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**Regeneration Rates of the Brittle Star *Ophiocoma echinata* Under Conditions
of Near-Future Ocean Acidification and Temperature**

By

Steven E. Bruzek

**A thesis submitted in partial fulfillment
of the requirements of the
University Honors Program
University of South Florida, St. Petersburg**

April 29, 2014

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CERTIFICATE OF APPROVAL

Honors Thesis

This is to certify that the Honors Thesis of

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**has been approved by the Examining Committee
on April 29, 2014
as satisfying the thesis requirement
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Abstract:

Ocean acidification is the result of uptake of atmospheric CO₂ in the oceans. Anthropogenic CO₂ emissions have increased rapidly since the start of the Industrial Revolution. Along with an increase in acidity, the ocean's average temperature is rising from global warming. This study investigates the near-future (year 2100) effects of acidification and temperature on regeneration rates of the brittle star *Ophiocoma echinata*. Using HCl to induce acidification, regeneration rates for near-future conditions (pH 7.8 and temperature 30°C) did not decrease significantly from present conditions. Regeneration increased significantly with an increase in temperature alone, suggesting a complex interaction between temperature and acidity.

Introduction

Levels of anthropogenic CO₂ have been rapidly increasing in the atmosphere since the Industrial Revolution. The oceans have absorbed approximately half of all the anthropogenic atmospheric CO₂ (Siegenthaler & Sarmiento 1993). Due to this phenomenon, it is estimated that the global average seawater pH has decreased by about 0.1 units since the beginning of the Industrial Revolution and is predicted to decrease by another 0.4 units by the end of the century (IPCC, 2007). The amount of CO₂ being absorbed by the ocean has multiple consequences. When CO₂ reacts with seawater, it produces hydrogen ions that increase the acidity of the water, lowering the pH (Wood et. al., 2008). Elevated pCO₂ will lower the saturation levels of calcium carbonate (CaCO₃), along with aragonite and calcite, two polymorphs of calcium carbonate (Fabry et. al., 2008; Kurihara et. al., 2004). Calcite and aragonite comprise shells or spicules of sponges, hard corals, barnacles, molluscs, echinoderms, foraminifera, and other calcifying marine invertebrates (Fabry et. al., 2008; Schram et. al., 2011). Echinoderms produce their skeletons from

magnesium-rich calcite that is even more susceptible to ocean acidification than aragonite (Andersson et al., 2008; Gayathri et al., 2007).

The phylum Echinodermata describes spiny skinned, coelomate invertebrates that are derived from sessile ancestors (Hyman 1955). There are three defining characteristics of Echinoderms: a calcium endoskeleton that becomes the dermis of adults, tube feet that are used for locomotion, feeding and sensory receptors, and pentameric symmetry to one degree or another (Harmon 2005). Brittle stars and basket stars comprise the class Ophiuroidea. Defining characteristics of this class include: no exterior ambulacral grooves, lack of pedicellaria and respiratory extensions, and movement that is independent of hydraulic action of the tube feet (Harmon 2005).

An important characteristic of Echinoderms is their ability to regenerate, which involves altering calcification rates (Bannister et. al., 2005; Bowmer & Keegan, 1983; Wood et. al., 2008). This process is most observable in the sea stars (Asteroidea) and brittle stars (Ophiuroidea). Predation is a large cause of mortality, so many Ophiuroids utilize regeneration as a predator avoidance technique to acquire a sublethal injury, such as partial arm loss, and still survive (Sides, 1987). The Ophiuroids can willingly self-amputate their arms to get away from a predator in a process called autonomy. This evolutionary adaptation has allowed the Ophiuroids to be a highly successful class in the Echinoderm phylum. Lindsay (2010) showed that the percentage of Ophiuroids found in the wild with injuries and regeneration was between 62.4 and 72.0 % among 26 different species. Ophiuroids demonstrated the largest percentage of individuals with injury and regeneration among all Echinoderms (Lindsay 2010). Regeneration is clearly an integral part of Ophiuroid survival. Because regeneration of Ophiuroid arms is a calcification process, ocean acidification has the potential to negatively affect regeneration, potentially having widespread effects among Ophiuroids and other calcifying organisms.

Research into the effects of ocean acidification on regeneration rates in Echinoderms is still relatively new. Schram et al., (2011) found that ocean acidification did not significantly affect the regeneration rate of the sea star *Luidia clathrata*. The brittle star *Amphiura filiformis* did not have a significant reduction in the rate of regeneration, but it did experience some muscle wastage attributed to higher acidity (Wood et. al., 2008). There is a potential for even more effects when the problem of ocean acidification is compounded with other consequences of global climate change. Along with an increased acidity in the oceans, global climate changes will bring about a rising global average temperature, presenting the dual challenges of increased seawater temperature and decreased pH on marine fauna (Wood et. al., 2011). For this experiment, ambient acidity and temperatures were chosen based off Tampa Bay surface water measurements made by Yates et al. (2002).

Global average seawater temperature is predicted to rise by 3-4°C by the year 2100 (IPCC, 2007). This rise in temperature could have large implications for marine organisms all over the world, especially in areas such as the arctic where temperature fluctuations are very limited throughout the year (Wood et al., 2011). Consequently, this study is to investigate the effects of both an increased acidity and an increased temperature, reflecting conditions that are predicted for the next century, on the regeneration rates of the brittle star *Ophiocoma echinata*.

Materials and Methods

Animal Collection and Care

All experimentation was carried out between December 2013 and April 2014 at the Knight Oceanographic Research Institute in St. Petersburg, Florida. *Ophiocoma echinata* (N=14) were purchased from Quality Goldfish Inc., Clearwater, Florida. The specimens were collected by

Quality Goldfish from the waters in and around Tampa Bay, Florida. Specimens were transferred from Quality Goldfish and immediately placed into a quarantine tank of salinity 35‰, temperature 28° C, and pH 8.1. The specimens were then transferred to the respective tanks for experimental procedures.

Specimens were kept without food intake for an incubation period of 3 days to establish a nutritional standardization. After nutritional standardization, brittle stars were maintained on a diet of *Shellfish Diet 1800*, *Instant Algae* and fed 3 times a week. Food intake was monitored to assure that an equal amount of food was consumed by each individual. The brittle stars responded with vigorous feeding following introduction of algae and typically fully consumed the food within two hours.

Tank Setup

Three 37-56 L glass aquariums were set up and filled with seawater that had been cycling through a recirculating live rock system to maintain stable chemical and biological conditions. Partial water changes (15% aquarium volume) were made every week to maintain proper water conditions. Lids were placed on each tank to minimize water loss due to evaporation. Twenty gallon aquarium filters were placed in each individual aquarium. CaribSea Arag-Alive Reef Sand was used as substrate for each tank to assist in maintaining proper biological conditions. Cylindrical glass containers (diameter = 15cm; height = 15cm) were placed in each of the tanks to isolate individual brittle stars. To simulate natural conditions, live rock and a 1cm thick layer of Arag-Alive substrate were placed in each container. Specimens mainly resided under the live rock, exposing just the ends of their arms to catch passing food, as is typical wild behavior. To

keep brittle stars contained in the individual containers, screen was placed on top of each container and held in place by rubber bands.

AZOO tube heaters and temperature controls were placed in all three tanks. Three temperature treatments were used (25° C, 27° C, and 30° C) and temperature was monitored daily using a Hanna Instruments pH/Temperature probe. If temperature measurements deviated from experimental parameters, appropriate adjustments of temperature controls were made. Room temperature remained steady at approximately 22° C. Room lighting was maintained at a low level due to the nocturnal nature of *Ophiocoma echinata*. Salinity was kept constant at 35‰ and measured using a refractometer.

Acidity was controlled using HCl purchased from Thermo Fisher Scientific and diluted down to a concentration of 0.1M. Acid was stored in 2L Aquadose containers held above the tanks. Flow rate was controlled using Air Tech flow rate controllers. Acidity was measured daily using a Hanna Instruments pH/Temperature probe. Proper adjustments to flow rate were made according to probe readings.

Experimental Setup

Treatments				
1	2	3	4	5
Ambient pH Low Temp.	Ambient pH Ambient Temp.	Ambient pH High Temp.	Low pH Ambient Temp.	Low pH High Temp.

Temperatures: Low=25° C; Ambient =27° C; High =30° C

pH: Ambient= 8.1; Low= 7.8

Mean \pm standard deviation for the 5 experimental parameters was calculated using daily measurements. Low temperature: $24.95 \pm 0.38^\circ \text{C}$. Normal temperature: $27.28 \pm 0.18^\circ \text{C}$. High temperature: $29.75 \pm 0.73^\circ \text{C}$. Normal pH: 8.07 ± 0.14 . Low pH: 7.81 ± 0.07 .

Arm Amputation

Brittle stars were initially anesthetized using a solution of MS-222 (2.5g/L). After brittle stars responded negatively to the anesthesia, including self-amputating multiple arms, further amputations were carried out under no anesthesia. Only brittle stars with all arms fully intact were chosen for experimentation. One arm was chosen at random for partial amputation. Amputation was carried out by using a scalpel blade and cutting between arm segments at a distance of 50% the total arm length. Certain specimens that had 4 of their arms fully intact and one that was more than 50% intact were also used, with the regenerating arm given a fresh amputation at 50% length of average arm. Brittle stars were taken out of their individual container and placed out of water to make the amputation. Upon completion of amputation, the brittle star was placed back in the glass container. Exposure time out of water was minimal and did not exceed 30 seconds for any specimens.

Arm Measurements

Brittle stars were exposed to experimental conditions for a period of 28 days. Upon completion of experimental period, brittle stars were put in individual plastic bags and placed in freezer. After 24 hours, specimens were removed and thawed. Once fully thawed, measurements could be accurately made. This method was preferred to live measurements due to the fact that when alive, some specimens self-amputated both previously regenerating and non-regenerating arms and caused accurate measurements to be difficult to obtain. All measurements were carried out

using Vernier calipers accurate to 0.5mm. The length of regenerated arm was measured. The length of regenerating arm was clearly distinguishable from the non-regenerating arm by size (Fig. 1). The lengths of the four intact arms were then measured and averaged. The percent of arm regrown was calculated by dividing the length of regeneration by the average length of all other arms. This method was to standardize the measurement of regeneration across different sizes of brittle stars.

Results

Some specimens were found to have self-amputated one or more of their arms during the experimental period. These were taken out of the experiment and not measured. A linear relationship was demonstrated in a comparison of disc length versus arm length (Fig. 2), and size was not factored into choosing specimens for different treatments.

The percentage of arm regenerated did not significantly differ between the values of pH and temperature for today (ambient pH and ambient temp) and those predicted in the year 2100 (low pH and high temp) (Fig. 3). Lowering the pH at ambient temperature of 27°C slightly, but not significantly, increased regeneration rates (Fig. 3). Temperature increase from ambient to high showed a significant increase in regeneration rates (Fig. 4) at ambient pH.

At high temperature, lowering pH caused a significant decrease in regeneration rates (Fig.4), but this value doesn't significantly differ from current conditions. Temperature increase as predicted by the year 2100 showed a large increase in regeneration, but no significant decrease was found from current conditions in any of the treatments. The percent regeneration of the arm was calculated using the average measurements of all the other arms, to achieve a percentage value

that can be compared over all samples. The percent regenerated was significantly different between treatments with a significant interaction between pH and temperature as shown by a Two-way ANOVA (Table 1). Temperature and pH alone did not show significant variation between treatments (Table 1). Larger specimens were found to have a larger percentage of arm regenerated after the trial (Fig. 5).

Discussion

Ophiocoma echinata regenerated their arms at about the same rate under current conditions of pH 8.1 and temperature 27°C and predicted conditions in the year 2100 of pH 7.8 and temperature 30°C. There was actually a slight increase in regeneration. This difference was not significant and only translated to about a 3% increase. This indicates that there might not be a large detrimental effect on regeneration rates in *Ophiocoma echinata* under conditions of near-future ocean acidification and warming. This is similar to other studies that found that the sea star *Luidia clathrata* (N=33) regenerated their arms at similar rates under ambient pH 8.2 and CO₂-enriched pH 7.8 seawater (Schram et al. 2011) and the brittle star *Amphiura filiformis* (N=80) has similar or accelerated regeneration in lowered pH treatments when compared to the ambient pH 8.0 (Wood et al. 2008). Results are highly variable as arm regeneration was found to be significantly reduced under conditions of low pH in the polar brittle star *Ophiocten sericeum* (N=108) (Wood et al. 2011).

Under conditions of ambient pH of 8.1, increasing the temperature from 27°C to 30°C had a significant increase in regeneration rates. This translated to about a 9% increase in regeneration rates between treatments (Fig. 3). Similarly, an increase in regeneration and differentiation rate was found to be faster at higher temperatures in the brittle star *Amphiura filiformis* (Thorndyke et

al., 2003) and *Ophiecten sericeum* (Wood et al., 2011). Interestingly, Wood et al. (2011) found that this increase in regeneration in was only found at ambient pH, and not at low pH, suggesting that the lowered pH slows regeneration and cancels out the increased rate of regeneration induced by the temperature. The same situation was found in this study, where an increase in temperature at ambient pH caused a significant increase in regeneration rate, but at low pH caused a slight decrease in regeneration rate. This could indicate that pH has a larger effect on regeneration at lower levels than current ones.

When temperature was compounded with acidification, variable results were found. Gianguzza et al., (2013) found that temperature appeared to modulate the impact of low pH on sea urchin *Arbacia lixula* larvae where low temperature had a positive effect and high temperature had a negative effect on growth. Wood et al., (2010, 2011) found that an increase in temperature showed up-regulation of metabolism, increased movement speed, and increased regeneration rates of the brittle star *Ophiura ophiura*, as well as seeing an up-regulation of metabolism in the Arctic brittle star *Ophiecten sericeum*. Often ocean acidification studies are only carried out with a change in pH, but there is clearly a complex interplay between many physical factors that will be changing along with the acidity.

Ocean acidification has been found to have negative impacts on many different marine fauna. Coccolithophores, the most abundant calcareous phytoplankton, experience decreased rates of calcification with elevated CO₂ (Iglesias-Rodrigues et al., 2008; Langer et al., 2006; Riebesell et al., 2000; Zondervan et al., 2001). Pteropods have a 28% decline in aragonite shell calcification when exposed to a pH decrease of 0.34, within the range predicted in the next century (Comeau et al., 2009). A change in pH of 0.4 units had a negative effect on the development of sperm, embryos, and larvae of two species of sea urchins (Havenhand et al., 2008). Brittle stars have

been found to have reduced larval development, growth, and survival at a pH predicted in the next century (Dupont et. al., 2008). Crustose coralline algae showed a 91% growth reduction (Kuffner et. al., 2007), gastropods had a significant reduction in height and weight (Shirayama and Thornton, 2005), mussels and oysters showed a 25% and 10% reduction, respectively, and corals showed a 56% decrease in calcification (Maier et. al., 2009) when exposed to conditions of near-future ocean acidification.

Any decline of regeneration or calcification rates could potentially affect brittle star survival due to the numerous uses of their arms including locomotion, food capture, and predator avoidance. Brittle star arms consist of many ossicles, controlled by muscles connecting adjacent ossicles (Lawrence, 1987). They move by controlled movement of each individual arm to apply forces to the substrate beneath them (Astley 2012). They also have photoreceptors, chemoreceptors, and mechanoreceptors on their arms (Astley 2012). Unlike asteroids, which have important organs like the pyloric caeca and gonads extending into their arms (Lawrence, 2010; Schram et al., 2011), brittle stars have all important organs contained within their central disc. This allows them to use their arms as a predator avoidance technique as mentioned above. Their arms are also used for capturing food and righting themselves. Arm loss or slower regeneration rates could compromise their ability to perform these functions (Ramsay et al., 2001).

This study used HCl to induce acidity as opposed to the more common technique of CO₂-induced acidity. The chemistry behind the changing acidity in HCl and CO₂-induced experiments is different and could potentially have affected results of this experiment. It has been shown that using HCl to induce acidity introduces changes to the seawater chemistry such as a comparatively lower level of bicarbonate (HCO₃⁻) (Cornwall et al., 2012) and a lowered total alkalinity (Gattuso et. al., 2010). Kurihara et al., (2004) found that there was no significant

difference between using HCl and CO₂ with regard to the fertilization rate, the effects on early cleavage, and the effects on the pluteus larvae of the sea urchins *Hemicentrotus pulcherrimus* and *Echinometra mathaei*. However, Watanabe et. al., (2001) compared the survival rates of copepods in acidified water at the same pH by both CO₂ and HCl and found that there was a greater impact when using CO₂. Li et al., (2011) showed that acute toxicity of acidification on several marine copepods was significantly higher in CO₂-induced acidification than in HCl-induced acidification. Lead toxicity of the fish species *Pimephales promelas* was more affected by CO₂-induced acidification than HCl-induced acidification, indicating that CO₂ overestimated the impact of pH on lead toxicity (Esbaugh et al., 2012). No experiments have shown the effects of using HCl versus CO₂ in echinoderms. Clearly there is a complex interplay between methods of induced acidification, seawater chemistry, and physiological responses of organisms, but HCl can still give a general picture of how these brittle stars will respond to acidic conditions in the year 2100.

This study was done with a small sample size of N=14 (Table 2). This small of a sample size created a lot of error in the measurements of the regeneration rates (Fig. 6). A trend can still be concluded and statistical significance still achieved for certain comparisons. The results could have been more conclusive with a larger sample size and longer regeneration times. Another more acidic treatment could have been interesting to compare the results to an environment predicted in a more long-term scenario. More research will need to be done to determine the broad range of effects that ocean acidification and rising temperature will have on Echinoderms. Many differing results could mean that responses are species and location specific. Ocean acidification is happening at such an alarming rate that more information will need to be gathered to reach potential solutions to the problems that organisms will face in the near future.

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Tables and Figures

Figure 1. Regenerated arm clearly identifiable by size.

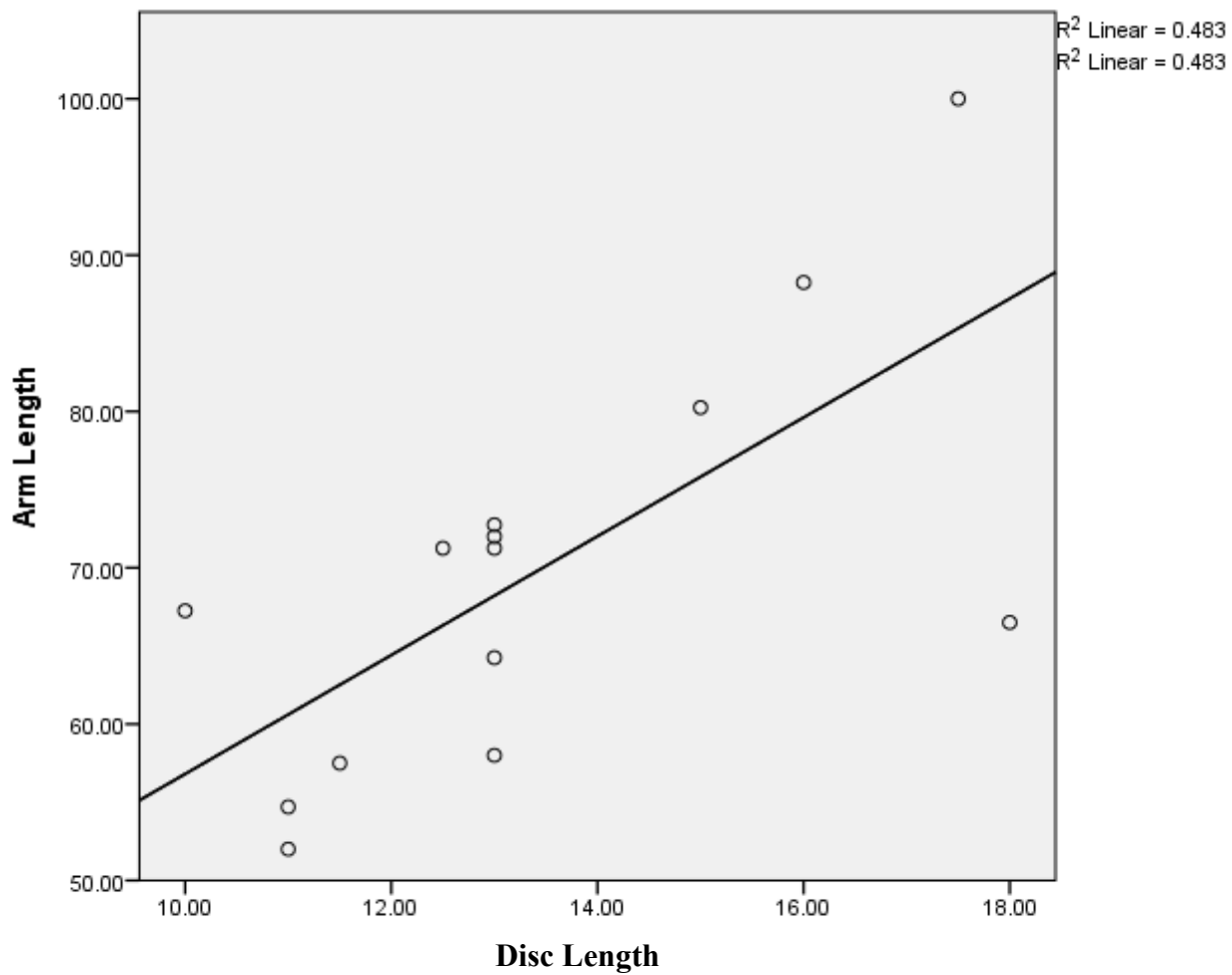


Figure 2. Regression analysis showing the relationship between disc length (mm) and average arm length (mm). $R^2 = 0.483$.

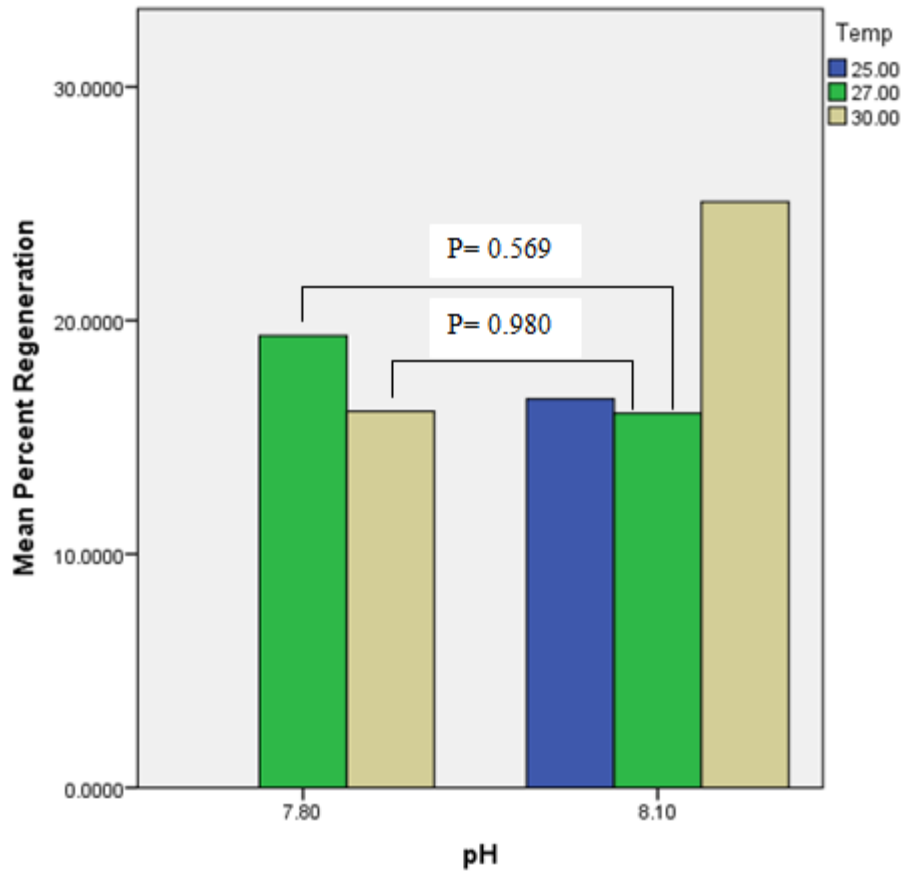


Figure 3. Mean percent regeneration at pH treatments of ambient (8.1) and low (7.8) and temperature treatments of high (30°C), ambient (27 °C), and low (25 °C). Statistical significance as determined by Student *t* test shown.

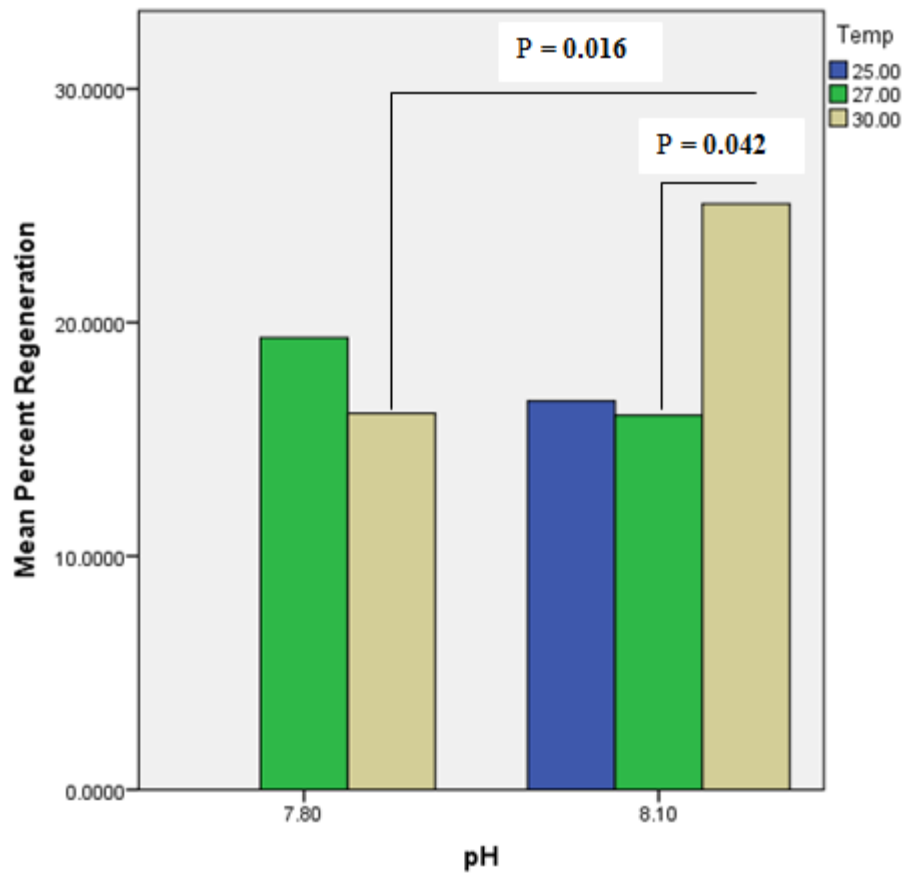


Figure 4. Mean percent regeneration at pH treatments of ambient (8.1) and low (7.8) and temperature treatments of high (30°C), ambient (27 °C), and low (25 °C). Statistical significance as determined by Student *t* test shown.

Table 1. Two-way ANOVA results indicating the impact of pH and temperature (Temp) on percentage arm regeneration.

Source	<i>df</i>	Mean Square	F	P
pH	1	.002	1.231	.296
Temp	2	.002	1.356	.306
pH*Temp	1	.011	5.826	.039
Error	9	.002		
Total	14			

Comparison of both pH (8.1, 7.8) treatments: analysis includes all three temperatures of 25, 27, and 30°C

Significant P value indicated in bold

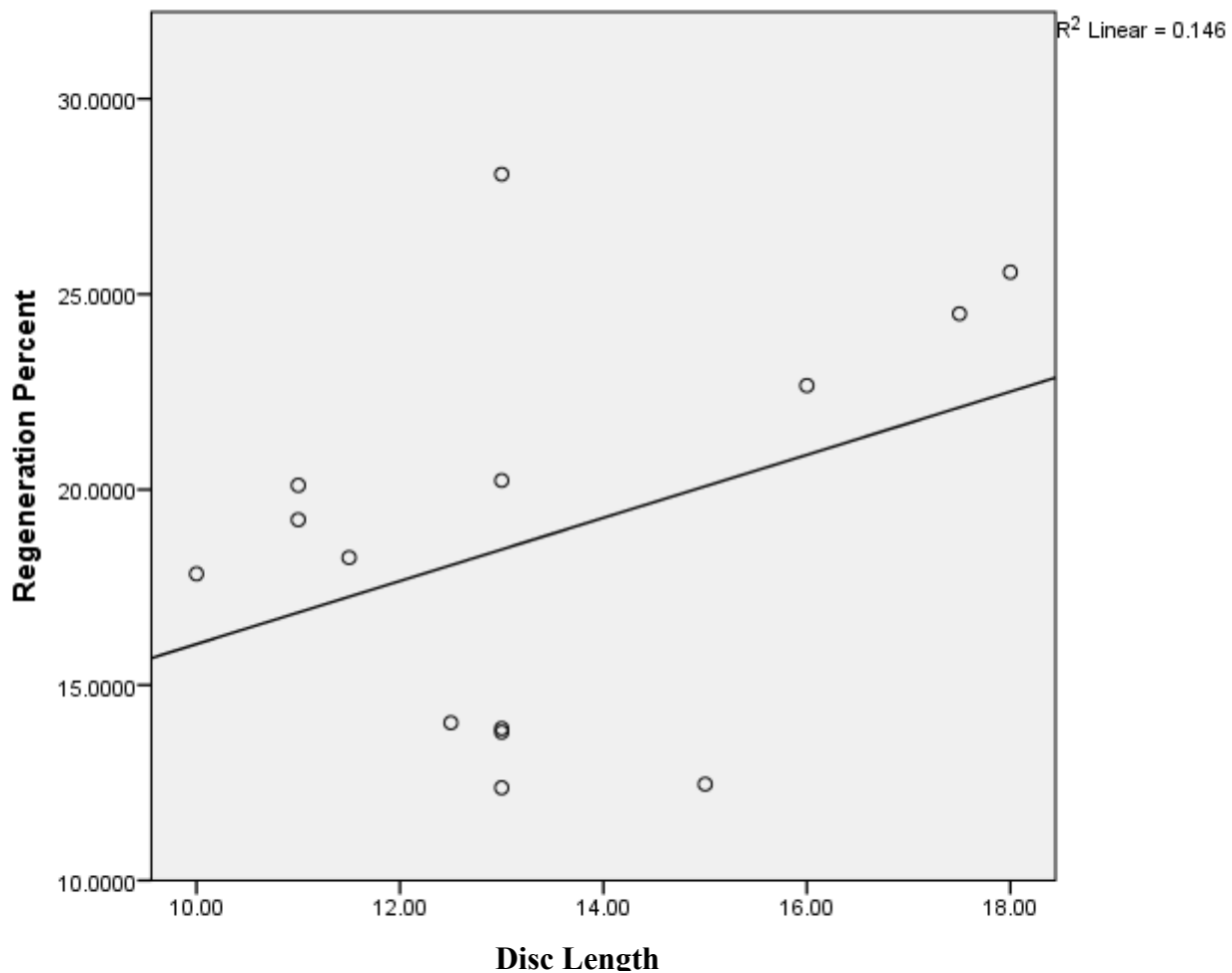


Figure 5. Regression analysis showing the relationship between disc length (mm) and regeneration percent with line of best fit. $R^2 = 0.146$.

Table 2. Sample size for each individual treatment.

Treatment	1: pH-8.1 T-25°C	2: pH-8.1 T-27°C	3: pH-8.1 T-30°C	4: pH-7.8 T-27°C	5: pH-7.8 T-30°C
Sample Size	2	2	3	3	4

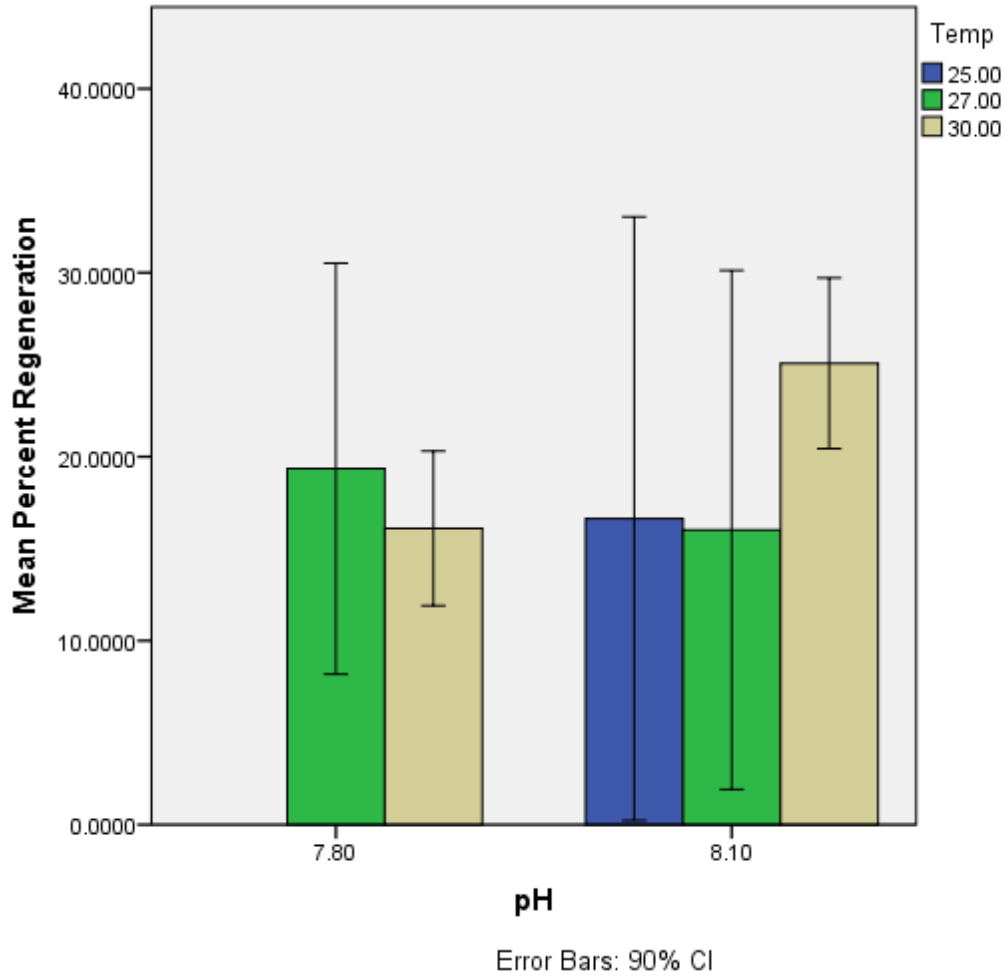


Figure 6. Mean percentage regeneration ($\pm 90\%$ confidence intervals) at pH treatments of ambient (8.1) and low (7.8) and temperature treatments of high (30°C), ambient (27 °C), and low (25 °C).