Investigation of behavioral and cognitive traits affecting dispersal tendencies in eastern mostquitofish (Gambusia holbrooki)

Jennifer Dougherty

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INVESTIGATION OF BEHAVIORAL AND COGNITIVE TRAITS AFFECTING
DISPERsal TENDENCIES IN EASTERN MOSTQUITOFISH
(Gambusia holbrooki)

by

JENNIFER DOUGHERTY

A THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science
in
The Department of Biological Sciences
to
The School of Graduate Studies
of
The University of Alabama in Huntsville

HUNTSVILLE, ALABAMA

2022
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\[ \text{Jennifer Doakfey} \qquad 6/22/2022 \]
\[ \text{(student)} \qquad \text{(date)} \]
Submitted by Jennifer Dougherty in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences and accepted on behalf of the Faculty of the School of Graduate Studies by the thesis committee.

We, the undersigned members of the Graduate Faculty of The University of Alabama in Huntsville, certify that we have advised and/or supervised the candidate on the work described in this thesis. We further certify that we have reviewed the thesis manuscript and approve it in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences.

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ABSTRACT

The School of Graduate Studies
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Degree __ Master of Science __
College/Dept. __ Biological Sciences __

Name of Candidate __ Jennifer Dougherty __

Title __ Investigation of behavioral and cognitive traits affecting dispersal in Eastern Mosquitofish (Gambusia holbrooki) __

Dispersal plays an important role in ecological and evolutionary processes. Despite being important to population and invasion dynamics, more information is needed to understand what traits act as drivers of dispersal tendencies. Here, we examined how different behavioral, environmental, and cognitive traits predicted dispersal tendency in the Eastern mosquitofish (Gambusia holbrooki). We examined how chronic exposure to an antidepressant affects different behaviors and dispersal in wild-caught G. holbrooki. We then tested additional behaviors and their relationship to dispersal tendency. We found that exposure to fluoxetine did not affect performance in any of the behavioral assays or on dispersal. In our second experiment, we found that sociability and body size act as predictors of dispersal, with more asocial and smaller fish dispersing farther. These findings have implications for understanding how species move into environments, though more work is needed to understand how this might affect future invasion, population, and disease dynamics.

Abstract Approval: Committee Chair __ 6/22/22 __
Department Chair __ 6/27/22 __
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ACKNOWLEDGEMENTS

I would like to thank my committee for their continued mentorship and guidance throughout my time at UAH, allowing me to grow and improve as a researcher. I would also like to thank the state of Florida for permission to collect fish. I am especially grateful to Noah Daniels, Patricia Gardner, Christine Hansen, Walker Pierce, and JD Bingman Sr. for their time and effort in the construction and running of my artificial stream system and behavioral assays. Finally, I would like to thank my family and friends for the words of encouragement, the constant proof-reading of grant proposals, and the care-packages that allowed me to spend long days in the lab.
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Chapter 1. Effects of Fluoxetine

Effects of a common anti-depressant on behavior and dispersal in the globally invasive freshwater eastern mosquitofish (*Gambusia holbrooki*).

1.1 Introduction

Despite increasing global efforts to address threats to ecosystems, there several of environmental concerns that are not well studied. One such issue is rising levels of anthropogenic pollutants in aquatic systems (Bernhardt *et al.* 2017, Bertram *et al.* 2018, Brodin *et al.* 2014, Dulawa *et al.* 2004). These pollutants can have negative effects by becoming more concentrated over time, interacting with other chemicals in the environment, and accumulating throughout the food chain (Bernhardt *et al.* 2017). An additional problem is that most water treatment systems were not designed to capture many of the chemicals/compounds that are now routinely found in waste-water effluents (Freydina *et al.* 2016), allowing these putative stressors to reach and accumulate in aquatic systems despite being recognized as hazardous or potentially harmful to wildlife. Most water treatment systems are created to remove a specific type of material, such as chlorine, solid waste, or gasoline (Freydina *et al.* 2016). However, there are many other materials, such as pesticides, medical waste, and even hormonal birth control (Benotti and Brownawell 2009) that escape into local water systems via agricultural and landfill runoff, improper disposal, or groundwater contamination (McDonald 2017).

Understanding the effects of contaminants not targeted by existing waste-water treatment on natural populations is important because a variety of these compounds have been shown to have negative impacts on wild animals (Whitacre 2011, Saaristo *et al.*
2018, Zala and Penn 2004, Brodin et al. 2014, Cheung et al. 2002). For example, atrazine, a common pesticide, has been linked to hormone imbalances in bird species (Zala and Penn 2004); in addition, exposure to copper in water systems affects foraging behavior and feeding success in some gastropods (Cheung et al. 2002). A particular area of growing concern is that of medical waste runoff which often includes high concentrations of daily medications ranging from over-the-counter pain relievers to oral antidepressants (Benotti and Brownawall 2009). One specific example is that of the chemical compound fluoxetine, a commonly prescribed oral antidepressant.

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) that is often prescribed to treat depression by affecting the serotonin transport molecule and uptake pathway (McDonald 2017). SSRIs are an interesting group to study, because the serotonin uptake receptor is conserved across all vertebrate species, suggesting that it could have far-reaching effects on biodiversity (Caveney et al. 2006). Due to improper disposal methods, SSRIs are detected in many surface water systems and have been shown to cause biological effects in various aquatic organisms, including sticklebacks, zebrafish, betta fish, mussels, and gastropods (Ford et al. 2016, Dzieweczynski and Hebert 2012). In contrast to how humans take a prescribed amount of an SSRI once a day, aquatic organisms are exposed to an SSRI or a mixture of SSRIs in their environment and have no control over “dosage” (i.e., exposure concentrations: Rand-Weaver et al. 2013). In addition, fluoxetine is known to have a long half-life, which means that organisms in contact with it are likely to receive chronic exposure (Silva et al. 2012). It is therefore important to understand the effects of such chemicals, as they may result in changes to ecologically relevant behaviors critical to reproduction and survival,
since even small amounts can elicit physiological change (Kuster and Adler 2014, Martin et al. 2019). Effects on animal physiology and behaviors could have broader impacts if they are related to key ecological processes such as dispersal (Castillo-Chavez and Yakubu 2001). To understand how fluoxetine affects traits that could potentially influence dispersal, we focused on a globally invasive freshwater fish exposed to a field-relevant concentration of fluoxetine in a controlled setting.

The eastern mosquitofish, *Gambusia holbrooki* (Family: Poeciliidae), is a small livebearing fish native to the Eastern United States ranging from New Jersey to Florida (Figure S1). They are invasive outside of this native range, having become widespread across the globe, to all continents except Antarctica (Global Invasive Species Database- http://www.issg.org/database, accessed 1 April 2020). The species has been a successful invader largely due to its ability to inhabit a wide variety of environments exhibiting considerable variation in physiochemical characteristics such as nutrient levels, conductivity, dissolved oxygen levels, and salinity (Lee et al. 2017). *Gambusia holbrooki* is a useful system for studying the effects of androgenic endocrine disrupting chemicals (EDCs) because of its widespread, cosmopolitan distribution in shallow freshwater habitats in both urban and agricultural areas likely to be affected by wastewater discharge and run-off (Saaristo et al 2013). Furthermore, previous studies have demonstrated links between certain behaviors and dispersal or movement in *Gambusia* (Rehage and Sih 2004; Cote et al. 2010). In particular, sociability correlates with dispersal tendencies in *G. affinis* (Cote et al. 2010), the sister species to *G. holbrooki*. Aside from behaviors like boldness, exploration, and sociability, it is reasonable to expect that cognitive abilities could also impact dispersal ability as cognition could affect an individual’s ability to
navigate a complex environment. Since fluoxetine is an antidepressant that targets the neuroendocrine system, it could impact behavioral or cognitive traits that affect individual dispersal decisions or ability.

This study examined the effects of exposure to field-detected levels of fluoxetine on ecologically relevant behaviors such as sociability and cognition, as well as patterns of dispersal in an artificial stream to test several hypotheses. If fluoxetine has therapeutic affects against anxiety in humans by allowing increased serotonin uptake and the serotonin uptake pathway is conserved across vertebrate species, then fish treated with fluoxetine should exhibit decreased anxiety and therefore display decreased sociability as the motivation to shoal for safety will have also decreased. On the other hand, there is no clear a priori prediction of how fluoxetine would impact cognitive flexibility. If fluoxetine reduces anxiety, then individuals may be less motivated to find a way around the barrier to reach a social reward. However, if fluoxetine reduces depressive tendencies, then individuals may be focused on trying to move forward in lieu of searching for a solution around the barrier. If either sociability or cognitive flexibility influence dispersal, then either or both traits should be correlated to individual scores and dispersal tendencies in an artificial stream. We predicted that fish that were more asocial and had greater cognitive flexibility would disperse a greater distance in the artificial stream.

1.2 Methods

Adult *Gambusia holbrooki* were collected from Wakulla County, Florida (Google maps: 30.211, - 84.179), and transported to the lab in thermally insulated containers with water from their collection site. In the lab, fish were randomly divided amongst 40L aquaria at equal densities and 1:1 (F:M) sex ratios. Fish were allowed to acclimate to lab
conditions for 1 month. During acclimation and throughout the experiment, aquaria contained sponge filters, had a 12:12 (L:D) cycle, received weekly 25% water changes, and were fed ad libitum once daily with premium flake food. Following the acclimation period, adult males and females were randomly assigned to either a control or fluoxetine treatment group. Fish were assigned to new 40L experimental tanks at a ratio of 4F:4M per tank (total = 8 fish per tank). Due to space and time constraints experiments were conducted in three groups with a total of 22 experimental tanks (11 controls and 11 treatment; total N = 176 fish). Since it was necessary to track individual IDs within each of the experimental groups of 8 fish throughout the experiment, uniquely colored visible implant elastomer tags (Northwest Marine Technology Inc.) were implanted just below the scales near the caudal peduncle of all individuals.

1.2.1 Fluoxetine exposure

Control groups in the experiment received 0.00 ng/L fluoxetine whereas the treatment groups were exposed to a constant concentration of 440 ng/L fluoxetine (Bertram et al. 2017, Martin et al. 2019). This concentration corresponds to ranges tested in previous experiments and is considered high, though these levels have been documented in the wild (Pelli and Connaughton 2015). Fluoxetine powder (Sigma Aldrich Inc.) was used to prepare a 1 mg/mL stock solution in nuclease-free water. Although fluoxetine has been shown to have a long half-life and experiences limited photolytic breakdown compared to other SSRIs (Kwon and Armbrust 2006), the stock solution was stored in darkness at 4 °C for the duration of the experiment. At the start of the experiment, treatment tanks received 16μL of stock solution to achieve a final concentration of 440 ng/L. Both the control and treatment groups were exposed to their
respective conditions for 28 days following methods for chronic exposure as in previous studies (Hughes et al. 2013). Chronic exposure was chosen over acute exposure as fluoxetine typically takes multiple weeks to have an effect (Martin et al. 2019, Bertram et al. 2018). Chronically elevated dosage more closely represents the exposure pattern in the wild (Kwon and Armbrust 2006). To ensure constant fluoxetine concentrations in treatment tanks across the exposure period, 4 µL of fluoxetine stock solution was added to the treatment tanks during each weekly water exchange.

1.2.2 Sociability

Assays of sociability generally followed methods originally described by Cote et al. (2010), and exactly matched the approached described by Culumber (2022). Briefly, the experimental arena consisted of a 20 L acrylic aquarium placed on top of a 1 cm grid to aid in tracking the focal fish. Clear, 4 L holding tanks were placed outside of both ends of the testing arena. One holding tank was filled with only water, and the other was filled with water and a small stimulus shoal consisting of conspecific individuals (N= 4 females, 2 males). This allowed for only visual interaction, but no physical contact or olfactory communication (Figure S4 and S5). The testing area was surrounded by black curtains to prevent outside disturbances. A webcam was set up overhead of the testing arena to record the position of the focal individual (Logitech HD 1080, set at a height of 25.4 cm above the tank).

Focal fish from a given experimental tank were physically and visually isolated from one another the evening before the assay to control for potential effects of recent social experience (Figure S2 and S3). Fish were isolated in small enclosures within their respective experimental tank such that fish in the treatment group remained exposed to
fluoxetine during isolation. On the day of testing, sociability assays were conducted on one focal individual at a time with the order of testing randomized among individuals within an experimental tank. The focal individual was placed in the arena and allowed to acclimate for five minutes during which they could swim freely. Opaque dividers visually isolated the focal fish from the holding tanks during the acclimation period. Following the acclimation period, the opaque barriers were removed, and the camera recorded the position of the focal individual for 15 minutes. A distance of 4cm from the stimulus shoal was used as the threshold for sociability. This distance corresponds to approximately 1 - 2 body lengths for the fish in our study (Total length range: 1.9-5.9cm). The total time the focal individual spent within the 4cm interaction zone was recorded and used as the measure for an individual’s sociability score where more time spent within the 4cm zone indicated greater sociability. Since all fish might exhibit some degree of heightened sociability immediately following social isolation and introduction to a novel environment (the testing arena), the first 5 minutes of the recording were discarded, and sociability was measured from the final 10 minutes of the assay (Culumber 2022). The focal individual was returned to their isolation chamber in their testing tank following the completion of the sociability assay and allowed to rest for 1 hour before the detour task.

1.2.3 Cognitive flexibility

One hour after the sociability assay, cognitive flexibility was measured using a detour task similar to that described by Cummings et al. (2020). The task was to move from one end of the detour tank to the other, which required navigating around a clear barrier and then through an opening in the center of the tank to reach a social reward. The social reward was a conspecific female in a transparent plastic cup at the far end of the
arena. This stimulus represented a reward as females of this species will want to move towards a conspecific to shoal and find safety and males will want to move toward the reward fish to attempt to shoal and/or copulate. The detour arena consisted of an 80 L tank (57 cm x 31 cm x 40 cm) and the obstacles were made from either black or clear acrylic sheets (Figure S6 and S7). The arena was surrounded by opaque blue paper to prevent outside distractions, including movement of human observers, and a webcam was placed overhead to record movement. The focal fish was placed in an opaque chamber and allowed to acclimate for five minutes. Following acclimation, the chamber was lifted remotely as to not overly disturb the focal fish. To complete the task the focal fish first had to move down a central corridor, navigate around a clear barrier blocking their ability to swim directly to the social reward, then navigate back to the center of the arena to reach the reward at the other end (Figure S6 and S7). To promote movement in the desired direction, an “aggravator” male fish from an unfamiliar population was placed in a clear container outside of the testing tank. Females should want to move away from the male to avoid harassment and males should want to move away to avoid competition (Cummings et al. 2020). The length of time it took the focal fish to reach the barrier was considered a measure of motivation, and cognitive flexibility was scored as the time between reaching the barrier and the social reward. Trials were terminated after 10 minutes. Any fish that did not reach the reward by that time received a maximum score of 600 seconds. Following this trial, the focal individual was placed back into its original tank. The aggravator male and reward female were changed every two trials for a new pair.
1.2.4 Dispersal

One hour following the detour task, dispersal tendency of each experimental group of 8 fish was tested using an artificial stream. Similar artificial streams have been used to test dispersal tendencies in *Gambusia* and other small stream fishes (Martin *et al.* 2019, Rehage and Sih 2004, McDonald 2017, Cote *et al.* 2010, Bonte and De la Pena 2009). The stream was constructed with eight “pools” consisting of 114L plastic tubs (40cm x 74cm x 30cm) connected by 1.5m “riffles” made from lengths of PVC pipe (15cm diameter: Figure S2). The top 12cm of each riffle tube was removed to allow light to enter. All pools and riffles were covered with fine screen mesh to prevent fish from jumping out. To avoid accidental dispersal and more closely represent natural habitats, we did not generate water flow. However, in order to prevent an individual from dispersing back “upstream”, we attached a funnel (15 cm base diameter, 3.8 cm tip diameter, 15 cm total length) to the downstream end of each riffle such that if an individual dispersed to a downstream pool, the small opening of the funnel into open water would reduce the chance of an individual returning upstream (Figures S8-S11). The total stream length was 15.24m. Each pool was filled with 95L of treated tap water providing enough volume to cover the top of the end of the funnels.

Each tub had various PVC pipe sections and half flowerpots to use as structure for environmental enrichment. The first pool in the stream array contained a stimulus shoal of conspecifics (N= 4 females, 2 males) that were contained within a 4L plastic tank submerged within the first pool. This was to ensure that all focal fish dispersed at will, and not because of being left alone with no shoaling partners. Each tub also contained a transparent mesh barrier midway between the ends of the pool and suspended from above
(Figure S11), similar to the barrier in the detour task assay. This barrier was designed to prevent a fish from leaving the funnel of one pool and swimming in a direct line at the surface to the next connecting tube, encouraging exploration before making the decision to disperse further in the stream. Water temperature was measured continuously throughout each trial using a HOBO logger (Onset Corp.) at 15-minute intervals.

One hour after all fish from an experimental group finished the detour task, they were transferred as a group to the uppermost (i.e., first) pool of the artificial stream and allowed to acclimate for 5 minutes. A removeable barrier between the first pool and the rifle leading to the next downstream pool ensured none of the focal fish dispersed prematurely. After acclimating for 5 minutes, the barrier was removed, and fish were allowed to freely disperse for 45 minutes. This length of time was determined based on preliminary trials with individuals that were not a part of the experiment from both the focal and a non-focal, conspecific population. Following the trial, dispersal scores were determined based on the position in the stream at which each fish is located (i.e., pool 1-8). At the end of the trial, barriers were placed at the entrance to each riffle to ensure that fish from adjacent pools cannot move while others are being retrieved. The pool that each individual was located was used as their dispersal score (1 - 8).

Following the dispersal trial, each group was placed back into their original experimental aquaria either with or without fluoxetine. The individuals in the opposite stimulus shoal were randomized for each new dispersal assay. The total body length (cm) of each fish was measured by photographing each individual next to a ruler. Their lengths were determined using ImageJ software, measuring from the tip of the nose to the farthest part of the caudal fin, utilizing the dashed-line option to account for any bend along the
lateral line. In order to test repeatability, all fish were returned to their original experimental tanks following their dispersal assay and allowed to rest for one week. All fish were then retested in the same three sociability, cognition, and dispersal assays one week later following the same methods and order as described above.

1.2.5 Statistical Analyses

We conducted separate analysis of covariance (ANCOVA) for the sociability and cognitive flexibility assays. Both ANCOVAs included fixed effects of sex and experimental group (control or fluoxetine) with body size as a covariate. Aside from cognitive flexibility, we also conducted an ANCOVA on the motivation score in the detour task as fluoxetine could impact motivation to reach the barrier and/or the ability to actually complete the task. This ANCOVA consisted of the same effects described above. For the ANCOVAs, we started with all possible interaction terms and conducted backwards elimination of any non-significant interaction term with a cutoff of $P > 0.1$. Finally, we used a generalized linear model (GLM) with a cumulative logit link to test for relationships between sex, treatment, body size, sociability, and cognitive flexibility with variation in dispersal. To avoid model overfitting given the large number of possible interaction terms, we initially included all possible two-way interactions between fixed effects. Backwards elimination of interaction terms was performed as described above. Repeatability was tested using the intraclass correlation coefficient using the first and second trials for all individuals. All analyses were conducted in SPSS v26 (IBM Inc.).
1.3 Results

Repeatability was 0.2 or greater for all traits (Table 1.1). Dispersal had the highest repeatability (ICC = 0.669) and was the only trait to be statistically significant (P < 0.01). Scores for the remaining traits were still well above 0.1 but did not reach the threshold for significance (Sociability: ICC: 0.198, P = 0.12; Cognitive flexibility: ICC: 0.211, P = 0.105). ANCOVA was run on variation in total sociability score; this exhibited no significant effects of treatment on sociability or sex, (F1,144 = 0.059, P = 0.81) nor an effect of sex (F1 = 3.56, P = 0.061, Table 1.2). The ANCOVA for cognitive flexibility also revealed no significant effect of treatment (F1 = 0.78, P = 0.38) nor an effect of sex (F1 = 0.94, p= 0.335, Table 1.3). Lastly, the generalized linear model for dispersal revealed that treatment did not affect dispersal ($X^2 = 0.108$, DF = 1, P = 0.74), or sociability ($X^2 = 0.057$, DF = 1, P = 0.81) or cognitive flexibility ($X^2= 3.54$, DF = 1, P = 0.06; Tables 1.4 and 1.5).

Table 1.1: Repeatability (Intraclass Correlation Coefficient: ICC) of behavioral assays estimated from a generalized linear mixed model. The standard error (SE), 95% confidence intervals, and p-values are also provided.

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<th>SE</th>
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<th>Sig.</th>
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<td>5.04</td>
<td>[0.44, 0.82]</td>
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Table 1.2: ANCOVA for sociability score (time spent next to shoaling group) versus sex, treatment, body size, and an effect of sex by body size.

<table>
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<th>DF2</th>
<th>F</th>
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<td>Treatment</td>
<td>1</td>
<td>144</td>
<td>0.059</td>
<td>P = 0.81</td>
</tr>
<tr>
<td>Body Size</td>
<td>1</td>
<td>144</td>
<td>0.83</td>
<td>P = 0.36</td>
</tr>
<tr>
<td>Sex * Body Size</td>
<td>1</td>
<td>144</td>
<td>4.15</td>
<td>P = 0.04</td>
</tr>
</tbody>
</table>
Table 1.3: ANCOVA for cognitive flexibility (time from barrier to finish) versus sex, treatment, and body size.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF1</th>
<th>DF2</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>147</td>
<td>0.94</td>
<td>P = 0.34</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>147</td>
<td>0.78</td>
<td>P = 0.38</td>
</tr>
<tr>
<td>Body Size</td>
<td>1</td>
<td>147</td>
<td>0.36</td>
<td>P = 0.55</td>
</tr>
</tbody>
</table>

Table 1.4: GLM for dispersal comparing sex, treatment, body size, sociability, and cognitive flexibility to dispersal based on bin dispersed into (1-8). Showing the degrees of freedom, significance, beta value, standard error, 95% confidence interval, X^2, and DF.

<table>
<thead>
<tr>
<th>Effect</th>
<th>B</th>
<th>SE</th>
<th>95% CI</th>
<th>Sig</th>
<th>X^2</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.17</td>
<td>0.48</td>
<td>[-0.76, 1.10]</td>
<td>P = 0.72</td>
<td>0.13</td>
<td>1</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.12</td>
<td>0.32</td>
<td>[0.74, 0.14]</td>
<td>P = 0.71</td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>Body size</td>
<td>-0.31</td>
<td>0.29</td>
<td>[-0.87, 0.25]</td>
<td>P = 0.28</td>
<td>1.16</td>
<td>1</td>
</tr>
<tr>
<td>Sociability</td>
<td>-0.000021</td>
<td>0.0011</td>
<td>[-0.002, 0.002]</td>
<td>P = 0.98</td>
<td>0.00</td>
<td>1</td>
</tr>
<tr>
<td>Cognitive flexibility</td>
<td>-0.002</td>
<td>0.0008</td>
<td>[-0.004, 0.00]</td>
<td>P = 0.021</td>
<td>5.32</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1.5: GLM for dispersal comparing sex, treatment, body size, sociability, and cognitive flexibility to dispersal based on whether or not the individual dispersed from the first bin (0-1). Showing the degrees of freedom, significance, beta value, standard error, 95% confidence interval, X^2, and DF.

<table>
<thead>
<tr>
<th>Effect</th>
<th>B</th>
<th>SE</th>
<th>95% CI</th>
<th>Sig</th>
<th>X^2</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-0.11</td>
<td>0.50</td>
<td>[-1.1, 0.87]</td>
<td>P = 0.82</td>
<td>0.51</td>
<td>1</td>
</tr>
<tr>
<td>Treatment</td>
<td>-0.11</td>
<td>0.35</td>
<td>[-0.79, 0.56]</td>
<td>P = 0.74</td>
<td>0.11</td>
<td>1</td>
</tr>
<tr>
<td>Body size</td>
<td>0.30</td>
<td>0.31</td>
<td>[-0.31, 0.91]</td>
<td>P = 0.34</td>
<td>0.91</td>
<td>1</td>
</tr>
<tr>
<td>Sociability</td>
<td>0.000</td>
<td>0.0012</td>
<td>[-0.002, 0.003]</td>
<td>P = 0.81</td>
<td>0.06</td>
<td>1</td>
</tr>
<tr>
<td>Cognitive Flexibility</td>
<td>0.002</td>
<td>0.0009</td>
<td>[-0.00007291, 0.004]</td>
<td>P = 0.060</td>
<td>3.54</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 1.1: ANCOVA for treatment and sociability. Each bar represents the number of seconds that an individual spent within 4cm of the conspecific shoaling group, based on their treatment. While it is not significant, fluoxetine treated fish tended to spend slightly more time near the shoaling group.

Figure 1.2 ANCOVA for sex and sociability. Each bar represents the number of seconds that an individual spent within 4cm of the conspecific shoaling group, based on their sex. While it is not significant, male fish tended to spend slightly more time near the shoaling group.
Figure 1.3: ANCOVA for treatment and cognitive flexibility. Each bar represents the number of seconds that an individual took to reach the social reward in the detour task, based on their treatment. While it is not significant, fluoxetine treated fish tended to take slightly longer to complete the task.

Figure 1.4: ANCOVA for sex and cognitive flexibility. Each bar represents the number of seconds that an individual took to reach the social reward in the detour task, based on their sex. While it is not significant, male fish tended to take slightly longer to complete the task.
Figure 1.5: GLM for dispersal: body size, sociability, cognitive flexibility as covariates. Each bar represents the average dispersal score based on treatment group, with a possible score of 1-4. Again, while not significant, fluoxetine treated fish dispersed slightly more than control fish.

1.4 Discussion

Endocrine-disrupting chemicals are widespread pollutants in our water systems. We examined how chronic exposure to a field-relevant concentration of fluoxetine, a common antidepressant, affects behavior and dispersal tendency in *G. holbrooki*. We were also interested to determine whether variation in our focal traits was predictive of patterns of dispersal in an artificial stream. We found that fluoxetine exposure did not influence fish social behavior or cognitive flexibility. The fluoxetine treatment also had no effect on dispersal distance in the stream. In addition, we did not find any strong association between social behavior or cognitive flexibility with dispersal in either the control or treatment groups. Regardless of what underlying behavioral or cognitive traits predict dispersal in *G. holbrooki*, these results suggest that even a relatively high dosage of fluoxetine may have little or no effect on patterns of fish movement.
Given the medicinal impacts of fluoxetine in humans and that the associated receptor is conserved across all vertebrate species, we predicted that exposure may affect behavior in a wild vertebrate. For example, previous studies have found effects of fluoxetine on anxiety, sexual selection, and reproductive behaviors in *Gambusia* (Martin et al. 2019, Bertram et al. 2018). Studies in other species, including newts, mice, and minnows, have found that fluoxetine also affects locomotion, boldness, and feeding habits (Clotfelter et al. 2004, Giacomini et al. 2015, Weinberger and Klaper 2014). In some cases, even acute exposure has been found to have large effects on behaviors (Henry and Black 2008). In partial contrast to the findings of some of these other studies, we did not observe any effects of fluoxetine exposure on the traits that we tested in eastern mosquitofish.

Our finding that fluoxetine exposure did not affect sociability is consistent with a prior study in *G. holbrooki* that also observed no appreciable effect of high levels of exposure on social behavior (Meijide et al. 2018). However, that study used a 14-day exposure which does not reach the threshold generally considered to be chronic exposure. Our results suggest that even longer exposure times may not elicit an effect on social behavior. To our knowledge, ours is the first study to test effects of fluoxetine on cognitive flexibility in the context of a detour task with a social motivation. Although we found no effect of exposure on time to solve a detour task, this does not necessarily indicate that fluoxetine does not affect other aspects of cognitive performance. In *G. holbrooki*, we also found that exposure do not affect an individual’s actual motivation to solve the detour task. Solving the task involves navigating around the barrier to reach the reward, but motivation to solve the task is measured just in how long it takes to get to the
barrier in first place. We posited that if fluoxetine affects traits related to anxiety it could affect motivation to solve the task, but this was also not the case (data not shown). It is possible that fluoxetine just does not affect the specific traits we chose to target. Even so, there could be other behaviors not tested here that are affected to some extent by exposure to fluoxetine. Other experiments have reported variety of exposure concentrations ranging from 40ng/L up to 400ng/L (Bertram et al. 2018, Dzieweczynski and Herbert 2012, Giacomini et al. 2015, Weinberger and Klaper 2014). It is possible that our chosen concentration was not enough to elicit a response in social behavior and cognition. However, 400ng/L chosen for this study is the highest-field relevant concentration reported.

A prior study showed that social tendencies may predict dispersal tendencies in the closely related G. affinis (Cote et al. 2010). In G. affinis - the sister species to G. holbrooki - individuals that were more asocial tended to disperse farther in an artificial stream (Cote et al. 2010). We found no such relationship between social behavior and dispersal for G. holbrooki. Our findings are consistent with work done on root voles and skinks, which found that social behavior was not correlated to dispersal tendency (Hoset et al. 2011, Michelangeli et al. 2017). On the other hand, studies performed on different species of lizards found that social preferences were related to dispersal tendencies (Galliard et al. 2015, Cote and Clobert 2007). We also found no relationship between cognitive flexibility and dispersal tendency.

Finally, fluoxetine has been shown previously to affect a variety of behaviors and responses in different species, but it does not seem to affect every physiological or behavioral process. In a study on betta fish, fluoxetine had no effect on nest guarding or
copulatory behaviors, suggesting that certain behaviors may be controlled by processes that are not affected by serotonin (Dzieweczynski and Hebert 2012). The equivocal results across studies involving different species - sometimes including closely related species as is the case for *Gambusia* - may indicate that it is not easy to generalize relationships between dispersal and underlying behavioral traits. However, regardless of what behaviors may predict dispersal for *G. holbrooki*, our results suggest that exposure to fluoxetine even at the highest field-relevant concentration does not affect dispersal as there was no difference in dispersal between control and treatment groups irrespective of their social and cognitive performance. This is important for *G. holbrooki* given its invasive nature and potential to act as a vector for disease transmission.

Two results in our study stand in contrast to results in related poecilid species. First, that there was no relationship between sociability and dispersal (Cote et al. 2010) and second that there was no difference between the sexes in cognitive flexibility (Lucon-Kiccatò and Bisazza 2017). With respect to sociability and dispersal, we generally followed the experimental protocol used for *G. affinis*. It is possible that there are interspecific differences in what traits predict dispersal. While we cannot rule this out, it seems unlikely given the close evolutionary relationship, as these are sister species. Although we maintained similar fish densities, due to space constraints our stream length and pool size were smaller than that of Cote et al. (2010). It is possible that even though densities were similar between the two studies, simply having a larger group size may have been a motivating factor for asocial fish to leave the group and disperse downstream in the *G. affinis* experiment. A second difference is that our stream did not utilize flow, primarily because *G. holbrooki* generally inhabit low to no flow areas of streams,
swamps, and lakes (Global Invasive Species Database). However, it is also unclear why flow would generate different dispersal patterns between social and asocial individuals.

Our second contrasting result was a lack of differences between males and females in cognitive flexibility. A previous study, using a similar detour task, found a difference between male and female guppies in solving a similar task, suggesting there may be a difference in learning ability or spatial recognition (Lucon-Xiccato and Bisazza 2017). A similar study also found that female *Gambusia* performed differently than males in a test of cognitive flexibility, suggesting that certain traits may be sex-specific (Wallace et al. 2020). It is possible that females may be more motivated to solve a task to escape harassment and shoal with conspecifics for safety, whereas males may be less motivated due to competition. However, we found no difference between males and females in ability to solve the detour task. It is unclear what explains differences in performance across closely related species, and additional studies are needed to better understand these contrasting results. Specifically, comparative studies that utilize identical methods for all species could be especially insightful.

In summary, we examined how chronic exposure to fluoxetine effects sociability, cognitive flexibility, and dispersal in the eastern mosquitofish. We found that exposure to fluoxetine did not have a significant effect on behavior or dispersal. Other studies involving different species suggest that fluoxetine still may affect other areas of development, physiology, and behavior. Further studies into the interaction between fluoxetine and other relevant pollution found in aquatic systems could help to explain if fluoxetine acts alone, or if it is more effective in tandem with other environmental conditions. In addition, it may be helpful to examine how fluoxetine and other associated
SSRIs affect aquatic organisms at a genetic level in terms of cortisol and hormone levels, as well as if fluoxetine levels accumulate within organisms over time, and throughout the food web. Lastly, comparative studies across species could provide new information on why studies exhibit contrasting results into effects of fluoxetine on certain behaviors and the relationships between certain behaviors and dispersal.
Chapter 2: Predictors of dispersal

Testing predictors of dispersal tendency in the eastern mosquitofish

(*Gambusia holbrooki*)

2.1 Introduction

Movement away from the natal range (*i.e.*, dispersal) plays a role in many ecological and evolutionary processes (Sih *et al.* 2014). Dispersal is a part of population dynamics, as introducing new individuals to an area increases gene flow and diversity and is highly correlated to environmental factors (Michelangeli *et al.* 2017, Paradis *et al.* 1999). Dispersal also plays a part in disease ecology, as organisms are often vectors to parasites, bacteria, and fungus (Castillo-Chavez and Yakubu 2001). This can also lead to implications for species and population management and policy decisions, as understanding the factors that contribute to movement and invasion can contribute to species and environment conservation efforts (Roberston *et al.* 2006).

Dispersal has been studied in a variety of species and models. For example, several environmental factors, such as stream conditions and flow, affect the dispersal rate and gene flow of a headwater salamander (Lowe 2003), while bird population sizes are directly related to dispersal distance (Paradis *et al.* 1999) where larger population densities lead to greater dispersal. The ability to disperse may increase a species survival and fecundity during habitat fragmentation or destruction (Bonte and De la Pena 2009). The decision and ability to disperse may be selected upon for reproduction and are thought to be highly correlated with other physiological and morphological traits (Roff...
and Fairbairn 2001, Duckworth 2008). Though various causes, such as resource availability and breeding pressures, are important to movement (Duckworth 2008), the factors explaining variation in dispersal tendencies are not well understood (Spiegel et al. 2016).

It is clear that animal behavior could play an important role in explaining dispersal decisions and abilities. Indeed, several studies have discussed the potential importance of behavioral variation in dispersal (Cote et al. 2010, Bonte and De La Pena 2009, Sih et al. 2012, Wolf and Weissing 2012, Rehage and Sih 2004). A variety of individual-level traits may explain variation in dispersal tendencies but remain poorly understood (Cote et al. 2010, Spiegel et al. 2017). Bridging this gap in knowledge can address the unresolved role of animal behavior in both basic and applied arenas from population divergence due to gene flow or genetic drift to invasion dynamics and success. Here, we studied traits related to dispersal in a freshwater fish species that is known to be highly invasive.

The eastern mosquitofish, *Gambusia holbrooki* (Family: Poeciliidae), is a small livebearing fish native to the Eastern United States ranging from New Jersey to Florida (Figure S1). These fish are invasive outside of this native range, having become widespread across the globe, in all continents except Antarctica (Global Invasive Species Database- http://www.issg.org/database, accessed 1 April 2020). The species has been a successful invader largely due to its ability to inhabit a wide variety of environments exhibiting considerable variation in physiochemical characteristics such as nutrient levels, conductivity, dissolved oxygen levels, and salinity (Lee et al. 2017). *Gambusia holbrooki* is a useful system to study because of its widespread, cosmopolitan distribution
in shallow freshwater habitats in both urban and agricultural areas (Saaristo et al 2013). Furthermore, previous studies have shown that behaviors are linked to dispersal and movement in Gambusia (Rehage and Sih 2004; Cote et al. 2010).

This study examined how different behaviors and individual cognition may act as predictors of dispersal tendency. Asocial individuals are less inclined to be a part of a group and therefore more inclined to leave native habitat and move to a new area. Given that dispersal involves moving into new, previously unobserved habitats several other traits may influence dispersal tendency or ability. For example, the ability to move throughout an environment may reflect an individual’s ability to navigate in a novel or complex setting. If an individual is able to solve a spatial task more quickly, then those individuals may be capable of more efficient dispersal. Similarly, testing behaviors such as exploration, activity, and boldness in a novel setting could shed insight into traits that predict dispersal. If an individual is more active, exploratory, or bold in a novel environment, then it may be more likely to disperse to new areas. Indeed, we predicted that individuals who were more active, more explorative, and were bolder would disperse farther in an artificial stream setting.

2.2 Methods

Adult Gambusia holbrooki were collected from the Aucilla river in Aucilla county, Florida (Google maps: 30.49, -83.73) and transported to the lab at UAH in thermally insulated containers with water from their collection site. In the lab, fish were randomly divided amongst 40L aquaria with equal densities and 1:1 (F:M) sex ratios. Fish were allowed to acclimate to lab conditions for 1 month. During acclimation and throughout the experiment, aquaria contained sponge filters, had a 12:12 (L:D) cycle,
received weekly 25% water changes, and were fed ad libitum once daily with premium flake food. Following the acclimation period, fish were assigned to new 40 L experimental tanks at a ratio of 4F:4M per tank. The experiments were conducted with a total of 8 experimental tanks (N = 64 fish). Since it was necessary to track individual IDs within each of the experimental groups throughout the experiment, uniquely colored visible implant elastomer tags (Northwest Marine Technology Inc.) were implanted just below the scales of all individuals.

2.2.1 Sociability

Assays of sociability generally followed methods originally described by Cote et al. (2010), and exactly matched the approached described by Culumber (2022). Briefly, the experimental arena consisted of a 20 L acrylic aquarium placed on top of a 1 cm grid to aid in tracking the focal fish. Clear, 4 L holding tanks were placed outside of both ends of the testing arena. One holding tank was filled with only water, and the other was filled with water and a small stimulus shoal consisting of conspecific individuals (N = 4 females, 2 males). This allowed for only visual interaction, but no physical contact or olfactory communication. The testing area was surrounded by black curtains to prevent outside disturbances (Figure S4 and S5). A webcam was set up overhead of the testing arena to record the position of the focal individual (Logitech HD 1080, set at a height of 25.4 cm above the tank).

Focal fish from a given experimental tank were physically and visually isolated from one another the evening before the assay to control for potential effects of recent social experience (Figure S2 and S3). Fish were isolated in small enclosures within their respective experimental tanks. On the day of testing, sociability assays were conducted
on one focal individual at a time with the order of testing randomized among individuals within an experimental tank. The focal individual was placed in the arena and allowed to acclimate for five minutes during which they could swim freely. Opaque dividers visually isolated the focal fish from the holding tanks during the acclimation period. Following the acclimation period, the opaque barriers were removed, and the camera recorded the position of the focal individual for 15 minutes. A distance of 4 cm from the stimulus shoal was used as the threshold for sociability, which corresponds to approximately 1 - 2 body lengths for the fish in our study (total length range: 1.9 - 5.9cm). The total time the focal individual spent within the 4cm interaction zone was recorded and used as the measure for an individual’s sociability score where more time spent within the 4cm zone indicated greater sociability. Since all fish might exhibit some degree of heightened sociability immediately following social isolation and introduction to a novel environment (the testing arena), the first 5 minutes of the recording were discarded, and sociability was measured from the final 10 minutes of the assay (Culumber 2022). The focal individual was returned to their isolation chamber in their testing tank following the completion of the sociability assay and allowed to rest for 1 hour before the detour task.

2.2.2 Cognitive flexibility

One hour after the sociability assay, cognitive flexibility was measured using a detour task similar to that described in Cummings et al. (2020). The task was to move from one end of the detour tank to the other, which required navigating around a clear barrier and then through an opening in the center of the tank to reach a social reward. The social reward was a conspecific female in a transparent plastic cup at the far end of the arena. This stimulus represented a reward as females of this species will want to move
towards a conspecific to shoal and find safety and males will want to move toward the reward fish to attempt to shoal and/or copulate. The detour arena consisted of an 80 L tank (57 cm x 31 cm x 40 cm) and the obstacles were made from either black or clear acrylic sheets (Figure S6 and S7). The arena was surrounded by opaque blue paper to prevent outside distractions, including movement of human observers, and a webcam was placed overhead to record movement. The focal fish was placed in an opaque chamber and allowed to acclimate for five minutes. Following acclimation, the chamber was lifted remotely as to not overly disturb the focal fish. To complete the task the focal fish first had to move down a central corridor, navigate around a clear barrier blocking their ability to swim directly to the social reward, then navigate back to the center of the arena to reach the reward at the other end (Figure S6 and S7).

To promote movement in the desired direction, an “aggravator” male fish from an unfamiliar population was placed in a clear container outside of the testing tank. Females should want to move away from the male to avoid harassment and males should want to move away to avoid competition (Cummings et al. 2020). The length of time it took the focal fish to reach the barrier was considered a measure of motivation, and cognitive flexibility was scored as the time between reaching the barrier and the social reward. Trials were terminated after 10 minutes. Any fish that did not reach the reward by that time received a maximum score of 600 seconds. Following this trial, the focal individual was placed back into its original tank. The aggravator male and reward female were changed every two trials for a new pair.
2.2.3 Open field trial

To not over-stress/exhaust the fish and to make sure we were getting an accurate measure of their behavior, this and the following dispersal assay were run the day after sociability and cognitive flexibility. An open field trial (OFT) was used to measure anxiety (proximity to the wall, distance from the center of the arena), boldness (total amount of time spent in the center of the arena), exploration (total number of quadrats moved through), and activity (total amount of time spent moving). The open field trial arena was a circular opaque tub (53cm diameter) with 17 sections marked with opaque lines on the bottom (Figure S12 and S13). A webcam (LogiTech HD 1080) was set 120cm above to record all movement. The focal fish was placed in the arena and allowed to acclimate for 30 seconds. Following acclimation, the camera recorded the next 5 minutes of movement. After completing the trial, the focal individual was placed back into its original tank. Each trial was scored based on the total number of quadrats an individual crossed into, the number of times they crossed through the center section, the total amount of time spent in the center, and the total amount of time spent moving. Following this trial, the individual was placed back into its original tank.

2.2.4 Dispersal

One hour following the OFT trial, dispersal tendency of each experimental group of eight fish was tested using an artificial stream. Similar artificial streams have been used to test dispersal tendencies in Gambusia and other small stream fishes (Martin et al. 2019, Rehage and Sih 2004, McDonald 2017, Cote et al. 2010, Bonte and De la Pena 2009). The stream was constructed with eight “pools” consisting of 114L plastic tubs (40cm x 74cm x 30cm) connected by 1.5 m “riffles” made from lengths of PVC pipe.
(15cm diameter: Figure S2). The top 12cm of each riffle tube was removed to allow light to enter. All pools and riffles were covered with fine screen mesh to prevent fish from jumping out. To avoid accidental dispersal and more closely represent natural habitats, we did not generate water flow. However, in order to prevent an individual from dispersing back “upstream”, we attached a funnel (15 cm base diameter, 3.8 cm tip diameter, 15 cm total length) to the downstream end of each riffle such that if an individual dispersed to a downstream pool, the small opening of the funnel into open water would reduce the chance of an individual returning upstream (Figure S8 - S11). The total stream length was 15.24m. Each pool was filled with 95L of treated tap water providing enough volume to cover the top of the end of the funnels.

Each tub had various PVC pipe sections and half flowerpots to use as structure for environmental enrichment. The first pool in the stream array contained a stimulus shoal of conspecifics (N= 4 females, 2 males) that were contained within a 4L plastic tank submerged within the first pool. This was to ensure that all focal fish dispersed at will, and not because of being left alone with no shoaling partners. Each tub also contained a transparent mesh barrier midway between the ends of the pool and suspended from above (Figure S11), similar to the barrier in the detour task assay. This barrier was designed to prevent a fish from leaving the funnel of one pool and swimming in a direct line at the surface to the next connecting tube, encouraging exploration before making the decision to disperse further in the stream. Water temperature was measured continuously throughout each trial using a HOBO logger (Onset Corp.) at 15-minute intervals.

One hour after all fish from an experimental group finished the detour task, they were transferred as a group to the uppermost (i.e., first) pool of the artificial stream and
allowed to acclimate for 5 minutes. A removeable barrier between the first pool and the rifle leading to the next downstream pool ensured none of the focal fish dispersed prematurely. After acclimating for 5 minutes, the barrier was removed, and fish were allowed to freely disperse for 45 minutes. This length of time was determined based on preliminary trials with individuals that were not a part of the experiment from both the focal and a non-focal, conspecific population. Following the trial, dispersal scores were determined based on the position in the stream at which each fish is located (i.e., pool 1 - 8). At the end of the trial, barriers were placed at the entrance to each riffle to ensure that fish from adjacent pools cannot move while others are being retrieved. The pool that each individual was located was used as their dispersal score (1 - 8).

Following the dispersal trial, each group was placed back into their original experimental aquaria either with or without fluoxetine. The individuals in the opposite stimulus shoal were randomized for each new dispersal assay. Following the end of the dispersal trial, all fish were photographed to measure total length (cm) using ImageJ software (Schneider et al. 2012). All fish were then retested in the same three sociability, cognition, and dispersal assays one week later following the same methods and order as described above.

2.2.5 Statistical analyses

Because the OFT tested multiple behaviors within one assay, we reduced the dimensionality of the OFT variables by running a principal component analysis (PCA). A single PC axis had an Eigenvalue greater than 1.0 and explained 63% of the variation in the data. All traits on that axis had positive loadings >0.4 such that individuals with positive scores were more active, more explorative, and bolder (Table S1). Each
individual’s PC score along that axis were used as an independent variable in subsequent analyses of dispersal.

We then used a generalized linear model (GLM) to test for fixed effects of sex, body size, sociability, cognitive flexibility, and PC1 from the OFT on variation in dispersal. We first ran the GLM first using dispersal score based on an individual’s distance traveled in the stream (1 - 8). However, given the limited number of individuals that actually dispersed out of the first “pool” at all, we re-ran the model a second time using a binary score if they chose to disperse from the first pool of the stream (0 - 1) in order to increase our power to detect a pattern. In order to avoid model overfitting given the large number of possible interaction terms, we initially included all possible two-way interactions between all fixed effects and conducted a backwards elimination of interaction terms based on a cut-off of P > 0.1. Repeatability was tested using the intraclass correlation coefficient using the first and second trials for all individuals. All analyses were conducted in SPSS v26 (IBM Inc.)

2.3 Results

Analysis of repeated trials found that several traits were repeatable through time (Table 2.1). Several traits were highly repeatable (activity: ICC: 0.625, P <0.01; exploration: ICC: 0.526, P <0.01; dispersal: ICC: 0.669, P<0.01) whereas others were non-significant but still well above 0.1 (Sociability: ICC: 0.198; cognitive flexibility: ICC: 0.211). Boldness was not repeatable (ICC: -0.066, p=P = 0.105; Table 2.1). The generalized linear model for dispersal (0-1 if dispersed or not) revealed an effect of sociability ($X^2 = 4.431$, DF = 1, $P = 0.035$), body size ($X^2 = 3.963$, DF = 1, $P = 0.047$), PC1 ($X^2 = 4.193$, DF = 1, $P = 0.041$) and an interaction of PC1 with body size ($X^2 = 31$
4.292, DF = 1, P = 0.038; Table 2.2 No other term was significant (Table 2.2). The generalized linear model for dispersal distance (bin 1-8) revealed an effect of only body size ($X^2 = 4.782, DF = 1, P = 0.029$), but no other significant term (Table 2.3).

Sociability and cognitive flexibility were not significant.

Table 2.1. Repeatability (Intraclass Correlation Coefficient: ICC) of behavioral assays estimated from a generalized linear mixed model. The standard error (SE), 95% confidence intervals, and p-values are also provided.

<table>
<thead>
<tr>
<th>Trait</th>
<th>ICC</th>
<th>SE</th>
<th>95% CI</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>0.63</td>
<td>4.33</td>
<td>[0.38, 0.79]</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Boldness</td>
<td>-0.066</td>
<td>0.876</td>
<td>[-0.38, 0.26]</td>
<td>P = 0.65</td>
</tr>
<tr>
<td>Cognitive</td>
<td>0.21</td>
<td>1.54</td>
<td>[-0.12, 0.50]</td>
<td>P = 0.11</td>
</tr>
<tr>
<td>Sociability</td>
<td>0.20</td>
<td>1.49</td>
<td>[-0.14, 0.049]</td>
<td>P = 0.12</td>
</tr>
</tbody>
</table>

Table 2.2: GLM for dispersal comparing sex, body size, sociability, cognitive flexibility, and PC1 against whether or not an individual dispersed beyond the first bin (0-1). Showing the degrees of freedom, significance, beta value, standard error, 95% confidence interval, $X^2$, and DF.

<table>
<thead>
<tr>
<th>Effect</th>
<th>B</th>
<th>SE</th>
<th>95% CI</th>
<th>Sig.</th>
<th>$X^2$</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>51.0</td>
<td>27.3</td>
<td>[-2.47, 104]</td>
<td>P = 0.062</td>
<td>3.50</td>
<td>1</td>
</tr>
<tr>
<td>Body Size</td>
<td>18.0</td>
<td>7.78</td>
<td>[2.77, 33.3]</td>
<td>P = 0.047</td>
<td>3.96</td>
<td>1</td>
</tr>
<tr>
<td>Sociability</td>
<td>-0.010</td>
<td>0.0048</td>
<td>[-0.019, -0.001]</td>
<td>P = 0.035</td>
<td>4.43</td>
<td>1</td>
</tr>
<tr>
<td>Cognitive</td>
<td>-0.005</td>
<td>0.0030</td>
<td>-0.011, 0.001]</td>
<td>P = 0.096</td>
<td>2.78</td>
<td>1</td>
</tr>
<tr>
<td>PC1</td>
<td>24.5</td>
<td>11.8</td>
<td>[1.35, 47.6]</td>
<td>P = 0.041</td>
<td>4.19</td>
<td>1</td>
</tr>
<tr>
<td>Sex * PC1</td>
<td>7.84</td>
<td>4.8</td>
<td>[-0.739, 16.4]</td>
<td>P = 0.073</td>
<td>3.20</td>
<td>1</td>
</tr>
<tr>
<td>Body Size * PC1</td>
<td>-10.3</td>
<td>4.97</td>
<td>[-20.0, -0.555]</td>
<td>P = 0.038</td>
<td>4.29</td>
<td>1</td>
</tr>
<tr>
<td>Sex * Body Size</td>
<td>-19.2</td>
<td>10.1</td>
<td>[-39.0, 0.505]</td>
<td>P = 0.056</td>
<td>3.65</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 2.3: GLM for dispersal comparing sex, body size, sociability, cognitive flexibility, and PC1 against how far an individual dispersed (1-8). Showing the degrees of freedom, significance, beta value, standard error, 95% confidence interval, $X^2$, and DF.

<table>
<thead>
<tr>
<th>Effect</th>
<th>B</th>
<th>SE</th>
<th>95% CI</th>
<th>Sig.</th>
<th>$X^2$</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-0.718</td>
<td>1.92</td>
<td>[-4.47, 3.04]</td>
<td>$P = 0.71$</td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>Body Size</td>
<td>-19.9</td>
<td>9.10</td>
<td>[-37.7, -2.063]</td>
<td>$P = 0.029$</td>
<td>4.78</td>
<td>1</td>
</tr>
<tr>
<td>Sociability</td>
<td>-0.067</td>
<td>0.0385</td>
<td>[0.142, 0.009]</td>
<td>$P = 0.084$</td>
<td>2.98</td>
<td>1</td>
</tr>
<tr>
<td>Cognitive Flexibility</td>
<td>0.003</td>
<td>0.0385</td>
<td>[-0.003, 0.008]</td>
<td>$P = 0.33$</td>
<td>0.95</td>
<td>1</td>
</tr>
<tr>
<td>PC1</td>
<td>-14.4</td>
<td>7.47</td>
<td>[29.0, 0.255]</td>
<td>$P = 0.054$</td>
<td>3.73</td>
<td>1</td>
</tr>
<tr>
<td>Sex * PC1</td>
<td>-4.02</td>
<td>2.34</td>
<td>[-8.60, 0.570]</td>
<td>$P = 0.086$</td>
<td>2.95</td>
<td>1</td>
</tr>
<tr>
<td>Body Size * PC1</td>
<td>6.14</td>
<td>3.19</td>
<td>[-0.114, 12.4]</td>
<td>$P = 0.054$</td>
<td>3.70</td>
<td>1</td>
</tr>
<tr>
<td>Body Size * Sociability</td>
<td>0.030</td>
<td>0.0164</td>
<td>[-0.002, 0.062]</td>
<td>$P = 0.066$</td>
<td>3.74</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 2.1: GLM for dispersal. Each bar represents how an individual dispersed. Score of 0 means that an individual did not disperse out of pool 1, while a score of 1 means that an individual did disperse out of pool 1. The number of each sex that dispersed compared to the total number of fish of each sex is shown above the bars.
Figure 2.2: GLM for dispersal and sociability. This graph shows the relationship between the number of seconds an individual spent near the conspecific shoal and how far they dispersed. Score of 0 means that an individual did not disperse out of pool 1, score of 1 means that an individual did disperse out of pool 1.

Figure 2.3: GLM for dispersal and PC1 x SL with four equally spaced bins for body size to better illustrate variation in behavior across body size. The PC1 axis examined boldness, activity, and exploration, with each graph showing a different bin of standard length (body size). Score of 0 means that an individual did not disperse out of pool 1, score of 1 means that an individual did disperse out of pool 1.
Figure 2.4: GLM for dispersal and SL (body size). This graph shows the relationship between the size of an individual and the distance dispersed. Score of 0 means that an individual did not disperse out of pool 1, score of 1 means that an individual did disperse out of pool 1.

Figure 2.5: GLM for dispersal and PC1. PC1 refers to the boldness, activity, and exploration scores from the open field trial. This graph shows the relationship between these behaviors and the distance dispersed. Score of 0 means that an individual did not disperse out of pool 1, score of 1 means that an individual did disperse out of pool 1.
2.4 Discussion

Dispersal is an ecologically important behavior, with the potential to affect gene flow, disease ecology, and invasion dynamics, among other important ecological and evolutionary processes. Here we examined how a suite of behaviors and cognitive ability may predict tendency to disperse in *G. holbrooki*. As in our previous experiment (Chapter 1), we did not observe a relationship between cognitive flexibility and dispersal in this trial. We did find that sociability, PC1 (boldness, exploration, and activity), and body size were correlated with dispersal. This suggests that by understanding variation in certain individual traits – including behaviors – we may be able to make predictions about dispersal tendencies. Nonetheless, there are likely to be additional traits that also influence dispersal but have yet to be evaluated in a comparative manner.

Previous studies examining *G. affinis* and electronic model systems have suggested that an individual’s tendency to shoal near a conspecific group may predict their tendency to disperse (Cote *et al.* 2010, Blanchard *et al.* 2021). These studies found that more asocial individuals tend to disperse more. Conversely, we found that more social individuals of *G. holbrooki*, the sister species of *G. affinis*, chose to disperse in our assay. It is unclear why social individuals would disperse farther in *G. holbrooki* but not in *G. affinis*. Since different studies have found differing results, it may not be possible to generalize behaviors across species or specific habitats with varying conditions. Dispersal is also typically studied within a short timeframe or scale, and it has been suggested that these results may change given a longer range of dispersal over a longer time period (Blanchard *et al.* 2021). It is also possible that differences in experimental protocol led to different results. In *Gambusia*, the sister species differed in the relationship between
sociability and dispersal. It is possible that there are simply species differences in how certain traits predict dispersal. While we cannot rule this out, it seems unlikely given the very close evolutionary relationship and similarities in biology between these two species. It is also possible that it is the absolute rather than relative number of fish present in the artificial stream. Although we attempted to maintain similar fish densities, due to space constraints our stream length and pool size was smaller than that of Cote et al. (2010). Therefore, the absolute number of fish study was smaller for any one dispersal assay. Having a larger absolute group size could be a motivating factor for asocial fish to leave the group and disperse downstream in the *G. affinis* experiment. This does not, however, explain why the pattern would be completely reversed in our experiment rather than just finding no pattern or a weaker pattern. A second difference is that our stream did not utilize flow, primarily because *G. holbrooki* generally inhabit low to no flow areas of streams, swamps, and lakes (Global Invasive Species Database). However, it is unclear why flow would generate differential dispersal patterns between social and asocial individuals.

Cognitive flexibility, demonstrated in this experiment by using a detour assay, did not show an effect upon dispersal scores. That is consistent with our prior work studying effects of fluoxetine (Chapter 1), suggesting that this measure of cognitive performance is not a strong predictor of dispersal tendency. Even though this measure of cognition does not affect dispersal, there may be other measures of cognitive performance that do affect movement, so additional studies would be necessary to determine the relationship.

In our case, fish that were more bold, more explorative, and more active (positive loadings on PC1) were more likely to disperse. It is possible that individuals who explore
an area and are more active are more likely to come across new areas into which they can disperse. Individuals who are more bold face the risk of predation by leaving the safety of their known habitat, but also may show a selective advantage by first colonizing new areas and being able to utilize resources. A previous study on bluebirds found that more aggressive and bold individuals were more likely to disperse and colonize, and that these individuals showed increased success and fecundity (Duckworth 2008). Earlier models have found that organisms who explore an area more, such as in the meadow brown butterfly (Condradt et al 2000), are more likely to come across a potential new habitat to utilize. Our findings support these previous studies, as more bold and explorative individuals seem to be more likely to find and take advantage of resource opportunities.

In comparing our results from the OFT to that of Cote et al. (2010) with the western mosquitofish, we again find a difference. In that study, only sociability and not performance in the OFT (activity, boldness, exploration) explained variation in dispersal. In the case of the eastern mosquitofish, we found that variation in these behavioral traits did in fact explain dispersal and in the predicted direction. Similar to the contrasting results for sociability, it is difficult to explain differences in our results if not for differences in artificial stream design or subtle differences in species biology or ecology.

We also found effects of sex and body size on variation in dispersal. Differences between the sexes in dispersal may be expected if the sexes differ in their motivation to disperse such as to avoid resource competition or to find new reproductive opportunities/lower reproductive competition (Bowler and Benton 2005). Previous work on the hummingbird flower mite showed that males responded to sex ratios within a group by moving from a male-biased group to a females-biased group when available
Our results are consistent with previously observed sex differences in movement. In our study, only 3 of the 25 female *Gambusia* dispersed (12%), whereas 11 of the 23 male *Gambusia* dispersed (48%). The sex differences observed in our study may be related to another trend observed in the data. *Gambusia holbrooki* is known to be sexually dimorphic: males are smaller in body size with determinate while females are generally – and sometimes considerably – larger in size with indeterminate growth (Henry and Black 2008, Martin et al 2019). We found an effect of body size on dispersal with smaller individuals being more likely to disperse at all. Whether sex differences explain body differences or vice versa remains unclear. Therefore, it is difficult to know the cause of either result for sure beyond only speculation. What is also difficult to explain is the interaction between body size and PC1. Both terms were significant on their own interacted significantly. There is no clear trend in the relationship between PC1 and dispersal across body sizes. It is possible that this interaction is driven by one individual in the largest body size group generating the strongest positive slope of any of the body size bins. If this is the case, it would seem to make more sense to interpret those two factors individually rather than to attempt to interpret weak and equivocal variability in patterns across body size.

Previous studies have suggested that dispersal may be driven by factors like kin selection, inbreeding avoidance, and habitat variability (Bowler and Benton 2005). In an effort to find out what else may affect dispersal tendency, we examined a suite of individual-level traits including sociability, cognitive flexibility, boldness, activity, and exploration. We found that dispersal can be predicted by some of these traits in *G. holbrooki*, suggesting some degree of predictability of potential movement tendencies.
from underlying traits. Future work examining other causes of dispersal, such as aggression, mate choice, and cognitive and physiological traits may be useful into developing a more completely understanding of the proximate causes of dispersal dynamics. Finally, examining reproductive success and fecundity may also be helpful in explaining how dispersal affects population dynamics and invasion success.
REFERENCES


Behavioural Brain Research, 296, 301–310.


Environmental Pollution, 261, 114-150.


Archives of Environmental Contamination and Toxicology, 54(2), 325–330.

Hoset, K. S., Ferchaud Al. L., Dufour, F., Mersch, D., Cote, J., Galliard, J. Natal dispersal correlates with behavioral traits that are not consistent across early life stages, 


Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 369(1656), 20130587.


Environmental Toxicology and Chemistry, 25(10), 2561–2568.

Lee, Finnbar; Simon, Kevin S.; Perry, George L. W. (2018). Prey selectivity and ontogenetic diet shift of the globally invasive western mosquitofish (Gambusia affinis) in agriculturally impacted streams. 

Ecology of Freshwater Fish, 27(3), 822-833.


Ecology, 84(8), 2145-2154.

Lucon-Xiccato, Tyrone; Bisazza, Angelo (2017). Sex differences in spatial abilities and cognitive flexibility in the guppy. 

Animal Behaviour, 123, 53–60.


APPENDIX A

IACUC PERMIT
April 27, 2020

TO: Zach Culumber

SUBJECT: Notice of Approval

Principal Investigator: Zach Culumber

Approval Date: April 27, 2020

UAH Approval Number and Proposal Titles:

<table>
<thead>
<tr>
<th>Year</th>
<th>Code</th>
<th>Short Title</th>
<th>Principal Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td>2020</td>
<td>R002</td>
<td>Personality</td>
<td>Zach Culumber</td>
</tr>
<tr>
<td>2020</td>
<td>R003</td>
<td>Fluoxetine</td>
<td>Zach Culumber</td>
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<tr>
<td>2020</td>
<td>R004</td>
<td>Thermal</td>
<td>Zach Culumber</td>
</tr>
</tbody>
</table>

The applications described above have been reviewed and approved by the UAH Animal Care and Use Committee.

The committee notes that you may need a DEA permit for the Fluoxetine studies. Also, the committee notes that you should include decapitation as a means of euthanasia on the cover sheet for the Fluoxetine study. Lastly, the committee notes that you may still want to chill the decapitated fish (or decapitate the chilled fish) to slow down biochemical changes in the tissues.

This approval will be in effect for three years from the date of approval. If you have any significant amendments to make (change in PI, change in number or species, significant change in protocol, etc.) please let me know as soon as possible.

Best regards,
Roy Magnuson, Chair, UAH IACUC,
Associate Professor, Department of Biological Sciences,
University of Alabama in Huntsville
SC 369K, 301 Sparkman Drive, Huntsville, AL 35899
Email: Roy.Magnuson@uah.edu  Cell: 256-724-0704
APPENDIX B

SUPPLEMENTARY MATERIALS

Table B.1: Loadings for PC1.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Component 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploration</td>
<td>0.945</td>
</tr>
<tr>
<td>Boldness</td>
<td>0.456</td>
</tr>
<tr>
<td>Activity</td>
<td>0.897</td>
</tr>
</tbody>
</table>

Figure B.1: Range map of *G. holbrooki*. 
Figure B.2: Side view of isolation setup.

Figure B.3. Top view of isolation setup.
Figure B.4: Illustration of the top view of the sociability arena.

Figure B.5: Photo of the top view of the sociability arena.
Figure B.6: Illustration of the top view of the detour arena.

Figure B.7: Photo of the top view of the detour arena.
Figure B.8: Illustration of the top view of the entire artificial stream set-up.

Figure B.9: Illustration of the side view inside of each dispersal pool, depicting examples of the funnel, barrier, and PVC tube.
Figure B.10: Photo of the top view of an individual pool, showing an example of the funnel, barrier, flowerpots, and fake plants in each pool.

Figure B.11: Photo of the top view of a pool when filled with water, showing an example of the funnel, barrier, and PVC sections.
Figure B.12: Illustration of the top view of the open field trial arena.

Figure B.13: Photo of the open field trial arena and webcam placement.