to reach behavioural decisions based on collective mechanisms (Sumpter et al., 2008; Ward et al., 2008; Ward et al., 2012). This may allow for the development of greater variability in cognitive performance as disadvantageous phenotypes can follow the group reducing the costs of poor decisions. Furthermore, it is possible that the social group favours the development of large differences between individuals in process similar to social niche partitioning (Montiglio et al., 2013; von Merten et al., 2017).

In conclusion, this study revealed phenotypic plasticity of inhibitory control in relation to the social environment experienced by developing guppies. Socially-driven phenotypic plasticity, as well as plasticity mediated by other factors (van Horik et al., 2019), might therefore interact with genes (Langley et al., 2020) in determining individual differences in inhibitory control in natural populations. Furthermore, the present study showed that the effect of social complexity on inhibitory control in the guppy is the opposite of what has been reported in mammals and birds (Asthon et al., 2018; Johnson-Ulrich, et al., 2020), suggesting that the relationship between sociality and cognition may not be straightforward and that the particulars of a species ecology, biology, or life history may have important effects which deserves attention in future research.

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Declarations of interest

None.

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Tables

Table 1. Post-hoc analysis of the effect of group size treatment on inhibitory control (experiment 1). In the GLMs, α level for significance was corrected with Bonferroni method ($P = 0.005$). Bold indicates significant results.

Minute	GLM	Tuckey's post-hoc test
1	**$X^2_2 = 101.410$, $P < 0.0001$**	**Single vs. Pair: $P < 0.0001$** Single vs. 6 fish: $P = 0.290$ **Pair vs. 6 fish: $P < 0.0001$**
2	**$X^2_2 = 15.375$, $P = 0.0005$**	Single vs. Pair: $P = 0.564$ Single vs. 6 fish: $P = 0.075$ **Pair vs. 6 fish: $P < 0.001$**
3	**$X^2_2 = 18.423$, $P < 0.0001$**	Single vs. Pair: $P = 0.964$ **Single vs. 6 fish: $P = 0.010$** **Pair vs. 6 fish: $P < 0.001$**
4	$X^2_2 = 10.050$, $P = 0.006$	-
5	$X^2_2 = 7.798$, $P = 0.021$	-
6	**$X^2_2 = 20.464$, $P < 0.0001$**	**Single vs. Pair: $P = 0.001$** **Single vs. 6 fish: $P < 0.001$** Pair vs. 6 fish: $P = 0.958$
7	**$X^2_2 = 45.200$, $P < 0.0001$**	**Single vs. Pair: $P < 0.0001$** **Single vs. 6 fish: $P < 0.0001$** Pair vs. 6 fish: $P = 0.988$
8	**$X^2_2 = 24.464$, $P < 0.0001$**	**Single vs. Pair: $P < 0.0001$** **Single vs. 6 fish: $P < 0.0001$** Pair vs. 6 fish: $P = 0.988$
9	**$X^2_2 = 28.269$, $P < 0.0001$**	**Single vs. Pair: $P < 0.001$** **Single vs. 6 fish: $P = 0.036$** **Pair vs. 6 fish: $P = 0.007$**
10	**$X^2_2 = 17.292$, $P = 0.0002$**	**Single vs. Pair: $P < 0.001$** **Single vs. 6 fish: $P = 0.004$** Pair vs. 6 fish: $P = 0.489$

Table 2. Post-hoc analysis of the effect of group stability treatment on inhibitory control (experiment 2). In the GLMs, α level for significance was corrected with Bonferroni method ($P = 0.005$). Bold indicates significant results.

Minute	GLM
1	**$X^{21} = 46.131$, $P < 0.0001$**
2	**$X^{21} = 10.259$, $P = 0.001$**
3	**$X^{21} = 9.102$, $P = 0.003$**
4	**$X^{21} = 25.586$, $P < 0.0001$**
5	**$X^{21} = 16.099$, $P < 0.0001$**
6	**$X^{21} = 10.720$, $P = 0.001$**
7	**$X^{21} = 10.405$, $P = 0.001$**
8	**$X^{21} = 19.880$, $P < 0.0001$**
9	$X^{21} = 0.600$, $P = 0.439$
10	$X^{21} =3.689$, $P = 0.055$

Figures

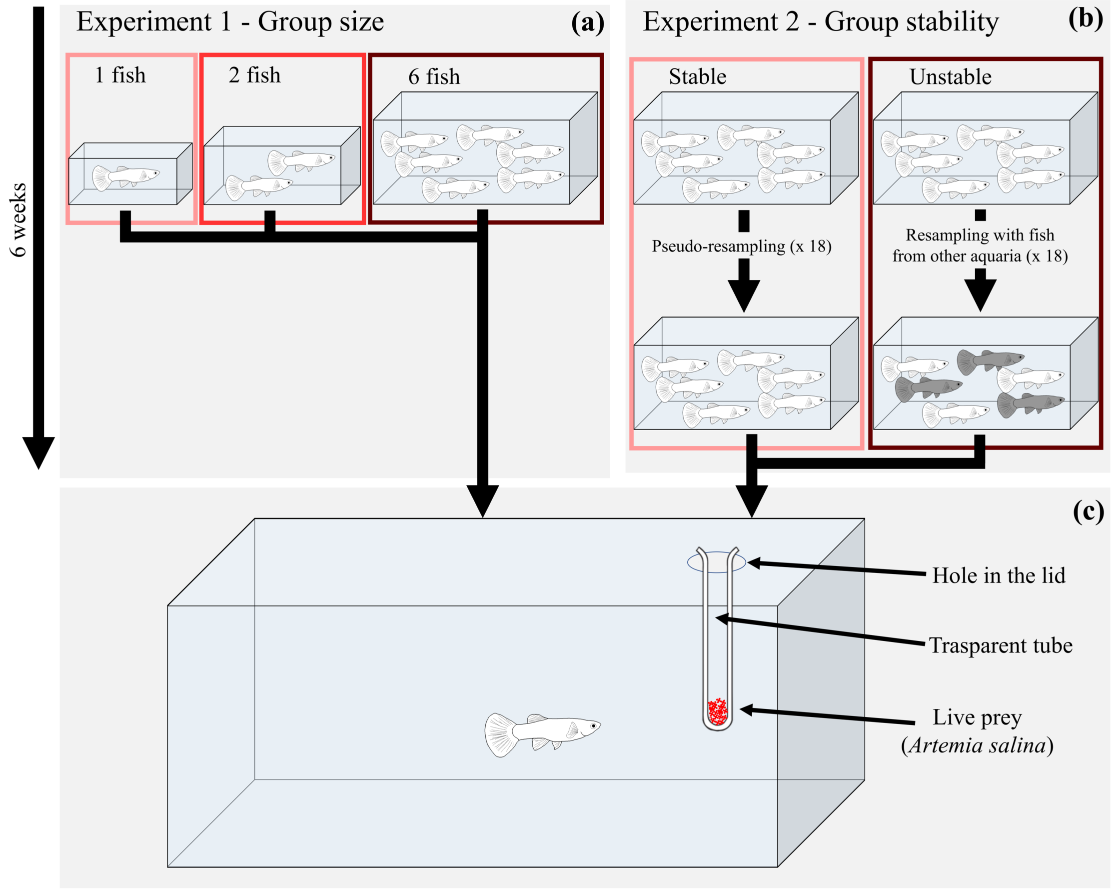

Figure 1

Overview of the experimental methods. (a) Scheme of experiment 1; (b) scheme of experiment 2; and (c) lateral view of the apparatus used for the inhibition task.

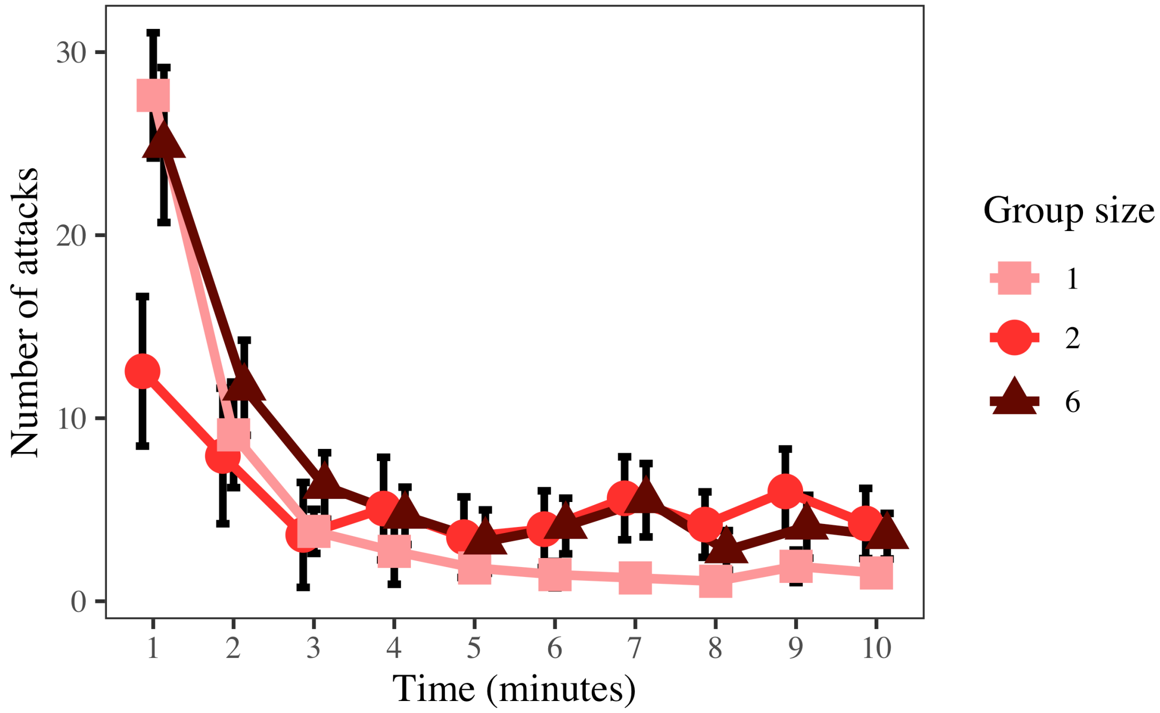

Figure 2

Effects of group size on inhibitory control (experiment 1). Points represent mean number of attacks toward the prey and error bars represent SEM.

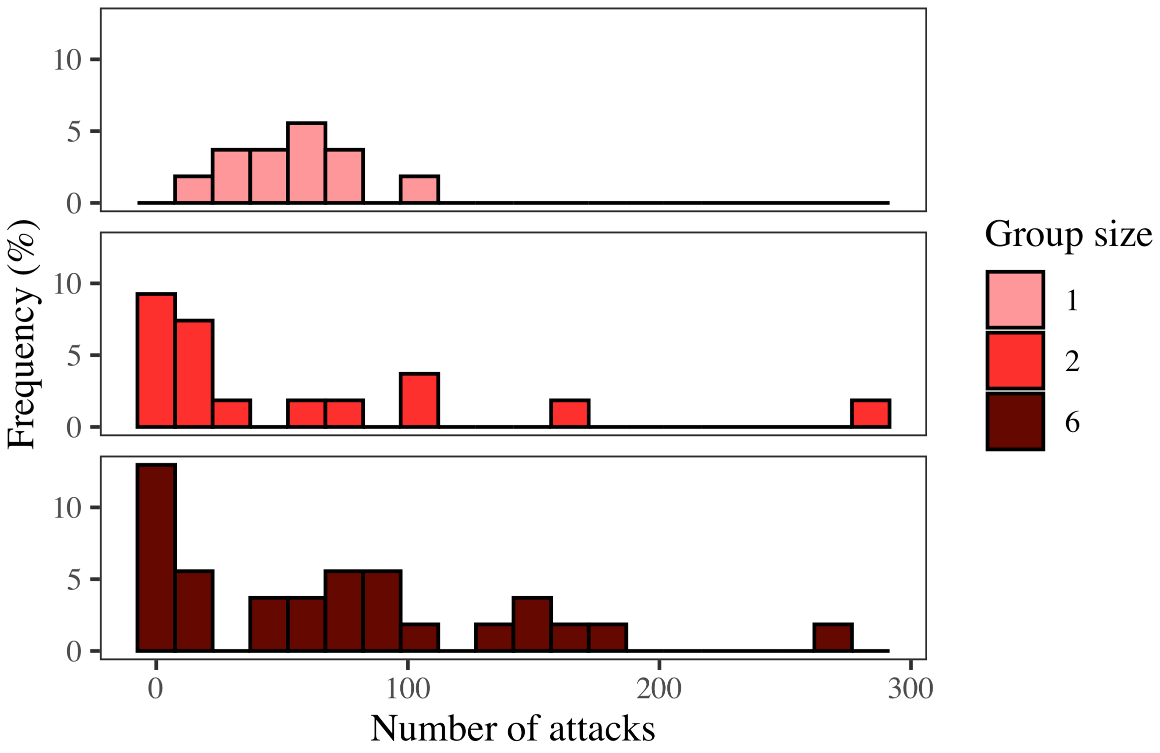

Figure 3

Variability of individual differences in inhibitory control due to group size (experiment 1). Frequency distribution plot of number of attacks toward the prey performed by guppies in the entire test.

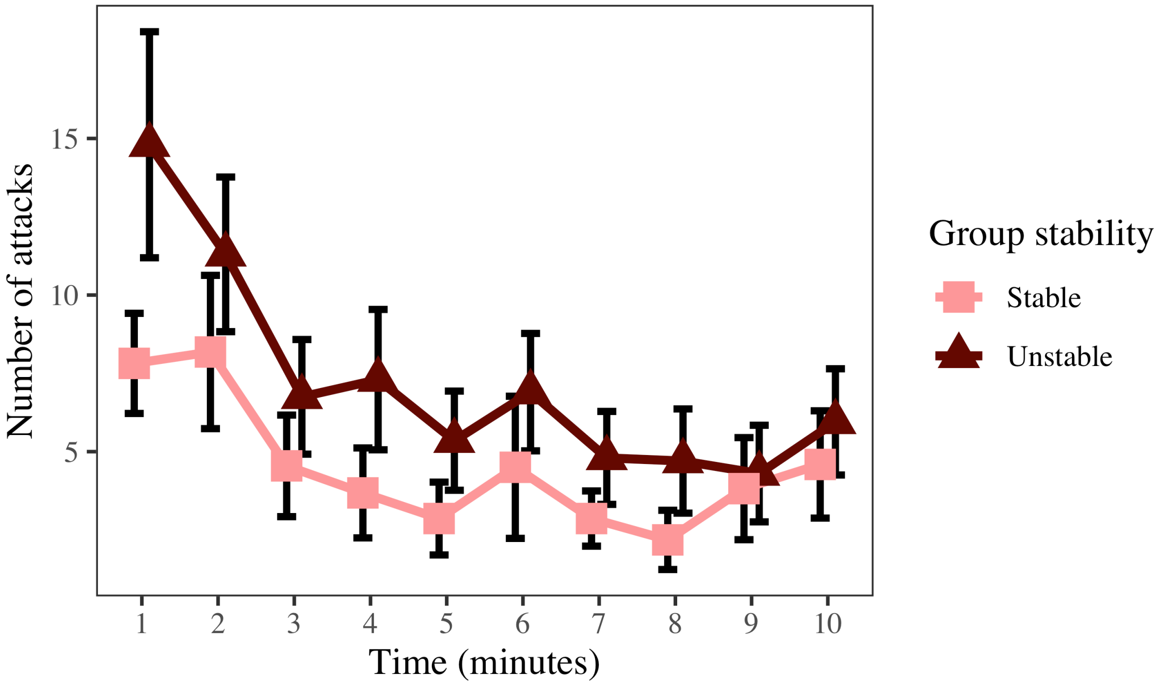

Figure 4

Effects of group stability on inhibitory control (experiment 2). Points represent mean number of attacks toward the prey and error bars represent SEM.