

Comparison of the somatic growth of juvenile sea urchin *Paracentrotus lividus* (Lamarck, 1816) fed with experimental and commercial pre-growth diets Comparación del crecimiento somático de juveniles del erizo de mar *Paracentrotus lividus* (Lamarck, 1816) alimentados con dietas experimentales y comerciales de precrecimiento

Noelia Tourón¹ , Estefanía Paredes², Damián Costas¹

¹ Marine Research Center (CIM), University of Vigo, ECIMAT, 36331 Vigo, Spain

² Coastal Ecology Laboratory (ECOCOST) Ecology and Animal Biology Department. Marine Research Center (CIM)- University of Vigo. Spain

Correspondencia: Noelia Tourón **E-mail:** noelia.touron.besada@uvigo.es

Original article | Artículo original

Keywords

Echinodermata,
Grow out diets,
growth increase,
size,
weight

ABSTRACT | There is need to develop grow out diets for juveniles of the sea urchin *Paracentrotus lividus* allowing a reduction of hatchery time. In this study the increase in size and weight of juvenile *P. lividus* was measured after feeding them with 4 specifically designed diets, 2 commercial diets and a control based on the brown macroalga *Laminaria* sp. The experimental diets were prepared with tuna (HA) or insect flour (HI), using pork gelatin (HA-G, 31.3% protein; HI-G, 31.6%) or Agar (HA-A, 21.8%; HI-A, 21.6%) as gelling agent; commercial diets H had 21.8% protein, N 20.95% and the macroalga 8.2%. Four replicates with 40 juvenile sea urchins were used per treatment, with average initial diameter and weight of 4.8 mm and 0.06 g, respectively. The experiment lasted 6 month and diets were provided ad libitum three days per week; water was exchanged 18 times/day; photoperiod 12 L: 12 D, oxygen saturation maintained at 8 - 10 mg/L and pH at 8 - 8.5. Significant differences were found in diameter and weight increase among treatments, with largest increase in size and weight with diet HA-A 21.4±2.1 mm (an increase of 128%) and 4.4±1.3 g, followed by HA-G> HI-G = HI-A> Ctrl > H = N. Final size in control diet was 16.6±1.6 mm and 2.4±0.8 g. The time needed to produce juveniles suitable for restocking (20 mm in diameter) after feeding the juvenile sea urchins with HA-A diet, could be reduced by more than 35% according to calculations made with the linear growth rate data.

Palabras clave

Echinodermata,
dietas de engorde,
crecimiento,
talla,
peso

RESUMEN | Se requiere desarrollar dietas de crecimiento para juveniles de *Paracentrotus lividus* que permitan reducir el tiempo en hatchery. En este estudio se midió el aumento de tamaño y peso de juveniles de *P. lividus* alimentados con 4 dietas diseñadas específicamente, 2 dietas comerciales y un control basado en la macroalga parda *Laminaria* sp. Las dietas experimentales se prepararon con harina de atún (HA) o de insectos (HI), utilizando gelatina de cerdo (HA-G, 31,3% proteína; HI-G, 31,6%) o Agar (HA-A, 21,8%; HI-A, 21,6%) como agente gelificante; las dietas comerciales tuvieron H 21,8% de proteína, N 20,95% y la macroalga 8,2%. Se utilizaron 4 repeticiones con 40 erizos por tratamiento, con diámetro y peso promedio inicial de 4,8 mm y 0,06 g, respectivamente. El experimento duró 6 meses y las dietas se proporcionaron *ad libitum* tres días por semana; el agua se reemplazó 18 veces al día; fotoperiodo 12 L: 12 D, saturación de oxígeno mantenida en 8 - 10 mg/L y pH en 8 - 8,5. Se encontraron diferencias significativas en el incremento de diámetro y peso entre tratamientos, siendo el mayor aumento en tamaño y peso con la dieta HA-A 21,4±2,1 mm (un aumento del 128%) y 4,4±1,3 g, seguido de HA-G>HI-G. = HI-A> Ctrl > H = N. El tamaño final en la dieta control fue de 16,6±1,6 mm y 2,4±0,8 g. El tiempo necesario para producir juveniles aptos para la repoblación (20 mm de diámetro) con la dieta HA-A, podría reducirse en 35% en base a la tasa de crecimiento lineal estimada.

INTRODUCTION

The objective of developing sea urchin aquaculture at an industrial level is to have the capacity to supply the growing demand for sea urchin gonads in the market, especially in countries such as Japan, Canada, France, or United States of America, since the overexploitation of the natural banks of this species may not allow its satisfaction soon. If the aim is to achieve optimal results in terms of both somatic growth and increase in gonadal index, thus reducing the time of juvenile sea urchins in the hatchery, it is necessary to develop different types of grow out diets, allowing a feeding pattern that ensures an optimal and fastest possible production of sea urchins. In this sense, numerous studies have been carried out developing diets that cover the energy needs of sea urchins (Klinger *et al.*, 1986), which promote their somatic growth (McCarron *et al.*, 2009), and also improve the

characteristics of the gonads of numerous species: *Paracentrotus lividus* (Lawrence *et al.*, 1991; Fernández & Caltagirone, 1994; Fernández *et al.*, 1995; Fernández & Pergent, 1998; Fernández & Boudouresque, 2000; Spirlet *et al.*, 2001; Cook *et al.*, 2007; Rey-Méndez *et al.*, 2015), *Lytechinus variegatus* (Klinger *et al.*, 1994; Hammer *et al.*, 2006), *Evechinus chloroticus* (Barker *et al.*, 1998; Woods *et al.*, 2008), *Loxechinus albus* (Olave *et al.*, 2001), *Psammechinus miliaris* (Kelly *et al.*, 1998; Pantazis *et al.*, 2000; McLaughlin & Kelly, 2001; Cook *et al.*, 2007), *Strongylocentrotus droebachiensis* (De Jong-Westman *et al.*, 1995; Pearce *et al.*, 2002), *Hemicentrotus pulcherrimus* (Nagai & Kaneko, 1975) and finally, *Mesocentrotus franciscanus* (McBride *et al.*, 1999).

The development of successful sea urchin aquaculture, therefore, requires a basic understanding of the dietary requirements during the sea urchin life stages, specifically after metamorphosis. The progressive variation in sea urchin size leads to a consequent variation in dietary requirements and food energy allocation (Grosjean *et al.*, 1998; Boudouresque & Verlaque, 2020; Grosso *et al.*, 2022). Several studies demonstrate the influence of the quantity and quality of available food on the morphology and physiology of sea urchins (Lawrence *et al.*, 1991; Fernández & Pergent, 1998; Daggett *et al.*, 2005; Liu *et al.*, 2007; Cook & Kelly, 2007). Other factors, such as the nutritional qualities of the diet, size of the individuals, water salinity and temperature (Santos *et al.*, 2020, 2022), and duration of the experiment, also affect the nutritional requirements.

The protein content of the supplied diet is essential for the growth and development of sea urchins, especially during the early stages of development, since it constitutes the main component of all their organs at a biochemical level (Fernandez & Pergent, 1998; Mc Bride *et al.*, 1998; Fernandez & Boudouresque, 2000; Kennedy *et al.*, 2001; Pearce *et al.*, 2002; Arce & Luna-Figueroa, 2003; Senaratna *et al.*, 2005; Schlosser *et al.*, 2005). It is believed that this high protein requirement in the early stages of development is necessary for the formation of the exoskeleton or would be required for the formation of an intrastromal matrix on its inner face (Dubois & Chen, 1989). Grosso *et al.* (2022) confirm that the protein requirement in the supplied diet is higher in juvenile individuals (15-25 mm in diameter) than in subadults and adult sea urchins.

The nutritional qualities of the diet used for growing out the urchins can have a decisive influence on the biochemical composition of the adult individuals (Fernández, 1997); the use of a diet with a high protein content (above the protein requirements of the species) will favor the storage of reserve substances in the gonads in the form of lipids and carbohydrates (McBride *et al.*, 1997), while the somatic grow out diets that work best, in general, have a protein content around 20% of its total composition (Eddy *et al.*, 2012).

The composition of the supplied diet affects the biochemical composition of the sea urchins, as shown by various studies in which stocks collected from the natural environment were compared with those from culture tanks and fed with elaborated diets (Fernández & Caltagirone, 1994; McBride *et al.*, 2004). Pigments such as echinone or carotenoids (the most important is β -carotene which brings orange color) are also essential to obtain good gonadal coloration in cultivated sea urchins (Pantazis, 2006; Symonds *et al.*, 2007).

Prior to designing the composition of the diet, it is necessary to consider various factors, for example, that there is an adequate proportion of proteins, carbohydrates, fatty acids, pigments, mineral salts, etc., which will affect the subsequent growth of the urchins and in the formation of the exoskeleton. The carbohydrate content of the diet, as well as the rate of food intake, are inversely related to the level of nutrient absorption by the urchins (Fernandez & Boudouresque, 2000). Heflin *et al.* (2016) suggest that *L. variegatus* may consume stored excess carbohydrates to compensate for the possibility of a later deficit.

It is also necessary to consider the stability of the pellets in the water, that is, their ability to maintain the initial texture and the insolubility of its ingredients, remaining intact for as long as possible; they must also have a good acceptance by the urchins (Dworjanyn *et al.*, 2007). In this study, different experimental diets focused on increasing the somatic growth of juvenile *P. lividus* individuals were designed, to compare which protein source promotes greater growth in juvenile urchins, as well as which type of gelling agent used to compact diets is better accepted by urchins.

MATERIALS AND METHODS

Experimental bioassay

Four experimental diets were designed to increase the somatic growth (diameter and weight) of juvenile sea urchins *Paracentrotus lividus* (Lamarck, 1816) kept in captivity. Four different diets were elaborated, 2 of them with tuna meal and another 2 with insect meal in their composition. Two gelling agents were used in these diets, commercial pork gelatin and Agar-Agar. These gelling elements were selected for their resistance in seawater, maintaining the consistency of the diets in good condition for at least 48 h.

On the other hand, 2 commercial dry diets focused on the growing out of sea urchins and abalone were purchased, the first one aimed at growing out the sea urchin *Strongylocentrotus droebachiensis* (Diet N), and the other one was produced in Mexico and aimed at feeding abalone (*Haliotis* sp.), consisting of pressed and extruded algae crackers (Diet H). These diets were introduced to verify their effectiveness in promoting the growth of juvenile *P. lividus* and to compare the results with the diets designed in this study.

The experimental design was based on 6 treatments or pre-growth diets supplied, with 4 replicates and one control per treatment consisting of sea urchins fed exclusively with the brown macroalga *Laminaria* sp. Two hundred juvenile urchins were selected per treatment, with average size of 4.76 mm in diameter and average weight of 0.06 g. There were no statistically significant differences in the initial measurements among the sets of juvenile urchins.

Subsequently, 40 juvenile sea urchins were introduced per replica in PVC containers made expressly and provided with a mesh at the bottom (1 mm mesh size) that prevented the sea urchins from escaping but allowed unconsumed food residues to pass through, preventing proliferation of bacteria inside the experimental containers. Each treatment was assigned a different color in order to facilitate the tasks of feeding and identifying the urchins, and the containers were randomly distributed in culture boxes at a rate of 6 containers per box (Fig. 1), in an open circuit, with an average of 18 seawater renewals/day and a photoperiod of 12 L: 12 D; an individual water intake system was also implemented for each experimental container (Fig. 2C). The oxygen saturation of the water was maintained between 8 and 10 mg/L and the pH values ranged between 8 and 8.5 throughout the experimental period.

The experiment lasted 180 days, during which the sea urchins were fed *ad libitum* with the corresponding diet 3 times per week on alternate days (Monday, Wednesday, and Friday), previously cleaning the residues accumulated in the culture boxes using a siphon. Bi-monthly data on the weight and size of the juveniles fed with each diet were collected (n=50), and they were analyzed once the experiment was finished.

A Mitutoyo brand digital caliper with 0.01 mm precision was employed for size measurements, and a GRAMP ST-360 model scale with precision 0,001 g, was employed for weight data. The selected sea urchins were previously allowed to dry for 10 s on absorbent paper before being weighed.

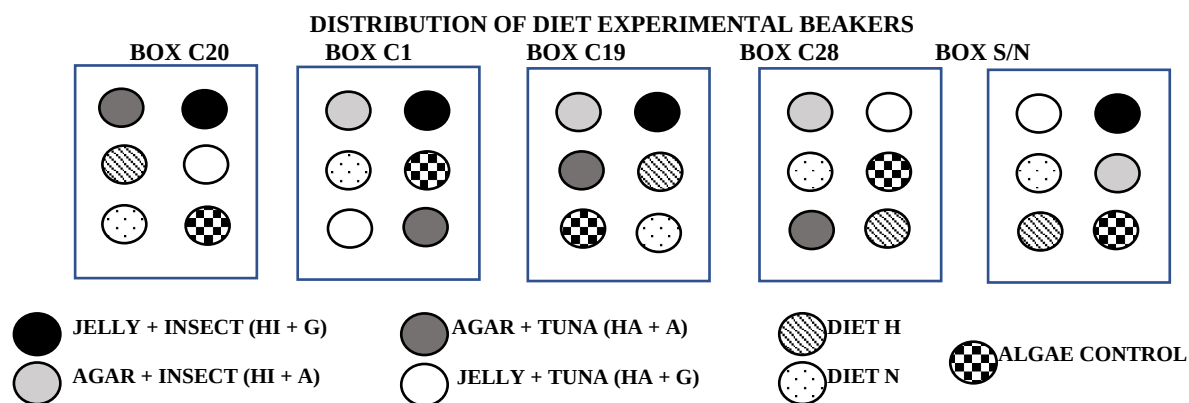


Figure 1.- Distribution of the experimental containers in the culture boxes and color and letter code used to identify the diets.

In Figure 2, the experimental culture boxes can be observed with the distribution of the containers inside (Fig. 2A, 2B) and the individual water intake system implemented, which allowed for an optimal circulation of the water inside the experimental containers (Fig. 2C).



Figure 2.- A) Shelf with the experimental boxes of the diet experiment. B) Boxes with experimental containers. C) Experimental container of the HA + A diet (yellow color).

Figure 3 shows the appearance of the diets once prepared, whole and divided into portions to freeze at -20°C , thus ensuring their optimal conservation.

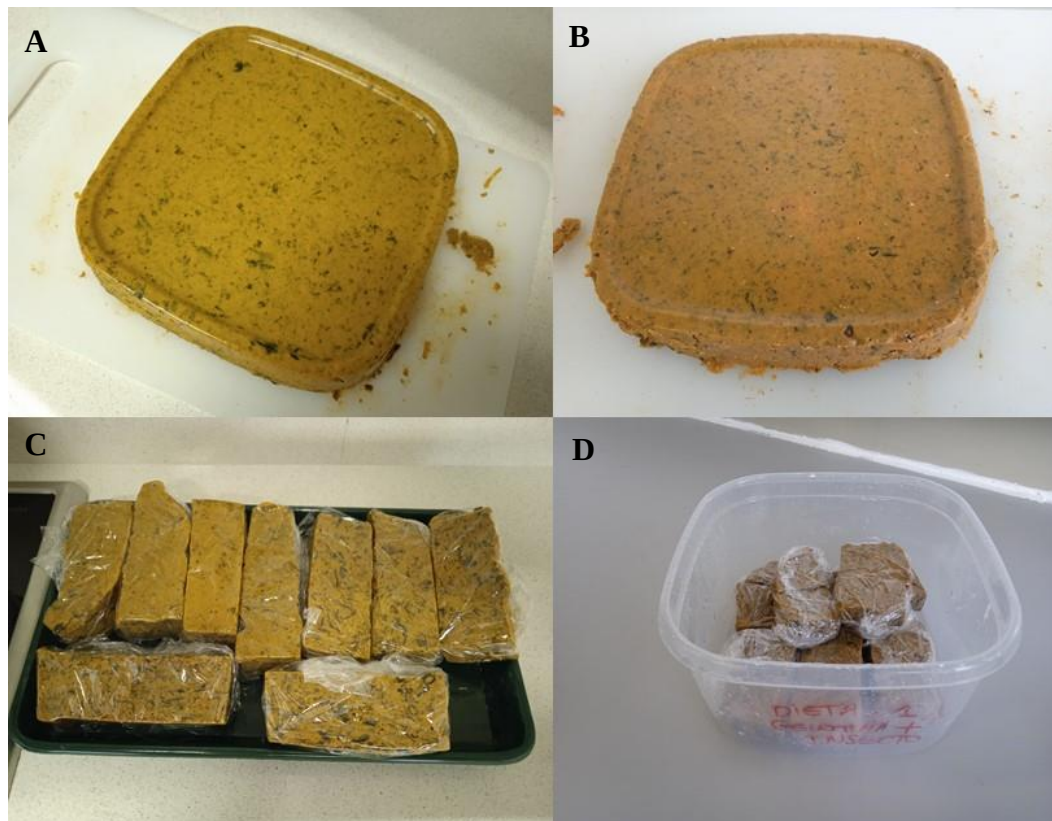


Figure 3.- A and B) Solidified processed diets; C and D) portions of the diets wrapped in film and prepared for freezing.

Biochemical composition of processed diets

The four diets prepared for the grow out of juvenile sea urchins, as well as samples of the commercial diets H and N, were lyophilized and sent to the Scientific and Technological Support Center for Research of the University of Vigo for their composition analysis.

In Table 1 we can see the biochemical composition (lipids, proteins, carbohydrates, etc.) of each diet supplied to *P. lividus* juveniles produced in the hatchery (samples 1-6).

Table 1. Chemical composition of the experimental diets used to grow out the *Paracentrotus lividus* juveniles.

Sample	Diet	Total Lipids (%)	Carbohydrates (%)	% N	% Protein ¹
1	Jelly + Insect (HI + G)	8.48	44.14	5.00	31.3
2	Jelly + Tuna (HA + G)	5.70	38.30	5.06	31.6
3	Agar + Tuna (HA + A)	6.13	40.96	3.45	21.6
4	Agar + Insect (HI + A)	8.55	43.02	3.49	21.8
5	Diet H	2.72	39.31	3.49	21.81
6	Diet N	4.57	36.52	3.35	20.95
7	Control ²	1.2	61.5	1.28	8.2

1: Estimated as 6,25 x %N; 2, Data on *Laminaria* sp. bromatology after Palasí Mascarós (2015).

This experiment was based on the comparison of the growth of the different groups of sea urchins fed with each pre-growing diet. For this purpose, the absolute (LGR) and relative (TCR) growth rates were calculated according to the formulas proposed by Busacker *et al.* (1990). The linear growth rate, expressed in $\mu\text{m}/\text{day}$, and the instantaneous growth rate (Ricker, 1979), which is also called the specific growth rate (SGR), expressed in % weight gain/day, were also calculated.

$$AG = Y_2 - Y_1$$

$$RG = AG / Y_1 \cdot 100 = (Y_2 - Y_1) / Y_1 \cdot 100$$

$$LGR = AG / (t_2 - t_1) = (Y_2 - Y_1) / (t_2 - t_1)$$

$$RGR = Y_2 - Y_1 / Y_1 \cdot (t_2 - t_1)$$

$$SGR (\% / \text{day}) = (\ln Y_2 - \ln Y_1) / t_2 - t_1 \cdot 100$$

Where: AG is the absolute growth; RG the relative growth, LGR is the absolute or linear growth rate expressed in $\mu\text{m}/\text{day}$; RGR the relative growth rate expressed as a percentage; SGR is the specific growth rate, expressed in % growth/day; Y_1 and Y_2 are the diameter or weight at the beginning and at the end of the experimental period; t_1 and t_2 are the initial and final times or the duration in days of the experiment.

Statistical analysis

Significant differences in the growth of juvenile sea urchins were tested using the Kruskal-Wallis test, since the data did not meet the assumption of normality and homoscedasticity after performing the Shapiro-Wilk and Levene tests, respectively. Differences were regarded as significant at $P < 0.05$. All results are given as mean $\pm$ SE. Statistical analyses were carried out using SPSS V15 software.

RESULTS

Morphometric measurements results

The highest size and weight increments, as well as linear growth rates (LGR), related to the increase in the diameter of the test, were obtained in the sea urchins fed with the four designed diets, especially with the diets composed of tuna meal and Agar (HA + A) and by insect meal and gelatin (HI + G) (Table 2). The sea urchins fed a diet based solely on *Laminaria* sp. showed an intermediate LGR, and the lowest LGR were observed in the urchins fed with the commercial diets N and H.

In the case of the specific growth rate (SGR) in weight, the highest values were obtained in the urchins fed with the HA + A diet once again, followed by the HI + G and HA + G diets, with which very similar SGR values were obtained. The urchins fed the Control diet obtained a slightly higher SGR than those fed the HI + A diet, and the worst SGR results were obtained in the urchins fed the commercial H and N diets.

Table 2.- Morphometric measurements of juvenile sea urchins *Paracentrotus lividus* fed with different diets during the experiment.

GROUP	HA + G	HA + A	HI + G	HI + A	DIET H	DIET N	CONTROL DIET
Initial diameter (mm)	4.8 $\pm$ 0.32	4.78 $\pm$ 0.34	4.8 $\pm$ 0.29	4.9 $\pm$ 0.24	4.63 $\pm$ 0.22	4.62 $\pm$ 0.22	4.78 $\pm$ 0.01
Final diameter (mm)	19.2 $\pm$ 1.5 ^b	21.38 $\pm$ 2.1 ^a	19.53 $\pm$ 3.4 ^b	18.65 $\pm$ 1.8 ^c	14.3 $\pm$ 1.62 ^e	14.35 $\pm$ 1.67 ^e	16.61 $\pm$ 1.57 ^d
Diameter increment (%)	300	347.28	306.88	280.61	208.86	210.61	247.49
Initial wet weight (g)	0.0616 $\pm$ 0.02	0.0649 $\pm$ 0.03	0.0638 $\pm$ 0.02	0.0729 $\pm$ 0.02	0.0508 $\pm$ 0.02	0.0506 $\pm$ 0.03	0.06 $\pm$ 0.02
Final wet weight (g)	3.31 $\pm$ 0.81 ^b	4.35 $\pm$ 1.26 ^a	3.44 $\pm$ 0.76 ^b	2.89 $\pm$ 0.85 ^c	1.56 $\pm$ 0.59 ^e	1.57 $\pm$ 0.61 ^e	2.42 $\pm$ 0.75 ^d
Weight increment (%)	5273.38	6602.62	5291.85	3846.33	2970.87	3002.77	3933.33
Linear growth rate ($\mu\text{m}/\text{day}$)	78.98	92.2	81.84	76.4	53.67	54.06	65.73
Specific growth rate (%/day)	2.21	2.34	2.22	2.04	1.9	1.91	2.05

Different letters on parameters in a row mean significant differences, $P < 0.05$

Evolution of sea urchin diameter with each grow out diet

The highest final sizes were obtained with the four diets specifically designed to increase the somatic growth of juvenile sea urchins, especially with the diet based on tuna meal with Agar as a gelling element (HA + A) (Fig. 4). The diameter of the sea urchins fed the H and N diets was less than the diameter of the control group (sea urchins fed with algae).

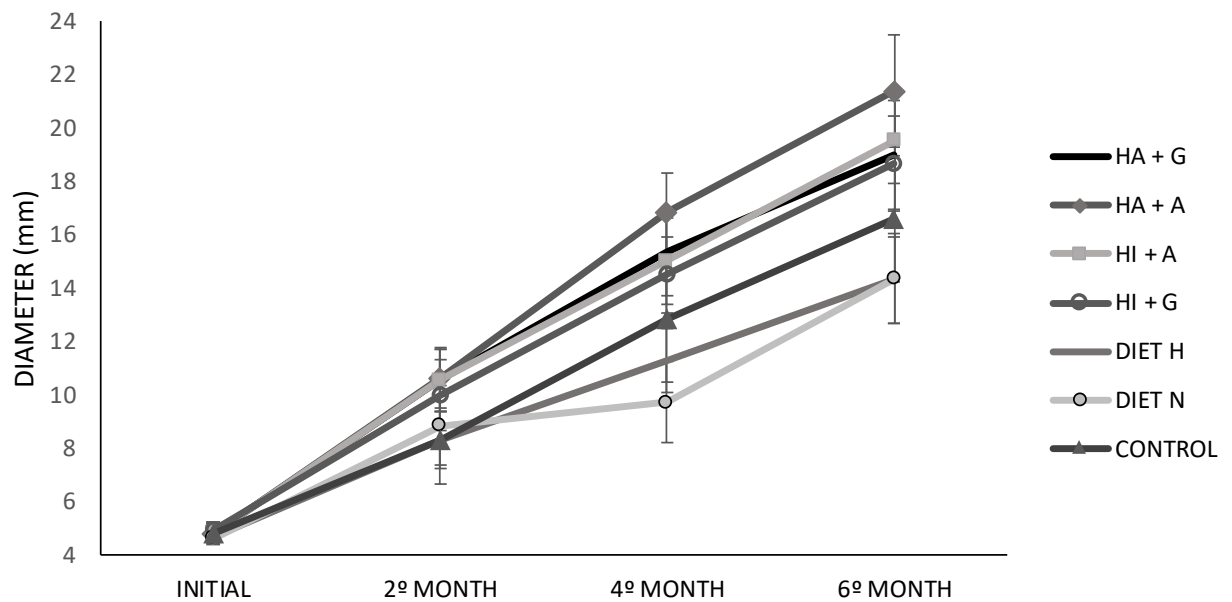


Figure 4.- Evolution of the diameter of the sea urchin *Paracentrotus lividus* with each diet.

Evolution of sea urchin weight with each grow out diet

In the case of weight evolution (Fig. 5), the same trend was observed as in the diameter evolution data, with the sea urchins fed the HA + A diet achieving a greater weight at the end of the experiment, followed by the urchins fed the other three diets designed (HI + A, HA + G, HI + G). The sea urchins fed with H and N diets reached lower weights than the sea urchins of the control group fed with algae.

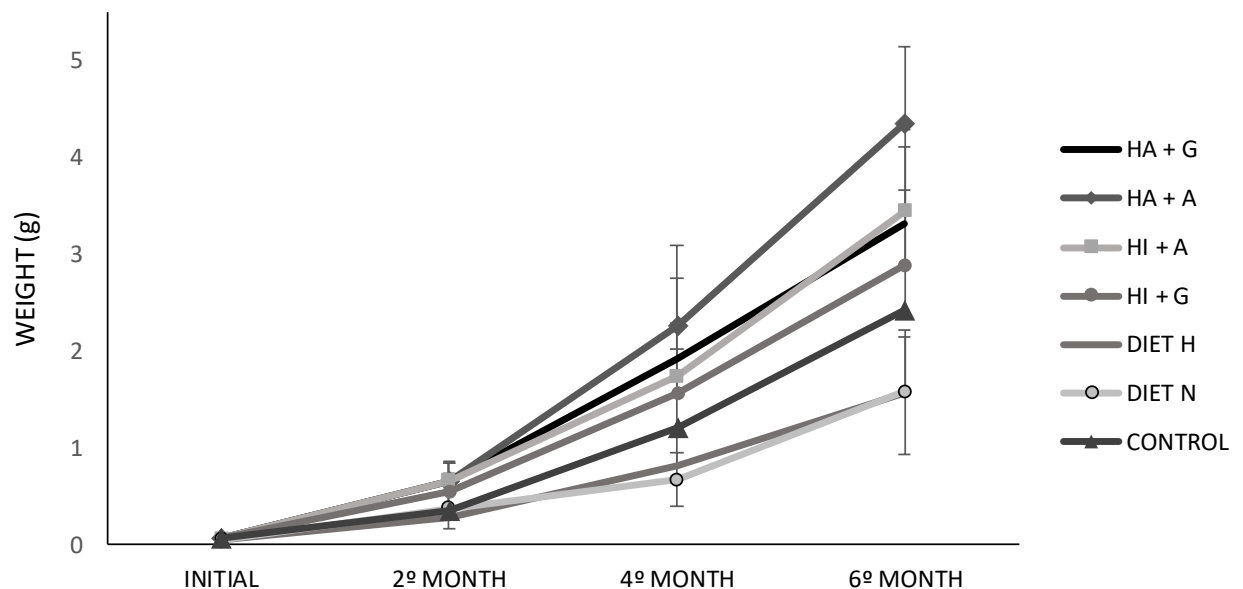


Figure 5.- Evolution of the weight of the sea urchin *Paracentrotus lividus* with each diet.

Increase in the size of juvenile sea urchins with each diet

The increases in size obtained for each diet followed the same trend as the data on the temporal increase of diameter throughout the experiment, obtaining a significantly greater increase in the sea urchins fed with the tuna meal diet with Agar as a gelling element; followed by the other 3 designed diets, the control and, in the end, the smallest increases in size were obtained with the H and N diets.

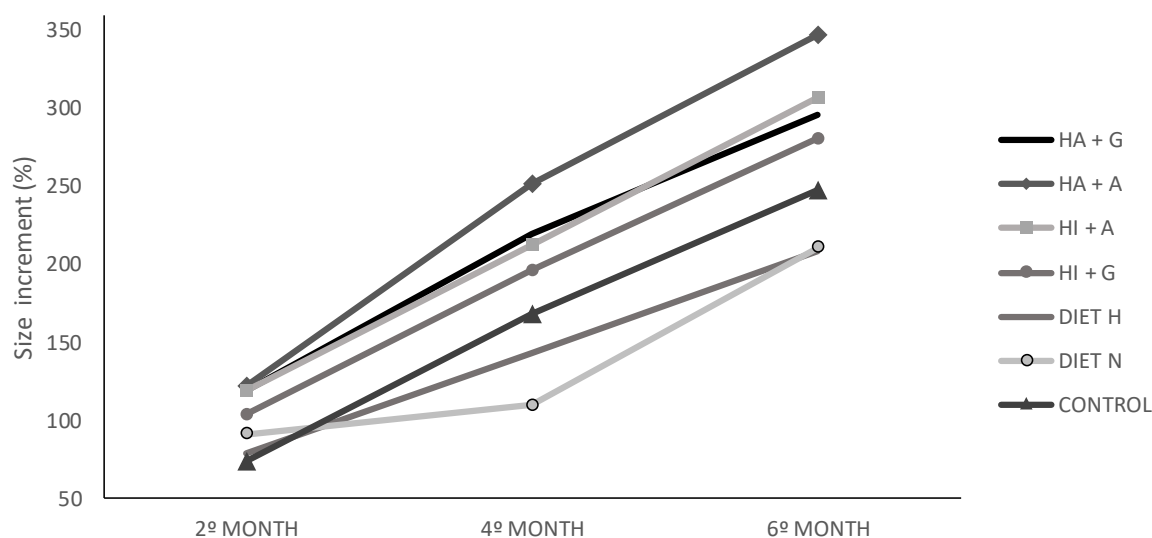


Figure 6.- Increment in the diameter of the sea urchin *Paracentrotus lividus* with each diet.

Increase in the weight of juvenile sea urchins with each diet

Considering the weight increase throughout the experiment, four different data groups could be observed, the greatest increase in the weight of the sea urchins was observed once again with the diet of Tuna meal + Agar (HA + A), while the weight gains obtained with the HA + G and HI + A diets showed very similar weight gain values at the end of the experiment, higher than the gains obtained with the HA + G and HI + A diets, HI + G and Control (Algae), also with very similar values of weight increase. In the end, the H and N diets were grouped with the lowest values of weight gain.

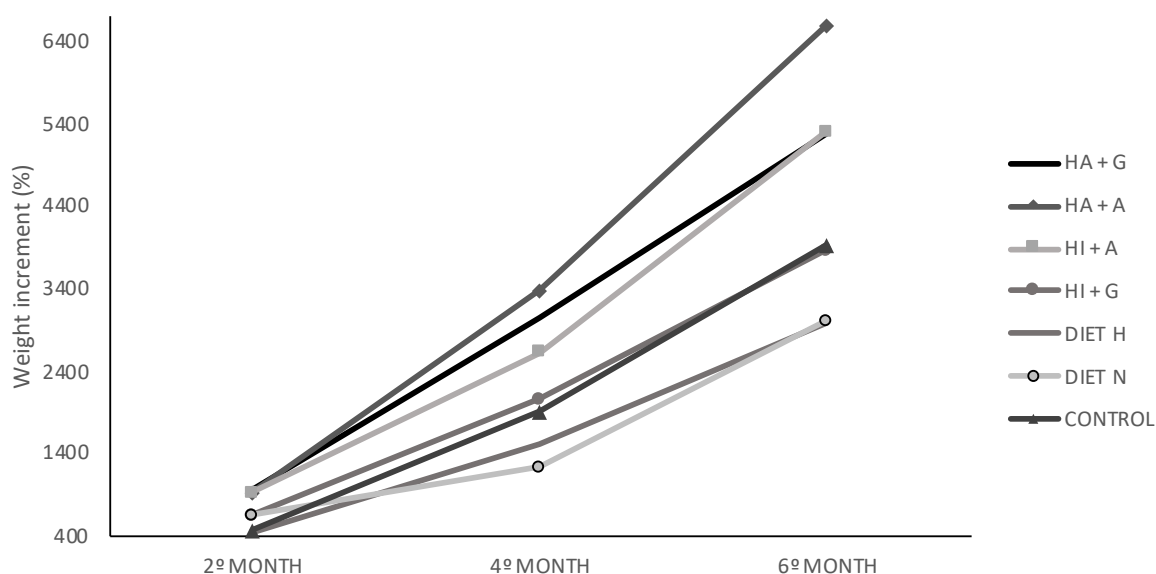


Figure 7.- Increment in the weight of the sea urchin *Paracentrotus lividus* with each diet.

Linear growth rate obtained with each diet

The highest linear growth rates were obtained in urchins fed the HA + A diet, closely followed by the other 3 designed diets (Fig. 8).

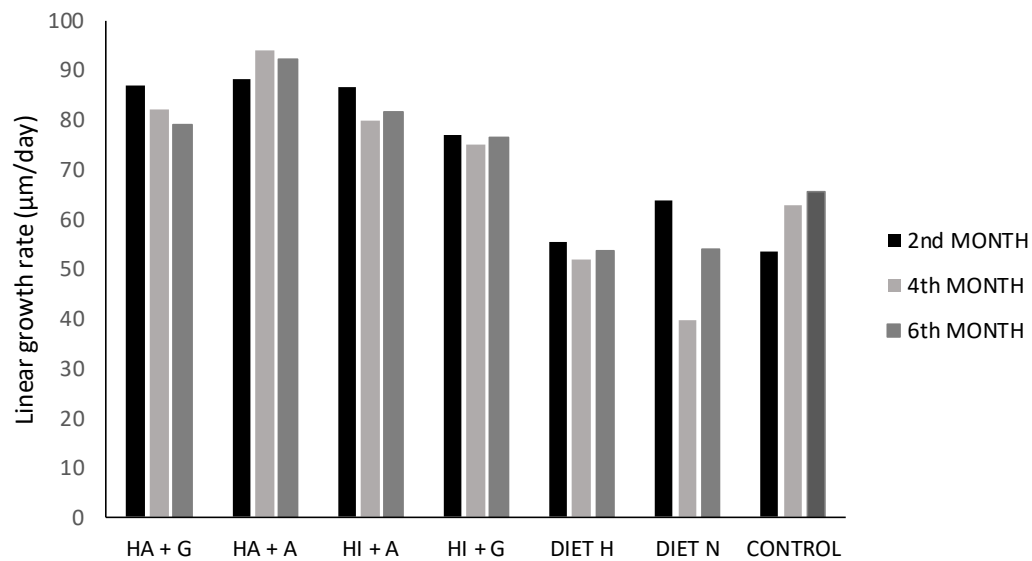


Figure 8.- Linear growth rate of the sea urchin *Paracentrotus lividus* fed with each diet.

Specific growth rate obtained with each diet

The highest final SGR were obtained in the urchins fed the HA + A diet, with a value of 2.34%, followed by the HI + A and HA + G diets with 2.22 and 2.21% SGR, respectively (Fig. 9). The SGR of the H and N diets were below the value of the urchins fed with the Control diet, with a SGR of 1.9 and 1.91, respectively.

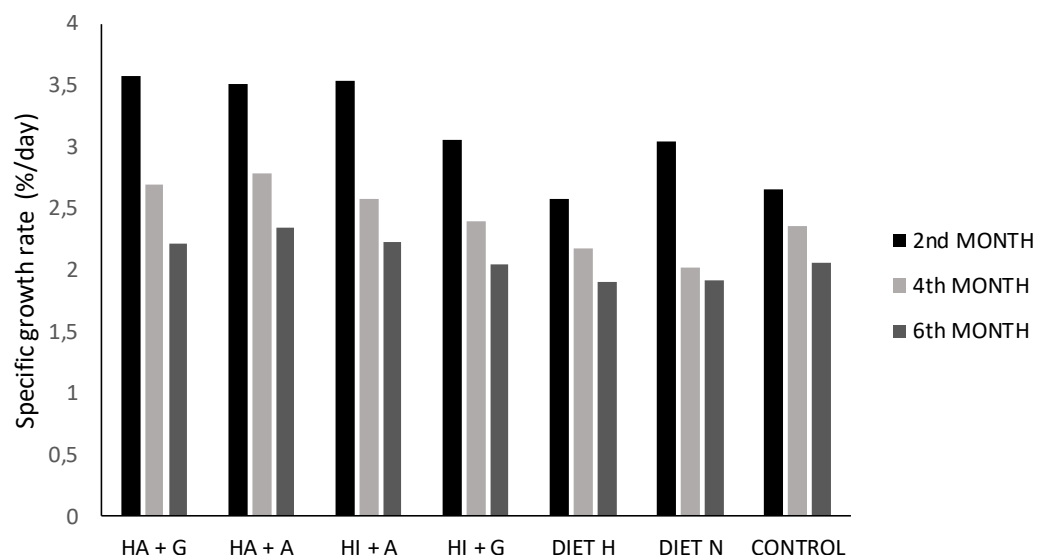


Figure 9.- Specific growth rate of the sea urchin *Paracentrotus lividus* fed with each diet.

Urchins grew with all the diets supplied. However, the diameter and weight gains were significantly higher with the prepared diets compared with urchins fed with the Control diet based on macroalgae, which were above those of urchins fed with the H and N diets.

DISCUSSION

There were four well-differentiated groups among the diets when examining the increases obtained in the growth of the sea urchins throughout the experiment, both in the increases in size and weight obtained with each diet. The diet composed of tuna meal and Agar (HA + A) showed significantly higher total increases in size and weight than the diets of group two (HI + A and HA + G); the diet composed of insect meal and gelatin (HI + G) followed and the Control diet based on macroalgae. Finally, the H and N diets showed the lowest values of size and weight increase of juvenile sea urchins, probably due to the lower food intake associated with the taste of these diets for the urchins, since they were diets designed for abalone pre-grow out and another species of sea urchin (*Strongylocentrotus droebachiensis*), respectively. The differences in the diets of the four groups appear to be related to their biochemical composition and nutritional value. The prepared diets tended to become softer over time, and presumably easier to ingest, when soaked in seawater.

Although data on the ingestion rate of the diets were collected, there were problems in differentiating the recovered diet remains from the feces of the sea urchins. Therefore, the recovery data obtained were not reliable and were not included in the analyses. However, a considerably lower consumption rate of the commercial diets was observed with the naked eye than in the case of the specifically designed diets and the Control diet, so we suppose that the lower growth of the sea urchins fed the two commercial diets may be related to its organoleptic characteristics focused on feeding abalone and another species of sea urchin, which may have not been to the like of *Paracentrotus lividus*.

It was also observed that the amount of the specifically designed diets consumed by the sea urchins was 4 to 5 times lower than the amount of macroalgae ingested, so they would be also a good alternative for feeding the sea urchins in case of not having sufficient algae biomass in the hatchery, whose availability varies greatly seasonally. Lawrence (1975), Rowley (1990) and Frantzis & Gremare (1992) have all concluded that quality of ingested food affects the growth rates of the urchins, and when the food is not limiting, as in this study, quality of feed is more important than its quantity. A commercial culture facility would benefit financially by using a diet that is consumed at low rates while supporting maximal growth.

Generally, diets focused on the somatic growth of sea urchins have a protein content around 20%, which proved to be more effective in increasing the somatic growth (Akiyama *et al.*, 2001; Eddy *et al.*, 2012), while a diet with 30% protein content is focused on increasing of the gonadal index in diets specifically designed for gonadal maturation (Baiao *et al.*, 2019).

In several studies, the growth of juvenile sea urchins fed with designed diets compared to macroalgae, normally higher growth rates were obtained with the macroalgal diet, as in the case of Dagget *et al.* (2005) with juveniles of *Strongylocentrotus droebachiensis*, or in that of De la Uz *et al.* (2018) with juveniles of *P. lividus*. Vizzini *et al.* (2018) fed small-sized specimens of the sea urchin *P. lividus* with fresh discards of the lettuce *Lactuca sativa* during a 24-week experiment, getting a good test diameter and total weight increments of 35% and 56%, respectively.

Results obtained by Tourón (2013), where the growth of juveniles of *P. lividus* fed with two commercial diets, a designed diet and two others based on macroalgae (*Laminaria* sp. and *Ulva* Sp.), were compared, confirm this trend of higher somatic growth obtained in the group of sea urchins fed macroalgae. The results obtained by Castilla-Gavilán *et al.* (2019) suggest that *P. lividus* adults can be reared on dried macroalgae thalli without detriment to their somatic growth, especially over short time periods.

De la Uz *et al.* (2018) compare the somatic growth of juvenile *P. lividus* in captivity for 175 days, with a mean initial size and weight very similar to those of the present study. In their study, the somatic growth obtained in juvenile sea urchins fed with two semi-moist feeds (Feed 1: based on *Ulva* Sp. dehydrated and finely chopped and Feed 2: same as Feed 1 but with gelatin supplement), was compared to the growth observed in juveniles fed with a macroalgal diet based on *Ulva* sp., finding no significant differences in the growth of the sea urchins fed with the different diets. The linear growth rates ($\mu\text{m/day}$) and specific growth ($\%/day$) obtained in this study were also higher in the sea urchins fed the macroalgal diet than in those fed the two specially prepared feeds supplied, and in all cases the value obtained by Feed 2 which had the protein supplement, was higher than the value achieved by

Feed 1, although the protein content of Feed 2 was still low, of 3.7% protein content. The protein supplement, supplied in the form of gelatin, provided greater growth to the sea urchins, mainly affecting their weight, although it was not enough to increase the growth of the sea urchins compared to those that were fed the macroalgal diet, which suggests that the protein content of the diet is not the only determining factor influencing the somatic growth of juvenile *P. lividus*.

Kennedy *et al.* (2007) provided evidence that lack of appropriate dietary minerals and pigments is a likely contributing factor to deficiencies in prepared feeds in those cases where natural seaweed diets have produced better somatic growths than prepared diets. Other studies have begun defining the gross levels of protein and carbohydrates required for somatic growth by urchins.

McBride *et al.* (1998) observed no significant differences in the growth of *S. franciscanus* fed prepared diets with protein levels of 30, 40, and 50%, but they observed a decrease in the feeding rate with increased protein levels, since sea urchins and other animals have an upper threshold for protein intake that results in decreased food intake with increasing dietary protein. Fernandez and Pergent (1998) compared the growth of juvenile *P. lividus* given three feed types varying in quality (“vegetable,” “mixed,” and “animal”), and found that the higher protein feeds (“mixed” and “animal”) with relatively lower carbohydrate levels (28.9% protein/35.3% carbohydrate and 47.2% protein/15.9% carbohydrate, respectively) gave better results than the “vegetable” type feed (12.7% protein/58.2% carbohydrate). Hammer *et al.* (2006) observed similar results in a feeding study with the sea urchin *L. variegatus*, where they determined that a 20% protein diet was more efficient than either a 9% protein or a 31% protein diet to enhance the urchin’s somatic growth.

In other study performed by Lourenço *et al.* (2020) with *P. lividus* adult individuals, they observed that a protein content between 20 and 45% at a fixed lipid level (7%) did not affect *P. lividus* growth. However, Zupo *et al.* (2019) reported different results, finding that a high protein diet (47%) promoted higher size increments when compared to diets with 22-35% protein in the same species. Some authors consider that a macroalgae-restricted diet offers low levels of protein and energy to support the high growth rates and gonad yield required for a profitable sea urchin production (Prato *et al.*, 2018, Lourenço *et al.*, 2021). In the study of Lourenço *et al.* (2021) in which *P. lividus* juveniles were fed with 3 grow out diets for 15 weeks, one based on fishmeal, another based on cereals and the third based on macroalgae, they obtained greater somatic growth with the diet composed of fishmeal, although data were not statistically significant.

In the present study, the diets made with neutral pig gelatin as a gelling element had a protein content around 30%, and showed to be equally effective in favoring the somatic growth of juvenile sea urchins obtained at the ECIMAT (Toralla Marine Science Station), obtaining linear and specific growth results of the sea urchins fed with them similar to the results obtained with diets designed with a protein content of around 20%. Eddy *et al.* (2012) compared the growth of juvenile sea urchins of *Strongylocentrotus droebachiensis* (5,5 mm in test diameter) fed different diets in captivity for 6 months; the composition of the diets varied between 16 and 40% protein content and 29 and 49% carbohydrate content, finding a greater growth of the juveniles with the low protein diet (16-22%) and high carbohydrate levels (> 40%). Previous studies showed that carbohydrate and protein levels of 40% and 20%, respectively, provide the proper amount of energy and essential amino acids needed to faster growth and reproduction of sea urchins (Hammer *et al.*, 2012; Ciriminna *et al.*, 2018). Similar results were obtained in the present study with *P. lividus*, since the diets that most increased both the size and weight of the juvenile urchins were HA + A and HI + A, with a protein content of 21.6 and 21.8 % and a carbohydrate content of 40.96 and 43.02%, respectively.

There are several studies that have evaluated the effects of the protein and carbohydrate contents in diets designed to feed sea urchins (Fernandez & Pergent, 1998; Hammer *et al.*, 2006; Woods *et al.*, 2008; Eddy *et al.*, 2012), although there are very few studies that assess the effects of lipid levels in the grow out experimental diets. Although there is no reference to previous studies concerning the effects of dietary lipids in *P. lividus*, Gibbs *et al.* (2009) observed that diets with 7-11% lipid composition produced greater fat deposition in the gonads of male *Lytechinus variegatus* compared to a diet with 3% lipid composition, but no differences were observed in females, suggesting different metabolic pathways between sexes. Furthermore, Hammer *et al.* (2012) showed that in *L. variegatus*, high dietary levels of lipids (> 9%) negatively influenced to growth of the sea urchin.

In the study performed by Baiao *et al.* (2019), four extruded diets were formulated to evaluate their effect on the growth and gonadal index of adult sea urchins of *P. lividus*. The diets contained 30 or 50% dry matter protein, designated by low (LP) or high (HP) protein level, and 6 or 11% lipid, designated by low (LL) or high (HL) lipid levels. The growth results obtained with the supplied diets were similar, with no significant differences between them in specific growth rate or protein efficiency ratio among diets. Weight gain, total body composition, and nutrient gain remained similar between the different treatments. The diet that worked best to increase the gonadal index of sea urchins was the one composed of 30% protein and 6% lipids.

In contrast to the results obtained by Hammer *et al.* (2012), the study performed by Baiao *et al.* (2019) showed that a dietary lipid level of 11% did not affect sea urchin's growth nor gonad yield, but a protein-sparing effect could not be observed either.

The diets that most increased the growth of the juvenile urchins in the present study had a lipid content of between 5.7 and 8.55%; the diet that worked best (HA + A) contained 6.13% of lipids in its composition, not being observed a clear influence of this factor on the growth of the urchins, although the diets that least increased urchin's growth had a lipid content lower than 5%.

In addition to the level of lipids in the diet, necessary for normal metabolic function, the quality of these lipids must also be considered. The fatty acids composition in the diet is as important as the percentage of protein that composes it for the growth of juvenile urchins (Akiyama *et al.*, 2001). Sea urchins can synthesize long-chain polyunsaturated fatty acids (LC-PUFA) such as arachidonic acid (ARA) and eicosapentaenoic acid (EPA) from their precursors, but other essential fatty acids must be supplied in the diet to improve the urchin growth (Carboni *et al.*, 2015).

The linear and specific growth rates obtained in this study were related to the qualities of each of the seven individual diets supplied, since each diet treatment tested was supplied individually in the experiment, without the urchins having another choice, it was physically available throughout the experiment and was supplied in separate tanks from the other feeding treatments, that is, there were no interactions among treatments.

A pattern of temporary decrease in the linear growth rate is generally observed in *P. lividus*, since after 8 months of age the linear growth rate of this species gradually decreases until it reaches the commercial size (Rey-Méndez *et al.*, 2015). However, the duration of this experiment was not long enough for significant temporal differences to be observed. In the case of the specific growth rate (SGR), its decrease in the juvenile sea urchins was clearly observed as they grew.

The present results clearly show that all designed experimental diets were effective in promoting somatic growth of *P. lividus*, suggesting that a 30% dietary protein with low lipid level (around 6%) can not only promote the increase of the gonadal index of sea urchins up to a 233% (as shown by Rey-Méndez *et al.*, 2015), but also to significantly increase the somatic growth of juveniles of this species of sea urchin.

CONCLUSION

The four diets prepared specifically to increase the growth rate of juvenile *P. lividus* individuals and provided *ad libitum*, performed better than the Control diet, based on the macroalga *Laminaria* sp., since significantly higher mean values of final size and weight were obtained than those obtained with the Control diet. The mean size of the sea urchins in the Control group was 16.61 mm in diameter after 6 months of culture, while the size obtained in the juveniles fed with the diet based on tuna flour and Agar as a gelling element (HA + A) was 21.38 mm, which represents an increase of 128.7%. The mean weight of the sea urchins of the Control group was 2.42 g, while in the juveniles fed with the HA + A diet was 4.35 g, which represents an increase of 179.8% in the weight of the juveniles. The time needed to produce juveniles suitable for restocking (20 mm in diameter) after feeding the juvenile sea urchins of this species with the HA + A diet, could be reduced by more than 35% according to calculations made with the linear growth rate data.

Acknowledgements

We are grateful to the staff of the Toralla Marine Science Station (ECIMAT) belonging to the Centro de Investigación Marina-Universidade de Vigo (CIM) and to the San Xosé de Cangas do Morrazo Fishermen's Association. We are also grateful to Galician Marine Aquaculture (GMA) company, who collaborated selflessly with the OCIMER project.

Funding information: This work has been developed within the framework of the OCIMER Project "Optimization of the integral cultivation of the sea urchin *Paracentrotus lividus*", financed by the PLEAMAR program of the Biodiversity Foundation with funds from the FEMP.

REFERENCES

- Akiyama T., Unuma T., Yamamoto T. (2001). Optimum protein level in a purified diet for young red sea urchin *Pseudocentrotus depressus*. *Fisheries Science*, 67(2):361-363.
- Arce E., Luna-Figueroa J. (2003). Efecto de dietas con diferente contenido proteico en las tasas de crecimiento de crías del Bagre del Balsas *Ictalurus balsanus* (Pisces: Ictaluridae) en condiciones de cautiverio. *Revista AquaTIC*, 18:39-47.
- Baião L.F., Rocha F., Costa M., Sá T., Oliveira A., Maia M.R., Fonseca A.J.M., Pintado M., Valente L. M. (2019). Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus* (Lamarck, 1816). *Aquaculture*, 506:127-138.
- Barker M.F., Keogh J.A., Lawrence J.M., Lawrence A.L. (1998). Feeding rate, absorption efficiencies, growth, and enhancement of gonad production in the New Zealand sea urchin *Evechinus chloroticus* Valenciennes (Echinoidea: Echinometridae) fed prepared and natural diets. *Journal of Shellfish Research*, 17:1583-1590.
- Busacker G.P., Adelman I.A., Goolish E.M. (1990) Growth. In Schreck C.B., Moyle P.B. (Eds.) *Methods for fish biology*. American Fisheries Society: Bethesda, Maryland, USA, pp. 363-364.
- Carboni S., Hughes A.D., Attack T., Tocher D.R., Migaud H. (2015). Influence of broodstock diet on somatic growth, fecundity, gonad carotenoids and larval survival of sea urchin. *Aquaculture Research*, 46(4):969-976.
- Ciriminna L., Signa G., Vaccaro A.M., Messina C.M., Mazzola A., Vizzini S. (2020). Formulation of a new sustainable feed from food industry discards for rearing the purple sea urchin *Paracentrotus lividus*. *Aquaculture Nutrition*, 26(4):1046-1057.
- Cook E.J., Kelly M.S. (2007). Enhanced production of the sea urchin *Paracentrotus lividus* in integrated open-water cultivation with Atlantic salmon *Salmo salar*. *Aquaculture*, 273(4):573-585.
- Cook E.J., Hughes A.D., Orr H., Kelly M.S., Black K.D. (2007). Influence of dietary protein on essential fatty acids in the gonadal tissue of the sea urchins *Psammechinus miliaris* and *Paracentrotus lividus* (Echinodermata). *Aquaculture*, 273(4):586-594.
- Daggett T.L., Pearce C.M., Tingley M., Robinson, S.M.C., Chopin T. (2005). Effect of prepared and macroalgal diets and seed stock source on somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*). *Aquaculture*, 244(1-4):263-281.
- De Jong-Westman M., March B.E., Carefoot T.H. (1995). The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin *Strongylocentrotus droebachiensis*. *Canadian Journal of Zoology*, 73:1495-1502.
- De la Uz S., Carrasco J.F., Rodríguez C. (2018). Crecimiento somático del erizo de mar *Paracentrotus lividus* alimentado con dos piensos semihúmedos frente a una dieta macroalgal. *XI Foro dos Recursos Mariños e da Acuicultura das Rías Gallegas*, 9-10.
- Dworjanyan S. A., Pirozzi I., Liu W. (2007). The effect of the addition of algae feeding stimulants to artificial diets for the sea urchin *Tripneustes gratilla*. *Aquaculture*, 273:624-633.
- Dubois P., Chen C. (1989). Calcification in echinoderms. In: Jangoux, M., Lawrence, J.M. (eds). *Echinoderm studies*, 3. Balkema: Rotterdam, p.p 109–178.

- Eddy S.D., Brown N.P., Kling A.L., Watts S.A., Lawrence A. (2012). Growth of juvenile green sea urchins, *Strongylocentrotus droebachiensis*, fed formulated feeds with varying protein levels compared with a macroalgal diet and a commercial abalone feed. *Journal of the world Aquaculture Society*, 43(2):159-173.
- Fernandez C. (1997). Effect of diet on the biochemical composition of *Paracentrotus lividus* (Echinodermata: Echinoidea) under natural and rearing conditions (effect of diet on biochemical composition of urchins). *Comparative Biochemistry and Physiology*, 118A(4):1377-1384.
- Fernandez C., Boudouresque C.F. (2000). Nutrition of the sea urchin *Paracentrotus lividus* fed different artificial food. *Marine Ecology Progress Series*, 204:131-141.
- Fernandez C., Caltagirone A. (1994). Growth rate of adult *Paracentrotus lividus* in a lagoon environment: the effect of different diet types. In: David B., Guille, A., Féral J.P., Roux M. (Eds.). *Echinoderms trough time*. Balkema: Rotterdam, pp. 655-660.
- Fernandez C., Dombrowski E., Caltagirone A. (1995). Gonadic growth of adult sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) in rearing: the effect of different diet type. In Emson R., Smith A., Campbell A. (Eds.). *Echinoderms Research 1995*. Rotterdam: Balkema, pp. 269-275.
- Fernandez C., Pergent G. (1998). Effect of different formulated diets and rearing conditions on growth parameters in the sea urchin *Paracentrotus lividus*. *Journal of Shellfish Research*, 17(5):1571-1581.
- Frantzis A., Grémare A., Vétion G. (1992). Growth rates and RNA: DNA ratios in *Paracentrotus lividus* (Echinodermata: Echinoidea) fed on benthic macrophytes. *Journal of Experimental Marine Biology and Ecology*, 156(1):125-138.
- Gibbs V.K., Watts S.A., Lawrence A.L., Lawrence J.M. (2009). Dietary phospholipids affect growth and production of juvenile sea urchin *Lytechinus variegatus*. *Aquaculture*, 292(1-2):95-103.
- Grosjean P, Spirlet C, Gosselin P, Vaïtilingon D, Jangoux M. (1998). Land-based, closed-cycle echinoculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): A long-term experiment at a pilot scale. *Journal of shellfish Research*, 17: 1523-1531.
- Gross L., Rakaj A., Fianchini A., Tancioni L., Vizzin S., Boudouresque C.F., Scardi M. (2022). Trophic requirements of the sea urchin *Paracentrotus lividus* varies at different life stages: comprehension of species ecology and implications for effective feeding formulations. *Frontiers in Marine Science*, 9:865450.
- Hammer B.W., Hammer H.S., Watts S.A., Lawrence J.M., Lawrence A.L. (2006). The effect of dietary protein and carbohydrate concentration on the biochemical composition and gametogenic condition of the sea urchin *Lytechinus variegatus*. *Journal of Experimental Marine Biology and Ecology*, 344:109-121.
- Hammer H.S., Powell M.L., Jones W.T., Gibbs V.K., Lawrence A.L., Lawrence J.M., Watts S A. (2012). Effect of feed protein and carbohydrate levels on feed intake, growth, and gonad production of the sea urchin, *Lytechinus variegatus*. *Journal of the World Aquaculture Society*, 43(2):145-158.
- Heflin L.E., Raubenheimer D., Simpson S.J., Watts S.A. (2016). Balancing macronutrient intake in cultured *Lytechinus variegatus*. *Aquaculture*, 450:295-300.
- Kelly M.S., Brodie C.C., McKenzie J.D. (1998). Somatic and gonadal growth of the sea urchin *Psammechinus miliaris* (Gmelin) maintained in polyculture with the Atlantic salmon. *Journal of Shellfish Research*, 17:1557-1562.
- Kennedy E.J., Roinson S.M.C., Parsons G.J., Castell J. (2001). Studies on feed formulations to maximize somatic growth rates of juvenile green sea urchins (*Strongylocentrotus droebachiensis*). *Aquaculture Association of Canada Specific Publications*, 4:68-71.
- Kennedy, E. J., Robinson, S. M., Parsons, G. J., Castell, J. D. (2007). Effect of dietary minerals and pigment on somatic growth of juvenile green sea urchins, *Strongylocentrotus droebachiensis*. *Journal of the World Aquaculture Society*, 38(1): 36-48.
- Klinger T.S., Hsieh H.L., Pangallo R.A., Chen C.P., Lawrence J.M. (1986). The effect of temperature on feeding, digestion, and absorption of *Lytechinus variegatus* (Lamarck) (Echinodermata: echinoidea). *Physiological Zoology*, 59(3):332-336.

- Klinger T.S., Lawrence J.M., Lawrence A.L. (1994). Digestive characteristics of the sea-urchin *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) fed prepared feeds. *Journal of the World Aquaculture Society*, 25(4):489-496.
- Lawrence J.M. (1975). The effect of temperature–salinity combinations on the functional well-being of adult *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). *Journal of the Experimental Marine Biology and Ecology*, 18:271-275.
- Lawrence J.M., Fenaux L., Corre M.C., Lawrence A. (1991). The effect of quantity and quality of prepared diets on production in *Paracentrotus lividus* (Echinodermata: Echinoidea). Scalera-Liaci, L., Canicatti, C. (Eds.). *Echinoderm Research*, Balkema, Rotterdam. pp. 107-110.
- Liu H., Kelly M.S., Cook E.J., Black K., Orr H., Zhu J.X., Dong S.L. (2007). The effect of diet type on growth and fatty-acid composition of sea urchin larvae, I. *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). *Aquaculture*, 264(1-4):247-262.
- Lourenco S., Jose R., Andrade C., Valente L. M. (2020). Growth performance and gonad yield of sea urchin *Paracentrotus lividus* (Lamarck, 1816) fed with diets of increasing protein: energy ratios. *Animal Feed Science and Technology*, 270:114690.
- Lourenço S., Cunha B., Raposo A., Neves M., Santos P.M., Gomes A.S., Tecelão C., Ferreira S.M.F., Baptista T., Gonçalves S.C., Pombo A. (2021). Somatic growth and gonadal development of *Paracentrotus lividus* (Lamarck, 1816) fed with diets of different ingredient sources. *Aquaculture*, 539:736589. <https://doi.org/10.1016/J.AQUACULTURE.2021.736589>
- McBride S.C., Pinnix W.D., Lawrence J.M., Lawrence A.L., Mulligan T.M. (1997). The effect of temperature on production of gonads by the sea urchin *Strongylocentrotus franciscanus* fed natural and prepared diets. *Journal of World Aquaculture Society*, 28:357-365.
- McBride S.C. (1998). The effect of protein concentration in prepared feed on growth, feeding rate, total organic absorption, and gross assimilation efficiency of the sea urchin *Strongylocentrotus franciscanus*. *Journal of Shellfish Research*, 17:1563-1570.
- McBride S.C., Lawrence J.M., Lawrence A.L., Mulligan T.M. (1999). Ingestion, absorption, and gonad production of adult *Strongylocentrotus franciscanus* fed different rations of a prepared diet *Journal of World Aquaculture Society*, 30:364-370.
- McBride S.C., Price R.J., Tom P.D., Lawrence J.M., Lawrence A.L. (2004). Comparison of gonad quality factors: color, hardness, and resilience, of *Strongylocentrotus franciscanus* between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery. *Aquaculture*, 233(1-4):405-422.
- McCarron E., Burnell G., Mouzakis G. (2009). Growth assessment on three size classes of the purple sea urchin *Paracentrotus lividus* using continuous and intermittent feeding regimes. *Aquaculture*, 288:83-91.
- McLaughlin G., Kelly M.S. (2001). Effect of artificial diets containing carotenoid-rich microalgae on gonad growth and color in the sea urchin *Psammechinus miliaris* (Gmelin). *Journal of Shellfish Research*, 20(1):377-382.
- Nagai Y., Kaneko K. (1975). Culture experiments on the sea urchin *Strongylocentrotus pulcherrimus* fed an artificial diet. *Marine Biology*, 29:105-108.
- Olave S., Bustos E., Lawrence J.M., Cárcamo P. (2001). The effect of size and diet on gonad production by the Chilean sea urchin *Loxechinus albus*. *Journal of the World Aquaculture Society*, 32:210-214.
- Palasí Mascarós J.T. (2015). Caracterización físico-química y nutricional de algas en polvo empleadas como ingrediente alimentario. Tesis doctoral, Universitat Politècnica de València, España.
- Pantazis P.A. (2006). Carotenoid profiles of two echinoids from central Greece, *Psammechinus microtuberculatus* and *Echinus esculentus*. *Journal of the World Aquaculture Society*, 37(3):339-344.
- Pantazis P.A., Kelly M.S., Connolly J.G., Black D. (2000). Effect of artificial diets on growth, lipid utilization, and gonad biochemistry in the adult sea urchin *Psammechinus miliaris*. *Journal of Shellfish Research*, 19(2):995-1001.

- Pearce C.M., Daggett T.L., Robinson S.M.C. (2002). Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture*, 214(1-4):307-332.
- Prato E., Fanelli G., Angioni A., Biandolino F., Parlapiano I., Papa L., Denti G., Secci M., Chiantore M. Kelly M., Ferrati M.P., Addis P. (2018). Influence of a prepared diet and a macroalga (*Ulva* sp.) on the growth, nutritional and sensory qualities of gonads of the sea urchin *Paracentrotus lividus*. *Aquaculture*, 493:240-250.
- Rey-Méndez M., Tourón N., Rodríguez-Castro B., Rama-Villar A., Fernández-Silva I., González N., martinez D., Ojeda J., Catoira J.L. (2015). Growth rate and gonadal index improvement in sea urchin culture *Paracentrotus lividus* (Echinoida: Echinidae). *Revista de Biología Tropical*, 63:261-272.
- Ricker W.E. (1979). Growth rates and models. In: Hoar W.S., Randall D.J., Brett J.R., (Eds). *Fish physiology*. Academic Press: London, pp. 677–743.
- Rowle R.J. (1990). Newly settled sea urchins in a kelp bed and urchin barren ground: a comparison of growth and mortality. *Marine Ecology Progress Series*, 62:229-240.
- Santos M., Albano P., Raposo A., Ferreira S.M., Costa J.L., Pombo A. (2020). The effect of temperature on somatic and gonadal development of the sea urchin *Paracentrotus lividus* (Lamarck, 1816). *Aquaculture*, 528:735487.
- Santos P.M., Silva J.A., Costa J.L., Pombo A. (2022). Effect of salinity on somatic growth and gonadal enhancement of the sea urchin *Paracentrotus lividus* (Lamarck, 1816). *Aquaculture*, 560:738593. <https://doi.org/10.1016/j.aquaculture.2022.738593>
- Schlosser S.C., Lupatsch I., Lawrence J.M., Lawrence A.L., Shpigel M. (2005). Protein and energy digestibility and gonad development of the European sea urchin *Paracentrotus lividus* fed algal and prepared diets during spring and fall. *Aquaculture Research*, 36(10):972.
- Senaratna M., Evans L.H., Southam L., Tsvetnenko E. (2005). Effect of different feed formulations on feed efficiency, gonad yield and gonad quality in the purple sea urchin *Heliocidaris erythrogramma*. *Aquaculture Nutrition*, 11:199–207.
- Spirlet C., Grosjean P., Jangoux M. (2001). Cultivation of *Paracentrotus lividus* (Echinodermata: echinoidea) on extruded feeds: digestive efficiency, somatic and gonadal growth. *Aquaculture Nutrition*, 7(2):91-99.
- Symonds R.C., Kelly M.S., Caris-Veyrat C., Young A.J. (2007). Carotenoids in the sea urchin *Paracentrotus lividus*: Occurrence of 9'-cis-echinenone as the dominant carotenoid in gonad colour determination. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 148(4):432-444.
- Tourón Besada, N. (2013). Caracterización genética de poblaciones de *Paracentrotus lividus* (Lamarck, 1816) en Asturias, Galicia y Canarias y desarrollo de sistemas de cultivo en batea. Tesis doctoral. Universidad de Santiago de Compostela, España.
- Woods C.M.C., James P.J., Moss G.A., Wright J., Siikavuopio S. (2008). A comparison of the effect of urchin size and diet on gonad yield and quality in the sea urchin *Evechinus chloroticus* Valenciennes. *Aquaculture International*, 16:49-68.
- Zupo V., Glaviano F., Paolucci M., Ruocco N., Polese G., Di Cosmo A., Constantin, M., Mutalipassi M. (2019). Roe enhancement of *Paracentrotus lividus*: Nutritional effects of fresh and formulated diets. *Aquaculture nutrition*, 25(1):26-38.

