Effect of formulated diets on the proximate composition and fatty acid profiles of sea urchin Paracentrotus lividus gonad

Ermelinda Prato[1] & Mariachiara Chiantore[2,3] & Maeve S. Kelly[4] & Adam D. Hughes[4] & Philip James[5] & Maria Paola Ferranti[2] & Francesca Biandolino[1] & Isabella Parlapiano[1] & Benedetto Sicuro[6] & Giovanni Fanelli[1]

Abstract Three formulated diets were tested to evaluate their effects on gonad quality in Paracentrotus lividus. Experiments were conducted in parallel by the Consiglio Nazionale delle Ricerche (CNR) of Taranto (trial 1) and the University of Genoa (trial 2), in land-based systems. In both trials, somatic and gonadsomatic index (GSI) were measured and the nutritional profile of the sea urchins has determined significant variations in the biochemical composition. Sea urchins fed the experimental diets contained higher levels of nutrients (protein and lipid and carbohydrate) compared to wild sea urchins. However, total polyunsaturated fatty acids (PUFAs), especially EPA and DHA, and the n-3/n-6 ratio were lower in urchins fed with formulated diets. In both trials, sea urchins fed with diet 2 (SABS) showed a similar profile with PUFAs higher than SAFAs and MUFAs, the highest UNS/SAT ratio, although the highest n3/n6 ratio was observed in the group fed diet 3 (CNR). Atherogenicity, thrombogenicity, and hypocholesterolemic/hypercholesterolemic indices showed the best values in sea urchins fed diet 2 in both trials.

* Ermelinda Prato
linda.prato@iamc.cnr.it

1 CNR-IAMC Istituto per l'Ambiente Marino Costiero, UOS Taranto, Via Roma 3, 74121 Taranto, Italy

2 Università degli Studi di Genova, DiSTAV, 16132 Genoa, Italy

3 CNR-IBF, Institute of Biophysics, UOS Genova, 16149 Genoa, Italy

4 Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Oban, Argyll PA37 1QA, UK

5 Norwegian Institute of Fisheries and Aquaculture Research, 9291 Tromso, Norway

6 Dipartimento di Scienze Veterinarie, Università di Torino, Grugliasco, 10095 Torino, Italy

Keywords Echinoculture · Paracentrotus lividus · Formulated diets · Growth ·

Introduction

The edible sea urchin, Paracentrotus lividus, is the most commercially exploited echi-noid in Europe (FAO 2012). It is a widespread species found along the European coasts of the North Atlantic and throughout the Mediterranean Sea (Boudouresque and Verlaque 2007).

In recent years, the increasing demand for sea urchin gonads has led to a decline of natural stocks in many European countries (Boudouresque and Verlaque 2007; Pearce et al. 2004). For this reason, there is considerable interest and investment in the development of sea urchin aquaculture as a new aquaculture industry and as a means of meeting the market demand and preserving natural stocks.

Sea urchin roe are sold largely for consumption in the sushi restaurant trade. Enhancing growth and quality of the gonads from wild or cultured urchins is vital to increase the competitiveness and profitability of future echinoculture industries. One factor key to affecting an improvement in roe quality is the availability of optimal formulated diets that maximize gonad growth, color, taste, textures, and nutritional value (Pearce et al. 2002).

An optimal diet provides nutritional elements in a balanced way, bringing together in a single food all the necessary nutrients. Formulated feeds can be nutritionally more complete than natural foods and are essential for commercially maximizing gonad production (Lawrence et al. 2011).

Research into sea urchin aquaculture, referred to as roe enhancement, has been undertaken using natural and formulated diets, or a combination of the two (Shpigel et al. 2005; Hammer et al. 2012). Studies have mainly focused on the effect of formulated diets on somatic and gonadic growth (de Jong-Westman et al. 1995; Pearce et al. 2002, 2004; James et al. 2007; Woods et al. 2008), on the effects of feed on organoleptic characteristics of the gonads (McLaughlin and Kelly 2001; Robinson et al. 2002; Pearce et al. 2004; McBride et al. 2004; Siikavuopio et al. 2007; Woods et al. 2008), and on how the formulated diet modifies their biochemical composition (Liyana-Pathirana et al. 2002; Hughes et al. 2006; Carboni et al. 2013).

Nutritionally, sea urchin gonads are a good source of proteins with a low fat and carbohy-drate content, such as most edible marine species. From the human health point of view, gonads are a rich source of polyunsaturated fatty acids (PUFAs) that provide a variety of health benefits against cardiovascular diseases (CVDs), including well-established hypotriglyceridemic and anti-inflammatory effects (Garaffo et al. 2011).

The objective of this study was to examine the effects of three formulated diets on somatic growth, gonadosomatic index (GSI), and biochemical composition of the gonad of the purple sea urchin, Paracentrotus lividus, held in land-based culture facilities, in two different Mediterranean localities. The results are relevant for the promotion of echinoculture in the Mediterranean area.

Materials and methods

Urchin collection and experimental conditions

Experiments were performed in parallel by the Consiglio Nazionale delle Ricerche (CNR) team in Taranto (trial 1) and the University of Genoa team (trial 2), operating in the CNR laboratory located in Camogli (Genoa, Italy).

Trial 1

Wild sea urchins (mean test diameter ± SD 30.98 ± 2.56 mm, mean wet weight ± SD 12.88 ± 2.84 g) were harvested from the subtidal zone in the Ionian Sea (40° 26′ 73″ N, 17° 13′ 50″ E; Mediterranean Sea), by SCUBA diving and transported in aerated plastic boxes with ambient seawater to the laboratory within 2–3 h from collection.

In the laboratory, they were placed into an aerated acclimation tank with flow-through filtered seawater with a temperature of 22 °C ± 1 °C. During the following 4 weeks of the acclimatization process, the urchins were starved to standardize their nutritional status prior to the start of the experiment and to allow the removal and replacement of any critically injured specimen from the experimental group.

After starvation, the feeding experiment was carried out for 3 months (from June to September 2014). Six sea urchins were randomly distributed and maintained in separate mesh pots inside of rectangular tanks. In total, four replicates for each of three dietary treatments were carried out. The system was exposed to a natural photoperiod in the land-based facility.

Trial 2

Wild sea urchins (mean test diameter ± SD 23.51 ± 6.48 mm, mean wet weight ± SD 7.32 ± 5.37 g) were harvested from a subtidal (~ 2 m depth) rocky shore zone, characterized

by the presence of macroalgae in the Ligurian Sea (44° 20′ 58″ N, 09° 09′ 17″ E; Mediterranean Sea) and transported to the laboratory (in aerated plastic boxes with ambient seawater) located in Camogli (Genoa, Italy). In the laboratory, the urchins were placed into an aerated acclimation aquarium and starved for 2 weeks before the start of the experiment.

The feeding experiment lasted 4 months (from June to October 2014). Five sea urchins were randomly distributed and maintained in separate mesh pots inside of rectangular tanks. In total, six replicates for each dietary treatment were carried out.

The experimental conditions for trials 1 and 2 were as closely as possible: the urchins were collected in the same season and kept in similar experimental conditions (Table 1). A random sample of 30 wild P. lividus (TØ) was taken at the start of the experiment to know the basic conditions of all studied parameters of the sea urchins gonads (somatic growth, gonadosomatic index, proximate, and fatty acid composition). Three experimental diets were tested in trial 1 and two in trial 2. Sea urchins were fed twice a week at a rate of approximately 1.0% of the body weight per day of the feed. The tanks were cleaned before each feeding event by removing standpipes and allowing the tanks to drain and washing the uneaten food and feces away with seawater.

Experimental diets

Diet 1, namely Nofima, tested in both trials, is a proprietary commercial diet, developed as an extruded dry feed, based on macroalgae, at the Norwegian Institute of Fisheries and Aquacul-ture Research Ltd in Tromsø, the exact composition of which is protected by a patent. To briefly summarize, diet 1 was characterized by high level of palmitoleic (16:1; 11%), oleic (18:1n9; 21%), linoleic (18:2n6; 14%), α-linolenic (18:3n3; 12%), and low levels of docosahexaenoic (DHA; 22:6n 3), eicosapentaenoic (EPA; 20:5n3), and arachidonic (ARA; 20:4n6) acids. Diet 2, SABS diet, used in both trials, was provided by the Scottish Association for Marine Science (UK), which mainly consists of soybean meal (21.27%), wheat (21.14%), rapeseed meal (21.27%), potato flour (19.84%), and gelatine (7.28%), and the remaining 9.2% was constituted by sodium alginate, flax oil lecithin, vitamin C, Algro Natural, Paradigmox, and inositol. It was characterized by the high level of protein and by 30% of the oleic and linoleic acids, 17% of the α-linolenic, and the low level of EPA and the absence of ARA and DHA. Diet 3: supplied by University of Turin, Italy, utilized only in trial 1 at the CNR. Briefly, this diet consisted of Krill

Table 1 General experimental conditions

	Trial 1	Trial 2
Start of the experiment	25 June 2014	19 June 2014
Duration of the trials	16 weeks	17 weeks
Replicate (n)	4	6
Specimen average size at TØ (mm)	30	24
Seawater temperature (°C)	26 ± 1.0 °C	23 ± 2.5 °C
Salinity	39 ± 1‰	37 ± 0.67‰
Photoperiod	Ambient	12 h light/12 h dark
Replicate tank volume (liters)	10 L	5.5 L
Replicate tank surface area (m^2)	0.1 m^2	0.1 m^2
Source of urchins	Wild	Wild
Flow rate	40 l/h	40 l/h
Feeding rate	1% body weight/day	1% body weight/day
Cleaning	Every 2–3 days	Every 2–3 days

(30%), Chaetomorpha linum (15%), starch (50%), vitamin supplement (1%), mineral supple-ment (3%), and celite (1%). It is characterized by a high protein content and a high level of myristic (14:0; 15%), palmitoleic (9%), oleic (11%), EPA (15%), and DHA (8%).

The complete proximate and fatty acid composition (% of the total fatty acids) of the diets used, to examine the impact of dietary fatty acids on sea urchin gonad, are reported in Table 2.

Somatic growth and gonadosomatic index

At the beginning (TØ) and at the end of the experiment, the test diameter (TD) was measured with Vernier calipers (± 0. 1 mm); the total wet weight and the gonad wet weight were weighed to the nearest 0.01 g.

Table 2 Proximate and fatty acid composition of the three experimental diets

	Diet 1	Diet 2	Diet 3
Moisture (%)	11.23 ± 0.07	5.01 ± 0.11	10.75 ± 5.18
Ash (%)	11.85 ± 0.14	8.15 ± 0.21	17.28 ± 1.50
Protein g/100 g	18.25 ± 1.85	26.62 ± 1.50	22.25 ± 2.00
Carbohydrate g/100 g	8.78 ± 3.15	5.95 ± 2.16	5.63 ± 1.38
Lipid g/100 g	4.61 ± 0.72	4.38 ± 1.49	5.48 ± 0.72
C14:0	10.82 ± 0.15	0.52 ± 0.15	15.13 ± 1.09
C15:0	0.52 ± 0.01	0.15 ± 0.03	0.66 ± 0.35
C16:0	18.85 ± 0.17	16.66 ± 1.36	26.51 ± 0.64
C17:0		0.45 ± 0.30	1.04 ± 0.62
C18:0	1.42 ± 0.03	4.22 ± 0.26	1.41 ± 0.38
C20:0	–	–	0.21 ± 0.07
C21:0	–	–	0.16 ± 0.09
∑SAFA	31.61 ± 0.33	21.85 ± 1.22	45.12 ± 2.15
C14:1	0.40 ± 0.02	–	0.50 ± 0.04
C15:1	0.11 ± 0.01	–	–
C16:1	10.82 ± 0.12	0.76 ± 0.16	8.71 ± 0.22
C17:1	0.70 ± 0.06	–	0.98 ± 0.27
C18:1n9t	–	–	0.28 ± 0.07
C18:1n9c	21.00 ± 0.43	29.94 ± 0.41	10.53 ± 0.72
C20:1n9	6.43 ± 0.09	–	0.49 ± 0.11
C24:1n9	–	–	–
∑MUFA	39.46 ± 0.59	30.69 ± 0.57	21.49 ± 0.82
C18:2n6c	13.90 ± 0.49	29.89 ± 1.10	4.91 ± 0.08
C18:3n6	0.20 ± 0.01	0.25 ± 0.05	0.51 ± 0.19
C18:3n3	11.75 ± 0.16	16.97 ± 0.84	3.03 ± 0.38
C20:2	–	–	–
C22:0 + 20:3n6	–	–	–
C20:3n3 + 22:1	–	0.25 ± 0.01	0.49 ± 0.11
C20:4n6	0.56 ± 0.02	–	0.79 ± 0.14
C20:5n3	1.78 ± 0.19	0.16 ± 0.02	15.32 ± 1.64
C22:6n3	0.74 ± 0.08	–	8.33 ± 1.51
∑PUFA	28.93 ± 0.92	47.45 ± 1.78	33.39 ± 2.36
∑n3	14.27 ± 0.42	17.31 ± 0.75	27.17 ± 2.72
∑n6	14.66 ± 0.51	30.14 ± 1.16	6.21 ± 0.36
n3/n6	0.97 ± 0.00	0.57 ± 0.02	4.40 ± 0.68
DHA + EPA	2.52 ± 0.27	–	23.64 ± 3.16
PUFA/SAFA	0.91 ± 0.04	2.18 ± 0.21	0.74 ± 0.08
UNS/SAT	2.16 ± 0.03	3.58 ± 0.26	1.22 ± 0.10

Each fatty acid is expressed as a percentage of total fatty acids (mean ± SD, n = 3). Moisture and ash are expressed as percentage of diet dry. Protein, lipid, and carbohydrate are given per gram of the diet dry weight

Gonad weight gain (GWG) and GSI, using the following formula, were calculated:

GSI ¼ gonad wet weight=whole urchin wet weight 100

Proximate analysis

Moisture (dried at 105 °C to constant weight), ash (ashing in a muffle furnace by heating at 550 °C until constant weight), and crude protein (Kjeldahl method; nitrogen to protein conversion factor = 6.25) of the sea urchin roe samples were analyzed, in triplicate, according to the methods of the Association of Official Analytical Chemists (AOAC 1995).

Lipids were extracted by direct elution with chloroform and methanol and determined gravimetrically according to Folch et al. (1957). The gonadal carbohydrate content was determined using the phenol–sulfuric acid method (Dubois et al. 1956).

Lipid classes

Triacylglycerols (TAGs) and total cholesterol (CHL) were measured by the colorimetric enzymatic Trinder method (1969), using a commercial kit (SGM, Rome, Italy). Phospholipids (PLs) were quantified by a colorimetric enzymatic method (Takayama et al. 1977) with a commercial kit (SGM). TAG, PL, and CHL levels were expressed as a percentage of total lipids. All analyses were performed in triplicate.

FA analysis

Fatty acids (FA) of total lipids were transesterified to methyl esters (FAMEs) in a boron trifluoride-catalyzed methanol: benzene solution (1:2, v/v). The mixture was shaken and then heated in boiling water for 45 min. Samples were allowed to cool, and 1 ml of distilled water was added followed by vigorous shaking. FAMEs were recovered in the upper benzene phase. Benzene phases were concentrated under nitrogen and kept at − 20 °C until further analysis.

Analysis of FAMEs was performed by gas chromatography (GC) using an HP 6890 series GC (Hewlett Packard, Wilmington, DE, USA) equipped with a flame ionization detector. FAMEs were separated with an Omegawax 250 capillary column (Supelco, Bellefonte, PA, USA) (30-m long, 0.25-mm internal diameter, and 0.25-mm film thickness). Helium was used as the gas carrier at a flow rate of 1 ml/min. The column temperature program was as follows: 150 to 250 °C at 4 °C/min and then held at 250 °C. FAMEs were identified by comparing retention times with a standard (Supelco 37 Component FAME Mix). FAs were quantified by integrating areas under peaks in the GC traces, with calibration derived from an external standard containing different methyl esters.

Lipid nutritional quality indices

To assess the nutritional quality of Paracentrotus lividus gonads for a human consumer, the data on fatty acid composition were used to determine the following three indices:

1. Atherogenicity index (AI), showing the inhibition of the aggregation of plaque and diminishing the levels of esterified FA, cholesterol, and phospholipids, thereby

preventing the appearance of micro- and macrocoronary diseases, determined as follows:

AI ¼ ð C12 : 0 þ 4 C14 : 0 þ C16 : 0Þ=ðSum MUFAs þ Sum PUFAsÞ ð Ulbricht and Southgate 1991Þ:

2. Thrombogenicity index (TI), showing the tendency to form clots in the blood vessels:

TI ¼ ½ð C14 : 0 þ C16 : 0 þ C18 : 0Þ=ð 0:5 Sum MUFAs þ 0:5 Sum n6 PUFAs þ 3 Sum n3 PUFAs þ ð n3=n6ÞÞ&

ðUlbricht and Southgate 1991Þ:

3. Fatty acids hypocholesterolemic/hypercholesterolemic ratios (HH), showing the hypocholesterolemic effect of PUFA:

HH ¼ ðC18 : 1cis9 þ C18 : 2n6 þ C20 : 4n6 þ C18 : 3n3 þ C20 : 5n3 þ C22 : 5n3 þ C22 : 6n3Þ=ð C14 : 0 þ C16 : 0Þ

ðFernández et al:2007Þ:

Statistical analyses

Homogeneity of data was explored with the Bartlett test or Cochran's C test, and the normality was determined with the Kolmogorov–Smirnov test. Analysis of variance (one-way ANOVA) and the post hoc Tukey's test or the Student–Newman–Keuls test were applied to test significant diet effects ($p \leq 0.05$).

Variables tested by ANOVA were test diameter (mm), gonad wet weight (g), gonadosomatic index, individual components of the proximate composition, and fatty acids. When requirements for normality were not met, the non-parametric Kruskal–Wallis test on ranks was applied ($p \leq 0.05$). All analyses were performed using R (R Development Core Team 2008) and the package software XLSTAT (version 2008.4.01).

Results

Trial 1

Somatic growth and gonadosomatic index In trial 1, somatic growth of sea urchins fed the experimental diets remained almost constant ($p > 0.05$), while with respect to initial samples, a significant increase in gonad wet weight was observed ($p < 0.05$; Fig. 1). By the end of the trial, GSI significantly increased ($p < 0.05$), from an initial value of 1.43 ± 0.21% in wild (TØ) to a maximum value of 15 ± 4.48% in the diet 2.

Proximate composition

All diets produced variations in the biochemical composition of the sea urchins' gonads. The proximate composition of the gonads at the end of the experiments is reported in Table 3. During trial 1, the moisture content of gonads did not change ($p > 0.05$), while

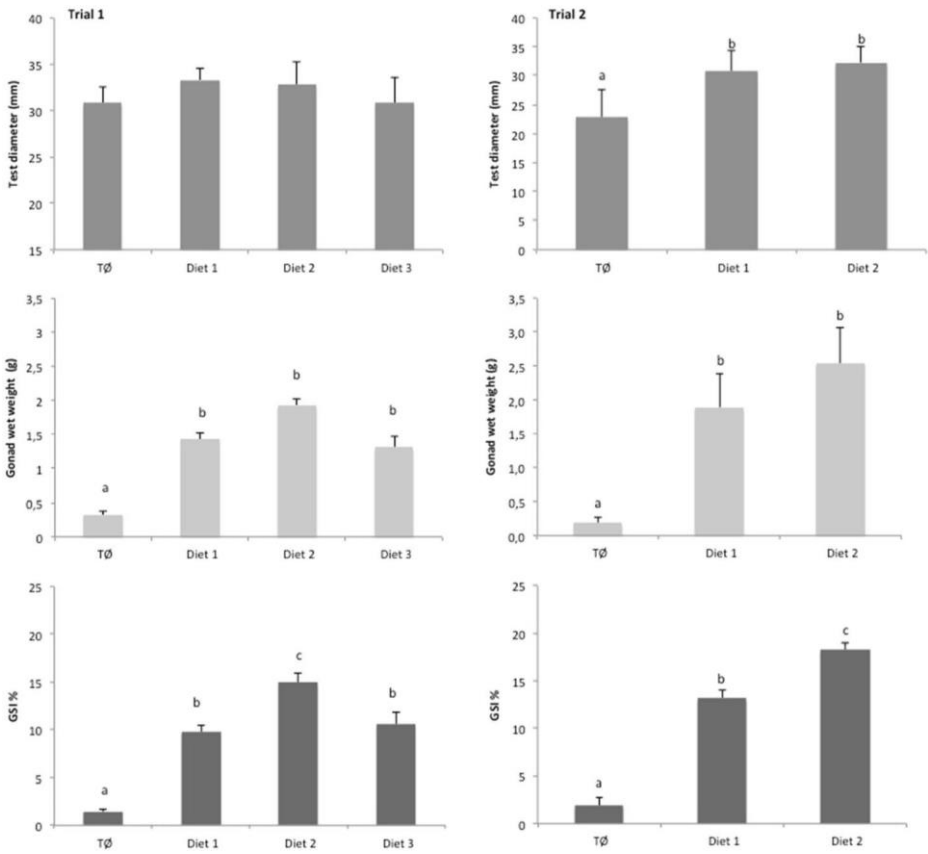

Fig. 1 Representative histograms of test diameter (mm), gonad wet weigh (g), and gonad somatic index (GSI %) (mean ± SE) of wild Paracentrotus lividus (TØ) and urchin fed with experimental diets. Columns with the same letter are not significantly different ($p > 0.05$). On the left, we report charts on the trial 1, on the right those of trial 2. Diet 1 = Nofima, diet 2 = SABs, and diet 3 = University of Turin

the ash content was significantly higher in the TØ group than in any of the experimental feeding trials ($p < 0.05$).

The overall proximate profile of sea urchin gonads was characterized by high protein level and low carbohydrate and lipid contents. The highest protein content was observed in sea urchins fed diet 2 for both trials (23.12 ± 0.38 g/100 g dry weight (dw)) ($p < 0.001$).

Sea urchins fed diets 1 and 2 had higher values for total lipids, while the diet 3 was not significantly different from the TØ group ($p < 0.001$).

Also carbohydrates changed significantly among different feeding treatments, ranging from 0.22 ± 0.03 g/100 g in sea urchins fed diet 2 to 2.21 ± 0.26 g/100 g in those fed diet 3 ($p < 0.001$).

Lipid classes

After the feeding treatments, the gonads of sea urchins did not show any significant difference with the TØ group, ($p > 0.05$). TAG represented the major lipid classes for all treatments, except for diet 3 that showed a significant decrease of TAG and an increase in PL ($p < 0.05$).

Table 3 Proximate composition of P. lividus gonad fed with experimental diets

	Wild	Trial 1			Trial 2	
	TØ	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2
Moisture (%)	74.50 ± 1.69a	74.51 ± 1.72a	74.40 ± 1.70a	73.01 ± 0.38a	69.34 ± 0.20b	68.67 ± 0.17c
Ash (%)	5.34 ± 0.25a	2.10 ± 0.16b	1.85 ± 0.13b	2.20 ± 0.35b	2.32 ± 0.07b	2.71 ± 0.08c
Protein g/100 g	11.12 ± 0.10a	14.87 ± 0.21b	23.12 ± 0.38c	15.75 ± 0.38b	15.96 ± 0.20b	17.63 ± 0.58c
Lipid g/100 g	1.36 ± 0.25a	3.5 ± 0.22b	3.5 ± 0.71b	2.27 ± 0.40a	2.63 ± 0.37b	2.10 ± 0.22b
Carbohydrate g/100 g	0.22 ± 0.03a	1.77 ± 0.33b	0.22 ± 0.03a	2.21 ± 0.26b	3.97 ± 0.68b	4.89 ± 0.22c

Values in the same row with different lowercase letters are significantly different ($p < 0.05$)

The second represented class was the PL that remained almost constant during the feeding treatments. The cholesterol content was very low, and no significant difference was observed among dietary groups (Fig. 2).

Fatty acids

The fatty acid compositions of gonads, expressed as percentage of total fatty acids, are reported in Table 4. At the start (TØ) of trial 1, PUFAs represented the highest proportion, contributing to more than 44.7% of the total gonad FAs. Saturated fatty acids (SAFAs) were the second group with 35.6% of the total gonad FAs, while the monounsaturated fatty acids (MUFAs) represented 19.6%.

After the dietary treatments, the FAs' profile significantly changed. The lowest proportions of SAFAs were found in sea urchins fed diet 2, contributing to 32% of the total FAs. Sea urchins fed diet 1 showed higher values for SAFAs (59.0% of the total FAs) ($p < 0.05$), followed by diet 3 (53 of the total FAs) ($p > 0.05$).

The major SAFAs were C14:0 and C16:0, followed by C18:0 and C21:0. Palmitic acid (C16:0) was the dominant, accounting for about 20.6% of the total FAs at the start of the experiment. In trial 1, after the feeding treatment, palmitic acid increased in sea urchins fed diet 1 and diet 3 with 31 and 28% of the total FAs, respectively (Tab. 4).

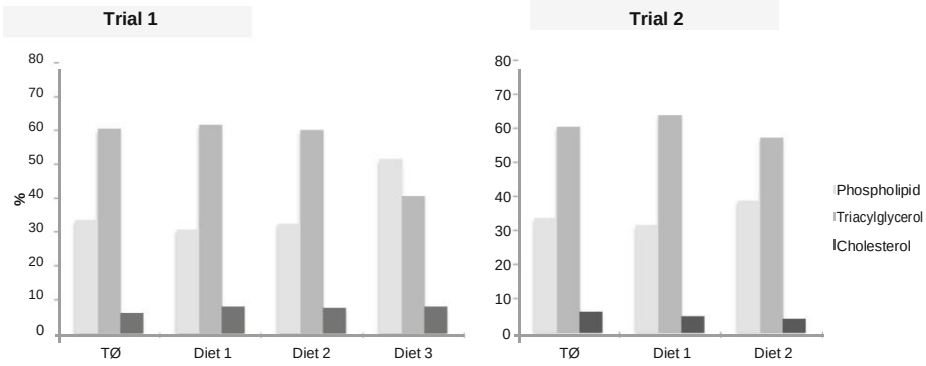

Fig. 2 Lipid classes composition (%) of wild Paracentrotus lividus (TØ) and urchins fed with experimental diets, in the two trials

Table 4 Fatty acid composition of Paracentrotus lividus wild (TØ) and at the end of the feeding trials

	Wild	Trial 1			Trial 2	
Fatty acids	TØ	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2
C14:0	6.46 ± 0.45	20.95 ± 0.28	5.12 ± 0.09	17.33 ± 0.37	21.79 ± 1.16	3.91 ± 0.25
C15:0	1.24 ± 0.27	1.30 ± 0.45	0.68 ± 0.02	1.22 ± 0.04	0.58 ± 0.05	0.38 ± 0.07
C16:0	20.61 ± 0.62	30.71 ± 0.72	19.39 ± 0.18	28.03 ± 0.01	25.89 ± 0.16	16.13 ± 0.42
C17:0	0.98 ± 0.24	0.55 ± 0.16	0.35 ± 0.02	0.51 ± 0.05	0.26 ± 0.08	–
C18:0	2.72 ± 0.36	3.03 ± 1.19	2.90 ± 0.10	3.32 ± 0.51	1.65 ± 0.01	2.97 ± 0.21
C20:0	0.66 ± 0.10	0.36 ± 0.21	0.35 ± 0.04	0.52 ± 0.07	0.25 ± 0.15	0.29 ± 0.10
C21:0	2.98 ± 1.18	2.02 ± 0.42	3.64 ± 0.23	1.92 ± 0.19	1.07 ± 0.02	1.00 ± 0.06
∑SAFA	35.65 ± 1.57a	58.93 ± 0.60b	32.10 ± 0.35a	52.86 ± 1.83c	51.49 ± 1.38c	24.70 ± 0.55d
C14:1	–	–	0.09 ± 0.01	–	0.80 ± 0.15	0.04 ± 0.00
C16:1	10.14 ± 0.27	8.29 ± 0.54	2.23 ± 0.04	6.31 ± 0.95	7.61 ± 0.40	1.79 ± 0.22
C17:1	–	–	–	0.56 ± 0.05	0.41 ± 0.03	
C18:1n9t	–	1.81 ± 1.55	–	0.73 ± 0.01	0.91 ± 0.38	–
C18:1n9c	9.52 ± 0.36	7.75 ± 2.25	11.20 ± 0.75	8.51 ± 4.10	8.79 ± 0.30	11.44 ± 0.21
C20:1n9	–	–	–	–	1.67 ± 0.02	5.52 ± 0.17
C24:1n9	–	–	0.42 ± 0.22	–	–	–
∑MUFA	19.66 ± 0.52a	17.85 ± 2.39a,b	14.09 ± 0.77c	15.74 ± 1.34b,c	20.19 ± 0.67a	18.80 ± 0.31a
C18:2n6c	3.86 ± 0.20	8.07 ± 0.70	21.88 ± 1.13	4.21 ± 1.31	8.99 ± 0.09	22.89 ± 0.27
C18:3n6	1.06 ± 0.16	–	0.44 ± 0.17	0.47 ± 0.14	–	–
C18:3n3	8.16 ± 0.50	7.66 ± 1.07	18.20 ± 1.39	5.21 ± 3.61	9.56 ± 0.54	21.79 ± 0.26
C20:2	–	–	0.21 ± 0.01	–	–	–
C22:0 + 20:3n6	1.01 ± 0.15	0.93 ± 0.12	1.23 ± 0.22	0.69 ± 0.32	1.07 ± 0.02	1.70 ± 0.09
C20:3n3 + 22:1	3.79 ± 0.32	0.91 ± 0.07	2.53 ± 0.14	1.69 ± 0.17	1.00 ± 0.02	3.31 ± 0.18
C20:4n6	10.03 ± 0.13a	2.58 ± 0.41b	3.81 ± 0.28c	2.95 ± 0.34b	3.41 ± 0.03b,c	4.08 ± 0.42c
C20:5n3	14.36 ± 0.79a	2.23 ± 0.33c	2.55 ± 0.10c	11.44 ± 1.69b	3.23 ± 0.11c	2.68 ± 0.12c
C22:6n3	2.40 ± 0.52a,b	0.85 ± 0.54a	3.08 ± 2.30a,b	4.72 ± 0.64b	1.05 ± 0.04b	0.03 ± 0.00c
∑PUFA	44.68 ± 2.07a	23.22 ± 2.40b	53.84 ± 0.45c	31.40 ± 3.02d	28.31 ± 0.80b	56.50 ± 0.67c

Values are mean (n = 6, ± SD). Different letters indicate significant differences for data in the same row ($p < 0.05$)

Also, MUFAs changed during feeding treatments ($p > 0.05$) and were mainly represented by C16:1 and C18:1n9 that had similar levels, except for the diet 2 (Table 4).

The sum of PUFAs was higher in the gonads of sea urchins fed diet 2, contributing to 53.84% of the total FAs. The most dominant PUFAs in this feeding group were linoleic acid (C18:2n6c), representing the 22% (trial 1), and α-linolenic linolenic acid (C18:3n3, ALA), representing the 18% of the total FAs. On the contrary, sea urchins fed diet 1 showed the lowest PUFA content (23% of the FAs) ($p < 0.05$).

At TØ, eicosapentaenoic (C20:5n3, EPA), docosahexaenoic (C22:6n3 DHA), α-linolenic (C18:3n-3, ALA), and linoleic acids (C18:2n6) were the major PUFAs in the sea urchin gonads (Table 4). EPA and DHA sum were found to be lower in all diet treatments compared to that observed in sea urchins at TØ.

As far as the unsaturated/saturated (UNS/SAT) ratio (Table 5), sea urchins fed diet 2 exhibited the highest values ($p < 0.001$), although the best n3/n6 ratio was observed in the group fed diet 3 ($p < 0.05$).

The PUFA/SAFA ratio always showed recommended threshold values (0.45) except in gonads of sea urchins fed diet 1. (0.40 ± 0.04; Table 5).

Significant changes in the lipid nutritional quality indices were also observed ($p < 0.05$). In particular, AI and TI showed the lowest values in gonads at TØ and in those fed diet 2, while the highest values were recorded in sea urchins fed diet 1 (trials 1 and 2). As regards the HH index, the gonad of sea urchins fed diet 2 showed higher value (Table 5).

Trial 2

Somatic growth and gonadosomatic index

Sea urchins fed diets 1 and 2 significantly increased in the test diameter with respect to initial samples. Similarly, a significant increase in gonad weight was observed by the end of the experiment ($p < 0.05$). Over the course of the trial, GSI increased from a value of 2.5% in the initial sample to a mean value of 18.3% (Fig. 1).

Proximate composition

All the experimental diets appeared to have a beneficial effect on proximate composition roe compared to initial gonad samples ($p < 0.05$) (Table 3). In trial 2, moisture and ash contents were significantly different between the groups fed the two different diets (moisture: $p < 0.001$; ash: $p < 0.001$).

The highest protein content was observed in sea urchins fed diet 2 for both trials (17.63 ± 0.58 g/100 g dw) ($p < 0.001$). In trial 2, the gonads of sea urchins fed with both diets showed higher values than wild ones ($p < 0.05$).

Also, carbohydrates changed significantly: the gonads of urchins fed diet 2 showed higher values for carbohydrate than those fed diet 1 ($p < 0.05$).

Lipid classes

By the end of the trial, there was no significant difference with the TØ group ($p > 0.05$). TAG represented the major lipid class in all treatments with values ranging from 57.2 to 63.75 of the total lipids, in diets 2 and 1, respectively. The second represented class was the PL that

Table 5 Nutritional quality indexes of P. lividus wild and at the end of the feeding trials

	Wild	Trial 1			Trial 2	
	TØ	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2
∑n3	28.72 ± 2.14a	11.64 ± 1.87b	26.36 ± 1.16a,c	23.07 ± 1.11c	14.84 ± 0.69b	27.81 ± 0.09a
∑n6	15.97 ± 0.07a	11.58 ± 1.23b	27.37 ± 1.02c	8.33 ± 2.12d	13.47 ± 0.13a,b	28.68 ± 0.76c
n3/n6	1.80 ± 0.13a	1.03 ± 0.05b	0.96 ± 0.08b	2.84 ± 0.59c	1.10 ± 0.04b	0.97 ± 0.03b
UNS/SAT	1.81 ± 0.18a	0.70 ± 0.00c	2.11 ± 0.04d	0.89 ± 0.02b,c	0.94 ± 0.05b	3.05 ± 0.09e
PUFA/SAFA	1.26 ± 0.11a	0.40 ± 0.04b	1.68 ± 0.01d	0.60 ± 0.08c	0.55 ± 0.03b,c	2.29 ± 0.08e
AI	0.72 ± 0.05a	2.79 ± 0.06b	0.58 ± 0.01a	2.07 ± 0.09c	2.33 ± 0.16d	0.42 ± 0.02e
TI	0.28 ± 0.02a	1.09 ± 0.09b	0.27 ± 0.01a	0.58 ± 0.02d	0.79 ± 0.05c	0.21 ± 0.01a
HH	1.79 ± 0.11a	0.56 ± 0.07d	2.48 ± 0.16b	0.81 ± 0.20c	0.55 ± 0.04d	2.00 ± 0.06a

Values are mean (n = 6, ± SD). Different letters indicate significant differences for data in the same row ($p < 0.05$)

remained almost constant during the feeding treatments with value of 31.6% of the total lipids in diet 1 and 38.7% in diet 2. Cholesterol content was very low, and no significant difference was observed among the dietary groups (Fig. 2).

Fatty acids

After the dietary treatments, the FAs' profile significantly changed. The lowest proportions of SAFAs was found in sea urchins fed diet 2, contributing to 25% of the total FAs, while diet 1 showed a values of 51.5% of the total FAs ($p > 0.05$). The major SAFAs were C16:0, followed by C18:0 and C21:0. Palmitic acid (C16:0) accounted for 26% in the gonad of sea urchins fed diet 1 and only for 16% in those fed diet 2 (Table 4).

There were no significant differences between MUFA content at the end of experiments ($p > 0.05$). They were mainly represented by C16:1 and C18:1n9 that had similar levels for the same diets in both trials (Table 4).

The sum of PUFAs was higher in the gonads of sea urchins fed diet 2, contributing to 56.5% of the total FAs. The most dominant PUFAs in this feeding group were linoleic acid (C18:2n6c), representing the 23%, and α-linolenic linolenic acid (C18:3n3, ALA), representing the 22% of the total FAs. Also in trial 2, EPA and DHA sum was found to be lower in all diet treatments compared to that observed in sea urchins at TØ. As far as the UNS/ SAT ratio (Table 5), sea urchins fed diet 2 exhibited the highest values (3.05 ± 0.09) ($p < 0.001$), although there were no significant differences between treatments for the n3/n6 ratio ($p > 0.05$).

The PUFA/SAFA ratio, in both feeding groups, always showed recommended threshold values (Table 5).

AI and TI showed similar value to that of trial 1, with favorable lowest values in gonads of the sea urchin fed diet 2. As observed in trial 1, the HH index showed the highest value in the gonad of sea urchins fed diet 2 (Table 5).

Discussion

It is well documented that sea urchins fed nutritionally better quality food may have higher gonadosomatic indices (Hammer et al. 2012). Only one trial in this study produced an increase in test diameter, and this was not surprising given the relatively short trial periods and larger size of sea urchins to begin for trial 1. In both trials, the tested diets produced significantly higher final gonad weights and gonadosomatic indices. This result is attributable to the intensification of gametogenesis and to the storage of nutritive phagocytes consistent with the accumulation of nutrients before the start of gametogenesis (Marsh and Watts 2007). We were unable to perform histological analyses of reproductive conditions, but at the end of 12 weeks, nearly all the urchins (approx. 95%) were observed to be ripe/spawning. In this study, the obtained GSI values were consistent with those reported for other sea urchins fed with formulated diets, showing an increase in gonad weight of about 1% per week (Shpigel et al. 2005).

Several studies have examined the effect of formulated diets and macroalgae on gonad growth of different sea urchin species, showing that sea urchins produce higher gonad growth with formulated diets compared to natural macroalgae (McLaughlin and Kelly 2001; Shpigel et al. 2005; Siikavuopio et al. 2007; Woods et al. 2008; Lawrence et al. 2009).

To date, the market evaluation of a high quality gonad is mainly a matter of color, taste, firmness texture, and gonad yield (Pearce et al. 2002; Agatsuma et al. 2005) and less on the nutritional value. However, the increasing consumer attitudes toward healthy food choices make of the knowledge of nutritional quality an added value.

In this study, all diets used in this feeding experiment caused variations in the nutritional composition of the sea urchin gonad. This suggests that sea urchins have assimilated the new diets and, overall, they acquired important reserves of proteins, a relative abundance of carbohydrate and low quantities of lipids. Protein content of a diet has been identified as a major factor affecting gonad production in echinoids (Frantzis and Gremare 1992). Determination of dietary protein requirements for optimal growth and gonad production in sea urchins is a complex challenge. Previous studies have reported that a formulated diet containing a moderate protein level (20%) produces an improvement of somatic growth rates and gonad production efficiency (Hammer et al. 2012), while a diet with a relative low protein content, as vegetal-based diets, does not support somatic and gonadal growth of sea urchins (Fernandez and Boudouresque 2000; Cook et al. 2007). Our results additionally support this hypothesis, since diet 2 with higher protein content produced the highest GSI (Table 2).

Carbohydrates were identified as a primary source of energy for gonad growth and gametogenesis in sea urchins (Montero-Torreiro and Garcia-Martinez 2003). In trial 1, an increase of carbohydrates content was observed for all feeding treatments, except for those fed diet 2 (Table 4). Whereas in trial 2, urchins fed diet 2 had higher carbohydrate content than those fed diet 1, and for both diets, the carbohydrate content was higher than that of the wild sea urchins and feeding groups of trial 1.

Lipids are an important storage nutrient for the development and maturity of sea urchin gonads (Gonzalez-Duran et al. 2008). Results show that total lipids in P. lividus gonads are similar to those reported by Mol et al. (2008) for P. lividus (3.05% ± 0.5).

None of the experimental diets caused a change in the proportion of the major lipid classes, except for diet 3. It is well known that TAG is the major lipid class supplying energy to fuel the development of many echinoderm and mollusc eggs (Carboni et al. 2013). The cholesterol was recorded in small amount in all dietary groups, indicating a beneficial effect for the health of a human consumer, as high cholesterol levels are associated with an increased risk of cardiovascular disease, such as heart disease and stroke (Horrocks and Yeo 1999).

Fatty acid profiles represent a good indication of food quality and well-being of organisms in aquatic ecosystems (Galloway et al. 2015). They are important structural and physiological components of cell membranes, and their concentrations in natural or formulated diets affect survival, growth and development of specific tissues, and reproductive performance (Hyne et al. 2009).

In both trials, sea urchins fed diet 2 showed a similar profile, with PUFAs higher than SAFAs and MUFAs, reflecting the same proportions of the diet composition (Table 2). This is in accordance with De la Cruz-Garcia et al. (2000), who reported for P. lividus PUFAs to be the most abundant FA class, accounting for 44.7%, followed by SAFAs 35% and MUFAs 20.3% of the total FAs. However, Cook et al. (2007) showed similar amounts of SAFAs and PUFAs (33.9 and 35.1%, respectively), while MUFAs showed a lower abundance (19.7%).

The FA profiles of P. lividus gonads showed large proportions of palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1n9), linoleic (C18:2n6), α-linolenic (C18:3n3), arachidonic (C20:4n-6), and eicosapentaenoic (C20:5n-3) acids. Palmitic acid (C16:0) is an important component of the lipid fraction in the P. lividus gonads. It is synthesized in plant chloroplasts through the synthesis of malonil-CoA, with further desaturation and elongations leading to

biosynthesis of C18:2 n6 and C18:3 n3 in a first instance and later to C20:4 n6, C20:5 n3, and C22:6 n3 (Angioni and Addis 2014). None of these latter FAs can be synthesized in animals and can only come from the diet.

Among monounsaturated, palmitoleic (C16:1), and oleic (C18:1n-9) acids, both precursors in the formation of PUFAs, were the most abundant, evidencing a clear effect of the diets. In particular, oleic acid increased in the gonads of sea urchins fed diet 2 compared to wild sea urchins (TØ), in both trials. This reflects the highest abundance of these FAs in the diet 2 (Table 4).

Cook et al. (2007) stated the importance of dietary protein levels in influencing gonad levels of long-chain PUFA that are crucial to reproductive performance and development of specific tissues. This was confirmed in the present study for sea urchins fed diet 2 with high protein content (Table 3). Therefore, this aspect should be taken particularly into account when formulating diets for echinoculture.

The highest dietary inputs (diets 1 and 2) of FA substrates (18:2n-6 and 18:3n-3) of the lipoprotein cholesterol (LC)-PUFA biosynthesis pathway and their content in the gonads of sea urchins, fed these diets, clearly show that the tissue levels of these two fatty acids are influenced by their contents in the diets.

The presence in the gonads of some fatty acids that are not detected in the diets such as 20:3n-6, not found in all diets and 20:3n-3 not found in diet 1, and ARA and DHA not found in diet 2, suggests that the sea urchin may have the ability to synthesize LC-PUFA (Carboni et al. 2013).

Epidemiologic studies suggest that people at risk for coronary heart disease (CHD) benefit from consuming n-3 fatty acids, especially EPA (C20:4 n-6) and DHA (C22:6 n-3). Although the ideal amount to take is not established, evidence from prospective prevention studies suggest that intakes of EPA + DHA ranging from 0.5 to 1.8 g per day significantly reduce the number of deaths from heart disease (Kris-Etherton et al. 2009). The n-3/n-6 ratio is considered a good index for comparing relative nutritional value of marine organisms, with a higher ratio being of great importance in prevention of coronary heart diseases, plasma lipid levels, and cancer risks (Department of Health and Social Security 1984). Very high intake of n-6 was recognized as undesirable in the human diet due to their associated negative health impacts such as an increased risk of cardiovascular disease and autoimmune diseases (Simopoulos 2006).

The recommended n-3/n-6 ratio differs between authors, but it is always higher than one (Simopoulous 2006). In this study, the best n-3/n-6 ratio was exhibited by sea urchins fed diet 3 (2.84), although acceptable values were also showed by the others feeding treatments. These data suggest that P. lividus could be categorized as beneficial to human health consumption.

Another useful key factor for the evaluation of nutritional quality is the PUFA/SAFA ratio. Values of the PUFA/SAFA ratio higher than 0.45 are recommended by the Department of Health (1994). The present results meet this requirement, and higher PUFA/SAFA ratios were found for all tested diets (except for diet 1–trial 1 that showed slightly lower values).

The dietetic value of seafood is also determined by the lipid quality indices, which depend on the relative proportions of some individual saturated and unsaturated fatty acids. These indices indicate the global dietetic quality of lipids and their potential effects on the development of coronary disease (Stanec et al. 2011). The lower values of AI and TI recorded indicated that diet 2 produces gonads more healthy for human consumption. Also, the values obtained by the HH index confirmed that diet 2 was the best (trial 1 2.48 ± 0.16; trial 2 2.00 ± 0.06) indicating that regular consumption may exert a hypocholesterolemic effect (Table 5).

Modern aquaculture products have decreased n-3 FAs compared to wild, and higher levels of terrestrial plant-originating C18: 2n-6 present in manufactured feeds affect the n-3/n-6 ratio in the edible part (Grigorakis 2007).

In the present study, this effect was evident: in both trials, the essential fatty acids from the n-3 family were significantly lower than in the wild sea urchins (TØ). In this study, the sea urchins fed on diet 1 showed the lowest n-3 (Table 5).

The best compromise between the best biochemical profile and the GSI was obtained with diet 2. The implications of improving nutritional quality and GSI after 12 weeks of feeding emphasize the possibility of applications in commercial aquaculture of these formulated diets for roe enhancement of urchins from harvest fisheries, where the low gonad yield of the animals results in a significantly reduced price per unit.

Conclusions

The fundamental agreement of the analytical data obtained in two independent feeding trials produced a consistent picture on the use of formulated diets for Paracentrotus lividus to promote echinocolture in the Mediterranean area. GSI and also the gonad nutritional quality are important issues in any commercial sea urchin aquaculture. The diets used in this experiment brought significant changes in all the parameters tested. After only 3 months of feeding treatments, the gonads showed an enhancement of GSI. Sea urchins fed with exper-imental diets clearly contained higher levels of nutrients (i.e., proteins and lipids and carbo-hydrates) compared with wild sea urchins. The fatty acid profile of the wild sea urchins could be more advantageous with regard to the n-3/n-6 ratio; however, the total lipid content was only 1.4%. The n-3/n-6 ratio of the sea urchins fed the formulated feeds was lower; however, the absolute amounts were higher due to the overall higher lipid content, up to 3.5%. The results suggest a good starting point, but further research is needed to determine the optimum concentrations of dietary PUFA (in particular EPA and DHA) to enhance their assimilation and deposition in gonads of sea urchins commercially farmed. The growing of cultured sea urchins to marketable size would enable the production of this valuable product and reduce the ever-increasing worldwide pressure of sea urchin fisheries on wild stocks.

Funding information This research has been supported by ResUrch project: BREsearch and technological development to improve economic profitability and environmental Sustainability of sea URCHin farming^ (http://resurchproject.com) funded by the EU under the FP7 framework.

References

Agatsuma Y, Sato M, Taniguchi K (2005) Factors causing brown colored gonads of the sea urchin Strongylocentrotus nudus in northern Honshu, Japan. Aquaculture 249:449–458

Angioni A, Addis P (2014) Characterization of the lipid fraction of wild sea urchin from the Sardinian sea (Western Mediterranean). J Food Sci 79:155–162

AOAC (1995) AOAC official methods of analysis, 16th edn. Association of Official Analytical Chemists, Washington DC

Boudouresque CF, Verlaque M (2007) Ecology of Paracentrotus lividus. In: Lawrence JM (ed) Edible sea urchins: biology and ecology. Elsevier Science, Amsterdam, pp 243–283

Carboni S, Hughes AD, Atack T, Tocher DR, Migaud H (2013) Fatty acid profiles during gametogenesis in sea urchin (Paracentrotus lividus): effects of dietary inputs on gonad, egg and embryo profiles. Comp Biochem Physiol A 164:376–382

Cook EJ, Hughes AD, Orr H, Kelly MS, Black KD (2007) Influence of dietary protein on essential fatty acids in the gonadal tissue of the sea urchins Psammechinus miliaris and Paracentrotus lividus (Echinodermata). Aquaculture 273:586–594

De la Cruz-Garcia C, Lopez-Hernandez J, Gonzalez-Castro MJ, Rodriguez-Bernaldo De Quiros ARB, Simal-Lozano J (2000) Protein, amino acid and fatty acid contents in raw and canned sea urchin (Paracentrotus lividus) harvested in Galicia (NW Spain). J Sci Food Agric 80:1189–1192

Department of Health and Social Security Report on Health and Social (1984) Subjects No. 28. Diet and Cardiovascular Disease HMSO, London. ISBN 0 11 320859 6

Department of Health, Nutritional Aspects of Cardiovascular Disease (1994) Report on Health and Social Subjects no. 46. London: H.M. Stationery Office. ISBN 10: 0113218753 ISBN 13: 9780113218752

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

FAO (2012) The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome

Fernandez C, Boudouresque CF (2000) Nutrition of the sea urchin Paracentrotus lividus (Echinodermata: Echinoidea) fed different artificial food. Mar Ecol Prog Ser 204:131–141

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Frantzis A, Gremare A (1992) Ingestion, absorption and growth rates of Paracentrotus lividus (Echinodermata: Echinoidea) fed different macrophytes. Mar Ecol Prog Ser 95:169–183

Galloway AWE, Brett MT, Holtgrieve GW, Ward EJ, Ballantyne AP, Burns CW, Kainz MJ, Muller-Navarra DC, Persson J, Ravet JL, Strandberg U, Taipale SJ (2015) A fatty acid based Bayesian approach for inferring diet in aquatic consumers. PLoS One, 10(6) doi: 10.1371/journal.pone.0129723

Garaffo MA, Vassallo-Agius R, Nengas Y, Lembo E, Rando R, Maisano R, Dugo G, Giuffrida D (2011) Fatty acids profile, atherogenic (IA) and thrombogenic (IT) health lipid indices, of raw roe of blue fin tuna (Thunnus thynnus L.) and their salted product BBottarga^. Food Nutr Sci 2:736–743

Gonzalez-Duran E, Castell JD, Robinson SMC, Blair TJ (2008) Effects of dietary lipids on the fatty acid composition and lipid metabolism of the green sea urchin Strongylocentrotus droebachiensis. Aquaculture 276:120–129. https://doi.org/10.1016/j.aquaculture.2008.01.010

Grigorakis K (2007) Compositional and organoleptic quality of farmed and wiled gilthead sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) and factors affecting it: a review. Aquaculture 272:55–75

Hammer HS, Powell ML, Jones WT, Gibbs VK, Lawrence AL, Lawrence JM, Watts SA (2012) Effect of feed protein and carbohydrate levels on feed intake, growth, and gonad production of the sea urchin, Lytechinus variegatus. J World Aquacult Soc 43(2):145–158

Horrocks LA, Yeo YK (1999) Health benefits of docosahexaenoic acid (DHA). Pharmacol Res 40:211–225 Hyne

RV, Sanchez-Bayo F, Bryan AD, Johnston EL, Mann RM (2009) Fatty acids composition of the estuarine Amphipod, Melita plumulosa (Zeidler): link between diet and fecundity. Environ Toxicol Chem 28:123–132

Hughes AD, Kelly MS, Barnes DKA, Catarino AI, Black KD (2006) The dual functions of sea urchin gonads are reflected in the temporal variations of their biochemistry. Mar Biol 148:789–798

James P, Heath P, Unwin MJ (2007) The effects of season, temperature and initial gonad codition on roe enhancement of the sea urchin Evechinus chloroticus. Aquaculture 270:115–131

de Jong-Westman M, March BE, Carefoot TH (1995) The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin Strongylocentrotus droebachiensis. Can J Zool 73:1495–1502 Kris-

Etherton PM, Grieger JA, Etherton TD (2009) Dietary reference intakes for DHA and EPA. Prostaglandins Leukot Essent Fat Acids 81:99–104

Lawrence JM, Cao X, Chang Y, Wang P, Yu Y, Lawrence AL, Watts SA (2009) Temperature effect on feed consumption, absorption, and assimilation efficiencies and production of the sea urchin Strongylocentrotus intermedius. J Shell Res 28:389–395

Lawrence J, Chang YQ, Cao XB, Lawrence A, Watts S (2011) Potential for uni production by Strongylocentrotus intermedius using dry formulated feeds. J World Aquac Soc 42:253–260

Liyana-Pathirana C, Shahidi F, Whittick A (2002) The effect of an artificial diet on the biochemical composition of the gonads of the sea urchin (Strongylocentrotus droebachiensis). Food Chem 79:461–472

Marsh AD, Watts SA (2007) Biochemical and energy requirements of gonad development (Chapter 3). In: Lawrence JM (ed), Edible sea urchins: biology and ecology. Elsevier, Amsterdam, pp. 35–53

McBride SC, Price RJ, Tom PD, Lawrence JM, Lawrence AL (2004) Comparison of gonad quality factors: colour, hardness and resilience, of Strongylocentrotus franciscanus between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery. Aquaculture 233:405–422

McLaughlin G, Kelly MS (2001) Effect of artificial diets containing carotenoid-rich microalgae on gonad growth and color in the sea urchin Psammechinus miliaris (Gmelin). J Shellfish Res 20:377–382
Mol S, Baygar T, Varlik C, Tosun ŞY (2008) Seasonal variations in yield, fatty acids, amino acids and proximate compositions of sea urchin (Paracentrotus lividus) roe. J Food Drug Anal 16:68–74 https://www. researchgate.net/publication/286203572
Montero-Torreiro MF, Garcia-Martinez P (2003) Seasonal changes in the biochemical composition of body components of the sea urchin, Paracentrotus lividus, in Lorbe´(Galicia, north-western Spain). J Mar Biol Assoc UK 83:575–581
Pearce CM, Daggett TL, Robinson SMC (2002) Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, Strongylocentrotus droebachiensis. Aquaculture 214:307–332
Pearce M, Daggat TL, Robinson SMC (2004) Effect of urchin size and diet on gonad yield and quality in the green sea urchin (Strongylocentrotus droebachiensis). Aquaculture 233:377–367
R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. URL http://www.R-project.org Accessed 08 Feb 2008
Robinson SMC, Castell JD, Kennedy EJ (2002) Developing suitable colour in the gonads of cultured sea urchins (Strongylocentrotus droebachiensis). Aquaculture 206:289–303
Shpigel M, McBride SC, Marciano S, Ron S, Ben-Amotz A (2005) Improving gonad colour and somatic index in the European sea urchin Paracentrotus lividus. Aquaculture 245:101–109
Siikavuopio SI, Christiansen JS, Sæther BS, Dale T (2007) Seasonal variation in feed intake under constant temperature and natural photoperiod in the green sea urchin (Strongylocentrotus droebachiensis). Aquaculture 272:382–334
Simopoulos AP (2006) Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomed Pharmacother 60:502–507
Stanec M, Borejszo Z, Dabrowski J, Janicki B (2011) Fat and cholesterol content and fatty acid profiles in edible tissue of spiny-cheek crayfish, Orconectes limosus (Raf) from Lake Goplo (Poland). Arch Pol Fish 19:241–248. https://doi.org/10.2478/v10086-011-0030-7
Takayama M, Itoh S, Nagasaki T, Tanimizu I (1977) A new enzymatic method for determination of serum choline-containing phospholipids. Clin Chim Acta 79:93–98
Trinder P (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J Clin Pathol 22:158–161
Ulbricht TL, Southgate DAT (1991) Coronary heart disease: seven dietary factors. Lancet 338:985–992 Woods CM, James P, Moss G, Wright J, Siikavuopio SI (2008) A comparison of the effect of urchin size and diet on gonad yield and quality in the sea urchin Evechinus chloroticus Valenciennes. Aquac Int 16:49–68