30 PPT YIELDS HIGHEST RATE OF LIMB REGROWTH IN *LUIDIA CLATHRATA*

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ABSTRACT: Sea stars have the ability to self-amputate limbs in the presence of danger (known as autotomy). How quickly those limbs can be regrown is vital to their survival, since having fewer limbs compromises sea stars' ability to hunt. Donachy (1987) illustrates that variations in salinity impact a sea star's ability to regrow shed limbs. Since 1950, average salinity fluctuations have increased by 5.3% on a global scale, becoming more extreme in both directions, probably due to climate change (Skliris et al. 2014). In light of this, the effects of salinity on limb regrowth is becoming a more and more urgent subject of research. For reference, salinity is a quantification of the percentage of salt (normally sodium chloride) present in water, measured in parts per thousand (ppt). This paper will research the question: What salinity yields the highest rate of limb regrowth in starfish (specifically Luidia clathrata). This comparative study looked at two research papers testing how variations in the surrounding salinity affect the amount (length, mass, dry weight) of limb regrown (Kaack and Pomory 2011; Honeycutt and Pomory 2019). The studies tested salinities of 20, 25, and 30 ppt; both studies found that 30 ppt yielded the most limb regrowth per unit time and, consequently, the highest rate of regrowth. Kaack and Pomory (2011) recorded 1.6 mm/week, and (Honeycutt and Pomory (2019). 2.0 mm/week for a 30 ppt treatment. The more oceanic salinity veers from the average of 35 ppt, the slower regrowth will occur, making hunting and healing slower and more difficult for these animals, compromising starfish survival.

Keywords: Salinity, limb regeneration, Luidia clathrata, comparative study, regrowth

Introduction

Being relatively slow-moving animals, starfish often will utilize autotomy when defending themselves from predators, so regeneration is crucial for recovery (Kaack and Pomory 2011; Wilkie 2001). If salinity levels fluctuate excessively from normal (35 ppt), that regenerative property can be slowed or entirely arrested. Regeneration is the vital second half to autotomy, and without the ability to regenerate shed limbs due to a hypo/hypersaline environment, sea stars could shed body parts until there is nothing left (Wilkie 2001). Starfish are vital to the oceanic ecology, being keystone predators. Their presence maintains crucial diversity among the seafloor residents, keeping one species from dominating the others (Checon et al. 2019).

As global warming statistics rise and surface ice continues to melt, oceanic salinity fluctuations are increasing (Liu et al. 2019; Skliris et al. 2014). Especially in areas where freshwater mixes with saltwater (such as coastal regions), salinity is deviating more from previously recorded averages (Liu et al. 2019). Starfish largely reside in these areas of costal mixing (Kaack and Pomory 2011). Like many other members of the echinoderm phylum, starfish can regenerate their limbs, but that process is influenced by environmental factors like salinity (Honeycutt and Pomory 2019). Understanding the relationship between limb regrowth and salinity can allow aquariums and other artificial environments to manipulate the surrounding salinity to promote natural recovery at its optimal rate for injured animals.

Scientists have typically used starfish as a research model for echinoderm regeneration and have looked in-depth at the specific mechanisms involved. Ferrario et al. (2017) studied the specific chemical processes of limb repair, looking step by step at how the wound closes and the new arm emerges. Cortes et al. (2014) examined gene expression and sediment preference in two species of *Luidia,* finding that *Luidia* tend to prefer soft, muddy soil to sand or loose stones. Lawrence and Pomory (2008) examined the rates of regrowth in *Luidia clathrata,* exploring the relationship between how much limb was cut off and how much limb was regrown over a standard period of time. They discovered that when more limb was cut off, the limb would grow back faster; so if only a tip of an arm was cut off, it would grow back slower than if an entire arm was removed. The University of West Florida has frequently investigated *Luidia clathrata*, examining the effects of salinity on arm length (Kaack and Pomory 2011) as well as the effects of salinity and food availability on arm regrowth (Honeycutt and Pomory 2019), finding in both studies that near 30 ppt is the best salinity to foster regeneration. Interestingly, in hyposaline environments, there is no significant difference between an excess or a dearth of food for starfish; the rate of regrowth is the same (Honeycutt and Pomory 2019).

All this aside, we still do not know how oceanic changes caused by climate change will affect limb regeneration. How will rising temperatures and/or ocean acidification impact limb regrowth? Considering everything, from reproductive success to hunting speed, and especially internal mechanisms (such as limb repair), there are plenty of unanswered questions which scientists are just beginning to explore.

My goal was to conduct a comparative study looking specifically at the role of salinity and its effect on arm regeneration. Already there is extant data studying the impact of various salinities on regrowth (Kaack and Pomory 2011; Honeycutt and Pomory 2019), and the relationship between salinity and arm regrowth. I took those data sets, looked for trends across the experiments, and found the optimal salinity to foster limb regrowth. This will enable us to manipulate the environment of a starfish to maximize arm regrowth, but more importantly, create a model allowing us to predict and potentially counteract the effects of fluctuations in oceanic salinity.

Methods

I retrieved two peer-reviewed articles on starfish using the Web of Science, and compared the two most relevant articles as my results. Using the phrase "'starfish' and 'regeneration*' and 'salinity,'" I searched all topics in the entire timeframe and through all indexes in the main search bar of Web of Science. From this, I garnered five results (see Table 1).

To evaluate, I first removed all duplicate papers published from the same study. (Two results offered by Web of Science [Honeycutt and Pomory Jun 2019; Honeycutt and Pomory Jan 2019] were conducted by the same scientists, had the same title, and were published within months of each other, so I eliminated the earlier published option). For this experiment, I limited the results to only those published after 2000, eliminating Donachy (1987) from consideration, being too out of date. Likewise, many of the results tested other factors as well as the effects of salinity on arm regrowth (such as Cortes et al. (2015)); for simplicity, I removed any articles dealing with three or more variables. By process of elimination, two articles remained that dealt predominantly with salinity-dependent regeneration, were published since 2000, and were not from the same study: Honeycutt and Pomory (2019) and Kaack and Pomory (2011).

Honeycutt and Pomory (2019) tested seven salinity treatments ranging from 15 ppt to 45 ppt in 5 ppt increments, and for each treatment, used four specimens of *Luidia clathrata.* Note: all animals from the 45 ppt treatment died immediately, so that data is not included in the results. Likewise, all the animals in the 15 ppt group did not regenerate any limb at all, although they remained alive, and that treatment's data is still included in the results. Kaack and Pomory (2011) tested three salinities ranging from 20 ppt to 30 ppt in 5 ppt increments,

Table 1: Results from Web of Science using Above Key Words

	Abridged Title	Author(s)	Date Published	Starfish Species Tested
1	Effects of salinity and feeding on arm regeneration in the starfish *Luidia clathrata*	Nicholas Honeycutt, Christopher Pomory	Jun 2019	*Luidia clathrata*
2	Effects of salinity and feeding on arm regeneration in the starfish *Luidia clathrata*	Nicholas Honeycutt, Christopher Pomory	Jan 2019	*Luidia clathrata*
3	Sediment preference, salinity tolerance and COX-1 genetic differences in two purportive species of Luidia	M. Cortes et al.	May 2015	Two species tested
4	Salinity effects on arm regeneration in *Luidia clathrata*	Katrina Kaack, Christopher Pomory	2011	*Luidia clathrata*
5	Effect of Salinity Stress and Arm Regeneration On Na, others In *Asterias-Forbesi*	J. Donachy	1987	*Asterias forbesi*

and for each treatment, used eight specimens of *Luidia clathrata*. Since both experiments used the same genus of starfish and tested comparable salinities, the data were reasonably equivalent, and I utilized these sets for my results. However, Kaack and Pomory's (2011) experiment lasted 12 weeks, and Honeycutt's and Pomory's (2019) experiment was conducted over 10 weeks, so I created a second figure comparing the rates (mm of regrowth divided by the amount of time in weeks) of regrowth for each salinity treatment (Figure 2).

Results

Out of all the salinities that Honeycutt and Pomory (2019) tested, they found the 30 ppt treatment to be the most successful, enabling the most arm regeneration over the ten-week period. Salinity <30 ppt experienced a sharp decline in the amount of limb regenerated, and, as stated, none of the animals from the 15 ppt treatment regenerated at all. As salinity was increased above 30 ppt, the amount of limb regrown was also less, but it tapered off more gradually (see Figure 1). Kaack and Pomory's (2011) data also demonstrates that 30 ppt is the most ideal salinity for limb regrowth, regenerating an average of nearly 25 mm of limb over the 12-week period (see Figure 1). For the other treatments (20 and 25 ppt), the length of limb regrown was again less, decreasing sharply near the 20 ppt point.

Statistical analyses were provided by the authors, demonstrating how consistent the data points were. Honeycutt and Pomory (2019) used a one-way ANOVA regression line for their statistical analysis, yielding an F2,18 value of 27.63 and a *p-value* of 0.000003. Kaack and Pomory (2011) also used a one-way ANOVA regression line for their analysis, yielding an F2,21 value of 1.12 and a *p-value* of 0.35.

Discussion

There were slight discrepancies in the two studies. Kaack and Pomory's (2011) data, on average, yielded higher rates of regrowth for all salinities tested. For example, for 30 ppt, Kaack and Pomory's data showed a regrowth rate of 2 mm per week, as opposed to Honeycutt and Pomory's which only yielded 1.58 mm/week. But Kaack and Pomory also tested their specimens over a longer period of time (12 weeks versus of Honeycutt and Pomory's 10 weeks) which could account for an inflated rate. As noted by Honeycutt and Pomory (2019), 30 ppt is (curiously) lower than average oceanic salinity of 35 ppt and higher than the salinity of the bay during collection, 27 ppt. That is quite interesting, since it would be reasonable to suspect that sea stars would either prefer the salinity of their natural environment or the native salinity of the ocean, so there is potentially more to be tested as to why 30 ppt tests as the optimal salinity. Cortes et al. (2014) tested salinity preference using a righting test (how long it takes a starfish to flip itself after being turned upside down) and their findings concur that *Luidia clathrata* are most active in salinities near 30 ppt.

Figure 1. This figure depicts the length of each arm regrown (in mm) by the animal in each treatment, with an error bar showing the standard deviation. Note that Kaack and Pomory tested salinities over a period of 12 weeks, while Honeycutt and Pomory tested over a period of 10 weeks.

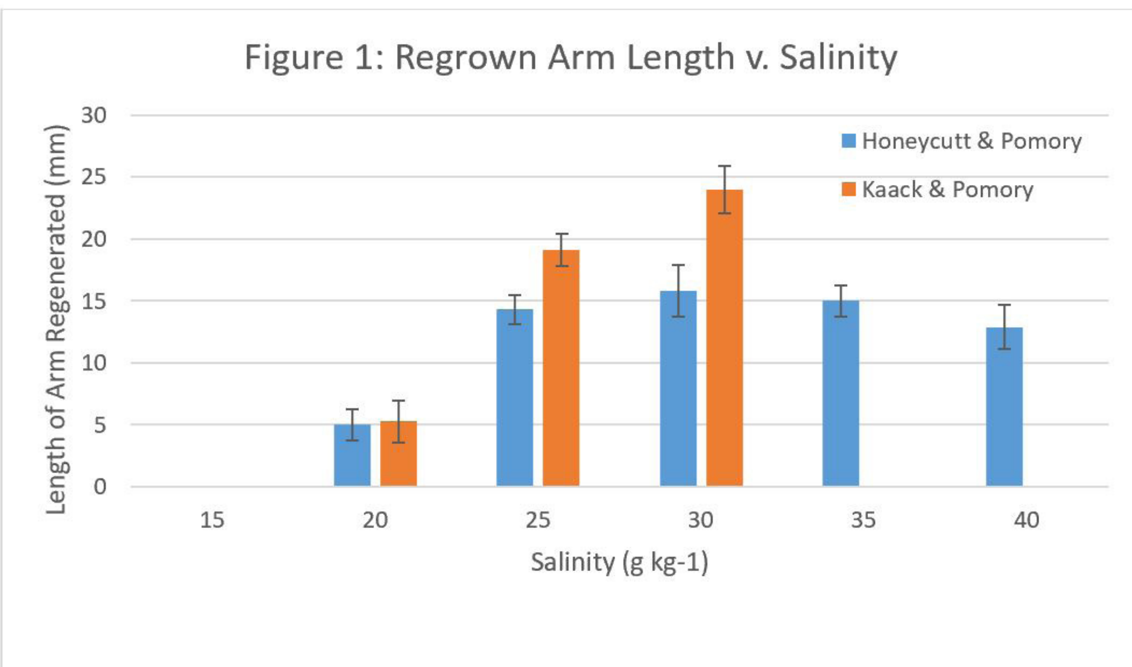
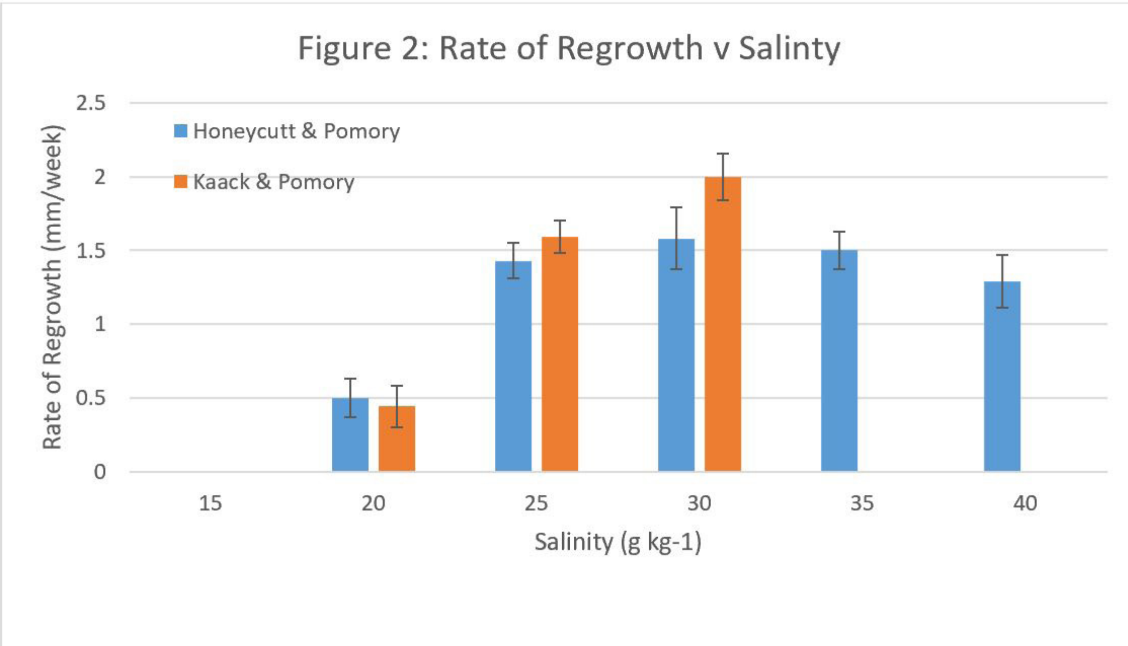


Figure 2. This figure shows the rate of regrowth (in mm per week) per each salinity treatment with an error bar of the standard deviation.



Although not the same as regeneration, this shows that *Luidia clathrata* are most active in similar salinities.

This study connects multiple years of research done at the University of South Florida (Kaack and Pomory 2011; Honeycutt and Pomory 2019), and compares it with other research done in linked fields (Cortes et al. 2014). Looking at all three of these studies, it appears that regeneration is not a separate function of starfish (i.e., not requiring a unique salinity to operate different from that required for regular functioning). However, this finding is not surprising. Other research has been done into the effects of salinity on starfish functioning; Allen et al. (2017) found that decreasing the salinity environment of the Crown-of-Thorns sea star delayed hatching times and reduced fertility in those specimens. Talbot and Lawrence (2002) found that lowering salinity slowed the respiration and regenerative ability in *Ophiophragmus filograneus* (a fellow echinoderm). Therefore , it is not surprising that decreasing salinity slows down limb regrowth in *Luidia clathrata* as well.

It must be noted that this experiment has a number of limitations. My conclusions are confined to *Luidia clathrata*, speaking only for that particular species of starfish, rather than the whole genus. And being a relatively obscure topic, there are almost no data sets relating salinity to arm regeneration in starfish. The ones I did manage to find (Kaack and Pomory 2011; Honeycutt and Pomory 2019) come from the same university, test the same species, and most importantly, their data ranges do not fully overlap. Kaack and Pomory (2011) only tested salinities from 20 to 30 ppt (in 5 ppt increments) and Honeycutt and Pomory (2019) tested a range from 15 to 45 ppt (in 5 ppt increments). Further, Kaack and Pomory (2011) tested regeneration over a 12-week interval, while Honeycutt and Pomory (2019) tested over ten weeks. Dividing the length of the arm by the timeframe can yield a comparable rate, but that is only under the assumption that arms regrow in a linear fashion, which may not be the case, and could affect my analysis. There are plenty of further questions for study. Instead of just testing *Luidia clathrata*, the question could be posed, how does salinity affect regeneration across different species? Do different species of starfish have different relationships with salinity and regeneration? What regression model does regeneration follow? What are the rate-determining factors of regeneration? These questions are the next step in understanding the qualitative attributes of limb regrowth in echinoderms.

As global salinity fluctuations increase with climate change, these keystone predators will become slower and slower, and will not heal as quickly when wounded, impacting the marine ecosystem in ways we have yet to understand.

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