The effect of sex, age, and boldness on inhibitory control

Running header: Predictors of inhibitory control

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Abstract

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 non-human studies have investigated how different factors are associated with variation in inhibitory control, those studies rarely considered how these factors interact. Here we investigate 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 post-maturity 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.

Keywords: cognitive abilities, inhibitory control, detour test, fish cognition, sex-differences, cognitive aging, problem-solving

Introduction

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 and 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 socially dominant individual (Johnson-Ulrich and 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 2018; MacLean et al 2014). Within species there is often high variability in inhibitory control among individuals, as seen in mammals (Johnson-Ulrich and Holekamp 2020), birds (Meier et al 2017, Kabadayi et al 2017a), and fish (Savaşçı et al 2021, Macario 2021, Lucon-Xiccato 2020). This variation is sometimes associated with key life-history traits (e.g. development (Diamond et al 1990)), personality traits (Dougherty and Guillette 2018, Griffin et al 2015), and measures of cognitive performance such as learning ability (Thornton and Samson 2012, Rasolofoniainia et al 2021). 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 (Lazarowski et al 2020), and ravens (Kabadayi et al 2017a)), but, as with most cognitive functions, it eventually declines late in life due to senescence (Sadoun et al 2019, Hu et al 2018). A well-studied non-human model for cognitive aging are zebrafish (see Adams and Kafaligonul 2018), where initial cognitive improvement and late-life cognitive impairment are both observed over their approximately three-year lifespan (Ruhl et al 2016). Unfortunately, most studies of non-human animals test individuals over a far shorter time frame than their natural lifespan, which reduces the likelihood of detecting cognitive senescence.

Sex differences in inhibitory control vary strikingly across species. Some species exhibiting clear sex differences such as humans (Mansouri et al 2015) and some fishes (Lucon-Xiccato and Bisazza 2017, Lucon-Xiccato et al. 2019a,b), whereas others do not (non-human primates: see Henke-von der Malsburg et al 2021, pheasants: van Horik et al. 2018, Clark's nutcrackers: Vernouillet et al. 2016, robins: Shaw 2017, or 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 et al. 2019a, Keagy et al 2019, Brandão 2019, but see Savaşçı et al. 2021). There are other explanations too. For example, sex differences in inhibitory control in sticklebacks 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 also generate variation in measures of inhibitory control between the sexes, but also among individuals within each sex. A recent meta-analysis reports 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 and Guillette 2018). Although some studies find a link between personality traits (e.g. boldness or exploration tendency) and inhibitory control (Savaşçı et al 2021, Gomes et al 2020, Lucon-Xiccato et al 2019b, Ferland et al 2014), other studies do not (van Horik et al 2018, Guillette et al 2015, Stow et al 2018, Rasolofoniaina et al 2021).

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 and Call 2015), other mammals (e.g. Junttila et al 2021, Juszczak and Bobrowska 2020), birds (e.g. Wascher 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 reduced 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-effects in fish tend to exclude individuals at older ages that equate to the natural lifespan.

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 livebearing fish. Males constantly attempt to coercively mate, and females continually try to evade and/or attack males (Bisazza and 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 7 week old (post maturation) males and females 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 only live as adults for a single breeding season of 16-24 weeks (Kahn et al. 2013). Our study is 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 account 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 if inhibitory control changes with age in *G. holbrooki*; (2) to test if boldness is correlated with the level of inhibitory control. (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

Fish were collected from the wild as juveniles, held in 90 L stock tanks in the aquarium facility at the Australian National University (≤ 50 fish per 90 L aquarium) and reared to maturity. They were kept at a constant temperature (28 °C ± 1 °C) on a 14:10 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 L aquarium. They were then elastomer-tagged to identify individuals throughout the study (see Booksmythe et al 2013).

Experimental procedure

At 7 weeks adult age, we randomly assigned focal test fish to one of three social reward treatments: a group of 6 male conspecifics, a group of 6 female conspecifics, or a mixed group of 3 male and 3 female conspecifics. The 'stimulus' fish were randomly drawn from stock tanks of non-test 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 re-tested 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 cm x 42 cm) containing a transparent barrier directly between the start and social reward tank (Figure 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 mins 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. Out of 258 fish tested, 251 fish 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 three 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, the fish 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 two, three, or four, 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 videoed and data were then collected by an observer (IV) 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 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 starting zone) as a measure of 'solving time'.

Statistical analysis

We pre-registered our plan for statistical analysis on OSF (https://osf.io/eb5pn). The analysis was run in R v4.1.0 using the packages GLMMadaptive and glmmTMB.

We ran separate hurdle lognormal mixed models 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 starting zone.

We ran three separate linear mixed effect models using 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 interaction 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 non-significant two-way interactions. The reason for excluding non-significant 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 male 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 OFS prior to being conducted.

Results

Differences in boldness

There was no significant sex difference in how the interaction between age and reward stimulus type affected boldness (GLMM hurdle, $\chi2 = 11.259$, $P = 0.187$), but there was a sex difference how age affected boldness (GLMM hurdle, $\chi2 = 16.018$, $P = 0.003$), suggesting that females, but not males, became bolder as they aged. We therefore ran separate models for each sex (Table 1, Table S4). Reward stimulus did not significantly interact with age for either males (GLMM hurdle, $\chi2 = 9.103$, $P = 0.334$) or females (GLMM hurdle, $\chi2 = 7.017$, $P = 0.071$).

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 ($P = 0.650$) (Figure 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, $\chi2$ = 8.425, *P* = 0.393). Similarly, there were no significant two-way interactions (GLMM hurdle, sex*age: $\chi2$ = 0.714, *P* = 0.700; age*stimulus: $\chi2$ = 7.964, *P* = 0.241; sex*stimulus: $\chi2$ = 0.837, *P* = 0.658). Females spent significantly less time than males trying to swim through the barrier (GLMM hurdle, $\chi2$ = 4.913, *P* = 0.027) (Table 2), but there was no sex difference in the likelihood of immediately detouring around the barrier in a given trial (GLMM hurdle, $\chi2$ = 0.451, *P* = 0.502) (Table S2).

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) (Figure 3), but there was no significant difference between 14 and 21 week old fish (pair-wise 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, χ^2

=4.993, *P* = 0.082) or on the likelihood of approaching it (GLMM hurdle, χ^2 =5.402, *P* = 0.067).

The reward stimulus did not predict either the 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, $\chi2$ = 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 (Figure 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, $\chi2$ = 2.210, *P* = 0.066) or females (LMEM, $\chi2$ = 2.339, *P* = 0.054).

Discussion

We investigated how inhibitory 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 *Gambusia 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 among individuals in their performance.

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 post-sexual maturation development in vertebrates that improve cognitive skills, including working memory, flexibility, and inhibitory control (Bunge and Wright 2007, Davidson et al 2006). Older individuals show improved inhibitory control in several taxa, including primates (e.g. Diamond et al 1990) and birds (Kabadayi et al 2017a). In fish, improvement in cognitive abilities with adult age have been shown in guppies for numerical skills (Bisazza et al 2010) and shoaling behaviour (Miletto Petrazzini et al 2012), in zebrafish for shoaling behaviour (Buske and Gerlai 2011), and Savaşçı et al (2021) recently demonstrated greater inhibitory control by older guppies.

An alternate 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 does not improve with repeated training in a cichlid fish (Kotrschal and Taborsky 2010), and learnt foraging skills in sticklebacks were retained only for two days (Croy and Hughes 1991, but see Brown 2001 and Triki and 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, non-significant (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 (Sadoun et al 2019, Hu et al 2018). For example, performance in associative learning tasks declines in zebrafish after two years (Yu et al 2006, Ruhl et al 2016); 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 lifespan 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 and 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 have been reported in guppies *P. reticulata*

(Corral-López et al 2020), Western mosquitofish *G. affinis* (Wallace et al 2020), and swordtails *Xiphophorus multilineatus* (Griebling et al 2020); and female guppies 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 and Healy 2005). 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 persistent (Bisazza and 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 and Gerlai 2008, Sovrano et al 2018, Santacà et al 2019). However, researchers rarely test if 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 shoal 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 and 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. Misslin and Cigrang 1986; see also: Brown and 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 phenomena 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 female immediately left the start zone. Over all, 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 if 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 and 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 where the rewards of greater risk-taking when residual reproductive value is low (Smith and 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 2014). 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 and Godin 2009, Wisenden et al 2011). Indeed, they are often treated as a boldness–exploration syndrome (Mazué et al 2015). It should be noted, however, that this explanation does not account for the non-significant (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 and Guillette 2018). In *G. holbrooki* we found that bolder fish had slightly, but non-significantly, 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 use learning as their measure of cognitive performance. Personality traits have rarely been found to be correlated with inhibitory control (Dougherty and Guillette 2018). There is a positive relationship between boldness and inhibitory control in zebrafish, guppies, and waxbills, (Lucon-Xiccato et al 2020, Gomes et al 2020), a negative relationship in rats (Ferland et al 2014), and no relationship in guppies (Savaşçı et al 2021). Compared to these studies, we had a substantially larger sample size (*N* = 251 vs <50 individuals) which strengthens our claim that there is no relationship between boldness and self-control in *G. holbrooki*. Methodological differences among studies should, however, be considered. Even studies on fish use a range of methods to measure inhibitory control (e.g. detour test: Lucon-Xiccato & Bizassa 2017; open-field test: Montalbano et al. 2020; cylinder reaching task: Lucon-Xicatto et al. 2019a,b;

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 generalise 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 post-maturation. In contrast, boldness, the most widely measured personality trait in animal studies (Dougherty and 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.

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Tables

Table 1. Factors that predict boldness in mosquitofish *Gambusia holbrooki*.

Predictor	Estimate	SE	χ^2	*P*
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

Parameter estimates for hurdle lognormal mixed effects model predicting boldness (time spent in the starting zone) for male and female mosquitofish *Gambusia holbrooki*. Model output is shown for fixed effects. Estimates and standard errors (SE) were obtained from the model summary. Chi-square and *P* values were calculated with a likelihood ratio test. The reward stimulus is a shoal of female (FF), male (MM), or mixed-sex (MF) conspecifics. Statistically significant results are 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.

Table 2. Factors that predict inhibitory control in mosquitofish *Gambusia holbrooki*.

Predictor	Estimate	SE	χ^2	*P*
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**

Parameter estimates for hurdle lognormal mixed effects model predicting the time spent trying to swim through transparent barrier (i.e. inhibitory control) in mosquitofish *Gambusia holbrooki*. Model output is shown for fixed effects. Estimates and standard errors (SE) were obtained from the model summary. Chi-square and *P* values were calculated with a likelihood ratio test (LRT). Stimulus is a shoal of female (FF), male (MM), or mixed-sex (MF) conspecifics visible by a focal fish through the barrier. Statistically significant results are emboldened ($P < 0.05$). Zero-part coefficients (i.e. likelihood of the fish immediately detouring around the barrier) are presented in Table A2.

Table 3. Factors that predict solving time in mosquitofish *Gambusia holbrooki*.

Predictor	Estimate	SE	χ^2	*P*
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

Parameter estimates for a linear mixed effects model predicting solving time in an inhibitory control test in male and female mosquitofish *Gambusia holbrooki*. Estimates and standard errors (SE) were obtained from the model summary, while Chi-square and *P* value were calculated from Type III ANOVA. Stimulus is a shoal of females (FF), males (MM), or mixed-sex (MF) conspecifics visible by a focal fish at the start zone. Statistically significant results are emboldened ($P < 0.05$).

Figure 1. Diagram of the inhibitory control test apparatus inside a glass tank (60×42x40 cm). Solid and dotted lines indicate opaque and transparent walls, respectively. Each focal fish starts its trial in the starting chamber (A). The time taken to leave the starting chamber (i.e. fully cross the border) is a measure of "boldness". A small transparent plastic tank (D) (30x19x20 cm) containing a group of conspecifics (6 males, 6 females, or 3 males and 3 female) is located opposite the starting chamber, behind a 15 cm transparent barrier (B). The total time a fish spends within 2.5 cm of the barrier is a measure of inhibitory control. The time it takes a fish to reach the stimulus (i.e. cross the line) in the goal zone (C) after leaving the starting chamber is a measure of solving time.

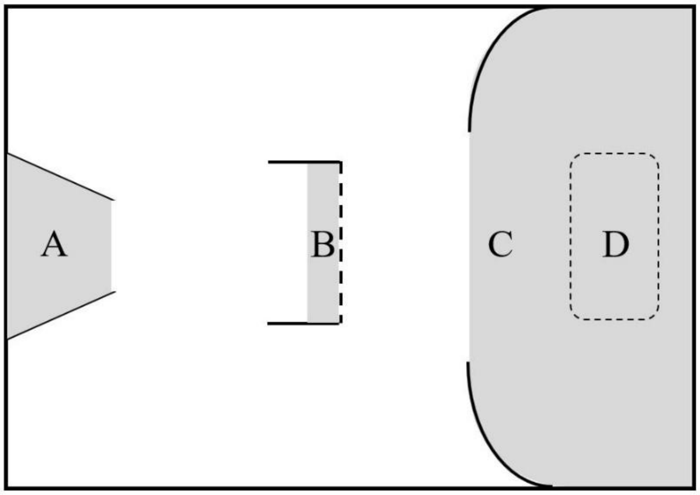


Figure 2. Boldness of male (blue) and female (red) mosquitofish Gambusia holbrooki at 7, 14 and 21 weeks adult age in an inhibitory control test. Boldness was measured as the time taken to leave the starting zone. The size of circles is proportional to the number of observations. Horizontal lines show standard errors, with group means in between the lines. Standard errors were calculated using non-zero values only, since the zero-part coefficients were analysed separately in a hurdle model. Data is 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 full figure see Fig. 1A).

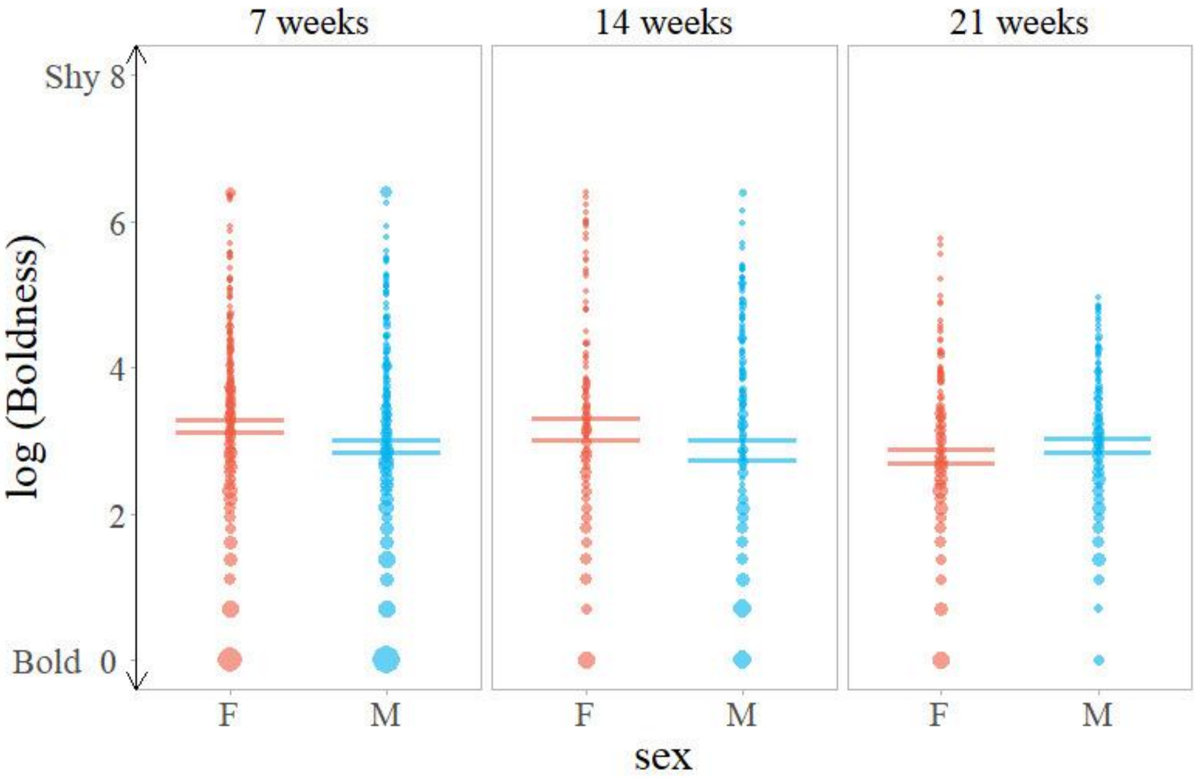


Figure 3. Inhibitory control of male (blue) and female (red) mosquitofish Gambusia holbrooki at three ages (7, 14 and 21 weeks adult age). Inhibitory control was measured as the time a fish spent within 2.5 cm of a transparent barrier that blocked their direct path to a shoal of conspecifics. The size 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 non-zero values only, since zero-part coefficients were analysed separately in a hurdle model. Data is 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

Fig. 2A).

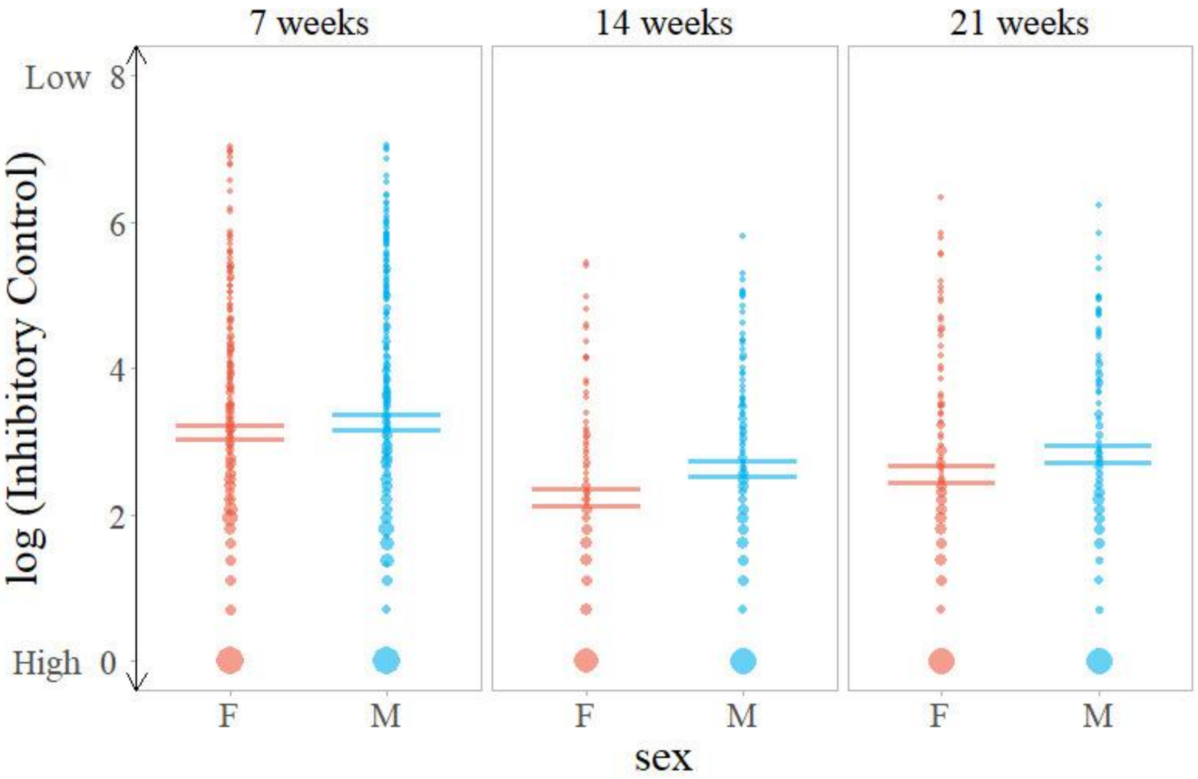
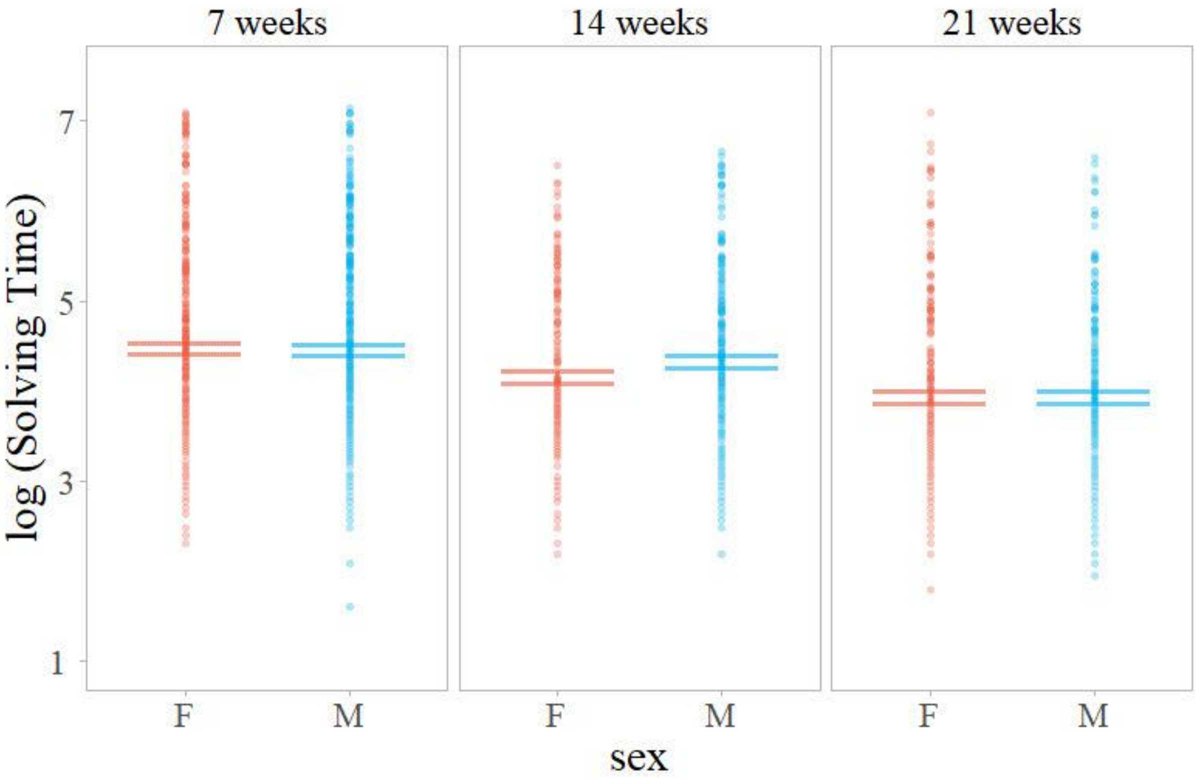


Figure 4. Solving time of male (blue) and female (red) mosquitofish Gambusia holbrooki at three age (7, 14, 21 weeks old) in an inhibitory control test. Solving time measures how quickly a fish reached the reward stimulus. Horizontal lines show standard errors, with group means in between the lines. Data is 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 s.e. are presented in Fig. 3A).



Appendix Figures

Figure A1. Boldness of male (blue) and female (red) mosquitofish *Gambusia holbrooki* over four consecutive trials at three ages (7, 14 and 21 weeks post-maturity) exhibited during an inhibitory control test. Boldness was measured as the time a fish takes to leave the starting 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 non-zero values only, since the zero-part coefficients were analysed separately in a hurdle model. Data is pooled for tests with three different reward stimuli: group of females, group of males, or a mixed sex group

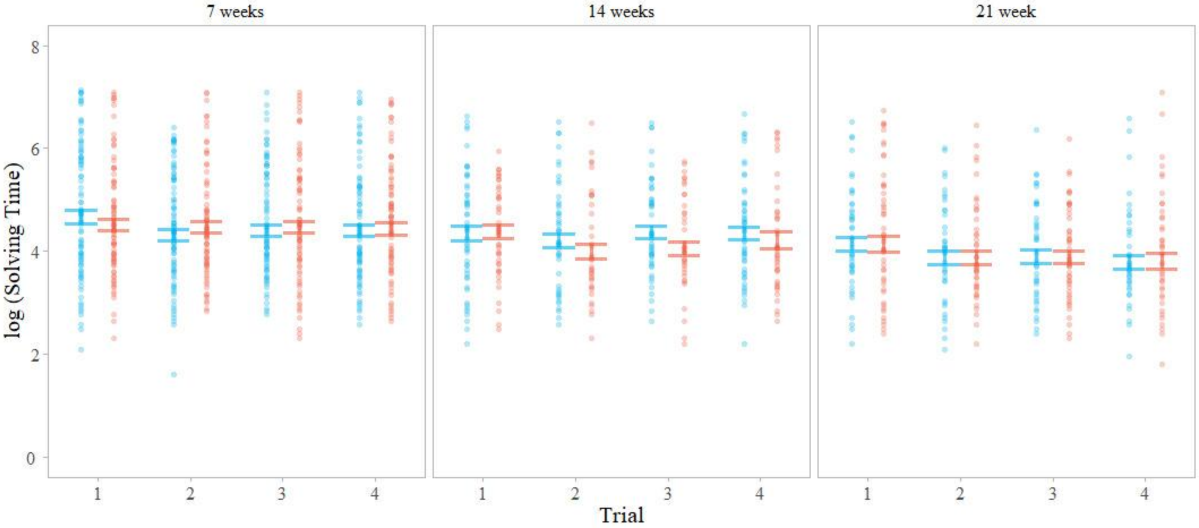


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Figure A2. Inhibitory control of male (blue) and female (red) mosquitofish *Gambusia holbrooki* over four consecutive trials at three ages (7, 14 and 21 weeks post-maturity). Inhibitory control was measured as the time a fish spent within 2.5 cm of 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 non-zero values only, since zero-part coefficients were analysed separately in a hurdle model. Data is pooled for tests with three different reward stimuli: group of females, group of males, or a mixed sex group.

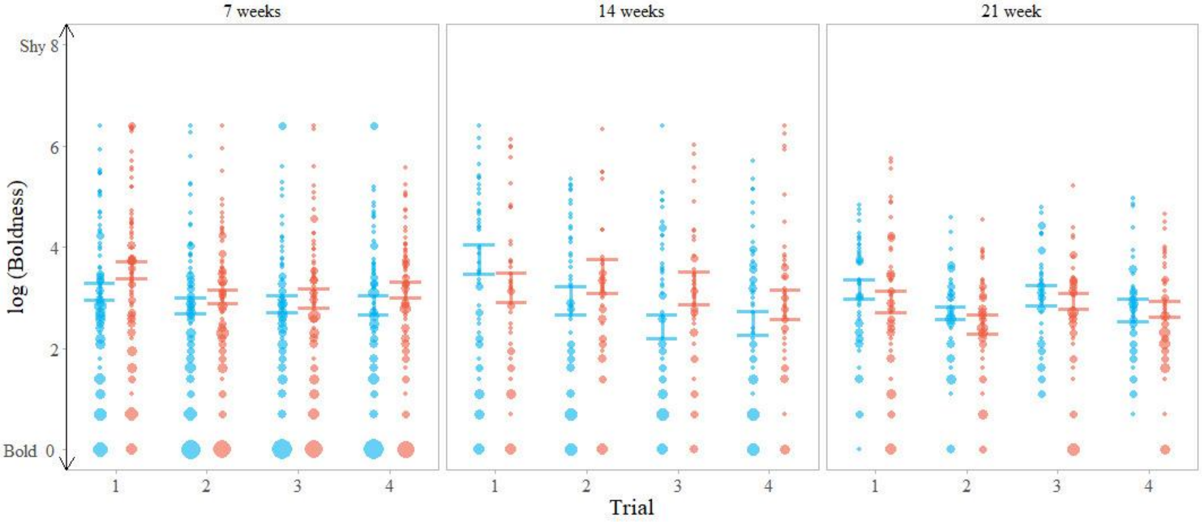
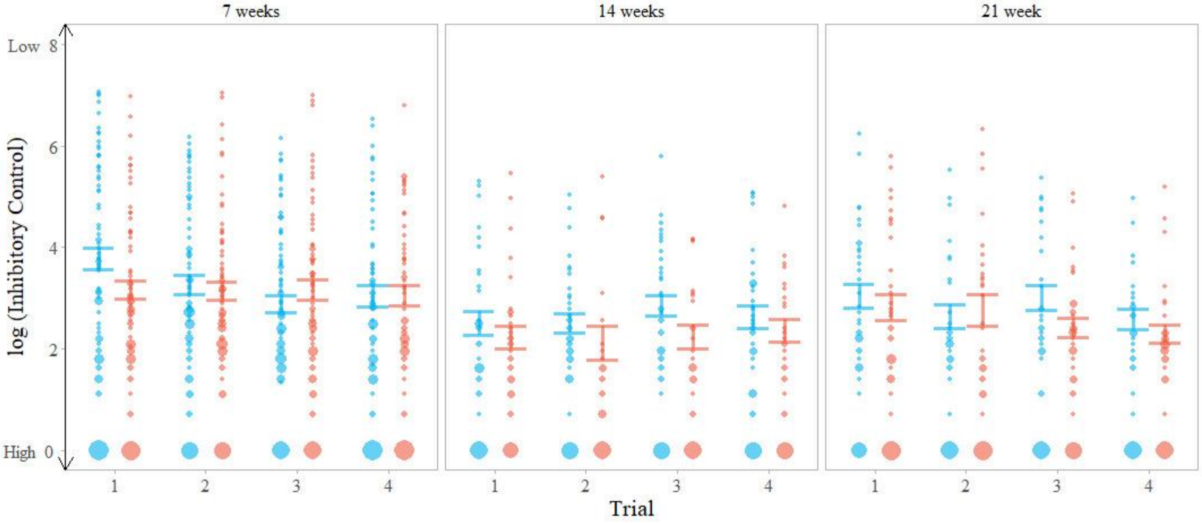


Figure A3. Solving time of male (blue) and female (red) mosquitofish *Gambusia holbrooki* over four consecutive trials at three ages (7, 14, 21 weeks post-maturity) in an inhibitory control test. Solving time measures how soon a fish reached the reward stimulus. Horizontal lines show standard errors,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



Appendix tables

Table A1. Zero-part coefficients for model predicting likelihood of fish leaving the start zone immediately in mosquitofish *Gambusia 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

Zero-part coefficients for hurdle lognormal mixed effects model predicting likelihood of boldness being 0 (i.e. when a fish leaves the starting zone immediately) for male and female mosquitofish *Gambusia holbrooki*. Estimates and standard errors (SE) were obtained from the model summary. Chi-square and *P* values were calculated with a likelihood ratio test. Stimulus is a shoal of female (FF), male (MM), or mixed-sex (MF) conspecifics visible by a focal fish from the start zone. Statistically significant results are shown in bold ($P < 0.05$).

Table A2. Zero-part coefficients for model predicting likelihood of fish not approaching transparent barrier in mosquitofish *Gambusia 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

Zero-part coefficients for hurdle lognormal mixed effects model predicting likelihood of 0 values in inhibitory control (when a fish does not approach a transparent barrier) in mosquitofish *Gambusia holbrooki*. Estimates and standard errors (SE) were obtained from the model summary. Chi-square and P values were calculated with a likelihood ratio test (LRT). Stimulus is a shoal of female (FF), male (MM), or mixed-sex (MF) conspecifics visible by a focal fish through the barrier. Statistically significant results are shown in bold ($P < 0.05$).