

The Effect of Temperature on the Regenerative Abilities of *Pleurobranchia bachei*

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7/15/2026

1 - Introduction

The sea gooseberry (*Pleurobranchia bachei*) is a common sight at the surface of the waters around Friday Harbor, WA. As comb jellies of the phylum Ctenophora, their small bodies are fragile and vulnerable to exterior damage (van Paridon, 2024). Fortunately, they have also evolved extremely fast regenerative capabilities, with some species even able to fully regenerate up to 75% of their body mass from just a small portion in under 48 hours (van Paridon, 2024). The extent of *P. bachei*'s ability to regenerate is not as well documented, so I wanted to examine how these regenerative abilities are tested against increased stress from environments more extreme than what they're used to. Over the course of several days, I looked at how well *P. bachei* regenerates its tissue while under a drastic increase in ambient water temperature.

2- Methods

Seven individuals of *Pleurobranchia bachei* were collected from the water off of FHL's dock, late in the afternoon with low current, high tide, and sunny weather. Each was given a number from 1-7, put into their own medium sized mason jar and had their jar's contents fully replaced with fresh seawater every 24 hours. 3 jars were placed in the control/cold treatment in one of the lab's seatables, that was kept at around 12 degrees celsius, or roughly the temperature of the seawater they were found in. The other 4 were placed into the "warm" treatment; an ice chest full of freshwater hooked up to a heater chiller that kept the temperature of the ice chest at 17 degrees celsius. A jar full of saltwater was also kept in the ice chest to be used for the warm treatments' water changes, so that new water that was added would be the same temperature. For both treatments, the water level of the treatment environments went up about 3/5ths of the jars' heights.

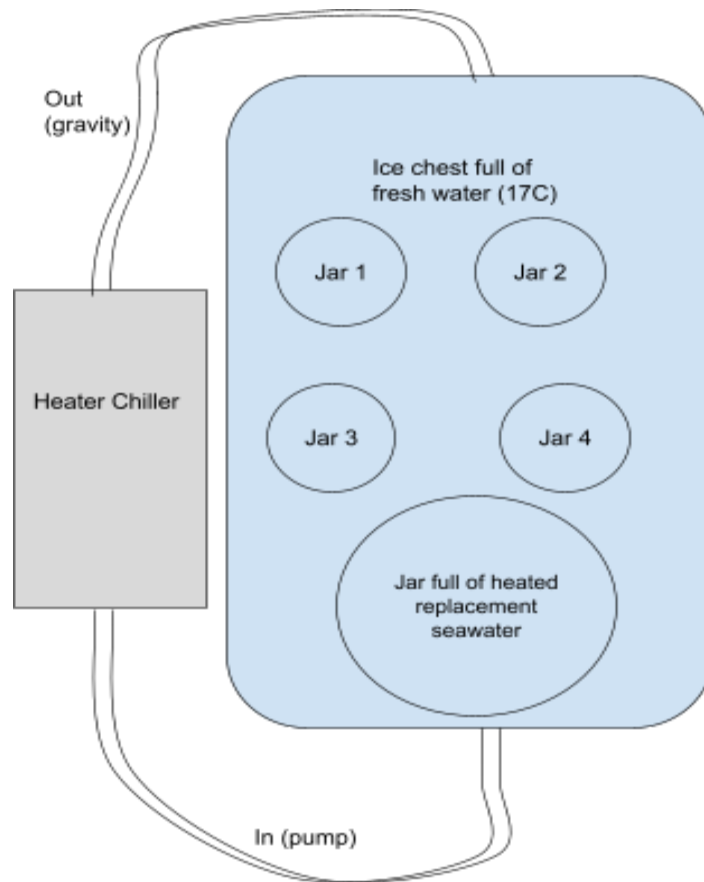


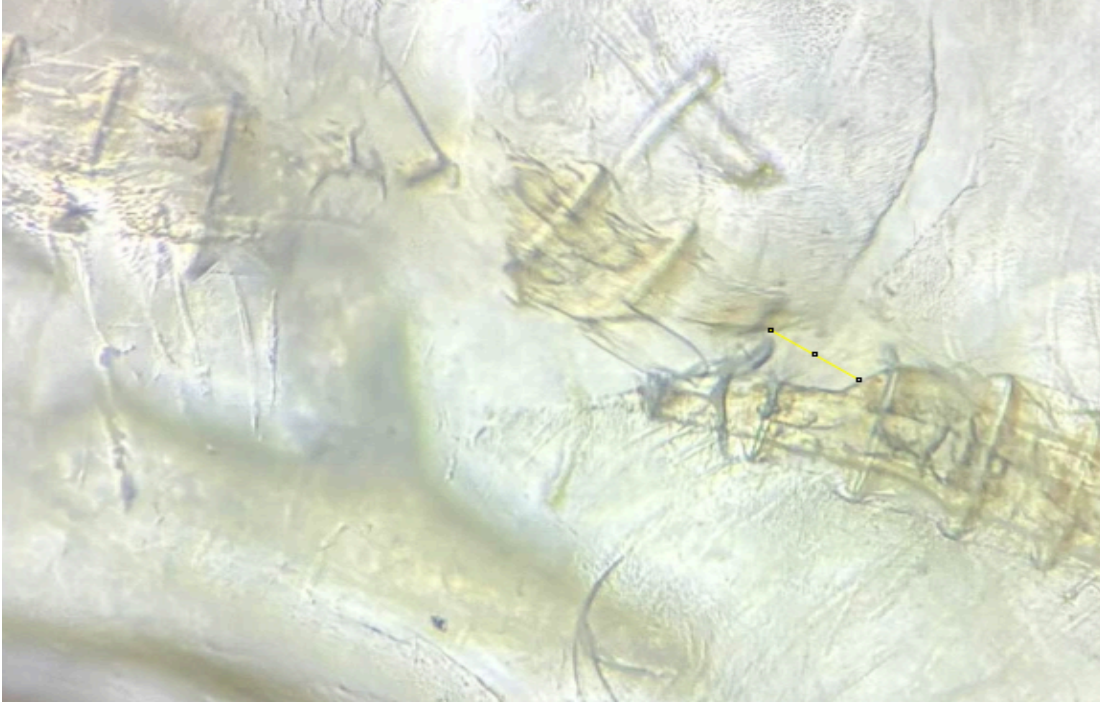
Diagram of the equipment used for the warm water treatment. The 5 jars were partly submerged in the warmed freshwater brought up by the heater chiller. Due to the heater chiller's inflow pump being considerably faster than the outflow pump, the outflow pump could not be used to make a system with a steady water line. To remedy this, a hole was drilled in the side of the tank with a tube attached so the water would flow down to the heater chiller by way of gravity instead.

First, each jelly was left alone in their jars for 24 hours to acclimate to their treatment's temperature. After that, the contents of each individual's jars were spilled out into a medium sized tank, and the individual fished out by using a fingerbowl to scoop up from underneath it (I found this to be much easier than trying to fish it out of the jar by hand). Once in a fingerbowl and under a dissection scope, "before" pictures were taken of each individual, before soft forceps and cutting implements were used to carefully attempt to cut out a portion of each individual's ctenes (combs). Photos were taken of each individual after an incision was made and sorted under "0hrs." For the next 24, 48, and 72 hours after this was done, each individual was extracted from their jar and had their incisions photographed and sorted under the correct timeframe.

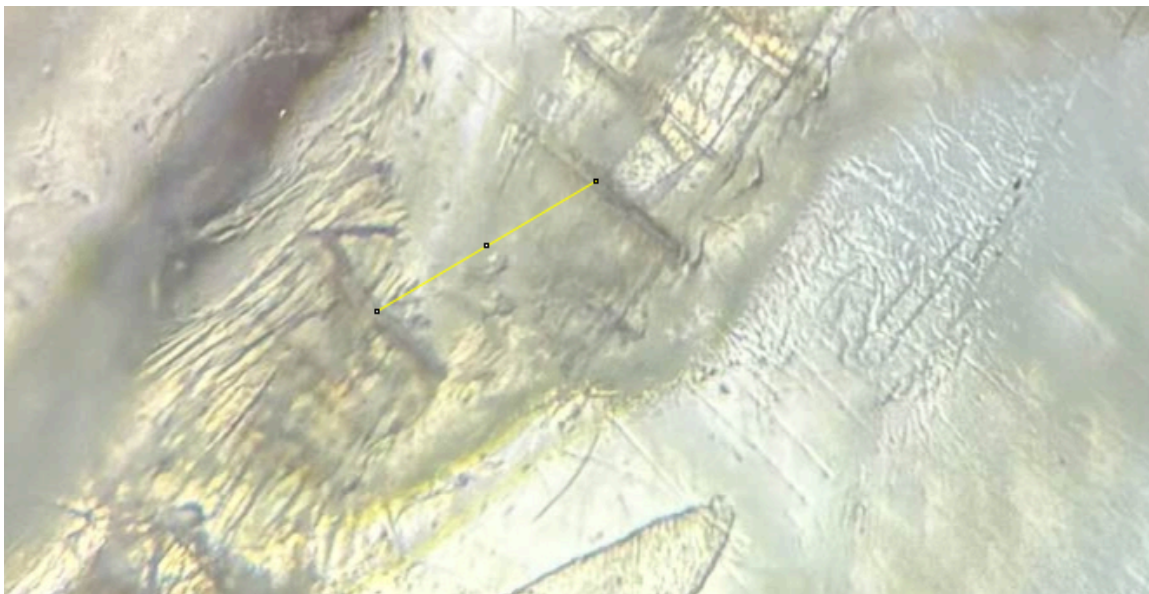
Once 4 photos had been taken of all 7 individuals, each photo was loaded into ImageJ for the closure progress of the wounds to be measured. In the absence of a consistent magnification level or unit of measurement across photos, the gaps between individual cilia on a specimen's ctenes were used to standardize a ratio of pixels to units for each individual photo, seeing as such gaps were, for all intents and purposes, taking up the same fraction of space of each individual's surface area. Using this, the distance between two healthy ctenes was measured across each individual's wounds and recorded as that day's current wound size. After all data was collected, measurements were analyzed and processed into percentages of how much of the original wound had been healed after each day.



ImageJ's line tool being used to measure the gap between cilia. Using the "set scale" option under "Analyze," the program was able to turn this length of pixels into a set ratio of pixels to real-world units so photos could be compared against one another regardless of magnification. Due to the healing process of ctenes causing them to stretch out, using the most "normal" and in-focus gap in cilia visible (such as the one pictured) was important to keep some degree of consistent standard across photos measurements.



In cases where there was accidentally an “island” made between two separate incisions, the size of the wounds on either side of the “island” were measured and added together to be that day’s wound closure progress. Fortunately, these worked out to be quite comparable in total size to the standard wound sizes.



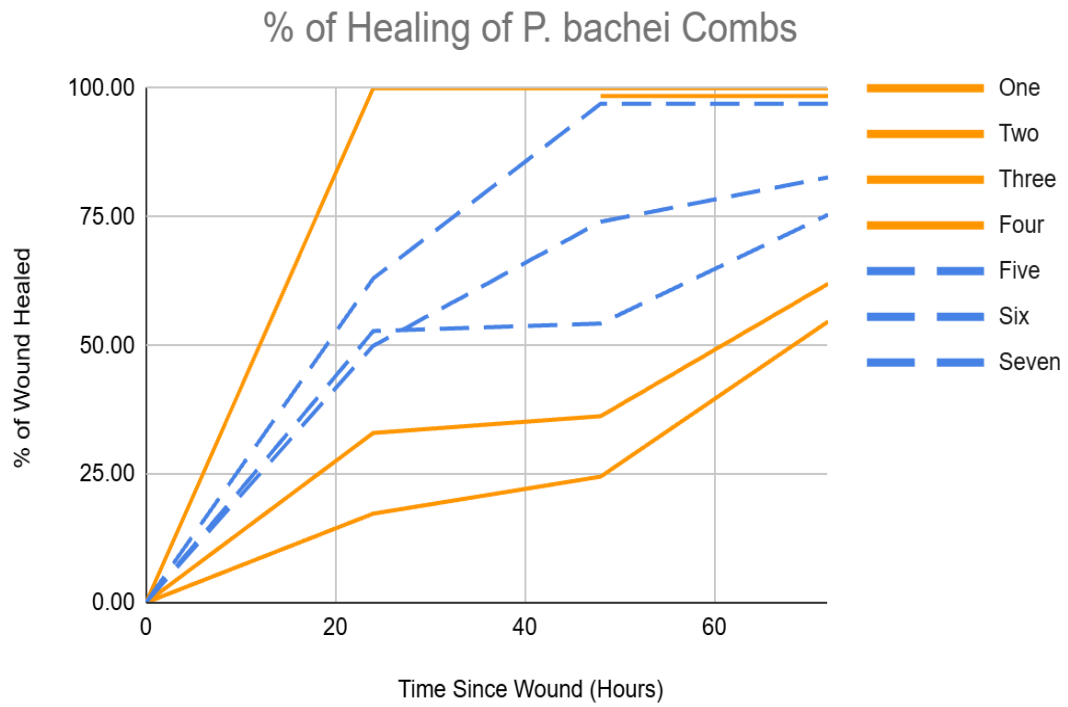
As the healthy ctenes on either side of the wound attempt to close the gap and meet each other in the middle, it’s rare that the angle of the two will allow them to intersect perfectly dead-on with one another. In these situations, the wound measurement was made from the midpoint of each ctene, and the healed wound would resemble a jagged lightning bolt.

Results

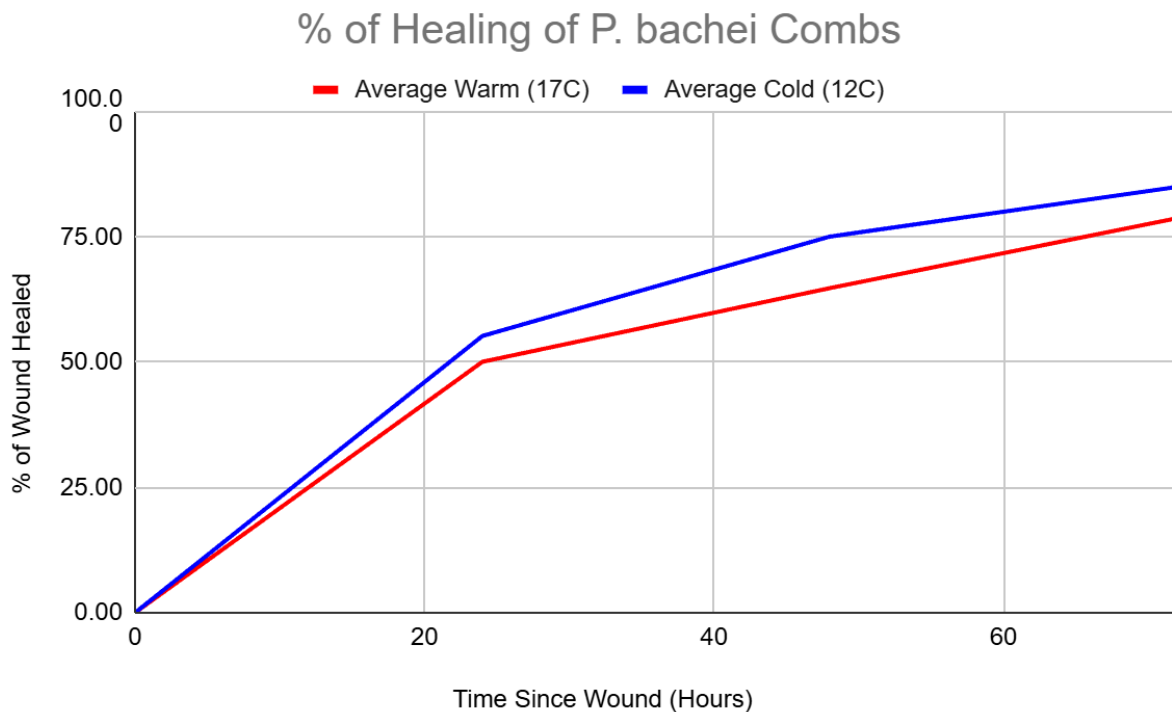
This table shows how much of the original wound had healed across all seven individuals and all 3 periods after the incision was made. When a wound shows '100.00' it means that at that time the wound had fully sealed shut. The average regeneration progress that day for each treatment is also displayed. The photo that would be used for the third specimen's 24 hour mark was missing and thus could not be counted.

Hours:	One	Two	Three	Four	Average Warm	Five	Six	Seven	Average Cold
24	100.00	17.32	Missing	32.99	50.10	63.04	49.95	52.81	55.26
48	100.00	24.50	100.00	36.25	64.81	100.00	74.06	54.27	75.11
72	100.00	54.65	100.00	61.99	78.79	100.00	82.69	75.41	85.03

Over the course of the experiment, all individuals exhibited a consistently positive wound regeneration rate, though some individuals healed faster than others, and some healed more in a day than others. On average, the cold treatment (12C) healed faster and was able to heal more in the experiment's time frame than the warm treatment (17C). Though notably, the first and third specimens, as a part of the warm treatment, were able to regenerate just as fast or faster than the fastest regenerating member of the cold treatment, with the former completely healing its wound in under 24 hours.



This graph shows how fast each specimen was able to regenerate the wound made to its ctenes. The warm treatments are in solid orange, and the cold treatments are the dashed lines in blue. Some of the warm treatment healed more, faster than the cold treatment, but the rest of the warm treatment performed measurably worse than the cold treatment.



Graphed comparison of the averages of the two treatments' wound closure progress. Both were fairly close throughout, though the cold treatment consistently healed more than the warm treatment did. Notably, while both treatments seemed to slow down over time, the warm treatment slowed down faster than the cold treatment.

Conclusion

Comb jellies are made from three distinct layers of tissue, an outer epidermal layer and inner gastrodermal layer with a loose filling of cellular material between them known as the mesoglea (Martindale and Henry, 1996). When a comb jelly sustains damage to its exterior, the local epithelial and mesoglea tissue move in to fill in and seal the wound site. To heal the wound itself, these local cells proliferate out and differentiate into what is needed to repair the organism to working order (Henry and Martindale, 2000), and we can see this in the way the ctenes stretch themselves out to race to fix the wound site.

It's likely that the effects of increased heat specifically affected the cellular material of the mesoglea's ability to patch up the wounds. The mesoglea is made up of proteins, enzymes, and dense collagen fibers (Tamm, 2012), which could have experienced some level of denaturation in the increased heat, greatly reducing wound regeneration speed in some individuals. Other species such as *Mnemiopsis leidyi* have been shown to survive in a remarkable range of temperatures from 2 to 32 degrees Celsius, with lower temperatures and salinities

limiting their survivability instead of higher ones (Purcell et al., 2001). A similar level of adaptability in *Pleurobrachia* could explain why the gap in healing rate between the two treatments was so marginal despite the large difference in temperature. As climate change continues to heat up the world's oceans, it will likely take a long time before ctenophores like *Pleurobrachia* are majorly affected by the increase in temperature.

For this study, some limitations were how far apart data points were spaced, the cold water treatment not being as stable around its target temperature, the misplaced photo of jelly #3, and the parasite on #2 that all could have contributed to making the data less reliable or not as granular as I would have liked. Future studies could compare different species together, examine the salinity tolerance of *P. bachei*, compare its resilience under higher and lower temperatures, and/or look at how they regenerate from different (i.e. tentacles) or more severe injuries more frequently or over longer periods of time.

Acknowledgements

I'd like to thank Willem Weertman for being an invaluable help with the design, construction, and set up of the equipment used for the warm water treatment, I would not have gotten very far at all without his help. I'd also like to thank my professors Dr. Rebecca Varney and Dr. Will Ballentine for helping me with the idea for my project and encouraging me to pursue it, because I probably wouldn't have had the confidence to go through with it otherwise.

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