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Turning waste into gold: Sustainable feed made of discards from the food industries promotes gonad development and colouration in the commercial sea urchin *Paracentrotus lividus* (Lamarck, 1816)

Laura Ciriminna[a,b], Geraldina Signa[a,b,*], Antonino Maurizio Vaccaro[a], Giulia Visconti[a], Antonio Mazzola[a,b], Salvatrice Vizzini[a,b]

[a] *DISTEM, Dipartimento di Scienze della Terra e del Mare, Università degli Studi di Palermo, via Archirafi 18, Palermo, Italy*
[b] *CoNISMa, Consorzio Nazionale Interuniversitario per le Scienze del Mare, Piazzale Flaminio 9, Rome, Italy*

A R T I C L E I N F O

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A B S T R A C T

Development of sustainable aquaculture practices is a suitable solution to reduce the pressure on overexploited stocks of the Mediterranean sea urchin, *Paracentrotus lividus*, and to respond to the increasing market demand. To move forward the Blue Growth and following the principles of circular economy, a three-month feeding experiment was conducted to test a sustainable feed based on food processing discards on sea urchins. Two feed formulations differing on the proportions of the two main ingredients (endive outermost leaves and European anchovy discards in a ratio of 60:40 and 80:20 respectively) were prepared and tested on *P. lividus* gonad yield, development and quality. The results were compared with those of wild sea urchins to assess the differences with natural patterns. Both feed formulations promoted gonad growth resulting in a significantly higher percentage increase in gonad biomass compared with wild specimens (490%, 330% and 78% increase in gonad weight in the feed 60/40, 80/20 and wild sea urchins respectively). Similarly, GSI of reared sea urchins varied by about 3–12% and 14% for sea urchins fed with feeds 80/20 and 60/40 respectively, while that found in wild sea urchins varied by about 3–5%. Gonad development was also boosted by the provision of the sustainable feed, as sexual maturation was faster in reared specimens than in wild ones. At the end of the trial, reared sea urchins showed also a very high (> 90%) frequency of marketable gonad colour. Lastly, the formulation with a more balanced vegetal/animal ratio (feed 60/40) gave the best results overall, combining the highest GSI and the best gonad colouration. Outcomes of this study confirm the suitability of food processing discards as ingredients for sea urchin feeds, although further research is needed to evaluate the effects on nutritional quality and organoleptic features of sea urchin gonads.

1. Introduction

Sea urchin gonads, also known as roe, are a prized seafood product worldwide. Consequently, a dramatic reduction of natural stocks of sea urchin commercial species, due to uncontrolled over-exploitation, has been recently observed (Lawrence, 2013; Pais et al., 2011; Yeruham et al., 2019). In many countries of the Mediterranean area, *Paracentrotus lividus* (Lamarck, 1816) is the most exploited sea urchin for commercial purposes, with substantial detrimental impacts on local populations and the whole ecosystems (Guidetti et al., 2004; Pais et al., 2007). In particular, France has one of the oldest artisanal sea urchin fisheries, but also in Spain and Italy purple sea urchins are widely harvested as gonads are traditionally considered a delicacy (Stefánsson et al., 2017). The introduction in the last century of new fishing methods, together with the spread of illegal and unlicensed fishing, contributed to the over-exploitation of *P. lividus* natural populations (Addis et al., 2012; Carboni et al., 2014; Fernández-Boán et al., 2012), despite the existence of local laws for managing and regulating its catches (Fernández-Boán et al., 2012; G.U.R.I, 1995).

The development of sustainable and effective aquaculture practices could be a valuable solution to reduce the pressure on natural sea urchin stocks and satisfy its market demand (Carboni et al., 2013). However, today echinoculture is still based on harvesting sea urchins, due to the lack of proper sea urchin hatcheries able to provide juveniles (Rubilar

* Corresponding author at: DISTEM, Dipartimento di Scienze della Terra e del Mare, Università degli Studi di Palermo, via Archirafi 18, Palermo, Italy.
E-mail address: geraldina.signa@unipa.it (G. Signa).

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and Cardozo, 2021) and the low growth rates of juveniles to reach marketable size (Boudouresque and Verlaque, 2007). Moreover, another critical bottleneck for echinoculture, and for aquaculture in general, is the exploitation of natural resources as ingredients for feed production (e.g., kelp, seaweeds, fish oils and meals). While the consumption of natural resources is critical to obtain high-quality gonads, their use in the feed industry is not sustainable for several ecological, economic and management reasons (Pearce et al., 2002). Size, colour, taste, and firmness of the gonads are strictly related to the proper nutritional quality of the diet provided and, at the same time, they strongly influence the gonad market value (Baião et al., 2019; Cuesta-Gomez and Sánchez-Saavedra, 2018; Stefánsson et al., 2017). At the same time, large-scale harvesting of natural resources is very expensive, may compromise their population dynamics, viability and ecosystem functioning, and may promote conflicts with other marine users (Pearce et al., 2002). Therefore, the reuse of by-products and wastes, by reducing the exploitation of natural resources, is to be considered as a valuable solution to move towards a sustainable approach in echinoculture, in agreement with the circular economy principles (de la Caba et al., 2019).

Food loss and waste have been steadily increasing worldwide, reaching about 1.3 billion tons per year, with one-third of food for human consumption being lost or wasted throughout the food retail chain, with strong socio-economic and environmental implications (Gustavsson et al., 2011). A large amount of food loss consists of fruits and vegetables, whose inedible parts are discarded during collection, handling, transportation, and processing steps (Gustavsson et al., 2011). This high amount of vegetal biomass with a high fibre, protein and mineral content might instead be recycled and returned to the food chain as raw material (Laufenberg et al., 2003). Similarly, fish processing discards, such as organs, skin, bones, cut-offs, and damaged or spoiled fish from fisheries and aquaculture industries, are a global issue nowadays, as they amount to about a third of the fish mass processed (Kim and Mendis, 2006). Despite the low economic value, these by-products have a high nutritional content, due to a high amount of proteins, minerals and lipids, as well as being an extremely valuable source of bioactive molecules (Esteban et al., 2007; Kim and Mendis, 2006; Olsen et al., 2010). In particular, fish processing discards are natural sources of essential fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are required by fish for growth, reproduction and disease resistance (Glencross, 2009). Under the assumption that food processing discards have a high potential to be exploited, their use in aquaculture feed production is increasingly taken into account (Bimbo, 2007). First promising attempts to use fish processing discards as valuable resources in aquaculture date from about the year 2000 (Kotzamanis et al., 2001; Turchini et al., 2003). Similarly, increasing attention was focused on the exploitation of vegetal discards as an ingredient for sustainable feeds. Kang et al. (2010) highlighted higher growth rates in the juveniles of the white-leg shrimp *Litopenaeus vannamei* (Bonne, 1931) fed with papaya wastes. Luo et al. (2014) showed an improvement of gonads flavour in the sea urchin *Strongylocentrotus intermedius* (Agassiz, 1863) fed with banana peels. More recently, Mo et al. (2020) recorded weight gain for the grass carp *Ctenopharyngodon idellus* (Valenciennes, 1844) fed with a mix of cereal, meat and fruit wastes. These studies confirmed that the introduction of practices of circular economy in feed production may allow turning wastes into resources. This approach could indeed reduce the reliance on high-cost and qualitatively scarce resources, such as fish oil and meal, matching the purpose of Blue Growth, namely the socio-economic growth based on sustainability and biodiversity protection of marine systems and resources (Eikeset et al., 2018). In this context, Ciriminna et al. (2020) assessed the suitability of food processing discards as ingredients for *P. lividus* diet. Outermost leaves of endive *Cichorium endivia* (Linnaeus, 1753), usually discarded before the sale, and processing discards of the European anchovy *Engraulis encrasicolus* (Linnaeus, 1758), mainly fins, skin, bones and offal, were used as the main ingredients based on the high content in carbohydrates (especially from endive leaves: ~ 65%), proteins and lipids (especially from anchovy discards: ~ 40% and 15% respectively) (Ciriminna et al., 2020). A new sustainable feed was produced and tested as a sea urchin diet, showing very promising results in nutritional content and quality (e.g. ratio ω3/ω6 fatty acids from 2.5 to 4), good stability in seawater (mean feed loss < 40% after 72 h), palatability (mean ingestion rate: 107 mg day^{-1}) and absorption efficiency (31%), confirming its suitability for feeding sea urchins (Ciriminna et al., 2020). Here, the suitability of the sustainable feed for rearing sea urchins was further evaluated by testing its effects on gonad production, development and colour in adult *P. lividus* reared indoor for 3 months. In more detail, the performance of two formulations of the new sustainable feed on gonad somatic index, gonad maturity stages and colour of reared sea urchins was tested, and results were compared with those obtained from wild specimens. The main goals were: i) to evaluate the potential of the new sustainable feed as a sea urchin diet, compared to natural patterns observed in wild sea urchins collected in the same sampling area; ii) to assess the best proportion of vegetal and animal ingredients from food industry discards promoting the best production and quality of sea urchin gonads.

2. Materials and methods

2.1. Feed formulations

A sustainable feed based on food processing discards was produced following the protocol developed by Ciriminna et al. (2020). Briefly, two feed formulations were prepared using outermost leaves of endive *Chicorium endivia* (Linnaeus, 1753) and European anchovy *Engraulis encrasicolus* (Linnaeus, 1758) processing discards (mainly fins, skin, bones and offal) plus a low amount (2.5 %) of agar (Agar-Agar fine powder 100% Food Grade, Intra Laboratories, UK) as a binder. The two main ingredients were mixed in different proportions to prepare the two feed formulations: (i) the "feed 60/40" was characterised by overall equilibrated proportions of the two ingredients (i.e. about 600 and 400 g kg^{-1} dry weight of endive and anchovy discards respectively), and (ii) the "feed 80/20" was characterised by a higher relative amount of vegetal ingredients (i.e. about 800 and 200 g kg^{-1} of endive and anchovy discards respectively). To prepare the feed, agar was dissolved into boiling MilliQ distilled water (385 g l^{-1}). Then, the solution was allowed to cool to about 60 °C and mixed with the main ingredients. The obtained mixtures were manually transformed into cylindrical pellets (0.5 cm diameter, ~2 cm length, ~1 g wet weight), using 35 ml syringes, air-dried at room temperature (24 °C) for 24 h and then stored a −20 °C until provision.

The proximate composition of the feed formulations was determined according to Ciriminna et al. (2020) and highlighted a high nutritional value of both formulations with the one with the higher amount of

Table 1
Ingredients and proximate composition of the two feed formulations.

	Feed Formulation	
Ingredients (g kg^{-1} dw)	**Feed 60/40**	**Feed 80/20**
Cichorium endivia	587.5	787.5
Engraulis encrasiculos	387.5	187.5
Agar	25.0	25.0
Proximate Composition (g kg^{-1} dw)		
Lipid	138.0	96.2
Protein	294.8	264.9
Carbohydrates	321.3	423.2
Ash	245.9	215.9

Mean proximate composition of the feed ingredients from Ciriminna et al. (2020): *Cichorium endivia* outermost leaves: lipid: 38.0, protein: 191.4, carbohydrates: 644.2, ash: 126.3 g kg^{-1} dw. *Engraulis encrasicolus* processing discards: lipid: 140.1, protein: 405.8, carbohydrates: 43.4, ash: 410.7 g kg^{-1} dw.

vegetable ingredient (feed 80/20) being richer in carbohydrates, and the other (feed 60/40) richer in protein and lipid content (Table 1).

Lipids were measured following a slightly modified version of the Bligh and Dyer (1959) method: a solution of MilliQ distilled water, methanol (CARLO ERBA Reagents, Chaussée du Vexin, France) and chloroform (Panreac Quimica Sau, Barcelona, Spain) ratio1:2:1 (v:v:v) respectively, with 0.01% of butylated hydroxytoluene (Sigma-Aldrich®, St. Louis, United State of America) as an antioxidant, was added to ground sub-samples of freeze-dried feed bars. Samples were then sonicated to improve lipid extraction and centrifuged twice to separate the lipid phase from the aqueous phase. The lipid extracts were evaporated to dryness under a gentle nitrogen stream and weighed. Protein content was estimated by analysing the total nitrogen content in an Elemental Analyzer (FlashEA® 1112, Thermo Fisher Scientific, Monza, Italy), which was subsequently converted in protein content by applying a conversion factor of 6.25 (Horowitz and Latimer, 2006). Ash content was assessed by combustion in a muffle furnace (ZB/1, Asal s.r.l. Milan, Italy) at 550 °C for 4 hr according to Nielsen (2010). Carbohydrates were indirectly determined according to Baião et al. (2019), by applying the following formula: carbohydrates = (100 – (ash + protein + lipid)).

2.2. Feeding trial

The feeding trial was conducted on adult sea urchins, as they allocate more energy for reproduction than juveniles (Fabbrocini and Adamo, 2010; Fernandez and Boudouresque, 2000). In the experiment, sea urchins fed on the two new formulations were compared with the natural development of wild sea urchins. Natural diets (i.e. macroalgae) were not included in the experiment because of (i) the low performance of algal diets on *Paracentrotus lividus* (Lamarck, 1816) gonad yield and quality, as already found in a previous study conducted under the same experimental conditions (Vizzini et al., 2014); (ii) the low sustainability of algal diets in aquaculture and hence the low relevance to the objectives of the present study.

In October 2015, about 160 wild specimens of *P. lividus* of similar size (mean test diameter TD ± standard deviation: 41.2 ± 5.3 mm) were collected by SCUBA divers at Cala Rossa, Terrasini (38°8'34.47" N; 13°4'15.99" E; northern Sicily, Italy, Mediterranean Sea) and transported to laboratories within seawater-filled oxygenated containers. The sampling area was overall characterised by a meadow of *Posidonia oceanica* (L.) Delile, 1813, patches of the macroalgae *Cystoseira* (C. Agardh, 1820) spp. and *Dictyota* (J.V. Lamouroux, 1809) spp. growing on a rocky bottom (author's observations). In the laboratory, 20 specimens were randomly collected, weighed and sacrificed to evaluate the initial gonad conditions in terms of biomass, gonad somatic index, maturity stage and colour (T0 - Wild). Other 140 specimens were transferred into a 150 L tank and kept fasting for 15 days, for acclimatising to laboratory conditions and standardising the relative food appetence before the onset of the feeding trial without affecting gonad production (Pearce et al., 2002). After the fasting period, other 20 specimens were randomly collected and sacrificed to evaluate the potential effects of fasting (T0 - Fast) on the gonad conditions. The remaining 120 sea urchins were randomly divided into two groups of 5 tanks with 80 L capacity (in total, 10 tanks with 12 specimens per tank) in a recirculating aquaculture system (RAS) (Fig. 1). Each group corresponded to a feed formulation (feed 60/40 and feed 80/20, respectively). The animals were fed *ad libitum* every 72 h, for 12 weeks (~ 3 months, October 2015 - January 2016), which was considered a suitable period for assessing the effects of controlled feeding according to the literature (Carboni et al., 2015; Schlosser et al., 2005; Vizzini et al., 2014). Before each round of feed provision, feed particle leftovers and sea urchin faeces were carefully siphoned from each tank. The RAS was equipped with common sand filter, bio-filter and protein skimmer to maintain optimal water quality conditions. Moreover, the environmental conditions in the tanks were kept stable throughout both the fasting period and the feeding trial, with seawater temperature: 20.0 ± 1.0 °C, salinity: 38.0 ± 0.5, photoperiod: 8 h light and 16 h dark and continuous water flow in/out: 5 L min^{-1}. Ammonia levels (mean ± s.d.: 0.034 ± 0.005 mg l^{-1}), pH (8.07 ± 0.04) and oxygen saturation (always > 90%) were measured daily in effluent water from each tank.

Every 4 weeks (T1, T2, T3 corresponding respectively to November, December 2015 and January 2016), 4 specimens were randomly collected from each tank, for a total of 20 specimens for each feed formulation. At the same experimental times, wild sea urchins of similar size (43.1 ± 3.7 mm) were also collected (15–20 specimens each time depending on availability in nature) from the same coastal site, to allow the comparison over time between reared and wild specimens. After each sampling, wild sea urchins were kept for 24 h in an 80 L tank of the same experimental RAS, to empty their gut and avoid biases in the next weight measurements. For the same reason, reared sea urchins were collected and measured before feeding provision. No mortality was recorded throughout the experimental period.

Total weight (TW) measurement was performed from T0 to T3 using an electronic balance (Sartorius BL120S d ±0.01 mg). Then, the weighted sea urchins were sacrificed to record the gonad wet weight (GW) and calculate the gonad somatic index (GSI) as:

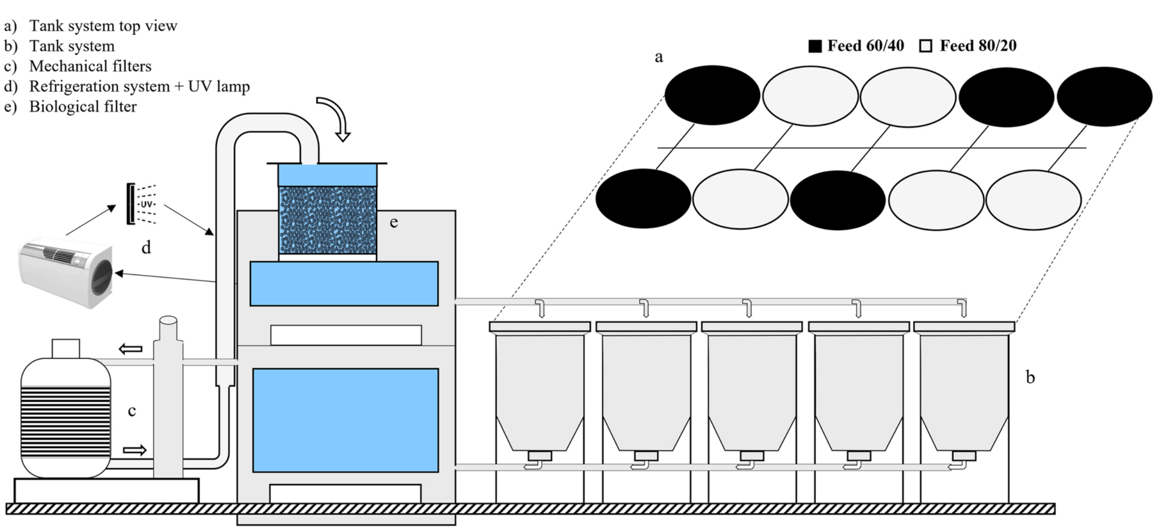

Fig. 1. Scheme of the indoor recirculating aquaculture system (RAS) used for the feeding trial with *Paracentrotus lividus* Lamarck (1816). Five 80 L tanks were randomly assigned to both experimental feed formulations (feed 60/40 and feed 80/20).

GSI = GW/TW x 100.

After TW and GW measurement, one of the five gonads of each sacrificed sea urchin was taken for microscopic (Leitz DMRB, Leica, Wetzlar, Germany) determination of sex. Then, a subsample of 6 female specimens and 5 male specimens for each formulation was randomly selected for histological analysis aiming at confirming the sex and the maturity stage. Selected gonads were dehydrated, embedded in paraffin, dissected in 7 μm thick slices using a microtome (5040 Rotary Microtome, Bright Instuments, Huntingdon, United Kingdom) and stained with the alcian blue-periodic acid Schiff reagent (AB/PAS) method. Gonads (both ovaries and testis) were preliminarily categorised according to morphologically criteria on a scale of gamete maturity phases and nutritive deterioration of gonads (Byrne, 1990). Therefore, the stages of the *P. lividus* gametogenic cycle were classified into six categories: stage I, recovery; stage II, growing; stage III, premature; stage IV, mature; stage V, partly spawned; stage VI, spent (Byrne, 1990).

To assess the colour, gonads were placed in clean Petri dishes and compared with Pantone® colour standards chart (Colour Formula Guide 1000, 1991) under standard artificial daylight (Reer, 4000 K) by three expert observers, according to the literature (Symonds et al., 2007; Vizzini et al., 2014, 2019). The observers assigned each specimen to a single colour category among those defined by Shpigel et al. (2005): dark orange (DO), pale yellow (PY), bright orange (BO), yellow-orange (YO) and mango orange (MO), which were then classified in three quality categories (I: inadequate, A: acceptable and E: excellent) following Symonds et al. (2009).

2.3. Statistical analysis

Differences in gonad wet weight (GW) and gonad somatic index (GSI) between sea urchins across time were tested using univariate permutational analysis of variance (PERMANOVA, Anderson et al., 2008). The factors Feed and Time were both fixed and orthogonal, the former with 3 levels (Feed: Wild, feed 60/40 and feed 80/20) and the latter with 4 levels (Time: T0, T1, T2, T3). Analyses were based on untransformed data resembled using Euclidean distance using the software PRIMER 6 v3.1.10 & PERMANOVA+β20 (Plymouth, UK; Anderson et al., 2008). When significant differences were found, pair-wise tests were run as a posteriori check for significant effects.

3. Results

3.1. Gonad growth

Permutational analysis of variance (PERMANOVA) carried out on gonad wet weight (GW) and gonad somatic index (GSI) data showed significant differences for both factors Feed and Time and their interaction (Table 2a). In particular, GW showed a significant gradual increase across time only in reared sea urchins, while GW of wild specimens was significantly higher just at T3 than at T0 (Fig. 2a). On the other hand, while the three treatments did not differ significantly at T0, sea urchins fed with the feed 60/40 presented significantly higher GW than wild ones at all times starting from T1, while those fed with the feed 80/20 from T2. Moreover, the feeding treatment 60/40 showed also the fastest increment of gonad weight over the trial (percentage GW increase from T0 to T3 = 78.1%, 329.0% and 488.8% for wild, feed 80/20 and feed 60/40 respectively), as well as the highest GW values at T3.

Similar to GW, wild sea urchins showed significant differences in GSI only between T3 and T0, but also a fluctuating trend over the experimental period (Fig. 2b). In contrast, all reared sea urchins presented a

Table 2
Results of permutational analysis of variance (PERMANOVA) testing for differences in gonad weight (GW) and gonad somatic index (GSI) of *Paracentrotus lividus* Lamarck (1816) between feed formulations, times and their interaction.

a) MAIN TEST	**GW**				**GSI**		
Source of variation	**df**	**MS**	**Pseudo-F**	**P(perm)**	**MS**	**Pseudo-F**	**P(perm)**
Feed	2	50.34	13.85	**0.001**	412.26	33.60	**0.001**
Time	3	86.39	23.77	**0.001**	507.63	41.38	**0.001**
Feed x Time	6	11.72	3.22	**0.004**	84.38	6.87	**0.001**
Residual	219	3.6			12.26		
b) PAIR-WISE TESTS		**GW**			**GSI**		
Within time between feeds		**t**	**P(perm)**	**Unique perms**	**t**	**P(perm)**	**Unique perms**
T0	**WILD vs FAST**	1.25	0.215	901	0.58	0.541	995
T1	**WILD vs FEED 60/40**	1.82	**0.029**	963	1.82	0.071	998
	WILD vs FEED 80/20	1.56	0.122	944	0.96	0.360	999
	FEED 60/40 vs FEED 80/20	0.50	0.696	976	0.73	0.480	997
T2	**WILD vs FEED 60/40**	3.19	**0.003**	982	5.72	**0.001**	999
	WILD vs FEED 80/20	2.67	**0.014**	973	4.18	**0.001**	998
	FEED 60/40 vs FEED 80/20	1.16	0.274	957	1.37	0.178	997
T3	**WILD vs FEED 60/40**	4.13	**0.001**	984	6.50	**0.001**	997
	WILD vs FEED 80/20	2.60	**0.018**	985	4.31	**0.001**	996
	FEED 60/40 vs FEED 80/20	2.06	**0.047**	982	2.11	**0.048**	997
Within feed between times							
WILD	**T0 vs T1**	0.25	0.808	913	1.97	0.066	996
	T0 vs T2	0.43	0.646	686	0.97	0.363	996
	T0 vs T3	2.01	**0.043**	975	1.96	**0.032**	995
	T1 vs T2	0.22	0.843	960	0.80	0.440	998
	T1 vs T3	1.80	0.053	962	0.38	0.732	998
	T2 vs T3	1.36	0.193	972	0.99	0.353	995
FEED 60/40	**FAST vs T1**	2.44	**0.001**	960	3.19	**0.003**	997
	FAST vs T2	4.60	**0.001**	956	6.81	**0.001**	998
	FAST vs T3	7.68	**0.001**	975	9.98	**0.001**	994
	T1 vs T2	1.26	0.255	966	3.49	**0.002**	996
	T1 vs T3	3.44	**0.001**	978	6.40	**0.001**	996
	T2 vs T3	2.34	**0.025**	968	2.78	**0.011**	996
FEED 80/20	**FAST vs T1**	2.36	**0.024**	960	2.19	**0.038**	996
	FAST vs T2	4.41	**0.001**	942	5.06	**0.001**	997
	FAST vs T3	6.34	**0.001**	973	7.00	**0.001**	998
	T1 vs T2	1.10	0.264	956	2.60	**0.013**	997
	T1 vs T3	2.81	**0.009**	978	4.43	**0.002**	998
	T2 vs T3	1.95	0.059	965	1.85	0.075	998

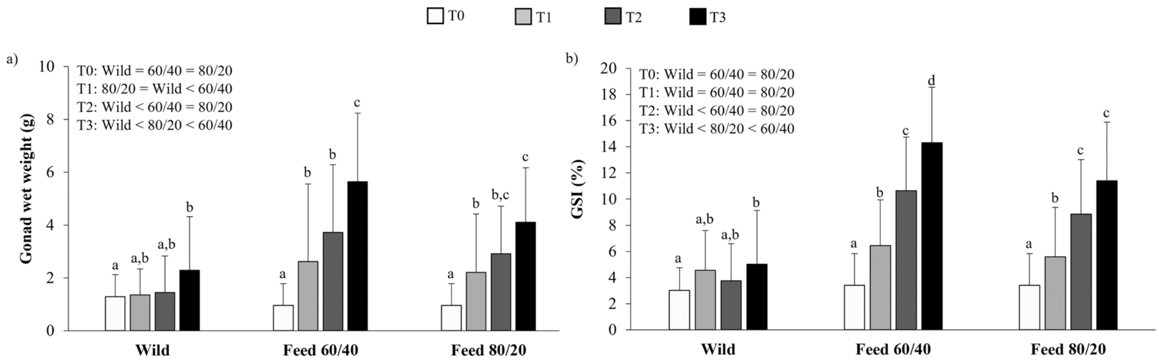

Fig. 2. (a) Gonad wet weight (GW, mean ± standard deviation) and (b) gonad somatic index (GSI, mean ± standard deviation) of sea urchins collected from the natural environment (wild), and sea urchins fed with the two experimental feed formulations (feed 60/40, feed 80/20) across experimental times (T0-T3). Lowercase letters on each panel indicate significant differences between times within feeds. The boxes on both panels indicate significant differences between feeds within times.

significant increase in GSI throughout the trial, mirroring the patterns of GW. The comparison between treatments highlighted comparable values at T0, significant differences between wild and reared specimens since T2 and between reared specimens (feed 60/40 > feed 80/20) only at T3.

3.2. Gonad development

Histological analysis revealed overall a slower gonad development in wild specimens than reared sea urchins (Figs. 3 and 4). Wild females showed a gradual maturation across time, consisting of a decrease of the frequency of the gonads found in the recovery stage (I), up to disappearance at T3, and an increase of those found in the premature stage (III) (Fig. 3). On the other hand, reared females showed a sharper progression in sexual development, achieving the mature stage (IV) at T2, with a frequency of 50% of all the observed sea urchins, and remained stable up to T3, regardless of the feed formulation provided (Fig. 3).

Similarly to females, wild males showed a decrease in the recovery stage (I) and an increase in the premature stage (III) from T0 to T3, but the mature stage (IV) was not achieved (Fig. 4). Moreover, unlike females, reared males showed different patterns according to the formulation provided. In more detail, the specimens fed with the feed 60/40 showed a faster development than the others (feed 80/20), with the achievement of the premature stage (III) just one month after the start of the feeding trial (T1), followed by a further increase up to achieve the

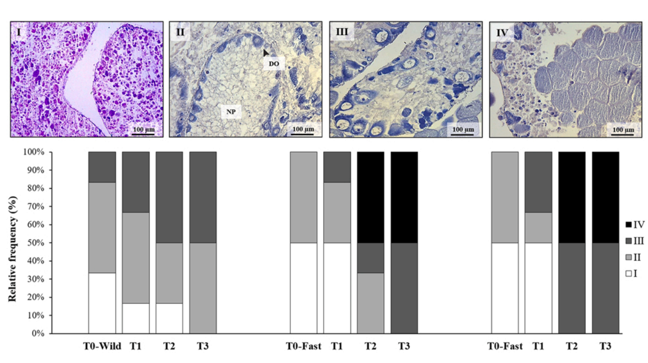

Fig. 3. Above: histological sections of female gonads. Stage I (recovery): nutritive phagocytes begin forming a meshwork across the ascini. Stage II (growing): nutritive phagocytes (NP) and early vitellogenic oocytes attached to the ascinal wall (DO). Stage III (premature): oocytes at all stages of development. Stage IV (mature): ovary packed with ova. Below: relative frequency (n = 6) of the gametogenic stages of female sea urchins collected from the natural environment (wild), and sea urchins fed with the two experimental formulations (feed 60/40, feed 80/20) across experimental times (T0-T3).

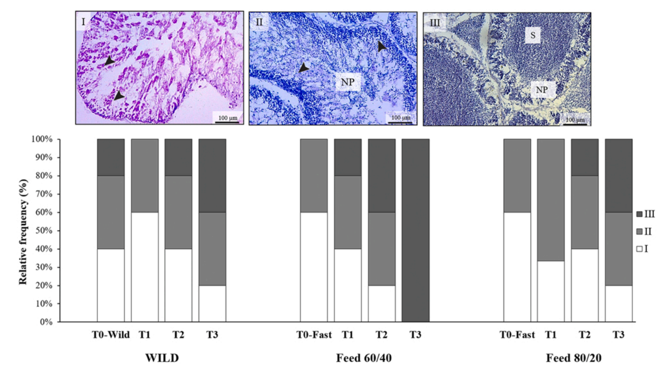

Fig. 4. Above: histological sections of male gonads. Stage I (recovery): primary spermatocytes along ascinal wall (arrowheads). Stage II (growing): testis with columns of developing spermatocytes (arrowheads) in the meshwork of nutritive phagocytes (NP). Stage III (premature): premature testis with spermatozoa (S) in the ascinal lumen and nutritive phagocytes (NP) around the periphery. Below: relative frequency (n = 5) of the gametogenic stages of male sea urchins collected from the natural environment (wild), and sea urchins fed with the two experimental formulations (feed 60/40, feed 80/20), across experimental times (T0-T3).

100% of all the observed gonads at the end of the trial (T3). In contrast, the male specimens fed with the feed 80/20 achieved the premature stage at T2, followed by a lower increase at T3, when immature gonads were also found (Fig. 4).

3.3. Gonad colour

The assessment of gonad colour showed an excellent colouration (E) at the beginning of the trial (T0) in almost 70% of both wild and fasted sea urchins. This value increases up to about 90% if the categories "E: excellent" and "A: acceptable" are summed together (Fig. 5). Wild specimens showed an overall worsening of the gonad colour over time, consisting of a sharp decrease of the frequency of the gonads with excellent and acceptable colour (E + A) (from 90% to 47% at T0 and T3 respectively) coupled with a marked increase of those classified as inadequate (I) (from 10% to 53% at T0 and T3 respectively). In contrast, despite both groups of reared sea urchins showed an initial colour worsening, especially those fed with the feed 80/20 (E + A gonad frequency: from 90% to 55% at T0 and T1 respectively), all the sea urchins fed with the sustainable feed highlighted a subsequent and overall improvement of the gonad colour (A+E gonad frequency: 95% at T3 for both groups of reared sea urchins). However, the frequency of the gonads with excellent colour was always higher in those fed with the feed

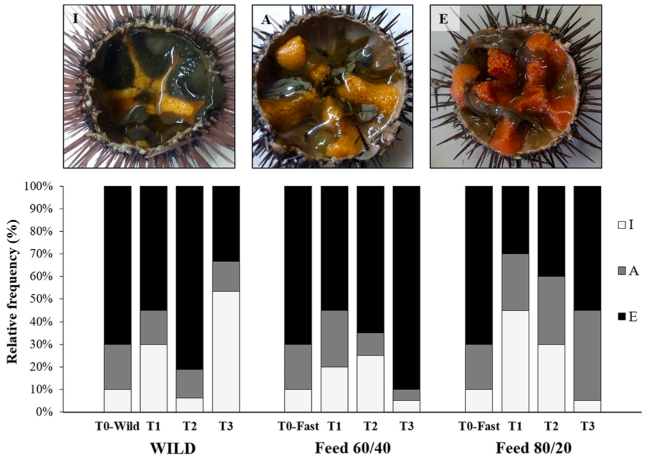

Fig. 5. Relative frequency of the gonad colour categories of sea urchins collected from the natural environment (wild), and sea urchins fed with the two experimental formulations (feed 60/40, feed 80/20), across experimental times (T0-T3). Values are expressed as the percentage of specimens with gonads within each colour category (E: excellent, A: acceptable, I: inadequate).

60/40 than in those fed with feed 80/20. In particular, at T3, the sea urchins fed with the feed 60/40 showed about 90 + 5% of excellent and adequate gonads, while those fed with feed 80/20 only about 55 + 40% of excellent and adequate gonads. Less than 10% belonged to the inadequate (I) category in both groups of reared sea urchins (Fig. 5).

4. Discussion

A sustainable feed prepared by recycling processing discards of endive and common anchovy from the food retail chains was provided to adult sea urchins *Paracentrotus lividus* (Lamarck, 1816), in controlled conditions, to assess the potential of sustainable feeds based on the circular economy principles for echinoculture. In more detail, sea urchins were fed for 3 months with two feed formulations characterised by a different ratio of vegetal vs. animal ingredients to evaluate the most effective formulation promoting gonad production, maturation and quality. The results were compared with those obtained in wild sea urchins, aiming to evaluate differences with natural patterns. Overall, both formulations led to a greater increase in gonad production in reared *P. lividus* than in wild specimens, in terms of gonad wet weight (GW) and gonad somatic index (GSI). Similarly, reared sea urchins showed faster sexual maturation and a better gonad colouration than wild specimens, confirming the high suitability of the sustainable feed as a diet for sea urchins under farming conditions.

At the beginning of the trial, no significant differences in both GW and GSI were highlighted between fast and wild sea urchins, indicating that the fasting period did not lead to significant changes in gonad biomass (Guillou et al., 2000; Raposo et al., 2019) and therefore confirming that is suitable for standardising the initial experimental conditions without affecting gonad production (Pearce et al., 2002). Afterwards, the significant increase in GW and GSI observed in the sea urchins fed with the two experimental formulations after just one month, suggests a rapid conversion of the provided feed nutrients into gonad biomass, contributing to the higher gonad than somatic growth. Indeed, gonads are the main nutrient storage organ as the nutritive phagocytes of the gonads are responsible for both nutrient accumulation and transfer into the developing gametes (Fabbrocini et al., 2012; Marsh et al., 2013; Walker et al., 2015). For this reason, GSI is considered a good indicator of diet nutritional quality, and high-quality feeds are commonly associated with high GSI values (Carboni et al., 2015; Cuesta-Gomez and Sànchez-Saavedra, 2016).

In contrast, the patterns of GW and GSI observed in wild sea urchins did not match perfectly and indicated a much more gradual and lower increase over time, maybe due to a lack of a proper nutritional supply across time (Byrne, 1990; Shpigel et al., 2004; Tenuzzo et al., 2012). Although the growth of sea urchin gonads into the wild may benefit from the low winter temperature and the short daylight period (Byrne, 1990; Shpigel et al., 2004), it is also strongly influenced by food quality and availability, which are typically fluctuant in the natural environment (Cook and Kelly, 2007) differently from the constant and controlled rearing condition. Although wild *P. lividus* can remove large amounts of vegetal biomass to satisfy their nutritional requirements (Klinger, 1984; Lawrence et al., 2020), their favourite food items (i.e. macroalgae) are characterised by low concentrations of macro and micronutrients (Cook et al., 2000; Fernandez and Boudouresque, 2000; Vizzini et al., 2014). Therefore, it is likely that the ingestion of algal biomass (*Cysoseira* spp. and *Dyctiota* spp. are abundant in the collection site) might have led to scarce energy intake and consequently an imbalance between gonad and somatic growth (Schlosser et al., 2005; Shpigel et al., 2005).

In contrast, although the controlled and stable condition of the rearing system used for the feeding trial may have plaid a positive role, the influence of diet on gonad growth is further confirmed by the comparison between the two feeding treatments. Comparing the results from reared sea urchins, it was evident, indeed, that the nutritional differences between the two formulations played a key role in modulating gonad production. Although *P. lividus* is considered an herbivore (Boudouresque and Verlaque, 2007), the sea urchins fed with the formulation characterised by a more balanced vegetal/animal ratio (i.e. feed 60/40) showed higher GW values and a greater gonad increment over time than those fed with the formulation with a higher vegetal content (i.e. feed 80/20). GSI was also boosted by the feed 60/40, consistently with Fernandez and Boudouresque (1998, 2000), who also found the highest GSI following the provision of a balanced diet, made of 60% of vegetal ingredient and 40% of fish meal. These findings confirm the key role of animal proteins and lipids as important storage compounds of the nutrients needed for gonad growth, development and maturation (Fernandez and Boudouresque, 2000; González-Durán et al., 2008; Grosso et al., 2021). Furthermore, the European anchovy discards are richer in fatty acids than endive leaves, especially in omega-3 long chain polyunsaturated and essential fatty acids (Ciriminna et al., 2020), which are vital for reproductive fitness and a multitude of physiological functions, including gonad development and enhancement (Glencross, 2009; Liu et al., 2007; White et al., 2016). In contrast, the higher relative amount of vegetal ingredients of the feed 80/20 may have led to a slower absorption of nutrients. Vegetal meals have, indeed, a high abundance of insoluble carbohydrates (Bach Knudsen, 1997; Esteban et al., 2007) and fibres (Plazzotta et al., 2017), which are usually poorly digested by sea urchins (Fernandez and Boudouresque, 2000; Powell et al., 2020). However, good performances of artificial diets based on terrestrial vegetables on *P. lividus* GSI have been recently highlighted by several authors. Raposo et al. (2019), Santos et al. (2020) and Sartori and Gaion (2015) recorded high, while different, performances (mean GSI = 9%, 9% and 19% respectively) feeding *P. lividus* with maize and spinach, while Vizzini et al. (2014) obtained a high mean GSI (10%) providing lettuce to *P. lividus*. The results obtained in this study are overall comparable to these, but also slightly better than others related to sea urchins fed with macroalgae or mixed diets (Prato et al., 2018; Vizzini et al., 2014; Zupo et al., 2019). This confirms the higher potential of the proposed sustainable feed formulation based on balanced vegetal and animal discards (i.e. feed 60/40) in promoting gonad growth, compared to natural or macroalgae-based diet.

Gonad maturation is another crucial issue in echinoculture, because marketable high-quality gonads, in terms of firmness, colour and taste, are obtained when the ratio between nutritive phagocytes (abundant in the recovery and growth phases) and gametes (abundant in the mature and generation phases) are in favour of the former (Böttger et al., 2006; Walker et al., 2001). Most of the sea urchins collected at the beginning of the trial were in the early reproductive stages, consistent with the annual reproductive cycle of *P. lividus* in the Mediterranean area (Lozano et al., 1995). While the fasting period seems to have induced a partial regression in the reproductive stages, consistent with Guillou et al. (2000) and Raposo et al. (2019), the gonad maturation patterns of reared sea urchins revealed a subsequent progression in the reproductive stages. In more detail, both formulations promoted similar gonad development in female sea urchins, while the feed 60/40 showed the best performance in males, achieving the totality of specimens in the premature stage at the end of the trial. Different response of females and males may be related to different specific requirements during sexual maturation and gametes production. Indeed, the reproductive effort is greater in females than in males, as the development of embryos and larvae depends on the maternal provisioning of nutrients (Carboni et al., 2015). A suitable advancement in sexual maturation is fundamental for echinoculture purposes. A low progression may result in a limited increase in gonad biomass, due to a slow nutritive phagocytes growth, while a too fast gonad maturation could lead to spawning events with a consistent loss in gonad biomass due to the emission of gametes (Marsh et al., 2013; Walker et al., 2001, 2015). Nevertheless, in this study, the gonad development was faster in reared sea urchins than in wild specimens. These results contrast with those from Fabbrocini et al. (2019), who found a quicker progression in wild specimens than in sea urchins fed with agar-based pellets. These differences, however, may be related to the different environmental conditions (off-shore cages) and the short duration of the trial (one month) conducted by Fabbrocini et al. (2019). Here, both the experimental formulations fostered a suitable gonad development for echinoculture goals, indicating an appropriate energy supply, which is one of the most limiting factors for gonad development in growing and mature stages of the reproductive cycle (Schlosser et al., 2005). Indeed, during these stages, sea urchins need to store great amounts of nutrients for the development of gametes (Walker et al., 2015).

Lastly, the analysis of gonad colour, which is a key factor in determining both the quality of the gonads and its economic value (Stefánsson et al., 2017), revealed also positive effects of the new feed formulations on the sea urchin gonads. The vast majority of reared sea urchins achieved, indeed, a marketable colouration after 12 weeks, differently from wild sea urchins that showed an initial colour improvement, followed by a clear worsening over the experimental period. Colour in echinoid gonads is driven mostly by carotenoid dietary intake, and particularly by echinenone, which sea urchins synthesise from β-carotene (Shpigel et al., 2005). Outermost endive leaves have a greater concentration of carotenoids (lutein and β-carotene), compared with young leaves (de Azevedo-Meleiro and Rodriguez-Amaya, 2005), resulting in well suited dietary sources for obtaining high-quality gonads. The temporal pattern observed for gonad colour, however, suggests that sea urchins need time to absorb carotenoids from the diet, synthesise echinenone and store it in the gonads, consistent with previous studies (Plank et al., 2002; Shpigel et al., 2005). Nevertheless, the overwhelming majority ($\sim$ 95%) of the gonads produced by *P. lividus* in 12 weeks presented an excellent and acceptable (E + A) colour, confirming the efficacy of both formulations in producing marketable gonads. Similarly, Santos et al. (2020) obtained marketable gonad colour from sea urchin fed with diets based on spinach, maize and pumpkin, while Luo et al. (2014) found a better performance in promoting gonad colour in sea urchins fed with kelp-based diet than those fed with pumpkin or banana peel. Moreover, Vizzini et al., (2018, 2019) highlighted the effectiveness of lettuce vegetal discards mixed with animal lipids and proteins (i.e., white eggs and commercial pellet) in improving the gonad colour of *P. lividus* (70–90% of gonads in E + A colour). Here, the present findings suggest that the use of animal discards is also highly functional to improve gonad colour and that vegetal discards may replace added carotenoids, usually considered one of the most expensive complements in feed formulation (Cuesta-Gomez and Sánchez-Saavedra, 2018).

5. Conclusion

The present study showed that the outermost leaves of *Cichorium endivia* (Linnaeus, 1753) and *Engraulis encrasicolus* (Linnaeus, 1758) processing discards from the food retail chains have a great potential to be recycled as dietary ingredients in echinoculture. The new sustainable feed based on food processing discards promoted gonad growth and development and contributed to improving the gonad colour of the purple sea urchins *Paracentrotus lividus* (Lamarck, 1816), the most important commercial sea urchin in the Mediterranean Sea (Stefánsson et al., 2017). In this way, food processing discards are transformed into new valuable biomass that might naturally replace traditional ingredients (e.g., fish oils and meals) in aquaculture feeds. This would reduce the environmental and economic impact of the food discard production and disposal, under the principles of circular economy and sustainability, toward the Blue Growth. Moreover, recycling food processing discards may significantly reduce the pressure exerted on marine organisms (macroalgae and fish) for the production of aquaculture feeds. However, since sea urchin gonads are a high-quality niche product, further research is needed to assess the effect of the new sustainable feed on nutritional value and organoleptic features of gonads, as well as to evaluate their performance in aquaculture, compared with commercial feeds.

CRediT authorship contribution statement

Laura Ciriminna: Formal analysis, Writing – original draft, Writing – review & editing. **Geraldina Signa:** Writing – original draft, Writing – review & editing. **Antonino Maurizio Vaccaro:** Conceptualization, Methodology, Validation, Investigation. **Giulia Visconti:** Validation, Investigation. **Antonio Mazzola:** Resources, Funding acquisition, Project administration. **Salvatrice Vizzini:** Resources, Funding acquisition, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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