Effect of temperature on growth and survival of *Nanomia bijuga* (Cnidaria; Hydrozoa, Siphonophora)

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ABSTRACT

The common siphonophore, *Nanomia bijuga,* is a complex colonial organism found in the midwater habitat and is important to the oceanic food web. Since 2022 the Monterey Bay Aquarium (MBA) has successfully raised multiple generations of *N. bijuga.* The MBA collaborated with the Monterey Bay Aquarium Research Institute (MBARI) to open ***Into the Deep/En lo profundo*****,** an exhibition highlighting the deep sea that includes live animals from the Monterey Canyon. Since the availability of *N. bijuga* is inconsistent due to limited ship time for collections, the MBA wants to cultivate them to display all year around. To achieve this, we want to determine the optimal conditions for raising *N. bijuga*. We will focus on how temperature affects the growth and survival of *N. bijuga* juveniles by raising them at two different temperatures. To analyze the survival, we will count the number of siphonophores present in each tube. In addition, photos will be taken under a microscope and analyzed using the application ImageJ. We will measure the length and number of the nectophores and count the number of tentacles present to observe the growth over a period of time. After collecting the data, we will use a t-test to compare the data at each temperature. From the results we hope to improve the cultivation of *N. bijuga* by finding

the best temperature to raise them and provide animals for the deep-sea exhibition at the MBA.

INTRODUCTION

Since spring 2022, the Monterey Bay Aquarium (MBA) has an ongoing exhibition: *Into the Deep*, that displays live animals from the deep waters around the world. They also work closely with the Monterey Bay Aquarium Research Institute (MBARI), which is a nonprofit oceanographic research center with the mission of advancing marine science and engineering to understand the changing ocean. Their research has made it possible to learn more about the species that inhabit the water column, midwater, and the deep ocean. In addition, MBARI is the MBA's research and technology partner, and MBA is MBARI education and conservation partner. Together they create a great impact on the community and contribute to marine science innovation. In addition, one of their missions is to inspire people to conserve the ocean and continue the education from one generation to the other. This *Into the Deep* exhibition cost approximately $15 million to build and gives visitors a journey into the mysterious depths of the ocean. Exhibits highlight the unique ecology and adaptations experienced by animals living in the deep sea. This includes vertical migration, a daily migration in which some deep-sea animals travel to the surface at night for food. Additionally, the exhibit highlights bioluminescence, a process in which light is produced through a chemical reaction. About 2 million visitors per year stroll through the corridors of each exhibit. The exhibit shows them the wonders of the ocean and inspires them to conserve and protect the ocean. This exhibit provides the opportunity for visitors to observe what is impossible to see without the support of advanced marine science technology like remotely operated vehicles (ROVs). Into the Deep is the largest exhibition of ocean life in North America at approximately 10,000-square-feet and took five years of development. The exhibit possesses a complex life support system; It is equipped with sand filters, ultraviolet light filter, and a temperature of 11°C to keep water in exhibits cold and the oxygen levels low. It features a total of 8 tanks displaying midwater deep sea

animals. An exhibition as complex as this one is supported by a team of 8 aquarists and 1 curator to take care of the exhibit.

The Midwater is the part of the ocean below the surface and above the seafloor (Drazen, J. C. et al., (2020). This mesopelagic zone extends from 200 meters (660 feet) to 1,000 meters (3,300 feet). At these depths sunlight disappears and the magic of the organisms that inhabit it is ignited (Robison, 2009). Colors disappear and the temperature increases deeper down (below 5º Celsius). As does the pressure which at 2,000 meters equals about 200 times the atmospheric pressure at sea level. A significant part of the animals that inhabit this ecosystem are tiny, an example of which might be the microscopic crustaceans: copepods, amphipods, worms, sea butterflies, and more unusual species (Smithsonian institute). This environment is considered one of the most difficult in which organisms might coexist. However, each one of them has developed the ability to adapt to these harsh conditions. In the exhibition, visitors view the wonders of the deep in each of the tanks, which reveal secrets and unravel mysteries that lay hidden in the depths of the ocean.

The common siphonophore *Nanomia bijuga* is a pelagic hydrozoan (Cnidarian) with a complex morphology (Genzano *et al.,*2014). *N. bijuga* is an important component of the oceanic food web as a major predator in the ocean and one of the most common physonect siphonophores off the west coast of the United States. *N. bijuga* is hermaphroditic and has both sexual and asexual reproduction. It is a predator and is a complex colonial organism composed of morphologically distinct zooids with functional specializations working together (Church *et al*., 2015). *N. bijuga* is found between 200 to 400 meters above the surface. The pneumatophore is a tissue filled with carbon monoxide that supports the floatability and the nectophore is a zooid specialized for swimming. The gastrozooid is specialized for feeding and ingests food. The palpon is derived from gastrozooids and contains digestive functions. Male gonodendron contains male gametes and is the site of spermatogenesis. Female gonophores contain the female gametes and are the site of oogenesis (Church. *et al*., 2015).

Much of the biology of this species remains unknown. A literature review has revealed that *N. bijuga* has a global distribution. However, more research is needed to determine if the global population is only one species or more than one. To examine the life history, research and experiments have been carried out to achieve the cultivation of this species in the laboratory (Sherlock and Robison, 2000). In this way we can make observations and learn a little more about its life cycle. To avoid the cost and uncertainty associated with live collections, the Monterey Bay Aquarium would like to be able to cultivate *N. bijuga* through several generations providing a constant source of animals that can be placed into the exhibition. However, MBA has been able to successfully raise *N. bijuga* to the 2nd generation within the last few years. Therefore, MBA would like to work on increasing their success at raising the young. This research project is designed to determine the optimal temperature for raising the common siphonophore, *N. bijuga*, in the laboratory. Specifically, this research project will look at the growth and survival of *N. bijuga* at different temperatures.

MATERIALS AND METHODS

Collection:

The collection of *N. bijuga* was held on May 31, 2023, and was performed on the research vessel (RV) *Rachel Carson* using a remotely operated vehicle (ROV) *Ventana* and the suction sampler and carousel of collection jars on the ROV. Upon returning to the aquarium, the specimens were placed in a diffusion tube overnight to spawn at a temperature of 16 °C. Spawning occurs naturally in the diffusion tubes and planulae will develop and become visible within a couple days.

Experimental design:

However, before being transferred to the diffusion tube, it was acclimatized to avoid a significant change in temperature shock. There will be two temperatures (13°C and 16 °C) with 3 replicate diffusion tubes for each temperature. Based on the past experiences of MBA, the *N. bijuga* are fragile and get damaged in traditional jelly kreisels. The diffusion tubes are a tube within a tube. The inner tube has mesh on the bottom to allow

the water through but keep the *N. bijuga* inside. The inner tube sits within the outer tube on a riser. A piece of tubing sits between both tubes and supplies water. These tubes are placed on a wet table with support of recirculating filtered seawater (Fig.3 A & B). The tubes provide a passive flow for water turnover but keep the *N. bijuga* intact. Each tube was fed 7 days a week, with different concentrations of live animals (Patry *et al*., 2020). Starting with 25 mL *Americamysis bahia* (Aquatic Indicators St. Augustine, FL, USA), every day, 25 mL of *Acartia tonsa* (Algagen, LLC Sustainable Mass Production of Plankton, Vero Beach, FL, USA), Monday, Wednesday, Friday, and Saturday and *Parvocalanus crassirostris* (Reed Mariculture Inc., Campbell, CA, USA) Sunday, Tuesday, and Thursday.

Data collection:

In each cylindrical tube were placed 3 juvenile *N. bijuga* (a total of 18 individuals in the 3 replicates of both temperatures (Fig.3 A & B, every three days a week the survival in both temperatures was verified. In addition, each tube was observed through the acrylic, the tube was illuminated with a flashlight and an individual was randomly selected to count the number of nectophores present on the body. However, this counting process must be done with particular caution and as quickly as possible since light disturbs the *N. bijuga*. These midwater species do not like light as a whole the water could become hot if too much light is thrown on them, and the animal will be stressed. Based on observations and past experience the species under stress begins to swim in random directions causing it to attach to the edges, fall to the bottom or expel parts of their bodies (nectophores).

ANALYSIS:

To analyze the survival of *N. bijuga*, the number of siphonophores present in each tube will be counted the animals inside. Then the individuals will be selected from each tube 2 or 3 times per weekend and count the number of nectophores present in the three *N. bijuga* in each tube. After collecting the data, we will use a t-test, to compare the means of results at each temperature, compare the growth of the *N. bijuga* at two different temperatures and compare the number of nectophores at two different temperatures.

RESULTS

The number of overall survivors was 13 individuals out of a total of 18 individuals. The number of survivors totaled 6 for the replicates at 13℃ and 7 survivors for 16 °C. The mortality rate at both temperatures was not significantly different. We found in 16 °C that the mean number of nectophores was 6.68 (fig.4) and the mean for 13℃ was 5.61. After obtaining these results we conducted a t-test to compare the means of both groups. The t-test showed a p-value of 0.00936, which indicates that there was a significant difference between the means of groups 13℃ and 16 °C. However, based on personal observation, our animals at 16 °C showed some developmental differences in body morphology.

During our observations we noticed that animals developing in warm water had a more compact body. Unlike animals developing in cold water, which had a small space between growing nectophores. In addition, the animals in warm water seem more resistant to transfer from one tank to another. However, these observations opened us to think about other possibilities that could significantly affect their survival. Does growing fast mean that they developed well, or did they just grow morphologically? Did the animals in cold water grow slower but develop better? These are some of the many questions that arose as we observed their development over the weeks.

DISCUSSION

Experimental attempt one:

In the first stage of our research, new *N. bijuga* were collected in order to spawn them and use their larvae. They were spawned on July 4, 2023, at a temperature of 16 °C and 12 planulae (larvae) were moved to each of the six tubes (three for each of the two temperatures) for a total of 72 planulae. Before placing them in the tubes, they were acclimatized in a water bath to avoid the drastic change in temperature. However,

multiple factors did not allow their development and the vast majority. Based on the observations and results, we concluded that moving them too fast affected them because they may have been too weak to withstand the change in temperature. This could have stopped their growth and development in both temperatures. Initially, we sought to be able to track the growth and development of *N. bijuga* by taking photographs under the microscope (Zeiss stereo Zoom V16, Canon EOS Rebel t5i) two to three times per week. Each photo would be analyzed and measured using the ImageJ application (Patry *et al*., 2020). The length of tentacles, nectosome and the number of nectophores (swimming bells) present in each animal randomly selected from each tube of both temperatures would be measured.

Experimental attempt two:

Taking into consideration the first attempt of our experiment, we collected six adult animals on the research vessel (RV) *Rachel Carson* on July 12, 2023. We used the suction sampler on the remotely operated vehicle (ROV) Ventana and the carousel of collection jars on the ROV. The *N. bijuga* were transferred into the tubes on the same day to spawn them. However, even in this way no reproduction was observed by the animals inside the tubes, so we were unsuccessful. This shows us once again how unpredictable spawning can be for this species and how complex its life cycle can be.

Experimental attempt three (final):

Once we restarted our experiment, we chose to use juvenile N. *bijuga* from a successful spawning of adults that were collected on May 31, 2023. We transferred them to the experimental tubes on July 14, 2023. Since we wanted to analyze how temperatures affect their growth and development, we hoped to start from the larval stage. However, there was not enough time for more spawning attempts. We continued to analyze growth and development but later in the life cycle, so we did not show the complete development from the very beginning. Every other day we counted the survivors in each tube. In addition, we observed their development and the number of nectophores present in each

animal. The process of counting the nectophores in the diffusion tubes is a complicated one since we are counting gelatinous species in transparent tubes apart from the water flow. Based on these observations made during three weeks, it was possible to take note of the development of each animal inside each tube. The mortality rate at both temperatures was not significantly different. However, their development did, our animals within the three replicates with a temperature of 16 degrees Celsius showed a significant difference in the development of their bodies. This is strange because the *N. bijuga we* collect on MBARI (RV) Rachel Carson live at temperatures less than 10°C.

It is possible that this could be supported by vertical migration or upwelling, which brings cold water for the deep sea to the surface and provides nutrients that could favor the growth of the species. Which brings up the question: how does it reproduce in warm water and live in cold water? There are multiple factors that could support why and too many that we are not aware of at this time. The ocean currents bring animals from offshore to the coast, so animals may experience changes in temperature as the current moves them. The coastal upwelling occurs along the entire west coast of the United States and Baja California (Huyer, 1983). These animals might be born in warm waters and the currents bring them to the coast of Monterey Bay where the waters are cooler. These animals are planktonic and perform a daily vertical migration, which means that these animals may be able to withstand the change in temperatures. They must have the ability to adapt, but it is unknown exactly how much of a temperature change they can tolerate.

We recommend conducting more research involving these organisms. Next time we will need more than 3 weeks to observe and collect data. In addition, we would like to start earlier in their life cycle, so that we can document how different temperatures affect their development. It would be ideal to conduct this experiment at different temperatures to see how warm water affects the species. More replicates are needed to increase statistical significance of the data. Also, put one animal per tube to collect data for each animal because we had to take the average of three animals.

The ocean is a critical component of the Earth and through the decades has undergone a significant change in this ecosystem which has forced many species to adapt or cause their total or partial extinction. As the ocean warms, it would be good to know how this increasing temperature might impact the species that live in the deep sea. Culturing *N. bijuga* will provide animals for the deep-sea exhibition at the Monterey Bay Aquarium. Bringing this exhibit helps to accomplish one of the missions of the Aquarium and MBARI which is to inspire people to conserve the ocean. As we educate the community, they will understand the vitality behind the animals that are difficult to see in the wild without an ROV.

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Tables and Figures

Figure 1. Assembly instruction for diffusion tubes. Source: (Patry *et al*., 2020).

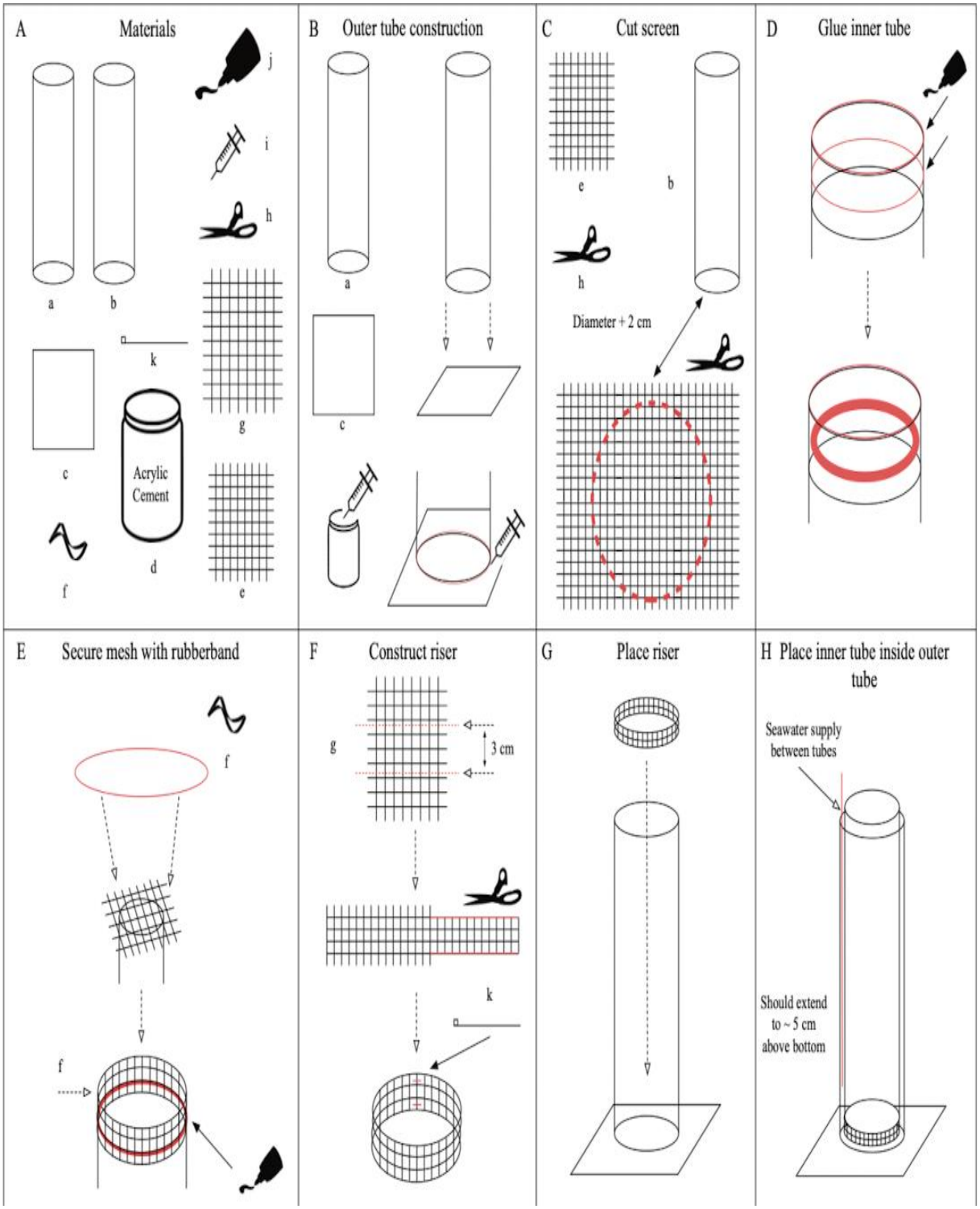


Figure 2. Schematic of *Nanomia bijuga* colony. Source: http://commons.wikimedia.org/wiki/File:Nanomia_bijuga_whole_animal_and_growth_zones.svg, by Freya Goetz.

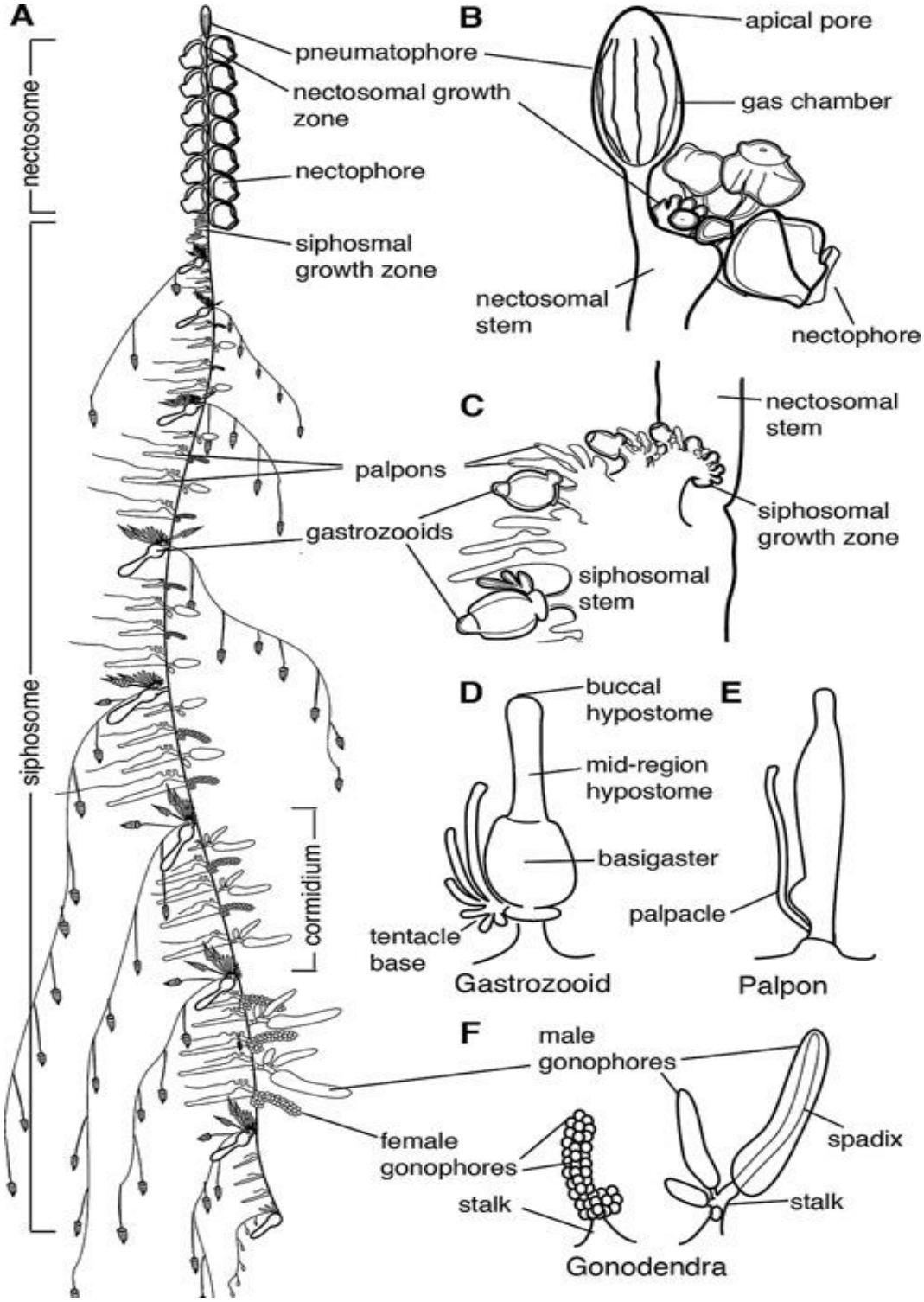
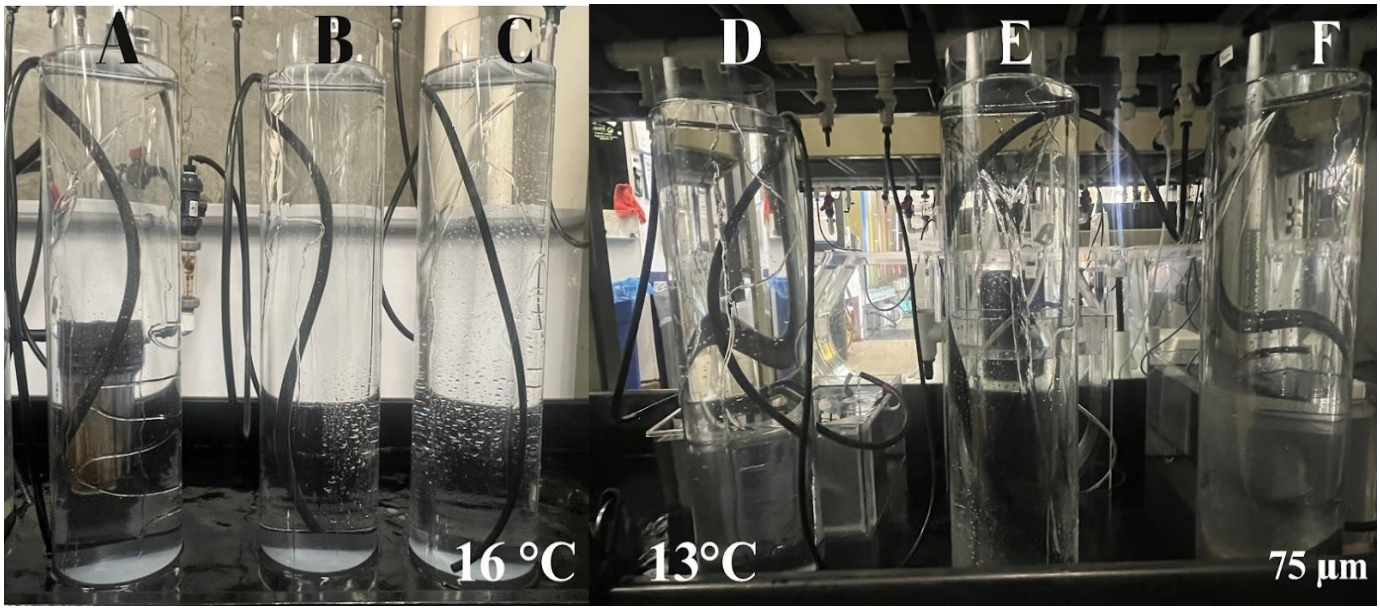


Figure 3. Experimental diffusion tubes.

(A) Tubes setting in Drifters Lab. Design: (Patry *et al*., 2020).



(B) References drawing of the tubes setting. Design: Audrey Sauble.

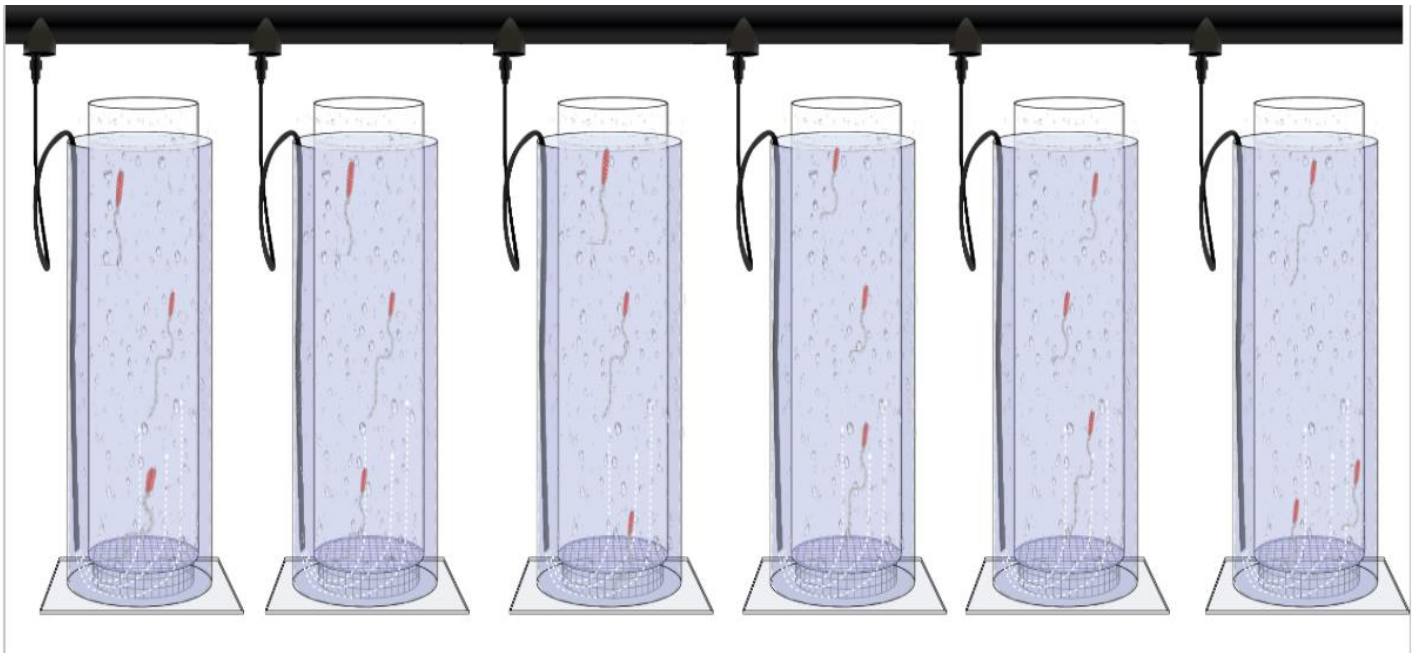


Figure 4. Box plot: In this analysis we obtained a mean of 5.61 in group 13℃ represented in blue and the mean for group 16 °C represented in yellow is 6.82. These results show the average number of nectophores at the end of our experiment for each temperature. and then we conduct a t-test in the R studio that shows there is a true difference between group 13℃ and 16 °C because the p- value is less than 0.

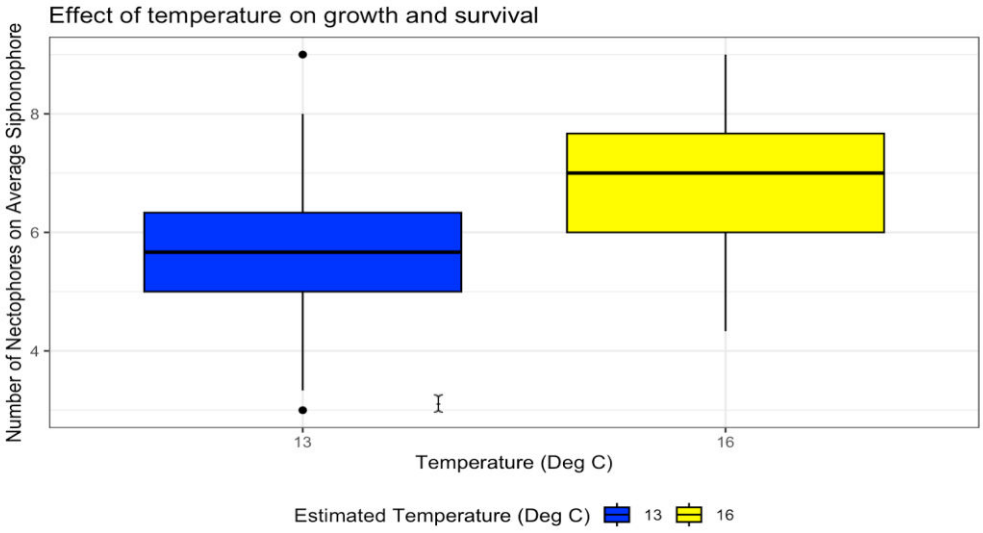


Figure 5. Average of number of nectophores during the study. The dashed line represents the mean in group 13°C represented in blue and the mean for group 16 °C represented in yellow. The choppy area represents the margin of error between between group 13°C and 16 °C.