

Impact of Acidic Water on Aquatic Communities

Jonathon W. Bitner

SENIOR HONORS THESIS

Submitted In Partial Fulfillment of Requirements of the
College Scholars Honors Program
North Central College

22 April 2014

Approved: _____ Date: _____
Thesis Director Signature

Dr. Gregory Ruthig

Approved: _____ Date: _____
Second Reader Signature

Dr. Thomas Cavenagh

Table of Contents

Section Title	Page
I. Abstract.....	2
II. Marine Ecosystems.....	2-10
i. Coral Degradation.....	3
ii. Increased Coral Predation.....	4
iii. Sea Star Decreased Activity.....	5
iv. Reduced Calcification Levels.....	5
v. Community Level Impacts of Acidification in Marine Ecosystems.....	8
III. Freshwater Ecosystem.....	11-23
i. Introduction.....	11
ii. Materials and Methods.....	18
iii. Results.....	20
iv. Discussion.....	22
IV. References.....	24-25

Abstract

Acidic water is a threat to aquatic species in many parts of the world. The ability to withstand acidic conditions varies between species. I conducted a literature review to determine how the impact of acidification on one group of organisms can alter trophic level interactions throughout the community. In conjunction with this literature review on marine ecosystems, an experiment was also conducted to determine if *Poecilia reticulata*, a pivotal species in many freshwater ecosystems, are susceptible to acidic conditions. I found that lower water pH can decrease the survival and growth rates of key species, such as coral, that are integral to the success of the entire ecosystem. When some species are removed from an ecological community, the rest of the community will see a significant decline in survival rates as vital characteristics of a healthy ecosystem, such as nutrients and shelter, disappear,

Marine Ecosystem Introduction

Many factors are contributing to the changing landscape of the ocean, and these issues often occur on a massive scale. The rising sea level, for example, is not a simple problem to solve, and will impact people and animals around the globe. As shorelines are swallowed up by the ocean, new habitats will be created for aquatic species as terrestrial habitats are destroyed. While this changing sea level is an important factor that will impact aquatic communities, the increase in acidity will likely play a more significant role in the reshaping of these ecosystems.

Ocean acidification is the process that is slowly lowering the pH of the oceans. Currently, the ocean has a pH value of around 8.1, depending on the specific location (White 2014). Researchers have predicted that within the next century, this value could drop more than 0.5, which is a drastic change from rates in previous centuries. Most of this is due to human driven activities, such as the burning of fossil fuels. This in turn releases carbon dioxide into the

atmosphere, and over 25% of the molecules are absorbed by the ocean, where they can undergo a chemical reaction with water to produce excess hydrogen ions. This steady build up of hydrogen is the driving force behind acidification (White 2014).

Most species in the ocean have evolved to handle very specific conditions, and are unable to adapt to the pH shifts since they are occurring so rapidly. Even slight shifts in pH, as little as 0.3, can significantly decrease the reproductive success of many species (White 2014). Very few studies attempt to see how an entire ecosystem will be impacted as the ocean becomes more and more acidic (Pinnegar 2011, White 2014). Ecosystem relationships are often complex and it is difficult to predict how future habitat conditions will change these interactions. However, it is important to realize that species are not isolated from each other and the well being of one species usually contributes to the well being of its neighbors (Pinnegar 2011). As species susceptible to ocean acidification begin to dwindle, species that rely on these organisms will need to adapt and find a suitable replacement, or they too could face a massive decline in population. This review will look specifically at the loss of one of the ocean's most fundamental species, coral, and how its decline will impact various other creatures in the habitat.

Coral Degradation

One of the most dramatic factors contributing to coral species loss is their inability to continue to produce a calcium carbonate skeleton. This skeleton is a vital component of their success, and its production is not energetically favorable. Calcium carbonate is secreted through the outer most layer of their skin; however, this process requires coral to obtain calcium carbonate from their environment (Doropoulos 2012). As the pH drops in the ocean and more carbon dioxide becomes dissolved in the water, fewer of these molecules will be available for use. A reaction between carbon dioxide, water, and carbonate produces bicarbonate; a molecule

that is not useful to coral. As levels of carbon dioxide rise in the water, less and less carbonate is available for coral to produce their skeletons, impeding on their success rates. In fact, pH drops of as little as 0.3 have been shown to reduce coral settlement rates by over 45% (Doropoulos 2012). Not only is the coral itself impacted, but beneficial algae that is usually associated with a healthy coral community has also been shown to settle less frequently in more acidic water (Doropoulos 2012). The synergistic effects of these two factors will cause coral reefs to rapidly decline in the coming decades. In the following pages, the fitness of several different species will be examined in relation to ocean acidification, and then the overall community health based on these results will be predicted.

Increased Coral Predation

Unlike most of the other species that will be mentioned, *Clinona orientalis* (sea sponge) has the ability to thrive as the ocean pH drops (Wisshak 2012). *Clinona orientalis* obtains nutrients through the erosion of coral reefs. This is done by excreting a molecule that acidifies the surrounding water and breaks down the coral. As the pH naturally becomes more acidic, less energy needs to be spent eroding the coral, and therefore it will be able to cover a larger area and breakdown more of the reef. Currently, the sea sponge can erode $2.23\text{kgm}^{-2}\text{yr}^{-1}$. When the pCO_2 is slightly elevated (8.02), this number increases by 17%, and at highly elevated levels (7.63) it increases again by 61% (Wisshak 2012). This research did not take into account the weakened calcium carbonate skeleton of coral as the ocean acidifies, therefore we can realistically expect these percentages to increase in the natural environment. As the skeletons are weaker, they become easier to erode, and as the water naturally becomes more acidic, sea sponges need to expend less energy to break them down. The crown of thorns sea star (*Acanthaster planci*) is another species that relies on the coral reefs as a food source. Currently,

they have sporadically appeared as major nuisances on coral reefs, as they are excellent at harvesting the coral. In fact, they have become a significant issue in the Great Barrier Reef and in the Indo-Pacific region (Uthicke 2013). These creatures eat by forcing their stomach outside of their body and using the acids to breakdown the coral. While this function will likely become easier as the ocean becomes more acidic, their overall reproductive success will significantly drop (Uthicke 2013).

Sea Star Decreased Activity

Studies have shown that reproduction in these organisms decreases by 29% at pH 7.9 and by 75% at pH 7.6 (Uthicke 2013). The major concern with *A. planici* is the motility and velocity of sperm. As the water's hydrogen concentration increases, the sperm is not as successful as it is under conditions today. In fact, both sperm velocity and motility decline with pH, two significant factors in determining reproductive success (Uthicke 2013). Although researchers believe the decrease in population size of *A. planici* might help alleviate some of the pressure on coral reef systems, they do not think it will be enough to restore them to pre-acidification levels (Uthicke 2013). This research may also be applicable to other species that reproduce in a similar method to *A. planici*, meaning this information may have a larger impact on the community that it seems at first glance.

Reduced Calcification Levels

Similar to coral, calcification is a critical process for crab survival. Calcification is the process by which rigid structures, such as shells and bones, are formed by the accumulation of calcium. The production and maturation of exoskeletons heavily relies on calcium, and when the proper chemicals are lacking, this process is stalled. If exoskeleton production is held off for too long, a series of negative side effects can occur, such as reduced growth, weakened carapace,

or possibly death if the crab is still growing and cannot produce a new exoskeleton in the proper amount of time (Long 2013).

Both King and Tanner crabs were examined at different pH treatments (Control (8), 7.8, and 7.5). After growing in these conditions for ~200 days, their survival rates and exoskeleton health were determined. The king crab mortality rates were the lowest in the control, as expected, however it steeply increased at pH 7.8 (104% higher) and pH 7.5 (997% higher) (Long 2013). In fact, the king crabs in the treatment with pH 7.5 were so vulnerable that none survived past 95 days in the experiment. Tanner crabs showed a similar trend (130% higher in 7.8 and 400% higher in 7.5) (Long 2013).

King crabs also gained significantly more weight after each molt when in the control replicate than in the pH 7.8 or 7.5 trial. In fact, the 7.5 trial had so few molts and such a low survival rate that no data on this topic could be calculated. After 200 days, Tanner crabs held at pH 7.5 were significantly smaller than those held at pH 7.8 and the control of 8.0, suggesting that growth rates were stunted (Long 2013).

Yet another group of organisms negatively impacted by ocean acidification is the bivalves. Researchers claim that the harvest of these creatures garners over a billion dollars in sales each year in the United States; that alone makes them an important group to study (Gobler 2014). Specifically, this research identified how *Acrgopecten irradians* and *Mercenaria mercenaria* are being affected by the decline in ocean pH.

While this research provides interesting results that identify how bivalves will react to acidification, it also examines hypoxic conditions and whether or not this will alter their growth rates. As more carbon dioxide is absorbed into the water and more oxygen is used up by metabolic processes, hypoxic conditions are bound to occur (Gobler 2014). This information

allows the researchers to examine the synergistic affects that these two factors may have on marine organisms. Previous studies have not taken this in to account, therefore many of the values proposed by past research may in fact be more extreme than first anticipated.

The species of interest were tested under four different conditions; acidic water only, hypoxic water only, hypoxic/acidic water, and then a control. A low pH for the experiment was around 7.4 for two month old *M. mercenaria* and 7.5 for four month old *M. mercenaria*, while the controls were 7.9 and 8.0 respectively. Hypoxic conditions were near 50 uM dissolved oxygen for both age groups, and the control was much higher at 250uM dissolved oxygen (Gobler 2014). Two different age groups were tested to help determine if there was a critical period during development in which bivalves are more susceptible to environmental factors. *A. irradians* larvae were also exposed to these conditions. Control levels were of pH 8.0 and oxygen levels of 250uM, hypoxic conditions contained only 40uM of dissolved oxygen, and low pH conditions were measured to be 7.5 (Gobler 2014).

Results show that *A. irradians* living in acidic conditions had a significantly different survival rate than those both the control group and the low oxygen only group. In fact, after two weeks the survival rates for the control and hypoxic group only were nearly identical at just above 40% (Gobler 2014). Meanwhile, low pH groups showed an average survival of around 25% and the group with both hypoxic and acidic water had a slightly lower survival rate of 21% (Gobler 2014).

The research with *M. mercenaria* produced results that those at the age of two months old (7um/day) are much more susceptible to acidic conditions than those four months of age (12um/day). Low oxygen and acidic conditions; however, significantly stunted growth in four month of *M. mercenaria* (7um/day) compared to two month old (11 um/day) (Gobler 2014).

Low oxygen allow did not impact growth rate in either of these groups.

Community Level Impacts of Acidification in Marine Ecosystems

All of the organisms previously discussed rely on each other in one way or another. Whether it be shelter, food, protection, or some other symbiotic relationship, the survival of one species is integral to another. After analyzing how all of these community members will be impacted by ocean acidification, a more complex web of associations can be created between each group.

The first, and arguably most significant step in the process, is the degradation of coral through an increased amount of carbon dioxide in the ocean. However, acidification is not the only factor contributing to coral loss. Sea sponges, as discussed earlier, can more easily break down coral under low pH conditions. This means that not only is acidification directly impacting coral loss, it is also indirectly affecting its survival thorough the increased activity of sea sponge activity.

Another indirect impact is the decrease in sea star reproduction. While this may end up being a positive outcome (since sea stars also consume coral), it also means there could potentially be an increase in sea sponge population. If fewer sea stars are present, less coral will be eaten, meaning there will be more left for the sea sponges, allowing them to support more offspring. However, this relationship has not been directly tested, therefore it is difficult to predict what will actually occur.

Regardless of the outcome, coral will still see a significant decline in total ocean coverage. Not only will it be less prevalent, but it will also be weaker and less structurally stable due to a decrease in calcium carbonate. In turn, this means loss of habitat for numerous species, including crabs and bivalves. Loss of habitat usually leads to either an outflow of species, as

they search for a suitable environment, or the predation of these animals since they have fewer places to hide. Crab species will not only be affected by acidification through habitat loss, but also due to a decrease in their ability to calcify and create their exoskeletons. These two factors combined will make it extremely difficult for their current population numbers to persist, and they will eventually feel this environmental pressure.

Bivalves are another species that rely on coral as a suitable habitat. These creatures are filter feeders, and the large number of microorganisms that surround reefs make it easy to find nourishment. As the reefs begin to shrink, fewer microorganisms will be able to be sustained, and that in turn means fewer filter feeders such as bivalves.

Finally, as both bivalves and crabs disappear from the community, the large predator species will also begin to fade from the community, and the ecosystem be completely changed. This impact becomes exponentially more detrimental as the ocean becomes more acidic, since new species are affected as the pH continues to drop. For example, coral is typically the first species to feel the impact, as drops as small as 0.2 (a pH of about 7.8) can alter coral settlement abilities. If the pH drops a bit more, to around 7.7, that is when Sea Star reproduction is altered. However, this drop still affects coral as well, making it even more difficult for them to settle and calcify. At around 7.5 and 7.4, crabs and bivalves are impacted, along with the previously mentioned species. Sea Sponge activity continually increases with acidification, therefore there is another layer of depth to the issue. Simply put, the more acidic the ocean becomes, the faster the coral communities will decline.

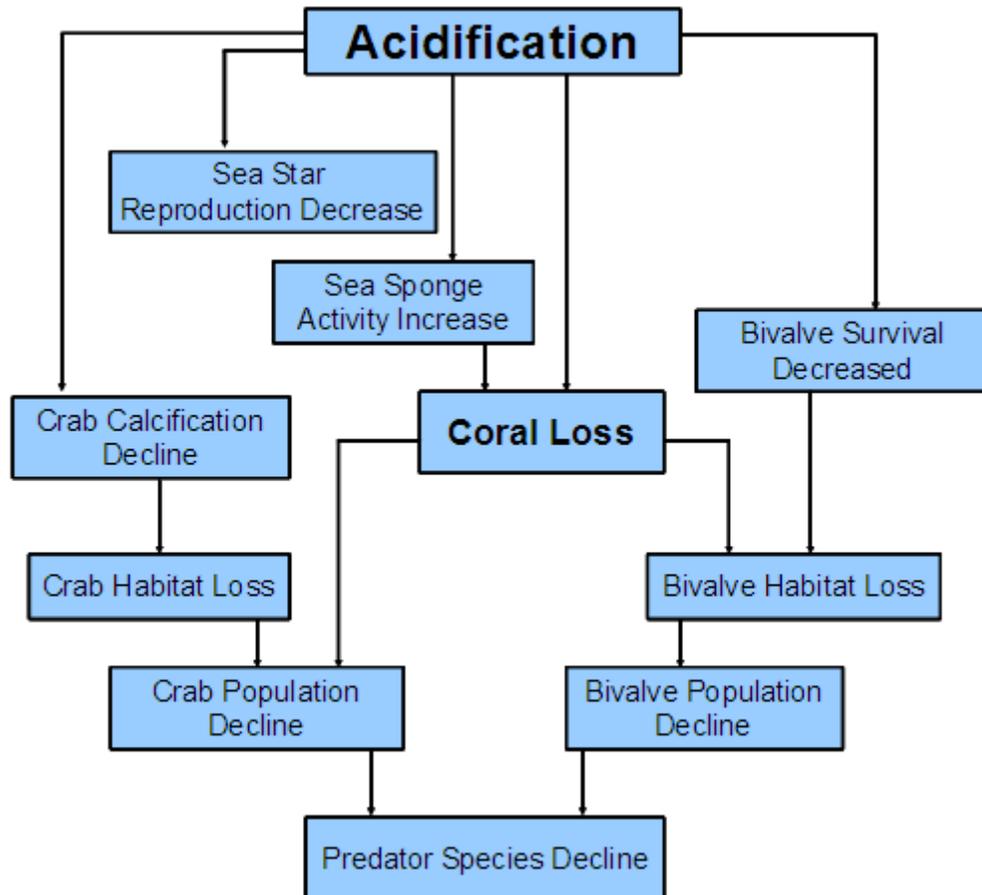


Figure 1. A simplified outline of the impact of acidification on an aquatic community. The driving factor in this relationship is coral loss; however, as the ocean becomes more acidic the impact becomes much more pronounced and impacts more species. This chart shows the expansive reach of acidification and how more than one organism can be affected by one process.

Freshwater Ecosystem Introduction

Freshwater fish play an important role in maintaining the structure of numerous ecosystems around the globe. Most fish serve as both predator and prey, significantly impacting the abundance and health of animals and plants in their associated habitat. Beyond these typical aquatic environments, however, fish have a wide range of habitats they impact. Forests, mountains, and even plains need these creatures to remain balanced. Without fish, many of the predators higher on the food chain would not have sufficient nourishment to behave normally. Populations would decline, and the habitat would likely begin to collapse (Gjedrem and Rosseland, 2011).

Pollution and climate change are the main culprits that threaten wildlife survival (Gjedrem and Rosseland 2011). Typically, these threats are seen by the public as oil spills in the ocean or the rising sea levels due to increased temperature. These two factors can radically alter the acidity of water, a variable that can diminish a fish's ability to grow.

To determine how low pH impacts fish, I performed a controlled study to test lowered pH on the development in *Poecilia reticulata*, the guppy. Guppies are members of the Poeciliidae family, a family that shares the unusual trait of bearing live fish. Due to their bright colors (in nature this is only seen in the males) and small size, *P. reticulata* is one of the most common aquarium pets across the United States, making it a fish that has garnered much commercial interest. Understanding how pH impacts these fish could help aquarists raise healthier fish, giving consumers a household pet that is easier to maintain. They are native to several tropical locations such as Brazil, Venezuela, and Guyana, and serve an important role on the food chain.

Any alterations in this fish's growth could be detrimental to many other species that rely on it as a primary source of food, as a decrease in mass likely correlates to a decrease in nutritional value.

Poecilia reticulata has also become an invasive species in many parts of the world. After being artificially introduced to certain habitats to help control mosquito populations, they unfortunately began damaging the integrity of the entire ecosystem (Teo and Chen 1993). If this species is more susceptible to acidity than other fish, it may be possible to use this information to develop a plan to remove them from the environment, or at least decrease their abundance. By altering the pH to levels which favor other species' growth and impedes that of *P. reticulata*, the habitat could potentially return to normal. Further research must be done to determine if this is truly a viable option, however it is still worth consideration.

Over the course of several weeks, *Poecilia reticulata* were raised in tanks that were artificially manipulated to different pH levels (7.0, 6.0, and 5.0). Previous studies have shown that acidic conditions may impact nutrient intake, leading to a decrease in overall growth rate of fish. Other researchers have found that oxygen uptake by guppies is stunted in acidic waters, however this research was only performed over the course of a few hours (Aride 2007) and may not hold true over the course of several weeks. Numerous other studies list similar physiological pathways that may impact fish development, yet none of them have used *Poecilia reticulata* in the studies. It is hypothesized that fish in the most acidic condition will show stunted growth and low survivorship, while those in more neutral water will exhibit faster growth and higher survival rates.

Since the impact of acidic water on fish has been studied numerous times over the

years, it is beneficial to review these experiments before proceeding. The majority of research deals with fish other than *Poecilia reticulata*, however they can still garner valuable information that can assist in developing a suitable experiment for these animals.

In one related study by Nchedo and Chijioke (2012), the effects of pH on hatchling success and larval survival are examined. In Nigeria, African catfish ranks very high in consumer preference, making it a species that is often studied. If scientists understand how it develops under various conditions, it becomes easier for fisheries to grow them in larger quantities and at higher quality.

In order to test the impact of acidic water on egg development, fertilized eggs were placed into 51 circular plastic tanks (30cm diameter). Tanks consisted of aged tap water which was then altered by adding two molar hydrochloric acid to lower the pH or sodium hydroxide to raise the pH. The reasoning behind using hydrochloric acid is never discussed, however this poses a potential threat to the animals. Chlorine is known to be detrimental to fish health and in high enough doses can even be lethal. In fact, part of the reason for letting tap water age is to remove this compound from the liquid before introducing it to the fish. Since it was used consistently to lower the pH of water throughout the experiment it may not skew the results from trial to trial, however the overall results might yield a survival rate and growth rate that are lower than if chlorine was not present. Each pH had three replicates, and 50% of the water was replaced on a daily basis to remove waste material that could have built up.

The range of pH 6.5 to 8.5 produced the highest hatching rates and did not yield any abnormalities during development. Again, this range between 6.5 and 8.5 showed the highest survival rate while the extremes of 4.5 and 9.5 produced the most deaths. Nchedo points out that

the impact of pH varies from species to species and cites zebra fish, atlantic salmon, perch, and brook trout as a few examples of animals that are not resistant to pH during development. Interestingly enough, some brook trout actually do appear resistant, however there is not enough research to explain this anomaly.

pH can also impact common carp (*Cyprinus carpio L.*) growth and survival (Heydarnejad 2012). This experiment required 21 tanks and tested 7 different pH levels (6.0 - 9.0 with intervals of 0.5), each with three replicates. The fish were first held in a tank with a pH of 7.0 for two weeks to acclimate to their new environment. Experimental tanks were set up using sulfuric acid or sodium hydroxide for pH adjustments and were continuously aerated. Groups of 20 fish (all similar size) were then placed in each tank for 21 days. They were fed a strict diet and food eaten by each tank was monitored, with any excess food being removed after a half hour to be weighed. This allowed the researchers to determine several variables related to growth. As with Nchedo's study, 50% of the water in each tank was replaced daily. The fish were also placed under fluorescent lights that simulated a natural day/night cycle.

Results show that no fish survived at pH 9.0, in fact, none of these fish survived past 14 days. At pH levels of 7.5 and 8.0 all variables were the highest (weight and length gain, survival, etc.) as expected. Other results show that there may be growth potential in some alkaline environments, as near the end of the study the standard growth rate in these conditions began to rise. Perhaps these fish were finally beginning to acclimate to the environment and found ways to take advantage of the slightly higher pH. Overall, however, it appears that common carp grows best in the same conditions that yield high rates of African catfish hatching success from Nchedo's research.

While it may appear that most fish prefer fairly neutral water, some fish in fact can use acidic water to their advantage. A New Zealand researcher has reported that some Galaxiid fish are capable of living in these seemingly disadvantageous conditions. While there are no experiments directly aimed at monitoring the movement of Galaxiids, field studies have often shown the fish swimming into acidic water to avoid salmonids (Glover 2012). Most salmonids are known to be very sensitive to water pH, therefore they typically will not pursue Galaxiids when they relocate.

Glover and his team of researchers wanted to understand how these fish are able to survive in water with pH levels as low as 4.0. To do this, they investigated the sodium metabolism of Galaxiids in different conditions. Sodium is exchanged through the body and environment via a Sodium/Hydrogen pathway. As acidity increases in the environment, so does the hydrogen concentration. This means that hydrogen can less easily leave the fish and in turn inhibits sodium intake. Low counts of sodium in fish can eventually lead to hyponatremia and eventually death. It was expected that there would be some physiological change that occurred as fish acclimated to the new environment, allowing improved ion flow to maintain this vital equilibrium. Inanga (Common Galaxias) was chosen as the model organism, as they are already known to survive in poor quality water (high salinity, hypoxic conditions, etc.).

Fish were collected from neutral and acidic lakes, along with water samples for use in the lab. Any pH adjustments were completed using Potassium Hydroxide and Hydrochloric Acid, however researchers tried to keep this to a minimum. Results showed a 46% drop in influx from pH 7.2 to 5.7 when 100uM of sodium was present. In fact, at pH 4.4 there was a 99% decrease in ion movement.

Defying expectations, the inanga did not undergo any significant physiological changes. Similar to most other fish, when placed in acidic conditions their net ion flow began to decrease. More sodium was leaving their body than they were taking in, and the Sodium/Hydrogen exchangers did not appear to adjust to help maintain equilibrium. However, unlike other fish outside the Galaxiid family, the inanga were still able to survive. Glover explains that although inanga did not undergo any changes during the experiment, they are born with a significant modification that allows for survival in low pH environments. When compared to other fish species, inanga naturally have a much higher sodium influx affinity than other fish. Simply put, they are able to pull sodium into their bodies at a higher rate than other fish, regardless of the condition. Therefore, although the influx of sodium did drop when placed in acidic conditions, the baseline influx of sodium is so much higher than other species that they are still able to transfer enough to survive. Inanga also prefer environments rich in sodium, giving sodium influx a slight increase due to the concentration gradient. In fact, the ion flux rates did not show significant drops when placed in acidic water with very high sodium concentrations (1000uM).

Although Glover did not pursue this genetic basis theory during his research, T. Gjedrem and B.O. Rosseland (2011) did. These scientists examined the genetic variation for tolerance to acidic water in salmonids. Although they did not perform any formal experiments, they compiled information from numerous other studies to provide a comprehensive guide. In the 1960's and throughout the 1970's, low pH depleted most of the fish stocks in Norway. Snow melted off of mountains at a much faster rate than normal, and as it melted it brought minerals and chemicals into the surrounding water systems. The pH in several areas plummeted below 4.0, a level which is much too acidic for most salmonids, if not all. By 1999 almost 84,000km²

had been affected and over 18 *S. salar* populations were lost to poor water quality. A similar event happened in the Adirondack Mountains in New York, impacting over 200 lakes. This research was compiled to determine how genetics can alter survivability, and to help guide future studies to try and prevent such massive loss from these dramatic events.

Each species within the salmonid group shows a different level of tolerance towards acidic water. For example, rainbow trout appear to have very low tolerance while brook trouts appear to survive quite well in these conditions. While this may be an over simplified answer to a broad question, it does tend to hold true. Of course, variation does exist within each species, allowing for some discrepancies.

Two groups of *Salmo salar* (Canadian population and Norwegian population) were examined to determine water quality that led to optimal growth. The Canadian population fared the best when pH was about 5.0, while the population from Norway needed at least a pH of 6.2. It is believed that the higher quantity of organic content in the Canadian habitat allows for better aluminum binding, which in turn allows these fish to survive lower pH. Aluminum is a common pollutant that often is found in areas with high acidity. After performing numerous breeding experiments to determine if genetics played a role in acid tolerance or if it was primarily caused by aluminum binding organic matter, the results showed that genetics do play an important factor. However, fish that were collected from acidic sites did not appear to have any more success in these conditions than fish from neutral waters (although these results conflict with Glover's study, this may be due to the different species being examined). This suggests that a genetic component is persisting throughout the species and that physiological changes do not occur based on environmental conditions.

The effect of pH on growth rate and survival time was observed using *O. mykiss* and *S. alpinus*. They were reared for three months at pH levels of 4.8-5.0, 5.5, and 6.1-6.2. At the conclusion of the experiment, fish of the same species showed no significant differences in mass or length when kept at pH 5.5 and 6.1-6.2. Both species raised in 4.8-5.0 were much smaller than the aforementioned groups. The study also reports that size does not play a major factor in pH vulnerability, however the age of the fish and stage of development do impact their susceptibility. Younger fish tend to display more exaggerated effects when in acidic water, while adult fish usually can negate some of the impact.

Since oxygen is carried through the blood stream and plays a vital role in animal growth, many researchers use a hematocrit to help determine physiological impacts. Cuvier was examined in different pH levels by Paulo Henrique Rocha Aride (2007), as this species is often said to be 'Acid Resistant'. After exposing fish to pH levels of 4.0, 6.0, and 8.0 (adjusting water levels over three hours to allow fish to slowly acclimate), the results support this theory. In contrast to many species of fish, hematocrit, hemoglobin, and red blood cell count all decreased under alkaline conditions. Standard growth rate was also higher in acidic water. pH often affects osmoregulation in fish, which may be related to an increase in mucus secretion. As the levels of oxygen decrease in the blood, this means less oxygen ultimately is received by the body tissues. Consequently, slowed rates of growth should be seen. This theory will be tested in the following experiment, as juvenile *Poecilia reticulata* will be exposed to a variety of pH levels and their growth will be monitored over the course of three weeks.

Materials and Methods

Identical habitats were set up for all replicates. Fish tanks (29cm X 14.5cm) were

prepared using reconstituted pond water (1.0 mg/L Ca²⁺, 0.5 mg/L Mg²⁺, 0.5mg/L K⁺, and 1.0mg/L Na⁺) with no filter. This water is an approximation of the natural environment, however it allows for more control over the conditions and reduces the amount of variation between trials.

Once the tanks were prepared, fish were introduced to the system within a day of being born. *Poecilia reticulata* were bred specifically for this experiment to eliminate many other variables (age, initial water quality, genetics). Tanks were adjusted using 1.0M KOH and 0.5M H₂SO₄ prior to fish introduction, since these chemicals have little to no side effects on fish development and are commonly used in animal studies. Most replicates required less than 0.500mL of H₂SO₄ to bring the tank to the proper pH, therefore if any side effects were caused by the chemicals, they would not be severe due to the small dose size.

Each day after the initial setup, fish were fed in excess (TetraMin Goldfish Flakes), water was cleaned and pH was adjusted as needed to maintain a consistent environment. Since the fish were extremely small, the water height was kept around 3.0-5.0cm.

Measurements were taken once a week for three weeks. Fish length was obtained through photo analysis, and activity was measured in total movements and total distance covered in 10 minutes. The activity measurements helped determine if pH made the fish lethargic over time, and also served as a rudimentary way to assess health beyond growth rate. Total body length measurements were taken from the tip of the caudal fin to the beginning of the mouth. Fish were fed prior to these measurements, therefore stress from handling did not impact food intake.

After three weeks, final length and weight measurements were taken, and total

growth rate could be determined. Between replicates, tanks were thoroughly rinsed with warm water to remove any traces of the prior trial.

Results

Due to complications when breeding the fish, only two replicates were run for each condition. In the first replicate, the fish in pH 5.0 did not survive past one week. However, the second replicate had a survival rate of 100% for all tanks. Using the data from all surviving fish, there is not a significant difference between treatments since the F values are small (Length : 0.356, Activity: 0.720, Distance: 2.871) and the p values are large (Length: 0.764, Activity: 0.637, Distance: 0.381). Fish in pH 5.0 grow at a rate very similar to fish in pH 7.0 and 6.0. The same held true for total activity and distance traveled. Even without the ANOVA analysis, a brief comparison of the mean growth rate and the corresponding standard deviation shows that that is little different between the three groups (Figure 2).

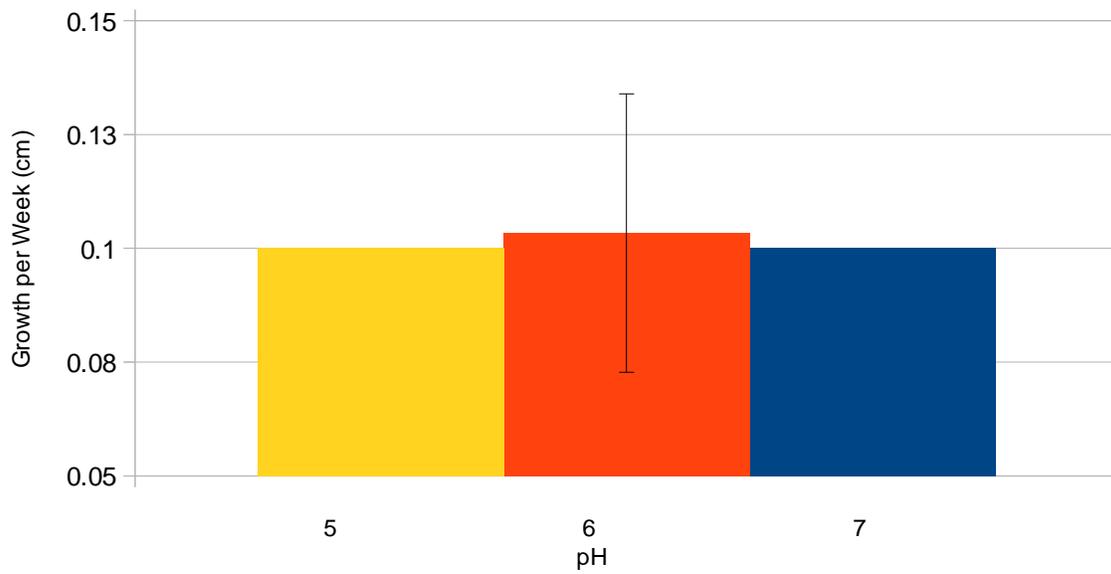


Figure 2. Growth rate (length) did not vary between the treatments. The results shown indicate that growth rate averages around 0.1cm per week for each condition. Error bars represent one standard deviation.

Discussion

pH does not appear to significantly impact *Poecilia reticulata* growth, total activity, or distance traveled. Acidic water (pH = 5.0) did result in a 50% mortality rate, however pH 6.0 and 7.0 saw a 100% survival rate. Since only two replicates were used, it is difficult to make any definitive conclusions as to whether or not this survival rate is due to the acidic nature of the water. The results do show that this is a topic worth investigating, since the one fish that did survive made it through the entire experiment and appeared to be just as healthy and grow at the same rate as the other fish held in less acidic water.

Poecilia reticulata are originally from tropical conditions, where flash floods are a common occurrence. With the increase in water level comes a change in water acidity, since nutrients and other chemicals are running off into the lakes and rivers. If fish are regularly exposed to changing water conditions they may have evolved to deal with these conditions. This would account for the survival of all fish in the second replicate, as it is possible that the sodium/hydrogen pathway discussed in Glover's research with Galaaxids was not compromised, allowing for sufficient uptake of nutrients from the environment. However, the death observed in the first replicate conflicts with this statement and may support the theory that *Poecilia reticulata* cannot regulate ion/nutrient flow as well in acidic conditions. Further study is needed to determine whether or not this is a viable model for their survival and growth, and to observe if there is another physiological pathway that is being manipulated.

Slowed oxygen uptake was another factor that may have been detrimental to fish growth (Teo and Chen 1993), however since growth was the same for all replicates it did not appear to contribute to fish health or development. Previous studies in oxygen uptake only lasted for a few hours (Teo 1993). While it is possible that oxygen intake was initially lower for the

first few hours in acidic conditions and the fish acclimated to the new environment, it eventually evened out over the course of several days, since no significant decrease in growth was recorded throughout the three weeks. This is another area for future study; determining how oxygen use changes during acclimation to new environments, and how long it takes to return to baseline levels.

Further study, involving more replicates and more pH intervals, will help determine an accurate pH in which these animals' growth is stunted. As it stands, growth between pH 7.0, 6.0, and 5.0 are all very similar, however pH 5.0 produces a higher mortality rate, likely due to acclimation issues, or random variability due to lower replicate size. Because these fish are usually able to acclimate to a wide range of pH values, it may be difficult to remove them from areas where they have become invasive simply by altering the conditions of the water. This trait also makes them easy for aquarists to raise, since they do not need to worry about acutely acidic water impacting how fast they grow.

References

- Copatti, Carlos, Luciano Garcia, et al. "Low water hardness and pH affect growth and survival of silver catfish juveniles." *Ciencia Rural*, Santa Maria. 41.8 (1482): 1487. Web. 24 Apr. 2013.
- Doropoulos, Christopher. "Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions." *Ecology Letters* 15(4): 338-346. Print.
- Gjedrem, T., and B.O. Rosseland. "Genetic variation for tolerance to acidic water in salmonids." *Journal of Fish Biology*. 80. (2012): 1-14. Web. 24 Apr. 2013.
- Glover, Chris, Katherine Donovan, and Jonathon Hill. "Is the Habitation of Acidic-Water Sanctuaries by Galaxiid Fish Facilitated by Natural Organic Matter Modification of Sodium Metabolism?." *Physiological and Biochemical Zoology*. 85.5 (2012): 460-469. Web. 24 Apr. 2013. <<http://www.jstor.org/stable/10.1086/666903>>.
- Gobler, Christopher. "Hypoxia and Acidification Have Additive and Synergistic Negative Effects on the Growth, Survival, and Metamorphosis of Early Life Stage Bivalves." *PLoS ONE* 9(1): 1-10. Print.
- Heydarnejad, Saeed. "Survival and growth of common carp (*Cyprinus carpio* L.) exposed to different water pH levels." *Turkish Journal of Veterinary & Animal Sciences*. 36.3 (21207012): 245-249. Web. 4 May. 2013. <doi:10.3906/vet-1008-430>.
- Long, William. "Effects of Ocean Acidification on Juvenile Red King Crab (*Paralithodes camtschaticus*) and Tanner Crab (*Chionoecetes bairdi*) Growth, Condition, Calcification, and Survival." *PLoS ONE* 8 (4): 1-10. Print.
- Nchedo, Ariole, Chijioke Okpokwasili. "Effect of pH on Hatching Success and Larval Survival of African Catfish (*Clarias gariepinus*)." *Nature and Science*. 10.8 (2012): 47-52. Print.
- Pinnegar, John K. "The potential impacts of ocean acidification: scaling from physiology to fisheries*." *Fish and Fisheries* 13: 333-344. Print.
- Rodd, Helen, and David Reznick. "Life History Evolution in Guppies: III. The Impact of Prawn Predation on Guppy Life Histories." *Oikos*. 62.1 (1991): 13-19. Web. 24 Apr. 2013. <<http://www.jstor.org/stable/3545440>>.
- Romeu, Garcia, and J. Maetz. "The Journal of General Physiology." *Proceedings of the National Academy of Sciences*. 47. (1964): 1195-1207. Print.
- Teo, Leng-Hong, and Chen Ti-Wen. "A study of metabolic rates of *Poecilia reticulata* Peters under different conditions." *Aquaculture and Fisheries Management*. 24. (1993): 109-117. Print.
- Uthicke, Sven. "Impacts of Ocean Acidification on Early Life-History Stages and Settlement of the Coral-Eating Sea Star *Acanthaster planci*." *PLoS ONE* 8(12): 1-9. Print.
- Venn, Alexander, Eric Tambutte, et al. "Impact of seawater acidification on pH at the tissue-skeleton interface and calcification in reef corals." *Proceedings of the National Academy of Sciences*. 110.5 (2012): 1634-1639. Print.
- Wisshak, Max. "Ocean Acidification Accelerates Reef Bioerosion." *PLoS ONE* 7(9): 1-8. Print.

