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Seaweed inclusion in artificial diet of purple sea urchin Paracentrotus lividus: effects on somatic and gonadal development

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ABSTRACT

This work evaluated the inclusion of seaweed in artificial diet of *Paracentrotus lividus* sea urchins weighing between 11 and 14 g to test its effect on growth and gonadal yield. Initially, multiple-choice feeding preference assays were conducted using 20% fresh and dried specimens of *Laminaria digitata*, *Sargassum muticum*, and *Ulva lactuca* seaweeds incorporated into an artificial diet. Then, over the course of 20 weeks, experiments comprised of four different diets were carried out, including one for each seaweed incorporated into the artificial diet. Afterwards, sea urchins were weighed to evaluate somatic growth. From the eighth week onwards, animals were selected for gonadosomatic index and gonad coloration analyses. In the feeding preference test, fresh and dried *L. digitata* showed the best results. In test with seaweed incorporated into the artificial diet, the preference was for diets containing *L. digitata* and *S. muticum.* Both diets also promoted a higher gonadosomatic index than that of the control at the end of the experiment. Therefore, it can be concluded that the inclusion of *L. digitata* and *S. muticum* in the artificial diet of sea urchins can act as a food stimulant to improve growth performance.

Keywords: Gonadosomatic index; Food preference; Echinoderm culture; Mariculture; Artificial diet; *Laminaria digitata*.

Inclusão de macroalgas na dieta artificial do ouriço-do-mar roxo Paracentrotus lividus: efeitos no desenvolvimento somático e gonadal

RESUMO

Este trabalho avaliou a inclusão de algas marinhas na dieta artificial de ouriços-do-mar *Paracentrotus lividus* com peso entre 11 e 14 g para testar seu efeito no crescimento e rendimento gonadal. Inicialmente, foram realizados ensaios de preferência alimentar de múltipla escolha utilizando 20% de espécimes frescos e secos de *Laminaria digitata*, *Sargassum muticum* e *Ulva lactuca* incorporados a uma dieta artificial. Em seguida, ao longo de 20 semanas, foram realizados experimentos compostos de quatro dietas diferentes, incluindo uma para cada alga incorporada à dieta artificial. Depois, os ouriços-do-mar foram pesados para avaliação do crescimento somático. Da oitava semana em diante, os animais foram selecionados para análise do índice gonadossomático e da coloração gonadal. No teste de preferência alimentar, *L. digitata* fresca e seca apresentaram os melhores resultados. No teste com algas incorporadas à dieta artificial houve preferência por dietas contendo *L. digitata* e *S. muticum*. Ambas as dietas também promoveram índice gonadossomático superior ao controle no fim do experimento. Portanto, pôde-se concluir que a inclusão de *L. digitata* e *S. muticum* na dieta artificial de ouriços-do-mar pode atuar como estimulante alimentar para melhorar o desempenho de crescimento.

Palavras-chave: Índice gonadossomático; Preferência alimentar; Maricultura; Cultura equinoderma; Dieta artificial; *Laminaria digitata*.

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INTRODUCTION

Sea urchin fisheries have become ecologically unsustainable in many regions, particularly along the Mediterranean and Atlantic coasts of Europe, where unregulated harvesting of the purple sea urchin, *Paracentrotus lividus*, has caused drastic population declines beyond the point of natural recovery (FAO, 2020; Giglioli et al., 2021; Lawrence, 2013; Stefánsson et al., 2017) While some studies suggest that global warming have also contributed to this decline (Yeruham et al., 2019), overfishing remains the primary driver, fueled by high demand in countries such as Japan, Spain, Italy, Chile, and France (Stefánsson et al., 2017). Japan alone accounts for 80–90% of the global consumption, while countries like Chile, New Zealand, and the Philippines, as well as several European nations, have smaller domestic markets (Stefánsson et al., 2017).

In Europe, *P. lividus* is especially significant, being the most harvested species in the Mediterranean and Northeastern Atlantic, from Scotland to Morocco (Boudouresque & Verlaque, 2013). It is a major focus for aquaculture in Southern Europe; however, achieving commercially desirable gonad quality in aquaculture remains challenging (Bertocci et al., 2014; Harry & Eddy, 2015). Despite the demand being concentrated in Italy, France, and Spain, with a market size estimated between 3,000 and 3,500 tons, the lack of detailed market data and the presence of illegal fishing complicate the picture (Stefánsson et al., 2017). The increasing global demand over recent decades has intensified harvesting pressures, leading to significant depletion of natural stocks worldwide (Sartori et al., 2016). Gonadal characteristics, such as size, color, texture, and flavor, are essential for the market value of the product (McBride, 2005; Sakai et al., 2003). Therefore, a balanced diet that ensures these qualities, along with animal growth, is crucial for successful sea urchin farming (Cyrus et al., 2015a; Lawrence et al., 1997; Tomšić et al., 2015). Adequate levels of protein, carbohydrates, and digestible energy influence both somatic growth and gonadal development (Cook et al., 2007; Heflin et al., 2012; 2016b; Lawrence, 2001; Schlosser et al., 2005), while carotenoid intake is crucial for gonad coloration (Lourenço et al., 2021; Santos et al., 2020; Symonds et al., 2009). Several studies have attempted to modulate the coloration of *P. lividus* gonads by adding carotenoids from natural and synthetic sources to dried diets (Baião et al., 2019; Shpigel et al., 2006) or through a combination of diets based on seaweed (Shpigel et al., 2005).

However, achieving commercially desirable gonad development remains challenging in aquaculture (Harry & Eddy, 2015). Gonadal characteristics, such as size, color, texture, and flavor, are essential for product value (McBride, 2005; Sakai et al., 2003). Consequently, a balanced diet that ensures these qualities, along with animal growth, is crucial for successful sea urchin farming (Cyrus et al., 2015a; Lawrence et al., 1997; Tomšić et al., 2015). Protein, carbohydrate, and digestible energy levels influence somatic growth and gonadal development (Cook et al., 2007; Heflin et al., 2012; 2016b; Lawrence, 2001; Schlosser et al., 2005), while carotenoid intake affects gonad coloration (Lourenço et al., 2021; Santos et al., 2020; Symonds et al., 2009). Several studies have aimed to modulate the gonad coloration of *P. lividus* by adding carotenoids from natural and synthetic sources to dried diets (Baião et al., 2019; Shpigel et al., 2006) or through a combination of diets based on seaweed (Shpigel et al., 2005).

Although artificial diets have influenced the animal's growth, they do not seem to be as effective on gonads color, essentially because of the low commercial quality caused by the pale color (Azad et al., 2011; McBride, 2005; Pearce et al., 2004; Robinson et al., 2002; Shpigel et al., 2005). The inclusion of seaweeds in artificial diets has also had a positive effect on the growth of sea urchins (Cyrus et al., 2015a; Dworjanyn et al., 2007).

Apart from these promising results, experiments to evaluate food preference are still relevant for the preparation of diets and for the inclusion or replacement of ingredients. The factors that do or do not affect the intake of a specific diet by an organism may be restrictive (Dong et al., 2016; Fleming et al., 1996; Ribeiro & Nuñer, 2008). Studies on diet consumption is another important step to maximize the growth and gonadal development of sea urchins (Seymour et al., 2013).

The study aimed to identify the most effective diet–fresh seaweed, dried seaweed or an artificial formula–for enhancing the growth and overall health of purple sea urchin in aquaculture. A cost-efficient diet that promotes gonadal development while preserving key qualities like color, texture, and flavor is crucial for the sustainable success of sea urchin farming (Symonds et al., 2007). To achieve this, the study conducted food preference tests to evaluate which diet *P. lividus* preferred, and further analyzed how these different diets impacted somatic growth, the gonadosomatic index, and gonad coloration.

MATERIAL AND METHODS

Developing a cost-effective diet that enhances gonadal growth while maintaining the desired color, texture, and flavor of the gonads is essential for the sustainability of sea urchin aquaculture (Symonds et al., 2007). In this context, the present study aimed to evaluate the dietary preferences of the purple sea urchin, *P. lividus*, when provided with diets of fresh seaweed, dried seaweed, and an artificial formula, and to assess the effects of these diets on somatic growth, gonadosomatic index, and gonad coloration.

Biological material and experimental diets

Paracentrotus lividus were harvested in Bantry Bay (51°38'48"N; 9°34'53"W), Ireland, and transported to Bantry Marine Research Station (BMRS). Animals were fed a diet consisting of the seaweed *Laminaria digitata* (Hudson) JV Lamouroux (Ochrophyta, Phaeophyceae). The animals were acclimated in 5,000-L tanks with aeration and constant water flow until selection for subsequent experiments.

The seaweeds *L. digitata*, *Sargassum muticum* (Yendo) Fensholt (Ochrophyta, Phaeophyceae), and *Ulva lactuca* Linnaeus (Chlorophyta, Ulvophyceae) were harvested in the same site as that of the sea urchins. Excess water was removed with a paper towel before drying at 40°C for 16 h. After this period, seaweeds were weighed every hour until a constant weight was reached and then ground and sieved (600 μm).

Following Fernandez and Pergent (1998), a simple artificial diet was prepared for the control (DC). Three experimental artificial diets were prepared with 20% of each seaweed *L. digitata* (DL), *S. muticum* (DS), and *U. lactuca* (DU) (Table 1). The ingredients were mixed, and hot water was added. The artificial diets were pressed, cut into blocks of approximately 2 x 2 x 0.5 cm, and dried in an oven at 50°C for 16 h. After this period, the diets were weighed every half hour until the weight remained constant.

All experiments were performed at BMRS with water directly pumped from Bantry Bay. No heaters were used during the experimental period. Temperature and salinity were monitored from Monday to Friday by a digital thermometer and a refractometer. Arithmetic means based on measurements recorded on weekdays were calculated for the weekend.

Table 1. Composition (%) of artificial diets.

Ingredients (g 100·g^{1})	DC	DL	DS	DU
Corn flour	43	33	33	33
Wheat flour	43	33	33	33
Sunflower oil	9	9	9	9
Gelatin	5	5	5	5
Ground *Laminaria digitata*	0	20	0	0
Ground *Sargassum muticum*	0	0	20	0
Ground *Ulva lactuca*	0	0	0	20
Crude protein (dry base)	14.5	16.2	15.1	14.5

DC:control diet; DL: diet with *L. digitata*; DS: diet with *S. muticum*; DU: diet with *U. lactuca*.

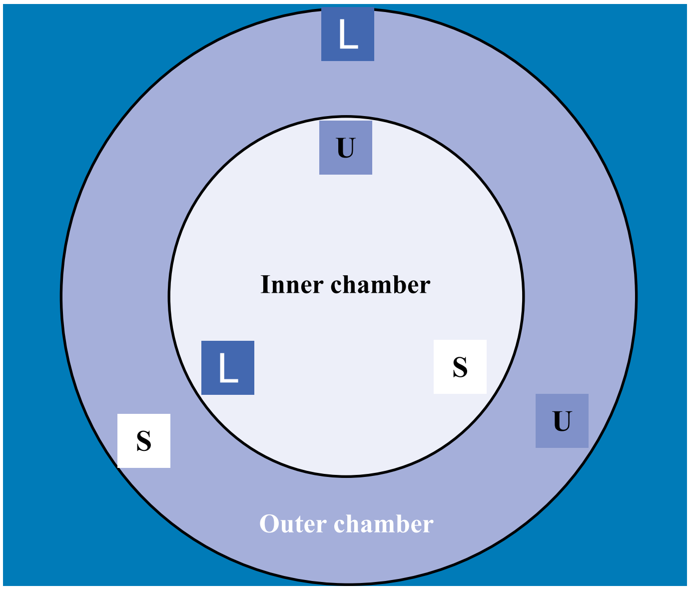
Food preference experiments

Following Prince et al.'s (2004) methodology, a multiple-choice food preference assay was undertaken with fresh seaweed, dry seaweed, and artificial diet (Fig. 1).

Experiment 1

For the experiment with fresh seaweed, eight sea urchins weighing between 11 and 13 g were selected (n = 8/each animal a replica). They were transferred to a 40-L tank with constant water flow (approximately 0.51 $L \cdot min^{-1}$) and kept without food for three days.

As shown in Fig. 1, each experimental unit was composed of a 1.5-L (inner chamber) round receptacle within another 3-L (outer chamber) receptacle, while water was constantly flowing from the inner to the outer chamber (n = 8/each animal a replica/ each sea urchins in its experimental unit). One random piece of fresh *L. digitata*, *S. muticum*, and *U. lactuca*, each weighing between 1 and 1.5 g fresh weight, was held by wire on the wall of the inner chamber, while another random piece of each seaweed of the same weight was held in the outer chamber such that the segments of seaweed were equidistant. A sea urchin was placed in the inner chamber of each experimental unit so that it could access the piece of seaweed of the inner chamber, without access to the external chamber, a model representing control



L: *Laminaria digitata*; S: *Sargassum muticum*; U: *Ulva lactuca*.

Figure 1. Food preference assay. Two pieces of each seaweed are each kept in both inner and outer chambers. Meanwhile, a sea urchin is kept in the inner chamber without access to the outer one.

over changes by autogeny. After 16 h, the seaweed from the inner chamber was removed, dried on paper, and weighed on a digital scale (± 0.001 g). Thereafter, consumption rate (C) was calculated as Eq. 1:

$$C = (Si \times SCf / SCi) - Sf \qquad (1)$$

Where: Si: the weight of the seaweed in the inner chamber at the beginning of the experiment; Sf: the weight of the seaweed in the inner chamber at the end of the experiment; SCi: the weight of seaweed in the outer chamber at the beginning of the experiment; SCf: the weight of seaweed in the outer chamber at the end of the experiment.

Experiment 2

The experiment with dry seaweed followed the same methodology as that for fresh seaweed with one difference (n = 8/each animal a replica). The seaweed was dried in an oven at 40°C, following the method previously described, and pieces from 0.5 to 1.0 g were fixed inside the chambers. After the experimental period (16 h), seaweed samples were dried once more and weighed until the weight was constant. The consumption rate was then calculated according to Eq. 1.

The same methodology was used for artificial diets. After a 16 h experimental period, feeds were dried in an oven at 50°C until they reached constant weight. The consumption rate was then calculated according to Eq. 1.

Experiment 3: somatic and gonadal growth

Purple sea urchins (n = 65 animals for each treatment) weighing between 11 and 14 g were randomly selected and placed in rectangular (13 × 22 × 60 cm) cages with a 1-cm^2 mesh size to compare weight, gonadosomatic index (GSI), and gonad hue. Five cages, one for each treatment plus control, were placed horizontally on the surface of a round 2,000-L tank with constant water flow (approximately 8 L•min^{-1}) and exposed to a natural photoperiod (from May to October). Tanks were siphoned daily.

Treatments consisted of four artificial diets (DC, DL, DS, and DU). Control diet consisted of fresh *L. digitata* (FA) cut into strips measuring 15 × 4 cm. Treatment and control diets were replaced daily. Animals were weighed on a digital scale every four weeks for 20 weeks. Dead sea urchins were removed daily from the cages.

Fifteen animals from each cage were randomly selected prior to the start of the experiment for GSI analysis. Then, after weighing at the eighth week, the animals were again selected for GSI analysis every four weeks until the end of the 20-week experimental period. Gonads were removed and weighed after excess water was extracted.

GSI was calculated as Eq. 2:

$$GSI\ (\%) = (Wg / Wa) \times 100 \qquad (2)$$

Where: Wg: the wet weight of the gonad; Wa: the wet weight of the animal.

Gonadal coloration was analyzed by a market-based color scale, following Symonds et al. (2009), and classified as follows: excellent (bright yellow and bright orange); acceptable (light orange and dark orange); and unacceptable (light yellow and dark brown).

Statistical analysis

All data, except for gonad coloration, were subjected to the Shapiro-Wilk's test to verify normality and to Levene's test to verify homoscedasticity (α = 0.05). The data on feeding preference and GSI were transformed using the arcsine square root transformation for normalization and then subjected to analysis of variance (ANOVA0 and Tukey's test (α = 0.05). For the non-parametric data related to sea urchin weight, the Kruskal-Wallis' test was applied, followed by Bonferroni's adjustment for multiple comparisons, correcting the significance level (α = 0.05). Data on gonad color were analyzed using Pearson's χ^2 test (α = 0.05). All analyses were conducted using Statistica 13 software.

RESULTS

Food preference

The sea urchin *P. lividus* showed a significant preference for *L. digitata* (Fig. 2), fresh or dry. Dry seaweed rehydrated immediately after being submerged in water and showed a pale hue. However, sea urchins presented low intake of rehydrated seaweed, including *L. digitata* (< 0.1 g), when compared to artificial diet fresh seaweed. Compared to control feed (DC), sea urchins demonstrated a greater preference for artificial diets comprised of *L. digitata* (DL) and *S. muticum* (DS). In the food preference assays, temperature ranged between 12 and 13°C, and salinity varied between 31 and 33‰.

Somatic growth and gonadal development

Somatic growth increased during the experimental period, whereas GSI demonstrated growth only during the final period, after falling from the GSI recorded at the initial analysis prior to the start of the experiment. At the end of the experiment, animals fed with DL and DS diets weighed more when compared to those fed with DC and fresh algae (FA). Significant differences occurred from the eighth week onwards regarding the FA diet and from the 16th week onward with regard to the DC diet (Fig. 3).

At the end of the experimental period, sea urchins fed with DL and DS diets had the highest GSI when compared to those fed with the DC diet (Fig. 4).

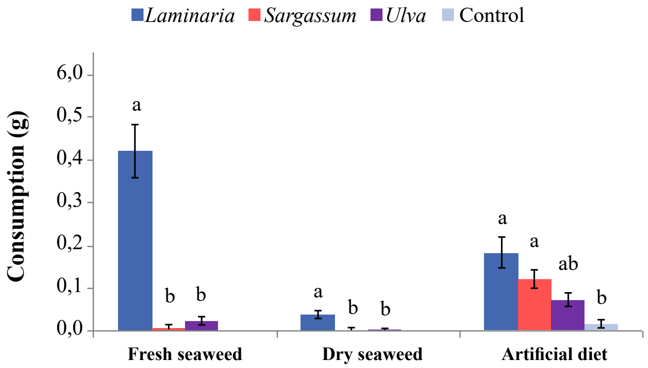


Figure 2. Food intake by the sea urchin *Paracentrotus lividus* after 16 h of fresh and dry seaweed (both with *Laminaria digitata, Sagassum muticum* and *Ulva lactuca*) and artificial diets (control diet and with 20% *L. digitata, S. muticum*, and *U. lactuca*). Data are expressed as mean ± standard error (n = 8). Different letters indicate statistical difference in separate assays (α = 0.05) (analysis of variance/Tukey post-hoc).

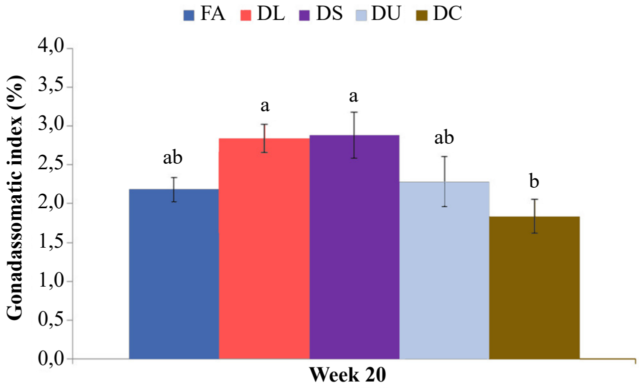


Figure 4. Gonadosomatic index of the sea urchin *Paracentrotus lividus* fed different types of diets (FA: fresh seaweed *Laminaria digitata*; DL: artificial diet with *L. digitata*; DS: artificial diet with *Sagassum muticum*; DU: artificial diet with *Ulva lactuca*; artificial diet DC control). Data are expressed as mean ± standard error (n = 15). Different letters indicate statistical difference in each assay (α = 0.05) (analysis of variance/Tukey post-hoc).

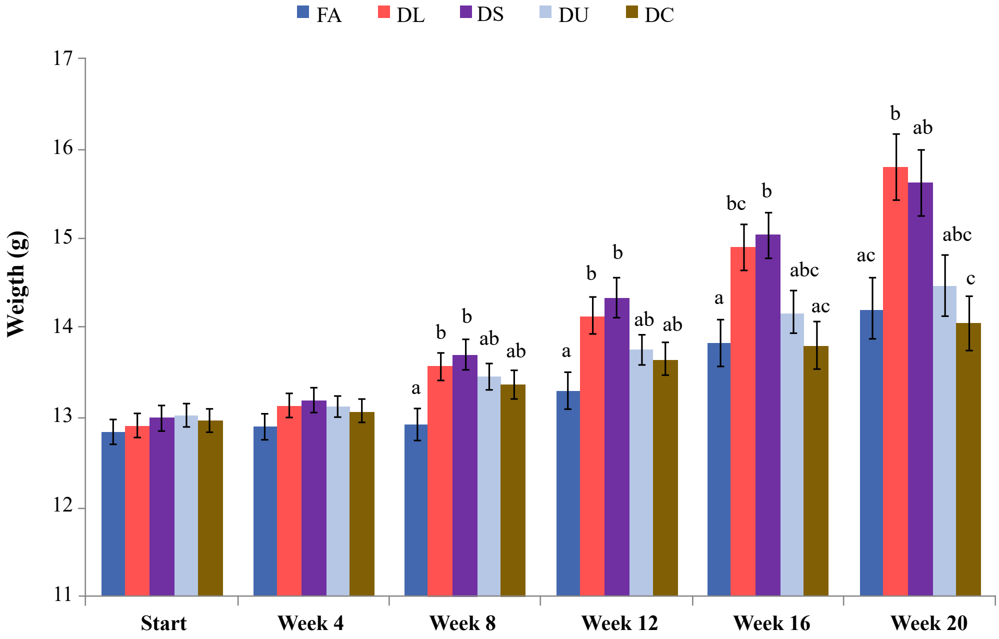


Figure 3. Weight of sea urchin *Paracentrotus lividus* fed different types of diet (FA: fresh seaweed *Laminaria digitata*; DL: artificial diet with *L. digitata*; DS: artificial diet with *Sagassum muticum*; DU: artificial diet with *Ulva lactuca*; artificial diet DC control). Data are expressed as mean ± standard error (n = 65 up to the eighth week; n = 50 up to the 12th week; n = 35 up to the 16th week; n = 20 up to the 20th week). Different letters indicate statistical difference among treatments (α = 0.05) (Kruskal-Wallis / Bonferroni post-hoc).

Since gonad hue showed no statistical difference, it was apparent that the diets failed to have any effect on this parameter (Fig. 5). At the 16th week, sea urchins fed the FA diet showed a high number of gonads of slightly over 50%, which can be classified as excellent.

During the assay on animal growth, the temperature of the tank ranged between 10 and 18°C (mean = 13.4 ± 1.5°C), with salinity between 30 and 35‰ (mean = 32.7 ± 1.2‰). Mortality data included one animal from the *L. digitata* artificial diet cage and two from the control diet cage during the first four weeks. During the next four weeks, one animal from each cage with artificial diets *L. digitata* and *U. lactuca* and fresh diet with *L. digitata* died. No other deaths occurred during the other months.

DISCUSSION

The sea urchin *P. lividus* showed a strong preference for the seaweed *L. digitata*, both fresh and dry, in comparison with the other two evaluated seaweeds. When all seaweed species were offered simultaneously, sea urchins fed mainly on *L. digitata*. In Bantry Bay, Symonds et al. (2007) reported that the main food sources for *P. lividus* were seaweeds of the genera *Laminaria* and *Palmaria palmata*, possibly the result a preference for one seaweed over another or the abundance of one species over another in the region. However, using different species of dry and fresh seaweed, such as *P. palmata*, *Saccharina latissima*, *L. digitata* and *Grateloupia turuturu*, Castilla-Gavilán et al. (2019) observed that sea urchins consumed the same amount of seaweed, fresh or dry.

As in the case of *S. muticum*, *P. lividus* seems to avoid consuming exotic species when offered fresh seaweed (Monteiro et al., 2009; Tomas et al., 2011). However, Cardoso et al. (2020) carried out a study using different species of brown seaweed, such as *Laminaria ochroleuca*, *Saccorhiza polyschides*, and *Undaria pinnatifida*, and red seaweed, such as *Chondrus crispus*, *Mastocarpus stellatus*, and *Grateloupia turuturu*. They found that *P. lividus* made no distinction between native and non-native seaweed, a result contrary to that observed in our study.

In comparison to the DC diet, *P. lividus* showed a preference for both DL and DS diets. Although *S. muticum* was almost never consumed, either fresh or dried, it was still accepted in the presence of *L. digitata* when included in an artificial diet. No significant difference was seen in the consumption of DL when compared to DS and DU diets, as could have been expected by the food preference assays with fresh and dried seaweed. The addition of seaweed to an artificial diet does not seem to affect the chemical and sensory properties of the sea urchin (Cyrus et al., 2015b).

Herbivory-triggered ecological pressure has caused many seaweeds to develop defensive morphological and chemical adaptations (Duffy & Hay, 1990; Paul & Puglisi, 2004). Secondary metabolites, such as polyphenols, florotanins, acetogenins, terpenoids, and halogenate compounds, have toxic effects on animals or lessen the palatability of seaweed (Cacabelos et al., 2010; Pereira & Vasconcelos, 2014). However, because of coevolution, several herbivores have also acquired

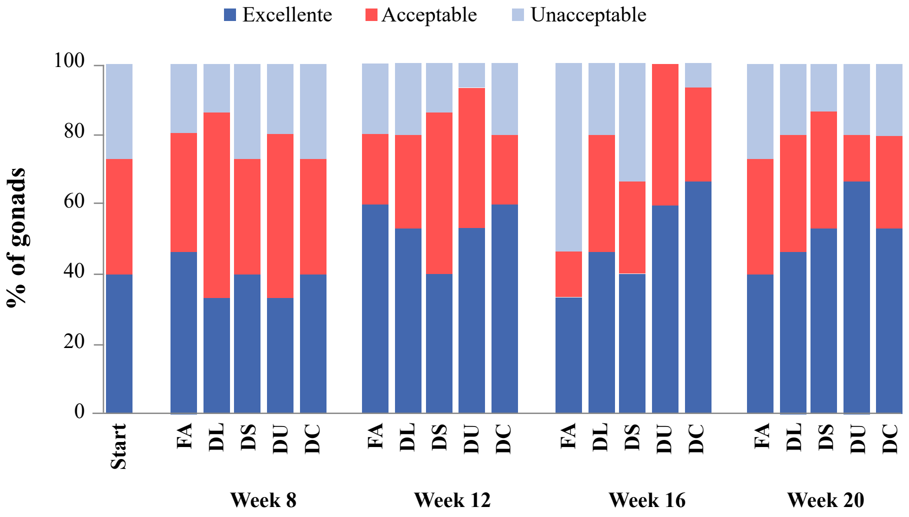


Figure 5. Classification of gonads of sea urchin *Paracentrotus lividu*s according to hue when fed different types of diets (FA: fresh seaweed *Laminaria digitata*; DL: artificial diet with *L. digitata*; DS: artificial diet with *Sagassum muticum*; DU: artificial diet with *Ulva lactuca*; artificial diet DC control). No statistical difference was found (α = 0.05) [Pearson (χ^2)].

adaptations and tolerance to seaweed defenses, affecting preference for certain types (Seymour et al., 2013; Sotka & Gantz, 2013; Steneck et al., 2017).

The drying and grinding of seaweeds for their inclusion in artificial food eliminates structural and morphological differences among the species. This fact may also affect preference for certain seaweeds over others. However, when several ground seaweeds mixed in agar were provided, the sea urchin *Lumbriculus variegatus* showed food preferences like those in which fresh seaweed was offered as a food choice. Thus, it can be said that food preference of the sea urchin is predominantly mediated by chemical interactions (Souza et al., 2008), which are not affected, at least in part, by the processing of algae. Like *L. lividus* in the present work, the sea urchin *Tripneustes gratilla* showed the same food preference between fresh and dried seaweed, but it had a lower intake of dried seaweed over fresh. Nonetheless, when included in the artificial diet, no differences in consumption of diets with seaweeds (Dworjanyn et al., 2007).

In the present work, GSI did not reach 4%. *P. lividus*, which was harvested at the end of summer in Bantry Bay, provided an index of approximately 5% (Symonds et al., 2007). Schlosser et al. (2005) reported GSI of 5% in sea urchins cultivated in the autumn and 18% in the spring. The GSI fell significantly when compared to the percentage at the start of the experiment. This indicates that the animals spawned, partly owing to experimental handling. High density in cages also caused a lower growth rate during the first months. This fact may have caused the decrease in GSI and thus provided lower growth rates and feed conversion (Goebel et al., 2017; Siikavuopio et al., 2007). Owing to the density decrease throughout harvest, weight gain and GSI rates tended to rise. According to Blount et al. (2017), density decrease in wild populations of the sea urchin improved the size and hue of gonads in a few weeks by the greater access to feed. This strategy could be extended to farm populations.

The diet with only fresh *L. digitata* provided a low somatic growth rate when compared to DL and DS diets, but the same gonadosomatic indexes were obtained when sea urchins were fed on artificial diets. It is a well-known fact that artificial diets increase the somatic and gonadal growth of sea urchins (Baião et al., 2019; Lourenço et al., 2021). Somatic growth rates and gonad production are directly related to protein levels in the diet (Heflin et al., 2016a, 2016b; Lourenço et al., 2022). The protein level in *Laminaria* spp. may vary between 7 and 13% (Dawczynski et al., 2007; Kennedy et al., 2005), whereas DL and DS provided the highest protein rates, respectively 16.5 and 15.1%, forecasting the best dietary results. Although diets with over 20% protein levels cause higher growth rates, higher levels may also increase food conversion rates and thus production costs (Cuesta-Gomez & Sánchez-Saavedra, 2017; Fernandez & Boudouresque, 2000; Hammer et al., 2006; Heflin et al., 2016a). However, the protein source may have a greater influence on GSI rates than concentration (Pearce et al., 2002).

The results from the FA diet revealed no significant difference in gonadosomatic index when compared to other artificial diets and could have been the product of low protein levels in the artificial diets employed. Phillips et al. (2010) registered significant differences between GSIs of sea urchins fed on artificial diets and those fed seaweed only in diets with a higher protein level (23%). No difference was observed in diets with lower (18%) protein levels. However, Tomšić et al. (2015) reported higher GSI rates in artificial seaweed diets with protein levels ranging between 12.5 and 22.5%. Several artificial diets have not resulted in maximum gonadal yield in sea urchins (Cyrus et al., 2014), and the mechanisms related to GSI increase and feeding remain unknown (Cyrus et al., 2015b). Results may have been also affected by low GSI, lack of mineral vitamin complex in artificial diets, and greater access to feed in cages with the FA diet. Such increased access could be attributed to the greater area of alga stalks when compared to artificial feed, especially considering the high density characterizing the experiment.

The DL and DS diets had the best GSI and somatic growth when compared to the DC diet. Other studies have confirmed the improvement in these two parameters caused by the inclusion of seaweed (Shpigel et al., 2005). This result is mainly related to an increase in consumption rates and absorption of diets with seaweed, which can stimulate feeding (Cyrus et al., 2015b; Dworjanyn et al., 2007; Pearce et al. 2003; Purbiantoro et al., 2014). Cyrus et al. (2015a) reported improvement in diets with 20% *Ulva* when compared to diets without seaweed, but only in somatic growth, not GSI rate. Cyrus et al. (2014) did not observe any improvement in somatic growth or GSI, even though an increase in consumption rate was reported. Increase in consumption rate causes a greater ingestion of nutrients with correspondingly greater weight gain and GSI. However, seaweed species can influence sea urchin performance. For example, the inclusion of *Ulva* seaweed in the diet, termed as DU, showed no differences relative to the control group. This probably occurred because of lower attractiveness and, consequently, lower consumption, as also observed in the food preference experiment. Cyrus et al. (2015a) reported increased growth and feed absorption efficiency in sea urchins fed their preferred seaweed.

The inclusion of seaweed in the diet may have enhanced gonadal growth and development through a supply of minerals required by the sea urchin, a supply which may have been missing in the artificial diets used in the current experiment. Seaweeds are known for being richer in minerals and trace elements than land plants. Many food supplements provide the required nutrients not found in these plants (Evans & Critchley, 2014; Ito & Hori, 1989; MacArtain et al., 2007; Rupérez, 2002).

No difference in gonad hue was reported among the supplied diets. The diet containing *L. digitata* alone failed to improve this characteristic, at least in the gonad developmental phase, when compared to other artificial diets. Symonds et al. (2007) reported a broad variety in the gonad hue of *P. lividus* harvested at the same time and place.

Previous studies (Cyrus et al., 2014, 2015a; Shpigel et al., 2005) have shown that sea urchins fed an artificial diet have gonads with inferior coloration when compared to those fed only seaweed. However, in the present study, this difference was not significant, even though the artificial diet (DC) produced more than 50% of gonads with excellent coloration by the 16th week, according to our scoring system, as noted before. Cyrus et al. (2015a) reported a noticeable improvement in gonad coloration after feeding sea urchins with seaweed, while Pearce et al. (2002, 2003) found no significant difference in the inclusion or exclusion of seaweed in the diet. Thus, the effects on gonad coloration from the inclusion of seaweed in the artificial diets of sea urchins is a topic requiring further investigation. Seaweeds have nutrients, mainly carotenoids, which can have positive effects on gonad color. In Lourenço et al.'s (2020) study, for instance, it was observed that the concentration of carotenoids and the resultant color attributes of sea urchin gonads are mainly influenced by the sea urchin's sex and gonad size. The study also revealed that the mechanisms of carotenoid metabolism and storage in females help achieve the desired color in the market, irrespective of the dietary source of carotenoids.

Some studies have linked the desired colors of sea urchin gonads with the presence of equinone, the main carotenoid found in this organ. Carotenoids ingested by sea urchins accumulate in the intestinal walls and can be degraded or synthesized into new substances. Β-carotene is metabolized to equinone in the gonads themselves or, in part, in the intestine and then transferred to the gonads. Other ingested carotenoids that accumulate in the intestine do not accumulate in large cups in the gonads (Kelly & Symonds, 2013). However, other studies did not find a direct relationship between equinone and gonad color, suggesting the involvement of other factors, such as lipid concentration, the presence of other carotenoids, and animal size (Symonds et al., 2007, 2009). In practice, the inclusion of β-carotene in the diet has shown positive results in obtaining gonads with better colors (Pearce et al., 2003; Robinson et al., 2002; Suckling et al., 2011), while the intake of other carotenoids did not show the same effect (Shpigel et al., 2006; Suckling et al., 2011).

A proposed approach to improve the production of high-value commercial gonads and reduce production costs is the use of two diets during the sea urchin cultivation cycle. One diet would be used to promote somatic growth, while the other, rich in β-carotene, would be provided during the final gonad developmental phase. This method would allow sea urchins to quickly utilize available resources to produce larger-sized eggs with better coloration in a short period of time.

Previous research by Blount et al. (2017), Cyrus et al. (2015b), and Sartori et al. (2016) also emphasized that improving diet quality results in sea urchins capable of producing higher-quality eggs in a reduced timeframe. These findings have important implications for the sea urchin aquaculture industry, suggesting that specific feeding strategies can be adopted to optimize the production of valuable gonads.

By understanding the urchins' dietary preferences, the study aimed to optimize feeding strategies that support gonadal development without compromising the quality of the final product in terms of color, texture, and flavor, which are critical for market viability (Symonds et al., 2007). Furthermore, assessing food preferences is important for ensuring the overall well-being of the sea urchins. Providing a diet that the urchins are inclined to consume not only improves growth and reproductive outcomes, but also enhances animal welfare by reducing stress and promoting natural feeding behaviors. Animal welfare considerations in aquaculture involve creating conditions that mimic the natural environment as closely as possible, including offering diets that are palatable and nutritionally adequate. By evaluating both preference and the effects of different diets on somatic growth and the gonadosomatic index, the study could identify feeding protocols that balance productivity and welfare.

Although *P. lividus* showed a preference for the seaweed *L. digitata*, the exotic alga *S. muticum* revealed similar results when added to artificial diets. This is relevant since the harvest of this species to produce artificial feed for the cultivation of *P. lividus* may control its proliferation and impact, as well as avoid harvest pressure on natural banks of *L. digitata.*

CONCLUSION

The current study showed that brown seaweed can be used as an ingredient in artificial diet of sea urchin *P. lividus*.

Its inclusion in the diet is a feed stimulant and directly affects the attractiveness of the diet. It also gives better results in gonadosomatic index and in the animal's somatic growth, reducing the overall farming time.

CONFLICT OF INTERESTS

Nothing to declare.

DATA AVAILABILITY STATEMENT

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AUTHORS' CONTRIBUTION

Conceptualization: Pontinha, V.A, Maguire, J., Hayashi, L.; **Investigation:** Pontinha, V.A., Maguire, J.; **Resources:** Pontinha, V.A.; **Writing – original draft:** Pontinha, V.A.; **Writing – review & editing:** Simioni, C., Vieira, F.N., Hayashi, L.; **Final approval:** Simioni, C.; **Formal Analysis:** Vieira, F.N., Hayashi, L.; **Supervision:** Vieira, F.N., Hayashi, L.

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