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# A new sustainable formulated feed based on discards from food industries for rearing the sea urchin *Paracentrotus lividus* (Lmk)

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## Abstract

During a 18-week experiment in a sea-based system, *Paracentrotus lividus* was fed with two formulations of a new sustainable feed whose main ingredients were food farming discards, mostly outermost lettuce leaves, in different percentage. Egg white was added as protein source and binder, and a little amount of commercial fish feed was added as lipid source. At the end of the experiment, a high survival rate (>80%) and an increase in test diameter (22%), total weight (130%), gonad weight (240%) and gonad index expressed as size-adjusted gonad weight (288%) were recorded. Gonads achieved a good colour and high levels of essential and polyunsaturated fatty acids, hence representing a commercially valuable product. Comparing the two feed formulations, the one with the lower lettuce content (57% vs. 67%) led to the best product quality, in terms of gonad features (gonad index and colour) and content of essential fatty acids. In order to move towards a higher sustainability of the aquaculture sector, this study proposes a low-cost feed, produced with cheap and sustainable ingredients such as food farming discards.

**KEYWORDS**
bio-economy, blue-economy, echinoculture, fatty acids, gonads, nutritional quality

## 1 | INTRODUCTION

The purple sea urchin *Paracentrotus lividus* (Lamarck) is an ideal candidate to satisfy the demand of sea urchin roe in the European market. Unfortunately, in the last decades, the increment of its request has resulted in the overexploitation of natural populations and a consequent decline of stocks in the Mediterranean Sea (Gianguzza et al., 2006; Guidetti, 2004; Pais, Serra, Meloni, Saba, & Ceccherelli, 2012). Aquaculture production of sea urchins may represent an ecologically sustainable alternative to satisfy the market demand, preserving natural populations. Indeed, recently much effort has been put to set-up rearing protocols for gonad enhancement (Kelly & Chamberlain, 2010; McCarron, Burnell, Kerry, & Mouzakitis, 2010; Sartori, Pellegrin, Macchia, & Gaion, 2016; Spirlet, Grosjean, & Jangoux, 2000). Nevertheless, one of the main gaps in echinoculture is the identification and development of a high nutritional quality diet to boost the production of high-quality gonads in terms of biomass, colour, texture and taste and, at the same time, economically effective and environmental sustainable. Several studies worldwide reported that sea urchins fed only with macroalgae show improved gonad colour and taste, but low somatic and gonadal growth, while those fed only with high-protein-formulated feeds show overall higher somatic growth and gonad yield but pale gonad colour and bitter taste (Carrier, Eddy, & Redmond, 2017; Daggett, Pearce, Tingley, Robinson, & Chopin, 2005; Shpigel, McBride, Marciano, Ron, & Ben-Amotz, 2005; Unuma, Sakai, Agatsuma, & Kayaba, 2015). Both options, however, are scantily sustainable as they are based on the exploitation

  

of natural resources, which may lead to detrimental ecological effects (Carrier et al., 2017; Daggett et al., 2005). A good combination of gonad colour and yield has been obtained by feeding *P. lividus* first with a formulated diet and then with algal diets (Shpigel et al., 2005). The formulation of a diet with both vegetal and animal ingredients may maximize sea urchin somatic growth reducing time to market and lead to high gonad quality and yields.

To date, several attempts have been made to formulate an alternative diet to natural one by using a balanced mix of vegetable and animal sources in order to obtain a good market product, combining high somatic and gonadal growth with high gonad nutritional and organoleptic quality (Kennedy, Robinson, Parsons, & Castell, 2005; Otero-Villanueva, Kelly, & Burnell, 2004; Pantazis, Kelly, Connolly, & Black, 2000; Schlosser, Lupatsch, Lawrence, Lawrence, & Shpigel, 2005; Spirlet, Grosjean, & Jangoux, 2001). Nonetheless, to date, little consideration has been given to create a profitable while ecologically sustainable echinoculture industry (Fabbrocini et al., 2012; Vizzini, Miccichè, Vaccaro, & Mazzola, 2015; Vizzini, Visconti, Vaccaro, & Mazzola, 2018). Therefore, nowadays, the formulation of sea urchin feeds and the development of cost-effective feeding protocols are priorities for satisfying the economy needs to develop sustainable echinoculture. In this context, the exploitation of constantly available land-based vegetables seems to be a good option to replace other vegetable-based components in the diet of *P. lividus* (i.e., macroalgae). Indeed, recent laboratory and sea-based feeding trials reported that fresh discards of the lettuce *Lactuca sativa* (L.) increased gonadal growth and improved gonad colour in *P. lividus* (Vizzini et al., 2015, 2018). Moreover, lettuce has a high percentage of C18 fatty acids (FA; Le Guedard, Schraauwers, Larrieu, & Bessoule, 2008), which are known as potential precursors of (n-3) and (n-6) polyunsaturated C20 FAs in sea urchins (Bell, Dick, & Kelly, 2001; Castell et al., 2004). For these reasons, lettuce may be used in low-cost and environmentally sustainable feeds for sea urchin aquaculture, but, as previously mentioned, the use of only vegetable sources does not assure a sufficient gonad yield for obtaining a marketable product. Hence, the addition of animal proteins in the formulation of feeds is necessary.

Main aim of this study was to assess the performance of a new sustainable formulated feed including, as main ingredients, different percentages of food farming discards of both vegetal (*L. sativa* leaves) and animal origin (egg white), and, to a lesser extent, commercial fish feed. In more detail, we tested the response of *P. lividus* to the new formulated feed in terms of survival rate, test diameter, total weight, gonad index expressed as size-adjusted gonad weight (sensu Ebert, Hernandez, & Russell, 2011), gonad colour, and essential and polyunsaturated fatty acids, especially those belonging to the *omega*-3 family. Despite the importance of fatty acid content and profile in the diets provided to reared species, including sea urchins (Carboni, Hughes, Atack, Tocher, & Migaud, 2013; Kelly, Carboni, Cook, & Hughes, 2015; Liu et al., 2007), there are only few studies reporting the role of FA content in natural, industrial or formulated feeds on the nutritional and commercial quality of sea urchin roe (Cook, Bell, Black, & Kelly, 2000; Pantazis et al., 2000; Prato et al., 2018).

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental feed formulations

A new sustainable feed was prepared in two formulations differing for the percentage of the main ingredients. In more detail, *Lactuca sativa* discards (fresh outermost leaves) were used as the main ingredient in both formulations, in percentages of 57% (2L formulation) and 67% (3L formulation). Egg white was added in both formulations (2L: 29%; 3L: 22%) to provide animal proteins, which are the major determinant of gonadal growth and taste for edible sea urchins (Walker et al., 2015) and for its natural binding features (Bukola, Fashina Bombata, & Elegbede, 2015). Commercial pelletized fish feed (ALTERNA MARINE 1P, Skretting Italia SpA, Loc. Vignetto, Mozzecane [Vr], Italy) was also added in lower amounts in both formulations (2L: 14%; 3L: 11%) to ensure a high supply of lipids and essential fatty acids (EFAs). Ingredients were mixed in a blender, and then, bar-shaped feeds were obtained by oven cooking the mixture (180°C). Afterwards, the bar-shaped feeds were stored at −20°C until further use.

### 2.2 | Sea urchin feeding experiment

The experiment was carried out in a sea-based system at the fish farm "Ittica S. Giorgio srl" (Licata, Southern Sicily: 37°5′12.82″N 13°56′28.69″E). 1,080 specimens of *Paracentrotus lividus* of comparable size (22.6 ± 1.2 mm) were located in 6 plastic cages (60 × 50 × 20 cm; internal surface area = 1.20 $m^2$), 180 specimens in each cage, and kept starved for one month prior to the onset of the feeding treatment (Cipriano-Maack, Wood, & Culloty, 2017; Spirlet et al., 2000). Cages were kept at a depth of 2 m, hanged up along a 60-m linear rope, which was maintained at the water surface by means of buoys, and moored to the sea bottom with ballasts. The experiment started on 1st December 2011 and ran until 12th April 2012 (18 weeks). Three cages were randomly assigned to each of the two feed formulations and then represent replicates of each formulation. Before feed provision, cages were always cleaned by faeces and any feed remains, and then, in each cage, sea urchins were fed ad libitum.

At the beginning of the experiment ($T_0$) and three further dates distant approximately 50 days each other ($T_1$, $T_2$ and $T_3$), test diameter was measured in 50 sea urchins per cage through a vernier calliper (±0.1 mm). On the same dates, 3 sea urchins were randomly collected from each cage and total wet weight (TW) and gonad wet weight (GW) were measured through a Sartorius electronic balance (Genius Me 235S ±0.01 g), and test diameter (TD) using a Vernier calliper (±0.1 mm). The same three sea urchins were also used to assess the colour of the roe according to Shpigel et al. (2005) and adjusted in three categories (I: inadequate, A: acceptable and E: excellent) following Symonds, Kelly, Suckling, and Young (2009). The same observer used a Pantone® colour standards chart (Colour Formula Guide 1000, 1991) in natural daylight to assign each specimen to a single category.

In addition, on the same dates ($T_0$, $T_1$, $T_2$, $T_3$), two sea urchins were randomly sampled from each cage for the analysis of fatty acid profiles of the gonads.

At the end of the experiment, survival rate (%) was calculated as follows:

$$S = (s_f/s_i) * 100$$

where $s_f$ is the number of surviving sea urchins at the end of experiment and $s_i$ is the initial number of sea urchins.

During the whole experiment, surficial sea water temperature and dissolved oxygen were recorded every day using a multi-parameter probe (Hydrolab-DS5).

## 2.3 | Fatty acid analysis

Fatty acid (FA) analysis was carried out on sea urchin gonads, the two feed formulations and their ingredients (*L. sativa* and fish feed). Egg white was not analysed for FAs because it is made by only water and proteins. Samples were freeze-dried and ground. Following a modified version of the Bligh and Dyer (1959) method, lipids were extracted using a MilliQ distilled water:methanol:chloroform mixture (1:2:1 v:v:v) with 0.01% BHT (butylated hydroxyltoluene) to avoid lipid oxidation. Samples were then sonicated to improve lipid extraction and then centrifuged twice to separate the lipid phase from the aqueous phase. The lipid extracts were evaporated to dryness under gentle nitrogen stream, weighed and expressed as percentage of total lipids. Then, lipid extracts were subjected to acid-catalysed transesterification using methanolic hydrogen chloride to obtain fatty acid methyl esters (FAMEs), which were then analysed by a gas chromatograph (GC-2010, Shimadzu) equipped with a BPX-70 capillary column (30 m length; 0.25 mm ID; 0.25 μm film thickness, SGE Analytical Science) and detected by a flame ionization detector (FID). Peaks were identified by retention times from mixed commercial standards (37FAME from Supelco; QUALFISH from Larodan). Tridecanoic and tricosanoic acids (C13:0 and C23:0) were used as surrogate standards, while pentacosanoic acid methyl ester (ME C25:0) was used as internal standard for FAME quantification.

## 2.4 | Data elaboration and statistical analysis

The occurrence of a cage effect in influencing sea urchin growth and nutritional quality was tested at univariate level using a randomized complete block design ANOVA (STATISTICA V.10) setting cage, feed formulation and time as predictive factors for each variable of interest. Differences in test diameter of *Paracentrotus lividus* were tested through a two-way repeated ANOVA (n: 50 non-independent replicates) followed by Tukey's *post hoc* comparison tests (STATISTICA V.10). Normality and homogeneity of variance were previously tested using respectively Shapiro–Wilk and Cochran's test, and transformation of data was not necessary.

The relationship between sea urchin gonad weight and size was assessed using an allometric growth model with an adjustment for size when gonads begin to develop (Ebert et al., 2011):

$$GW{:}\alpha\,(TD-C)^{\beta}$$

where GW is gonad weight (g), TD is test diameter (cm), $C$ is the size when gonads begin to develop (0.5 cm, following Spirlet, Grosjean, & Jangoux, 1994). After ln transformation, ANCOVA was run using general linear models (GLM, STATISTICA V. 10) (Ebert et al., 2011), to assess the opportunity to use a single slope $\alpha$ and a single exponent $\beta$ in the computation of the size-adjusted gonad weight. To do this, ln GW was set as dependent variable, sampling times as categorical predictor and ln (TD−$C$) as covariate. Separate ANCOVAs were run for each feed formulation (2L, 3L).

Permutational multivariate analysis of variance (PERMANOVA, 9,999 permutations) was used to test differences in total wet weight and size-adjusted gonad weight between feed formulations (Factor Feed: 2 levels, 2L and 3L) and sampling times (Factor Time: 4 levels, $T_0$, $T_1$, $T_2$ and $T_3$) using PRIMER-E software (Plymouth, UK). Both factors were fixed and orthogonal.

Gonad samples collected at $T_2$ for fatty acids analysis were lost and consequently not analysed and not included in the statistical tests. Individual FAs were expressed as percentage of total FAs and resembled using Bray–Curtis similarity after arcsine square root function transformation. PERMANOVA was carried out on transformed data to test differences in FA profile between feed formulations (Factor Feed: 2 levels, 2L and 3L) and sampling times (Factor Time: 3 levels, $T_0$, $T_1$ and $T_3$). Both factors were considered fixed and orthogonal. Canonical analysis of principal coordinates (CAP, Anderson & Willis, 2003) was run on the gonad FA profiles based on the interaction of factors "Feed × Time." The main classes of FAs, together with those considered as important indicators of nutritional quality in aquaculture, namely EFA such as arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), *omega*-3 and *omega*-6 PUFA and the ratio *omega*-3/*omega*-6 (Gago, Luis, & Repolho, 2009; Sargent, Bell, McEvoy, Tocher, & Estevez, 1999) were superimposed to the graph. The analysis of percentage similarity (SIMPER) on untransformed data was used to identify the FAs that contributed more to the similarity within, and the dissimilarity between the two formulated feeds and between sea urchins fed with the two feeds across time.

# 3 | RESULTS

Throughout the experiment, mean temperature of surface water ranged between 12.31 ± 0.36°C in February and 16.01 ± 1.87°C in December, while dissolved oxygen between 6.72 ± 0.42 mg/L in December and 7.73 ± 0.29 mg/L in March (Figure 1).

Cage effect was not significant ($p > 0.05$) for any of the tested variables. Survival rates of sea urchins at the end of the experiment were high for both feed formulations (2L: 88.5 ± 6.9%; 3L: 88.1 ± 4.6%). Somatic growth had also a similar trend. Both test diameter and total weight significantly increased during the experiment, with no significant differences between the two feed formulations (Figure 2a, b, Table 1).

The relationship between gonad weight and test diameter (adjusted for size when gonads begin to develop) of the sea urchins fed

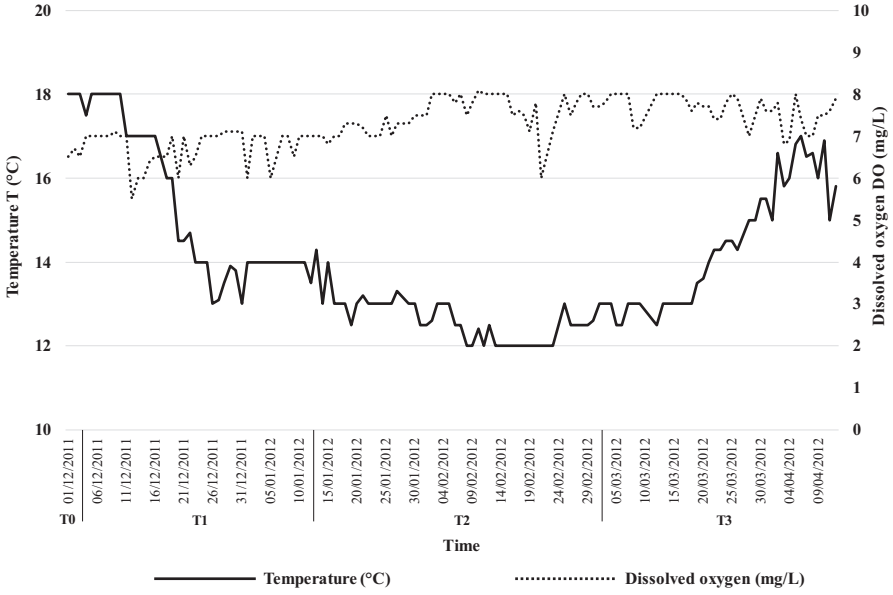


**FIGURE 1** Temperature (°C) and dissolved oxygen (mg/L) measured in sea water during the feeding experiment

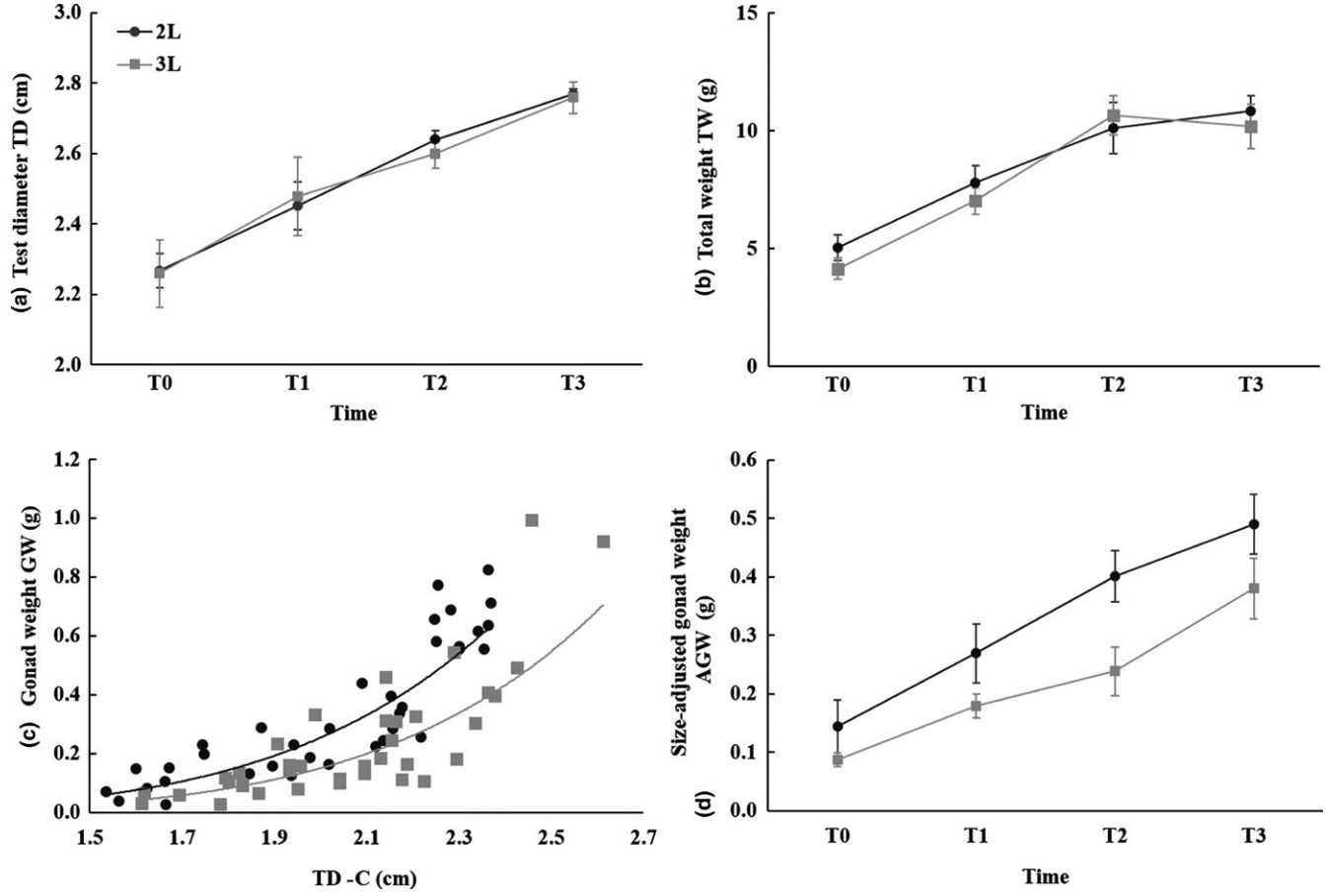


**FIGURE 2** (a) Test diameter (mean ± *SE*), (b) total weight (mean ± *SE*), (c) allometric regression between gonad weight and test diameter (adjusted for size when gonads begin to develop); parameters of the regressions are indicated in the text and (d) size-adjusted gonad weight (mean ± *SE*) of *Paracentrotus lividus* fed with the two feed formulations, 2L and 3L, during the experiment

with the two feed formulations well fitted the allometric growth model (Figure 2c; 2L: $R^2 = 0.779$; 3L: $R^2 = 0.679$). ANCOVA revealed that the difference in ln GW for the interaction of the terms ln (TD – C) and Time was not significant for both feed formulations (2L: MS = 0.11, $F_{(3,28)} = 0.68$, $p = 0.57$; 3L: MS = 0.27, $F_{(3,28)} = 1.77$, $p = 0.18$); therefore, $\alpha$ and $\beta$ were considered the same for all

**TABLE 1** (a) Repeated ANOVA results testing the effects of the fixed and orthogonal factors Feed (2L and 3L) and Time ($T_0$–$T_3$) on test diameter of *Paracentrotus lividus*; (b) PERMANOVA results testing differences in total weight and size-adjusted gonad weight of *P. lividus* between feed formulations across time

| **(a) *Repeated* ANOVA** | | | | | | |
|---|---|---|---|---|---|---|
| **Source** | ***df*** | **MS** | ***F*-value** | ***p*-value** | | **Tukey's test** |
| Test diameter | | | | | | |
| Feed | 1 | 0.05 | 0.01 | 0.910 | | |
| Time | 3 | 27.45 | 61.48 | **<0.001** | | $T_0 < T_1 < T_2 < T_3$ |
| Feed × Time | 3 | 0.12 | 0.27 | 0.841 | | |
| **(b) PERMANOVA** | | | | | | |
| **Source** | ***df*** | **MS** | **Pseudo-*F*** | ***P*(perm)** | **perms** | **Pair-wise tests** |
| Total weight | | | | | | |
| Feed | 1 | 3.53 | 0.69 | 0.406 | 994 | |
| Time | 3 | 141.62 | 27.6 | **<0.001** | 998 | $T_0 < T_1 < T_2 = T_3$ |
| Feed × Time | 3 | 2.01 | 0.39 | 0.745 | 997 | |
| Size-adjusted gonad weight | | | | | | |
| Feed | 1 | 0.20 | 12.59 | **<0.001** | 998 | 3L < 2L |
| Time | 3 | 0.33 | 21.13 | **<0.001** | 999 | $T_0 < T_1 < T_2 < T_3$ |
| Feed × Time | 3 | 0.00 | 0.55 | 0.663 | 998 | |

*Note*. Tukey's test and pair-wise tests indicate significant post hoc differences.
Significant differences are highlighted in bold characters.

samples per formulation (2L: $\alpha = 0.006$, $\beta = 5.387$; 3L: $\alpha = 0.003$, $\beta = 5.775$). Moreover, mean ln (TD −C) was 0.967 b and 0.719 for 2L and 3L, respectively, mean ln GW was −1.347 and −1.739 for 2L and 3L, and the standard error of the estimate $SE_{est}$ was 0.398 and 0.393, respectively. Size-adjusted gonad weight showed significant differences between feed formulations and times, but not for their interaction, with significantly higher values in the sea urchins fed with 2L than in those fed with 3L, and a significant gradual increment across the whole experiment (Figure 2d; Table 1).

The best result in terms of gonad colour was obtained in the sea urchins fed with the 3L formulation with a peak of 100% of the sum of excellent (E) and acceptable (A) categories at $T_2$, while 2L showed a peak of 90% of E and A categories at the end of the experiment (Figure 3).

Fatty acid profiles of the new feed formulations were significantly different (PERMANOVA: Pseudo-$F_{(1,4)}$: 2,410.4; $p < 0.001$). Overall, the two formulations were characterized by a different relative amount of saturated fatty acids (SFA: 2L > 3L) and monounsaturated FAs (MUFA: 2L < 3L), while the percentage of polyunsaturated FAs (PUFA) was overall comparable (Table 2). Within MUFA and PUFA, the FAs 18:2 n6 (linoleic acid, LA), 18:1(n-9) and 20:5(n-3) (eicosapentaenoic acid, EPA) were responsible of the main differences, as revealed by the SIMPER analysis (Supporting information Data S1a). Among the other FAs identified by SIMPER, also 16:1(n-7), 16:0, 22:6(n-3) (docosahexaenoic acid, DHA) and 18:3(n-3) (α-linolenic acid, ALA) contributed to the differences observed between the two feed formulations (Supporting information Data S1a). Looking at the biomarkers of nutritional quality, the sum of (n-3) PUFA and (n-3) HUFA was higher in the 2L than in the 3L formulation, while ∑(n-6) PUFA showed the opposite trend. All the essential fatty acids (EFAs), namely EPA, ARA and DHA, were also higher in 2L than in 3L, as well as the ratio EPA/ARA, in contrast to the ratio DHA/EPA. The main ingredients of the new formulated feed, *Lactuca sativa* and the commercial fish feed, showed very different profiles, being the former almost exclusively constituted by 18:3(n-3) (ALA), 18:2(n-6) (LA) and 16:0, consistently with the literature (Le Guedard et al., 2008), and the latter by a high relative abundance of 18:1(n-9), 18:2(n-6) (LA) and 16:0, and also EFAs, in particular DHA and EPA (Table 2). Lipid content was much higher in fish feed than in lettuce, while it was overall comparable between the two feed formulations (Table 2).

Fatty acid profiles of gonads were significantly different for the interaction between factors Feed and Time (Table 3). Pair-wise tests revealed that they did not differ between feed formulations at $T_0$, while they differ at both $T_1$ and $T_3$. Moreover, FA profiles of the gonads of the sea urchins fed with the two formulations differed across all the sampling times (Table 3). SIMPER analysis revealed that 18:3(n-3) (ALA) contributed mostly to the differences between the gonads of sea urchins fed with 2L and 3L at both $T_1$ and $T_3$, and also that all the three EFAs (i.e., EPA, ARA and DHA) contributed to dissimilarity at $T_1$, but only EPA and ARA at $T_3$ (Supporting information Data S1b). Moreover, the dissimilarity of the FA profile from the onset to the end of the feeding experiment ($T_0$–$T_3$) was higher in the sea urchins fed with 2L than with 3L, and mainly driven by ALA, which decreased across time, followed by EPA and ARA, which, in contrast, increased across time (Supporting information Data S1c).

Canonical analysis of principal coordinates (CAP) of the FA profiles of the sea urchin gonads showed a clear separation of $T_3$ from

**TABLE 2** Fatty acid profiles and lipid content (mean ± *SD*) of the two feed formulations 2L and 3L, and the main ingredients: *Lactuca sativa* and commercial fish feed [ALTERNA MARINE 1P, Skretting Italia SpA, Loc. Vignetto, Mozzecane (Vr), Italy]

| | Feed formulations | | | | Ingredients | | | |
|---|---|---|---|---|---|---|---|---|
| | 2L | | 3L | | Lettuce (*L. sativa*) | | Fish feed | |
| FAs | m | ds | m | ds | m | ds | m | ds |
| 12:0 | 0.17 | 0.00 | 0.16 | 0.01 | 0.21 | 0.01 | 0.14 | 0.00 |
| 14:0 | 4.41 | 0.05 | 2.17 | 0.03 | 0.38 | 0.01 | 2.97 | 0.00 |
| 15:0 | 0.38 | 0.00 | 0.27 | 0.01 | 0.16 | 0.01 | 0.31 | 0.00 |
| 16:0 | 16.04 | 0.13 | 12.96 | 0.04 | 12.05 | 0.34 | 14.69 | 0.03 |
| 17:0 | 0.35 | 0.00 | 0.24 | 0.01 | 0.16 | 0.01 | 0.32 | 0.00 |
| 18:0 | 3.70 | 0.02 | 2.86 | 0.02 | 1.47 | 0.07 | 3.72 | 0.02 |
| 20:0 | 0.39 | 0.00 | 0.41 | 0.01 | 0.48 | 0.02 | 0.40 | 0.00 |
| 22:0 | 0.30 | 0.00 | 0.38 | 0.01 | 0.91 | 0.05 | 0.25 | 0.00 |
| LCFAs (>22:0) | 0.21 | 0.01 | 0.24 | 0.01 | 1.29 | 0.04 | 0.12 | 0.00 |
| ∑ SFA | 25.96 | 0.17 | 19.69 | 0.10 | 17.13 | 0.54 | 22.91 | 0.06 |
| 14:1(n-5) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.16 | 0.00 |
| 16:1(n-7) | 5.65 | 0.09 | 2.53 | 0.01 | 0.38 | 0.06 | 3.80 | 0.00 |
| 17:1(n-7) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 |
| 18:1(n-7) | 2.64 | 0.02 | 2.02 | 0.02 | 0.83 | 0.06 | 2.85 | 0.00 |
| 9t-18:1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.35 | 0.01 |
| 18:1(n-9) | 16.33 | 0.10 | 24.23 | 0.08 | 1.79 | 0.28 | 26.61 | 0.05 |
| 20:1(n-9) | 0.83 | 0.00 | 1.72 | 0.03 | 0.14 | 0.02 | 2.55 | 0.01 |
| 20:1(n-11) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.21 | 0.02 |
| 22:1(n-9) | 0.16 | 0.01 | 0.27 | 0.01 | 0.11 | 0.00 | 0.41 | 0.00 |
| 24:1(n-9) | 0.28 | 0.00 | 0.33 | 0.01 | 0.46 | 0.04 | 0.39 | 0.00 |
| ∑ MUFA | 25.89 | 0.17 | 31.10 | 0.02 | 3.70 | 0.41 | 37.36 | 0.06 |
| 6t-18:2 | 0.05 | 0.00 | 0.06 | 0.01 | 0.00 | 0.00 | 0.05 | 0.01 |
| 18:2(n-6) | 20.76 | 0.05 | 29.35 | 0.26 | 22.61 | 1.27 | 16.52 | 0.01 |
| 18:3(n-3) | 4.20 | 0.05 | 6.87 | 0.27 | 56.05 | 2.12 | 3.88 | 0.01 |
| 18:3(n-6) | 0.28 | 0.00 | 0.17 | 0.01 | 0.00 | 0.00 | 0.22 | 0.00 |
| 18:4(n-3) | 1.71 | 0.07 | 1.10 | 0.02 | 0.00 | 0.00 | 1.31 | 0.02 |
| 20:2(n-6) | 0.26 | 0.00 | 0.34 | 0.01 | 0.26 | 0.02 | 0.52 | 0.00 |
| 20:3(n-3) | 0.10 | 0.00 | 0.14 | 0.01 | 0.17 | 0.01 | 0.24 | 0.00 |
| 20:3(n-6) | 0.20 | 0.00 | 0.14 | 0.01 | 0.08 | 0.01 | 0.25 | 0.00 |
| 20:4(n-3) | 0.57 | 0.02 | 0.47 | 0.01 | 0.00 | 0.00 | 0.72 | 0.00 |
| 20:4(n-6) - ARA | 0.63 | 0.01 | 0.32 | 0.02 | 0.00 | 0.00 | 0.80 | 0.01 |
| 20:5(n-3) - EPA | 9.86 | 0.14 | 4.08 | 0.07 | 0.00 | 0.00 | 5.83 | 0.01 |
| 22:4(n-6) | 0.12 | 0.00 | 0.10 | 0.01 | 0.00 | 0.00 | 0.19 | 0.00 |
| 22:5(n-3) | 1.42 | 0.04 | 0.97 | 0.01 | 0.00 | 0.00 | 1.65 | 0.00 |
| 22:6(n-3) – DHA | 7.97 | 0.14 | 5.11 | 0.13 | 0.00 | 0.00 | 7.55 | 0.01 |
| ∑ PUFA | 48.15 | 0.25 | 49.22 | 0.10 | 79.17 | 0.83 | 39.73 | 0.01 |
| ∑ (n-3) PUFA | 25.85 | 0.28 | 18.74 | 0.33 | 56.23 | 2.11 | 21.18 | 0.02 |
| ∑ (n-6) PUFA | 22.30 | 0.04 | 30.47 | 0.27 | 22.94 | 1.29 | 18.55 | 0.01 |
| (n-3)/(n-6) | 1.16 | 0.01 | 0.98 | 0.31 | 1.54 | 0.67 | 1.15 | 0.01 |
| ∑ (n-3) HUFA | 21.55 | 0.24 | 11.73 | 0.23 | 0.00 | 0.00 | 17.06 | 0.02 |
| ∑ EFA | 18.47 | 0.28 | 9.51 | 0.21 | 0.00 | 0.00 | 14.18 | 0.02 |

*(Continues)*

**TABLE 2** (Continued)

| | Feed formulations | | | | Ingredients | | | |
|---|---|---|---|---|---|---|---|---|
| | 2L | | 3L | | Lettuce (*L. sativa*) | | Fish feed | |
| FAs | m | ds | m | ds | m | ds | m | ds |
| DHA/EPA | 0.81 | 0.00 | 1.25 | 0.01 | – | – | 1.29 | 0.00 |
| EPA/ARA | 15.62 | 0.04 | 12.85 | 0.56 | – | – | 7.33 | 0.08 |
| Lipid content (mg/g dw) | 139.22 | 24.82 | 143.58 | 8.10 | 48.93 | 6.22 | 206.18 | 8.45 |

*Notes*. Main biomarkers of nutritional quality are also indicated. ARA: arachidonic acid; DHA: docosahexaenoic acid; EFA: essential fatty acid; EPA: eicosapentaenoic acid; HUFA: highly unsaturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

the other sampling times along the first canonical axis (Figure 4). Samples at $T_3$ were also distinct along the second canonical axis based on the feed formulation. A high PUFA content characterized sea urchins fed with 3L, which clustered at the top right, while high ARA, EPA (and the sum of total EFAs), as well as the sum of (n-3) HUFA and (n-6) PUFA, drove the separation of the sea urchins fed with 2L at the bottom right. Unlike $T_3$, all the sea urchins kept starved in the cages subsequently intended for the feeding experiment ($T_0$) and only 3L at $T_1$ were grouped at the top left, while 2L at $T_1$ clustered on the bottom left driven by the high abundance of DHA. Although the high inter-individual variability, lipid content of the gonads tended to increase across time for both feed formulations, peaking at $T_3$ in the sea urchins fed with 2L (Supporting information Data S2).

## 4 | DISCUSSION

Both formulations of the new sustainable feed composed by food farming discards, and to a lesser extent by commercial fish feed, resulted palatable and effective for rearing the sea urchin *Paracentrotus lividus*, leading to high somatic and gonadal growth, and good gonad quality in a 18-week experiment. The new formulated feed led to an averaged increment in test diameter (22%), total weight (130%), and especially in gonad weight (240%) and gonad index, expressed as size-adjusted gonad weight (288%) sensu Ebert et al. (2011). Moreover, the relationship between gonadal and somatic growth was well described by an allometric model, consistent with previous studies on sea urchin growth (Ebert et al., 2011; Ouréns, Freire, & Fernández, 2012) and energy allocation in medium-sized sea urchins (Barker, Keogh, Lawrence, & Lawrence, 1998; McCarron, Burnell, & Mouzakitis, 2009), such as those used in the present experiment (2.5 ± 0.2 cm). Indeed, medium-sized sea urchins are characterized by higher somatic growth than larger sea urchins, in which, instead, gonadal growth is fostered because of decoupling of body and gonadal growth (Lawrence, 2000). Nevertheless, here, size-adjusted gonad weight underwent a threefold mean increase over the experiment, highlighting that the sea urchins fed with the new manufactured feed had good feed intake, digestion efficiency and nutrient conversion in a short time period (18 weeks). At the beginning of the experiment, both measured and size-adjusted gonad weight showed low values, mirroring the combination of the reproductive stage and starving. Indeed, in fall season, sea urchins are overall in their recovery and spent stages (i.e., gonads almost devoid of sexual cells; Spirlet et al., 2000; Sánchez-España, Martínez-Pita, & García, 2004; Schlosser et al., 2005), reflecting the beginning of the gonadal maturation process in the Mediterranean Sea (Lozano et al., 1995). In addition, the starvation regime prior to the experiment may have led

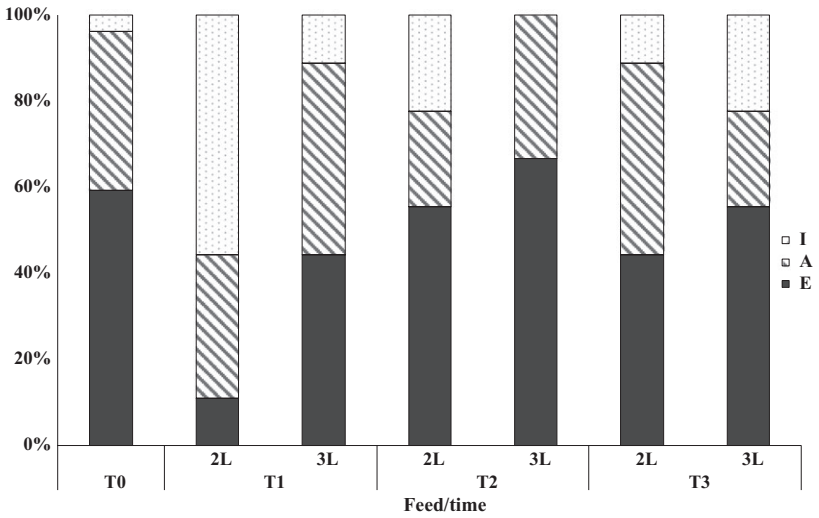


**FIGURE 3** Gonad colour of *Paracentrotus lividus* fed with the two feed formulations (2L, 3L) across time ($T_0$–$T_3$). Values are represented as the per cent of individuals with gonads in each colour category (E: excellent, A: acceptable, I: inadequate)

**TABLE 3** PERMANOVA results testing the effects of the fixed and orthogonal factors Feed (2L, 3L) and Time ($T_0$, $T_1$, $T_3$) on FA profiles of *Paracentrotus lividus*

| PERMANOVA | | | | | |
|---|---|---|---|---|---|
| **a) Main test** | | | | | |
| **Source** | ***df*** | **MS** | **Pseudo-*F*** | ***P*(perm)** | **perms** |
| Feed | 1 | 107.75 | 8.50 | **<0.001** | 9,927 |
| Time | 2 | 176.72 | 13.94 | **<0.001** | 9,922 |
| Feed x Time | 2 | 28.47 | 2.25 | **<0.01** | 9,915 |
| **b) Pair-wise tests** | | | | | |
| **Differences between feeds within times** | | | | | |
| **Time** | | **Feed** | ***t*** | ***P*(perm)** | **perms** |
| $T_0$ | | Starved | 1.28 | 0.125 | 462 |
| $T_1$ | | 2L versus 3L | 2.17 | **<0.01** | 462 |
| $T_3$ | | 2L versus 3L | 2.42 | **<0.01** | 462 |
| **Differences between times within feeds** | | | | | |
| **Feed** | | **Time** | ***T*** | ***P*(perm)** | **perms** |
| 2L | | $T_0$ versus $T_1$ | 3.48 | **<0.01** | 462 |
| | | $T_0$ versus $T_3$ | 3.82 | **<0.01** | 462 |
| | | $T_1$ versus $T_3$ | 2.08 | **<0.01** | 462 |
| 3L | | $T_0$ versus $T_1$ | 2.15 | **<0.01** | 462 |
| | | $T_0$ versus $T_3$ | 2.83 | **<0.01** | 462 |
| | | $T_1$ versus $T_3$ | 2.22 | **<0.01** | 462 |

*Notes*. (a) Main test, (b) Pair-wise tests.
Significant differences are highlighted in bold characters.

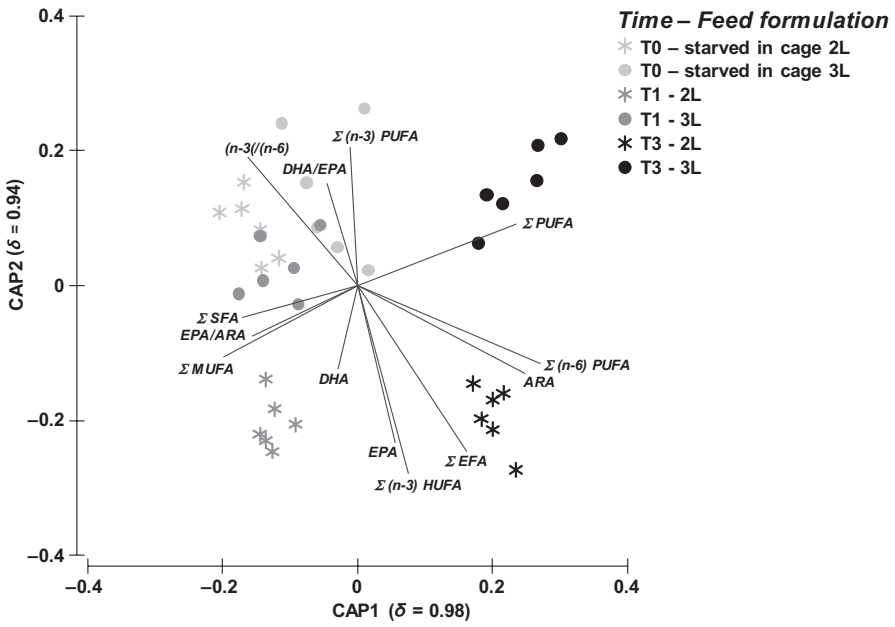


**FIGURE 4** Canonical analysis of principal coordinates (CAP) of the fatty acid profiles of *Paracentrotus lividus* fed with the two feed formulations, 2L and 3L, across time. The main classes of FAs and the main indicators of nutritional quality are superimposed to the graph. The meaning of the acronyms is reported in Table 4

to the resorption of the gonadal tissue (Spirlet et al., 2001). Once fed again, sea urchins rapidly start recovering from starvation through multiplying gametocytes and storing nutrients in phagocytes (Spirlet et al., 2001), leading to an increase. Anyway, by comparing sea urchins fed with the two feed formulations, it is evident that gonadal growth was fostered by the 2L feed formulation, while somatic

growth did not result influenced by both formulations. Indeed, size-adjusted gonad weight significantly increased over time and was also significantly higher in the sea urchins fed with the 2L feed than those fed with the 3L feed. In contrast, test diameter significantly increased across sampling times in a comparable way for all reared sea urchins.

*P. lividus* gonads also displayed an acceptable and excellent colour in both feed formulations. This result confirms the suitability of lettuce as a major ingredient in the formulation of a new feed for obtaining a good roe product, due to their high concentration in carotenoids (Caldwell & Britz, 2006), as previously observed in adult *P. lividus* reared under laboratory conditions (Vizzini et al., 2015) and in small *P. lividus* reared in a sea-based system (Vizzini et al., 2018). Furthermore, this finding indicates that the egg white content (about 20%–30%) was suitable for the purpose stated, and not excessive to induce a worsening of the product. Indeed, although proteins are crucial to maintain physiological functions (Watts, Lawrence, & Lawrence, 2013), previous studies showed that high-protein diets causes pale off-white gonad colour (Robinson, Castell, & Kennedy, 2002; Shpigel et al., 2005). Moreover, also in this case, the comparison of the two feed formulations revealed a better performance of the 2L formulation, yielding a good colour quality coupled with a higher improvement of gonad features.

As regards fatty acids (FA), the two feed formulations reflected the ingredient contribution, proportionally to their lipid content: Despite the lower content of commercial fish feed compared to that of lettuce, its fourfold higher lipid content explains the high essential fatty acid (EFA) amount in the feed. Furthermore, comparing the two formulations, 2L, featured by a lesser amount of lettuce and a higher amount of fish feed than 3L, showed the higher content of all EFAs: arachidonic acid (ARA, 20:4(n-6)), eicosapentaenoic acid (EPA, 20:5(n-3)) and docosahexaenoic acid (DHA, 22:6(n-3)), and the lowest content of the C18 FAs, typical of *L. sativa* (Le Guedard et al., 2008, present results). Same patterns were observed in the gonads of the sea urchins fed with 2L, compared to those fed with 3L, indicating that dietary FA content influenced the gonad composition through maternal provision, confirming the few available data (Carboni et al., 2013; Gago et al., 2009). Therefore, despite PUFA and EFA increased across time in the sea urchins fed with both the feed formulations, the higher levels of (n-6) and (n-3) PUFA and EFA at the end of the experiment with the 2L formulation reflect unambiguously the dietary input, indicating also a higher nutritional quality. Indeed, EFA, in addition to having important physiological functions in gamete development and reproductive performance (Cook, Hughes, Orr, Kelly, & Black, 2007; Watts et al., 2013), has widely acknowledged beneficial effects on human cardiovascular and nervous systems, playing also a key role in the response to inflammation (Ward & Singh, 2005). Therefore, nowadays, EFAs are important targets in pharmaceutical and nutraceutical sectors; they are also considered indicators of high nutritional quality in aquaculture products, have a huge request in the seafood market sector and therefore would represent an added value in the sea urchin market.

The higher protein level (i.e., higher egg white content) of the 2L formulation compared to 3L one may also have contributed to the higher accumulation of EFA in the gonadal tissues of the sea urchins fed with 2L, due to the close link between lipid and protein metabolism, as previously observed in *P. lividus* by Cook et al. (2007). The high ARA level may also be linked to the ability of sea urchins to synthesize de novo PUFAs from their precursors. Likewise the patterns observed by Carboni et al. (2013), the much higher content of ARA in the gonads than in the feed, especially at the end of the experiment, as well as the decrease in linoleic acid (18:2(n-6)), is likely to be the result of the ability of sea urchins to synthesize ARA from its precursor [18:2(n-6); Bell et al., 2001; Carboni et al., 2013]. Anyway, despite it is acknowledged that gonad FA composition results from endogenous synthesis plus exogenous input, dietary accumulation seems to be the predominant FA pathway in the gonads.

Overall, the results of this study demonstrate that the combination of *L. sativa*, egg white and commercial fish feed was able to assure a good growth, to maintain the organoleptic characteristics and in particular the gonad colour of reared specimens (Vizzini et al., 2015) and to improve the nutritional quality of sea urchin roe. Moreover, the ingredients used are available all year round and are easy to store, frozen or fresh, making easier the formulation of sustainable feeds. Lastly, comparing the two feed formulations, present findings indicated that the formulation featured by a lesser amount of lettuce and a higher amount of egg white and commercial fish feed (namely 2L) has led to the best response in terms of sea urchin gonadal growth, colour improvement and abundance of essential fatty acids, indicators of high nutritional quality. These results show the importance of a well-balanced ingredient selection (vegetable and animal) in the production of formulated echinoculture feeds, to ensure a proper provision of pigments, proteins and lipids (notably fatty acids) for obtaining a good quality product. Therefore, the new formulated feed would represent a good choice for echinoculture to satisfy the need of a high-quality product coupled with a sustainable cost-effective feed formulation.

## 5 | CONCLUSION

This study highlighted that food farming discards, namely the outermost leaves of the lettuce *Lactuca sativa* and egg white, are optimal ingredients for producing sustainable feeds for rearing herbivorous/omnivorous invertebrates such as sea urchins. These ingredients, coupled to only a small amount of commercial fish feed, resulted performant in terms of sea urchin survival, growth and gonad improvement, encouraging the exploitation of food discards for the development and use of new eco-friendly feeds in aquaculture. Such evidence of the good conversion potential of discards from food industries into biomass of commercial value in the aquaculture sector meets the requirements of bio-economy and blue-economy, which promote circular processes and sustainable development leading to the reduction in the use of natural resources and waste production. Also, sea-based rearing

systems may represent a promising activity for echinoculture in the Mediterranean Sea and, through the diversification of reared species, may represent also an additional income for local farmers guaranteeing a good quality product with a reduced environmental and economic impact.

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