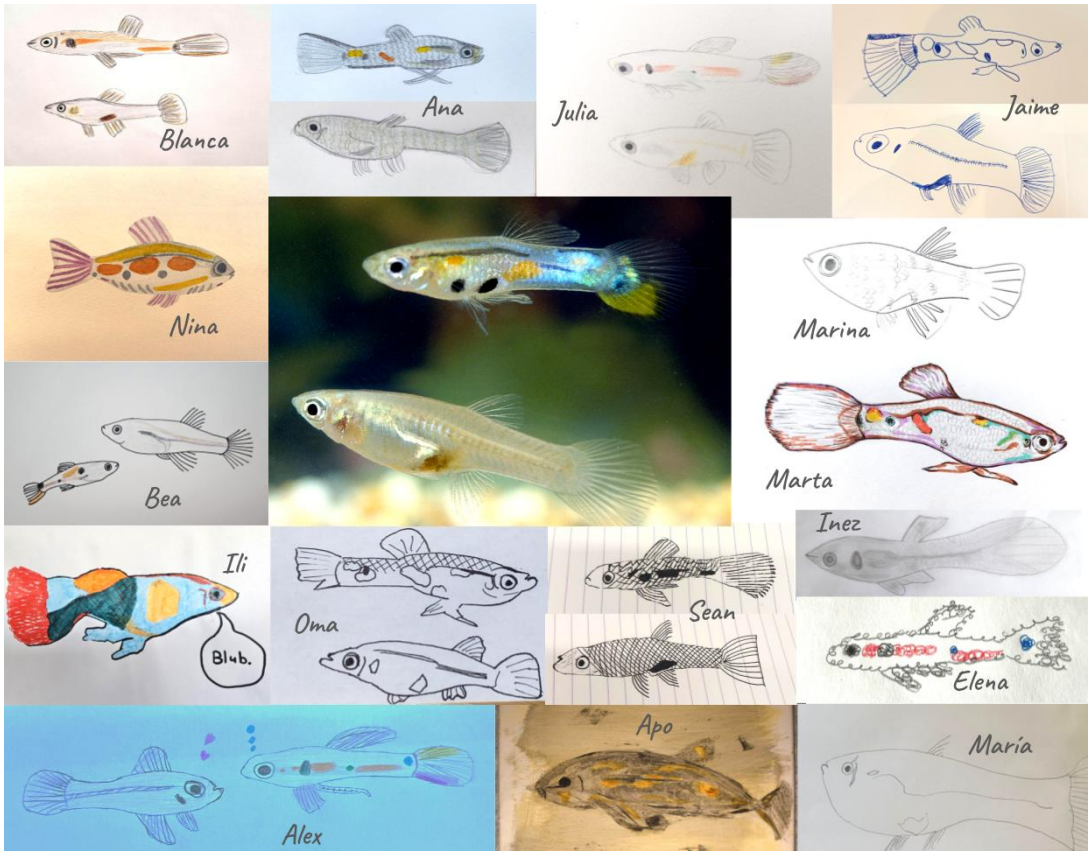


# A great escape: How do predation pressures influence the dispersal behaviour of guppies

Beatriz Ramos Miranda (1416847)
Master thesis: BHE80336
Behavioural Ecology (BHE)
Alexander Kotrschal (WUR)
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WAGENINGEN
UNIVERSITY & RESEARCH

**ABSTRACT**

Predation pressures can lead to new adaptations in prey, enhancing their antipredator traits and reducing risks through morphological and behavioural changes. In Trinidadian guppies (*Poecilia reticulata*) their colour, number of offspring or size changes depending on the presence or absence of a predator. However, it remains unclear how predation pressures can shape the dispersal behaviour of this fish. Dispersal behaviour is a fundamental characteristic that determines different processes and patterns of an organism, such as their geographical distribution or the structure of their population. But it also is highly costly for the individual since it needs to find a new habitat to settle and face the risks that come with it. Here, we compared the dispersal response of guppies under varying predation pressures. Four important factors for the ecology of this fish were researched: males and females, with and without a predator odor, single or in groups, fish selected for better antipredator traits or non-selected fish. For this I designed a semi natural environment with relatively small tanks connected with a water flow so that the fish would move in between. Males and females had different dispersal responses when they had a past selection for better antipredator traits where females. Nevertheless, both males and females increased their dispersal behaviour when they were single in a tank or when they were in presence of a predator. We conclude that how or when guppies disperse in response to a predator is a consequence of the evaluation of the benefits and disadvantages of this behaviour.

**KEY WORDS**

Antipredator traits, dispersal behaviour, guppy adaptation, predation pressures, selection lines.

## INTRODUCTION

Dispersal can be defined as the movement or propagation of an individual to a new habitat (Borges et al., 2021; Clobert et al., 2012). It plays a crucial role in the evolution and ecology of living organisms (Burgess et al., 2015). Dispersing can influence several aspects of an individual such as gene flow, local adaptation, population dynamics and species geographical distributions (Burgess et al., 2015; Clobert et al., 2012; Crosby et al., 2023; Gonzalez et al., 2009; Kokko & Lopez-Sepulcre, 2006; Ronce, 2007). It includes three stages: 1) the departure from a current habitat, 2) the transfer to a new habitat, 3) the settlement into that novel place (Borges et al., 2021; Clobert et al., 2009; Clobert et al., 2012; Saastamoinen et al., 2017). Whether or not, and how far, an individual moves from the initial place is, can be determined by selection pressures and it can bring important fitness consequences (Borges et al., 2021; Clobert et al., 2012; Ronce, 2007).

Predation is a major force, shaping the ecology of prey through lethal and non-lethal effects (Fraimout et al., 2022; Fraser & Gilliam, 1987; Hasenjager & Dugatkin, 2017; Lima & Bednekoff, 1999; Reznick´ & Endler, 1982). Predator-mediated selection favours individuals with traits that enable them to survive long enough to reproduce at least once (Alcock, 2005; Kjernsmo, 2014). Preys adapt to predation pressure by changing different traits, whether it's their morphology (Caro, 2005; De Waele et al., 2024b), such as exoskeletons, spines, and various types of protective coloration; or their behaviour (De Waele, 2024a, Magurran, 2005) such as avoiding dangerous areas, forming groups, or seeking refuge in safe places (Kjernsmo, 2014). Most animals encounter the threat of predators at some point in their lives. The danger creates a trade-off between finding food and mates and avoiding becoming prey themselves. Besides the impact on mortality, the fear of predators can also elicit strong responses that can affect the life histories of the animals (Hasenjager & Dugatkin, 2017; Magurran, 2005; Ripple & Beschta, 2004). As the threat can variate across space and time, their anti-predator defences can also be plastic, allowing them to minimize the cost while adapting their response (Brown et al., 2013; Hasenjager & Dugatkin, 2017). For example, larger and more cohesive groups are formed when the threat levels are increased. In the wild, animals with predations and without predation can evolve different characteristics. For instance, in areas with low predation, males have brighter colors. But other traits like body shape, nº of offspring or ability to escape can be selected. In nature, predators have a strong effect on species densities and can alter migration rates (Sih & Wooster, 1994).

In the presence or absence of a predator, the defence strategy of guppies changes. There is a higher personality dependent response in absence of a predator, but in presence of a predator the risk increases, and their response aims for minimizing the risk. However, there is also a predator-dependent response, where, for example, if the predator selects for shoals, those individuals that are alone might have a higher survival rate, making this strategy more appropriate. Overall, prey is expected to be found where there is less risk (Mattingly & Butler, 1994; Werner & Gilliam, 1984). For guppies, when there is high predation, streams offer protection. Streams have less resources but are a safe area in case of risk. In the upper stream there is no shoaling, because there is less predation and mainly *Rivulus hartii*, a pike cichlid that selects for shoals. In contrast, downstream there is a tendency of shoaling, because there is higher predation. Also, the population stays shallow to avoid predation (Mattingly & Butler, 1994). Effective dispersal can enhance individual fitness and population survival, but high risks are also faced in the dispersal process (Hogan et al., 2014).

In this study we investigated the effect of predation pressure in guppy traits, specifically in their dispersal behaviour. Therefore, I used Trinidadian guppies that were artificially selected for adult survival under predation pressure (De Waele et al., 2024b). These fish were placed in a tank with a

predator to select for better antipredator traits, while another group was not selected but in similar condition without a predator. In previous studies with this selected fish, the fish that were selected relied more on personal information than the control group (De Waele, 2014a). The individuals from the selected group seem to rely less on the shoal. Therefore, we expected the control group to disperse less. Usually, dispersers are believed to be consistently more bold, exploratory, asocial or aggressive than residents (Far, 1975).

Guppies have a highly structured social network (Croft et al., 2004) and like to explore in a cooperative way (Croft et al., 2006). I also studied the fish in a situation where they were single and a scenario where they were with other individuals. In this second scenario, only the main fish could leave because the rest of the shoal was behind a net. We expected the individuals that were alone to be more dispersals than the fish with the group. Since guppies in the wild tend to form shoals (Seghers, 1974; Croft et al., 2006), we expected the fish in the second scenario to rather stay with the shoal.

I tested the difference between males and females. Since males' main drive is mating (Croft, 2003a), I expected them to disperse more than females. Male guppies allocate a significant proportion of their time and energy on mating (Guevara-Fiore, 2010; Magurran & Seghers, 1994), but they also are known to be constantly looking for females (Croft et al., 2003a, Croft et al., 2003b; Griffiths & Magurran, 1998; Houde, 1997). In contrast, females are likely to school with familiar individuals and show site fidelity (Griffiths & Magurran, 1998; Guevara-Fiore, 2010), male guppies tend to switch between shoals which results in high rates of shoal encounters (Croft et al. 2003b; Guevara-Fiore, 2010).

Lastly, I included the presence of a predator odor into half of the experiments to see how the presence of a predator influences this behaviour. Earlier studies have shown that predation increases dispersal rates (Otsuki & Yano, 2014). Predators have a strong effect on the ecology of species and can alter their dispersal (Sih & Wooster, 1994). However, dispersing can have a higher cost than staying if the predators, for example, have a wide distribution. When comparing populations in presence or absence of a predator, guppies are more cohesive when there is a predator (Farr, 1975) and in absence of a predator they school less (Reznick & Endler, 1982). It also depends on the size of the predator and their strategy to hunt on prey. In this scenario, we expect the fish to swim less from one tank to another in presence of a predator.

The experiments included semi-natural conditions, with a water flow between tanks, to see if the fish wanted to leave an initial tank, how far and when. Both, forming shoals and avoiding predation are relevant behaviours to the ecology of guppies and they are displayed by both males and females, adapting to the different scenarios. Here I tested four factors that can help to understand how different scenarios can shape the response of these fish. The hypothesis is that there are differences between factors: 1) males and females, 2) single and group, 3) selected for better antipredator traits and control, 4) presence and absence of a predator odor. Cross interactions between these factors can also play a role, however these were not explored in great detail. With these four factors we could see the dispersal response of guppies under varying predation pressures.

## METHODS

### Study species:

Guppies (*Poecilia reticulata*) are one of the most widely distributed tropical fish and they are mainly found in shallow areas and streams (Magurran, 2005). To simulate their natural environment, we chose relatively small tanks connected with a water flow. The presence of predators drives guppies to rapidly

develop new adaptations in response to the pressures that they face (Magurran, 2005). Therefore, studying the response to different predation pressures is easy in *P. reticulata* due to their rapid evolution. In absence of a predator there is a mainly personality-dependent response, where dispersers are more bold, exploratory, asocial or aggressive than residents or locally born individuals. But in presence of a predator the personality-dependent dispersal is cancelled, and dispersers and residents show a similar response (Cote et al., 2013).

Our study fish were collected in 2005 from high-predation populations in the Quare River in 2005. The fish used in the current study belong to the 4th generation of those Trinidadian guppies, after a sex specific selection experiment. In this experiment, a group of males and a group of females were placed into a semi-natural tank with a predator on it and similar to a guppy's natural habitat. From these individuals some were placed behind a barrier where they could not be predated, while the remaining fish were placed with the pike cichlid, where they could be consumed by it. When around 80% were eaten males and females were separated and placed together so that they could produce the next generation. The same procedure was carried out when the offspring reached maturity. Three replicates of the experiment were made, with a predation-selected population and a control population in each replicate. This was done in the University of Wageningen for three generations (De Waele et al., 2024b).

For all the tests, I initially studied males and females together, but for all there were significant differences between them, so I decided to separate sexes. In the wild males and females differ in behaviour. Since guppy females' reproductive success is mainly limited by food, they are more active when foraging (Laland & Reader, 1999). This is in contrast with males, whose limitation is the access to females and therefore their main drive (Houde, 1997; Croft, 2003a). Thus, males invest more of their energy on mating (Guevara-Fiore, 2010; Magurran & Seghers 1994) and are known for being constantly looking for females (Croft et al., 2003a, Croft et al., 2003b; Griffiths & Magurran, 1998; Houde, 1997).

For the predator odor I used individuals of oscars (*Astronotus ocellatus*). The selection of the antipredator traits was made with a major guppy predator, a pike cichlid (*Crenicicla alta*) (De Waele et al., 2024b). In this study I did not manage to get this cichlid, but I used oscars. These fish are not natural predators of guppies but have been used for previous studies, where guppies also responded to their presence (Poulin et al., 1987). These oscars were placed in another tank, where its water flow was connected to half of the guppy tanks. This way the predator odor was present on the water systems.

**Experimental set-ups and methodology**

To study dispersal in a seminatural set-up I created a water flow with relatively small, connected tanks. With these set-ups I aimed to see the response of the guppies in an environment as close as possible to a real-life scenario. I placed the fish in an initial compartment or tank so that the guppies, to go from one tank to the next one, had to swim against the water flow. If they did not leave the first tank, I considered that there was no dispersal.

In the first tank where the fish started, there could be either one fish or with a group of fish separated by a net. This way there was one individual that could swim but the rest of the fish (2 males and 2 females), were behind the net and could not follow this first individual (Fig. 1). On some occasions they would jump over the net, or the males would try to push the net until they could fit through it. In this scenario I would put the fish back behind the net so that again only one fish could move. The fish were covered with a blanket so as not to be disturbed by the rest of the people in the laboratory.

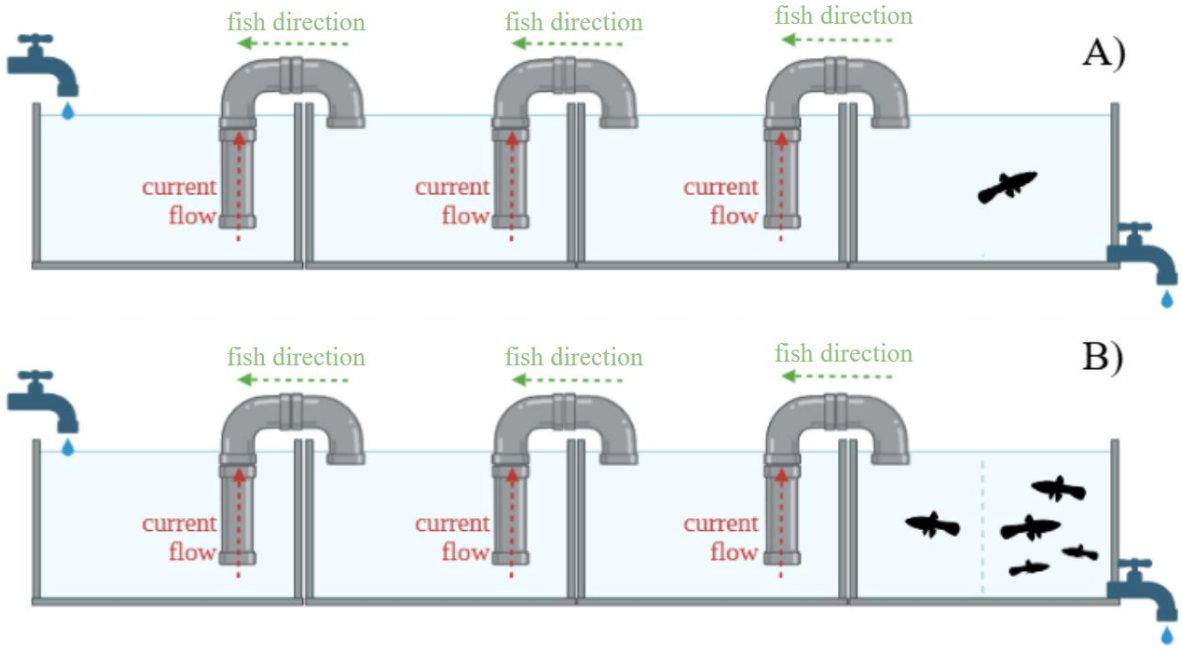


**Figure 1. Scheme of the set ups.** On top, the representation of the fish when they were single (A). On the bottom, the fish that were in groups separated from the rest with a net (dashed line) (B).

I carried out the experiments in Wageningen University & Research, in the laboratory of the building Carus. I connected four tanks with tubes that had a water stream in between them. The water flow of the tubes was 54.75 ± 18.04 L/h. The tubes where the fish swam through were 25 mm diameter and 30 cm long. The entering tube was shallower in the initial than the exit site so that returning to the first tank was more complicated than moving against the current. The tanks were 23 x 15 cm, and I made a lid for the 4 connected tanks with holes for the tubes. To maintain it close I would close it with tape, and they had a plastic limit in the corners so that the lid could not open. The tank position where the fish were over the time was recorded.

To remove the water from the tubes that connected the tanks I placed a needle with a small tube in the needle so that I could swallow the air from the tubes until there was only water. Then the tube ending was under the water so that there was no water coming in. These smaller tubes were 25 cm long the middle one and 35 cm long the lateral ones. Sometimes the needle would move, and the air would come in creating a bubble or avoiding the fish to move inside that tube (Fig. 2). In that case, I would cover with nail polish so that the hole was covered and no more air would come in, avoiding bubbles that disturb the water flow and consequently fish dispersal.


**Figure 2. Dispersal set-ups.** Four tanks were placed in the laboratory connected with tubes (A). In the group of fish, the individual that was our target was separated with a net (B), so that the other fish could not follow it. To remove the air I used a needle, and when it got an air bubble, I covered it with nail polish (C). Pictures taken in the lab of Carus, in Wageningen University & Research.

I did a try-out week, placing the fish on tanks and seeing their response to determine what timings would be best to carry out the experiments. I decided to observe the fish every two hours so as not to disturb them often. When they were observed more frequently than that, the fish would disperse less because they would be vigilant to the human presence. When the individuals were left only one day they would take some time before they were adapted and decided to move. Therefore, I stabilised to leave the fish one night to adapt to the new environment. Then I would, on the first day, collect the fish and place them in the corresponding tank. Afterwards, I would connect the tanks so that the water of all of them was running at 16. The following morning, I would check how far they had gotten every two hours (8:00, 10:00, 12:00, 14:00, 16:00) and fix if a fish moved or a tube got a bubble. Lastly, I would leave them another night and the following morning I would remove the fish at 8:00.

I used 16 set-ups with 4 different treatments (Fig. 3): if the fish were selected for antipredator behaviour (predation vs. control), if there was a predator odor (present vs. absent), if the fish was grouped or not (single vs. group) and the sex of the fish (male vs. female). Each selection line had 3 replicates, so I tested each replicate 7 times each (n=336).

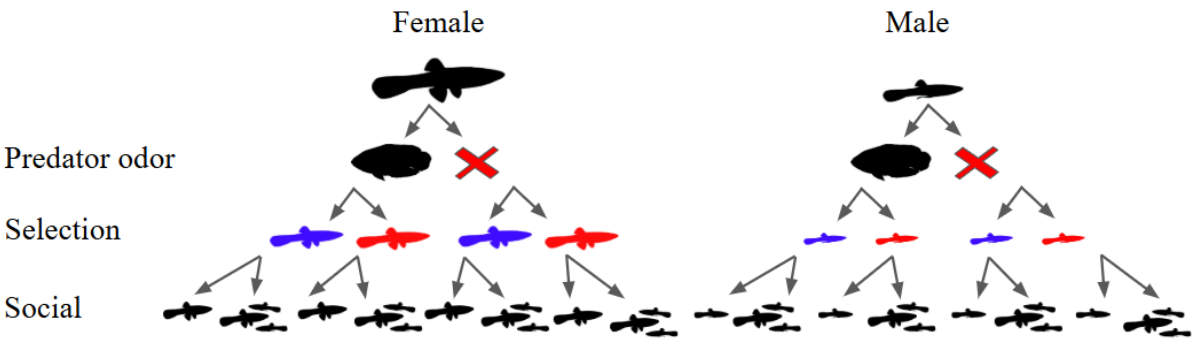


**Figure 3. Diagram of the study groups.** I studied both males (on the right), and females (on the left). Both had situations with a predator odor and without. In this case the predators were oscars (*Astronotus ocellatus*). Additionally, I tested both groups from the selection lines. The control group (in blue), and the predation (in red). The predation group was selected for the best antipredator traits, while the control

was not. Finally, also tested the individuals in scenarios where they were alone, but also in groups where only the main fish could leave the first tank because the rest was separated with a net.

I tested whether there were differences in the number of individuals that moved. However, this was a binomial response and did not show the variation in time. For that reason, I also compared the tank position to the fish over time. But some individuals managed to go back so I calculated the total times that they moved from a tank and compared the different groups. This way I could see whether or not they disperse, when and how much.

**Statistical analysis**

All analyses were performed in R (version 4.4.1). Since females moved more than males and their behaviour was different, I decided to separate them and test them individually. I used a GLM (Generalized Linear Model) to compare the dispersal response. For all test, the fixed factors in this case were the presence of the predator odor (Predator: present, absent), if the fish were alone in the tanks or with the group separated by the net (Social: single, group), if they were selected for the antipredator traits (Selection: predation, control) and lastly, I included the number of the replicate from which they belonged (Replicate: 1, 2, 3).

For testing the difference in groups on their dispersal behaviour I categorised them with if they moved from the initial tank or not. I carried out a binomial generalized linear model with Moved (yes, no) as a response variable. Furthermore, I wrote in which tank the fish were over time, from zero if they remained in the first tank, to three if they reached the last one. I performed a Poisson generalized linear model with measurement (0, 1, 2, 3) as a response variable. This variable indicates the tank position where the fish were at that time. Here I also have time as a fix factor with the six-time measurements I had. Lastly, some fish managed to return after moving forward, so I calculated the total distance that they moved by summing the total number of movements (e.g. from the first tank to the second and then back would be two movements). I test the difference of the total distance with a Poisson generalized linear model. In this case distance was the response variable, with values from zero to eight times that a fish moved from a tank to another.

## RESULTS

In our experiment, I sampled 336 fish, but some fish escaped or moved through the net during the whole experiment. Consequently, those individuals that escaped the tank or that were pushing the net and going to the other side through the whole experiment were discarded. I had 300 valid data records, and instead of having 168 samples per group we had fewer. In the control group I had 144 valid samples, and in the predation I had 156. For the females I end up having 144 samples and for males 156. I also had 143 samples of the fish that were in groups and 157 of those who were alone. Additionally, I had 146 samples of the individuals with the predator odor present and 154 with the odor absent. From all the individuals whose response was considered valid, 140 individuals moved from their initial tank and 63 managed to return at least one tank back.

**Table 1. Statistical analysis outcome.** GLM results from R studio for the test 1: if the fish moved from the initial tank or not; test 2: the tank position where the fish were over time; test 3: the total number of times that a fish moved from one tank to another one. Both males and females include the significant interactions (n = 300).

| FEMALE | | | | | MALES | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Test 1: Moved (binomial GLM)** | | | | | | | | | |
| **Coefficient** | **Estimate** | **Std. Error** | **z value** | **Pr(>\|z\|)** | **Coefficient** | **Estimate** | **Std.Error** | **z value** | **Pr(>\|z\|)** |
| **(Intercept)** | 0.146 | 0.549 | 0.265 | 0.791 | **(Intercept)** | -1.498 | 0.655 | -2.287 | 0.022* |
| **Predator odor present** | 0.578 | 0.345 | 1.675 | 0.094 . | **Predator odor present** | 0.068 | 0.502 | 1.93 | 0.054 . |
| **Selection Predation** | -0.298 | 0.344 | -0.867 | 0.386 | **Selection Predation** | 1.625 | 0.625 | 2.6 | 0.009** |
| **Social single** | 0.575 | 0.344 | 1.672 | 0.095 . | **Social single** | 1.024 | 0.506 | 2.025 | 0.043* |
| **Replicate** | -0.193 | 0.208 | -0.926 | 0.355 | **Replicate** | 0.016 | 0.207 | 0.075 | 0.94 |
| | | | | | **Predatorpresent: SelectionPredation** | -1.663 | 0.685 | -2.429 | 0.015* |
| | | | | | **SelectionPredation: Socialsingle** | -1.519 | 0.687 | -2.21 | 0.027* |
| **Test 2: Tank position over time (Poisson GLM)** | | | | | | | | | |
| **Coefficient** | **Estimate** | **Std. Error** | **z value** | **Pr(>\|z\|)** | **Coefficient** | **Estimate** | **Std. Error** | **z value** | **Pr(>\|z\|)** |
| **(Intercept)** | -1.703 | 0.179 | -9.512 | < 0.001 *** | **(Intercept)** | -3.11 | 0.25 | -12.432 | <0.001 *** |
| **time** | 0.048 | 0.004 | 10.813 | < 0.001 *** | **time** | 0.046 | 0.007 | 6.469 | <0.001 *** |
| **Predator odor present** | 0.662 | 0.177 | 3.736 | < 0.001 *** | **Predator odor present** | 0.596 | 0.176 | 3.391 | <0.001 *** |
| **Selection Predation** | -0.921 | 0.207 | -4.449 | < 0.001 *** | **Selection Predation** | 1.154 | 0.206 | 5.589 | <0.001 *** |
| **Social single** | 0.506 | 0.173 | 2.931 | 0.003 ** | **Social single** | -0.326 | 0.277 | -1.179 | 0.239 |
| **Replicate 2** | -0.069 | 0.116 | -0.592 | 0.554 | **Replicate 2** | 0.816 | 0.16 | 5.1 | <0.001 *** |
| **Replicate 3** | -0.157 | 0.113 | -1.391 | 0.164 | **Replicate 3** | 0.741 | 0.159 | 4.674 | <0.001 *** |
| **Predatorpresent: SelectionPredation** | 0.603 | 0.197981 | 3.045 | 0.002 ** | **Predatorpresent: SelectionPredation** | -0.833 | 0.232 | -3.586 | <0.001 *** |
| **Predatorpresent: Socialsingle** | -1.089 | 0.206 | -5.288 | < 0.001 *** | **time: Socialsingle** | 0.032 | 0.011 | 2.977 | <0.001 *** |
| **SelectionPredation: Socialsingle** | 0.826 | 0.203 | 4.066 | < 0.001 *** | **SelectionPredation: Socialsingle** | -1.2 | 0.238 | -5.032 | <0.001 *** |
| **Test 3: Total distance swam (Poisson GLM)** | | | | | | | | | |
| **Coefficient** | **Estimate** | **Std.Error** | **zvalue** | **Pr(>\|z\|)** | **Coefficient** | **Estimate** | **Std.Error** | **zvalue** | **Pr(>\|z\|)** |
| **(Intercept)** | 0.199 | 0.216 | 0.922 | 0.357 | **(Intercept)** | -1.12 | 0.299 | -3.743 | <0.001*** |
| **Predator odor present** | 0.729 | 0.22 | 3.316 | 0.001*** | **Predator odor present** | 0.367 | 0.235 | 1.565 | 0.118 |
| **Selection Predation** | -0.505 | 0.218 | -2.32 | 0.020* | **Selection Predation** | 0.824 | 0.298 | 2.768 | 0.006** |
| **Social single** | 0.596 | 0.24 | 2.478 | 0.013* | **Social single** | 0.54 | 0.241 | 2.239 | 0.025* |
| **Replicate 2** | -0.133 | 0.162 | -0.819 | 0.413 | **Replicate 2** | 0.841 | 0.232 | 3.631 | <0.001*** |
| **Replicate 3** | -0.094 | 0.155 | -0.608 | 0.543 | **Replicate 3** | 0.733 | 0.232 | 3.167 | 0.002** |
| **Predatorpresent: Socialsingle** | -0.917 | 0.274 | -3.347 | 0.001*** | **Predatorpresent: SelectionPredation** | -0.645 | 0.332 | -1.944 | 0.052 . |
| **SelectionPredation: Socialsingle** | 0.592 | 0.272 | 2.175 | 0.030* | **SelectionPredation: Socialsingle** | -1.0844 | 0.34 | -3.186 | 0.001** |

Here I tested if there were differences in the dispersal behaviour of females by comparing the number of fish that left the initial tank. I found that the presence of a predator odor suggests evidence of having a positive effect on the fish dispersal. I also compared the individuals with better antipredator traits, and it had similar responses to the control group that was not selected to have those traits. Additionally, I found suggested evidence of the effect of the sociality of the fish. For instance, single and group fish seem to have different responses. Lastly, the replicate number also did not show a difference between them (see Table 1, test 1 females).

Afterwards I compared the dispersal of the females over time, and I found some differences between treatments. I saw that females increased their tank position over time. Also, the presence of a predator odor increased the response compared to when the odor was absent. In contrast to males, females that were selected for better antipredator traits showed a decrease in the response, compared to the control group. On the other hand, single fish swam more compared to those that were grouped together (Fig. 4). But like in the previous test and in contrast to males, females did not show differences between replicates. Lastly, I also found significant interactions, where the presence of a predator odor combined with the predation group had larger tank positions and also the combination of the being from the predation and having better antipredator traits with the fish being single. However, the presence of a predator when the fish were single showed smaller numbers (see Table 1, test 2 females).

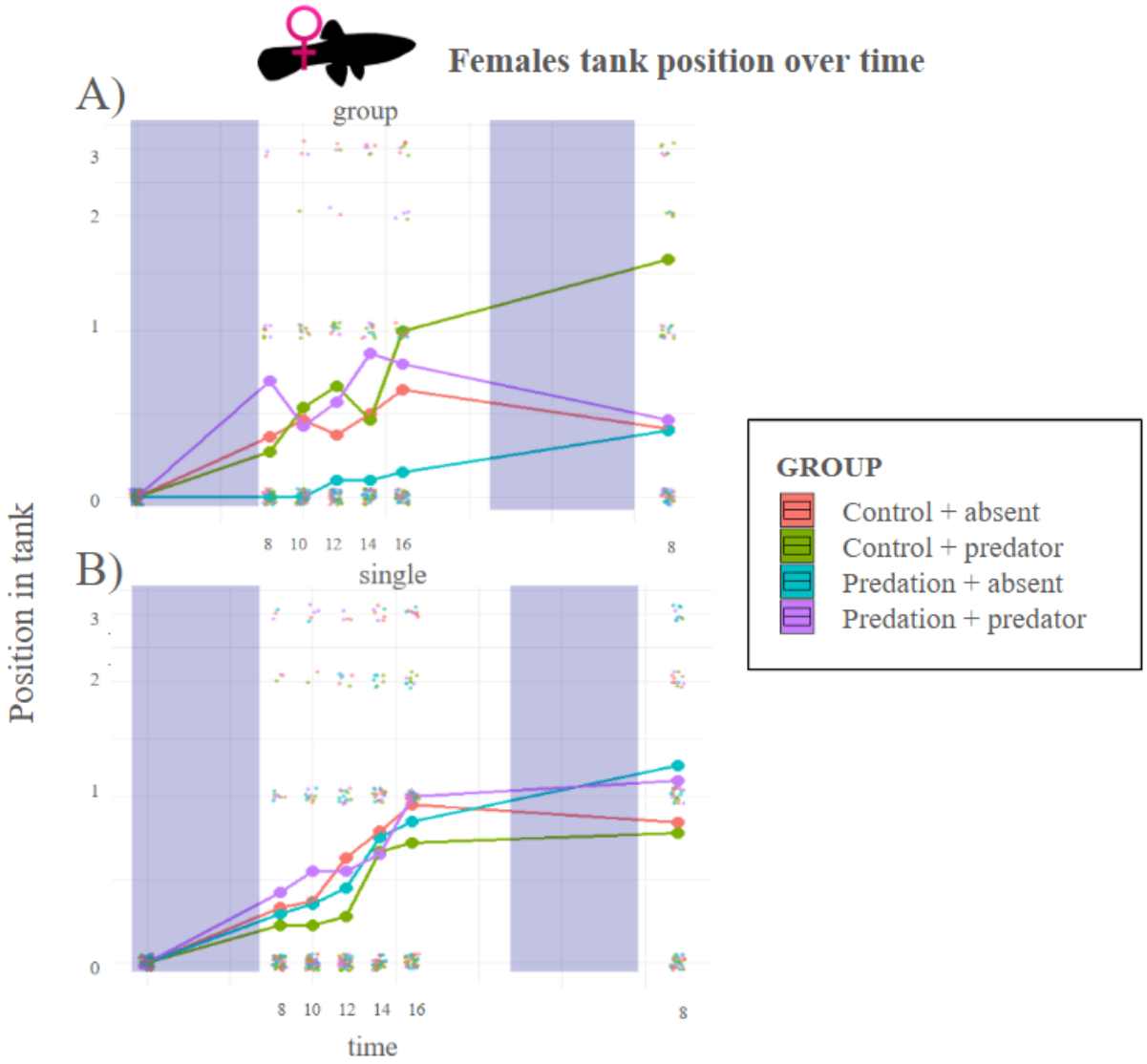


**Figure 4. Females position over time.** This plot shows the position of the females in the tanks over time. Since they showed different behaviour alone than in groups, I plotted them separately. On top (A), the fish in group are plotted and, on the bottom (B), the single fish. Since most values are below one, the y-axis is costumed so that the lower in the axis the bigger the axis is. For these values greater or equal to one are log-transformed and shifted. The darker frame means nighttime.

Lastly, I wanted to compare how far female guppies swam, so I calculated the total times they moved from one time to another. If they swam and returned, I counted that as two. Females' response in presence of a predator was swimming larger distances than without the odor. Additionally, when the fish were single the total distance was larger than the group fish (Fig. 5). As shown in the previous test, females from the predation group had smaller distances than the control and there were no differences between replicates. Finally, I saw the same significant interactions than when I compared the tank position over time. I saw there was less distance travelled when the predator was present, but the fish were single. I also found an interaction between the selection group and the sociality. In this case, the single fish swam more in the predation group (see Table 1, test 3 females).

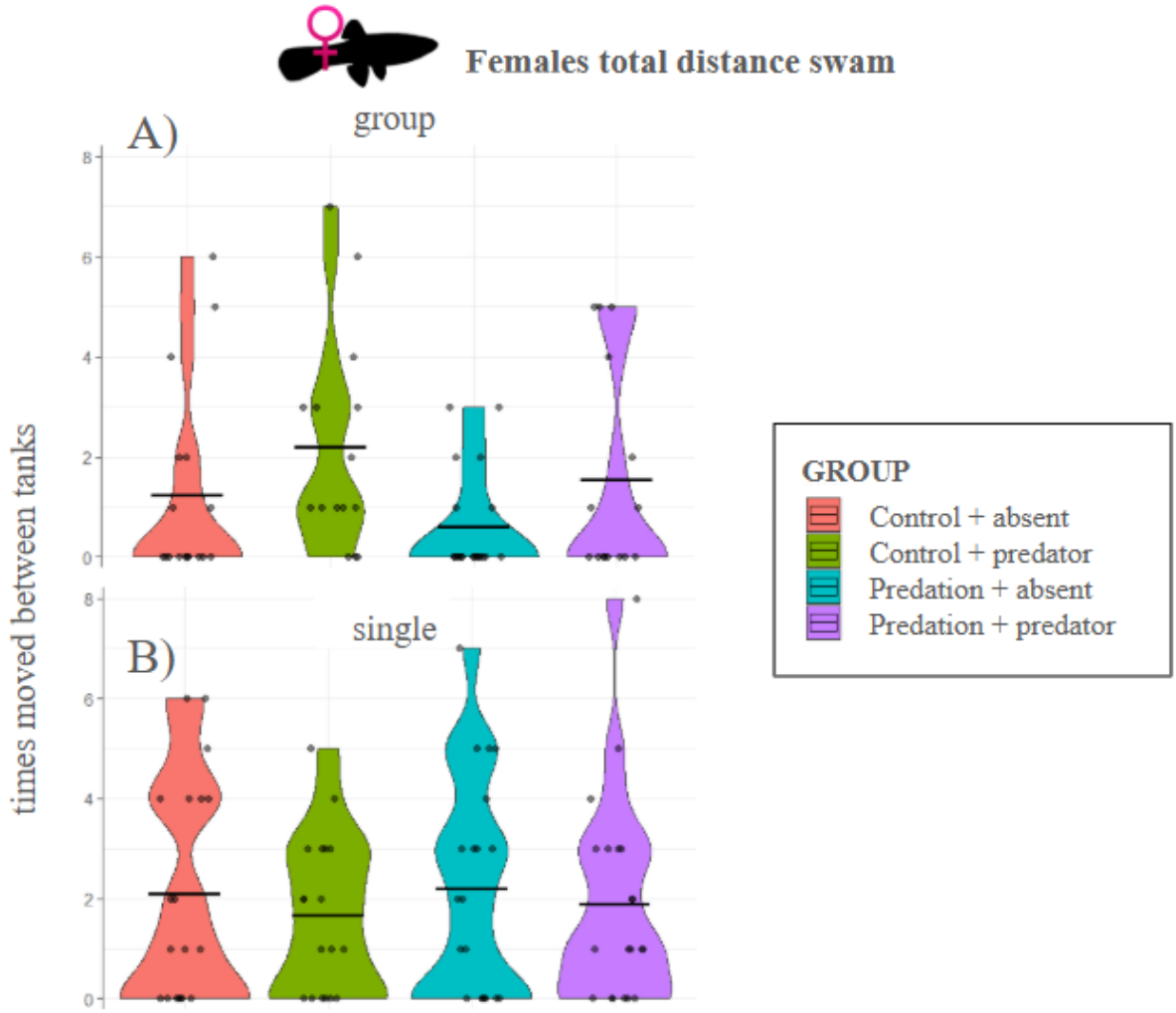


**Figure 5. Total distance swam by females.** After calculating the sum of the times that they moved from one tank to another one, I plotted the results for females. Since they showed different behaviour alone than in groups, I plotted them separately. The top results (A) are the ones of the grouped females, and the bottom ones (B) are the single fish.

For the males I did the same tests. When comparing if they left their initial tank, I found suggested evidence of the effect of the presence of a predator versus the absence. Furthermore, when I compared the predation group to the control group, I noticed that more fish moved in the predation group. Additionally, when the fish were single there were more individuals that left their initial compartment than when they were in groups. For the different replicates I found no differences. However, there are also significant interactions. In the presence of a predator the predation group left the tank less. Also, the predation group when the fish were single showed less dispersal (see Table 1, test 1 males).

When testing in males the differences of the tank position over time I found that time, the presence of a predator odor and having antipredator traits showed larger numbers. On the other hand, I found similar tank positions when comparing the single with the grouped individuals. In contrast to females, I did find significant differences between the replicates. Lastly, there were smaller numbers when combining the effect of the predator present with the predation group and with the predation population when the fish were single. But there was an increase of the dispersal behaviour in the single fish over time (see Table 1, test 2 males, Fig. 6).

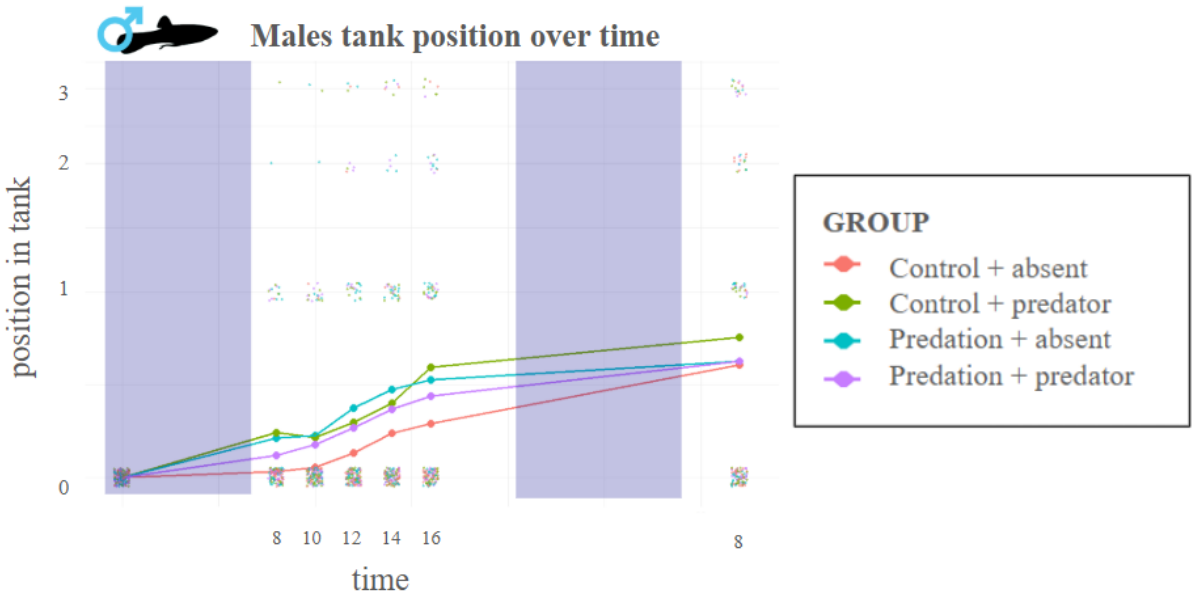


**Figure 6. Males position over time.** The figure shows the tank position where every male was over time. Single fish and group fish showed similar numbers, so I plotted them together. Since most values are below one, the y-axis is costumed so that the lower in the axis the bigger the axis is. For these values greater or equal to one are log-transformed and shifted. The darker frame means nighttime.

When comparing the total times that males moved, I found out that they did not differ on the total distance swam when the predator was present. However, the predation group showed larger distances compared to the control group. Single fish also had larger distances compared to the ones that were in groups (Fig. 7), and different replicates showed different distances. Lastly, as I saw in the two other tests, the response was swimming smaller distances when the predation control was alone in a tank.

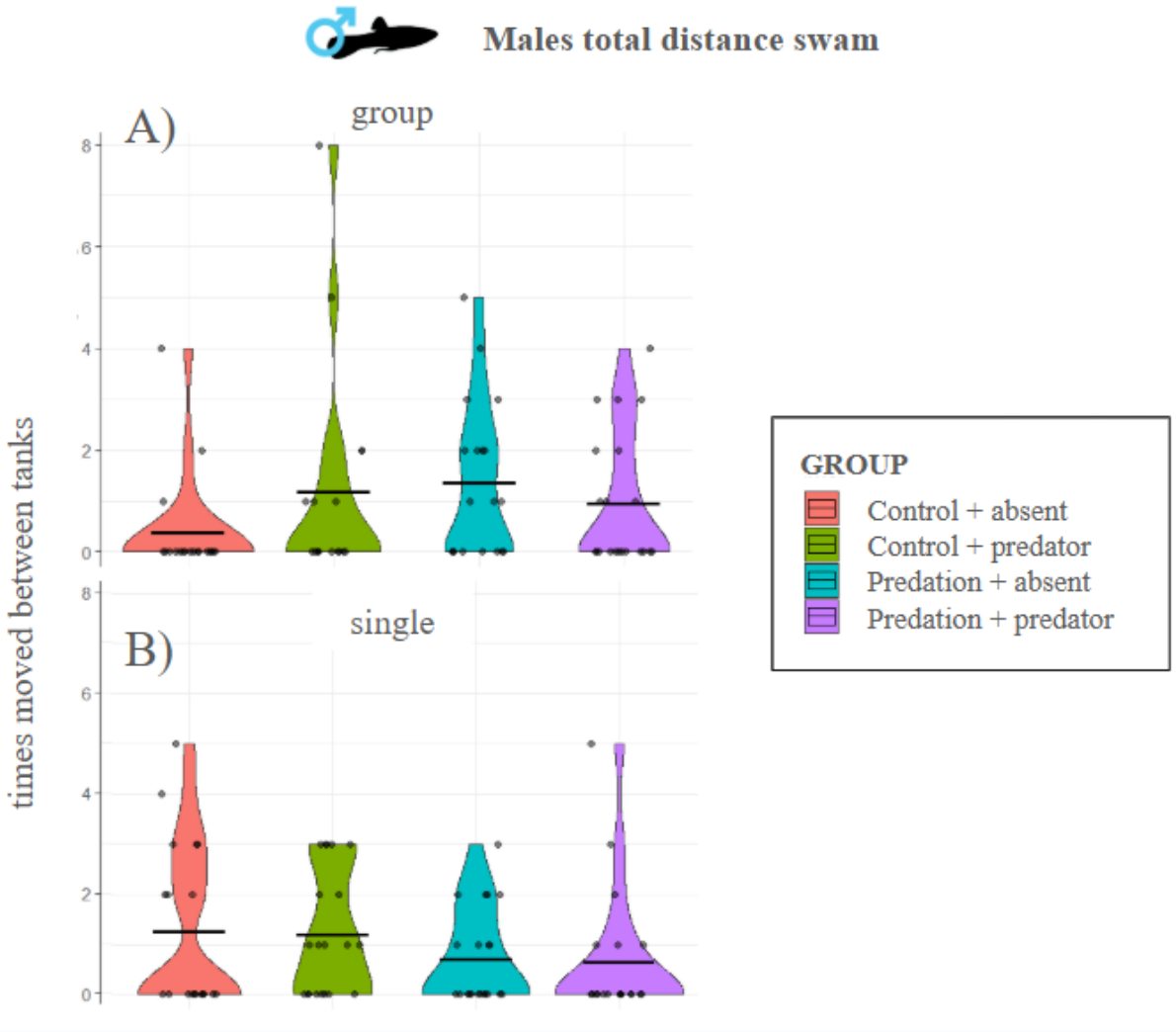


**Figure 7. Total distance swam by males.** For the total times that the males moved from one tank to another, I found that they showed different behaviour alone than in groups, so I plotted them separately. The top results (A) show the males in groups and the bottom ones (B) the fish when they were studied single.

**Table 2. Main results of dispersal behaviour.** The table shows GLM results for the main fixed factors of both females and males. The comparison between the individuals that left their initial tank is shown in the first test, the tank position over time in the second one and the total times that the fish moved in the third. "NS" means that there were no significant differences, "+" with green background that there was an increase and "-" with red background that there was a decrease. For the replicates, I indicate with "S" if there were significant differences because it contained three different levels. In some cases, the p-value was between 0.05 and 0.1 and those situations are indicated with a "NS*".

| Test | Moved | Time | Total distance |
|---|---|---|---|
| **Main fixed effects** | | ***Female*** | |
| Predator odor | NS* | + | + |
| Predation | NS | - | - |
| Single | NS* | + | + |
| Replicate | NS | NS | NS |
| | | ***Male*** | |
| Predator odor | NS* | + | NS |
| Predation | + | + | + |
| Single | + | NS | + |
| Replicate | NS | S | S |

**DISCUSSION**

Here I wanted to study how the predation pressures influence the dispersal behaviour of guppies. While males showed larger numbers on their dispersal behaviour when the individuals where selected for better antipredator traits that non selected, females that were not selected showed greater numbers that females that were selected. However, both males and females showed higher dispersal numbers in presence of a predator odor than in absence, and when the fish were single compared to the scenarios where the fish were in groups (see table 2).

Dispersal behaviour is an important characteristic of an organism, as it determines most of their patterns and processes (Walters, 2000). The realization of this behaviour is a result of evaluating the fitness cost and benefits of moving from the actual habitat patch to a new one (Bonte et al., 2012; De Bona, 2019; Clobert et al., 2009; Matthysen, 2012). This behaviour can have several benefits, such as escaping from poor conditions, increasing the chance of mating or the access to resources, it can reduce competition or increase the variance in fitness from the distribution of the offspring over several conditions (Matthysen, 2012; Prather, 2022). Nonetheless, there are also costs in dispersing (Hogan et al., 2014), such as increasing the risk or the energy that is used in moving (Comte & Olden, 2018). Understanding what drives guppies to disperse enables researchers to predict the spread of the population based on the ecological conditions (De Bona, 2019).

The four factors that were studied did seem to differ. However, not all the results were aligned to our expectations. We did not consider the combination effect of the factors in our predictions. For instance, in the case of males, both predation and the presence of a predator increased their dispersal. Nonetheless, the interaction of these two, so the presence of a predator when the male was from the predation group, there was a decrease of the dispersal. This could be due to a stronger response to the presence of a predator and better antipredator skills of the predation fishes. Besides, for both males and females, there was a decrease when the fish were from the predation and single. This could be explained by the fact that the predation relies more on their personal information (De Waele, 2024a) (see table 1).

Contradicting our expectations that males disperse more than females (Croft et al., 2003a), I saw that females showed larger numbers. However, both sexes showed different dispersal responses, thus I decided to test them individually. It has been studied that males rely more on females' response regarding predation risk rather than their own personal information (Brusseau et al., 2025). Additionally, while males respond mainly to the presence of females, females respond to the access to

other resources such as food (De Waele et al., 2024b; Kotrschal et al., 2015). Lastly, our fish had different scenarios where they were single or alone and with or without a predator odor, and this combined with the previous explanations could explain why our expectations differ with our results.

Additionally, also not aligned with the expected results, males and females showed larger numbers in presence of a predator odor compared to the absence. Different ecological contexts, where the predator uses different strategies, can lead to several responses in the prey (Cote et al., 2013). The pros and cons related to dispersal are often different between strategies, as well as stages of dispersal (departure, transfer or settlement). For instance, at the departure stage, dispersal is generally predicted to increase with higher predation risks (Prather, 2022; Weisser, 2001). Besides, streams offer protection to guppies when there is high predation (Mattingly & Butler, 1994), so moving against the water flow can be a sign of them looking for refuge. An important factor that conditions the antipredator response is the predator from whom they have to escape (Botham et al., 2006). If the predator selects for shoals, those individuals that are alone might have a higher survival rate. This is the case of the predator *Rivulus hartii* (Farr, 1975). But in this study, I used oscars, which are cichlid fish that establish dominance hierarchy and defend their territory (Beeching, 1997; Gonçalves-de-Freitas & Mariguela, 2006).

In agreement with our predictions, I saw that guppies that were alone in a tank had larger dispersal behaviours than grouped guppies. Guppies in the wild tend to form shoals (Croft et al., 2005; Seghers, 1974) because they are stable associations between individuals with different social networks. Guppies have a highly structured social network (Croft et al., 2004) and like to explore in a cooperative way (Croft et al., 2003a). When comparing if they would disperse alone rather than staying with the shoal, I assumed that they would rather stay with the shoal.

Lastly, when comparing the selection lines, as guppies in the selected group relied more on personal information than the control group (De Waele, 2024a), I expected the control group would be less dispersal. This response was seen in males, but females from the control group showed greater results than the ones selected for better antipredator traits. Female guppies invest heavily in offspring and are the liming sex. In comparison, males invest relatively little, and they compete for mating. Guppy females are more active and original when foraging (Laland & Reader, 1999), probably as a consequence of their reproductive success being mainly limited by food, whereas in males, the access to females is the limitation (Houde, 1997). Thus, there is strong selection for males to maximize their reproductive success by moving along with the females (Croft et al., 2003a; Greenwood, 1980). When females were selected for better antipredator behaviour, there was also a switch in their energy investment, such as greater offspring number in the first brood (De Waele et al., 2024b). This switch in investments could also have decreased the female movement. On the other side, male selection could have been shifted to a higher necessity from mating to increase their fitness.

Although we did not use the same predator than the one used for the selection lines previous to our experiment, we used another cichlid. Our predator has a similar ecology to blue acara (*Aequidens pulcher*), which is a natural predator of the guppy in Trinidad (Godin & Davis, 1995; Liley & Seghers, 1975). Using a different predator than the one that was used for making the selection lines ensures that the response is not only to one type of predator but to the presence of a predator.

Nonetheless, it is difficult to simulate “natural” experiments. Although previous studies have explored the effects that predators have on prey, factors such as predator species (Botham et al., 2006) or habitat size (Millar, 2006) are difficult to simulate and can limit the interpretation of the findings. Furthermore, the effect on prey differs across the animal life stages and within different predators (Botham et al.,

2006; Cote et al., 2013; Folkestad, 2005). Thus, our scenario can be limiting when researching in laboratory set ups effect of the presence or absence of a predator on different experimental populations.

Guppies allocate their energy on dispersal, mating or foraging, based on evaluating the advantages and disadvantages of each behavioural strategy. This decision of how or when to disperse is influenced by a combination of factors (Cote et al., 2013). Whether the animal is alone, whether there is a predator and what predator are different drivers of this response. My results support that depending on the scenario a better antipredator response is chosen. Prey are able to use predator fish odors as an indicator of increased predation risk (Brown et al., 2000; Jachner, 2001; Kats & Dill, 1998; Magurran, 1990; Ylönen et al., 2007). Even though prey can react quicker to chemical cues than visual cues (Brown et al., 1997), how other predation signals make fish express other dispersal responses will need to be clarified in future experiments.

**AI statement**

This thesis was not generated with AI. The use of AI was exclusively for correcting RStudio codes.

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## APPENDIX I

Additional tables with the total number of responses. Table 1 shows count of the total fishes that moved at least once from the initial tank. Table 2 has also the number of individuals that moved from the initial compartments but also managed to go back in any of the tank positions.

**Table 1. Summary of the fishes that moved organised by groups.**

| Sum of moved | Column Labels | | |
|---|---|---|---|
| **Row Labels** | **Female** | **Male** | **Grand Total** |
| **Control** | **40** | **30** | **70** |
| **absent** | **18** | **11** | **29** |
| group | **7** | **3** | **10** |
| single | **11** | **8** | **19** |
| **present** | **22** | **19** | **41** |
| group | **11** | **7** | **18** |
| single | **11** | **12** | **23** |
| **Predation** | **38** | **32** | **70** |
| **absent** | **18** | **19** | **37** |
| group | **6** | **10** | **16** |
| single | **12** | **9** | **21** |
| **present** | **20** | **13** | **33** |
| group | **7** | **8** | **15** |
| single | **13** | **5** | **18** |
| **Grand Total** | **78** | **62** | **140** |

**Table 2. Summary of individuals that swam and returned separate by groups.**

| | **Female** | | **Male** | | **Total Sum of moved** | **Total Sum of return** |
|---|---|---|---|---|---|---|
| **Row Labels** | **Sum of moved** | **Sum of return** | **Sum of moved** | **Sum of return** | | |
| **Control** | **40** | **19** | **30** | **12** | **70** | **31** |
| **absent** | **18** | **12** | **11** | **5** | **29** | **17** |
| group | 7 | 5 | 3 | 1 | 10 | 6 |
| single | 11 | 7 | 8 | 4 | 19 | 11 |
| **present** | **22** | **7** | **19** | **7** | **41** | **14** |
| group | 11 | 2 | 7 | 3 | 18 | 5 |
| single | 11 | 5 | 12 | 4 | 23 | 9 |
| **Predation** | **38** | **20** | **32** | **12** | **70** | **32** |
| **absent** | **18** | **10** | **19** | **8** | **37** | **18** |
| group | 6 | 3 | 10 | 5 | 16 | 8 |
| single | 12 | 7 | 9 | 3 | 21 | 10 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **present** | **20** | **10** | **13** | **4** | **33** | **14** |
| group | 7 | 5 | 8 | 2 | 15 | 7 |
| single | 13 | 5 | 5 | 2 | 18 | 7 |
| **Grand Total** | **78** | **39** | **62** | **24** | **140** | **63** |

## APPENDIX II

I did additional tests in R studio to add on into our results. Figure 1 and table 1 shows the results of the individuals that moved. I filtered the individuals that moved and only kept those and tested the differences over time. I did the same for males, see figure 2 and table 2. Additionally, we recorded what was the furthest tank that the fish reached, from 0 until 3. We tested the differences in both females (table 3, figure 3) and males (table 4, figure 4).

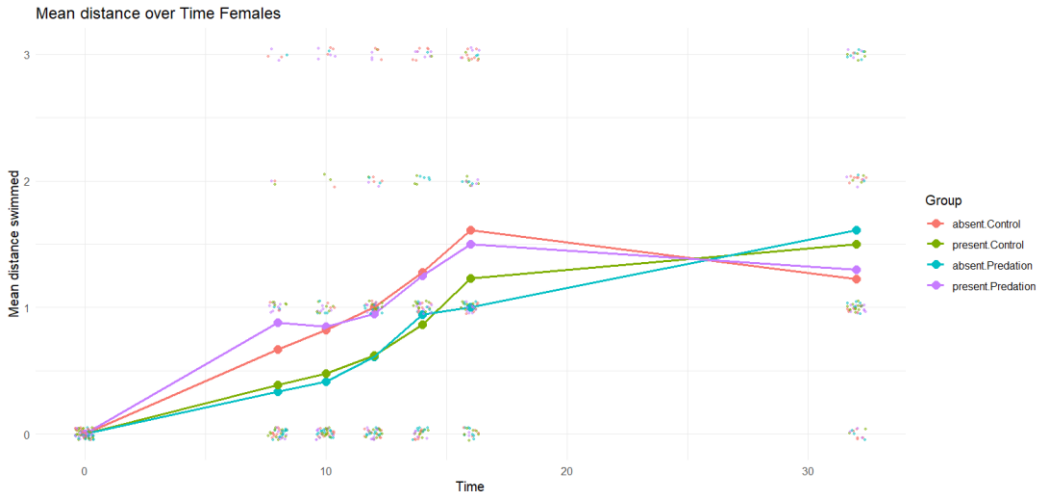


**Figure 1. Females that moved over time.** I filtered only the females that swim and removed the ones that stayed in the initial tank and then I plot them over time. Both single and grouped fish are plotted together since they did not show significant differences.

**Table 1. Females that moved over time.** Table of the results of the GLM with a Poisson distribution of the tank position of the females that moved over time, excluding the fish that did not.

| **Coefficient** | **Estimate** | **Std.Error** | **zvalue** | **Pr(>\|z\|)** |
|---|---|---|---|---|
| **(Intercept)** | -0.928 | 0.173 | -5.362 | <0.001*** |
| **time** | 0.046 | 0.005 | 10.022 | <0.001*** |
| **Predator odor present** | 0.070 | 0.183 | 0.383 | 0.701 |
| **Selection Predation** | -0.288 | 0.146 | -1.975 | 0.048* |
| **Social single** | 0.303 | 0.156 | 1.941 | 0.052 . |
| **Replicate 2** | -0.067 | 0.117 | -0.575 | 0.565 |
| **Replicate 3** | -0.046 | 0.119 | -0.383 | 0.702 |
| **Predatorpresent:SelectionPredation** | 0.592 | 0195 | 3.032 | 0.002** |
| **Predatorpresent:Socialsingle** | -0.538 | 0.203 | -2.645 | 0.008** |

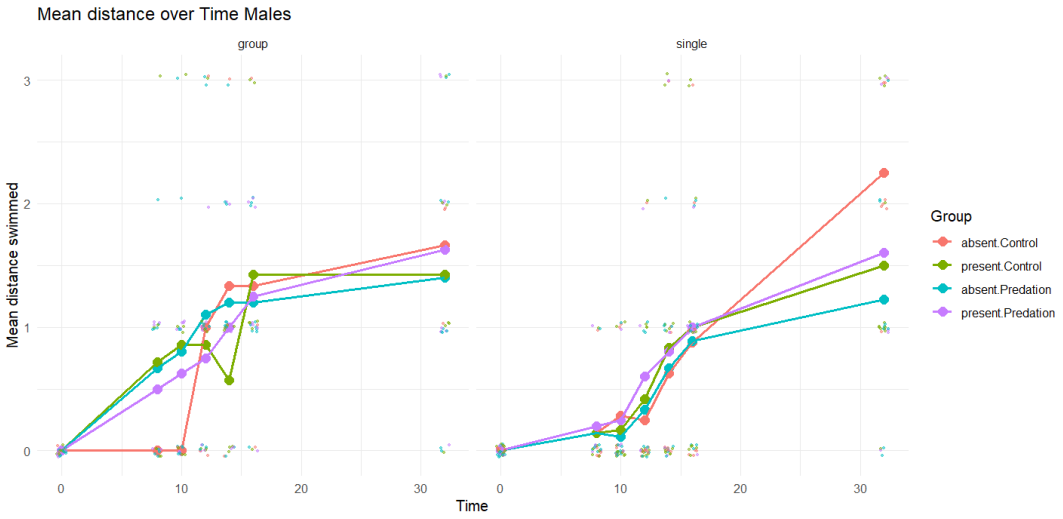


**Figure 2. Males that moved over time.** I filtered only the males that swim and removed the ones that stayed in the initial tank and then I plot them over time. On the top image (A) all males that moved are shown, but since there are different behaviours between the single and the group, I plot them separately. The bottom left (B), the group males are shown and, on the right, the single (C).

**Table 2. Males that moved over time.** Table of the results of the GLM with a Poisson distribution of the tank position of the males that moved over time.

| **Coefficient** | **Estimate** | **Std.Error** | **zvalue** | **Pr(>\|z\|)** |
|---|---|---|---|---|
| **(Intercept)** | -1.301 | 0.229 | -5.676 | <0.001*** |
| **time** | 0.047 | 0.007 | 6.267 | <0.001*** |
| **Predator odor present** | 0.032 | 0.120 | 0.270 | 0.787 |
| **Selection Predation** | 0.009 | 0.123 | 0.072 | 0.943 |
| **Social single** | -0.964 | 0.249 | -3.873 | <0.001*** |
| **Replicate 2** | 0.485 | 0.161 | 3.017 | 0.002** |
| **Replicate 3** | 0.555 | 0.162 | 3.420 | <0.001*** |
| **time:Socialsingle** | 0.031 | 0.011 | 2.754 | 0.006** |

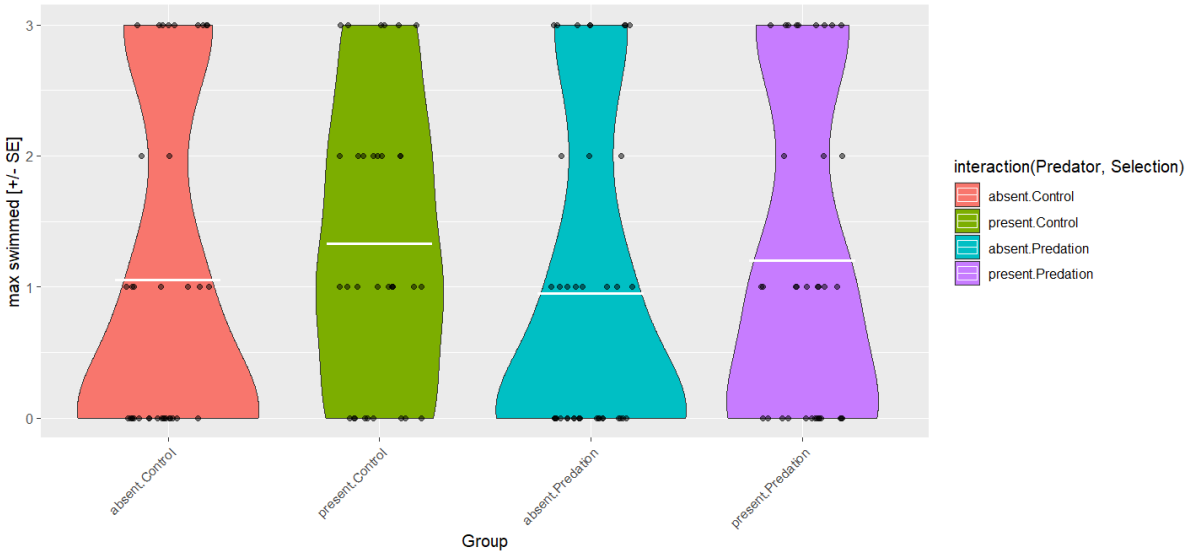


**Figure 3. Furthest tank reached by females.** There were 4 tanks, so the y-axis shows form 0 (initial tank), to 3 (last tank). The furthest tank that the females reached is shown.

**Table 3. Furthest tank reached by females.** Table of the results of the GLM with a Poisson distribution of the furthest tank that the females swam to.

| Coefficient | Estimate | Std.Error | zvalue | Pr(>\|z\|) |
|---|---|---|---|---|
| **(Intercept)** | -0.248 | 0.266 | -0.931 | 0.352 |
| **Predator odor present** | 0.736 | 0.269 | 2.742 | 0.006** |
| **Selection Predation** | -0.536 | 0.267 | -2.012 | 0.044* |
| **Social single** | 0.506 | 0.296 | 1.705 | 0.088 . |
| **Replicate 2** | -0.020 | 0.197 | -0.102 | 0.919 |
| **Replicate 3** | -0.033 | 0.191 | -0.171 | 0.865 |
| **Predatorpresent:Socialsingle** | -0.831 | 0.334 | -2.488 | 0.013* |
| **SelectionPredation:Socialsingle** | 0.697 | 0.333 | 2.090 | 0.037* |

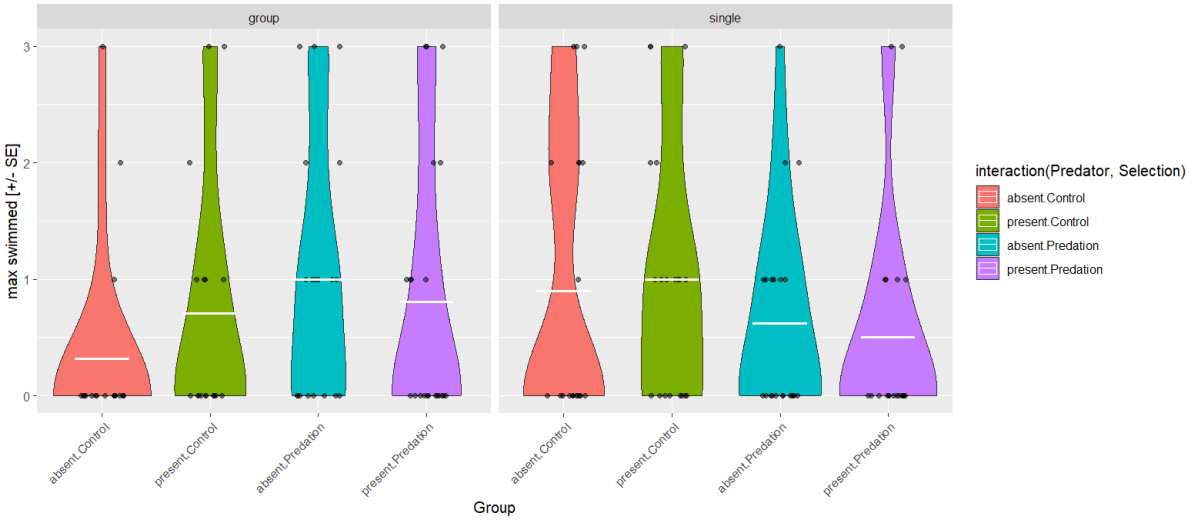


**Figure 4. Furthest tank reached by males.** On the top violin graph (A), all the males are plotted. But since there are significant differences, I plot them separately. In the left the group males (B) and in the right the single (C). There were 4 tanks, so the y-axis shows form 0 (initial tank), to 3 (last tank). The furthest tank that the females reached is shown.

**Table 4. Furthest tank reached by males.** Table of the results of the GLM with a Poisson distribution of the furthest tank that the males swam to.

| **Coefficient** | **Estimate** | **Std.Error** | **zvalue** | **Pr(>\|z\|)** |
|---|---|---|---|---|
| **(Intercept)** | -1.271 | 0.322 | -3.953 | < 0.001 *** |
| **Predatorpresent** | 0.038 | 0.187 | 0.204 | 0.839 |
| **SelectionPredation** | 0.647 | 0.204 | 2.235 | 0.025* |
| **Socialsingle** | 0.702 | 0.289 | 2.449 | 0.014* |
| **Replicate 2** | 0.763 | 0.287 | 2.991 | 0.003** |
| **Replicate 3** | 0.625 | 0.255 | 2.434 | 0.015* |
| **SelectionPredation:Socialsingle** | -1.184 | 0.392 | -3.020 | 0.003** |

## APPENDIX III

In this chapter the measurements of the lids are added. The plexiglass was bought and afterwards cut and added to the limits (see figure 1). Additionally, a picture of both study species and predator are added (Fig. 2). The pictures were taken in the laboratory of Carus.

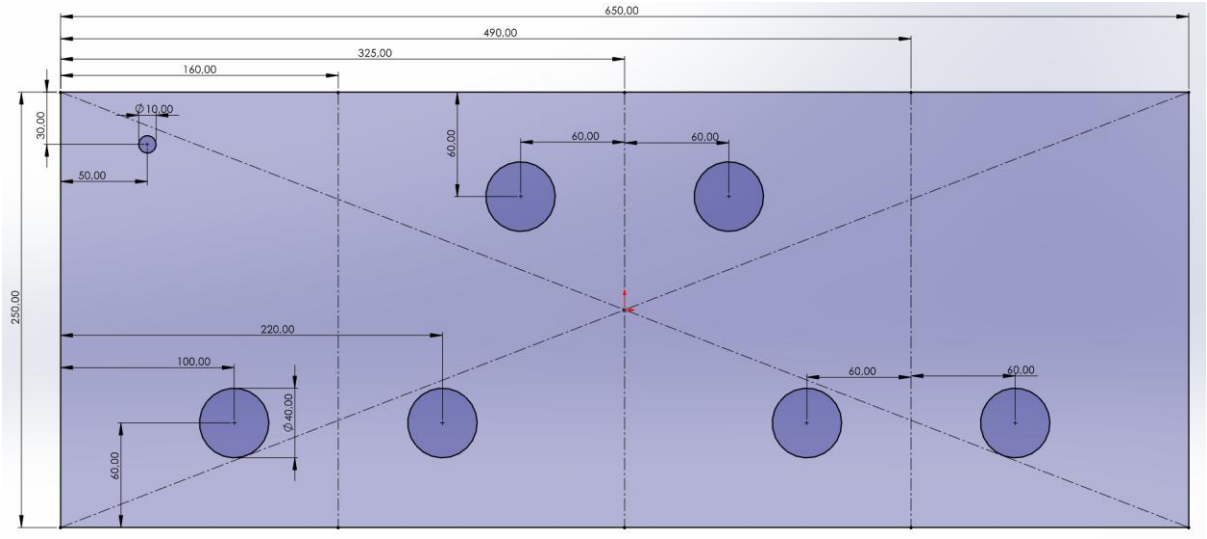


**Figure 1. Lids measurements.** A plexiglass lids were cutted in order to fit the tubes in between the tanks. The lids were 650 x 250 mm and the tubes were altering sides so that the fishes would have a larger distance to swim.

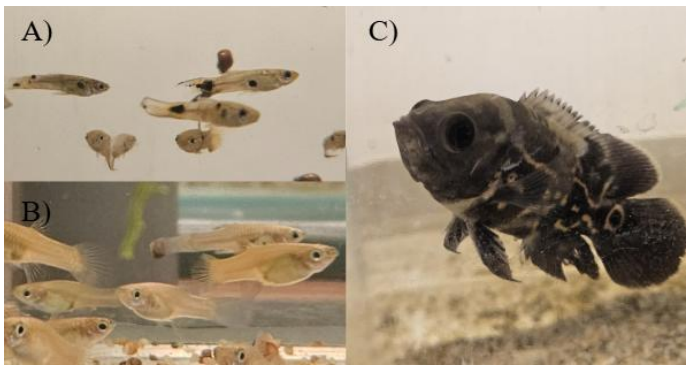


**Figure 2. Study species.** On the left there is our main study species, on top male guppies (A) and on the bottom the females (B). Females are much bigger but less colourful than males. In the right image (C) there is a picture of an oscar. All pictures were taken in our laboratory in Carus.