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Gonad quality of sea urchin *Paracentrotus lividus* cultured in an offshore pilot-scale trial on the south-east Italian coast

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Abstract

A pilot project aimed at testing roe enhancement strategies based on offshore *Paracentrotus lividus* cultures was conducted off the south-east coast of Italy (Apulia Region). Adult sea urchins were reared in sea cages located 700 m offshore at a depth of 12 m for 3 months. The animals were fed once a week on two formulated diets, prepared mixing nutrients with agar 20 g/Kg and differing only in terms of the protein source: anchovy flour (Diet A) or krill flour (Diet K). At the end of the rearing trial, the gonad somatic index of sea urchins fed on formulated diets significantly exceeded that of wild sea urchins. Total FAA content in the gonads of wild sea urchins and Diet A-fed sea urchins was similar, whereas in Diet K-fed sea urchins it was significantly higher. In terms of fatty acids, the gonads contained SFAs, MUFAs and PUFAs. In visual and sensory assessment of gonads by panel test and electronic nose, the gonads of reared sea urchins were rated as being of better size, while no differences were recorded for coloration, taste and odour. This study shows that under these experimental conditions, commercial-grade *Paracentrotus lividus* roe enhancement can be achieved after 3 months in sea cages.

KEYWORDS

fatty acids, free aminoacids, offshore rearing, *Paracentrotus lividus*, roe enhancement, sensory assessment

1 | INTRODUCTION

Sea urchin gonads (roe) are a luxury food and much sought-after worldwide. Sea urchin fishing is mainly practised on the Atlantic coasts of Europe, the Mediterranean, in north-east Asia (Japan and Korea), New Zealand and Chile (Bouduresque & Verlaque, 2013; Micael, Alves, Costa, & Jones, 2009). Among the edible species of sea urchin, *Paracentrotus lividus* (Lamarck, 1816) is highly appreciated by consumers, due to its high roe content. However, this species is threatened by intense capture pressure, which has reduced wild stocks (Bertocci et al., 2014; FAO, 2012; Ouréns, Naya, & Freire, 2015), although the increase in sea water temperature due to climate change has probably contributed to the collapse of *P. lividus* in the Mediterranean Sea (Yeruham, Rilov, Shpigel, & Abelson, 2015). This situation has long called for conservation measures. *P. lividus* is included in the IUCN Red List (Barcelona Convention Annex III) and, as a consequence, its exploitation is now regulated (http://www.iucnredlist.org/). In Italy, this species is particularly appreciated in Sardinia, Sicily and Apulia, where high demand has led to a dramatic diminution of wild stocks (Guidetti, Terlizzi, & Boero, 2004; Pais, Serra, Meloni, Saba, & Ceccherelli, 2012). Therefore, urgent action is required in order to recover the local populations of *P. lividus* and manage the ecological effects of *Arbacia lixula* (inedible sea urchin) prevalence on rocky bottoms (Wangensteen, Turon, Pérez-Portela, & Palacín, 2012).

In spite of the growing interest in sea urchin rearing systems, this activity lags behind other species, mainly because formulated diets frequently lead to poor gonad quality in terms of texture, firmness,

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colour and taste (Walker et al., 2015), with consequent low marketability of the product, mainly intended for gourmet consumers.

In general, the quality of the gonads is determined by taste, colour, consistency and compactness, which are in turn strongly influenced by the lipid, carbohydrate, carotenoid and protein content of the diet (Cuesta-Gomez & Sánchez-Saavedra, 2017; Taylor, Heflin, Powell, Lawrence, & Watts, 2017). From a commercial point of view, the highest value gonads are those in which the phagocytes, just before the start of the process of gamete maturation, have reached their maximum size, since gonads at this stage have high levels of protein, carbohydrates and lipids. The development of phagocytes is closely linked to the assimilation of nutrients from the diet. Recent echiniculture studies have therefore focused on the development of artificial diets designed to increase gonadal mass but also to obtain gonadal texture, taste and colour comparable to those of wild sea urchins (Cyrus, Bolton, & Macey, 2015; Takagi, Murata, Inomata, Endo, & Aoki, 2017; Zhang et al., 2017).

Feed stability is considered a crucial requirement for the development of sea urchin rearing systems. Sea urchins are grazers and need time to eat the offered feed, so it must remain intact for several days, in order to limit the loss of nutrients and to make rearing management easier (Mortensen, Siikavuopio, & Raa, 2003; Pearce, Daggett, & Robinson, 2002b). Agar has promising features as a binder in the preparation of formulated diets for the sea urchin *P. lividus* since, besides giving good results in terms of gonad growth, it produces biocomposites that were completely eaten within a few days, thus reducing to a negligible level the amount of wasted feed (Barker, Keogh, Lawrence, & Lawrence, 1998; Fabbrocini, Volpe, Coccia, D'Adamo, & Paolucci, 2015; Fabbrocini et al., 2012). Moreover, the use of binders to generate biocomposites allows the inclusion in the formulated feed of a wide range of nutrient factors as well as additives enhancing gonadal organoleptic qualities (depending on the specific rearing needs), making them a promising starting point for the formulation of innovative and highly versatile formulated diets in rearing systems.

In recent years, an increasing number of studies have demonstrated the feasibility of offshore rearing of sea urchins, which also includes co-culture in integrated multitrophic aquaculture systems (Lamprianidou, Telfer, & Ross, 2015). Since one of the highest costs of offshore rearing systems arises from the need to feed the sea urchins frequently, employing technical personnel, in this study, we applied our previous experience in manufacturing agar-based feed that can remain in water up for many days without loosening consistency (Paolucci, Fasulo, & Volpe, 2015). Thus, the purpose of this study was to investigate the feasibility of an offshore culture system for *P. lividus* adult specimens given formulated feed in the form of agar-based biocomposites once a week, in order to obtain roe that meets the taste requirements of habitual consumers. Two feed formulations were tested. Based on alternative marine protein sources (krill and anchovy flour), they differed in terms of sweet and bitter amino acid content. Reared and field-collected sea urchins were compared. Since the aim of this study was the production of high-quality gonads, rather than rapid gonad yield, the main evaluation parameters were the fatty acid (FA) and free amino acid (FAA) profiles and the carotenoid content, which are the main determinants of the gonad's organoleptic characteristics. Moreover, the gonads were assessed with an electronic nose and a panel test evaluation by habitual consumers. Despite being less objective and less sensitive than the instrumental analyses, they give an overall picture of gonad quality and thus marketability.

2 | MATERIALS AND METHODS

2.1 | Cages and stocking density

Six cages measuring 1 m × 1 m × 0.5 m were constructed using steel bars for the structural framework and covered with plastic wire with a mesh size of 3 cm. The top of each cage was removable. The bottom of each cage was lined with a rigid plastic sheet to isolate it from the sandy seabed. The experimental cages with test animals were randomly arranged in two lines of three cages. Each line was anchored to a block of cement at both ends. Each cage was stocked with 30 adult sea urchin (*P. lividus*) specimens with size range of 35–40 mm. Cages were sited 700 m offshore at a depth of 12 m in waters off Ugento (Lecce, Apulia, South-East Italy, 39.7365397, 17.9396599).

2.2 | Sea urchin collection

Paracentrotus lividus sea urchins were collected by scuba divers at the end of May 2013 (postspawning—stasis phase). The sea urchins used for the roe enhancement experiments came from the same area as the wild specimens used for comparison. At this site and depth, the immediate dietary source was *Posidonia oceanica*. A random sample of 20 sea urchins from the original collection ("wild group T0") provided an initial baseline for measurements of test diameter (TD), body weight, gonad weight and gonad maturation phase. Twenty sea urchins were later collected in the same area as the initial collection at the end of the roe enhancement trial and formed the control group eating a wild diet ("wild group T3"). A calliper and an ordinary weighing scale with 0.2 g accuracy were used to measure TD and individual weight respectively.

2.3 | Diet formulation and preparation

The sea urchins in the cages were fed two formulated diets which differed in terms of the source of the marine protein: one was made with anchovy flour (Diet A), and the other with krill flour (Diet K). The formulations of the two diets were based on data available in the literature and differed only in terms of the source of the fish meal, anchovy flour being less expensive than krill flour. The feed was made into moist blocks (2 cm × 3 cm × 2 cm) consisting of 100 g/Kg dried nutrients dissolved in 20 g/Kg agar solution. Previous studies by the present authors (Paolucci et al., 2015) were carried out with varying percentages of nutrients (50–250 g/Kg) and polysaccharides of marine origin (10–20–30 g/Kg) in order to select the biocomposites with the best network strength. The best performing

biocomposites, showing the lowest percentage of water absorption and nutrient release, were those manufactured with 20 g/Kg agar and 100 g/Kg nutrients. The biocomposites were prepared in accordance with Fabbrocini et al. (2012), and the blocks were stored in vacuum-sealed plastic bags at −20°C. The ingredients and nutrient composition of the formulated diets are shown in Table 1. The amino acid content and fatty acid content are shown in Tables 2 and 3, respectively.

2.4 | Roe enhancement trial

The roe enhancement trial lasted 3 months, from the beginning of June to the end of August 2013. A quantity of feed equivalent to 5 g/Kg of the total biomass was added every 7 days. Visual inspection carried out by scuba divers revealed that there were still pieces of the feed blocks in the cages on day 6, but there was no feed left after the 7th day. At the end of the trial, the sea urchins were sacrificed for the nutritional, sensory and olfactory analyses.

2.5 | Gonad index and gonadal stage

The sea urchins were allowed to drip for approximately 5 min, weighed (0.2 mg accuracy) and then dissected; the gonads were extracted and weighed for evaluation of the gonad somatic index (GSI): GSI = gonad wet weight (*g*)/sea urchin wet weight (*g*) × 100. Histological analysis was performed as reported in Fabbrocini et al. (2012). Gonadal stage was assigned with reference to Byrne's nomenclature (1990): stage I (recovery), stage II (growing), stage III (premature), stage IV (mature), stage V (partly spawned) and stage VI (spent).

TABLE 1 Composition of the feed formulation of manufactured diets

	Diet A	Diet K
Ingredients (g/Kg in diet)		
Anchovy meal	190.0	–
Krill meal	–	190.0
Pea flour	270.0	270.0
Corn flour	250.0	250.0
Kelp (dry)	250.0	250.0
Agar	20.0	20.0
Fish oil	18.0	18.0
Vitamin premix[a]	1.0	1.0
Mineral premix[b]	1.0	1.0
Proximate composition (g/kg dry weight)		
Total protein	171.6 ± 15.8	162.5 ± 14.7
Total carbohydrate	370.3 ± 32.1	366.6 ± 34.4
Total fat	51.7 ± 4.2	51.9 ± 3.8

Diet A contained anchovy flour while Diet K contained krill flour as fish meal. Proximate composition analysis was carried out on three batches for each diet (n = 3). Values are reported as mean ± *SEM*.

[a]Zoofast, Italy.

[b]Mineral premix contains (mg/kg): Mg 100; Z, 60; Fe 40; Cu 5; C, 0.1; I 0.1; and antioxidant (BHT) 100. Vitamin premix contains (mg/kg): E 30; K 3; thiamine 2; riboflavin 7; pyridoxine 3; pantothenic acid 18; niacin 40; folacin 1.5; choline 600; biotin 0.7; and cyanocobalamin 0.02.

2.6 | Carotenoid analysis

Fresh gonad tissue (1 g total wet weight) was homogenized in AR acetone (1:1, w/v) using an Ultra Turrax mixer (USA) in the absence of light in order to minimize photo-oxidation of carotenoids. The homogenate was transferred to a 15-ml amber tube and centrifuged at 6,000 *g* for 20 min at 4°C. The supernatant was transferred to a 15-ml amber vial and evaporated to dryness with oxygen-free nitrogen. The residue was reconstituted in 2 ml of diethyl ether and 2 ml of methanolic potassium hydroxide 100 g/Kg and left overnight for lipid saponification. Thereafter, 4 ml of sodium chloride 5 g/Kg was added and the vials were flushed with oxygen-free $N_{2,}$ shaken vigorously for 5 min and left to phase separate. The upper solvent phase was removed, and the carotenoid concentration was measured by UV–vis spectrophotometer at 470 nm.

2.7 | Amino acid analysis

Gonads were extracted with formic acid 1.0 g/Kg for 1 hr. The extract was clarified at 18,000 *g* for 60 min and filtered first through 5 μm and then 0.45-μm Millipore filters, and analysed with a cation-exchange column (100 × 6 mm AG50WX8-H+ Bio-Rad). Amino acids were eluted with 3.0 M NH_3 and collected. The eluate was evaporated to dryness, recovered with 0.1 M HCl, and filtered through 0.45-μm filters. Samples of feed were lyophilized and hydrolysed for 24 hr at 110°C with 6 M HCl. HCl was removed under vacuum, and the dried sample was reconstituted with a lithium citrate buffer (0.2 M, pH 2.2) for analysis. Quantification of free amino acids was performed by reverse-phase (RP) HPLC. A Waters chromatograph (model 2,690) equipped with a fluorescence detector (model 474) was employed. Samples (10 μl) were derivatized with the Waters ACCQ-Fluor reagent in accordance with Van Wandelen and Cohen (1997), using quaternary HPLC eluent systems for separating 6-amin oquinolyl-N-hydroxysuccinimidyl carbamate-derivatized amino acid mixtures. Quantification was based on the peak area of fluorescence emission intensity with excitation at 350 nm, recording fluorescence emission at 395 nm. Amino acids were identified on the basis of their retention times and quantified by comparison of the corresponding peak area with the respective calibration curve. Amino acid standards were as follows: Amino Acid Standard H (Thermo Scientific, cod. 20088). 3-Aminobutanoic acid was used as an internal standard.

2.8 | Lipid analysis

Lipids from sea urchin gonads were extracted by the Soxhlet method in accordance with the methods of analysis used for chemical checks on food conducted by the Italian National Institute of Health (Reports ISTISAN 96/34). A sample of 20 g was extracted using

TABLE 2 Free amino acid composition of the formulated diets (g/kg dry weight) and free amino acid content of *Paracentrotus lividus* gonads (g/kg dry weight) at the end of the roe enhancement trial

	Formulated diets		Experimental groups		
	Diet A	Diet K	Wild T3	Diet A	Diet K
Aspartic acid	nd	0.041 ± 0.029	0.210 ± 0.010^{a}	0.110 ± 0.010^{b}	0.155 ± 0.006^{b}
Glutamic acid	0.603 ± 0.052	1.144 ± 0.081	1.260 ± 0.023^{b}	1.462 ± 0.062^{b}	2.432 ± 0.093^{a}
Serine	0.223 ± 0.052	0.347 ± 0.029	1.913 ± 0.053^{b}	1.327 ± 0.055^{c}	2.719 ± 0.084^{a}
Glycine	0.134 ± 0.015	0.694 ± 0.058	28.266 ± 0.403^{a}	28.566 ± 0.255^{a}	29.274 ± 0.871^{a}
Glutamine	nd	nd	3.747 ± 0.0970^{b}	3.665 ± 0.138^{b}	5.371 ± 0.239^{a}
Histidine	0.268 ± 0.022	nd	1.250 ± 0.037^{b}	1.357 ± 0.072^{b}	4.025 ± 0.219^{a}
Threonine	nd	nd	0.530 ± 0.026^{b}	0.600 ± 0.031^{b}	1.771 ± 0.077^{a}
Arginine	nd	nd	10.207 ± 0.380^{b}	6.738 ± 0.176^{c}	13.329 ± 0.30^{a}
Asparagine	4.226 ± 0.365	nd	nd	nd	nd
Alanine	0.335 ± 0.023	1.203 ± 0.103	3.453 ± 0.083^{c}	4.223 ± 0.155^{b}	5.239 ± 0.203^{a}
Proline	nd	nd	17.067 ± 0.037^{ab}	2.074 ± 0.079^{a}	1.387 ± 0.093^{b}
Tyrosine	nd	0.295 ± 0.022	0.750 ± 0.017^{c}	1.757 ± 0.062^{b}	4.819 ± 0.264^{a}
Valine	0.231 ± 0.023	0.413 ± 0.037	0.340 ± 0.003^{b}	0.562 ± 0.020^{b}	2.542 ± 0.126^{a}
Methionine	nd	nd	0.143 ± 0.001^{b}	0.179 ± 0.010^{b}	0.455 ± 0.029^{a}
Cysteine	3.006 ± 0.030	2.937 ± 0.258	nd	nd	nd
Isoleucine	1.458 ± 0.120	1.255 ± 0.111	0.230 ± 0.010^{b}	0.333 ± 0.010^{b}	1.758 ± 0.093^{a}
Leucine	nd	1.358 ± 0.118	0.413 ± 0.020^{b}	0.572 ± 0.020^{b}	2.558 ± 0.077^{a}
Lysine	1.287 ± 0.112	2.620 ± 0.229	10.273 ± 0.330^{b}	9.352 ± 0.165^{b}	14.658 ± 0.361^{a}
Phenylalanine	0.364 ± 0.022	0.443 ± 0.037	0.303 ± 0.003^{c}	0.531 ± 0.010^{b}	1.213 ± 0.042^{a}

Wild T3, urchins caught and analysed at the end of the roe enhancement trial; Diet A, urchins fed the diet containing anchovy as fish meal; Diet K, urchins fed the diet containing krill as fish meal.
Values are reported as mean ± *SEM*. Superscript letters indicate significant differences among groups.

diethyl ether as solvent, for 6 hr. The solvent was then removed with a rotary evaporator (Mod. Hei VAP Value, Heidolph, Germany), and the residue was dried to constant weight.

The fatty acid profile was determined by gas chromatography, as fatty acid methyl esters (FAMEs), in accordance with method AOAC 996.06 (AOAC, 1997). The fatty acid content of sea urchin gonads was determined using an SP-2560 (100 m × 0.25 mm × 0.20 μm, Supelco) capillary column. The sample was introduced using the split-splitless injection system in split mode (ratio 1:100). The oven temperature program was started at 140°C (held for 5 min), linearly increased to 260°C (4°C/min) and kept at this temperature for the remainder of the analysis time. Fatty acid composition was obtained by comparison with the retention times of standard mixture FAMEs (fatty acid methyl esters; 37 Component FAME Mix—Supelco, USA) and was expressed as a percentage. Data were recorded and processed by ChromQuest 5.0 software (Thermo, Rodano, Italy).

2.9 | Olfactory analysis

A commercial portable electronic nose (PEN 3), including the Win Muster software for data mining, made by Airsense Analytics Inc. (Schwerin, Germany), was used to analyse the olfactory characteristics of gonad samples. The instrument was equipped with an array of 10 metal oxide semiconductors (MOS) acting as chemical sensors, differing in thickness and chemical composition in order to ensure selectivity towards volatile compound classes as previously reported (Laurienzo et al., 2013). The sensor response is expressed as resistivity (Ω). MOS sensors rely on changes in conductivity induced by the adsorption of molecules in the gas phase and on subsequent surface reactions. They consist of a ceramic substrate coated in a metal oxide semiconducting film, which is heated by a wire resistor. Due to the high operating temperatures (200–500°C), the organic volatiles transferred to the surface of the sensors are totally combusted to carbon dioxide and water, leading to a change in the resistance. The high temperature also allows no interference from water and fast response and recovery times (Kohl, 1992).

In this study, the hot sensors' detection limit was in the range of 1 mg/kg as indicated by the instrument supplier. For sample extraction, the coatings were gently removed (except for uncoated samples), and three cube-shaped pieces of 1 g of each sample were placed in airtight 45-ml glass vials, sealed with a PTFE/silicone septum and a screw cap, stored at 25°C for 1 hr to equilibrate, and analysed at the same temperature. The measurement device sucked the gaseous compound from the headspace of the sample through the sensory array at 400 ml/min for 200 s. The period of measurement allowed a steady state in the sensors' response. A second

TABLE 3 Fatty acid (g/kg dry weight) composition of formulated diets and fatty acids in the gonads of *Paracentrotus lividus* at the end of the roe enhancement trial

	Formulated diets		Experimental groups		
	Diet A	Diet K	Wild T3	Diet A	Diet K
Caprylic, C8:0	nd	nd	0.0017 ± 0.0006	0.0019 ± 0.0003	0.0015 ± 0.0004
Tridecanoic, C13:0	nd	nd	0.0005 ± 0.0002	nd	0.0004 ± 0.0002
Myristic, C14:0	0.0425 ± 0.0039	0.0518 ± 0.0017	0.0208 ± 0.0006	0.0224 ± 0.0009	0.0183 ± 0.0005
Pentadecanoic, C15:0	nd	nd	0.0033 ± 0.0005	0.0024 ± 0.009	0.0017 ± 0.0002
Palmitic, C16:0	0.1275 ± 0.0083	0.1261 ± 0.0022	0.051 ± 0.003^{a}	0.0535 ± 0.0022^{b}	0.0485 ± 0.0006^{b}
Palmitoleic, C16:1	0.0766 ± 0.0055	0.0905 ± 0.0027	0.0084 ± 0.0008	0.0099 ± 0.0013	0.0080 ± 0.0007
Heptadecanoic, C17:0	nd	nd	0.0012 ± 0.0006	0.0017 ± 0.0010	0.0008 ± 0.0005
cis-10-Heptadecanoic, C17:1	nd	nd	0.0022 ± 0.0006	0.0019 ± 0.0008	0.0007 ± 0.0004
Stearic, C18:0	0.0249 ± 0.0032	0.0201 ± 0.0033	0.0175 ± 0.0008	0.0168 ± 0.0007	0.0157 ± 0.0008
Elaidic, C18:1 n9t	nd	nd	0.0005 ± 0.0001	0.0020 ± 0.0006	0.0010 ± 0.0004
Oleic, C18:1 n9c	0.1126 ± 0.0075	0.1178 ± 0.0012	0.0046 ± 0.0006^{c}	0.0076 ± 0.0002^{b}	0.0135 ± 0.0004^{a}
Linolelaidic, C18:2 n6t	nd	nd	0.0016 ± 0.0007	0.0019 ± 0.0006	0.0006 ± 0.0004
Linoleic, C18:2 n6c	0.0776 ± 0.0037	0.0520 ± 0.0033	0.0077 ± 0.0004^{c}	0.0120 ± 0.0009^{b}	0.0258 ± 0.0004^{a}
Arachidonic, C20:0	nd	nd	0.0015 ± 0.0006	nd	0.0006 ± 0.0005
γ-Linolenic, C18:3 n6	nd	nd	0.0021 ± 0.0006	0.0021 ± 0.0006	0.0009 ± 0.0004
cis-11-Eicosenoic, C20:1	0.0408 ± 0.030	0.0562 ± 0.0018	0.0084 ± 0.0006^{b}	0.0137 ± 0.0007^{a}	0.0093 ± 0.0004^{b}
Linolenic, C18:3 n3	0.0123 ± 0.0020	0.0017 ± 0.0019	0.0126 ± 0.0007^{a}	0.0079 ± 0.0004^{b}	0.0075 ± 0.0004^{b}
cis-11,14-Eicosadienoic, C20:2	0.0211 ± 0.0040	0.0028 ± 0.0016	0.0099 ± 0.0006^{a}	0.0073 ± 0.0009^{b}	0.0084 ± 0.0005^{a}
Behenic, C22:0	nd	nd	0.0005 ± 0.0001	nd	0.0010 ± 0.0005
cis-8,11,14-Eicosatrienoic, C20:3 n6	nd	nd	0.0019 ± 0.0004	0.0031 ± 0.0009	0.0005 ± 0.0001
Erucic, C22:1 n9	0.0115 ± 0.0025	0.0126 ± 0.0015	0.0064 ± 0.0005	0.0054 ± 0.0006	0.0039 ± 0.0004
cis-11,14,17- Eicosatrienoic, C20:3 n3	nd	nd	0.0057 ± 0.0004^{a}	0.0033 ± 0.0006^{a}	0.0024 ± 0.0004^{b}
cis-5,8,11,14,17-Eicosapentaenoic C20:5 n3	0.0615 ± 0.0047	0.0573 ± 0.0027	0.0533 ± 0.0015^{a}	0.0511 ± 0.0011^{b}	0.0321 ± 0.0007^{c}
Arachidonic, C20:4 n6	0.0036 ± 0.0037	0.0046 ± 0.0037	0.0328 ± 0.0008^{a}	0.0242 ± 0.0006^{b}	0.0227 ± 0.0006^{b}
cis-4,7,10,13,16,19-Docosahexaenoic, C22:6 n3	0.0612 ± 0.0023	0.0512 ± 0.0024	0.0043 ± 0.0006^{c}	0.0188 ± 0.0005^{a}	0.0080 ± 0.0006^{b}

Wild T3, urchins caught and analysed at the end of the roe enhancement trial; Diet A, urchins fed the diet containing anchovy as fish meal; Diet K, urchins fed the diet containing krill as fish meal. Data are expressed as means ± *SEM*.
Superscript letters indicate significant differences among groups.

pump transported filtered air to the sensor array at 600 ml/min for 400 s to rinse the system between two consecutive samples. The analyses were performed on recorded data in the steady state. The results are displayed in a two-dimensional view (Gardner & Bartlett, 1991).

2.10 | Visual and sensory descriptive analysis

Gonad size, taste and colour were assessed by five independent tasters, each tasting one gonad from the same sea urchin. Ten gonads collected from each cage (Diet A and Diet K groups), and the wild group (T3) were assessed at the end of the trial. The sea urchins were delicately shaken to remove excess water and cracked open, and the gonads were removed and presented for analysis without delay for blind testing. Gonads were assessed using a scale of 1-5 criteria based on size, colour, taste and odour, as shown in Table 4, in accordance with Pearce, Daggett, and Robinson (2004), with some modifications.

2.11 | Statistical analysis

Data are expressed as mean ± *SEM*, calculated using MS-Excel. Analysis of variance (one-way ANOVA) followed by Bonferroni's test was performed to evaluate the effect of dietary treatments. To ensure compliance with the assumptions of ANOVA, before analysis data were arcsine-transformed and tested for normality with Shapiro–Wilks normality test and for homogeneity of variance with Cochran's test; p-values <.01 were considered significant. For the

TABLE 4 Criteria for taste-test assessment of gonad of *Paracentrotus lividus* subjected to roe enhancement (according to Pearce et al., 2004, with some modifications)

Attribute	Scale	Description
Size (subjectively by eye)	1	Small
	2	Small–medium
	3	Medium
	4	Medium–big
	5	Big
Colour (subjectively by eye without paint samples)	1	Bright yellow or orange
	2	Paler yellow or orange, mustard
	3	Light yellow-brown, orange-brown, red-brown, cream
	4	Yellow-brown, orange-brown, red-brown, cream
	5	Dark brown, orange-brown, red-brown, cream
Taste (subjectively)	1	Excellent (very sweet)
	2	Very good (very sweet, but <1)
	3	Good (sweet)
	4	Satisfactory (bland: not sweet, not bitter)
	5	Poor (bitter)
Odour (subjectively)	1	Excellent (strong marine)
	2	Good (mild marine)
	3	Satisfactory (seaweed)
	4	Poor (fishy)
	5	Very poor (putrid)

electronic nose tests, six independent measures were performed for each sample (n = 5).

The data were processed using Win Muster software, obtaining the correlation matrix (CM). The CM measures the separability of classes, assigning discrimination index (DI) values in the range of 0 to 1, in accordance with Volpe et al. (2014). Values lower than 0.5 indicate poor separability of the samples, while values ≥0.950 are significant.

3 | RESULTS

3.1 | Gonad index and gonadal stage

Gonad weight and GSI were significantly higher (p < .01) in formulated diet-fed sea urchins (both Diet A and Diet K) than in wild specimens (Table 5). Histological analysis of the gonads showed that the reproductive cycle progressed during the roe enhancement trial. Wild sea urchins at the beginning of the trial (T0) were in stage I (recovery), with the meshwork of nutritive phagocytes beginning to appear in the centre of the acini and no developing gametes along the acinal wall. At the end of the trial, the gonads of both wild (T3) and cultured sea urchins (Diet A and Diet K groups) appeared to be in late stage I and early stage II (growing), with the lumen entirely occupied by a thick meshwork of nutritional phagocytes and developing gametes along the acinal wall.

3.2 | Gonad carotenoid content

Carotenoid content was expressed as mg of beta carotene equivalent/Kg of dry gonad. It was significantly (p < .01) higher in the gonads of the wild sea urchins collected at the end of the roe enhancement trial (wild T3; 0.961 ± 0.007) than in the gonads of sea urchins fed Diet A (0.509 ± 0.003) and Diet K (0.388 ± 0.002).

3.3 | Free amino acids in the gonads

Almost all the essential amino acids were detected in the gonads of both wild (T3) and cultured sea urchins (Diet A and Diet K; Table 2), and there were no qualitative differences in the groups' FAA profiles. The most abundant FAAs were glycine, lysine, arginine and alanine.

TABLE 5 Total and gonad weight, GSI and gonad stage of *Paracentrotus lividus* specimens at the beginning (Wild T0) and at the end of the trial

	Total weight (*g*)	Gonad weight (*g*)	GSI	Stage
Wild T0	31.14 ± 3.46	0.32 ± 0.17^{b}	1.03 ± 0.31^{b}	I
Wild T3	28.33 ± 4.87	0.30 ± 0.18^{b}	1.06 ± 0.43^{b}	I–II
Diet A	34.78 ± 4.72	0.81 ± 0.34^{a}	2.33 ± 0.62^{a}	I–II
Diet K	35.36 ± 4.21	0.95 ± 0.53^{a}	2.70 ± 0.50^{a}	I–II

10 specimens of Paracentrotus lividus were caught from the wild at the biginning of the trial (Wild T0) and at the end of the trial (Wild T3). 10 specimens were randomly taken from the cages of the experimental groups Diet A (sea urchins fed the diet containing anchovy as fish meal) and Diet K (urchins fed the diet containing krill as fish meal). Values are reported as mean +/- SEM. Gonad stages are classified according to Byrne (1990). Superscript letters indicate significant differences among groups.

Glycine was the dominant amino acid in both wild and cultured sea urchins, with no statistically significant differences among groups. Lysine, arginine and alanine concentrations were significantly higher in the sea urchins fed Diet K ($p < .001$). Arginine concentrations were significantly lower in the sea urchins fed Diet A ($p < .001$).

3.4 | Fatty acids

The FA composition of the gonads of the wild and cultured sea urchins (Diet A and Diet K) is shown in Table 3. The main PUFAs present in *Paracentrotus lividus* gonads were cis-11,14,17-eicosapentaenoic acid (C20:5n-3, EPA), arachidonic acid (C20:4n6, AA), linolenic (C18:3n-3), cis-11,14-eicosadienoic (C20:2), linoleic (C18:2n-6), cis-11,14,17-eicosatrienoic (C20:3n-3), cis-4,7,10,13,16,19-docosahexaenoic (C22:6n3). The most abundant SFAs were palmitic acid (C16:0), myristic acid (C14:0) and stearic acid (C18:0), and the most abundant MUFAs were palmitoleic acid (C16:1), oleic acid (C18:1n9c), cis-11-eicosenoic acid (C20:1) and erucic acid (C22:1n9). Of the SFAs, only palmitic acid differed significantly between wild (T3) and cultured sea urchins, being higher in the former. The most important difference regarding MUFAs was the higher level of oleic acid in the gonads of cultured sea urchins. Of the PUFAs, linoleic acid and DHA were statistically more abundant in the gonads of cultured sea urchins. Linolenic acid, arachidonic acid and EPA were significantly higher in the wild sea urchins.

3.5 | Electric nose analysis

The analysis of volatile substances in the sea urchin gonads shows that there were no significant differences between wild (T3) and cultured sea urchins. The CM DI values are shown in Table 6. The DIs of the samples are low, indicating no significant differences. PCA (Figure 1) shows the volatile substances of the gonads of wild and cultured sea urchins grouped in a single cluster.

3.6 | Sensory attributes

Sensory attributes are shown in Figure 2. The gonads differed significantly across groups in terms of size, but not colour, taste or odour. Visual assessment rated the gonads of cultured sea urchins as being of better size than the gonads of wild sea urchins. Colour, taste and odour were perceived as similar across groups.

TABLE 6 Correlation Matrix among the *Paracentrotus lividus* groups

	Diet A	Diet K	Wild T3
Diet A	0.000		
Diet K	0.218	0.000	
Wild T3	0.302	0.396	0.000

Wild T3, urchins caught at the end of the roe enhancement trial; Diet A, urchins fed the diet containing anchovy as fish meal; Diet K, urchins fed the diet containing krill as fish meal.
The numbers indicate the DI. Values of DI ≥ 0.95 are significant.

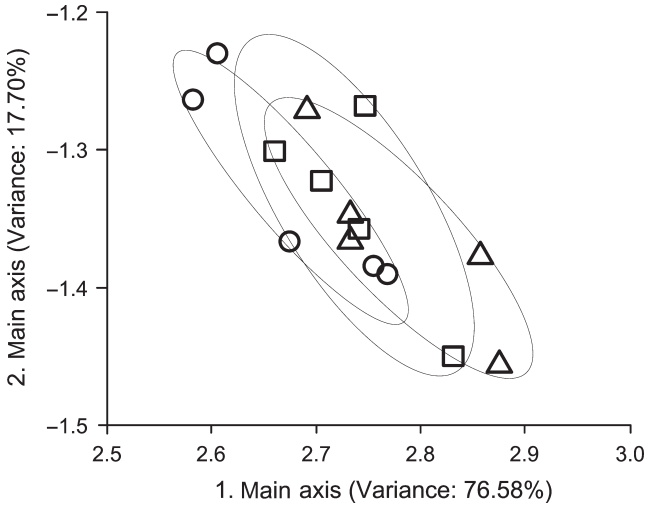

FIGURE 1 PCA analysis of the volatile substances of the gonads of wild and cultured urchins Circle = T3; square = Diet A; triangle = Diet K

4 | DISCUSSION

The present offshore pilot-scale trial was carried out off the south-east Italian coast from May to August. In this area, the *P. lividus* annual reproductive cycle is characterized by a long period during which the gonads have developing, mature and spawning gametes, and a brief resting period from July to September (Fabbrocini & D'Adamo, 2010a; Fabbrocini, Maurizio, & D'Adamo, 2016; Tenuzzo, Zaccarelli, & Dini, 2012). In this study, the histological analysis showed that gonads progressed from the recovery phase to early growing in both cultured sea urchins and wild specimens used as field controls. At the end of the roe enhancement trial, the gonads of both wild and cultured sea urchins were in late stage I and early stage II. The main change occurring in the gonad structure during the experimental period was the increased thickness of the phagocytic meshwork, which was more evident in the cultured sea urchins and was probably responsible for their increased GSI values. Specifically, our experimental conditions saw the GSI double in cultured sea urchins, in contrast to the field controls, in which the GSI remained almost constant, despite the slight progression in the reproductive cycle.

It is well known that sea urchin gonad growth mainly depends on dietary regime (Cuesta-Gomez & Sánchez-Saavedra, 2017; Marsh, Powell, & Watts, 2013), since gonads, specifically nutritive phagocytes, are the main nutrient storage organ (Carboni, Hughes, Atack, Tocher, & Migaud, 2015; Spirlet, Grosjean, & Jangoux, 2000; Takagi et al., 2017; Walker et al., 2015). In fact, a lipid-rich diet in *P. lividus* has been shown to increase GSI by increasing gonad reserve storage (Carboni et al., 2015), while *Strongylocentrotus purpuratus* specimens fed high-carbohydrate diets showed a more efficient use of proteins (Cuesta-Gomez & Sánchez-Saavedra, 2017). Carbohydrates seem to be the preferred source of energy for sea urchins (Hammer et al., 2012;

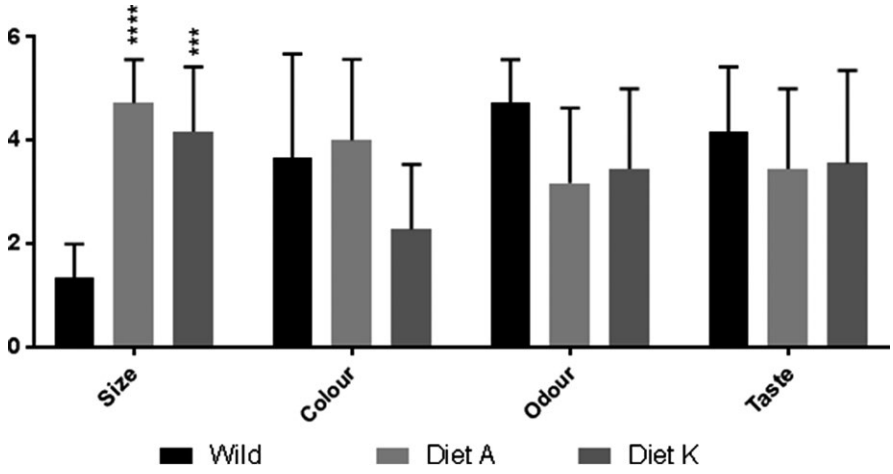

FIGURE 2 Urchin gonads sensory attributes. Data are expressed as means ± *SEM*. Statistical analysis was performed by one-way ANOVA followed by the Bonferroni post hoc test. *p* value indicates significant difference between the wild and the cultured groups (***$p < .0005$; ****$p < .0001$). Wild T3 = urchins caught and analyzed at the end of the roe enhancement trial. Diet A = urchins fed the diet containing anchovy as fish meal. Diet K = urchins fed the diet containing krill as fish meal. The scale on the ordinate is arbitrary

Marsh et al., 2013), while proteins are considered the main dietary component responsible for *P. lividus* somatic growth (Carboni et al., 2015). However, as observed by Spirlet et al. (2000) and by Fabbrocini and D'Adamo (2010b, 2011), in *P. lividus* high-protein commercial feed leads to an immediate metabolic response, causing the switching of postspawned gonads towards active gametogenesis without the intermediate conspicuous accumulation of nutrients in the nutritive phagocytes. Moreover, in *Strongylocentrotus droebachiens*, the taste of gonads tends to deteriorate as the protein level is increased (Pearce, Daggett, & Robinson, 2002a). Formulated diets should therefore provide enough energy in the form of dietary carbohydrates to meet the energy needs of sea urchins, while sparing the more expensive nutrients such as proteins (Cyrus et al., 2015; Hammer et al., 2012). A further consideration is the environmental impact of protein-based aquafeeds (Basto Silva, Valente, Matos, Brandão, & Belmira, 2017). In formulating our experimental diets, the nutritional composition was carefully dosed to take account of all these issues. Moreover, nutrients were entrapped in an agar matrix in order to minimize their loss in water, and thus their impact on the environment. The possibility of administering nutrients immersed in a matrix that prevents dispersion and disaggregation in water is a response to a long-standing criticism (Pearce et al., 2002b). Like other algae-derived polysaccharides (alginates, k-carrageenans), agar has a good binding capacity due to its ability to form hydrogels (Paolucci, Fabbrocini, Volpe, Varricchio, & Coccia, 2012), and it has proved to be very suitable as a binder in biocomposites for sea urchin feed due to its palatability and water resistance (Fabbrocini et al., 2012, 2015). In our roe enhancement trial, biocomposites consisted of agar 20 g/Kg and nutrients 100 g/Kg. These percentages showed a good consistency, low water absorption and limited nutrient dispersion (Paolucci et al., 2015). Preliminary tests conducted on various types of biopolymer extracted from algae, used alone in several percentages or even in blends, with varying percentages of nutrients, have shown that agar can stay in water for up to 14 days without showing obvious signs of structural collapse (Paolucci et al., 2015). Based on scuba diver observations, it was found during the present trial that all biocomposites were consumed within a week, a period of time during which disaggregation was limited, and it can be safely assumed that all feed was consumed. Indeed, in addition to doubling their size with respect to wild sea urchin gonads, the gonads of caged sea urchins progressed in the reproductive cycle just as fast as wild ones, as previously observed in confined conditions using similar experimental feed formulations (Fabbrocini et al., 2015).

The instrumental evaluations are consistent with the results of the panel test regarding taste and odour, which were found to be similar for cultured and wild sea urchins. It should be stressed here that the panel was composed of regular consumers of sea urchin roe, since the purpose of the evaluation was to verify that in the eyes of the habitual consumer, the gonads obtained from our experiment were comparable to those of wild specimens. Such equivalence depends on various factors, mainly the free amino acid and lipid content (Agatsuma, Sato, & Taniguchi, 2005; Pearce et al., 2004; Takagi et al., 2017). In many species of sea urchin, glycine and alanine are known to be sweet amino acids (Osako et al., 2007; Phillips et al., 2010; Takagi et al., 2017). When they are not present or present in low concentrations, there is an alteration of the characteristic gonad flavour and the bitterness is more pronounced. Valine, leucine, isoleucine (Osako et al., 2007; Phillips et al., 2010; Verachia et al., 2012) and a sulphur-containing amino acid, pulcherrimine (Murata et al., 2001), are known to confer a bitter taste, while glutamine contributes to the umami taste (Osako et al., 2007). In this study, the most abundant FAAs were glycine, lysine, arginine and alanine. Among the sweet amino acids, glycine was dominant in both the wild and cultured sea urchins, with no statistically significant differences among groups, while alanine was statistically higher in cultured specimens, an outcome that probably explains the similarity in taste perceived by the panel. Regarding the sulphur-containing AAs cysteine and methionine, it is interesting to note that the former was present in the feed but not in the gonads, whereas the latter was not in the feed but was present in the gonads. Methionine is an essential amino acid, which animals cannot synthesize, but plants can. Methionine biosynthesis can be catalysed by two different enzymes. Mammals possess only type I cobalamin-dependent methionine synthase (Banerjee & Matthews, 1990), whereas plants have only the type II

cobalamin-independent enzyme (Ravanel et al., 2004). Bacteria have both. Interestingly, the *Strongylocentrotus purpuratus* genome appears to encode both types of enzymes (Goel & Mushegian, 2006). This would explain the presence of methionine in *P. lividus* gonads in the absence of methionine in the diet. Why cysteine is absent in *P. lividus*, in spite of its presence in the diet, can only be hypothesized. On the one hand, cysteine could be used as a source of energy and rapidly metabolized to pyruvate. On the other hand, the ability to produce cysteine, as occurs in mammals, can be affected if the diet does not contain sufficient amounts of folic acid, methionine and group B vitamins (Mahmood, 2014).

The fatty acid composition of *P. lividus* gonads in the present study is in general agreement with the literature (Arafa, Chouaibi, Sadok, & El Abed, 2012; Martinez-Pita, Garcia, & Pita, 2010; Serrazanetti, Pagnucco, Conte, & Cattani, 1995; Siliani et al., 2016). Stearic acid, palmitic acid, EPA and ARA are the most abundant fatty acids in *P. lividus* gonads. Variations in the lipid composition of sea urchin gonads has been found, related to both endogenous and exogenous factors such as water temperature, season, growing stage and sampling area (Siliani et al., 2016; Zárate, Díaz De Vivar, Avaro, Epherra, & Sewell, 2016). It has also been suggested that dietary lipids play a key role in establishing the fatty acid profile of sea urchin gonads (Carboni, Hughes, Atack, Tocher, & Migaud, 2013; Marsh et al., 2013; Martinez-Pita et al., 2010). However, a recent study by Kabeya et al. (2017) showed that *P. lividus* possesses all the enzymatic machinery necessary for fatty acid biosynthesis, limiting the contribution of diet. Specifically, *P. lividus* can perform all the desaturation reactions required to convert the C18 PUFA precursors ALA (18:3n-3) and LA (18:2n-6) into the physiologically important EPA (20:5n-3), ARA (20:4n-6) and DHA, consistent with the fact that the two most abundant LC-PUFAs in *P. lividus* lipids are usually EPA and ARA (Carboni et al., 2013; Siliani et al., 2016), as also seen in this study. This outcome may partly explain why high levels of ARA were found in the gonads of *P. lividus* individuals fed diets with low ARA content (Carboni et al., 2013; present study). These observations also support the general similarity between the fatty acid composition of wild and cultured sea urchin gonads in this study. The wild sea urchins here used as field controls live in a meadow of *Posidonia oceanica*, which is one of their most strongly preferred feeds (Bouduresque & Verlaque, 2013). Further studies to biochemically characterize *P. lividus* and its epiphytic community will help to achieve a more tailored formulation of the biopolymer-based feed.

The development of artificial diets for sea urchin cultivation is also important for the colour of the gonads. Sea urchins fed artificial diets often produce gonads that are large but pale (Walker et al., 2015), while diets based on algae with the addition of natural β-carotene result in smaller gonads but of better colour (Pérez, Lattuca, Fraysse, & Malanga, 2015). Studies of the effect of dietary protein and carbohydrate content on sea urchin gonadal sensory qualities in *S. droebachiensis* have produced contrasting results (Pearce et al., 2002b; Siikavuopio, Trine, & Carleho, 2007). However, it should be noted that, besides diet composition, the colour of the gonads is affected by a number of variables, such as the pigments and the binders used in the formulated diets (Agatsuma et al., 2005; Pearce et al., 2002b). In this study, no carotenoids were added to the diet; however, the feed included a seaweed that could have affected the sensory properties of the roe, including the colour. Surprisingly, no significant differences in gonad coloration were perceived across groups. The colour of the gonads is most affected by carotenoids, especially echinone. However, the levels of dietary carotenoids cannot be directly linked to qualitative assessment of gonad colour, since coloration can be seen with both very low and high levels of carotenoids (Symonds, Kelly, Caris-Veyrat, & Young, 2007). A possible explanation of the limited differences between the colour of the gonads observed in this study is the short wavelength range in which the human eye perceives the colour orange.

Finally, the analysis of the gonads' volatile substances carried out using the electronic nose shows that the olfactory fingerprints of cultured and wild sea urchin gonads broadly overlapped, in agreement with the panel's perception of the odour. The principle of electronic nose operation differs significantly from that of the commonly used analytical instruments (GC, GC-MS, HPLC, HPLC-MS, etc.). Indeed, it does not analyse the volatile fraction of the food, responsible for most of the aroma perception, separating and identifying the various components, but gives an overall evaluation that can be defined as an olfactory fingerprint. In this respect, although less specific, it provides a rapid and low-cost evaluation that can predict the sensory quality of the analysed gonads.

A number of sea urchin species are reared throughout the world, and enhancement of their gonad quality is of great interest, due to the importance of the sea urchin market in many countries from Europe to Asia and America (Walker et al., 2015). The results of the present study, especially regarding the agar-based experimental feed, may also be of interest to the improvement of rearing techniques for species other than *P. lividus*, as the nutrient formulation can be modified to suit the species-specific requirements without altering its efficiency.

5 | CONCLUSIONS

In conclusion, this study shows that with the adopted feed formulation and schedule, *P. lividus* roe enhancement can be achieved for specimens reared in sea cages. Visual and sensory assessment of gonads showed that those of the formulated diet-fed sea urchins were of better size, while no differences were recorded for coloration, taste and odour. Moreover, the experimental biocomposites were consumed in a week, reducing the amount of waste produced and nutrient dispersal, thus optimising feed efficiency and reducing the environmental impact and the costs of cage management.

Although there remains much room for improvement, mainly regarding the formulation of biocomposites based on more environmentally and economically sustainable nutrient sources, the rearing conditions tested here represent a promising echiniculture system that is able to produce *P. lividus* gonads of a sensory quality that meets the taste of consumers.

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