

Spring 5-1-2011

Relative Abundance of Mitochondria Rich Cell Types in Threespine Stickleback: Interpopulation Differences and Salinity Effects

Dante Paolino

University of Connecticut - Storrs, dante.paolino@gmail.com

Follow this and additional works at: https://opencommons.uconn.edu/srhonors_theses



Part of the [Evolution Commons](#), and the [Integrative Biology Commons](#)

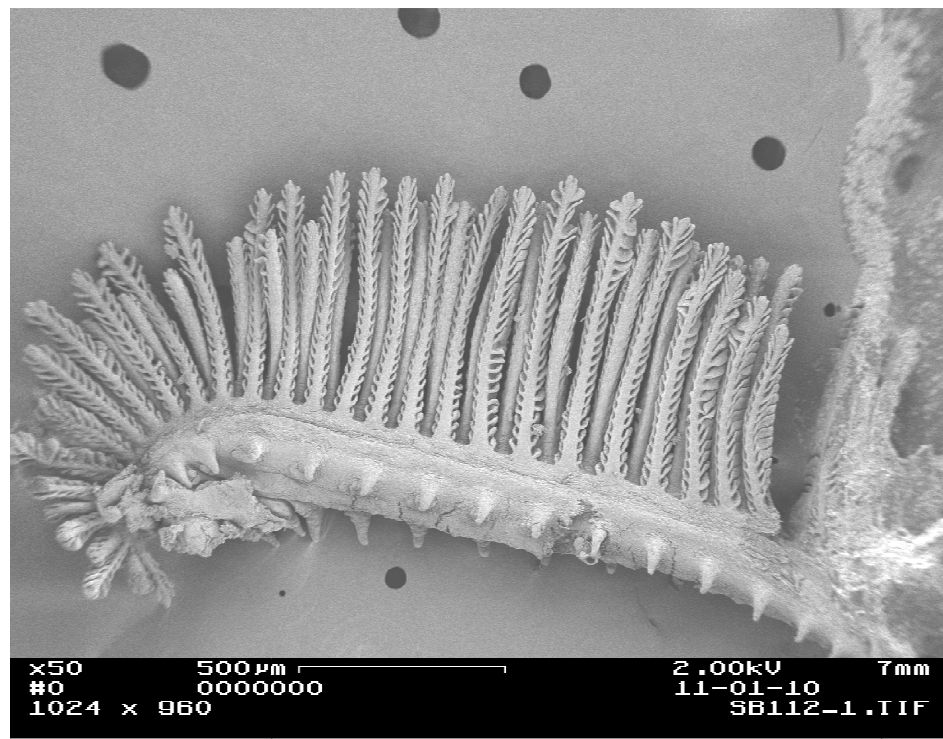
Recommended Citation

Paolino, Dante, "Relative Abundance of Mitochondria Rich Cell Types in Threespine Stickleback: Interpopulation Differences and Salinity Effects" (2011). *Honors Scholar Theses*. 391.

https://opencommons.uconn.edu/srhonors_theses/391

**Relative Abundance of Mitochondria Rich Cell Types in Threespine
Stickleback: Interpopulation Differences and Salinity Effects**

**By
Dante Paolino, Biological Sciences B.S.**



University of Connecticut

Honors Thesis

May, 2011

Abstract:

The objective of this study is to determine whether there are evolutionary trade-offs among populations isolated to different salinities in nature with regards to mitochondria rich (MR) cell production. We expected differences among populations in MR cell abundances due to the energy requirements needed to maintain and produce these cells. Eggs from three populations of threespine stickleback (*Gasterosteus aculeatus*) were hatched and reared for 10 weeks and then the fish were put into one of two salinity challenges: either 0.4 ppt freshwater or 35 ppt saltwater for 13 days. The populations consisted of one isolated to freshwater, one isolated to saltwater, and one that is anadromous. Mitochondria rich cells were characterized by the categorization scheme of Lee et al. (1996) as either deep hole (ion secreting cells), shallow basin (potential transitional cell type), or wavy convex (ion absorptive cells). Scanning electron microscopy was used to determine cell densities. There were strong salinity effects for deep hole cells as they were absent in freshwater and present in salt, and wavy convex cells which were nearly absent in saltwater but abundant in fresh, however there were no differences in cell densities of any cell type between populations. This indicates that either the populations never lost their ability to osmoregulate in different salinities or that measuring cell abundances is not an effective way to measure osmoregulatory ability. Analyses were also conducted on the along filament position of MR cells, and we found that cell densities were significantly lower at the base and the tip of the filament. We also found that in freshwater, MR cells are more abundant toward the distal half of the filament and in saltwater, MR cells are more abundant toward the proximal half.

Introduction:

Some aquatic animals live in environments with varying salinities and they must either conform to ambient conditions or regulate internal concentrations of solutes at levels different from the environment. The approaches taken to cope with these osmotic changes can be better understood by studying the osmoregulatory physiologies of individual fish and how they adjust to these challenges. Teleosts have the ability to maintain ion concentrations in their bodies that are different from their external environments and this internal balance of ions and water is called osmoregulation. The main site of osmoregulation in fish is at the gill where ions are actively transported into the fish in freshwater (FW), or are secreted into the environment in saltwater (SW; Evans et al. 2005). Many studies have been done on bony fish such as salmon, tilapia, and mullet, to test how their physiologies change in response to variations in environmental salinity (McCormick et al. 2009; Chang et al. 2001; Hossler 1979).

There have been few studies, however, on what micro-evolutionary changes occur in euryhaline fish that have become isolated to one level of environmental salinity. A specific example of this environmental isolation is the land-locking of a population of threespine stickleback (*Gasterosteus aculeatus*) that were once migratory. Diadromous fishes that become landlocked are confined to a FW environment indefinitely and must osmoregulate in FW during all times of the year and at all life stages. Studies on arctic charr show that some SW function may be lost as a result of becoming landlocked (Staurnes 1992). This study focuses on whether microevolution has occurred in populations that are isolated to different salinities.

The threespine stickleback has been a model species for investigating changes in body form and behavior upon colonization of FW habitats. The marine populations of this species

regularly colonize FW environments causing changes in morphology, behavior, and physiology (McKinnon & Rundle 2002). Previous studies show genetic differences in morphology and physiology between FW and SW stickleback (McCairns & Bernatchez 2009). The existence of SW, FW, and anadromous populations make this organism the ideal specimen to test for intra-species evolutionary changes in osmoregulatory physiology. Another advantage to working with threespine stickleback is that their entire genome has been sequenced. This offers the potential for further analysis of genetic modifications related to osmoregulatory evolution. Finally, threespine stickleback are readily reared.

Three strategies for osmoregulation exist in fish, and some fish, such as euryhaline teleosts, shift between methods depending on the conditions of their environment (Marshall & Grosell 2005; Kaneko & Hiroi 2008). The first method is osmoconformity which keeps an internal environment isotonic to the external environment. A fish that has evolved to live this way will allow its blood to equilibrate with the surrounding water with regards to concentrations of solutes and water. The second method is hyper-osmoregulation in which a fish will maintain the ions in its extracellular fluid at a concentration higher than that of the surrounding water. This generally occurs in FW habitats where there are few free-floating ions in the surrounding environment. Hyper-osmoregulators must deal with constant diffusive fluxes of ion loss and water gain across the gills. In order to deal with these fluxes, hyper-osmoregulators produce very dilute urine and have MR cells on their gill surfaces to absorb ions in the water. The third technique of osmoregulation is called hypo-osmoregulation. This process is usually found in SW fish who maintain internal concentrations of solutes at levels lower than their surrounding environment. Hypo-osmoregulators must constantly deal with diffusive fluxes of ions into their blood and water out of their blood. These fish constantly drink SW and have MR cells that

secrete ions into the environment. Threespine stickleback populations that have become land-locked are confined to FW environments and must hyper-osmoregulate all year round. Conversely, those populations which remain in SW habitats are always hypo-osmoregulating. Anadromous populations systematically travel between salinities and are known to shift from hypo to hyper-osmoregulation.

MR cells, also known as chloride cells or ionocytes, are cells on the surfaces of gill filaments that work to either absorb ions in FW fish, or secrete ions in SW fish. The model for ion secretion in SW suggests that NaCl secretion is performed by the transport of NaCl into an MR cell from the extracellular fluid across the basolateral membrane by the use of $\text{Na}^+\text{-K}^+\text{-ATPase}$ (NKA). This is coupled with the apical exit of Cl^- via channels and the paracellular release of Na^+ , both of which are operated down their respective electrochemical gradients (Hwang & Lee 2007). The key transporters associated with NaCl secretion are agreed to be NKA, the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter (NKCC), and the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel (Marshall 2002; Hirose et al. 2003; Evans et al. 2005). In FW fish must absorb ions from the environment, but the model for apical absorption of Na^+ into MR cells is uncertain. One current theory states that an electroneutral exchange of Na^+ and H^+ occur via an apical Na^+/H^+ exchanger (NHE; Hwang & Lee 2007). Compared with studies on Na^+ uptake, those for Cl^- uptake are much rarer, and much more remains unclear. The central mechanism that has been proposed is one involving the absorption of Cl^- linked with the secretion of HCO_3^- via apical anion exchangers, but very few studies have been done on this pathway (Evans et al. 2005; Tresguerres et al. 2006).

Differences in FW and SW MR cell function are reflected in cell morphology. The different types of MR cells have a confused nomenclature that differs among nearly all studies

on MR cells. One method to categorize these cells is by describing the surface morphology which has distinguishing features that can be viewed under scanning electron microscopy. Lee et al. (1996) described three different types of MR cells that have three unique apical membranes: deep hole cells (primarily found in SW), shallow basin, and wavy convex (primarily found in FW). It is still unknown whether an MR cell can transition between all three types, or if individual MR cells close and open in different salinities. Some studies have shown that shallow basin type cells have altogether different functions than deep hole and wavy convex such as Ca^{2+} absorption (Lee et al. 1996) or that they are dormant forms of another cell type with different mechanisms for absorption or secretion (Hiroi 2005).

Many studies exist on MR cells, but their relative abundances have never been compared between different populations isolated to different salinities in nature. We aimed to determine whether the response to varying salinity may have changed as populations evolved over time to different salinity regimes. In this study, threespine stickleback from three populations were put through salinity challenge tests and their gills were examined under scanning electron microscopy. Densities of MR cells were taken by counting different types of MR cells (according to the Lee et al. 1996 categorization scheme) along individual filaments and calculating cell densities over the entire surface area of the filaments. The three populations of threespine stickleback that we hatched were from three completely different environments: one isolated to SW, one isolated to FW, and one population that was anadromous. Evolutionary trade-offs among populations were also expected due to the energy requirements needed to maintain and produce MR cells. If these trade-offs exist, we expect to see differences in salinity response among the SW and FW populations.

Materials and Methods:

Source Populations: Breeding adults from three populations of threespine stickleback were trapped in Alaska. One population was anadromous from the Rabbit Slough stream which is part of the Cook Inlet (lat: 61°52'60.09; lon: -149°26'68.21; elev: 21 ft.). A second population was a marine population from Resurrection Bay (lat: 60°12'17.85; lon:-149°40'51.01; elev:17 ft). The third population was a land-locked fresh water population taken from Frog Lake (lat: 61°36'56.88; lon: -149°44'02.10; elev: 305 ft.).

Gametes taken from males and females from each population were combined to create 7-9 clutches of full-sibling families (mean clutch size = 185 embryos). These crosses were performed at the University of Alaska in Anchorage by lab personnel and embryos were shipped over-night to Clark University two days post-fertilization, and immediately taken to the Atwater Aquatics Facility at the University of Connecticut (Storrs, CT).

Rearing Conditions: General stickleback rearing followed standard methods provided by colleagues at Clark University (e.g., Wund et al. 2008) and was performed in accordance with University of Connecticut Institutional Animal Care and Use Committee Protocol A10-013. We split each family by placing 25 embryos into Petri dishes (90-mm dia), filled with reverse osmosis (RO) water constituted to a salinity of 0.5 ppt made by adding Instant Ocean. Embryos were segregated by family and maintained in Petri dishes until they were fed for 3-5 days after hatching. After this time fry were transferred to 0.85-L glass jars and acclimated to 3 ppt salinity to deter infections. After five weeks post-fertilization we consolidated the fry by family into 35L aquaria. We made 50% water changes at least every three days. We kept the stickleback at a photoperiod of 14L:10D and an average temperature of 19°C.

We fed fry freshly hatched Platinum Grade® *Artemia* nauplii two to three times daily for the entire study *ad libitum*. Plastic plants and bio-bricks were added to rearing containers to provide habitat structure.

Salinity Challenge Experiment: After 10 weeks of development, we performed a salinity challenge on randomly selected stickleback from each family in 0.85L jars containing either freshwater (FW; 0.4ppt) or saltwater (SW; 35ppt). Two replicate jars were created per salinity treatment. Fish were kept at a density of 10 fry per jar. The trial lasted 13 days, during which time salinities were periodically monitored and maintained during water changes. Prior to feeding, we reconstituted brine shrimp suspensions to match the treatment salinities of the jars. Mortalities were monitored daily.

Processing of Specimens: We randomly assigned surviving fish to one of several analyses, such as dry mass, mRNA isolation, and immunoblotting. From each replicate jar, we designed one fish for SEM analysis (N = 2 fry / family and treatment). After euthanizing with MS222, one member of the workup crew measured, weighed, and then handed off the specimen designated for all but dry mass analysis to a dissector who removed the complete branchial basket. The entire branchial basket of each juvenile fish was excised under a dissecting microscope, rinsed with distilled water, and then gills for SEM analysis were placed in vials containing a fixative of 2% glutaraldehyde, 2% paraformaldehyde, in a 0.1M phosphate buffer (pH 7.4). We carried out all of this on ice, and we stored the vials in ice and refrigerator at 4° C. The fixative volume was dependent on the size of the sample, but always contained at least a 20:1 ratio of fixative to tissue.

Scanning Electron Microscopy Procedures: I washed each branchial basket in 0.1M phosphate buffer (pH 7.4) kept at 4°C for 10 minutes three times. After the second rinse, I removed one gill arch from the branchial basket and then only this arch continued through the process. I then placed this arch in a 2% osmium solution with 0.1M phosphate buffer for 16 hours at 4°C. After 24 hours, I washed the samples with dH₂O (4°C) for 10 minutes 3 times, followed by increasing concentrations of 4°C ethanol for drying (30%, 50%, 70%) for 15 minutes each. After this process, I brought the samples up to room temperature and further dried them with 95% ethanol and four times with 100% ethanol (both at room temperature) for 15 minutes each. I stored the samples in 100% ethanol for 24 hours and then critical point dried and incubated them in liquid CO₂ for 1 hour and 30 minutes using a Polaron E3000 Series Critical Point Drying Apparatus. I mounted the samples for SEM immediately following critical point drying, and then sputter coated and silver painted them using a Polaron Series II Sputtering System Type E5100.

I chose gill filaments that were located toward the middle of the gill arch to be examined in the SEM. I took pictures on the SEM from the base of the filament to the tip. I used a Zeiss/Leo EM982 Field Emission Scanning Electron Microscope (FESEM) with an accelerating voltage of 2kV.

Data Collection: I counted cells along each photographed section (2000X magnification) of each filament and then measured the length and surface area of each segment using SigmaScan Pro 5 software. The total number of cells was summed as was the total surface area. The sum of all cells, and each individual cell type was divided by the surface area of the complete filament and the density of cells was calculated.

We calculated average cell diameters for each MR cell type by taking the average of two diameters of the apical openings of 90 cells (30 of each type). The two measured segments were perpendicular to each other. Both measurements per cell were averaged and the difference was taken between the two lengths. The larger the difference between the two diameters indicated a more oblong shape, and the closer the two lengths were to each other indicated a more circular apical opening.

We determined the placement of cells along a filament by apportioning the filament into 10 segments since filaments were not the same lengths and had different numbers of photos. The sum of all cells were counted along these 10 segments and then averaged among all 30 fish to determine where the highest densities of cells were located. This was also split among FW and SW treatments and then among the three cell types in the same way.

Statistical Analysis:

We modeled the along-filament distribution of cells using a nested two-way analysis of variance. The response variable was the estimated density of cells in individual images; we square root transformed because the untransformed data were right-skewed. We had multiple images for each individual. Individual was a random effect, nested within population-salinity combinations. Salinity and population of origin were crossed main effects.

We modeled the effect of salinity and population on total MR cell density, shallow basin and wavy convex cell density using a two-way analysis of variance. The response variable was estimated cell density over an entire filament. The transformation varied with cell type: total cell density and shallow basin cell density were not transformed but wavy convex cell density was square root transformed. Because we imaged only one filament per individual fish, individuals

provided replication for each level of salinity and population. Salinity and population of origin were crossed main effects.

We modeled the effect of population only on deep hole cell density because virtually no deep hole cells were found in freshwater treatment groups. We therefore used a one-way analysis of variance. Deep hole cell density was not transformed.

Results:

Three types of mitochondria rich cells were observed and counted using the terminology of Lee et al. (1996). Deep hole MR cells have apical membranes that are not visible because they create a deep hole looking pore on the surface of the gill epithelium (Fig. 1). Shallow basin cells have a shallower apical membrane that is more visible than the deep hole, but are still not flush with the surrounding pavement cells. Shallow basin cells have characteristic micro-villi that come out of the apical membrane (Fig. 2). Wavy convex cells have apical surfaces that look flush with the pavement cells that surround them (Fig. 3).

The diameters of MR cell apical openings also helped categorize the cells (Fig. 4). The cell type with the largest presentation was the wavy convex cell which had an average diameter of about 2.6 microns wide. The cell with intermediate presentation was the shallow basin cell, which had an average diameter of 1.5 microns. The cell with the smallest apical opening was the deep hole cell which had an average diameter of 1.1 microns. Cells differed in the shape of their apical presentation, as indicated by ‘difference in diameter.’ The wavy convex cells had the most variation between diameters meaning that they were mostly oblong, whereas the deep hole cells had a much smaller difference between diameters meaning that they were more circular in shape.

A non-uniform distribution of MR cells was observed along gill filaments. Statistical analysis indicates that there is a significant difference in density with position along filament (nested two-way ANOVA: effect of position along filament on total MR cell density $p < 0.0001$). MR cells appeared less likely to appear near the base and the tip, but they are roughly uniform through the middle (Fig. 5). Deep hole cells occurred in smaller proportions than both shallow basin and wavy convex cells, and these proportions remained consistent from filament to filament (Fig. 6). In SW, MR cells were more concentrated toward the base of the filament and in FW, MR cells were more concentrated toward the tip of the filament (Fig. 7). Significant differences were observed between salinity treatments in the distribution of MR cells along filament (nested two-way ANOVA: position*salinity interaction $p = 0.019$).

The effects of salinity, population, and the interaction between salinity and population were analyzed on each cell type. The density including all MR cells was not affected by salinity and did not differ among populations. MR cell sum density remained consistent between about 0.38-0.46 cells per 100 sq microns (Fig. 8). There was no effect of population ($p = 0.76$), salinity ($p = 0.90$), nor interaction ($p = 0.51$). Deep hole cell densities did not differ among populations, but did differ in salinity treatments. Statistical analysis indicated that there was no effect of population (one-way ANOVA : $p = 0.92$). Deep hole cell densities remained at about 0.15 cells per 100 sq microns across populations in salt water and were all practically zero in fresh water (Fig. 9). Shallow basin cell densities did not vary among populations or between salinities. Densities remained between 0.15 and 0.3 cells per 100 sq microns (Fig. 10). A two-way ANOVA indicates that there was no effect of population ($p = 0.13$), no effect of salinity ($p = 0.55$), nor interaction of the two ($p = 0.45$). Wavy convex cells showed an effect of salinity but did not show a population effect. Densities remained between 0.16 and 0.32 cells per 100 sq

microns in FW, but nearly absent in all populations in SW (Fig. 11). A two-way ANOVA indicates that there is no effect of population ($p = 0.31$), a strong effect of salinity ($p = 0.0001$), and no interaction ($p = 0.48$).

Discussion:

Literature on osmoregulation has focused on the MR cell response to different salinities, but responses among different populations isolated to unique environments has never been documented. This study determined that marine fish and land-locked fish were equally capable of producing the same abundances of wavy convex cells and deep hole cells as each other even though both populations would never need this ability in their natural habitats. There were no differences among the three populations in the ability to produce any cell type, but there were significant differences as an effect of salinity in the abundances of wavy convex and deep hole cells.

Recent studies on MR cells have shown that more than one cell type exist and that each cell type is more common in a specific salinity (table 1 in Hwang & Lee 2007). Lee et al. (1996) characterized three different cell types that they encountered in their study on tilapia, and these types had surface identities that were also observed in other reports on different species of euryhaline fish. These three types (wavy convex, shallow basin, and deep hole) were observed in our threespine stickleback but with different sizes of apical openings than what Lee et al. (1996) noted due to the differences in anatomical size between the two species. The tilapia wavy convex cells were 6 microns wide whereas our stickleback's were 2.6; tilapia shallow basin were 4-6 microns wide whereas our stickleback's were 1.5; tilapia deep hole cells were 2 microns wide

and our stickleback's were 1.1 (Lee et al. 1996; Fig. 4). The surface identities were consistent in our SEM images however, so we kept the descriptive titles that Lee et al. (1996) used. Wavy convex cells were still the largest out of the three followed by shallow basin cells and deep hole cells respectively (Fig. 4).

The presence of large surface areas in the wavy convex cells and shallow basin cells leads us to believe that these cells are used for some sort of ion absorption. Shallow basin cells contain micro-villi which are known to have absorptive functions, but what they are absorbing is still undetermined. Lee et al. (1996) indicated that Ca^{2+} absorption may be a function of these cells, but this is still debated. The larger surface exposure of the wavy convex cells indicates that these cells are meant for some sort of absorption as well. Since more of the surface is visible, more of the apical membrane is in contact with the environment and more molecules can be absorbed. Deep hole cells are observed to create a pit between surface pavement cells, and the apical membranes of these cells are not actually visible under SEM. It is generally understood that Cl^- is secreted from the junctions between these MR cells and surrounding pavement cells by the vibrating probe technique (Foskett & Scheffey 1982). Different studies show that the deep pits actually contain accessory cells along with the deep hole cells and this creates a larger channel for more ions to escape into the environment (Kaneko & Hiroi 2008).

MR cell along-filament position may not tell us much about the differences among populations' abilities to tolerate different salinities, but these findings may have some significance in determining whether an MR cell can morph between types. One study shows that MR cells from SW fish moving into FW first convert to FW cells and then are gradually replaced by newly generated FW cells (Katoh & Kaneko 2003). Perhaps something similar occurs when a fish is transferred from FW to SW. Studies on tilapia embryos show that upon transition to SW

from FW, FW type MR cells are enlarged and multi-cellular complexes form producing accessory cells from undifferentiated cells (Hiroi et al. 1999). This indicates that FW cell types can transform into SW multi-cellular complexes giving evidence that there is plasticity in the ion-regulating functions of MR cells. Our study shows that in SW, MR cells are more abundant toward the base of the filament and in FW, the cells are more abundant toward the tip (Fig. 7). A closer look at this data shows that in FW, a higher density of wavy convex cells were located toward the distal half (toward the tip) of the filament in all fish, and deep hole cells were absent. In SW, the densities of deep hole cells were highest towards the proximal half (toward the base) of the filament and wavy convex cells were nearly absent. The densities of shallow basin cells in SW were highest in the proximal half of the filament like those of the deep hole cells. Perhaps these shallow basin cells are the temporary conversions of wavy convex cells upon transition into SW. This study was done after 13 days of acclimation and perhaps at different times, the cells would be distributed differently. Lee et al. (1996) showed that reversible changes of all MR cell types occurred after 24 hours however, so maybe these cells are simply hidden under pavement cells until they are needed.

Data on MR cell position along the gill filaments also shows us that there are significantly fewer MR cells at the extreme ends of the filament. The densities remain constant throughout the middle portion of the filament, but dramatically drop off at both the tip and the base. One study shows that the overall resistance to flow towards the tips of the filaments are relatively large so that water flow mainly occurs through the basal and middle inter-lamellar spaces (Hughes 1972). If there is less water flow across the tips of the filaments it seems as if the fish have evolved to have fewer cells on this portion of the filament in order to not waste energy.

Our findings showed that the density of all cells did not differ among salinities in any population (Fig. 8) giving us reason to believe that there are a fixed number of cells visible on each filament. Deep hole cells were completely absent in FW (Fig. 9) and wavy convex cells were nearly absent in SW (Fig. 11). Shallow basin cells were not affected by population or salinity treatment suggesting that they may serve some other function aside from NaCl absorption or secretion. If shallow basin cells do have a different function, they may not necessarily transition between all three types. As mentioned earlier, different studies have shown that shallow basin type cells may have functions different than deep hole and wavy convex altogether such as Ca^{2+} absorption (Lee et al. 1996).

After this study we found that mitochondria rich cell abundances were not different among the three populations that were tested, but they did differ among salinities. There was a strong salinity effect on deep hole MR cell densities in SW, and wavy convex MR cell densities in FW. This provides further evidence that deep hole cells have a function in ion secretion in SW and that wavy convex cells have a function to absorb ions in FW. We hypothesized that deep hole cell densities would decrease in the land-locked population due to an evolutionary response from the fish being isolated to FW all year round, but this did not occur. We also expected to find a lower wavy convex cell density among the bay population because they rarely come in contact with FW, but this did not occur either. Since the MR cell densities did not differ among populations this study suggests that either osmoregulatory abilities are the same across populations or that the differences in fishes' abilities to osmoregulate are not reflected by MR cell abundance.

There is some indication from graphs that there may be some differences among populations (Fig. 11), but this study states that they are not significant differences. A power

analysis determined that we need about 160 samples in order to see if there are significant differences among populations in this study. This sample size would give us a nominal power of 0.8 which would provide a credible result. Survival curves showed differences in survival among populations, so for future studies it may be beneficial to get some samples before any mortality occurs. Perhaps there is variability from fish to fish or family to family in the rate of which they turn on appropriate MR cells. Those fish that are slower in their rate or weaker in their intensity of response die, so we only studied those that had the appropriate response. Imaging the filaments of the fish that are going to die may show some differences in MR cell abundances between populations since there were differences in survival among the populations in different salinities. Perhaps these fish that are about to die show some signs of impending mortality, and if someone watches the tanks for this behavior we can procure some live samples before they die. Our survival curves indicate that virtually all of the mortality would occur in the first two days of the experiment. Further assays are also being completed on stickleback gills from the same experiment such as western-blotting, immuno-histochemistry, mRNA analysis, and ATPase analysis to determine whether there are evolutionary trade-offs among the three populations. Simply having the same density of MR cells may not be an appropriate measure of the population's salinity tolerance.

Acknowledgements:

Eric Schultz, Jonathan Velotta, and Jeff Divino for their help with the experiment, and through the writing process.

Stephen Daniels and Marie Cantino for their assistance in the Electron Microscopy Lab.

The UConn Honors Thesis Life Science Grant Program for funding for this study.

Figures:

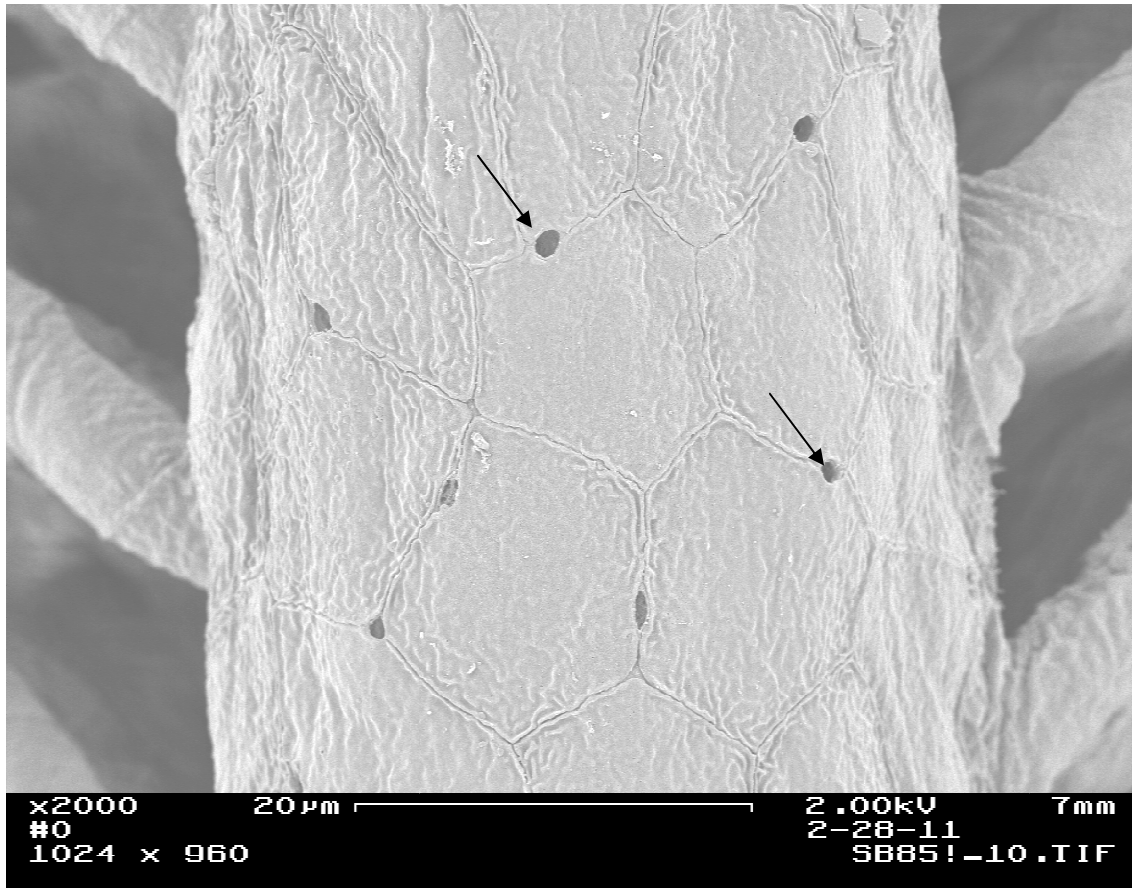


Fig. 1: Arrows pointing to deep hole MR cells on the surface of a stickleback gill filament. Deep hole cells are only visible on the surface by the openings between surrounding epithelial pavement cells. The actual apical membrane of these MR cells is not visible because they are too deep to be seen by SEM.

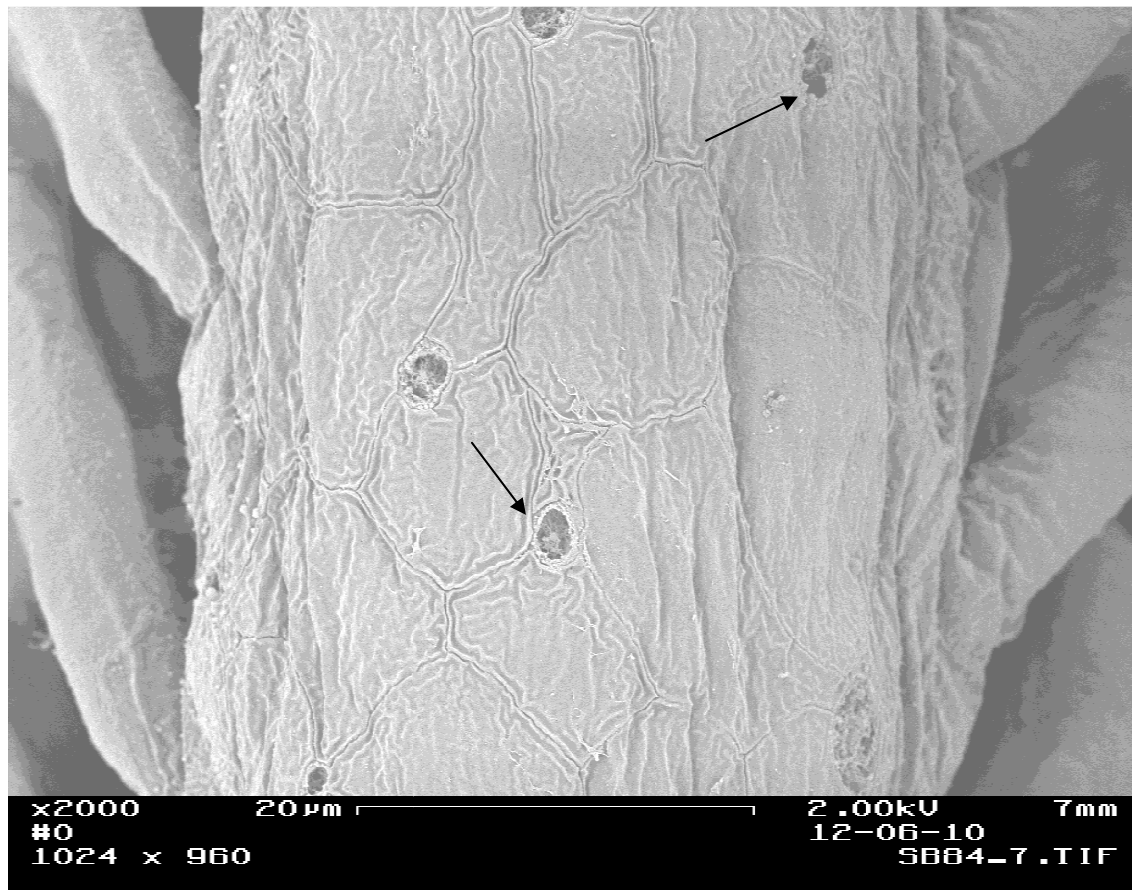


Fig. 2: Arrows pointing to shallow basin MR cells on the surface of a stickleback gill filament. The apical membranes of these cells are visible under SEM and are shallower than deep hole cells. Micro-villi protrude from the apical surfaces of these cells.



Fig. 3: Arrows pointing to wavy convex cells on the surface of a stickleback gill filament. Wavy convex cells are flush with the surrounding pavement cells. These cells are bigger in size than shallow basin and deep hole.

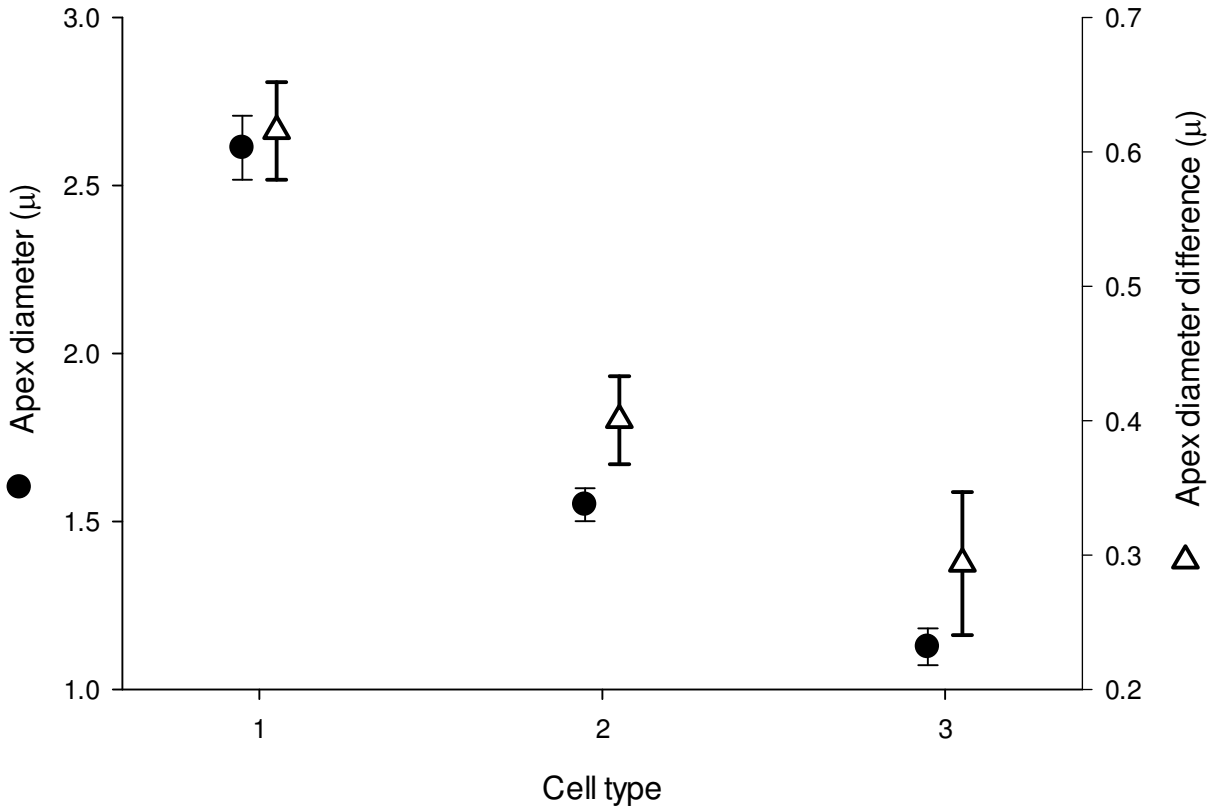


Fig. 4: Averages of cell type diameters and the differences between perpendicular diameters of each cell. Cell type 1 represents wavy convex cells, type 2 represents shallow basin cells, and type 3 represents deep hole cells. The average diameter is plotted on the left hand y-axis and the diameter difference is plotted on the right hand y-axis. Apex diameter difference indicates the shape of the cell. A larger difference between the two diameters measured per cell corresponds to a more oblong shape, and a difference closer to zero indicates a more circular apical opening.

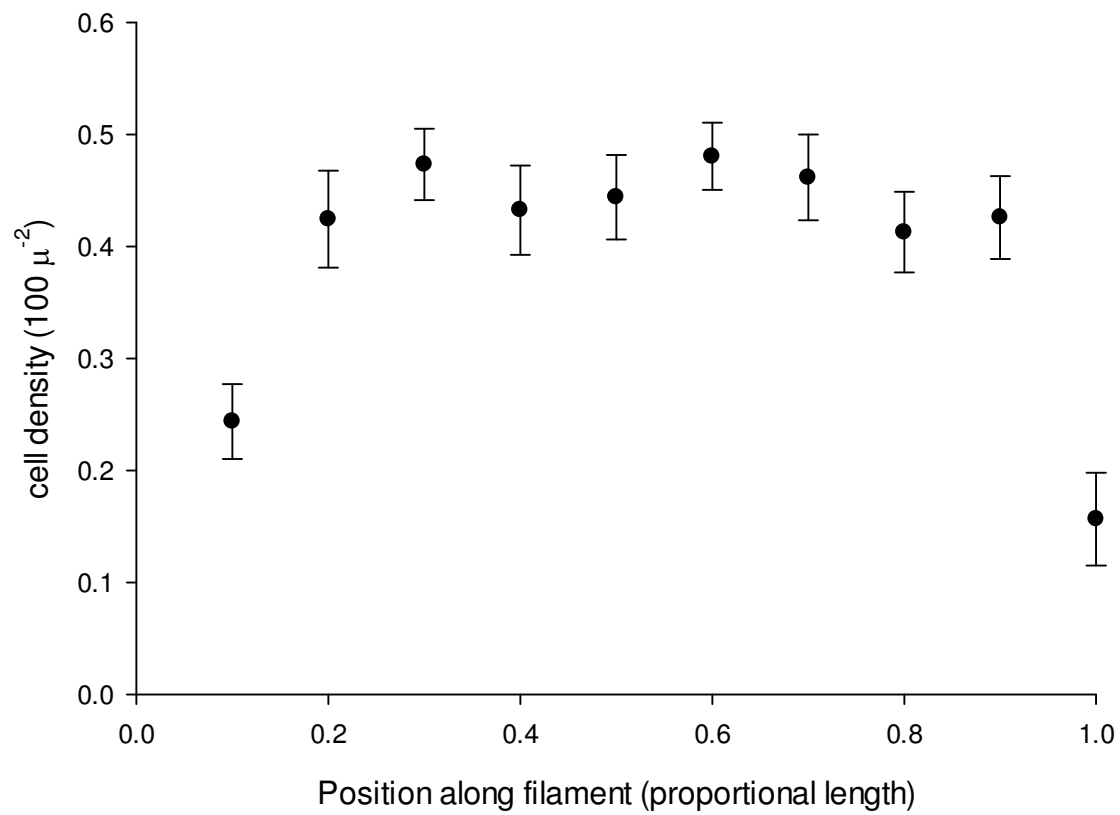


Fig. 5: Position of cell densities along different proportions of a gill filament. The y-axis provides the cell density per 100 sq. microns and the x-axis gives the proportional position along the filament from the base (0.1) to the tip (1.0).

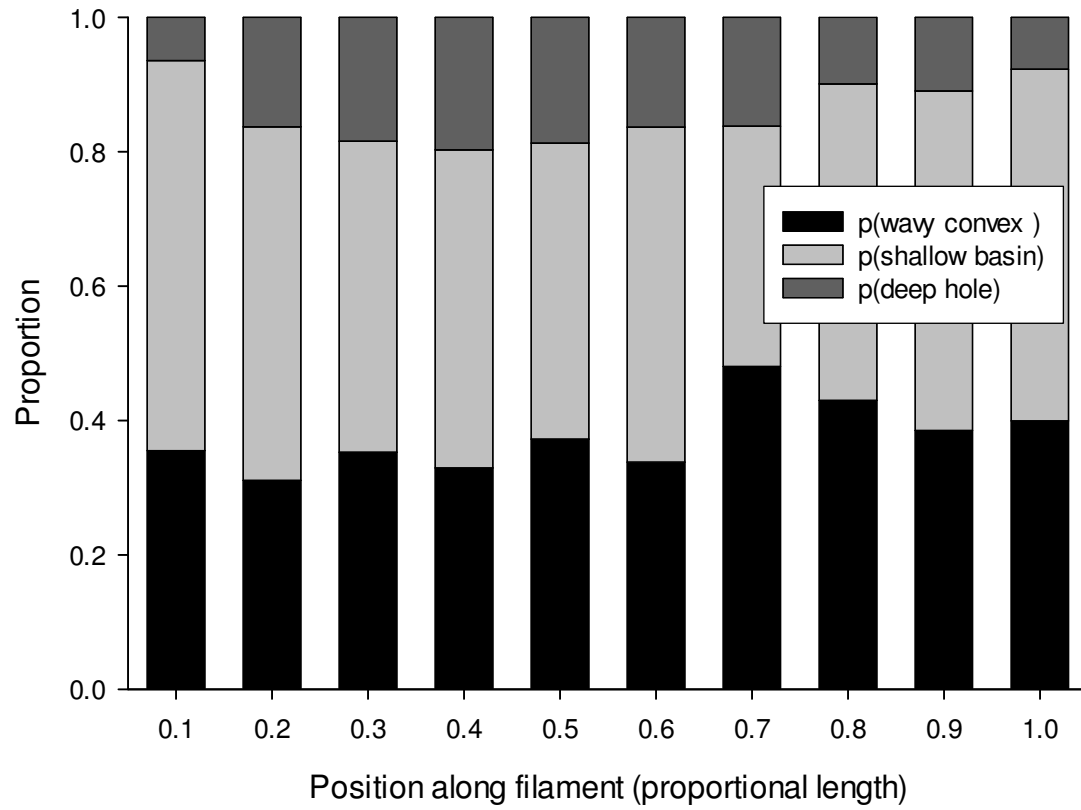


Fig. 6: Proportional densities of all three cell types along a gill filament. The y-axis is the proportion of each cell type out of the total cells scored and the x-axis is the proportional position along the gill filament.

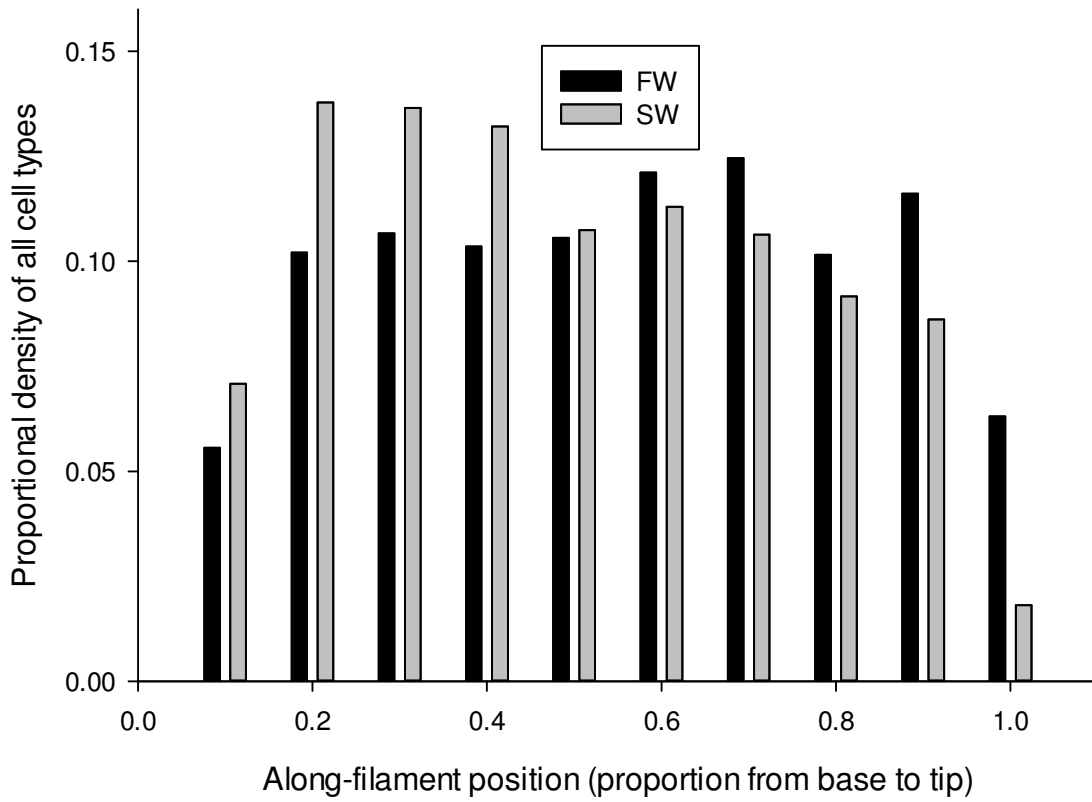


Fig. 7: Along filament proportional densities of cells between treatments. Each bar represents the proportion of cells scored that is located on each proportion of the filament.

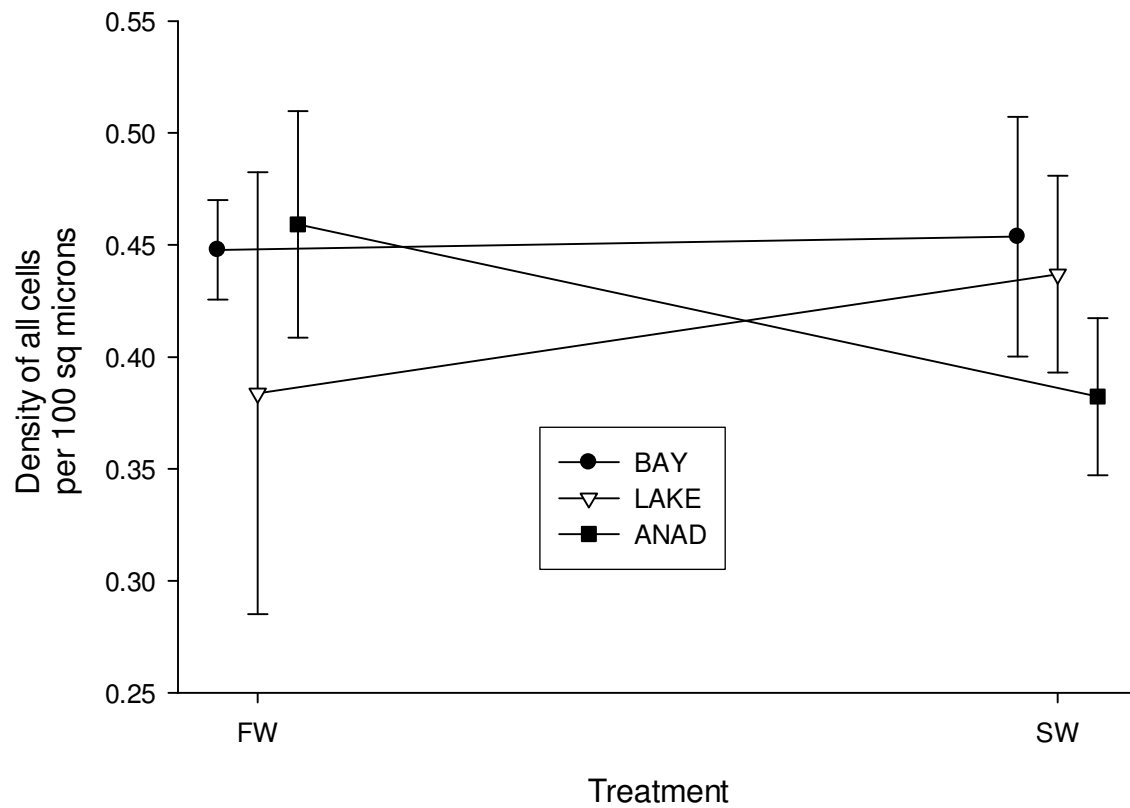


Fig. 8: Density of all cells among populations and salinity treatments. Different populations are indicated by the symbols: BAY represents the marine population, LAKE represents the land-locked FW population, and ANAD represents the anadromous population. The FW salinity treatment corresponds to 0.4 ppt freshwater and the SW treatment corresponds to 35 ppt saltwater.

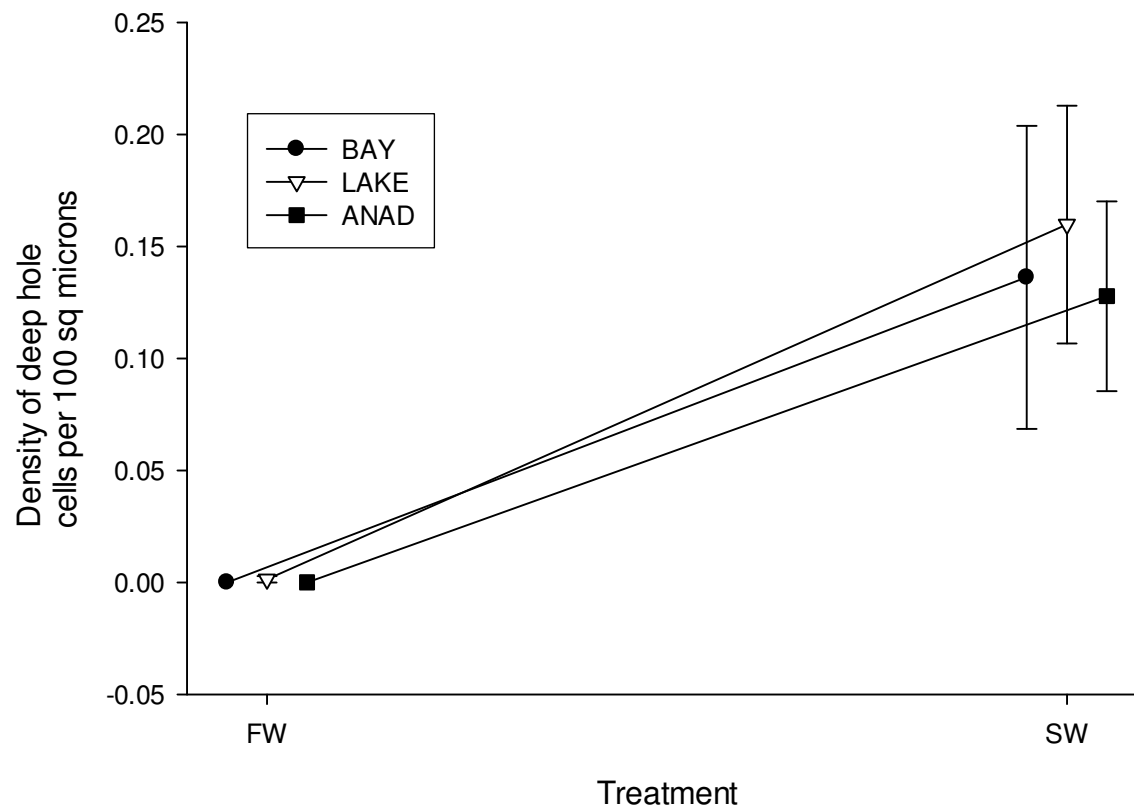


Fig. 9: Density of deep hole cells among populations and salinity treatments. Different populations are indicated by the symbols: BAY represents the marine population, LAKE represents the land-locked, FW population, and ANAD represents the anadromous population. The FW salinity treatment corresponds to 0.4 ppt freshwater and the SW treatment corresponds to 35 ppt saltwater.

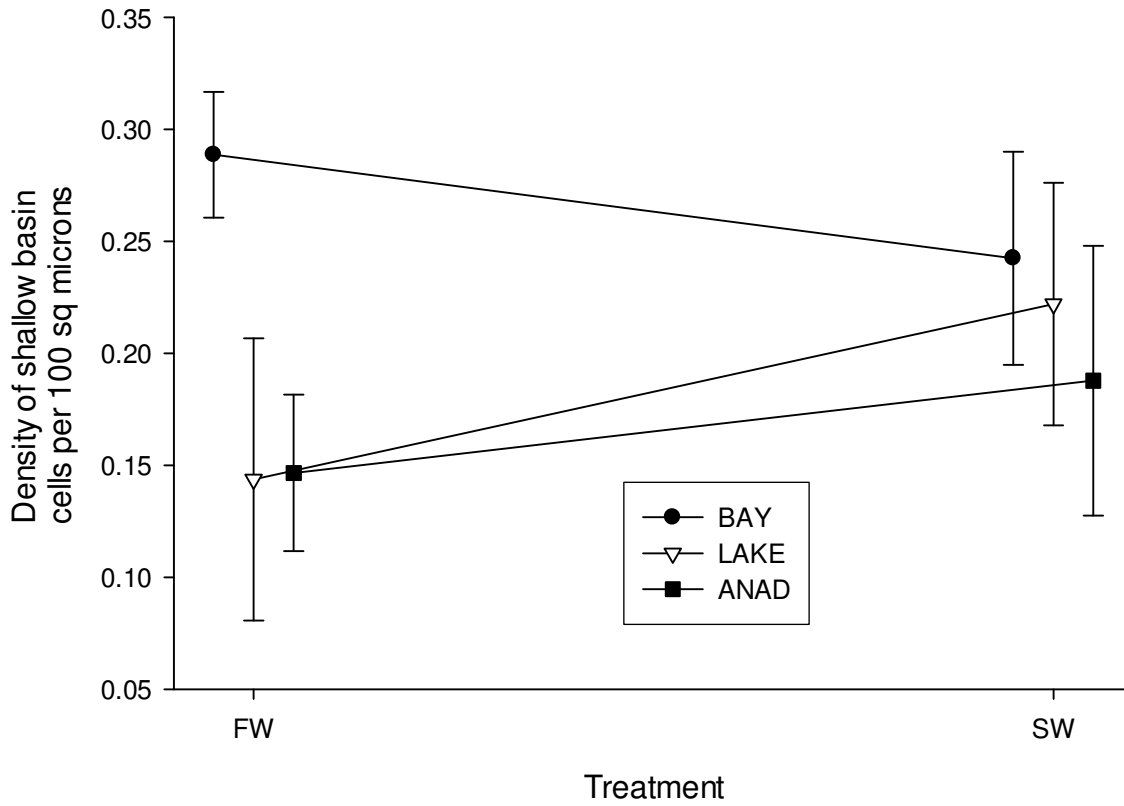


Fig. 10: Density of shallow basin cells among populations and salinity treatments. Different populations are indicated by the symbols: BAY represents the marine population, LAKE represents the land-locked FW population, and ANAD represents the anadromous population. The FW salinity treatment corresponds to 0.4 ppt freshwater and the SW treatment corresponds to 35 ppt saltwater.

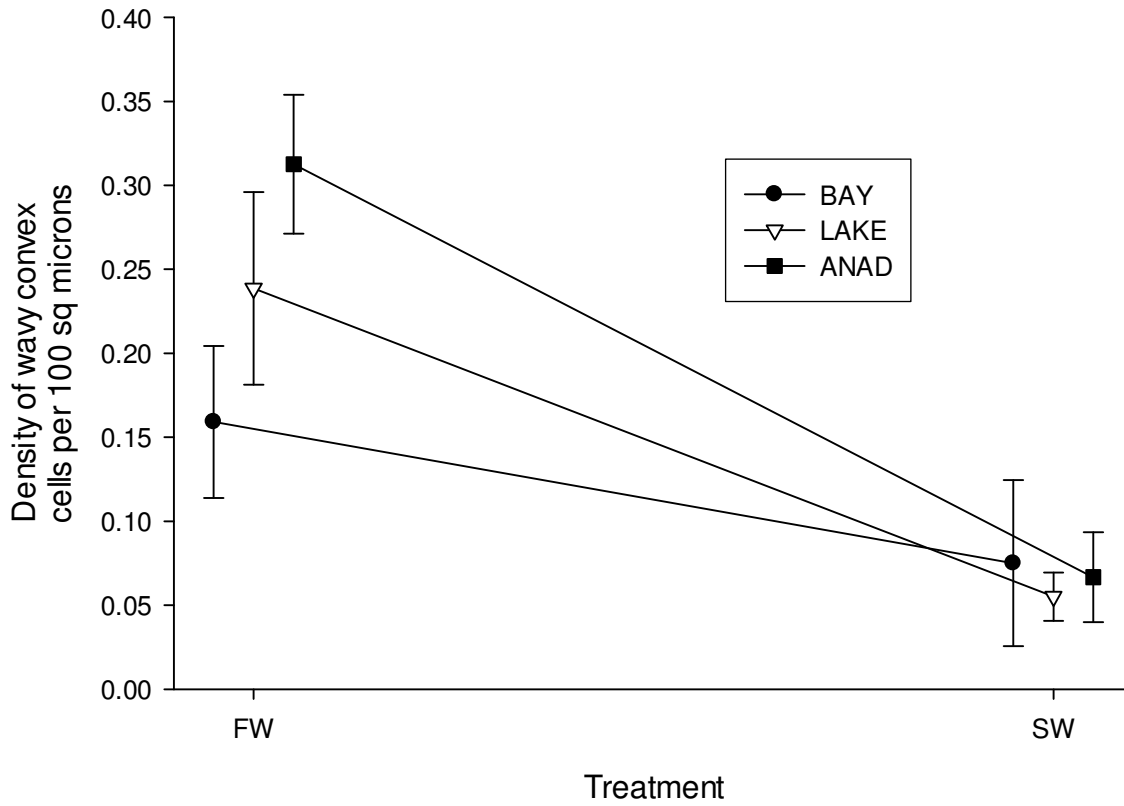


Fig. 11: Density of wavy convex cells among populations and salinity treatments. Different populations are indicated by the symbols: BAY represents the marine population, LAKE represents the land-locked FW population, and ANAD represents the anadromous population. The FW salinity treatment corresponds to 0.4 ppt freshwater and the SW treatment corresponds to 35 ppt saltwater.

References:

- Chang, I.C., Lee, T.H., Yang, C.H., Wei, Y.W., Chou, F.I., Hwang, P.P., 2001. Morphology and function of gill mitochondria-rich cells in fish acclimated to different environments. *Physiol. Biochem. Zool.* 74, 111–119.
- Evans, D. H., P. M. Piermarini, and K. P. Choe. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews* 85(1): 97-177.
- Foskett KJ, Scheffey C., 1982. The chloride cell: definitive identification as the salt secreting cell in teleosts. *Science* 215: 164-166.
- Hiroi J., Kaneko T., Tanaka M., 1999. In vitro sequential changes in chloride cell morphology in the yolk-sac membrane of Mozambique tilapia (*Oreochromis mossambicus*) embryos and Larvae during seawater adaptation. *J. Exp Biol* 202: 3485-3495.
- Hiroi, J., McCormick, S.D., Ohtani-Kaneko, R., Kaneko, T., 2005. Functional classification of mitochondrion-rich cells in euryhaline Mozambique tilapia (*Oreochromis mossambicus*) embryos, by means of triple immunofluorescence staining for Na⁺/K⁺-ATPase, Na⁺/K⁺/2Cl⁻ cotransporter and CFTR anion channel. *J. Exp. Biol.* 208, 2023–2036.
- Hirose, S., Kaneko, T., Naito, N., Takei, Y., 2003. Molecular biology of major components of chloride cells. *Comp. Biochem. Physiol. B* 136, 593–620.
- Hossler, F.E., Ruby, J.R., McIlwain, T.D., 1979. Gill Arch of the mullet, *Mugil cephalus*. 2. modification in surface ultrastructure and Na,K-ATPase content during adaptation to various salinities. *J. Exp. Zool.* 208, 399–405.
- Hughes, G.M., 1972. Morphometrics of fish gills, *Respir. Physiol.* **14**, pp. 1–25.
- Hwang PP, Lee TH. 2007. New insights into fish ion regulation and mitochondrion-rich cells. *Comp Biochem Physiol A Mol Integr Physiol* 148:479–497.

- Kaneko, T., and J. Hiroi. 2008. Osmo- and ionoregulation. Pages 163-183 in R. N. Finn, and B. G. Kapoor, editors. Fish larval physiology. Science Publishers, Enfield, New Hampshire, USA.
- Katoh, F., and Kaneko T. 2003. Short-term transformation and long-term replacement of branchial chloride cells in killifish transferred from seawater to freshwater, revealed by morphofunctional observations and a newly established “time-differential double fluorescent staining” technique. *J Exp Biol* 206: 4113–4123.
- Lee T-H, Hwang P-P, Lin H-C, Hung F-L. 1996. Mitochondria-rich cells in the branchial epithelium of the teleost, *Oreochromis mossambicus*, acclimated to various hypotonic environments. *Fish Physiol. Biochem.*, 15(6): 513-523.
- Marshall WS, Grosell M. Ion transport, osmoregulation and acid-base balance. In: Evans DH, editor. *The Physiology of Fishes*. CRC Press; Boca Raton: 2005. pp. 177–230.
- Marshall, W.S., 2002. Na⁺, Cl⁻, Ca²⁺ and Zn²⁺ transport by fish gills: retrospective review and prospective synthesis. *J. Exp. Zool.* 293, 264–283.
- McCairns, R. J. S., and L. Bernatchez. 2010. Adaptive divergence between freshwater and marine sticklebacks: Insights into the role of phenotypic plasticity from an integrated analysis of candidate gene expression. *Evolution* 64(4):1029-1047.
- McCormick, S. D., A. M. Regish, and A. K. Christensen. 2009. Distinct freshwater and seawater isoforms of Na⁺/K⁺-ATPase in gill chloride cells of Atlantic salmon. *Journal of Experimental Biology* 212(24):3994-4001.
- McKinnon, J. S., and H. D. Rundle. 2002. Speciation in nature: the threespine stickleback model systems. *Trends in Ecology & Evolution* 17(10):480-488.

- Staurnes, M., Sigholt, T., Lysfjord, G., Gulseth, O.A., 1992. Difference in the seawater tolerance of anadromous and landlocked populations of Arctic char *Salvelinus alpinus*. Can. J. Fish. Aquat. Sci. 49, 443–447.
- Tresguerres, M., Katoh, F., Orr, E., Parks, S.K., Goss, G.G., 2006. Chloride uptake and base secretion in freshwater fish: a transepithelial ion-transport metabolon? Physiol. Biochem. Zool. 79, 981–996.