Exploring the Impact of Dissolved Organic Carbon on Bluegill (*Lepomis macrochirus*) Behavior: Why Live Subjects are Important for Scientific Study

BIOS 35502: Practicum in Environmental Field Biology

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Abstract

Global browning in aquatic systems is a phenomenon, augmented by anthropogenic climate change, that is caused by increasing concentrations of dissolved carbon, making bodies of water brown, decreasing visibility. Dissolved organic carbon (DOC) is a term used to describe the decomposed organic matter from plant and animal tissue that incorporates into bodies of water. Higher levels of DOC alter the absorbance of solar radiation and heat distribution throughout a water column. Organisms living in the water column, especially visual predators, may be impacted, potentially exhibiting altered behaviors to adapt to these variations in visibility and temperature. The objective of this study was to explore the effects of varying concentrations of DOC upon the aggressive and explorative behaviors of bluegill (*Lepomis macrochirus*) fish. Bluegill, caught from Bay Lake on the University of Notre Dame Environmental Research Center property, were kept in nine mesocosms containing three different concentrations of dissolved organic carbon. After an acclimation period of five weeks, two behavioral assays focusing on aggression and boldness/exploration were conducted upon each fish. I found that latency time was not significantly related to DOC concentration for both the aggression and boldness/exploration assays (p=0.4658 and p=0.2294, respectively). Due to minimal replication, these findings highlight a need for further studies both of bluegill as well as expansion to other fish species to determine the impacts of increasing DOC concentrations on fish behavior.

Introduction

The ecological impact of anthropogenic activity has focused mainly on the increases in atmospheric carbon concentrations from fossil fuels emissions. As a result, terrestrial carbon uptake has also been found to increase, cycling excess carbon out of the atmosphere and into other planetary systems (Joos et al. 2001). In both marine and freshwater systems, phytoplankton play a significant role in CO_2 uptake through photosynthesis in what is often called the biological pump (Engel 2002; Low-Decarie et al. 2014). Phytoplankton convert the inorganic, atmospheric carbon into organic carbon through photosynthesis and their subsequent decomposition, over time, increases levels of dissolved organic carbon (Basu and Mackey 2018). Heightened levels of atmospheric carbon dioxide also prompt greater uptake by terrestrial plants. Terrestrial systems have been historically known as major carbon sinks, and this trend extends to today with the augmentation of climate change (Ciais et al. 1995; Ciais et al. 2019). Therefore, carbon concentrations in the decomposed matter have led to greater levels of dissolved organic carbon (DOC) in lakes and other bodies of water via runoff (Monteith et al. 2007). Although DOC occurs naturally, these increased concentrations due to global warming can alter the structure of lake ecosystems. (Solomon et al. 2015).

Dissolved organic carbon serves as a fundamental control in aquatic ecosystems through the alteration of the vertical distribution of light and temperature throughout a body of water (Kirk 1994). DOC absorbs varying wavelengths of incoming solar radiation. Therefore, higher levels of DOC limit light penetration at greater depths, concentrating heat absorbance towards the surface of a water column (Pérez-Fuentetaja 1999; Houser 2006). Primary producers within these systems then receive altered levels of light for their energy production (Thrane 2014). Phytoplankton are known to migrate vertically through a water column to gain greater access to light and necessary nutrients while avoiding predators (Salonen et al. 1984). Changes in light availability also may impact phytoplankton behavioral patterns, which may also affect the concentrations of essential nutrients as the abundance of consuming phytoplankton fluctuate in the water column over time. Aquatic plants also exhibit phenotypic changes in response to light and nutrient availability; root to shoot length ratios change based on an organism's access to the nutrients it needs to grow (Cronin and Lodge 2003). Previous studies have also found that there will be increased production of floating leaves to increase the surface area exposed to sunlight when there are higher DOC and lower light availability in the water (Cronin and Lodge 2003). Consumers' predator-prey interactions might also be affected by faster light extinction due to reliance on visual stimuli (Horpplia 2004). Previous studies have found fish to be more aggressive and territorial as well as less tolerant of other individuals near them with higher visibility (Valdimarsson et al. 2001). Visibility, especially in captivity, has been found to affect the stress responses of fish; lower visibility leads to lower activity levels, higher metabolic rates, and altered swimming performance (Strand et al. 2007; Barton and Schreck 1987; Bonga 1997). In this way, other behaviors of consumers may be influenced by the concentrations of DOC in a water column.

In this study, I analyzed the relationship between concentrations of DOC and the behavioral response of bluegill fish (*Lepomis macrochirus*), focusing on aggression, boldness, and exploration. I hypothesized that bluegill exposed to lower levels of DOC would be more aggressive and more explorative due to their higher visibility in comparison to the bluegill accustomed to greater concentrations of organic carbon.

Materials and Methods

Study Site

This experiment was wholly conducted on the property of the University of Notre Dame Environmental Science Center (UNDERC), which is a private research facility covering around 3035 hectares overlapping the state lines of Wisconsin (Vilas County) and the Upper Peninsula of Michigan (Gogebic County:46° 13' N, 89° 32' W). Bluegill collection occurred at Bay Lake, a 67.3-hectare lake with a maximum depth of approximately 40 meters that is primarily oligotrophic and low DOC (absorbance of 0.162 at 440nm). Bay Lake is mainly surrounded by the research facility and one other private property; it is fished regularly with low fishing pressure.

Bluegill Collection

Bluegill were collected using Fyke nets set near shore at seven locations over 18 days from June 2, 2019 to June 19, 2019. Due to difficulty capturing the number of fish needed for the study, sites to place the nets were chosen based off of known areas of high bluegill abundance. Individuals less than sixteen centimeters long were released.

Experimental Design

After collection in the field, the fish were maintained and studied at the UNDERC Aquatic Lab for three weeks. At the lab, fish were processed by caudal fin clippings, nose to tail length measurements, and sex determination for later identification. They were then randomly sorted into nine mesocosms (3 fish/mesocosm), which were prepared during the week before bluegill collection. Each of the nine mesocosms (Pipe Top Round Tanks, Freeland Industries Inc., Portage, WI) was filled with water from Tenderfoot Lake. There were three levels of DOC that are controlled by three varied amounts of “Super Hume” (Lennon et al. 2013). The lowest DOC level contained water from Tenderfoot Lake without any of the Super Hume (absorbance of 0.154 at 440 nm). The moderate DOC level had an average absorbance of 0.7073 at 440 nm and, the highest DOC level had an average absorbance of 1.338 at 440 nm (Spectronic GENESYS 2, Thermo Electron Co., Madison, WI). Twice per week, this water was changed, the mesocosms were scrubbed clean, and three cups of salt were added after each water change. Salt aids stressed fish by adding further electrolytes to their environment and by helping to eliminate harmful bacteria in the tanks (C. Dassow, personal communication). Halfway through the acclimation period, every tank was cleaned with bleach due to the observation of bacterial fin rot in several of the tanks. Fish within each mesocosm were uniquely marked from subsequent identification via unique caudal fin clips within each mesocosm. The bluegill were left to acclimate to their respective DOC level for three weeks while being fed freeze-dried bloodworms (approximately 0.3191g, Tetra GmbH, Melle, Germany). During this period, there were a series of bluegill fatalities due to the stress of being placed in the mesocosms. As a result, I had added new fish to the mesocosms to take their place. These fish were each kept in tanks with equivalent DOC concentrations before being added to the experimental mesocosms.

Behavioral Analysis

To determine whether DOC impacted the behavioral response of these bluegill, I conducted aggression and boldness/exploration assays. I conducted a mirror image assay, which tests individuals for aggressive behavior (Balzarini 2014). I determined that a fish had recognized its reflection when they turned their eyes towards the mirror and then back away from or approach the mirror. A mirror was placed at one side of the container and, the fish was added to the other side of the study container. Fish acclimated in the study container for two minutes with the mirror concealed. Thereafter, I observed the fish for ten minutes, recording the latency time to recognize itself in the mirror and exhibit any aggressive behavior with a maximum time of 10 minutes. Additionally, I recorded whether this display was overt or restrained (Balzarini 2014) (Table 1, Figure 2). Overt aggression was displayed through ramming or biting the reflection in the mirror. Restrained behaviors still were displays of aggression but did not involve causing harm to the opponent in the form of the reflection. Some examples of restrained aggression included swimming back and forth along the mirror or displaying the lateral view. In total, nine behaviors were looked for with each fish.

To test boldness/exploration, fish were placed into a container with an opaque divider in the middle, obscuring their view of the other side of the container (Wilson and Godin 2009). On the side opposite the fish, I placed a novel object, a Lego car attached to a rock. Each fish was allowed a two-minute acclimation period. Then, I removed the divider and recorded the latency time for the fish to enter the novel environment as well as whether it interacted with the novel object (Figure 2), with a maximum observation time of ten minutes.

Statistical Analyses

To analyze the results of the aggression assay, a Kruskal-Wallis one-way ANOVA was used to determine the relationship between time taken to exhibit aggression and DOC level, sex, and expression of overt or restrained aggression. The relationship between fish length and time taken to demonstrate aggression was analyzed using simple linear regression.

Similarly, the boldness/exploration assay was analyzed using a Kruskal-Wallis one-way ANOVA to determine the relationship between time taken to explore the novel object and DOC level, sex, and whether contact was made with the Lego car. The relationship between fish length and the time taken to explore the novel object was analyzed using simple linear regression. All analyses were conducted in R Programming version 1.2.1335.

Results

Mirror Test of Aggression

There was no significant difference in latency time between DOC levels (mean ± SE; low DOC, 133 ± 78.7; medium DOC, 364 ± 112; high DOC, 214 ± 100; H=1.5278; p=0.4658; Figure 3). No significant difference was found in latency time between sexes (mean ± SE; male, 370 ± 68.4; female, 355 ± 95.5; H=1.7646; p=0.1841; Figure 4). There was a significant difference found in latency time between individuals that expressed none, overt, or restrained aggression (mean ± SE; none, 600 ± 0; restrained, 57.6 ± 12.5; overt, 53.4 ± 11.7; H=13.885; $p<0.001$; Figure 5). A post-hoc Dunn's Test showed that no aggressive behavior differed significantly from the overt and restrained aggression (Z=3.182; p=0.00146 and Z=3.270; p=0.00108, respectively). However, latency times of overt and restrained aggression did not differ significantly from each other (Z=0.0878; p=0.930). There was no functional relationship observed between latency time and the length of the individual ($R^2=0.00493$; p=0.762; Figure 6).

Novel Object Test of Boldness/Exploration

There was no significant difference found in latency time between DOC concentrations (mean ± SE; low DOC, 234 ± 79.1; medium DOC, 465 ± 90.0; high DOC, 395 ± 103; H=2.9444; p=0.2294, Figure 7). There was no significant difference found in latency time between sexes (mean ± SE; male, 370 ± 68.4; female, 355 ± 95.5; H=2.7153; p=0.09939; Figure 8). There was no significant difference found in latency time between individuals that did and did not make physical contact with the novel object (mean ± SE; contact, 389 ± 57.3; no contact, 131 ± 12.5; H=1.3022; p=0.2538; Figure 9). There was no functional relationship observed between latency time and the length of the individual (R^2=0.000866; p=0.899, Figure 10).

Discussion

In this study, I aimed to determine if variation in concentration of dissolved organic carbon altered aggression and boldness/exploration in bluegill. With the global browning phenomena being perpetuated by climate change, it is crucial to understand how aquatic organisms' behaviors may change and influence food web dynamics. The results obtained from this study did not support my hypotheses; there does not appear to be an effect of DOC level upon bluegill aggression and boldness/exploration. However, with greater replication and minimization of stress from the mesocosms in future studies, this hypothesis may gain more support.

I expected to find that bluegill exposed to low DOC with high visibility would have lower latency times and more overt aggression, shown in the results of the mirror test. However, I found no significant relationships in this study that contributed to answering this question. Although significance was found in latency time between aggression exhibited (none, overt, or restrained), this is because the individuals that exhibited no remarkable behavior had a set latency time of 600 seconds with no error or deviation. Therefore, no aggression exhibited was significantly different, but overt and restrained were not significantly different from each other, which is the more important result for this study. Fish in the medium DOC concentration exhibited the highest latency times, which was in direct contrast to my expected results. One potential explanation for this is that numerous individuals across all DOC concentrations were exposed to stress when they were transported to the laboratory, resulting in high bluegill mortality due to bacterial infection and fin erosion.

In the novel object test of boldness/exploration, I expected to find lower latency time and more contact with the novel object from bluegill in the low DOC level. Similarly to the aggression assay, there were no significant results found. This is likely due to the same concerns and stresses that were previously mentioned that affected the aggression assay results. Although latency times were lower in both tests with low DOC fish, no significance was able to be determined.

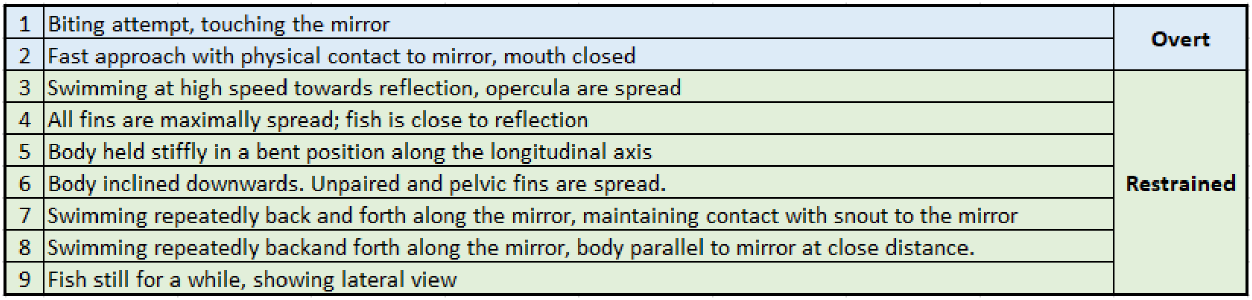
When removing an organism from its natural habitat and placing it into a new environment that may not meet all its needs, it is natural for the individual to be put under stress, especially with younger organisms (Kowakoski et al. 2012). Fish, especially, are known for their inability to tolerate high levels of stress when placed into mesocosms, such as what was used in this study (Marçalo et al. 2008). Stressed fish altered metabolic rates and are less likely to be confrontational to conserve energy for flight (Sloman et al. 2000; Barton and Schreck 1987). Being in stressful conditions also suppresses fishes' immune systems, leaving them more susceptible to bacterial and fungal infections (Maule et al. 2989; Snieszko 1974; Walters and Plumb 1980). These issues related to stress induction were evident in this study with several study organisms dying from extensive "fin rot." Fin rot may occur from several sources such as physical abrasion from contact with rough surfaces, aggression from other fish, nutritional deficiencies, or bacterial and fungal infection (Latremouille 2003; Schneider and Nicholson 1980). Although individuals with fin rot were not used in this study, individuals not visibly impacted by fin rot themselves may, however, have been affected by the proximity of deteriorating bluegill in the same mesocosms, although not much research has been done to support this. To combat the continued spread of fin rot, I changed the water in every mesocosm twice per week and added three cups of salt to each mesocosm after the water change. Although bluegill are freshwater fish, adding salt to the mesocosms provides the fish with further electrolytes to better handle the stressful environment and aid in the elimination of bacteria or fungus (Carneiro and Urbinati 2001; Tomasso et al. 1980). There was a significant decrease in mortality of the fish in every DOC level, and some fish even showed signs of recovery over time. During the second week of acclimation, I bleached and scrubbed every mesocosm to eliminate as much bacterial or fungal buildup as possible.

I observed that the bluegill in the medium DOC level were the most affected by the fin erosion and the most fatalities were from the three mesocosms in this level. The reasoning for this is unclear; however, this is likely the reasoning that their latency times were unexpectedly high in comparison to the extreme DOC levels. Uncertain but natural variability such as aforementioned can be mitigated by greater replication, which should be implemented in future studies.

In addition to the use of more replicates, future studies should attempt to minimize the stress of individuals in mesocosms using larger containers and including elements of the bluegills' natural habitats in them such as rocks and vegetation. As opposed to mesocosms, future studies could sample bluegill from bodies of water with a variety of DOC levels and test their aggression, boldness, and exploration, observing specific behaviors and latency time. That way, fish are likely under the lowest amount of stress because they are being tested right from their natural environment. Greater replication in future studies also may aid in coming to significant conclusions and determine if DOC truly influences fish behavior.

Despite the obstacles of this study, further studies that analyze how aquatic organisms are impacted by increasing DOC is of great importance, especially as global browning is expected to continue to increase with climate change. With atmospheric CO_2 being a primary anthropogenic climate change driver, levels of dissolved carbon in both marine and freshwater systems are also increasing (Hoegh-Guldberg et al. 2007; Caldeira and Wickett 2003; Mitsch et al. 2013). Greater dissolved carbon causes further global browning, and this is suspected of having effects on the behaviors of both producers and consumers (Creed et al. 2018). Ecosystem-wide behavioral fluctuation can lead to changes in food web dynamics and biodiversity in freshwater systems globally (Modenutti et al. 2010). Understanding these impacts is essential for aquatic management in response to climate change, and they also may affect freshwater aquaculture, directly impacting human activity. In the realm of how DOC affects secondary and tertiary consumers, there has not been extensive study. However, as time progresses, we must consider these impacts more closely to maintain the beauty and biodiversity of our natural, freshwater ecosystems for future generations to study and appreciate.

Tables



1	Biting attempt, touching the mirror	**Overt**
2	Fast approach with physical contact to mirror, mouth closed	
3	Swimming at high speed towards reflection, opercula are spread	**Restrained**
4	All fins are maximally spread; fish is close to reflection	
5	Body held stiffly in a bent position along the longitudinal axis	
6	Body inclined downwards. Unpaired and pelvic fins are spread.	
7	Swimming repeatedly back and forth along the mirror, maintaining contact with snout to the mirror	
8	Swimming repeatedly backand forth along the mirror, body parallel to mirror at close distance.	
9	Fish still for a while, showing lateral view	

Table 1: Table of the behaviors focused on in the mirror assay of aggression. Biting and ramming are the two behaviors considered to be overtly aggressive, meaning to do some sort of harm to an opponent.

Figures

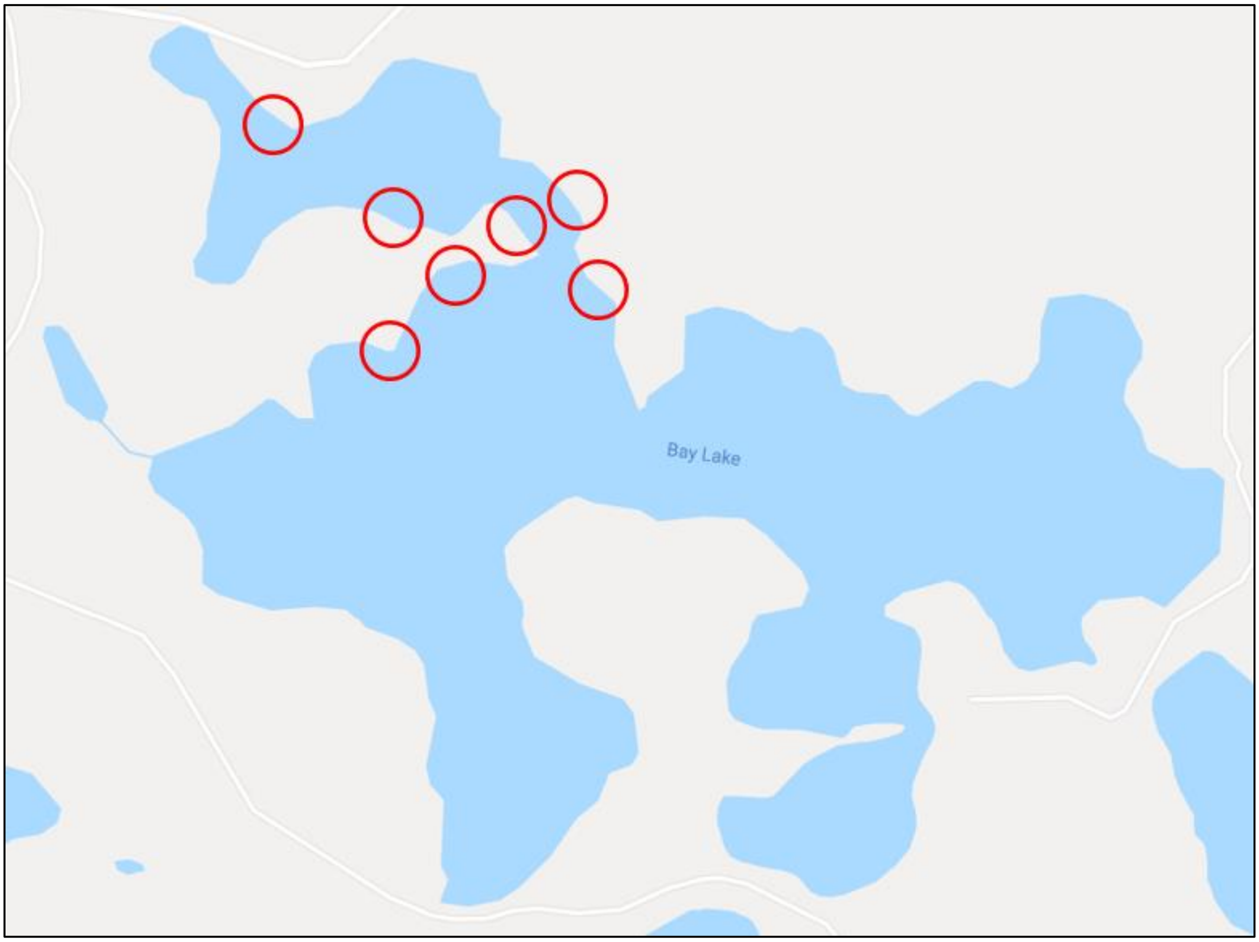


Figure 1. Map of Bay Lake, where all bluegill collections occurred. At each of the red circles, Fyke nets set there caught fish that were utilized in this study.

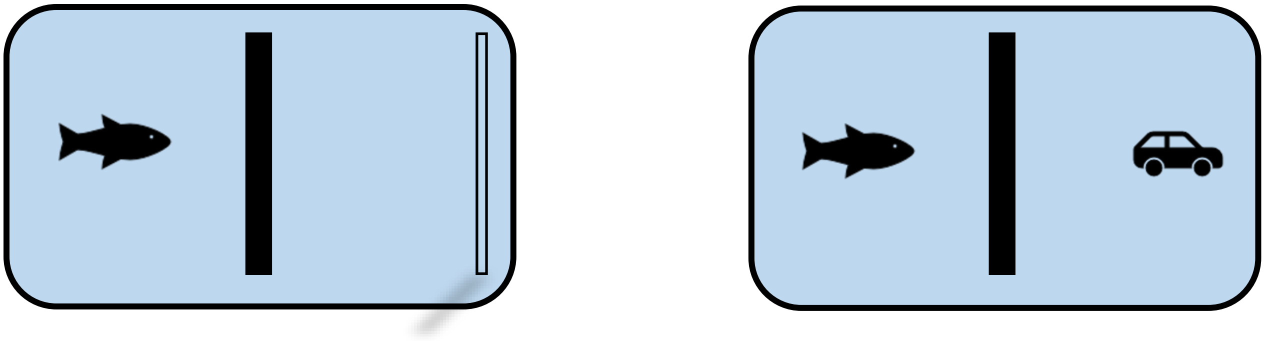


Figure 2. Diagram of the set up for the mirror assay of aggression (left) and the novel object assay of boldness/exploration (right). For the mirror test, the fish was initially placed the container in the left side of the partition and the mirror was on the opposite side. Similarly, in the novel object test, the fish was initially placed on the left side of the partition and the novel object (Lego car) was placed on the opposite side.

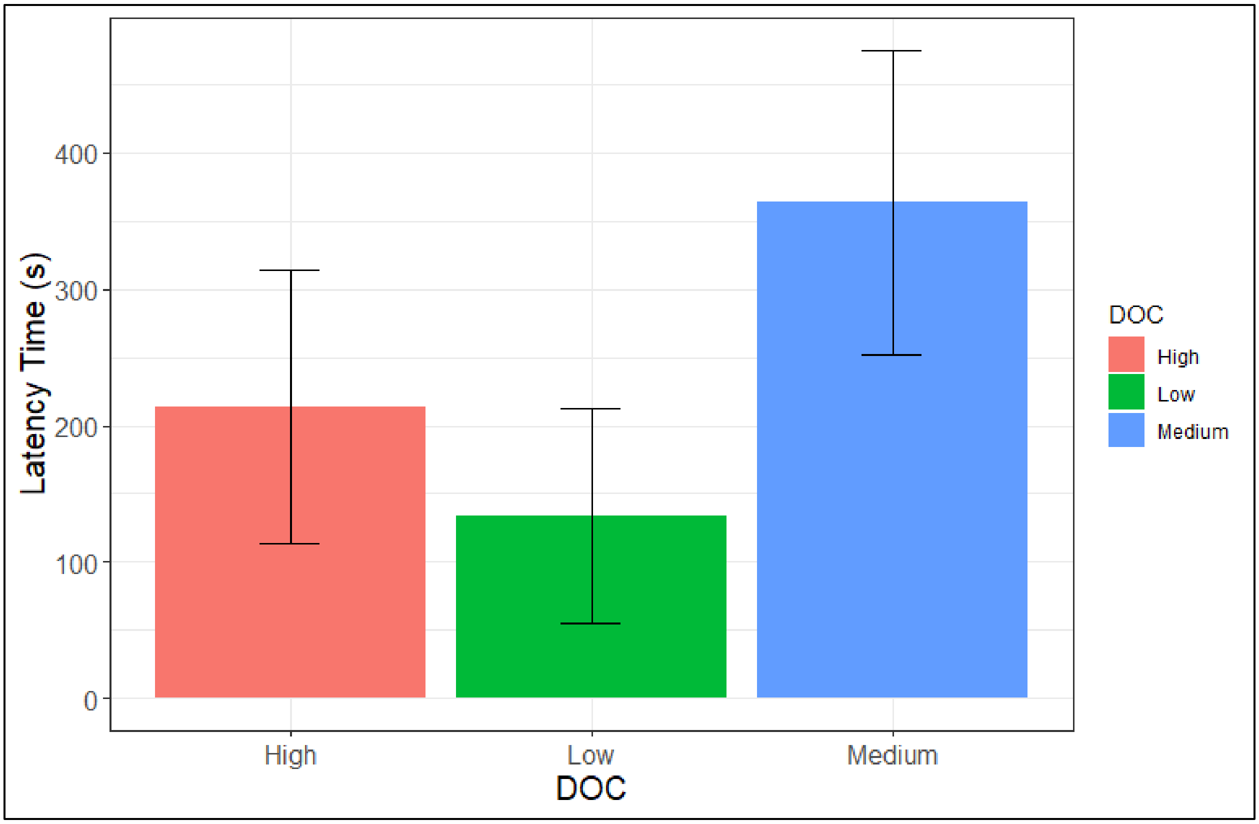


Figure 3. Bar plot of latency times in the mirror assay of aggression by DOC level. Results of a Kruskal-Wallis test revealed no significant difference in latency times between the DOC levels (H=1.5278, p=0.4658).

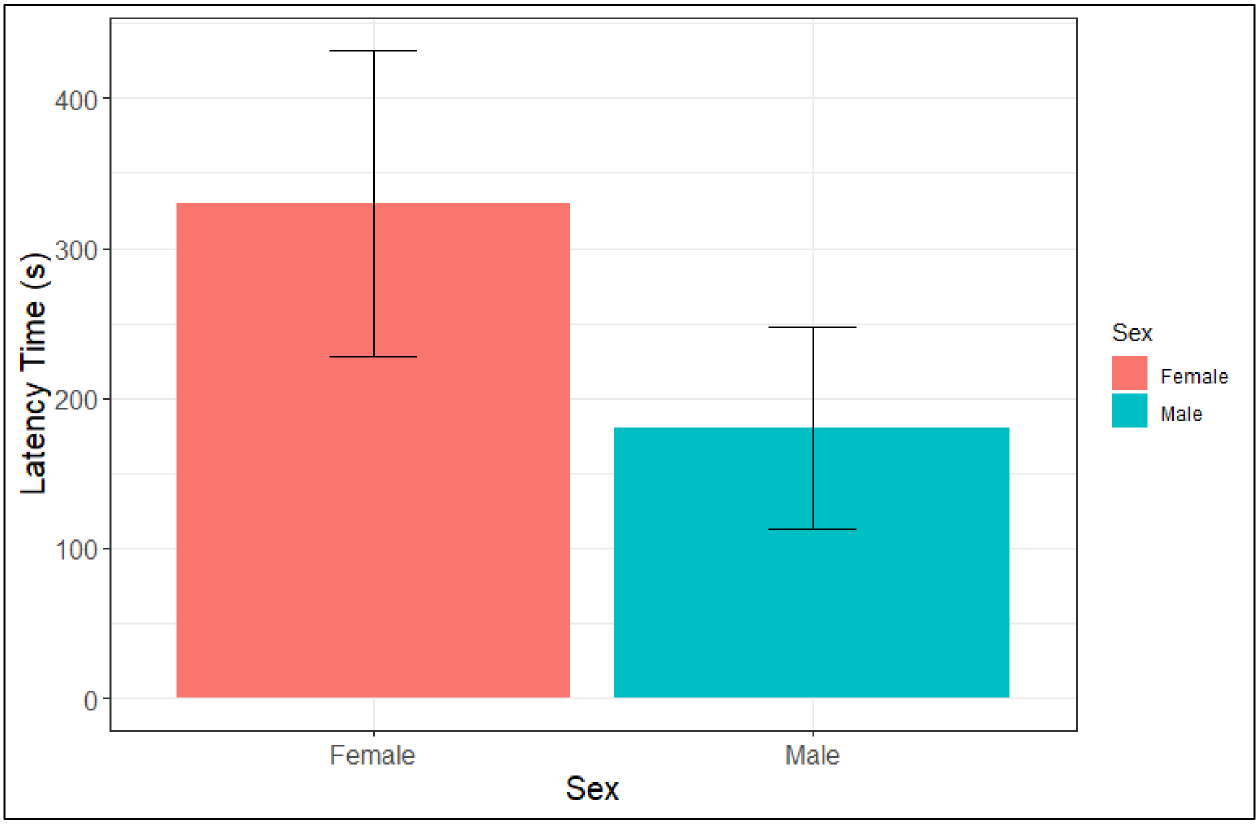


Figure 4. Bar plot of latency times in the mirror assay of aggression between sexes. Results of a Kruskal-Wallis test revealed no significant difference in latency times between male and female individuals (H=1.7646, p=0.1841).

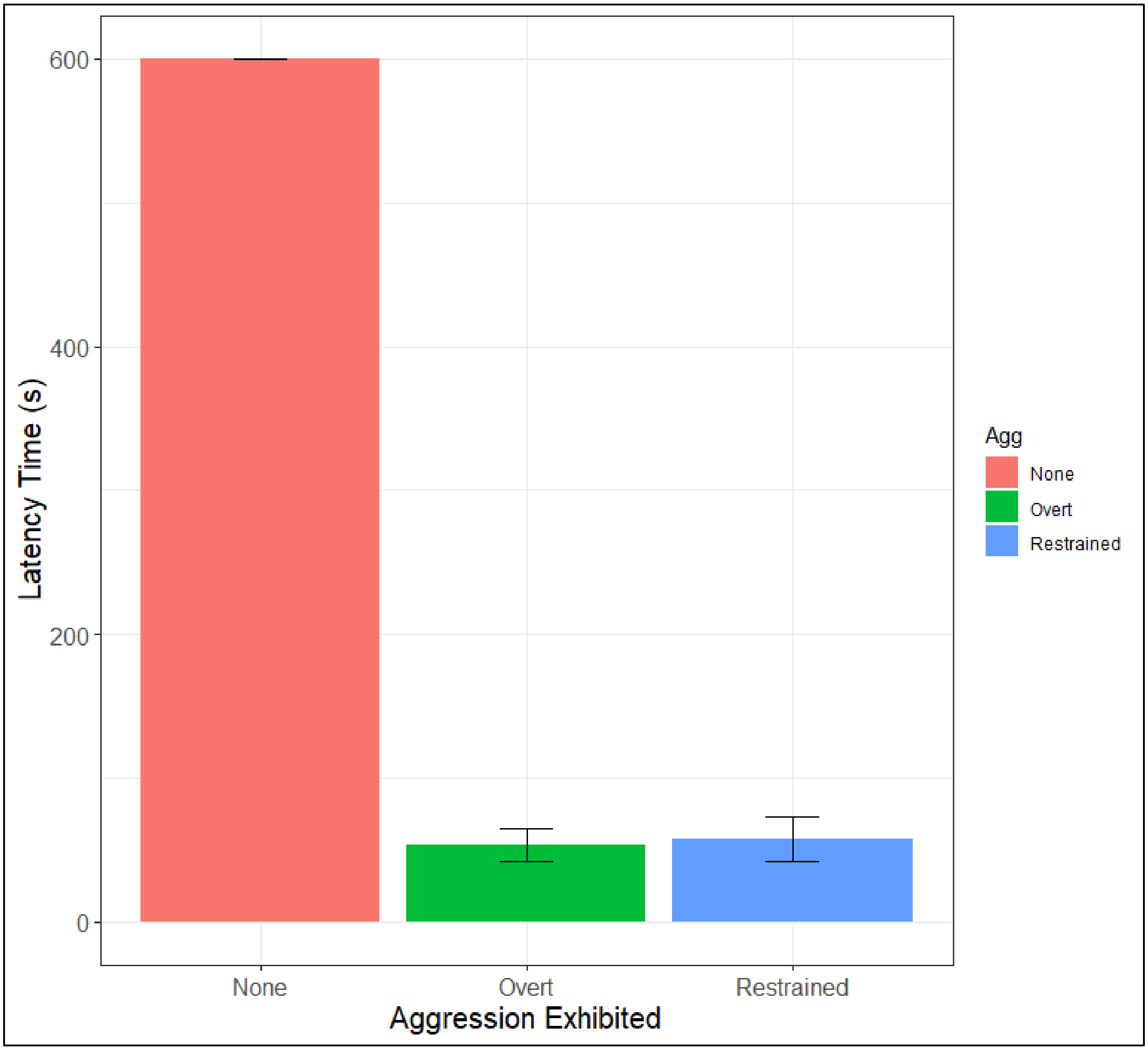


Figure 5. Bar plot of latency times in the mirror assay of aggression between individuals that exhibited no, overt, or restrained aggression. Results of a Kruskal-Wallis test did reveal a significant difference in latency times between no aggression and both the overt and restrained aggression (H=1.5278, p=0.4658; Dunn's test, Z=3.182; p=0.00146 and Z=3.270; p=0.00108, respectively). No significant difference was found between overt and restrained aggression (Z=0.0878, p=0.930).

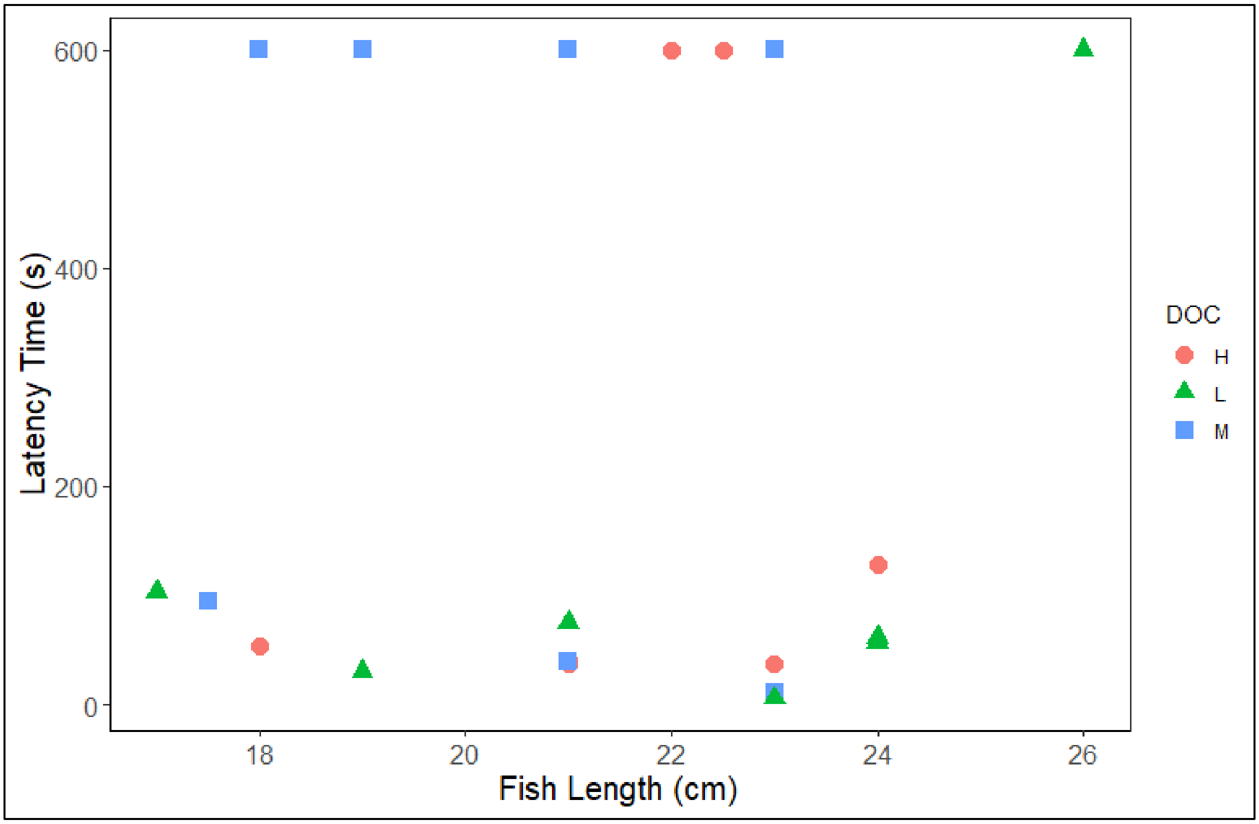


Figure 6. Plot of linear regression between the latency times and fish lengths from the mirror test of aggression. No significant relationship was revealed by the linear regression model (R^2=0.00493; p=0.762).

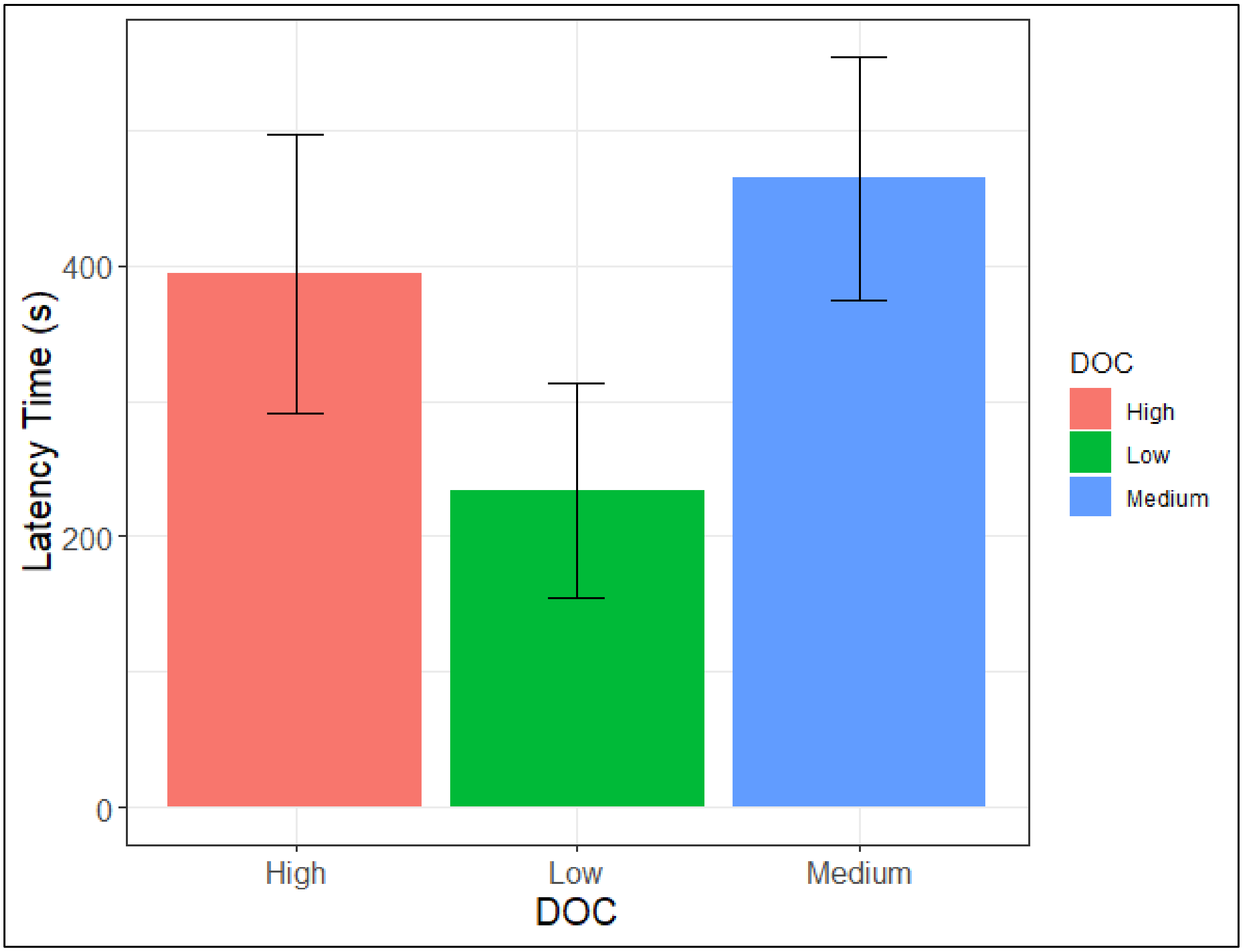


Figure 7. Bar plot of latency times in the novel object assay of boldness/exploration between DOC levels. Results of a Kruskal-Wallis test revealed no significant difference in latency times between low, medium, and high DOC levels (H=2.9444, p=0.2294).

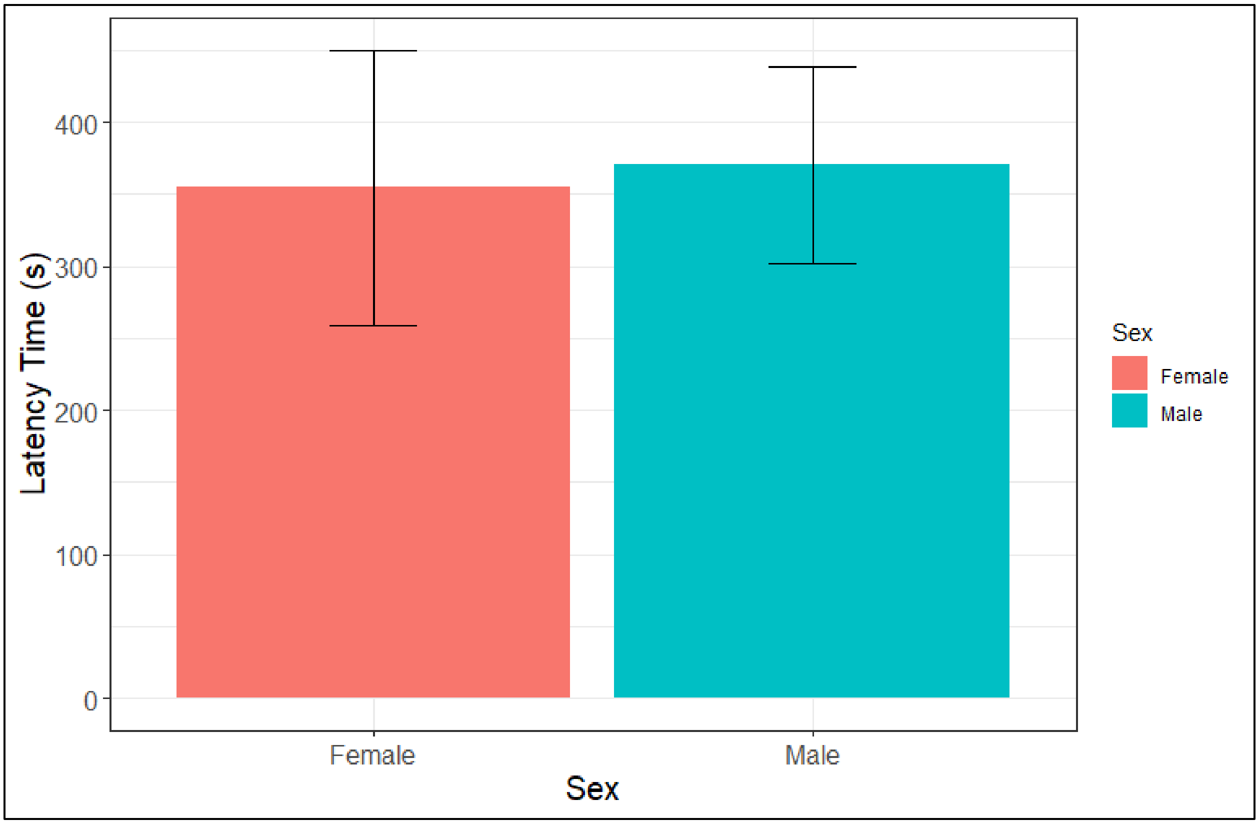


Figure 8. Bar plot of latency times in the novel object assay of boldness/exploration between sexes. Results of a Kruskal-Wallis test revealed no significant difference in latency times between male and female individuals (H=2.7153, p=0.09939).

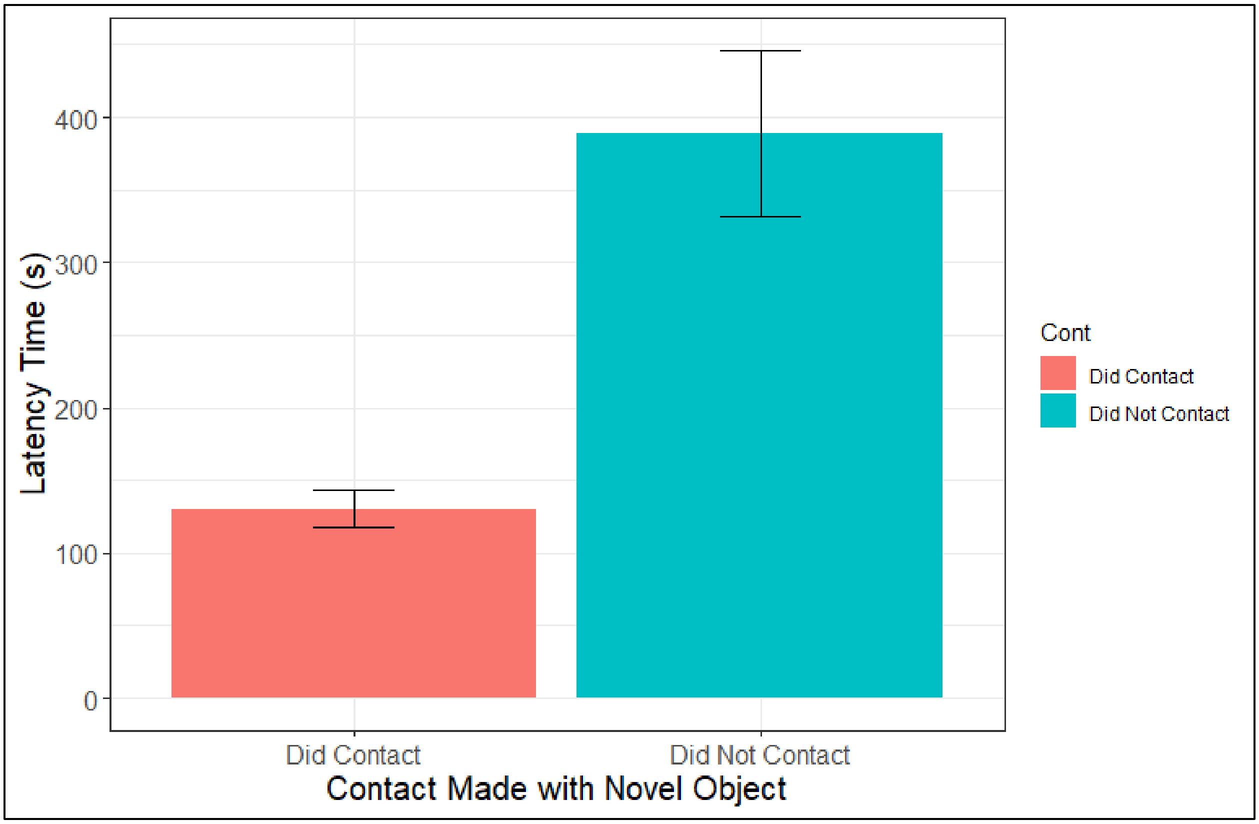


Figure 9. Bar plot of latency times in the novel object assay of boldness/exploration between individuals that did or did not make physical contact with the novel object. Results of a Kruskal-Wallis test revealed no significant difference in latency times (H=1.3022, p=0.2538).

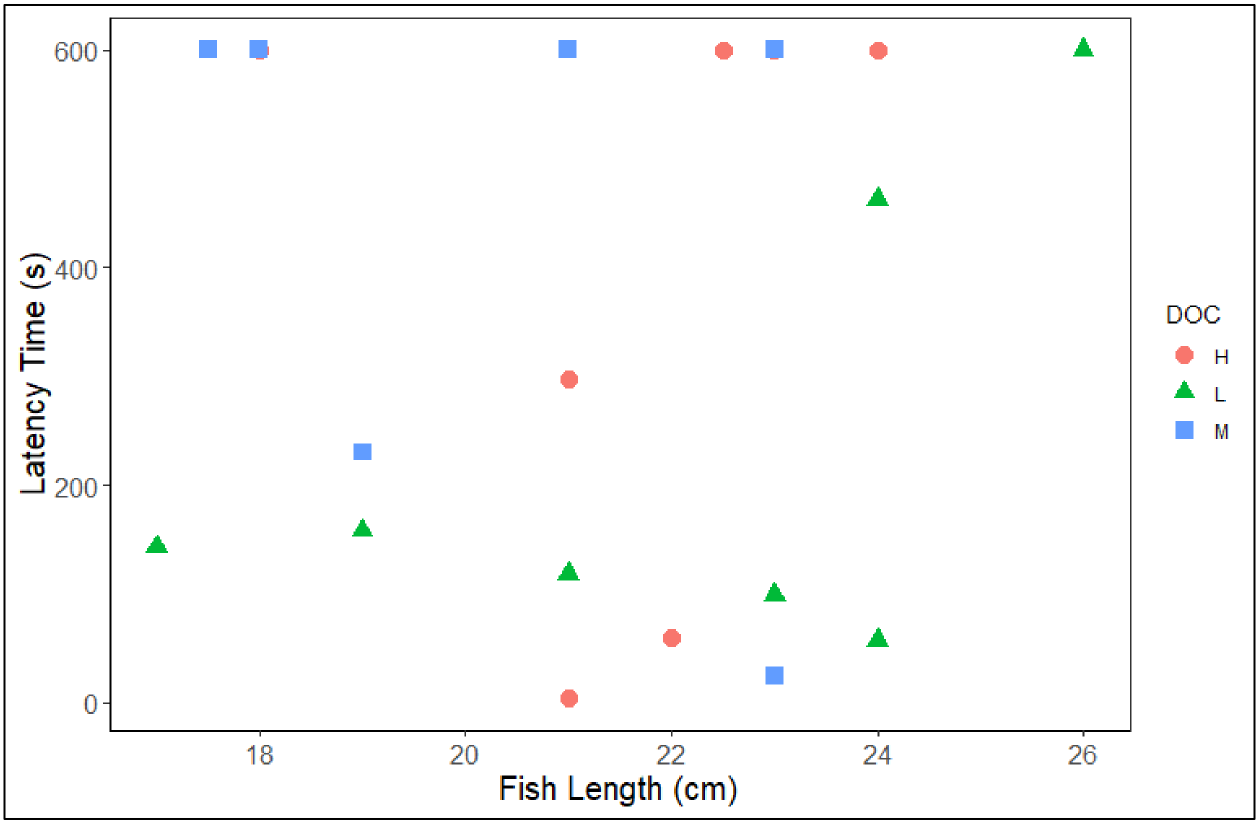


Figure 10. Plot of linear regression between the latency times and fish lengths from the novel object assay of boldness/aggression. No significant relationship was revealed by the linear regression model (R^2=0.000866; p=0.899).

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Appendix – Raw Data

Mirror Assay of Aggression

Aggression Exhibited: R = Restrained, O = Overt, N = None

DOC	Aggression Exhibited	Sex	Length (cm)	Time (s)
H	R	M	21	37
H	O	F	21	40
H	R	M	24	128
H	O	F	18	54
H	N	F	22	600
H	N	M	22.5	600
H	O	M	23	38
M	N	F	18	600
M	R	M	17.5	95
M	N	F	19	600
M	R	F	21	40
M	N	M	23	600
M	R	M	23	11

M	N	F	21	600
L	O	F	17	104
L	N	M	26	600
L	R	M	19	30
L	R	M	24	62
L	O	M	21	75
L	O	M	23	6
L	O	M	24	57

Novel Object Assay of Boldness/Exploration

DOC	**Contact Made?**	**Sex**	**Length (cm)**	**Time (s)**
H	N	M	23	600
H	N	M	21	298
H	N	F	22	60
H	N	F	18	600
H	N	M	22.5	600
H	N	M	24	600
H	N	F	21	4
M	N	F	18	600
M	N	M	23	24
M	N	F	21	600
M	N	F	19	230
M	N	M	23	600
M	N	M	17.5	600
M	N	F	21	600
L	N	M	19	158
L	Y	M	21	118
L	N	M	24	462
L	N	M	23	99
L	N	M	26	600
L	N	M	24	57
L	Y	F	17	143