



The effect of sex, age and boldness on inhibitory control

Ivan M. Vinogradov^{a,*}, Michael D. Jennions^a, Eleanor van Veen^a, Claudia Fichtel^b, Peter M. Kappeler^{b,c}, Rebecca J. Fox^a

^a Division of Ecology & Evolution, Research School of Biology, The Australian National University, Canberra, ACT, Australia

^b Behavioral Ecology and Sociobiology Unit, German Primate Center – Leibniz Institute for Primate Research, Göttingen, Germany

^c Dept of Sociobiology/Anthropology, Johann-Friedrich-Blumenbach Institute of Zoology and Anthropology, University Göttingen, Göttingen, Germany

ARTICLE INFO

Article history:

Received 11 June 2022

Initial acceptance 13 July 2022

Final acceptance 18 July 2022

Available online 7 October 2022

MS number 22-00288

Keywords:

cognitive ability
cognitive ageing
detour test
fish cognition
inhibitory control
problem solving
sex difference

Inhibitory control requires an individual to suppress impulsive actions in favour of more appropriate behaviours to gain a delayed reward. It plays an important role in activities such as foraging and initiating mating, but high within-species variation suggests that some individuals have greater inhibitory control than others. A standard index of inhibitory control used in many taxa is measuring how long an animal persists in trying to move itself or an appendage (e.g. its hand) through a transparent barrier to reach a reward. Although recent nonhuman studies have investigated how different factors are associated with variation in inhibitory control, these studies have rarely considered how these factors interact. Here we investigated how sex, age, personality (boldness) and the type of reward stimulus interact to predict the degree of motor inhibitory control in eastern mosquitofish, *Gambusia holbrooki*. We measured inhibitory control using a standard detour assay, 'boldness' (time to emergence in a novel environment), and the rate of learning. There were three different reward stimuli: a shoal of females, a shoal of males or a mixed-sex shoal. Individuals were tested in four consecutive trials, always with the same reward type, to quantify short-term learning. These measures were repeated at 7, 14 and 21 weeks after maturation to examine the effect of age. Females had significantly greater inhibitory control than males. Regardless of sex, older fish had significantly greater inhibitory control than younger fish, and boldness predicted learning ability. The type of reward stimuli had no sex-specific effect on inhibitory control. We discuss the biological significance of these sources of variation in inhibitory control, and the importance of accounting for them in studies examining individual differences in cognitive abilities.

© 2022 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Inhibitory control allows individuals to inhibit impulsiveness to obtain delayed rewards (Diamond, 2013), and it can elevate fitness by facilitating efficient foraging (Coomes et al., 2021; Rosati, 2017; Ryer & Olla, 1991) or increasing mating success (Keagy et al., 2019; Minter et al., 2017). For example, greater inhibitory control is advantageous when it is beneficial to behave flexibly (Coomes et al., 2021), such as to delay feeding in the presence of a socially dominant individual (Johnson-Ulrich & Holekamp, 2020), or refrain from engaging in sexual behaviour at inappropriate times (Rodriguez-Nieto et al., 2019). Strong inhibitory control is associated with greater intelligence in humans (Shamosh et al., 2008), and improved behavioural flexibility and larger brain size in primates (Amici et al., 2008; MacLean et al., 2014). Within species there is often high variability in inhibitory control among individuals, as seen in mammals (Johnson-Ulrich & Holekamp, 2020), birds

(Kabadayi, Jacobs, et al., 2017; Kabadayi, Krasheninnikova, et al., 2017; Meier et al., 2017) and fish (Lucon-Xiccato et al., 2020; Macario et al., 2021; Savaşçı et al., 2021). This variation is sometimes associated with key life history traits (e.g. development, Diamond, 1990), personality traits (Dougherty & Guillette, 2018; Griffin et al., 2015) and measures of cognitive performance such as learning ability (Rasolofoniaina et al., 2021; Thornton & Samson, 2012). Two other major sources of variation in inhibitory control within species are age and sex (e.g. Lucon-Xiccato, 2022).

The effect of age on inhibitory control has been a recent focus of interest. Inhibitory control tends to improve with age (e.g. primates, Vlamings et al., 2010; but see Henke-von der Malsburg et al., 2021; dogs, *Canis lupus familiaris*, Lazarowski et al., 2020; ravens, *Corvus corax*, Kabadayi, Jacobs, et al., 2017), but, as with most cognitive functions, it eventually declines late in life due to senescence (Hu et al., 2018; Sadoun et al., 2019). A well-studied nonhuman model for cognitive ageing is the zebrafish, *Danio rerio* (see Adams & Kafaligonul, 2018), in which initial cognitive improvement and late-life cognitive impairment are both observed over the

* Corresponding author.

E-mail address: ivan.vinogradov@anu.edu.au (I. M. Vinogradov).

approximately 3-year life span (Ruhl et al., 2016). Unfortunately, most studies of nonhuman animals have tested individuals over a far shorter time frame than their natural life span, which reduces the likelihood of detecting cognitive senescence.

Sex differences in inhibitory control vary strikingly across species. Some species exhibit clear sex differences such as humans (Mansouri et al., 2016) and some fishes (Lucon-Xiccato & Bisazza, 2017; Lucon-Xiccato, Gatto, et al., 2019; Lucon-Xiccato, Montalbano, et al., 2019), whereas others do not (nonhuman primates, Henke-von der Malsburg et al., 2021; pheasants, *Phasianus colchicus*, van Horik et al., 2018; Clark's nutcrackers, *Nucifraga columbiana*, Vernouillet et al., 2016; North Island robins, *Petroica longipes*, Shaw, 2017; dogs, Vernouillet et al., 2018). Sex differences where females show greater inhibitory control than males have been attributed to males being under stronger selection to mate indiscriminately, with negative pleiotropic effects on their inhibitory control (Lucon-Xiccato, Gatto, et al., 2019; Keagy et al., 2019; Brandão et al., 2019; but see Savaşçı et al., 2021). There are other explanations too. For example, sex differences in inhibitory control in three-spined sticklebacks, *Gasterosteus aculeatus*, were attributed to lower neophobia in males (Keagy et al., 2019). Males were more likely than females initially to approach a transparent test barrier, which resulted in lower inhibitory control measures based on time to reach a reward. This implies that personality traits might generate variation in measures of inhibitory control not only between the sexes, but also among individuals within each sex. A recent meta-analysis reported that cognitive traits and personality characteristics tend to be correlated, although the nature of the relationship varies greatly among species and can differ between the sexes (Dougherty & Guillette, 2018). Although some studies found a link between personality traits (e.g. boldness or exploration tendency) and inhibitory control (Ferland et al., 2014; Gomes et al., 2020; Lucon-Xiccato, Montalbano, et al., 2019; Savaşçı et al., 2021), other studies have not (Guillette et al., 2015; Rasolofoniaina et al., 2021; Stow et al., 2018; van Horik et al., 2018).

A classic method to measure inhibitory control is to present an individual with a reward (usually food or access to conspecifics) that is visible through a transparent barrier. The individual must then inhibit its impulse to go directly to the target, and instead take the extra time to detour around it. The number of attempts and/or time spent trying to pass through the barrier and the total time taken to reach the reward are common measures of inhibitory control. Taxon-appropriate versions of inhibitory control tests have been used to study primates (e.g. Manrique & Call, 2015), other mammals (e.g. Junttila et al., 2021; Juszczak & Bobrowska, 2020), birds (e.g. Wascher et al., 2021), reptiles (e.g. Szabo et al., 2020) and fish (e.g. Savaşçı et al., 2021).

Recent studies on inhibitory control suffer from three limitations. First, most studies have small sample sizes (usually fewer than 30 fish) which reduce the statistical power to detect true effects of focal factors on inhibitory control. Second, factors of interest tend to be investigated individually in separate experiments: interactions between factors are rarely tested. Third, tests of age-related effects in fish tend to exclude individuals at older ages that equate to the natural life span.

Here we investigated the effect of sex, age, the type of reward stimulus, a personality trait ('boldness') and the interactions between these factors on inhibitory control in eastern mosquitofish, *Gambusia holbrooki*. Mosquitofish are a sexually dimorphic, freshwater live-bearing fish. Males constantly attempt to coercively mate, and females continually try to evade and/or attack males (Bisazza & Marin, 1995). These sex differences make *G. holbrooki* an ideal model to test for sex differences in boldness and inhibitory control (see also Michelangeli et al., 2020). We expected males to have low inhibitory control and to be bolder than females because

they mate indiscriminately and may benefit more from risk-taking behaviours due to a stronger link between mating and reproductive success (Janicke et al., 2016). We initially tested males and females at 7 weeks after maturation in a detour assay where a focal individual was presented with one of three reward stimuli: a shoal of females, a shoal of males or a mixed-sex shoal. We expected that the motivation of male and female *G. holbrooki* to join a shoal would differ depending on how many males or females it contained: males prefer to approach females and females tend to avoid males (Agrillo et al., 2006). The focal individual had to inhibit its impulse to swim through a transparent barrier that blocked the direct route to the shoal, and instead had to detour around it to reach the shoal. We recorded three variables: (1) the time taken to leave the start zone as a measure of boldness; (2) the time spent trying to swim through the transparent barrier as a measure of inhibitory control; and (3) the total time taken to reach the shoal once the fish left the start zone. Each individual was tested in four consecutive trials to quantify short-term learning (i.e. a decrease in solving time). We then repeated the experiment on the same individuals at 14 and 21 weeks to test for cognitive senescence. In our source population most fish live as adults for only a single breeding season of 16–24 weeks (Kahn et al., 2013). Our study was designed to gain a better understanding of age-related variation in inhibitory control than other longitudinal studies by repeated testing of males and females. In addition, we accounted for potential sex-specific effects of reward shoal composition, personality (i.e. boldness) and, importantly, how these factors interact with sex.

We had three main aims: (1) to test whether inhibitory control changes with age in *G. holbrooki*; (2) to test whether boldness is correlated with the level of inhibitory control; and (3) to test for sex differences in inhibitory control and whether these depend on the type of reward shoal (e.g. males might show less inhibitory control than females when presented with females). For aims (1) and (2) we were also interested in testing for a sex difference.

METHODS

Origin and Maintenance of Fish

We used hand nets to collect juvenile fish from the wild in Canberra, Australia. We transported fish in water from the collection site. Fish were moved to 90-litre stock tanks in the aquarium facility at the Australian National University (≤ 50 fish per 90-litre aquarium) and reared to maturity. They were kept at a constant temperature (28 ± 1 °C) on a 14:10 h light:dark cycle, and fed twice a day on commercial fish flake and *Artemia* nauplii. Five weeks after maturation (i.e. 5 weeks 'adult age'), fish were randomly assigned to holding tanks at a 1:1 sex ratio with 30 fish per 90-litre aquarium. They were then immobilized in cold water and subcutaneously injected with an elastomer tag to identify individuals throughout the study (see Bookmythe et al., 2013). Individuals regained normal behaviour within a minute of returning to a stock tank and were not tested for at least 3 days after injection.

Experimental Procedure

At 7 weeks adult age, we randomly assigned focal test fish to one of three social reward treatments: a group of six male conspecifics, a group of six female conspecifics or a mixed group of three male and three female conspecifics. The 'stimulus' fish were randomly drawn from stock tanks of nontest fish every morning and returned to the stock tanks at the end of the day. To examine the effects of age on inhibitory control, the focal fish were retested at 14 and 21 weeks adult age. Each individual was tested with the same social reward type at all three ages.

The individual being tested was placed in the 'start zone' of a large tank (60×42 cm and 40 cm high) containing a transparent barrier directly between the start and social reward tank (Fig. 1). The fish could leave the 'start zone' immediately or initially stay there, providing us with a measure of boldness (i.e. willingness to enter a novel environment). The focal individual then had to inhibit its impulse to swim through the transparent barrier which appeared to offer a direct route to the shoal of conspecifics. Instead, the individual had to detour around the barrier to reach the shoal (which was defined as entering the 'goal zone' around the reward tank). The trial began when the focal individual left the start zone and ended when it reached the goal zone, or after 20 min had elapsed. If the fish reached the goal zone within 20 min, it was left to interact with the six conspecifics for 5 min (the 'reward' time), after which it was returned to the start zone for the next trial. Each fish was tested in four consecutive trials.

We had predetermined exclusion criteria for fish that failed to solve the task. Of 258 fish tested, 251 contributed data to the analysis. If a fish did not reach the stimulus within 20 min on its first trial, it was removed from the apparatus and retested the next day. If a fish failed its first trial on 3 consecutive days, it was removed from the experiment. If a fish did not reach the 'goal zone' within 20 min on its second, third or fourth trial, it was moved to the goal zone and given a 5 min reward, after which the trial was repeated immediately. If a fish failed four consecutive attempts for trials 2, 3 or 4, it was removed from the experiment. If an individual was removed from the experiment, its data were discarded from the relevant analysis for that age group. Our sample sizes for fish tested at 7 weeks of age were 129 males, of which 41, 43 and 45 were tested with male (MM), female (FF) and mixed-sex (MF) stimuli groups, respectively, and 122 females, of which 46, 43 and 33 were tested with MM, FF and MF conspecific stimuli groups, respectively. These numbers declined to 48 males (MM = 15, FF = 12, MF = 21) and 54 females (MM = 21, FF = 14, MF = 19) tested at 21 weeks of age due to natural mortality or failure to complete trials.

All trials were videorecorded and data were then collected by an observer (I.V.) blinded to stimulus type and focal fish ID (elastomer tags are only visible under UV light). The three dependent variables that we recorded were: (1) time taken to leave the start zone, which

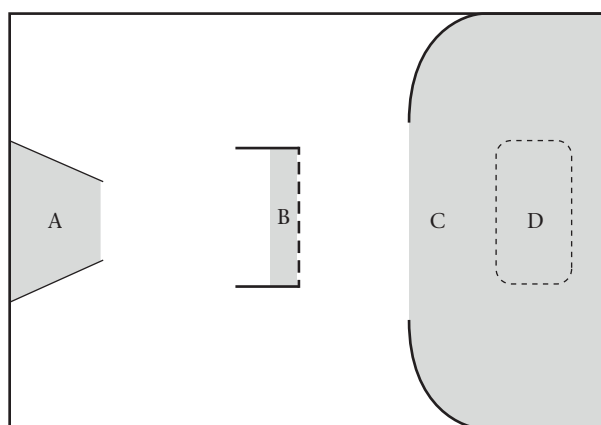


Figure 1. Diagram of the inhibitory control test apparatus inside a glass tank (60×42 cm and 40 cm high). Solid and dotted lines indicate opaque and transparent walls, respectively. Each focal fish started its trial in the start zone (A). The time taken to leave the start zone (i.e. fully cross the border) was a measure of 'boldness'. A small transparent plastic tank (D; 30×19 cm and 20 cm high) containing a group of conspecifics (six males, six females or three males and three females) was located opposite the start zone, behind a 15 cm transparent barrier (B). The total time a fish spent within 2.5 cm of the barrier was a measure of inhibitory control. The time it took a fish to reach the stimulus (i.e. cross the line) in the goal zone (C) after leaving the start zone was a measure of solving time.

we describe as a measure of 'boldness' (White et al., 2013); (2) time spent actively trying to pass through the barrier as a measure of 'inhibitory control' (including 0 values for fish that did not approach the barrier at all); and (3) overall time taken to reach the goal zone (excluding the time spent in the start zone) as a measure of 'solving time'.

Statistical Analysis

We preregistered our plan for statistical analysis on OSF (<https://osf.io/eb5pn>). The analysis was run in R v4.1.0 (R Core Team, 2019) using the packages GLMMadaptive and glmmTMB.

We ran separate hurdle lognormal mixed models (GLMM) in the GLMMadaptive package (Rizopoulos, 2021) to quantify the effect of sex, age, stimulus type and trial order on inhibitory control and boldness, respectively. Trial order was treated as a continuous variable in this and all subsequent models. We ran hurdle models because boldness and inhibitory control had zero-inflated distributions. When inhibitory control was 0 (i.e. a fish did not try to swim through the transparent barrier), the hurdle component of the model calculated the likelihood of an individual not approaching the barrier. When boldness was 0, the hurdle component calculated the likelihood of an individual immediately leaving the start zone.

We ran three separate linear mixed-effect models (LMEM) using the glmmTMB package to analyse the effect of sex, age, stimulus type and trial order on boldness, inhibitory control and solving time, respectively. Boldness was also included as a covariate in the models analysing variation in solving time and inhibitory control. In each model, trial order, age, sex, stimulus type and boldness were treated as fixed factors, while fish ID was a random factor to account for repeated testing of the same individuals. We initially included all two-way and three-way interactions between age, sex and stimulus type in our models. When the three-way interaction was not significant, it was dropped from the model. The same process was then repeated for nonsignificant two-way interactions. The reason for excluding nonsignificant interactions is to report the main effects correctly. If any of the models showed one or more significant interactions involving sex, we ran separate models for males and females to test for any sex-specific effects of trial order, age, stimulus type and boldness on response variables.

Finally, we ran two models to investigate variation in solving time and inhibitory control that explicitly tested for a sex difference in the effect of an individual's boldness. We again included trial order, age, sex and stimulus type, but also included the two-way interaction between boldness and sex. We emphasize that all these analyses were planned and registered on OSF prior to being conducted.

Ethical Note

The collection of animals was conducted under a Scientific Licence from the Australian Capital Territory (ACT) Government, granted under Section 21 of the Fisheries Act 2000, licence number FS20188. Collection, housing and experimental work conducted as part of the study followed the ASAB/ABS guidelines for the treatment of animals in behavioural research. Housing conditions, handling and experimental monitoring were conducted so as to maximize the animals' welfare. Fish were returned to stock tanks after the experiment. All experimental procedures were carried out under approval from ANU Animal Ethics Committee (Approval #A2018/27) and complied with existing laws regulating the treatment of vertebrates in Australia.

Table 1
Parameter estimates for hurdle lognormal mixed-effects model predicting boldness (time spent in the start zone) for male and female mosquitofish, *G. holbrooki*

Predictor	Estimate	SE	χ^2	<i>P</i>
Males				
Stimulus (MF)	0.027	0.189	1.752	0.416
Stimulus (MM)	0.235	0.194		
Age 14 weeks	0.081	0.132	0.862	0.650
Age 21 weeks	0.122	0.133		
Trial order	-0.070	0.042	2.790	0.095
Females				
Stimulus (MF)	0.006	0.189	0.014	0.993
Stimulus (MM)	-0.017	0.175		
Age 14 weeks	0.038	0.133	7.818	0.020
Age 21 weeks	-0.284	0.123		
Trial order	-0.062	0.040	2.381	0.123

Model output is shown for fixed effects. Estimates and SEs were obtained from the model summary. Chi-square and *P* values were calculated with a likelihood ratio test. The reward stimulus was a shoal of female (FF), male (MM) or mixed-sex (MF) conspecifics visible to a focal fish through the barrier. Statistically significant result is shown in bold ($P < 0.05$). The zero-part coefficients (i.e. likelihood of boldness being 0) from the model are presented in [Table A1](#).

RESULTS

Differences in Boldness

There was no significant sex difference in how the interaction between age and reward stimulus type affected boldness (GLMM hurdle: $\chi^2 = 11.259$, $P = 0.187$), but there was a sex difference in how age affected boldness (GLMM hurdle: $\chi^2 = 16.018$, $P = 0.003$), suggesting that females, but not males, became bolder as they aged. We therefore ran separate models for each sex ([Table 1](#), [Appendix Table A1](#)). Reward stimulus did not significantly interact with age for either males (GLMM hurdle: $\chi^2 = 9.103$, $P = 0.334$) or females (GLMM hurdle: $\chi^2 = 7.017$, $P = 0.071$).

Males were significantly more likely to leave the start zone immediately in later trials ($P = 0.020$), but trial order had no significant effect on the measure of boldness for males that delayed leaving the start zone ($P = 0.095$). In contrast, females were not

significantly more likely to immediately leave the start zone in later trials ($P = 0.109$), but, as with males, there was no significant effect of trial order on our measure of boldness for females that delayed leaving the start zone ($P = 0.123$).

Younger males were significantly more likely than older males to immediately leave the start zone ($P < 0.001$), but age had no significant effect on our measure of boldness when males delayed leaving the start zone ($P = 0.650$; [Fig. 2](#)). In contrast, older females were significantly bolder than young ones when they delayed leaving the start zone ($P = 0.020$), but, as with males, age had no effect on the probability of immediately leaving the start zone ($P = 0.90$).

The reward stimulus had no significant effect on our measure of boldness for either sex (males: $P = 0.416$; females: $P = 0.993$) or on the likelihood of immediately leaving the start zone (males: $P = 0.934$; females: $P = 0.734$).

Differences in Inhibitory Control

There was no significant sex difference in the interaction between age and stimulus that affected the time spent trying to swim through the transparent barrier (GLMM hurdle: $\chi^2 = 8.425$, $P = 0.393$). Similarly, there were no significant two-way interactions (GLMM hurdle: sex*age: $\chi^2 = 0.714$, $P = 0.700$; age*stimulus: $\chi^2 = 7.964$, $P = 0.241$; sex*stimulus: $\chi^2 = 0.837$, $P = 0.658$). Females spent significantly less time than males trying to swim through the barrier ([Table 2](#)), but there was no sex difference in the likelihood of immediately detouring around the barrier in a given trial ([Appendix Table A2](#)).

Over the four consecutive trials, the time spent trying to swim through the transparent barrier decreased significantly ($P = 0.003$), while the likelihood of immediately detouring around the transparent barrier did not ($P = 0.335$). The 7-week-old fish were more likely to approach the barrier than 14- or 21-week-old fish ($P < 0.001$), and, when they did so, 7-week-old fish spent significantly longer than older fish trying to swim through the transparent barrier ($P < 0.001$; [Fig. 3](#)), but there was no significant

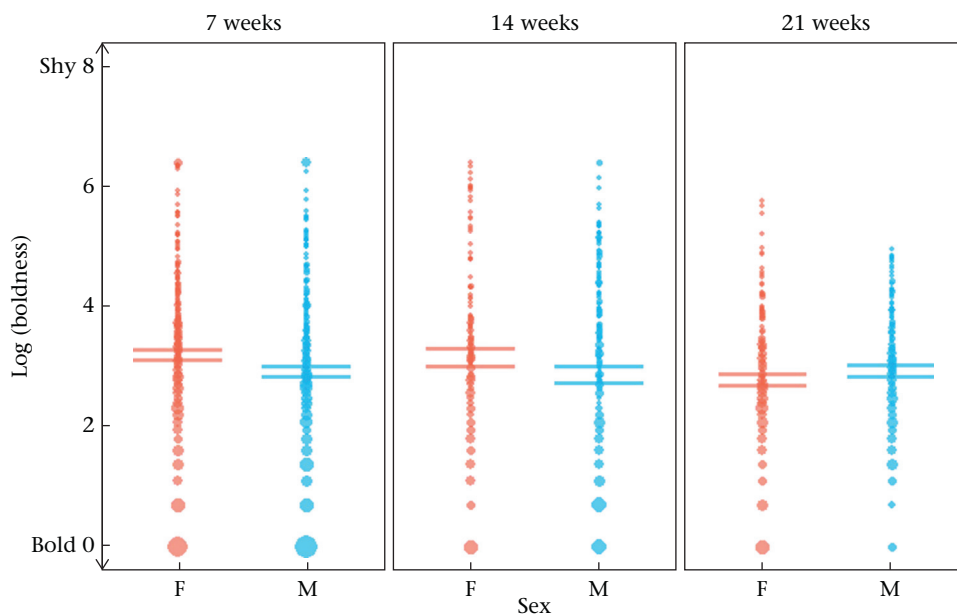


Figure 2. Boldness of male (M) and female (F) mosquitofish, *G. holbrooki*, at 7, 14 and 21 weeks adult age in an inhibitory control test. Boldness was measured as the time taken to leave the start zone. The size of circles is proportional to the number of observations. Horizontal lines show standard errors, with group means in between the lines. Standard errors were calculated using nonzero values only, since the zero-part coefficients were analysed separately in a hurdle model. Data were pooled for four consecutive trials and for tests with three different reward stimuli: group of females, group of males or a mixed-sex group (for the full figure see [Appendix Fig. A1](#)).

Table 2

Parameter estimates for hurdle lognormal mixed-effects model predicting the time spent trying to swim through transparent barrier (i.e. inhibitory control) in mosquitofish, *G. holbrooki*

Predictor	Estimate	SE	χ^2	<i>P</i>
Stimulus (MF)	-0.054	0.130	0.405	0.817
Stimulus (MM)	0.027	0.129		
Age 14 weeks	-0.774	0.118	46.901	<0.001
Age 21 weeks	-0.520	0.115		
Sex (M)	0.235	0.105	4.914	0.027
Boldness	-0.053	0.031	2.928	0.087
Trial	-0.120	0.040	9.016	0.003

Model output is shown for fixed effects. Estimates and SEs were obtained from the model summary. Chi-square and *P* values were calculated with a likelihood ratio test. The reward stimulus was a shoal of female (FF), male (MM) or mixed-sex (MF) conspecifics visible to a focal fish through the barrier. Statistically significant results are in bold ($P < 0.05$). Zero-part coefficients (i.e. likelihood of the fish immediately detouring around the barrier) are presented in Table A2.

difference between 14- and 21-week-old fish (pairwise comparison: $P = 0.057$). There was no effect of age on the likelihood of approaching the transparent barrier ($P = 0.880$).

Boldness did not predict the time spent trying to swim through the transparent barrier ($P = 0.087$) or the likelihood of approaching it ($P = 0.461$). When we ran a separate model to test explicitly for an interaction between sex and boldness, we did not find a sex difference in the effect of boldness on the time spent trying to swim through the barrier (GLMM hurdle: $\chi^2 = 4.993$, $P = 0.082$) or on the likelihood of approaching it (GLMM hurdle: $\chi^2 = 5.402$, $P = 0.067$).

The reward stimulus did not predict either the time spent trying to swim through the barrier ($P = 0.817$) or the likelihood of approaching it ($P = 0.555$).

Differences in Solving Time

There was a significant sex difference in the interaction between age and stimulus that affected solving time (i.e. time to reach the goal zone; LMEM: $\chi^2 = 10.426$, $P = 0.032$). Males tested with a

mixed-sex shoal stimulus reached the reward shoal faster when they were older, while females did not (Fig. 4). We therefore ran separate models for males and females (Table 3). There was no significant interaction between age and reward stimulus for either males (LMEM: $\chi^2 = 2.210$, $P = 0.066$) or females (LMEM: $\chi^2 = 2.339$, $P = 0.054$).

DISCUSSION

We investigated how inhibitory control was affected by sex, age, the type of reward stimulus, 'boldness' as a measure of personality and interactions between these factors using data from 251 mosquitofish, *G. holbrooki*. There were three main findings. First, older fish showed significantly greater inhibitory control and a faster solving time than younger fish. There were no sex differences in the effect of age on inhibitory control. Second, females had significantly greater inhibitory control than males; however, there was no evidence that the type of social reward stimulus had a sex-specific effect on inhibitory control or solving time. Third, bolder fish of both sexes had a significantly faster solving time. In sum, we found sex differences in a measure of inhibitory control in *G. holbrooki*, and that age and boldness explained some of the variation in performance among individuals.

Effect of Age

Inhibitory control improved with age in *G. holbrooki*, as indicated by a lower probability of approaching the transparent barrier at least once, less time spent trying to pass through it and a shorter time to reach a reward stimulus. For both sexes, the youngest adults performed less well than the two older age groups. This is consistent with changes in brain structure during development after sexual maturation in vertebrates that improve cognitive skills, including working memory, flexibility and inhibitory control (Bunge & Wright, 2007; Davidson et al., 2006). Older individuals show improved inhibitory control in several taxa, including primates (e.g. Diamond, 1990) and birds (Kabadayi, Jacobs, et al., 2017).

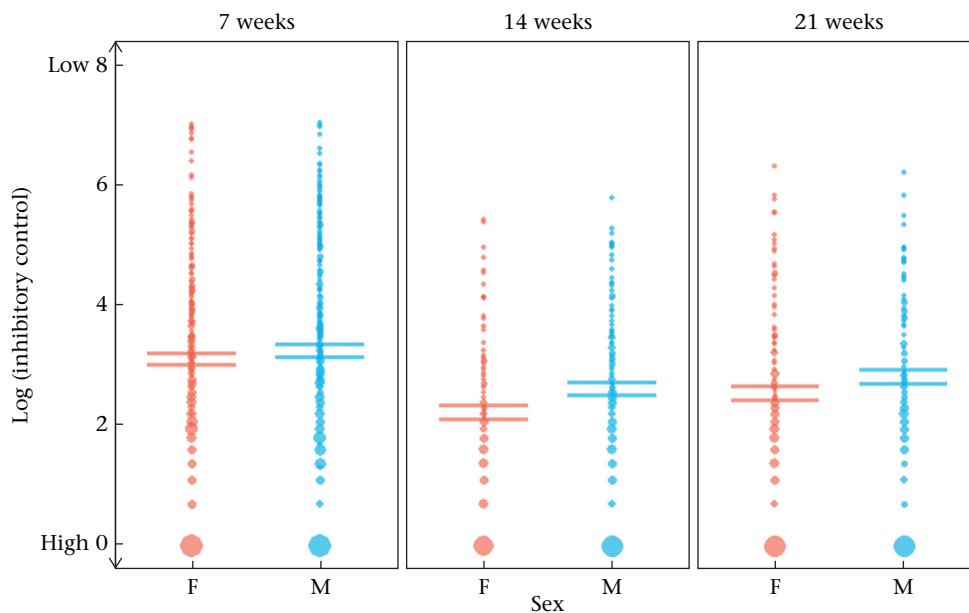


Figure 3. Inhibitory control of male (M) and female (F) mosquitofish, *G. holbrooki*, at 7, 14 and 21 weeks adult age. Inhibitory control was measured as the time a fish spent within 2.5 cm of a transparent barrier that blocked its direct path to a shoal of conspecifics. The size of the circles is proportional to the number of observations. Horizontal lines show standard errors, with group means in between the lines. Standard errors were calculated from nonzero values only, since zero-part coefficients were analysed separately in a hurdle model. Data were pooled for four consecutive trials and for tests with three different reward stimuli: group of females, group of males or a mixed-sex group (for the full figure see Appendix Fig. A2).

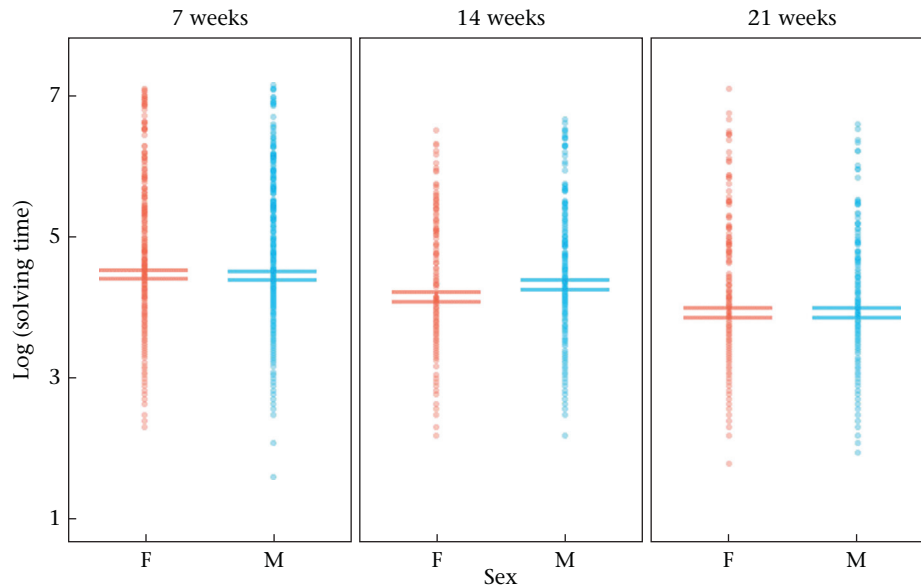


Figure 4. Solving time of male (M) and female (F) mosquitofish, *Gambusia holbrooki*, at 7, 14 and 21 weeks adult age in an inhibitory control test. Solving time measured how quickly a fish reached the reward stimulus. Horizontal lines show standard errors, with group means in between the lines. Data were pooled for four consecutive trials and for tests with three different reward stimuli: group of females, group of males or a mixed-sex group (unpooled means and SEs are presented in [Appendix Fig. A3](#)).

Table 3

Parameter estimates for a linear mixed-effects model predicting solving time in an inhibitory control test in male and female mosquitofish, *G. holbrooki*

Predictor	Estimate	SE	χ^2	<i>P</i>
Males				
Stimulus (MF)	0.040	0.117	1.126	0.569
Stimulus (MM)	0.127	0.121		
Age 14 weeks	-0.120	0.093	25.914	<0.001
Age 21 weeks	-0.482	0.096		
Boldness	0.002	0.0005	8.367	0.004
Trial	-0.045	0.031	2.1338	0.143
Females				
Stimulus (MF)	0.016	0.128	1.417	0.490
Stimulus (MM)	0.131	0.119		
Age 14 weeks	-0.285	0.101	27.049	<0.001
Age 21 weeks	-0.490	0.094		
Boldness	0.001	0.0004	5.097	0.024
Trial	-0.040	0.032	1.573	0.208

Estimates and SEs were obtained from the model summary. Chi-square and *P* values were calculated from a Type III ANOVA. The reward stimulus was a shoal of female (FF), male (MM) or mixed-sex (MF) conspecifics visible to a focal fish through the barrier. Statistically significant results are in bold ($P < 0.05$).

In fish, improvement in cognitive abilities with adult age has been shown in guppies, *Poecilia reticulata*, for numerical skills (Bisazza et al., 2010) and shoaling behaviour (Miletto Petrazzini et al., 2012) and in zebrafish for shoaling behaviour (Buske & Gerlai, 2011); Savaşçı et al. (2021) also recently demonstrated greater inhibitory control by older guppies.

An alternative explanation for improved inhibitory control by older fish is their increased familiarity with the test apparatus due to prior testing. However, as fish were tested in single-day blocks that were 7 weeks apart, it seems unlikely that they would have gained enough training in the test apparatus to improve their performance due to learning. For example, learning ability did not improve with repeated training in a cichlid fish (Kotrschal & Taborsky, 2010), and learnt foraging skills in sticklebacks were retained only for 2 days (Croy & Hughes, 1991; but see Brown, 2001; Triki & Bshary, 2020). We therefore suggest that the most plausible explanation for improved inhibitory control by older

G. holbrooki is cognitive maturation since associative learning in fish usually requires repeated training over many days.

We found very weak evidence for cognitive senescence in *G. holbrooki*, with only a small, nonsignificant ($P = 0.057$) decline in inhibitory control between fish tested at 14 and 21 weeks of age. In vertebrates, cognitive impairment is usually only detected in very old adults (Hu et al., 2018; Sadoun et al., 2019). For example, performance in associative learning tasks declines in zebrafish after 2 years (Ruhl et al., 2016; Yu et al., 2006); and inhibitory control improves early in life but eventually declines in older fish (i.e. there is cognitive senescence; Ruhl et al., 2016). Most eastern mosquitofish live for a single breeding season (Meffe, 1992) and their natural adult life span in our study population is estimated to be 16–24 weeks (Kahn et al., 2013). To our knowledge, no studies have yet reported cognitive decline in *G. holbrooki*, but our results suggest that senescence does not occur in the first 21 weeks of adulthood.

Effect of Sex and Reward Stimulus

Female *G. holbrooki* had significantly greater inhibitory control than males. Although both sexes were initially equally likely to try to swim through the transparent barrier, males spent more time persisting in doing so rather than detouring around. This supports the hypothesis that sex differences in cognition arise in species with strong sex-specific selection (Gaulin & FitzGerald, 1986; reviewed in Jones et al., 2003). In poeciliid fishes, sex differences in cognition have been attributed to males and females having highly divergent reproductive roles that generate sex-specific selection (reviewed in Cummings, 2018). For example, better female than male performance in associative learning has been reported in guppies (Corral-Lopez et al., 2017), western mosquitofish, *Gambusia affinis* (Wallace et al., 2020) and swordtails, *Xiphophorus multilineatus* (Griebing et al., 2020); female guppies also tend to outperform males in reverse-learning tasks (Miletto Petrazzini et al., 2017). More specifically, it has been hypothesized that lower inhibitory control by males is due to selection to persist in their mating attempts (Rowe & Healy, 2014). In support of this,

Lucon-Xiccato et al. (2020) found that male guppies were less successful than females at completing inhibitory tasks, which is similar to our findings for *G. holbrooki*. Since males constantly harass females to mate, sex differences in inhibitory control might be due to selection on males for greater persistence (Bisazza & Marin, 1995).

In many fish, shoaling with conspecifics is advantageous, and shoals are therefore used as a reward stimulus in cognitive studies (e.g. Al-Imari & Gerlai, 2008; Santacà et al., 2019; Sovrano et al., 2018). However, researchers rarely test whether shoal composition affects the outcome of cognitive tests. We expected that the motivation of male and female *G. holbrooki* to join a shoal would differ depending on how many males or females it contained. For example, in studies of shoaling preferences, female *G. holbrooki* prefer to school with females rather than males, presumably to avoid the costs of sexual harassment (Agrillo et al., 2006; Chung et al., 2021). Conversely, males prefer female-only shoals to increase their likelihood of mating (Booksmythe et al., 2013). In our current study, however, there was little evidence that the sex ratio of the shoal affected the test fish's behaviour. There was also no significant interaction between either the age or sex of the test fish and the composition of the shoal that affected its behaviour. We suggest that our detour barrier test might have elicited a different response to that seen when fish choose which shoal to join because any shoal, regardless of its composition, is preferable to being alone in an unfamiliar environment, thereby generating an equally strong motivation to school (also see Gatto et al., 2018).

Measures and Effect of Boldness

Boldness is broadly defined as a willingness to take risk, for example by being near a potential predator or entering a novel environment (Smith & Blumstein, 2008). We operationally measured boldness as the time taken to leave the shelter of the start zone. This is a standard measure, also known as an 'emergence test'. Many fish emerged slowly, but some departed straight away. Immediate emergence could reflect an initial negative flight response to being handled with a net, rather than boldness (e.g. Mislin & Cigrang, 1986; see also Brown & Braithwaite, 2004; Näslund et al., 2015). We found that male, but not female, *G. holbrooki* were significantly less likely to leave the start zone immediately when older, and were less likely to do so in later trials at a given age. One interpretation is that males became less fearful with successive trials due to habituation to the test apparatus (Oosten et al., 2010). The same phenomenon of longer-term familiarity might also explain the effect of male age (but see our previous comments about learning). These explanations do not, however, account for the lack of an effect of age or trial order on whether females immediately left the start zone. Overall, there was no sex difference in the likelihood of immediate departure, so our findings for *G. holbrooki* differ from those in other fishes where females show higher anxiety-like behaviour than males (e.g. Hegab et al., 2018; dos Santos et al., 2021). Investigating whether an immediate fear response to being handled affects common methods to assess cognitive performance could be a profitable line of future investigation.

When fish do not immediately leave the start zone, the time until exiting is a clearer signal of boldness. In such cases, we found that female, but not male, *G. holbrooki* became bolder with age, but there was no effect of trial order for either sex. By definition, personality traits, such as boldness, are repeatable behavioral tendencies that vary among individuals (Sih et al., 2004) but can still show adaptive plasticity in response to the environment experienced during development (Nettle & Bateson, 2015). In *G. holbrooki*, repeatable personality differences have been reported at 20 weeks after birth under laboratory conditions (Polverino et al., 2016).

Although Polverino et al. (2016) also found evidence for sex differences in personality, they found no sex-specific effect of age, which contrasts with our results. A parsimonious explanation for the observed sex difference in the effect of age is sex-specific selection. Since greater boldness is often correlated with increased mortality, it is possible that this is maladaptive for young females with high reproductive value, and only adaptive for older females with a low residual reproductive value (Smith & Blumstein, 2008). In contrast, bolder males are likely to benefit regardless of their age because mating is a zero-sum game and they are more likely to acquire mates (Janicke et al., 2016).

For both sexes, bolder fish reached the reward stimulus sooner, even though boldness did not affect the time spent trying to swim through the barrier. The simplest explanation is that more active (i.e. bolder) individuals make decisions faster (Sih et al., 2004). We therefore suggest that bolder individuals moved around the test apparatus more rapidly and thereby reached the stimulus shoal faster even if they did not spend less time at the barrier. Boldness and exploratory behaviour or activity are often highly correlated in fishes (e.g. Fraser et al., 2001; Wilson & Godin, 2009; Wisenden et al., 2011). Indeed, they are often treated as a boldness–exploration syndrome (Mazué et al., 2015). Note, however, that this explanation does not account for the nonsignificant ($P = 0.087$) effect of boldness on time spent at the barrier. That is, higher exploration should also have led bolder fish to find a way around the barrier sooner.

Cognitive abilities and the personality trait of boldness are, on average, only correlated when boldness is measured as a response to a predator cue, and significant correlations are more often found for males than females (meta-analysis: Dougherty & Guillette, 2018). In *G. holbrooki* we found that bolder fish had slightly, but nonsignificantly, weaker inhibitory control, but there was no sex difference in the relationship. It is noteworthy that most studies that have tested for a correlation between personality and cognition used learning as their measure of cognitive performance. Personality traits have rarely been found to be correlated with inhibitory control (Dougherty & Guillette, 2018). There is a positive relationship between boldness and inhibitory control in zebrafish, guppies and waxbills, *Estrilda astrild* (Gomes et al., 2020; Lucon-Xiccato et al., 2020), a negative relationship in rats, *Rattus norvegicus* (Ferland et al., 2014), and no relationship in guppies (Savaşçı et al., 2021). Compared with these studies, we had a substantially larger sample size ($N = 251$ versus <50 individuals) which strengthens our claim that there is no relationship between boldness and self-control in *G. holbrooki*. Methodological differences between studies should, however, be considered. Even studies on fish use a range of methods to measure inhibitory control (e.g. detour test, Lucon-Xiccato & Bisazza, 2017; open-field test, Montalbano et al., 2020; cylinder reaching task, Lucon-Xiccato et al., 2019; Lucon-Xiccato, Montalbano, et al., 2019; Savaşçı et al., 2021). The effect of boldness on inhibitory control should ideally be measured using a range of test designs, including different rewards/threats to generalize findings.

Conclusion

Sex and age affected inhibitory control in *G. holbrooki*. Females had stronger inhibitory control than males, and it improved with age for both sexes, with minimal evidence for senescence in fish that were 21 weeks after maturation. In contrast, boldness, the most widely measured personality trait in animal studies (Dougherty & Guillette, 2018), was not correlated with inhibitory control. Future research should test whether sex and age differences in inhibitory control can be explained by selection on mating strategies in other species with divergent sex roles. In sum, our

study is among only a handful to consider interactions between sex, age and personality traits as factors that can explain variation in cognitive abilities that affect standard measures of inhibitory control.

Author Contributions

I.M.V., M.D.J., E.V., C.F., P.T. and R.J.F. conceived and designed the study. I.M.V., E.V. and R.F. collected the data, I.M.V. and E.V. analysed the data. All authors interpreted the data, cowrote the manuscript and gave permission for publication.

Declaration of Interest

The authors declare no competing interests.

Data Availability

The data sets and R code generated during the current study are available in the Supplementary material.

Acknowledgments

We thank the staff of ANU Animal Services and U. Aich, M.-H. Chung, and L. Harrison for assistance with fish husbandry. The study was funded by the Australian Research Council (DP190100279 to M.D.J.).

Supplementary Material

Supplementary material associated with this article is available, in the online version, at <https://doi.org/10.1016/j.anbehav.2022.09.011>.

References

- Adams, M. M., & Kafaligonul, H. (2018). Zebrafish—A model organism for studying the neurobiological mechanisms underlying cognitive brain aging and use of potential interventions. *Frontiers in Cell and Developmental Biology*, 6(135). <https://doi.org/10.3389/fcell.2018.00135>
- Agrillo, C., Dadda, M., & Bisazza, A. (2006). Sexual harassment influences group choice in female mosquitofish. *Ethology*, 112(6), 592–598. <https://doi.org/10.1111/j.1439-0310.2006.01188.x>
- Al-Imari, L., & Gerlai, R. (2008). Sight of conspecifics as reward in associative learning in zebrafish (*Danio rerio*). *Behavioural Brain Research*, 189(1), 216–219. <https://doi.org/10.1016/j.bbr.2007.12.007>
- Amici, F., Aureli, F., & Call, J. (2008). Fission-fusion dynamics, behavioral flexibility, and inhibitory control in primates. *Current Biology*, 18(18), 1415–1419. <https://doi.org/10.1016/j.cub.2008.08.020>
- Bisazza, A., & Marin, G. (1995). Sexual selection and sexual size dimorphism in the eastern mosquitofish *Gambusia holbrooki* (Pisces Poeciliidae). *Ethology Ecology & Evolution*, 7(2), 169–183. <https://doi.org/10.1080/08927014.1995.9522963>
- Bisazza, A., Piffer, L., Serena, G., & Agrillo, C. (2010). Ontogeny of numerical abilities in fish. *PLoS One*, 5, Article e15516.
- Booksmythe, I., Backwell, P. R. Y., & Jennions, M. D. (2013). Competitor size, male mating success and mate choice in eastern mosquitofish, *Gambusia holbrooki*. *Animal Behaviour*, 85(2), 371–375. <https://doi.org/10.1016/j.anbehav.2012.11.009>
- Brandão, M. L., Fernandes, A. M. T. d. A., & Gonçalves-de-Freitas, E. (2019). Male and female cichlid fish show cognitive inhibitory control ability. *Scientific Reports*, 9(1), Article 15795. <https://doi.org/10.1038/s41598-019-52384-2>
- Brown, C. (2001). Familiarity with the test environment improves escape responses in the crimson spotted rainbowfish, *Melanoetaenia duboulayi*. *Animal Cognition*, 4(2), 109–113. <https://doi.org/10.1007/s100710100105>
- Brown, C., & Braithwaite, V. A. (2004). Size matters: A test of boldness in eight populations of the Poeciliid *Brachyrhaphis episcopi*. *Animal Behaviour*, 68(6), 1325–1329. <https://doi.org/10.1016/j.anbehav.2004.04.004>
- Bunge, S. A., & Wright, S. B. (2007). Neurodevelopmental changes in working memory and cognitive control. *Current Opinion in Neurobiology*, 17(2), 243–250. <https://doi.org/10.1016/j.conb.2007.02.005>
- Buske, C., & Gerlai, R. (2011). Shoaling develops with age in zebrafish (*Danio rerio*). *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 35(6), 1409–1415. <https://doi.org/10.1016/j.pnpb.2010.09.003>
- Chung, M.-H. J., Jennions, M. D., & Fox, R. J. (2021). Quantifying the costs of pre- and postcopulatory traits for males: Evidence that costs of ejaculation are minor relative to mating effort. *Evolution Letters*, 5(4), 315–327. <https://doi.org/10.1002/evl3.228>
- Coomes, J. R., Davidson, G. L., Reichert, M. S., Kulahci, I. G., Troisi, C. A., & Quinn, J. L. (2021). Inhibitory control, exploration behaviour and manipulated ecological context are associated with foraging flexibility in the great tit. *Journal of Animal Ecology*, 91, 320–333. <https://doi.org/10.1111/1365-2656.13600>
- Corral-Lopez, A., Bloch, N., Kotschal, A., van der Bijl, W., Büchel, S., Mank, J., & Kolm, N. (2017). Female brain size affects the assessment of male attractiveness during mate choice. *Science Advances*, 3, Article e1601990. <https://doi.org/10.1126/sciadv.1601990>
- Croy, M. I., & Hughes, R. N. (1991). The role of learning and memory in the feeding behaviour of the fifteen-spined stickleback, *Spinachia spinachia* L. *Animal Behaviour*, 41(1), 149–159. [https://doi.org/10.1016/S0003-3472\(05\)80510-X](https://doi.org/10.1016/S0003-3472(05)80510-X)
- Cummings, M. E. (2018). Sexual conflict and sexually dimorphic cognition—reviewing their relationship in poeciliid fishes. *Behavioral Ecology and Sociobiology*, 72(4), 73. <https://doi.org/10.1007/s00265-018-2483-9>
- Davidson, M. C., Amso, D., Anderson, L. C., & Diamond, A. (2006). Development of cognitive control and executive functions from 4 to 13 years: Evidence from manipulations of memory, inhibition, and task switching. *Neuropsychologia*, 44(11), 2037–2078. <https://doi.org/10.1016/j.neuropsychologia.2006.02.006>
- Diamond, A. (1990). Developmental time course in human infants and infant monkeys, and the neural bases of, inhibitory control in reaching. *Annals of the New York Academy of Sciences*, 608(1), 637–676. <https://doi.org/10.1111/j.1749-6632.1990.tb48913.x>
- Diamond, A. (2013). Executive functions. *Annual Review of Psychology*, 64(1), 135–168. <https://doi.org/10.1146/annurev-psych-113011-143750>
- Dougherty, L. R., & Guillette, L. M. (2018). Linking personality and cognition: A meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1756), Article 20170282. <https://doi.org/10.1098/rstb.2017.0282>
- Ferland, J.-M. N., Zeeb, F. D., Yu, K., Kaur, S., Taves, M. D., & Winstanley, C. A. (2014). Greater sensitivity to novelty in rats is associated with increased motor impulsivity following repeated exposure to a stimulating environment: Implications for the etiology of impulse control deficits. *European Journal of Neuroscience*, 40(12), 3746–3756. <https://doi.org/10.1111/ejn.12748>
- Fraser, D. F., Gilliam, J. F., Daley, M. J., Le, A. N., & Skalski, G. T. (2001). Explaining leptokurtic movement distributions: Intrapopulation variation in boldness and exploration. *American Naturalist*, 158(2), 124–135. <https://doi.org/10.1086/321307>
- Gatto, E., Lucon-Xiccato, T., & Bisazza, A. (2018). Factors affecting the measure of inhibitory control in a fish (*Poecilia reticulata*). *Behavioural Processes*, 157, 11–17. <https://doi.org/10.1016/j.beproc.2018.08.003>
- Gaulin, S. J., & FitzGerald, R. W. (1986). Sex differences in spatial ability: An evolutionary hypothesis and test. *American Naturalist*, 127(1), 74–88. <https://doi.org/10.1086/284468>
- Gomes, A. C. R., Guerra, S., Silva, P. A., Marques, C. I., Trigo, S., Boogert, N. J., & Cardoso, G. C. (2020). Proactive common waxbills make fewer mistakes in a cognitive assay, the detour-reaching task. *Behavioral Ecology and Sociobiology*, 74(3), 31. <https://doi.org/10.1007/s00265-020-2809-2>
- Griebling, H. J., Rios-Cardenas, O., Abbott, J., & Morris, M. R. (2020). A study of tactical and sexual dimorphism in cognition with insights for sexual conflict. *Animal Behaviour*, 170, 43–50. <https://doi.org/10.1016/j.anbehav.2020.10.006>
- Griffin, A. S., Guillette, L. M., & Healy, S. D. (2015). Cognition and personality: An analysis of an emerging field. *Trends in Ecology & Evolution*, 30(4), 207–214. <https://doi.org/10.1016/j.tree.2015.01.012>
- Guillette, L. M., Hahn, A. H., Hoeschle, M., Przyszluski, A.-M., & Sturdy, C. B. (2015). Individual differences in learning speed, performance accuracy and exploratory behaviour in black-capped chickadees. *Animal Cognition*, 18(1), 165–178. <https://doi.org/10.1007/s10071-014-0787-3>
- Hegab, I. M., Qian, Z., Pu, Q., Wang, Z., yukun, K., Cai, Z., Guo, H., Wang, H., Ji, W., Hanafy, A. M., & Su, J. (2018). Gender difference in unconditioned and conditioned predator fear responses in Smith's zokors (*Eospalax smithii*). *Global Ecology and Conservation*, 16, Article e00503. <https://doi.org/10.1016/j.gecco.2018.e00503>
- Henke-von der Malsburg, J., Kappeler, P. M., & Fichtel, C. (2021). Linking cognition to ecology in wild sympatric mouse lemur species. *Proceedings of the Royal Society B: Biological Sciences*, 288(1963), Article 20211728. <https://doi.org/10.1098/rspb.2021.1728>
- van Horik, J. O., Langley, E. J. G., Whiteside, M. A., Laker, P. R., Beardsworth, C. E., & Madden, J. R. (2018). Do detour tasks provide accurate assays of inhibitory control? *Proceedings of the Royal Society B: Biological Sciences*, 285(1875), Article 20180150. <https://doi.org/10.1098/rspb.2018.0150>
- Hu, S., Ide, J. S., Chao, H. H., Castagna, B., Fischer, K. A., Zhang, S., & Li, C. R. (2018). Structural and functional cerebral bases of diminished inhibitory control during healthy aging. *Human Brain Mapping*, 39(12), 5085–5096. <https://doi.org/10.1002/hbm.24347>
- Janicke, T., Häderer, I. K., Lajeunesse, M. J., & Anthes, N. (2016). Darwinian sex roles confirmed across the animal kingdom. *Science Advances*, 2(2), Article e1500983. <https://doi.org/10.1126/sciadv.1500983>

- Johnson-Ulrich, L., & Holekamp, K. E. (2020). Group size and social rank predict inhibitory control in spotted hyaenas. *Animal Behaviour*, *160*, 157–168. <https://doi.org/10.1016/j.anbehav.2019.11.020>
- Jones, C. M., Braithwaite, V. A., & Healy, S. D. (2003). The evolution of sex differences in spatial ability. *Behavioral Neuroscience*, *117*(3), 403–411. <https://doi.org/10.1037/0735-7044.117.3.403>
- Junttila, S., Huohvanainen, S., & Tiira, K. (2021). Effect of sex and reproductive status on inhibitory control and social cognition in the domestic dog (*Canis familiaris*). *Animals*, *11*(8), 2448. <https://doi.org/10.3390/ani11082448>
- Juszczak, G. R., & Bobrowska, A. (2020). Assessment of problem-solving skills and inhibitory control in mice using water escape detour test. *Current Protocols in Mouse Biology*, *10*(3), e82. <https://doi.org/10.1002/cpmo.82>
- Kabadayi, C., Jacobs, L., & Osvath, M. (2017). The development of motor self-regulation in ravens. *Frontiers in Psychology*, *8*(2100). <https://doi.org/10.3389/fpsyg.2017.02100>
- Kabadayi, C., Krasheninnikova, A., O'Neill, L., van de Weijer, J., Osvath, M., & von Bayern, A. M. P. (2017). Are parrots poor at motor self-regulation or is the cylinder task poor at measuring it? *Animal Cognition*, *20*(6), 1137–1146. <https://doi.org/10.1007/s10071-017-1131-5>
- Kahn, A. T., Kokko, H., & Jennions, M. D. (2013). Adaptive sex allocation in anticipation of changes in offspring mating opportunities. *Nature Communications*, *4*(1), 1603. <https://doi.org/10.1038/ncomms2634>
- Keagy, J., Minter, R., & Tinghitella, R. (2019). Sex differences in cognition and their relationship to male mate choice. *Current Zoology*, *65*. <https://doi.org/10.1093/cz/zoz014>
- Kotrschal, A., & Taborsky, B. (2010). Environmental change enhances cognitive abilities in fish. *PLoS Biology*, *8*(4), Article e1000351. <https://doi.org/10.1371/journal.pbio.1000351>
- Lazarowski, L., Krichbaum, S., Waggoner, L. P., & Katz, J. S. (2020). The development of problem-solving abilities in a population of candidate detection dogs (*Canis familiaris*). *Anim Cogn*, *23*(4), 755–768. <https://doi.org/10.1007/s10071-020-01387-y>
- Lucon-Xiccato, T. (2022). The contribution of executive functions to sex differences in animal cognition. *Neuroscience & Biobehavioral Reviews*, *138*, Article 104705. <https://doi.org/10.1016/j.neubiorev.2022.104705>
- Lucon-Xiccato, T., & Bisazza, A. (2017). Sex differences in spatial abilities and cognitive flexibility in the guppy. *Animal Behaviour*, *123*, 53–60. <https://doi.org/10.1016/j.anbehav.2016.10.026>
- Lucon-Xiccato, T., Bisazza, A., & Bertolucci, C. (2020). Guppies show sex and individual differences in the ability to inhibit behaviour. *Animal Cognition*, *23*(3), 535–543. <https://doi.org/10.1007/s10071-020-01357-4>
- Lucon-Xiccato, T., Gatto, E., & Bisazza, A. (2019). Male and female guppies differ in problem-solving abilities. *Current Zoology*, *66*(1), 83–90. <https://doi.org/10.1093/cz/zoz017>
- Lucon-Xiccato, T., Montalbano, G., & Bertolucci, C. (2019). Personality traits covary with individual differences in inhibitory abilities in 2 species of fish. *Current Zoology*, *66*(2), 187–195. <https://doi.org/10.1093/cz/zoz039>
- Macario, A., Darden, S. K., Verbruggen, F., & Croft, D. P. (2021). Intraspecific variation in inhibitory motor control in guppies, *Poecilia reticulata*. *Journal of Fish Biology*, *98*(1), 317–328. <https://doi.org/10.1111/jfb.14608>
- MacLean, E. L., Hare, B., Nunn, C. L., Addessi, E., Amici, F., Anderson, R. C., Aureli, F., Baker, J. M., Bania, A. E., Barnard, A. M., Boogert, N. J., Brannon, E. M., Bray, E. E., Bray, J., Brent, L. J. N., Burkart, J. M., Call, J., Cantlon, J. F., Cheke, L. G., et al. (2014). The evolution of self-control. *Proceedings of the National Academy of Sciences*, *111*(20), E2140. <https://doi.org/10.1073/pnas.1323533111>
- Manrique, H. M., & Call, J. (2015). Age-dependent cognitive inflexibility in great apes. *Animal Behaviour*, *102*, 1–6. <https://doi.org/10.1016/j.anbehav.2015.01.002>
- Mansouri, F. A., Fehring, D. J., Gaillard, A., Jaberzadeh, S., & Parkington, H. (2016). Sex dependency of inhibitory control functions. *Biology of Sex Differences*, *7*(1), 11. <https://doi.org/10.1186/s13293-016-0065-y>
- Mazué, G. P. F., Dechaume-Moncharmont, F.-X., & Godin, J.-G. J. (2015). Boldness–exploration behavioral syndrome: Interfamily variability and repeatability of personality traits in the young of the convict cichlid (*Amatitlania siquia*). *Behavioral Ecology*, *26*(3), 900–908. <https://doi.org/10.1093/beheco/aru030>
- Meffe, G. K. (1992). Plasticity of life-history characters in eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) in response to thermal stress. *Copeia*, *94*–102.
- Meier, C., Pant, S. R., van Horik, J. O., Laker, P. R., Langley, E. J., Whiteside, M. A., Verbruggen, F., & Madden, J. R. (2017). A novel continuous inhibitory-control task: Variation in individual performance by young pheasants (*Phasianus colchicus*). *Animal Cognition*, *20*(6), 1035–1047. <https://doi.org/10.1007/s10071-017-1120-8>
- Michelangeli, M., Cote, J., Chapple, D. G., Sih, A., Brodin, T., Fogarty, S., Bertram, M. G., Eades, J., & Wong, B. B. M. (2020). Sex-dependent personality in two invasive species of mosquitofish. *Biological Invasions*, *22*(4), 1353–1364. <https://doi.org/10.1007/s10530-019-02187-3>
- Miletto Petrazzini, M. E., Agrillo, C., Piffer, L., Dadda, M., & Bisazza, A. (2012). Development and application of a new method to investigate cognition in newborn guppies. *Behavioural Brain Research*, *233*(2), 443–449. <https://doi.org/10.1016/j.bbr.2012.05.044>
- Miletto Petrazzini, M. E., Bisazza, A., Agrillo, C., & Lucon-Xiccato, T. (2017). Sex differences in discrimination reversal learning in the guppy. *Animal Cognition*, *20*(6), 1081–1091. <https://doi.org/10.1007/s10071-017-1124-4>
- Minter, R., Keagy, J., & Tinghitella, R. M. (2017). The relationship between male sexual signals, cognitive performance, and mating success in stickleback fish. *Ecology and Evolution*, *7*(15), 5621–5631. <https://doi.org/10.1002/ece3.3091>
- Misslin, R., & Cigrang, M. (1986). Does neophobia necessarily imply fear or anxiety? *Behavioural Processes*, *12*(1), 45–50. [https://doi.org/10.1016/0376-6357\(86\)90069-0](https://doi.org/10.1016/0376-6357(86)90069-0)
- Montalbano, G., Bertolucci, C., & Lucon-Xiccato, T. (2020). Measures of inhibitory control correlate between different tasks but do not predict problem-solving success in a fish, *Poecilia reticulata*. *Intelligence*, *82*, Article 101486. <https://doi.org/10.1016/j.intell.2020.101486>
- Näslund, J., Bererhi, B., & Johnsson, J. I. (2015). Design of emergence test arenas can affect the results of boldness assays. *Ethology*, *121*(6), 556–565. <https://doi.org/10.1111/eth.12368>
- Nettle, D., & Bateson, M. (2015). Adaptive developmental plasticity: What is it, how can we recognize it and when can it evolve? *Proceedings of the Royal Society B: Biological Sciences*, *282*(1812), Article 20151005. <https://doi.org/10.1098/rspb.2015.1005>
- Oosten, J. E., Magnhagen, C., & Hemelrijk, C. K. (2010). Boldness by habituation and social interactions: A model. *Behavioral Ecology and Sociobiology*, *64*(5), 793–802. <https://doi.org/10.1007/s00265-009-0896-1>
- Polverino, G., Cigliano, C., Nakayama, S., & Mehner, T. (2016). Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. *Behavioral Ecology and Sociobiology*, *70*(12), 2027–2037. <https://doi.org/10.1007/s00265-016-2206-z>
- R Core Team. (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>
- Rasolofoniaina, B., Kappeler, P. M., & Fichtel, C. (2021). Wild narrow-striped mongooses use social information to enhance behavioural flexibility. *Ethology*, *127*, 253–266. <https://doi.org/10.1111/eth.13123>
- Rizopoulos, D. (2021). *Glmmadaptive: Generalized linear mixed models using adaptive Gaussian quadrature*. GitHub repository. <https://drizopoulos.github.io/GLMMadaptive/>
- Rodriguez-Nieto, G., Emmerling, F., Dewitte, M., Sack, A. T., & Schuhmann, T. (2019). The role of inhibitory control mechanisms in the regulation of sexual behavior. *Archives of Sexual Behavior*, *48*(2), 481–494. <https://doi.org/10.1007/s10508-018-1283-7>
- Rosati, A. G. (2017). Foraging cognition: Reviving the ecological intelligence hypothesis. *Trends in Cognitive Sciences*, *21*(9), 691–702. <https://doi.org/10.1016/j.tics.2017.05.011>
- Rowe, C., & Healy, S. D. (2014). Measuring variation in cognition. *Behavioral Ecology*, *25*(6), 1287–1292. <https://doi.org/10.1093/beheco/aru090>
- Ruhl, T., Jonas, A., Seidel, N. I., Prinz, N., Alabayram, O., Bilkei-Gorzo, A., & von der Emde, G. (2016). Oxidation and cognitive impairment in the aging zebrafish. *Gerontology*, *62*(1), 47–57. <https://doi.org/10.1159/000433534>
- Ryer, C. H., & Olla, B. L. (1991). Information transfer and the facilitation and inhibition of feeding in a schooling fish. *Environmental Biology of Fishes*, *30*(3), 317–323. <https://doi.org/10.1007/BF02028847>
- Sadoun, A., Rosito, M., Fonta, C., & Girard, P. (2019). Key periods of cognitive decline in a nonhuman primate model of cognitive aging, the common marmoset (*Callithrix jacchus*). *Neurobiology of Aging*, *74*, 1–14. <https://doi.org/10.1016/j.neurobiolaging.2018.10.003>
- Santacà, M., Busatta, M., Lucon-Xiccato, T., & Bisazza, A. (2019). Sensory differences mediate species variation in detour task performance. *Animal Behaviour*, *155*, 153–162. <https://doi.org/10.1016/j.anbehav.2019.05.022>
- dos Santos, B. E., Giacomini, A. C. V. V., Marcon, L., Demin, K. A., Strekalova, T., de Abreu, M. S., & Kalueff, A. V. (2021). Sex differences shape zebrafish performance in a battery of anxiety tests and in response to acute scopolamine treatment. *Neuroscience Letters*, *759*, Article 135993. <https://doi.org/10.1016/j.neulet.2021.135993>
- Savaşçı, B. B., Lucon-Xiccato, T., & Bisazza, A. (2021). Ontogeny and personality affect inhibitory control in guppies, *Poecilia reticulata*. *Animal Behaviour*, *180*, 111–121. <https://doi.org/10.1016/j.anbehav.2021.08.013>
- Shamosh, N. A., DeYoung, C. G., Green, A. E., Reis, D. L., Johnson, M. R., Conway, A. R. A., Engle, R. W., Braver, T. S., & Gray, J. R. (2008). Individual differences in delay discounting: Relation to intelligence, working memory, and anterior prefrontal cortex. *Psychological Science*, *19*(9), 904–911. <https://doi.org/10.1111/j.1467-9280.2008.02175.x>
- Shaw, R. C. (2017). Testing cognition in the wild: Factors affecting performance and individual consistency in two measures of avian cognition. *Behavioural Processes*, *134*, 31–36. <https://doi.org/10.1016/j.beproc.2016.06.004>
- Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: An ecological and evolutionary overview. *Trends in Ecology & Evolution*, *19*(7), 372–378. <https://doi.org/10.1016/j.tree.2004.04.009>
- Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: A meta-analysis. *Behavioral Ecology*, *19*(2), 448–455. <https://doi.org/10.1093/beheco/arm144>
- Sovrano, V. A., Baratti, G., & Potrich, D. (2018). A detour task in four species of fishes. *Frontiers in Psychology*, *9*(2341). <https://doi.org/10.3389/fpsyg.2018.02341>
- Stow, M. K., Vernouillet, A., & Kelly, D. M. (2018). Neophobia does not account for motor self-regulation performance as measured during the detour-reaching cylinder task. *Animal Cognition*, *21*(4), 565–574. <https://doi.org/10.1007/s10071-018-1189-8>
- Szabo, B., Hofer, S., & Whiting, M. J. (2020). Are lizards capable of inhibitory control? Performance on a semi-transparent version of the cylinder task in five

- species of Australian skinks. *Behavioral Ecology and Sociobiology*, 74(10), 118. <https://doi.org/10.1007/s00265-020-02897-y>
- Thornton, A., & Samson, J. (2012). Innovative problem solving in wild meerkats. *Animal Behaviour*, 83(6), 1459–1468. <https://doi.org/10.1016/j.anbehav.2012.03.018>
- Triki, Z., & Bshary, R. (2020). Long-term memory retention in a wild fish species *Labroides dimidiatus* eleven months after an aversive event. *Ethology*, 126(3), 372–376. <https://doi.org/10.1111/eth.12978>
- Vernouillet, A., Anderson, J., Clary, D., & Kelly, D. M. (2016). Inhibition in Clark's nutcrackers (*Nucifraga columbiana*): Results of a detour-reaching test. *Animal Cognition*, 19(3), 661–665. <https://doi.org/10.1007/s10071-016-0952-y>
- Vernouillet, A., Stiles, L., McCausland, J., & Kelly, D. (2018). Individual performance across motoric self-regulation tasks are not correlated for pet dogs. *Learning & Behavior*, 46. <https://doi.org/10.3758/s13420-018-0354-x>
- Vlamings, P. H., Hare, B., & Call, J. (2010). Reaching around barriers: The performance of the great apes and 3–5-year-old children. *Animal Cognition*, 13(2), 273–285.
- Wallace, K. J., Rausch, R. T., Ramsey, M. E., & Cummings, M. E. (2020). Sex differences in cognitive performance and style across domains in mosquitofish (*Gambusia affinis*). *Animal Cognition*, 23(4), 655–669. <https://doi.org/10.1007/s10071-020-01367-2>
- Wascher, C. A. F., Allen, K., & Szpl, G. (2021). Learning and motor inhibitory control in crows and domestic chickens. *Royal Society Open Science*, 8(10), 210504.
- White, J. R., Meekan, M. G., McCormick, M. I., & Ferrari, M. C. O. (2013). A comparison of measures of boldness and their relationships to survival in young fish. *PLoS One*, 8(7), Article e68900. <https://doi.org/10.1371/journal.pone.0068900>
- Wilson, A. D. M., & Godin, J.-G. J. (2009). Boldness and behavioral syndromes in the bluegill sunfish, *Lepomis macrochirus*. *Behavioral Ecology*, 20(2), 231–237. <https://doi.org/10.1093/beheco/arp018>
- Wisenden, B. D., Sailer, C. D., Radenic, S. J., & Sutrisno, R. (2011). Maternal inheritance and exploratory-boldness behavioural syndrome in zebrafish. *Behaviour*, 148(14), 1443–1456. <http://www.jstor.org/stable/41444677>
- Yu, L., Tucci, V., Kishi, S., & Zhdanova, I. V. (2006). Cognitive aging in zebrafish. *PLoS One*, 1(1), e14. <https://doi.org/10.1371/journal.pone.0000014>

Appendix

Table A1

Zero-part coefficients for hurdle lognormal mixed-effects model predicting likelihood of boldness being 0 (i.e. when a fish leaves the start zone immediately) for male and female mosquitofish, *G. holbrooki*

Zero-part coefficients	Estimate	SE	χ^2	P
Males				
Stimulus (MF)	0.118	0.478	0.137	0.934
Stimulus (MM)	-0.058	0.490		
Age 14 weeks	-0.810	0.337	36.316	<0.001
Age 21 weeks	-2.728	0.574		
Trial	0.246	0.113	5.477	0.020
Females				
Stimulus (MF)	-0.259	0.450	0.618	0.734
Stimulus (MM)	-0.334	0.417		
Age 14 weeks	-0.433	0.359	4.821	0.090
Age 21 weeks	-0.756	0.350		
Trial	0.175	0.119	2.575	0.109

Estimates and SEs were obtained from the model summary. Chi-square and P values were calculated with a likelihood ratio test. The reward stimulus was a shoal of female (FF), male (MM) or mixed-sex (MF) conspecifics visible to a focal fish from the start zone. Statistically significant results are shown in bold ($P < 0.05$).

Table A2

Zero-part coefficients for hurdle lognormal mixed-effects model predicting likelihood of 0 values in inhibitory control (when a fish did not approach a transparent barrier) in mosquitofish, *G. holbrooki*

Zero-part coefficients	Estimate	SE	χ^2	P
Stimulus (MF)	-0.176	0.170	1.178	0.555
Stimulus (MM)	-0.047	0.167		
Age 14 weeks	0.778	0.145	42.901	<0.001
Age 21 weeks	0.800	0.142		
Sex (M)	-0.094	0.137	0.459	0.498
Boldness	-0.029	0.039	0.544	0.461
Trial	0.047	0.049	0.929	0.335

Estimates and SEs were obtained from the model summary. Chi-square and P values were calculated with a likelihood ratio test. The reward stimulus was a shoal of female (FF), male (MM) or mixed-sex (MF) conspecifics visible to a focal fish through the barrier. Statistically significant result is shown in bold ($P < 0.05$).

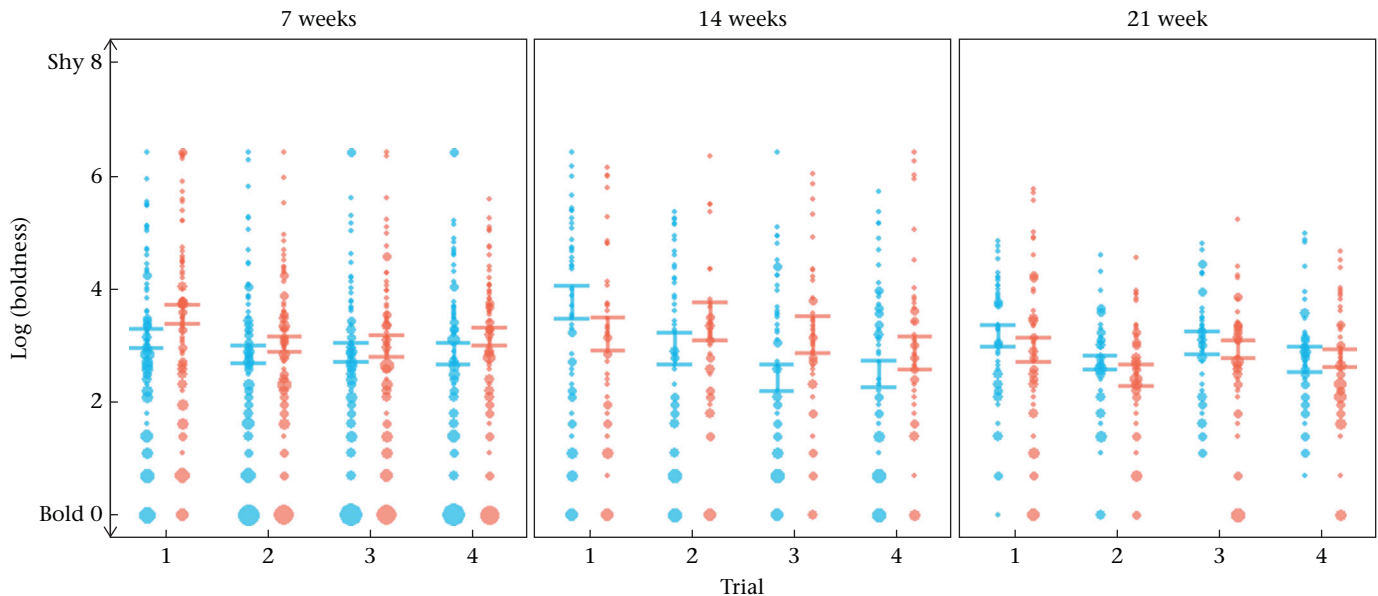


Figure A1. Boldness of male (blue) and female (red) mosquitofish, *G. holbrooki*, over four consecutive trials at 7, 14 and 21 weeks adult age in an inhibitory control test. Boldness was measured as the time taken to leave the start zone. The size of circles is proportional to the number of observations. Horizontal lines show standard errors, with group means in between the lines. Standard errors were calculated using nonzero values only, since the zero-part coefficients were analysed separately in a hurdle model. Data were pooled for tests with three different reward stimuli: group of females, group of males or a mixed-sex group.

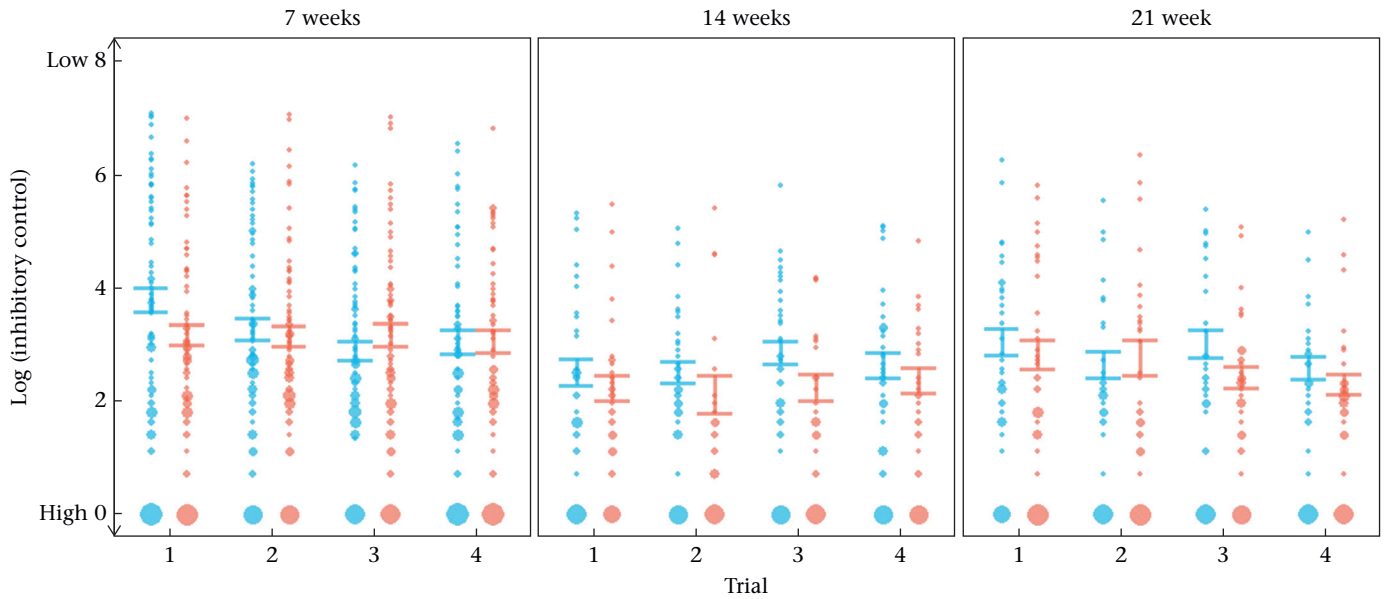


Figure A2. Inhibitory control of male (blue) and female (red) mosquitofish, *G. holbrooki*, over four consecutive trials at 7, 14 and 21 weeks adult age. Inhibitory control was measured as the time a fish spent within 2.5 cm of a transparent barrier that blocked the direct path to a shoal of conspecifics. The size of circles is proportional to the number of observations. Horizontal lines show standard errors, with group means in between the lines. Standard errors were calculated from nonzero values only, since zero-part coefficients were analysed separately in a hurdle model. Data were pooled for tests with three different reward stimuli: group of females, group of males or a mixed-sex group.

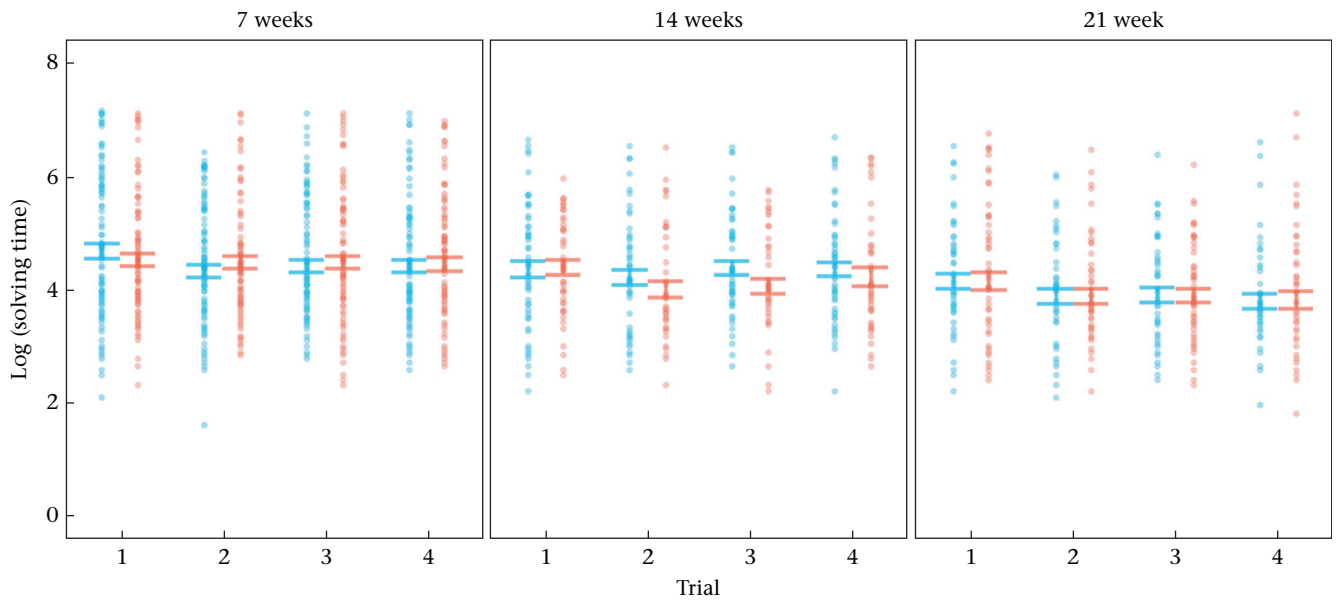


Figure A3. Solving time of male (blue) and female (red) mosquitofish, *G. holbrooki*, over four consecutive trials at 7, 14 and 21 weeks adult age in an inhibitory control test. Solving time measured how soon a fish reached the reward stimulus. Horizontal lines show standard errors, with group means in between the lines. Data were pooled across tests with three different reward stimuli: group of females, group of males or a mixed-sex group.