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High dietary protein, *n* − 3/*n* − 6 ratio and *β*-carotene enhances *Paracentrotus lividus* (Lamarck, 1816) larval development

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Abstract

The nutritional characteristics of microalgae affect the growth, survival and fatty acid composition of sea urchin larvae. This study aimed to evaluate the influence of nutritive characteristics of single microalgal diets in *Paracentrotus lividus* (Lamarck, 1816) larval development, growth, and condition. Larvae of *P. lividus* were fed with three monospecific microalgal diets, *Rhodomonas* sp. (Rho), *Dunaliella tertiolecta* (Duna) and the diatom *Chaetoceros calcitrans* (Chae), and their development and growth were analysed until competence. Additionally, the fatty acid (FA) profile of larvae was analysed at competence and compared with the FA profile of the correspondent diet. The three groups of larvae attained competence simultaneously with differences in growth performance. The larvae fed with Chae attained the largest stomach and the shortest post-oral arm. The larvae were able to accumulate long-chain polyunsaturated fatty acids (PUFA), such as docosahexaenoic (DHA, C22:6*n* − 3), eicosapentaenoic (EPA, C20:5*n* − 3) and arachidonic (ARA, C20:4*n* − 6) acids, either by assimilation and retention of dietary FA or by the synthesis from α-linolenic acid (ALA, C18:3*n* − 3) and linoleic acid (LA, C18:2*n* − 6). Furthermore, the low DHA/EPA ratio and high EPA/ARA and *n* − 3/*n* − 6 ratios of Rho and Chae and the high levels of the β-carotene present in Chae improved larval growth and development. In conclusion, the results indicated that of the three microalgal diets tested, *C. calcitrans* provided important nutritional characteristics, especially in terms of FA composition and carotenoids, improving *P. lividus* larval growth and condition.

KEYWORDS
echinoculture, fatty acid profile, larval condition, larviculture, nutrition, PUFA

1 | INTRODUCTION

Sea urchins are a highly valuable marine resources due to their gonads, greatly appreciated as a culinary delicacy worldwide. In recent years, the increasing market demand for this gastronomic delicacy have been leading several wild populations to overexploitation in many coastal regions of Europe, North Asia (specially Japan) and Chile (Boudoresque & Verlaque, 2020). In Europe, the purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) is the most consumed species, mainly in France and Spain (Carboni et al., 2014; Monfort, 2002; Stefánsson et al., 2017). This species has a wide distribution covering the European Atlantic coast, South of Morocco, Macaronesia islands and the Mediterranean Sea (Boudoresque & Verlaque, 2020).

The aquaculture of sea urchins can offer a sustainable alternative to the exploitation of the wild stocks, meeting the current market demand and at the same time promoting the restoration of local populations through restocking (Lawrence, 2007; McBride, 2005; Paredes et al., 2015). However, the high mortality rates occurring during larval development combined with high running costs of larval rearing systems and juvenile production are important

bottlenecks to the full-life cycle production of *P. lividus* (Brundu et al., 2016; Carboni et al., 2012; Dworjanyn et al., 2007; Hannon et al., 2017). Therefore, it is necessary to develop experimental protocols to control larval production to increase the survival success during the settlement phase and to rear post-metamorphic juveniles (De La Uz et al., 2013; Rial et al., 2018). These new protocols must involve the optimization of several factors such as diet type, dosage, nutritional value, stocking density, rearing temperature, salinity, dissolved oxygen and settlement cues that may increase efficiency in medium- to-large scale systems (Azad et al., 2010).

Diet nutritional value is one of the dominant factors influencing directly the larval growth, metamorphosis and survival (Araújo et al., 2020; Jimmy et al., 2003; Kelly et al., 2000). The nutritional quality of microalgae selected as food for larvae and post-larvae is determined by several aspects, including ingestibility (cell size/shape), digestibility (related to cell wall structure and composition) and biochemical composition (proteins, lipid, carbohydrates, suitable polyunsaturated fatty acids—PUFA—and carotenoids content) (Guedes & Malcata, 2012; Liu et al., 2009). Within favourable nutritive conditions, the larval condition is improved—showing short arms and large stomach—and the larval development rates increase—presenting shorter planktonic stages (Byrne et al., 2008; Gomes et al., 2021; Schiopu et al., 2006).

Numerous microalgae species have been tested as feed for *P. lividus* larvae with highly variable results in terms of survival (0% to 85%) and age-at-competence (10 to 29 days) (Carboni et al., 2012; Gomes et al., 2021; Liu et al., 2007a for a review). Several studies attempted to correlate the nutritional value of microalgae with sea urchin larvae biochemical profile (Carboni et al., 2012; Castilla-Gavilán et al., 2018; Krishnan et al., 2020; Liu et al., 2007a, 2007b; Schiopu et al., 2006). However, only a few analysed the reciprocal relationship of fatty acids (FA) profile between *P. lividus* larvae and its diet. Liu et al. (2007a) showed that larvae fed with *Dunaliella tertiolecta* (Butcher, 1959) had higher proportion of *n* − 3 PUFA in comparison to the artificial diets tested, which may have improved larval growth. Carboni et al. (2012) showed that the high levels of docosahexaenoic acid (DHA, C22:6*n* − 3) present in *Pleurochrysis carterae* (Braaud & Fagerland) T. Christensen, 1978 (Guiry & Guiry, 2021) and *Cricosphaera elongata* (Droop, Braarud, 1960) improved larval performance. These studies also showed that *P. lividus* larvae has specific dietary requirements such as high levels of lipids, including DHA, eicosapentaenoic acid (EPA, C20:5*n* − 3), low DHA/EPA and high EPA/ARA ratios (Carboni et al., 2012; Liu et al., 2007a). In the study conducted by Schiopu et al. (2006), the high content of EPA of *Rhodomonas* sp. improved survival and growth of *Dendraster excentricus* (Eschscholtz, 1831) larvae, suggesting that the use of this microalga could be beneficial for the development of *P. lividus* larvae.

The present study aimed to evaluate the influence of the nutritive characteristics of monospecific microalgae diets in the sea urchins' larval development, growth and condition. To conduct the present study, were selected three monospecific diets of microalgae with different nutritional characteristics known to enhance *P. lividus* larval development (Gomes et al., 2021). For the first time, it was assessed the reciprocal influence of *Rhodomonas* sp. and diatom *Chaetoceros calcitrans* (Takano, 1968) FA profile on *P. lividus* larval FA composition and their influence on larval growth, development and condition. Considering that the larval development of *P. lividus* beneficiates from mix microalgae diets, this study will help to understand the role of each microalga to fulfil the nutritional requirements of *P. lividus* larvae and, ultimately enable the optimisation of larval rearing protocols.

2 | MATERIALS AND METHODS

ETHICS STATEMENT

The present study did not involve vertebrates, protected or endangered species. All experimental procedures on sea urchins were conducted in compliance with the Portuguese law and the Directive 2010/63/EU.

2.1 | Microalgae cultures

The microalgae cultures of *Rhodomonas* sp. (Rho), *C. calcitrans* (Chae) and *D. tertiolecta* (Duna) were grown in 250 ml, 1, 2 and 6 L glass flasks, with autoclaved seawater (121°C during 25 min) enriched with a commercial F/2 culture medium (Nutribloom Plus, Nécton, Portugal). For the diatom Chae, the F/2 medium was enriched with silicates. Cultures were maintained in batch lines at 20°C, exposed to a continuous fluorescent light and supplied with aeration in an environmental controlled room.

2.2 | Microalgae sampling

Microalgae cultures were sampled in the last day of their exponential growth phase from each glass flasks for chemical analysis. The culture concentration was assessed at that day (Rho: 5.53×10^6 cells/ml; Chae: 1.10×10^7 cells/ml; Duna: 6.8×10^6 cells/ml), and samples were collected by centrifugation at 10,000 rpm during 10 min at 21°C (Eppendorf Centrifuge 5810 R; Eppendorf AG, Hamburg, Germany). Afterwards, all samples were stored at −80°C and freeze-dried.

2.3 | Broodstock husbandry

P. lividus broodstock ($N = 45$) with a test diameter (TD) of approximately 33.5 ± 0.25 mm (excluding spines) were collected manually, in the intertidal zone of Peniche, Portugal (39°19′N; 9°21′W) in November 2019. The urchins were reared in MARE-Polytechnic of Leiria aquaculture facilities until gonad maturation. They were kept within a density of 26.46 ± 5.16 g/L in a recirculating aquaculture system (RAS) equipped with three 60 L tanks and a 70 L sump tank. The broodstock was fed, ad libitum, every two days for 8 weeks, with

a mixture diet composed of equal proportions of frozen maize kernels and fresh spinach (Sartori et al., 2016) until spawning induction. The uneaten food remains in the tanks were removed before each feeding. During this time, seawater temperature was maintained at 22 ± 2°C, salinity at 33.85 ± 1.52 mg/L, 7.95 ± 0.32 average pH and average dissolved oxygen of 7.98 ± 0.25 mg/L, monitored daily with a handheld multiparameter meter (YSI Incorporated, Yellow Springs, USA). Lighting for the experiments was provided by fluorescent lights at a intensity of 11.3 ± 0.16 $\mu E/m^2/s^1$ and a constant photoperiod of 12 h light:12 h dark. Ammonia and nitrite concentrations in the rearing system were monitored daily before and after a 10% water change and were kept near undetectable values.

The sea urchins (6 males and 6 females) were induced to spawn by injecting potassium chloride 0.5 M, in the proportion of 40 µl/g WW, into the coelom through the peristomial membrane. The fertilization was conducted as described by Gomes et al. (2021).

2.4 | Larval culture and sampling

2.4.1 | Larval rearing

The larval rearing experiment was carried out in triplicate tanks, using nine 50 L cylindroconical tanks (42 × 70 cm, diameter × height) in a closed system and arranged in a Latin square design. Larvae were stocked at a density of 6 ind./ml ($n \approx 3$ million larvae) in continuous fluorescent light (11.14 ± 0.23 $\mu E/m^2/s^1$). The larvae were reared in aerated static seawater previously filtered (80 µm) and UV treated (filtered sea water, FSW). During the larval rearing, seawater was approximately maintained within an average temperature of 19.08 ± 0.25°C, average salinity of 35.33 ± 1.33 mg/L salinity, average pH 8.00 ± 0.26 and average dissolved oxygen of 8.01 ± 0.22 mg/L. Ammonia and nitrite concentrations in seawater were monitored daily before and after a 10% water change and were kept near undetectable values. The larvae were fed every two days with the three microalgae during cultures exponential growth phase. The feeding doses were standardized according to cell volume to supply equal bio-volumes of microalgae and adapt to larval development stages (Table 1). To determine microalgae culture concentration, the cell counts were carried out daily using a Neubauer counting chamber under 40× magnification. It was considered that larvae attained competence to settle when the rudiment was as larger as the stomach, and age-at-competence was defined as the number of days post-fertilization (DPF) required for at least 75% of the larvae reach competence for settlement. Prior to FA analyses, larvae were fasted during 24 h, to allow the removal of the gut content and then, larval samples (about 1000 larvae) from each microalgal treatments were collected after filtration through a 100 µm sieve, frozen at −80°C until freeze-drying.

2.4.2 | Larval survival

Larval survival rate (%) was determined by counting the live larvae in three 20 ml samples collected below water surface of each rearing tank. Larval survival was calculated as the total number of living larvae divided by the number of larvae initially transferred to the tank. Prior to sample collection, the rearing water was stirred to ensure a random sample of larvae.

2.4.3 | Larval growth performance

The larval development was assessed by checking the presence of new arms and rudiment under the microscope (20× magnification) following the criteria defined by Liu et al. (2007a) and Carboni et al. (2012). Prior to sample collection, the rearing water was stirred to ensure a random sample of larvae. For these observations, a minimum sample of 10 larvae of each tank were analysed. A new development stage was considered when at least 75% of the observed larvae attained that stage. When a new stage was achieved, a minimum of 10 larvae were photographed with a camera Zeiss AxioCam MRc3 (Carl Zeiss, Germany) coupled to the microscope. The larval body length (BL), body width (BW), post-oral arm length (POAL) and stomach length (SL) were measured using the image analysis software Zen 2.6 lite (Carl Zeiss, Germany).

TABLE 1 Shape, bio-volume and feeding ration of microalgae used to feed *Paracentrotus lividus* larvae at each larval development stage. The cell densities in the three diets were standardized to supply equal biovolume of microalgae

Species	*Rhodomonas* sp. (Rho)	*Chaetoceros calcitrans* (Chae)	*Dunaliella tertiolecta* (Duna)
Shape[a]	Cone half sphere	Half-elliptical prism	Prolate spheroid
Diameter (µm)	12	3	5
Bio-volume (μm^3)	180	88	65
Ratio to Rho	1:1	2:1	2:1
Feed ration (cells/ml/day)			
4 - arms larvae	3600	7200	7200
6 - arms larvae	7200	14,400	14,400
8 - arms larvae	14,400	28,800	28,800

[a]Hillebrand (1999).

2.5 | Proximate composition analysis

The microalgal diets were characterized in terms of protein, lipid, carbohydrate and carotenoid content. The FA composition was determined for all microalgal diets tested and for *P. lividus* larvae.

Protein content in microalgae was quantified following the Kjeldahl method (Kjeldahl, 1883). A sample of 0.5 g of each diet was placed in a digestion tube, with one catalyst tablet (1.05 g NAS, VWR Chemicals, USA) and 25 ml of 97% H_2SO_4 (VWR Chemicals, USA) with a blank control using 1 ml of distilled water. The mixture was digested (2006 Digestor Unit DS6, FOSS, Hilleroed, Denmark) at 400°C for 90 min. After cooling, the mixture was mixed with 70 ml of distilled water and distilled (Kjeltec 2100 Distillation Unit, FOSS, Denmark) under alkaline conditions. The distillate was collected in 30 ml of 4% boric acid and titrated with 0.1 M chloridric acid (VWR Chemicals, USA). Protein content was calculated as follows: % protein = $100\times((Va - Vb)\times[HCl]\times6.25\times0.014)$/sample weight (g), where *Va* is the volume of titrant used for the sample (ml) and *Vb* is the volume of titrant used for of the blank (ml).

Carbohydrate content in microalgae was analysed following Dubois et al. (1956) with modifications described in Lourenço et al. (2021). Microalgae samples of 30 mg were hydrolysed with 8 ml H_2SO_4 1 M at 90°C (water bath) during 60 min. After cooling, the hydrolysate volume was adjusted to 10 ml. For colour reaction, 200 µl of each hydrolysate was mixed with 500 µl of H_2SO_4 97% and heated for 15 min at 90°C. Then, 100 µl of 5% phenol was added to the mixture and stirred. Three aliquots of 200 µl were transferred to a 96 × 96 flat bottom microplate, and the absorbance was read at 490 nm (Synergy H1 Hybrid Reader Biotek® Winooski, USA). The concentration of carbohydrates in hydrolysate solution was obtained by interpolation of samples absorbance in increasing D-glucose (>98.0%, VWR chemicals, Belgium) concentrations calibration curve (0.0–0.2–0.3–0.4–0.5–0.6–0.7–0.8 mg/ml). The amount of total carbohydrates in experimental microalgae diets was expressed as % DM of glucose.

Quantification of total lipids in microalgae was performed following the methodology proposed by De Coen and Janssen (1997) with the adaptations reported by Lourenço et al. (2021). Freeze-dried samples (≈ 30 mg) were homogenized with 1.5 ml of a chloroform: methanol: water mixture (1:1:1) and centrifuged (Eppendorf Centrifuge 5810 R, USA) at 2000×*g* and 4°C for 10 min for phase separation. The lower organic phase was recovered and conveniently diluted with chloroform ($CHCl_3$, Prolabo® VWR, South Africa). Afterwards, 100 µl of diluted sample were mixed with 500 µl of 97% sulfuric acid and heated at 200°C for 10 min. After cooling, 1.5 ml of ultra-pure water were carefully added to the mixture. Finally, 300 µl aliquots (in triplicate) were transferred to a 96 × 96 flat bottom microplate and samples absorbance read at 375 nm. The calibration curve was prepared with tripalmitin (ACROS Organics™) standard solutions in chloroform with increasing concentrations from zero (0) to 2.6 mg/ml, treated as described for the samples. The results of lipid content were expressed as percentage of dry matter (% DM).

2.5.1 | Carotenoid pigments analysis

Carotenoids were extracted from 30 mg frieze-dried microalgae samples with acetone and quantified by high-performance liquid chromatography (HPLC). The carotenoids present in the extract were detected and quantified under reversed-phase conditions, using a Merck-Hitachi Elite LaChrom HPLC system equipped with a L-2450 DAD detector, a L-2200 autosampler and a L-2130 pump. Separation of the carotenoids was achieved at room temperature with an ACE Advanced Chromatography Technologies HPLC C18 column (250 × 4.6 mm diameter size, 5 µm particle size, 100 Å pore size) equipped with a matching guard cartridge. The eluent was 100% methanol at a flow rate of 1 ml/min for lutein and b-carotene. For fucoxanthin, a gradient consisting of methanol (A), water (B) and ethyl acetate (C) was used as follows: 0–3 min, 90% A, 10% B; 3–10 min, 100% A; 10–23 min, 60% A, 40% C; 23–35 min, 90% A, 10% B. The detection wavelength used for all carotenoids was 450 nm. Identification of the carotenoids was conducted by comparison with analytical references of lutein/zeaxanthin, all-trans echinenone and all-trans β-carotene. Calibration and quantification were made using the external calibration standard method and the peak areas. The obtained chromatograms were processed with EZChrom and OpenChrom software.

2.5.2 | Fatty acid analysis

Fatty acids profile of microalgae and larvae was analysed by gas chromatography (GC). FA methyl esters (FAME) were obtained by direct acid transmethylation following Fernández et al. (2015). The freeze-dried samples (≈ 50 mg) were mixed, in screw cap glass tubes, with 2 ml of methanol (CH_3OH, HiPerSolv, CHROMANORN, Prolabo® VWR, Lyndhurst, South Africa) containing 2% H_2SO_4 and heated at 80°C (water bath) for 2 h. Afterwards, 1 ml of Mili-Q water and 2 ml of n-hexane were added to the mixture, stirred, and centrifuged at 1500×*g* during 5 min to phase separation. Finally, the upper hexane phase was recovered into GC vials and analysed in a GC (Finnigan Ultra Trace) equipped with a Thermo TR-FAME capillary column (60 m × 0.25 mm ID, 0.25 µm film thickness), an auto sampler (AS 3000, Thermo Electron Corporation) and a flame ionization detector (FID). Oven temperature was set at 100°C for 1 min, followed by an increase at 9°C/min to 180°C (maintaining for 10 min) and a second increase at 2°C/min to 235°C (during 5 min). Temperatures of injector (splitless) and the detector were 250 and 280°C, respectively. Helium (1.5 ml/min) was used as carrier gas. Air and hydrogen were supplied to the detector at flow rates of 350 and 35 ml/min, respectively. Standard mixtures (SUPELCO 37, PUFA N°1 from Marine source and PUFA N°3 from Menhaden oil, SUPELCO, Bellefonte, PA, USA) were used to identify the FAMEs in samples. FA content was expressed as percentage in respect to total identified area (% Total FA).

2.6 | Statistical analyses

The effect of the microalgal diets in larval biometric parameters and biochemical analyses were tested by one-way analysis of variance (ANOVA). Data were initially tested for the assumptions of normal distribution, using the Shapiro-Wilk test, and homogeneity of variances by the Levene's test. When the assumptions failed, it was applied the nonparametric Kruskal–Wallis test. Whenever statistically significant differences were found, multiple pairwise comparisons between groups were conducted using the post-hoc Tukey's honestly significant difference (HSD) test or the non-parametric Games-Howell test when the assumptions for normal distribution and variances homogeneity were not fulfilled (Zar, 2010). In addition, to assess the effect of the microalgal diets and DPF in the development, it was used Pearson's chi-square (χ^2) test (Zar, 2010). All the results were expressed as mean ± (standard deviation, SD). The larval condition was assessed empirically by analysing the deviation of the individual larvae size from the expected tendency obtained by fitting linear models to the biometric data BW ~ BL (model A), SL ~ BL (model B) and POAL ~ SL (model C) following the methodological approach defined in Gomes et al. (2021). A significance level of $p < 0.05$ was used for all analyses. These analyses were conducted with IBM SPSS™ Statistics 25 (IBM Corporation, Armonk, New York, USA). The FA content with mean concentration higher than 1% Total FA were normalized by log $(x+1)$ transformation. The normalized FA matrix was then used to evaluate the main effect of diet through MANOVA. A principal component analysis (PCA) was then conducted in the FA correlation matrix to evaluate which fatty acids were more influential in separating the different larval groups by diet. In all cases, significant differences were considered when $p < 0.05$. The PCA analysis was performed using the Canoco software (Version 4.5).

3 | RESULTS

3.1 | Microalgae proximate composition

The three microalgae cultures presented different nutritional composition (Tables 2 and 3). Statistically significant differences were observed in total protein ($H_{KW} = 7.20$, $df = 2$, $p < 0.001$), carbohydrates ($F = 40.05$; $df = 2$, $p < 0.001$) and lipidic content ($F = 36.54$, $df = 2$, $p < 0.001$) among microalgae cultures. Rho presented the highest protein content (43.09% DM) and the lowest carbohydrate content (10.60% DM), while Chae had the lowest lipid content (4.75% DM). Regarding the carotenoid content (Table 2), Chae presented the highest β-carotene content (122.51 μg/g) and is the only species storing fucoxanthin (36.55 μg/g).

The three microalgae show different FA profiles (Table 3). Rho contained the highest content in lauric acid (C12:0, 0.98%), erucic acid (C22:1*n* − 9, 0.61%) and DHA (C22:6*n* − 3, 6.10%) when compared with the other two microalgae. Similarly, total PUFAs (65.34%), DHA/EPA ratio (0.46) and EPA/ARA ratio (109.75) were highest in Rho, while containing the lowest proportion of total SFA (21.62%) and MUFA (13.04%). Chae presented the highest content of myristic acid (C14:0, 16.86%), pentadecanoic acid (C15:0, 0.62%), stearic acid (C18:0, 0.94%), palmitoleic acid (C16:1*n* − 7, 23.96%), ARA (C20:4*n* − 6, 1.11%), EPA (14.44%) and hexadecatetraenoic acid (C16:4*n* − 1, 11.72%). Chae was the poorest microalgae in linoleic (LA, C18:2*n* − 6, 0.44%) and α-linolenic (ALA, C18:3*n* − 3, 0.04%). Furthermore, Chae presented high proportion of total SFA (37.14%), MUFA (30.14%) other PUFA (FA that are neither *n* − 3 or *n* − 6; 15.66%) and the lowest DHA/EPA (0.04) and EPA/ARA (13.04) ratios. Duna presented the highest content of palmitic acid (C16:0, 21.50%), oleic acid (C18:1*n* − 9, 3.83%), vaccenic acid (C18:1*n* − 7, 4.79%), LA (11.40%), γ-linolenic acid (C18:3*n* − 6, 2.44%), ALA (37.54%), hexadecatrienoic acid (C16:3*n* − 4; 5.00%) and total *n* − 6 PUFA (14.03%). Duna lacked EPA content and presented the lowest value for *n* − 3/*n* − 6 ratio (2.72).

3.2 | Larval growth performance and survival

The development of new arms and rudiment occurred simultaneously in the three dietary treatments (Table 4). The larvae developed the 6-arm stage at 14 DPF, 8-arm stage at 16 DPF and the rudiment at 17 DPF. Larvae fed with Chae showed survival rates significantly higher at 14 DPF ($H_{KW} = 36.67$, $df = 2$, $p < 0.001$), 16 DPF ($H_{KW} = 36.68$, $df = 2$, $p < 0.001$) and 17 DPF ($H_{KW} = 32.89$, $df = 2$, $p < 0.001$) in relation to the other two diets (Table 4). The competence for settlement was reached by larvae

TABLE 2 Proximate composition (% dry matter, DM) and carotenoids pigments content (μg/g) of the microalgae *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna) used to feed the *Paracentrotus lividus* larvae.

	Rho	Chae	Duna	*p*-value
Protein content (% DM)	43.09 ± 0.13[a]	1.55 ± 0.01[b]	0.86 ± 0.01[b]	<0.001
Carbohydrates (% DM)	10.60 ± 0.31[b]	13.19 ± 0.17[a]	14.01 ± 0.19[a]	<0.001
Total lipid content (% DM)	8.07 ± 0.55[a]	4.75 ± 0.01[b]	8.34 ± 0.56[a]	<0.001
Pigments (μg/g)				
Lutein	n.d.	n.d.	n.d.	–
ß-carotene	6.50 ± 1.02	122.51 ± 2.93	41.64 ± 5.31	–
Fucoxanthin	n.d.	36.55 ± 4.08	n.d.	–

The results are reported as value ± SD. N.d., Not detected.Statistically significant different groups ($p < 0.05$) are represented by superscript letters.

TABLE 3 Fatty acids profile (% Total FA) of the microalgae *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna) used to feed the *Paracentrotus lividus* larvae.

Diets	Rho	Chae	Duna	*p*-value
SFA (% Total FA)				
C12:0	0.98 ± 0.09^{a}	0.48 ± 0.09^{b}	n.d.	<0.001
C13:0	n.d.	0.66 ± 0.28	n.d.	–
C14:0	8.19 ± 0.14^{b}	16.86 ± 0.09^{a}	0.24 ± 0.00^{c}	<0.001
C15:0	0.40 ± 0.03^{b}	0.62 ± 0.02^{c}	n.d.	<0.001
C16:0	11.12 ± 0.11^{c}	11.83 ± 0.15^{b}	21.50 ± 0.13^{a}	<0.001
C17:0	0.26 ± 0.01^{b}	4.84 ± 0.06^{a}	5.05 ± 0.33^{a}	<0.001
C18:0	0.50 ± 0.02^{b}	0.94 ± 0.02^{a}	n.d.	<0.001
C20:0	n.d.	0.67 ± 0.02	n.d.	–
C24:0	0.18 ± 0.01	0.22 ± 0.03	n.d.	0.200
∑ Total SFA	21.62 ± 0.28^{c}	37.14 ± 0.42^{a}	26.79 ± 0.33^{b}	<0.001
MUFA (% Total FA)				
C14:1*n* − 5	0.14 ± 0.01^{b}	0.50 ± 0.08^{a}	0.93 ± 0.45^{a}	0.020
C15:1*n* − 5	2.06 ± 0.70	1.30 ± 0.18	1.23 ± 0.96	0.303
C16:1*n* − 7	5.70 ± 0.05^{b}	23.96 ± 0.25^{a}	2.46 ± 0.21^{c}	<0.001
C17:1*n* − 7	0.08 ± 0.01	n.d.	n.d.	–
C18:1*n* − 9	0.71 ± 0.04^{b}	0.32 ± 0.14^{c}	3.83 ± 0.12^{d}	<0.001
C18:1*n* − 7	3.08 ± 0.05	3.22 ± 0.21	4.79 ± 0.15^{a}	<0.001
C20:1*n* − 9	n.d.	0.35 ± 0.09^{b}	0.79 ± 0.01^{a}	<0.001
C22:1*n* − 9	0.61 ± 0.03^{a}	n.d.	0.31 ± 0.01^{b}	<0.001
C22:1*n* − 11	n.d.	0.48 ± 0.06	n.d.	–
∑ Total MUFA	13.04 ± 0.53^{c}	30.14 ± 0.34^{a}	14.37 ± 0.86^{b}	<0.001
***n* − 6 PUFA (% Total FA)**				
C18:2*n* − 6 (LA)	4.63 ± 0.03^{b}	0.44 ± 0.02^{c}	11.40 ± 0.20^{a}	<0.001
C18:3*n* − 6	1.17 ± 0.03^{b}	0.43 ± 0.01^{c}	2.44 ± 0.09^{a}	<0.001
C20:3*n* − 6	1.49 ± 0.02	–	–	–
C20:4*n* − 6 (ARA)	0.12 ± 0.0^{c}	1.11 ± 0.08^{a}	0.19 ± 0.00^{b}	<0.001
∑*n* − 6	7.41 ± 0.03^{b}	1.98 ± 0.07^{c}	14.03 ± 0.19^{a}	<0.001
***n* − 3 PUFA (% Total FA)**				
C18:3*n* − 3 (ALA)	14.40 ± 0.03^{b}	0.04 ± 0.05^{c}	37.54 ± 0.94^{a}	<0.001
C18:4*n* − 3	22.99 ± 0.17	n.d.	n.d.	–
C20:3*n* − 3	0.04 ± 0.03	n.d.	n.d.	–
C20:5*n* − 3 (EPA)	13.17 ± 0.12^{b}	14.44 ± 0.17^{a}	n.d.	0.011
C22:6*n* − 3 (DHA)	6.10 ± 0.09^{b}	0.61 ± 0.02	0.61 ± 0.01	<0.001
∑*n* − 3	56.70 ± 0.30^{b}	15.09 ± 0.17^{c}	38.16 ± 0.96^{b}	<0.001
Other PUFA (% Total FA)				
C16:2*n* − 4	n.d.	0.76 ± 0.05	n.d.	–
C16:3*n* − 4	0.36 ± 0.01^{c}	2.70 ± 0.01^{b}	5.00 ± 0.19^{a}	<0.001
C16:4*n* − 1	0.86 ± 0.03^{c}	11.72 ± 0.11^{a}	1.65 ± 0.18^{b}	<0.001
C18:2*n* − 4	n.d.	0.48 ± 0.01	n.d.	–
∑ Other PUFA	1.22 ± 0.04^{c}	15.66 ± 0.15^{a}	6.65 ± 0.37^{b}	<0.001
∑ Total PUFA	65.34 ± 0.32^{a}	32.73 ± 0.32^{c}	58.84 ± 0.81^{b}	<0.001
***n* − 3/*n* − 6**	7.65 ± 0.04^{a}	7.64 ± 0.20^{a}	2.72 ± 0.04^{b}	<0.001
DHA/EPA	0.46 ± 0.01^{a}	0.04 ± 0.00^{b}	–	<0.001
EPA/ARA	109.75 ± 10.88^{a}	13.04 ± 0.93^{b}	–	<0.001

The results are reported as value ± SD. N.d., Not detected.Statistically significant different groups ($p < 0.05$) are represented by superscript letters.

for all treatments at 18 DPF. At competence, the survival rate for larvae fed with Rho, was 2.03 ± 3.29%, for larvae fed with Chae was 4.88 ± 6.29% and for larvae fed with Duna was 1.67 ± 0.60%, with no statistically significant differences among among dietary groups ($H_{KW} = 7.44$, $df = 2$, $p = 0.841$).

The larval biometric parameters (i.e., BL, BW, SL and POAL) were significantly different among dietary treatments. Relatively to BL, it was observed significant differences between treatments at 8 DPF ($F = 5.16$, $df = 2$, $p < 0.001$), 12 DPF ($F = 19.80$, $df = 2$, $p < 0.001$) and 16 DPF ($F = 4.88$, $df = 2$, $p < 0.001$). At 8 DPF, the larvae fed with Rho presented the largest BL (240.41 μm). At 12 DPF and 16 DPF, the larvae fed with Chae presented the smallest BL (12 DPF: 242.76 μm; 16 DPF: 286.51 μm) (Figure 1). Significant differences in BW were observed between treatments at 12 ($H_{KW} = 29.97$, $df = 2$, $p < 0.001$) and 16 DPF ($F = 4.79$, $df = 2$, $p < 0.001$). Larvae fed with Chae showed a shorter BW at 12 DPF (182.09 μm) and 16 DPF (253.06 μm) in relation to the other diets (Figure 2). The stomach (SL) (Figure 3) showed the same pattern with significant differences between treatments at 12 DPF ($F = 14.92$, $df = 2$, $p < 0.001$) and at 18 DPF ($F = 6.45$, $df = 2$, $p < 0.001$). At 12 DPF, the larvae fed with Chae presented the smallest stomach (SL: 82.07 μm), but at 18 DPF these larvae showed the largest stomach (SL: 147.54 μm) when compared with the other dietary groups. Concomitantly, for the POAL, there were identified statistically significant differences among the dietary treatments, namely at 12 DPF ($F = 52.51$, $df = 2$, $p < 0.001$), at 16 DPF ($F = 15.56$, $df = 2$, $p < 0.001$) and at 18 DPF ($F = 28.42$, $df = 2$, $p < 0.001$). For this parameter, it was observed an initial increasing trend until 12 DPF, slowing down afterwards until 16 DPF, and decreased thereafter. At 18 DPF, larvae fed with Chae presented the lower POAL (319.68 μm) followed by larvae fed with Rho (354.36 μm) and with Duna (389.58 μm) (Figure 4).

The analysis of linear regression standard residuals produced by condition model A (BW ~ BL) showed that the larvae fed with the Chae presented the smallest BW. The model B (SL ~ BL) residuals analysis showed that the larvae fed with Duna presented in average the smallest stomachs. While model C (POAL ~ SL) showed that the larvae fed with Duna presented the longest post oral arms (Table 5).

TABLE 4 Development (DPF—Days post fertilization), percentage and survival (%) of 6-arm, 8-arm, rudiment and competence larval stage of *Paracentrotus lividus*. Larvae were fed with *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna). Data are expressed as mean ± SD

Diet	Larval stage	DPF	Mean % (± SD)	Survival % (± SD)
Experiment I				
Rho	6-arm	14	81.20 ± 0.12	5.25 ± 3.23
	8-arm	16	86.39 ± 0.05	4.43 ± 2.22
	Rudiment	17	95.51 ± 0.02	3.51 ± 2.26
	Competence	18	100 ± 0.00	2.03 ± 3.29
Chae	6-arm	14	79.82 ± 0.03	37.78 ± 11.75
	8-arm	16	83.12 ± 0.04	22.74 ± 11.59
	Rudiment	17	94.02 ± 0.01	11.26 ± 6.31
	Competence	18	100 ± 0.00	4.88 ± 6.29
Duna	6-arm	14	76.12 ± 0.09	6.11 ± 0.72
	8-arm	16	76.67 ± 0.05	2.72 ± 0.60
	Rudiment	17	92.65 ± 0.05	2.01 ± 0.81
	Competence	18	100 ± 0.00	1.67 ± 0.60

3.3 | Larval fatty acid composition

The FA profile of *P. lividus* larvae varied between dietary treatments (Table 6 and Figures 5 and 6). The PCA biplot showed that the first axis explained about 66.7% of the variation observed (Figure 5) and larvae fed with Rho was strongly correlated with C22:1*n* − 9 (4.09%), and DHA (7.65%). Larvae fed with Chae

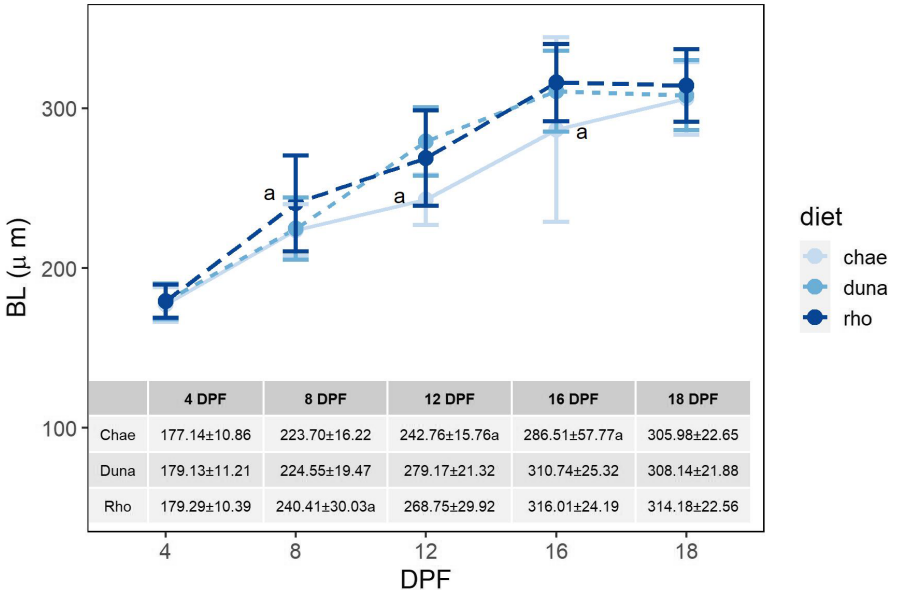
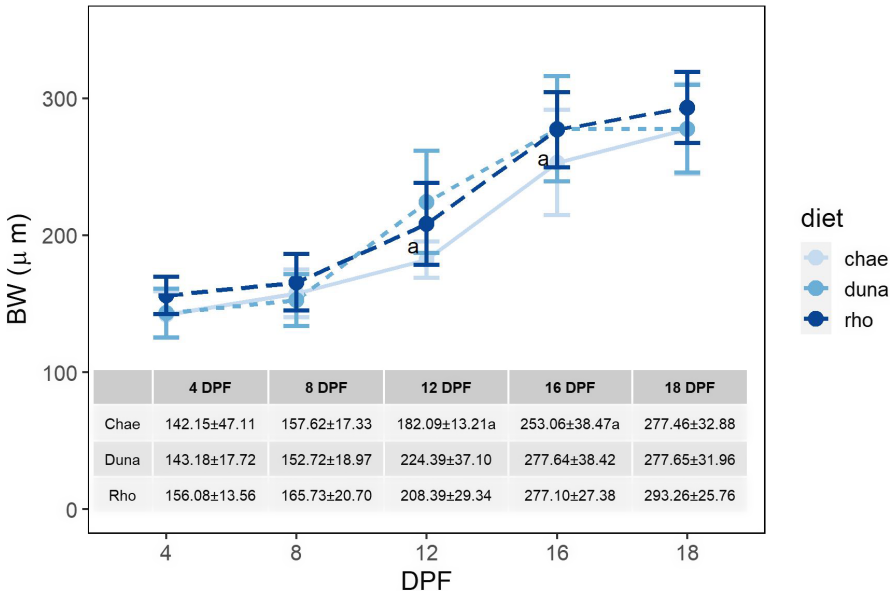
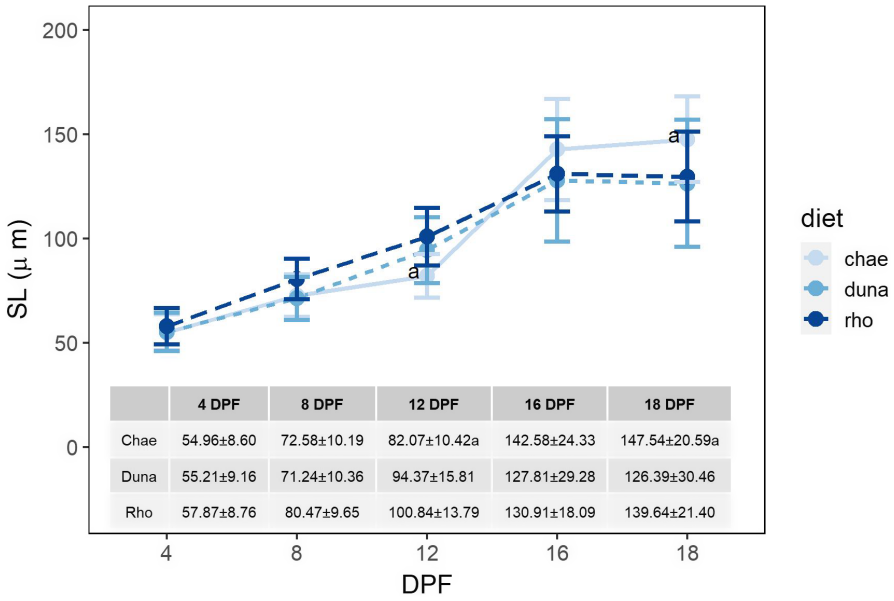


FIGURE 1 Mean (± standard deviation bars) ($n = 3$) body length (BL, μm) evolution from 4 to 18 DPF (days post-fertilization) of *Paracentrotus lividus* larvae fed with *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna). Statistically significant different groups ($p < 0.05$) are represented by superscript letters.



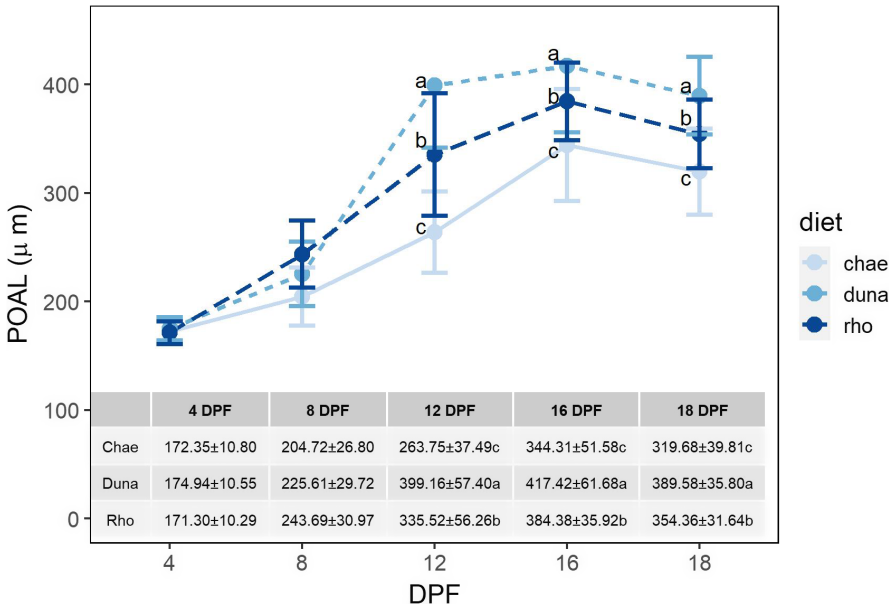
	4 DPF	8 DPF	12 DPF	16 DPF	18 DPF
Chae	142.15±47.11	157.62±17.33	182.09±13.21a	253.06±38.47a	277.46±32.88
Duna	143.18±17.72	152.72±18.97	224.39±37.10	277.64±38.42	277.65±31.96
Rho	156.08±13.56	165.73±20.70	208.39±29.34	277.10±27.38	293.26±25.76

FIGURE 2 Mean (± standard deviation bars) ($n = 3$) body width (BW µm) evolution from 4 to 18 DPF (days post-fertilization) of *Paracentrotus lividus* larvae fed with *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna). Statistically significant different groups ($p < 0.05$) are represented by superscript letters.



	4 DPF	8 DPF	12 DPF	16 DPF	18 DPF
Chae	54.96±8.60	72.58±10.19	82.07±10.42a	142.58±24.33	147.54±20.59a
Duna	55.21±9.16	71.24±10.36	94.37±15.81	127.81±29.28	126.39±30.46
Rho	57.87±8.76	80.47±9.65	100.84±13.79	130.91±18.09	139.64±21.40

FIGURE 3 Mean (± standard deviation bars) ($n = 3$) stomach length (SL µm) evolution from 4 to 18 DPF (days post-fertilization) of *Paracentrotus lividus* larvae fed with *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna). Statistically significant different groups ($p < 0.05$) are represented by superscript letters.



	4 DPF	8 DPF	12 DPF	16 DPF	18 DPF
Chae	172.35±10.80	204.72±26.80	263.75±37.49c	344.31±51.58c	319.68±39.81c
Duna	174.94±10.55	225.61±29.72	399.16±57.40a	417.42±61.68a	389.58±35.80a
Rho	171.30±10.29	243.69±30.97	335.52±56.26b	384.38±35.92b	354.36±31.64b

FIGURE 4 Mean (± standard deviation bars) ($n = 3$) post-oral arm length (POAL µm) evolution from 4 to 18 DPF (days post-fertilization) of *Paracentrotus lividus* larvae fed with *Rhodomonas* sp. ho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna). Statistically significant different groups ($p < 0.05$) are represented by superscript letters.

TABLE 5 Output of the larval condition models and standard residuals analysis by diet in each larval development experiment. Larvae of *Paracentrotus lividus* were fed with *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna). The model variables BW, BL, SL and POAL represent, respectively, the larval body width, body length, stomach length and post-oral arm length. The goodness-of-fit analysis outputs for models a, B and C are presented by the coefficient of determination (r^2) and by the ANOVA results (*F* statistics and *p*)

	Model A (BW ~ BL)		Model B (SL ~ BL)		Model C (POAL ~ SL)	
	R_{std} (*p*-value)	*t*-student (*p*-value)	R_{std} (*p*-value)	*t*-student (*p*-value)	R_{std} (*p*-value)	*t*-student (*p*-value)
Rho	0.12 ($p = 0.81$)	1.24 ($p = 0.23$)	0.08 ($p = 0.96$)	1.12 ($p = 0.96$)	−0.15 ($p = 0.003$)	−3.15 ($p < 0.001$)
Chae	−0.03 ($p = 0.008$)	−3.17 ($p = 0.008$)	0.04 ($p = 0.24$)	0.64 ($p = 0.24$)	−0.11 ($p = 0.87$)	−2.98 ($p = 0.87$)
Duna	0.13 ($p = 0.97$)	2.33 ($p = 0.01$)	−0.14 ($p = 0.045$)	−2.48 ($p = 0.048$)	0.22 ($p = 0.002$)	3.95 ($p < 0.001$)
Model Fit	$R^2 = 0.89$, $F = 109.93$, $p < 0.001$		$R^2 = 0.86$, $F = 61.99$, $p < 0.001$		$R^2 = 0.69$, $F = 12.86$, $p < 0.001$	

was correlated with higher content of C18:0 (8.73%), C18:1*n* − 7 (10.16%), stearidonic acid (C18:4*n* − 3; 1.96%), hexadecatrienoic acid (C16:3*n* − 4; 2.74%), C16:4*n* − 1 (6.05%) and EPA (26.01%) (Table 6). In addition, larvae fed with Duna was correlated with higher abundance of C18:1*n* − 9 (1.09%), ARA (15.28%), ALA (5.69%) and LA (2.27%) (Table 6).

The biplot representing the scores and variables loadings on the principal components in Figure 6 indicated that total PUFA (46.06%), other PUFA (8.27%), total MUFA (25.62%) and EPA/ARA ratio (5.37) were correlated with higher abundance in larvae fed with Chae, but negative correlated with DHA/EPA ratio (0.11). Similarly, larvae fed with Rho showed a positive score associated to high *n* − 3/*n* − 6 ratio (6.92) and *n* − 3 PUFA (34.97%). Furthermore, total SFA (38.55%) and *n* − 6 PUFA (18.19%) were correlated with higher abundance in larvae fed with Duna.

4 | DISCUSSION

The biochemical characteristics of microalgae, such as protein, lipids, carbohydrates, and carotenoids, are important factors to promote larval development. Microalgae with a high protein content has been reported as fuel to the development of sea urchin larvae (Dupont et al., 2010; Fernández-Reiriz et al., 1989; Volkman et al., 1989) as these are not able to use carbohydrates as energy source (Whitehill, 2012). Nevertheless, results obtained by Castilla-Gavilán et al. (2018) showed that carbohydrates could still play a significant role by enhancing the specific growth of larvae. In fact, the larval growth and metamorphosis of other species like the oysters were improved by the presence of carbohydrates in microalgal diets (Haws & DiMichele, 1993). In the present study, Rho presented the highest protein content, while Chae and Duna presented the highest content in carbohydrates. A suitable diet for echinopluteus should also provide an high level of carotenoids such as β-carotene and xanthophylls (e.g., fucoxanthin) because these play an important role in larval survival and development (De Jong-Westman et al., 1995). The three microalgae supply β-carotene to the larvae; however, Chae is the most important source of this pigment and the only microalgae providing fucoxanthin.

Moreover, the FA present in microalgae play an important role in sea urchins' larval development (Cárcamo et al., 2005). The FA profile of the microalgae cultures used in this study was comparable with those reported previously for Rho (Fernández-Reiriz et al., 1989; Pinto, 2018; Schiopu et al., 2006; Volkman et al., 1989), Chae (Krishnan et al., 2020; Méndez-Martínez et al., 2018) and Duna (Carboni et al., 2012; Liu et al., 2007a), with Chae presenting the lowest DHA/EPA ratio and the highest *n* − 3/*n* − 6 ratio and Rho presenting the highest EPA/ARA ratio.

Despite the low larval survival in more advanced larval stages (2%–5%), all microalgal diets promoted larval growth. The survival rate of larvae fed with Chae (≈ 5%) was higher than the results reported by Ahmed et al. (2016). In that study, *P. lividus* larvae attained competence with low survival rates (below 1%) when fed with the same diet and reared at the same stocking density as the present study (6 larvae/ml). Nonetheless, the survival rates obtained for larvae fed with both Rho and Duna (≈ 2%) were lower than reported in similar studies using the same diets (Carboni et al., 2012; Castilla-Gavilán et al., 2018; Liu et al., 2007a; Suckling et al., 2018). The relatively low survival rate observed can be related with the high stocking density. In fact, the larval survival rate is apparently inversely correlated with density. Several studies indicate that larvae raised in lower densities show higher survival rates at competence (Brundu et al., 2016; Castilla-Gavilán et al., 2018; Suckling et al., 2018). Here, the decision of initiate the experiment with a high larval density in prejudice of larval survival, provided the required number of larvae at competence to conduct the fatty acid analysis.

For all microalgal treatments, larval competence was achieved at 18 DPF indicating that age-at-competence was independent of the microalgae nutritional value. The age-at-competence was achieved earlier than observed by Liu et al. (2007a) and Carboni et al. (2012, 2014), in which *P. lividus* larvae were fed with a Duna monospecific diet at a rearing temperature of 18°C. This fact suggests that rearing temperature could be a controling factor for larval development. On the other hand, in the study conducted by Castilla-Gavilán et al. (2018), *P. lividus* larvae fed with a single Rho diet attained competence at 15 DPF, using a rearing temperature of 20°C, a feed ration identical to that used in the present study and a rearing density of 1 larvae/ml in 50 L tanks, six times lower to the used in the present

TABLE 6 Fatty acid profile (% Total FA) of larvae of *Paracentrotus lividus* fed with *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna). The results are reported as value ± SD. N.d., Not detected. Statistically significant different groups ($p < 0.05$) are represented by superscript letters.

Diets	Rho	Chae	Duna	*p*-value
SFA				
C14:0	4.47 ± 2.40^{a}	7.29 ± 1.74^{a}	1.66 ± 1.31^{b}	<0.001
C15:0	1.14 ± 0.91	1.35 ± 0.16	0.27 ± 0.32	0.086
C16:0	9.42 ± 0.06	13.69 ± 0.55	15.76 ± 1.86	0.209
C17:0	5.28 ± 2.95	3.19 ± 0.96	2.33 ± 1.90	0.307
C18:0	7.89 ± 2.88	8.73 ± 0.22	7.80 ± 0.50	0.947
C20:0	1.34 ± 0.18	0.21 ± 0.70	1.07 ± 0.66	0.487
C21:0	3.12 ± 1.03^{a}	0.71 ± 0.61^{b}	4.15 ± 0.52^{a}	<0.001
C22:0	5.48 ± 1.02	n.d.	4.60 ± 0.50	0.100
C23:0	0.64 ± 0.73	1.05 ± 0.91	0.82 ± 0.84	0.812
C24:0	0.08 ± 0.02	0.28 ± 0.49	0.08 ± 0.20	0.683
∑ Total SFA	32.82 ± 3.93^{b}	28.31 ± 5.12^{b}	38.55 ± 1.08^{a}	<0.001
MUFA				
C14:1*n* − 5	2.07 ± 1.54	1.00 ± 0.87	0.54 ± 0.45	0.322
C15:1*n* − 5	0.12 ± 0.04	n.d.	n.d.	–
C16:1*n* − 7	4.43 ± 3.83	6.61 ± 1.47	1.42 ± 1.35	0.304
C18:1*n* − 7	6.10 ± 3.95^{ab}	10.16 ± 1.44^{a}	3.38 ± 0.86^{b}	<0.001
C18:1*n* − 9	n.d.	n.d.	1.09 ± 1.01	–
C20:1*n* − 9	8.14 ± 3.10	7.57 ± 0.14	7.49 ± 0.72	0.862
C22:1*n* − 1	n.d.	n.d.	3.02 ± 1.51	–
C22:1*n* − 9	4.09 ± 1.83^{a}	0.29 ± 0.50	n.d.	<0.001
C24:1*n* − 9	n.d.	n.d.	0.18 ± 0.12	–
∑ Total MUFA	23.42 ± 1.63^{a}	25.62 ± 2.56^{a}	17.12 ± 1.60^{b}	<0.001
***n* − 6 PUFA**				
C18:2*n* − 6 (LA)	1.45 ± 0.62^{b}	1.25 ± 1.09^{b}	2.27 ± 0.31^{a}	0.05
C18:3*n* − 6	0.21 ± 0.23	n.d.	0.64 ± 0.54	0.181
C20:4*n* − 6 (ARA)	8.98 ± 2.93	4.81 ± 0.60	15.28 ± 1.97^{a}	<0.001
C22:2*n* − 6	0.38 ± 0.30	n.d.	n.d.	–
∑*n* − 6	8.78 ± 5.20	6.05 ± 1.50	18.19 ± 2.20^{a}	<0.001
***n* − 3 PUFA**				
C18:3*n* − 3 (ALA)	2.95 ± 0.78	n.d.	5.69 ± 0.41^{a}	<0.001
C18:4*n* − 3	0.52 ± 0.60	1.96 ± 0.02^{a}	0.32 ± 0.36	<0.001
C20:3*n* − 3	1.31 ± 0.92^{a}	0.36 ± 0.63^{b}	2.27 ± 0.49^{a}	<0.001
C20:5*n* − 3 (EPA)	21.76 ± 3.48^{a}	26.01 ± 1.49^{a}	12.60 ± 0.64^{b}	<0.001
C21:5*n* − 3	0.52 ± 0.06	n.d.	1.00 ± 0.78	0.324
C22:5*n* − 3	0.26 ± 0.30	n.d.	n.d.	–
C22:6*n* − 3 (DHA)	7.65 ± 0.82^{a}	2.70 ± 0.59^{b}	6.05 ± 1.55^{a}	<0.001
∑*n* − 3	34.97 ± 2.73^{a}	30.67 ± 5.77^{ab}	24.90 ± 2.74^{b}	<0.001
Other PUFA (% Total FA)				
C16:3*n* − 4	n.d.	2.74 ± 1.05	1.23 ± 0.09	0.184
C16:4*n* − 1	n.d.	6.05 ± 0.59	n.d.	–
C18:2*n* − 4	n.d.	1.07 ± 1.05	n.d.	–
C18:3*n* − 4	n.d.	0.65 ± 0.12	n.d.	–
∑ Other PUFA	–	8.27 ± 0.36^{a}	1.23 ± 0.09	<0.001

(Continues)

TABLE 6 (Continued)

Diets	Rho	Chae	Duna	*p*-value
∑ Total PUFA	43.75 ± 1.71	46. 06 ± 3.75	44.33 ± 1.92	0.480
***n* − 3/*n* − 6**	6.92 ± 1.16[a]	5.19 ± 1.05[a]	1.40 ± 0.31[b]	<0.001
DHA/EPA	0.36 ± 0.10[a]	0.11 ± 0.03[b]	0.25 ± 0.28[c]	<0.001
EPA/ARA	2.77 ± 0.82[b]	5.37 ± 0.45[a]	0.83 ± 0.09[c]	<0.001

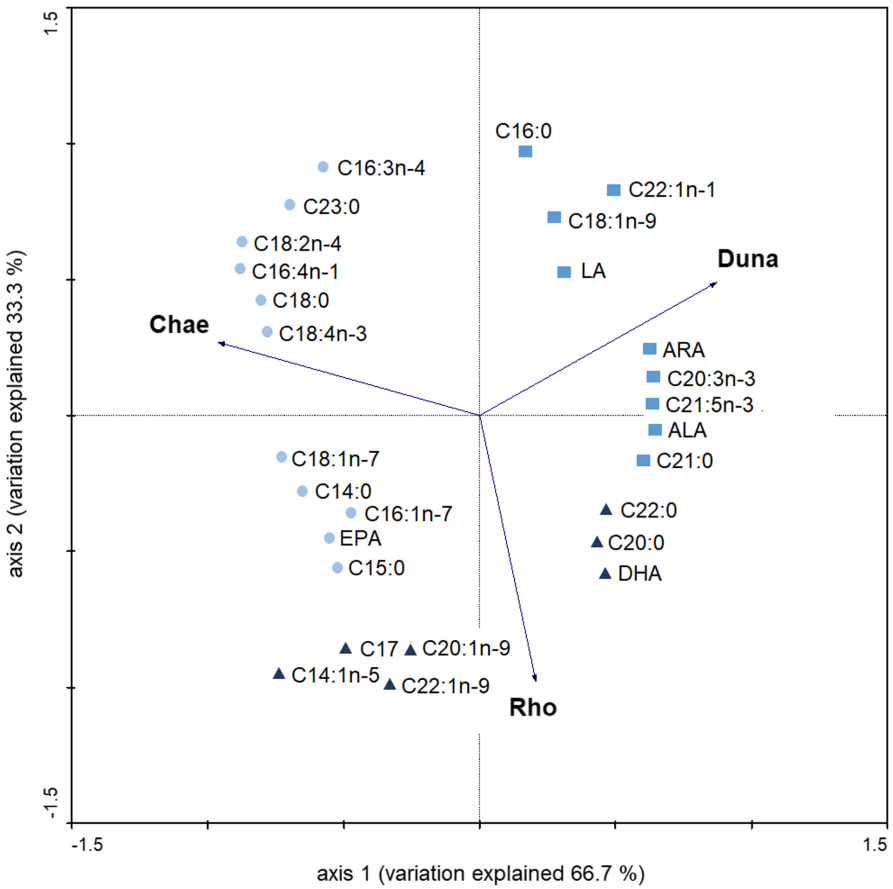


FIGURE 5 Biplots of principal component analysis of correlation (PCA) based in fatty acid profile of *Paracentrotus lividus* larvae. Larvae were fed with *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna).

study. This difference in the age of competence within studies also suggests that besides the rearing temperature, the stocking density, as proxy of food availability, affects the larval development. This effect of food availability is supported by the results obtained by Ahmed et al. (2016). In that study, *P. lividus* larvae were reared at a stocking density of 6 larvae/ml with a fixed ration of 5000 cells/ml/day of Chae and attained competence at 10 DPF with low survival rates. Due to the high mortality rates and the fixed diet ration, the remaining larvae had more food available, increasing their chance to attain competence. Even though Chae based diet led to the poorest survival rates when compared to *Tetraselmis suecica* (Kylin Butcher, 1959) and *Nannochloropsis oculata* (Hibberd, 1981).

The analysis of the larval biometric parameters showed that both BL and BW increased steadily until reaching competence independently the dietary treatment. Nonetheless, larvae fed with Chae presented a larger stomach in relation to the other dietary treatments, which contrast with the previous results reported by Gomes et al. (2021). In that study, larvae fed with Chae monospecific diet presented the smallest stomachs even when compared with larvae fed with Rho monospecific diet. The present results could be influenced by the cell concentration (cells/ml/day) used as feed ration. Since microalgae cell concentration was adapted to larval development but not to survival, the high growth performance resulted of a relatively higher food availability per larva when density numbers dropped (Gomes et al., 2021). Generally, it was observed the shortening of POAL for all the diets tested, which is a sign of normal larval development, as reported by Liu et al. (2007a) and by Fenaux et al. (1994). On the other hand, larvae fed with Duna presented a relatively larger POA, indicating that this diet is nutritionally poorer in comparison to the other diets tested (Cárcamo et al., 2005; Strathmann et al., 1992). Similar results were reported by Castilla-Gavilán et al. (2018) for *P. lividus* larvae and by George et al. (2004) for *Lytechinus variegatus* (Lamarck, 1816) larvae, using Duna as a monospecific diet.

The analysis of larval condition models showed that larvae fed with Chae presented lower growth during development, showing to be thinner to what expected by the model A (BW ~ BL). Nevertheless, the larvae fed with Rho and Chae presented larger stomachs in relation to their length by model B (SL ~ BL). According to Qi et al. (2018),

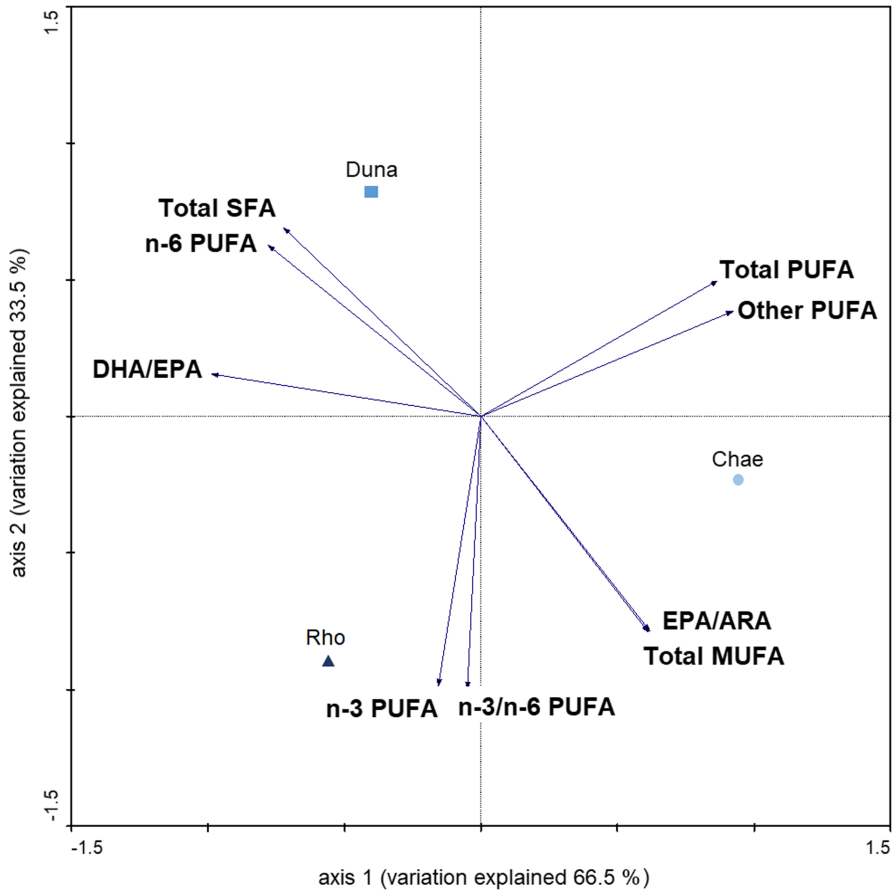


FIGURE 6 Biplots of principal component analysis of correlation (PCA) based in bioindicators analysis of *Paracentrotus lividus* larvae. Larvae were fed with *Rhodomonas* sp. (Rho), *Chaetoceros calcitrans* (Chae) and *Dunaliella tertiolecta* (Duna).

larvae fed with more suitable diets present relatively larger stomachs than those fed with nutritionally poorer diets. The result obtained reflect the digestive ability and the nutritional quality of the diets provided (George et al., 2008; Qi et al., 2018; Schiopu et al., 2006). Model C explores the concept that the POAL is the best indicator of development response to food quality (McEdward & Herrera, 1999; Strathmann et al., 1992). In fact, the average standard residuals obtained in model C for larvae fed with Rho showed a shorter arm in relation to the larval stomach, supporting the idea Rho fulfils the nutritionally requirements for larval development.

The growth and condition of *P. lividus* larvae were significantly affected by the biochemical composition of their microalgal diets. As previously mentioned, the high content of proteins present in Rho certainly have promoted the larval growth. The presence of high levels of carbohydrates in Chae clearly had impact on larval growth, by showing the largest stomach and shortest POA at competence. This reflects the energy efficiency of this microalga. Further, the results of this study also suggest that the high levels of β-carotene present in Chae improved the larval development and condition. In the sea urchin *Strongylocentrotus droebachiensis* (Müller, 1776), increasing levels of β-carotene in a diet had a positive effect on larval performance (De Jong-Westman et al., 1995). In the present study, the fatty acid profile of *P. lividus* larvae reflected the assimilation of the microalgal diets provided. Like other marine invertebrates, *P. lividus* can synthesize ARA and EPA from dietary LA and ALA (Kabeya et al., 2017). *Rhodomonas* sp. presented the highest content of C22:1*n* − 9, DHA and PUFA and median levels of LA and ALA in comparison with the two other microalgae. Concomitantly, larvae fed with Rho showed high content of C22:1*n* − 9 and DHA. Chae was rich in C18:0, C16:4*n* − 1, ARA, EPA, but particularly poor in LA and ALA influencing the FA profile of the larvae of *P. lividus* fed with this microalga, which presented also higher content of C18:4*n* − 3 and C16:4*n* − 1. These larvae were characterized by a relatively high content of total PUFA (46.06%), other than *n* − 3 and *n* − 6 PUFA (8.27%), total MUFA (25.62%) and EPA/ARA ratio (5.37), but low DHA/EPA ratio (0.11). Duna had high content of C16:0, C18:1*n* − 9, ALA and LA, which was also observed by Liu et al. (2007a). This microalga also presented a high content of total *n* − 6 PUFA (14.03%) and the lowest content of *n* − 3/*n* − 6 ratio (2.72). Concomitantly, the larvae fed with Duna showed high abundance of C18:1*n* − 9, ARA, ALA and LA. Despite EPA being not detected in Duna, a relative high abundance of EPA was found when larvae were fed with this diet. The increase in EPA and ARA levels in larvae suggests active biosynthesis of EPA and ARA from LA and ALA through the "Δ8 pathway" as evidenced by Carboni et al. (2012) and Kabeya et al. (2017). Overall, it was observed high levels of DHA in larvae fed with all diets, reflecting the selective retention of dietary DHA, which was also reported by Carboni et al. (2012). Among PUFA, larvae presented a high *n* − 6 PUFA content when compared with their respective diets, especially for larvae fed with Duna. This fact suggests that the larvae actively accumulate these FA from dietary and retained them in their tissues (Liu et al., 2007a). In relation to *n* − 3 PUFA content, a higher proportion of these PUFA were found in *P. lividus* larvae fed with Rho and Chae, which may have improved larval growth (short POA) and condition. The relatively higher levels of C20:1 *n* − 9 and C22:1*n* − 9 present in larval tissue compared

to the quantity present in the diets, indicated that they were elongated from C18:1*n* − 9. This result was also evidenced by Liu et al. (2007a) and by Castell et al. (2004) in *S. droebachiensis* juveniles. Moreover, larvae fed with Chae presented high levels of C18:4*n* − 3 and C16:4*n* − 1. While the presence of C16:4*n* − 1 confirms the assimilation of FA present in this diatom, the presence of C18:4*n* − 3 apparently benefits the larval growth of *P. lividus* (Liu et al., 2007a).

It has been shown that for a good larval development, the ratios DHA/EPA and EPA/ARA are more important than the FA absolute values (Carboni et al., 2012; Liu et al., 2007a; Schiopu et al., 2006). Larval performance (larger sizes and condition) was better when the dietary DHA/EPA ratio was lower as seen in Chae and EPA/ARA higher as seen in Rho diet, confirming the findings of Liu et al. (2007a). Another useful key factor for the evaluation of nutritional quality is the *n* − 3/*n* − 6 ratio. The recommended *n* − 3/*n* − 6 ratio differs between authors, but is always higher than one (Prato et al., 2018), indicating that the synthesis pathway of EPA and DHA is prioritized in larvae with good condition. Despite the low level of LA and ALA, Chae showed high *n* − 3/*n* − 6 ratio and the larvae fed with this diet presented the best growth performance (larger stomach and shortest POAL). Similar results were also observed in *Salmacis bicolor* (Krishnan et al., 2020), *Strongylocentrotus nudus* (A. Agassiz, 1864) (Qi et al., 2018) and *Loxechinus albus* (Molina, 1782) larvae (Cárcamo et al., 2005). Rho also presented a high *n* − 3/*n* − 6 ratio, improving larvae fed with this diet to develop a short POA. On the other hand, Duna had the lowest *n* − 3/*n* − 6 ratio, influencing larvae to develop a longer POA as already observed by Carboni et al. (2012) in larvae fed with similar diet.

The present study discussed the effects of the nutritional characteristics of three monospecific microalgal diets on the development of *P. lividus* larvae. Despite the low survival obtained for the more advanced larval stages (rudiment and competence), the data suggested that high content in *n* − 3 PUFA, low DHA/EPA and high *n* − 3/*n* − 6 ratios present in *C. calcitrans* enhanced growth and condition of larvae. The carotenoid content present in this microalga certainly had an important role on larval development. In the present study, the larval biomass was a limiting factor to evaluate other nutritional parameters. Further studies on the nutritional condition of *P. lividus* must include the studies on the protein, lipidic and energetic content of this early life stage to measure and quantify the larval dietary assimilation.

Our results indicate that the inclusion of *C. calcitrans* in mixed microalgal diets will promote higher survival and growth (larger stomach and shortest POA), since it provides a more balanced nutrient profile compared to monospecific diets. Further research should look to a larger number of phytoplankton species to determine what nutritional characteristics should be supplied to enhance larval development and condition.

In the future, the larval metabolism should also be addressed, since it could provide insights on which microalgal species to focus on, because echinoplutei adjust a suite of genes associated to different metabolic responses when exposed to different microalgae.

Nonetheless, these findings will be helpful towards the commercial of sea urchin larval production for achieving high aquaculture potential, under captive rearing conditions.

AUTHOR CONTRIBUTION

ASG contributed to this study with methodology, investigation, formal analysis, original draft writing, review and editing. SL contributed with study conceptualization, methodology, formal analysis, results visualization and draft review. PMS contributed with investigation, draft review and editing. MN, PA and CT contributed with investigation, formal analysis, draft review and editing. AP contributed with study conceptualization, resources allocation, funding acquisition and with the draft reviewing and editing.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

Agassiz, A. (1864). Synopsis of the echinoids collected by Dr. W. Stimpson on the North Pacific exploring expedition under the command of captains Ringgold and Rodgers. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *15*, 352–361.

Ahmed, H. O., Elmasry, E., El-Sayed, A. F. M., & Abdel Razek, F. A. (2016). Larval growth and metamorphosis of south eastern Mediterranean Sea urchin *Paracentrotus lividus* (Echinodermata:

Echinoidea) fed different microalgal diets. *Journal of Fisheries and Aquatic Science*, *11*, 287–295. https://doi.org/10.3923/jfas.2016.287.295

Araújo, J., Candeias-Mendes, A., Monteiro, I., Teixeira, D., Soares, F., & Pousão-Ferreira, P. (2020). The use of diatom *Skeletonema costatum* on aquaculture-produced purple sea urchin (*Paracentrotus lividus*) larvae and post-larvae diet. *Aquaculture Research*, *51*, 2545–2554. https://doi.org/10.1111/are.14597

Azad, A. K., Mckinley, S., & Pearce, C. M. (2010). Factors influencing the growth and survival of larval and juvenile echinoids. *Reviews in Aquaculture*, *2*, 121–137. https://doi.org/10.1111/j.1753-5131.2010.01030.x

Boudouresque, C. F., & Verlaque, M. (2020). Paracentrotus lividus. *Sea Urchins: Biology and Ecology*, *447–485*. https://doi.org/10.1016/b978-0-12-819570-3.00026-3

Braarud, T. (1960). On the coccolithophorid genus *Cricosphaera* n. gen. *Nytt Magasin for Botanikk*, *8*, 211–212.

Brundu, G., Vian Monleón, L., Vallainc, D., & Carboni, S. (2016). Effects of larval diet and metamorphosis cue on survival and growth of sea urchin post-larvae (*Paracentrotus lividus*; Lamarck, 1816). *Aquaculture*, *465*, 265–271. https://doi.org/10.1016/j.aquaculture.2016.09.014

Butcher, R. W. (1959). *An introductory account of the smaller algae of British coastal waters. Part I: Introduction and Chlorophyceae* (pp. 1–74). Fisheries Investigations.

Byrne, M., Sewell, M. A., & Prowse, T. A. A. (2008). Nutritional ecology of sea urchin larvae: Influence of endogenous and exogenous nutrition on echinopluteal growth and phenotypic plasticity in *Tripneustes gratilla*. *Functional Ecology*, *22*, 643–648. https://doi.org/10.1111/j.1365-2435.2008.01427.x

Carboni, S., Kelly, M. S., Hughes, A. D., Vignier, J., Atack, T., & Migaud, H. (2014). Evaluation of flow through culture technique for commercial production of sea urchin (*Paracentrotus lividus*) larvae. *Aquaculture Research*, *45*, 768–772. https://doi.org/10.1111/are.12019

Carboni, S., Vignier, J., Chiantore, M., Tocher, D. R., & Migaud, H. (2012). Effects of dietary microalgae on growth, survival and fatty acid composition of sea urchin *Paracentrotus lividus* throughout larval development. *Aquaculture*, *324–325*, 250–258. https://doi.org/10.1016/j.aquaculture.2011.10.037

Cárcamo, P. F., Candia, A. I., & Chaparro, O. R. (2005). Larval development and metamorphosis in the sea urchin *Loxechinus albus* (Echinodermata: Echinoidea): Effects of diet type and feeding frequency. *Aquaculture*, *249*, 375–386. https://doi.org/10.1016/j.aquaculture.2005.03.026

Castell, J. D., Kennedy, E. J., Robinson, S. M. C., Parsons, G. J., Blair, T. J., & Gonzalez-Duran, E. (2004). Effect of dietary lipids on fatty acid composition and metabolism in juvenile green sea urchins (*Strongylocentrotus droebachiensis*). *Aquaculture*, *242*, 417–435.

Castilla-Gavilán, M., Buzin, F., Cognie, B., Dumay, J., Turpin, V., & Decottignies, P. (2018). Optimising microalgae diets in sea urchin *Paracentrotus lividus* larviculture to promote aquaculture diversification. *Aquaculture*, *490*, 251–259. https://doi.org/10.1016/j.aquaculture.2018.02.003

De Coen, W. M., & Janssen, C. R. (1997). The use of biomarkers in *Daphnia magna* toxicity testing. IV. Cellular energy allocation: A new methodology to assess the energy budget of toxicant-stressed daphnia populations. *Journal of Aquatic Ecosystem Stress and Recovery*, *6*, 43–55. https://doi.org/10.1023/A:1008228517955

De Jong-Westman, M., Qian, P.-Y., March, B. E., & Carefoot, T. H. (1995). Artificial diets in sea urchin culture: Effects of dietary protein level and other additives on egg quality, larval morphometrics, and larval survival in the green sea urchin, *Strongylocentrotus droebachiensis*. *Canadian Journal of Zoology*, *73*, 2080–2090. https://doi.org/10.1139/z95-245

De La Uz, S., Rodrfguezi, J. C., Carrasco, F., & Anadon, N. (2013). Metamorphosis, growth and survival of early juveniles of *Paracentrotus lividus* (echinodermata: Echinoidea): Effects of larval diet and settlement inducers. *Cahiers de Biologie Marine*, *54*, 691–695.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356. https://doi.org/10.1021/ac60111a017

Dupont, S., Ortega-Martínez, O., & Thorndyke, M. (2010). Impact of near-future ocean acidification on echinoderms. *Ecotoxicology*, *19*, 449–462. https://doi.org/10.1007/s10646-010-0463-6

Dworjanyn, S. A., Pirozzi, I., & Liu, W. (2007). The effect of the addition of algae feeding stimulants to artificial diets for the sea urchin *Tripneustes gratilla*. *Aquaculture*, *273*, 624–633. https://doi.org/10.1016/j.aquaculture.2007.08.023

Eschscholtz J. F. (1831). Zoologischer Atlas - Beschreibungen neuer Thierarten, während des Flottcapitains von Kotzebue zweiter Reise um die Welt, auf der Russisch-Kaiserlichen Kriegsschlupp Predpriaetië in den Jahren 1823-1826. *G. Reimer, Berlin pt. 4*: 1-19, pl. 16-20.

Fenaux, L., Strathmann, M. F., & Strathmann, R. R. (1994). Five tests of food limited growth of larvae in coastal waters by comparison of rates of development and form of echinoplutei. *Limnology and Oceanography*, *39–1*, 84–98. https://doi.org/10.4319/lo.1994.39.1.0084

Fernández, A., Grienke, U., Soler-Vila, A., Guihéneuf, F., Stengel, D. B., & Tasdemir, D. (2015). Seasonal and geographical variations in the biochemical composition of the blue mussel (*Mytilus edulis L.*) from Ireland. *Food Chemistry*, *177*, 43–52. https://doi.org/10.1016/j.foodchem.2014.12.062

Fernández-Reiriz, M. J., Perez-Camacho, A., Ferreiro, M. J., Blanco, J., Planas, M., Campos, M. J., & Labarta, U. (1989). Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. *Aquaculture*, *83*, 17–37. https://doi.org/10.1016/0044-8486(89)90057-4

George, S. B., Fox, C., & Wakeham, S. (2008). Fatty acid composition of larvae of the sand dollar *Dendraster excentricus* (Echinodermata) might reflect FA composition of the diets. *Aquaculture*, *285*, 167–173. https://doi.org/10.1016/j.aquaculture.2008.08.010

George, S. B., Lawrence, J. M., & Lawrence, A. L. (2004). Complete larval development of the sea urchin *Lytechinus variegatus* fed an artificial feed. *Aquaculture*, *242*, 217–228. https://doi.org/10.1016/j.aquaculture.2004.06.024

Gomes, A., Lourenço, S., Santos, P. M., Raposo, A., Mendes, S., Gonçalves, S. C., Ferreira, S. M. F., & Pombo, A. (2021). Effects of single and mixed-diatom diets on growth, condition, and survival of larvae of the sea urchin *Paracentrotus lividus* (Lamarck, 1816). *Aquaculture International*, *29*, 1069–1090. https://doi.org/10.1007/s10499-021-00676-8

Guedes, A.C., & Malcata, F. X. (2012). Nutritional value and uses of microalgae in aquaculture. *Aquaculture*, *10*, 59–78.

Guiry, M.D. & Guiry, G.M. (2021). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway (taxonomic information republished from AlgaeBase with permission of M.D. Guiry). Pleurochrysis carterae (Braarud & Fagerland) T.Christensen, 1978. Accessed through: Costello, M.J.; Bouchet, P.; Boxshall, G.; Arvanitidis, C.; Appeltans, W. (2021) European Register of Marine Species. https://www.marbef.org/data/aphia.php?p=taxdetails&id=235969

Hannon, C., Officer, R. A., & Chamberlain, J. (2017). Evaluation of the efficacy of algal-conditioned substrates for inducing settlement of *Paracentrotus lividus* larvae. *Aquaculture Research*, *48*, 1968–1973. https://doi.org/10.1111/are.12959

Haws, M. C., DiMichele, L., & Hand, S. C. (1993). Biochemical changes and mortality during metamorphosis of the eastern oyster, *Crassostrea virginica*, and the Pacific oyster, *Crassostrea gigas*. *Molecular Marine Biology and Biotechnology*, *2*, 207–217.

Hibberd, D. J. (1981). Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym

Xanthophyceae). *Botanical Journal of the Linnean Society*, *82*, 93–119.

Hillebrand, H. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, *35*, 403–424.

Jimmy, R. A., Kelly, M. S., & Beaumont, A. R. (2003). The effect of diet type and quantity on the development of common sea urchin larvae Echinus esculentus. *Aquaculture*, *220*, 261–275. https://doi.org/10.1016/S0044-8486(02)00193-X

Kabeya, N., Sanz-Jorquera, A., Carboni, S., Davie, A., Oboh, A., & Monroig, O. (2017). Biosynthesis of polyunsaturated fatty acids in sea urchins: Molecular and functional characterisation of three fatty acyl desaturases from *Paracentrotus lividus* (Lamark 1816). *PLoS One*, *12*, 1–15. https://doi.org/10.1371/journal.pone.0169374

Kelly, M. S., Hunter, A. J., Scholfield, C. L., & McKenzie, J. D. (2000). Morphology and survivorship of larval *Psammechinus miliaris* (Gmelin) (Echinodermata: Echinoidea) in response to varying food quantity and quality. *Aquaculture*, *183*, 223–240. https://doi.org/10.1016/S0044-8486(99)00296-3

Kjeldahl, J. (1883). Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. *Zeitschrift für Analytische Chemie*, *22*, 366–382. https://doi.org/10.1007/BF01338151

Krishnan, M. G., Rajasree, S. R. R., Aranganathan, L., & Karthih, M. G. (2020). Comparative analysis of mono and combined microalgal diets on growth parameters of *Salmacis bicolor* larvae. *Thalassas: An International Journal of Marine Sciences*, *36*, 621–632. https://doi.org/10.1007/s41208-020-00241-9

Lamarck, J. B. M. (1816). *Histoire naturelle des animaux sans vertèbres*. Tome troisième.

Lawrence, J. M. (2007). Chapter 1 - Edible Sea urchins: Use and life-history strategies. *Developments in Aquaculture and Fisheries Science*, *37*, 1–9. https://doi.org/10.1016/S0167-9309(07)80065-2

Liu, H., Kelly, M. S., Cook, E. J., Black, K., Orr, H., Zhu, J. X., & Dong, S. L. (2007a). The effect of diet type on growth and fatty-acid composition of sea urchin larvae, I. *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). *Aquaculture*, *264*, 247–262. https://doi.org/10.1016/j.aquaculture.2006.12.021

Liu, H., Kelly, M. S., Cook, E. J., Black, K., Orr, H., Zhu, J. X., & Dong, S. L. (2007b). The effect of diet type on growth and fatty acid composition of the sea urchin larvae, II. *Psammechinus miliaris* (Gmelin). *Aquaculture*, *264*, 263–278. https://doi.org/10.1016/j.aquaculture.2006.12.022

Liu, W., Pearce, C. M., Alabi, A. O., & Gurney-Smith, H. (2009). Effects of microalgal diets on the growth and survival of larvae and post-larvae of the basket cockle, *Clinocardium nuttallii*. *Aquaculture*, *293*, 248–254. https://doi.org/10.1016/j.aquaculture.2009.04.032

Lourenço, S., Cunha, B., Raposo, A., Neves, M., Santos, P. M., Gomes, A. S., Tecelão, C., Ferreira, S. M. F., Baptista, T., Gonçalves, S. C., & Pombo, A. (2021). Somatic growth and gonadal development of *Paracentrotus lividus* (Lamarck, 1816) fed with diets of different ingredient sources. *Aquaculture*, *539*, 736589. https://doi.org/10.1016/j.aquaculture.2021.736589

McBride, S. C. (2005). Sea urchin aquaculture. *American Fisheries Society Symposium*, *46*, 179–208. https://doi.org/10.1201/9780203970881.ch48.

McEdward, L. R., & Herrera, J. C. (1999). Body form and skeletal morphometrics during larval development of the sea urchin *Lytechinus variegatus* lamarck. *Journal of Experimental Marine Biology and Ecology*, *232*, 151–176. https://doi.org/10.1016/S0022-0981(98)00106-3

Méndez-Martínez, Y., García-Guerrero, M. U., Lora-Vilchis, M. C., Martínez-Córdova, L. R., Arcos-Ortega, F. G., Alpuche, J. J., & Cortés-Jacinto, E. (2018). Nutritional effect of artemia nauplii enriched with *Tetraselmis suecica* and *Chaetoceros calcitrans* microalgae on growth and survival on the river prawn *Macrobrachium americanum* larvae. *Aquaculture International*, *26*, 1001–1015. https://doi.org/10.1007/s10499-018-0264-0

Molina, J. I. (1782). Saggio sulla storia naturale del Chile, del Signor Abate Giovanni Ignazio Molina. *Bologna*, 367.

Monfort, M. C. (2002). Fish roe in Europe: Supply and demand conditions. *FAO/GLOBEFISH Research Programme*, *72*, 47.

Müller, O.F. (1776). *Zoologiæ Danicæ Prodromus, seu Animalium Daniæ et Norvegiæ indigenarum characteres, nomina, et synonyma imprimis popularium*. Havniæ (Copenhagen). xxxii (pp. 274).

Paredes, E., Bellas, J., & Costas, D. (2015). Sea urchin (*Paracentrotus lividus*) larval rearing - Culture from cryopreserved embryos. *Aquaculture*, *437*, 366–369. https://doi.org/10.1016/j.aquaculture.2014.12.022

Pinto, H. (2018). *Efeito dos modos de preservação da microalga Rhodomonas baltica (Karsten, 1898) no cultivo do copépode Acartia tonsa (Dana, 1849)* (Doctoral dissertation). Instituto Politecnico de Leiria (Portugal).

Prato, E., Fanelli, G., Angioni, A., Biandolino, F., Parlapiano, I., Papa, L., Denti, G., Secci, M., Chiantore, M., Kelly, M. S., Ferranti, M. P., & Addis, P. (2018). Influence of a prepared diet and a macroalga (Ulva sp.) on the growth, nutritional and sensory qualities of gonads of the sea urchin *Paracentrotus lividus*. *Aquaculture*, *493*, 240–250. https://doi.org/10.1016/j.aquaculture.2018.05.010

Qi, S., Zhao, X., Zhang, W., Wang, C., He, M., Chang, Y., & Ding, J. (2018). The effects of 3 different microalgae species on the growth, metamorphosis and MYP gene expression of two sea urchins, *Strongylocentrotus intermedius* and *S. nudus*. *Aquaculture*, *492*, 123–131. https://doi.org/10.1016/j.aquaculture.2018.02.007

Rial, D., Rial, P., Casal, A., Costoya, N., & Costas, D. (2018). Induction of settlement, growth and survival of juveniles of *Paracentrotus lividus*. *Aquaculture*, *483*, 16–20. https://doi.org/10.1016/j.aquaculture.2017.10.005

Sartori, D., Pellegrini, D., Macchia, S., & Gaion, A. (2016). Can echinoculture be a feasible and effective activity? Analyses of fast reliable breeding conditions to promote gonadal growth and sexual maturation in *Paracentrotus lividus*. *Aquaculture*, *451*, 39–46.

Schiopu, D., George, S. B., & Castell, J. (2006). Ingestion rates and dietary lipids affect growth and fatty acid composition of *Dendraster excentricus* larvae. *Journal of Experimental Marine Biology and Ecology*, *328*, 47–75. https://doi.org/10.1016/j.jembe.2005.06.019

Stefánsson, G., Kristinsson, H., Ziemer, N., Hannon, C., & James, P. (2017). Markets for sea urchins: a review of global supply and markets. *Skýrsla Matís*, *45*, 10–17. https://doi.org/10.13140/RG.2.2.12657.99683

Strathmann, R. R., Fenaux, L., & Strathmann, M. F. (1992). Heterochronic developmental plasticity larval sea urchins. *Evolution*, *46*, 972–986.

Suckling, C. C., Terrey, D., & Davies, A. J. (2018). Optimising stocking density for the commercial cultivation of sea urchin larvae. *Aquaculture*, *488*, 96–104. https://doi.org/10.1016/j.aquaculture.2018.01.022

Takano, H. (1968). On the diatom *Chaetoceros calcitrans* (Paulsen) emend and its dwarf form pumilus forma nov. *Bulletin Tokai Regional Fisheries Research Laboratory*, *55*, 1–7.

Volkman, J. K., Jeffrey, S. W., Nichols, P. D., Rogers, G. I., & Garland, C. D. (1989). Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, *128*, 219–240. https://doi.org/10.1016/0022-0981(89)90029-4

Whitehill, E. (2012). *The effects of temperature on energy utilization by marine invertebrate larvae*. All Dissertations. (p. 137).

Zar, J. H. (2010). *Biostatistical analysis* (5th ed.). Prentice-Hall/Pearson.

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