# Salinity Preference in Killifish (*Fundulus* spp.)

By

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## Abstract

Fish normally live in salinities ranging from 0 ppt (freshwater) to ~35 ppt (seawater) but most fish can only inhabit fresh or saltwater making salinity a main factor in determining their distribution. While euryhaline fish can tolerate the osmoregulatory challenges that occur with changes in environmental salinity, they exhibit preference for particular salinities. The Common Killifish (*Fundulus heteroclitus*) is a small estuarine fish that normally prefers brackish water (~20 ppt). In brackish water where they overlap, *F. heteroclitus* can co-occur and hybridize with Banded Killifish (*Fundulus diaphanus*), a fish that prefers freshwater. The first goal of this thesis was to set-up and optimize the Loligo® ShuttleBox system to measure salinity preference of the two species to compare this new, more accurate system to previously published data. The second goal was to determine the salinity preference of wild juvenile F1 *F. diaphanus* x *F. heteroclitus* hybrids. The system consists of two connected choice tanks and a video-tracking system that allows salinity to be automatically increased or decreased depending on fish location. I predicted that the salinity preference of *F. heteroclitus* would be 20 ppt, *F. diaphanus* would be 1 ppt, and wild juvenile hybrids would be ~9.5 ppt, consistent with an additive genetic basis for salinity preference. Future work should include completing additional trials using wild *F. heteroclitus, F. diaphanus*, and hybrids as well as lab bred pure and reciprocal F1 hybrid crosses to gain a better understanding of the role of parental effects on salinity preference.

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# 1. Introduction

## 1.1 Habitat Preferences

To meet growth, survival, and reproductive needs, organisms exploit the resources available to them within their environment (Levin, 1992). Ideally, mobile organisms should choose to occupy a particular habitat because the abiotic conditions, such as temperature or salinity, as well as biotic conditions, such as food availability, allow for maximum fitness (Svärdson, 1949; Kearney and Porter, 2004). However, there may be changes in conditions such as increased competition or predation in an otherwise optimal habitat that induce stress and decrease fitness in this location (Schulte, 2014). These biotic and abiotic conditions can be either dynamic or static (Hirzel and Lay, 2008; Schulte 2014) and may force the organism to decide between staying or dispersing (Caughley, 1994; Svärdson, 1949; Kearney and Porter, 2004). Thus, organisms need to be capable of surviving in variable environments or relocating when conditions become unfavourable (Nguyen et al., 2013; McManus et al., 2014). Increased energy use, risk of injury, and death include some of the costs that are associated with dispersing to new habitat, while escaping unfavourable conditions and gaining new resources are some of the benefits associated with dispersal (Bonte et al., 2012; Caughley, 1994). If the costs associated with dispersal are greater than the benefits the organism is expected to remain in its current habitat (Caughley, 1994). Considering that organisms can sense variation in many abiotic factors and learn which environments are preferable, they should be capable of selecting environments that are physiologically optimal (Kültz 2015).

## 1.2 Salinity and Osmoregulatory Challenges

Salinity, the amount of dissolved salt in the water, is an abiotic factor that has a major influence on the distribution of aquatic organisms, including fishes (Kültz 2015). This because salinity to impacts the thermodynamic properties of water such as density and solvent capacity and because the internal systems of fish are greatly affected by salinity (Kültz 2015). Dissolved inorganic ions such as $Na^+$ and $Cl^-$ determine the ionic strength of most water, often measured as osmolality (millosmoles of solute per kilogram of water (Kültz 2015).

Fish normally live in salinities ranging from 0 ppt (freshwater) to ~35 ppt (seawater) but most fish can only inhabit fresh or saltwater making salinity a main factor in determining their distribution (Edwards and Marshall, 2013; Kültz 2015). Regardless of whether fish live in salt or freshwater, they have osmoregulatory challenges (reviewed by Edwards and Marshall, 2012). Because they are in direct contact with the water, variation in salinity requires a fish to mount a physiological response to maintain a constant internal osmolality of approximately 10-14 ppt (Evans and Claiborne, 2009). For example, fish in saltwater must work against passive ion gain and water loss to the hyper-osmotic environment; they do this by actively excreting ions and taking up water to maintain homeostasis (reviewed by Edwards and Marshall, 2012). Conversely, fish in freshwater must work against passive ion loss and water gain and do this by actively taking up ions and excreting water to maintain homeostasis (reviewed by Edwards and Marshall, 2012).

When changes in salinity occur, fish are capable of sensing these changes physiologically which is a process referred to as osmosensing (Kültz, 2013). This includes perceiving the change in salinity using a variety of sensory mechanisms and the relay of information from sensors to

effector cells via a number of different signal transduction mechanisms (Kültz, 2013). With this information, fish are then able to select the salinity where they live (McCain et al., 2020).

Euryhaline fish are capable of tolerating a wide range of salinities (McCormick et al., 2013) and are commonly found in estuaries and intertidal zones with frequently changing salinities (Kültz 2015). Being euryhaline provides an advantage as these fish can inhabit new and unique niches that stenohaline fish, those limited to a small range of salinities, cannot (Kültz 2015). Coastal and intertidal zones are resource-rich which helps to offset the costs of osmoregulation faced by fish in areas of varying salinity (Kültz 2015). This may increase the fitness of these fishes and their ability to compete for resources over stenohaline fish (Kültz 2015).

## 1.3 Salinity Preference in the Genus *Fundulus*

A genus commonly used as a model organism in evolutionary and environmental science is *Fundulus*; this genus contains 38 species that live in North and Central America in coastal and inland areas (Whitehead 2010). They are found in all osmotic environments: hypersaline marine, marine, brackish, and freshwater (Whitehead 2010). This makes them a good genus for studying the relationship between ecology, evolution, and physiology of salinity adaptation (Griffith 1974).

*Fundulus heteroclitus*, commonly known as the mummichog, is a particularly well-known model organism in the genus *Fundulus* (Dawley, 1991). *F. heteroclitus* is euryhaline and lives in marine environments such as salt marshes and estuaries along the Atlantic coast of the USA and Canada, where salinity varies daily because of the tides (Fritz and Garside, 1974a; Potter et al., 2010). *F. heteroclitus* has evolved osmotic plasticity that allows it to tolerate salinities ranging from freshwater (0 ppt) to a salinity three times that of seawater (120 ppt)

(Whitehead, 2010). Fritz and Garside (1974a) found that adult mummichogs had a salinity preference for 20 ppt and a weaker preference for 8 ppt that corresponds with their distribution in the wild (Fritz and Garside, 1974a). *Fundulus diaphanus* is another member of the genus *Fundulus* (Fritz and Garside, 1974a). Although it belongs to the same genus as *F. heteroclitus*, it typically lives in very different habitats (Fritz and Garside, 1974a). *F. diaphanus* lives mainly in freshwater environments such as lakes and rivers; adults have a salinity preference of 0 ppt (Fritz and Garside, 1974a). Fritz and Garside (1974a) also found that the salinity preferences of adult *F. diaphanus* and F. *heteroclitus* were not affected by acclimation salinity, suggesting that preference may have low plasticity in these species (Fritz and Garside, 1974a).

*F. diaphanus* and *F. heteroclitus* sometimes inhabit the same geographical area in coastal regions (Fritz and Garside, 1974b). In some of these populations, *F. diaphanus* and *F. heteroclitus* interbreed to produce mostly female clonal hybrids (Dawley, 1991; Fritz and Garside, 1974b). One of the locations where these hybrids are located is in Porters Lake, Nova Scotia (Dawley 1991). Porters Lake has a salinity gradient that ranges from freshwater (0.5 ppt) in the Northern end of the lake where it is furthest from the Atlantic Ocean to brackish (~16 ppt) in the Southern end of the lake where it merges with the Atlantic Ocean (Merette et al., 2009). The hybrid populations are located near the center of the lake where the salinity is ~5 – 15 ppt (Jonah, 2019; Merette et al., 2009). In Porters Lake, 95.6% of the clonal hybrids have *F. diaphanus* mitochondrial genome (Merette et al., 2009; Dalziel et al., 2020). Most of the hybrids in Porters Lake seem to be F1 clones, although there is evidence for some sexual hybrids in other locations in the Maritimes (Dawley et al., 1999; Hernández Chávez & Turgeon, 2007; Mérette et al., 2009).

Salinity preference has previously been studied in adult *F. heteroclitus and F. diaphanus* (Bucking et al., 2012; Fritz and Garside, 1974a). Fritz and Garside (1974a) used two different systems to study salinity preference (Fritz and Garside, 1974a). One system consisted of a vertical salinity gradient with salinities ranging from 0-31 ppt at intervals of 5-8 ppt (Fritz and Garside, 1974a). The other system was a horizontal system where the fish was only able to choose between two predetermined salinities (Fritz and Garside, 1974a). The predetermined salinities included salinities the fish showed some preference for, 31, 20, 14 or 8 ppt for *F. heteroclitus* and 0 ppt or 14 ppt for *F. diaphanus*, and either the salinity that the fish had been acclimated to or another preferred salinity (Fritz and Garside, 1974a). Although these systems appear to work well, there are horizontal systems that allow the fish to choose between a continuous range of salinities (Bucking et al., 2012). This is what we will be using for this study to determine a more accurate salinity preference for these species.

## 1.4 Research Objectives

This study focused on setting up the Loligo® ShuttleBox and optimizing its use for studying salinity preference in the genus *Fundulus*. I also aimed to determine if the salinity preference of these *Fundulus* species could be determined in two hours with the Loligo® ShuttleBox. While the salinity preference of adult *F. heteroclitus* and adult *F. diaphanus* is known, there is no data on juvenile fish salinity preference or the salinity preference of the hybrids in any life stage (Fritz and Garside, 1974a); thus, another objective of my study was to determine the salinity preference of *F. heteroclitus*, *F. diaphanus*, and *F. diaphanus* x *F. heteroclitus* wild-caught juveniles.

# 2. Materials and Methods

## 2.1 Fish collection and lab acclimation

Juvenile killifish were collected from Porters Lake, Nova Scotia (44.785135°N, -63.360340°W) (see figure 2.1) in September 2020. The fish were collected from two locations within the lake; location A and location B (see figure 2.2) with salinities of 5 ppt and 4.5 ppt, respectively (A and B in figure 2.2). Minnow traps and a seine net were used to collect the fish following the Animal care protocols approved by the Saint Mary's University Animal Care committee (SMU ACC AUPF 19-08A1). Upon collection, fish were identified using measuring methods by Mérette (2009) that have 90% accuracy (Mérette, 2009). Calipers were used to take three measurements: one from the caudal peduncle dorsal end to the dorsal fin anterior insertion (A in Figure 2.3), one from the caudal peduncle ventral end to the anal fin posterior insertion (B in Figure 2.3), and another from the caudal peduncle ventral end to the caudal peduncle dorsal end (C in figure 2.3). Based on the length of these three measurements, the probability that the fish is each of the three. species can be determined (Mérette, 2009).

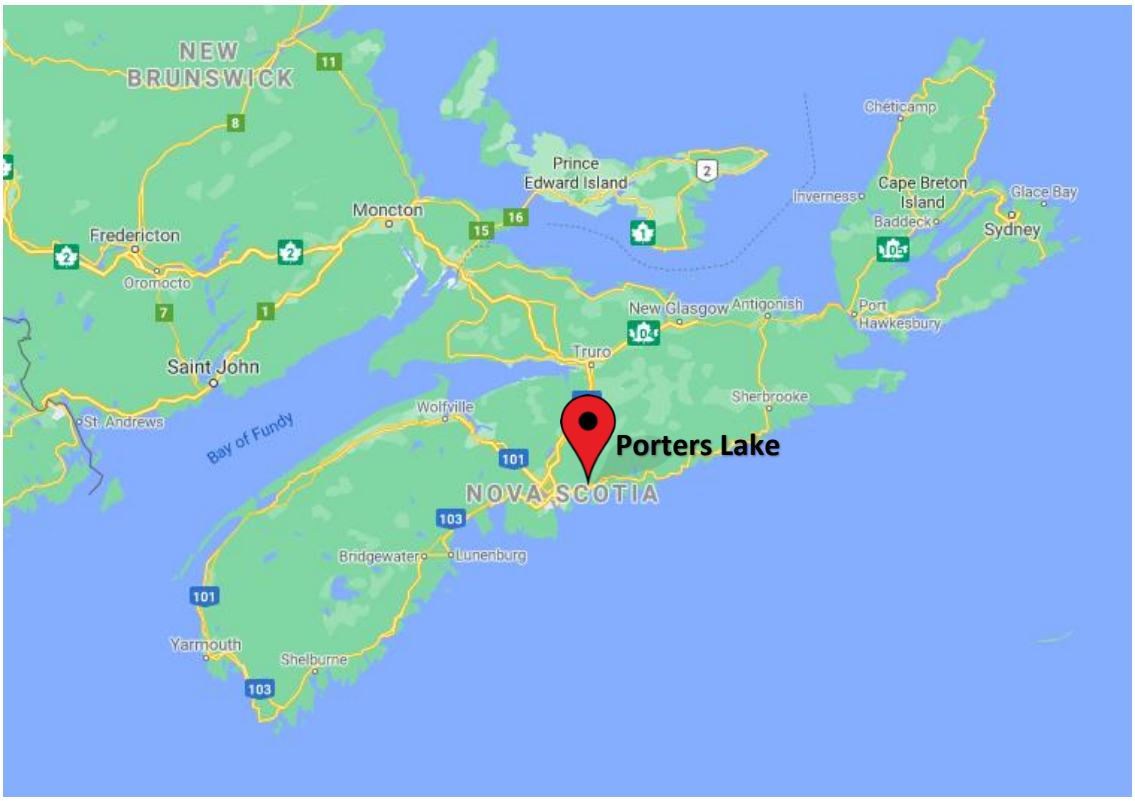


**Figure 2.1** A map of Nova Scotia indicating the location of Porters Lake (screen shot taken from Google Maps ©).

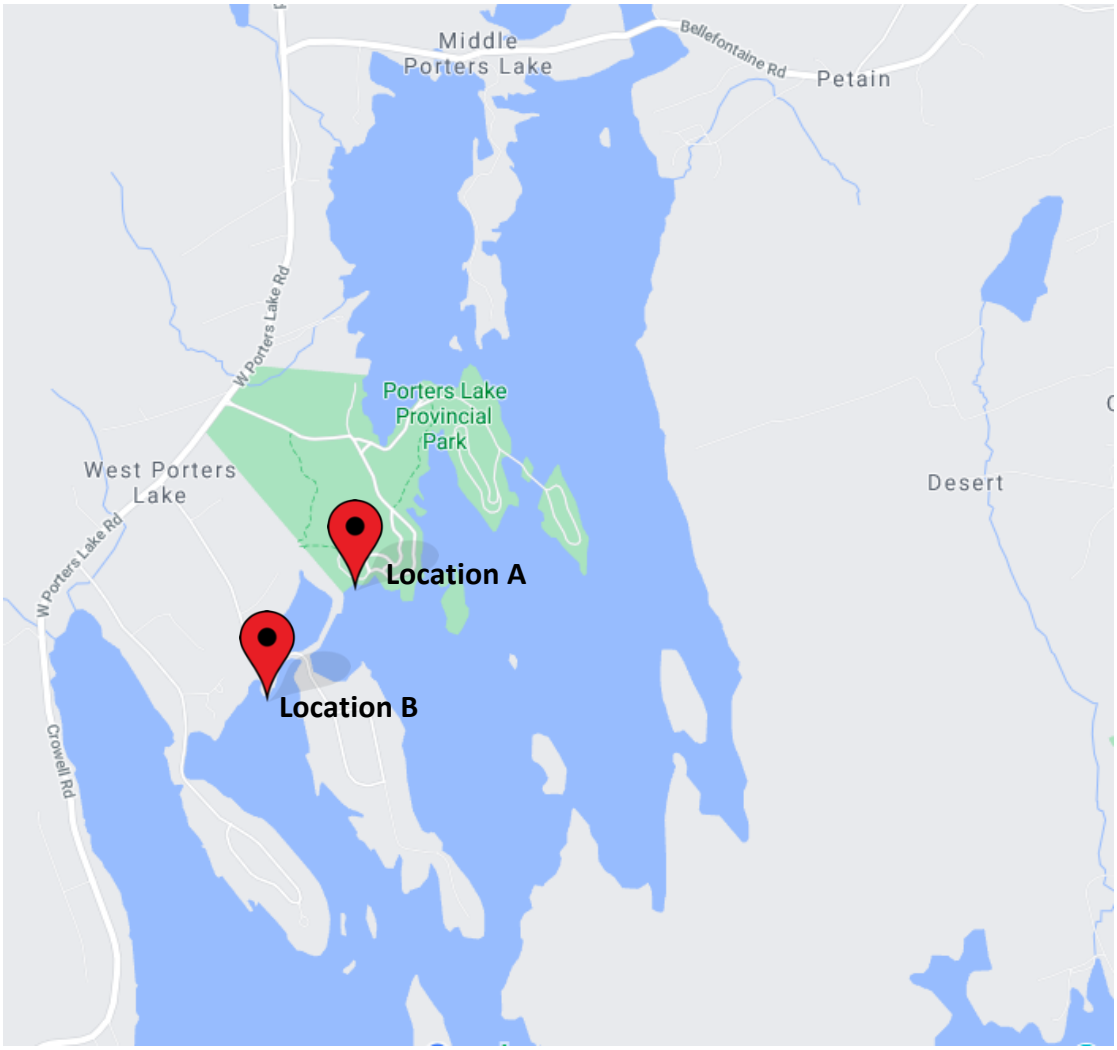


**Figure 2.2** A map indicating the two sampling locations within Porters Lake; location A and location B (screen shot taken from Google Maps ©).

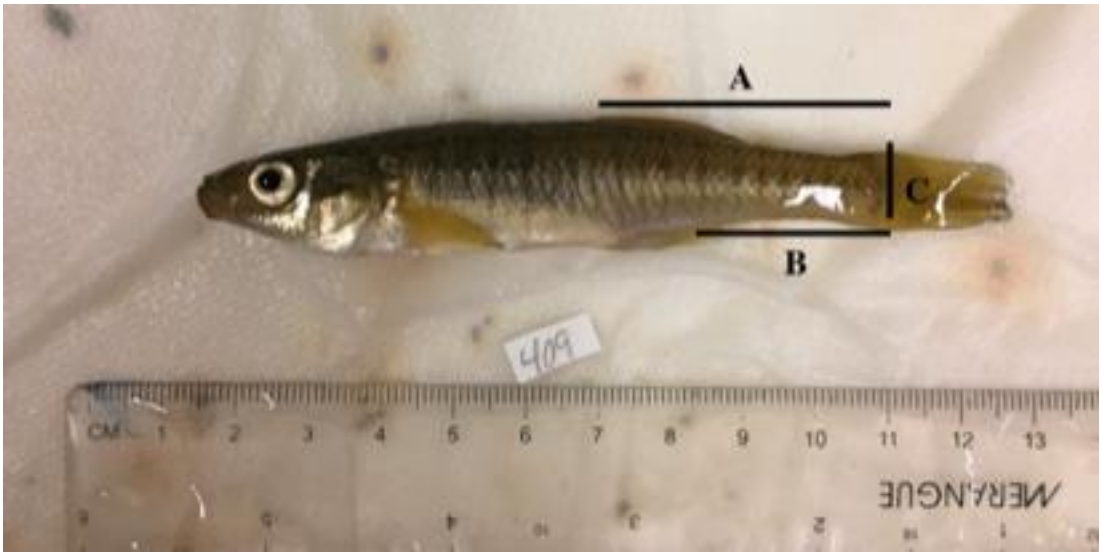


**Figure 2.3** *F. diaphanus* with three measurements required for Killifish identification based on Mérette (2009) methods. Photo from Jonah (2019).

Fish were transported to Saint Mary's University Aquatic Facility where they were acclimated for approximately seven months before beginning the experiment. During this time, they were held in 10-gallon tanks. The water in the tanks was kept at a salinity of 10 ppt +/- 1 ppt (measured with a Hanna probe; Hanna Instruments, Woonsocket, RI). The tanks were aerated with air stones and contained artificial plants, gravel, and sponges for habitat enrichment. The photoperiod throughout acclimation was set to dark:light 9h:15h and the room was kept between 18- 21 °C. Fish were fed a diet of mysis shrimp, bloodworms, and crushed beef heart flakes once daily. Water quality analysis and water changes of ≥20% occurred at least once a week. The analyses included tests of pH (API, Mars Fishcare Inc., Chalfont PA), nitrite (API, Mars Fishcare Inc., Chalfont PA), ammonia (API, Mars Fishcare Inc., Chalfont PA), and nitrate (Fluval, Rolf C. Hagen Inc., Montreal, QC).

## 2.2 Experimental set-up

Experiments occurred in a room separate from the fish housing facility. The room had a photoperiod of dark:light 9h:15h of and a temperature of 22-23 °C. White plastic garbage bags were placed around the outside perimeter of the experimental tank to prevent outside stimuli from affecting the location of the fish. The experimental tank used was a custom-designed ShuttleBox (ShuttleBox, Loligo, Viborg, DK; figure 2.4). The tank consisted of two circular choice tanks with diameters of 38 cm which were connected by a channel 10 cm in length and 6.5 cm in width. The depth of the tank was 19.8 cm but only 16 cm to overflow. The salinity of the choice tanks could be changed through the addition of freshwater (0.1 ppt dechlorinated tap water) or saltwater (30 ppt dechlorinated tap water reconstituted with Red Sea salt©). The water came from two 30-gallon reservoir tanks and then flowed through the two buffer tanks, one which increased salinity and one which decreased salinity of the experimental tank. The flow rates were monitored and adjusted to ensure they were equal by using manually adjusted clamps. The choice tanks were initially filled with water of 10 ppt and were measured by salinity meters within probe vessels (Cond 3310, WTW, Weilheim, DE). The ShuttleBox was monitored using a USB 2.0 camera (UI-1640SE-C-GL, IDS, Massachusetts, USA) to record fish location and adjust salinity input accordingly (see Section 2.3). The two meters and camera were connected to a computer along with a DAQ-M (data acquisition system) (DAQ-M, Loligo, Viborg, DK) which acted as a relay for the four dosage pumps.

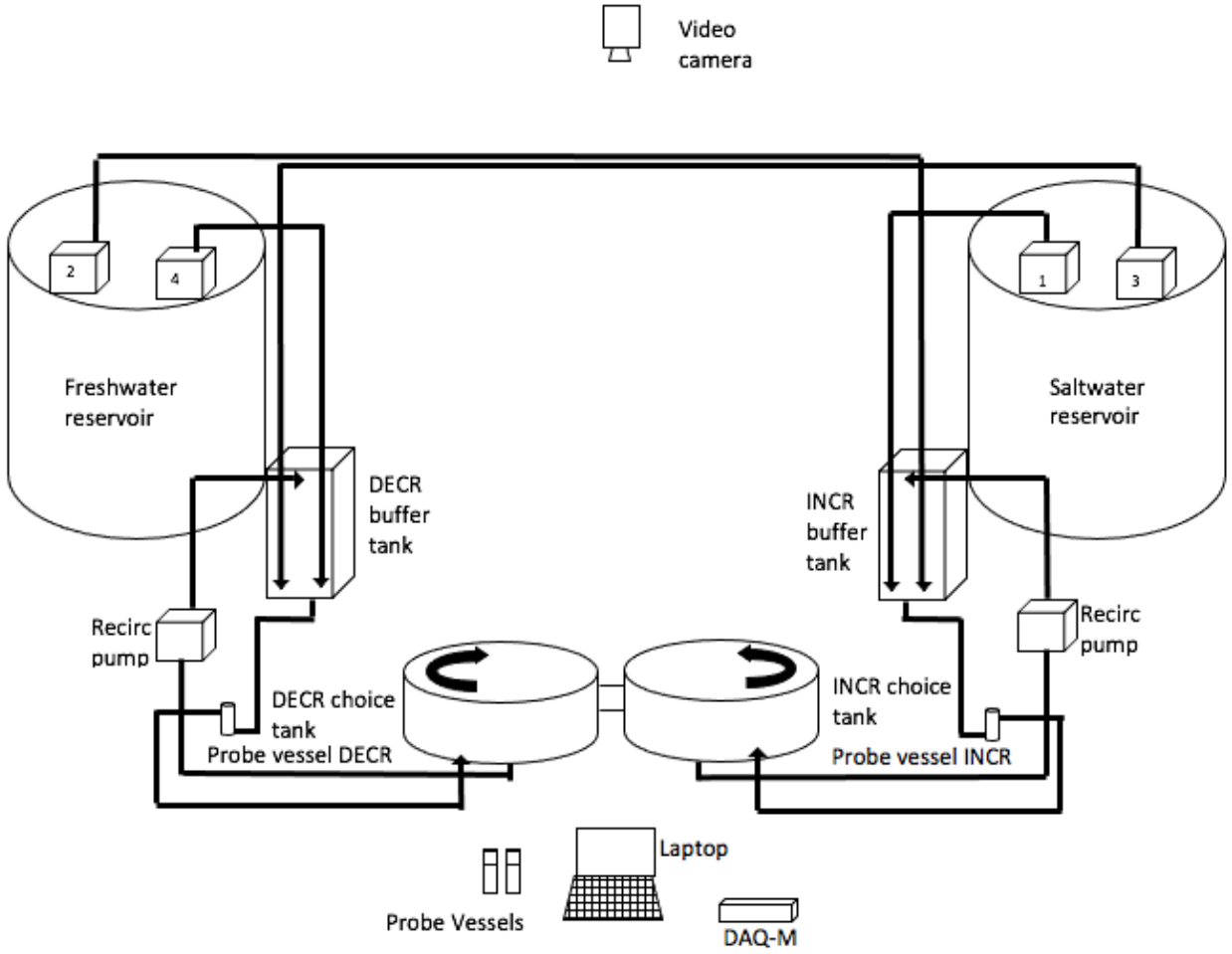


**Figure 2.4** A diagram of the ShuttleBox salinity system depicting the flow of water through the system. INCR (increase) and DECR (decrease). Water flow: ➡

## 2.3 Trials

I did not get the opportunity to run a complete trial, but if I had, the procedure would be as follows. Before experimentation, the fish would have been fasted for approximately 24 hours. One fish at a time would be transferred from their housing tank into the experimental tank from a small plastic transport container containing water of 10 ppt. The Loligo ShuttleSoft software would monitor the position of the fish and save this information to determine salinity preference at the end of the trial. Each choice tank would be designated to either increase or decrease

salinity by triggering the pumps in the reservoirs in the presence of the fish. The two sides of the experimental tank in the ShuttleBox called choice tanks would be predefined as either increasing or decreasing salinity which would allow for the pumps within the reservoir tanks (either freshwater or saltwater) to be activated in the presence of a fish. If a fish had been in the decrease choice tank, the salinity of the ShuttleBox choice tanks would decrease and when the fish was in the increase choice tank, the salinity would increase in the ShuttleBox choice tanks. The ShuttleSoft software would keep track of these salinity changes via the probe vessels measuring the salinity of the water moving between the buffer and choice tanks. If a fish was in the channel between the tanks the salinity would not be changed. The hysteresis would be set at a salinity of 0.2 to give the water adequate time to mix and the salinity difference between the two tanks would be set to 3 ppt at all times during the experiment, following the procedure of Christensen and Grosell (2018). Each fish would be acclimated to the ShuttleBox for 15 minutes and then kept in the ShuttleBox tank for two hours. I would have completed this process with eight *F. heteroclitus*, eight *F. diaphanus*, and eight *F.diaphanus* x *F. heteroclitus*. The fish would have been re-measured and tagged after each trial to keep track of what fish had been used and to double-check what species the fish was. To determine if salinity preference can be determined in two hours in *Fundulus*, one adult *F. heteroclitus* and one adult *F. diaphanus* would have been run through a trial in the ShuttleBox.

## 2.4 Statistical Analysis

The time each fish spent on each side of the Shuttlebox would have been calculated by the ShuttleSoft software to obtain the mean and median salinity preference. The total activity levels for each fish would also be recorded. R studio version 1.3.1093 and R version 4.0.4 would be used to conduct the data analysis. A one-way ANOVA would be used to test if there was a

statistically significant difference in mean salinity preference between *F. heteroclitus, F. diaphanus,* and *F. diaphanus* x *F. heteroclitus F*1 hybrids.

# 3. Results

The Loligo® ShuttleBox was set-up and optimized for use with *Fundulus* species in our lab. To determine the salinity preference of wild juvenile *F. heteroclitus, F. diaphanus*, and F1 hybrids, salinity preference trials would have been completed. I also would have determined whether or not it is possible to determine the salinity preference of *Fundulus* species in two hours. The salinity preference trials consisted of placing individual fish in a Loligo® ShuttleBox that consisted of two connected choice tanks where salinity could be increased or decreased depending on which 'choice tank' the fish was located.

## 3.1 ShuttleBox Optimization for *Fundulus*

To determine salinity preference in Fundulus species, the Loligo® ShuttleBox described previously had to be set up. This included downloading software (ShuttleSoft, LabView, uEye-camera, WTW conductivity instruments) to a Windows 10 computer to allow for communication between all components of the system (DAQ-M, uEYE video camera, Cond 3310 salinity meters, TetraCon 325 salinity probes, Ehiem pumps, Windows computer). It also included setting up the physical system and then optimizing the entire system to be compatible with *Fundulus* species while overcoming system malfunctions and other issues.

## 3.2 Expected Results of Determining *Fundulus* Salinity Preference in Two Hours

It is expected that by using adult *F. heteroclitus* and *F. diaphanus* salinity preference can be determined in two hours because Marshall et al. (2016) successfully completed 30-minute salinity preference trials with *F. heteroclitus* using a vertical salinity gradient with a lower layer at 28 ppt and an upper layer at 3 ppt (Marshall et al., 2016).

## 3.3 Expected Salinity Preference

I was unable to collect salinity preference data for these fish, so I have included the expected results for salinity preference. If I had collected the data I would have then tested it for differences in salinity preference (response variable) among species (manipulated variable) using a one way ANOVA. I predict that the juvenile *F. heteroclitus* will have an average salinity preference of 20 ppt, the juvenile *F. diaphanus* will have an average salinity preference of 1 ppt, the juvenile *F. diaphanus* x *F. heteroclitus* hybrids will have an intermediate salinity preference with an average of 9.5 ppt.

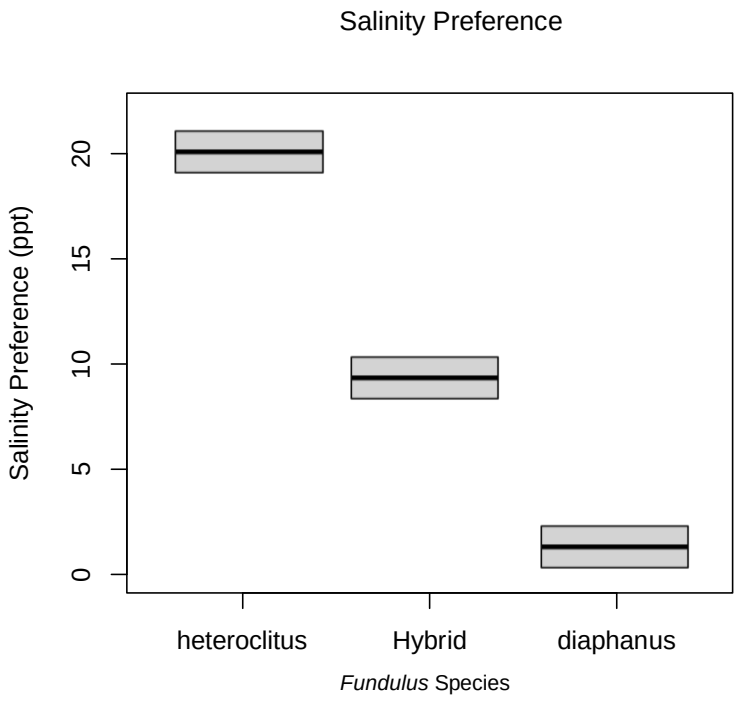


**Figure 3.** Predicted average salinity preference of juvenile *F. heteroclitus, F. diaphanus,* and the F1 hybrid. For each *Fundulus* species, I planned to study eight individuals.

*F. heteroclitus* adults are expected to show a salinity preference of 20 ppt and *F. diaphanus* adults are expected to show a salinity preference of 0 ppt as these are their known salinity preferences and would confirm that the system can determine their salinity preference in two hours.

# 4. Discussion

One of the goals of this study was to set up and optimize the Loligo® ShuttleBox for use with *Fundulus*. I also wanted to determine if salinity preference in *Fundulus* can be determined within two hours and I wanted to know the salinity preference of juvenile *F. heteroclitus*, *F. diaphanus,* and F1 hybrids. My expected results were that juveniles would prefer the same salinity as the

adults, and that the hybrids would show intermediate preference (i.e., *F. heteroclitus* prefers 20 ppt, *F. diaphanus* prefers 1 ppt, and the F1 hybrid prefers 9.5 ppt).

## 4.1 Loligo® ShuttleBox Optimization for *Fundulus* spp.

The Loligo® ShuttleBox for salinity has been set up and optimized for testing 'dynamic' fish salinity preference in three previous studies (Bucking et al., 2012; Christensen and Grossell, 2018; Serrano et al., 2010), including one study of how *F. heteroclitus* diet influences salinity preference (Bucking et al., 2012). Dynamic experiments allow the fish to pick a precise salinity. The only other study examining salinity preference in both adult *F. diaphanus* and *F. heteroclitus* used two different systems lacking this precision; one was a static vertical salinity gradient from 0-31 ppt at intervals of 5-8 ppt, and the other was a static horizontal salinity gradient experiment in which the fish could only choose between two different predetermined salinities (Fritz and Garside, 1974a). The vertical salinity gradient made it difficult to determine whether Fritz and Garside (1974a) were observing a salinity preference or a water depth preference in the fish, which is why they also tested a horizontal gradient. This is a benefit to horizontal systems like the ShuttleBox because previous studies have demonstrated a water depth preference in other fish species (Yu and Lee, 2002). Additionally, by giving the fish a choice between two specific salinities, one can get an estimate of their salinity preference, but it is difficult to determine their exact salinity preference without a constant salinity gradient like with the ShuttleBox.

Using Christensen and Grossell (2018) as a guide I was able to set up most of the ShuttleBox along with the other corresponding components. I was then able to optimize the system for use with *Fundulus*. This included adjusting the lighting to allow the camera to observe fish the size of Killifish. I found that the system does have size limitations on specimens.

The lab-bred juveniles I originally aimed to use were too small for the system, so I transitioned to using larger wild juveniles. The setup also included setting up water reservoirs of the two salinities (0 ppt and 30 ppt). The ShuttleBox is constantly pumping water; therefore, I had to ensure sufficient amounts of 0 ppt and 30 ppt water. The freshwater reservoir could simply be filled up when it began to run low, but the saltwater was not as simple. This required two saltwater reservoirs to allow for one to fill up and be prepared with commercially available sea salt while the other pumped water into the ShuttleBox. This is why the two-hour trials were desirable, as there is no constant seawater source at Saint Mary's University. Due to the small size of Killifish, I also had to use mesh to block the input, output, and overflow holes in the ShuttleBox to prevent fish from escaping from the system. To prevent external stimuli from affecting the position of the fish, I used white garbage bags to block the perimeter of the ShuttleBox. I also adjusted the tubing through trial and error to be an appropriate length and in an appropriate position for optimal water flow. I also had to overcome some setup steps that were either not mentioned in the ShuttleSoft salinity manual or were not sufficiently explained, which required more time than originally predicted.

## 4.2 Determining Salinity Preference in Two Hours

Based on the previous knowledge of salinity preference of adult *F. heteroclitus* and *F. diaphanus* I predict that the ShuttleBox is capable of determining salinity preference in two hours. This will be supported if the calculated salinity preferences of adult *F. heteroclitus* and *F. diaphanus* are similar to their known salinity preferences. These shorter trials are beneficial to longer ones because they can be run while using less sea salt (preferable in labs without a constant seawater source). Additionally, shorter trials greatly reduce the time needed to run the experiments and allow for more time to be able to run a greater number of trials in less time. This

allows for more data that can give a better idea of the salinity preference of the population as a whole.

*F. heteroclitus* and *F. diaphanus* are both euryhaline (Fritz and Garside, 1974)a. This may allow them to adjust to the changing salinity quickly enough to calculate salinity preference within two hours. Further, *F. heteroclitus* is an estuarine fish that experiences a daily variation in salinity due to the changing tides (Fritz and Garside, 1974a). Because *F. heteroclitus* is adapted to an environment with a constantly changing salinity it may allow for it to sense and respond to the changing salinity of the system especially quickly. Additionally, a previous salinity preference study done on *F. heteroclitus* successfully used a 15-minute acclimation period with a 30-minute trial (Marshall et al., 2016). A study completed on *F. heteroclitus* observed that cells can react to changes in salinity within two minutes which further suggests that salinity preference in this fish may be calculated within 2 hours (Fougere et al., 2020).

## 4.3 Determining Salinity Preference

Based on the previous knowledge of *Fundulus* spp. salinity preference, I predicted that *F. heteroclitus* juveniles would have a salinity preference of 20 ppt and *F. diaphanus* juveniles would have a salinity preference of 1 ppt, similar to adults (Fritz and Garside, 1974a). Finally, I predicted that the F1 hybrid would have an intermediate salinity preference between the parent species, around 9.5 ppt, suggesting an additive genetic basis for preference. Although there is little data on the genetic basis of salinity preference in fish, this is the salinity where the species is found in the wild as the sites, we collected fish from include 9.5 ppt in their salinity gradient. Further, Jonah (2019) found that the F1 hybrids have a salinity tolerance that is intermediate of the two parental species (Jonah, 2019). The expected results align with the salinity preference of the adult *Fundulus* species and the salinities where they are most commonly found in the wild.

Typical habitat salinity is where most teleosts have the lowest energetic costs associated with osmoregulation (Ern et al., 2013). Osmoregulation is an energy-consuming process, so fish selecting salinities where osmoregulation is easiest may be at an advantage (Ern et al., 2013). This would allow for them to use less energy for osmoregulation and more for other activities such as seeking food or evading predators.

## 4.4 Experimental Limitations

### 4.4.1 Salinity Preference

Due to time constraints, I was unable to complete sufficient trials to determine the salinity preference of juvenile *F. heteroclitus*, *F. diaphanus*, and the F1 hybrid. Initially, I was faced with software and equipment issues and malfunctions that reduced my time to run trials. Further, due to Covid-19 physical distancing restrictions, we were rarely able to work in the lab as a team. This caused tasks to take longer than expected and impaired communication because we could not observe and work on the system at the same time.

### 4.4.2 Salinity Preference Trial Length

Initially, our goal was to run 24-hour salinity preference trials with eight *F. heteroclitus*, eight *F. diaphanus*, eight *F. heteroclitus* x *F. diaphanus*, and eight *F. diaphanus* x *F. heteroclitus* juveniles. After working with the system, I quickly realized that to complete trials of such a length I would need a constant seawater source which I did not have access to. I had access to a constant freshwater source, but seawater had to be made. As a result of time constraints and the lack of a constant saltwater source, I changed my trial length to two hours with a one-hour acclimation period and one hour of trial.

### 4.4.3 Experimental Organism

The original goal was to use lab-bred juveniles for the experiment. After some trial and error, I discovered that these fish at their current stage would be too small for the uEYE camera to track. To overcome this, I tried using the larger lab-bred juveniles, but these were still too small to be tracked. I also made the base of the ShuttleBox as white and uniform as possible to make it easier for the camera to track the fish, but this was still not sufficient to track the lab-bred juveniles. As a result, I changed my methods to use the wild-caught juveniles which were much larger and capable of being tracked by the camera.

## 4.5 Future Directions

I think that when this experiment is run it would be most beneficial to run salinity preference trials with lab-bred juveniles. With F1 hybrid lab-bred juveniles, the mother and father's species are known, whereas for wild hybrid fish this would require mitochondrial genotyping. However, 96% of the wild F1 hybrids have *a F. diaphanus* mother and a *F. heteroclitus* father (Dalziel et al., 2020). Running trials with the lab-bred fish would allow us to determine if parental effects are related to salinity preference and if salinity preference is a maternally or paternally inherited trait. Further, it could be beneficial to run trials using wild *Fundulus* juveniles caught from different sites within Porters Lake to determine if those potentially bred and reared at different salinities have different salinity preferences.

# References

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamonien, M. et al. (2012). Costs of dispersal. *Biological Reviews*, 290-312.

Brennan, R.S., Galvez, F., and Whitehead, A. (2015). Reciprocal osmotic challenges reveal mechanism of divergence in phenotypic plasticity in the Killifish . *Journal of Experimental Biology*, 1212-1222.

Bucking, C., Wood, C.M., and Grosell, M. (2012). Diet influences preference on estuarine fish, the killifish *Fundulus heteroclitus*. *The Journal of Experimental Biology*, 1965-1974.

Caughley, G. (1994). Directions in conservation biology. *Journal of Animal Ecology*, 215-244.

Christensen, E.A.F., and Grosell, M. (2018). Behavioural salinity preference of juvenile yellow perch *Perca flavenscens*. *Journal of Fish Biology*, 1620-1626.

Cossins, A.R., and Crawford, D.L. (2005). Fish as models for environmental genomic. *Nature Review Genetics*, 324-333.

Dalziel, A.C., Tirbhowan, S., Drapeau., H.F., Power, C., Jonah, L.S., Gbotsyo, Y.A., and Dion-Cote, A. (2019). Using asexual vertebrates to study genome evolution and animal physiology: Banded ( *Fundulus diaphanus* ) x Common Killifish ( *F. heteroclitus* ) hybrid lineages as a model system. *Evolutionary Applications*, 1-26.

Dawley, R. (1991). Clonal hybrids of the common laboratory fish *Fundulus heteroclitus*. *Proceedings of the National Academy of Sciences*, 2485-2488.

Dawley, R.M., Chrzanowski, K.L., Beaulieu, K., and Goddard, K.A. (1999). Allozyme Analysis of Clonal Diversity Among Unisexual Hybrids of the Killifishes *Fundulus diaphanus* and *F. heteroclitus*. *The Journal of Heredity*, 607-612.

Edwards, S.L., and Marshall, W.S. (2012). Principles and Patterns of Osmoregulation and Euryhalinity in Fishes. In S. F. McCormick, *Euryhaline Fishes* (pp. 2-32). Waltham: Academic press.

Ern, R., Huong, D.T.T., Cong, N.V., Bayley, M., and Wang, T. (2014). Effect of salinity on oxygen consumption in fishes: a review. *Journal of Fish Biology*, 1210-1220.

Evans, D.H. and Claiborne, J.B. (2009). Osmotic and ionic regulation in fishes. In D. Evans, *Osmotic and Ionic Regulation: Cells and Animals* (pp. 295-366). Boca Raton: Taylor and Francis Group.

Fougere, B., Barnes, K.R., Francis, M.E., Claus, L.N., Cozzi, R.R.F., and Marshall, W.S. (2020). Focal adhesion kinase and osmotic responses in ionocytes of F*undulus heteroclitus*, a euryhaline teleost fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 207-217.

Fritz, E.S., and Garside, E.T. (1974)a. Identification and description of hybrids of *Fundulus heteroclitus* and *F. diaphanus* (Pisces: Cyprinodontidae) from Porters Lake, Nova Scotia, with evidence for absence of backcrossing. *Canadian Journal of Zoology*, 1433-1442.

Fritz, E.S., and Garside, E.T. (1974)b. Salinity preferences of *Fundulus heteroclitus* and *F. diaphanus* (Pisces: Cyprinodontidae): their role in geographic distribution. *Canadian Journal of Zoology*, 997-1003.

Griffith, R. (1974). Environmental and salnity tolerance in the genus *Fundulus*. *Copeia,* 319-331.

Hernandez-Chavez, C., and Turgeon, J. (2007). Asexual and sexual hybrids between *Fundulus diaphanus* and *F. heteroclitus* in the Canadian Atlantic region. *Molecular Ecology*, 1467-1480.

Hirzel, A.H., and Le Lay, G. (2008). Habitat suitability modelling and niche theory. *Journal of Applied Ecology*, 1372-1381.

Jonah, L. (2019). Evolutionary variation in salinity tolerance among species of Killifishes (Fundulus spp.). *Master's Thesis. Saint Mary's University, Halifax.*

Kültz, D. (2012). The Cominatorial Nature of Osmosensing in Fishes. *Physiology*, 259-275.

Kültz, D. (2013). Osmosensing. In S. F. McCormick, *Euryhaline Fishes* (p. 46). Waltham: Academic Press.

Kültz, D. (2015). Physiological mechanisms used by fish to cope with salinity stress. *Journal of Experimental Biology*, 1907-1914.

Kearney, M. and Porter, W.P. (2004). Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. *Ecology*, 3119-3131.

Levin, S. (1992). The problem of pattern and scale in ecology. *Ecology*, 1943-1967.

Marshall, W.S., Tait, J.C., and Mercer, E.W. (2016). Salinity Preference in the Estuarine Teleost Fish Mummichog (*Fundulus heteroclitus)*: Halocline Behavior. *Physiological and Biochemical Zoology*, 225-232.

McCain, S.C. Kopelic, S., Houslay, T.M., Wilson, A.J., Lu, H., and Earley, R.L. (2020). Choice consequences: salinity preferences and hatchling survival in the mangrove rivulus (*Kryptolebias marmoratus*). *Journal of Experimental Biology*, 1-10.

McManus, L.C., Yurek, S., Teare, P.B., Dolan, T.E., and Serafy, J. (2014). Killifish habitat suitability as a measure of coastal restoration performance: integrating field data, behavioral trials and simulation. *Ecological Indicators*, 173-181.

Merette, D., Bourret, A., and Turgeon, J. (2009). Diversity and distribution of gynogenetic hybrids between *Fundulus diaphanus* and *Fundulus heteroclitus* in Porter's Lake (Nova Scotia) in relation to salinity. *Journal of Fish Biology*, 1115-1122.

Nguyen, L.P., Nol, E., Abraham, K.F., anf Lishman, C. (2013). Directional selection and repeatability in nest-site preferences of Semipalmated Plovers (*Charadrius semipalmatus*). *Canadian Journal of Zoology*, 646-652.

Potter, I.C., Chuwen, B.M., Hoeksema, S.D., and Elliot, M. (2010). The concept of an estuary: a definition that incorporates systems which can become closed to the ocean and hypersaline. *Estuarine, Coastal and Shelf Science*, 497-500.

Schulte, P. (2014). What is environmental stress? Insights from fish living in a variable environment. *Journal of Experimental Biology*, 23-24.

Svardson, G. (1949). Competition and habitat selection in birds. *Oikos*, 157-174.

Whitehead, A. (2010). The evolutionary radiation of diverse osmotolerant physiologies in Killifish (Fundulus Sp.). *Evolution*, 1-16.

Yu, S.L., and Lee, T.W. (2002). Habitat Preference of the Stream Fish, S*inogastromyzon puliensis* (Homalopteridae). *Zoological Studies*, 183-187.