**The Effect of Social Isolation on Goldfishes’ Ability to Retain Spatial Memory**

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Spatial memory refers to an organism’s ability to create a mental map of its surroundings. This type of memory is often stored as long-term memory, and can be further classified as explicit or declarative memory, meaning that it requires conscious thought. Spatial memory relates to personal experience, which allows it to be further identified as a type of episodic memory (Freberg, 2016). The formation and retention of spatial memory is an essential function of many organisms including humans. Without spatial memory, one would not be able to recall the location of objects or know how to navigate from one location to another.

The hippocampus creates and stores spatial memory. This structure is located in the telencephalon, which is part of the forebrain (Portevella et al., 2002). Forebrain configurations vary substantially among vertebrate groups; members of infraclass Teleostei, which include most ray-finned fishes, have a greatly reduced forebrain that lacks a hippocampus altogether (Yamamoto, 2009). Due to this, teleost fish were once believed to be incapable of forming spatial memories. Field observations and laboratory studies have debunked this idea. Some teleost fish, such as Pacific salmon (*Oncorhynchus tshawytscha*), use environmental cues to navigate during long seasonal migrations (Dittman & Quinn, 1996). Similarly, many scientists have successfully taught goldfish (*Carassius auratus*) to complete spatial tasks in laboratory settings.

Churchill (1916) created a maze that required goldfish to swim through three compartments to obtain a food reward. After undergoing several trials, the fish were able to significantly reduce the amount of time it took to complete the maze, suggesting that the fish used spatial memory to reach their goal, and did not swim to the correct compartment by chance. Additionally, a few fish successfully completed retention tests after thirteen days lapse of practice. After examining the strategies goldfish use to complete spatial tasks, Lopez et al. (1999) concluded that teleost fish, like mammals and birds, use separate systems for spatial learning and memory. Vargas et al. (2004) discovered that goldfish are able to encode geometric and featural information simultaneously to create a map-like representation of their environment. This is an activity that rats, and even adolescent humans, may be incapable of performing. These experiments have confirmed that teleost fish can form and retain spatial memories and have navigational abilities that rival those of mammals (Vargas et al., 2004).

Teleost fish, then, must possess brain structures that are homologous to the mammalian hippocampus. Both mammals and fish have a pallium, which makes up the dorsal portion of the telencephalic lobes (Huesa et al., 2009). Salas et al. (1996) taught goldfish to swim through a four-arm maze and then performed telencephalic ablations on some of his test subjects. Results showed ablated fish were no longer able to use place strategies to complete the maze. Further research demonstrated that lesions made to the pallium of goldfish impaired the animals’ spatial learning abilities, supporting the idea that the teleost fish lateral pallium is homologous to the mammalian hippocampus (Portevella, 2002). Additionally, single-cell recording techniques have provided the first evidence that teleost fishes have place cells, a type of hippocampal neuron that functions in spatial memory (Canfield & Mizumori, 2004).

Atrophy of the hippocampus is characteristic of humans who have been diagnosed with Alzheimer’s disease (Juottonen et al., 1999). Additionally, irregular place cell firing has been observed in Alzheimer’s patients (Mably et al., 2016). These facts make it unsurprising that a loss of episodic memory, specifically spatial memory, is a hallmark of the onset of Alzheimer’s disease (Serino et al., 2014). Hsiao et al. (2012) noted that humans who undergo social isolation are at a higher risk of developing Alzheimer’s disease. Social isolation decreases the level of neurotransmitters present in the hippocampus (Shao et al. 2015). Therefore, it appears likely that social isolation may correlate with impaired retention of spatial memory, however, few studies have been conducted on this topic. Pisu et al. (2011) and Schrijver et al. (2004) tested the effects of social isolation on the *formation* of spatial memory using rats as a model. Pisu et al. concluded that rats who were kept in social isolation formed new spatial memories quicker than rats kept in social groups while Schrijver et al. concluded that social isolation has no effect on the formation of spatial memories. Quan et al. (2010) tested the effects of social isolation on the *retention* of spatial memory using rats as a model and concluded that social isolation impairs spatial memory retention. It does not appear as though teleost fish have ever been used as a model for testing the effects of social isolation on the retention of spatial memory.

*Research Question*

Does social isolation impair goldfishes’ ability to retain spatial memory?

*Hypothesis*

Although teleost fish lack hippocampi, I predict that living in social isolation will alter the fishes’ brain function, resulting in impaired retention of spatial memory.

**Method**

**Subjects**

Twenty-four goldfish were acquired from Carolina Biological Supply and kept according to Malone University’s *Psychology Vivarium/Animal Care Facility Guidelines*. The fish were housed in two separate 10 gallon glass aquariums named Tank A1 and Tank A2. Twelve fish were housed in each aquarium, which allowed for approximately one gallon of water per centimeter of fish. The vendor ships fish in sets of twelve; this housing method was chosen to maintain the schools the fish arrived in.

The ten gallon aquariums were set up five days prior to the fish's arrival to ensure a stable temperature and pH were capable of being maintained. Throughout the experiment, the water in each aquarium was filtered, maintained at approximately 20 degrees Celsius, and maintained at a pH between 7.35 and 8.88. A correlation has been found between enhanced spatial learning and memory in goldfish and the presence of environmental enrichment (Abreu et al., 2019). Due to this, a green and a purple plastic aquarium plant were placed in the southeast and southwest corners of each tank respectively.

A 12:12 light-dark cycle was implemented for the duration of the experiment (Sánchez-Vázquez et al., 1996). The photophase took place between 08.15 and 20.15, and the goldfish were fed a 1% wet body weight ratio of *TetraFin* goldfish flakes once a day at 14.15 (Tinoco et al., 2012). The flakes were stirred in water prior to being fed to the fish to prevent air-gulping while the subjects ate. The 1% wet body weight ratio was determined using the following equation: [total number of subjects x the average weight of the subjects in grams x 0.01] (Deal & Volkoff, 2021). The average body weight of all subjects was obtained by placing each fish in a beaker of water on a tared scale and recording its weight (Priestley et al., 2006).

The fish were acclimated to the new aquariums according to Carolina Biological Supply’s “Freshwater Fish Acclimation'' protocol (<https://www.carolina.com/teacher-resources/Video/how-to-acclimate-freshwater-fish-video/tr11220.tr>). Upon arrival, the closed plastic bags that the fish arrived in were floated on the surface of their respective aquariums for a duration of thirty minutes. The bags were then opened and floated for an additional ten minutes. To complete the acclimation process, one quarter of the water from each bag was replaced with aquarium water once every ten minutes. This process continued for one hour, resulting in a total of six water replacements for each bag. Once fully acclimated, the fish were transferred into their respective aquariums using a net. No water from the shipping bags was introduced into the aquariums. During the first forty-eight hours of being introduced to the new aquariums, the lights in the laboratory were kept off allowing only natural light in, and the fish were fasted. Further, the fish were allowed to acclimate to their new environment for fourteen days before the experiment commenced. The conditions of tanks and fish were recorded daily and are available for inspection upon request.

**Apparatus**

The maze was modeled after Saito and Watanabe’s (2005) modified version of a Morris water maze, and was constructed out of a 75 x 75 x 10 cm clear plexiglass container. The maze was filled with 5 cm of water kept at approximately 20 degrees Celsius and a pH between 7.25 and 8.65. The water in the maze was unfiltered to prevent currents from creating intramaze cues. A piece of 75 x 75 cm acrylic board was used as the floor. Sixteen holes were drilled into the acrylic board forming a 4 x 4 lattice pattern. Each hole was 15 cm away from all other holes, and no hole was closer than 15 cm from the nearest wall. A clear, BPA free, recyclable plastic tube with a diameter of 9 cm was used as a start box (Figure 1). The following process will henceforth be referred to as “the start box process”: at the commencement of each trial, the tube was placed inside the maze, either against the middle of the west wall or against the middle of the east wall. After the start box was positioned, a fish was removed from its aquarium using a net and lowered through the top of the tube. Each fish was allowed to sit in the start box for fifteen seconds to acclimate to its surroundings. The tube was then lifted off the floor of the maze to release the fish. The tube was completely removed from the maze after the fish was released to prevent the subjects from using the tube as an intramaze cue.

Goldfish rely on allocentric navigation and extramaze cues to complete spatial tasks (Rodriguez et al., 1994). Four pieces of 75 x 10 cm poster board acted as extramaze cues in this experiment. Goldfish are tetrachromats (Neumeyer, 1992), and the maximum pigment absorbance within their cones has been determined to be between 440 and 470 nm, 525 and 530 nm, and 220 and 230 nm, which appear as blue, green, and orange respectively (Yager & Thorpe, 1970). Each piece of poster board was painted a unique color and placed up against one of the maze’s walls. The poster board facing the north wall was blue, the poster board facing the west wall was green, the poster board facing the south wall was black, and the poster board facing the east wall was orange.

**Figure 1**

*Depiction of the Maze Apparatus*

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*Note.* Depiction of the maze apparatus (not drawn to scale). The fifteen small dark gray circles represent the holes drilled into the floor of the maze, the star indicates the baited hole, and the two large light grey circles represent the start box placements.

**Procedures**

Experimentation consisted of three stages, habituation trials, acquisition trials, and retention trials. Some of the methods used in the habituation and acquisition trials were adopted from Saito and Watanabe’s (2005) study. Habituation trials began on the fifteenth day of the experiment, immediately after the fourteen-day acclimation period has ended. The fish were fasted on the last day of the acclimation period in an attempt to motivate them during habituation trials; the fish were also refused flake food for the duration of habituation trials to increase the likelihood that they would consume bloodworms while in the maze. Each fish went through one habituation trial a day for four days. A plastic divider was used to separate each ten gallon tank at the commencement of trials. All fish began on the south side of the divider, but were placed on the north side of the divider after they completed their habituation trial for the day. After every fish completed its habituation trial, the dividers were removed from the tanks.

During habituation trials, eight of the sixteen holes in the floor of the maze were baited with *Tetra* freeze dried bloodworms. The bloodworms were sectioned and weighed to ensure that the fish did not receive more than a 1% wet body weight ratio of food. Each fish was introduced to the maze using the start box process. During the first and third day of habituation trials, the start box was positioned in the middle of the west wall of the maze, and during the second and fourth day, the start box was placed in the middle of the east wall. Upon entering the maze, each fish was allowed to eat bloodworms for a duration of ten minutes. Time started being recorded the moment the tube was raised.

After habituation trials ended, the fish participated in two acquisition trials a day for fourteen days. Each day, prior to the commencement of trials, 1,500 mL of water was removed from the maze and replaced with 1,500 mL of water from one of the ten gallon tanks (A1). This was done in an effort to reduce the chance of osmotic shock when introducing the fish to a new environment. All the fish from tank A1 were tested in random order. To reduce the amount of water mixed between the two ten gallon tanks, an additional 2,000 mL of water was removed from the maze and replaced with 2,000 mL of water from the other ten gallon tank (A2) before the fish from tank A2 were introduced to the maze. After the water change was complete, all fish from A2 were tested in random order. In an attempt to reduce confounds, the order in which the fish were tested was counterbalanced. For example, all fish from tank A1 were tested before the fish from tank A2 on odd numbered days and all the fish from tank A2 were tested before the fish from tank A1 on even numbered days.

 During each acquisition trial, only a single hole was baited with a bloodworm (Figure 1). The location of the baited hole did not change for the duration of the experiment. At the beginning of each acquisition session, both ten gallon aquariums were divided. All fish began on the south side of the divider. Each fish was transferred from the ten gallon aquarium to the start box using a net; once inside the start box, the fish were introduced to the maze using the start box process. The order of start box placement alternated every day. For example, on odd numbered days, the start box was placed on the west wall of the maze for each fish’s first trial, but was positioned on the east wall for each fish’s second trial, however on even numbered days, the start box was placed on the east wall of the maze for each fish’s first trial, but was positioned on the west wall for each fish’s second trial. After being released from the start box, each fish was allowed to search for the hidden bloodworm for two minutes. Time began being recorded the moment the tube was raised and stopped being recorded the moment a fish touched the bloodworm. If a fish did not find the bloodworm in the allotted time, it was removed from the maze at the end of the two minutes. Regardless of whether or not a fish found the bloodworm, all fish were transferred from the maze to a 500 mL beaker at the end of their first trial. Each fish remained in the beaker for ten minutes before being returned to the maze for the second trial. The second trial was conducted in the same way as the first, except the start box was placed on the opposite wall of the maze. After completing the second acquisition trial, each fish was returned to the north side of the divided ten gallon tank it came from. After all fish finished both acquisition trials, the dividers were removed from the aquariums.

After acquisition trials were completed, the two schools of goldfish were randomly assigned to one of two experimental groups: the Social Group and the Isolation Group. To maintain the schools they arrived in, the fish who lived in Tank A2 acted as the Social Group and the fish who lived in Tank A1 acted as the Isolation Group. Each fish assigned to the Isolation Group was transferred to a separate ten gallon filtered glass aquarium where they lived in solitude. In an attempt to reduce confounds, the fish assigned to the Social Group were also transferred from to a new ten gallon filtered glass aquarium where they continued to live in a group. Each new aquarium had an identical set up to the original aquarium the fish lived in; a filter was positioned on the north wall, a green plastic aquarium plant was placed in the southeast corner, and a purpleplastic aquarium plant was placed in the south west corner. Blackout material was placed on the west wall of each aquarium to prevent the fish in isolation from seeing each other. Blackout material was also placed on the west wall of the aquarium housing the Social Group to eliminate confounds. The water in each aquarium was kept at approximately 20 degrees Celsius and maintained at a pH between 7.62 and 9.3.

After the fish were separated into their respective treatments, they no longer entered the maze on a daily basis. Instead, they underwent a thirteen-day period of lapse of practice followed by two retention trials on the fourteenth day. Retention trials were conducted in the same way as acquisition trials with the following exception: each fish was given five minutes to locate the hidden bloodworm instead of two minutes. The pattern of a thirteen-day rest period followed by two retention trials on the fourteenth day continued for twenty-eight days, resulting in each fish undergoing a total of four retention trials on two separate days.

**Results**

The total number of fish living in Tank A1 and Tank A2 who consumed bloodworms in the maze on each day of habituation trials is displayed in Table 1. A Chi-square test was used to analyze the data gathered from each day of habituation trials to determine if a difference in performance existed between the fish living in Tank A1 and the fish living in Tank A2. For each Chi-square test performed, no significant differences were found between the two groups, *X2* (1, *N* = 20) = 0.09, *p* = 0.76 on day one, *X2* (1, *N* = 20) = 0.21, p = 65 on day two, *X2* (1, *N* = 19) = 0.88, *p* = 0.35 on day three, and *X2* (1, *N* = 19) < 0.01, *p* = 0.96 on day four.

The fishes' latencies throughout acquisition trials were analyzed using a mixed design analysis of variance (ANOVA). The experimental group that each fish was in (Social versus Isolation) was used as the between-subjects factor and the day of the trial (1 through 14) was used as the within-subjects factor. Each day, the individual fishes’ performance in the maze was rated on a scale from 0-2. Fish who did not find the bloodworm during either one of the two trials for that day were given a rating of 0, fish who found the bloodworm during one of the two trials were given a rating of 1, and fish who found bloodworms during both of the trials were given a rating of 2. Mauchly’s test of sphericity indicated that variance was similar across all days of acquisition trials, *Mauchly’s W* (90) = 0.00 *p* = < 0.10, allowing the data analysis to be continued with univariate tests and using the calculated statistics with sphericity assumed. Both the between-subjects and within-subjects effects were significant, *F*(1, 18) = 8.84, *p* < 0.01, and *F*(13, 234) = 3.10, *p* < 0.01 respectively. The interaction was also significant, *F*(13, 234) = 3.01, *p* < 0.01.

At the commencement of the study, I planned to calculate the average time it took each fish in the Social Group to complete the maze during each retention trial and the average time it took each fish in the Isolation Group to complete the maze during each retention trial and analyze the average using a T-test. However, no fish successfully completed the maze during retention trials, preventing the analysis from being conducted.

**Table 1**

*Total Number of Subjects from Each Tank that Consumed Bloodworms During Each Day of Habituation Trials.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Day 1 | Day 2 | Day 3 | Day 4 |
| Tank A1 | 1 | 2 | 2 | 3 |
| Tank A2 | 1 | 2 | 1 | 4 |

**Discussion**

 The results of the Chi-square tests indicate that the fish from Tank A1 and Tank A2 performed equally on all habituation trials. The fact that there was no significant difference between the two tanks was expected, as the fish had not yet been separated into the Social and Isolation Groups and were, therefore, living in equivalent environments and receiving equivalent care. Despite this, the observable behavior exhibited by the fish in the maze was unexpected. After being released from the start box, each fish favored the perimeter of the maze and rarely, if ever, swam through the center of the maze where the bloodworms were located. When fish did leave the perimeter to swim through the center of the maze, they often ignored the worms and holes entirely, quickly swimming to the opposite wall. Their swift swimming and lack of interest in food, even when it was visible, suggest that the fish disliked the exposed nature of the center of the maze. In a study conducted by Vigayan et al. (2019), goldfish spent half as much time foraging in open areas as opposed to covered habitats in the presence of predators. The tendency of the goldfish to stay near the walls of the maze and avoid the exposed center may relate to their natural history and innate predator avoidance strategies (Vigayan et al., 2019).

 The significant results of the ANOVA performed on the data gathered from acquisition trials indicate that there was a difference in both, the mean scores of the fish across the days of acquisition and the two experimental groups’ performances in the maze. As with habituation trials, the fish generally avoided the center of the maze throughout acquisition trials. This resulted in a total of four different fish successfully completing the maze and receiving a score of 1. Of these fish, two completed the maze on two separate days, and two only completed the maze on one occasion. No fish ever received a score of 2. The significance found in the within-subjects factor is due to the fact that a majority of the fish received a score of 0 during each day of acquisition trials; a single fish received a score of 1 on Day 1, three fish received a score of 1 on Day 5, one fish received a score of 1 on Day 6, and one fish received a score of 1 on Day 11. The significance found in the between-subjects effect can be attributed to the fact that only fish from Tank A1 successfully completed the maze throughout acquisition trials, with the interaction representing the fluctuation of performance across days, but only within the group of fish living in Tank A1. This was unexpected as the fish had not yet been separated into their respective treatments and were, therefore, living in equivalent environments and receiving equivalent care.

 During a majority of both habituation and acquisition trials, it was noted that the fish not only stayed near the perimeter, but also repeatedly swam headfirst into the plexiglass walls of the maze. A study conducted by Hess and Gallup (2013) suggested that goldfish prefer to spend time with mirror-images of themselves rather than be alone or interact with conspecifics. This behavior displayed by the fish in the maze suggests that the fish were able to see and were attracted to their reflections, which likely contributed to their lack of motivation to enter the center of the maze.

A greater number of individuals were expected to locate bloodworms throughout both habituation and acquisition trials. The results of previous studies indicate that goldfish are able to successfully complete a variety of mazes. For example, goldfish have been shown to be capable of completing plus mazes during two separate studies conducted by Abreu et al. (2019) and Rodrigues et al. (1994). Likewise, goldfish used in a spatial experiment designed by Churchill (1916) learned how to swim through a series of compartments to reach a food reward, and both Lopez et al. (1999) and Vargas et al. (2004) demonstrated that goldfish were capable of using visual cues to differentiate among several compartments in a maze. Additionally, goldfish learned to complete the maze constructed by Saito and Watanabe (2005), which I modeled my own apparatus after. If goldfish are capable of creating and retaining spatial memory as these studies suggest, there must have been errors in my experimental design.

There are several aspects of this study I would like to change if I were given the opportunity to conduct it for a second time. Primarily, I would alter the design of my maze apparatus entirely to reduce the amount of open space; this may be accomplished by using a T-maze, plus maze, or radial arm maze. In an attempt to eliminate the possible reflections produced by the clear plexiglass, I would use colored, opaque plexiglass to compose the arms of the new maze. I would personally like to reattempt this experiment using a plus maze, as this model has been successfully used by both Abreu et al. (2019) and Rodriguez et al. (1994). In addition, using a plus maze would enable me to keep my four original extramaze cues: the north, west, south, and east arms of the maze would be composed of blue, green, black, and orange opaque plexiglass, respectively.

With the elimination of open spaces and mirrored reflections provided by the new maze design, I would seek to further improve my study by making the following alterations to the experimental procedure: A single hole would be drilled in the floor of the maze at the end of each arm. During habituation trials, all holes would be baited with a food reinforcer.

Of the four fish who located bloodworms throughout the course of my study, only two fully consumed the bloodworms; the other two fish spit out the worm after having it in their mouth for a few seconds. This behavior suggests that the bloodworms may have had a low reinforcing value, resulting in an overall lack of interest in the fish locating them. It is also possible that the fish were experiencing food neophobia, as they had not been exposed to bloodworms prior to trials in the maze. In an attempt to prevent this from happening in future studies, I would suggest baiting the holes in the maze with a food that the fish have been previously exposed to and find palatable.

If I were given the opportunity to revise my protocols, I would introduce the goldfish to the maze using the same start box process I used in my original experiment, however I would like to increase the amount of time spent in the start box from fifteen seconds to one minute in an attempt to give the fish more time to acclimate to the new environment (Abreu et al. 2019). In my original experiment, start box placement alternated between the center of the west wall of the maze and the center of the east wall of the maze. If I were to use a plus maze for future experiments, I would place the starbox in the center of the four arms, rather than alternating between the ends of the west and east arms (Abreu et al. 2019). After being released from the start box, each fish would be given ten minutes to swim through the maze and locate the reinforcers (Saito & Watanabe, 2005). Time would begin being recorded the moment the start box is raised. Instead of conducting habituation trials for a predetermined number of days, I would prefer to conduct habituation trials until each of the subjects have located the food reinforcer in all four arms of the maze on at least one occasion. After this criterion has been met, acquisition trials would commence.

Acquisition trials would be conducted in the same manner as the revised habituation trials with the following exceptions: Only the hole at the end of the north arm would be baited, and each fish would be given two minutes to locate the food reinforcer. Time would begin being recorded the moment the start box is raised and would stop being recorded the moment a fish touches the reinforcer. Any fish who does not locate the reinforcer in the allotted time would be removed from the maze at the completion of two minutes. During my original experiment, fish were put through two acquisition trials a day. Given the opportunity, I would like to revise this procedure by putting each fish through twenty trials a day to match the protocol used by Vargas et al. (2004). Instead of conducting acquisition trials for a predetermined number of days, I would like to continue conducting acquisition trials until a majority of the fish reached 70% accuracy in the maze, completing fourteen of the twenty trials in under two minutes (Vargas et al., 2004).

After acquisition trials cease, the fish would undergo a thirteen day lapse of practice followed by a single retention trial on the fourteenth day. The pattern of a thirteen-day rest period followed by one retention trial on the fourteenth day would continue for fifty-six days, resulting in each fish undergoing a total of four retention trials on four separate days.

The present study was unsuccessful in producing the intended data making it unclear whether social isolation has an effect on goldfishes’ ability to retain spatial memories. Implementing the aforementioned changes in the maze apparatus or the procedure may make this data obtainable. Future studies should focus on altering the design of this experiment with the intention of improving the subjects’ performances in the maze during both habituation and acquisition trials.

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**Appendix A: Timetable of Study**

Week 1-3: During the first day of week one, the fish tanks and the maze were set up. Goldfish were introduced to the tanks on day three of week one. The goldfishes’ acclimation period began on day three of week one and extended through day three of week three. Habituation trials commenced on day four of week three and extended through day seven of week three.

Week 4-5: Acquisition trials took place from day one of week four to day seven of week five.

Week 6-9. Fish were divided into Social and Isolation Groups on day 6 of week six. Each fish participated in two retention trials on day seven of week seven, and on day seven of week nine.

Week 11: Conduction of data analysis.

Week 12: Thesis defense

Week 19: Thesis defense and presentation.

**Appendix B: Effects of Stress**

A total of six fish died before the subjects were divided into their respective experimental groups. Five of the thirteen fish (38.46%) living in Tank A1 (which was to become the Isolation Group) died during the two week acclimation period and one of the twelve fish (8.34%) living in Tank A2 (which was to become the Social Group) died after the second day of habituation trials. The results of a Chi-square test indicated that there was not a significant difference in mortality between the fish living in Tank A1 and the fish living in Tank A2, *X2* (1) = 3.47, *p* = 0.06. However, the effect borderlines significance, suggesting that there may have been a trend of mortality in tank A1 during the acclimation period.

After the fish had been divided into the Social Group and the Isolation Group, mortality rates increased. A total of six subjects (46.15% of the original A1 sample) from the Isolation Group died between the time they were put into isolation and the time they underwent the second retention trial, however no fish (0% of the original A2 sample) from the Social Group died during this time. One week after the second retention trial was conducted, the two remaining fish (15.38% of the original A1 sample) from the Isolation Group had died and four individuals (33.34% of the original A2 Sample) from the Social Group had died. Results from a Chi-square test reveal that there was a statistically significant difference between the number of fish that died in the Isolation Group versus the Social Group, *X2* (1) = 8.89, *p* < 0.01. The elimination of the Isolation Group paired with the rapid decline of health observed in the Social Group resulted in early termination of the experiment.

During the time this study was conducted (September through December), day length decreased dramatically. Although an artificial light was used to keep the fish on a 12:12 light-dark cycle, the room that the fish were kept in had several large windows, exposing the subjects to a substantial amount of natural light. Day length and feeding schedule both act as zeitgebers that cue goldfish to enter a state of dormancy during winter months (Nisembaum et al., 2021). The fish used in this study were exposed to decreasing day length, but their feeding schedules remained constant. This may have caused internal desynchrony which is associated with cardiovascular health (de Oliveira et al., 2019). A necropsy was performed on every fish that died after their separation into Social and Isolation Groups. Necropsy reports indicated that an enlarged heart was found in each deceased subject.

It is speculated that the increase of deaths in each experimental group can be attributed to cardiovascular diseases caused by the conflicting zeitgebers that were created as day length decreased. Likewise, it is surmised that the compound effects of the conflicting zeitgebers and the stress of living in isolation resulted in the Isolation Group having significantly higher mortality than the Social Group.