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Salinity Tolerance of *Gambusia affinis*

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Salinity Tolerance of *Gambusia affinis*

by

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Abstract

Developmental plasticity refers to changes during development as a result of environmental contributions. Salinity is a varying environmental condition in freshwater and estuarine habitats that can mediate developmental plasticity in *Gambusia affinis*, which can increase their tolerances as an invasive species. For my study, estuarine and freshwater populations of *Gambusia affinis* were sampled in March of 2017 using dip nets. Both populations were then brought back to the laboratory where pregnant females were acclimated to three different salinities (0‰, 15‰, 25‰) until they gave birth. I predicted that the estuarine population of *Gambusia affinis* would have a greater salinity tolerance than the freshwater population of *Gambusia affinis* and that for both populations, the offspring reared at the highest salinity would have a greater salinity tolerance than the offspring reared at the lower salinities. Their offspring remained in their tanks where they were birthed until they reached maturity and then were acclimated back to 0‰ for two weeks to look specifically at developmental plasticity. After the two-week acclimation period, the offspring were directly transferred into 24-hour experimental trials run at 20‰, 25‰, and 30‰ and survivorship was assessed. It was determined that salinity does mediate genetic and, specifically, developmental plasticity effects. The estuarine population of *Gambusia affinis* does have a greater salinity tolerance than the freshwater population of *Gambusia affinis* and that for both populations, the offspring reared at the highest salinity had greater salinity tolerance than the offspring reared at the lower salinities. If developmental plasticity is playing a larger role than genetics in determining individual tolerances, this can increase the survivorship and increase its distribution as an invasive species to even more non-native habitats because of these nonreversible effects and adaptable tolerances.

Keywords: *Gambusia affinis*, developmental plasticity, invasive species, salinity tolerance

Dedication

I dedicate this thesis to Dr. Jake Schaefer. His guidance and constant support throughout my project was much appreciated and contributed so much to the success of my thesis. I am excited that his lab is continuing this project and look forward to seeing where they take my research.

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Introduction

Developmental plasticity is the ability for an organism to alter its phenotype in response to varying environmental conditions (McCairns & Bernatchez, 2009). Varying environmental conditions can be either abiotic or biotic factors that can cause an organism to either lose unnecessary characteristics or develop new, complex features that increase fitness (Zimmer & Emlen, 2016). Developmental plasticity is usually defined as phenotypic changes that are not reversible due to genetic and environmental contribution invoked during development to produce a phenotype. In comparison, phenotypic plasticity is the ability for a single genotype to produce varying phenotypes when exposed to different environmental conditions; however, phenotypic plasticity is not limited to only occurring during development (Reed et. al., 2010). The adult phenotype has the ability for short term acclimation and reversible plasticity. Most studies look for genetic effects alone through phenotypic plasticity while controlling for acclimation effects; however, my study examined genetic effects between populations with controlling short term acclimation while also looking at development plasticity due to environmental contributions.

Temperature and salinity are important abiotic factors that can invoke a plastic response in an organism (Schaefer & Ryan, 2006). A study performed by Schaefer and Ryan (2006), showed that Zebrafish that had a thermal history during development experienced irreversible changes in their thermal tolerance as adults (Schaefer & Ryan, 2006). As for salinity exposure, when a population is exposed to waters with varying salinity, their ability to tolerate the concentration of salt ions will have a direct correlation with their level of fitness and will lead to their classification as either a primary, secondary, or salt tolerant organism because of the irreversible phenotypic changes they experienced during development (Myers, 1949; Schaefer & Ryan, 2006).

When a population is introduced to a new environment, individuals of the population can develop characteristics that allow them to survive and outcompete native individuals of a populations (Mooney & Cleland, 2001). Invasive species with these traits can be problematic. Invasive species are organisms that are not indigenous to the area they are inhabiting (Jimenez-Valverde et. al., 2011). According to Mooney and Cleland (2001), negative impacts that invasive species have exhibited are predation, competition, hybridization, and changing the way native species evolve, which can ultimately lead to the extinction of the native species (Mooney & Cleland, 2001). Therefore, it is important to understand invasive species' tolerances and their damaging effects on the new environment they are invading and the native organisms that reside there. Many invasive species are limited in distribution by their tolerance to the environmental conditions. For example, invasive species that have the ability to tolerate waters with a higher salinity content allowing for flexibility in their behavior and interactions with noninvasive species which can aid in the success of their invasion (Mooney & Cleland, 2001).

Gambusia affinis and *Gambusia holbrooki,* commonly known as Mosquitofish, are two examples of invasive species which have gained world-wide distribution in efforts to control mosquito populations (Chervinski, 1983; Alcaraz et. al., 2007). These two species of *Gambusia* are native to the Southeastern region of the United States. Both of these species have been introduced to over 50 countries (Alcaraz et. al., 2007). *Gambusia affinis* is known as the Western Mosquitofish and are native to the Mississippi River basin, the northern Gulf Coast, and west of Peninsular, Florida to the Rio Grande River (Lee et. al., 1980). *Gambusia holbrooki* is known as the Eastern Mosquitofish and is native to northern Florida, Georgia, and seen along the Atlantic coast of the United States (Wooten et. al., 1988).

Since *Gambusia affinis* and *Gambusia holbrooki* have been introduced to different environments world-wide, they have had ecological impacts on native fish and amphibians in those environments (Alcaraz et. al., 2007). *Gambusia* has an aggressive nature which increases its survivorship in these new environments they are invading (Cote et. al., 2010). The Barren Topminnows (*Fundulus julisia*) exists in the Barren Plateau region of Tennessee but is now considered one of the most critically endangered fish species in the eastern United States (Hurt et. al., 2017). One of the most serious threats to the Barren Topminnow is *Gambusia affinis* because of their invasive introduction and aggressiveness towards this species. *Gambusia affinis* have established successful populations in this area which has reduced suitable habitats for the Barren Topminnow. A study done by Laha and Mattingly (2007), demonstrated the effects *Gambusia affinis* have on the Barren Topminnow offspring (Laha & Mattingly, 2007). They conducted a 24-hour trial to assess the survivorship of the Barren Topminnow young, and their results showed 0% survivorship which was a direct result of the predation and aggressive behavior by *Gambusia affinis* (Laha & Mattingly, 2007). Along with their aggressive nature, *Gambusia* has the ability to occupy a variety of waters, thrive in hostile environments, tolerate a wide range of temperatures, tolerate waters with low oxygen levels, and can withstand a wide range of salinities which has increased their distribution (Hubbs, 2000). *Gambusia* is in the order Cyprinodontiformes which is an order comprised of freshwater fish that have remarkable tolerances for temperature and salinity (Haney & Walsh, 2003). For example, their temperature tolerance is one of the highest for north American fishes and they have been recorded in hypersaline environments with a salinity of 55‰ (Haney & Walsh, 2003). Within the order Cyprinodontiformes, *Gambusia* is a member of the Poeciliidae family and is considered to be a secondary freshwater fish which makes its ability to tolerate high salinities for a certain period of time a unique characteristic (Myers, 1949). Myers (1949) classified all families based on their tolerances, therefore, salt-tolerant freshwater fishes, such as *Gambusia*, were classified as secondary freshwater fishes instead of primary because of the limitations of primary freshwater fishes (Myers, 1949). Secondary freshwater fish have the ability to tolerate waters with a higher salinity (between 0.05‰ and 30‰) while primary freshwater fish cannot tolerate waters with a salinity content higher than 0.05‰ (Meyerson et al., 1999). A study conducted by Chervinkski (1983) showed that *Gambusia affinis* were able to tolerate 100% sea water for seven days and could even tolerate the transfer back to freshwater (Chervinkski, 1983). Another native species that has been affected by the invasion of *Gambusia holbrooki* in Italy is the Mediterranean Toothcarp (*Aphanius fasciatus*) (Alcaraz et. al., 2007). Because of the *Gambusia holbrooki's* aggressive behavior and their ability to tolerate a wide range of salinity, they were able to invade waters in which the Mediterranean Toothcarp was native, causing this endangered species to take refuge in waters with exceedingly high salinity where *Gambusia holbrooki's* success is limited.

The ability for *Gambusia* to tolerate a broad range of salinities brings back the theory of natural selection. Over the course of time, natural selection plays a role in the survival of the fittest among organisms. When environments change, species either migrate to new environments or adapt to the changes of the current environment in which they reside (Sollid & Nilsson, 2006). As previously stated, *Gambusia* is native to the southeastern region of the United States. In particular, *Gambusia affinis* reside in the coastal marshes of the northern Gulf of Mexico which has experienced sea-level rises that have had dramatic impacts on salinity conditions in these areas (Purcell et. al., 2008; Purcell et. al., 2008). There have been numerous studies on the salinity tolerance of *Gambusia* that have demonstrated their history with salinity exposure, lethal salinity stress, and their rates of survival in these areas (Purcell et. al., 2010).

According to Purcell et. al. (2010), they tested the salinity tolerance on both freshwater and estuarine populations of *Gambusia affinis* and then reared both in a common garden to test for genetic effects between populations (Purcell et. al., 2010). Their study showed that populations of *Gambusia affinis* with previous exposure to waters with higher salinity, such as estuaries, had a higher salinity tolerance, which increased their survival rate, compared to populations that were found in freshwater environments with no previous exposure (Purcell et. al., 2010). The difference between these two populations could be related to genetic adaptation and that natural selection is playing a role in increasing the survival rate of the estuarine population when compared to the freshwater population (Purcell et. al., 2008).

The methods used in the Alcaraz et. al. (2007) study on how salinity mediates the competitive interactions between invasive mosquitofish and the endangered Mediterranean Toothcarp were used as a reference for my experiment (Alcaraz et. al., 2007). Their study tested the aggressive behavior and food competition between *Gambusia holbrooki* and Mediterranean Toothcarp, as well as the role salinity plays, whereas my study only experimented on *Gambusia affinis*. The results from the Alcaraz et. al. (2007) study showed that due to the higher salinities, *Gambusia holbrooki* captured less prey and had reduced aggressive behavior towards the Mediterranean Toothcarp (Alcaraz et al., 2007). Their experiment was unique because it gave the first experimental evidence that salinity does mediate the competitive interactions between these two species, causing the Mediterranean Toothcarp to take refuge in waters with a higher salinity (Alcaraz et al., 2007). The novelty in my work is that I tested for genetic and developmental plasticity effects by rearing *Gambusia affinis,* from both estuarine and freshwater environments, at different salinities and then acclimated them back to 0‰ to look specifically at development plasticity. This was done to support the hypothesis that individuals exposed to higher salinities during development would have a greater salinity tolerance as adults. Genetic effects were also assessed and the hypothesis that the estuarine population of *Gambusia affinis* would have a higher salinity tolerance than the freshwater population of *Gambusia affinis* was formulated, which has been supported by numerous studies.

Gambusia affinis were taken from freshwater and estuarine environments, and their offspring were reared in a common garden at three different levels of salinity. The purpose of my experiment was to observe developmental plasticity in *Gambusia affinis* when reared at different experimental salinities. Estuarine environments have a higher salinity content than freshwater environments, so it was predicted that the individuals in the *Gambusia affinis* estuarine population would have the capacity for a greater plastic response. By comparing the two different populations, any genetic differences driven by selection could be observed. By comparing within the populations across rearing salinities, developmental plasticity could be observed where it was expected that the rearing environments permanently change the individual tolerances, which would occur after acclimating back down to 0‰. The first prediction was that the estuarine population of *Gambusia affinis* would have a greater salinity tolerance than the freshwater population of *Gambusia affinis*. The second prediction was that for both populations, the offspring reared at the highest salinity would have a greater salinity tolerance than the offspring reared at the lower salinities.

Methodology

The freshwater and estuarine populations of *Gambusia affinis* were collected in March of 2017 using dip nets. The freshwater population was sampled from Lake Thoreau in Hattiesburg, Mississippi (31.348921 °N, -89.417336 °W) and the estuarine population was sampled from the upper portion of Simmons Bayou in Ocean Springs, Mississippi (30.3815881 °N, -88.7711368 °W). All the fish were brought back to the wet laboratory at The University of Southern Mississippi, where each population was placed in separate tanks at a salinity of 0‰. Both populations were given two weeks to acclimate to laboratory conditions before experimental procedures began. For the entirety of the acclimation period and experimental procedures, the water temperature was kept constant at 26°C, air pumps and sponge filters were present in all experimental tanks and population tanks, and the photoperiod was constant at 16:8. Once they were mature enough, for both populations, they were fed a combination of freeze dried blood worms, frozen adult brine shrimp that had to be defrosted, and *Drosophila melanogaster*, commonly known as the fruit fly. They were fed newly hatched brine shrimp (nauplii) when they were first birthed until they were mature enough to eat the larger food sources, which took approximately three to four weeks. Every week, tanks were refilled and salinity was measured using a YSI Model Professional Plus to maintain the appropriate range. Growth rate pictures were taken of offspring each week using an iPhone 7. When the offspring were first birthed, their first picture included all offspring. In weeks following, only two to three were randomly selected from each trial to assess growth. Each picture was taken with a ruler for scale and size was measured using TPsDig software.

Once the two-week acclimation period to the laboratory conditions was completed for both populations, twelve 10 L tanks were set up at three different salinities (0‰, 15‰, 25‰).

The salinity range for the tanks at 0‰ was maintained at 0.5‰-1‰. The salinity range for the tanks at 15‰ was maintained at 14.1‰-16.3‰. The salinity range for the tanks at 25‰ was maintained at 23.1‰-25.5‰. To keep each trial's data consistent, four tanks per salinity were used, with two tanks containing a freshwater population pregnant female and two tanks containing an estuarine population pregnant female. Pregnant females from both populations were randomly selected and put into one of the three salinity treatments, where they were placed in a plastic breeder chamber until they gave birth. Gender was determined by observing the size of the fish because *Gambusia affinis* females are relatively larger than the males and the ovaries of the *Gambusia affinis* female are visible. Pregnant females that were transferred from the 0‰ population tank to the 0‰ trial tank were given fifteen minutes to acclimate to their new conditions. Pregnant females that were transferred to 15‰ and 25‰ were acclimated to their new conditions by slowly increasing the salinity from 3‰ to 6.3‰ for each over the course of three to five days until the appropriate salinity was reached.

After the pregnant females gave birth, they were preserved in formalin tubes and their offspring were reared at the same salinity they were birthed in until they reached maturity. Maturity was determined based on size. The offspring were considered mature when they reached 20-30mm or approximately half an index finger, which could be physically observed in the lab. It took approximately five to eight weeks for the offspring to reach maturity. Because my study was testing for developmental plasticity, after the offspring reached maturity they were acclimated from their trial salinity back down to 0‰. The offspring that were reared at 0‰ were transferred from the 0‰ trial tank to the 0‰ acclimation tank and given fifteen minutes to adjust to their new conditions. The offspring that were reared at 15‰ and 25‰ were adjusted to their new conditions by slowly decreasing the salinity from 2‰ to 4‰ for each tank over the course of four to seven days. Once the offspring were acclimated back to 0‰, they were given a two-week acclimation period before the experimental trials were conducted.

After the two-week acclimation period at 0‰, three 10 L tanks were set up at salinities consisting of 20‰, 25‰, and 30‰. The salinity range for the experimental tank at 20‰ was maintained at 20‰-20.8‰. The salinity range for the experimental tank at 25‰ was maintained at 23.9‰-25.1‰. The salinity range for the experimental tank at 30‰ was maintained at 29.4‰-30.2‰. Before the fish entered the experimental trials, they were starved for 24-hours. After the starvation period, they were directly transferred into the varying experimental tanks, where their survivorship was assessed after a 24-hour experimental trial period. LD_{50} is a term used to represent the estimated minimum exposure of salinity that is fatal to 50% of the population that the treatment is administered to (Weil, 1952). In my experiment, I estimated the salinity level at which half the population was deceased (lethal dose for 50% of the population). To calculate the LD_{50}, salinity was regressed against observed mortality to determine the slope and intercept, which was then used to interpolate the salinity level at which 50% mortality was expected. I used LD_{50} estimates to compare genetic effects for populations (pooling all rearing condition trials), and development plasticity (pooling populations) for rearing effects. The trials that resulted in zero mortality were excluded from the LD_{50} estimate except for a single zero mortality trial for the population reared at 25‰ to allow for a linear progression.

After the 24-hour experimental trials, the offspring were preserved in formalin tubes. To make sure that the transferring process did not affect the experimental results, a fourth experimental tank was set up as a control group at 0‰ and was tested four times with 0% mortality. Preliminary trials were done at salinities greater than 30‰ and there was 100% mortality.

Results

There were 17 trials conducted with 17 pregnant females. There were four experimental tanks set up as control groups at 0‰ and they were tested four times with 0% mortality; therefore, control data was excluded from other analyses after these trial results. Preliminary trials were done at salinities greater than 30‰ and there was 100% mortality with all trials performed. Growth was also assessed by measuring the offspring each week from birth until they entered the experimental trials. How long it took for the pregnant females to give birth, the number of offspring birthed, and how long it took for the offspring to reach maturity before entering the experimental trials were recorded (Table 1).

From the experimental procedures performed, the results showed that the estuarine population of *Gambusia affinis* had a greater salinity tolerance than the freshwater population of *Gambusia affinis* (Figure 1). LD_{50} was calculated for each population to observe the minimum amount of salinity estimated to kill half of the population. The LD_{50} calculated for the estuarine population was 33.3‰ and 28.8‰ for the freshwater population.

Both populations of *Gambusia affinis*, estuarine and freshwater, were reared at 0‰, 15‰, and 25‰ and then tested in experimental salinities of 20‰, 25‰, and 30‰. The results showed that both populations reared at the highest salinity of 25‰ had the greater salinity tolerance when compared to the populations of offspring that were reared at 0‰ and 15‰ (Figure 2). The LD_{50} was calculated for each rearing salinity to observe the minimum amount of salinity estimated to kill half of the population. For the populations reared at 0‰, their LD_{50} was calculated to be 27.8‰. For the populations reared at 15‰, their LD_{50} was calculated to be 31.25‰. Lastly, for the populations reared at 25‰, their LD_{50} was calculated to be 37.5‰.

Discussion

The results from my experiment support both the hypotheses that (1) the estuarine population of *Gambusia affinis* would have a greater salinity tolerance than the freshwater population of *Gambusia affinis* and (2) that for both populations, the offspring reared at the highest salinity would have a greater salinity tolerance than the offspring reared at the lower salinities. The first hypothesis regarding the estuarine population of *Gambusia affinis* was a prediction that is supported by various literature done by previous experimenters. The Alcaraz et. al. (2008) study on salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish, the Purcell et. al. (2008) study on adaptation as a potential response to sea-level rise: a genetic basis for salinity tolerance in populations of a coastal marsh fish, the Chervinkski (1982) study on salinity tolerance of the mosquitofish, *Gambusia affinis*, and the Nordlie and Mirandi (1996) study on salinity relationships in a freshwater population of eastern mosquitofish are all examples of literature that supports my hypothesis (Alcaraz et. al., 2008; Purcell et. al., 2008; Chervinkski, 1982; Nordlie & Mirandi, 1996). The LD_{50} was calculated for each population to observe the minimum amount of salinity estimated to kill half of the population. The LD_{50} calculated for the freshwater population was 28.8‰, which means that 28.8‰ is the minimum amount of salinity estimated to kill half of the freshwater population of *Gambusia affinis*. The results found in my study are similar to the Nordlie and Mirandi (1996) results found on the salinity relationship in freshwater populations of eastern Mosquitofish (Nordlie & Mirandi, 1996). Their results showed that the survival rate of the freshwater population dropped from 73.0% at 20‰ to 60.3% at 25‰ and that only 37.4% survived when introduced to 30‰ (Nordlie & Mirandi, 1996). The LD_{50} for Nordlie and Mirandi's results was calculated to be 26.9‰ which is less than the LD_{50} calculated from my study; however, the

LD_{50}s are still relatively close in terms of being the minimum amount of salinity estimated to kill half the freshwater population of *Gambusia affinis*. These results do follow the same structure as seen in my results that as the salinity increased from 20‰ to 30‰, the freshwater population experienced a decline in survival rates (Figure 1). Though these results show similarities in terms of survivorship, one substantial difference is that Nordlie and Mirandi (1996) acclimated their fish to the testing salinity over a 14-day acclimation period and then assessed survivorship, whereas in my study there was a 14-day acclimation period at 0‰ and then a direct transfer into the testing salinity where survivorship was assessed after 24-hours (Nordlie & Mirandi, 1996). The LD_{50} calculated for the estuarine population was 33.3‰, which means that 33.3‰ is the minimum amount of salinity estimated to kill half of the estuarine population of *Gambusia affinis*. Estuarine environments have a higher salinity content than freshwater environments and based on the results in my experiment and the literature, the estuarine populations have evolved through exposure to higher salinities, which has allowed individuals in the population to acclimate to higher salinities through plastic responses due to exposure of higher salinities. These results are specifically supported by the results found in the Purcell et. al. (2008) study on the adaptation as a potential response to sea-level rise: a genetic basis for salinity tolerance in populations of a coastal marsh fish (Purcell et. al., 2008). Their research showed fish from brackish and intermediate marshes had an increased tolerance to a salinity of 25‰ when compared to fish from freshwater environments, and as one can see from the results in my experiment, the estuarine population had 0% mortality at 25‰, whereas the freshwater population had 26.7% mortality at 25‰ (Figure. 1) (Purcell et. al., 2008). Therefore, the results for genetic effects between the populations compare similarly to other studies conducted. Purcell et. al. (2008) concluded that populations with a history of high salinity exposure have adaptations that increase survival and the term "adaptations" is being referred to as a tolerance advantage (Purcell et. al., 2008).

By comparing within the populations across the rearing salinities of 0‰, 15‰, and 25‰, developmental plasticity was observed and the second hypothesis was supported that the populations reared at 25‰ would have a higher salinity tolerance. Based on the results found from the second prediction, there were plastic responses due to their developmental environment that invoked irreversible changes in the adults. The LD_{50} was calculated for each rearing salinity to observe the minimum amount of salinity estimated to kill half of the population. For the populations reared at 0‰, their LD_{50} was calculated to be 27.8‰. For the populations reared at 15‰, their LD_{50} was calculated to be 31.25‰. Lastly, for the populations reared at 25‰, their LD_{50} was calculated to be 37.5‰. These results reiterate that the populations of offspring reared at this salinity showed the greater salinity tolerance as adults. This clearly shows that in waters with a salinity of 27.8‰, half of the population of *Gambusia affinis* reared at 0‰ will be deceased, whereas it requires approximately 10‰ higher to kill half the population of *Gambusia affinis* reared at 25‰. The range in LD_{50} values when comparing within the populations (28.8‰-33.3‰) is wider than the comparison among populations (27.8‰-37.5‰), which indicates developmental effects are larger than genetic effects in my study.

There were 17 trials conducted to gather the results for my experiment. Four of the trials were used as control groups at a salinity of 0‰ to verify that the direct transferring process did not affect the experimental trial results. These trials resulted in 0% mortality, indicating that the transfer process did not affect the experimental trials. There were also three preliminary trials done at salinities greater than 30‰. These trials resulted in 100% mortality, which helped set the experimental salinity trial range from 20‰ to 30‰. Therefore, there were very few experimental trial replicates, but enough data to form the conclusion that both of my hypotheses could be supported, especially if more replicates were to be performed in the future. Replication is important in science because it allows researchers to check their work, and getting the same result further supports the conclusions drawn. Collecting more data in the future can determine if these hypotheses can continue to be supported or if there were experimental errors that could lead to the rejection of the hypotheses made. One unforeseen result when testing the rearing effects on both of the populations was that my data shows that at the experimental salinity of 25‰, the populations reared at 0‰ had a lower mortality rate than the populations reared at 15‰. This could be due to the fact that three trials were run on the populations reared at 0‰ at the experimental salinity 25‰, whereas only two trials were run on the populations reared at 15‰ at the experimental salinity 25‰. This just reiterates further that more data and replicates are necessary to have a more accurate representation and better understanding of the salinity tolerance of these populations at the varying salinities.

Growth measurements were also recorded every week from the time the offspring were born until they entered the experimental trial. Only two to three offspring were randomly selected from each trial to assess growth. Each picture was taken with a ruler for scale, and size was measured using TPsDig software. There was no statistical difference and no effect of population on growth. However, there was a big difference between the twelve tanks that the offspring were reared in. This could be due to the density effects regarding the amount of fish present in each tank. If more fish were present, it could have resulted in lower growth due to more competition for food. This is a prediction that could be tested in future studies.

In conclusion, the main hypothesis that (1) the estuarine population of *Gambusia affinis* would have a greater salinity tolerance than the freshwater population of *Gambusia affinis* and

(2) that for both populations, the offspring reared at the higher salinity would have a greater salinity tolerance than the offspring reared at the lower salinities, were both accepted based upon the results found in my study. By testing these hypotheses, it was determined that salinity does mediate genetic and, specifically, developmental plasticity effects, which brings back the concern of *Gambusia affinis* being a successful invasive species. If developmental plasticity is playing a larger role than genetics in determining individual tolerances, this can increase the survivorship of this species and increase its distribution to even more non-native habitats because of these nonreversible effects and adaptable tolerances. Since the results from the comparison between populations were consistent with previous studies, it would be beneficial to focus on development plasticity within the populations when moving forward with this study. As previously stated, replication and collecting more data would further support the hypothesis already made. It would also be interesting to test the developmental plasticity on not only the first generation, but across several generations to show true genetic adaptations in reference to salinity tolerance.

Tables

Table 1. Trial data on pregnant females: how long it took the pregnant females to give birth, number of offspring, and time between birth and maturity.

Trial Number	Population	Time Between Acclimation & Birth	Number of Offspring	Time Between Birth & Maturity
1	Fresh	21 days	11	42 days
3	Est	51 days	9	40 days
4	Est	39 days	13	20 days
5	Fresh	26 days; 41 days	7	41 days; 26 days
6	Est	50 day	2	57 day
7	Fresh	38 days	18	34 days
9	Est	32 days	6	61 days
10	Fresh	2 days	9	47 days
11	Fresh	28 days	9	42 days
12	Est	24 days; 38 days; 39 days	16	35 days; 28 days; 27 days
13	Est	25 days	7	36 days
14	Est	3 days	7	50 days
15	Fresh	3 days	5	52 days
16	Est	8 days	3	50 days
17	Fresh	10 days	11	31 days
18	Fresh	4 days	8	58 days
22	Fresh	14 days	9	32 days

Figures

Figure 1. Population effects between estuarine and freshwater populations of *Gambusia affinis* at varying experimental salinities.

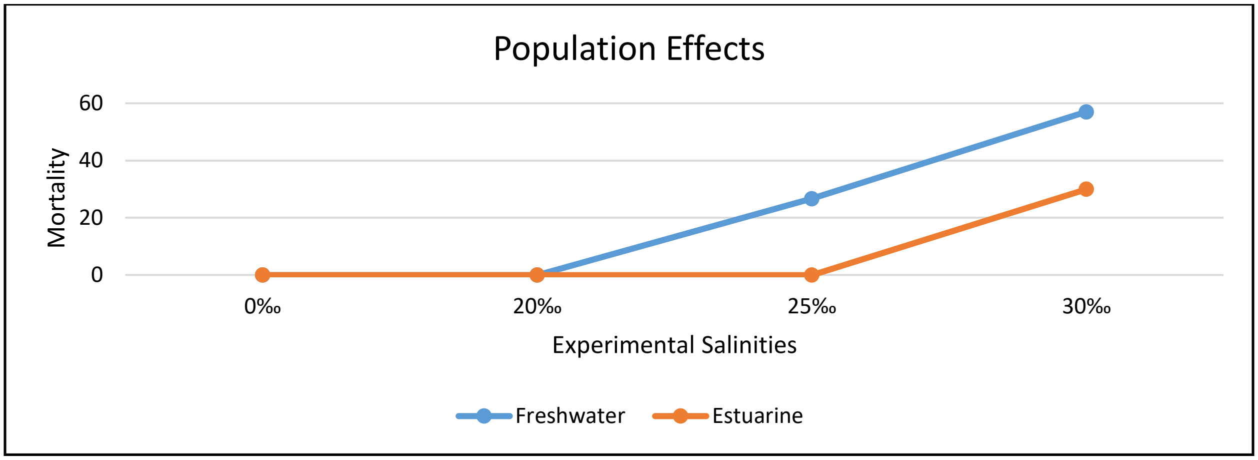
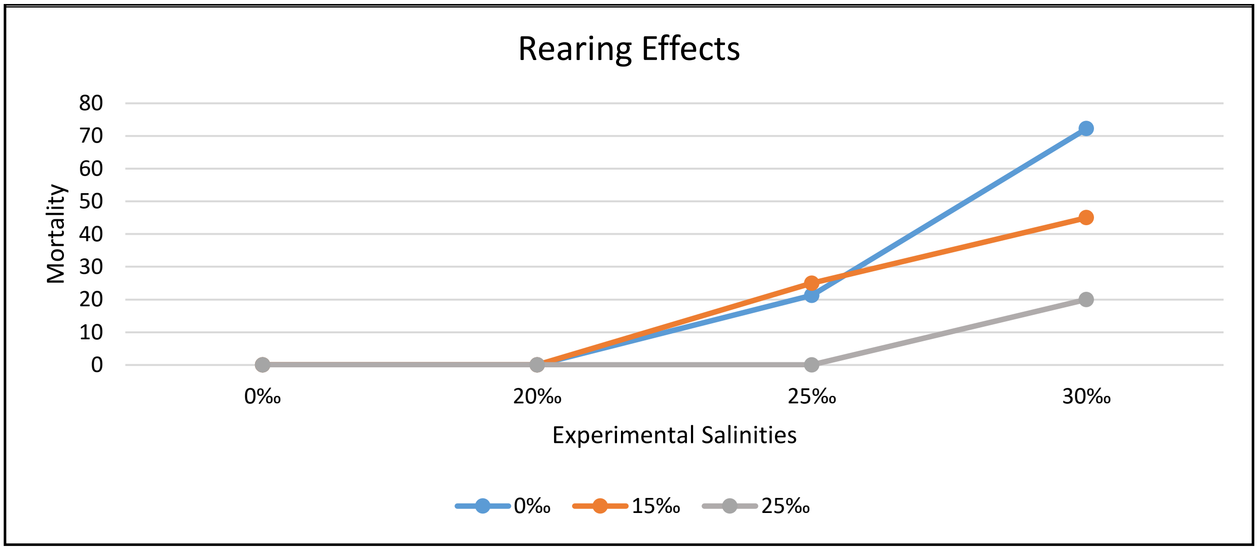


Figure 2. Rearing effects of estuarine and freshwater populations of *Gambusia affinis* reared at 0‰, 15‰, and 25‰ and tested at varying experimental salinities.



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