Sheltering & Foraging Behavior in Relation to Mating Season in the Crayfish *F. virilis*

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Abstract

      In this experiment, we studied the foraging and sheltering behavior in the freshwater crayfish *F. virilis.* We investigated the effect of sex and reproductive readiness on these behaviors, the individual repeatability of these behaviors, and attempted to identify evidence of a behavioral suite among these two behaviors. Crayfish were tested in two sets of three rounds, one set in the summer non-reproductive season and the other in the fall reproductive season, in assays designed to quantify aspects of shelter use and foraging behavior. We found that reproductive crayfish were more likely than nonreproductive crayfish to engage in the risky but rewarding behaviors of leaving their shelter and foraging for food. We also found that during the summer, when the crayfish are non-reproductive, females are also more likely than males to engage in those behaviors of leaving their shelter and foraging for food. Crayfish with longer carapace lengths were more likely to touch their food, though this was only statistically significant in reproductive crayfish. Nonreproductive males exhibited relatively high repeatability for sheltering times compared to females and reproductive males. All crayfish exhibited relatively high repeatability for latency to touch their food regardless of sex or reproductive season. The foraging and sheltering behaviors did not correlate between individual crayfish, showing no evidence of individual behavioral suites. However, behavioral patterns were found to differ between population groups, suggesting that factors such as sex or reproductive readiness influence behavior more than individual differences.

Table of Contents

Authorship

All team members contributed to all parts of the project.

Acknowledgements

1.0 Introduction

Behavioral plasticity and behavioral repeatability are related concepts in the study of animal behavior. Behavioral plasticity is when an organism can produce alternate behaviors based on different environmental factors (Gherardi et al., 2012). Oftentimes, the potential plasticity of an animal is dependent on its morphology and physiology. Physical capability can affect plasticity, such as the strength of a sense of smell allowing an animal to change its behavior when the scent of a predator is present (Gherardi et al., 2012). The conditions an individual is exposed to in its environment can also alter behavioral plasticity; a wider range of environmental conditions can lead to a wider range of plasticity. These individual environmental factors can play as large a role in determining the potential plasticity of an individual as genetic factors do (Breck et al., 2019).

Behavioral repeatability is a measure of the consistency of a certain behavioral response, and could be considered as the reverse of behavioral plasticity. The repeatability of a behavior can reflect the degree to which that behavior has ecological importance as an adaptive response, whereas a lack of behavioral repeatability can point to a lack of selective pressure on certain behaviors over others (McEvoy et al., 2015). With a lack of selective pressure acting on behavioral traits, behavioral plasticity may become the default condition. On the other hand, it could be that behavioral plasticity is helpful to animals in responding to environmental changes. Whether plasticity or repeatability is more adaptive can depend on the environmental context. Behavioral plasticity can help animals respond to environmental changes in the short term, while behavioral repeatability can be seen as a consequence of selection for optimal behavior in a relatively constant environment (Roche et al., 2016).

Behavioral repeatability is related to another concept in animal behavior, known as animal personality. Animal personality can be defined as "individual behavioral differences [that] are consistent over time and/or across situations" (Réale et al., 2007, p. 294). Research in animal personality has improved our understanding of how and when differences in behavior in populations may be adaptive (Réale et al., 2007). However, the presence of "personality" does not imply the absence of phenotypic behavioral plasticity (Roche et al., 2016). There is both a broad-sense concept of animal personality and a narrow-sense concept of animal personality. Broad-sense personality can be defined as any observable behavior that is consistently different between individuals of the same population. Narrow-sense personality can be defined as behaviors that are repeatedly measured by tests that are standardized across individuals (Roche et al., 2016). Since narrow-sense personality only encompasses behaviors measured in the lab, it can be argued that the lab conditions might not capture as much variation as would be shaped by natural selection. In relation to using artificial conditions, habituation to experiment can also affect the repeatability of behaviors. Familiarity with the experimental conditions can result in altered behavior and thus lower repeatability (Tan et al., 2018). It becomes especially important then to consider what is needed to validate whether behavior that is observed in standardized artificial conditions is what determines life-history traits and fitness-related behaviors in the natural environment of the test species. This type of validation is an important area of research in the field of behavioral ecology (Roche et al., 2016).

When behaviors are found to be repeatable, it is possible for them to exist in suites of correlated behaviors, which are known as behavioral syndromes. Behavioral plasticity precludes the occurrence of behavioral syndromes. The behaviors that comprise behavioral syndromes exist either within a behavioral context, such as correlations in foraging in different habitats, or across behavioral contexts, such as correlations between foraging and mating (Sih et al., 2004). In summary, behavioral plasticity and behavioral repeatability are reverses of one another, while behavioral repeatability is what comprises animal personality and behavioral syndromes can be seen as a term similar to animal personality in its measure of related behaviors.

The organism in which we studied behavioral repeatability, plasticity, and behavioral syndrome was *F. virilis,* a species of freshwater crayfish. *F. virilis* can be found in a vast range of different flowing and still water habitats, although they tend to prefer rivers and streams with rocky bottoms. They hide under rocks and gravel for cover, and if the substrate allows it they may dig burrows. They are polytrophic omnivores, and will eat aquatic plants, fish eggs, mollusks, and crustaceans, although most of their nutrition comes from scavenging dead animals. They are also prey for a wide variety of mammals, birds, and fish (Caldwell & Bovbjerg, 1969). The mating season of *F. virilis* is in September and October, and readiness for mating is conferred with morphological changes. Changing between the non-reproductive form, Form II, and the reproductive form, Form I, happens by molting, although the crayfish do also molt outside of the transition between seasons without changing their reproductive form. The molt into Form I occurs in the late summer, and the molt into Form II occurs in the late spring (Aiken, 1965). Reproductive females have wider abdomens and swollen white glair glands, and reproductive males differ from non-reproductive males by the structure of the first pleopods and robustness and size of the exoskeleton (Wetzel, 2002; Tierney et al., 2008).

Crayfish are solitary and territorial animals, and aggression is common amongst all crayfish behaviors (Kubec et al., 2019). Social hierarchy is an important aspect of crayfish social behavior. This hierarchy is generally determined by strength, but can also be influenced by other factors such as sex and size (Kubec et al., 2019). There is aggressive competition for shelter that is influenced by social hierarchy. Lower ranked *F. virilis* crayfish are less likely to use shelter than higher ranked ones when competing for shelter, and in *F. rusticus* crayfish, shelter availability impacts agonistic encounters and habitat choice (Fero & Moore, 2014; Chibucos et al., 2015). Reproductive behavior is also influenced by social hierarchy; in red swamp crayfish *Procambarus clarkii,* females eavesdrop on fights and decide to mate with the dominant male (Aquiloni et al., 2008). Crayfish behavior can be influenced by the season, sex, and by size; for example, male crayfish are more likely to engage in agonistic encounters with other males than females are with other females, and female-female encounters are less fatal (Kubec et al., 2019). Form II *F. rusticus* crayfish have exhibited more frequent sheltering compared to Form I animals, and Form II males have also exhibited less intraspecies aggression than Form I males (Tierney et al., 2008).

Freshwater crayfish can also be useful models for examining behavioral repeatability and plasticity, as well as behavioral syndromes. Pintor et al. (2008) performed a study to investigate the presence of behavioral suites in crayfish. They conducted behavioral assays to

compare four different behaviors: aggression, foraging voracity, threat response, and boldness to forage under predation risk. These assays were conducted on four different populations: in its native and invaded range, and in the presence and absence of the Shasta crayfish. They predicted that crayfish living in the invaded range and those living with the Shasta crayfish would be generally more aggressive across the four observed behaviors. Their findings about the behaviors of individual populations differed somewhat from their predictions, but more pertinent to our study, they found that signal crayfish have a positive correlation among three of the four observed behaviors: intraspecific aggression, novel prey foraging, and boldness to forage under risk of predation, suggesting the presence of an aggression behavioral suite. The authors stated that this suite could have evolved in these crayfish in order to ensure that invasive crayfish are capable of establishing a population, which would require aggression across multiple different behaviors. Although the crayfish we were investigating have many differences from the populations studied by Pintor et al. (2008), that investigation does show that crayfish are capable of exhibiting behavioral syndromes.

With all of the above background information in mind, our experiment was designed to quantitatively measure the intra-individual repeatability of foraging and sheltering behaviors in crayfish, and to identify if those behavioral tendencies have any correlation with a crayfish's physical characteristics. Our experimental design consisted of observing the behavioral responses of each individual crayfish in two separate tests. The first tested their tendency to seek and remain in a shelter when placed in a novel environment, and the second tested their latency to seek food, which may be a measure of risk-taking behavior. To quantify sheltering behavior, we compared the amount of time spent outside the shelter versus inside the shelter in a series of identical trials. Risk taking behavior was quantified by the time it took crayfish to approach food when it was presented concurrently with the "wake up" signal of a dropped rock. The experiments were repeated for each individual crayfish with a week between trials in order to identify repeatability of behavioral responses.

2.0 Methodology

2.1 Collection & Care of Crayfish

The crayfish were collected by hand from the Mill River in the Blackstone River watershed in late May 2021 (by L. Mathews) and brought to the WPI greenhouse. We took over the general crayfish care regimen in September. Each crayfish was kept in its own individual tank (28 cm x 13.3 cm) to prevent them from directly interacting with each other physically or exchanging chemical cues. Each tank included a PVC tube about 15 cm in length for use as a shelter. Sections of polystyrene egg crate were used to cover the containers to prevent crayfish from escaping. Fluorescent lights were set on a timer based on the natural day-night cycle of that period of time (timings were updated every two weeks). To maintain the health of the crayfish, we replaced about 50% of the water in their tanks with fresh tap water twice per week. The crayfish were each fed one food pellet three times per week: twice with rabbit pellets and once with shrimp pellets.

2.2 Experimental Design & Execution

The experiment took place in a set of two series of trials, one done in the summer when crayfish were in non-reproductive form, and one in the fall when they were in reproductive form. The summer trials were performed by L. Mathews and the fall trials were performed by our group. Each series of trials used identical methods. If any crayfish was discovered to have molted or died when we went in to feed or water change, its identifying number was recorded. Crayfish that died or molted during the experiment, or within seven days of the beginning or end of the experiment, were excluded from the data.

The fall phase of the experiment had a starting sample size of 96 crayfish, which were divided into four groups of replicates each containing 24 crayfish; these groups were made solely for the purpose of increasing the ease of testing, as we were only able to complete 24 replicates per day. We tested each crayfish once every seven days for a total of 3 rounds, with the four replicate groups being tested on consecutive days. Each group had a scheduled water change approximately 48 hours before testing and a feeding approximately 24 hours before testing. We set up a recording room with four digital video cameras adjacent to where the crayfish were being held. The recording room also contained fluorescent lights set on the same day/night cycle timer as the holding room. During the experiment, each replicate group was divided into four subgroups of six, with one of the four cameras recording each subgroup for 30 minutes.

For part 1 of the experiment, we placed crayfish individually into 24 experimental tanks identical in size to the holding tanks. Each tank was filled with fresh tap water to a depth of approximately 7.6 cm, and a PVC pipe shelter was placed in the center of the tank. Experimental tanks were labeled by replicate number, denoted by tape placed on the floor beside each set of six. Before crayfish were transferred to experimental tanks, black craft foam was fitted between each tank to prevent the crayfish from seeing each other. The 24 crayfish were removed from their holding tanks and each was placed into a plastic cup labeled with their identification number. Unique cups were used for each crayfish to prevent the transfer of chemical signals. After starting the recording on each camera, the crayfish were quickly transferred from their cups into their corresponding experimental tanks. We then left the recording room and the behavior of all 24 crayfish was recorded undisturbed for 30 minutes. When recording was complete, experimental tanks were covered with sections of egg crate to prevent crayfish from escaping, and they were left in the recording room overnight.

Part 2 of the experiment took place approximately 24 hours after part 1. The coverings were removed from the tanks, and the initial location of each crayfish (inside/outside the shelter) was recorded. To carry out the experiment, recording was initiated on all four cameras, and each researcher gathered six small rocks and six shrimp pellets in hand. For each subgroup of six crayfish, one rock and one shrimp pellet were simultaneously dropped into the same location of each tank; we worked quickly to minimize the amount of time between each drop. The crayfish were then recorded undisturbed for 30 minutes. Following the completion of part 2, all 24 crayfish in that group were collected in their unique cups and returned to their corresponding

holding tanks. The PVC shelters and rocks were then removed from each experimental tank and rinsed with fresh tap water. Tanks were then drained and rinsed, and 24 new experimental tanks were set up in the same configuration to carry out part 1 of the experiment on the next group of replicates.

Table 1 describes the schedule of experimentation. The schedule was composed of 5 consecutive days of running experiments and 2 "rest" days, during which feedings and water changes still took place, but no experiments were carried out. This was repeated a total of three times to create three consecutive sets of data for each replicate group, with each set of data separated by one week. We checked crayfish for molting or death when we fed the crayfish and changed their water. If a crayfish died before or during the experimental trials, we eliminated them from the dataset and if a crayfish molted during the experimental trials, we eliminated them from the dataset as well. After the 10-day post-experiment observation period, all surviving crayfish were re-released into the Mill River where they had been collected.

Table 1: Experimental Schedule

Day	Experiment	Group Water Changed	Group Fed
1		A	C
2		B	A, D
3	Group A Part 1	C	B
4	Group A Part 2; Group B Part 1	D	C
5	Group B Part 2; Group C Part 1		D
6	Group C Part 2; Group D Part 1		A
7	Group D Part 2		B

2.3 Video Scoring

Videos for part 1 and part 2 of the experiment were scored with different sets of criteria. Because recording was typically initiated before everything was in place (for ease of experimentation), the time at which the experiment officially began (all six crayfish in tanks for part 1, all six rocks and pellets dropped for part 2) was noted when scoring each video, and this time was then subtracted from

Figure 1: A dried crayfish with approximately 50% of its body in a shelter.

subsequent time notations during scoring to standardize the start time of all videos. For both parts, the time was noted for each instance of a crayfish entering or exiting their shelter. A crayfish entering or exiting its shelter was defined as when 50% of its body was inside or outside the shelter, as shown in the example in Figure 1. For part 1, this data was used to calculate the amount of time each crayfish spent inside vs. outside of its shelter when first placed into the experimental tank.

Video scoring for part 2 was similar to part 1; however, instead of noting every instance of a crayfish entering or exiting their shelter, only the first instance of a crayfish exiting their shelter was recorded (though some crayfish began the experiment outside of their shelter). Additionally for part 2, we recorded each instance of a crayfish interacting with the rock or with the food pellet. Interaction with either object was defined as a crayfish appearing to deliberately touch one of the stimuli with its claws or legs, to minimize the chances of falsely recording an accidental interaction (e.g. brushing the rock with their tail while feeding on the pellet).

Each video was observed and scored by one team member. The videos were divided equally among us in a way that ensured that no individual crayfish was scored by the same person more than once. This was done to ensure that any biases (e.g. differing interpretations of a crayfish entering/exiting a shelter) a researcher may have would be randomly distributed throughout the data.

2.4 Data Analysis

We first examined the data for general effects of factors like sex and body size on the following dependent variables: time in shelter (for part 1), whether or not crayfish touched the food (for part 2), and for those that did touch the food, the latency to do so. For latency to touch food in part 2, we only included crayfish that touched the food in all three rounds. To do the analysis, we created generalized linear mixed effect models (GLMMs) that used individual identity as a random effect to control for individual variability in behavioral responses. In these models, we used sex, carapace length, and round as fixed effects. Separate GLMMs were performed for the summer data and the fall data to determine if there were variations in these effects between seasons. For the dependent variables of time spent in shelter and time to touch the food, our models assumed a gamma error distribution, while for the dependent variable of whether or not the crayfish touched the food, we used the binomial distribution. All models were created using the R-package lme4.

We next looked at the repeatability of sheltering and foraging behaviors among individuals to determine if each crayfish tended to behave the same way across repeated experiments, and to determine if there was any variation between sex or season. Part 1 of the experiment focused on sheltering behavior, while part 2 focused on foraging behavior. To do this analysis, we used the following dependent variables: total time spent in shelter (for part 1), and latency to touch the food (for part 2). Only crayfish that touched the food in all three rounds were considered for part 2. Each model used individual identity as a random effect, with carapace length and round as fixed effects. Separate models were performed based on sex and season,

for a total of four analyses (one for each sex in each season). All repeatability values (R, also called ICC or intraclass correlation coefficient) were calculated by hand from equation 3.15 in Nakagawa et al. (2017) for gamma-distributed data, based on data from generalized linear mixed effect models.

We conducted a Pearson's correlation analysis to determine if there was any relationship between the sheltering behavior examined in part 1 (total time spent in shelter) and the foraging behavior examined in part 2 (latency to touch food). To do this, we used an online calculator (https://www.socscistatistics.com/tests/pearson/) using data from round 1 separately by sex and by season. As a result, this analysis excluded all crayfish that did not touch the food in round 1 of part 2.
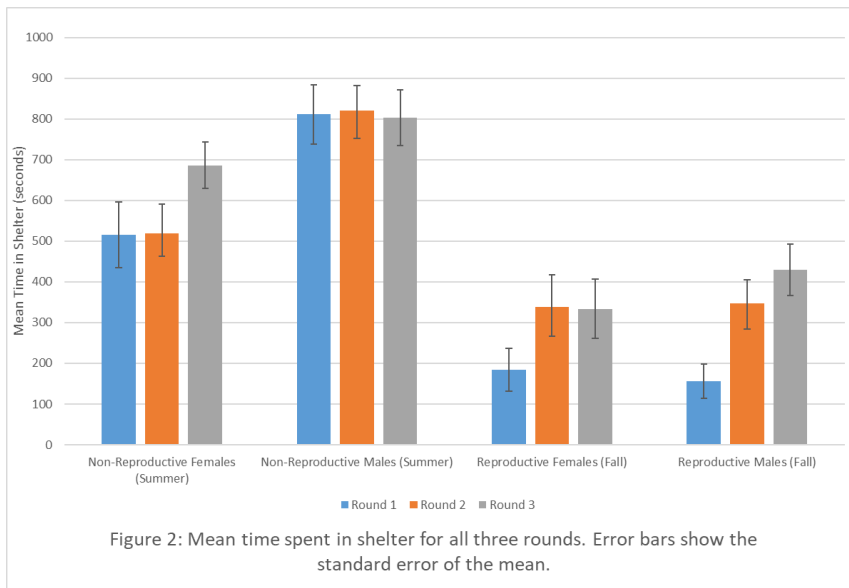
3.0 Results

3.1 Results Overview

Of the 118 crayfish collected for the summer experiments, 43 were excluded because they molted during the experiment or within a seven day period following the experiment, and four of the crayfish were excluded because they died during the experiment or within a seven day period following the experiment. In the fall rounds there were 96 total replicates. Five of those crayfish were excluded because they molted during the experiment or within a seven day period following the experiment, while three of the crayfish were excluded because they died during the experiment or within a seven day period following the experiment.

3.2 Part 1: Sheltering Time

For the dependent variable of total time that the crayfish spent in shelter in part 1 of the experiment, the only significant fixed effect in the fall was round number, reflecting that following the first round, both female and male crayfish had a higher mean sheltering time in the two final rounds. This may be evidence of the crayfish becoming acclimated to their environments, rather than it being evidence of a behavioral syndrome or behavioral repeatability. In the summer, the only significant fixed effect was

Figure 2: Mean time spent in shelter for all three rounds. Error bars show the standard error of the mean.

sex. Females were less likely to spend time in the shelter during the summer compared to males (Figure 2).

3.3 Part 2: Foraging Behavior

During the summer rounds but not the fall rounds, whether or not the crayfish touched the food was dependent on sex (Table 2). In the summer dataset, the proportion of female crayfish that touched the food was significantly higher than the proportion of male crayfish that touched the food (Figure 3). During the fall rounds, whether or not the crayfish touched the food was dependent on carapace length (Figure 4, Table 2). Crayfish with longer carapace lengths were significantly more likely to touch the food than those with shorter carapace lengths.

For those crayfish that did touch the food in all three summer rounds, their latency to touch the food during the summer was not impacted by any of the fixed effects (Table 2). However, their latency to touch the food in the fall was dependent on which round it was (Table 2). Figure 5 shows that the females in the fall took significantly less time to touch the food during round three than the previous rounds.

Figure 3: Proportions of crayfish that touched the food. This dataset includes only crayfish that touched the food in part 2 of all three rounds.

Figure 4: Mean latency to touch the food for crayfish that touched the food in all three rounds. Error bars are based on standard error of the mean.

Figure 5: Mean carapace size of crayfish that touched food and did not touch food. The carapace size was a significant fixed effect in the fall. Mean summer carapace size included for comparison. Error bars are based on the standard error of the mean.
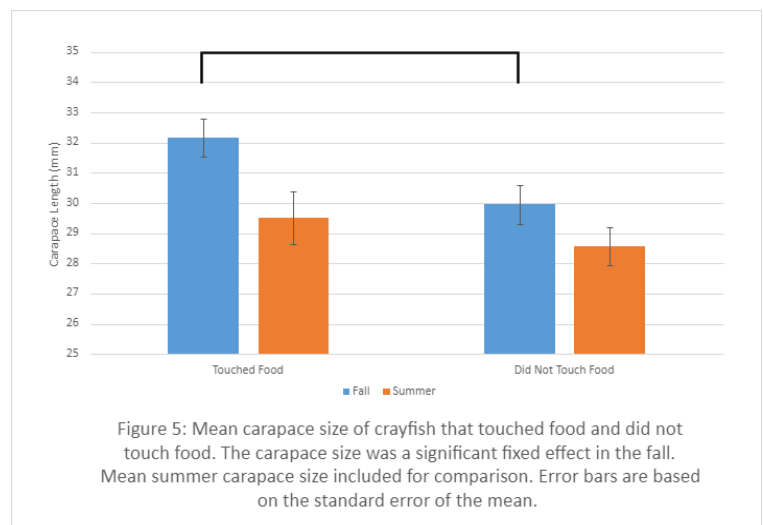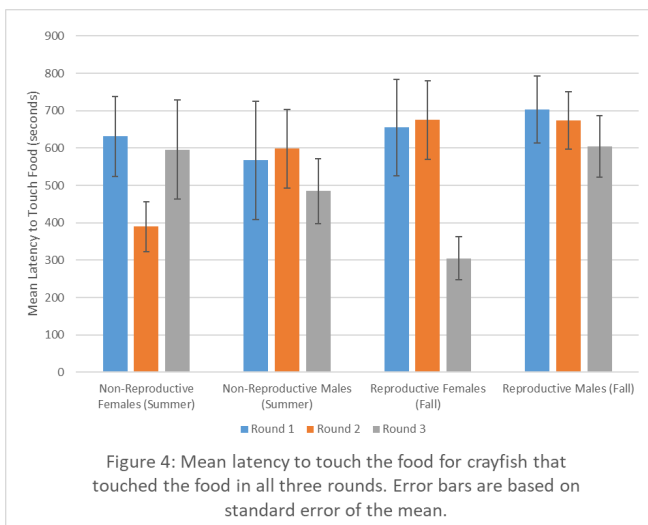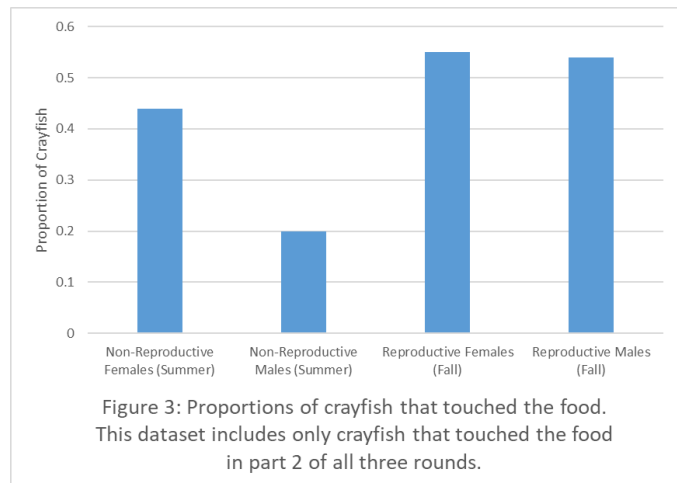
Table 2: Fixed effects that were included in models for three dependent variables in parts 1 and 2 of the experiment. Crayfish identity was set as a random effect for all models. For the third dependent variable, latency to touch the food, the analysis only included replicates where the crayfish touched the food within the trial time in all three rounds, which resulted in smaller sample sizes. Significant P values (< 0.05) are bolded.

Dependent variable	Season	N (Female, male)	Fixed effect	Estimate (SE)	P
Time spent in the shelter in part 1 of the experiment	Summer	69 (25, 44)	Sex	0.37 (0.148)	**0.012**
			Carapace length	-0.014 (0.017)	0.423
			Round	0.048 (0.085)	0.569
	Fall	78 (27, 51)	Sex	0.060 (0.366)	0.871
			Carapace length	-0.011 (0.039)	0.772
			Round	0.432 (0.218)	**0.047**
Whether or not crayfish touched food in part 2 of the experiment	Summer	69 (25, 44)	Sex	0.916 (0.417)	**0.028**
			Carapace length	-0.055 (0.046)	0.233
			Round	0.184 (0.192)	0.338
	Fall	78 (27, 51)	Sex	0.563 (0.532)	0.290
			Carapace length	-0.171 (0.066)	**0.010**
			Round	0.095 (0.219)	0.663
Latency to touch food in part 2 of the experiment	Summer	20 (11, 9)	Sex	0.040 (0.191)	0.835
			Carapace length	-0.008 (0.025)	0.759
			Round	-0.041 (0.097)	0.676
	Fall	43 (15, 28)	Sex	0.156 (0.211	0.460
			Carapace length	0.022 (0.026)	0.383
			Round	-0.149 (0.074)	**0.044**

3.4 Repeatability of Sheltering Time & Latency to Touch Food

Repeatability estimates for the dependent variables of time in shelter and latency to touch the food are shown in Table 3. For female crayfish, their repeatability value for sheltering was slightly lower in the fall than in the summer. The repeatability for males between seasons showed higher variation. Males appear to have more consistent sheltering behavior in the summer, but lose that consistency in the fall. Females appear to have the same lack of consistency in sheltering behavior in both the summer and the fall. Between seasons, there was a large difference in the *R* values for females, but the *R* value for males remained very similar between the summer and the fall. Females generally showed higher repeatability than males in both seasons, though these differences were small in magnitude.

Table 3: Repeatability (R) values for time in shelter and latency to touch food.

	Shelter Behavior	Latency to Touch Food
Non-Reproductive Females (Summer)	0.15	0.44
Non-Reproductive Males (Summer)	0.43	0.37
Reproductive Females (Fall)	0.13	0.37
Reproductive Males (Fall)	0.11	0.35

3.5 Correlation Between Sheltering Time & Latency to Touch Food

For males in the fall, there was a weak negative correlation of fall latency to touch food and fall sheltering time, while for summer males, there was a slightly higher positive correlation of summer latency to touch food and summer sheltering time. For females in the fall, there was a weak negative correlation of fall latency to touch food and fall sheltering time and for females in the summer, there was a weak positive correlation of summer latency to touch food and summer sheltering time. None of the correlations were statistically significant.
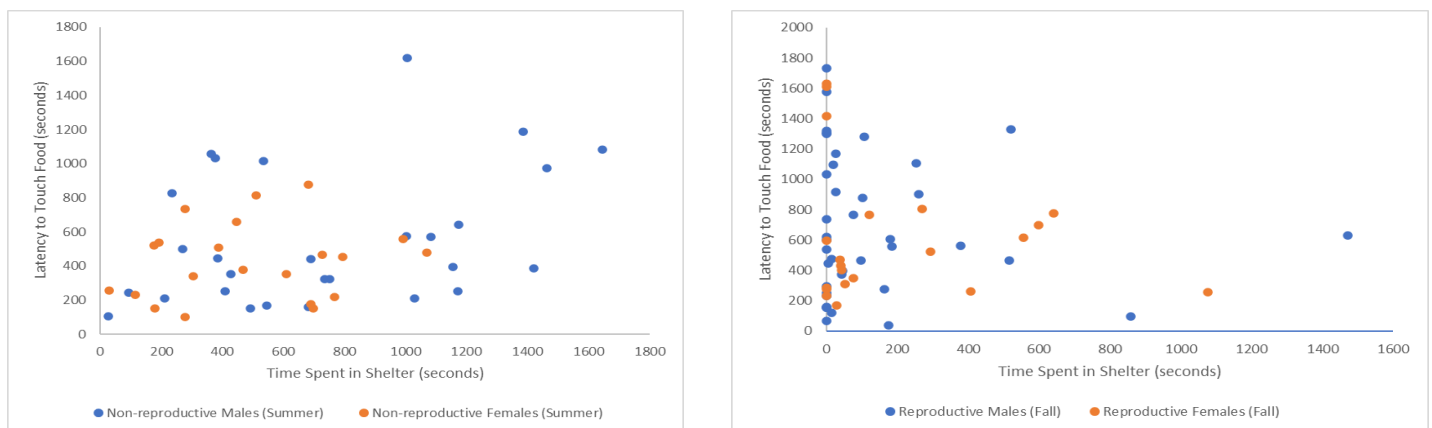

Figure 6: The time spent in shelter in part 1 compared to the latency to touch food in part 2 for summer non-reproductive (left) and fall reproductive males and females (right). This analysis includes only data from round 1 in each season, and excludes any crayfish that did not touch the food in round 1 of part 2 of the experiment.

Table 4: Pearson's correlation for the relationship between time spent in shelter and latency to touch food.

Season	Sex	N	Pearson's correlation coefficient	p
Summer (Non-reproductive)	Females	21	0.162	0.483
	Males	28	0.341	0.076
Fall (Reproductive)	Females	21	0.155	0.503
	Males	40	-0.072	0.663

4.0 Discussion

For prey animals like crayfish, sheltering and foraging behaviors are necessary behaviors that also increase their likelihood of predation, and can be a function of selection under conditions of predation and reproductive needs. Here, we report the results of an experiment to investigate the impact of reproductive physiology, sex, and carapace length on sheltering and foraging behaviors, as well as the individual repeatability of and correlation between those behaviors. Our experiment showed that both foraging and sheltering behavior varied based on a number of factors.

Between the summer non-reproductive season and the fall reproductive season, both female and male crayfish were more likely to spend less time in the shelter and to touch their food in the fall than in the summer. Both behaviors could be interpreted as risk-taking behaviors because leaving a source of protection, such as a shelter, whether in search of food or for another reason is more likely to expose the organism to predators and other environmental harms (Gherardi et al, 2012). This shows that the crayfish were more likely to engage in risk-taking behaviors in the fall, when they are in their reproductive form. Increased activity may have a general effect on the mating success of individuals during the reproductive season. The increased activity correlates with how male crayfish in their reproductive form are more likely to exhibit aggressive behaviors (Kubec et al, 2019).

During the non-reproductive season, the females in our study were more likely to engage in risk taking behavior than males. They had lower average sheltering time as well as having a higher likelihood to engage with food. Females overall required more energy and nutrients in the summer than males (Harlıoğlu & Farhadi, 2017). This could be because they need to produce eggs to prepare for the mating season in the fall, which would explain why they were more likely to forage for food in our experiment. This increased need to forage can also explain the decrease in sheltering times exhibited by the females in the summer, as they presumably need to leave their shelter to find food.

While both males and females showed differences in both sheltering and foraging between seasons, the magnitude of those differences was less for females than for males. In addition, the repeatability of behavior for females within each season was low, meaning that on an individual level, each female was not consistent in their behavior. Values that we interpreted as high or low repeatability were based on statements made by Waldron et al (2021) in their study about behavioral repeatability in salamanders, where latency was considered significantly repeatable if the R value was greater than 0.30. That study aligned with our experiment in its focus on behavioral repeatability and its measurement of latency to touch food as a foraging behavior. Consistently low repeatability means that the individual crayfish did not exhibit the same behavior multiple times, so the individual crayfish were not consistent in how quickly they touched the food. Foraging behavior may be strongly influenced by physiological state, which may not change over short periods of time (Harlıoğlu & Farhadi, 2017).

In comparison to individual repeatability, the differences of the mean values for behavior between the seasons was higher for males, specifically in regards to sheltering behavior. In the summer, males were the only group to have a high repeatability for sheltering behavior. All collected data indicates that male crayfish are unlikely to exit their shelter during the summer when they are in their non-reproductive form, as they spent more time in the shelter and were less likely to interact with food, and showed relatively high repeatability in these behaviors. The exact reason as to why the males in the summer behaved so differently from all other groups in the study is unknown.

During both seasons, it was also found that larger crayfish tended to engage more often in foraging. This was demonstrated during part 2 of the experiment, where we observed that crayfish with a greater carapace length were more likely to touch the food than those with a shorter length. However, this pattern was not observed with regards to sheltering behavior. We suggest that there may be multiple different factors influencing risk-taking behavior, because foraging behavior was correlated with size while sheltering behavior was not. It is possible that larger crayfish were more likely to engage in foraging behavior than smaller crayfish because larger crayfish are typically at an advantage when foraging as they are not as at risk by predators. It is also possible that larger crayfish were more likely to engage in foraging behavior than smaller crayfish because they needed more calories than smaller crayfish.

One of the shortcomings of this study is in regards to the sample sizes. While most of the sample sizes were reasonably large, the size for the latency to touch food in the summer was quite small, only including 20 individuals. Crayfish that molted too close to the experimental times, or during the experiments were excluded from the final data. As molting is known to affect the behavior of crayfish (Juneta-Nor et al., 2020), in particular in regards to appetite, not including those individuals would prevent their data from skewing the overall outcomes of the experimental sections. Any crayfish that died were also not included in the final data, even if they had been a part of some of the rounds of experimentation before their death. One final shortcoming is that the two groups of crayfish, the summer and fall groups, were not all the same individuals. Individuals that were not included in the summer group due to molting were included in the fall group, with the opposite being true as well. Also, crayfish that died between

seasons also contributed to the summer and fall groups being made of different individuals.

Future directions that this research could take might include using freshly caught crayfish for the summer and the fall experiments rather than using the same group of crayfish for both seasons. Since the same set of crayfish were used for the summer and fall experiments, it is possible that the observed differences in behavior between seasons could be a result of physiological or cognitive changes induced by prolonged exposure to laboratory conditions, rather than an inherent biological phenomenon. Along the lines of seasonality, future research could look at other behaviors beyond sheltering and foraging that might also differ between seasons, or at behaviors that are correlated with sheltering and reproductive morphologies. In addition, our study identified the potential that carapace length has an effect on foraging behavior, so more studies looking into the effect of size on behavior could help to explain the reasoning behind our observations.

5.0 References

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