Effects of thermal stress on the larval growth of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857)

Gara María Hernández Guerra

Course 2018/2019

Tutor: May Gómez Cabrera

Cotutor: Eugenio De Jesús Carpizo Ituarte

Research work to obtain Marine Science Degree

Effects of thermal stress on the larval growth of the purple sea urchin, *Strongylocentrotus Purpuratus* (Stimpson, 1857)

Student: Gara María Hernández Guerra

DNI: 78590941-X

Degree: Marine Sciences Degree

Tutor: Dr May Gómez Cabrera
Biology Department at Universidad de Las Palmas de Gran Canaria (ULPGC)

Cotutor: Dr. Eugenio de Jesús Carpizo Ituarte
Zoology Department at Instituto de Investigaciones Oceanológicas (IIO). Universidad Autónoma de Baja California.

Las Palms de Gran Canaria, 15 July, 2019

Abstract

The present study is focused in the study of the embryonic and larval development of purple sea urchin, Strongylocentrotus purpuratus, when are developed under stress at 3 different temperatures (19ºC, 22ºC and 25ºC), taking into account the parameters of dissolved oxygen and pH, in order to simulate the limits they can suffer in the environment due to the increase in seawater temperature . Using the calculation of the length and daily monitoring of the purple sea urchin larvae culture in the different temperature treatments, it was demonstrated that at 25ºC they were able to resist the fertilization process, but they did not survive the development. In the other hand at low temperatures had apparent positive effects on their development, the larvae length is greater and their morphology more regular in the treatment at 19ºC that at 22ºC.

1.- Introduction

Anthropogenic global warming results in an increase in the global average temperature, both in terrestrial and marine environments (González-Lozano, 2010). It represents one of the most serious threats to biodiversity since the emission of greenhouse gases and other human activities are causing a rapid change in the Earth's climatic system, which today are noticing the ecological effects of climate change (Gooding, et al. 2009).

In marine environments, the global average temperature of the sea water surface has increased 0.76 º C in the last 150 years and it is anticipated that, at the end of this century, it will increase between 1 and 4 º C. (Gooding, et al. 2009) (IPCC,2013). The biological importance of this factor varies among species, causing direct effects on the performance of individuals during their life cycle, through changes in physiology, morphology and behavior and even in the survival of marine organisms as they live, many of them, close to their thermal tolerances (Harley et al.,2006).

Global warming can have serious effects on the population of the sea urchin, an indicator organism used in research since by subjecting them to temperature changes within their habitat, they quickly show signs of stress. And it has been demonstrated, with exhaustive studies, that increasing this parameter decreases the appearance of embryos during fertilization (Byrne et al., 2009). Optimum temperature for fertilization and subsequent embryonic and larval development varies depending on the species of equinoidea, its habitat and breeding season. It is also related to the thermal tolerance limit that the organism has. (Rahman, 2014; Hammond, 2010; Fujisawa,1989).

2.- Justification and objectives

The temperature plays a fundamental role in the embryonic and larval development of the purple sea urchin, as well as in the ecosystem distribution, being able to tolerate wide temperature ranges, however if extreme variations occur, it can cause physiological problems in the species.

The objective of this TFT is to show how thermal stress, affects the embryonic and larval development of the purple sea urchin *Strongylocentrotus purpuratus*.

3.- Description of the species

Within the group of marine invertebrates, the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson, 1857), is a regular echinoderm that presents the following taxonomic unit (Workman,1999):

Kingdom: Animalia

Phylum: Echinodermata

Class: Echinoidea

Order: Echinoida

Genus: *Strongylocentrotus*

Species: *Strongylocentrotus purpuratus*

Morphologically, its body has a spherical shape, characterized by having a rigid shell and their locomotion is carried out by the presence of mobile spines (calcareous structures) and tube feet. Tube feet are involved in breathing, quimiorreception and locomotion. As for its digestive system, it is short, straight and radial; formed by the mouth (oral part), a small esophagus that communicates with a large stomach and a short intestine that goes from the stomach to the anus (aboral part) is a complex chewing device formed by 5 teeth called Aristotle's Lantern, which most regular sea urchins depend on to feed (Gil et al., 2014; Marshall and Williams, 1985) **(Figure 1**).

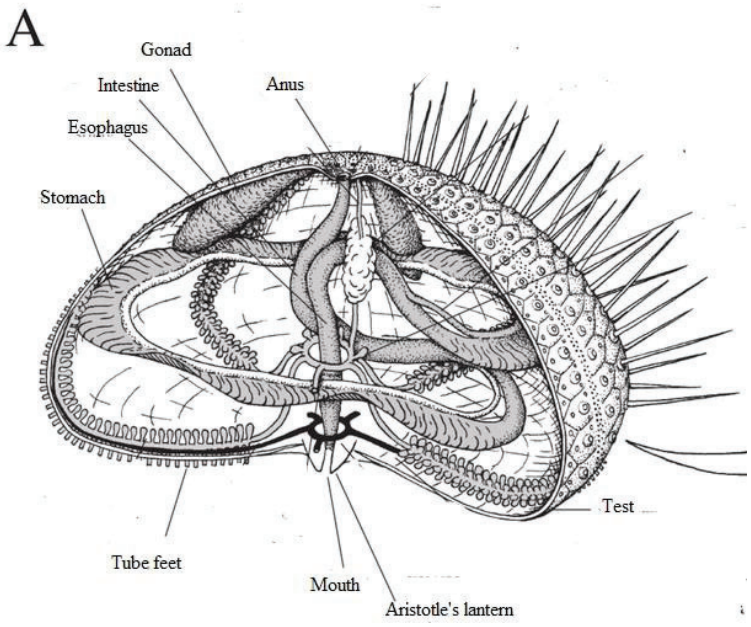

***Figure 1**. Internal anatomy of the sea urchin (Hickman et al. 2001).*

3.1.- Reproduction and larval development

With respect to its reproduction, *S. purpuratus* is a dioecious species that reaches its sexual maturity with an approximate size of 2.5 cm of the test diameter (Morris et al., 1980). Fertilization is external, so the eggs and sperm are released to the seawater and occurs between the months of September to July (Tegner, 2001).

Once the oocyte has been fertilized, embryonic development is produced from zygote to equinoderm larvae or equinoplutei (Mottet, 1976). The segmentation of the zygote originates a certain number of blastomeres that continues with a cellular displacement within the embryo and ends with its growth. The blastulae differ by presenting a central cavity limited by a layer of epithelial cells, and the gastrulae is characterized by the presence of the archenteron, embryonic cavity that is formed in the process of gastrulation and prefigures the future digestive tract (Olaechea, 2006). In the equinopluteus stage of 2 arms the intestine is made up of three parts: esophagus, stomach and intestine, but it is not functional. The muscles of the esophagus contract, the stomach increases. Subsequently, the pluteus larva of 4 arms is formed, with two arms postorales well-developed and with the complet digestive tract (Rahman et al.,2012) (**Figure 2**).

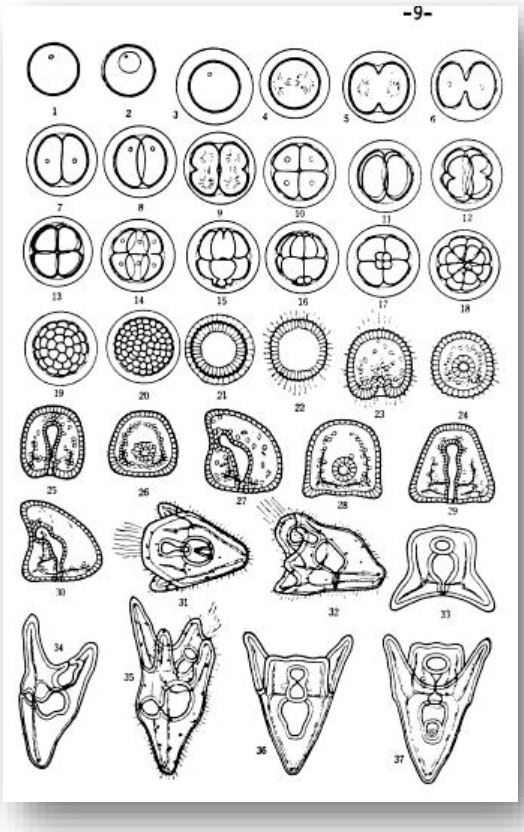

Figure 2. *Embryonic and larval development of the purple sea urchin Strongylocentrotus purpuratus (Workman, 1999)*

3.2.-Distribution and habitat

The purple sea urchin is common in the intertidal and subtidal zones due to its resistance to physical-chemical changes and the wave action, reaching 160 m depth (Morris et al. 1980). In some regions, they inhabit two communities, the coastal rocky reefs (caves and clefts), where they predominated but with low species diversity; and the kelp forest of *Macrocystis pyrifera* where the provide stability and structure to the community because in this case they have a high diversity of species, (Rogers-Bennett, 2007) and this is the most consumed food in adult stage for purple sea urchin (Ramirez-Félix, E. 2000).

They are distributed geographically in the temperate waters of the Pacific Ocean, from Cook Inlet, Alaska, USA, to the island of Cedros in Baja California, Mexico (Ebert, 1968) (**Figure 3a)**). They are able to tolerate a wide range of temperatures ranging from 4 º C in Alaska during winter and in southern California to 20ºC in summer. (Osovitz and Hofmann, 2005). If they are given time to acclimate, they survive at minimum temperatures of 2ºC and maximum of 23 º C, being the thermal limit at 25 º C, where they die within 24 hours. On the other hand, the eggs will be developed at temperatures between 13 and 20 º C, but they will have the capacity to tolerate the temporary cooling

at 5ºC and will continue to develop once the temperature is normal. However, the purple sea urchin is less tolerant to low levels of dissolved oxygen (Farmanfarmaian, 1963). In the study area, of the coast of Ensenada, Baja California, the average annual surface temperature is between 16 and 20 º C (Díaz-Pérez and Carpizo-Ituarte, 2011).

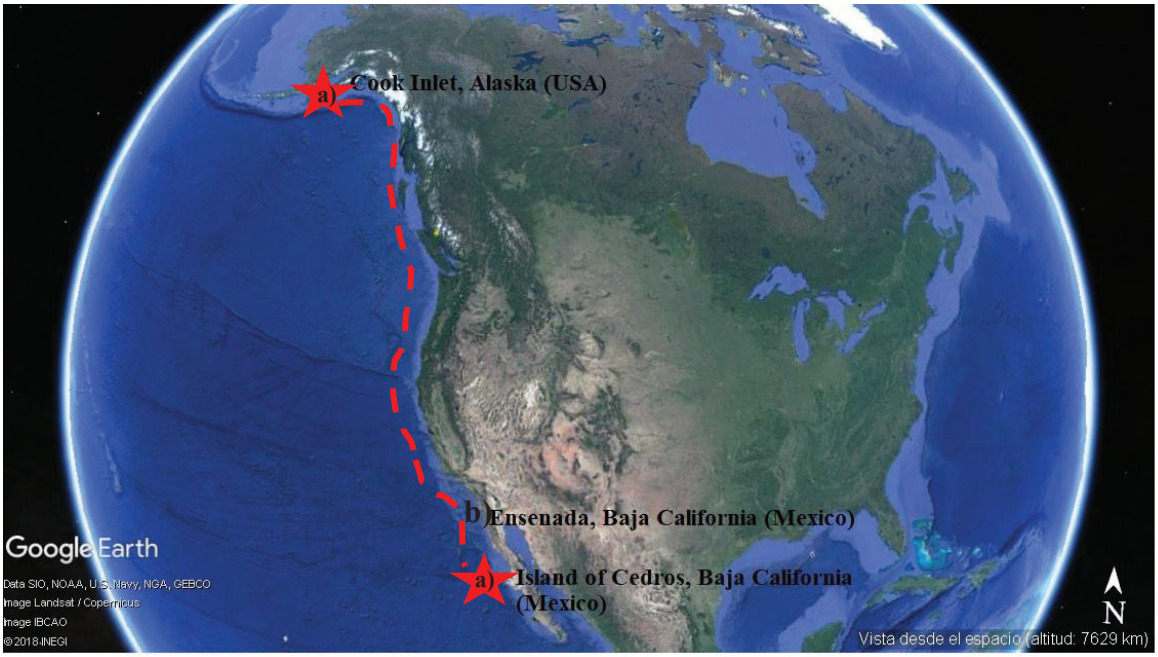

***Figura 3**. **a)** Geographical distribution of purple sea urchin of the Pacific Ocean **b)** Geographical location of the study area. Source: **Modified** image Google Earth.*

4.- Methodology

4.1.- Study area

10 organisms were collected around the island of Todos Santos, in front of the city of Ensenada (Baja California, Mexico) (31°47′59 ″ N 116 ° 47 ′ 20 ″ W) and transferred to the laboratory at the Instituto de Investigaciones Oceanológicas of the Universidad Autónoma de Baja California(31°51'46.7"N, 116°40' 02.5" W) (**Figure 3 b)**). They were kept in tanks of 900 liters, with continuous flows of water until they were used, at a temperature between 15 and 17ºC (+/-2 º C) and were fed with the brown seaweed *Macrocystis pyrifera*, obtained from the coast.

4.2.-Laboratory reproduction techniques

4.2.1.-Spawning and fertilization

Stimulation was carried out favoring the expulsion of the gametes in 2 adult sea urchins by injection of 3 mL of potassium chloride (KCl) with a concentration of 0.5 M in the celomic cavity (oral part) through the peristomal membrane (Strathmann, 1987). Organisms were placed with the oral side downward, in containers with seawater filtered up to 1 µm and irradiated with ultraviolet light lamp to prevent bacterial contamination. The expulsion of gametes began between two and three minutes after the injection of KCl, eggs in color yellow and grained in appearance, in contrast to the sperm observed in whitish color (Salas-Garza, A. et al. 2005) (**Figure 4**).

Once the gametes were obtained, fertilization was carried out, according to the method described by Carpizo-Ituarte et al. (2002). 3 containers of 3l of filtered seawater were used. The recipient was added 3 ml of sperm concentration and 3ml of eggs concentration, which were homogenized for 5 minutes so that fertilization would be satisfactory. Subsequently, a sample was taken to verify in the microscope the effectiveness of fertilization counting at least 10 fertilized eggs. The fertilization envelope was used as evidence of the fertilization (**Figure 4**).

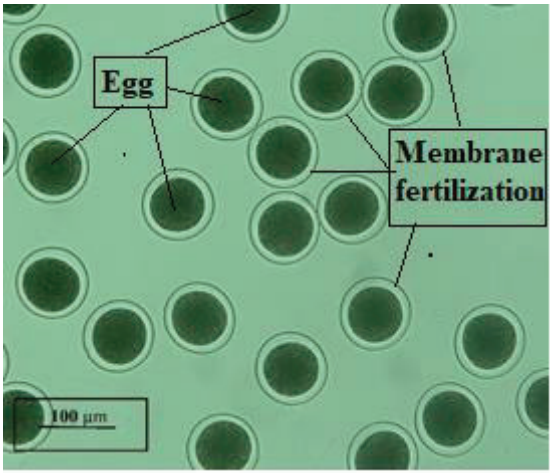

***Figure 4**. Fertilized eggs of the purple sea urchin (10x)*

4.2.3.- Quantification of embryos

To know the quantity of larvae existing in 3l at different temperatures a sample of a known volume was taken, in this case 0.050 ml, it is fixed with lugol, dissolution of molecular iodine (I2) and potassium Iodide (KI) in distilled water, and a count is performed on a stereo microscope. Once the larvae density was obtained at 0.050 mL,

using conversion factors, the total density of purple sea urchin larvae was calculated at 3 L dissolution. Subsequently, the volumes to be added to the crops were obtained as the purpose of this process was to add a density of 7 ml^{-1} larvae of purple sea urchin in each flask with continuous aeration at 19, 22 and 25ºC (**Table 1**).

***Table 1**. Added volume of the concentration of 3L to each flask to obtain a density of 7 larvae per mL^{-1} in each treatment of the two experiments.*

	Experiment 1	**Experiment 2**
Temperature (ºC)	**Volume (ml)**	
19	8	13
22	12	30
25	15	31

4.3.- Experimental design

Once fertilized, zygotes were distributed in 4, Erlenmeyer flasks filled with 1 L seawater filtered up to 1µm and irradiated with UV light (FSW). The experimental design consisted of 3 treatments (19,22 and 25ºC), with 4 replicates for each treatment for a total of 12 experimental containers. Continuous aeration was provided to each container introducing an airstone to each one of them. To keep the experimental temperatures the 4 replicates of each treatment were introduced in a additional tray with 10 liter of water and a summersible water heater. Treatment at 19 º C was considered as control, taking in consideration, the average sea water temperature that the urchins experience in Bahía de Todos Santos in Ensenada, B.C. To **subject** the larvae to thermal stress, we used 22 and 25 º C considering that the surface temperature of seawater throughout the year in Ensenada is between 16-20 º C (Díaz-Pérez and Carpizo-Ituarte, 2011). The water temperature was automatically recorded in each container every 30 minutes during experiments by temperature recorders (HOBO data logger). Also, the physical-chemical parameters were measured daily, using a multiparametric, dissolved oxygen and pH probe.

We performed 2 experiments with different batches of larvae and named experiment 1 and 2. Each experiment lasted 9 days and the water exchange was carried out daily. The larvae were fed daily, from day 4, with 5000 cel/ml of the microalgae *Rhodomonas sp*. Both experiments ended when the larvae reached the equinopluteus.

4.4.- Data Analysis

Monitoring of embryonic and larval development was made from fertilization, (considered as day 1), using a motorized compound microscope AxiosKop2 C Zeiss. Measurements were made from microphotographs taken with the microscope and were analyzed using the AxionVision Zeiss program. (AxioVs40 V4.8.0.0). The size of the eggs, blastula and gastrula were determined by measuring the diameter of 6 embryos (**Figure 5c**) In the case of pluteus larvae, total body length measurements (µm) were taken from the apex to the end where the arms begin (**Figure 5a**) and the body length(µm) without considering the arms (**Figure 5b**). We measured 6 larvae from each sample. The final size of each stage of development corresponds to the average of 6 measurements.

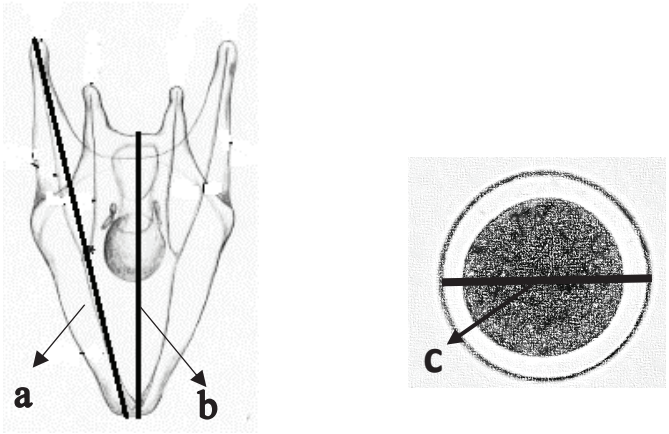

Figure 5. *Measurements (am) taken:* ***(a)*** *average body length considering the arms,* ***(b)*** *average body length without considering the arms of the purple sea urchin S. purpuratus (**Modified** by Smith et al.,2008).* ***(c)*** *diameter of purple sea urchin eggs.*

4.5.- Statistics

Statistical analyses were performed using the SigmaPlot 12.0 software. Once the average length of the larvae was obtained in the three treatments, they were analyzed to look for possible statistical among treatments. To this end, we used a Shapiro-Wilk normality test with a confidence limit of 95% considering significant differences when the value of $P < 0.05$. As for the treatments ± standard deviation, it was verified using the Mann-Whitney non-parametric test, where there were statistically significant differences when the value of $P < 0.001$.

5.-Results

5.1.-Physico-Chemical parameters

5.1.1.- Temperature

During cultivation, temperature varied. In experiment 1, established initially at 19ºC, temperature oscillated between the 17.76-18.8 ºC with an average value of 18.18ºC. At 22 º C, temperature was between 20.04ºC and 21.95ºC with an average of 21.05 º C. And lastly, at 25 ºC its minimum and maximum temperature ranged between 24.06 °C and 25.03 º C with an average value of 24.8 º C (**Table 2**).

In the second experiment, at 19 º C the temperature oscillated between 17.95 º C and 19.38 º C with an average of 18.31 º C. At 22 º C, its minimum and maximum temperature were around 18.43 º C and 23.48 º C with an average value of 21.36. To finish, at 25 º C its temperature oscillated the 23.29 º C and 25.03 º C with an average of 24.84 º C (**Table 2**).

***Table 2**. Range of experimental temperature during experiments of the larval development of the purple sea urchin S. purpuratus.*

		Temperature (ºC)			
		Minium	**Maximum**	**Average**	**Stand. Deviation**
Experiment 1	**19ºC**	17.76	18.81	18.18	0.177
	22ºC	20.04	21.95	21.05	0.098
	25ºC	24.06	25.03	24.80	0.119
Experiment 2	**19ºC**	17.95	19.38	18.31	0.238
	22ºC	18.43	23.48	21.36	0.944
	25ºC	23.29	25.03	24.84	0.153

If the temperature graphs of the two experiments are compared to each other and considering the aforementioned values, at 19 º C it can be seen that the temperature does not undergo drastic variations in the two replicates. They have a temperature peak of 0.4-0.5 º C but remain constant. (**Figure 6. a.1, a.2**). Despite these values, statistically there were not significant differences between the two treatments (Mann-Whitney; $P < 0.001$).

At 22 º C, the two graphs do have variations between them, in experiment 1 (**Figure 6 b. 1**) They remain relatively stable with respect to the time, around 21 º C, with some peaks of temperature of 0.2-0.3 º C. Instead, in experiment 2, it had very wide temperature ranges, due to setbacks during the experimental period. On day 3, as shown

in Figure 7b. 2, there was a drop in the temperatures, reaching a minimum of 18.43 º C, due to the breakage of the electric water heater. This was replaced by another, which caused the temperature to reach a peak of 23.48 º C, until it was stabilized on day 5 around 21 and 22 º C. So, there were significant differences between the different treatments (Mann-Whitney; $P < 0.001$). Subsequently, it will be assessed how this setback affected the growth of the larvae during their development

At 25ºC, the graphs show similarities based on their dispersion over time, presenting temperature drops around 0.5-1.0 º C on days 2, 4 and 8. During the other days, the temperatures remain constant with values between 24.7-24.9 º C (**Figure 6 c. 1., c. 2**), but differences were observed between the two treatments (Mann-Whitney;$P < 0.001$).

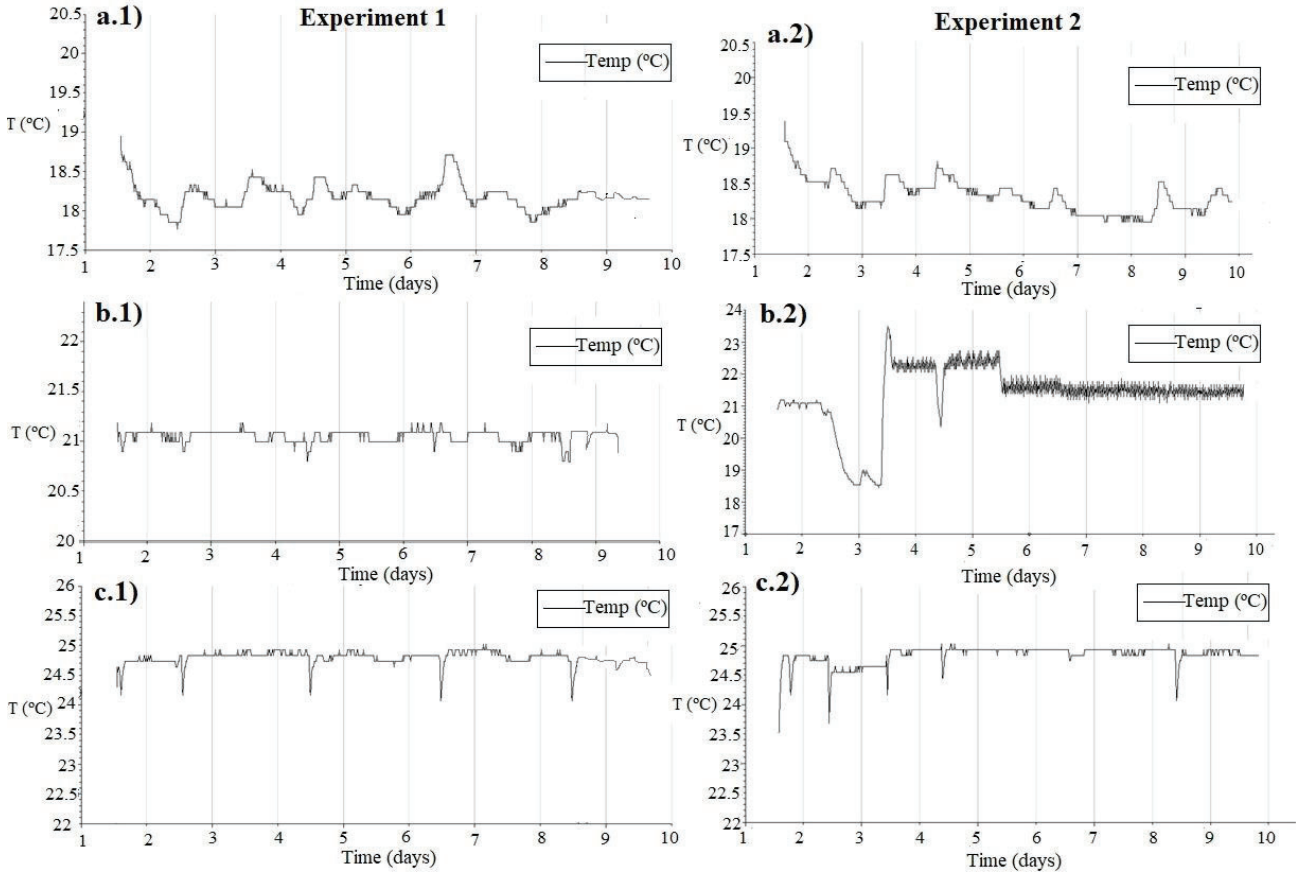

Figure 6*. Variation of the temperature in the different treatments and replications (experiments), during all the embryonic and larval development.* ***a.1), b. 1****),* ***c. 1)*** *temperature during cultivation in experiment 1;* ***a.2), b.2), c.2)****. Temperatures during the process in experiment 2.*

5.1.2.-Dissolved oxygen and pH

The solubility of oxygen with respect to temperature is very representative. In Experiment 1 (**Figure 7.a)**), it can be observed that at a higher temperature the average amount of dissolved oxygen decreases. At 19 º C, the highest dissolved oxygen value is given on day 1, 7.7 mg/L, where it is also observed that the standard error is greater, begins to decrease until day 3 with a value of 6.6 mg/l and then remains stable until the end of the experiment at 7.0 mg/L. At 22 º C the amount of dissolved oxygen is around 6.6 mg/l on Day 1, decreasing to 5.75 mg/L on day 2. Subsequently, exponential growth occurs until reaching values of 6.0 mg/L on the last day of the experiment. At 25 º C, dissolved oxygen values are around 5.25 mg/l on day 3, where growth occurs over the days until reaching the value of 6.4 mg/L highest peak on day 8.

In Experiment 2 (**Figure 7b)**), at 19 º C the highest solubility peak occurs on day 4 with an average value of 7.75 mg/L, having a low value on day 8 of 6.25 mg/L. At 22 º C, day 3 is given the highest dissolved oxygen value, 6.75 mg/L average and the minimum solubility occurred on day 8 with 5.75 mg/L. At 25ºC they have their highest point at 6.0 mg/L on day 3 and reaching a minimum value on day 8 with 5.25 mg/L.

If you compare figure 7 to each other, you can see that the two experiments fulfilled the rule that at a higher temperature the amount of dissolved oxygen decreases. The most significant difference is that in **Figure 7a)** from days 2 and 3 the dissolved oxygen values exponentially increase, however, in **Figure 7b)** from those days the solubility decreases until day 8, reaching its lowest point.

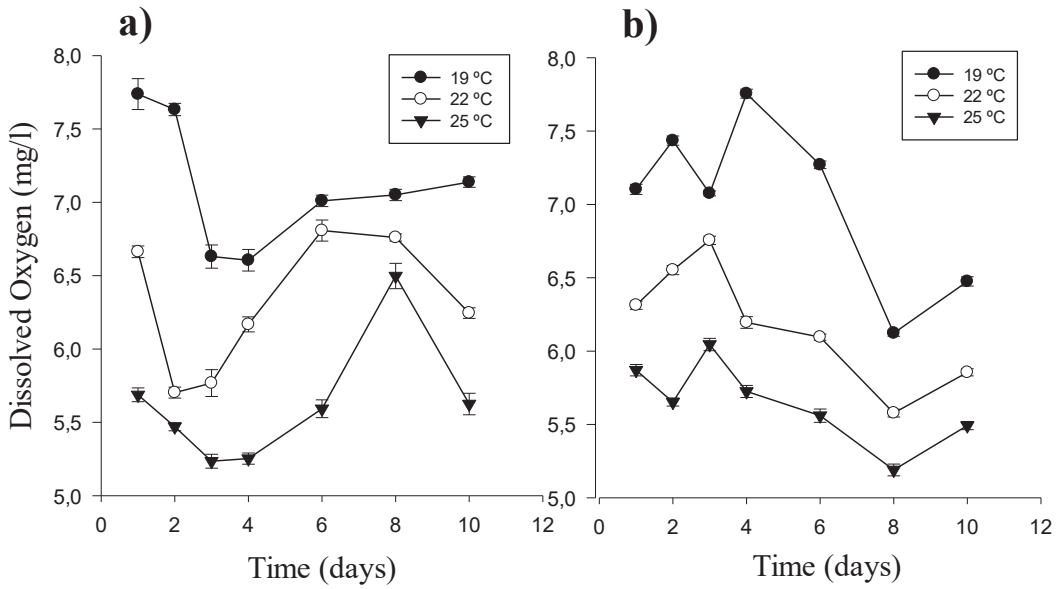

Figure 7. *Variation of oxygen solubility in different temperature treatments over the days. a) Experiment 1 b) Experiment 2. The variations of each point correspond to the standard error of 5 measurements.*

With respect to pH, in the cultive oscillated between 8.2-8.3 for each treatment, having the lowest value in the control temperature (19ºC).

5.2.-Larval development

On day 2, the embryonic development of the purple sea urchin in experiment 1 was in the different temperature treatments (19.1, 22.2 º C) in the blastulae stage. At 19.1 º C this stage was more developed when observing, in some embryos, the formation of the archenteron, that originates with the invagination of blastopore, which gave rise to the gastrulation, later. At 25.1 º C the limit of thermal tolerance was reached, so the growth of the embryos in 2n cells was stopped or even they did not arrive nor to develop because the inside of the membrane of fertilization was deformed (**Figure 8**).

In experiment 2, development was slower than the first. At 19.2 and 22.2 º C the purple sea urchin was in morula stage. Many of them were already getting out of their membrane and began to move with the help of the cilia. At 25.2 º C it occurred exactly the same as at 25.1 º C, the embryos died (**Figure 8**).

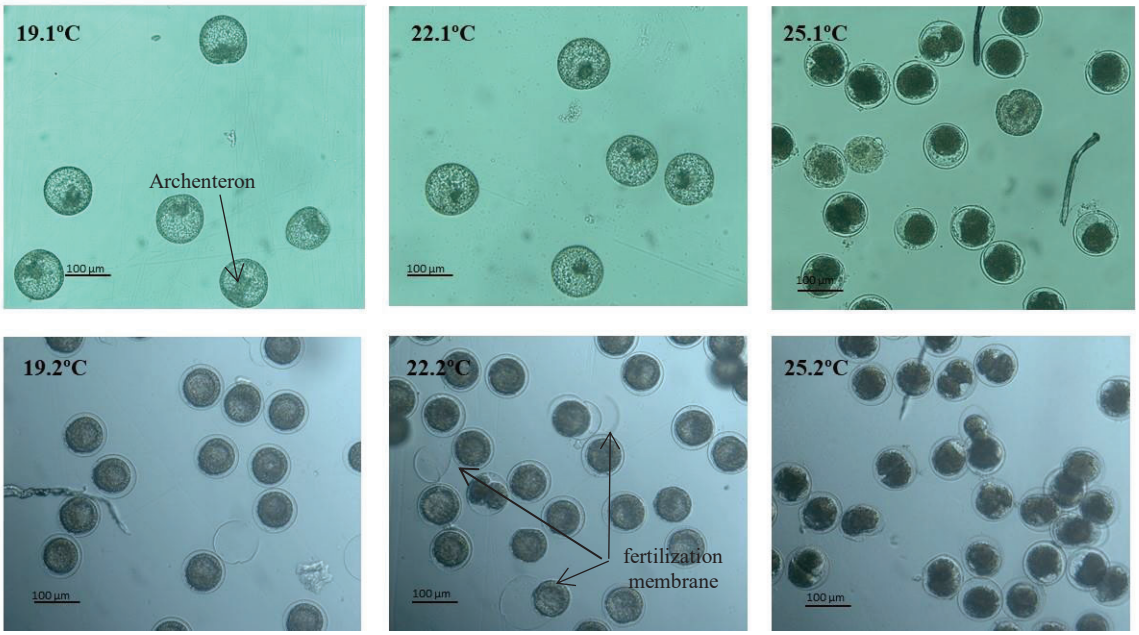

***Figure 8**. Sequence of images of the larval development of the purple sea urchin on day 2 in the different treatments and experiments. 19.1, 22.1 and 25.1 ºC corresponded to experiment 1 and 19.2, 22.2 and 25.2ºC to the experiment 2.*

From day 3, the results were no longer considered at 25 º C. In the samples of experiment 1 it was observed that embryonic development had already reached the initial

prism stage, since it still presented some embryos in the gastrula stage. The only difference was that at 22.1 º C the prism stage was more developed and began to observe the formation of the arms. In addition, the digestive system began to form. In the second experiment, embryonic development was in the gastrulae stage (**Figure 9**).

The equinopluteus larvae was formed on day 6. In the first experiment they **weew** clearly appreciated the 4 arms in addition to its digestive system, although at 22.1 º C, the larvae had some deformities. They had irregular shape, in some larvae the arms were not appreciated clearly and the development of the group of larvae of the treatment at 22.1º C was not equitable. The second experiment showed a significant difference between the two temperatures. At 19.2 º C, they were in equinopluteus stage but at 22.2 the development had slowed down, and the predominating stage was final prism, with the beginning of the arm formation (**Figure 9**).

At the end of the two experiments, on day 10, the larvae remained in equinopluteus stage of 4 arms, but already began to form two other arms, to arrive at larvae equinopluteus of 6 arms. The only exception was at 22.2 º C that its larval development had slowed down, since its stage continued in stage prism, starting of larvae equinopluteus of 2 arms (**Figure 9**).

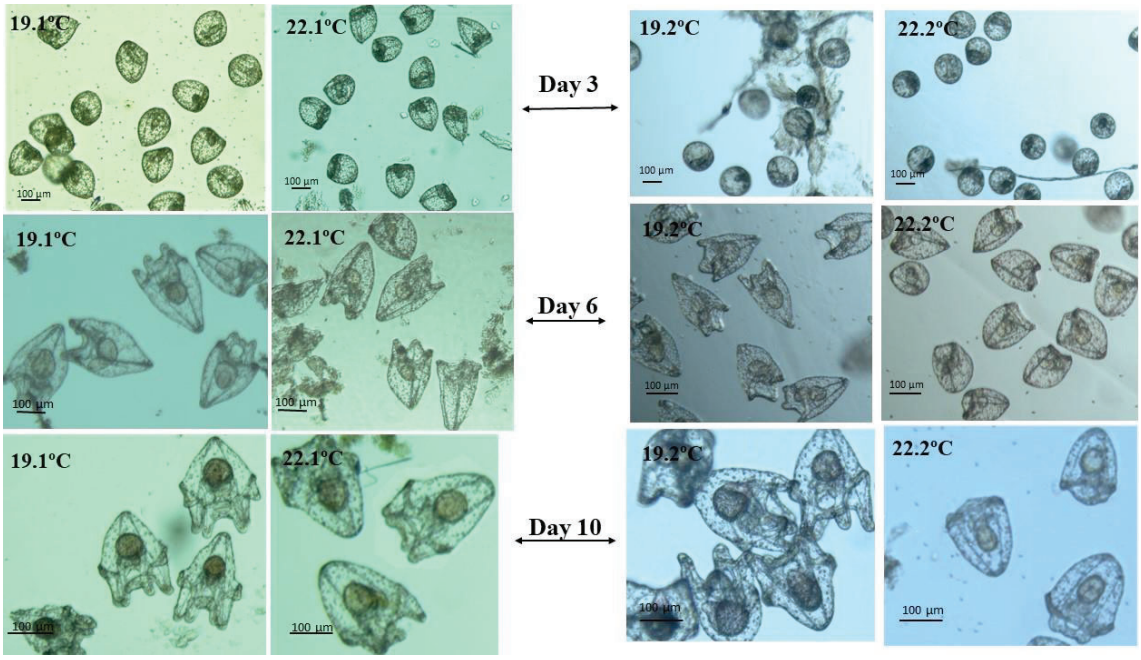

***Figure 9**. Sequence of images from days 3, 6 and 10 of the growth of the purple sea urchin in different temperatures and experiments. 19.1 and 22.1ºC corresponded to experiment 1. 19.2 and 22.2ºC to the experiment 2.*

5.3.- Morphometry

5.3.1- Length

The total average length during experiment 1 (figure 11.a) of the purple sea urchin on day 1, when the fertilization membranes occurred, was similar in all three treatments, around 105 µm for diameter. During day 2 drastic changes were noted, since at 25 ºC the embryos did not withstand the temperature treatment and died. On day 3 they reached the prism stage so at 19ºC the total average length was 163.05 ± 5.9 µm and at 22 º C, 159.02 ± 5.4 µm. As larval development began on day 4, growth was exponential at 19 and 22 º C, obtaining similar size average values of 409.49 ± 7.3 µm and 385.95 ± 3.4 µm at the end of the experiment. Treatment at 19 º C had a faster growth than at 22 º C, although there were intervals of time where they had a greater total length than at 19 º C; day 2 and 4. (**Table 3**) (**Figure 10a**). Therefore, there are no significant variations between them (P = 0.898; p > 0.05) throughout the experimental development.

In Experiment 2, from day 2 it can be seen that the average length decreases (**Figure 10b**), taking values of 87.32 ± 0.7 and 94.38 ± 2.5 µm in treatments of 19 and 22 º C respectively. This coincides with the morula stage, in which they begin to hatch from the fertilizing membrane. In addition, the embryos did not withstand the treatment at 25ºC and die. On day 3, there is an increase in size reaching 144.29 ± 2.5 µm at 19 º C and 143.56 ± 3.7 µm at 22 º C. The growth was exponential, from day 2, in both treatments reaching an average length of 388.53 ± 7.1 µm at 19 º C and 255.05 ± 7.9 µm at 22 º C. It should be noted that the higher temperature growth is slower as seen in Figure 11b). The difference in the average values of the two groups is not large, so statistically it does not present significant variations between these two treatments (P = 0295; P > 0.05) (**Table 3**) (**Figure 10b**)

If we compared the two experiments, in the treatment at 19 º C there are no significant variations with respect to their growth (P = 0749; p > 0.05) and at 22 º C it is observed that in experiment 2 the growth is slower than in the first replicate, having differences of up to 150 µm. Still, it does not present statistically significant variations (P = 0171; P >.05) (**Figure 10**).

***Table 3**. Total length of the larvae during the experimental period. Representation of the average of 6 length larvae measurements with its standard error in the different temperatures of experiments 1 and 2.*

	Length (µm)					
	Experiment 1			Experiment 2		
Time(days)	19ºC	22ºC	25ºC	19ºC	22ºC	25ºC
1	105.56 ± 0.8	105.42 ± 0.7	105.21 ± 1.2	106.1 ± 1.3	102.5 ± 0.4	102.8 ± 0.7
2	112.27 ± 0.9	121.43 ± 0.7	-	87.32 ± 0.7	94.38 ± 2.5	-
3	163.05 ± 5.9	159.02 ± 5.4	-	144.29 ± 2.5	143.56 ± 3.7	-
4	230.274 ± 6.2	242.32 ±8.1	-	190.57 ± 6.3	154.66 ± 5.2	-
6	307.15 ± 7.5	291.53 ± 9.6	-	285.98 ± 4.6	198.19 ± 3.7	-
8	352.24 ± 3.1	318.97 ± 4.9	-	332.16 ± 8.0	206.99 ± 3.0	-
10	409.49 ± 7.3	385.95 ± 3.4	-	388.53 ± 7.1	255.05 ± 7.9	-

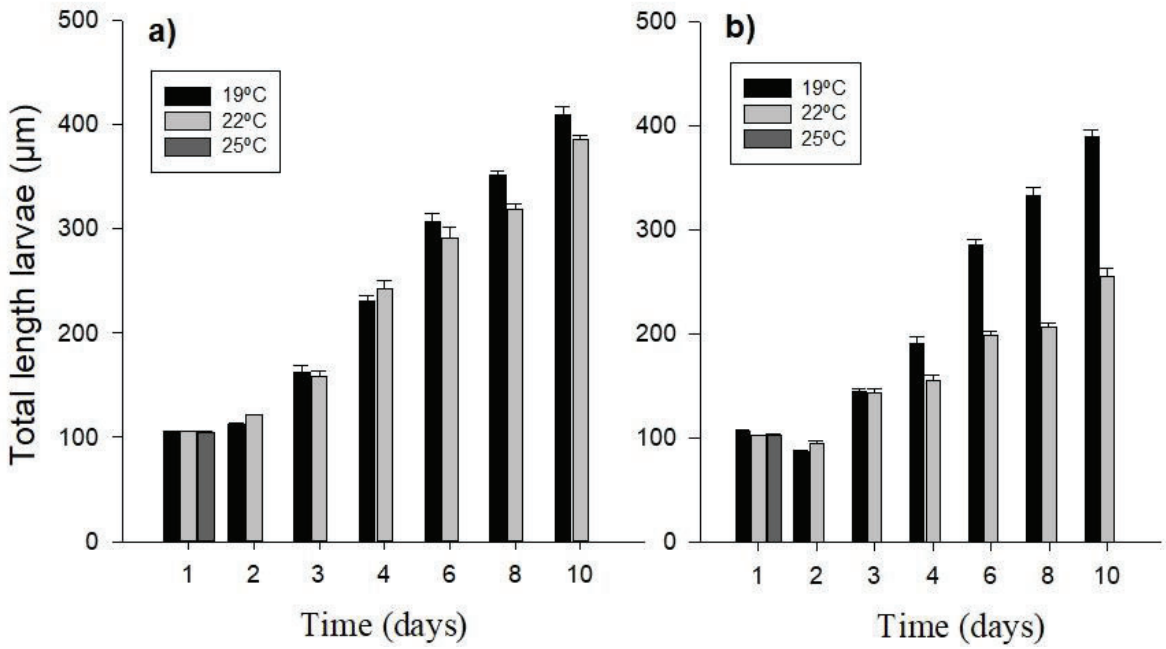

***Figure 10.** Total average length per day of the purple sea urchin during his larval development since fertilization occurred at different temperature treatments (19, 22 and 25ºC). Vertical lines indicate the standard error. a) First experiment b) Second experiment.*

5.3.2.-Body length

The average body length without taking into account the arms was valued from the moment in which the embryonic development was concluded in the prism stage, day 4.

With regard to experiment 1, on day 4 the different treatments presented an average length of 196.5 ± 8.0 µm and 206.13 ± 5.6 µm at 19 and 22 º C respectively. Because of the progress of the experimental period, growth values were increasing. On day 8, the values between the two temperatures were similar with 269.21 ± 8.4 µm at 19 º C and 261.72 ± 11.1 µm at 22 º C. And, finally, when the larvae had reached the equinopluteus stage of 4 arms, their length reached 356.19 ± 5.9 µm with a temperature of 19 º C and 313.09 ± 7.9 µm with a thermal value at 22 º C. With a temperature of 19 º C the larval growth, measured in average body length, will be greater than at 22 º C. (**Table 4**) (**Figure 11a)**). It should be noted that the difference in the average values of the two treatments is not large, so statistically it does not present significant variations (P = 0740; P > 0.05).

In experiment 2, day 4, the growth at 19 º C was 165.22 ± 6.2 µm and at 22 º C, 161.32 ± 4.5 µm. At 22 º C it was observed that the growth of the larvae was slower than at 19 º C but progressively continued the development. On day 6, the average length of the larvae was 264.53 ± 6.0 µm at 19 º C and 230.74 ± 12.7 µm at 22 º C. To conclude, on day 10, in the different treatments at 19 and 22 º C, a body length of 295.46 ± 5.5 µm and 239.82 ± 6.0 µm were obtained, respectively (**Table 4**) (**Figure 11b**). Therefore, there are no significant variations between temperature and length values (P = 0.352; P > 0.05)

If the two experiments are compared, it is verified that at 19 º C they present a linear growth, where their obtained values resemble and do not present significant differences between the variables (P = 0.536; P > 0.05). On the other hand, at 22 º C they have a less pronounced growth and there are no significant variations (P = 0157; P > 0.05). The growth values are lower in experiment 2. (**Figure 11**)

***Table 4**. Average body length during experiment 1 and 2. Representation of the average of 6 body length larvae measurements with its standard error in the different temperatures.*

	Body length larvae (µm)			
	Experiment 1		Experiment 2	
Time (days)	19ºC	22ºC	19ºC	22ºC
4	196.5 ± 8.0	206.13 ± 5.6	165.22 ± 6.2	161.32 ± 4.5
6	242.44 ± 4.0	226.6 ± 7.9	224.1 ± 2.8	180.41 ± 3.9
8	269.21 ± 8.4	261.72 ± 11.1	264.53 ± 6.0	230.74 ± 12.7
10	356.19 ± 5.9	313.09 ± 7.9	295.46 ± 5.5	239.82 ± 6.0

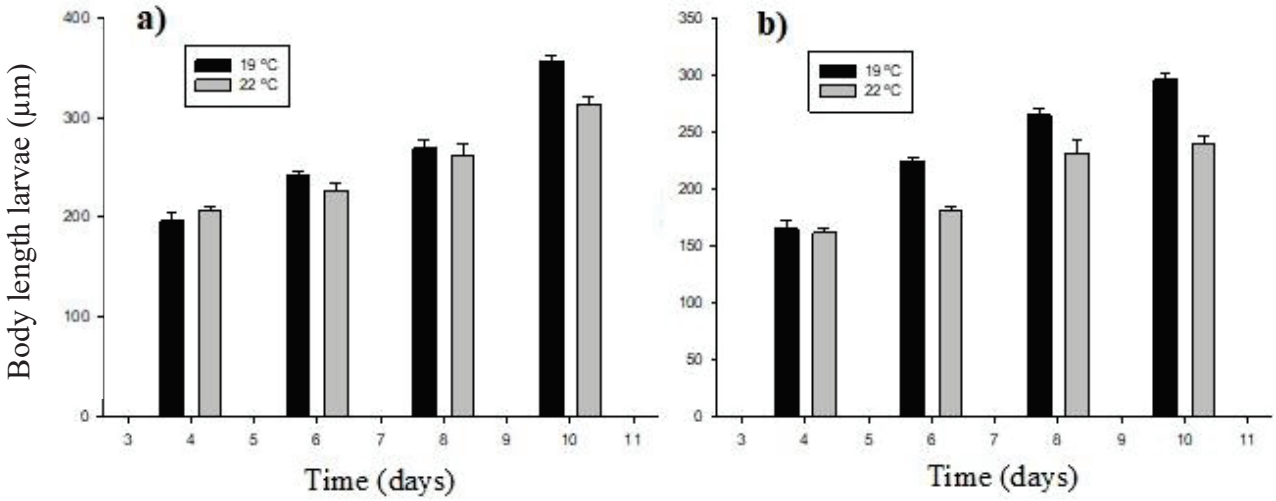

***Figure 11**. Average body length per day of the purple sea urchin since his larval development began at different temperature treatments (19, 22 and 25ºC). Vertical lines indicate the standard error. a) First experiment b) Second experiment.*

6.- Discussion

The development of *S. purpuratus* presented a thermal tolerance limit at 25ºC in experiments 1 and 2, where the larvae were unable to develop and died as embryos. These results could be explained from the described by Farmanfarmaian and Giese (1963), who verified that at 25ºC the gametes of the purple sea urchin were able to carry out fertilization and form fertilization membranes, but the cell division was abnormal, and no further development occurred. In the study of Byrne et al. (2009) on the species *Heliocidaris erythrogramma*, this same process occurred, but at 26ºC. This contrasts with studies of tropical equinoidea species in which at higher temperature ranges (24-34ºC)

can develop and reach the juvenile stage (Rahman et al, 2014; Rahman et al. 2012; Sewell et al. 1999).

The difference found in the 2 experiments is possibly linked to the parents that originate the embryos cultured; we used different pairs sea urchins for each experiment. Therefore, although they are of the same species, they present a standard of reaction in which, the genotype has a specific function that is to relate each phenotype with its environment. This means that there is phenotypic plasticity since the purple sea urchin do not present the same phenotype to environmental changes (Schmalhausen, 1949) Consequently, there will be more thermotolerant organisms than others, as in the case of experiment 1 with respect to experiment 2. These results coincide with Miner, (2007), who studied the growth of the sea urchin *Strongylocentrotus purpuratus* at the same temperature and there was no similarity in the two replicates performed.

The difference of the size of the larvae between the temperatures tested in the present work, may be due to the fact that according to Farmanfarmaian and Giese (1963) the thermal limit of the purple sea urchin is at 23.5 º C, so being close to this upper limit of tolerance, could affect its development. In the treatment at 22 º C of experiment 2, the electric heater was broken during day 2 and 4 causing temperature limits between 18.4-23.5 º C. For this reason, the length of the larvae during the experimentation phase underwent negative changes and their development was slowed. These values were not used to be compared with other authors, since their temperature range was unstable, with respect to the other treatments.

Thus, in order for the embryos to complete their development in a natural way, the most suitable temperature, among the different treatments studied, was 19 º C. Azad et al (2011) and Miner (2007) observed that the water temperatures most suitable for the development of the larvae were from 11 to 14 º C. Hinegardner (1969) used 15 º C for the cultivation of larvae *S. purpuratus* in laboratory. Farmanfarmaian and Giese (1963) observed that in order to produce a normal embryonic development of the purple sea urchin, the seawater temperature should be between 13 and 20 º C, with these values being in the range of the present study.

The time of development in our study is similar to study of Azad et al. (2011) at 17 º C, where the embryonic development of the *S. purpuratus* was 4 days with a fertilized egg diameter at 105.7 ± 0.8 µm and concluded in the prism stage at a length of 230 µm approximately. Embryonic development *in S. purpuratus* differs with studies made by Rahman et al. (2012) in which embryonic development lasted one day, and it was observed that the fertilization membrane measured 134.86 ± 5.35 µm and ended with the prism stage at 181.56 ± 3.99 µm in the species *Salmacis sphaeroides*.

As for larval development, the equinopluteus larvae of 4 arms had an average length between 307.15 ± 7.5-285.98 ± 4.6 µm at 19 º C and 291.53 ± 9.6 at 22 º C during day 6 of experimentation. Rosas et al (2009) in his study observed that the same day at 23 º C, the total length was 314.3 ±23.30 µm in species *Tripneutes ventricosus*. Azad et al. (2011) on day 8 of his study, the larvae of purple sea urchin reached the size of 360 µm at 17 º C that coincide with our results at 19 º C of total length average 352.24 ± 3.1-332.16 ± 8.0 µm. With respect to the length of the body, the larvae of our study presented a larger size than the study carried out by Azad et al. (2011) during days 4 and 8 (165 µm and 175 µm approximately). But they differ from the results obtained by Gómez et al (2005) which observed that the size of the body when the equinopluteus larvae was 4 arms reached the 400±2 µm in the species of different family *Lytechinus variegatus*. So, the larvae development to stage equinopluteus in the purple sea urchin have a shorter duration and reach smaller sizes in relation to other species of tropical urchins

Once the results obtained have been assessed, it can be deduced that, if the predictions of global warming forecasts were fulfilled in which there will be an increase in sea water temperature between 1.1º C and 4.1º C at the end of the 21st century (IPCC, 2013), it will affect the population of purple sea urchin in the coast of Ensenada (Baja California, Mexico). In this area, the relatively cold sea water of the California Current meets and the warmer of tropical and subtropical waters (Durazo et al., 2017) Surface water temperature, during the months of reproduction (September to July) of this species (Tegner, 2001), between 16-20 º C (Carpizo et al., 2011)(Locarnini et al, 2013). When the fertilization is carried out and taking into account the results obtained, the embryonic development would not occur and the species of the zone could disappear, which would mean the redistribution of the population *S. pupuratus*, focusing on the northern coast of California and Alaska, where they reach lower temperatures. Another possible option would be that, considering the phenotypic plasticity, some organisms present greater thermotolerance and extend its thermal limit to tropical temperatures, being able to develop normally to reach the adult stage.

Although with individuals of the same species are obtained certain variations, has had to compare *Strongylocentrotus purpuratus* with other species with similar tolerance ranges to observe the significant differences existing and the similarities between species of tropical origin and temperate waters.

7.- Conclusion

1. Once it is subjected to thermal stress to the embryos during its development, it can be observed that with a temperature at 25 º C, they are unable to grow and die during the next 24 hours.
2. If the treatments are compared at 19 and 22 º C, the total length and the body of the larvae are lower at higher temperature because being close to the thermal limit (23.5 º C) affects the development.
3. If they occur sudden changes in temperature values in the treatment at 22 º C, for reasons beyond the experiment, it can be determined that its development slows down and will need more time to complete it and grow.
4. After observing the results, treatment at 19 º C was considered the best temperature for the development of the larvae to be faster and more effective in addition to their growth being greater.
5. It should be noted that the differences in growth between different experiments with same treatment, is because embryos obtained from different parental sea urchin presents a different genotype. **As a result, some sea urchins will be more thermotolerant than others.**
6. Rising sea water temperatures due to climate change may affect the reproduction of the population of purple sea urchins on the coast of Baja California because of their proximity to the upper thermal limit of the species.

8.- References

Azad, A.K.; Pearce, C.M. and McKinley, R.S. (2011). Influence of stocking density and temperature on early development and survival of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857). Aquac Res, 43, 1577-1591

Byrne, M.H.M.; Selvakumaraswamy, P.; Nguyen, H.D.; Dworjanyn, S.A and Davis AR. (2009). Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. Proc. R. Soc. B 276: 1883–1888.

Carpizo-Ituarte, E.; Salas-Garza, A. and Parés-Sierra, G. (2002). Induction of metamorphosis with KCl in three species of sea urchins and its implications in the production of juveniles. Cienc. Mar. 28: 157–166.

Díaz-Pérez, L. and Carpizo-Ituarte, E. (2011). Effect of thermal stress on survival and delay of metamorphosis in larvae of the purple sea urchin *Strongylocentrotus purpuratus*. Ciencias marinas, 37(4a), 403-414.

Durazo, R.; Castro, R.; Miranda, L.E.; Delgadillo-Hinojosa, F. and Mejía-Trejo, A. (2017). Anomalous hydrographic conditions off the northwestern coast of the Baja California Peninsula during 2013–2016. Ciencias Marinas, 43(2), 81-92.

Ebert, T. A. (1968). Growth rates of the sea urchin Strongylocentrotus purpuratus related to food availability and spine abrasion. Ecology, 49(6), 1075-1091.

Farmanfarmaian, A. and Giese, A. (1963). Thermal Tolerance and Acclimation in the Western Purple Sea Urchin, *Strongylocentrotus purpuratus*. Physiological Zoology, 36(3), 237-243.

Fujisawa, H. (1989). Differences in Temperature Dependence of Early Development of Sea Urchins with Different Growing Seasons. Biological Bulletin. 176. 10.2307/1541576.

Gil, D.G.; Perez, A.F. and Rubilar, T. (2014). Echinodermata. 295-316.

Gómez, M.O. and Gómez G.A. (2005). Desarrollo embrionario y larval de *Lytechinus variegatus* (Echinoidea: Toxopneustidae) en condiciones de laboratorio en la Isla de Margarita-Venezuela. Revista de Biología Tropical, [en linea] 53(3), pp.313-318.

González-Lozano, C.P (2010). Estrés térmico durante el desarrollo ontogenético del erizo morado *Strongylocentrotus purpuratu*s. Universidad Autónoma de Baja California.

Gooding, R. A.; Harley, C.D. and Tang, E. (2009). Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. Proceedings of the National Academy of Sciences of the United States of America, 106(23), 9316-21.

Hammond, L.M. and Hofmann, G. E. (2010). Thermal tolerance of *Strongylocentrotus purpuratus* early life history stages: mortality, stress-induced gene expression and biogeographic patterns. Marine biology, 157(12), 2677-2687.

Harley, C.D.; Randall-Hughes, A.; Hultgren, K.M.; Miner, B.G.; Sorte, C.J.;Thornber, C.S.; Rodriguez, L.F.; Tomanek, L. and Williams, S.L. (2006), The impacts of climate change in coastal marine systems. Ecology Letters, 9: 228-241.

Hickman, C.P.; Roberts, R. and Parson, A. (2001). Integrated Principles of Zoology. 11ra ed. McGraw-Hill College St. Augustine, Florida. USA. pp 899

Hinegardner, R.T. (1969) Growth and development of the laboratory cultured sea urchin. Biological Bulletin 137, 465–475.

IPCC, (2013): "Resumen para responsables de políticas. En: Cambio Climático (2013) Bases físicas. Contribución del Grupo de trabajo I al Quinto Informe de Evaluación del Grupo Intergubernamental de Expertos sobre el Cambio Climático" [Stocker, T. F., D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex y P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, Reino Unido y Nueva York, NY, Estados Unidos de América

Locarnini, R.A.; Mishonov, A.V.; Antonov, J.I.; Boyer, T.P.; Garcia, H.E.; Baranova, O. K.; Zweng, M. M.; Paver, C. R.; Reagan, J. R.; Johnson, D.R.; Hamilton, M. and Seidov, D. (2013). World Ocean Atlas 2013, Volume1: Temperature. S. Levitus, Ed., A. Mishonov Technical Ed.; NOAA Atlas NESDIS 73, 40 pp.

Marshall, A.J., Williams, W.D. (1985). Zoología Invertebrados. Vol 1. 7º edición. Editorial Reverté, S.A. México 979 pp.

Miner, B. (2007). Larval feeding structure plasticity during pre-feeding stages of echinoids: Not all species respond to the same cues. Journal of Experimental Marine Biology and Ecology. 343. 158-165. 10.1016/j.jembe.2006.11.001.

Morris, R.H.; Abbott, D.P. and Haderlie, E.C. (1980) Intertidal invertebrates of California. Stanford University Press, Stanford.

Mottet, M.G. (1976). The fishery biology of sea urchins in the family strongylocentrotidae. Wash. Dep. Fish. Tech. Rep. 20: 1-66.

Olaechea,,P.; Panes, J.J. and González-Figueroa, H. (2006). Desarrollo embrionario de *Tetrapygus niger* (Molina, 1782) «erizo negro» en diferentes temperaturas. Biotempo, 6: 27-31.

Osovitz, C.J. and Hofmann, G.E. (2005) Thermal history-dependent expression of the hsp70 gene in purple sea urchins: Biogeographic patterns and the effect of temperature acclimation. J. Exp. Mar. Biol. Ecol. 327: 134–143.

Rahman, M.A.; Yusoff, F.M. and Arshad, A. (2014) First successful documentation on the embryonic, larval and juvenile development of the tropical sea urchin, *Diadema setosum* (Leske, 1778). In: International Conference on Agriculture, Food and Environmental Engineering (ICAFEE'2014), 15-16 Jan. 2014, Kuala Lumpur, Malaysia. (pp. 80-84).

Rahman, M.A.; Yusoff, F.M.; Arshad, A.; Shamsudin, M.N. and Amin, S.M. (2012). Embryonic, larval, and early juvenile development of the tropical sea urchin, *Salmacis sphaeroides* (Echinodermata: Echinoidea). The Scientific World Journal, 2012, 938482.

Ramírez-Félix, E. (2000). Análisis de la extracción de erizo rojo de mar (*Strongylocentrotus franciscanus*) según especie clave y conectividad en el área de Santo Tomás a Punta China, Baja California, México. INP. SAGARPA. México. Ciencia Pesquera No. 14

Rogers-Bennett, L. (2007). The Ecology of *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus* California Department of Fish and Game, Bodega Marine Laboratory, University of California, Davis, Bodega Bay, CA (USA)

Rosas, J.; Velásquez, A.; Fernández, S.; Mata, E. and Cabrera, T. (2009). Desarrollo larvario y supervivencia hasta la metamorfosis del erizo *Tripneutes ventricosus* (Lamarck)(Echinodermata: Echinoidea) alimentado con microalgas a dos temperaturas. Revista de biología marina y oceanografía, 44(2), 387-396.

Salas-Garza, A.; Carpizo-Ituarte, E.; Parés-Sierra, G.; Martinez-Lopez, R. and Quintana-Rodríguez, R. (2005). Juvenile production of the red sea urchin *Strongylocentrotus franciscanus* (Echinodermata: Echinoidea) in Baja California, Mexico. Revista de biología tropical. 53 Suppl 3. 345-55.

Schmalhausen, I.I.(1949). Factors of Evolution. Chicago University Press, Chicago.

Smith, M.M.; Cruz-Smith, L.; Cameron, R.A and Urry LA. (2008). The larval stages of the sea urchin *Strongylocentrotus purpuratus*. J. Morphol. 269: 713–733.

Stimpson, W. (1857). On the Crustacea and Echinodermata of the Pacific shores of North America. Boston Journal of Natura l History. 6: 444-532, Plates 18-23.

Strathmann, M.F. (1987). Reproduction and Development of Marine Invertebrates of the Northern Pacific Phylum Echinodermata: Class Echinoidea. In: Strathmann MF (ed.), Coast. University of Washington Press, Seattle, pp. 511–534.

Sewell, M. and Young, C. (1999). Temperature limits to fertilization and early development in the tropical sea urchin *Echinometra lucunter*. Journal of Experimental Marine Biology and Ecology. 236. 291-305.

Tegner, M.J. (2001). The ecology of *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*, in: Lawrence, J.M. (Ed.) Edible sea urchins:

biology and ecology. Developments in aquaculture and fisheries science, 32: pp. 307-331

Workman, G. (1999) A Review of the Biology and Fisheries for Purple Sea Urchin (*Strongylocentrotus purpuratus,* Stimpson,1857) and Discussion of the Assessment Needs for Proposed Fishery.

9.- TFT Report

❖ Actividades desarrolladas durante la realización del TFT.

Durante la realización del TFT pude hacer uso de las instalaciones del laboratorio de Embriología y Ecología del Desarrollo en el Instituto de Investigaciones Oceanológicas, con supervisión, para realizar un sistema de cultivo a pequeña escala monitorizado y creado por mí. Realicé su inicial fertilización, cuantificación y distribución de larvas de erizo morado. Hacía un seguimiento diario del experimento y de las larvas presentes en el cultivo mediante el uso del microscopio para la toma de microfotografías. También aprendí de forma autónoma a manejar el programa estadístico Sigmaplot (versión 11.0), y a mejorar el inglés y la redacción con la corrección.

❖ Formación recibida (cursos, programas informáticos, etc.)

Para este trabajo asistí a las clases que daba mi cotutor en la Universidad de Baja California (UABC) de Embriología para aprender la técnica de fertilización en diferentes especies de equinodermos y como realizar un sistema de cultivo eficiente. Además, recibí pautas y manuales sobre cómo utilizar el microscopio AxiosKop2 C Zeiss y el programa AxionVision Zeiss (AxioVs40 V4.8.0.0) para medir la longitud de las larvas y hacer seguimiento de su crecimiento.

❖ Nivel de integración e implicaciones dentro del departamento y con el personal.

En el laboratorio de Embriología y Ecología del Desarrollo trabajaba de manera autónoma y podía hacer uso de todas las instalaciones (limpieza de tanques, alimento de equinodermos, etc.). Es un equipo pequeño formado por mi cotutor Eugenio Carpizo, dos alumnos y un técnico, que durante mi estancia en

Ensenada no había, por lo que yo me hice cargo de todas sus funciones con total libertad y responsabilidad. Había alumnos realizando experimentos de fin máster por lo que yo los ayudaba a monitorizar sus cultivos y en lo que ellos me necesitaran. La relación tanto con ello como con mi tutor era excelente, nos entendíamos muy bien y me dio la total confianza, responsabilidad y libertad para hacerme cargo de su laboratorio.

❖ Valoración personal del aprendizaje conseguido a lo largo del TFT.

El hecho de estar en un laboratorio donde había un grupo de personas tan reducido y que mi cotutor me haya dado total libertad ha conseguido que me haya dado cuenta de que tengo la capacidad suficiente para realizar cualquier actividad de forma autónoma. He adquirido muchos conocimientos sobre equinodermos, embriología y técnicas de laboratorio. Mi cotutor solo me planteo la idea del experimento y yo me encargué de montar todo, así como de enfocar el TFT y valorar los resultados. De manera personal, estoy muy orgullosa de este documento por el trabajo que me ha llevado porque, al fin y al cabo, con poca ayuda, he conseguido sacarlo adelante.