

Aversive control of *Betta splendens* behavior using water disturbances: Effects of signaled and unsignaled free-operant avoidance and escape contingencies



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ABSTRACT

The vast majority of research on aversive control of behavior using animal models employs electric-shock based procedures with avian and mammalian species. Notwithstanding that pragmatic aspects explain this prominence in the field, there is still a need for testing well-established facts about shock-based aversive control using other, perhaps more ecologically valid or biologically relevant, forms of stimulation with other species. Following up on an observation that water disturbances appear to be aversive to *Betta splendens* (bettas), we developed a preparation for studying free-operant avoidance with this species in which water flows (WFs) replaced electric shocks. Fish changed compartments in a shuttle tank to escape or avoid 10-s WF, which were delivered with 30-s flow-flow and response-flow intervals. We tested the effect of adding a warning stimulus (curtains of air bubbles) to the last 5 s of the response-flow interval (i.e., signaled avoidance) on the bettas' temporal distribution of crossings and swimming patterns. Escape was the predominant response, which importantly reduced the exposure to the WF. Avoidance responses rarely exceeded the frequency of escape. The warning stimulus did not produce the expected postponement of responses to the last segment of the response-flow interval. Distinctive swimming patterns emerged, dissipated, and reappeared during baseline and treatment conditions. These findings confirm the aversive function of WF for bettas and expand the generality of negative reinforcement phenomena using non-shock-based procedures with a less-frequently studied species. Further developments of the WF paradigm show promise for replicating other aversive control phenomena (e.g., punishment), and analyzing spatiotemporal patterns produced by aversive contingencies.

1. Introduction

The vast majority of research on learning and behavioral processes regulated by aversive contingencies employs electric-shock procedures typically with mammalian and avian animal models (e.g., fear conditioning, avoidance, and conditioned suppression – Cabrera and Dos Santos, 2012; Curzon et al., 2009; Hurtado-Parrado et al., 2017; Izquierdo et al., 2016; Kenney et al., 2017). This prevalence of electric shock in aversive control research with birds and mammals could be explained by its convenience of use, its reliability, its broad range of intensities and durations, the feasibility of titrating shock levels for individual subjects, and the fact that it is aversive at levels that do not cause tissue damage (Barker et al., 2010; Baron, 1991; Crosbie, 1998). In addition, electric shock can be presented very briefly so that

processes such as avoidance and punishment can be studied independently of real or adventitiously reinforced escape behavior (Baron, 1991; Sidman, 1953). Although these pragmatic considerations must have played an important role in the success of preeminent research programs that exclusively employed shock-based procedures (e.g., Azrin and Holz, 1966), the decision to extensively use electric shock has been also based on "...the conviction that general principles will emerge" (Baron, 1991, p. 176) – i.e., that similar principles would have been established had other types of aversive stimuli been used. However, there is still a need for further testing and potentially replicating well-established facts about shock-based aversive control in species other than birds and mammals using other, perhaps more ecologically valid or biologically relevant, forms of stimulation. As Crosbie (1998) noted, organisms may respond differently to biologically

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relevant than to biologically irrelevant aversive stimuli, and although both types produce similar effects, "... it is uncertain whether they have similar patterns of adaptation and recovery, and produce similar by-products such as emotional behavior or overgeneralization." (Crosbie, 1998, p. 175)

1.1. Alternatives to electric shock in aversive control research

Efforts to develop alternatives to electric shock have originated from different sources: First, the dramatic decline of aversive control research during the last decades, mainly due to ethical debates (Catania, 2008; Pierce and Cheney, 2017) which have included the view that electric shock implies pain and therefore cruelty (Baron, 1991; Crosbie, 1998; Davis, 1981; Hineline and Rosales-Ruiz, 2013; Russell, 1997). Second, the recognition that electric shock has practical limitations, including disruption of sleep and social behavior and interference with electronic measurements (Barker et al., 2010). Third, criticisms of experimental models of anxiety and depression that involve electric shock, which claim that these models entail a pain component that does not have a counterpart in human emotional phenomena (Barker et al., 2010; McQuade et al., 1999).

Although some research with monkeys and rodents exposed to intense noise and light and tail pinches have supported generality of the effects of aversive stimuli (e.g., Barnes and Kish, 1957; Brodie and Boren, 1959; Clark et al., 1973; Kaplan et al., 1965; Rohles, 1965; Spealman, 1978; Weiss and Laties, 1966), few efforts along these lines have been conducted during the last few decades (e.g., Barker et al., 2010; Hurtado-Parrado, 2015; Morales-Mayer et al., 2015; Reed et al., 1995). Like electric shock, tail pinches have a pain component; perhaps for this reason, light and sound are the only stimuli that recently have been systematically tested as feasible alternatives to electric shock. In the case of sound, Reed and colleagues (Reed, 2011; Reed et al., 1995; Reed and Yoshino, 2001, 2005, 2008; Yoshino and Reed, 2008) demonstrated that brief loud tones (e.g., a 1.5 s tone of 105 dB) have a punishing effect on the behavior of rats. In the case of light, Barker et al. (2010) reported that bright light suppressed lever pressing maintained by food reinforcement, and produced reliable escape and signaled avoidance in rats. Similarly, Pear et al. (2008) found that bright light contingent on entry into a particular region of an experimental tank suppressed entries of a *Betta splendens* (betta) into that region.

Despite these promising findings, it appears that the feasibility of light and noise as alternatives to electric shock is limited. The suppressive effects of intense noise have been less pronounced than those observed in analogous studies with electric shock, and have only been observed under limited circumstances (e.g., schedules with low reinforcement rates; Reed and Yoshino, 2008). Also, Barker et al. could not reproduce unsignaled free-operant avoidance using intense light, and a recent replication of their procedure indicated that this form of stimulation produces retinal damage in Wistar rats (Hurtado-Parrado et al., under review).

1.2. Stimulus functions of water disturbances for the behavior of *Betta splendens*

Informal observations of betta behavior during research on spatio-temporal patterns produced by different non-aversive operant contingencies (i.e., the type of analysis described by Pear, 2004) indicated that water disturbances produced by air being pumped into the tank resulted in fish's agitation, followed by hesitation to approach the area where this stimulus was delivered (see sample video, Hurtado-Parrado, 2012). Observation of bettas in the lab and the fact that the natural habitats of wild bettas are quiet fresh water ponds where they establish a territory, build bubble nests, and breed (Jaroensutasinee and Jaroensutasinee, 2001; Monvises et al., 2009; Goldstein, 2004) suggested that water disturbances could be unconditioned aversive events for this species. This suggested further that using water disturbance as

an aversive event with these fish would potentially establish two types of generality of aversive conditioning phenomena: (a) generality from electric shock to water disturbance, and (b) generality from mammalian and avian species to a fish species. These considerations triggered the development of an experimental setup in which bettas were exposed to water disturbances in the form of water flows (WFs). Preliminary tests of the WF preparation (Hurtado-Parrado, 2015) showed its promise as an ecologically valid and presumably non-painful stimulus useful for testing replication of aversive control phenomena almost exclusively studied with electric shock in rats and pigeons. Two areas were of special relevance in this regard because of the lack of relevant research (a) systematic attempts to replicate behavioral phenomena produced by aversive stimuli only demonstrated in fish with shock-based procedures (i.e., free-operant signaled and unsignaled avoidance; Hurtado-Parrado, 2010), and (b) lack of an analysis of spatiotemporal patterns produced by different aversive contingencies (i.e., the type of analysis described for positively reinforced behavior by Pear, 2004). Here we present the results of the first systematic implementation of the WF protocol, which aimed to contribute to both these areas.

1.3. Overview of the study

The centrality of free-operant avoidance in aversive control research (Hineline and Rosales-Ruiz, 2013; Sidman, 1953), and the fact that the only fish species it has been demonstrated in is goldfish (*Carassius auratus*) using shocks (Behrend and Bitterman, 1963; Scobie, 1970), led us to first explore free-operant avoidance in bettas using the WFs preparation. Behrend and Bitterman (1963) and Scobie (1970) demonstrated free-operant signaled and unsignaled avoidance with goldfish using a shuttle tank. In the unsignaled conditions of their experiments, a 0.25-s shock was delivered with a shock-shock (S-S) interval that ranged between 15 and 60 s. Every time the fish changed compartments, the delivery of the shock was postponed in a response-shock (R-S) interval that ranged between 15 and 60 s. In the signaled conditions, a warning stimulus –light– came on during the last five seconds of the R-S interval. We used an adaptation of these procedures in which WFs replaced shocks to (a) assess the negative reinforcement function of water disturbances for the behavior of bettas; (b) investigate the generality of signaled and unsigned free-operant avoidance and escape behavior, which have been studied in fish exclusively with electric shock; and (c) characterize the spatial and temporal distribution of fish behavior under avoidance and escape contingencies.

2. Materials and methods

2.1. Subjects

Six non-wild adult male bettas (labeled C01, C02, C03, C04, C05, and C06) with mean length of 6 cm and selected on the basis of being dark enough to be detected by a video tracking system (refer to *Instruments* section) were obtained from a local pet store. Each subject was housed in an individual tank (40 cm × 20 cm × 30 cm), in a room separate from the room housing the experimental tank, and was transported to the experimental tank before each daily session. A 12/12 h. light/dark cycle (lights on at 8:00 AM) was in effect in the room housing the fish, and all the experimental sessions occurred between 10:00 AM and 3:00 PM. Fish were fed in their housing (or home) tanks 1 h. before the daily session was conducted. Fish C01, C02, and C04 were removed from the study on day 80, 76 and 82, respectively, due to health issues.

All experimental procedures and animal maintenance were approved by the Fort Garry Campus Animal Care Committee of the University of Manitoba (Protocol No. F12-024).

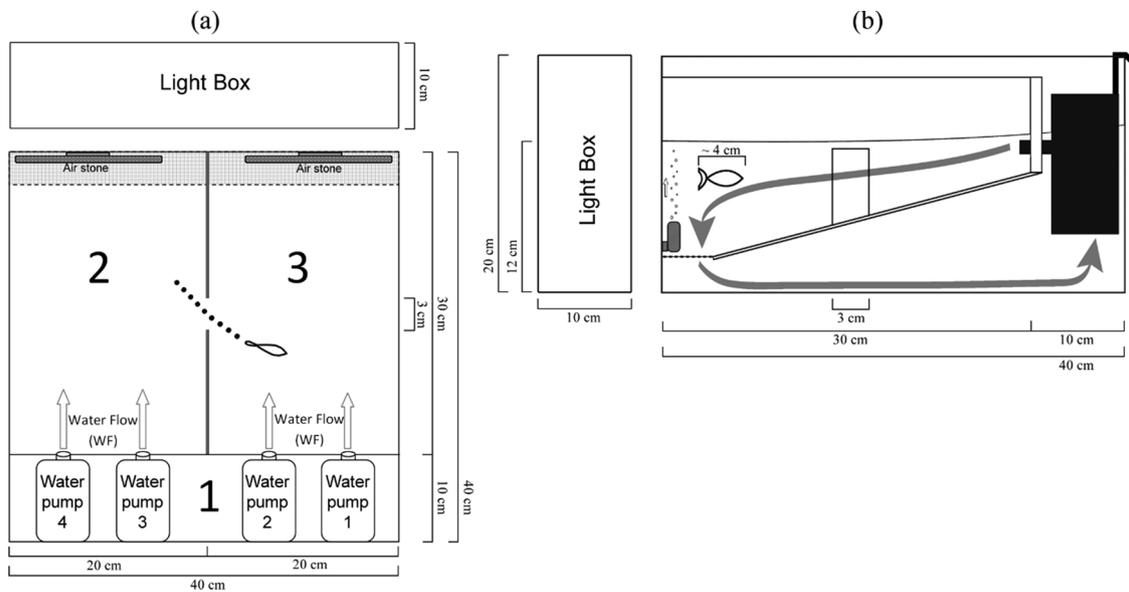


Fig. 1. Overhead view (a) and side view (b) of the experimental shuttle tank (40 cm × 40 cm × 20 cm). The tank was divided into 3 different compartments by opaque white plastic dividers (a). In compartment 1 (40 cm × 10 cm × 20 cm), four water pumps provided WFs to the equally-sized compartments 2 and 3 (20 cm × 30 cm × 20 cm each). The fish could swim freely and cross between areas 2 and 3 through a 3 cm wide opening in the divider connecting these compartments. Compartment 1 was inaccessible to the fish. One air stone was attached with suction cups near the lower back walls of compartments 2 and 3. The air stones were connected to a pump located outside the tank in order to produce curtains of air bubbles (CABs). Water was recycled by a channel created by a plastic divider and a wire mesh through which pumps forced the water (b). A light box containing two 15-watt bulbs and located 5 cm away from the tank was constantly on and, in combination with overhead fluorescent lights, evenly illuminated the inside of the tank.

2.2. Instruments

As diagrammed in Fig. 1, the experimental tank was a glass shuttle tank (40 cm × 40 cm × 20 cm), filled to a depth of 12 cm with dechlorinated water at a temperature between 26°–27 °C. The experimental tank was divided by white opaque plastic dividers into three compartments. Compartment 1 contained four AquaClear 50® water pumps and was inaccessible to the subject, which could only access compartments 2 and 3. A small opening (3-cm wide) connected these latter two equal-sized compartments. The water pumps introduced WFs into the compartments with a constant flow of approximately 3600 ml per minute. Water projected by the water pumps was recycled through a channel consisting of a plastic divider and a wire mesh. Due to a mechanical failure of one of the four water pumps, only pumps 1 and 4 were active during the initial sessions of the first experimental condition. Immediately after the technical issues were resolved, all four pumps were utilized for the remainder of the first treatment condition. Inspection of the data collected during this condition led to the deliberate use of just pumps 1 and 4 throughout the rest of the study. The shuttle tank was emptied, cleaned, and refilled before each fish was given its daily session.

Two air stones were attached by suction cups to the wall opposite to compartment 1. Air was pushed to these air stones from an air pump outside the tank to produce curtains of air bubbles CABs.

A custom-made 3D video tracking system (VTS; Pear, 2004; Pear and Eldridge, 1984; Pear et al., 1989) tracked the fish in real time throughout each session. Two cameras were positioned side-by-side, 80 cm apart, and attached to a scaffold over the shuttle tank. Both cameras scanned from the outer wall of compartment 3 to the outer wall of compartment 2. Since the VTS operated on the basis of brightness contrast, and the fish was the darkest object visible to the cameras, only the subject's position was tracked and recorded. The video output was processed to plot a single point location in three spatial coordinates every 1/10th of a second. This output was then run through another computer containing additional custom-made software ("Operant Behavior Laboratory", or "OBL"; Wayne S. Chan, 2011) that recorded the following behavioral measures: (a) number of crossings between

compartments; (b) amount of time that the subject was exposed to the WFs in each compartment, and (c) swimming trajectories of the fish. The OBL software also controlled the timing of WFs and CABs through relays connected to the water pumps and the air pump.

A light box with two 15-watt bulbs (located 5 cm away from the experimental tank; see Fig. 1) was constantly on during each session. This box illuminated the tank's interior evenly, with the aim of improving the tracking by reducing the occurrence of spurious dark areas.

The intrinsic delay of a few seconds between the activation of the water pumps that produced the WFs and the moment when these stimuli reached their maximum strength made it necessary to increase the length of each flow presentation, when compared to the shock duration used by Behrend and Bitterman and Scobie (i.e., less than 1 s). Because 10-s deliveries of WFs were effective in producing sizeable changes in betta behavior in preliminary experiments (Hurtado-Parrado, 2015), this duration of the WFs was utilized in the present study. It should be noted that any crossing that occurred during the activation of the water pumps was considered an escape response because it caused their immediate deactivation, and thus interruption of the WFs. This was the case even if the WFs had not reached their full strength, which occurred during the first seconds that followed the activation of the water pumps.

In lieu of S-S and R-S intervals, as in a standard free-operant schedule, the present study used flow-flow (F-F) and response-flow (R-F) intervals. The specific values of the R-F and F-F intervals implemented were derived from research with goldfish in which different configurations of the R-S and S-S intervals produced consistent signaled and unsignaled avoidance (Behrend and Bitterman, 1963; Pinckney, 1968; Scobie, 1970). The parameters chosen for the present study were R-F = 30 s and F-F = 30 s.

Unlike shock in traditional free-operant avoidance, the aversive stimulus in the present study had a nonzero duration. This prompted the arbitrary decision to deactivate the pumps when a crossing occurred during their activation. This decision permitted us to study escape in addition to avoidance behavior. In addition, we decided that the R-F interval following a crossing would start the instant that the crossing occurred, as this seemed most consistent with traditional free-operant avoidance.

Table 1
Sequence of conditions for each subject and number of sessions per condition (between brackets).

Fish	Conditions										
C01	BL-1 (15)	UA-1 (22)	BL-2 (6)	UA-2 (6)	BL-3 (8)	SA-1 (13)	BL-4 (5)	SA-2 (5)			
C02	BL-1 (11)	UA-1* (16)	BL-2 (7)	UA-2 (14)	BL-3 (5)	UA-3 (5)	BL-4 (9)	SA-1 (6)	BL-5 (3)		
C03	BL-1 (11)	UA-1* (17)	BL-2 (7)	UA-2 (9)	BL-3 (5)	UA-3 (7)	BL-4 (5)	SA-1 (8)	BL-5 (6)	SA-2 (12)	
C04	BL-1 (12)	SA-1* (16)	BL-2 (14)	SA-2 (10)	BL-3 (5)	SA-3 (8)	BL-4 (5)	UA-1 (11)			
C05	BL-1 (12)	SA-1* (16)	BL-2 (6)	SA-2 (9)	BL-3 (9)	SA-3 (6)	BL-4 (5)	UA-1 (9)	BL-5 (5)	UA-2 (12)	
C06	BL-1 (15)	SA-1 (23)	BL-2 (5)	SA-2 (6)	BL-3 (8)	SA-3 (6)	BL-4 (5)	UA-1 (9)	BL-5 (5)	UA-2 (7)	

Note: BL = Baseline; UA = Unsignaled Avoidance; SA = Signaled Avoidance; * = condition with two functional water pumps per compartment (two-pump configuration).

In contrast to Behrend and Bitterman's (1963) procedure, in which light was used as the warning stimulus, the signaled conditions in the present study entailed CABs during the last five seconds of the R-F interval. This decision resulted from consideration of the findings of studies with bettas that demonstrated operant discrimination only when CABs (as opposed to some other type of stimulus) signaled reinforcement – i.e., light and spatial cues failed to acquire that same discriminative function (Craft et al., 2007; Wirth et al., 2003).

2.3. Procedure

Daily sessions were 20 min long (including WF periods during treatment conditions), and only one subject was tested at a time. Before each session, the subject was removed from its home tank using a small container and was introduced to one of the compartments of the shuttle tank (assigned randomly). The opening between compartments 2 and 3 was obstructed to keep the fish in the assigned compartment until the start of the session, which occurred three minutes after the subject was introduced into the experimental tank.

Immediately after the session ended, OBL software automatically saved all the data, and the fish was returned to its home tank. Then, the experimental tank was emptied, cleaned, and refilled with new water as preparation for the next subject's session.

A single-case design was used (Perone and Hursh, 2013). The following are the details of each condition of the experiment.

2.3.1. Baseline

Each subject received 20-min baseline sessions in which WFs were not delivered. The number of crossings between compartments and the swimming trajectories of the fish were recorded for each baseline session.

2.3.2. Unsignaled avoidance (UA)

WFs were delivered every 30 s (F-F = 30 s) in the compartment where the fish was located, unless the fish crossed to the opposite compartment of the tank (i.e., response-dependent deliveries of WFs). With each crossing the timer was reset to 30 s (i.e., R-F interval = 30 s); if WFs were occurring at that moment they were immediately stopped. Additional to the measures described for baseline conditions, three more were recorded on each UA session: (a) number and percentage of WFs delivered (percentages were calculated assuming that a total absence of crossings would have resulted in the delivery of 30 WFs – i.e., 100%); (b) duration and percentage of exposure to WFs (percentages were calculated assuming that a total absence of crossings would have resulted in a total exposure to WFs of 300 s – i.e., 100%); and (c) frequencies of different types of crossings, as described below.

Crossing during any time that a WF was occurring was categorized as escape (Esc) and, as previously mentioned, initiated an R-F interval. This response immediately turned off the water pump and thus interrupted the WF. Crossings that occurred during R-F or F-F intervals were classified as avoidance, and likewise initiated an R-F interval. Note that F-F intervals occurred only following a full 10-s WF without any incidence of a crossing response. Crossings were further differentiated in order to obtain more detailed information about the temporal distribution of the avoidance responses. A crossing was recorded as: *early avoidance* (EA) if it occurred during the first 25 s of the R-F interval (i.e., fish changed compartment within 25 s of the last response); *late avoidance* (LA) if it occurred during the last 5 s of the R-F interval; and *Flow-Flow avoidance* (FF) if it occurred anytime during the F-F interval.

2.3.3. Signaled avoidance (SA)

The parameters and measures scheduled for the UA condition were the same as those for the signaled avoidance (SA) condition, with the addition of a warning stimulus (CABs) presented during the last 5 s of each R-F interval. This stimulus lasted for the remaining 5 s of the R-F interval, or until the fish changed compartments.

2.4. Design

Table 1 shows the sequence in which each subject was exposed to the different conditions of the experiment, which changed whenever a stable pattern of crossings was observed (see stability criteria below). Fish C01, C02, and C03 differed from C04, C05, and C06 regarding the sequence in which they were exposed to SA and UA conditions: the first were initially exposed to UA, while the latter were exposed to SA first.

During the first treatment condition of subjects C02, C03, C04, and C05 (marked with a "*" across the Table 1), WFs were produced by two water pumps per compartment (two-pump configuration), as originally planned. However, after considering the poor performance of these fish during their initial condition, it was decided to use a single water pump per compartment (one-pump configuration) for the rest of the study. Fish C01, C02, and C04 were removed from the study, due to illness, before completing all the programmed conditions.

2.5. Stability criteria

Based on criteria used by Critchfield et al. (2003); Green and Estle (2003), and Magoon and Critchfield (2008), each condition (baseline or treatment) terminated when no consistent trend was noticeable (no increase or decrease in responding for three consecutive data points, confirmed through visual inspection of the graphed data) and either: (a) the total number of crossings between compartments for a session fell within the average of the last 5 sessions by five (i.e., ± 5) responses, or (b) 20 consecutive sessions were run in a condition and there was no consistent trend when this 20 session cut off was reached.

2.6. Data analysis

Visual analyses of graphed data for each individual were conducted using guidelines for the type of experimental design implemented (e.g., differences in level and variations in trend across baseline and treatment conditions; Bourret and Pietras, 2013; Hurtado-Parrado and López-López, 2015).

3. Results

Fig. 2 shows the total number of crossings displayed by each fish across the baseline (BL) and treatment conditions (UA and SA) of Experiment 1. For each treatment condition, these data are broken down on the frequency of each type of crossing; namely, escape (ESC), early avoidance (EA), late avoidance (LA), and flow-flow (FF) responses. Note that fish C01, C02, and C03 differed from C04, C05, and C06

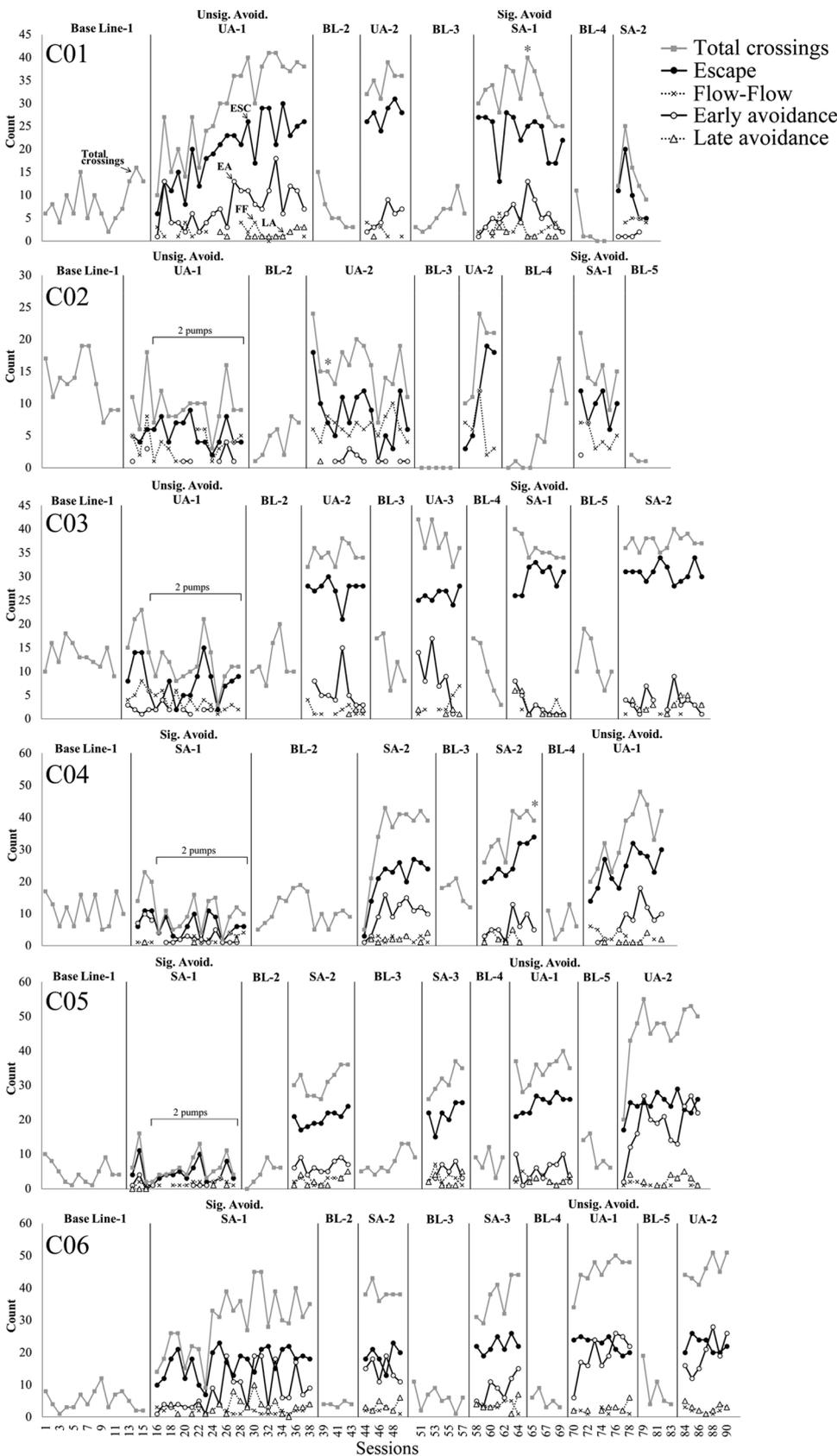


Fig. 2. Number of crossings displayed by each fish across daily sessions of baseline (BL) and treatment conditions (UA and SA) of Experiment 1. For each treatment condition, these data are broken down on the frequency of each type of crossing; namely, escape (ESC), early avoidance (EA), late avoidance (LA), and flow-flow (FF) responses. Note that fish C01, C02, and C03 differed from C04, C05, and C06 regarding the sequence in which they were exposed to SA and UA conditions (former were exposed to SA and UA conditions (former were exposed to UA first). As indicated in the corresponding graphs, fish C02-C05 were exposed to the 2-pump configuration during the last sessions of the first treatment condition. Throughout the rest of the experiment, only one water pump remained active for each compartment (see details in Method section). Note: BL = Baseline; SA = signaled avoidance; UA = unsignaled avoidance; ESC = escape; EA = early avoidance; LA = late avoidance; FF = flow-flow crossing; * = infection was detected on fish C01, C02, and C04.

regarding the sequence in which they were exposed to SA and UA conditions (the former 3 were exposed to UA first, the latter 3 to SA first).

3.1. Total number of crossings

All fish displayed a low level of crossing responses prior to any exposure to the treatment conditions – i.e., during the first baseline (BL-

1). A malfunction made it necessary to begin the first treatment condition using a single water pump for each compartment (see details in Method section). The increase in crossings of fish C02-C05 during these first sessions contrasted with the decrease observed when the two-pump configuration became active, which in some cases reached frequencies below the previous baseline. These data, together with the observation that fish struggled at escaping the WFs produced by the two-pump configuration, suggested that the one-pump configuration produced more crossings. Because fish C01 and C06 were the last to reach stability during baseline, and thus to start the first treatment condition, we tested these subjects to see if the one-pump configuration produced more frequent and consistent levels of responding. This unplanned manipulation produced a consistent ascending trend in the number of crossings displayed by C01 (UA-1) and C06 (SA-1), which reached from two to three times BL-1 frequencies.

The introduction of BL-2 produced a rapid decrease in the number of crossings displayed by C01 and C06, which reached levels similar to those observed during BL-1. The crossings for the remaining fish (C02, C03, C04, and C05) remained near the low levels observed throughout their first treatment condition. We thus tested to see if the increasing trend observed in the crossings of C01 and C06 with the one-pump configuration could be reproduced in all fish during a second treatment condition. Data displayed in the fourth panel of each fish in Fig. 2 corresponds to this manipulation, which shows that the increase in crossing responses observed initially in C01 and C06 were reproduced across these and the remaining fish. These data supported the notion that the two-pump configuration suppressed crossings, thus during the rest of the experiment only one pump remained active in each compartment. Apparently as a result of this manipulation, the total number of crossings remained well above baseline levels throughout the subsequent treatment conditions, reaching frequencies as high as three or four times those of the interspersed baselines.

A comparison of the subjects' performance during UA and SA conditions indicated that crossings during UA sometimes reached higher levels than during SA. Although this trend was observed at some point across all fish, it was more marked and consistent for fish C05 (UA-2) and C06 (UA-1 and UA-2). However, note that the data of C01, C02, and C04 are limited in this regard, since these subjects did not complete all the programmed conditions of the experiment due to illness.

3.2. Frequency of different types of crossings: escape, early avoidance, late avoidance, and flow-flow avoidance

As shown in Fig. 2, escape became the predominant type of crossing for fish C01 and C06 across their first treatment condition. This trend was accompanied by a somewhat irregular increment in the number of early avoidance responses. For fish C02, C03, C04, and C05 escape and early avoidance were also the predominant responses across the first treatment condition; however, frequency of these types of crossings was overall low and unsteady.

Once the one-pump configuration was established for all fish in the second treatment condition, the pattern of escape and early avoidance displayed by fish C03, C04, and C05 became similar to that observed in C01 and C06. Fish C02 initially showed higher frequencies of escape compared to the previous UA-1 condition, which resembled the trend observed in the other fish. However, the incidence of this response dropped during subsequent sessions, became unstable, and remained at levels that did not differ substantially from those in UA-1. Moreover, early avoidance in C02 occurred sporadically during UA-2, and virtually did not occur again during the rest of the experiment.

Throughout the treatment conditions that followed, escape remained the predominant response, and a slightly increasing trend in this crossing type was further observed across treatment conditions by all subjects except C01 and C02. Early avoidance was the second most frequent type of crossing. Although early avoidance in fish C05 and C06 closely matched or slightly exceeded the rate of escape at some points

of the experiment (especially during their last treatment conditions), this did not coincide with a substantial reduction in escape.

Late avoidance and flow-flow avoidance were the least consistent and most infrequent response types during the entire experiment. Although C02's frequency of flow-flow avoidance sometimes exceeded escape, this subject's data seems limited by the fact that the overall frequency of crossings only slightly surpassed baseline levels most likely because of early illness.

A comparison of SA and UA conditions shows that escape reached higher levels during UA conditions for all fish except C03, in which escape consistently occurred with more frequency during the SA conditions. Although early avoidance reached higher frequencies during the UA conditions of all fish, this was particularly evident and consistent in fish C05 and C06. Notwithstanding its low frequency the highest and most consistent frequencies of late avoidance were observed under SA for all fish except C02, which displayed a single instance of LA during the entire experiment. No clear distinction between the frequencies of flow-flow responses is evident when comparing SA and UA conditions.

3.3. Total WF frequency and duration

Each graph in Fig. 3 shows a given subject's data (C01-C06) regarding percentages of frequency and duration of WFs during baseline and treatment conditions (UA and SA). These percentages were calculated assuming that a total absence of crossings during an entire 20-min session would have resulted in the fish being exposed to 30 instances of WFs and a total of 300 s of flow time. For instance, if a fish in a given session received 15 instances of WFs and was exposed to a total of 200 s of flow time, these values would correspond to 50% of WFs ($15/30 \times 100 = 50\%$) and 66.7% of total flow time ($200/300 \times 100 = 66.7\%$).

Fig. 3 shows that the percentage of WFs per session rarely went below 80% throughout the experiment. This was specifically the case for C03, C05, and C06, which only seldom reduced the number of WFs per session to 24 instances out of the originally scheduled 30. In contrast, percentage of WFs very often reached values that were equal or above 100%, which implies that the fish received all or more than the 30 WFs originally scheduled. Unlike the low-to-absent reduction in the frequency of WFs, all fish except C02 produced large reductions in the time exposed to the WFs, although levels overall remained below 35% (~100 s out of the originally scheduled 300 s) and sometimes dropped below 25%.

The systematic decline in WF time observed in fish C01 and C06 during their first treatment condition was not observed in fish C02, C03, C04 and C05). It was only until the one-pump configuration became active during the second treatment condition, that the same pattern of WF frequencies and durations was replicated across all fish.

The fish overall managed to reduce more the exposure to the WFs, both in number and time, during UA conditions than during SA conditions.

3.4. Temporal distribution of crossings and swimming patterns

Fig. 4 shows the temporal distribution of crossings in terms of cumulative records and swimming patterns during the last session of each baseline and treatment condition for fish C03. These data are representative of the patterns observed in the other fish (Appendix A in supplementary materials shows the data for all fish). Although 3D data were obtained by the tracking system, the third dimension – depth – was not utilized because it did not provide any additional useful data for the purpose of this study.

3.4.1. Temporal distribution of crossings

As illustrated in the cumulative records of Fig. 4, crossings during the last sessions of baseline conditions, when stability was reached,

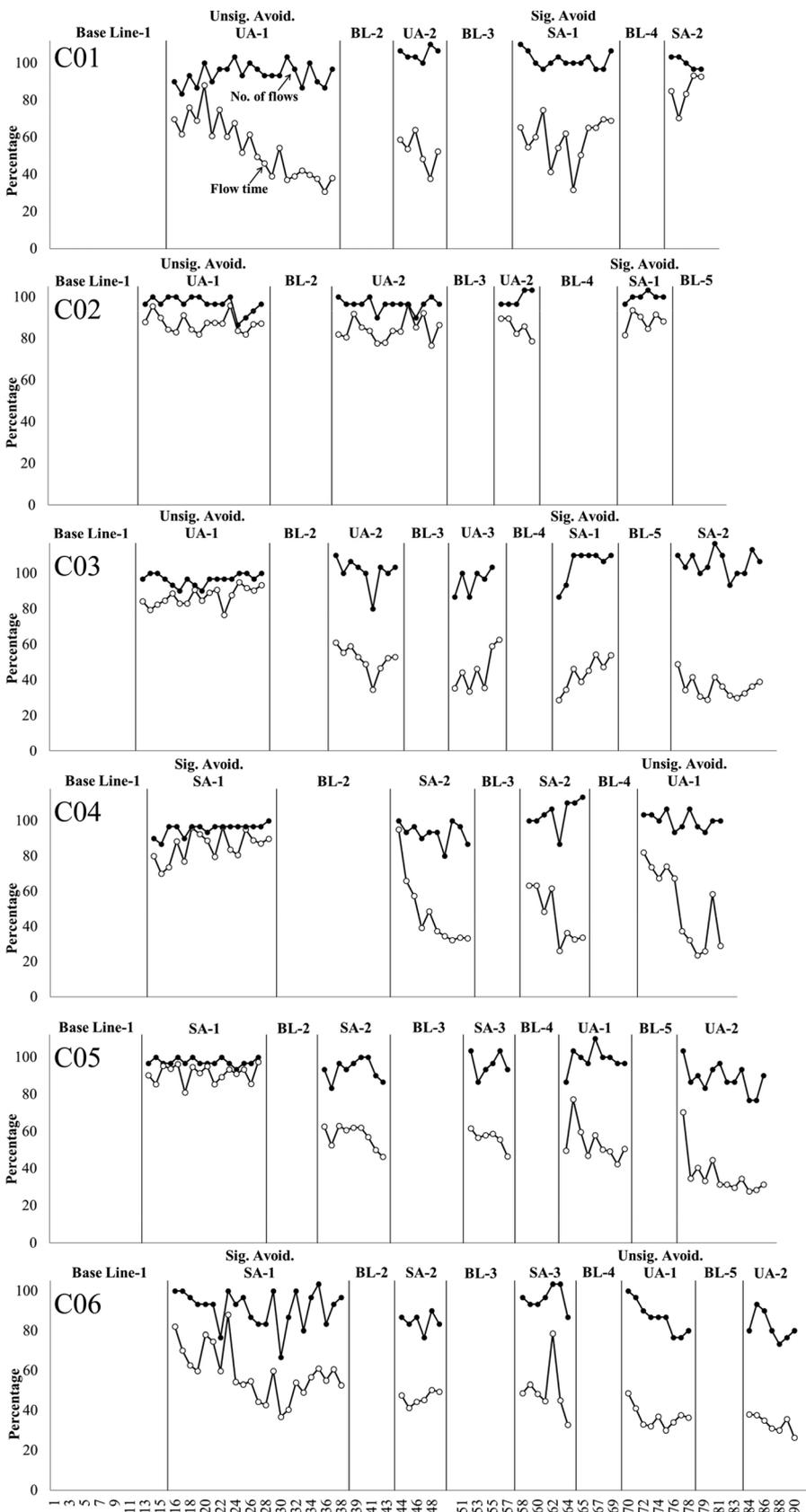


Fig. 3. Frequency and duration of WFs, expressed in percentages, during UA and SA conditions. There are no data points during baselines because WFs were not delivered during these conditions. A 100% refers to the total number of WFs (30 WFs) and flow time (300 s of flow time) given a total absence of crossings. It was thus possible for this percentage to exceed 100%, based on the subject’s crossing patterns. For instance, fish C03 was exposed to 32 instances of WFs (out of 30 originally planned) during the last session (day 88), representing 107% and 39% of total number of flows and flow time scheduled, respectively. Note: SA = signaled avoidance; UA = unsignaled avoidance. Details about the number of water pumps active during the first treatment condition and the infection of fish C01 and C02 are the same as for Fig. 2.

were low in number and were generally spread out over the session. In contrast, crossings during steady states of treatment conditions showed a continuously increasing “staggered” pattern and did not differ across

SA and UA conditions. This pattern consisted of the fish changing compartments at a very regular pace just after the beginning of each WF delivery. In those fish that began the experiment with the two-pump

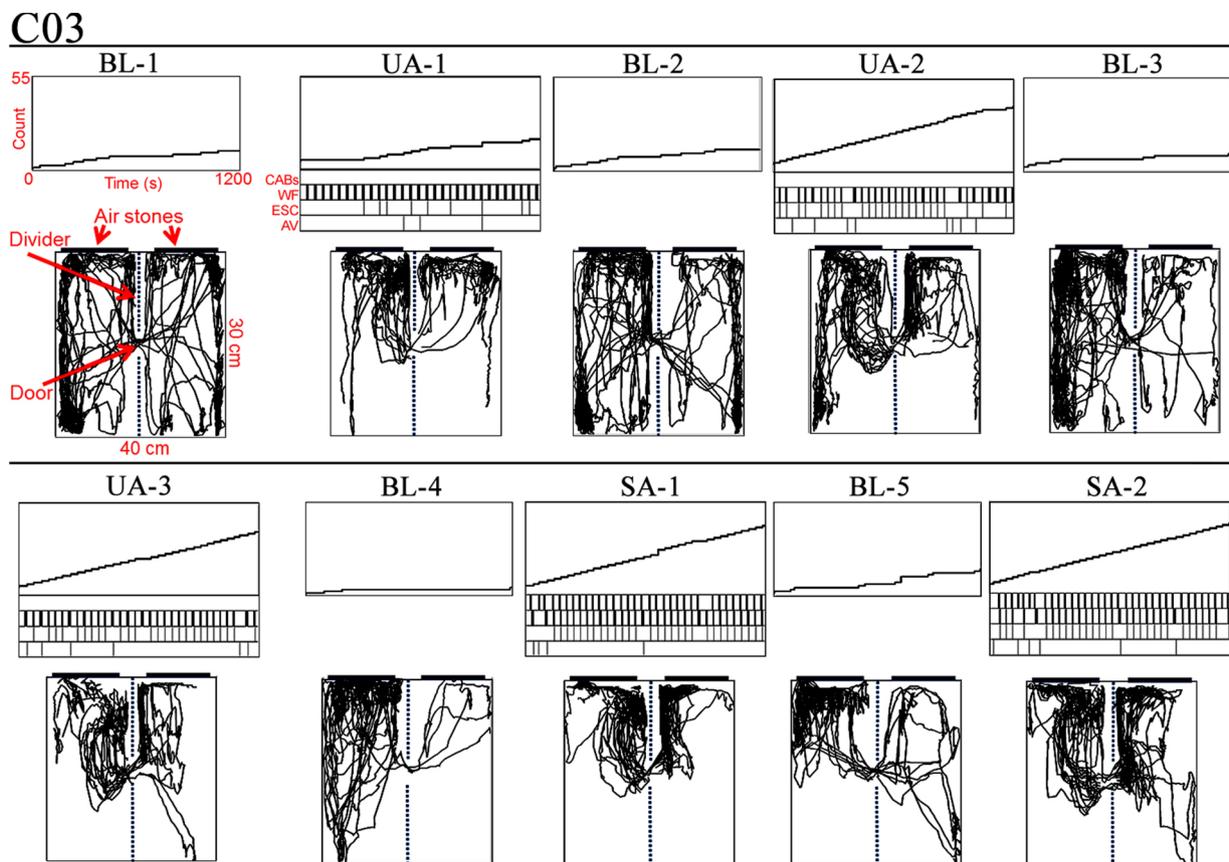


Fig. 4. Top row graphs show the cumulative records of fish C03's crossings during the last session (1200s) of each baseline (BL) and treatment condition (UA and SA). Horizontal bands below the cumulative records of UA and SA sessions show the occurrence of CABs, WF periods (WF), escape (ESC), and avoidance responses (AV). Bottom row graphs show XY (or "overhead") plots of the swimming trajectories described by the fish during each corresponding session. Dotted lines indicate the location of the divider and the door, and solid bars describe the location of the air stones.

configuration (C02-C05), this staggered pattern only emerged during the second treatment condition. Avoidance responses of any kind (EA, LA, FFA) were somewhat irregular and spread out over the sessions. The pattern of these responses did not seem to vary from UA to SA conditions.

3.4.2. Swimming patterns during baseline and treatment conditions

The swimming pattern that emerged during the first BL condition was very similar across all fish. It could be described as highly repetitive back-and-forth swimming alongside the glass walls of the experimental tank that run parallel to the divider (lap-swimming), which in many cases extended to the adjacent walls upon which the air stones were mounted (see the first overhead plot under BL-1 in Fig. 4). Lap-swimming was noticeable in subsequent BLs for all fish, and in some cases, it was also part of the swimming patterns developed during treatment conditions.

With regard to treatment conditions, two distinctive transitory swimming patterns were identified across all fish. These tended to fade during the interspersed BLs and reappear when treatment conditions were reinstated. The first pattern was most salient in fish C01, C04, and C05, and it is illustrated in Fig. 5 with data of C04 (see Appendix A for spatiotemporal data of all fish). This pattern could be described as a repeated "twisting" trajectory at a pace determined by WF deliveries (every 30 s, as dictated by the R-F interval). In this pattern the fish looped, crossed, and repeated a similar path in the other compartment.

The second swimming pattern that emerged during treatment conditions was observed in C03 and C06 and is illustrated in Fig. 6 with data of fish C03. This pattern could be described as repetitive swimming along the wall of the divider, from the connecting door to the wall where the air stones were attached (in many cases extending

underneath, in front, or above the air stones). The side of the divider in which the fish described this form of lap-swimming varied in accordance with WF deliveries (every 30 s, as determined by the R-F interval).

4. Discussion

The present study aimed at investigating the generality of two aversive control phenomena, previously studied almost exclusively with electric shock in mammals and birds: free-operant escape and avoidance. Although we did not directly compare the effects of shock with those of WFs, it is helpful at this point to discuss how the effects of these two stimuli may have compared with each other in the preparation used in this study. A clear similarity is that, on the basis of past research, including a study with bettas that learned to avoid electric shock (Otis and Cerf, 1963), the subjects in the present study would have learned to escape shock just as they learned to escape WFs. One obvious difference is that WFs physically moved the fish whereas shock does not. It is not known at present how this difference affected the results. Another obvious difference is that because shock is subjectively a painful stimulus whereas as far as we know WFs are not, they probably would have learned to escape shock more quickly – assuming that the opportunity to escape would have been available, which it typically is not in a standard free-operant avoidance preparation. It is not clear whether the fish in this study would have learned to avoid shock, as the subjects showed little evidence of learning to avoid flows in the present study. As mentioned in the literature review, however, previous research has found it difficult to establish avoidance in bettas. Indeed, rats frequently fail to learn free-operant avoidance when shock is used (M. Perone, personal communication, January 23, 2015), even though

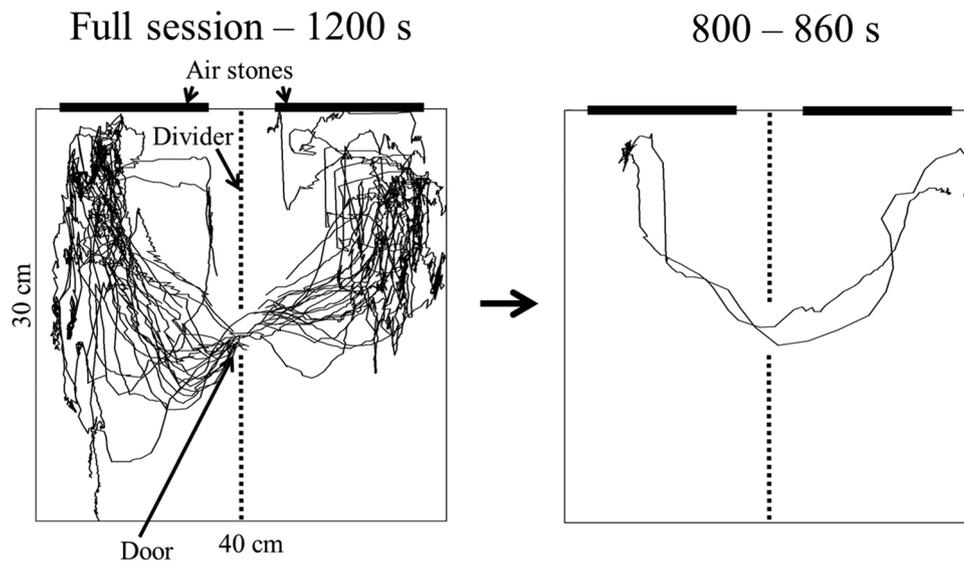


Fig. 5. The left overhead plot shows the swimming trajectories of fish C04 during the last session of SA-3, excluding WF periods. A 100-s segment of the same session (800–860 s) provided a representative detail and is shown in the right plot.

this is the standard preparation for studying free-operant avoidance. One approach to this problem in future studies may be to shape free-operant avoidance through positive reinforcement, as was done in a follow-up of the present study (Hunter et al., 2018).

A consistent and marked increase in crossing frequency during the conditions that had WFs scheduled, reaching three to four times the levels observed in interspersed baselines, was replicated within and across subjects without signs of habituation. Such a high number of crossings produced a steady reduction in total exposure time to WFs, in some cases reaching below 75% of the total WF duration scheduled per session. These findings confirm for bettas the aversive effects of WFs, the removal of which functions as a negative reinforcer. Moreover, these results expand the generality of a negative reinforcement phenomenon – escape – using a form of stimulation different than the typically used electric shock. In accordance with Johnston's (Johnston, 1979) terminology, the study extended the generality of a basic behavioral process across species and settings.

It is plausible that the changes in crossing responses observed in the present study could have been caused by the natural displacement of the fish through the opening in the divider due to the force of the WFs, and not as the result of the negative reinforcement contingency - i.e., increase in crossings were expected reactions to the flow and not the result of the operant contingency. An additional control condition in which the WFs were unavoidable and unescapable (e.g., noncontingent WFs occurring in both compartments of the experimental tank) could have provided the information necessary to test this alternative explanation. However, data on exposure time to the WFs supports a negative reinforcement interpretation (Fig. 3). Fish showed a progressive decline in the time they were exposed to the WFs across the different conditions. This reduction, which overall reached and maintained at levels below 35% (i.e., 100 s out of the 300 s originally scheduled per session), was the result of fish crossing faster after each activation of the WFs (escape latencies became shorter throughout the experiment).

As it will be discussed in detail in a later section, the fact that there

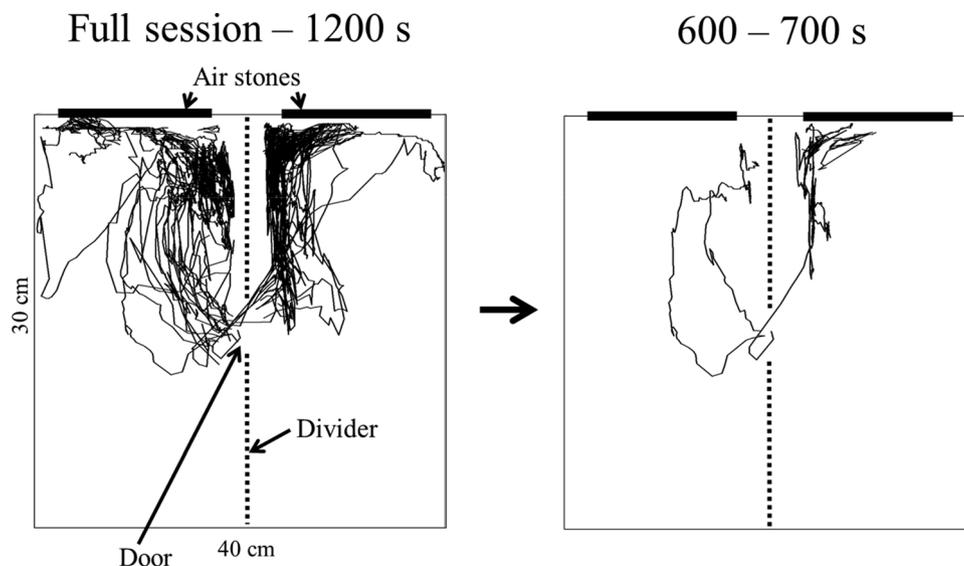


Fig. 6. The left overhead plot shows the swimming trajectories of fish C03 during the last session of SA-1, excluding WF periods. A 100-s segment of the same session (600–700 s) provides representative detail and is shown in the right plot.

was an inherent delay between the activation of the pump and WFs reaching the maximum strength, suggests that fish may have progressively reduced throughout the experiment not only the exposure time but also the intensity of the WFs to which they were exposed.

Free-operant avoidance phenomena observed in other species, including fish (Behrend and Bitterman, 1963; Scobie, 1970; Simon and Kreuz, 1985), were not reliably replicated in this study, notwithstanding the extensive number of sessions (in some cases surpassing 50 days of training). Whereas escape became the predominant response across all fish very early in the experiment, avoidance – primarily early avoidance – typically had a low frequency and only occasionally matched or slightly exceeded the frequency of escape (as observed in subjects C05 and C06 – see Fig. 2). Even in those cases in which there were substantial avoidance responses, they did not result in a sizable reduction in the overall frequency of WFs or of escape responses.

It is this predominance of escape behavior that explains the dissociation between the overall frequency and duration of WFs that was observed in the study. The fact that WF time substantially decreased over sessions, whereas WF frequency did not, was the result of the fish developing a highly regular response pattern that consisted of changing compartments during the first few seconds of each WF. As fish developed faster escape responses (evidenced in the progressive reduction in WF time across sessions), and avoidance remained low, the frequency of WFs delivered per session approached, or even surpassed, the 30 WFs originally scheduled per session. When an escape response occurred, a WF was cut short before its full 10 s, and thus the next WF occurred sooner than it would have otherwise. Only in cases where fish showed an increase in avoidance was WF frequency decreased a sizeable amount (e.g., UA conditions of C06 and C05 – see Fig. 3).

The finding that bettas engage in escape behavior from a non-shock aversive stimulus systematically replicates previous research with stimuli different from electric shock (e.g., intense noise or bright light – Barker et al., 2010; Knutson and Bailey, 1974). However, the lack of free-operant avoidance emerging in this experiment adds to a series of previous efforts in which replication of this phenomenon has been attempted with non-shock stimuli, but has only been demonstrated a few times. For instance, whereas Rohles (1965) and Clark et al. (1973) successfully demonstrated free-operant avoidance in monkeys using wind and artificial gravity, Knutson and Bailey (1974) and MacKenzie et al. (1993) could not replicate such phenomena in rats and domestic hens using intense noise; in fact, similar to the present study, subjects in MacKenzie et al.'s experiments learned to escape, but not to avoid the aversive stimulus. Moreover, in Barker et al.'s (2010) study with rats, punishment of lever pressing and escape responding (head entries into a hole) were successfully replicated using intense light as the aversive stimulus. However, free-operant avoidance could only be established and maintained when escape was prevented from occurring.

4.1. Effectiveness of WFs in controlling betta behavior: one-pump vs. two-pump configuration

High and reliable crossing behavior over baseline levels only occurred consistently when the one-pump configuration became functional. The results of this unplanned manipulation suggest that, similar to electric shocks (Boren et al., 1959; Das Graças de Souza et al., 1984; Glazer and Weiss, 1976; Lawry et al., 1978), the effects of WFs depend on their parameters, such as speed. It is possible that the force of the WFs produced by two water pumps competed with the swimming behavior of the fish, resulting in the inability to develop consistent crossing behavior. Further research that explores the effects of systematic manipulations of the WF parameters could test this interpretation. For example, future research could manipulate the volume of water released from Pumps 1 and 4. Another possible solution is using Pumps 2 and 3 only, as opposed to Pumps 1 and 4. It should also be noted that there is a potential problem with the one-pump procedure in that the fish could escape/avoid (or at least reduce the impact of) the

WF by staying close to Pump 2 or 3. This type of problem is not unique to our preparation. Azrin and Holtz (1966) pointed out that similar unplanned reduction in aversiveness can occur in experiments using shock as an aversive stimulus.

Of greater relevance is the fact that the crossing pattern that emerged in fish that went from a two-pump to a single-pump configuration did not seem to differ from that of other fish that started the experiment with the one-pump configuration (C01 and C06). All fish gradually acquired high rates of crossings throughout the conditions in which only one pump was functional for each compartment. This suggests that bettas exposed to uncontrollable WFs may not show the deficits in escape-avoidance learning that have been observed in dogs and rodents that have previously experienced uncontrollable electric shocks, a phenomenon known as *interference effect* (Anderson et al., 1979; Anisman and Merali, 2009; Glazer and Weiss, 1976; Lawry et al., 1978) or *learned helplessness* (Maier and Seligman, 1976). The fact that the interference effect has only been demonstrated using electric shock calls for further research using the WF paradigm, especially considering that Behrend and Bitterman (1963) provided preliminary evidence of such a phenomenon with shock in goldfish. To date, this effect has not been explicitly tested in fish of any kind.

4.2. UA versus SA conditions

In Scobie's (1970) and Behrend and Bitterman's (1963) experiments, unsigned shocks produced crossings that typically occurred during the first part of the R-S intervals; conversely, signaled shocks resulted in goldfish postponing crossing until near the end of the R-S interval, when the warning stimulus was present. These response patterns resulted in the frequency of crossings during unsigned shocks being higher than necessary to prevent all the scheduled shocks per session, as compared to signaled conditions. Extrapolating from these findings, we expected that betta crossing frequency would have been higher during unsigned than during signaled conditions. Considering that fish C01, C02, and C04 did not complete all the programmed UA and SA conditions of the experiment, only the data of C03, C05, and C06 are relevant to the discussion of this effect. As predicted, fish C05 and C06 showed higher levels of crossings during UA than during SA. However, the fact that this outcome was not replicated in C03, which experienced the UA at the beginning of the experiment, suggests that higher levels during the SA were caused by the extended training rather than by the presence or absence of the warning stimulus. This interpretation seems plausible considering that the higher levels of crossings for C03 occurred during its last condition of the experiment (SA-2).

Additional information regarding a possible difference in crossing frequency across SA and UA conditions results from analyzing the types of responses displayed by the fish (escape, early avoidance, late avoidance, and flow-flow crossings). These analyses are unique in that previous research in free-operant avoidance typically has not differentiated escape and avoidance behavior (the electric shocks used are typically too brief for escape to occur – indeed preventing escape from being a factor in free-operant avoidance has been one of the purposes of using brief electric shock in studies of free-operant avoidance; Baron, 1991; Sidman, 1953). Crossing-type data indicates that the difference in the overall frequency of crossings across UA and SA represents, in fact, two separate outcomes. First, fish tended to engage more in escape as the experiment progressed, which is the “training effect” previously alluded to. Second, scheduling a warning stimulus (CABs) near the end of the R-F interval seemed to have affected the incidence of avoidance to a certain degree; specifically, its presence appeared to have some control – although more would have been expected based on the findings of Behrend and Bitterman (1963) – while its absence appeared to have favored crossings during the first part of the R-F interval (early avoidance).

4.3. The role of the warning stimulus (CABs)

Despite the overall low incidence of late avoidance, the warning stimulus (CABs) apparently had some effect on the behavior of the bettas; the highest and most consistent frequencies of this response (late avoidance) were observed under SA conditions (in particular C03 and C06 – Fig. 2) and session videos indicated that on CABs onset, fish turned away from this stimulus, approached the air stones, swam towards the door, or air gulped. CABs did not produce noticeable agitation in the fish. Although the data of the present experiment do not provide the information necessary to formulate a satisfactory explanation for this behavior, we present some possible accounts that may be explored by further research.

A first interpretation is that the duration of the CABs (5 s) may have been too brief. This is supported by the fact that bettas showed fewer early avoidance responses during the signaled conditions, which indicates that subjects tended to postpone their crossings until near the end of the R-F interval (similar to Behrend and Bitterman's fish). However, subjects may not have been able to complete the change of compartment before the advent of the WF, so their responses occurred during the first seconds of the WF and were recorded as escape. Adding to this notion is the possibility that the response requirement in the present experiment was more challenging, and therefore more time consuming; fish were required to cross through a narrow door instead of, as in Behrend and Bitterman's (1963) and Scobie's (1970) experiments, swimming over a hurdle that divided the two compartments. Finally, session videos showed that fish often displayed incomplete crossings and swam toward the door during CABs. These observations provide additional support to this hypothesis, suggesting that fish on several occasions were "in the process" of crossing when the CABs period ended.

Data on the swimming patterns somewhat contradicts this account. The fact that fish typically were very close to the air stones and the door at the onset of the CABs suggests that they would have had enough time to experience the CABs and cross to the other compartment. Future tests in this regard are required, including increasing the length of the CABs and adapting the apparatus to allow for the use of a target response similar to Behrend and Bitterman's (1963) and Scobie's (1970).

A second interpretation is that bettas could have been exposed to the CABs prior to being acquired for the study (i.e., at the pet shop), which could have resulted in latent inhibition (Lubow, 1965) – i.e., unplanned CABs pre-exposure in the absence of WFs resulted in subsequent failure to condition their discriminative function during the experiment. Though further research could explore this possibility by controlling the pre-experimental history of the subjects, this notion is not supported by the fact that bettas did seem to respond to the CABs. As previously noted, during CABs bettas for instance approached the air stones, swam towards the door, or air gulped. An alternative interpretation of these responses is provided below.

A third interpretation is that the experimental procedure inadvertently favored the combined occurrence of sign- and goal-tracking effects. When a given event (conditioned stimulus – CS) is paired with a biologically relevant stimulus (unconditioned stimulus – US) the CS may evoke approach or contact with itself (or its location) or with the US (or its location). These effects are termed sign-tracking (Hearst and Jenkins, 1974), and goal-tracking (Boakes, 1977; Silva et al., 1992), respectively. Although both effects typically have been studied with appetitive contingencies involving food, and replicated in different species, including fish (Nilsson et al., 2008), there are reports that analogous effects could be reproduced when the US is an aversive stimulus, such as shock (Leclerc and Reberg, 1980). Among the variables that have been proposed as responsible for modulating goal- and sign-tracking effects is the spatial distribution of the stimuli, especially the distance between the CS and the US. In Silva et al.'s (1992) study, pigeons were exposed to different distances between a light (CS) and the site in which food (US) was presented (food hopper). Spatiotemporal

analyses showed that subjects sign-tracked when the CS was closest to the food hopper, goal-tracked when the CS was farthest from the site of the US, and "engaged in both sign- and goal-tracking (or something intermediate) at intermediate conditioned-stimulus-to-unconditioned-stimulus distances" (Silva et al., 1992, p. 17). Extrapolating from these findings, it seems plausible that fish in the present study engaged in a mixed pattern of sign- and goal-tracking as a result of the contingencies involved (CABs reliably predicted WFs) and the spatial configuration of relevant stimuli; namely, the water pump nozzles (i.e., WFs source), the air stones/CABs (located in the wall opposite to the nozzles), and the opening in the tank's divider wall (somewhat halfway between the air stones and the nozzles). It seems plausible that the paradoxical effect of the fish missing the opportunity to avoid the WF due to its engagement in responses during the CABs is a form of *omission responding* (Peden et al., 1977), reported in previous studies with other species, including fish (Bottjer et al., 1977). Namely, subjects miss the opportunity to access reinforcers that are available for limited periods of time as a result of responding towards a CS that has been paired with those same reinforcers (Peden et al., 1977). Future research should systematically explore this interpretation by manipulating spatial characteristics of the experimental procedure in order to see if, as in Silva et al.'s study, changing the location of the air stones (e.g., placing them closer to or farther from the door) affects the incidence of the different types of responses observed during the present study (related to the door, the air stones/CABs, or the source of the WFs).

4.4. WF frequency and duration

The effort to analyze dissociations between the frequency and duration of the fish' exposure to the aversive stimulus is relevant because in previous research the duration of the aversive stimulus (electric shock) typically has been negligible. Data from the three subjects that completed all programmed conditions (C03, C05, and C06) indicates that fish were more successful in reducing the number of WFs when no warning stimulus was presented (Fig. 3). Fish also achieved greater reductions in the time they were exposed to WF when no warning stimulus was presented (Fig. 3).

The contingencies in this experiment determined that frequency and duration of WFs depended on the patterns of crossings; that is, high and reliable avoidance responses reduced the frequency of WFs, whereas escape reduced the duration of WFs, but served to ultimately increase their frequency. Accordingly, it seems that the differences observed throughout the study regarding WF frequency and duration were the result of specific patterns of escape and avoidance that emerged across the experiment, and did not directly result from whether the condition was signaled or unsignaled. Consequently, an explanation of these patterns is inevitably linked to the understanding of what caused the variations in incidences of escape and avoidance. As will be discussed below, it was suspected that procedural aspects could explain such variations.

4.5. Swimming patterns

The swimming patterns that emerged during baseline conditions differed overall from those that developed during treatment conditions, and no differences were detected between SA and UA conditions. Whereas baseline patterns primarily consisted of stereotyped lap-swimming, two transitory behavioral patterns were identified during treatment conditions (twisting trajectories and repetitive swimming along the dividing wall), which tended to fade during the interspersed baselines and reappear when treatment conditions were reinstated. Interestingly, lap-swimming observed during baselines was occasionally noticeable in the swimming patterns displayed during treatment conditions. These outcomes replicate what has been shown in previous spatiotemporal research across different appetitive experimental paradigms (Pear, 2004); namely, aversive contingencies regulate not only

the incidence of discrete responses (crossings through an opening), but also the behavior that occurs between such discrete responses (e.g., Pear, 1985). Furthermore, the fact that the aversive contingencies did not explicitly target those swimming patterns, which still emerged, disappeared, and resurged in accordance with the scheduled treatment conditions, supports the hypothesis that behavior occurring between the reinforced responses (e.g., a pigeon's movements between reinforced key pecks or a fish's swimming trajectories between crossings) could be generated and maintained by adventitious reinforcement (Pear, 1985; Skinner, 1948).

Two aspects of the specific swimming patterns that emerged merit discussion. First, Martin (2010) reported that bettas typically developed highly stereotyped lap-swimming in front of a glass wall in which mirror images were presented in noncontingent schedules. The fact that bettas throughout the present study displayed similar swimming patterns, despite the absence of mirror images, suggests that unintended reflections may have been occurring in the glass walls. This would explain why fish consistently returned to lap-swimming behavior whenever baseline conditions were established.

Second, one particular swimming pattern, which emerged during treatment conditions, involved regular trajectories between the door and air stones areas (repetitive swimming along the wall of the divider, going from the connecting door to the air stones wall). The fact that the scheduled escape and avoidance contingencies did not explicitly require fish C03 and C06 to display these specific swimming trajectories suggests that other factors could have influenced the fish to respond very similarly to their environment. Sign-/goal tracking effects (Silva et al., 1992) and/or adventitious reinforcement (Pear, 1985; Skinner, 1948) could account for these homogeneous swimming patterns.

4.6. Why avoidance was not fully established?

One possible explanation for the low incidence of avoidance, the major unexpected outcome of the study, is that a *warm-up effect* (an initial low rate of response – the *warm-up* period – followed by its increase and stabilization; Sidman, 1955) could have been involved. Though it is possible that 20-min sessions were not long enough for the warm-up period to dissipate, this interpretation seems unlikely because the temporal distribution of crossings indicates that avoidance responses did not increase by the end of the session; in fact, these responses often appeared clustered at the beginning of the session. Moreover, Behrend and Bitterman (1963), using a different fish species (goldfish), successfully reproduced signaled and unsignaled free-operant avoidance implementing daily sessions of same length. Alternatively, a joint analysis of the data on temporal distributions of crossings, swimming patterns, and predominant response type provides leads to a comprehensive interpretation of the key findings of the present study.

Fish typically postponed crossing until the onset of each WF (thus the high incidence of escape), regardless of the treatment condition, which mirrored the effect that Behrend and Bitterman (1963) observed in goldfish exposed to signaled shock; i.e., their fish systematically postponed crossing until near the end of the R-S interval when the warning stimulus was present. This finding suggests that some form of stimulation, distinct from CABs, may have been functioning as a warning stimulus throughout both signaled and unsignaled experimental conditions. It is plausible that the purported stimulus – perhaps sound or vibrations – may have been produced during water pump activation (at start-up and/or while running). Considering that WFs take some time to gain full strength once they are turned on, bettas were able to avoid the full-strength disturbances by crossing during the first few seconds of the WF period.

This interpretation accounts for the predominance and regularity of crossings during the first seconds of each WF delivery, and classifies them not as escape, but as discriminated avoidance – i.e., an unintentional signaled avoidance contingency was scheduled throughout the

experiment. Furthermore, it explains why CABs exerted poor stimulus control; since the other stimuli related to the water pump activation and/or operation occurred more contiguous to the aversive stimulus, fish typically postponed their crossing past the CABs periods (first seconds of the WF interval). This is also consistent with the observation that the introduction of a warning stimulus during SA did not substantially change the incidence of late crossings (LA) – i.e., betta behavior was already under stimulus control.

Although it is unclear how the higher levels of early avoidance observed during UA conditions could relate to this proposed account, one possibility involves the biological relevance of CABs; that is, the potential of CABs for eliciting responses related to different behavioral functions, such as territorial, parental, or foraging. Craft et al. (2007) proposed that air bubbles could be a “more salient stimulus compared to color or location” (p. 576) for bettas due to the fact that males of this species construct bubble nests on the surface of the water to attract mates, which further serves as territorial marker. It is therefore plausible that, during signaled conditions, the fish showed fewer crossings during the first portions of the R-F interval (i.e., early avoidance) as a result of being engaged in CABs-ai R-S tones-related behavior. This seems consistent with the observation that CABs elicited different responses in the fish, and that they spent a considerable amount of time in the air stones areas, even during non-CABs periods, very often displaying repetitive swimming behavior.

5. Conclusions

Bettas exposed to a free-operant avoidance procedure involving water disturbance as the aversive stimulus reliably escaped periodically delivered WFs. Although escape improved with practice, bettas did not appear to learn to avoid the WFs, despite the fact that, based on escape behavior, WFs were clearly aversive. Though the addition of a warning stimulus to the free-operant procedure affected the behavior of the bettas to a certain degree, it did not result in more or more efficient avoidance behavior, as has been demonstrated in other species, including goldfish. It was also found that distinctive swimming patterns emerged during baseline and treatment conditions, which dissipated and reappeared as those conditions were removed and reinstated. These findings confirm the aversive function of WFs for bettas and expand the generality of negative reinforcement phenomena using non-shock-based procedures with a less-frequently studied species. Further developments of the WFs paradigm show promise for studying other aversive control phenomena (e.g., punishment) in bettas, and testing the generality of the aversive function of water disturbances for the behavior of other fish species. The growing popularity of zebrafish as a behavioral model, and the fact that free-operant avoidance has been only demonstrated with electric shock in one fish species (goldfish), offer potential courses of research that may benefit from the use of the WF paradigm.

Research on spatiotemporal patterns of behavior has demonstrated that a comprehensive understanding of behavioral processes is enhanced when it is not limited to the study of rates of discrete responses (Pear, 2004). Although the spatiotemporal analyses conducted did not provide satisfactory answers to the unexpected findings, there is a major heuristic value to this approach in terms of suggesting interpretations that are easily translatable to further research. As Sidman (1960) observed, “failure of replication will serve as a spur to further research rather than lead to a simple rejection of the original data” (p. 74).

An analysis of the different types of data suggests that (a) the short duration of the warning stimulus, (b) the spatial configuration of the sources of stimuli (e.g., the location of the air stones/CABs with regard to the door and the water pump nozzles), (c) the uncontrolled stimulation produced by the water pumps activation/operation, and (d) the use of a door between compartments instead of a hurdle, may explain the lack of replication of the expected negative reinforcement

phenomena, including the complete avoidance of WFs and control by the warning stimulus (CABs). Further research is needed to explore the effects of manipulating these factors on the establishment of discriminated and non-discriminated free-avoidance phenomena. Moreover, in the case of the spatial and temporal configuration of the air stones and CABs, future studies could test if certain behavioral phenomena that are rarely studied with aversive stimulation could be reproduced using WFs, namely sign- and goal-tracking effects (Boakes, 1977; Hearst and Jenkins, 1974; Silva et al., 1992).

An explanation of the psychobiological mechanisms by which WFs affect the behavior of bettas was beyond the purpose of this study. However, it is expected that further research will explore the possibility that the aversive function of WFs is related to territorial, bubble-nesting, or breeding activities, and/or could be associated with predatory risks or the presence of intruders. Such efforts to incorporate biological and ecological aspects of bettas into the understanding of the behavioral functions of WFs could also extend the scope of the interpretations for the unpredicted outcomes, especially the lack of free-operant avoidance. It should be noted that although some fish died during the experiment, this did not appear to be due to any direct or indirect physical effects of the WFs, such as tissue damage due to fish being forced against the walls of the test tank. First, we did not observe any such occurrence. In addition, Animal Care Staff of the Department of Psychology of the University of Manitoba checked the fish daily for any signs of illness or injury, and University of Manitoba veterinarians were available at all times to administer care to the fish if needed and to autopsy any fish that died. No trauma was observed in any of the fish used in this study either by the Department of Psychology Animal Care staff or by University of Manitoba Veterinarian Services while the fish were alive or at autopsy if deceased.

An important aspect to consider when interpreting the outcomes of the studies with the WF paradigm is that male bettas are highly territorial fish, especially in isolation (Monsives et al., 2009). Specifically, the procedure used here involved daily removal of an isolated subject from its individual home tank (i.e., its territory) and its introduction into an environment that was, to a certain degree, unfamiliar (e.g., between sessions, the experimental tank was cleaned, and its water was changed). It is possible that bettas react to this situation similarly to the way they would naturally respond when entering the territories of conspecifics and/or establishing their own territory in a novel environment (e.g., displaying activities of exploration, inspection, and/or defense). Betta adjustment to the contingencies of the present experiment could have been supported or constrained by the behavioral interactions triggered by the inherent characteristics of the apparatuses and procedures. For instance, it is plausible that the size of the shuttle tank and its compartments may have affected the swimming trajectories and crossing patterns of bettas, especially since it is uncertain to what extent their size corresponds to the area that a male would typically defend in the wild (i.e., there is a lack of information on the specific dimensions of a typical male betta's territory in the wild). In addition, uncontrolled mirror reflections occurring in the glass walls could have elicited territorial or defensive behavior patterns, to which lap-swimming, one of the stereotypical swimming responses observed in the present study, belongs (Martin, 2010).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.beproc.2018.10.021>.

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