Guppies show rapid and lasting inhibition of foraging behaviour

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Abstract

To cope with the variable environment, animals are continuously required to learn novel behaviours or, in certain cases, to inhibit automatic and previously learned behaviours. Traditionally, inhibition has been regarded as cognitively demanding and studied mostly in primates, other mammals and birds, using laboratory tasks, such as the cylinder task. Recent studies have also revealed that fish show high levels of inhibition in the cylinder task. However, conclusions on such results are undermined by evidence that the cylinder task may be inappropriate to compare such phylogenetically distant species. Here, we studied whether a fish, the guppy, *Poecilia reticulata*, could learn to inhibit behaviour using a different paradigm, which exploited spontaneous foraging behaviour and overcame some drawbacks that characterised the cylinder task. We exposed guppies to live brine shrimp nauplii, *Artemia salina*, enclosed within a transparent tube. Initially, the guppies attempted to attack the prey but over time showed a rapid decrease of the attacks. Control tests seemed to exclude the possibility that this behavioural trend was due to response to novelty or habituation, and suggested that the guppies were learning to inhibit the foraging behaviour. Memory tests indicated that guppies retained the inhibition of foraging behaviour for at least 24 h. Our study seems to indicate that teleost fish display rapid and durable inhibition of spontaneous foraging behaviour; this may be related to previous evidence, from the cylinder task, supporting efficient behavioural inhibition in this taxon.

Keywords: behavioural flexibility; fish cognition; inhibitory control; inhibition learning; memory.

1. Introduction

Learning is an important mechanism that allows animals to cope with fluctuating environments. Most studies on learning have focussed on how individuals learn to produce novel responses and manifest novel behaviours. However, animals also must learn to inhibit behaviours that were previously appropriate or to block automatic responses. For example, carnivores may benefit, becoming more successful predators, by inhibiting their prepotent responses towards pouncing on prey (MacNulty et al., 2007) and prey may inhibit foraging in the presence of predators (Ryer and Olla, 1991). Literature on humans and other primates suggests that these and similar processes are performed by a single core executive function often referred to as inhibitory control (Diamond, 2013).

Most research on inhibition has been conducted on humans, other mammals, and a few avian species (e.g., Beran and Hopkins, 2018; Diamond, 1990; Marshall-Pescini et al., 2015; Meier et al., 2017; Parrish et al., 2018). Studies on humans are chiefly interested in understanding the causes and consequences of dysfunctional ability to control impulses (e.g., Jasinska et al., 2012; Schachar et al., 1995). By contrast, studies on other species are generally focussed on drawing phylogenetic comparisons to understand the evolution of this cognitive ability (e.g., Amici et al., 2008; Kabadayi et al., 2016; MacLean et al., 2014). The main result of comparative research is an awareness that inhibiting behaviour is a difficult task and is therefore enhanced in species with large and complex nervous systems (MacLean et al., 2014). A recent study on the guppy, *Poecilia reticulata*, unveiled this tiny teleost fish as a remarkable exception to the foregoing rule. This species demonstrated inhibitory performance comparable to that of most mammals and birds tested with the same task (Lucon-Xiccato et al., 2017), despite of the significantly smaller size of the guppy’s brain, relative to those of endothermic vertebrates (MacLean et al., 2014).

Before dismissing the hypothesis of the positive correlation between brain size and inhibitory performance as invalid when applied to teleosts, it is important to consider a methodological issue. The paradigm used in the aforementioned studies, the cylinder task, is a version of the detour task (Kabadayi et al., 2017a), in which subjects are initially trained to eat a piece of food placed inside an opaque cylinder, after which they are tested with a transparent cylinder; subjects must inhibit the tendency to move directly towards the visible food item in favour of instead detouring to enter the cylinder from the open, lateral side. According to a growing number of studies, this paradigm may be inappropriate to compare species that are phylogenetically distant or exhibit diverse ecological adaptations (Kabadayi et al., 2017b; van Horik et al., 2018). For example, van Horik and colleagues (2018) showed that the performance of pheasants, *Phasianus colchicus*, on the cylinder task was affected by their prior experience and their motivation to acquire food, rather than their inhibitory control. Other researchers have suggested that performance on the cylinder task is related to brain size for mammals exclusively (Isaksson et al., 2018; Kabadayi et al., 2016).

These methodological concerns regarding the cylinder task beg the question of whether fish possess high inhibitory control or have other advantages in solve the cylinder task. The relevant literature seems to support the latter explanation. Fish have also been proven capable of solving other versions of the detour task that used social stimuli as goal (Gatto et al., 2018; Lucon-Xiccato et al., 2017; Lucon-Xiccato and Bisazza, 2017a). However, cleaner fish, *Labroides dimidiatus*, performed very poorly when tested in a diverse paradigm, the reverse-reward contingency task (Danisman et al., 2010), which requires individuals to choose a non-preferred food type (small food item) to obtain a preferred food type (large food item). No cleaner fish managed to learn to inhibit selection of the larger food item, except for one subject, tested on a simplified version of the task. It is therefore possible that fish are at an intrinsic advantage when solving detour tasks, perhaps because, in their environment, they must frequently detour around such objects as plants or rocks, or because they can rely on cues other than sight, such as the scent of the stimulus food in the water, to guide them along the right path to the target.

This study aimed to assess whether fish can solve an inhibition task other than the detour task, to deepen understanding of the inhibitory ability of this group. In experiment 1, we tested guppies, the fish species most studied in relation to inhibition (Gatto et al., 2018; Lucon-Xiccato and Bisazza, 2017a; Lucon-Xiccato et al., 2017; Santacà et al., 2019), with a procedure developed for cuttlefish, which involves live prey being placed inside a transparent tube, requiring the subjects to learn to inhibit the response of attacking the prey (Agin et al., 1998; Dickel et al., 2001; Messenger, 1973).

We chose the tube task for five reasons. First, as the stimulus is sealed in the tube, the subject is not exposed to chemical cues of the food, which would thereby minimize the advantage of the fish, if it reaches the stimulus by relying on chemicals dissolved in the water, rather than on visual information. Second, the tube task relies on inhibition of a spontaneous behaviour, as did the original version of the detour task with transparent objects (Diamond, 1990). Conversely, the aforementioned version of the cylinder task, has been modified and involves an initial training phase, and might be impacted by species differences in learning during the initial phase. Inhibition of spontaneous behaviours is thought to be controlled by inhibitory control, as well as inhibition of learned behaviour (Diamond, 2013). Hence, the tube and the cylinder tasks are expected to measure, at least in part, the same cognitive process, but in different ways. Third, the performance in the cylinder task is also affected by the spatial abilities of the species, because detouring an obstacle requires spatial competences with some extent (Kabadayi et al., 2017a). Fourth, the tube task is expected to be more challenging, relative to the cylinder task, which should help to determine whether fish possess high inhibitory abilities. Indeed, in the tube task, the subjects must completely cease the relevant behaviour, whereas in the detour task, the subjects merely have to modify their behaviour (i.e., they have to move laterally, rather than in a straight line to reach a target behind a transparent wall). Also, the initial training phase of the detour task is a supplement to the original methodology that makes the task easier to solve (Santos et al., 1999). Fifth and last, inhibiting a behaviour is intuitively harder when the stimulus is a live, moving prey, which compels strong attraction in many species (MacDonald, 1973; Thompson et al., 1981), relative to the piece of food typically used in the cylinder task.

These advantages notwithstanding, some limitations complicate the viability of the tube task. First, fish may be attracted by the tube instead of focussed on the prey inside the tube. Guppies and other fish species often show exploratory and neophilic behaviour towards unfamiliar objects (Hamilton et al., 2016; Lucon-Xiccato and Dadda, 2016) and this exploratory behaviour may cause them to swim within close proximity of the object and, eventually, to bite it (Lucon-Xiccato and Dadda, 2014; Rodd et al., 2002). We controlled for this possibility in a condition of experiment 1, in which we presented guppies with an empty tube to analyse their neophilic response to the novel object. We expected a reduced number of interactions with the empty tube compared to the number of attacks towards the tube with the prey. A second possible limitation of the tube task is that inhibition is not the only form of learning that may account for a decrease in the number of attacks. In the case of habituation, an individual is repeatedly exposed to a stimulus that usually causes an automatic response and shows a reduction of the response (Rankin et al., 2019). We tackle this possibility in experiment 2. A critical characteristic of habituation is that it occurs at faster rates when the relevant stimulation is greater (Rankin et al., 2019). By contrast, inhibitory processes tend to show the opposite trend: inhibiting a behaviour is harder when the lure is greater (Brucks et al., 2017; Bugnyar et al., 2012; Rosati et al., 2007).In experiment 2, we compared the behaviour of two groups of guppies, one exposed to a large amount of stimulus prey and one exposed to a small group of prey. In the case of inhibition learning, we expected that the group exposed to the greater amount of prey would show a slower decrease in attack rate.

In experiment 3 of this study, we investigated another aspect of inhibition, not yet addressed in fish. We asked whether guppies can maintain the learned inhibition of the foraging behaviour after an interval of time. Prior studies related to guppies' performance on the cylinder task consisted in a series of sequential trials (e.g., Lucon-Xiccato et al., 2017a). We tested guppies with trials of the tube task separated by 24 h and 72 h. If the learned inhibition of a strongly motivated behaviour, such as foraging, is maintained over time, this may further indicate that this species possesses a notable capacity of inhibition.

2. Materials and methods

2.1 Experimental design

Experiment 1 aimed to test whether guppies can learn to inhibit attacks on the prey inside the transparent tube and control for neophilia. We tested two groups of guppies, one group with the prey and another group without the prey, as a control. We observed the first group through 2 trials of 20 min each, in which the tube was filled with brine shrimps. The 2 trials were separated by a 1-h interval. In case of inhibition learning, we expected the guppies to curb the number of attacks over each 20-min trial or over the 20 min of trial 1, if learning occurred only in trial 1. We also expected to observe fewer attacks overall in trial 2, in case of learning. Regarding the control condition of experiment 1, we observed the number of attacks performed by the control guppies towards the empty tube for 20 min (one trial).

In experiment 2, we integrated a further control to exclude the possibility that the decrease in the number of attacks resulted from habituation rather than inhibition learning. We compared two groups of guppies in a single trial, with a large and a small quantity of brine shrimps, respectively. We expected to find that the amount of prey modulated the decrease in number of attacks over trial time.

In experiment 3, we sought to determine whether the learned inhibition was maintained over time, by testing two groups of guppies in two trials, as in experiment 1. We imposed 24-h and 72-h intervals, respectively, between the trials. If guppies could remember the learned inhibition after these two intervals, we expected to attain the same results cited in the account of experiment 1. If the memory window for the learned behaviour was shorter than both intervals, we expected the guppies to exhibit a different pattern of change in the number of attacks between experiment 3 and experiment 1. If the memory window was shorter than 72 h but longer than 24 h, we expected the guppies to show a higher number of attacks after the 72-h interval, relative to the 24-h interval. We performed the memory test in a separate experiment because, before performing experiment 1, it was impossible to determine whether guppies would be able to solve the tube task.

2.2 Subjects

The subjects were adult guppies of an outbred ornamental strain (total: $N = 44$; experiment 1: $N = 12$; experiment 2: $N = 16$; experiment 3: $N = 16$). These guppies were descended from 200 individuals purchased in 2012 and then reared in the laboratory in large mixed-sex groups. We used only females, because several studies involving guppies have suggested that the female possesses greater learning (reviewed in Lucon-Xiccato and Bisazza, 2017b). To avoid having the reproductive cycle impact female performance, we tested females at the same reproductive stage (1 week after partum) and with no evidence of pregnancy (i.e., abdominal distension). The maintenance tanks (100 × 70 cm, 400 L) were made of opaque grey plastic, with natural gravel bottoms, natural plants, and filters. Due to the opacity of these tanks, the fish had no experience with transparent surfaces before the experiment. Water temperature was kept at 26 ± 1 °C and fluorescent lamps provided 12 h of light (07.00-19.00 h) each day. We fed the fish twice per day, using commercial food flakes and brine shrimp, *Artemia salina*, nauplii. For the experiments, we haphazardly selected the subjects from the maintenance tanks with naïve guppies and we tested them only once (i.e., data from the different experiments and the different conditions of each experiment are independent). We performed no harmful manipulations on the fish, and none of the subjects showed signs of distress during the experiments. After test completion, we released the fish into other tanks for breeding.

2.3 Apparatus and stimulus

The apparatus was the same across the three experiments. We tested each subject in a plastic aquarium, filled with 4 L of water (33 × 13 cm, 15 cm height; Fig. 1a). We used multiple aquaria to run tests on multiple subjects simultaneously. These aquaria were divided into two sections by a grid net: the main sector (25 × 15 cm) that housed the experimental subject; and the minor sector (8 × 15 cm) that housed an air stone and 2 immature (2-month old) conspecifics as social companions because the guppy is a social species. We routinely use these social companions in our experiments (e.g., Lucon-Xiccato and Bisazza, 2014; Miletto Petrazzini et al., 2017) because they prevent social deprivation but do not demonstrably affect subjects' behaviour; adult females show aggressive behaviour toward smaller conspecifics approaching a food patch (Magurran and Seghers, 1991) but we did not observe this type of interaction with immature social companions in separate sectors. The walls of the experimental aquaria were covered with green plastic to prevent the fish from seeing beyond the walls of the tank and a lid of transparent plastic was placed atop the aquaria. The lid was rigged with a small circular hole (Ø 1.2 cm) to accommodate the tube (see below). An LED strip, placed 50 cm above the aquaria, provided illumination from 7.00

to 19.00 h. To record the behaviour of the subjects, we placed a webcam (Logitech) 50 cm above each aquarium. The camera was connected to a computer running custom-made recording software.

The prey stimulus consisted of 24 h-old brine shrimp nauplii, prepared according to standard protocol used in fish facilities. The guppies used in this study recognised brine shrimps as prey because brine shrimps were furnished to them daily during maintenance. The day before the experiment, we placed 2 g of *A. salina* cysts (Ocean Nutrition, HE 240.000 NPG) in a sedimentation cone with 2 L of water (kept at 28 °C using a heater) and 70 g salt. Employing this protocol, we obtained freshly hatched nauplii for the experiments. In experiments 1 and 3, to present the stimulus to the guppies, we inserted 4 ml of the nauplii-containing solution in a standard glass test tube (length: 10 cm; Ø: 1.2 cm). The number of nauplii in the tube was 470 ± 48 (mean ± SD, $N = 10$). In experiment 2, one group of guppies was presented with 2 ml of solution with nauplii, mixed with 2 ml of water, corresponding to half of the prey used in experiments 1 and 3 (small quantity condition). The other group of guppies of experiment 2 (large quantity condition) was exposed to the nauplii contained in 8 ml of the initial solution, which were re-suspended in 4 ml of water to fit the tube; this corresponded to double the amount prey used in experiments 1 and 3.

2.4 Habituation procedure

The three experiments followed the same general procedure, which consisted of two sequential phases, habituation and test. The habituation phase of the experiments lasted 3 d and served to habituate the subjects to the experimental aquaria and the feeding schedule necessary to conduct the test with the tube. This habituation was based on the habituation previously adopted in this species (Lucon-Xiccato and Dadda, 2017; Lucon-Xiccato et al., 2015). The first day, we moved each individual fish into an apparatus and after few minutes, we fed it twice (1-h interval between each feeding session). To feed the guppies, we inserted a Pasteur pipette into the water, through the hole in the transparent lid above the aquaria, and gently released a small amount of food. During the entire habituation phase, we used commercial flakes, crumbled and mixed with water as food, but not brine shrimps. This was done because guppies often fail to consume all the brine shrimps soon after delivery, because the brine shrimps may swim away. After the two feeding sessions, we left the fish undisturbed until the following day. On the second and third days of habituation, we fed the guppies 4 and 6 times, respectively, with a 1-h interval between each feeding session. At this point, we released the food from the pipette only when the fish was looking at it. This procedure allowed the fish to grow progressively accustomed to receiving food through the hole in the lid and, ultimately, come to grab the food as soon as it exited from the point of the pipette. At the end of the third day of habituation, we discarded those fish that failed to learn to feed from the pipette ($N = 5$) and substituted them with new subjects.

2.5 Test procedure

The test phase began the day after the habituation phase ended. In the control condition, with no prey, of experiment 1 and in experiment 2, it consisted of 1 trial. In the condition with prey of experiment 1 and in experiment 3, it consisted of 2 trials, separated by an interval. The interval between trials was 1 h in experiment 1 and either 24 h or 72 h in experiment 3. We performed the trials of the two conditions of experiment 1 simultaneously; we performed experiment 2 and experiment 3 separately, for logistical reasons. Trials always commenced at 10:00 h to avoid differences across the experiments due to circadian variation in the guppies' feeding motivation and cognitive abilities (Winocur and Hasher, 1999); the only exception was trial 2 of experiment 1, which took place at 11:00 h, because the interval between the two trials was set at 1 h. During each trial, we presented the tube filled with brine shrimps to the guppies for 20 min (Fig. 1b). Conversely, in the control condition of experiment 1, we presented the tube filled with water without brine shrimps, as control for neophilia. We inserted the tube from the hole of the lid, suspended 5 cm beneath the water surface during the trials by a support (Fig. 1b).

Because our experiment focussed on changes of fish behaviour over the test time, it was important to ensure that the behaviour of brine shrimps remained constant. To avoid disturbance to the fish, we established this control in a preliminary experiment. We observed the activity of brine shrimps in the tube for 20 min after immersion in empty aquaria. From recordings of brine shrimps with a webcam placed on the side of the aquaria, we counted the number of times that brine shrimps crossed a line that bisected the submerged part of the tube. This analysis showed that, after the first minute that the brine shrimps were highly active (perhaps in response to the pipette procedure used to fill the tube), the activity of the nauplii was constant and stable (table 1). In the experiments, we thus inserted the tube in the aquaria 1 min after the insertion of the brine shrimps; this ensured that the activity of brine shrimps was stable through the entire test phase and did not affect subjects' attack rate. A webcam recorded the test phase of the experiments and we analysed the behaviour of the guppies from the recordings played back on a computer. For each trial, we recorded each guppy's number of attempts to attack the prey, for each minute, resulting in 20 1-min blocks of time. Guppies were considered as attempting to attack the prey when they touched the glass tube with their snouts.

2.6 Statistical analysis

We performed statistical analysis using R version 3.4.0 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). We used two tailed tests and a significance threshold of $P = 0.05$. The dependent variable collected in the experiment was the number of attacks performed by each guppy subdivided in the 20 min of each trial. The number of attacks was a count of discrete events. These data have usually large variance and follow a Poisson distribution (i.e., the variance is equal to the mean); we therefore performed the statistical analyses with generalised linear mixed-effects models (GLMMs) with Poisson error distribution (‘glmer’ function from the ‘lme4’ package) that have been developed to handle this type of data.

In experiment 1, we initially fitted a GLMM on the number of attacks of the first trial to compare the condition with and without brine shrimps inside the tube to ensure that the behaviour of guppies was not due to exploration toward the tube. We included condition as fixed effect and fish ID as random effect to account for repeated measurement; we fitted the minute (from 1 to 20) as covariate because preliminary data plotting showed linear decrease in the dependent variable. After confirming the different behaviour in the two conditions, we fitted another GLMM on the number of attacks of the condition with brine shrimps only, using the data of both trial 1 and trial 2. The fixed effect in this model was trial (trial 1 or trial 2), and the random effect and the covariate were as described above. To verify that guppies learned to inhibit the predatory behaviours (see Experimental design), we aimed to test: the decrease in the number of attacks across time (significant effect of minute) due to learning; the reduction of the number of attacks in trial 2 compared to trial 1 (significant effect of trial); and eventually a different trend of the change in number of attacks between the two trials (significant interaction between trial and minute). We also performed change point analysis (for each trial separately) with the PELT exact method for multiple change points (‘cpt.mean’ function of the ‘changepoint’ R package); this analysis allowed to identify if and in which minute substantial changes in the number of attacks occurred.

In experiment 2, we analysed the number of attacks with a GLMM with minute and brine shrimps quantity (large or small) as fixed effects, and fish ID as random effect. In experiment 3, we initially fitted a GLMM as described for experiment 1, with the addiction of interval between the trials (24 h or 72 h) as fixed effect. After finding a significant 3-ways interaction, we run further GLMMs on the data set split for the interval between trials. We performed change point analysis of experiment 3 as described for experiment 1.

2.7 Ethical note

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed (Italy, D.L. 4 Marzo 2014, n. 26). The Ethical committee at University of Ferrara approved the experimental procedure (aut. n. 2/2018-TLX).

3. Results

3.1 Experiment 1 – Inhibition learning

The analysis on both conditions (first trial), showed that guppies attacked the tube more often in the condition with brine shrimps inside the tube compared to the control condition with no prey (GLMM: $\chi^2_1 = 7.431$, $P = 0.006$). In the condition with brine shrimps inside the tube, guppies attempted to attack the prey 28.83 ± 28.20 times in the first trial (mean ± SD). In the control test without prey inside the tube, 2 guppies did not contact the tube in the entire trial, 2 guppies contacted the tube once, 1 guppy twice, and 1 guppy contacted the tube 13 times (mean ± SD: 2.83 ± 5.03).

There was a significant change in the number of attacks across minutes (GLMM: $\chi^2_1 = 115.497$, $P < 0.0001$), but this change was different between the two conditions (condition × minute interaction: $\chi^2_1 = 36.405$, $P = 0.0001$). This indicates a substantial difference between the behaviour of guppies toward the tube filled with brine shrimps and the empty tube: the number of contacts with the tube significantly decreased across time when brine shrimps were present (GLMM: $\chi^2_1 = 154.090$, $P < 0.0001$; Fig. 2a); in sharp contrast, there was no change in the number of contacts with the empty tube across time (GLMM: $\chi^2_1 = 0.036$, $P = 0.850$; Fig. 2b).

In the second trial of the condition with brine shrimps, after 1 h interval, 2 guppies did not attempt to attack and the average number of attacks was 10.17 ± 13.95. The GLMM on the number of attacks toward the prey in both trials revealed a significant effect of trial ($\chi^2_1 = 27.763$, $P < 0.0001$) and a significant effect of minute within trial ($\chi^2_1 = 85.803$, $P < 0.0001$). However, there was also a significant interaction between trial and minute ($\chi^2_1 = 68.500$, $P < 0.0001$; Fig. 2a). This was due to the fact that in trial 2, guppies showed a constant number of attacks across all minutes (GLMM: $\chi^2_1 = 0.692$, $P = 0.406$; Fig. 2a) in contrast to the decrease of attacks observed in trial 1. The change point analysis confirmed the results of the GLMM. In trial 1, we detected a substantial change in the number of attacks between minute 1 and minute 2, and a second change between minute 6 and minute 7 (Fig. 2a). The change point analysis on trial 2 did not find changes in the number of attacks across minutes (Fig. 2a). The GLMM that compared the data of the last minute of the first trial and the first minute of the second trial did not find a significant difference ($\chi^2_1 < 0.001$, $P = 0.987$).

3.2 Experiment 2 – Different quantity of stimulus prey

Similarly to the condition with prey of experiment 1, guppies showed a substantial decrease in the number of attacks across minutes (GLMM: $\chi^2_1 = 280.362$, $P < 0.0001$). There was no significant main effect of brine shrimp quantity ($\chi^2_1 = 0.761$, $P = 0.383$). As expected, we found that the brine shrimp quantity affected the decrease in the number of attacks ($\chi^2_1 = 9.746$, $P = 0.002$): in line with the hypothesis of inhibition learning, guppies exposed to the larger quantity of brine shrimps showed reduced decrease in the number of attacks (Fig. 3).

3.3 Experiment 3 – Memory

The GLMM on the number of attacks toward the prey inside the tube revealed a significant effect of trial ($\chi^2_1 = 19.758$, $P < 0.0001$) and minute within trial ($\chi^2_1 = 193.826$, $P < 0.0001$), but there was not significant effect of the interval between the two trials ($\chi^2_1 = 0.196$, $P = 0.658$). Also, the trial × minute interaction was significant ($\chi^2_1 = 36.786$, $P < 0.0001$). More importantly, the three-way interaction between trial, minute and interval between the trials was significant ($\chi^2_1 = 19.202$, $P < 0.0001$), suggesting that the change in number of attacks across minute varied between the first and the second trial according to the time interval between the trials (Fig. 4). The remaining interactions in the GLMM were not significant (trial × interval: $\chi^2_1 = 0.031$, $P = 0.859$; minute × interval: $\chi^2_1 = 0.778$, $P = 0.378$).

To understand the three-way interaction in the previous GLMM, we separately analysed the data of guppies tested with different time interval between the trials. When the interval between trials was 24 h, the number of attacks changed according to trial ($\chi^2_1 = 10.000$, $P = 0.002$) and minute ($\chi^2_1 = 85.540$, $P < 0.0001$), and, critically, it showed a different pattern of decrease across minutes in the two trials ($\chi^2_1 = 52.897$, $P < 0.0001$): the number of attack was higher in the initial minutes of the trial 1, but it was constant in the entire trial 2 (Fig. 4). Similarly to what observed in experiment 1, the change point analysis for the 24 h interval showed a marked change in the number of attacks in the min 1 of the trial 1, but not changes in the number of attacks in the trial 2 (Fig. 4). Thus, with the 24 h interval, guppies showed to remember the inhibitory behaviour learned in trial 1 also during trial 2.

When the interval between trials was 72 h, the number of attacks was lower in trial 1 compared to trial 2 ($\chi^2_1 = 9.904$, $P = 0.002$) and it decreased with minute within trial ($\chi^2_1 = 108.332$, $P < 0.0001$). However, the interaction between trial and minute was not significant ($\chi^2_1 = 3.069$, $P = 0.080$), suggesting that the decrease in the number of attack was similar between the first and the second trial (Fig. 4). The change point analysis showed a marked change in the number of attacks in minute 1 of both trial 1 and trial 2 (Fig. 4). Thus, with the 72 h interval, guppies did not expressed the inhibitory behaviour learned in trial 1 at the beginning of trial 2, and during trial 2, they showed to learn again to inhibit the behaviour.

4. Discussion

Recent studies have reported that teleost fish can efficiently solve detour tasks with transparent obstacles, such as the cylinder task (Lucon-Xiccato et al., 2017), which is usually considered a measure of inhibitory control in higher vertebrates (MacLean et al., 2014). Yet, the remarkable performance of fish in the cylinder task may be due to the specific paradigm and do not reflect a general competence in inhibiting behaviours. The present study tested guppies, using a different paradigm to measure inhibition and showed that this fish can learn to inhibit the foraging response toward live prey very quickly.

In experiment 1, guppies were exposed to live brine shrimps sealed into a transparent tube or to an empty tube as control. In the condition with brine shrimps, guppies initially attempted to capture the prey and made contact with the transparent tube with high frequency (more than 1 attempt every 6 seconds during the first minute of the test). Conversely, in the absence of brine shrimps, the guppies undertook an extremely low number of 'attacks' toward the tube (< 1 per minute). The attack behaviour of guppies was therefore triggered by the presence of brine shrimps behind the transparent tube and not due to other motivations such as exploration toward novel objects inserted in the tank (Hamilton et al., 2016; Lucon-Xiccato and Dadda, 2014; Lucon-Xiccato and Dadda, 2016).

The number of attacks on brine shrimps decreased rapidly across the guppies' experience with the tube, reaching approximately 30 % of initial levels after only 1 min of testing and stabilising to a minimum, just over 1 attack per minute after only 6 min of test. This reduced number of attacks was maintained in a subsequent exposure to the tube (trial 2), which took place after 1 h. A similar decrease in the number of attacks is commonly observed in cuttlefish tested with the same paradigm and it is regarded as evidence of inhibition learning (Agin et al., 1998; Messenger, 1973). The animal experiences a situation in which spontaneous behaviour, attacking the prey, is not appropriate and does not provide the expected result (capture); the animal thus learn to inhibit this inappropriate attack behaviour. Arguably, in guppies, other types of learning can account for the same pattern of behavioural change, such as extinction or habituation. The type of learning observed with the tube task differs from extinction because extinction occurs when a conditioned response resulting from a learned predictive relationship between two events is abolished (Shettleworth, 2010). Regarding habituation, this usually occurs when an individual subjected to a sensory stimulation exhibit causes a certain motor response, and after repeated stimulations the response is decreased (reviewed in Rankin et al., 2019; Schmid et al., 2015; Shettleworth, 2010). Our experimental situation does not seem to fully fit this scenario, because we did not need repeated sensory stimulations and the decrease in attacks was also observed after a single exposure to prey. Further, the guppies reduced seemingly active foraging behaviour rather than an automatic motor response to stimulation. Aside from these considerations, in experiment 2, we furnished direct evidence that the learning process of guppies does not fit one of the main proprieties of habituation: habituation occurs more quickly when the stimulation is more intense (Rankin et al., 2019). Actually, guppies exhibited the opposite trend; subjects exposed to more intense stimulation, provided by a larger group of prey showed a decreased reduction in the number of attacks. This pattern is consistent with an inhibitory behaviour process, because inhibition is expected to occur at a slower rate when there is a greater lure involved (Brucks et al., 2017; Bugnyar et al., 2012; Rosati et al., 2007). Similar evidence of the absence of habituation effects in the tube task has been found in cuttlefish (Agin et al., 2006). Hence, we can reasonably conclude that the guppies exhibited fast and efficient inhibition of the foraging behaviour in the tube task.

A novel question addressed by our study concerns the length of time over which the fish can retain the learned inhibition of behaviour. In experiment 3, we showed that guppies tested with the 24-h interval exhibited a different learning pattern between the first and the second trials; like experiment 1, this pattern seems to indicate that the guppies learned to inhibit the behaviour in trial 1 (decrease in the number of attacks) and remembered this learned inhibition in trial 2 (reduced and constant number of attacks). Conversely, the guppies tested using the 72-h interval showed a learning pattern in the second trial that was similar to that of the first trial, suggesting that they had newly learned to inhibit the behaviour in trial 2. This indicates that the memory window for the inhibitory task falls between 24 h and 72 h. As far as we know, prior studies did not investigated the duration of memory for an inhibited behaviour in fish (Gatto et al., 2018; Lucon-Xiccato et al., 2017). Studies in other contexts have suggested that fish can sustain greater memory windows, but there is large variability between species. For instance, the retention of a learned foraging behaviour, for the 15-spined stickleback, *Spinachia spinachia*, starts to decrease after 2 days (Croy and Hughes, 1991), an interval similar to that observed in our study. On the other hand, the silver perch, *Bidyanus bidyanus*, has been reported to remember a learned foraging skill for up to 5 weeks (Warburton, 2003). Given the current state of research, it is not yet possible to determine whether the memory performance of guppies hinged on the specific task, as inhibiting predatory behaviour is very cognitively demanding. It should be also noted that rapid ‘forgetting’ of a learned inhibition, followed by re-attempting the performance of the original behaviour, could be elements of the high cognitive flexibility expressed by this species (Lucon-Xiccato and Bisazza, 2014).

From a comparative perspective, our results are relevant to understating the diffusion of inhibitory capacities across vertebrates, although it is difficult to render a precise comparison of the performance of guppies with that of birds and mammals because the tube task has not, to date, been used in these clades. Our study clearly does not support the premise that fish are, somehow, intrinsically advantaged in solving the cylinder task, because of methodological details. Instead, guppies seem have a quality of general effectiveness at inhibiting behaviours that allows them to solve various inhibitory tasks. This, along with findings in avian species (Isaksson et al., 2018; Kabadayi et al., 2016; Kabadayi et al., 2017b), suggests that the hypothesis of increased inhibitory abilities in large-brained species (MacLean et al., 2014) is not valid for the entire vertebrate taxon. Guppies, indeed, have brains substantially smaller than those of mammals or birds, but this species still demonstrates notable inhibitory abilities.

If brain size does not account for guppies' inhibitory performance, other possibilities should be considered. Complexity and variability of social system are also selective forces that may have driven the evolution of refined inhibitory control, at least in primates (Amici et al., 2008). Accordingly, guppies exhibit a complex social system, characterised by individual recognition (Griffiths and Magurran, 1999), reciprocity in interactions between group mates (Dugatkin and Alfieri, 1991; Cattelan et al., 2018), and, interestingly, high occurrence of fission-fusion events that render the composition of guppy shoals highly variable (Croft et al., 2003). It is also worth noting that prior studies have often reported a rather impressive repertoire of cognitive abilities in fish. Fish can, for instance, use tools (Brown 2012), develop cultural traditions (Helfman and Schultz, 1984), take 'Machiavellian' decisions in social contexts (Bshary, 2011), use numerical information (Miletto Petrazzini et al., 2015), and acquire complex spatial maps (de Perera, 2004). Therefore, it is possible that some general factors account for the cognitive abilities of fish, which includes their high inhibitory capacities. Among other possibilities, future studies should investigate the role of neuronal density (Herculano-Houzel, 2017) and the large number of genes implicated in cognition that underwent duplication in fish (Schartl et al., 2013). Certainly, inhibition and other cognitive abilities have played a role in the high diversity and ecological success of fish (Bshary and Brown, 2014).

In conclusion, guppies exposed to an unreachable prey exhibited a clear inhibition of foraging behaviour, which was observed in three independent experiments. The present study seems to substantiate early evidence of high inhibitory abilities in guppies with a novel task that might be considered demanding. To gain a thorough understanding of the evolution of this cognitive ability, it is important for us to subject other vertebrates to testing with the tube task and other inhibitory tasks, and try to determine whether the same neural substrates and the same cognitive processes (i.e. inhibitory control) underlie inhibitory behaviour in primates and other groups, for each specific task.

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Figure captions

Fig. 1 (a) Top view of the experimental apparatus and (b) lateral view of a guppy approaching the tube with live prey

Fig. 2 Number of attacks (mean ± SEM) towards the (a) prey and the (b) empty tube (control condition) in experiment 1 divided in minute blocks

Fig. 3 Number of attacks (mean ± SEM) toward the prey in experiment 2 of the guppies exposed to the two quantities of prey (large or small) divided in minute blocks

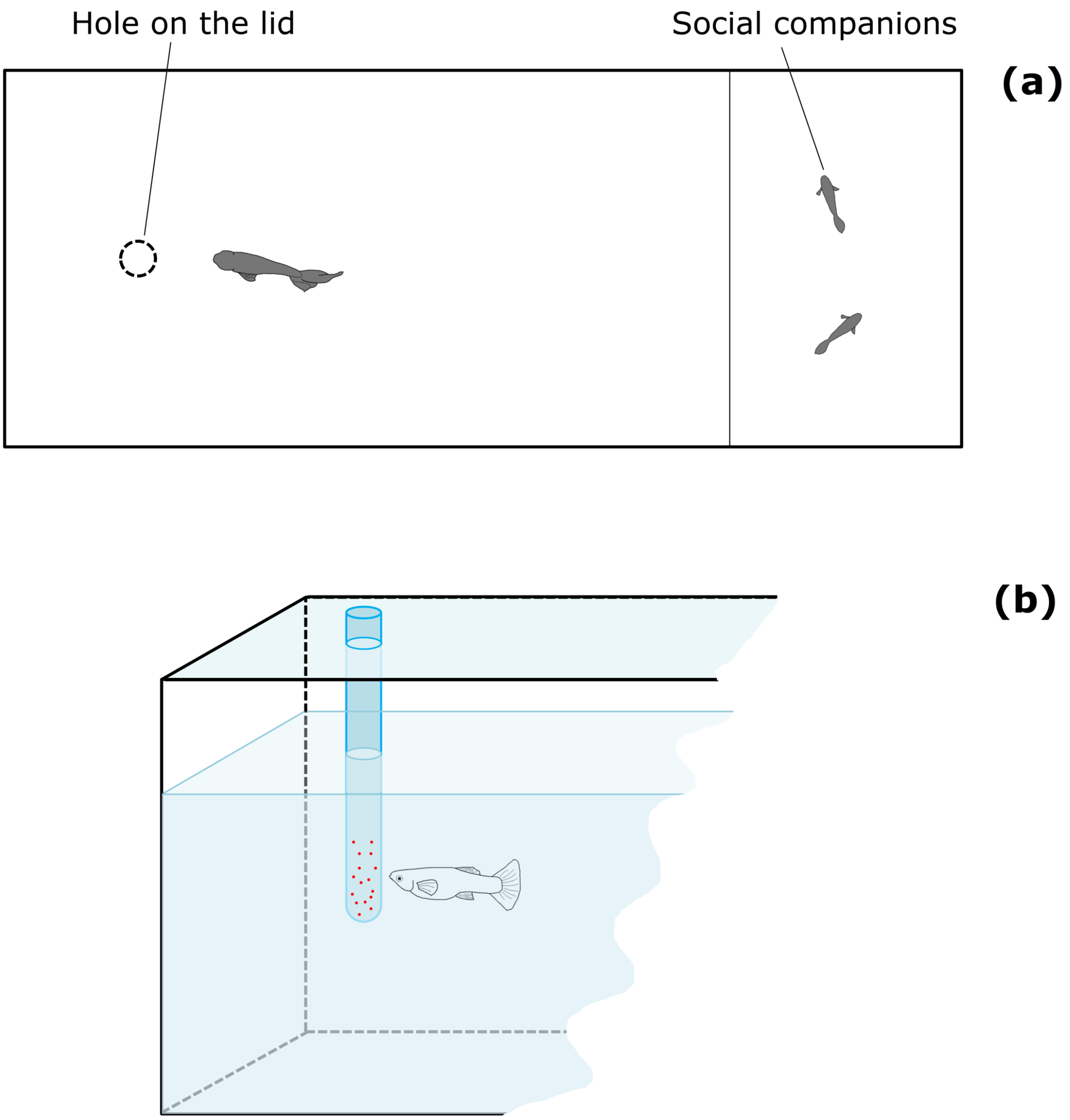
Fig. 4 Number of attacks (mean ± SEM) toward the prey in experiment 3 of the guppies exposed to the two intervals between trials (24 h or 72 h) divided in minute blocks

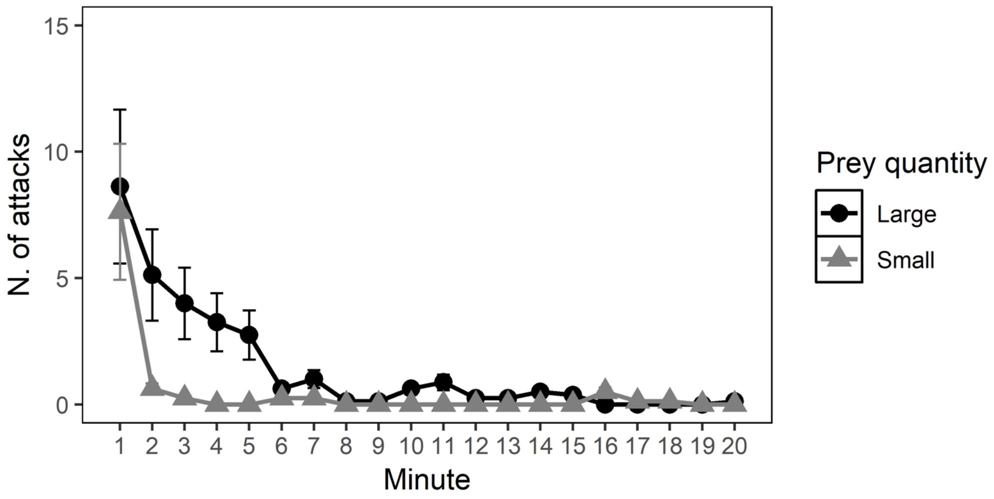
Tables

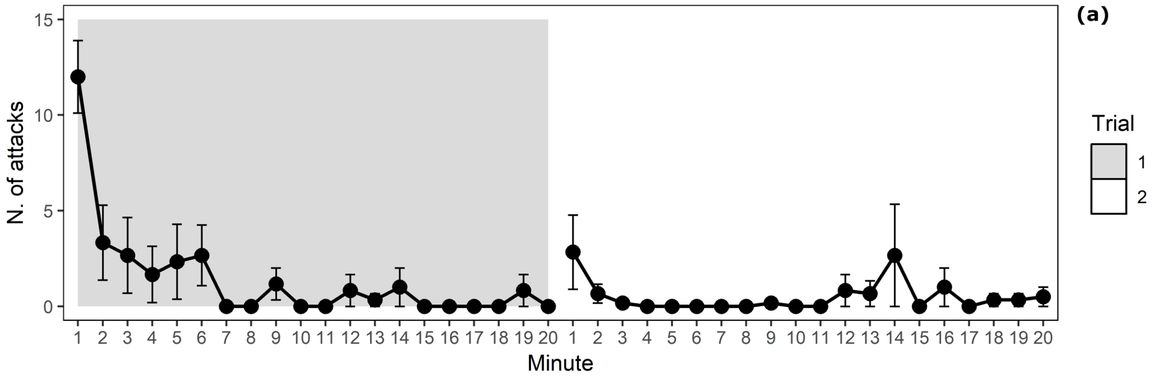
Table 1

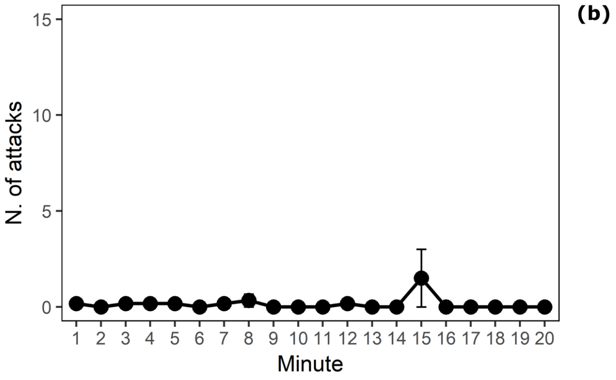
Activity of brine shrimps in the tube across time measured as number of crossing of the median line of the tube (mean ± SD).

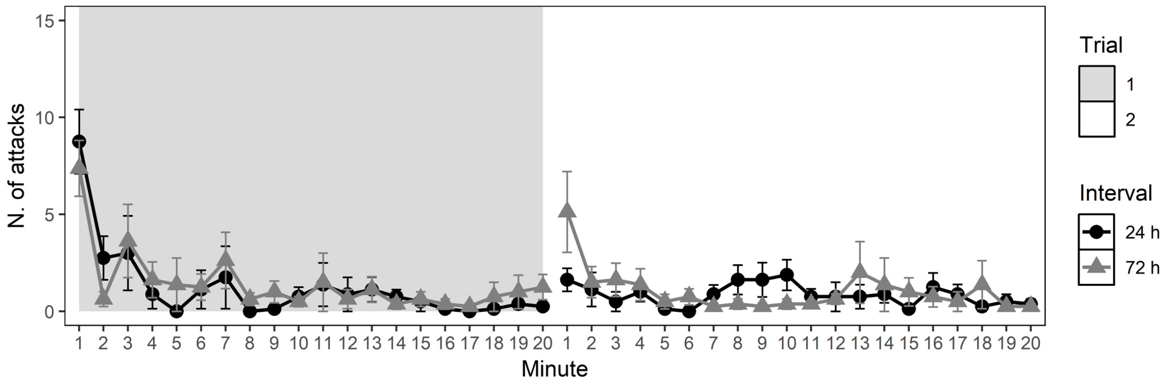
Minute	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mean	197.00	126.25	115.25	127.25	118.75	121.00	127.25	129.75	125.25	129.75	131.75	128.5	121.00	116.25	117.25	124.50	130.00	130.00	128.00	126.25
SD	45.35	14.31	15.84	8.06	8.66	18.40	9.11	14.27	21.65	27.87	15.20	25.67	9.20	9.32	6.40	6.76	17.64	11.46	11.46	8.10


Hole on the lid
Social companions
(a)
(b)


N. of attacks
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Minute
Prey quantity
Large
Small


(a)
N. of attacks
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Minute
Trial
1
2


(b)
N. of attacks
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Minute


N. of attacks
0
5
10
15
Minute
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Trial
1
2
Interval
24 h
72 h

732