The effect of predator presence on the grouping behavior of Trinidadian killifish from high predation and killifish only populations.

Mariah Noelle Cornelio, Emily Khuu, Katelyn McCoy, Emmanuel Reyes

Biology 2242 – Undergraduate Research Experience.

Professor Matthew Walsh

April 28, 2024

Abstract

Within a species, genetic variation arises from a number of factors, notably isolation and predation. These factors form a selective pressure that encourages adaptations to emerge. As such, the self-isolating killifish of Trinidad seem like perfect candidates to study these principles in action. *Anablepsoides hartii* is a species whose ability to jump upstream has led to a community or few different fish evolving new adaptations parallel to the first ones – namely, behaviors. The differences in these populations may be captured by experimentation and observation, such that potential behavioral differences can be determined. As such, our group performed exactly that, observing the occurrence of grouping behavior when stressed and not stressed for a randomized subset of each population type. Fish were settled into fish tanks and predator cue was poured into the tank environment to observe the reactions the fish had. Indeed, there were differences between high predation and killifish only populations. It was found that fish from the killifish only population were much more likely to group together, inconsistent with the hypothesis. What also was interesting was the severity of the reaction being different between populations from different rivers, with fish from the Arepo river becoming far more individualistic in comparison to the other river's population. It would seem that these traits corresponded with the different populations, suggesting behavioral changes related to their self-isolating behaviors.

Introduction

Given the natural dynamics of the food chain and the promotion of predator-hunt-prey interactions, grouping behavior in species is quite common. When organisms are met with stressors like predator presence, gathering numbers may provide a safety advantage to those in a group. A prime example of this anti-predator adaptation is observed when zebras travel in herds and utilize their stripes to confuse their hunters. In this way, the probability of an individual getting captured and killed significantly decreases by “diluting” themselves into the crowd (Ioannou 2017). Conversely, other species, such as the snow leopard, can exhibit independent behavior rather than grouping when confronted with a predator. Also, it has been observed in banded killifish that this species grouping mechanism is context dependent. The banded killifish tends to form smaller groups when food is available to reduce competition but gather in larger groups when there's a perceived threat to increase safety. (Hoare et al. 2004) In hindsight, it does not matter where an animal lies on the food chain. Animals as small as mice and beetles can be solitary hunters and may not rely on groups for protection or survival. In this scientific study, this behavior in killifish is investigated and analyzed to determine their preference for either solitary or group behavior in high-stress situations, particularly the presence of predators.

Rivulus hartii, recently reclassified as *Anablepsoides*. *hartii*, is a species of killifish native to the Caribbean island of Trinidad and Tobago, specifically the Arima and Aripo river systems. Members of this species are noted as having the unique skill to self-isolate (Gibb et al. 2011) via jumping up streams which leaves multiple distinct populations that develop apart from each other. These distinct populations continue to separate into either “high predation” or “killifish only”, each with evolutionary differences resulting from the population split, such as differences in brain and eye size. (Howell et al. 2020). Trinidadian stream systems are unique in that they are typically situated in the form of long, layered waterfalls where you may find different populations of *R. hartii* in longitudinal, shallow pools. Diversity is greatest in the lower-elevation pools, which house *R. hartii* as well as a multitude of other piscivorous species. Moving to mid-level elevations, the species diversity begins to thin out. These shallow pools mainly consist of *R. hartii* and guppies (*Poecilia reticulata),* and high-elevation sites contain only killifish species. (El-Sabaawi RW, et al. 2012) These observed differences could manifest in traits that are not just physical. It is possible that behavioral traits between the two populations are different, reflecting their different evolutionary developments.

To determine if there are behavioral differences in killifish responses, a stressor was added to a controlled environment, and the subsequent reactions observed. It was previously questioned whether these populations had different responses to the threat of predation. Thus, the experiment developed here intends to explore whether these different populations tend to hide in groups or alone and find trends between the types of locations they may choose.

A “predator cue,” composed of water in which a predating species resided, can simulate the presence of said predator and elicit a stress reaction. Said water contains a small amount of chemicals the Killifish can smell. The introduction of predator scents could trigger a reaction so powerful that an organism might opt to seek out a new shelter for their home. (Vail & McCormick 2011) When adding predator cue to the habitat, it should be noted that the concentration of the cues dissipates, causing little to no reaction after an extended period of time, therefore not affecting the measured grouping behavior of killifish when under stress (Chivers et al. 2013) (Pestanda et al. 2013). By analyzing these trends, the objective is to establish correlations in grouping behavior with either population.

Grouping together is generally an effective anti-predator strategy, and if grouping behavior corresponds with a more successful survival rate, this could be attributed to the Killifish only population developing a higher evolutionary pathway through adaptive behaviors. This is evident through the notion that a species becomes more apt for survival and proliferation through collectivism against a threat via evolutionary changes and natural selection. It is important to note that correlation is not necessarily causation, but the behavioral measurements will still provide insight on the development of killifish in relation to predation stressors. (Howell and Walsh 2022). With this information, the most logical basis of this experiment will be that high predation populations will tend to group together when hiding due to predator cue while the killifish-only populations will group less when exposed to it.

Materials and Methods

The equipment used for this experiment includes a 10-gallon bare-bottom glass tank to which we added 5.55 inches of water taken from a water system to which the fish were already accustomed. Using descendants of fish from the AP and AM rivers, with one set of 6 non-gender-specific fish chosen at random from a large sample size from a low predation area of the stream. Another set of 10 non-gender-specific fish chosen at random from a large sample size, is taken from a downstream site with high predation. The fish are provided with four sets of shelters, as follows: one large leafy plant, a trio of shorter plants using gravel to hold the plants to the bottom of the tank, a smattering of rocks to provide a substrate, and a small PVC pipe oriented so that one end faces the glass allowing observation of the inside of the PVC pipe during the experiment (Figure 1). All shelters are manufactured and not of natural origins. A cylindrical container with openings on both ends was placed into the water and the fish were placed in the container. The fish were allowed to acclimate for a minute and a half, then the container was lifted. Then the number of fish in each shelter spot was documented. The same treatment was administered in a separate tank for each group of fish with the addition of 100ml (about 3.38 oz) of water from a predator's tank added while the fish were in the cylinder.

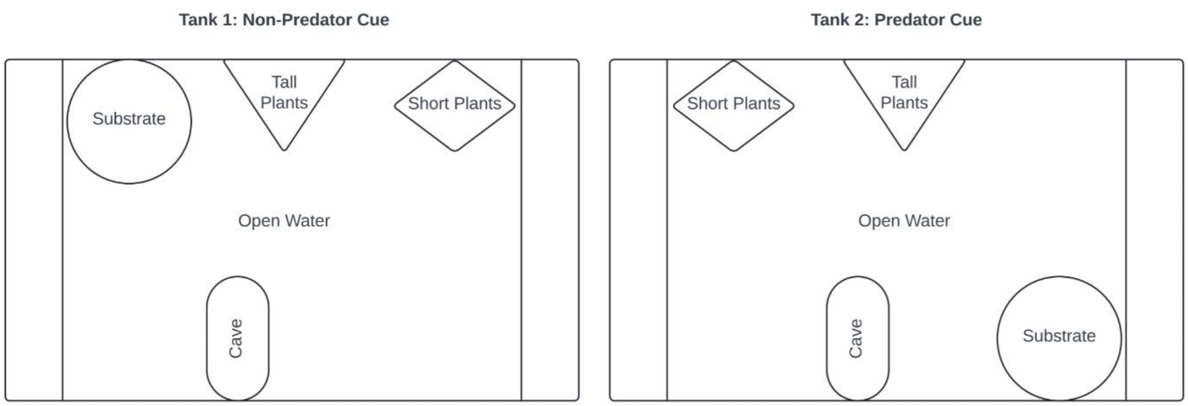

Figure 1. Placement of shelters in Tank 1 (non-predator cue tank) and Tank 2 (predator cue tank).

For the data processing and analysis procedure, each trial was filmed on an iPad Pro tablet settled on a tripod stand and then uploaded to the database. Counts of each fish location preference and other important details were placed in the metadata file created on Microsoft Excel. Further numerical analysis of the data was performed on the Python and R programs. For R, “ggplot2”, “ggrepel”, “readr”, “dplyr”, and “RColorBrewer” were the packages used to filter and curate the graphs shown in the results section of this paper. For Python, the data was cleaned and transformed by label encoding categorical variables. The "numpy” package was used for numerical analysis in python while “pandas” and “matplotlib” were used to create graphs for the analysis.

Results

Once all methods of experimentation were completed, results and data were analyzed. Statistical tests were performed to inspect the data found. An ANOVA F-test was done first to describe relationships between characteristics of the experiment which included population, river, total, experiment water, and location. This test was done to propose which variables would prove to be most significant in this study so further tests performed for analysis could detail the findings from this ANOVA test. In Table 1, population, experiment water, the relationship between population and river, and the relationship between river and experiment water display a p-value that is less than alpha 0.05. The F-statistic of these four categories highlighted in blue are greater than the right-tail critical value of the ANOVA test (Table 1). Furthermore, the rest of the categories on this table fail to contain a p-value that is less than alpha 0.05 (meaning that their p-value was greater than alpha) and their F-statistic falls to the left of the critical value.

Table 1. ANOVA F-test table results for comparison between all variables.

ANOVA Test	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Population	1	10.1250	10.1250	52.3777	1.55e-11
River	1	0.1250	0.1250	0.6466	0.4225
Total	1	0.0356	0.0356	0.1840	0.6685
Experiment_Water	1	3.1269	3.1269	16.1756	8.71e-05
Location	1	0.0002	0.0002	0.0011	0.9740
Population:River	1	1.1309	1.1309	5.8504	0.0166
Population:Total	1	0.0007	0.0007	0.0036	0.9521
River:Total	1	0.0173	0.0173	0.0896	0.7650
Population:Experiment_Water	1	0.1320	0.1320	0.6830	0.4097
River:Experiment_Water	1	1.1237	1.1237	5.8129	0.0170
Total:Experiment_Water	1	0.0026	0.0026	0.0135	0.9077
Population:Location	1	0.0002	0.0002	0.0011	0.9733
River:Location	1	0.0005	0.0005	0.0024	0.9612
Total:Location	1	0.0013	0.0013	0.0065	0.9357
Experiment_Water:Location	1	1.61e-05	1.61e-05	8.31e-05	0.9927
Population:River:Total	1	0.0008	0.0008	0.0040	0.9494
Population:River:Experiment_Water	1	0.1331	0.1331	0.6884	0.4079
Population:Total:Experiment_Water	1	0.0412	0.0412	0.2129	0.6451
River:Total:Experiment_Water	1	0.0018	0.0018	0.0094	0.9230
Population:River:Location	1	5.02e-05	5.02e-05	0.0003	0.9872
Population:Total:Location	1	0.0016	0.0016	0.0083	0.9273
River:Total:Location	1	0.4490	0.4490	2.3229	0.1294
Population:Experiment_Water:Location	1	0.0006	0.0006	0.0033	0.9542
River:Experiment_Water:Location	1	0.0095	0.0095	0.0491	0.8250
Total:Experiment_Water:Location	1	0.1631	0.1631	0.8439	0.3596
Population:River:Total:Experiment_Water	1	0.0345	0.0345	0.1785	0.6732
Population:River:Total:Location	1	0.3988	0.3988	2.0629	0.1528
Population:River:Experiment_Water:Location	1	0.0002	0.0002	0.0012	0.9725
Population:Total:Experiment_Water:Location	1	0.0862	0.0862	0.4462	0.5051
River:Total:Experiment_Water:Location	1	0.0185	0.0185	0.0958	0.7573
Population:River:Total:Experiment_Water:Location	1	0.2384	0.2384	1.2334	0.2683
Residuals	168	32.4756	0.1933	NA	NA

Table 2. Statistical test result summaries performed on three different experimental questions devised based on this experiment.

Statistical Test Result Summaries

Test 1 Question: Is there a relationship between population (KO vs HP) and grouping after predator cue is added?

Test	df	Statistic	p_value
Chi-Square Test	1	38.81704	4.654463e-10

Test 2 Question: Is there a relationship between water cue (non-predator vs predator) and grouping?

Test	df	Statistic	p_value
Chi-Square Test	1	11.54887	0.0006779037

Test 3 Question: Is the average of grouping behavior greater than zero?

Test	Statistic	P_value	df	Mean_of_x	Confidence_interval
One-sample T-Test	7.507136903	4.36E-09	39	2.86875	[2.09580624635972, 3.64169375364028]

Likewise, the chi-square and t-tests were also performed to answer the scientific questions asked in this experiment. Results of the first chi-square test to answer question one reveal that the p-value is significantly less than alpha. For this test being a one sample right-tailed t-test, the test statistic falls to the right of the critical value. The results of question two also show that the p-value is less than alpha, and the test statistic falls to the right of the critical value. The third test result is a one-sample right-tailed t-test with a p-value less than alpha and degrees of freedom of 39, meaning there are n=40 samples. The mean of this test falls between the confidence interval of [2.096, 3.6417] and the t-statistic falls to the right of the critical value (Table 2).

Pie charts using R were then generated based on the social behavior of comparisons between killifish-only versus high-predation and Arima versus Aripo river populations in the non-predator cue and predator cue samples. Regarding the killifish-only section in the “KO vs HP” pie charts, around 97% of the fish showed grouping in the non-predator water whereas that value reduced – but still surpassed individualistic behavior – to 60% in the predator water. In the high-predation section of the same chart, 40% showed grouping before predator cue was added and reduced significantly to only show 20% grouping (Figure 2). In Figure 2, the green color is more present in the "KO" section while the red color is more prominent in the "HP" segment.

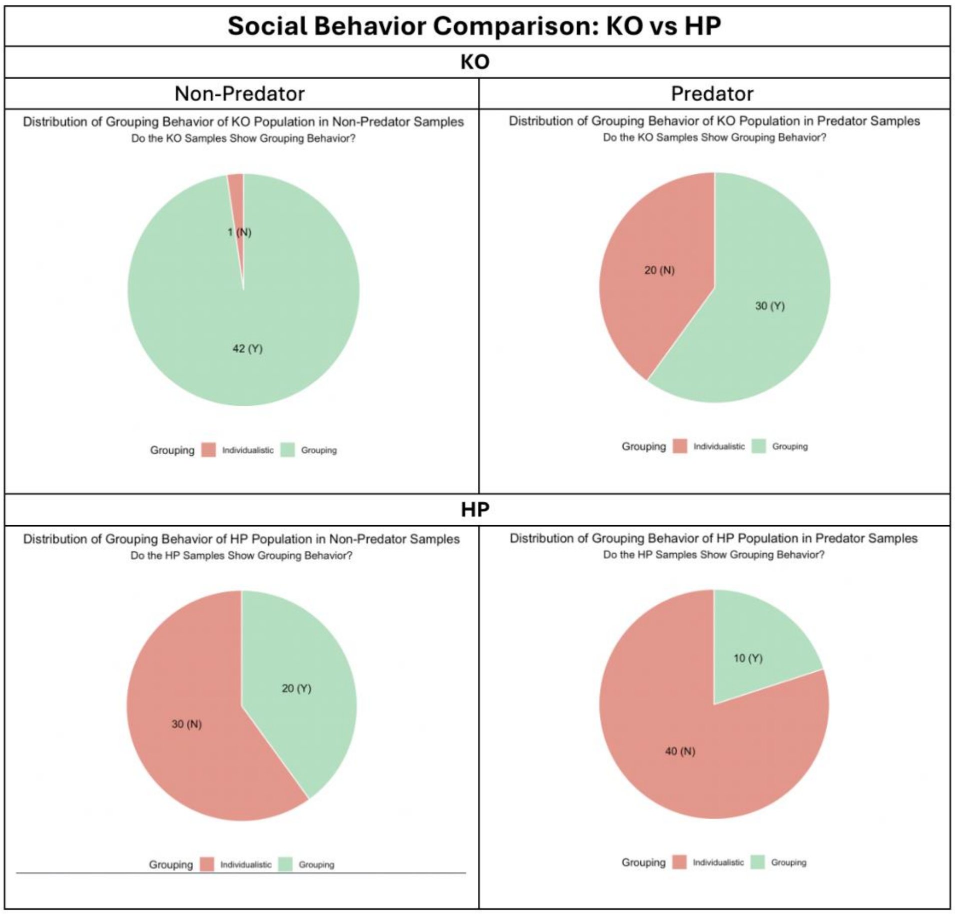

Figure 2. Comparison of social behavior (grouping or individualistic) between killifish-only (KO) and high-predation (HP) populations in non-predator cue versus predator cue samples.

Additionally, a "AM vs AP" pie chart was also created. In the Arima (AM) river section, approximately 62% of fish showed grouping behavior before predator presence and decreased to a 50-50 ratio of grouping and individualistic behavior after the cue was added. In the Aripo (AP) river segment, around the same ratio of 70% reflects grouping behavior in the previous "AM

Non-Predator" section. After the cue was added, the red overtakes green as the grouping behavior dropped to 30% which is the inverse of its non-predator counterpart (Figure 3).

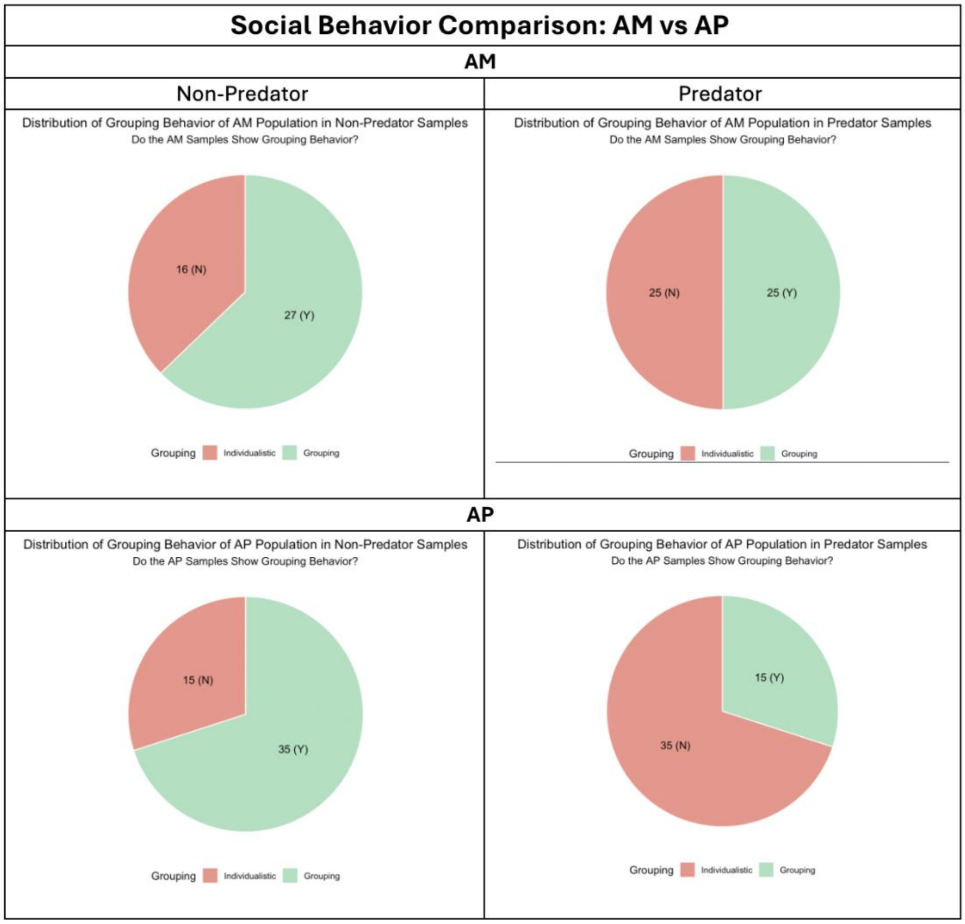

Figure 3. Comparison of social behavior (grouping or individualistic) between the Arima River (AM) and Aripo River (AP) populations in non-predator cue versus predator cue samples.

Furthermore, variances of the data were collected and analyzed. For “location by population”, the KO population exhibits more variance by about 1.8. In “grouping by population”, the HP population variance barely surpasses the KO bar by about .025 (Figure 4).

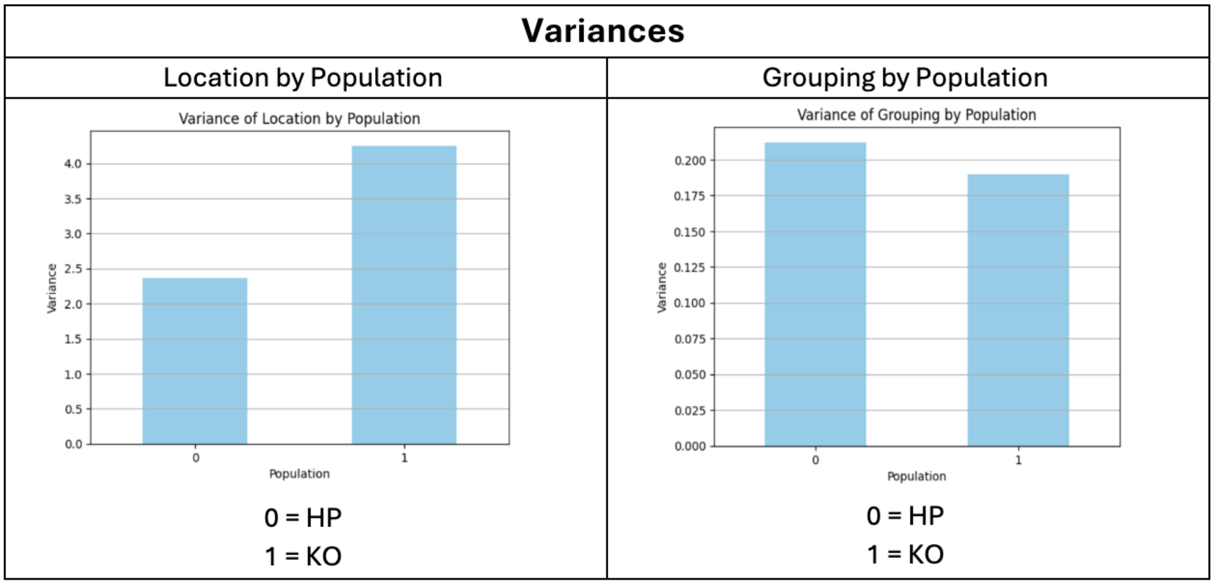

Figure 4. Variances of different variables by population using binary values.

Next, initial shelter preference was analyzed at 0 minutes signifying the event of once the fish were transferred to the tanks before and after predator cue was added. This analysis was done using bar charts for the "KO vs HP" and the "AM vs AP" population differentiation. Disregarding open water as a hiding spot, before the predator water was put into the tank, KO and HP fishes preferred the short plant. After the cue was added, KO fishes still preferred the short plant whereas HP fishes evenly preferred to hide in the tall plant in addition to the short (Figure 5). Moreover, for the "AM vs AP" section and also disregarding open water as a hiding place, Arima and Aripo fishes preferred the same hiding spots as the "KO vs AP" comparison before and after predator water was placed into the tanks (Figure 5).

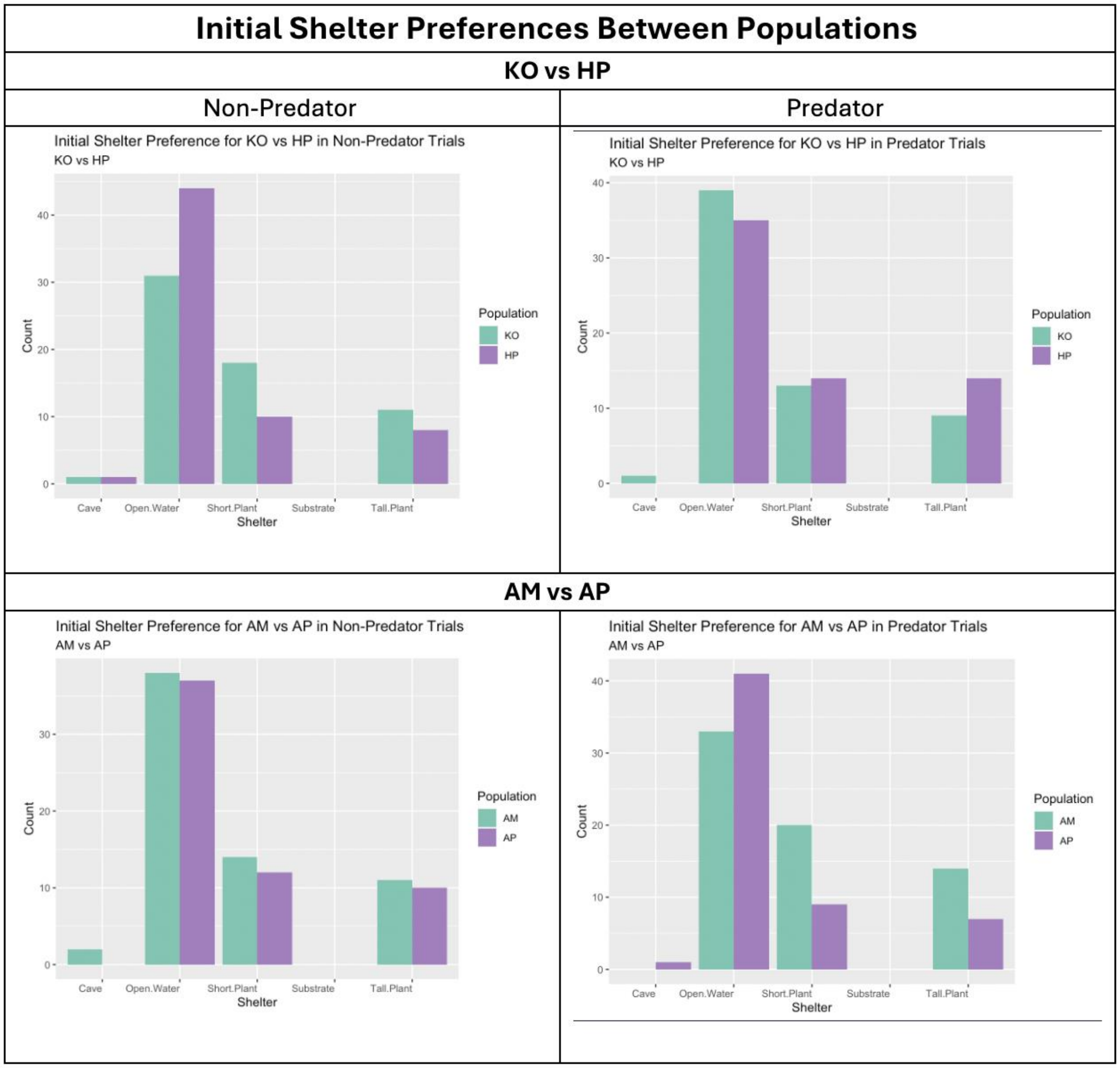

Figure 5. Initial shelter preferences (0 minutes) between KO vs HP and AM vs AP populations in non-predator cue versus predator cue samples.

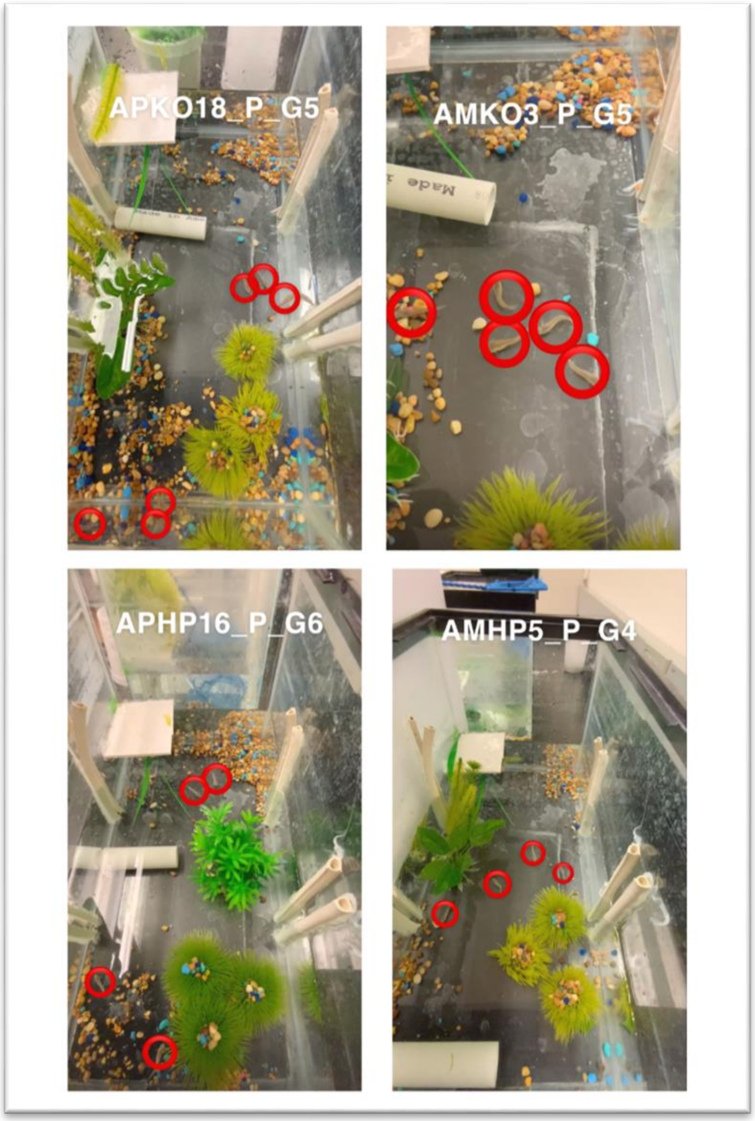

Figure 6. Screenshot analysis from video evidence of social behavior under stress of predation. Two samples come from KO sites (top) and the other two from HP sites (bottom). Screenshots were taken strictly within the first minute after predator cue was added so effects would not wear off as time went on.

To consolidate the results found, visual evidence taken from the videos recorded of each trial were taken to observe the social behavior of the fishes after the predator cue was added into the tank (Figure 6). “APKO18_P_G5” (top left) shows the killifish grouping in two groups of 3. As seen in the picture, 5 out of the 6 circles overlap. In the top right quadrant, “AMKO3_P_G5” shows 4 killifish close to one another situated in the open water. One is more to the left of the group. “APHP16_P_G6” (bottom left) shows two fish near the top of the tank together while the other two are at the bottom spread apart in two different places where the one on the bottom right is hiding slightly underneath the short bushes while the other one is in the substrate. “AMHP5_P_G4” (bottom right) appears to have 4 killifish spread out from each other where one seems to be going to the substrate, the next one to the tall plant, one in open water, and the last one facing the short plant.

Discussion

With the data collected and analyzed, overall results do not support our hypothesis that HP populations group more when there is predator presence, but rather the KO populations exhibit this behavior when there is a predator around. The discussion of the figures and tables below show and explain how our hypothesis is disproved and the alternate hypothesis of KO exhibiting grouping in predator presence is supported.

Statistical Analysis of Grouping Behavior:

Statistical testing was conducted to assess the statistical significance of variables influencing grouping behavior in killifish populations. ANOVA results revealed that population was the most significant predictor of grouping behavior, with an F value of 52.37 and a p-value of 0.0000000000155, indicating a highly significant relationship between population and grouping. (Table 1). This highlights the importance of population differences between KO and

HP in shaping killifish grouping behavior. Furthermore, chi-squared tests demonstrated significant associations between grouping behavior and both populations, with a p-value = 0.000000000465, reinforcing just how significantly the effect population type has on killifish grouping tendencies (Table 2). The null hypothesis for this test expressed that there is no relationship between population (KO vs HP) and grouping after predator cue is added while the alternative hypothesis expressed that there is a relationship between these two variables after predator cue is added. Because the p-value was less than alpha = 0.05, the null hypothesis was ultimately rejected, showing a connection between the variables stated. The close relationship between population and grouping was further supported by visual evidence, as depicted in Figure 6, where KO populations consistently exhibited grouping behavior in both pictures compared to HP populations which only showed one instance of grouping when the predator cue was added.

Another chi-squared test examined the next most statistically significant relationship found in the ANOVA with an F value of 16.18 and a p-value of 0.0000871 between grouping behavior and experiment water (Table 1). The null hypothesis for this test stated that there is no relationship between experimental water cue used and grouping while the alternative hypothesis stated that there is a relationship between those two factors. The chi-squared test revealed a statistically significant association with a p-value of 0.00068. With the null hypothesis being rejected, this suggests that the type of experiment water used, particularly the presence of predator cues in the water, significantly influence killifish grouping behavior. (Table 2.)

Lastly, a one-sample t-test was conducted on the variance/mean calculation of grouping behavior, yielding a p-value of .00000000436, indicating a high and statistically significant degree of grouping behavior observed across trials. The null hypothesis for this test stated that the average instances of grouping behavior would be equal to 0, indicating that there is no significant effect of grouping behavior while the alternative hypothesis stated that the average instances of grouping behavior would be greater than 0, indicating significant effects. With the p-value being less than alpha = 0.05, this rejects the null hypothesis and indicates that there is indeed a significant display of grouping behavior in the studied killifish populations (Table 2).

Effects of Population Differences on Grouping Behavior:

The analysis revealed notable differences in grouping behavior between killifish populations from KO and HP habitats. Killifish from KO populations exhibited a significantly higher inclination to grouping compared to those from HP populations, regardless of the presence or absence of predator cues. For instance, KO populations displayed grouping in 97.67% of trials without predator cues and 60% of trials with predator cues, whereas HP populations exhibited grouping in only 40% of trials without predator cues and 20% of trials with predator cues (Figure 2).

This disparity in grouping behavior may be attributed to evolutionary differences between KO and HP fish, as suggested by previous research indicating that male KO fish have slightly larger brains and potentially superior sensory and cognitive abilities compared to male HP fish (Walsh et al., 2016). These cognitive advantages could enable KO fish to effectively process visual and olfactory information, facilitating faster learning and adaptive behaviors such as grouping. Such behaviors are commonly observed across various animal species as a defense mechanism against predators, suggesting that grouping enhances the survival prospects of KO fish in their natural habitat.

Effects of River Origin on Grouping Behavior:

When considering the influence of river origin on grouping behavior, distinct patterns emerged between killifish populations from the AM river and AP river. The data indicates that killifish from the AM river displayed more consistent grouping behavior, with 62.79% of trials without predator cues and 50% of trials with predator cues exhibiting grouping. In contrast, killifish from the AP river exhibited the highest degree of grouping in trials without predator cues at 70%, but grouping was less prevalent in trials with predator cues, observed in only 30% of cases. (Figure 3).

Despite both rivers being situated within Trinidad and Tobago, the two rivers do differ in a few categories, including water speed and the amount of light available. For instance, the water speed from the KO region of the Aripo River is averaged at 2 L/S, while the HP area averages at 52.7 L/S, an increase of 2500%. Whereas the average for the KO region of the Arima river is 25 L/S, while the HP average is 32 L/S, only a 28% increase. Additionally, the amount of light in percentage of open canopy is similar between rivers, altering locations by HP and KO, where the KO has roughly half the amount of HP. (El-Sabaawi RW, et al. 2012) Furthermore, the Aripo River flows into the Atlantic Ocean, while the Arima River feeds into the Caroni River. These environmental disparities likely contribute to variations in habitat complexity, predation pressure, and resource availability, influencing the behavioral strategies adopted by killifish populations. Fish are known to change their life history tactics in response to environmental factors, with predation risk being a key driver. For example, populations experiencing high predation pressure may prioritize reproductive output over individual survival, potentially altering their behavioral responses to predators (Cavraro et al., 2014).

Despite the differences in river origin and population characteristics, a consistent trend emerges across trials: killifish exhibit less grouping behavior in the presence of predator cues. This suggests that while grouping may be an effective anti-predator strategy in certain contexts, it is not universally employed by killifish populations under predation pressure. Rather, grouping appears to be a context-dependent response influenced by a combination of intrinsic factors such as population differences and extrinsic factors such as predator presence.

Variance and Shelter Preference Analysis:

The variance analysis indicates that KO populations exhibit less variability in grouping behavior compared to HP populations, consistent with their higher propensity to group. Additionally, KO fish exhibits more variability when choosing a hiding location. (Figure 4) To be more precise, both KO and HP killifish displayed a strong preference for open water habitats, regardless of predator presence. This preference was consistent across trials conducted in both predator and non-predator conditions. The next favored hiding spots were the short plants, then the tall plants, followed by the cave.

In trials with predator cues, a notable deviation was observed: the cave was exclusively occupied by KO killifish, whereas HP killifish primarily utilized other hiding spots. This trend was consistent across trials conducted with killifish populations from the Arima and Aripo rivers, with both AM and AP populations displaying a similar preference for open water habitats followed by the short bush, then the tall bush, and the cave being predominantly occupied by one population in predator trials. (Figure 4)

The observed preference for open water over the substrate location is intriguing, considering killifish's natural inclination to choose hiding spots that allow them to match their background (Kjernsmo and Merilaita 2012). Killifish tend to select habitats that allow them to blend into their surroundings, allowing their tan-colored bodies to camouflage against brown and tan substrates. However, in our experiments, killifish exhibited an inclination to either forego hiding altogether or hide out of sight under plant leaves or within the cave. This deviation from expected hiding behavior could be attributed to the fish displaying an instinctual behavior of freezing instead of startling when in the presence of predators. (Fischer et al. 2014) With this in mind, it makes more sense that the fish stayed out in the open water as that was where they were intially deposited when the predator water was added to the tank. Additionally, killifish are known to show aggression towards each other in close quarters, which may have influenced their choice of hiding spots in the experimental setup after the initial freezing behavior (McGhee and Travis, 2010).

While this aspect of behavior is not directly central to our hypothesis regarding grouping behavior, it provides valuable insights into the complex interplay of ecological and behavioral factors shaping killifish responses to predation risk. Further research is warranted to explore the underlying mechanisms driving habitat selection and hiding behavior in killifish populations, elucidating the adaptive significance of these behaviors in predator-prey interactions.

Overview:

Overall, the results of several statistical tests and analysis indicate that not only is there is a statistically significant relationship between the grouping behavior and the population and experiment water which contributes to whether killifish will group or not, but the degree of grouping that is occurring is significant. These specific tests were used as the ANOVA table in Table 1 suggested a relationship between these factors and were furthermore solidified using the appropriate tests to aid in data visualization. Because the statistical tests performed indicated that population had the most statistically significant variable in relation to grouping but the aggregated experimental results show that fish from the KO population group more frequently and consistently, it can be concluded that the aggregated experimental tests do not support the hypothesis which states that killifish from the HP population exhibit more grouping behavior than KO when under the pressure of predation.

Implications and Limitations:

The observed behavior of killifish in response to predation cues provides valuable insight into the adaptive strategies employed by individualistic animals facing imminent danger. Killifish are not typically known to school but instead typically show aggression towards each other (McGhee and Travis, 2010). Our experiment corroborates this notion, as the fish exhibited more individualistic behavior in the presence of a predator, albeit with variations among populations. However, the emergence of grouping behavior, particularly in certain populations, suggests a potential for adaptive learning and collective defense mechanisms in response to predation threats.

If killifish can learn to employ grouping as an anti-predator behavior, it indicates that for similar grouping adaptations among other individualistic species facing predation threats. In fact, it is possible for species with inherent social tendencies to overcome their typical behaviors and band together in the face of danger. (Webster 2023). Such behavioral plasticity and adaptive responses are crucial for species survival in unpredictable environments, highlighting the importance of understanding and studying anti-predator strategies in diverse ecological contexts.

It is important to acknowledge certain limitations of our study, such as the inherent variability in natural populations and the controlled laboratory conditions. While carrying out the experiment, some constants were changed to get optimal results. For example, during the first week of data collection there were initially 10 fish per trial which decreased to 5 before finally settling on 6 fish per trial. The number of fish has inadvertently affected the data, because when performing a full table Anova, it was found that the number of fish in the trials had a significant effect on grouping. Additionally, a board was not used initially to separate the fish from the surrounding environment, meaning that movements from humans may have influenced the fish movement in the first day of data collection. Furthermore, the context of the experiment pushed the fish into situations where they may have had no choice but to group or to be near each other, which was counted as grouping. This experiment cannot fully conclude that grouping is a behavior commonly seen in killifish at all.

In summary, this research delves into the intricate social dynamics of killifish populations, particularly in response to predator cues and environmental conditions. Through a detailed combination of experimentation and statistical analysis, several key observations have been made. First and foremost, it becomes evident that the origin of killifish populations significantly influences their social behavior. Fish hailing from regions with an absence of natural predators tend to exhibit a higher propensity for group behavior when confronted with predator cues. This underscores the profound impact of evolutionary history and environmental context on social tendencies. Additionally, distinct variations in grouping behavior were observed among populations originating from different river systems. For instance, individuals from the Arima River demonstrated a greater inclination towards group behavior compared to their counterparts from the Aripo River. This research also revealed that the killifish from both populations and locations showed a propensity to seek shelter in the shorter plant area over the other shelters provided.

Moving forward, this study illuminates exciting avenues for future research. Exploring the underlying physiological mechanisms behind observed behavioral patterns promises to yield deeper insights into the adaptive strategies of killifish. Further research could focus on investigating the influence of other environmental variables, such as water quality and habitat composition. A greater understanding of these variables holds immense potential for enriching our understanding of aquatic ecosystems' dynamics. In essence, this research serves as a vital steppingstone in unraveling the complexities of killifish social behavior. By clarifying the complex interaction among genetic background, environmental conditions, and behavioral reactions to predation pressures as well as towards other fish, this study makes a substantial contribution to our broader comprehension of evolutionary ecology and animal behavior in aquatic settings.

References

Beston, S. M., & Walsh, M. R. (2019). Natural selection favours a larger eye in response to increased competition in natural populations of a vertebrate. Functional Ecology, 33(7), 1321–1331. https://doi.org/10.1111/1365-2435.13334

Cavraro F, Irini Daouti, Ioannis Leonardos, Torricelli P, Stefano Malavasi. 2014. Linking habitat structure to life history strategy: Insights from a Mediterranean killifish. Journal of sea research (Den Burg Print). 85:205–213. doi:https://doi.org/10.1016/j.seares.2013.05.004.

Chivers DP, Dixson DL, White JR, McCormick MI, Ferrari MC (2013). Degradation of chemical alarm cues and assessment of risk throughout the day. Ecology and Evolution. 2013;3(11):3925–3934. doi:10.1002/ece3.760

El-Sabaawi RW, Kohler TJ, Zandonà E, et al. 2012. Environmental and Organismal Predictors of Intraspecific Variation in the Stoichiometry of a Neotropical Freshwater Fish. Pols One. 7(3):e32713–e32713. doi:https://doi.org/10.1371/journal.pone.0032713.

Fischer EK, Schwartz AJ, Hoke KL, Soares D. 2014. Social Context Modulates Predator Evasion Strategy In Guppies. Foster S, editor. Ethology. 121(4):364–371. doi:https://doi.org/10.1111/eth.12345.

Gibb, A. C., Skelton, J., Lewis, M. C., Sheehan, T. F., Johnson, T. P., Quinn, T. P., & Kolmann, M. A. (2011). Fish out of water: Terrestrial jumping by fully aquatic fishes. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 315A(10), 649–653. https://doi.org/10.1002/jez.711

Hoare DJ, Couzin ID, Godin J-G .J, Krause J. 2004. Context-dependent Group Size Choice in Fish. Animal Behaviour. 67(1):155–164. doi:https://doi.org/10.1016/j.anbehav.2003.04.004.

Howell, K. J., & Walsh, M. R. (2022). Transplant experiments demonstrate that larger brains are favoured in high-competition environments in Trinidadian killifish. Ecology Letters, 26(1), 53–62. https://doi.org/10.1111/ele.14133

Howell, K. J., Beston, S. M., & Walsh, M. R. (2020). Coordinated evolution of brain size, structure, and eye size in Trinidadian killifish. Ecology and Evolution, 11(1), 365–375. https://doi.org/10.1002/ece3.7051

Ioannou, C. 2017. Grouping and Predation. In: Shackelford, T., Weekes-Shackelford, V. (eds) Encyclopedia of Evolutionary Psychological Science. Springer, Cham. https://doi.org/10.1007/978-3-319-16999-6_2699-1

Kjernsmo K, Merilaita S. 2012. Background choice as an anti-predator strategy: the roles of background matching and visual complexity in the habitat choice of the least killifish.

Proceedings of the Royal Society B: Biological Sciences. 279(1745):4192–4198. doi:https://doi.org/10.1098/rspb.2012.1547.

McGhee KE, Travis J. 2010. Repeatable behavioural type and stable dominance rank in the bluefin killifish. Animal Behaviour. 79(2):497–507. doi:https://doi.org/10.1016/j.anbehav.2009.11.037.

Pestana JLT, Baird DJ, Soares AMVM. Predator threat assessment in Daphnia magna: the role of kairomones versus conspecific alarm cues. Marine and Freshwater Research. 2013 [accessed 2024 Mar 20];64(8):679–686. https://www.publish.csiro.au/mf/MF13043. doi:10.1071/MF13043

Vail AL, McCormick MI. 2011. Metamorphosing Reef Fishes Avoid Predator Scent When Choosing a Home. Biology Letters. 7(6):921–924. doi:https://doi.org/10.1098/rsbl.2011.0380.

Walsh MR, Broyles W, Beston SM, Munch SB. 2016. Predator-driven brain size evolution in natural populations of Trinidadian killifish (Rivulus hartii). Proceedings of the Royal Society B: Biological Sciences. 283(1834). doi:https://doi.org/10.1098/rspb.2016.1075. [accessed 2021 Jan 6]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4947895/.

Webster MM. 2023 Mar 29. Social learning in non-grouping animals. doi:https://doi.org/10.1111/brv.12954.