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Growth, survival and fatty acids profile of sea urchins, *Paracentrotus lividus* juveniles fed with *Ulva* spp. and maize in aquaculture production. First results using G1 generation in Portugal

Abstract

Sea urchins are a marine resource whose value and demand presents a growing trend, the objective of this work was to evaluate growth and survival in aquaculture produced (G1) juveniles fed with two different diets and trying to stablish a protocol for a feasible production of *P. lividus*. A nutritional trial was defined to evaluate two different feeding protocols in G1 (captivity born) juveniles were fed for 4 months, September 2017 - January 2018) with two diets: diet A - macroalgae (*Ulva* spp.); diet M+A - 25 % of maize grains+ 75% of macroalgae (*Ulva* spp.) w/w. Alternative dietary solutions for *P. lividus* were shown to be viable, survival was 100% and juvenile growth, quite similar for the two treatments presented very good rate 2.25 and 2.13%. At the end of trial juvenile sea urchin presented already gonad differentiation, what was not expected in individuals of that class size 1.85 and 1.78 for diet A and M+A. FA profiles largely reflect the diets, macroalgae-based diet enabled a higher ω3/ω6 ratio. In this context, it is highly positive the revealed potential of this species to self-synthesize long chain PUFA (with 20 carbons) by incorporation of precursors (C16 and C18) of these FA directly from diet (either macroalgae-based only or in maize+macroalgae-based). Namely, EPA, an anti-inflammatory bioactive FA, was shown to be enriched in the gonads of sea urchins. This work evidence the need to develop a specific diet that may enhance somatic growth in detriment of gonad growth, avoiding gametogenesis in such young/smaller individuals.

Keywords: sea urchin rearing, *Paracentrotus lividus*, feeding table, fatty acid profile, *ulva spp*

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Introduction

Sea urchins are a marine resource whose value and demand presents a growing worldwide trend, particularly in Japan but also in others international markets, due to the world population growth but also the increasing interest in the product.[1] Sea urchin gonads - roe in anglo saxonic countries - the edible part, are considered a gourmet delicatessen valorized by size, firmness, colour, smell and taste,[2] even is considered as an umami food item, the designate 5th taste and may reach very high prices as 3€ per individual for a very fresh unprocessed product or until ca 50€.Kg-[1] for processed gonads in the Japanese market (Morikawa comm. pers.). This increased global demand led to a collapse in some fisheries due to overexploitation.[3,4] In Europe *Paracentrotus lividus*, purple sea urchin, is most appreciated in France, Spain and Italy but, the evolvement of the consumption has been felt in neighbourhoods countries, such is the case of Portugal. Indeed, the Portuguese national consumption of echinoid is low and confined to restricted zones but the fishing effort for exportation mainly to Spain and France (the nearest markets) have led to a considerable reduction in the natural abundance of the resource.[5,6]

Over the last two decades interest have been arising in echinoid aquaculture production to fulfil the requires.[1,7] Despite the research efforts in the echinoids rearing the intrinsic characteristic of the species have been limiting the production mainly: the larval rearing itself, the unpredictability success of metamorphose stage and posterior fixation that is reflected by a non-reliable supply of juveniles; and in the juvenile phase the very slow growth reported in literature, this key constraints posed serious economical limitations in the uptake of Echino culture by the industry as documented by Carboni,[8] and Zupo et al.[9]

Another issue is the lack of specific adapted feeds, therefore different facilities have been feeding urchins mainly with the macroalgae naturally present in the sea urchin collection site accordingly to the geographical locations, as the diet and the feeding habits of a specific population may vary accordingly to the habitat.[10] The use of algae, although may be the best food supply, implicates logistical and economically difficulties: the continuous availability and stocking of good quality algae. Some approaches in developing inert diets have been made[1] but until now no inert diet is commercially available. Yet, due to seaweed supply constraints, artificial feeds also have been tested and previous studies already described the feasibility of maize grain for feeding *P. Lividus* with comparable results and the advantages of an inert feed in terms of storage, supply, price and good behavior in salt water.[11, 12]

Also, an important dimension is its effect on the nutritional value of the farmed seafood. Namely, there may be substances with relevant biological activity, whose chemistry may range from hydrophilic components, such as peptides, to lipophilic substances, such as carotenoids and ω3 polyunsaturated fatty acids (ω3 PUFA). Within the ω3 PUFA, eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3) are linked to decreased morbidity and mortality from cardiovascular and other diseases.[13] In particular, EPA has been claimed to improve the anti-inflammatory activities of high-density

lipoprotein, among other actions.[14] In this context, a special attention should be dedicated to the FA profile of diets and *P. lividus*.

The objective of this work was to contribute to the establishment of a protocol for a feasible aquaculture production of *P. lividus*. A nutritional trial was made to evaluate two different diets in captivity born (G1) juvenile production.

Materials and methods

Sea urchin collections and adaptation

The sea urchin used in this work are offspring of wild specimens caught in Southeast coast of Portugal and reproduced in the Aquaculture Research Station (EPPO) from the Portuguese Institute for the Sea and Atmosphere (IPMA). Production trials of sea urchins, *P. lividus* at EPPO started using adults captured in the intertidal jetty rocks off the Algarve East coast (South of Portugal) in the autumn of 2016[15] and conditioned at the facilities. Wild brood stock were manually collected and transported in boxes maintaining certain level of humidity and avoid direct sunlight. At EPPO, animals were placed in fiber glass tanks in an open system with controlled water temperature (<23ºC) to avoid extreme summer warm. The provided diet was primarily green seaweed *Ulva spp*. that was readily accepted by urchins the day after capture. Other species of macroalgae were tested, such as *Saccorhiza polyschides*, *Cystoseira usneoides*, *Codium sp*. and *Asparagopsis armata*, collected in the intertidal zone on the southwest coast of Portugal.[16]

Spawning, fertilization and egg incubation

Sea urchins spawning was obtained by osmotic stimulation as already described by Gago et al.,[17] through peristomal membrane an injection of 1mL of potassium chloride (KCl) 0.5M, followed by manual shake during 1-2 minute to help diffuse the solution in the celomic space, after few minutes, if the sea urchin were maturated, gametes are released. Male and female gametes were collected and observed under microscope for evaluation. A ratio of 500:1 (male and female gametes respectively) were used for fertilization. Fertilization occurred in a 5L goblet with sterilized sea water during 2h, after that time fertilization success was monitored and fertilized eggs were transferred to 1500L cylinder fiberglass tanks in flow through systems with 10%.hour-1 renewal, bottom inlet and surface outlet, provided with a 55µm plankton mesh to avoid eggs loss.

Larval and juvenile rearing

After hatching larvae may be transferred to rearing tanks at a density of 1500-2000 larvae.L-1. At 22±1ºC larvae started exogeneous feeding at 24h. The diet on larval phase was a mixture of several microalgae species: *Isochrysisaff. galbana*, *Nannochloropsis occulata*, *Tetraselmis* sp. IMP3 and diatoms *Chaetoceros calcitrans*, *Phaeodactylum tricornutum*, *Skeletonema costatum*. The daily algae concentration was between 75,000 to 300,000 cells.mL-1 and supplied throughout the day (2-3 times). At 15DAH larvae started metamorphosis to benthonic transition. At this phase, diet was being gradually complemented and replaced by macroalgae (*Ulva* spp.) produced at EPPO earth ponds. Juveniles were kept in 1500 liters fiber glass tanks until the beginning of the nutritional experiment.

Trial experimental design and rearing conditions

A nutritional trial was defined to evaluate two different feeding protocols in juvenile production. G1 (captivity born) juveniles were fed during 4 months (110 days, September 2017 to January 2018) with an 2x4 design, two diets with four replicate tanks: diet A - macroalgae (*Ulva* spp.); diet M+A - 25% of maize grains +75% of macroalgae (*Ulva* spp.) w/w. Captivity born juvenile (121 DAH – days after hatch) sea urchins were distributed in eight rectangular flat bottom 110L fibers glass tanks, in an open system with filtered sea water. In order to facilitate food access, individuals were placed in smaller cylinder containers (∅ 0,52m; volume 10L) suspended inside each rearing tank (Figure 1). The containers walls were covered with a large mesh area to assure good water quality. Each container received 200 sea urchins with an initial weight of 0.220±0.022g and 0.207±0.009g for diet A and diet M+A respectively.

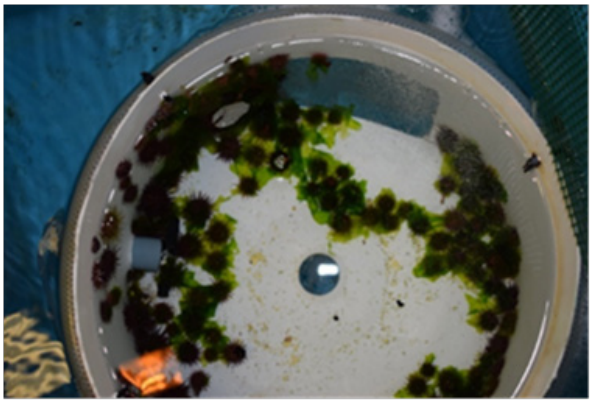

Figure 1 Floating structures for sea urchin (*Paracentrotus lividus*) juvenile culture trial (∅ 0,52m; volume 10L).

As individual grow, the containers were removed, and sea urchins were released in the rearing tank (110L). Water renewal was approximately 180%.h-1. Temperature recorded daily (17.0±1.9ºC) followed the geographically natural descending season (September to January). Photoperiod was set to 14h light: 10h dark. Tanks were cleaned every two days by siphoning, removing fecal pellets to maintain good water quality. Food supply was adjusted to maintain continuous available food reducing waste, therefore food items were supplied every two to three days depending on consumption. The feeding regime is described in Table 1. The values correspond to average feed supply (wet weight) adjust to the total biomass of urchin calculated at each sampling period. Differences in feeding during time is mainly justified by temperature reduction due to winter period.

Table 1 Feeding regime for each treatment. Average feed supply (% wetg/wet urchin biomass) for each treatment (A and A+M)

Daily average feed supply (% Sea urchin biomass)				
	Diet A	Diet A+M		
	Ulva spp.	Total	*Ulva* spp.	Maize
*t*1	22,1	17,4	13,0	4,4
*t*2	11,7	7,0	5,1	1,9
*t*3	6,7	4,7	3,4	1,3

*t*1- 121 to 151 DAH; *t*2- 152 to 182; *t*3- 183 to 223 DAH.

Fatty acid profile

The fatty acid profile was determined in the samples before and after digestion (bioaccessible fraction). Moreover, the fatty acid profile of the main lipid classes was also determined. Fatty acid methyl esters (FAME) of non-polar and polar lipids were prepared by acid-catalyzed transesterification using the methodology described by Bandarra et al.[18] Samples were injected into a Varian Star 3800Cp

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gas chromatograph (Walnut Creek, CA, USA), equipped with an auto sampler with a flame ionization detector at 250ºC. FAME were identified by comparing their retention time with those of Sigma–Aldrich standards (PUFA-3, Menhaden oil and PUFA-1, Marine source from Supelco Analytical). The FA content in total lipids (TL) deposited in sea urchin gonad was compared to the FA in TL in feed by calculating a deposition rate as:

Deposition rate =FA in sea urchin (gonad)/FA in diet.

A value of RD=1 corresponds to an FA being deposited in sea urchin gonad at the same rate as being fed to fish. RD<1 corresponds to a relative depletion of the FA, and RD>1 corresponds to a relative synthesis of the FA.

Sampling procedures and data management

Sea urchin were monitored over time for biometrical measures. A sample of 50 individuals were collected monthly for weight and length measurements at the age of 121, 152, 182 and 223 DHA. Test diameter (carapace diameter) was measured through photo analysis using Image J, a free ware Java-based image processing software.[19] Total weight of each individual was measured using a precision balance KERN EG 620-3NM, e=0.001 g (Kern & Sohn GmbH, Germany). Specific growth rate (SGR) was calculated as SGR (%)=[(ln WWt–ln WW0)/t]x100, where WWt and WWo are the final and initial wet weights, respectively and t the trial duration. Survival was determined at the end counting all the remaining individuals. At the end of the trial (223 DHA) gonads samples were taken from 9 sea urchins per each treatment for analyzing: fatty acids (FA) profile, gonadosomatic index GI (%)=[gonads weight (g wet)/ whole urchin (g wet)] x100.

All statistical analysis was performed using Sigma Plot a graphing and statistical analysis software developed by Systat Software, Inc., San Jose California USA, (www.systatsoftware.com). To test the normality and the homogeneity of variance of data, the Kolmogorov-Smirnov's test and Levene's F-test, respectively, were used. Data, which corroborate these assumptions, were analysed by one-way ANOVA distribution using the Student's t-test to determine the difference between farmed sea urchins with respect to the effects of diet (maize+algae vs algae). When data could not satisfy normal distribution and homo scedasticity requirements differences were analyzed with non-parametric analysis (Mann-Whitney's U-test).

Results

Broodstock adaptation to captivity and reproduction

Adaptation to captivity of wild broodstock do not pose problems if care is taken in the capture procedure. Already in the rearing tanks several macroalgae species were offered as describe above (section 2.1), however sea urchins did not have any interest in consuming any of these macroalgae and by far the best accepted was green seaweed *Ulva* spp., what possibly can be explained by the internal programming due to the characteristics of the origin site of collection of the specimens. The Southeast cost of Portugal (collecting site) is basically a sandy coast, with few rock points therefore there are few abundance and variability in macroalgae populations. Another practical advantage of using *Ulva* spp. as the main food was the availability of vast quantities of this seaweed in earthen ponds at the facility.

Nutritional trial results

Survival, growth and GSI

Survival during the trial period was 100%. At the end of the trial, 7 months of age (223DAH) sea urchin fed diet A (macroalgae) showed better somatic growth: test diameter, wet weight and specific growth rate (SGR) as shown in Table 2 although without significance p<0.05.

Table 2 Juvenile sea urchin *P. lividus* growth parameters at the beginning and at the end of the trial

	Initial		Final	
	Diet A	Diet M+A	Diet A	Diet M+A
Testdiameter (cm)	0.80±0.13[a]	0.81±0.14[a]	1.85±0.45[a]	1.78±0.44[a]
Wet Weigth (g)	0.21±0.09[a]	0.22±0.10[a]	2.49±1.46[a]	2.28±1.40[a]
SGR (%)	-	-	2.25±0.07[a]	2.13±0.17[a]
Total Biomass (g)	10.44±0.62[a]	10.92±0.84[a]	124.58±13.50[a]	114.07±14.61[a]
GSI (%)	-	-	2.66±1.57[a]	7.86±3.07[b]
Rearingdensity (g/m^2)	49.70	52.00	136.91	125.35

Diet A, macroalgae; Diet M+A, macroalgae+maize

Same letter in the line stand for no significative differences (p<0.05).

Indeed, growth was quite similar for the two treatments during the entire trial as best described in Figure 2, and only during the last month, sea urchin fed Diet A showed a slighter enhanced growth compared to diet M+A, yet not significant. Despite of the juvenile size some sea urchin (n=9) were sampled to asses gonad maturation. Surprisingly, some individuals showed already gonad maturation, 70 and 100% in treatment A and M+A respectively, however gonads were very small according also to individual's small size and mainly in State I.[20] Macroscopically, gonad from treatment M+A presented a good firmness that also is reflected in gonadosomatic index (GSI) presented in Figure 2 showing that sea urchin fed macroalgae+maize (diet M+A) had a significantly higher (p=0.002) index.

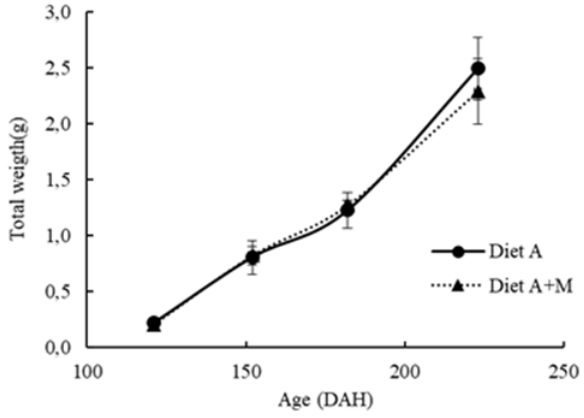

Figure 2 Sea urchin, *P. lividus*, juvenile weight evolution during the nutritional trial.

Fatty acids profile

The relative (in %) fatty acid profiles of the diets (A-macroalgae and M+A-maize grains/macroalgae) fed to the farmed sea urchins are presented in Table 3. The substitution of maize in the diet formulation, thereby reducing the proportion of macroalgae to 75%w/w, changed in various aspects the fatty acid profile of the feed. The most impressive change was the reduction of ω3 PUFA level and increase of ω6 PUFA

level, thus leading to a decrease of the ω3/ω6 ratio from 13.19±0.39 to 1.10±0.23. Moreover, there was a slight increase of total MUFA and total PUFA contents as well as a small reduction of total SFA content with maize incorporation in the feed. At a more detailed level, while there was a reduction of palmitic acid (16:0) content with maize incorporation, from 20.70±0.23 to 18.60±0.18% (of the total FA), oleic acid (18:1ω9) concentration augmented more than eight-fold due to the 25 % maize component, from 1.36±0.07 to 11.54±0.12%. It was also observed a marked reduction of the 16:3 ω3, 18:3 ω3, and 18:4 ω3 levels as well as of the other ω3 PUFA present at already low levels in the A diet as a result of maize incorporation. Contrastingly, linoleic acid (18:2 ω6) content was much higher in the maize-containing M+A diet, 22.79±0.06 vs 2.55±0.01 %, thus becoming the quantitatively most important FA in the farmed urchins' feed. With respect to the FA profiles of the gonads of farmed urchins fed the two alternative diets, A and M+A (Table 2), they resembled the FA profiles of the diets in the proportion of the SFA, MUFA, PUFA, and ω6 PUFA as well as in the relative importance of almost all main FAs. Besides, variations due to maize incorporation in the diet largely matched the changes already described for the diet's FA profile. Namely, the opposite variations of ω3 PUFA and ω6 PUFA levels and the steep increases of oleic and linoleic FA contents as a consequence of maize substitution in the feed closely reproduced the alterations already observed in the diet. However, there were insightful departures from the pattern of change in the diet's FA profile. Firstly, the ω3 PUFA content displayed a much more accentuated reduction, from 36.31±1.39 to 6.74±2.04%, in the urchin gonads than in the diet. Indeed, in the latter, ω3 PUFA had a 40% relative decline, while, in the former, this was more than 80 %. This fact was mainly due to steeper reductions of the 16:3 ω3, 18:3 ω3, and 18:4 ω3 contents in the gonads of urchins fed the maize-enriched diet than in the diet itself. The eicosapentaenoic acid (20:5 ω3, EPA) was remarkably significant in the gonads of urchins fed the 100% macroalgae diet, 7.48±0.06 %, particularly if compared with the EPA content in the M+A diet, 1.35±0.11%. FA contents in the gonads clearly above those in the diets were also observed for the myristic acid (14:0), the various isomers of 20:1, 20:4 ω3 in the urchins fed A diet, and 20:4 ω6 in the urchins fed M+A diet. On the other hand, especially for 16:3 ω3, 18:3 ω3, and 18:4 ω3, concentrations in the gonads were much lower than in the diets. Indeed, for these three quantitatively important ω3 PUFA, it was observed that their sum was in the 21-35% range in the diets, but only in the 3-20% range in the gonads of the urchins fed these different diets.

Table 3 Relative fatty acid profile (in % of total FA) of the diets (A-macroalgae and M+A-maize+macroalgae) given to the farmed sea urchins and in the gonads of farmed urchins fed with the alternative diets

Fatty acid (%)	Diet		Urchins	
	A	M+A	A	M+A
14:0	1.58±0.01^{A}	0.95±0.00^{B}	4.73±0.71^{a}	8.31±1.89^{a}
15:0	0.20±0.01^{A}	0.12±0.01^{B}	0.92±0.10^{a}	0.35±0.04^{b}
16:0	20.70 ±0.23^{A}	18.60±0.18^{B}	15.84±0.41^{a}	14.80±2.35^{a}
18:0	0.55±0.02^{A}	1.05±0.01^{B}	2.40±0.06^{a}	1.71±0.20^{b}
20:0	0.35±0.01^{A}	0.35±0.01^{A}	0.61±0.09^{a}	0.19±0.02^{b}
ΣSFA	28.72±0.23^{A}	24.35 ± 0.18^{B}	26.60± 0.08^{a}	26.42±4.36^{a}
16:1 ω9	0.14±0.00^{A}	0.09 ±0.00^{B}	0.15 ± 0.05^{a}	0.35±0.07^{b}
16:1 ω7	2.69±0.02^{A}	1.67±0.01^{B}	2.02±0.20^{a}	2.91±1.29^{a}
18:1 ω9	1.36 ±0.07^{A}	11.54 ±0.12^{B}	0.75±0.09^{a}	8.33±1.91^{b}
18:1 ω7	11.98±0.11^{A}	7.33 ±0.06^{B}	6.53 ±0.42^{a}	2.07±0.51^{b}
20:1 ω11	nd	nd	2.99 ±0.05^{a}	1.40 ±0.15^{b}
20:1 ω9	0.12±0.01^{A}	0.15±0.00^{B}	2.29 ±0.39^{a}	4.39 ±0.16^{b}
20:1 ω7	0.14±0.01^{A}	0.08 ±0.00^{B}	1.52 ±0.08^{a}	1.21±2.17^{a}
ΣMUFA	17.34 ± 0.04^{A}	21.41 ± 0.08^{B}	19.66±0.28^{a}	22.84 ±2.48^{a}
16:2 ω4	0.22±0.01^{A}	0.13±0.01^{B}	0.15±0.06^{a}	0.05±0.02^{a}
16:3ω3	9.93±0.08^{A}	5.96±0.05^{B}	5.77±0.09^{a}	0.68±0.20^{b}
16:3 ω4	nd	nd	2.96±3.90^{a}	0.06 ±0.01^{b}
18:2 ω6	2.55±0.01^{A}	22.79±0.06^{B}	0.75±0.04^{a}	20.41±5.69^{b}
18:3 ω3	8.96±0.06^{A}	6.00 ±0.03^{B}	3.68 ±0.26^{a}	1.20 ±0.08^{b}
18:3 ω4	nd	nd	0.42 ±0.05^{a}	0.12±0.05^{b}
18:4 ω3	15.64±0.18^{A}	9.38±0.11^{B}	10.39±0.26^{a}	1.40±0.39^{b}
20:2 ω6	0.06±0.05^{A}	0.04 ±0.03^{A}	0.84±0.04^{a}	2.72±0.72^{b}
20:3 ω3	0.15±0.01^{A}	0.09 ±0.01^{B}	2.75±0.41^{a}	0.25±0.05^{b}
20:4 ω3	1.14±0.07^{A}	0.68 ±0.04^{B}	4.06 ±0.94^{a}	0.40±0.11^{b}
20:4 ω6	0.19±0.02^{A}	0.12±0.01^{B}	1.03±0.13^{a}	6.45±1.24^{b}
20:5 ω3	1.35±0.11^{A}	0.81 ±0.06^{B}	7.48 ±0.06^{a}	1.66±0.44^{b}
22:5 ω3	2.92 ±0.03^{A}	1.75±0.02^{B}	1.88 ±0.08^{a}	0.44±0.06^{b}
22:6 ω3	1.47± 0.11^{A}	0.88±0.07^{B}	0.27±0.00^{a}	0.20±0.17^{a}
ΣPUFA	44.93 ± 0.27^{A}	48.84 ± 0.11^{B}	40.20 ± 1.08^{a}	36.75±5.59^{a}
Σω3	41.56 ±0.32^{A}	25.55 ±0.19^{B}	36.31±1.39^{a}	6.74±2.04^{b}
Σω6	3.15 ± 0.07^{A}	23.15 ± 0.08^{B}	2.71 ± 0.38^{a}	29.80±7.32^{b}
Σω3/Σω6	13.19±0.39^{A}	1.10±0.23^{B}	13.48±1.39^{a}	0.25± 0.13^{b}

Values are presented as average±standard deviation. nd – not detected. Different uppercase letters within a row correspond to statistical differences ($p<0.05$) between diets. Different lowercase letters within a row correspond to statistical differences ($p<0.05$) between gonads of farmed urchins fed with the alternative diets.

Discussion

Growth and reproductive development

Juvenile growth in this study presented very good rate when compared to others works in the literature.[21] Also, the rearing temperature play an important role as already described.[4,22] An exhaustive description of the reproductive cycle observed in several studies of the Mediterranean and Atlantic populations of *P. lividus* reports that the main period for development and maturation of the gonads is autumn-winter and ripening and spawning occurs during spring-summer,[23] although different patterns may be identified as a shorter period of gametes emission in spring and later in autumn or just at the summer, what may indicate the direct impact of oceanographic parameters like temperature (related to local specific currents and winds regimes) and consequently food availability in the sea urchin reproductive cycle. In this work the occurrence of mature individuals in such young and small individuals was not expect. Machado et al.,[23] comparing two different populations of the Atlantic Portuguese coast found that the smallest mature individuals were 26mm and have estimated 35.9mm as the size at 50% of the individuals (Td50) attains the first sexual maturity. Previous works in nearest location, Ouréns et al.,[4] have reported 20.4 for low and 27.9mm for high density populations. It is known that unstable environments may influence reproduction and maturation in a way that maturation may occur in

individuals smaller as 10-20mm,[24] in a strategy for survival favoring reproduction at expense of somatic growth, and food availability plays a key role mainly by its importance in gonad formation. As our environmental conditions were the same for the two treatments compared it seems obviously that the diet had an influence in the maturation results.

The GSI results obtained in diet M+A are comparable to the ones obtained by Sánchez-España *et al.,* [24] with urchins collected in very rich environments, such as is the case of areas influenced by estuaries with high nutrients inputs what gives us an indication that diet M+A may in a way, fulfill sea urchin basic nutritional requirements. Contradictory results have been found through different studies in the relations between food availability and favorable/unfavorable habitats leading to the understand that *P. lividus* may have a very plastic behavior in the switch between somatic growth and gonad maturation, and also that precocious maturation at smaller sizes does not necessarily means unfavorable environmental conditions. Studies with tropical green sea urchin *Lytechinus variegatus*[25,26] had described the role of stomach and intestine in the growth of gonad, revealing the overly complex interactions in gonad maturation.

The significantly higher gonadosomatic index (GSI) observed in juvenile fed with diet M+A can be explained by the supply of carotenoids present abundantly in maize, as carotenoids have been found in higher levels in sea urchin gonads, playing a very important role in reproduction[27] as also in biological defenses Kawakami *et al.,* De Jong-Westman *et al.,*[28] have already reported that a diet rich in protein and carotenoids had a positive impact in gonad development of the green sea urchin, *Strongylocentrotus droebachiensis*. More recently, Sartori and Gaion[1] showed the feasibility of a low-cost and easy-to standardize diet, such as that based on maize and spinach, compared with more elaborated diets as natural macroalgae or tropical fish pellets. A previous study of 9 weeks work in 2015 Sartori et al.,[1] have observed that *P. lividus* fed only on a natural macroalgae did not showed signs of gonad maturation, instead better results was obtained with a mixed diet of maize and spinach. In our work we also observed that maize had a positive impact in gonad formation, although at this age/size somatic growth was preferable as roe have a better commercial value when attaining bigger sizes not compatibles with the small size.

Fatty acid profile

The observed FA profiles of the A and M+A diets largely reflect the usual composition of the green seaweed *Ulva sp*. and maize. In the case of *Ulva* sp., similar FA profiles, including the quantitative importance of the 16:3 ω3, have been reported for different *Ulva* species.[29] However, in comparison to the A diet (solely *Ulva* sp.), these authors found lower levels of ω3 PUFA and higher levels of linoleic acid in the species studied by them. On the other hand, *U. lactuca* from the North California Coast presented 11 % 18:3 ω3 and 22 % of 18:4 ω3,[30] which surpass the percentages determined for current study's *Ulva*. Concerning maize, though the M+A profile combines 25 %, w/w, maize with 75 %, w/w, macroalgae, it is clear that the utilized maize FA profile was similar to the usual one, with high levels of oleic and linoleic acids.[31] Accordingly, the variation in the ω3/ω6 ratio is the necessary corollary of these FA profiles in *Ulva* sp. and maize (*Zea mays*).

The significant similarity between the FA profiles of the diets and the urchins' gonads shows that diet is a relevant determinant of the gonad lipids. This has also been observed in other similar studies with the same sea urchin species, *P. Lividus*.[32,33] Furthermore, the comparison to the wild sea urchins of the same species also reveals important departures from the wild FA profile,[34] which may ascribe to the particular diets. Namely, the wild FA profile is characterized by high levels of arachidonic acid (20:4 ω6, ARA), EPA, and 20:2 ω6, which do not surpass 6, 8, and 3 % of the total FA in the *P. lividus* of the present study. On the other hand, the levels of oleic and 18:3 ω3 are modest, and the linoleic acid does not even reach the limit of quantification in the wild *P. lividus*.[34] Hence, P. lividus seems to be quite efficient at incorporating and accumulating FA from the diet in the gonads, though there are differences in this efficiency between FA as already mentioned in the literature.[32]

However, the gonad FA profiles do not fully reflect the diet FA profiles, since there are some deviations large enough to be worthy of discussion. Indeed, regarding ARA and EPA, but also 20:2 ω6, 20:3 ω3, and 20:4 ω3, there was a higher quantitative importance in the gonads than should be expected on the basis of the diets, particularly the ω3 PUFA in the case of A diet and the v6 PUFA in the case of M+A diet. This duality seems to correspond to the relative richness of ω3 PUFA and ω6 PUFA in the diets. On the other hand, linoleic acid as well as 16:3 ω3, 18:3 ω3, and 18:4 ω3 contents in the urchins' gonads exhibited a lower quantitative importance than would be expectable taking into account the FA profile of the diets. These comparisons strongly suggest that *P. Lividus* is able to self-synthesize long chain PUFA (with 20 carbons) by incorporation of precursors (C16 and C18) of these FA directly from their diet, as previously observed in another study with *P. lividus*[33] and for other sea urchin species.[35] It has been observed a higher concentration of 18:3 ω3 and linoleic acid in the diet than in the gonads, indicating a possible use of these FA as substrate for energy or, precisely, for the synthesis of long chain PUFA.[32] This is supported by a recent study on *P. lividus* enzymes,[36] which has shown that this sea urchin species is able to carry out desaturation reactions, fundamental for the biosynthesis of long chain PUFA. The results of the current study seem to indicate a more efficient operation of this metabolic machinery consisting of elongases and dessaturases in the case of ω3 PUFA and of ARA. Moreover, large accumulation of myristic acid and some MUFA —as observed with the isomers of 20:1— has been reported in the literature, but for the case of ovaries of various echinoderm species.[37,38] These FA could function as an energy reserve for embryo development.[39]

Finally, from a nutritional point of view, the gonads of urchins fed only macroalgae seem to be better than those of the urchins with partial maize substitution. In fact, whereas for the former the ω3/ω6 ratio was much higher than one —a recommended value[40] that enables to consider A urchins beneficial to human health, for the latter theω3/ω6 ratio was clearly below one.[41]

Conclusions

In conclusion *P. Lividus* seems viable to produce in aquaculture. In this work growth rate, one of the bottle neck, presented better results that former reports what is very promising mainly because still much can be done in feeds nutrition's. Once that basic nutritional requirements can be satisfied with the diets described it seems plausible that more adjusted feeds can be achieved with some conducted studies Therefore, one of the major concerns in future works is developing a diet that may enhance somatic growth in detriment of gonad growth, avoiding gametogenesis in such young/smaller individuals. In fact, some work (not published) have been done by the authors using different leftover of vegetable raw material mainly rich in FA and carotenoids as beans or spinach were very promising in terms of balance on somatic and gonadal growth adding value to less valuable products in a circular approaching. Moreover, care must be taken

to find dietary solutions for *P. Lividus* that enhance their nutritional value, namely, through a healthier FA profile and a higher ω3/ω6 ratio. This is reinforced by the revealed potential of this species to self-synthesize long chain PUFA (with 20 carbons) by incorporation of precursors (C16 and C18) of these FA directly from diet. In this way, for instance, EPA, an anti-inflammatory bioactive FA, may be available in significant amounts in farmed sea urchins.

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Conflicts of interest

The author declares that there is no conflicts of interest.

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