Growth of juveniles of two species of sea urchins under three different diets

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ABSTRACT

The general observation that organisms are adapted to their environment lies at the foundation of biology (Yokota et al, 2002). The evolutionary and ecological framework implies to the organism a suite of adaptative responses (biochemical, physiological, behavioural) which together enable it to survive and to reproduce within a particular set of environmental conditions (Yokota et al, 2002). Marine coastal habitats are characterized by high environmental variability. It is believed that, due to adaptation or acclimation to natural environmental variability, intertidal species may have some capacity to recover from future changes (Yokota et al, 2002). Urchins are considered keystone species in ecosystems as their Aristotle's lantern is adapted for biting, tearing and scrasping and can even function to grab the substrate. They are herbivores although, some of them, in particular situations they behave like omnivores. The regular echinoids seem to have the widest spectrum of food types included soft-bodied organisms (plant and animals), hard surfaces (rock with incrusting or boring plants and animals) or hard animals (corals and bivalves), and soft substrate. The present study focuses on evaluating the effects of three different diets (the giant kelp, *Macrocystis pyrifera,* a formulated food and combination of both) on the growth of two species of juvenile sea urchin (*Lytechinus pictus* and *Arbacia stellata).* Both species are inhabitants of the Pacific coast of Baja California, with *A.* Stellata increasing their range extension to the north, due to the more common presence of warmer ocean waters in front of Baja California. For this purpose, measurements of weight and size of the organisms, were made every 15 days for three months. Aditionally, temperature, pH, oxygen, sea water flow and behavior of the sea urchins were monitored. In the present study we use *M. pyrifera*, since is an abundant natural food resource in Baja California used by different species of sea urchins. We also used a formulated mircoencaspulated diet because was expected to have a positive effect on the growth of sea urchins in captivity. Knowing the growth response to different diets of these common especies in Baja California, will help us to conduct future studies to understand its posible interaction in the ocean and predict posible impacts of the increasing presence of *A*. Stellata along the coast of Baja California.

1. Introduction

1.1. The sea urchins *Arbacia stellata* and *Lytechinus pictus*

Sea urchins live in different marine habitats, from intertidal to deep-sea environments up to 6800 m including the Artic and Antarctic Oceans (Yokota, 2002). The starry sea urchin *Arbacia stellata* (Figure 1), is distributed along the Pacific coast of North, Central and South America from southern California to Peru, and also includes the Galapagos Islands and the mainland and peninsular coasts of the Gulf of California (Mortensen, 1935; Clark 1948; Brusca, 1980; Houston 2006). Probably the populations of the sea urchin *Arbacia stellata* present in the Gulf of California and in the outer area of the Pacific coast are the result of the colonization of the Panama region (Díaz et al.,2017), a recruitment event in warmer waters (Burcham and Caruso, 2015). For the white sea urchin, *Lytechinus pictus* (Figure 2), occurs on Eastern Pacific. No collection records were found at depths greater than 200 m for the species *Lytechinus pictus* (Conejeros-Vargas et al, 2017). Both species thrive in the Gulf of California, in Peru and in the Galapagos Islands, but El Niño conditions in 1997 and 1998 and currents carrying their larvae brought them reportedly as far north as the Channel Islands off Southern California. When conditions are suitable and coastal currents transport larvae northward until southern California and beyond, seeing warm-temperate and subtropical

Figure1. Images of the aboral (left) and oral (right) views of a juvenile of *Arbacia stellata.*

Figure2. Images of the aboral (left) and oral (right) views of a juvenile of *Lytechinus pictus* used in the present study.

species is not uncomm (Clark, S, 2015).

1.2. Characteristics of sea urchins

Although sea urchins differ between species, their general characteristics are interesting. They present a globular body and a radial arrangement of organs, shown by five bands of pores running from mouth to anus over the test (internal skeleton) (Figure 3) (Encyclopædia Britannica, 2019). The pores accommodate tube feet (Encyclopædia Britannica, 2019), which is a hydraulic structure that echinoderms have, and the tube feet are also known for their roles in light sensitivity, respiration, chemoreception and locomotion (Lesser et al, 2011). From nodules on the test arise long, movable spines and pedicellariae (pincerlike organs) (Encyclopædia Britannica, 2019); whose functions include locomotion, sensing, and protection from physical trauma and predators (Tsafnat et al, 2012). The anus opens upwards and the mouth downwards.

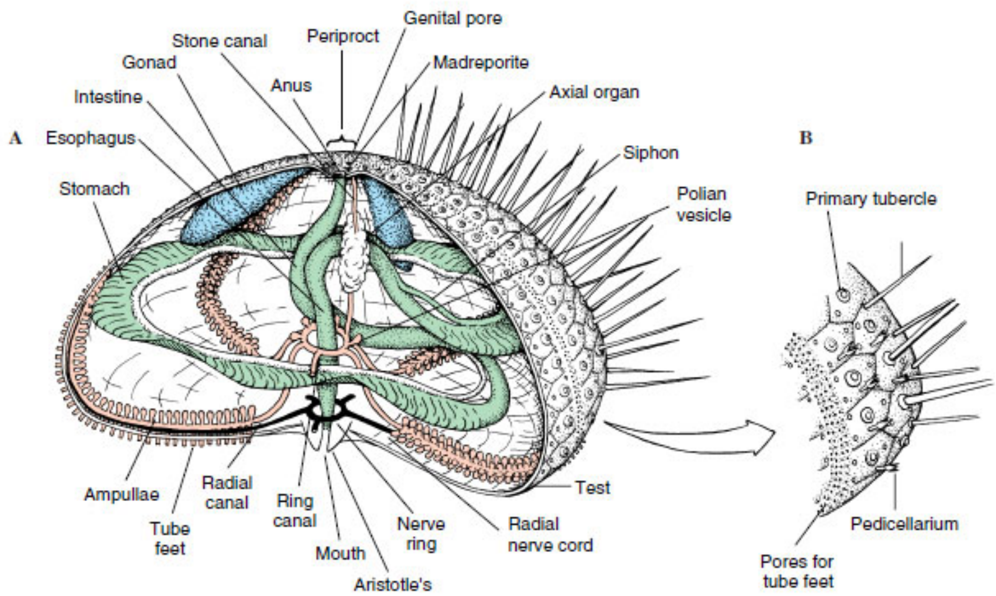

Figure 3. **A**, Internal structure of a sea urchin; water-vascular system in tan. **B**, Detail of portion of endoskeleton. Source: biocyclopedia Britanica.

The structure of the mouthpart in the sea urchin was analysed and described by Aristotle (384-322 BC), which he called the Aristotle's lantern. The mouth is composed of five protruding teeth. The Aristotle's lantern allows some urchins to excavate hiding places for example, in coral or rock (Encyclopædia Britannica, 2019). Furthermore, Aristotle's lantern also allows them have a wider spectrum of food types. Sea urchins consume the food that is available in the field, although they show preferences when they have a choice. Preference of certain food is from attractants, incitants, stimulants and deterrents present in potential food, and also its physical characteristics (Lawrence et al., 2013). Sea urchins form spatial agregations in response to the density of the food.

Urchins move further in areas where their algal food is less plentiful. This leads to the accumulation of urchins in areas with abundant algae (Abraham, 2007).

Due to its wide spectrum of food types, sea urchins are considered a keystone species. A keystone species is an organism that helps define an entire ecosystem (Society, 2019). Almost all examples of keystone species are animals that have a huge influence on food webs. In the case of sea urchins, they are important to combat invasive algae on corals reefs. Anthropogenic activities such as fishing can modify food webs and impact sea urchin populations (Duggins et al, 1989). The coral reefs affected by human fishing have more intense urchins-kelp interactions than those that are not affected, due to the increase of sea urchins in the first case (Hay, 1984). Sea otters also control certain species of sea urchins along the northeastern coast of the Pacific, creating new scenarios of seaweed and sea urchins, the same as storm events through the destructive removal of sea urchins or algae (Ebeling et al., 1985).

1.3. Growth of sea urchins

Growth supposes the change in weight or diameter of the sea urchins, which implies extension by calcification and production of soft tissues (Barrera, 2018). Echinoid skeletons consist of ossicles that include spines, limestone elements of the Aristotle lantern and plates that are joined together by small projections of collagen threads (Telford, 1985; Barrera, 2018). The growth is done by calcification around the individual plaques, also known as ossicles, as well as by the creation of new plaques at the anal end of the ambulacral and interambulacral rows (Barrera, 2018). The growth of the ossicles in echinoids is the result of the creation of an organic matrix in the calcification process (Dubois and Chen, 1989; Barrera, 2018). Factors such as temperature, water quality (Raymond and Scheibling, 1987) light, environmental and climatic variables (Russell, 1998), the density of individuals, food availability and composition of the diet (Hammer, 2005) can modify the growth rate of the sea urschins.

Correlation between adult growth and longevity may be related to the allocation of resources to growth and reproduction and to predictable recruitment (incorporation of individuals into a population) of new individuals (Ebert, 1975). Species that grow rapidly and allocate large amounts to reproduction may compromise resources available for maintenance. Because they are short lived as adult, these species would need to have predictable recruitment to persist. Species with unpredictable recruitment must live longer to persist, and thus many invest more resources in body construction-growing heavier skeletons, more slowly, while still allocating resources to reproduction (Ebert, 1982). Slower growing species also have a relatively high allocation to body wall and are associated with more exposed environments with high energy or water movement (Emlet, 2000). For example, *Lytechinus variegatus*, it has rapid growth, early reproductive maturity, and short longevity, characteristics of a species with a ruderal life-history strategy (Watts et al., 2013).

More studies are focused on adults than on juveniles. Although that does not mean that the adult stage is more important than the juvenile, since the recruitment process (incorporation of individuals into a population.) is very important for the conservation of a population.

This study focuses on the growth of two species of sea urchin *Lytechinus pictus* and *Arbacia stellata,* in the laboratory, using two sources of food (a macroalgae and a microencapsulated diet) combined in three different diets.

Composition of food

One food used was *Macrocystis pyrifera* (Figure 4). *M. pyrifera* from Baja California depending of the season presents 8.4-14.2 % of crude protein, 26.5%-33.3% of ash,4.8-7.2% of crude fibre, 0.4-1.0% of ether extract and 59.0-45.6% nitrogen-free extract (Serviere-Zaragoza et al, 2002). The maximun value of proteins in winter is 9.45% (Serviere-Zaragoza et al, 2002) in Ensenada.Regarding its lipid content, it contains 0.69 to 1.14% depending on the season (Vega-Villasant et al, 2006).

Figure 4. *Macrocystis pyrifera* Source: SIB-Aysen.

Other food used was Extruded Microdiet (Figure 5.), provided by Dr. María Teresa Viana Castrillón, who is a specialist in Nutrition and Physiology of the Instituto de investigaciones Oceanológicas at UABC (IIO-UABC). The Proximate composition (Table 1.) for this artificial food is of 48.11% of crude protein, 15.84 % of crude lipids and 8.09 of ash.

Figure 5. Extruded Microdiet. The artificial food is in a container full of sea water. A test was done to know if it remained undissolved after three days, it was effectively maintained.

Table 1. Ingredients of the microdiet were provided by Dr. T. Viana Castrtillón (IIO-UABC)

Ingredients	(%)	Proximate composition	(%)
Poultry by-producy	0.0	Crude Protein	48.11
Fishmeal	67.9	Crude lipids	15.84
Corn starch	0.0	Ash	8.09
Whole corn meal	16.0		
Fish oil	10.4		
DHA	1.9		
Taurine	0.9		
Rovimix	2.4		
stay c	0.2		
Sodium bonzoate	0.2		
BHT	0.1		
Sodium bonzoate	0.2		
BHT	0.1		
TOTAL	100.0		

2. Backgrownd

Studies that document feeding and growth in early juvenile sea urchins are scare and there are no published studies on the effect of different diets on growth on early juveniles of *Lytechinus pictus* and *Arbacia stellata.* Other studies with different species and different ages, showed results that demostrated the correlation between protein, lipids, carbohydrate content and growth of sea urchins.

In a recent study Heflin et al. (2016) showed that juvenile *Lytechinus variegatus* fed with formulated diets, with dietary protein levels below 23%, increased its wet weight more than sea urchins fed with diets containing 18% dietary carbohydrate, was significantly higher than the ones fed diets containing only 12% dietary carbohydrate. Juvenile *Lytechinus variegatus* appear to require a minimum of 20- 21% dietary protein for optimal growth (Hammer et al., 2004). In contrast, with high lipid content the growth of juvenile *Lytechinus variegatus* showed a negative correlation (Gibbs et al., 2009).

Cárcamo (2014) did the study of effects of different diets on the growth of juvenile of *Loxechinus albus*; the urchins feed with *Macrocystis pyrifera* showed the lowest growth rate in comparison with five algal foods and one animal food.

3. Objective

Our main objectives in this work are:

1. Contribute to the knowledge of feeding needs of juvenile *Lytechinus pictus* and *Arbacia stellata*.

2. Calculate the growth in weight and size of juveniles of *Lytechinus pictus* and *Arbacia stellata* with three different diets.

3. Document feeding behaviour under the conditions of this study.

4. Material and methods

In this experiment there were three treatments for each species of sea urchin. In treatment 1 they were fed with formulated food, in treatment 2 with *Macrocystis pyrifera* and in treatment 3 we offered a combination of formulated food and *M. pyrifera*. Each treatment consisted of three replicates with 20 sea urchins each one, for a total of 60 sea urchins/treatment. 360 sea urchins were provided by the Laboratorio de Ecología y Biología del Desarrollo (IIO-UABC) where this study was conducted. The sea urchins were chosen considering total wet weight. Because de initial populations has a range of sizes and weights, we considered 9 urchins of 0.03-0.04 mm, 6 of 0.04-0.05 mm and 5 of 0.05-0.06 mm, to make the intitial populations in each treatment, the closest in size and weight. The position of the tanks was chosen at random (Figure 6).

4.1. Maintenance

The urchins were feed three days a week, as well as cleaned before adding fresh food. The amount of food was calculated from their wet weight. First, the average weight of each replica was calculated. Secondly, The average weight was calculated by the number of sea urchins in each replic. Finally, we considered 5% of their wet weight to calculate the amount of microencapsulated to add for the dring the first 45 days of the experimental period. After the first 45 days, we increased the amount of food provided to 10% of their wet weight. To calculate the amout of *M. pyrifera* we did the next operation:

$$\text{Amount of } M.\ pyrifera = \frac{100g\ (wet\ weight) * average\ weight)}{20g\ (dry\ weight)}$$

In treatment 3, 50% of microencapsulated and 50 % of *M. pyrifera* were used.

To clean the recipients we followed a simple routine desccribed briefly as follows .1. All the recipients were emptied and their content, inlcuding the urchins, filtered through a 400 µm mesh light. 2. After sieving the content, recipients were siphoned and filled again with filtered seawater.

Figure 6. The containers where the sea urchins were maintained during the experiment. Each container had associated one flow of sea water and air.

4.2. Water quality measurements

Additionally, parameters such as water flows, temperature, pH, disolved O_2 and number of urchins on the walls were continuously monitored. Sea water flow were measured with the help of a graduated cylinder. We estimated the amount of wáter flow in 30 seconds and then estímate the amount of liters/hour. With electrodes Durafet pH sensor of the Honeywell Brand, pH was measured. The amount of O_2 (mg/L) was measured with multiparameter model pro1020 of the brand YSI. Temperature (^{o}C) was measured with a digital thermometer.

On the other hand, test diameter and whole wet weight measurements were taken every fifteen days. For the diameter, a Zeiss Stereoscopic C Microscope was used. This presents an eye ruler with divisions and for each zoom of the microscope it has its equivalent length in mm. Therefore, the diameter was calculated with the following operation:

Nº of divisions x equivalent length= diameter (mm)

Then, to calculate the weight a balance with a sensibility of 0.001 g was used (Figure 7).

Figure 7. Balance and microscope used for measurements.

The nonparametric method Kruskal-Wallis test was used to test if a group of data comes from the same population distribution. Then, Dunn's-test was used to determine significant differences between the distributions of treatments.

5. Results

5.1. Weight and size of *Lytechinus pictus*

In all the treatments the sea urchins presented an increase in mean weight at the end of the experiment (Figure 8. and Table 2.), although at 30 days with treatment 2 the mean weight remained (of mean = 0.046 sd. ± 0.002 to mean = 0.046 sd. ± 0.003), at 45 days showed a decrease (mean = 0.043 sd. ± 0.001) and then, it increased. The urchins in the treatment 1, at first, presented a greater increase in diameter, but at 60 days and at the end, the ones that showed a greater increase was those of in the treatment 3. With respect to size (Figure 9. and Table 3.) only in the treatment 2 the sea urchins presented a decrese at 15 days (of mean = 4.60 sd. ± 0.02 to mean = 4.59 sd. ± 0.07) , 30 days (mean = 4.52 sd. ± 0.08) and 45 days (mean = 4.46 sd. ± 0.02); then, it increased again. The urchins in treatment 1 also presented a greater increase in diameter, but at 45 days and at the end they showed a greater increase in treatment 3. As for the standard deviations (Figures 8. and 9., Tables 2. and 3.), these are negligible with respect to the mean. This means that the variation in weight and diameter of the sea urchins in this time was unequal .

Table 2. Mean weight (grams) of all sea urchins *Lytechinus pictus* with their respective standard deviations during the period (days) of the experiment in each treatment. In treatment 1 the food was formulated food, in treatment 2 was *Macrocystis pyrifera* and in treatment 3 was a combintation of both of them.

Treatment 1			Treatment 2		Treatment 3	
Days	Weight (g)	Desv	Weight (g)	Desv	Weight (g)	Desv
1	0.044	0.001	0.043	0.002	0.042	0.002
15	0.071	0.003	0.046	0.002	0.064	0.003
30	0.145	0.005	0.046	0.003	0.097	0.004
45	0.217	0.006	0.043	0.001	0.161	0.006
60	0.307	0.009	0.046	0.004	0.270	0.010
75	0.393	0.093	0.052	0.007	0.492	0.028

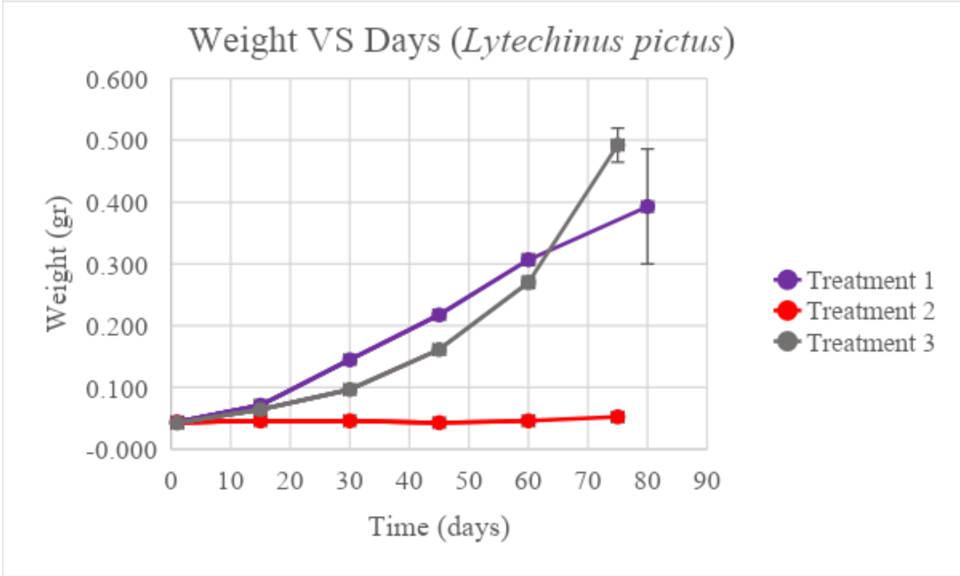

Figure 8. Mean weight variation (grams) of all sea urchins with their respective standard deviations during the period (days) of the experiment in each treatment. In treatment 1 the food was formulated food, in treatment 2 it was the *Macrocystis pyrifera* and in treatment 3 it was both of them.

In regards to water quality (Figure 10.), approximately T^a, O_2 and pH followed the same pattern in all treatments. The range of T^a in treatment 1 was of 14.07 to 18.23 ºC, in treatment 2 was of 14.0 to 18.23 ºC and in treatment 3 was of 14.13 to 18.37 ºC. The range of O_2 in treatment 1 was of 7.25 to 8.14 mg/L, in treatment 2 was of 7.58 to 8.18mg/L and in treatment 3 was of 7.20 to 8.18 mg/L. It is observed that the value of O_2 decreased with time, this may be due to the difference in size of sea urchins between treatments. Furthermore, in spite of the oxygen value following the same pattern for all treatments, throughout the experiment the oxygen value was lower in treatment 1 and higher in treatment 2. The range pH in treatment 1 was of 7.92 to 8.05, in treatment 2 was of 7.93 to 8.05 and in treatment 3 was of 7.93 to 8.05. The sea water flow values are different in some days, but in general they follow the same pattern too. The range of the sea water flow in treatment 1 was of 4.6 to 9.0 L/h, in treatment 2 was of 4.6 to 8.8 L/h, in treatment 3 was of 4.4 to 8.8 L/h. The parameter that does not follow the same pattern in all treatments is the number of sea urchins on the wall. The range of the number of sea urchins on the wall in treatment 1 was of 1 to 10, in treatment 2 was of 4 to 15 and in treatment 3 was of 0 to 13. It's notable that in treatment 2 the number of sea urchins on the wall decrease during the experiment, but in treatment 1 and 3 increased slightly.

Table 3. Mean diameter variation (millimeters) of all sea urchins *Lytechinus pictus* with their respective standard deviations during the period (days) of the experiment in each treatment. In treatment 1 the food was formulated food, in treatment 2 was *Macrocystis pyrifera* and in treatment 3 was a combintation of both of them.

	Treatment 1		Treatment 2		Treatment 3	
Days	Diameter (mm)	Desv	Diameter (mm)	Desv	Diameter (mm)	Desv
1	4.61	0.12	4.60	0.02	4.50	0.03
15	5.20	0.03	4.59	0.07	5.07	0.08
30	6.75	0.09	4.52	0.08	5.95	0.05
45	7.66	0.12	4.46	0.02	7.17	0.08
60	8.58	0.09	4.56	0.06	8.83	0.02
75	9.11	0.82	4.68	0.15	9.97	0.12

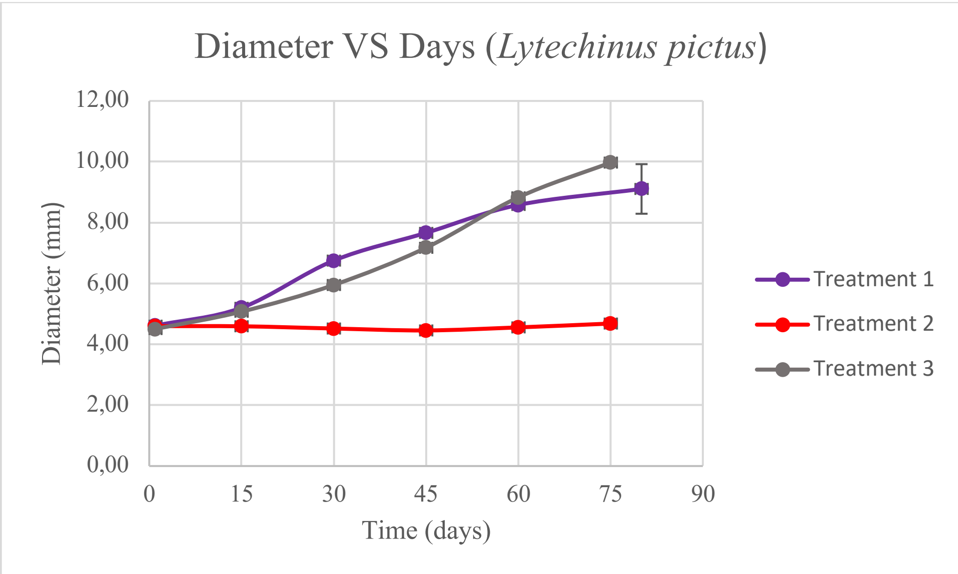

Figure 9. Mean diameter (mm) of all sea urchins with their respective standard deviations during the period (days) of the experiment in each treatment. In treatment 1 the food was formulated food, in treatment 2 was *Macrocystis pyrifera* and in treatment 3 was a combintation of both of them.

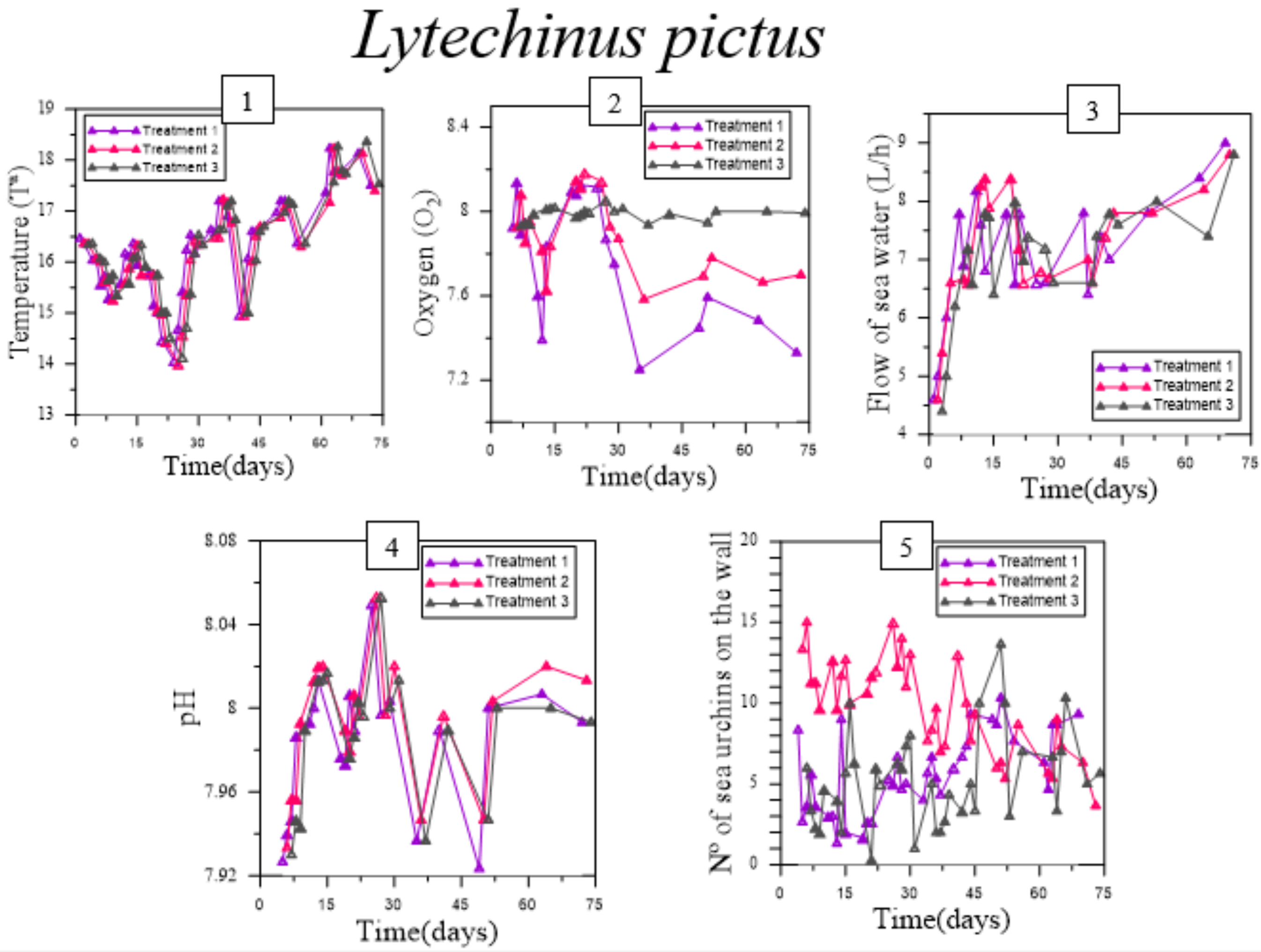

Figure 10. Values of temperature, oxygen, flow of sea water, pH and number of sea water on the wall during the experiment for each treatment.

Behavior of *Lytechinus pictus*

Lytechinus pictus is an active species, photos were taken in the morning (between 10:00-13:00 hours) and at night (between 22:00-01:00 hours), it was observed in all treatments that when sea urchins had food, in both periods they were scattered and possibly looking for food. Nevertheless, when the sea urchins had no access to food, most of them were on the wall in all the food most of them were on the wall in all the treatments (Figure 11.). It was also observed that *Lytechinus pictus* presents a random distribution.

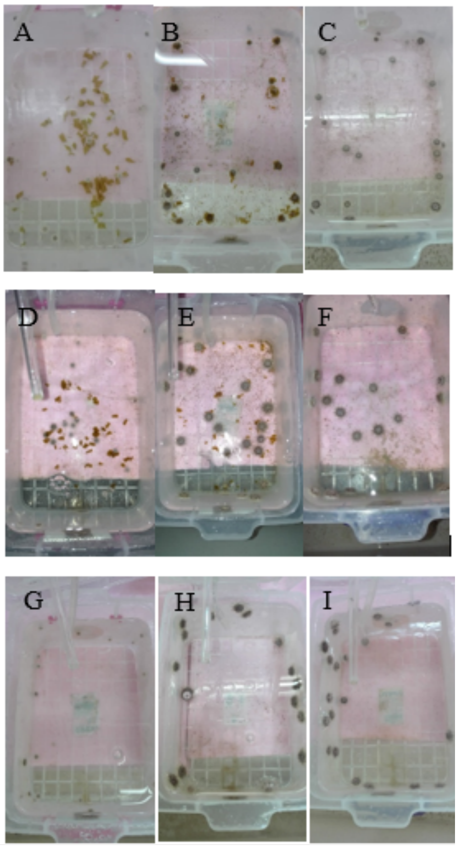

Figure 11. *Lytechinus pictus* when had food*:* A in treatment 2 in the morning, B in treatment 3 in the morning, C in treatment 1 in the morning, D in treatment 2 at night, B in treatment 3 at night, C in treatment 1 at night.

Lytechinus pictus when hadn't food: G in treatment 2 in the morning, H in treatment 3 in the morning, I in treatment 1 in the morning.

As curious observation, *Lytechinus pictus* presented *Macrocystis pyrifera* in the aboral part (Figure 12.). It's known that sea urchins present negative phototactism, so they to avoid high light intensities, they search for refuge between cracks and holes, also many may be placed themselves under shells or algae, as it is the case of *Lytechinus pictus.*

Figure 12. Algae is used by *Lytechinus pictus* to take refuge

5.2. *Arbacia stellata*

At 75 days of the experiment (Figure 13. and Table 4.), in all treatments the sea urchins presented an increase in mean weight, although at 30 days with *Macrocystis pyrifera* diet (treatment 2) the mean weight remained (of mean = 0.046 sd. ± 0.003 to mean = 0.046 sd. ± 0.003), at 45 days showed a decrease (mean = 0.038 sd. ± 0.000) and then, it increased. The sea urchins in treatment 1, at first, presented a greater increase in weight, but at 75 days they showed a greater increase in treatment 3. With respect to size (Figure 14. and Table 5.) only in treatment 2 the sea urchins presented a decrese in the diameter at 45 days (of mean = 4.40 sd. ± 0.08 to mean = 4.19 sd. ± 0.01), then, it increased again. The sea urchins in treatment 1 also presented a greater increase in diameter, but at 60 days and at the end they showed a greater increase in treatment 3. As for the standard deviations (Figures 13. and 14., Tables 4. and 5.), these are negligible with respect to the mean. This means that the variation in weight and diameter was unequal.

Table 4. Mean weight (grams) of all sea urchins *Arbacia stellata* with their respective standard deviations during the period (days) of the experiment in each treatment. In treatment 1 the food was formulated food, in treatment 2 was *Macrocystis pyrifera* and in treatment 3 was a combintation of both of them.

Weight and size of *Arbacia stellata*.

	Treatment 1		Treatment 2		Treatment 3	
Days	Weight (g)	Desv	Weight (g)	Desv	Weight (g)	Desv
1	0.041	0.001	0.042	0.002	0.044	0.004
15	0.059	0.001	0.046	0.002	0.068	0.014
30	0.121	0.005	0.046	0.003	0.101	0.006
45	0.208	0.016	0.038	0.000	0.172	0.008
60	0.401	0.024	0.044	0.003	0.343	0.017
75	0.657	0.164	0.050	0.002	0.687	0.013

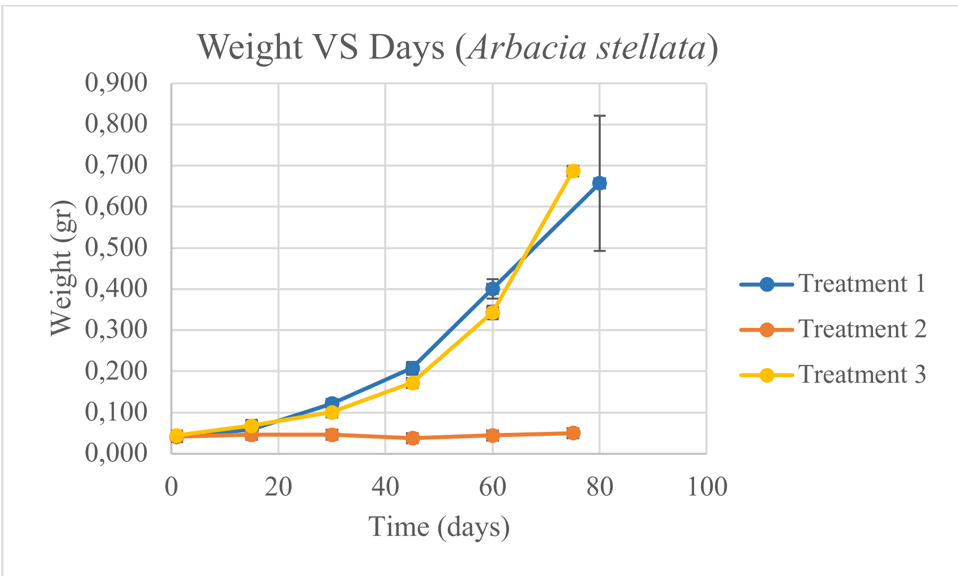

Figure 13. Mean weight (grams) of all sea urchins with their respective standard deviation during the period of the experiment in each treatment. In treatment 1 the food was formulated food, in treatment 2 was *Macrocystis pyrifera* and in treatment 3 was a combintation of both of them.

Table 5. Mean diameter (millimeters) of all sea urchins *Arbacia stellata* with their respective standard deviations during the period (days) of the experiment in each treatment. In treatment 1 the food was formulated food, in treatment 2 was *Macrocystis pyrifera* and in treatment 3 was a combintation of both.

	Treatment 1		Treatment 2		Treatment 3	
Days	Diameter (mm)	Desv	Diameter (mm)	Desv	Diameter (mm)	Desv
1	4.29	0.10	4.34	0.09	4.28	0.04
15	4.91	0.06	4.36	0.11	4.96	0.10
30	6.36	0.15	4.40	0.08	6.05	0.08
45	7.65	0.17	4.19	0.01	7.16	0.12
60	9.03	0.15	4.24	0.08	9.18	0.18
75	11.13	1.14	4.55	0.02	11.37	0.17

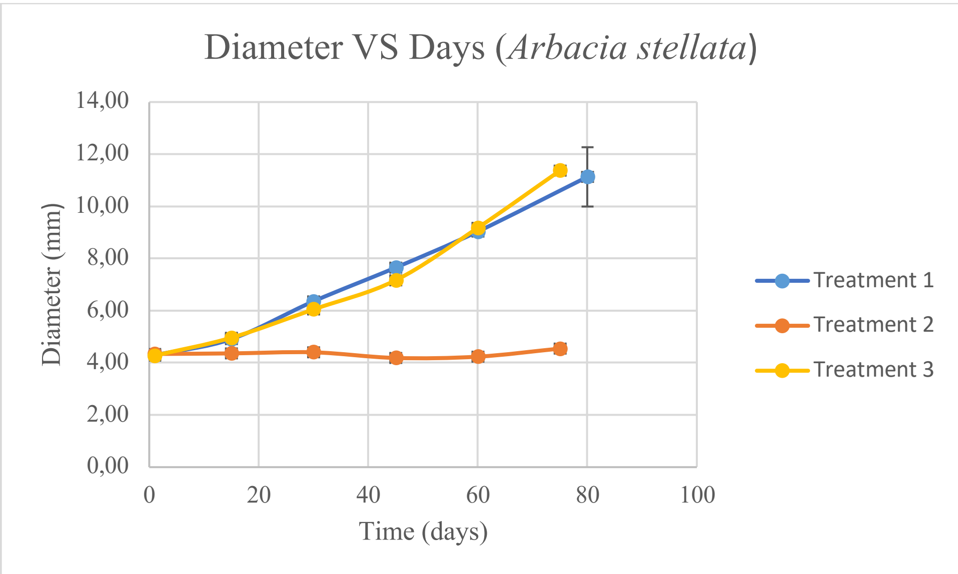

Figure 14. Mean diameter (mm) of all sea urchins with their respective standard deviation during the period of the experiment in each treatment. In treatment 1 the food was formulated food, in treatment 2 was *Macrocystis pyrifera* and in treatment 3 was a combintation of both of them.

With regard to water quality (Figure 15.), approximately Tª, O_2 and pH followed the same pattern in all treatments and the values were in a similar range. The range of Tª in treatment 1 was of 14.13 to 18.20 ºC, in treatment 2 was of 14.10 to 18.23 ºC and in treatment 3 was of 14.10 to 18.20 ºC. The range of O_2 in treatment 1 was of 7.09 to 8.17 mg/L, in treatment 2 was of 7.62 to 8.63 mg/L and in treatment 3 was of 7.14 to 8.14 mg/L. It is observed that the value of O_2 decreased with the time, this may be due to the difference in size of sea urchins between treatments. Furthermore, in spite of the oxygen value followed the same pattern for all treatments, throughout the experiment the oxygen value was lower in treatment 1 and higher in treatment 2.The range pH in treatment 1 was of 7.92 to 8.05, in treatment 2 was of 7.94 to 8.08 and in treatment 3 was of 7.94 to 8.06. The sea water flow values are different in some days, but in general they follow the same pattern too. The range of the sea water flow in treatment 1 was of 5.0 to 9.2 L/h, in treatment 2 was of 5.0 to 9.0 L/h, in treatment 3 was of 4.4 to 8.8 L/h. The parameter that does not follow the same pattern in all treatments is the number of sea urchins on the wall. The range of the number of sea urchins on the wal in treatment 1 was of 0 to 7, in treatment 2 was of 0 to 6 and in treatment 3 was of 0 to 7. Initially, there were more sea urchins on the wall in treatment 2 than in the other two treatments, but at the end it was the other way around.

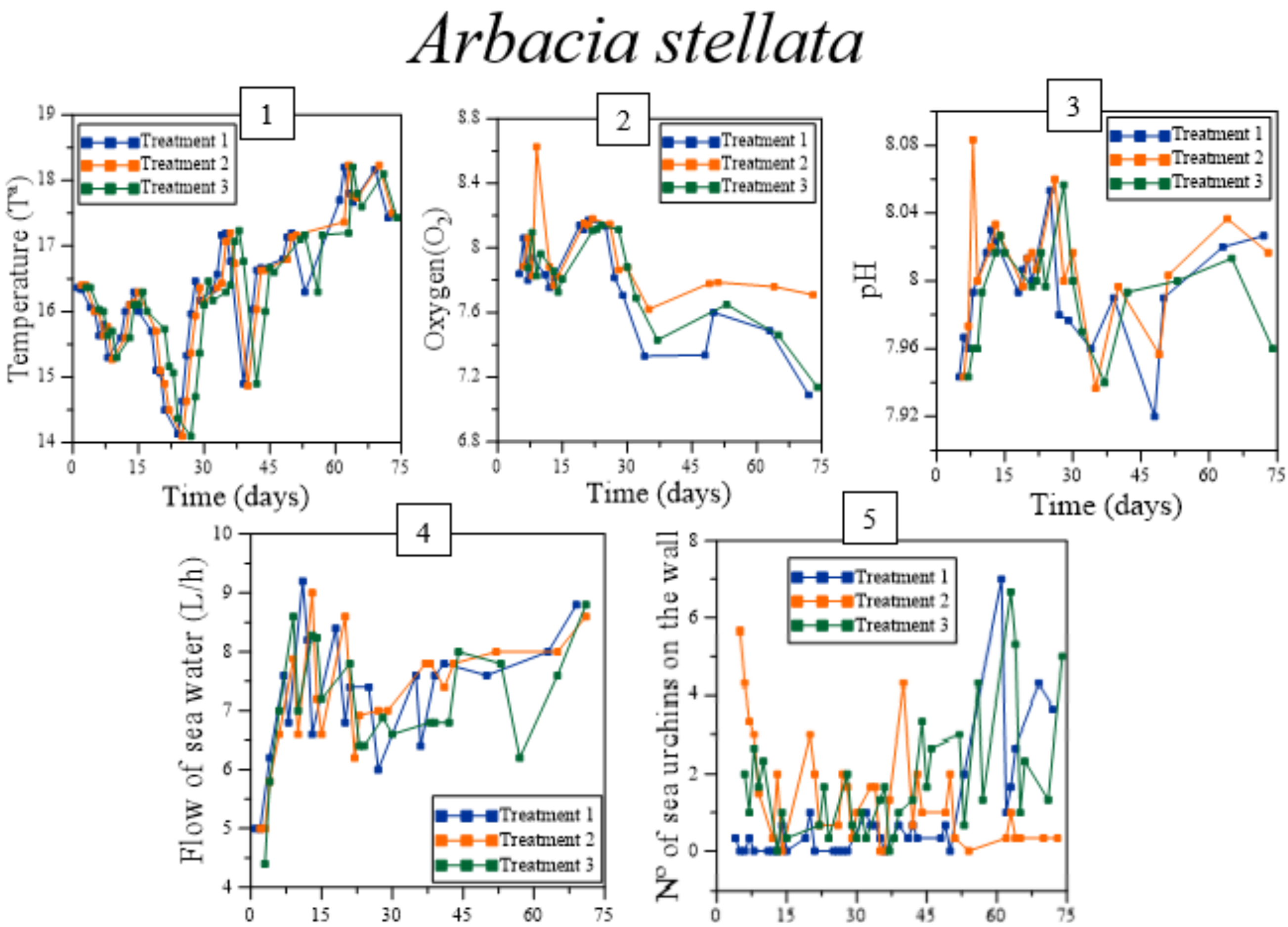

Figure 15. Values of temperature, oxygen, flow of sea water, pH and number of sea wáter on the wall during the experiment for each treatment.

Behavior of *Arbacia stellata*

Photos were taken in the morning (between 10:00-13:00 hours) and at night (between 22:00-01:00 hours) (Figure 16.). When the sea urchins had food, differences in their behavior were observed. In the treatment 1 and 3, at the night sea urchins were scattered while on the day they were close to the wall if they had food or not. In treatment 2, apparently in both periods the sea urchins had the same behavior, scattered. Nevertheless, when sea urchins did not have food, in treatment 2 they were close to the wall. It was also observed that *Arbacia stellata* presents an acute distribution.

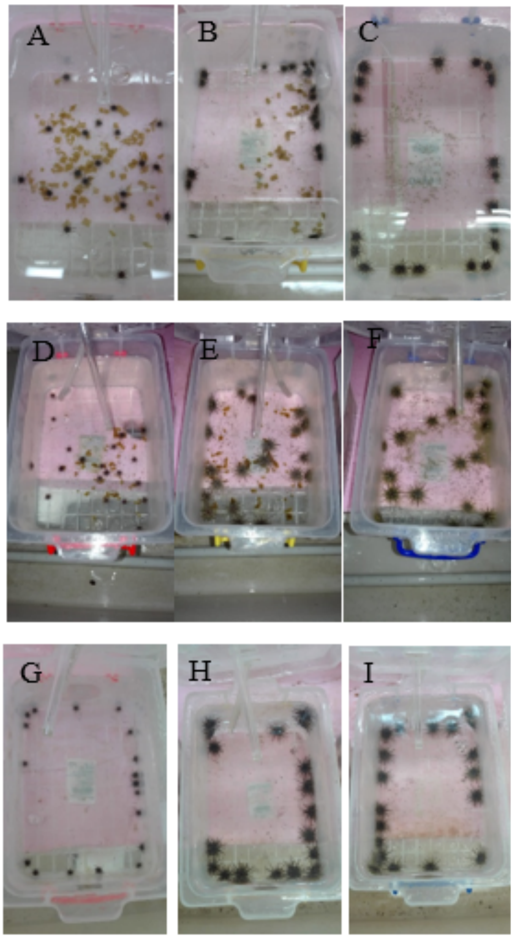

Figure 16 . *Arbacia stellata* when it had food*:* A in treatment 2 in the morning, B in treatment 3 in the morning, C in treatment 1 in the morning, D in treatment 2 at the night, B in treatment 3 at the night, C in treatment 1 at the night.

Arbacia stellata when it did not food: G in treatment 2 in the morning, H in treatment 3 in the morning, I in treatment 1 in the morning.

6. Discussion

Lytechinus pictus

Sea urchins may be attracted easier by strong smell of formulated food than by smell of *Macrocystis pyrifera*, this is reflected in the number of sea urchins on the wall. Where formulated food was present, there were less sea urchins on the wall since food was on the ground. Due to sea urchins were on the wall, they were less able to find the food. It should be noted that when the food was increased to 10% (at 45 days), the number of urchins on the wall was reduced in treatment 2. In addition, sea urchins find easier food when their size is greater due to they are more active, this happened in the treatment 1 and 3. Growth of sea urchins was also greater in treatment 1 and 3 than in treatment 2 due to difference between composition of food. Protein is an important macro-nutrient, that provides essential amino acids for growth, maintenance, and reproduction among sea urchins (Hammer et al. 2004; Hammer et al. 2006; 2012; Heflin et al. 2012; Heflin 2015). But an excess of a nutrient generally requires additional energy to process and either store or excrete, whereas a deficit of a specific nutrient will likely limit metabolic processes (Heflin et al, 2016). Moreover, Taylo (2017) concluded that consumption of excess protein should be avoided, as nitrogenous waste contributes greatly to water fouling (Basuyaux and Mathieu 1999) and excess dietary protein may contribute a bitter taste to the developing gonad (Pearce et al. 2002; Woods et al. 2008). Furthermore, with high lipid content the growth of juvenile *Lytechinus variegatus* showed a negative correlation (Gibbs et al., 2009*).* In this study formulated food content more lipids tan *Macrocystis pyrifera.* For that reason, growth of sea urchins was greater in treatment 3 than in treatment 1, because in treatment 3 the diet used is more balanced, without excess of a nutrient. Two sea urchins *Lytechinus pictus*, ones in treatment 1 and other in treatment 3 presented a bag in the anal part, the reason is not known since previous studies were not found (Figure 13.). Also it is noticed that *Lytechinus pictus* had more vertical growth in treatment 1 than in the treatment 3.

Figure 17. Sea urchin of *Lytechinus pictus* presented a bag in the anal part,

Arbacia stellata

Growth of *Arbacia stellata* in all treatments follows the same pattern that *Lytechinus pictus*. But if the results of both species are observed, gowth of *Arbacia stellata* was greater in treatment 1 and 3 than growth of *Lytechinus pictus*. However in treatment 2 the growth was parallel. Really, these results are expected since *Arbacia* is a genus known for its voracity. A possible cause of its greater growth is that *A.stellata* is less active than *L. pictus*, therefore, it spends less energy on mobility. In addition, *A. stellata* lacks tertiary spines, so it also saves energy expenditure in the growth of these spines, in contrast, *Lytechinus pictus* does have tertiary spines. *Arbacia* uses energy in the development of its thick spines and a strong shell. It would be interesting to make a comparison in the intake rate.

7. Preliminary conclusions

The type of food affected the growth of juveniles *A. stellata* and *L.pictus.* Urchins (*A. stellata and L. pictus*) in all dietary treatments grew and survival was 100%, indicating that all diets were adequate for maintenance and growth.

Our results shows reasonable growth the high content of protein and low content of lipids. Growth was highest when sea urchins were fed with artificial food and kelp at the same time (treatment 3). In treatment 3 had not excess of a nutrient, artificial food is high in protein and lipids content in comparison with *Macrocystis pyrifera* that it is low in protein and lipids content. Results of Hammer (2004) shows that juvenile *Lytechinus variegatus* appear to require a minimum of 20- 21% dietary protein for optimal growth. In this study the artificial food has 45% of protein crude and *Macrocystis pyrifera* presents 8.4-14.2 % of crude protein.

Taken in consideration that the conditions in the laboratory are not exactly those in the natural environment, this study suggests a first approximation of the growth in the marine ecosystems from Baja California. Since both species of this study are interesting due to their presence in Baja California. To ensure survival, sea urchins must have food available and high quantities if it *is Macrocystis pyrifera,* kelp present in Baja California.

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Table.S.1. Represents the results of the Dunn's-test, which compares the distributions of the treatment for each variable (diameter and weight) for *Lytechinus pictus*. If the value is closer to 1 than to 0, they have a more similar distribution.

Supplementary material

	Treatment 1	Treatment 2
(Diameter) treatment 2	0.0094	-
(Diameter) treatment 3	0.7456	0.0231
(Weight) treatment 2	0.023	-
(Weight) treatment 3	0.745	0.051

Table.S.2. Represents the results of the Dunn's-test, which compares the distributions of the treatment for each variable (diameter and weight) for *Arbacia stellata*. If the value is closer to 1 than to 0, they have a more similar distribution.

	Treatment 1	Treatment 2
(Diameter) treatment 2	0.0028	-
(Diameter) treatment 3	0.935	0.023
(Weight) treatment 2	0.023	-
(Weight) treatment 3	1.000	0.023

Información añadida

• Descripción detallada de las actividades desarrolladas durante la realización del TFT

En primer lugar, con ayuda del técnico de laboratorio, Marco, se preparó el escenario donde iba a tener lugar el experimento. Se pensó la mejor forma para organizar y como hacer los 20 pequeños tanques en los cuales iban a mantenerse los erizos de mar. Cada recipiente con un flujo de agua de entrada y otro de salida, al igual que un flujo de aire. Para esto fue se utilizó un traladro, gomas, tuppers, tubería, red de malla y cúter.

En segundo lugar, otras actividades que se llevaron a cabo fue el mantenimiento de los pequeños tanques y alimentar a los erizos de mar; así como los parámetros del agua (Tª, pH, O2 y flujo de agua de mar) y las medidas de los erizos de mar (talla y peso).

Por último, respecto a la realización por escrito del TFT, aprender a organizar mis ideas y plasmarlas en el papel fue un proceso progresivo en el que me ayudó el Doctor Eugenio. Una vez escrito el TFT, la Doctora María Dolores Gelado me ayudó a perfeccionar detalles del mismo.

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

En este experimento se utilizó el programa R y el programa Gapher, los cuales aprendí en la ULPGC. También se utilizó Excel y Microsoft Word.

• Nivel de integración e implicación dentro del departamento y relaciones con el personal.

La integración fue muy buena. Dentro del Laboratorio de Ecología y Biología del Desarrollo de respiraba compañerismo y profesionalidad. Cada lunes nos reuníamos todos los integrantes del laboratorio para hablar sobre como iban nuestros proyectos y para organizar las actividades de la semana.

• Aspectos positivos y negativos más significativos relacionados con el desarrollo del TFT

Como aspecto negativo decir que es difícil organizar las ideas y valorar que información está de más y cuál no.

En cuanto a aspectos positivos, todo el trabajo en sí fue positivo: las ganas, los compañeros, el lugar, el tema del trabajo…

• Valoración personal del aprendizaje conseguido a lo largo del TFT

Aprendí a saber lo que conlleva realizar un proyecto. Aprendí a organizar mis ideas y representarlas, a trabajar en equipo… Tuve libertad en el laboratorio y me ayudó a tener iniciativa en el trabajo.