The Effects of Salinity on Behaviors and Gill Epithelium of *Betta splendens*

A Report of a Senior Study

by

Shelby O'Brien

Major: Biology

Maryville College

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Date Approved_____, by_____

Faculty Supervisor

Date Approved_____, by_____

Editor

ABSTRACT

The purpose of this study is to better understand the osmoregulatory functions of *B. splendens* and to address whether or not higher salinity environments would be a healthy treatment to reduce stress, serve as an osmoregulatory aid, and manage a variety of ectoparasite, bacterial, and fungal diseases by examining both the natural and aggressive behaviors as well as their gill morphology. This study tested the hypothesis that salinity does not have a significant impact on the gill morphology itself, but may cause behavioral changes as well as possible dehydration in *B. splendens.* Behavioral tests were conducted for a 21 day period and then gills were removed and made into slides stained with hemotoxylin and eosin. Whereas salt-exposed fish tended to have more aggressive acts than control fish, there was no significant difference in the number of aggressive behaviors between the two groups over time ($p = 0.068$). In addition, neither group ($p = 0.512$) nor time ($p = 0.846$) independently had significant effects. Epithelial cell size was significantly reduced in salt-exposed individuals ($p = 0.0253$). This study's results rejected the previous hypothesis that behavior would significantly change and gill morphology would not be affected by salinity, although trends were seen towards accepting the behavioral portion.

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ACKNOWLEDGMENTS

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- All friends who gave a fish a home when the study reached its end

CHAPTER I

INTRODUCTION

OSMOREGULATION

Osmoregulation is the control of tissue osmotic pressure which drives the movement of water across biological membranes in order to create equilibrium between the concentrations of solutes within the organism and its external environment (Kay 1998). Animal cells cannot actively pump water; therefore, osmotic regulation requires the movement of solutes across membranes in order to alter osmotic gradients. Normal animal function depends on precise regulation of diverse physical relationships and biochemical processes, which are influenced by the environment. Animals maintain favorable concentrations of solutes and solutions in their intracellular and extracellular fluids by means of their epithelial tissues that form a barrier with their environment. They also use different tissues to control ion and water balance which is usually controlled by the renal tissues and extrarenal epithelium, such as gills, skin, and digestive mucosa of most animals (Moyes 2006).

All animals regulate the ionic profile of intracellular fluids, but they differ in the nature of extracellular fluid compartments. There are two main classifications for fish in this department: osmoconformer and osmoregulator (Kay 1998). An osomoconformer's internal osmolarity resembles that of its environment. If the external environment changes then the internal osmolarity changes parallel with it. These animals include marine invertebrates and primitive vertebrates. An osmoregulator maintains internal osmolarity regardless of the environment. Most marine and freshwater vertebrates are osmoregulators (Moyes 2006). An animal's ability to tolerate changes in its external osmolarity can also lead to the classification of the animal as

either a stenohaline or a euryhaline. Stenohalines can only tolerate a narrow range of salt concentrations, whereas euryhalines can tolerate wide variance in osmolarities (Kay 1998).

The gills and kidneys are the most important organs responsible for osmoregulation in fresh water and salt water fish, with the gills in direct contact with the external environment and the kidneys controlling the internal environment (Marshall and Grosell 2006). Fish gill lamellae are composed of mitochondria-rich chloride cells interspersed among two types of pavement cells, one rich in mitochondria and the other with a low mitochondrial content. These two types of mitochondrial rich cells carry out most of the transport functions of the gills (Moyes 2006).

FRESHWATER FISH

The gills are especially important in regulating ion concentrations in freshwater fish, which are hyposmotic to their environment. In order to obtain enough ions and perform osmoregulation, fresh water fish take in ions from their surrounding environment (see figure 1). The epithelial tissues in freshwater teleosts have many transporters that are frequently implicated in ion and water balance. The Na^+/K^+ ATPase is central to ion movements using the energy of ATP hydrolysis to export three Na^+ in exchange for importing two K^+. Various ion channels can open or close in response to mechanical, electrical, or chemical signals to permit specific ions to flow down electrochemical gradients (Moyes 2006). In freshwater fish, the current model for ion exchange links Cl^- uptake to HCO_3^- secretion using an anion exchanger. Meanwhile, Na^+ uptake is thought to occur via a Na^+ channel linked electrochemically to a coupled V-type H^+ ATPase on the apical membrane (Hawkings 2003). Some tissues use an H^+ ATPase to pump protons to change pH, a driving force for other transport processes (Moyes 2006). This model is based on the requirement to move both Na^+ and H^+ against their electrochemical gradients in low ionic

strength media (Hawkings 2003). In order to maintain plasma osmolality as well as ion and water balance, teleosts take in salts from fresh water through their gills, produce hypotonic urine to reabsorb salts, and expel excess water by the kidneys (Marshall and Grosell 2006). The gills of a freshwater fish must take up a Na^+, Ca^{2+}, and other ions from the water, frequently against steep electrochemical gradients. PNA- cells take up Na^+ through a channel. Although there is an unfavorable gradient for Na^+ uptake, these cells create local acidification using the H^+ ATPase which enhances the electrochemical driving force for sodium importation (Moyes 2006).When fresh water fish, such as rainbow trout, are exposed to salt water, there are some physiological changes that occur in order for adaptation due to the sudden increase in salinity. An initial period of increased drinking, together with diffusion of salts into the gills, results in an increased salt load, reflected in increased plasma and body salt content (Bath 1979). These fish end up failing to adapt to full strength sea water but after eight hours in two-thirds sea water a stabilization period begins, lasting some 7-10 days and eventually resulting in fully adapted fish which show normal plasma ion concentration, but increased cellular content of Na^+ and Cl^-, especially in muscle tissue (Bath 1979).

SALTWATER FISH

In contrast to freshwater fish, marine fish must avoid excess ion uptake and limit water loss (see Figure 1). Chloride cells are very important in saltwater fish for excreting ions. The combined actions of the Na^+/K^+ ATPase and the Na^+ /K^+-$2Cl^-$ cotransporter bring potassium and chloride into the cell from the blood. The Cl^- channels in the apical membrane allow Cl^- to escape into the seawater and basolateral K^+ channels allow K^+ to return to the blood (Moyes 2006). However, recent studies have revealed that intestinal anion exchange contributes significantly to Cl^- absorption, in exchange for HCO_3^- secretion (Grosell 2010). This also proves

that this exchange process is important for intestinal water absorption. In addition to contributing to solute coupled water absorption, intestinal anion exchange results in the precipitation of $CaCO_3$ which acts to reduce luminal osmotic pressure and also assists in water absorption. Most recently, activity of apical H^+ pumps, especially in distal segments of the intestine have been suggested to promote anion exchange and to reduce osmotic pressure in the lumen by preventing excess HCO_3^- concentrations from accumulating in intestinal fluids, also aiding in water absorption (Grosell 2010).

The Na^+ concentration gradient is favorable for linked electroneutral exchange in saltwater fish. The mechanism by which the protons are removed is believed to occur is through an electroneutral sodium/proton exchange (NHE) system, reducing the ATP requirement for acid–base regulation (Hawkings 2003). NHE works as a reversible transporter driven by the electrochemical pH gradient. (Moyes 2006). NHE isoforms have been identified by molecular and immunological methods in the gills of a marine species (*Myoxocephalus octodecimspinosus*) and the euryhaline killifish (*Fundulus heteroclitus*). In contrast to freshwater fish, marine fish excrete salts through the gills and produce a minimal volume of isotonic urine since their kidneys exhibit very low glomerular filtration or even lack glomeruli (Marshall and Grosell 2006). Because of this reason, water balance is a critical factor in the survival of euryhaline teleosts in different salinity environments (Cheng-Hao 2009). The following figure shows the key differences in how freshwater and saltwater fish take in both the ions and water during osmoregulation.

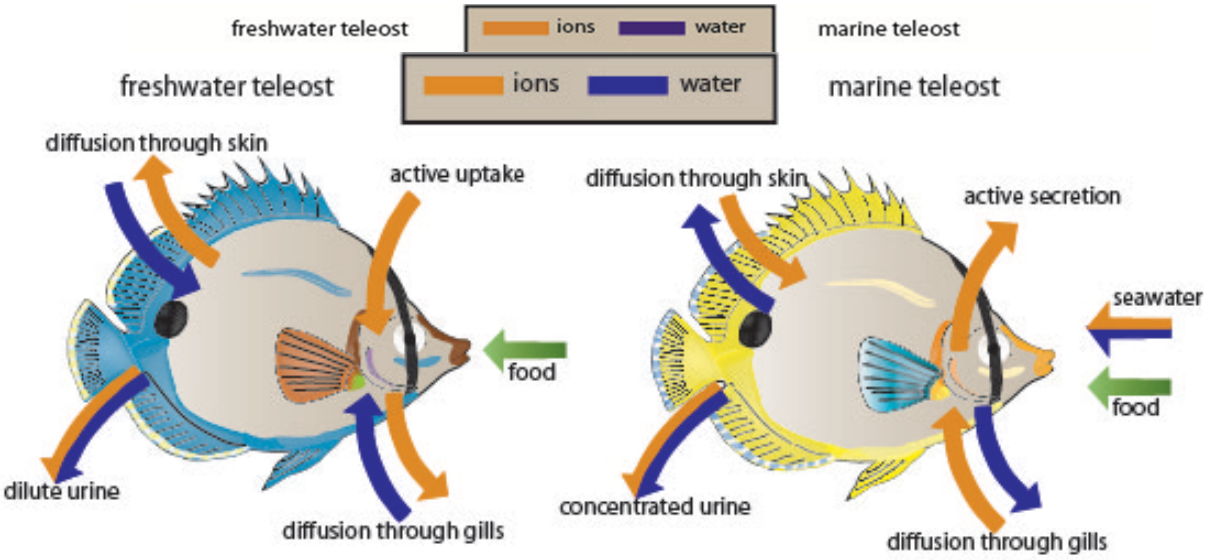

Figure 1: Freshwater and saltwater uptake of ions and water during osmoregulation. Designed by Amanda Burchett.

When it comes to saltwater fish in a freshwater environment, a study conducted on spotted halibut has shown that it may be possible for saltwater fish to survive in low salinity environments for a time, but that they do not fully adapt and that they do not survive when exposed to freshwater (Wada 2004). The halibut in this experiment that were exposed to over 4 ppt survived for 20 days, but all the fish exposed to 1 ppt died within 5 days. The halibut that were exposed to 4 ppt also attained almost the same body length as the control group at 20 days after transfer, but these fish showed an abnormally dark body color as well as delayed development. This suggests that they do not completely adapt to a lower salinity (Wada 2004). The percentage of prolactin-cell volume to pituitary volume was significantly higher in the lower salinity group and the chloride cells in the gill filaments were also significantly larger in the lower salinity group. These results suggest that juveniles could adapt to a low-salinity environment due to the activation of prolactin production and enlargement of chloride cells. It also suggests that late metamorphic larvae and early juveniles of certain saltwater fish may be

able to utilize a low salinity environment such as estuarine tidal flats or very shallow coastal areas as their nursery grounds (Wada 2004).

MIGRATORY FISH

Some species of fish are diadromous, which means they migrate between fresh water and salt water. Prior to the migration of species, such as salmon, from freshwater to saltwater, a process called smoltification occurs (Moyes 2006). This is when the gills of the fish undergo a dramatic cellular reorganization as the ion pumps of the gills prepare for exposure to the new environment. Seawater acclimation results in many changes in gill function including increases in the activity of Na^+/K^+ATPase in mitochondria rich gill cells, naturally associated reductions in H^+ ATPase, and the appearance of accessory cells on the gill epithelium (Hawkings 2003).

The adrenal-cortex hormone cortisol initiates many of the salinity-associated adaptations in fish, whereas the anterior pituitary hormone prolactin is associated with freshwater adaptations (McCormick 2001). Cortisol also promotes ion uptake and interactions with prolactin under some circumstances during acclimation to fresh water. The osmoregulatory actions of growth hormone and prolactin are antagonistic, and in some species, some thyroid hormones support the action of growth hormone and cortisol in promoting seawater acclimation (Moyes 2006).

BETTAS

Although considered strictly as a freshwater fish that lives in the rice fields of Asia, the Siamese fighting fish has a surprisingly high tolerance for saltwater (Sampio 2009). The wild Siamese fighting fish received its name due to its original location when it was discovered in Siam and its aggressive behavior. The Siamese fighting fish is specifically native to the Mekong basin in Southeast Asia which includes Thailand, Malaysia, Cambodia and Myanmar

(Jaroensutasinee 2008). *Betta* is a large genus of freshwater ray finned fishes. It is a member of the gourami family (family *Osphronemidae*), of order *Perciformes*, but was formerly classified among the *Anabantidae,* which means that it has a labyrinth organ (Lertpanich 2007; Jaroensutasinee 2008). The accessory air-breathing organs that are a part of these types of fish are alternative gas exchange organs that include a labyrinth organ, skin, lungs, respiratory gas bladders, digestive tracts, and structures derived from buccal, pharyngeal, and branchial cavities. These species have shown that they possess branchial and systemic circuits similar to a double-circuit circulatory system. The anterior gill arches receive blood from the heart and are the site for gas exchange, and the blood then flows to the labyrinth organ for further oxygen uptake before returning to the heart. The structural modifications and enlarged vessels in the posterior gill arches assist in moving the oxygenated blood from the heart into systemic circulation (Huang 2010).

The most well-known of the *Betta* species is *B. splendens* as it is traditionally kept as a fighting fish/pet (Lertpanich 2007). The various bettas can be divided into two groups, based on their spawning behavior: some build bubble nests, like *B. splendens*, while others are mouthbrooders, like *B. picta*. The mouthbrooding species are sometimes called "pseudo bettas", and are sometimes speculated to have evolved from the nest-builders in an adaptation to their fast-moving stream habitats (Lertpanich 2007). The wild bubble nesting betta is categorized into three species, which are *B. splendens*, *B. smaragdina* and *B. imbellis*. The name *B. splendens* means the brilliant warrior. Males of this colorful species perform an elaborate aggressive display when provoked. *B. splendens* has reddish body color and blue-green fin with red strip on the operculum as its important character. The word smaragdina, found in the name *B. smaragdina,* means the color emerald. This name indicates the color of the species. The

operculum of this species also has green scale shape. *B. imbellis* looks similar to *B. splendens* but with green strip on the operculum (Lertpanich 2007).

Figure 2: Bettas found in Thailand. (Left) *B.splendens,* (middle) *B. smaragdina,* (right) *B. imbellis*

Wild bettas are usually smaller with a length of 2.5-3 cm compared to the domesticated ones, which have a length of 3-6 cm. The body depth of the wild bubble nesting bettas has 2.8 to 3.6 folds of its body length (Lertpanich 2007). *B. splendens* inhabits in the shallow waters with muddy bottoms or rice paddy fields, shallow ponds, and even slow moving streams characterized by low pH, low O_2 concentration, high CO_2 concentration and high water temperature (Lertpanich 2007; Jaroensutasinee 2008). However, the domesticated fish grows well in 6.5-7.5 pH range, 75-100 mg of hardness and water temperature between 25-28°C (Lertpanich 2007).

Unlike domesticated fighting fish, wild fighting fish are small, inconspicuous, and dull brown or green in color. They hide beneath water plants, presumably to minimize predation from turtles, snakes, fish-eating egrets, herons and kingfishers. (Jaroensutasinee 2008). Male Siamese fighting fish have very aggressive social displays including gill cover erection, biting, tail beating, attacking and chasing. Fighting usually involves physical damage and can result in death. Females are duller in color and usually smaller than males. After the females finish laying

eggs, the males chase the mated females out of the bubble nest areas and solely provide parental care for developing eggs and larval fish (Jaroensutasinee 2008).

SALINITY TOLERANCE IN BETTAS

Sodium chloride has been used as a remedy for freshwater fish to reduce stress, serve as an osmoregulatory aid, and manage a variety of ectoparasite, bacterial, and fungal diseases (Burgdorf-Moisuk 2011). There are a variety of commercially available salts that can be used to treat fish including those used for recharging water softeners, artificial sea salts, aquarium salts, and any salt intended for human or animal consumption that does not contain iodine or added minerals. The anticaking agent found in some salts, such as yellow prussate of soda, and sodium iodide are both toxic to fish (Burgdorf-Moisuk 2011). Currently, most recommendations for treating fish with salt are dependent on the species and purpose of the treatment. These published doses and treatment durations vary from 0.015–35 parts per thousand, and indefinitely to 3 min, respectively. At higher concentrations and exposure durations, side effects can include agitation, increased opercular movements, loss of equilibrium, and death (Burgdorf-Moisuk 2011).

A study conducted in Brazil looked at how changes in salinity affected this specific type of ornamental fish. The salinity tolerance of *Betta splendens* was evaluated in this study by calculating the mean survival time and median lethal salinity. The chronic effect was also assessed by calculating the salinity survival maximum and median lethal salinity. Considering that the *B. splendens* is a freshwater species, this study suggested that this species demonstrated a high tolerance for water salinity (Sampio 2009). Other studies have also shown that when *B. splendens* undergoes a hypophysectomy, mucous cells in the skin decrease and the fish can no

longer survive in freshwater for an extended period of time, but can still survive in saltwater, dilute saltwater, or in a fish Ringer solution (Shukla 2009).

PURPOSE

Due to the fish's ability to survive in a higher salinity, both with and without severe hormonal changes, the purpose of this study is to better understand the osmoregulatory functions of *B. splendens* and to address whether or not higher salinity environments would be a healthy treatment to reduce stress, serve as an osmoregulatory aid, and manage a variety of ectoparasite, bacterial, and fungal diseases by examining both the natural and aggressive behaviors as well as their gill morphology. This study will test the hypothesis that salinity does not have a significant impact on the gill morphology itself, but may cause behavioral changes as well as possible dehydration in *B. splendens.*

CHAPTER II

METHODS

EXPERIMENTAL DESIGN

Eight *Betta splendens* specimens were obtained to examine behavioral, physiological, and anatomical influences of saline exposure. Four specimens were obtained from Fins N Skins in Knoxville, TN. The other four were obtained from Petsmart in Maryville, TN. The fish were then divided into the control group and the 5ppt saline group. Two fish from each store were exposed to the different groups. Figure 3 shows the treatment of the fish.

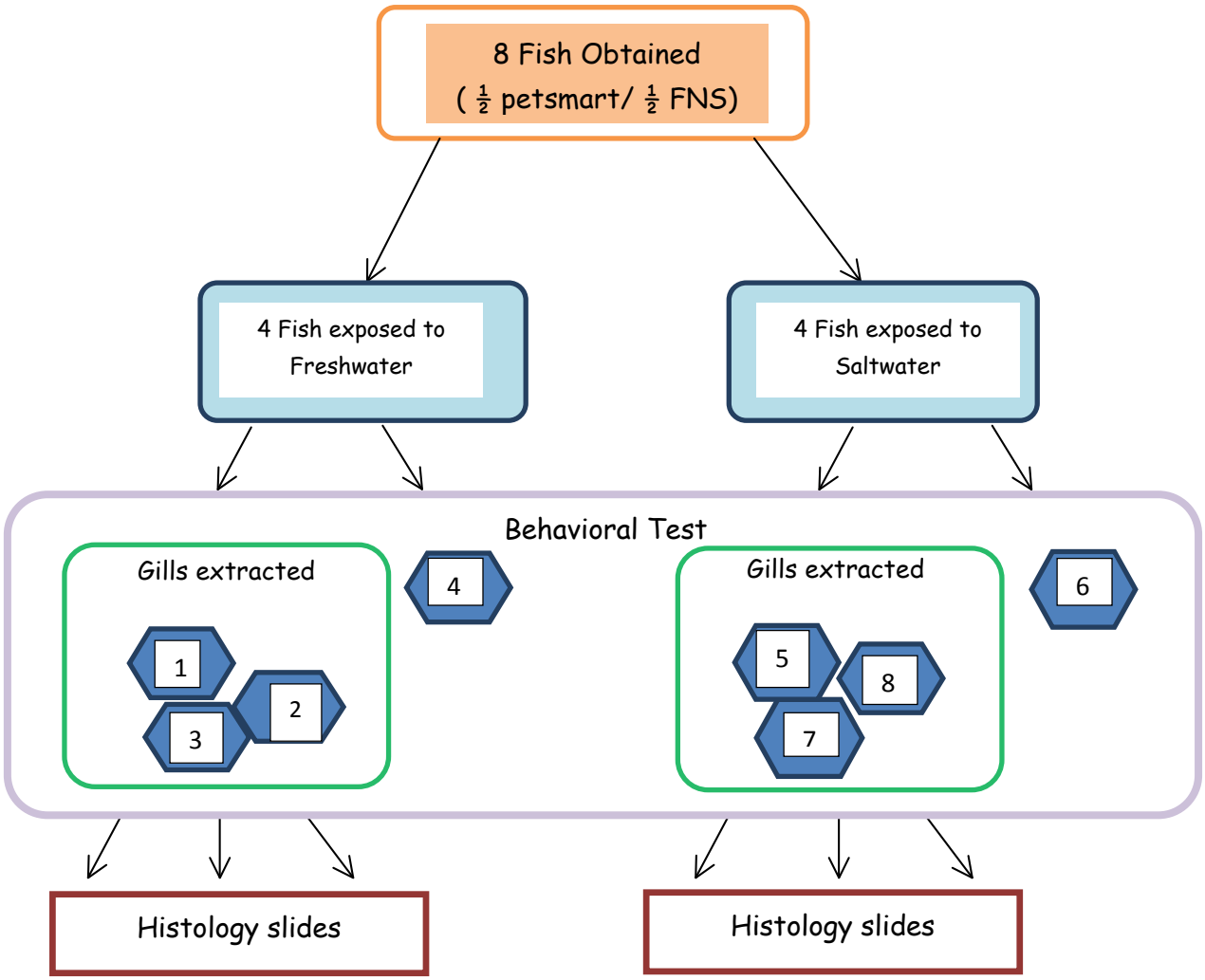

Figure 3. Treatment of the eight betta fish obtained from Petsmart and Fins N Skins. Two fish from each store were placed into either the saline or control group. Behavioral tests were conducted every other day for 21 days. Gills and blood were then extracted and examined.

Individuals were housed in ½ gallon tanks with dividers (¼ gallon exposure) and were fed betta pellets once a day. Four tanks had a salinity content of 5ppt, achieved by adding approximately 1/8 cup of Instant Ocean to 4 gallons of water and measured using a Vernier Lab Pro Salinity probe and distributed into the tanks. Both freshwater and saltwater were treated with a water conditioner in order to dechlorinate the water.

Behavioral Measurements

Each day, the fish were individually taken out of their tanks and placed into a five gallon tank. Those exposed to salinity were placed in a 5ppt salinity tank and those who were not exposed were placed in a freshwater tank. After five minutes of acclimatization, a mirror was placed in front of them and their behavior was observed for five minutes. They were then placed back into their original housing facility. This behavior was compared to a behavior ethogram every 20 seconds for a 5 min period and was conducted every other day for twenty-one days. The following figure shows the ethogram used to measure aggressive behavior.

Table 1. Ethogram used to measure betta aggressive behavior every 20 seconds for a 5 min period.

H	Hover- hangs in water column with fins not spread.	0
BR	Bottom rest.	0
B	Breathing (surfacing and gulping air)	0
FS	Fin Swim- slow swimming using pectoral fins; without fins in spread condition	0
SS	Serpentine Swim- rapid, uses entire body and S-shaped movements without fins in spread condition.	0
S	Shaking- shimmies the body, usually with fins and gills spread.	1
GS	Gill Spread- only indicate this if the behavior is not done as part of one of the next behaviors.	1
FSH	Fin Spread with body Horizontal to mirror. This may include periods of gill spreading as part of the action pattern.	1
FSP	Fin Spreading display with body Perpendicular to the mirror. This may include periods of gill spreading as part of the action pattern.	1
A	"Arches" body. This may include periods of gill spreading as part of the action pattern.	1
Ch	Charges mirror- approaches rapidly but does not touch. This may start from FSP. A charging fish may have its fins and gills spread. A movement towards the mirror out of a display makes the behavior a charge.	1
Ct	Contacts mirror- same as above but with actual contact. Watch approach before designating as either Ch or Ct. Probably a rare behavior for most fish.	1
L	Leave- swims away from the mirror.	0

GILL EPITHELIUM MEASUREMENTS

After the twenty-one day observation period, three of the control fish and three fish exposed to higher salinity concentrations were sedated using solution of Sigma Aldrich ethyl 3-

aminobenzoate methanesulfonate salt (MS-222; Aug 2005) and ddH_2O at 400mg/ml. Once sedated, the operculum was removed using small scissors under a dissection microscope. This exposed the gills of the fish. The gills were then removed by cutting through membranes at the top and bottom of the gill arches. The gill arches were removed and a single gill arch from each side of the fish was added to Ricca Chemical Co. Bouins solution (Cat no. 1120-16; Lot 2112023; exp. 05/2013). Gills were allowed to sit in the Bouins solution for 24 hours and then were cleared by rinsing with 70% ethanol three times. The gills were then dehydrated by moving the tissues through increasing percentages of alcohol- (80%, 95%, 100%, and 100%) and allowing them to sit for one hour in each percentage. Tissues were then placed in Fisher Scientific Protocol Safeclear for an hour and then placed in fresh Safeclear for another hour. The tissues were then infiltrated with paraffin wax by placing them into four different paraffin wax solutions for 45 min each under vacuum. The following are the waxes and the pressures at which the gills were housed: Wax I @ 12 psi; Wax II @ 15 psi; Wax III @ 21 psi; and Wax IV @ 25 psi. The tissues were placed in a wax pot and embedded in a wax block. The paraffin block was then trimmed around the tissue and mounted onto the microtome. Tissues were sectioned in 12μm ribbons, which were floated in a warm-water bath with a small amount of gelatin and mounted onto a slide.

Tissues were stained using the hematoxylin and eosin staining method (Presnell and Schreibman 1997). Slides were exposed to Safeclear for 10 minutes and then exposed to decreasing percentages of alcohol (100%, 95%, and 70%) for one minute in each percentage. They were then rinsed with running water for four minutes. After being hydrated the slides were stained in hematoxylin for four minutes. They were then rinsed again with running water for four minutes to wash away the stain. They were transferred into Scott solution for 2 min and then

rinsed once more in running water for four minutes. The slides were then stained in eosin for 3 minutes and then dipped twice in 70% and 95% ethanol. They were then placed into 100% ethanol solution for two minutes. The slides were then placed into another solution of 100% ethanol for two minutes and then placed into Safeclear for four minutes. This was then repeated in a fresh container of Safeclear (Presnell and Schreibman 1997). Once the slides finished this process, a slide cover was glued to the slides using paramount and the slides were examined. To measure the cells of the gill epithelium, the microscope used was standardized using a stage micrometer. The gills were then placed under 400X magnification and the height of the epithelial cells from plasma membrane to plasma membrane were measured. This was then converted using the micrometer conversion factor. These were then averaged and graphed.

STATISTICAL ANALYSIS

A two-way repeated measures ANOVA was conducted on the behavioral data to determine if there was a significant difference in behavioral changes when the fish were exposed to a higher salinity. This was done by running a balanced ANOVA in Minitab. The aggressive measurements were considered the response and the model was conducted as follows:

TREATMENT ID (TREATMENT) DAYS TREATMENT*DAYS

In this experiment, the treatments were divided into a control (A) and a 5ppt salt (B). The IDs were represented by the letters A-D for both treatments. The p-value for TREATMENT comes from using ID (TREATMENT) as the error term which is appropriate as TREATMENT is a between-subjects factor. The p-values for DAYS and TREATMENT*DAYS uses the error term which is appropriate for within-subjects factors. The simple squamous epithelial cells on the gill

lamellae were measured and a two sample t-test assuming equal variance was used in order to determine if there was any significant difference in the size of the cells.

CHAPTER III

RESULTS

The betta fish were observed for a 21 day period. The average number of aggressive behaviors over the 21 day period can be seen in figure 4. Whereas salt-exposed fish tended to have more aggressive acts than control fish, there was no significant difference in the number of aggressive behaviors between the two groups over time ($p = 0.068$). In addition, neither group ($p = 0.512$) nor time ($p = 0.846$) independently had significant effects.

After slides of the gills were completed, the simple squamous epithelial cells were measured to see if there was a difference in size due to osmosis. Epithelial cell size was significantly reduced in salt-exposed individuals ($p = 0.0253$).

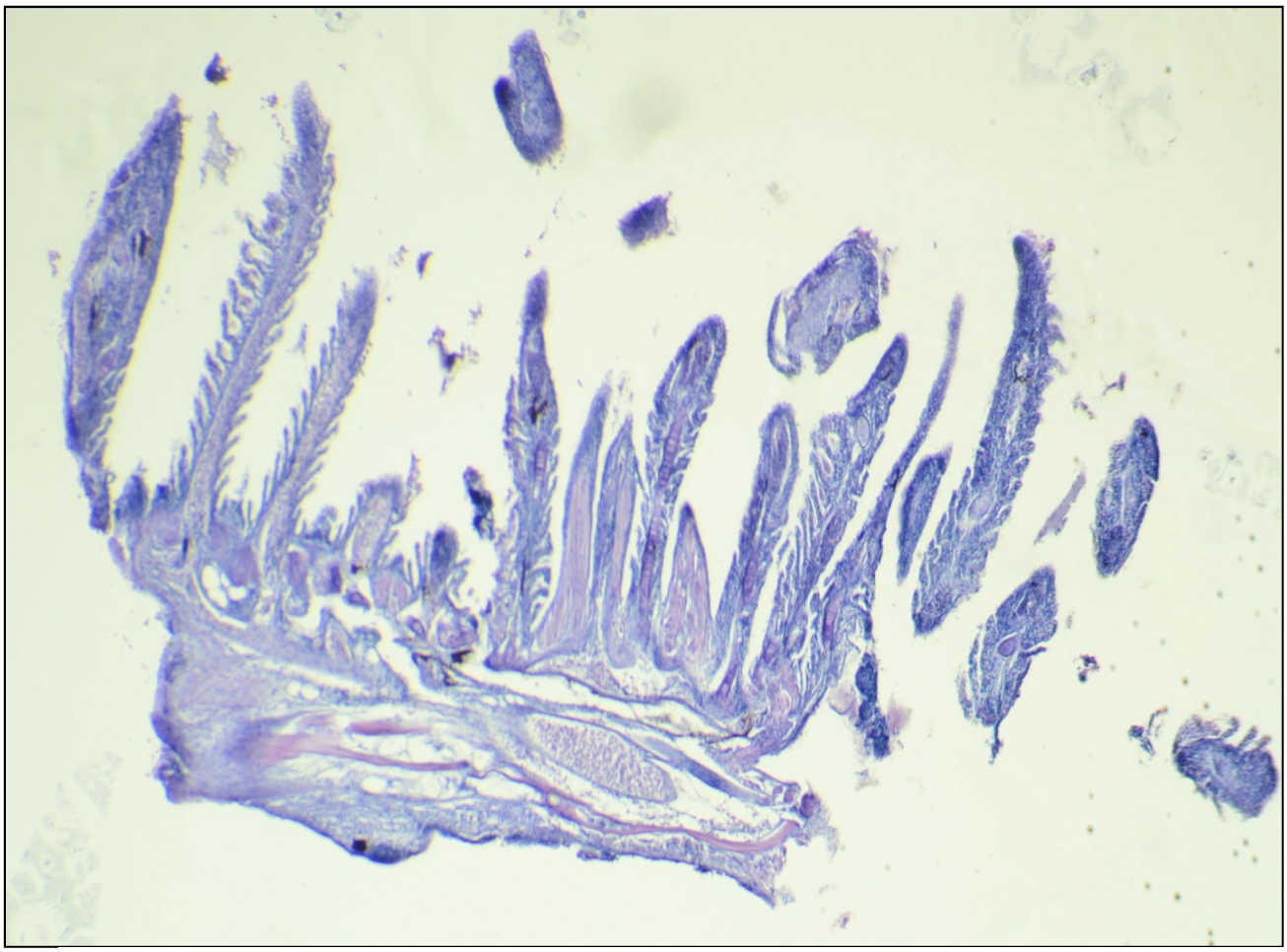

Figure 4. Gill filament and lamellae of a saltwater exposed betta fish gill. 1) primary lamellae. 2) secondary lamellae.

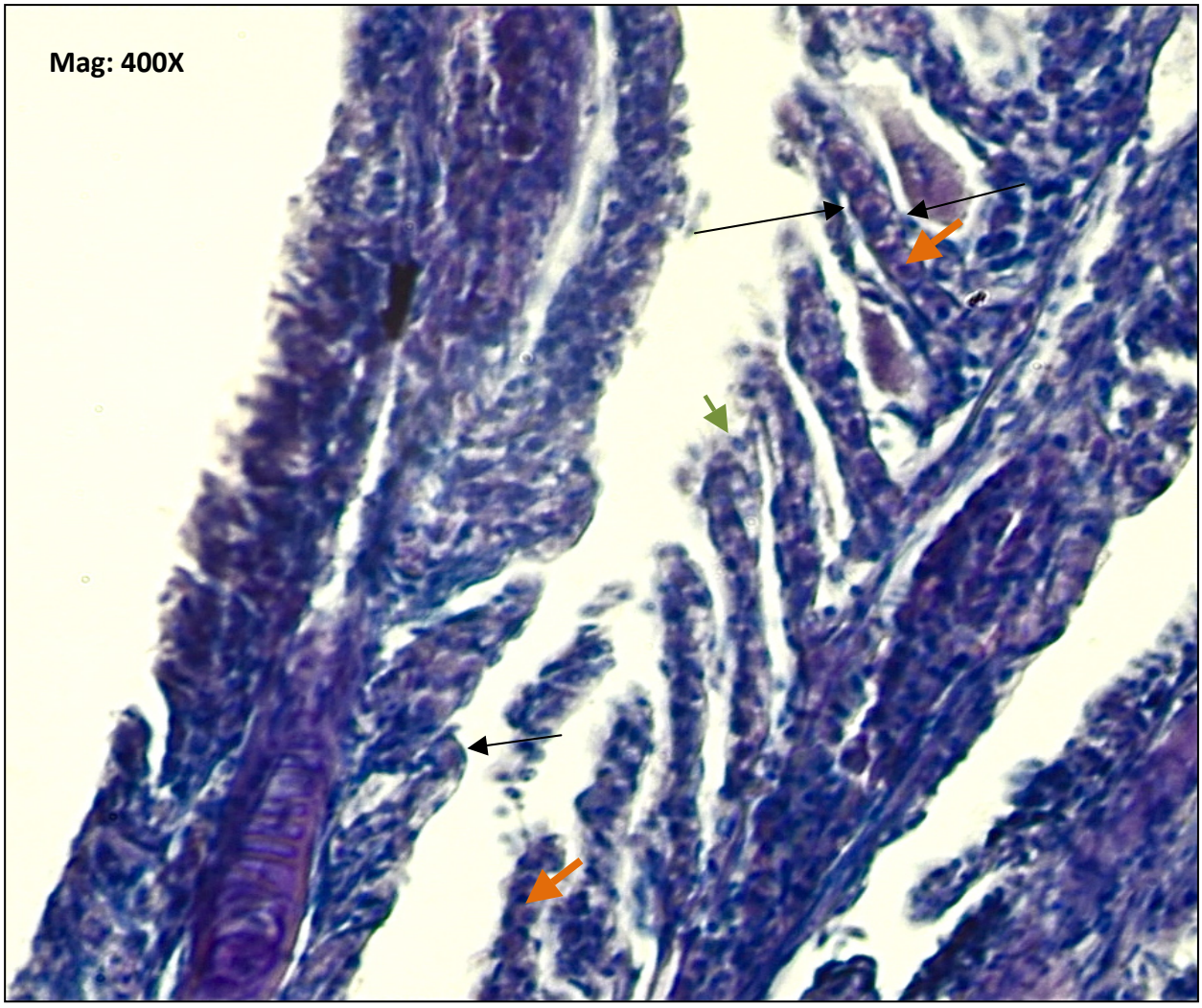

Figure 5. Close up of gill lamellae in saltwater exposed fish. Arrows indicate simple squamous epithelial cells. Orange arrows indicate erythrocytes found in the lamellar capillaries. Green arrow indicates mucous cell.

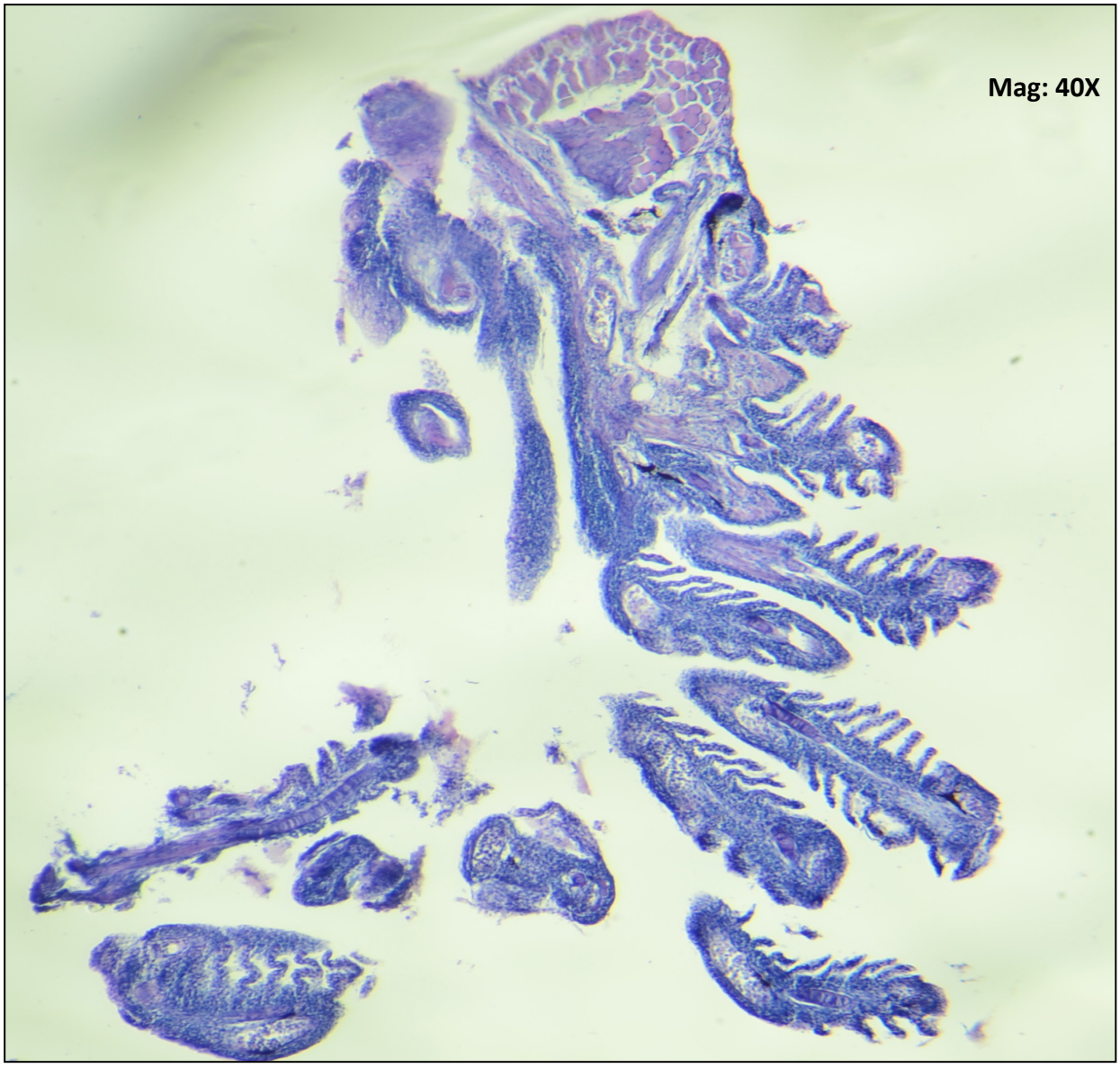

Figure 6. Gill filament and lamellae in control (freshwater) betta fish.

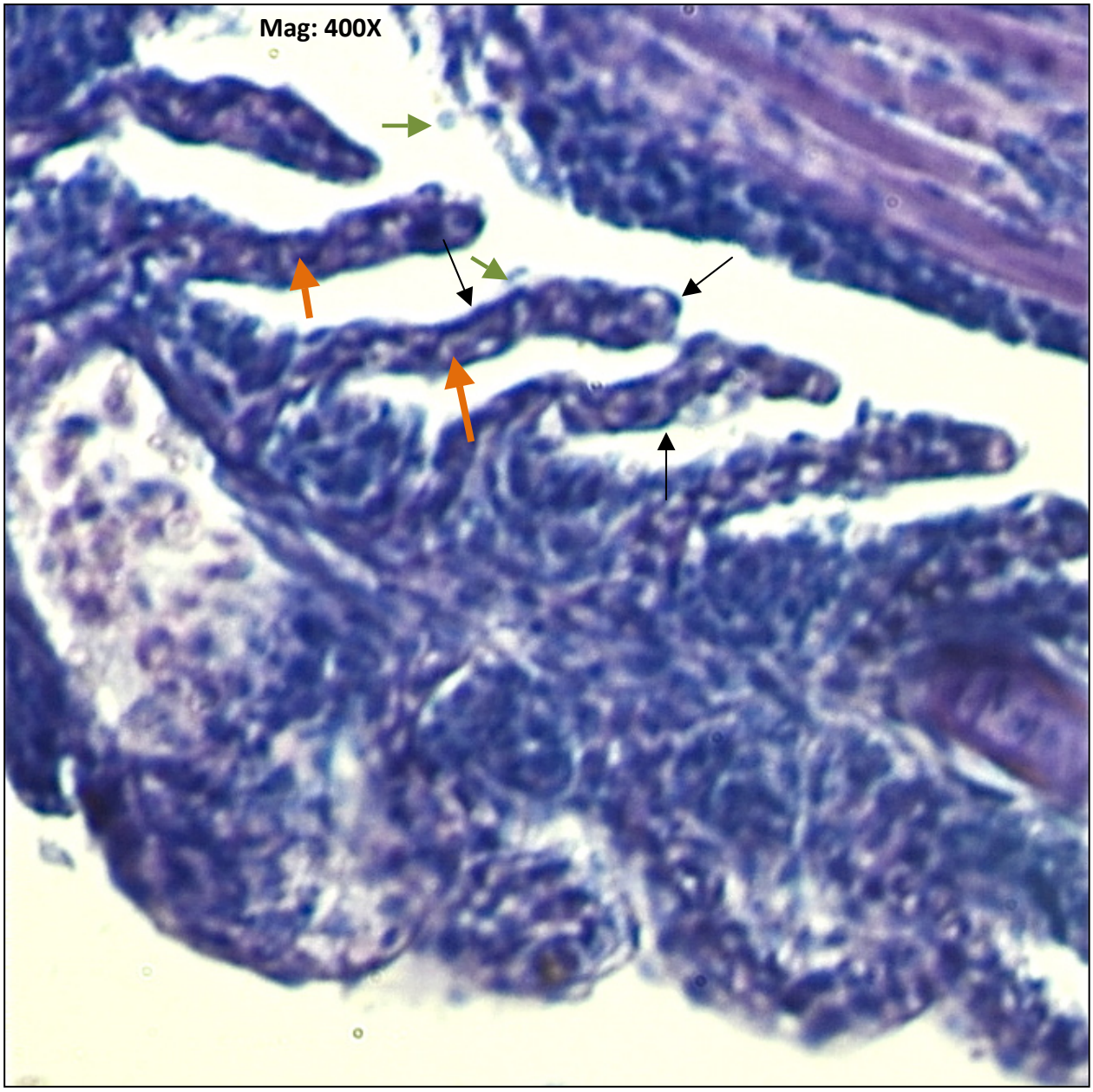

Figure 7. Close up of gill lamellae in freshwater fish. Arrows indicate simple squamous epithelial cells. Orange arrows indicate erythrocytes in lamellar capillary. Green arrows indicate mucous cells.

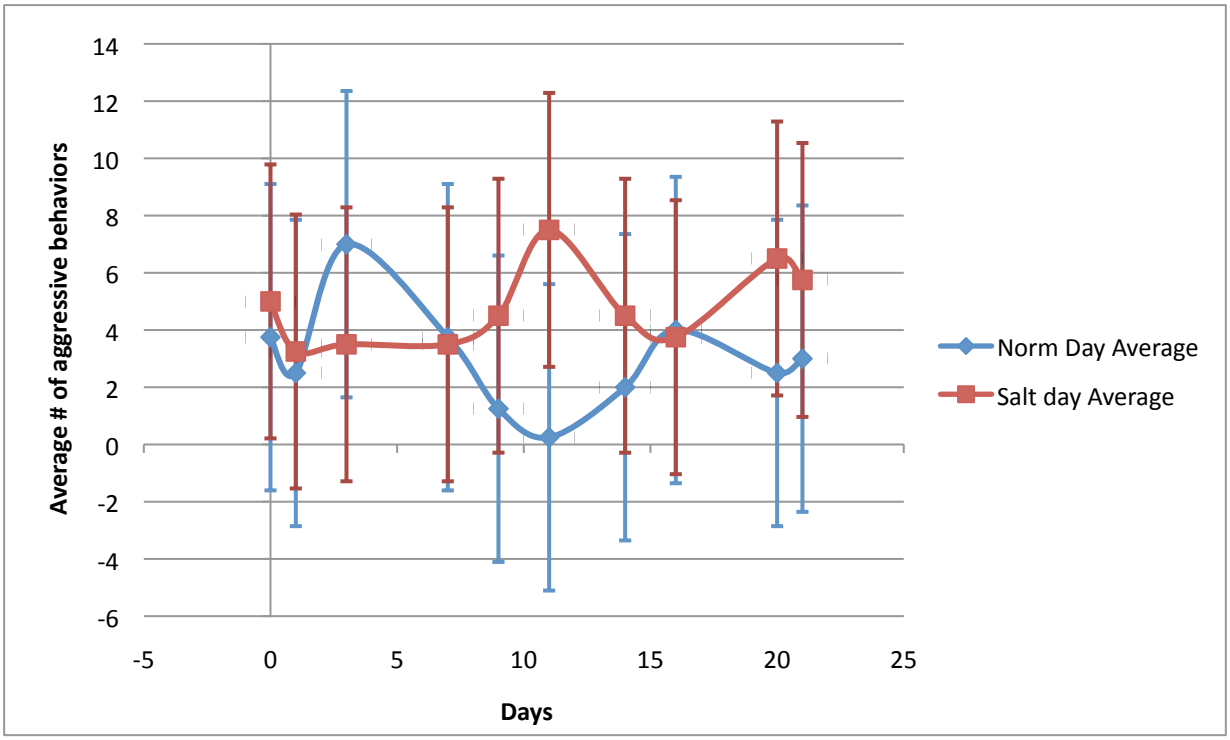

Figure 8. The average number of aggressive behaviors (+1 SE) for a 5 min period over 21 days.

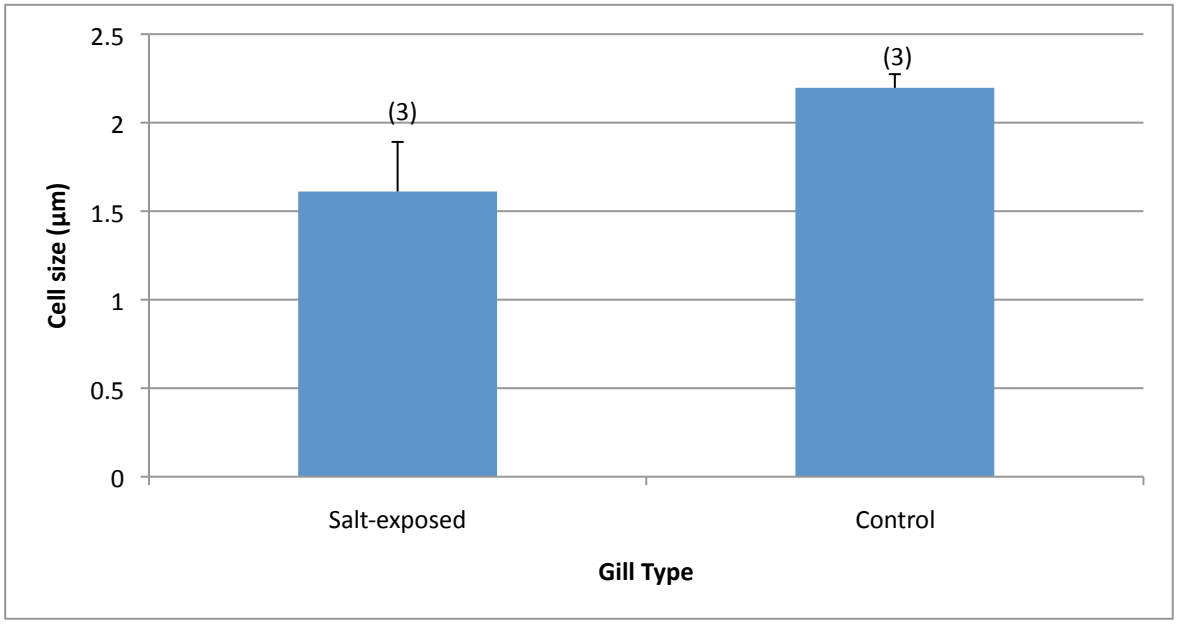

Figure 9. Average simple squamous epithelial size in the gill lamellae of the saltwater exposed betta fish and the control freshwater betta fish (p-value = 0.0253).

CHAPTER IV

DISCUSSION AND CONCLUSION

Although it was found that exposing the fish to a high amount of salinity for a three week period did not significantly change fish behavior, there were some behavioral trends among fish exposed to salt water. When the control fish were first introduced to their tanks, there was an increase in the amount of aggressive behavior, but as time went by and the halfway point was reached, that aggressive behavior seemed to wane and become almost nonexistent. There was a smaller increase in aggressive behavior closer to the end of the experiment, but it was not as high as it was before and eventually also waned. The saltwater exposed fish had almost the opposite effect. At the beginning, there was a lack of aggressive behavior, but as the experiment reached the halfway point, there was a definite increase in the number of aggressive behaviors. Closer to the end of the experiment, the aggressive behaviors had a small decrease, but then another small increase at the end. There was a lot of variation and high error due to individual personalities of the fish. There were some fish who were overall not at all aggressive and some who were overall very aggressive in both experimental groups. With this being said, it appears that there is some evidence to suggest, although not significant, that there may be a connection between the treatments and the number of days that they are exposed ($p= 0.068$). This may be seen due to a tendency for the measurements to increase over time for those fish exposed to salt and decrease over time for those in the control group.

There was, however, a decrease in the cell sizes of the saltwater fish gill cells when compared to the freshwater gill cells (p-value = 0.0253). Adaptive changes in the gill epithelium are considered to be critical in the process of high salinity adaptation by

allowing the secretion of the absorbed NaCl from the extracellular fluids. Studies of ion homeostasis have also shown that there needs to be a decrease in the osmotic permeability of the gill epithelium when exposed to high salinity environments (Laverty 2012). The mechanisms of this effect are not well known, although a decreased expression of one or more aquaporins (proteins that allow for the passage of water through a membrane) are a possibility for freshwater fish to adapt to a high salt environment (Laverty 2012).

In a study conducted on exposing juvenile sea bass to freshwater, Aquaporin (AQP) 1 was highly expressed in the digestive tract and kidneys of fish that were able to adapt to both salt water and freshwater, suggesting its involvement in water absorption. In the fish that were unable to adapt to freshwater, AQP1 transcript levels in the digestive tract were higher than in the freshwater adapted fish, suggesting its role in higher water absorption (Giffard-Mena et al. 2008). AQP3 transcript levels in gills were higher in those fish adapted to freshwater compared to those who were adapted to both fresh and salt water, suggesting a role in freshwater adaptation. AQP3 transcript levels in gills were higher in those who were unable to adapt to salt water than in the freshwater adapted fish, suggesting an increase in gill water permeability or other solutes. Transfer to fresh water was followed in the gills by an increase in Na^+/K^+-ATPase levels in the freshwater adapted and those unable to adapt, supporting the current model of ion absorption through the gills(Giffard-Mena 2008). This study and others have shown that salinity influences aquaporin expression levels in the gill, kidney and digestive tract which are the main osmoregulatory organs in fish. AQP1 may have a major osmoregulatory role in water transport in kidney and gut in salt water-acclimated fish, whereas AQP3 could be implicated in gill water transport in freshwater-acclimated fish (Giffard-Mena 2007).

During the smoltification of salmonoid species, the increase in Na^+/K^+ ATPase activity and water uptake in salt water is accompanied by decreased paracellular permeability suggesting a redirection of the fluid movement from a paracellular route in freshwater, to a transcellular route in saltwater (Sundell 2012). Increased transcellular fluid absorption are said to be achieved by the incorporation of aquaporins into the epithelial cell membranes and/or by changing the fatty acid profile of the epithelial lipid bilayer. The increased incorporation of unsaturated fatty acids into the membrane phospholipids increases the water permeability by enhancing the fluidity of the membrane, allowing water to leave the cells via osmosis (Sundell 2012). The occurrence of these changes and the decrease in the amount of water in the epithelial cells of the fish exposed to salt water may have been the cause of the increase in aggressive behavior.

Changes in behavior have also been seen in steelhead trout. In a study where trout were exposed to saltwater and stress was increased, Glucose concentrations increased throughout the experiment and lactate levels were elevated during the time spent in saltwater (Liebert 2006). Insulin-like growth factor-1 (IGF-1) did not show an immediate response to stress but after being exposed to saltwater, there were significantly higher concentrations for control fish at days 1 and 14. The positive correlations between IGF-1 and electrolyte levels in control fish may indicate its role for osmoregulation. The results suggested that feed intake was suppressed by the change of the media from freshwater to saltwater (Liebert 2006).

Future studies could include looking at the chloride, pavement, and other epithelial cells and to see how they are affected by saltwater. Other studies could include: increasing the time frame for measuring aggressive behaviors to see if trends continue; looking how increased salinity affects feeding behavior; looking at how aquaporin may affect cell size when exposed to increased salinity and the changes they may induce in the cell wall of epithelial cells; and

looking at how salt may affects other tissues and cellular components in a betta fish (kidneys and GI tract). Studies could also be conducted on how salt affects different types of bacteria that causes diseases in bettas and looking at the time frame and the salinity needed to kill the bacteria.

CHAPTER 5

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