Effects of Short Term Exposure to Elevated Predation Risk on the Learning of a Novel Foraging Task in Female Trinidadian Guppies (*Poecilia reticulata*)

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Abstract

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Predation pressure is an important selection pressure that shapes prey morphology, life history and behaviours. Long-term exposure to high predation risk is known to shape learning, but the effects of short-term exposure to high predation risk on learning are less understood. Short-term exposure to elevated predation risk (using conspecific alarm cues) induces neophobia, which can have direct survival benefits for prey. Neophobia is also naturally occurring in certain wild populations, and is known to shape foraging related learning. However, the effects of risk induced neophobia on foraging related learning are unknown. The aim of this thesis was to examine the effects of short-term exposure to elevated predation risk on the learning of a novel foraging task. I designed an experiment where wild caught Trinidadian guppies were exposed to different background levels of predation risk. They were subsequently trained to associate a food reward with a coloured object over 4 days, during which they received acute risk reinforcement stimuli. Results show that background risk had no effect on the learning of the novel foraging task. Both high and low predation risk treatments learned the foraging task equally well. However, while acute risk did constrain learning, it did not inhibit it. No evidence was found that the learned association could be generalized across contexts. However, a significant side bias in the data, where the right side of the tank was preferred over the left, prevented any firm conclusions. Future studies might disentangle the effects of short-term background risk and acute risk on the learning of a novel foraging task.

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Contribution of Authors

The concept of this thesis was designed by myself and Grant E. Brown. As first author, I collected all of the data, and wrote this thesis. I collaborated with Jean-Michel Matte on the statistical analysis part of this project.

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1. Introduction

Predation is a strong selective force acting on prey, shaping their morphology (Krueger and Dodson 1981; McCollum and Leimberger 1997), life histories (Crowl and Covich 1990; Stibor 1992), and behaviour (Lima and Dill 1990). As such, prey are forced to make trade-offs between predator avoidance and other fitness related activities. The ability to balance trade-offs requires the recognition of ecologically relevant threats (Brown *et al*. 2011). Recognizing threats can be difficult, as predation risk varies in space and time (Sih 1992; Sih *et al*. 2000). However, prey can improve their ability to evaluate varying threat levels by incorporating knowledge about diurnal or seasonal cycles (Ferrari and Chivers 2009), ontogenetic shifts in size due to gape limitations (Brönmark and Miner 1992), or selecting habitats with minimal predation risk (Werner and Gilliam 1984; Golub *et al.* 2005). Nevertheless, recognizing predation threats accurately is challenging for prey, given the high variability of these events, and the necessity of relying on cues and signals to detect predators (Chivers and Smith 1998). As such, prey often make behaviourally inappropriate (i.e. bad), and potentially costly, decisions (Dall 2010).

Inappropriate decisions can lead to costs of varying degrees for prey. For instance, displaying predator avoidance behaviours in the absence of an acute threat (i.e. false-positive) may result in lost-opportunities to engage in other fitness related activities; however, failing to respond to an acute threat (i.e. false-negative) can result in mortality. Therefore, there is an asymmetry between the costs of inappropriate behavioural decisions with regards to predation risk (Johnson *et al*. 2013). The nature of behavioural trade-offs means incurring some small cost is unavoidable; consequently, prey should "play it safe" when encountering anything new, in an attempt to incur lost-opportunity costs rather than increasing the risk of mortality.

Avoiding novel stimuli, or situations, as a means of protection from unknown risks is referred to as neophobia (Greenberg 2003). Neophobia has been studied in a variety of contexts including: object, gustatory, spatial, and predatory neophobia (Crane *et al.* 2020). These various forms of neophobia all share the common feature of increasing the chances of incurring lost-opportunity costs, rather than increasing the risk of predation mortality. Predator neophobia has been documented in wild populations, and has also been shown to be inducible over short time periods. Brown *et al*. (2013) showed that wild Trinidadian guppies (*Poecilia reticulata*) from high predation risk populations exhibited increased anti-predator behaviours when exposed to a novel chemical cue, a response not seen in individuals from a low risk population. However, when individuals from low predation risk populations were exposed to conditions of elevated background risk for several days (using conspecific alarm cues), they subsequently displayed the same behaviours. These results suggest that neophobic predator avoidance may function as a potential mechanism to help prey manage variability in predation risk, and the associated asymmetrical costs (Brown *et al*. 2013; Elvidge *et al*. 2016).

When faced with asymmetrical costs, incurring smaller lost-opportunity costs via neophobic behaviours is preferable. Lost-opportunity costs can be related to a number of different fitness related activities including mating, territory defence and foraging. For example, migratory new world black birds (Family *Icteridae*) are more neophobic than their resident counterparts are. When a novel object was placed next to a known food source, migratory birds took significantly longer to approach and start feeding than resident birds (Mettke-Hofmann *et al*. 2013). As migratory birds may experience novel situations more frequently than residents, displaying neophobic behaviours could protect them from potential risks. However, the benefit of protection is gained at the expense of lost-opportunities to forage.

Foraging is an important lost-opportunity cost as predators can impact prey fitness not solely through direct consumption effects, but also through indirect non-consumptive effects (Preisser and Bolnick 2008). For example, certain freshwater snails (*Physella virgate, P. heterostropha*) will spend more time on land, avoiding molluscivorous fish present in their aquatic feeding habitats. This reduction in foraging has a significant negative effect on the snails' fecundity (Langerhans and DeWitt 2002; McCollum *et al.* 1998). Foraging is therefore an important fitness activity, and can be indirectly affected by predation via non-consumptive effects.

As foraging is linked to fitness, the ability to learn novel foraging tasks can be beneficial. Learning over a short time period is defined as a change in behaviour based on recent experience (Brown 2012), allowing individual behavioural repertoires to be fined-tuned, or modified, to specific environments. Neophobia has been documented to constrain foraging related learning. Wild caught male starlings (*Stumus vulgaris*) that were more hesitant to feed in novel environments were also slower at learning a foraging task (Boogert *et al.* 2006). Seferta *et al.* (2001) also demonstrated a covariance between neophobia and learning in birds; individuals that were more neophobic, showed slower learning abilities in novel foraging tasks. These studies have examined the relationship between neophobia and learning, without including risk as a factor. Little is known of the relationship between risk induced neophobia and foraging related learning.

Risk induced neophobia results in the display of a suite of different behaviours, including reduced movement and foraging (Brown *et al.* 2015a,b), reduced exploration (Elvidge *et al.* 2016), and increased vigilance (Feyten and Brown 2018). Reduced movement and exploration would likely decrease the frequency of encountering novel foraging patches or tasks; increased

vigilance would likely decrease the time available to learn any new foraging tasks. We might therefore expect that inducing neophobia, via short term exposure to elevated predation risk, would constrain foraging related learning.

Long term exposure to elevated predation risk has already been documented to shape learning in some fish species. Different populations within the same species have been shown to have different learning capabilities based on differing predation regimes (Brown and Braithwaite 2005; Huntingford and Wright 1992; Brydges *et al.* 2008). Wild caught Panamanian bishop fish (*Brachyraphis episcopi*) from high predation risk populations learned a spatial task significantly slower than those from a low predation risk population (Brown and Braithwaite 2005). Conversely, high risk fish performed better on avoidance tasks than low risk fish. Using laboratory-bred descendants of wild caught three-spined stickelbacks (*Gasterosteus aculeatus*) Huntingford and Wright (1992) demonstrated that laboratory-bred first generation descendants of individuals from high predation populations learned to avoid a foraging patch faster when there was a simulated predation attack, compared to descendants from low predation populations. Despite predation risk showing both enhancing and reducing effects on learning, both these results can be argued to be adaptive for high risk populations. Slower spatial learning and heightened avoidance learning are both ways in which prey can protect themselves from potential risks.

While long term exposure to high levels of predation risk shapes learning (Brown and Braithwaite 2005; Huntingford and Wright 1992; Brydges *et al.* 2008), the effects of risk induced neophobia (through exposure to short term predation risk) on learning are still unknown. While long term exposure to predation risk can result in genetically based behavioural differences (Huntingford and Wright 1992), short term exposure to predation risk is likely to result in

phenotypically plastic behaviours (Brown *et al.* 2013; Brown et al. 2015a,b; Ferrari 2014). Given the relationship between neophobia and learned foraging tasks (Boogert *et al.* 2006; Seferta *et al.* 2001), and long term predation pressure and learning (Brown and Braithwaite 2005; Huntingford and Wright 1992; Brydges *et al.* 2008), a relationship between risk induced neophobia and learning would be expected. While studies have shown that risk induced neophobia interacts with learning with regards to predator identity (Brown *et al.* 2015a,b), whether risk induced neophobia interacts with the ability to learn novel foraging tasks is still unknown. As risk induced neophobia may be a potential mechanism for managing the costs associated with predation risk, any constraints it may impose on learning novel foraging tasks should be examined.

The aim of this thesis was to examine how short term exposure to elevated predation risk influenced the learning of a novel foraging task. The behaviours associated with risk induced neophobia, namely reduced movement and exploration (Brown *et al.* 2015a,b; Elvidge *et al.* 2016) and increased vigilance (Feyten and Brown 2018), would decrease the opportunities to encounter potentially new foraging opportunities. Consequently, I would expect this to negatively affect the ability of prey to learn a novel foraging task. I used wild caught Trinidadian guppies to test the hypothesis that short term exposure to elevated levels of predation risk influenced, either by inhibiting or delaying, the learning of a novel foraging task. I designed an experiment where individuals were taught to associate a food reward with one of two coloured objects. I exposed guppies to different levels of short term predation risk (hereafter referred to as background risk), and then trained them to associate a food reward with one of two coloured objects, during which they were exposed to different levels of acute risk. In order to determine if the learned colour association could be generalized across contexts, I tested them to

see if they would apply the learned association in a novel situation, where there was no food reward. No food rewards were given, as I did not want to continue to train the fish to associate a food reward with a specific colour. Rather, my goal here was to see how often they would search for food based on their trained colour and food association.

My general predictions for the training phase were that exposure to high levels of background risk would (i) increase the latency to approach a potential novel foraging opportunity, (ii) result in fewer attempts to complete the task, and (iii) create longer latencies for familiarization with the tasks, compared to low levels of background risk. For the testing phase, I predicted that exposure to high levels of background risk would inhibit the application of the learned task in a novel situation. I expected an interaction between background risk and trial number, where fish exposed to high levels of background risk would have (iv) longer latencies to approach the coloured object they were trained to associate a food reward with and (v) have fewer attempts to obtain food across all testing trials. I predicted the low background risk fish would increase their latencies and decrease their approaches across trials, as there was no food reward. I also predicted an (vi) interaction between background levels of risk, and acute risk reinforcement, where high background and high acute risks would have an additive effect, resulting in the longest latencies, and least number of entrances. Additionally, I predicted (vii) no effect of colour or (viii) side of the tank the rewarded object is on to influence the ability to learn the novel foraging task for both the training and testing phases.

2. Materials and Methods

2.1 Study species

This study was conducted in 2016 and 2017 using wild caught female Trinidadian guppies from the Northern Mountain Range of the Republic of Trinidad and Tobago (Figure 1).

Only females were used as they are more likely to display modified behaviour patterns with regards to foraging than males, due to higher parental investment requirements (Laland and Reader 1999; Reader and Laland 2000). Fishing permits were acquired from the Trinidadian Ministry of Agriculture, Land and Marine Resources. Approximately 300 female guppies were collected from the Upper Aripo River, a low predation site (Croft *et al.* 2006; Botham *et al.* 2008), on April 19th 2016. In 2017, approximately 120 female guppies were collected on April 23rd, and 240 on April 27th. All tests were conducted using the low predation fish. Female guppies were also collected from the Lopinot River, a high predation site (Deacon *et al.* 2018), in order to make conspecific alarm cue. Approximately 250 female guppies were collected on April 19th 2016, and 120 on April 24th 2017. Upon completion of data collection in both 2016 and 2017, all remaining fish were returned to their respective streams.

All fish were collected using seine nets, and temporarily held in 19 L plastic buckets, filled with approximately 8 L of stream water, and loosely covered with a plastic lid. Buckets were transported by truck to the laboratory facilities at the University of the West Indies, Saint-Augustine, Republic of Trinidad and Tobago. The guppies where held in 121 x 44 x 46cm (LxWxH) glass tanks filled with 168L of dechlorinated water at 22˚C, and equipped with an airstone as well as two box filters. Tanks were kept on a 12h:12h light/dark cycle. Fish were fed ad libitum once per day using commercial flake fish food.

2.2 Stimulus Preparation

To collect conspecific chemical alarm cues (AC), Lopinot River female guppies were euthanized via cervical dislocation (in accordance with Concordia University AREC protocol #30000255). Mean (± SD) of donors was 2.44 ± 0.41 cm (2016) and 2.27 ± 0.29 cm (2017). After the head (at the opercula) and tail (at the caudal peduncle) were removed, whole bodies

were homogenized in ~200 ml of dechlorinated water. A total of 89 (2016) and 59 (2017) guppies were used as cue donors. In both years, a final concentration of 0.1 cm^2ml^{-1} was generated with the addition of dechlorinated water. Alarm cues of this concentration are known to reliably elicit antipredator responses in Trinidadian guppies (Brown and Godin 1999).

2.3 Experimental Design and Data Collection

The main goal of this experiment was to explore the effects of background predation risk on the learning of a novel foraging task. My experimental design consisted of three different treatment axes: Background risk (high/low), acute risk (high/low), and colour associated with food reward (red/white) - (Figure 2). A sample size of nine (n=9, mean ± SD standard length = 23.20 ± 3.96mm) for each treatment combination was collected. The experimental protocol was performed over nine consecutive days (Figure 3). I collected the data over seven experimental blocks, four in 2016, and three in 2017.

During the pre-exposure phase, I conditioned guppies to differing levels of background risk (high/low) for three days. Elevated levels of predation risk were simulated using conspecific AC, while dechlorinated water (DW) was used as a control (i.e. low risk). Conspecific alarm cues are a reliable indicator of risk, as they are only detected within the water column after mechanical damage to the skin of the fish (Chivers and Smith 1998).

Following the pre-exposure phase, I moved onto the four day training phase. During this time I trained fish to associate a food reward with one of two coloured objects suspended within the water column, namely a red and white plastic cup. On each training day, the guppies were exposed to an acute risk reinforcement stimulus of either AC or DW in the morning and the

afternoon. This was done as neophobic behaviours induced by exposure to elevated background risk begin to wane without any reinforcement (Brown *et al.* 2015a,b).

The goal of the testing phase was to see whether the learned association could be generalized across contexts. I created a novel situation by changing the shape and location of the cups within the tanks.

2.4 Pre-exposure Phase

For each experimental block, I placed two shoals of 22 guppies into separate glass aquaria 60 x 30 x 30.5cm (LxWxH) containing 38 L of dechlorinated water at 22˚C. Each tank was equipped with an airstone, and held on a 12h:12h light dark cycle. Opaque white plastic was placed between the tanks to prevent any visual contact between treatment tanks. I injected 10 mL of stimulus three times a day (10am, 12pm, 2pm) for three consecutive days. Tanks being exposed to high levels of background risk received an AC stimulus, while those exposed to low levels of background risk received a DW stimulus. I injected the stimuli using a one-meter length of soft airline tubing that terminated directly above the airstone at the back of the tank. I flushed the stimulus through the tubing using 60ml of tank water. I fed the fish using commercial flake food ad libitum on the first two days, but not the third day to ensure they were hungry enough to forage the following day; food deprived guppies are more likely to forage innovatively (Laland and Reader 1999; Reader and Laland 2000).

2.5 Training Phase

The training phase took place over four days, starting the day after the pre-exposure phase was completed. Training took place in 44.5 x 30 x 31cm (LxWxH) glass aquaria filled with 15L of dechlorinated water at 22 ˚C. The tanks were equipped with an airstone, centered

against the back wall of the tank, and held on a 12h:12h light/dark cycle. Tanks were covered with opaque plastic on three sides to prevent any visual contact between tanks.

On the first training day, I transferred the fish from their pre-exposure tanks into their respective training tanks in shoals of three. I used shoals of three rather than singletons as newly learned foraging tasks spread through guppy populations based on shoaling tendency (Laland and Williams 1997). The fish were left to acclimate undisturbed for 30 minutes. I then injected the morning acute reinforcement stimulus of 5ml of either AC or DW through a one-meter length of soft airline tubing that terminated directly above the airstone in the tank. I flushed the stimulus through the tubing using 60ml of tank water. The fish were left undisturbed for an additional 30 minutes and then placed into a clear plastic cylinder (9 cm diameter) in the centre of the tank. I removed the airstone from the tank, and suspended the red and white cups upside down approximately four centimeters into the water column using wooden skewers. The bottoms of the cups were cut out to allow a food slurry to be dropped through the cup into the water column using a length of soft airline tubbing attached to a 60 ml syringe (Figure 4). The food slurry was prepared by mixing 125 mL of commercial flake food with 40 ml of dechlorinated water to create a viscous slurry. The fish were left to acclimate to the new set up in the clear cylinder for 10 minutes.

The training session began immediately after I lifted the clear cylinder from the tank, and lasted for five minutes. During this time, I recorded the latency of the first fish to enter the reward zone. The reward zone was the area directly beneath the rewarded coloured cup (Figure 4), as the fish needed to swim into this area in order to receive a single drop of food slurry as a reward. Each new entrance into the reward zone received a drop of food slurry. I also recorded

the total number of entrances into the reward zone. After five minutes, I removed the cups and any unconsumed food from the tank, and placed the airstone back into the water.

For each training session, I switched the side of the tank that the rewarded cup was on to avoid any possible side bias. Four training sessions were completed each day, with 60-90 minutes elapsing between sessions. Thirty minutes after the last training session of the day, I did a 50% water change on each training tank to remove excess food and waste. Twenty minutes after the water change was completed, I injected the afternoon acute reinforcement stimulus of 5ml of either AC or DW. A total of 16 training sessions were completed over the course of four consecutive days.

2.6 Testing Phase

Upon completion of the training phase, I left the fish in their training tanks with an airstone for a full day. During this time, no cups were placed in the tanks, they were not given any acute reinforcement stimuli, or any food. The testing phase took place the following day. The purpose of the testing phase was to determine if the learned colour association could be generalized across contexts. A novel situation was created by altering the shape and location of the cups. To alter the shape of the cups, I cut out a large section from each cup. I cut the cup in half vertically, starting from the top of the cup, and stopping two centimeters from the bottom of the cup, to leave the base intact.

On the testing day, I placed the fish into a clear acclimation cylinder (9 cm diameter), and removed the airstone from the tank. I placed the altered cups right side up in the tank, and anchored them to the bottom using small rocks (Figure 5). The fish were left to acclimate in the cylinders for 10 minutes. The testing trial lasted for five minutes, and began as soon as I

removed the acclimation cylinder. I recorded the same behaviours as during the training sessions, except no food reward was given. Here, a successful entrance into the reward zone was recorded when a fish entered the area directly inside the area of the cup that was cut out (Figure 5). Four testing trials were conducted per tank, with 60-90 minutes between trials. As with the training sessions, I switched the side of the tank that the trained colour rewarded cup was on for each trial.

2.7 Statistical methods

All data were analyzed using generalized linear mixed models. As data were collected in seven treatment blocks and included repeated measures, random effects were used in all models to account for non-independence between tanks and treatment blocks. All analyses were conducted in R (v. 3.5.3).

2.7.1 Training Phase

To test the prediction that high background risk increases the latency to approach a novel foraging opportunity, I modelled the relationship between the latency of entry to the reward zone to my various predictors of interest. These predictors include, background risk, acute risk reinforcement, colour of the rewarded cup, side of the tank the rewarded cup was on, and training session. I modelled the relationship using a zero-inflated generalized linear mixed model (Zuur *et al.* 2009) given that a high number of fish did not enter the reward zone (i.e. zeroes). This model was divided in two components. First, whether fish entered the reward zone or not (hereafter referred to as the probability of entry) was modelled using a Bernoulli distribution. Second, the latency of entry to the reward zone was modelled, only for fish which entered the reward zone, using a Gamma distribution. These models were also used to test the prediction that high background risk creates a longer latency for familiarization with the task. A significant

interaction between background risk and training session would potentially indicate support for this prediction.

To test the prediction that background risk affects the number of attempts to complete a foraging task, I modelled the number of entries in the reward zone to my various predictors using a Poisson distribution. It was not necessary to use zero-inflated modelling in this case because the Poisson distribution adequately includes zeroes (Zuur and Ieno 2016).

All model selection was conducted using forward selection using AICc (Zuur *et al.* 2009; Zuur and Ieno 2016), given that replication was too low to model a large number of interactions at once in a zero-inflated model. Model selection for the latency of entry to the reward zone was applied separately on each model component (Bernoulli and Gamma; Zuur *et al.* 2009). Post-hoc comparisons of factor levels were analyzed using the package *emmeans* (v. 1.3.3).

2.7.2 Testing Phase

For the analyses in this section my predictors are similar to the ones mentioned above for the training phase (section 2.7.1). However, they included trial number, rather than training session.

To test the prediction that high background risk inhibits the application of a learned task in a novel situation, I modelled the latency to enter the reward zone to my various predictors of interest. The modelling for this component of the analysis was conducted as described in section 2.7.1. For the same reasons as described above, two components consisting of a Bernoulli and Gamma distribution were used.

To test the prediction that high background risk results in fewer attempts to obtain food in the novel situation (based on the previous training), I modelled the number of entries in the

reward zone to my various predictors of interest. This model also used a Poisson distribution, as described for the number of entries in the reward zone in section 2.7.1

3. Results

3.1 Training phase

3.1.1 Latency to enter the reward zone

The first component using a Bernoulli distribution was necessary, as the proportion of fish entering the reward zone was often low and varied (Figure 6). Collectively, just under one third (29%) of training sessions had no fish enter the reward zone (i.e. zeroes). The second component of the model was modelled using a gamma distribution, as the data were determined (visually) to be normally distributed. As the data were collected in seven treatment blocks, and included repeated measures, both components of the zero-inflated model accounted for the non-independence of tanks and treatment blocks (Table 1).

After forward model selection, the most parsimonious zero-inflated mixed model for the probability of entering the reward zone (AICc = 1225.82, Table 1 & 5, Figure 8), provided no support for my initial hypothesis that background levels of risk would affect the learning of a novel foraging task. Background risk had no effect on the probability of entering the reward zone, and was not included in any parsimonious model. Rather, the acute risk reinforcement influenced the probability of entering the reward zone; fish were more likely to enter the reward zone when exposed to the DW than AC reinforcement stimulus (x^2 = 8.16, df = 1, p = 0.004, Table 5, Figure 8).

Contrary to my initial predictions, the colour of the trained cup did influence the probability of entry, where the probability was higher for the red than the white cup (x^2 = 8.78, df = 1, p = 0.003, Table 5, Figure 8). Also contrary to my initial predictions, the side of the tank

that the rewarded cup was on influenced the probability of entry. A significant interaction was found between the side of the tank the rewarded cup was on and training duration. The probability increased more rapidly with training duration when the cup was on the right side of the tank than on the left side (x^2 = 7.26, df = 1, p = 0.007, Table 5, Figure 8).

The second component of the model only included the entrances into the reward zone (Figure 7). Similarly, this model did not provide any support for my initial hypothesis. After forward model selection, the most parsimonious zero-inflated mixed model suggested that the latency to enter the reward zone (AICc = 2471.84, Table 1 & 6, Figure 9) was not affected by background risk. Neither background risk, nor acute risk reinforcement was included in any parsimonious model. As such, there was also no support for my prediction that background risk would influence the latency for familiarization with the task, as I found no significant interaction between background risk and training duration.

Partial support was found for my prediction that the colour of the reward zone would not be significant. This variable was also not included in parsimonious model. The latency to enter the reward zone did not differ between the red and white cups. Contrary to my initial prediction, the side of the tank the rewarded cup was on was once again significant. Analysis revealed a strong interaction between training duration and side of the tank the rewarded cup was on. The latency to enter the reward zone decreased more rapidly over training duration when the rewarded cup was on the right side of the tank than the left (x^2 = 9.12, df = 1, p = 0.0025, Table 6, Figure 9).

3.1.2 Number of entries in the reward zone

After forward model selection, the most parsimonious mixed model (AICc = 13266.93, Table 2 & 7, Figure 11), did not provide any support for my initial hypothesis. Background risk

had no effect on the number of entries in the reward zone, and was not included in any parsimonious model. Once again, it was the acute risk reinforcement stimulus that was included in the final model. The number of entries in the reward zone was higher for the DW reinforcement than the AC reinforcement stimulus (x^2 = 58.56, df = 1, p < 0.0001, Table 7). Additionally, there was no support for my prediction that background risk would influence the latency for familiarization with the task. No significant interaction between background risk and training duration was found.

My initial prediction concerning the colour of the rewarded cup was not supported, as the number of entries was higher for the red than the white cup (x^2 = 18.71, df = 1, p < 0.0001, Table 7). Additionally, my prediction about the side of the tank the rewarded cup was on was also not supported. A strong interaction between the side of the tank and training duration was found (x^2 = 98.20, df = 1, p < 0.0001, Table 7). The number of entries increased more with training duration when the rewarded cup was on the right side of the tank than the left. Figure 10 is a scatterplot of the raw data, while Figure 11 displays the least squares means and standard error derived from the final mixed model. It was necessary to account for the non-independence of tanks and treatment blocks (Table 2).

3.2 Testing Phase

3.2.1 Latency to enter the reward zone

The first component using a Bernoulli distribution was necessary, as the proportion of fish entering the reward zone was often low and varied (Figure 12). Collectively, over one third (36%) of trials had no fish entering the reward zone (i.e. zeroes). The second component of the model was modelled using a gamma distribution, as the data was determined (visually) to be normally distributed. As the data was collected in seven treatment blocks, and included repeated

measures, both components of the zero-inflated model accounted for the non-independence of tanks and treatment blocks (Table 3).

After forward model selection, the most parsimonious zero-inflated mixed model for the probability of entering the reward zone (AICc= 373.6584, Table 3&8, Figure14A), provided no support for my initial hypothesis. Background risk had no effect on the probability of entering the reward zone, neither did the acute risk reinforcement stimulus. Neither of these variables were included in any parsimonious model.

Partial support was found for my prediction that colour would be insignificant. This variable was also not included in any parsimonious model. The probability of entering the reward zone did not differ between the red and white cups. Surprisingly, the only variable that influenced the probability of entering the reward zone was the side of the tank the trained rewarded coloured cup was on. The probability of entering was higher when the cup was on the right side of the tank than the left side (x^2=6.97, df= 1, p=0.0083, Table 8).

Similarly, the second component of the model for the latency to enter the reward zone (AICc=597.1171 Table 3 & 9, Figure 14B) also provided no support for my initial hypothesis. Background risk had no effect on the latency to enter the reward zone, neither did the acute risk reinforcement stimulus. Neither variable was included in any parsimonious model. As background risk had no effect, I also did not find support for my prediction about the interaction between background risk and trial. Partial support for my initial prediction about colour was found, as this variable was also not included in any parsimonious model. Again, the only variable that affected the latency to enter the reward zone was the side of the tank the trained rewarded coloured cup was on. The latency to enter was shorter when the cup was on the right side of the tank than the left (x^2=11.28, df= 1, p=0.0008, Table 9). Indeed, Figure 13 indicates no consistent

pattern in the raw data, other than the effect of the side of the tank the trained rewarded coloured cup was on.

3.2.2 Number of entries into the reward zone

After forward model selection, the most parsimonious mixed model for the number of entries into the reward zone (AICc= 1453.015, Table 4 & 10, Figure 16), provided no support for my initial hypothesis. Background risk had no effect on the number of entries, and was not included in any parsimonious model. Similarly, acute risk reinforcement was also not included in the final model, as it had no effect on the number of entries.

Partial support for my prediction about the number of entries was found, as the number of entrances decreased with trial number (x^2=8.25, df=1, p=0.0040, Table 10). However, I was expecting a significant interaction between background risk and trial, where high risk individuals would have a low number of entrances across all trials, and the number of entrances for low risk individuals would start high and decrease over subsequent trials.

My predictions concerning colour and side were also not supported. The number of entries was higher for the white than the red cup (x^2=6.73, df=1, p=0.0095, Table 10), and was higher for the right than the left side (x^2=115.81, df=1, p<0.0001, Table 10). Figure 15 displays the raw data, while Figure 16 displays the least squares means derived from the final mixed model. This model accounted for the non-independence of tanks and treatment blocks (Table 4).

4. Discussion

The main goal of this thesis was to test the hypothesis that exposure to elevated predation risk over a period of a few days interferes with the learning of a novel foraging task. Overall, the results do not support my hypothesis. Background risk had no effect on the learning of a novel foraging task, and was not included in any parsimonious model. Fish exposed to high levels of

background risk were no slower to approach a novel foraging opportunity, did not have fewer attempts, and were no slower to become familiar with the task than those exposed to low levels of background risk. Both high and low background risk treatments learned the foraging task equally well. The latencies to enter the rewarded zone decreased, and the number of entrances increased consistently over the course of the 16 training sessions. While learning did occur, it is difficult to make any comments on the specific strength of the learned association. Most studies on foraging in guppies focus on the social transmission of foraging information (Reader et al. 2003; Reader and Laland 2000; Swaney et al. 2001). The only study involving the completion of a novel foraging task (without social transmission), was investigating the factors that influenced innovative behaviour in guppies, and did not include any repeated measures (Laland and Reader 1999). Despite the difficulty in making comparisons on the strength of the learned association, learning did indeed occur. The observed behavioural changes coincide with Brown's (2012) definition of learning; a change in behaviour over time based on recent experiences. The repeated training sessions allowed the fish to form a learned association between an object and a foraging reward, as seen in their decreased latencies and increased entrances over the course of the experiment. Thus, their behaviour changed over time to better suit their local environment.

The lack of effect of background risk was unexpected, given the relationship between neophobia and learning (Boogert *et al.* 2006; Seferta *et al.* 2001). Short term predation risk is commonly simulated using conspecific alarm cues (ex: Ferrari 2014; Mitchell *et al.* 2016), and exposure to conspecific alarm cues for multiple days has been effective in inducing neophobia in multiple species including: Trinidadian guppies and woodfrog tadpoles (*Rana sylvatica*; Brown *et al.* 2013), juvenile convict cichlids (Joyce *et al.* 2016), northern red-bellied dace (*Phoxinus eos*; Brown *et al.* in press), and fathead minnows (*Pimephales promelas*; Crane *et al.* 2015).

Given that these exposures were sufficient to induce neophobia, my results suggest that while neophobia was likely induced in the fish, it had no apparent impact on their ability to learn a novel foraging task.

Interestingly, exposure to acute levels of risk had more of an impact on learning than background risk. Shoals that were exposed to acute high risk reinforcement stimuli on training days showed a lower probability and greater latency to enter the reward zone than those exposed to acute low risk reinforcement stimuli. Additionally, they had fewer entries into the reward zone. While acute risk was significant in the modelling, it is important to note, that exposure to high acute risk did not inhibit learning. Over the course of the 16 training sessions, fish exposed to high acute risk still decreased their latencies and increased their number of entrances. The difference between low and high acute risk was simply in the averages, not the trends. The acute pulses of risk were much closer to the training sessions in time, compared to the pre-exposure phase, and so more recent events might hold more weight than earlier ones. Indeed, European minnows (*Phoxinus phoxinus*) are less likely to locate a foraging patch when they have visual contact with a predator than when they do not (Johnsonn and Sundström 2007). While my experimental set up did not include exposure to risk during the actual training sessions, the acute risk exposures did occur much closer to the training sessions than the background risk pre-exposure (i.e. on the same days). So perhaps the risk of predation experienced closer to the food locating portion of the experiment explains why acute risk had a mild effect on the ability to learn the foraging task, and background risk did not.

Most of the studies examining predation risk and learning have used wild populations from different predation environments. These populations have had longer exposure times to differing conditions than fish in a laboratory setting could experience. As such, there are likely to

be more significant differences between wild populations, such as genetic divergence, than between laboratory treatments. For example, brain mass is positively correlated with predation pressure in multiple populations of Trinidadian guppies (Kotrschal *et al.* 2017). However, I did not use wild fish from high risk populations, so the cognitive abilities that are selected for in harsher environments (Roth *et al.* 2010) may not be present after such a short exposure to elevated risk in a laboratory setting. This may explain why long term exposure to high levels of predation risk affects learning, but short term exposure does not. However, short term exposure to predation risk may simply affect learning in contexts other than foraging.

While neophobia naturally occurs in wild populations of guppies, its plasticity (Brown *et al.* 2013) allows for controlled experiments in a laboratory setting. In addition to wanting to examine short term exposure to elevated predation risk, my justification for using only low predation guppies was to control for other differing variables between populations. This would allow for conclusions to be drawn specifically about background risk, as other environmental factors can influence learning. For example habitat variation has been shown to influence learning (Girvan and Braithwaite 1998). Three-spined sticklebacks (*Gasterosteus aculeatus*) from river populations learned a spatial task slower (based on spatial cues) than those from ponds. Ponds are considered to be more stable in terms of habitat compared to rivers (Girvan and Braithwaite 1998). A follow up study demonstrated that habitat stability actually interacted with predation pressure to shape learning and memory in *B. episcopi* (Brydges *et al.* 2008). Factors such as competition and population density can vary significantly between high and low risk predation populations of Trinidadian guppies (Magurran 2005). Therefore, by inducing neophobia rather than using separate wild populations, I was able to control for other environmental aspects while manipulating background risk.

These claims about the effects of background and acute risks must be made cautiously however, as I did see a very significant effect of colour and which side of the tank the rewarded cup was on. The significance of the colour of the cups is not surprising in retrospect, as female guppies sexual selection is highly influenced by the carotenoid (orange-red) pigment in males (Kodric-Brown 1985). However, guppies are faster at learning to discriminate between colours than shapes (Lucon-Xiccato *et al.* 2019). While the guppies were more attracted to the red cup, they were still able to still learn with white cups. This demonstrates their ability to learn colour associations outside their sexual selection bias; while females are attracted to carotenoid pigments, they can still form associations with colours outside that part of the colour spectrum.

When the rewarded cup was on the right side of the tank, the fish had significantly higher probabilities to enter, had shorter latencies, and had a greater number of entrances. The interaction between training duration and side shows that learning occurred only when the cup was on the right side of the tank, and not the left. No learning occurred when the rewarded cup was on the left, as I observed no change in behaviour over time. This could possibly be due to cerebral lateralization, but limited evidence could be found to support this. Cerebral lateralization is the separation of various cognitive functions between the two hemispheres of the brain (Bibost and Brown 2014; Brown *et al.* 2007). Cerebral lateralization can often manifest in a variety of behavioural side preferences, collectively termed laterality (Brown and Magat 2011). For example, chimpanzees show a preference for which hand they use while foraging (Marchant and McGrew 1996), and fish can display turning biases (Ferrari *et al.* 2017). Laterality is shaped by predation risk, where fish from high predation populations display stronger laterality than those from low predation populations (Brown *et al.* 2004). Additionally, laterality is plastic, where short-term exposure to high predation risk results in stronger laterality (Broder and Angeloni

2014; Ferrari *et al.* 2015a; Jozet-Alves and Hébert 2013). Moreover, laterality has been shown to be positively correlated with cognitive performance (Bibost and Brown 2014). However, as my observed side bias was present across all treatments, and not only the high risk treatment, it is unlikely a result of laterality. While there is nothing in the experimental set up that would suggest a reason for the side bias, it is likely a factor that I was unable to identify.

The goal of the testing phase was to see if the learned foraging task could be generalized across contexts. Overall, the results do not support any of my predictions. Neither background risk nor acute risk reinforcement had any significant effects; the probability and latency of entry, and the number of entries did not differ regardless of background or acute risk. The significant side bias recorded for the number of entries once again makes it difficult to draw conclusions. Unexpectedly, it was the white cup that was preferred over the red cup in the testing phase. This reversal in colour preference was surprising, considering the strong preference for carotenoid (orange-red) pigments in female guppies (Kodric-Brown 1985) which was observed in the training phase. The only partial support for a prediction that I found was that trial number was significant, where the number of entries decreased as the trials increased. This was expected, as no food reward was given. However I was expecting a significant interaction between trial and background risk. Once again the significant side bias towards the right side of the tank, as well as the switch of preferred colours, means that I cannot draw any definitive conclusions about the ability of the fishes to generalize learned foraging tasks across contexts.

My results do not suggest that background predation risk in any way affected learned foraging behaviour to the extent that lost-opportunity costs were incurred. This was surprising as Ferrari *et al.* (2019) found that risk induced neophobia had consequences even when there was no predation event or stimulus detected. Damselfish (*Pomacentrus amboinensus*) that were

exposed to high risk conditions for several days were poorer competitors compared to those exposed to low risk conditions. Even in the absence of an acute threat, exposure to high background risk had carry-over effects (Ferrari *et al.* 2019). I observed no such carry-over effects. The lost-opportunity costs regarding foraging, that are associated with neophobia, may not manifest for learning novel foraging opportunities. It is possible however, that exposure to elevated levels of background risk does not influence the learning of a novel foraging task in guppies. If this is indeed the case, it could have potential positive impacts for conservation.

Exposing aquatic species to elevated levels of background risk, to induce neophobia, has been suggested as a tool for conservation efforts. Anthropogenic climate change has caused species distributions to expand and shift (Parmesan and Yohe 2003 ; Chen *et al.* 2011). Consequently, there has been an increase in invasive species (Rahel and Olden 2008; Mainka and Howard 2010). Invasive species can pose serious threats to local populations if native species do not recognize potentially novel predators. As neophobia has a direct survival benefit (Ferrari *et al.* 2015b), it may help prey deal with novel predators. If induced neophobia has minimal lost-opportunity costs for learning novel foraging tasks, it could potentially be induced in local populations to increase survival. As induced neophobia has potentially important conservation applications, it is important to elucidate the details of its expression, and potential carry over costs.

Future studies with refined experimental designs might help to provide a clearer picture of the relationship between background risk and lost-opportunity costs for foraging. Eliminating the side bias, as well as using population specific alarm cues may provide different, more accurate results. Moreover, conducting experiments under semi-natural conditions, with more ecologically relevant foraging tasks, may also provide a clearer picture of how lost-opportunity

costs due to short term exposure to elevated levels of background risk would manifest. As lost-opportunity costs relate to a range of fitness activities, exploring the relationship between background risk and behaviours such as mating and territory defence would provide a clearer picture, and develop a more comprehensive model of the interaction between predation risk, neophobia, and learning.

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Figures

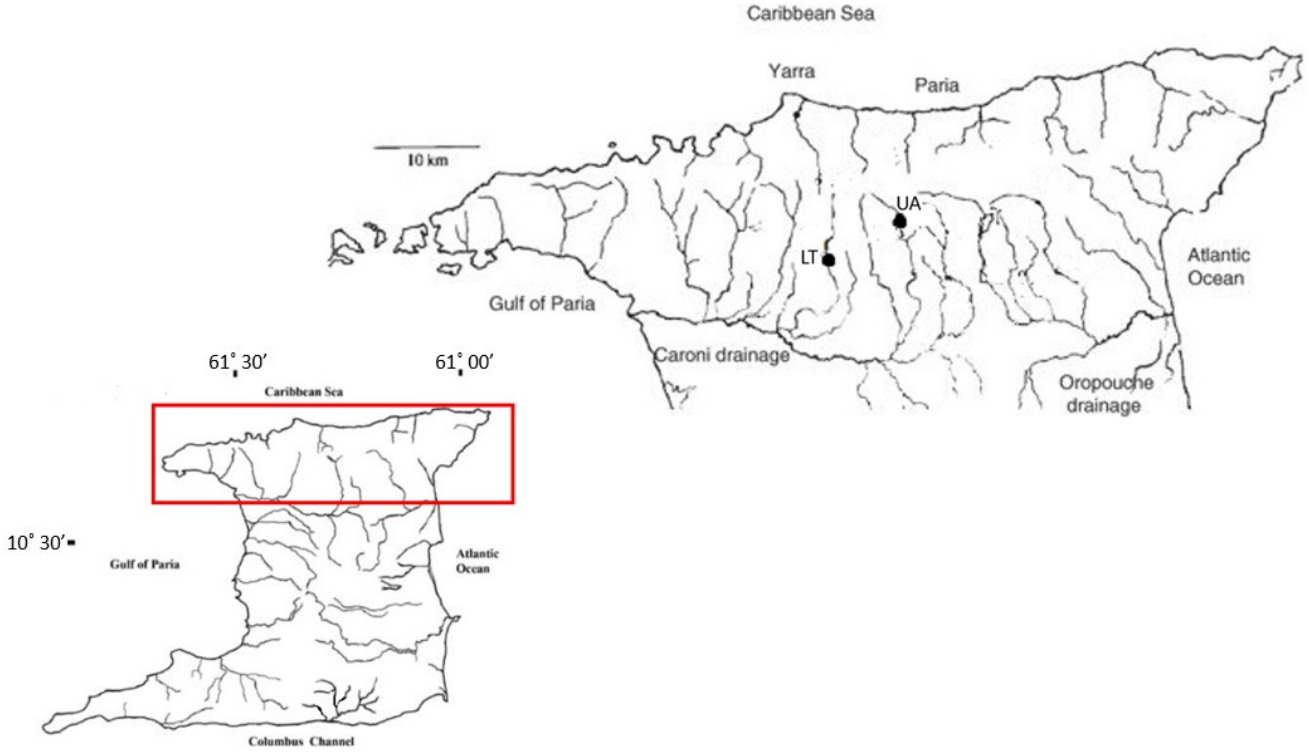

Figure 1: Map of the Northern Mountain Range in the Republic of Trinidad and Tobago, indicating various rivers including the Upper Aripo (UA) and Lopinot (LT) sampling sites. Map of the mountains adapted from Suk and Neff (2009). Map of the island of Trinidad adapted from Vanegas-Ríos and Phillip (2013).

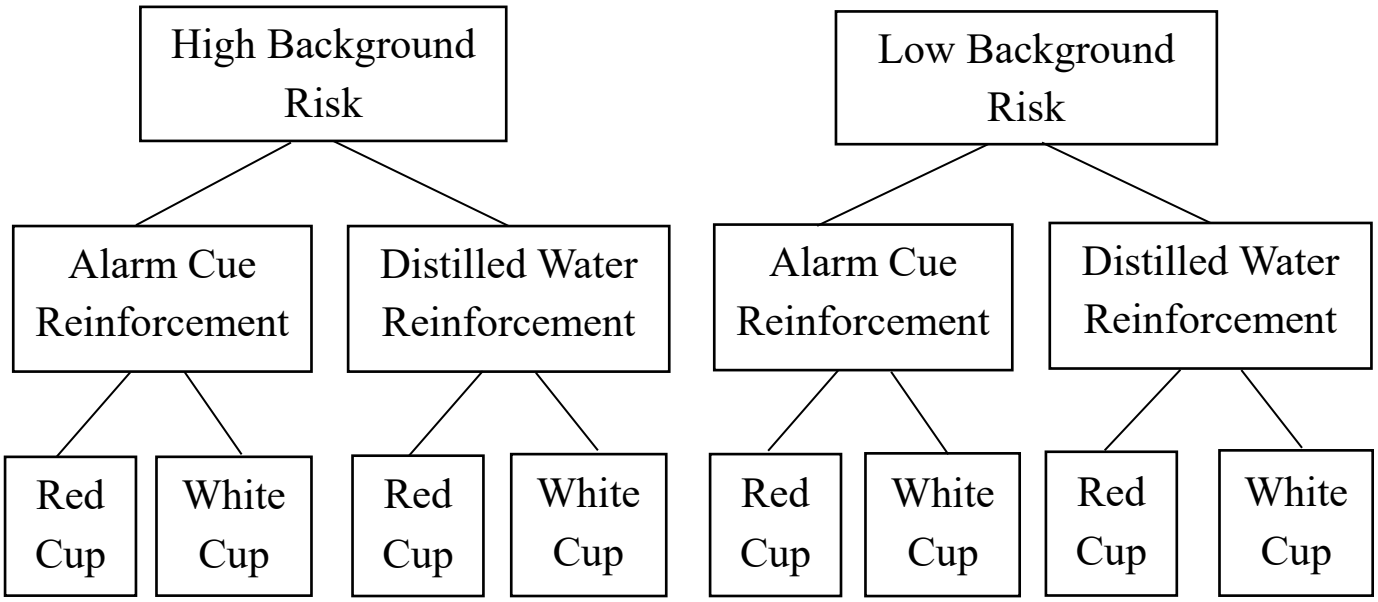

Figure 2: Fully factorial 2x2x2 experimental design illustrating the different levels of background risk (high= alarm cue /low=distilled water), acute reinforcement stimulus (Alarm cue=high /distilled water=low), as well as the colour of the reward cup (red/white) used for the training and testing.

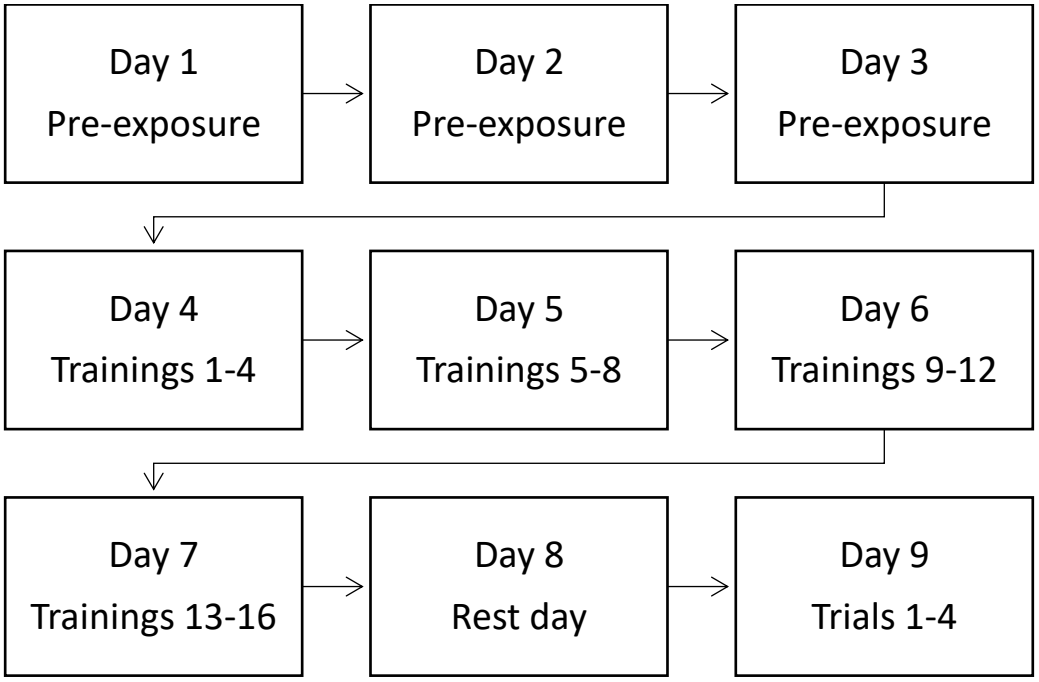

Figure 3: Flow chart for experimental time line for days one through nine

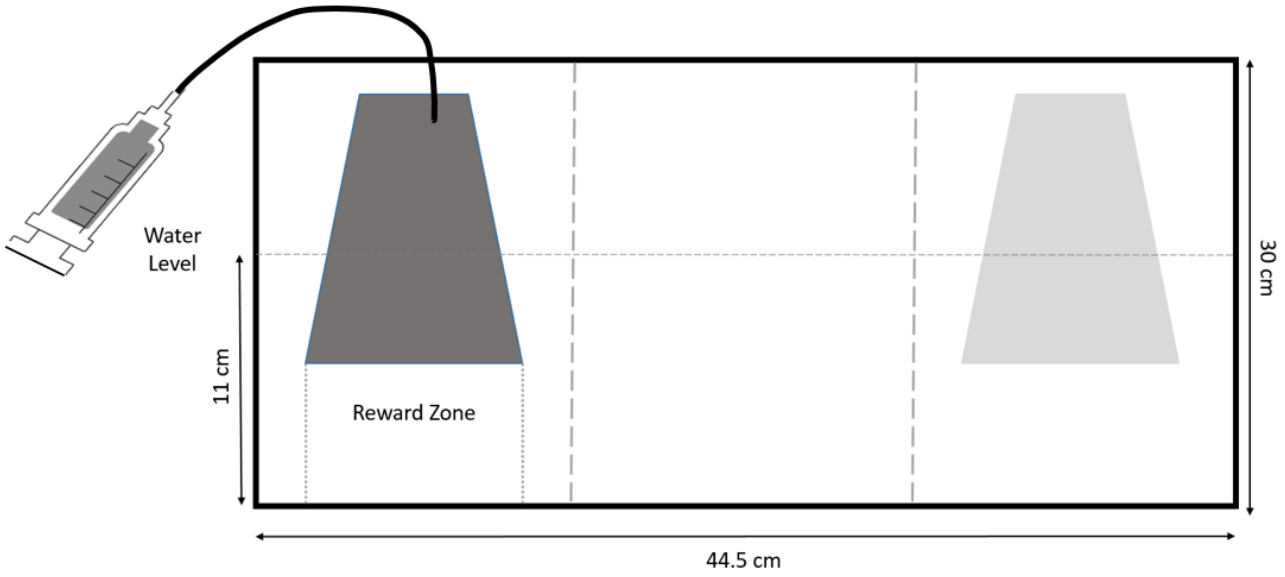

Figure 4: Diagram of a side view of the tanks used during the training phase of the experiment. The dark and light grey quadrilaterals represent the red and white cups used for training. The syringe containing the food slurry is attached to a length of sift airline tubing terminating just inside the cup.

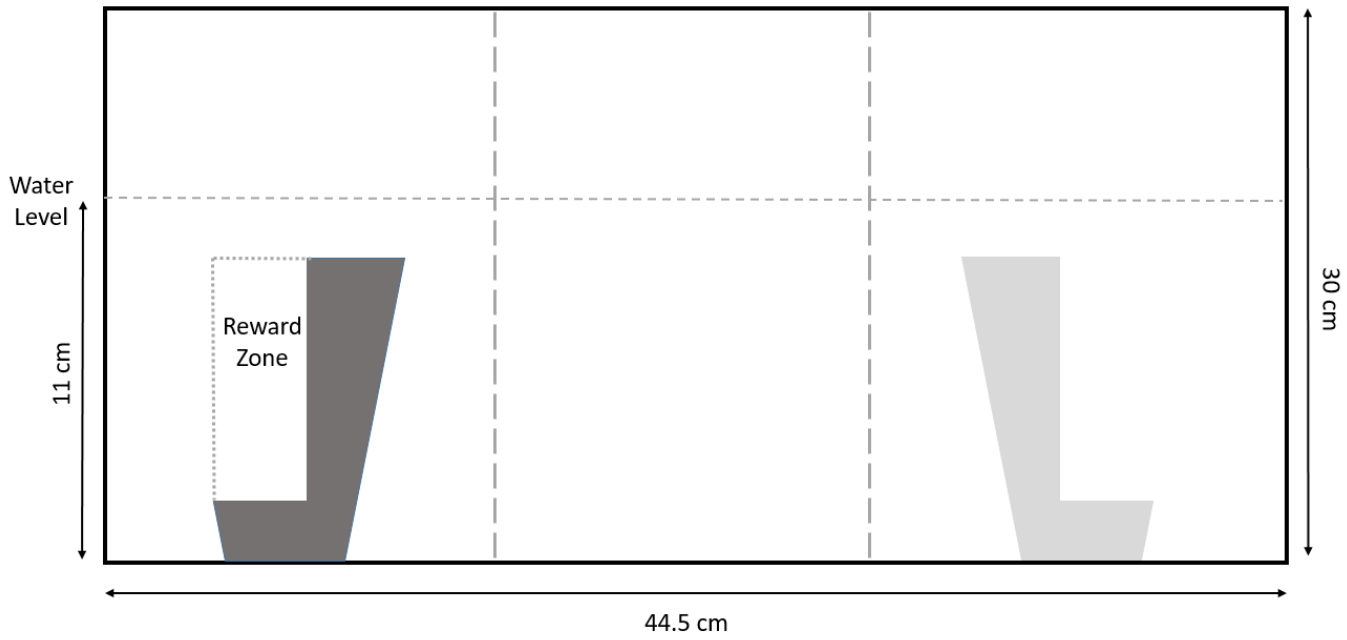

Figure 5: Diagram of a side view of the tank set up during the testing phase. Dark and light grey shapes represent the placement and configuration of the red and white cups.

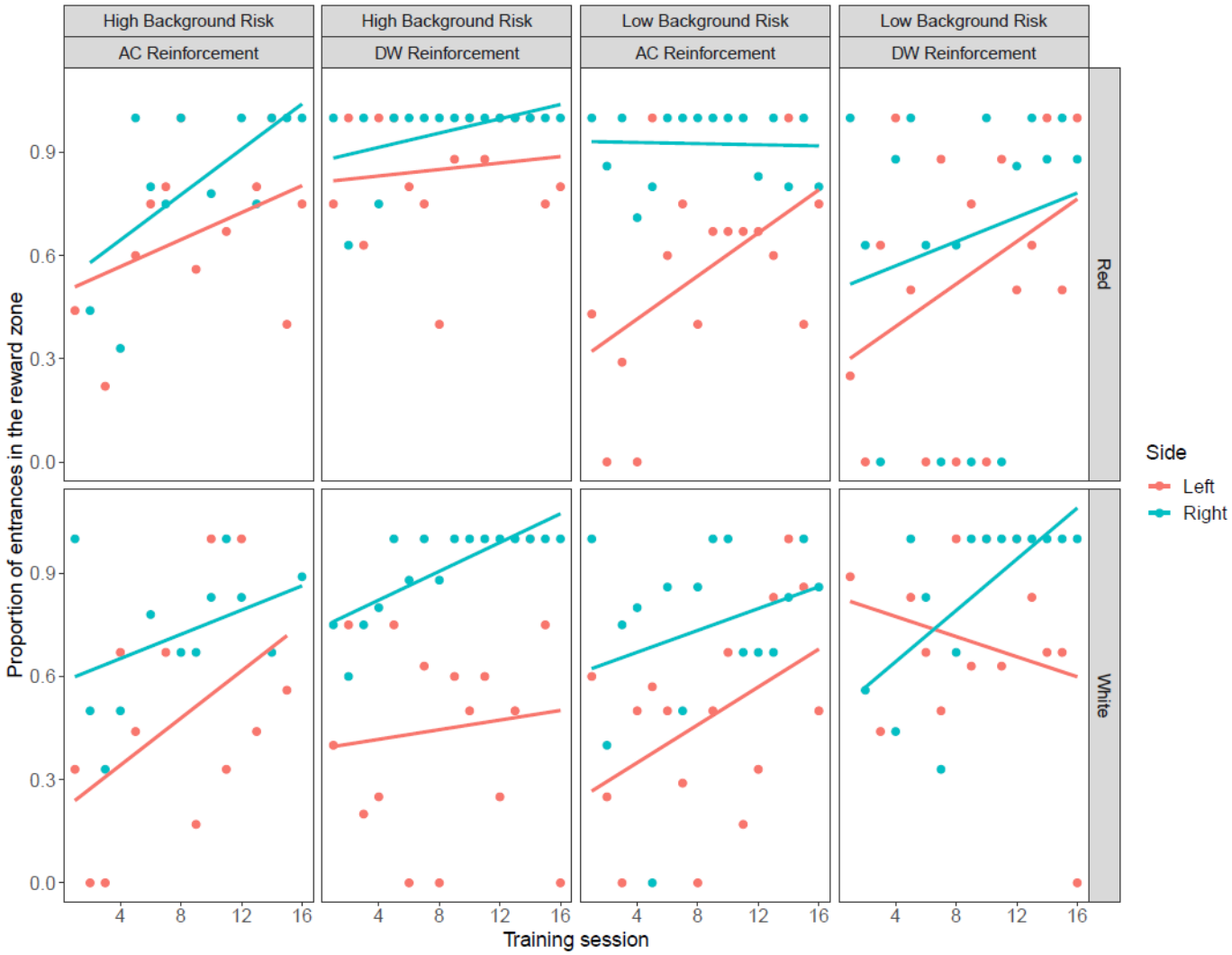

Figure 6: Scatterplot relating proportion of entrances into the reward zone to the number of training sessions, background risk (high/low), acute reinforcement (AC: alarm cue/ DW: Dechlorinated water), colour of the rewarded cup (red/white), and the side of the tank the rewarded cup was on (left/right).

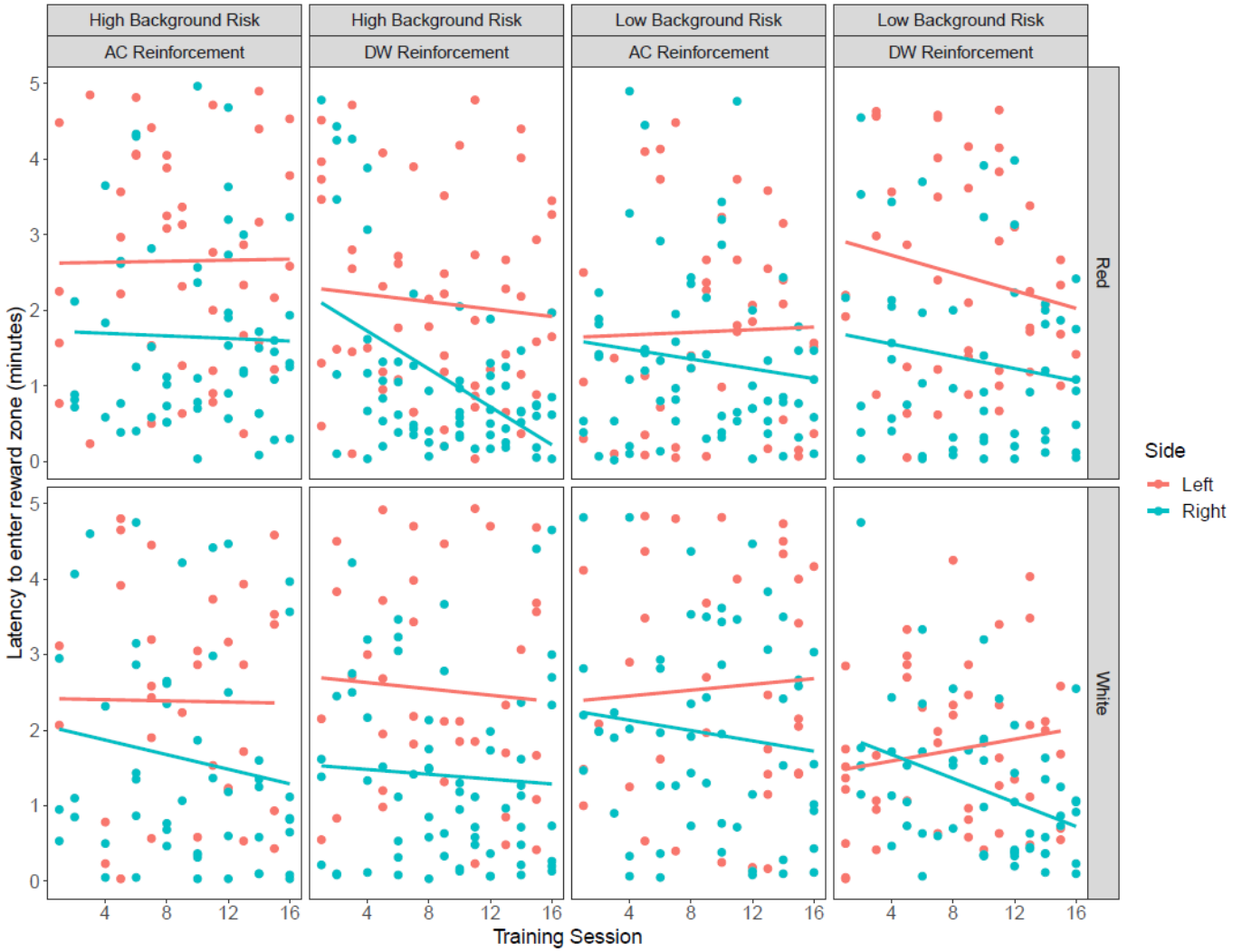

Figure 7: Scatterplot of the raw data of the entrances into the reward zone relating the latency to enter the reward zone (minutes) to the number of training sessions, background risk (high/low), acute reinforcement (AC: alarm cue/ DW: Dechlorinated water), colour of the rewarded cup (red/white), and the side of the tank the rewarded cup was on (left/right).

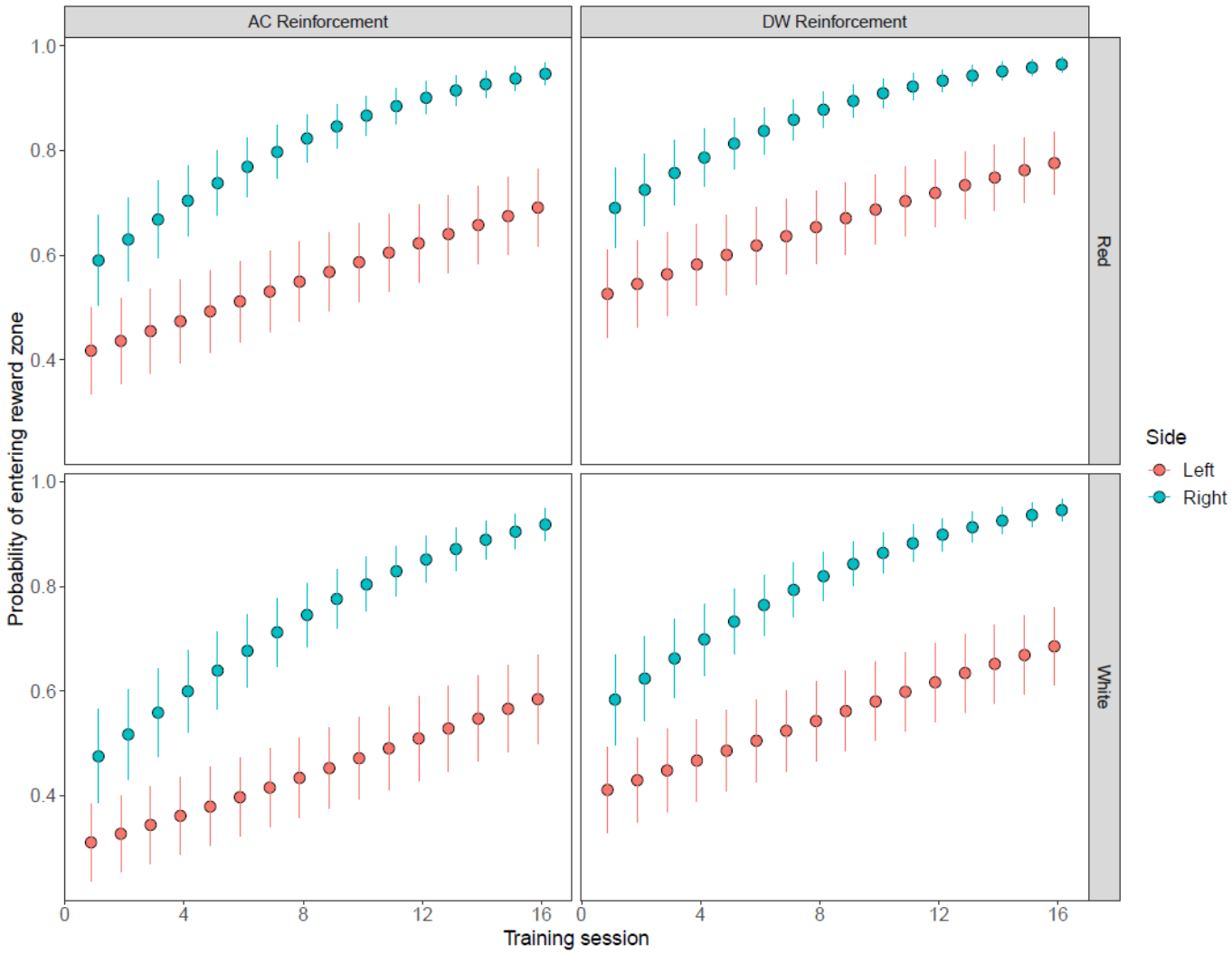

Figure 8: Least squares means of the zero-inflated generalized linear mixed model relating the probability of entering the reward zone to number of trainings, acute reinforcement (AC: alarm cue/ DW: Dechlorinated water), color of the reward zone (red/white), and the side of the tank the rewarded cup was on(left/right). Error bars represent standard error.

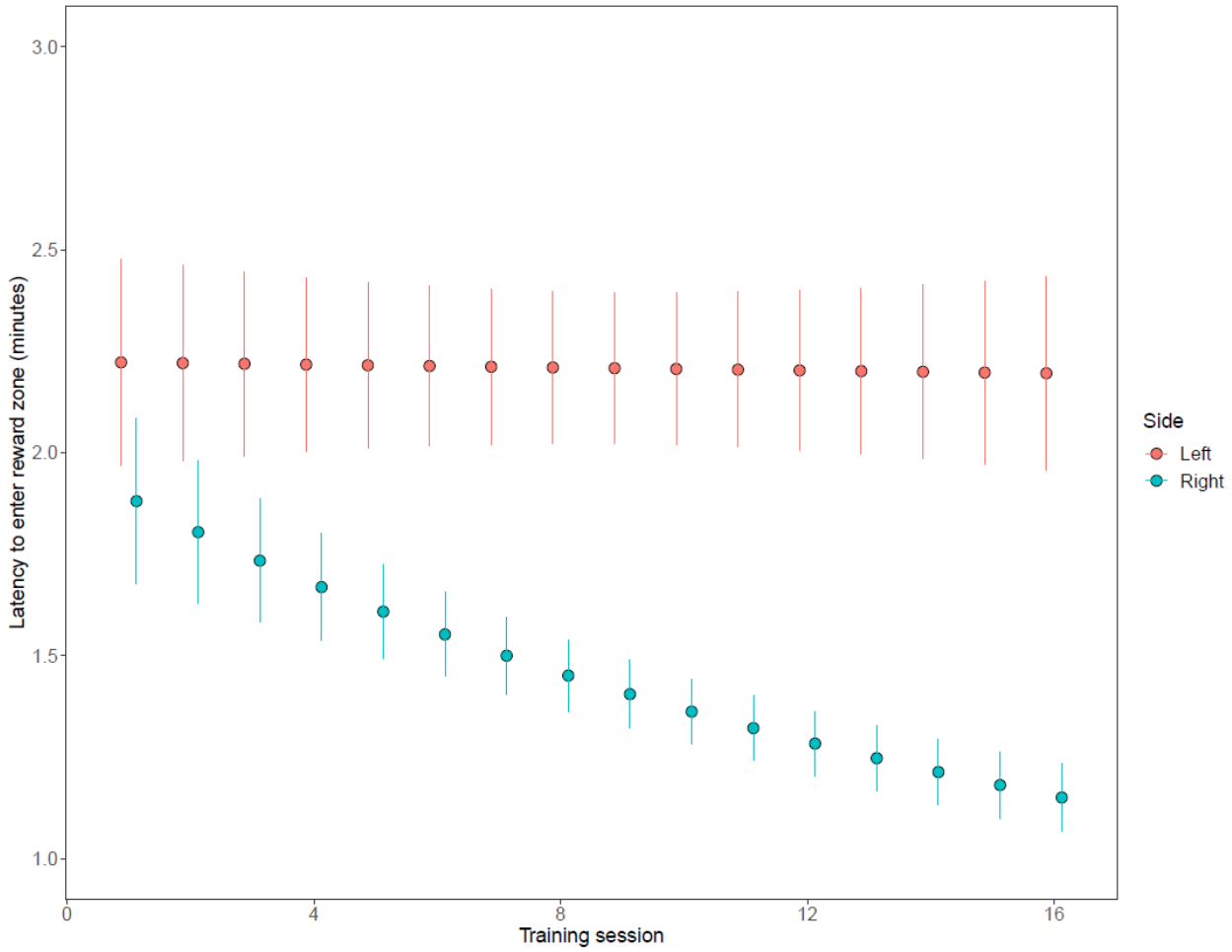

Figure 9: Least squares means of the zero-inflated generalized linear mixed model relating the latency to enter the reward zone to number of trainings and the side of the tank the rewarded cup was on (left/right). Error bars represent standard error.

Figure 10: Scatterplot of raw data relating the number of entrances in the reward zone to the number of training sessions, background risk (high/low), acute reinforcement (AC: alarm cue/ DW: Dechlorinated water), colour of the rewarded cup (red/white), and the side of the tank the rewarded cup was on (left/right).

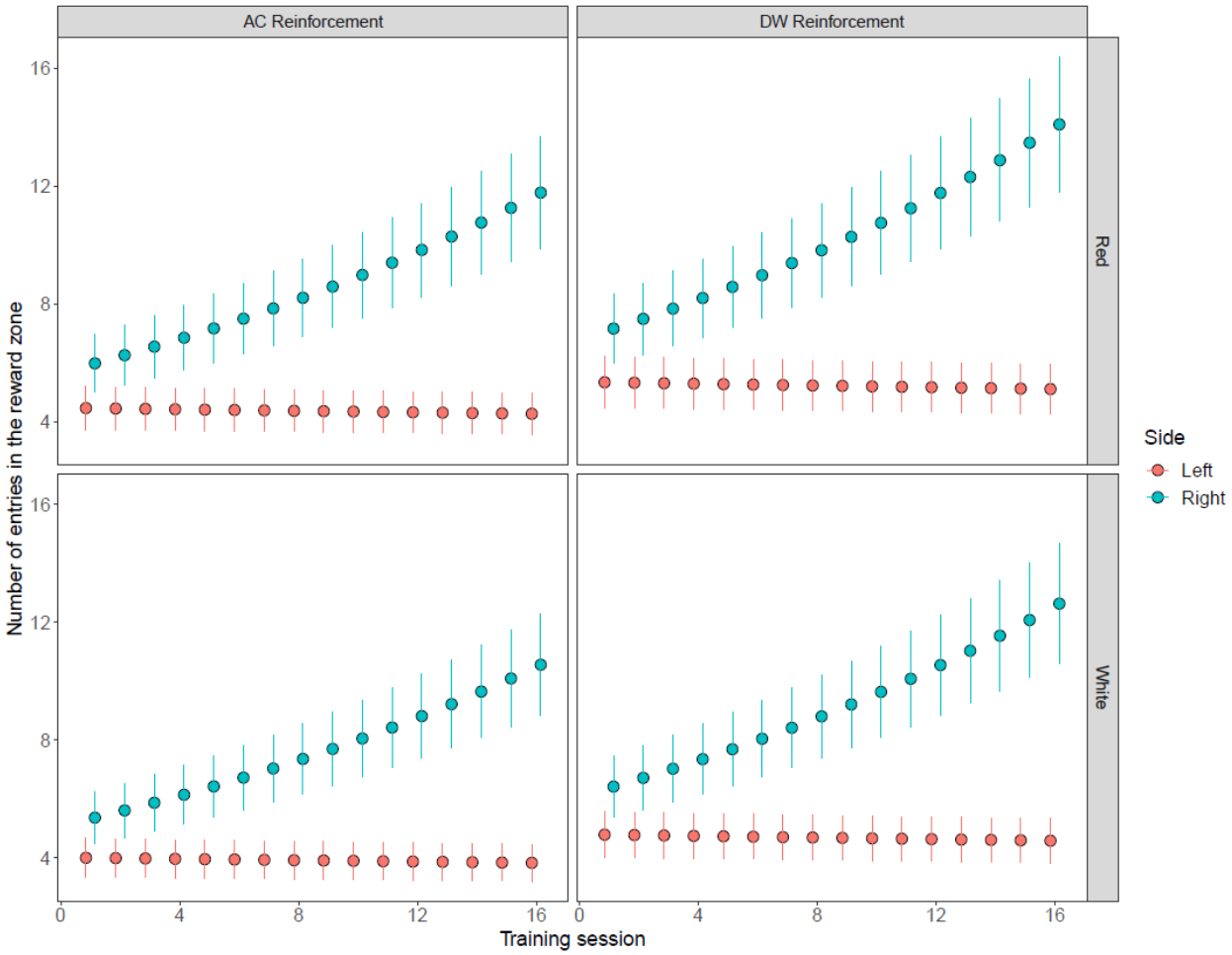

Figure 11: Least squares means of the generalized linear mixed model relating the number of entries in the reward zone to number of trainings, acute reinforcement (AC: alarm cue/ DW: Dechlorinated water), color of the reward zone (red/white), and the side of the tank the rewarded cup was on (left/right). Error bars represent standard error.

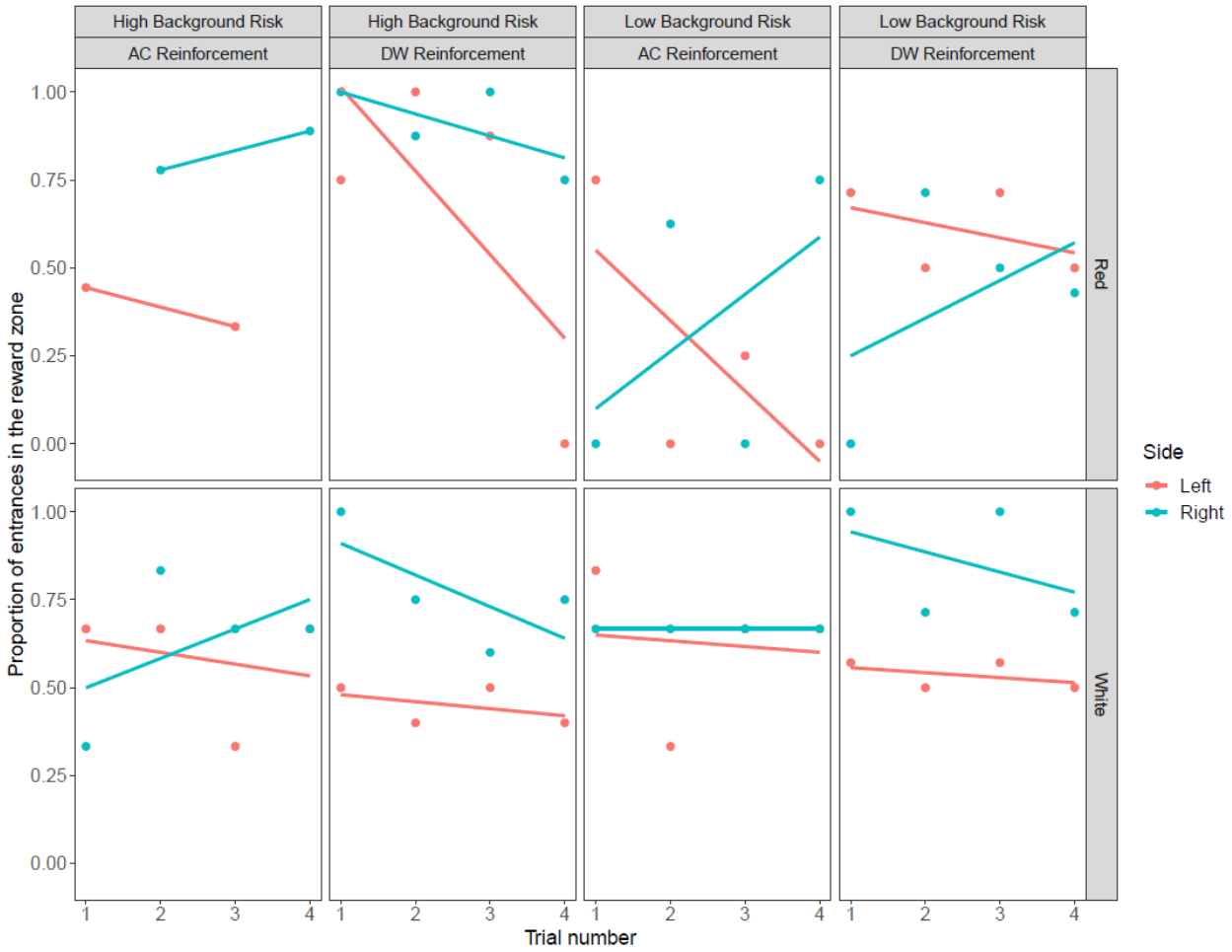

Figure 12: Scatterplot relating proportion of entrances into the reward zone to the trial number, background risk (high/low), acute reinforcement (AC: alarm cue/ DW: Dechlorinated water), colour of the rewarded cup (red/white), and the side of the tank the trained rewarded cup was on (left/right).

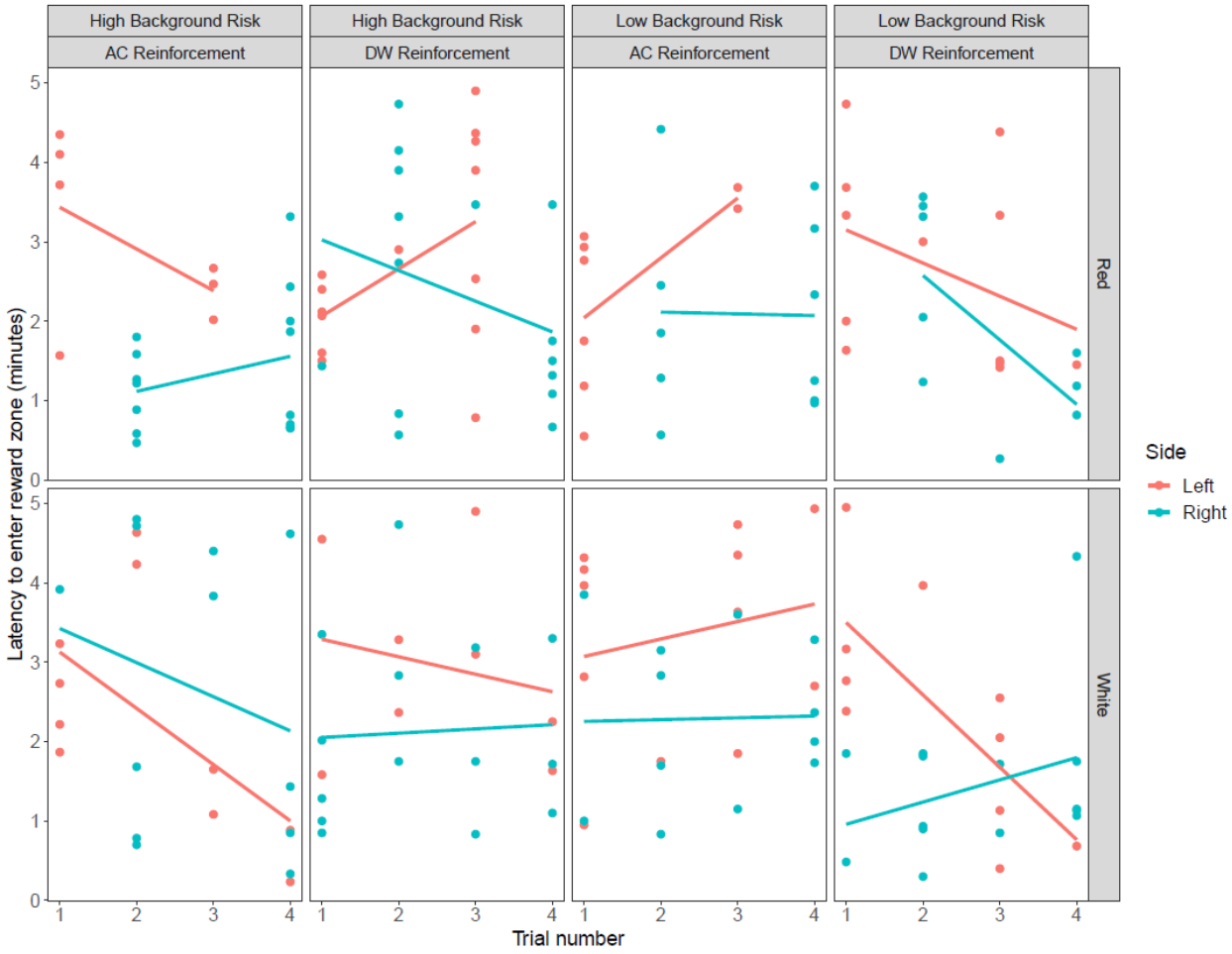

Figure 13: Scatterplot of the raw data of the entrances into the reward zone relating the latency to enter the reward zone (minutes) to trial number, background risk (high/low), acute reinforcement (AC: alarm cue/ DW: Dechlorinated water), colour of the rewarded cup (red/white), and the side of the tank the trained rewarded cup was on (left/right).

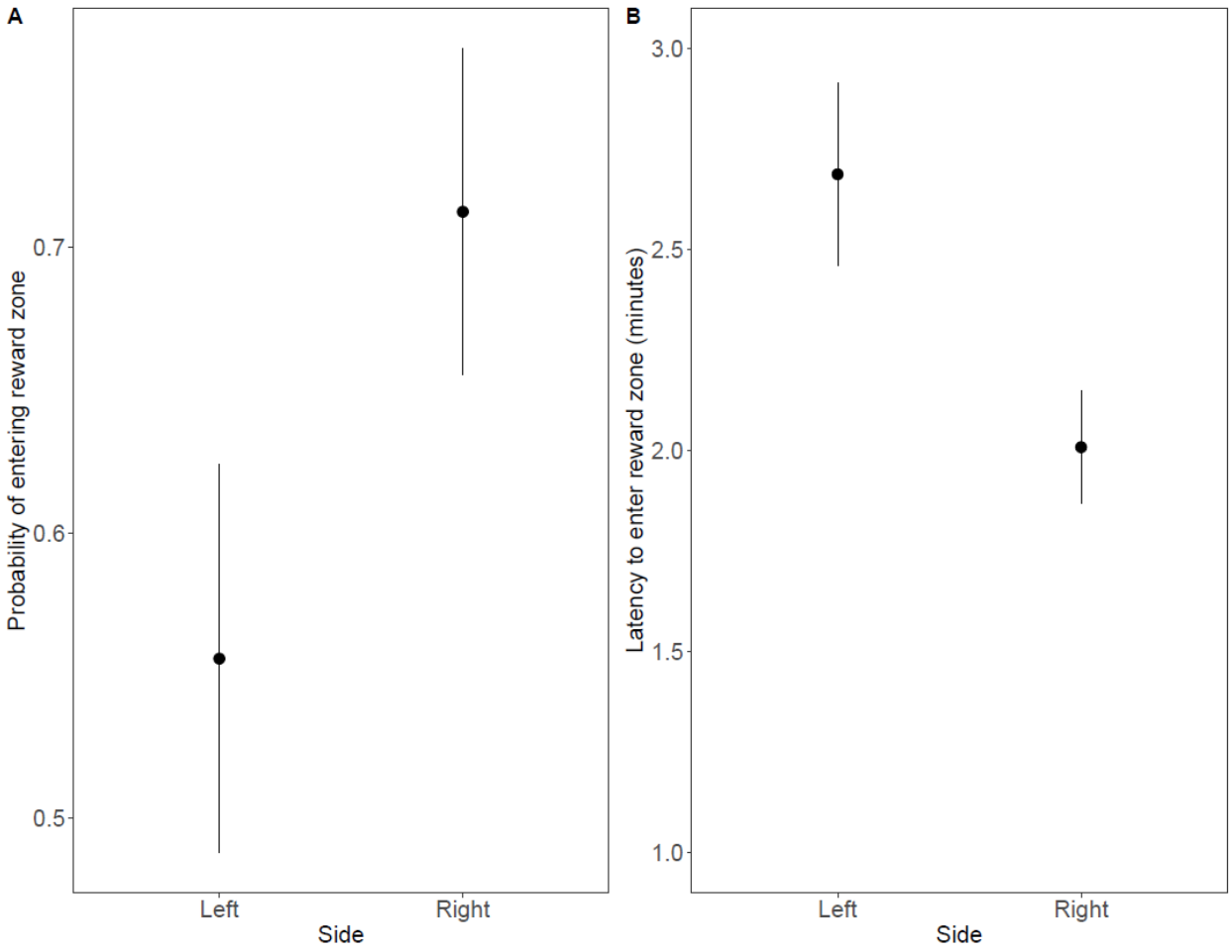

Figure 14: Least squares means of the zero-inflated generalized linear mixed model relating the A) probability of entering the reward zone and B) Latency to enter the reward zone to the side of the tank the trained rewarded cup (red/white) was on for the testing phase. Error bars represent standard error.

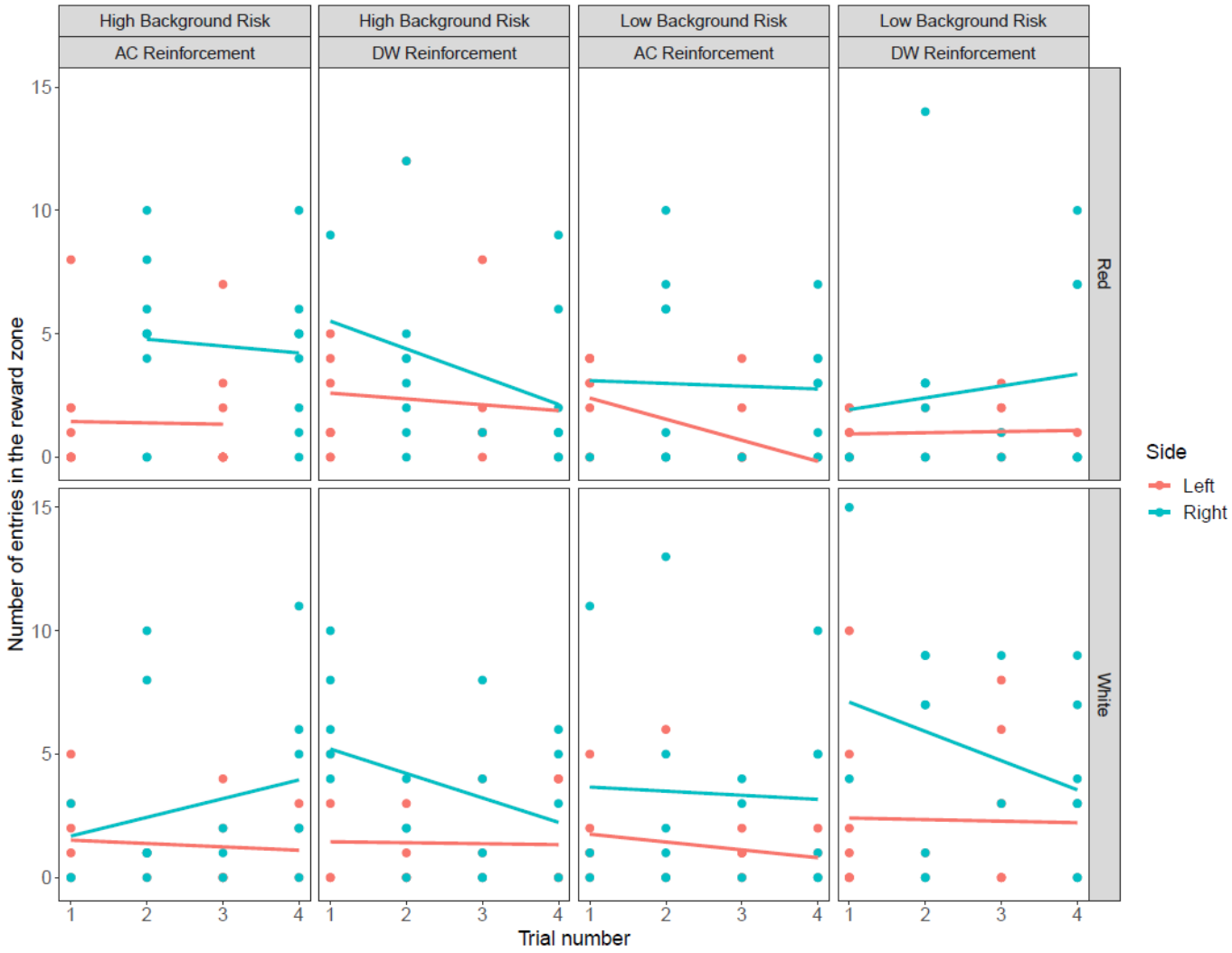

Figure 15: Scatterplot of raw data relating the number of entrances in the reward zone to the trial number, background risk (high/low), acute reinforcement (AC: alarm cue/ DW: Dechlorinated water), colour of the rewarded cup (red/white), and the side of the tank the trained rewarded cup was on (left/right).

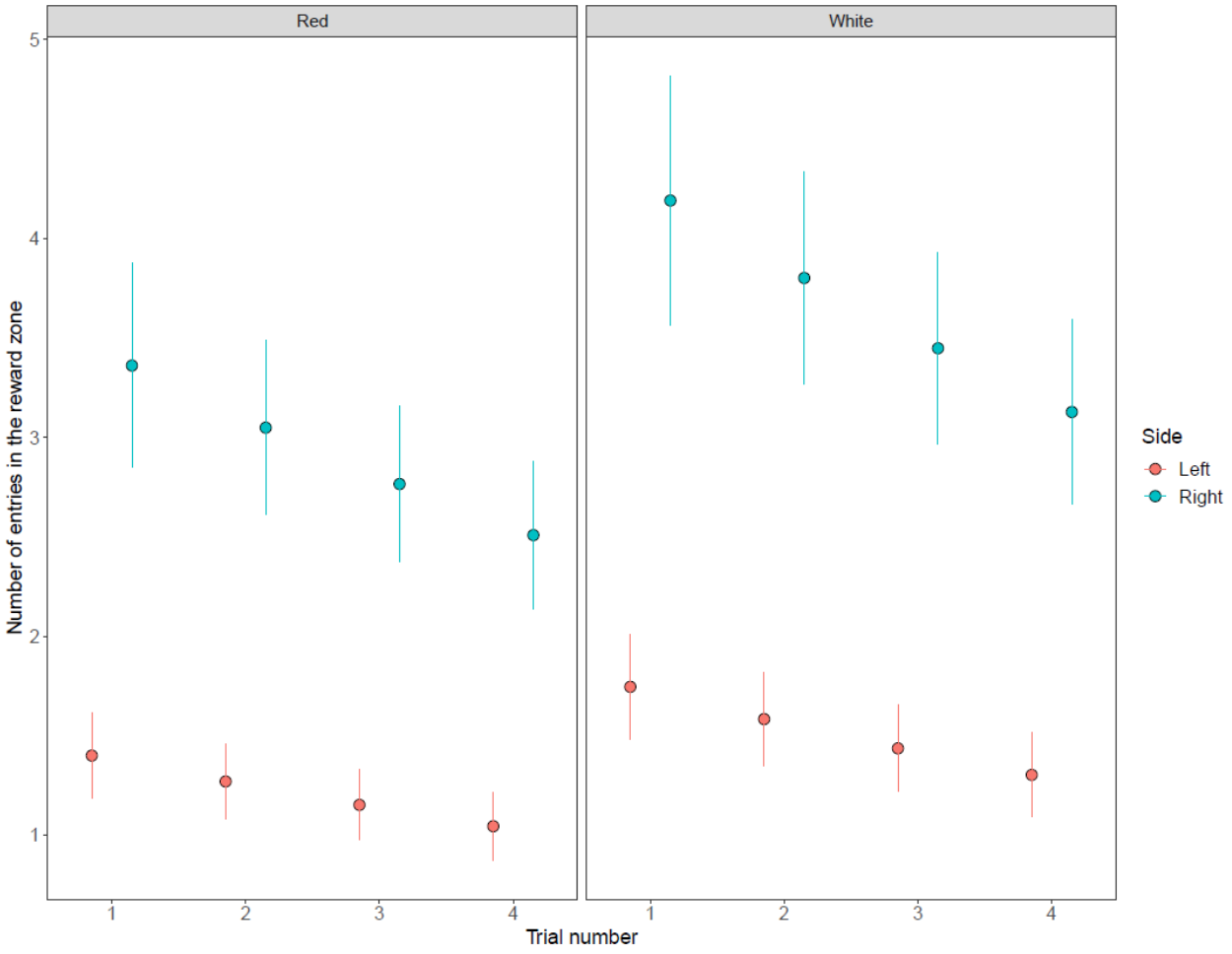

Figure 16: Least squares means of the generalized linear mixed model relating the number of entries into the reward zone to trial number, color of the reward zone (red/white), and the side of the tank the trained rewarded cup was on (left/right), for the testing phase. Error bars represent standard error.

Tables

Table 1: Forward selection of the two components of a zero-inflated generalized linear mixed model relating the latency of entry to the reward zone to various predictors for the training phase. Not all models that were tested are shown.

Model	Distribution	df	AICc	ΔAICc	Log-likelihood	Weight
LERZ.01 ~ 1 + 1|Tank + 1|Block	Bernoulli	3	1369.75		-681.87	0.00
LERZ.01~ B +1|Tank +1|Block	Bernoulli	4	1371.77	-2.02	-681.8665	0.00
LERZ.01 ~ S + 1|Tank + 1|Block	Bernoulli	4	1288.5	-81.25	-640.23	0.00
LERZ.01 ~ S*T + 1|Tank + 1|Block	Bernoulli	6	1236.98	-132.77	-612.45	0.36
LERZ.01~ S*T +B +1|Tank + 1|Block	Bernoulli	7	1238.99	-130.76	-612.45	0.13
LERZ.01 ~ S*T + R + 1|Tank + 1|Block	Bernoulli	7	1231.9	-137.85	-608.9	4.56
LERZ.01 ~ S*T + R + C + 1|Tank + 1|Block	**Bernoulli**	**8**	**1225.82**	**-143.93**	**-604.85**	**94.95**
LERZ ~ 1 + 1|Tank + 1|Block	Gamma	4	2534.06		-1263	0.00
LERZ ~ B+ 1|Tank + 1|Block	Gamma	5	2535.97	+1.91	-1261.95	0.00
LERZ ~ S + 1|Tank + 1|Block	Gamma	5	2482.18	-51.88	-1236.05	0.35
LERZ ~ S*T + 1|Tank + 1|Block	**Gamma**	**7**	**2471.84**	**-62.22**	**-1228.85**	**61.17**
LERZ~ B*S*T ++ 1|Tank + 1|Block	Gamma	11	2478.86	-55.2	-1228.26	1.84
LERZ ~ S*T + C + 1|Tank + 1|Block	Gamma	8	2472.90	61.16	-1228.36	36.29

Note: * indicates interactions (including main effects), bold indicates the most parsimonious model. Abbreviations: LERZ.01, Bernoulli distribution for entry into the reward zone (yes/no), LERZ, Latency of entry in the reward zone (minutes); B, Background risk (high/low); R, Acute Reinforcement stimulus (alarm cue/distilled water); S, Side of the tank with the reward zone (left/right); T, Training duration (1-16); C, Colour of the reward zone (red/white); 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 2: Forward selection of a generalized linear mixed model relating the number of entries in the reward zone to various predictors for the training phase. Not all models tested are shown.

Model	Distribution	df	AICc	ΔAICc	Log-likelihood	Weight
NERZ ~ 1 + 1|Tank + 1|Block	Poisson	3	14578.65		-7286.31	0.00
NERZ ~ B + 1|Tank + 1|Block	Poisson	4	14579.02	+0.37	-7285.49	0.00
NERZ ~ S + 1|Tank + 1|Block	Poisson	4	13582.01	-996.64	-6786.99	0.00
NERZ ~ S*T + 1|Tank + 1|Block	Poisson	6	13333.51	-1245.14	-6660.72	0.00
NERZ ~ S*T +B+ 1|Tank + 1|Block	Poisson	7	13333.65	-1245.00	-6659.78	0.00
NERZ ~ S*T + R + 1|Tank + 1|Block	Poisson	7	13283.59	-1295.06	-6634.75	0.02
NERZ ~ S*T + R + C + 1|Tank + 1|Block	**Poisson**	**8**	**13266.93**	**-1311.72**	**-6625.40**	**99.98**

Note: * indicates interactions (including main effects), bold indicates the most parsimonious model. Abbreviations: NERZ, Number of entries in the reward zone; B, Background risk (high/ low); R, Acute Reinforcement stimulus (alarm cue/distilled water); S, Side of the tank with the reward zone (left/right); T, Training duration (1-16); C, Colour of the reward zone (red/white); 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 3: Forward selection of the two components of a zero-inflated generalized linear mixed model relating the latency of entry to the reward zone to various predictors for the testing phase. Not all models tested are shown.

Model	Distribution	df	AICc	ΔAICc	Log-likelihood	Weight
LERZ.01 ~ 1 + 1|Tank + 1|Block	Bernoulli	3	378.7167		-186.32	2.47
LERZ.01 ~ B + 1|Tank + 1|Block	Bernoulli	4	380.2643	+1.5476	-186.06	1.14
LERZ.01 ~ S + 1|Tank + 1|Block	**Bernoulli**	**4**	**373.6584**	**-5.0583**	**-182.76**	**31.00**
LERZ.01 ~ S * B + 1|Tank + 1|Block	Bernoulli	6	374.3131	-4.4036	-181.01	22.34
LERZ.01 ~ S + R + 1|Tank + 1|Block	Bernoulli	5	375.0424	-3.6743	-182.41	15.52
LERZ.01 ~ S * C + 1|Tank + 1|Block	Bernoulli	6	377.3207	-1.3960	-182.51	4.97
LERZ.01 ~S + T +1|Tank+ 1|Block	Bernoulli	5	374.2932	-4.4235	-182.04	22.57
LERZ ~ 1 + 1|Tank + 1|Block	Gamma	4	606.1309		-298.95	0.47
LERZ ~ B + 1|Tank + 1|Block	Gamma	5	608.2432	+2.1123	-298.95	0.16
LERZ ~ R + 1|Tank + 1|Block	Gamma	5	608.1872	+2.0563	-298.92	0.17
LERZ ~ S+ 1|Tank + 1|Block	**Gamma**	**5**	**597.1171**	**-9.0138**	**-293.39**	**42.57**
LERZ ~ S * B + 1|Tank + 1|Block	Gamma	7	600.8831	-5.2478	-293.12	6.48
LERZ ~ S + T+ 1|Tank + 1|Block	Gamma	6	597.6400	-8.4909	-292.58	32.77
LERZ ~ S + C + 1|Tank + 1|Block	Gamma	6	598.9081	-7.2228	-293.22	17.38

Note: * indicates interactions (including main effects), bold indicates the most parsimonious model. Abbreviations: LERZ.01, Bernoulli distribution for entry into the reward zone (yes/no), LERZ, Latency to enter the reward zone (minutes); B, Background risk (high/low); R, Reinforcement stimulus (alarm cue/distilled water); C, Colour of the reward zone (red/white); S, Side of the tank with the reward zone (left/right); T, Trial number; 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 4: Forward selection of a generalized linear mixed model relating the number of entries in the reward zone to various predictors for the testing phase. Not all models tested are shown.

Model	Distribution	df	AICc	ΔAICc	Log-likelihood	Weight
NERZ ~ 1 + 1|Tank + 1|Block	Poisson	3	1578.935		-786.43	0.00
NERZ ~ B + 1|Tank + 1|Block	Poisson	4	1580.554	+1.629	-786.21	0.00
NERZ ~ R + 1|Tank + 1|Block	Poisson	4	1579.143	+0.208	-785.50	0.00
NERZ ~ S + 1|Tank + 1|Block	Poisson	4	1464.572	-114.363	-728.22	0.29
NERZ ~ S * B+ 1|Tank + 1|Block	Poisson	6	1468.284	-110.651	-727.99	0.05
NERZ ~ S + C+ 1|Tank + 1|Block	Poisson	5	1459.142	-119.793	724.46	4.45
NERZ ~ S+T + C + 1|Tank + 1|Block	**Poisson**	**6**	**1453.015**	**-125.92**	**720.36**	**95.21**

Note: * indicates interactions (including main effects), bold indicates the most parsimonious model. Abbreviations: NERZ, Number of entries into the reward zone; B, Background risk (high/low); R, Reinforcement stimulus (alarm cue/distilled water); C, Colour of the reward zone (red/white); S, Side of the tank with the reward zone (left/right); T, Trial number; 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 5: Post-hoc results from the final zero-inflated GLMM for the probability of entering the reward zone for the training phase

Final Model: LERZ.01 ~ Side*Training + Colour+ Reinforcement + 1|Tank + 1|Block

Predictor	**Chi Square**	**Df**	***P***
Training	43.34	1	<0.0001
Side	70.73	1	<0.0001
Colour	8.79	1	0.0030
Reinforcement	8.16	1	0.0043
Side*Training	7.26	1	0.0070

Note * indicates interactions (including main effects). Abbreviations: LERZ.01, Bernoulli distribution for entry into the reward zone (yes/no). 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 6: Post-hoc results from the final zero-inflated GLMM for the latency to enter the reward zone for the training phase

Final Model: LERZ~ Side *Training + 1|Tank + 1|Block

Predictor	Chi Square	Df	*P*
Side	52.33	1	<0.0001
Training	5.74	1	0.0166
Side*Training	9.12	1	0.0025

Note * indicates interactions (including main effects). Abbreviations: LERZ, latency to enter the reward zone (minutes). 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 7: Post-hoc results from the GLMM for the number of entries into the reward zone for the training phase

Final model: NERZ ~ Side*Training + Reinforcement +Colour + 1|Tank + 1|Block

Predictor	Chi Square	Df	*P*
Side	873.90	1	<0.0001
Training	152.87	1	<0.0001
Reinforcement	58.56	1	<0.0001
Colour	18.71	1	<0.0001
Side*Training	98.20	1	<0.0001

Note * indicates interactions (including main effects). Abbreviations: NERZ, number of entries in the reward zone. 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 8: Post-hoc results from the zero-inflated GLMM for the probability of entering the reward zone for the testing phase

Final model: LERZ.01 ~ Side + 1|Tank + 1|Block

Predictor	Chi Square	Df	*P*
Side	6.97	1	0.0083

Note abbreviations: LERZ.01, Bernoulli model for the latency to enter the reward zone (yes/no). 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 9: Post-hoc results from the zero-inflated GLMM for the latency to enter the reward zone for the testing phase

Final model: LERZ ~ Side + 1|Tank + 1|Block

Predictor	Chi Square	Df	*P*
Side	11.28	1	0.0008

Note abbreviations: LERZ, latency to enter the reward zone (minutes). 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.

Table 10: Post-hoc results from the GLMM for the number of entries in the reward zone for the testing phase

Final model: NERZ~ Side + Trial + Colour + 1|Tank + 1|Block

Predictor	**Chi Square**	**Df**	***P***
Side	115.81	1	<0.0001
Trial	8.25	1	0.0040
Colour	6.73	1	0.0095

Note abbreviations: NERZ, number of entries in the reward zone. 1|Tank and 1|Block are the random effects of tank and treatment block, respectively.