

Research Article

Diet of the land vegetable *Lactuca sativa* as an alternative to macroalgae for the growth of juvenile the sea urchin *Loxechinus albus* in northern Chile

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ABSTRACT. The sea urchin *Loxechinus albus* is of great commercial value due to its nutritional content. The main diet of the urchin in the north of Chile in its natural habitat is the macroalgae *Ulva lactuca*. This work evaluated the growth of *L. albus* juvenile fed diets based on vegetable lettuce *Lactuca sativa* and macroalgae *Ulva* sp. Ingestion, growth, survival, and proximal analysis of the diets were registered for 13 weeks. At the end of the experience (90 days), no mortality and greater consumption of *L. sativa* were observed. In the proximal analysis, *L. sativa* presented the highest percentage (lipids, proteins, ashes, carotenoids, chlorophyll). The greatest growth and weight gain were observed with the *Ulva* sp. diet. Finally, the incorporation of *L. sativa* in the diets of urchins would imply a supplement of minerals and carotenoids, so we propose the addition of this lettuce as a nutritional supplement during the first stage of the cultivation of the *L. albus*.

Keywords: *Loxechinus albus*; *Lactuca sativa*; *Ulva* sp.; nutritional supplement; sea urchin seeds; aquaculture

INTRODUCTION

The red sea urchin *Loxechinus albus*, the echinoderm of greater commercial value in Chile, is important in the world market. However, this species is dependent on algae as a source of food, which has been overexploited in Chile (SERNAPESCA 2006), mainly due to the export to Asian markets for the development of cosmetic and pharmaceutical products, for example, total algae landings of 259,320 t were recorded in 2018 increasing to 428,848 t in 2020 (SERNAPESCA 2018, 2020). The shortage of macroalgae influences the abundance of natural shelters for larvae and juveniles. On the other hand, the decrease in the extraction size (the legal minimum size is 70 mm) of the sea urchin impacts the reproduction of this species. In Chile, the export of the sea urchin started in the 1970s, after which the catches increased gradually; for example, 60,166 t were recorded in 2002 (SERNAPESCA 2003). In 2020, a decrease in disembarkation was observed, reaching 37,464 t (SERNAPESCA 2020). Efforts to maintain the ecological balance of this species have led to the imple-

mentation of extractive closures with a consequent economic impact on local fishers since its extraction is prohibited (SERNAPESCA 2020).

González et al. (2008) suggest that *L. albus* has differential feeding depending on age. The juveniles mainly assimilate the macroalgae *Ulva* sp., while adults mainly consume *Lessonia* sp. Sea urchins consume macroalgae tissues and the microfauna attached to the blades, such as small animals and several epiphytes (Mazzella et al. 1992). In addition, each sea urchin adapts to local ecological conditions and develops specific dietary patterns (Lawrence 2007).

The main bottleneck that has been identified in sea urchin cultivation is its dependency on local seaweed (Cipriano-Maack 2016). The long period it takes to reach commercial size (Bustos et al. 1990, Lawrence 2007, Cárcamo 2015) may be influenced by quantity, and quality (Fernández & Boudouresque 2000), and food frequency (Lawrence et al. 2003), affecting its physiology and growth. Food in the aquaculture industry represents 50% of the total production costs, emphasizing the need to attract various economic food

resources into production on a large scale to meet the high costs incurred by the supply of natural products (Zupo et al. 2019).

New research has proposed an initiative to incorporate terrestrial vegetables into the sea urchin diet, which are of commercial value in the settlement phase (Turchini et al. 2009). In this context, the constant exploitation of available terrestrial vegetables seems to be a good alternative to macroalgae for the sea urchin diet (Vizzini et al. 2015, 2018, 2019). Vizzini et al. (2015) evaluated terrestrial vegetables such as *Beta vulgaris* (beet), *Brassica oleracea* (cabbage), and *Lactuca sativa* (lettuce) as diets for the sea urchin *Paracentrotus lividus* and tested *L. sativa* as an agricultural discard. Vizzini et al. (2019) also evaluated new feed formulations for the sea urchin based primarily on vegetable leaves. Consequently, it is necessary to find alternative diets that promote the growth of organisms and obtain a product similar, equivalent, or superior to the wild product with low production costs according to the market's demands.

This study is the first to evaluate the use of lettuce as a diet for the sea urchin *L. albus* in northern Chile. In this work, the intake, growth, and survival during the early growth stages of *L. albus* fed with different diets of the terrestrial vegetable lettuce *L. sativa*, macroalgae *Ulva* sp., and a mixture of both in order to offer a new alternative for the diet of the sea urchin *L. albus* in northern Chile. Developing the cultivation of the sea urchin *L. albus* in controlled environments with alternative diets to macroalgae would improve the sustainability of this resource.

MATERIALS AND METHODS

Obtaining seeds and distribution of organisms

The seeds of red sea urchin *Loxechinus albus* ($n = 54$) with an average diameter of 13 mm, were obtained from the hatchery Bioinnovation Center of Antofagasta University. Nine PVC baskets of 160 mm diameter and 150 mm height were manufactured. The side faces had holes of 8 mm diameter, with four rectangle windows, two of 7×9 cm and two of 4×9 cm, to allow the flow of water, and the bottom, was covered with sieve mesh (Nylon Sefar Switzerland NUM 42 GR GG 450 μm). In addition, they contained three bases with proper heights (4 cm) for the flow of water (Fig. 1). Nine square black-bottom, curved edges ponds with a conical bottom of 250 L capacity contained 40 L of seawater filtered at 1 μm . The ponds were exposed to strong constant aeration and photoperiod of 12 h light:12 h dark (artificial). The specimens were weighed, grouped, and distributed equally (the biomass

per basket was similar). Eighteen urchins were used for each treatment and control, then three groups were divided into baskets with six specimens, and each replica was added to a pond (Fig. 2). A 100% weekly water change in each pond was performed, and every other day 50% of water filtered to 1 μm was renewed, and constant aeration was maintained. The experiment lasted 90 days, and intake, size, weight, survival, temperature, pH, and oxygen were recorded weekly using multiparameter equipment (Thermo scientific, Model Orion star A329).

Food

The macroalgae *Ulva* sp. was collected from the rocky intertidal shore at Antofagasta Bay 23°42'09.9"S, 70°25'29.3"W and used as food on the same day. Lettuce *Lactuca sativa* was obtained from a local supermarket. Before the experiment, the urchins were deprived of food for one week. A stainless steel bar was placed on them to prevent leaves from floating. The following diets were used: *Ulva* sp. (control), MIX *L. sativa* + *Ulva* sp. (treatment 1), and *L. sativa* without stem (treatment 2). In the MIX treatment, the same wet weight of macroalga and lettuce was added with a 1:1 (*L. sativa*: *Ulva* sp.) ratio. The urchins were fed with fresh leaves previously washed every seven days. In the first seven weeks, 10 g were added to each diet, and in the next six weeks, 20 g to each diet due to the increased consumption of the urchins (Table 1). All the food not consumed was replaced by fresh food at the end of the week.

Proximal analysis of diets

Lipids were determined weekly according to the modified method of Kochert (1978). About 100 mg of alumina was added to 100 mg of lyophilized sample (Labconco, Freezone 18 L console freeze dry system, serial 070267131). After lyophilization, the sample was milled, and the amounts of lipids extracted were determined by evaporating the solvents. Alumina was used to increase the adsorption capacity of lipids. It was quantified by weight differences on an analytical balance (Radwag AS 220/C/2), and its content is determined with the following equation:

$$\% \text{ Lipids} = \frac{(\text{final total weight} - \text{initial total weight})}{\text{biomass weight}} \times 100$$

Proteins were determined weekly using the colorimetric method of Lowry et al. (1951) modified by Herbert et al. (1971). The calibration curve was prepared using a bovine albumin standard with a concentration of 1000 mg L⁻¹, which was diluted to obtain concentrations in the range of 50-400 mg L⁻¹.

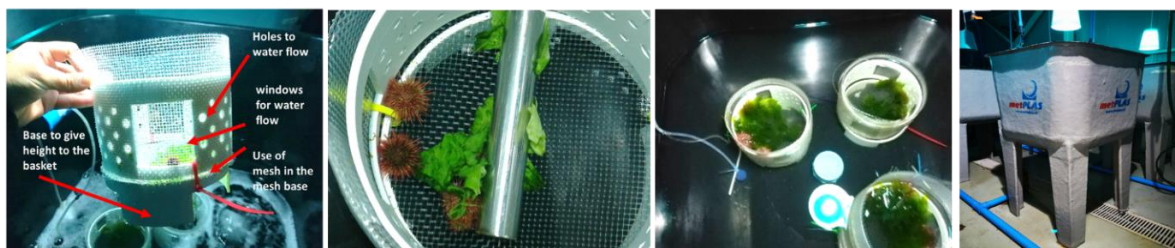


Figure 1. Experimental baskets and cultivating pond where urchins *Loxechinus albus* and food were maintained.

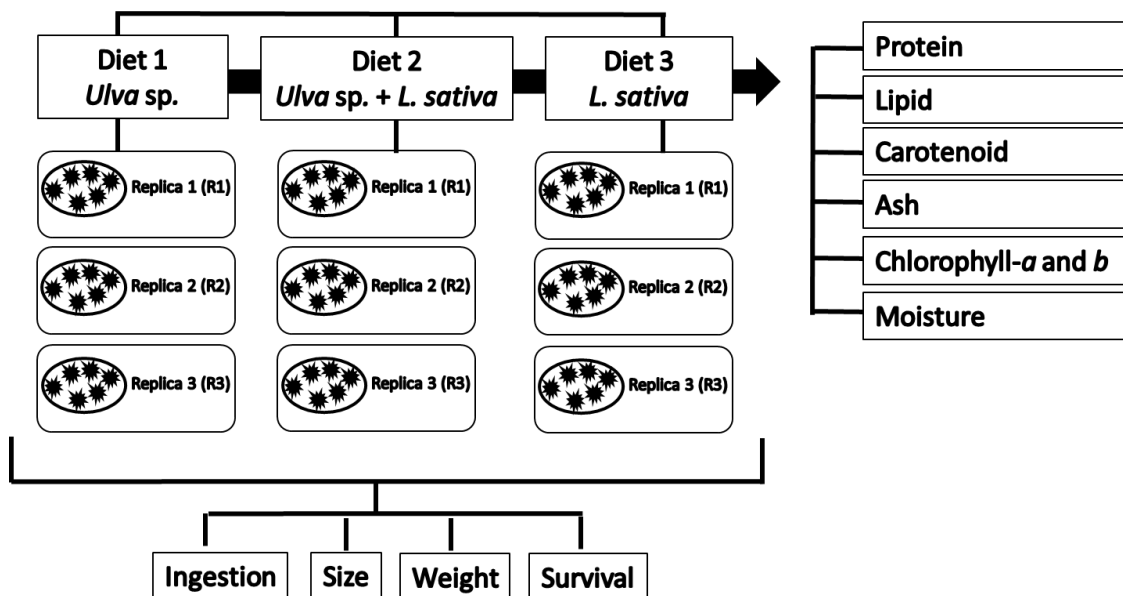


Figure 2. Experimental design. Treatments, control, and parameters were evaluated for 90 days.

Linear regression was applied to the absorbance values obtained from the protein concentrations in mg L^{-1} .

For ashes, the modified method of Daggett et al. (2005) was used. Approximately 0.01 g of lyophilized biomass was weighed and placed in the oven at 105°C (Memmert, MU 600-2400W) for approximately 2 h. It was then incinerated in a muffle (Thermolyne F6010) at 540°C for approximately 2 h until a constant weight was obtained. The sample's inorganic weight or ash content was obtained by weight difference (AOAC 2005). The ash log was taken every two weeks.

The Lichtenthaler & Welburn (1983) method was used to calculate concentrations. Carotenoids and chlorophyll-*a* and *b*, about 15 mg of the lyophilized biomass were weighed, added to 4 mL of methanol, and incubated in a water bath at 60°C for 15 min. The methanolic extract was centrifuged at 2800 rpm for 5 min. The supernatant was recovered, and the extract was measured using a spectrophotometer at 470, 653, and 666 nm (Halo RB-10 UV-VIS Ratio Beam). Registration was done once a week.

The moisture of around 3 g of biomass was weighed and placed in the oven at 105°C for approximately 4 h. It was then left in a desiccator to cool and weighed again. The moisture was determined by calculating the initial and final weight difference and expressed as a percentage. Registration was made every two weeks.

Feed intake

The acceptance of the different diets was evaluated weekly. According to Daggett et al. (2005), the intake rate was evaluated by recording the weight of food consumed by the urchins. For the MIX diet, the leaves were collected separately (*Ulva sp.* and *L. sativa*). The formula was used for each species, and the total intake was calculated by adding all values together.

Growth and survival rates

The growth rate in testa diameter (mm month^{-1}) was recorded weekly and evaluated using the method of Cook & Kelly (2009). The weight (g) was recorded weekly on a digital scale. Recordings were made for

Table 1. Food (g) added to *Loxechinus albus* seeds in the different diets.

Week	Control (<i>Ulva</i> sp.)	Treatment 1 (<i>Ulva</i> sp. + <i>L. sativa</i>)	Treatment 2 (<i>Lactuca sativa</i>)
1	10	5	5
2	10	5	5
3	10	5	5
4	10	5	5
5	10	5	5
6	10	5	5
7	10	5	5
8	20	10	10
9	20	10	10
10	20	10	10
11	20	10	10
12	20	10	10
13	20	10	10

each individual in treatments and control groups. The growth rate was assessed based on the difference in weight between the final and initial time. The survival of the individuals was evaluated at the end of the experiment according to the following formula:

Survival (%): (final individuals \times 100%) / initial individuals

Statistical analysis

The data were analyzed using the GraphPad Prism[®] 5.0 statistical software (GraphPad Software, Inc., San Diego, USA). The Shapiro Wilks test was used to assess the assumption of normality, and the Levene test for homoscedasticity. Differences were tested using one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison test. Statistical significance was set at $P < 0.05$.

RESULTS

The water temperature of the culture was maintained between 16 to 18°C during the experience. The pH and oxygen of the culture water were maintained at 8 and 9 mg mL⁻¹, respectively. The survival rate of the sea urchin *Loxechinus albus* was 100% during the experiment. The qualitative observation of sea urchins showed that they were healthy with good coloration and spines without discoloration or detachment (Fig. 3).

Proximal analysis of diets

The proximal analyses performed weekly on the different species used as food showed higher in *Lactuca sativa* concerning *Ulva* sp. in lipids, proteins, ashes, carotenoids, and chlorophyll except in moisture (Table 2).

Ingestion analysis

The acceptance of the different diets by the sea urchins was evaluated by weekly ingestion. After seven days, the uneaten leaves were collected. In the case of the MIX diets (*Ulva* sp. + *L. sativa*), the leaves were collected separately, the formula was applied for each species (macroalgae and plant), and the total intake of the MIX diet was added.

The sea urchins fed the diets showed that the highest food intake during all the experimental weeks was observed with the diet of the terrestrial vegetable lettuce *L. sativa* with an average intake range of 0.09 to 0.18 g d⁻¹. The MIX was the second preferred diet of the sea urchins during all the experimental weeks with an average intake of 0.07 to 0.12 g d⁻¹, followed by *Ulva* sp. with an average intake between 0.03 to 0.08 g d⁻¹.

The data obtained show that sea urchins mostly consumed the land lettuce. This preference is also demonstrated in the MIX diet, where it was observed that of the two options of lettuce leaves added in the MIX, the sea urchins mostly consumed the land lettuce *L. sativa*. During the 13 experimental weeks, significant differences ($P < 0.001$) in consumption were observed between *Ulva* sp. and *L. sativa*. The MIX did not present significant differences in consumption concerning *L. sativa* in weeks 7 and 10 ($P > 0.05$). Equally, during all the experimental weeks, no significant differences were observed in consumption between MIX and *Ulva* sp. ($P > 0.05$) (Fig. 4).

Growth analysis

At the end of the experience, significant differences in the weight were observed in the individuals fed with the different diets *Ulva* sp. vs. MIX ($P < 0.05$), *Ulva* sp. vs.

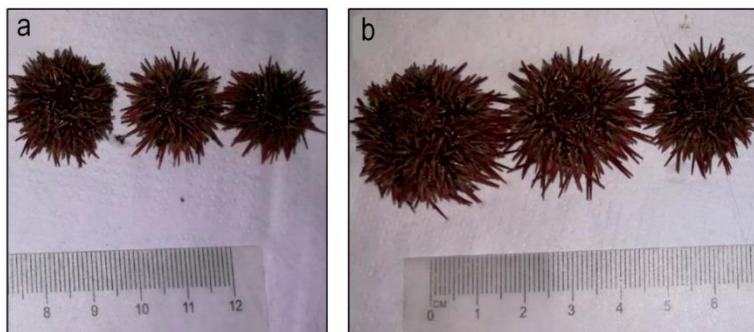


Figure 3. Size and morphology of sea urchins *Loxechinus albus*. a) Size of the sea urchins at the beginning of the experiment, b) size of sea urchins at the end of the experiment.

Table 2. Proximal analysis of the macroalgae *Ulva* sp. and vegetable *Lactuca sativa*. Values are the average of the 13 weeks in which food was to the urchins \pm standard deviation.

Analysis (%)	<i>U. lactuca</i>	<i>L. sativa</i>
Lipids	3 \pm 0.9	7 \pm 1.2
Proteins	15 \pm 0.5	21 \pm 0.9
Ashes	40 \pm 3.9	42 \pm 2.3
Carotenoids	0.1 \pm 0.0	0.2 \pm 0.0
Chlorophyll- <i>a</i>	0.1 \pm 0.0	0.3 \pm 0.0
Chlorophyll- <i>b</i>	0.1 \pm 0.0	0.2 \pm 0.0
Moisture	89 \pm 0.0	87 \pm 0.0

L. sativa ($P < 0.001$), and MIX vs. *L. sativa* ($P < 0.01$) (Fig. 5). The weight gain (g) was recorded every seven days, and a gradual increase was observed in all treatments during the experiment. The greatest gain (23 g) was obtained in the *Ulva* sp. diet, followed by the MIX with 20 g and the *L. sativa* with 14 g (Fig. 5). The growth of testa was 8 mm in diameter in all diets. The average growth rates were 22, 21, and 20 mm, for *Ulva* sp., MIX, and *L. sativa*, respectively. Due to urchin size variability at the beginning of the experiment, the average of the six urchins (by replica) was considered the initial diameter. Therefore, this result of growth was obtained by subtracting the testa diameter of the final group from the initial diameter; no significant differences ($P > 0.05$) were observed between different groups (Fig. 6). The growth rates in the sea urchin in mm month⁻¹ were 2.6, 2.7, and 2.4 for *Ulva* sp., MIX, and *L. sativa*, respectively.

DISCUSSION

The nutritional components of the diets of *Loxechinus albus* are fundamental to its metabolism and directly influence its growth, survival, and nutritional quality. The type of microalgae used as food and the supply

frequency directly and positively influence large-scale larval and juvenile production of *L. albus* (Cárcamo et al. 2005). Studies have been reported using vegetables to cultivate the sea urchin (Hur 1988, Robinson et al. 1997, Luo et al. 2014, Moreira dos Santos 2017). In this paper, the lettuce *Lactuca sativa* had a higher protein concentration (21%) and lower lipids content (7%). Similarly, Rasyid (2017) reported high protein and low fat in lettuce. Vizzini et al. (2018) found 19% protein and 9% lipids in *L. sativa*. In addition, the lettuce had high percentages of C18 fatty acid (Le Guedard et al. 2009), precursors of (n-3) and (n-6) (Castell et al. 2004). A 15% protein content recorded in the macroalga *Ulva* sp. was similar to that reported by 14% (Xiao-Ling et al. 2003), 16% (Ortiz et al. 2006), 37% (Abirami & Kowsalya, 2011), 13% (Abdel-Khaliq et al. 2014), and 18% (Rasyid 2017). The average lipid content of 3% was recorded in *Ulva* sp., higher than 1.2%, as reported by Xiao-Ling et al. (2003) and Abirami & Kowsalya (2011).

In *L. sativa*, 42% of the ash content was obtained, more than twice those reported by Fernández (2005), who reported 18%, and Zambrana & Vega (2016), who found ashes of 12% in *L. sativa* irrigated with drinking water. The average ash content for the macroalga *Ulva* sp., obtained from the results of the present study, was 40%. In comparison, the literature has reported different values such as 53% (Castro-González et al. 1996), 11% (Ortiz et al. 2006, Rasyid et al. 2017), and 27% (D'Armas et al. 2019). D'Armas et al. (2019) indicate that the high level of ash content is important for human nutrition and in the food industry.

Caldwell & Britz (2006) reported high concentrations of carotenoids in lettuce. The percentages of carotenoids in *Ulva* sp. were 0.1 and 0.2% for *L. sativa*. The average chlorophyll-*a* concentrations were 0.1% for *Ulva* sp. and 0.3% for *L. sativa*, while the average concentrations of chlorophyll-*b* were 0.1 and 0.2% for *Ulva* sp. and *L. sativa*, respectively. Caldwell & Britz

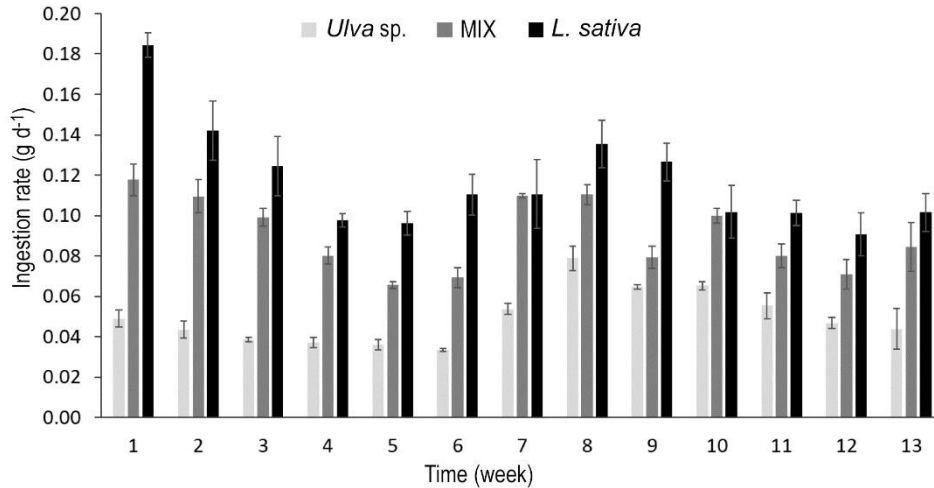


Figure 4. Ingestion rate of *Loxechinus albus* fed with the different diets, with $n = 18$ individuals for each treatment. Bars indicate standard error. Control: *Ulva* sp., Treatment 1: MIX (*Lactuca sativa* + *Ulva* sp.), and Treatment 2: *Lactuca sativa*.

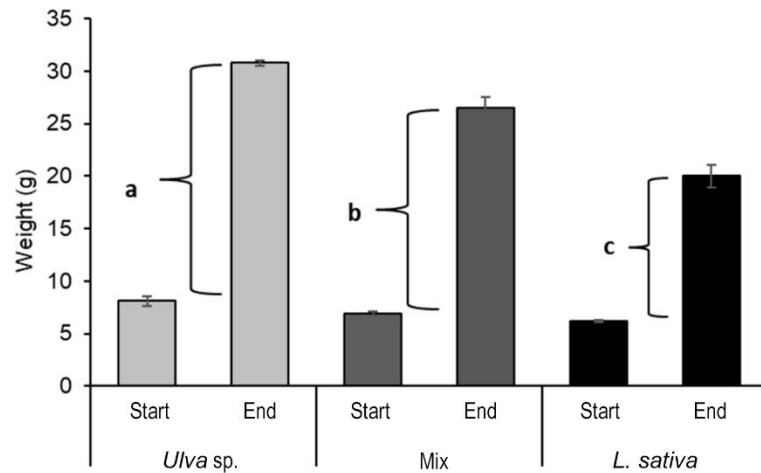


Figure 5. Comparison of weight (g) gained at the end of the experience (90 days) concerning the initial weight of *Loxechinus albus* fed with different diets, with $n = 18$ individuals for each treatment. Control: *Ulva* sp.; Treatment 1: MIX (*Lactuca sativa* + *Ulva* sp.), and Treatment 2: *Lactuca sativa*. Different letters indicate significant differences in the weight of organisms fed different diets *Ulva* sp. vs. MIX ($P < 0.05$), *Ulva* sp. vs. *L. sativa* ($P < 0.001$), and MIX vs. *L. sativa* ($P < 0.01$). Bars indicate standard error.

(2006) and Vizzini et al. (2015, 2018, 2019) confirmed that the sea urchin *Paracentrotus lividus* fed with the lettuce *L. sativa* under laboratory conditions, the color of the gonads was improved. The gonad coloration is derived mainly from carotenoid pigments, particularly β -equinenone, synthesized from β -caroteno by the sea urchin (Matsuno & Tsushima 2001, Shpigel et al. 2005).

In conventional agriculture, the published works on moisture for *L. sativa* lettuce register 92% (Fernández 2005). On the other hand, Zambrana & Vega (2016) reported 93% moisture in *L. sativa* irrigated with drinking water. This paper recorded the moisture

content of 87%, similar to that reported in the literature. The moisture contents reported by the literature are 12.6% (Ortiz et al. 2006), 17% (Rasyid 2017), and 21% (D'Armas et al. 2019) in the macroalga *U. lactuca*.

We obtained a value of 89% in *Ulva* sp., higher than that reported in the literature. In this regard, Rohani-Ghadikolalel et al. (2012) indicate that the moisture content is important for determining the shelf life and quality of processed seaweed foods since high moisture can promote the growth of microorganisms.

In this paper, no mortality was observed during the experiment. The water parameters, such as temperature and pH, were similar to those obtained by Vizzini et al.

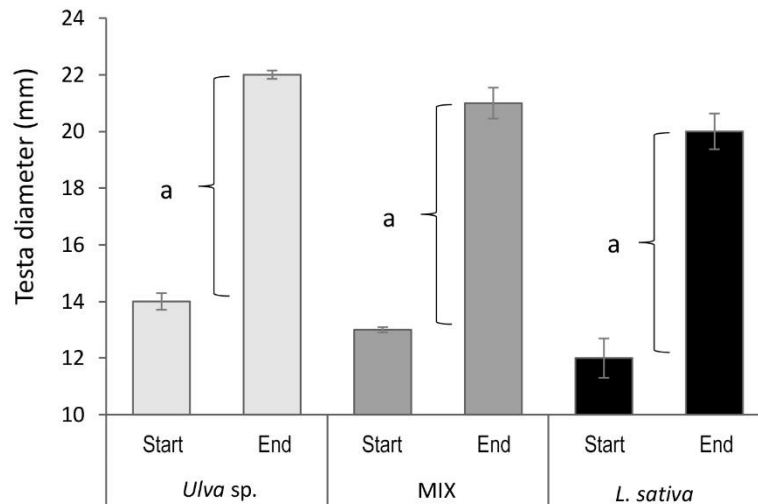


Figure 6. The average gain of testis diameter at the end of the experiment of *Loxechinus albus* organisms fed with the different diets, with $n = 18$ individuals for each treatment. Control: *Ulva* sp., Treatment 1: MIX (*Lactuca sativa* + *Ulva* sp.), and Treatment 2: *Lactuca sativa*. The letters (a) similarity indicates that no significant differences were observed in the diameter of the testis of the organisms fed with different diets. Bars indicate standard error.

(2015) and Moreira dos Santos (2017). The results of ingestion analysis showed that the sea urchin *L. albus* consumed mostly lettuce, followed by the MIX and the macroalgae diet. This preference can be because the texture of lettuce leaves changes and becomes softer after immersion in seawater compared to the macroalgae. In addition, bacterial colonization also can contribute to degrading the leaf and nutrients more rapidly (Egan et al. 2013) by producing enzymes involved in the degradation of polysaccharides (Kopel et al. 2014). These factors can provide greater ingestion of *L. albus*. Similarly, Vizzini et al. (2015) found that the lettuce *L. sativa* was the diet mostly consumed by the sea urchin *P. lividus* compared to beet, cabbage, and leaves of the macroalgae *U. lactuca*. Moreover, organisms fed with *L. sativa* maintained the gonadosomatic index (GI) and weight similar to the initial group.

This study observed the same growth in testis diameter for the sea urchin *L. albus* when consuming different diets. No significant differences in diets indicate that the consumption of macroalgae as a natural diet is fundamental for the growth of the testis. Other studies have reported variations in testis diameters and discussed that these differences are possibly genetic (Heflin et al. 2012). In this paper, the average initial testis diameter was 12-14 mm, with the growth rates of 2.57, 2.65, and 2.39 mm month⁻¹ for *Ulva* sp., MIX, and *L. sativa* diets, respectively. However, Cook & Kelly (2009) found that the initial testis diameter of the sea urchin *P. lividus* fed with the

macroalgae *Laminaria* spp. for 12 months fluctuated between 18-20 mm, with a growth rate of 1.9 mm month⁻¹. The values obtained in this study were greater than those reported in other studies on sea urchin species fed with macroalgae; the MIX and the *L. sativa* vegetable also increased the growth rate (mm month⁻¹). The biggest weight gain in the organism was obtained with the macroalgae, followed by the MIX and the lettuce. Although the proximal percentages were higher in the vegetable *L. sativa*, the highest weight was obtained with *Ulva* sp. It could indicate that other factors could influence the weight gain of sea urchins and the different diet components that could affect the nutritional quality of the gonad, the color of the gonad, and the GI.

Zupo et al. (2019) suggested food rich in carbohydrates for *P. lividus* from the early stages of growth till the sexual maturation and reported an increase in proteins for the development of the gonadal tissue, thus promoting the production of viable gametes and keeping lipids low. Baião et al. (2019) showed that the diet with low levels of protein (30%) and lipids (6%) had a higher yield of gonads in both sexes. Moreover, Lawrence (2007) indicates that carotenoids should be part of artificial diets because they influence pigmentation (Symonds et al. 2009, Suckling et al. 2011, Pilbrow et al. 2014).

The results obtained confirmed that terrestrial vegetables can be a viable alternative for the diets of *L. albus* in culture and thus meet the demand for macroal-

gae. The higher growth rate (g) obtained for the diet of the macroalgae *Ulva* sp. indicates that the macroalgae are an essential promoting factor in *L. albus* growth; however, the proximal analysis showed that lettuce has components that equally enhance the nutritional quality of the organism. Therefore, in addition to complementing the macroalgae diets, lettuce would help meet the demand for food in aquaculture centers. The results are positive and promising to continue studying the use of terrestrial vegetables as food for the sea urchin. However, further studies are required to analyze the quality and color of the gonad that meet the standards enforced by the market (Shpigel et al. 2018).

CONCLUSIONS

This study is the first in Chile to confirm the acceptance and preference of the lettuce *L. sativa* for the diet of the sea urchin *L. albus* as an alternative to the macroalgae *Ulva* sp. The growth in testa diameter did not show significant differences between the organisms fed with different diets. However, the diameters obtained were greater than those reported in another species. The weight gain exhibited significant differences between organisms fed with different diets. The *Ulva* sp. diet showed greater weight gain, indicating that nutritional components in the diet are directly related to the metabolism of the sea urchin, causing weight gain. The lettuce had high protein contents in the ranges reported in the literature. In this regard, the incorporation of lettuce would imply an added value since the protein content would not be reduced, and protein content is higher in lettuce than in the macroalgae. In addition, it provides minerals and carotenoids without increasing the product's calorie content. Other advantages of using the lettuce would be the annual availability, easy harvest, use of agricultural discards, the possibility of cultivation in sea urchin cultivation centers (e.g. aquaponics), and ease of consumption due to the quality of the leaf. Finally, based on the results obtained in this study, the lettuce *L. sativa* is proposed as a promising alternative food during the first stage of the cultivation of the sea urchin *L. albus*. Thus, it is considered a nutritious dietary component with low cost, easy maintenance, and easy ingestion.

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