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A blend of microalgae and cyanobacteria produced from industrial waste outputs for the enrichments of *Artemia salina*: Effects on growth performance, antioxidant status and anomalies rate of European seabass (*Dicentrarchus labrax*) larvae

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ABSTRACT

The recent fast development of the microalgae-related biotechnologies has enabled the availability of sustainable and nutrients-rich raw materials to be used as substitution of conventional sources in aquafeed formulation. However the cost of energy of the photobioreactors used to produce these microorganisms still constrained their wider inclusion into aquafeed compounds. By exploiting industrial waste outputs, *Nannochloropsis sp.* and *Spirulina sp.* were produced and utilised to formulate two experimental enrichments (LM1 and LM2). During a 60 days trial, their efficacy have been tested as *Artemia* metanauplii enrichments in the diet of European seabass (*Dicentrarchus labrax*) larvae, in comparison to a commercial control. Larvae were fed with isonitrogenous and isolipidic dietary treatments. Each dietary treatment presented a specific profile for the long chain polyunsaturated fatty acids ARA, DHA and EPA. Ratio of DHA/EPA in enriched *Artemia* of 4:1 as in the case of LM1 and LM2 in the presence of 1.4% of ARA (as a % on total fatty acid) guaranteed equal results to the control which displayed a ratio of DHA/EPA of 2:1 with 2.2% of ARA. No significant effects of the dietary treatments were detected in final survival, growth performance and incidence of skeletal anomalies. Regarding oxidative status, larvae fed with LM2 enrichment presented lower catalase activity than control larvae with no signs of oxidative damage, suggesting a potential antioxidant effect of LM2. The present study contributes to expanding the existing literature on successful utilisation of microalgae and cyanobacteria, used to produce valuable nutrients, in a perspective of circular nutrients economy.

1. Introduction

Aquaculture production of marine fish, such as European seabass (*Dicentrarchus labrax*), relies on the availability of a large number of healthy and fully weaned juveniles for the on-growing phase. The standard hatchery protocol involves feeding newly hatched larvae with live prey for a period before weaning them onto formulated dry feed completely. This initial reliance on live prey is necessary to provide essential nutrients and properly sized food for the small mouths of the altricial marine fish larvae. *Artemia* metanauplii (*Artemia salina)* are extensively used as live prey due to being cost-effective, easy-to-handle and polyvalent (Sorgeloos et al., 2001), despite their natural deficiency in long chain polyunsaturated fatty acids (LC-PUFAs) (Monroig et al., 2006; Ramos-Llorens et al., 2023). To overcome this bottleneck, enrichments are customarily performed to load up *Artemia* metanauplii with the adequate nutritional value for fish larvae (Boglino et al., 2012).

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In fact, marine fish have a limited capacity to synthesise LC-PUFAs, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) (Izquierdo, 2005), although these molecules are important for the synthesis of new cellular structures (Gisbert et al., 2005) and for the primary role on larval survival, growth and immune system modulation (Tocher et al., 2019; Roo et al., 2019; Betancor et al., 2021; Pham et al., 2023). On the other hand, due to their highly peroxidable nature, it has been suggested that an excess of dietary LC-PUFAs, without a proper balance of antioxidant nutrients, can lead to controversial effects on fish oxidative stress (Izquierdo et al., 2013; Betancor et al., 2015; Wischhusen et al., 2022; Pham et al., 2023). Oxidative stress has been linked to morphogenic and skeletogenic alterations during early development stages, which may result in various degree of skeletal anomalies in the larval development of hatchery-produced fish (Conceição et al., 2007; Lall and Lewis-McCrea, 2007; Izquierdo et al., 2013).

Standard LC-PUFAs rich ingredients used by aquaculture industry are derived either from wild marine fish, in the form of fish oil, or from a number of microorganisms including microalgae and cyanobacteria, which are the primary producers of EPA and DHA (Mutale-Joan et al., 2023). On one hand, the utilisation of marine fish oil is posing several environmental and economic concerns as this contributes to the over-exploitation of pelagic fish stocks (Egerton et al., 2020; Glencross et al., 2020) along with presenting very high and unstable prices, due to the competition with human nutrition and other livestock production. In addition, this finite commodity is susceptible to seasonal fluctuation in the fatty acids profile (Gámez-Meza et al., 1999; Zlatanos and Laskaridis, 2007) and to contamination with dioxin-like polychlorinated biphenyls (PCBs), persistent organic pollutants (POPs) and heavy metals, which bioaccumulate in animals at a higher trophic level (Sun et al., 2018; Santigosa et al., 2021; Zatti et al., 2023) and may interfere with normal larval physiological functions (Horri et al., 2018; Bogevik et al., 2023).

On the other hand, the exploitation of microalgae and cyanobacteria can address some of the concerns related to fish oil, despite their production is often constrained by the high costs associated with cultivation. These microorganisms can be produced by farming on non-arable land, reduce water demands and convert CO_2 and other waste products into valuable ingredients (Shah et al., 2018; Yarnold et al., 2019; Chen et al., 2021; Tocher et al., 2019; Conde et al., 2021). They are oxygenic photoautotrophs capable of producing LC-PUFAs together with a diverse array of unique and potent substances (Ma et al., 2020; Nagappan et al., 2021) such as some antioxidant molecules produced by Spriluina *spp* (Fernandes et al., 2023). Their incorporation into hatchery feeding regimens has been found to exhibit probiotic and antibacterial properties (Vadstein et al., 2018; Ognistaia et al., 2022), resulting in a beneficial effect on larval gut microbiota (Huang et al., 2023), positively influencing the production key performance indicators (Boglino et al., 2012; Paulo et al., 2020; Gui et al., 2022; Pan et al., 2022).

From a production perspective, industrial-scale cultivation of microorganisms can be achieved through outdoor systems, utilising ponds or tanks, or by employing controlled environments such as photobioreactors (Mutale-Joan et al., 2023). While outdoor systems can partially reduce energy costs by using sunlight as an energy input, seasonal environmental variations may affect the composition and quality of microorganism cells, potentially resulting in lower yields (Xu et al., 2020). On the other hand, photobioreactors, offering precise control over environmental conditions, require substantial energy inputs for lighting, temperature regulation, agitation, and nutrient supplementation. In the context of the circular economy, the reutilisation of industrial by-products as a source of energy in more advanced transformation processes is becoming increasingly important. In this context, European Union is funding several projects aimed at bridging the gap between research and industrial application. VAXA (VAXA Technologies Ltd.; Reykjavík, Iceland) has developed a new technology platform perfecting the cultivation of LC-PUFAs rich microorganisms by exploiting geothermal energy produced by a geothermal plant in Iceland. This allows to transform the waste outputs of a geothermal plant, hot and cold water and natural CO_2 emissions, to produce a number of autotrophic microorganisms such as *Nannochloropsis sp.* and *Spirulina sp*. This system results in a year-round microorganisms production, resilient to the environmental variation, free from chemical contamination and with 80% reduction in production cost due to geothermal energy utilisation. The aim of this study was to examine the effects of two products at development stage (LM1 and LM2) contained different proportions of *Nannochloropsis* sp. and *Spirulina* sp. produced with this more sustainable technology compared to a commercial lipid emulsion control (C), as *Artemia* metanauplii enrichments in the diet of European seabass larvae. Besides their effects on larval survival, growth performance and fatty acid profile, the antioxidant status and the incidence of skeletal anomalies were also evaluated.

2. Materials and methods

2.1. Larval origin and rearing methods

European seabass larvae at 7 days post-hatching (dph) coming from one spawning batch were obtained from a commercial marine hatchery (Ca Zuliani Soc Agricola Srl., Italy). Larvae were transferred to the Laboratory of Aquaculture at the University of Bologna (Cesenatico, Italy) into a 50 L liquid storage bag filled with 1/3 seawater and 2/3 compressed oxygen, placed inside a polystyrene box for thermal isolation. Once at the laboratory, larvae were randomly allocated into nine 50 L up-welling tanks (2200 larvae $tank^{-1}$) by using volumetric allocation (Parma et al., 2013). Tanks were supplied with seawater and connected to a closed recirculation system consisted of a mechanical sand filter (PTK 1200; Astralpool, Barcelona, Spain), ultraviolet lights (UV PE 45; Sita Srl, Barcelona, Spain) and a biofilter (PTK 1200; Astralpool, Barcelona, Spain). Water exchange rate in the tanks was initially of 0.5 L min^{-1}, increased to 2.5 L min^{-1} following larval development. Photoperiod was set at 12 h light/12 h darkness regime (with light period from 8 am to 8 pm), through artificial light at < 50 lux at the water surface. Temperature was maintained at 18 ± 2 °C. Dissolved oxygen in the tanks was maintained at > 9 mg/L through an automatic liquid oxygen system connected to a software controller (B&G Sinergia snc, Chioggia, Italy). Sodium bicarbonate was added to keep the pH constant at 7.8–8.0 and avoid alkalinity fluctuation. Salinity was maintained at 27 ± 2 g L^{-1}.

2.2. Experimental feeding regimes and live prey enrichments

Three different enrichment products were used to enrich *Artemia* metanauplii. Two experimental products, LM1 and LM2, consisting of microalgae and cyanobacteria blends with different proportions of *Nannochloropsis* sp. and *Spirulina* sp. produced by using geothermal resources (VAXA Farm, Reykjavík, Iceland) and a commercial control C (Red Pepper©, Bernaqua, Olen, Belgium). Both LM1 and LM2 were produced without addition of fish oil, LM2 contained a higher proportion of *Spirulina* compared to LM1. The feeding protocol was set, with some modifications, according to those used in commercial hatcheries for European seabass and based on previous published works (Villamizar et al., 2009; El-Dahhar et al., 2022). Wild caught *Artemia* cysts of EG strain coming from Great Salt lake in USA (Catvis B.V., 's-Hertogenbosch, The Netherlands) were incubated and hatched in seawater (salinity 30 g L^{-1}) at 28 °C for 18 hours. After this, *Artemia* metanauplii were harvested and enriched for 12 hours at 26 °C by transferring metanauplii at a concentration of 300 individuals ml^{-1} to new container filled with seawater and 750 mg L^{-1} of the enriching products. Triplicate groups of larvae were fed with non-enriched *Artemia* nauplii from 7 to 26 dph (5 nauplii ml^{-1}). Enriched *Artemia* metanauplii were then incorporated from 27 dph to 60 dph (7.5 metanauplii ml^{-1} from 27 to 50 dph and 10 metanauplii ml^{-1} from 51 to 60 dph). The feeding protocol with *Artemia* metanauplii without including formulated dry feed was

maintained until advanced larvae development to avoid any possible bias on the outcomes of this research. The proximate composition and the fatty acids profile of the enriched *Artemia* metanauplii are shown in Table 1.

2.3. Samplings and growth calculations

Before each sampling procedure, larvae were euthanised with 300 mg L^{-1} of MS222. At 7, 19, 26, 32, 39, 46 and 54 dph, 20 larvae tank $^{-1}$ were randomly collected in order to determine wet weight (WW) and dry weight (DW). At the end of the trial (60 dph), 100 larvae tank $^{-1}$ were collected to determine WW and DW. WW was measured after rinsing larvae with de-ionised water on a 400 μm sieve and removing the excess of moisture away from behind the sieve using lint-free paper towel (Bonaldo et al., 2011). DW was determined by oven-drying the larvae at 70 °C for 12 hours. At the end of the trial, all larvae were removed from each tank and counted to determine survival rate. Larvae were then rinsed in distilled water, and frozen (−80 °C) until biochemical analyses or fixed in 10% formalin buffered with phosphate buffer saline (PBS, pH 7.2) for 48 h at 4°C for skeletal anomalies analysis. Fixed samples were then washed in PBS (pH 7.5) for 48 h at 4°C and then stored in 70% ethanol until further processing. Growth rate was calculated as specific growth rate (SGR, % day^{-1}) according to the equation: SGR = 100 * (ln FBW - ln IBW)/days (where FBW and IBW represent the final and the initial body weights, respectively). Survival rate was calculated per tank as a percentage of the initial number of larvae. All experimental procedures were evaluated and approved by the Ethical-Scientific Committee for Animal Experimentation of the University of Bologna, in accordance with European directive 2010/63/UE on the protection of animals used for scientific purposes.

2.4. Lipid and fatty acids profiles

Lipids in both *Artemia* nauplii and metanaupli and European seabass larvae were extracted according to (Bligh and Dyer, 1959) by using methanol and chloroform as solvents. Fatty acids were subjected to a methylated ester method (FAMEs) following the procedures as described in (Lepage and Roy, 1986). Briefly, methylated-FAs (FAMEs) were extracted into toluene. The FAMEs were analysed by gas chromatography (GC/FID, Clarus 500, Perkin-Elmer) using an SP-2330 fused silica capillary column (30 m × 0.25 mm i.d., 0.20 μm; Supelco Inc., Bellefonte, USA).

Table 1
Proximate analysis (% of dry weight) and fatty acid composition (% of total fatty acids) of the non-enriched *Artemia* nauplii and enriched *Artemia* metanauplii with the experimental enriching products LM1, LM2 and C.

	Non-enriched	LM1	LM2	C	*P* value
Total protein	55.95 ± 0.47	55.18 ± 0.95	56.36 ± 0.62	55.71 ± 1.20	ns
Total lipids	20.07 ± 0.09	23.07 ± 0.32	23.19 ± 0.76	23.90 ± 0.41	ns
Total fatty acids	16.16 ± 0.53	18.24 ± 0.71	18.42 ± 0.25	18.94 ± 0.47	ns
Ash	8.54 ± 0.04	11.66 ± 1.38	10.33 ± 0.21	10.19 ± 0.06	ns
11:0	2.25 ± 0.00	2.29 ± 0.22	2.18 ± 0.08	2.03 ± 0.25	ns
14:0	0.81 ± 0.01	5.60 ± 0.18^{b}	5.68 ± 0.09^{b}	1.44 ± 0.08^{a}	0.000
15:0	0.22 ± 0.05	0.17 ± 0.02^{a}	0.18 ± 0.03^{a}	0.21 ± 0.01^{b}	0.001
16:0	12.04 ± 0.04	12.06 ± 0.11^{a}	12.07 ± 0.1^{a}	13.15 ± 0.12^{b}	0.000
17:0	0.83 ± 0.01	0.67 ± 0.05	0.68 ± 0.00	0.68 ± 0.02	ns
18:0	4.96 ± 0.00	4.46 ± 0.05	4.48 ± 0.03	4.43 ± 0.07	ns
20:0	0.25 ± 0.03	0.28 ± 0.02	0.25 ± 0.08	0.22 ± 0.06	ns
22:0	0.27 ± 0.04	0.40 ± 0.02^{a}	0.37 ± 0.04^{a}	0.54 ± 0.02^{b}	0.000
Σ saturated	21.63 ± 0.23	25.93 ± 0.28^{a}	25.89 ± 0.11^{a}	22.70 ± 0.41^{b}	0.000
14:1 n-5	0.58 ± 0.03	0.41 ± 0.04	0.40 ± 0.03	0.38 ± 0.01	ns
16:1 n-7	2.66 ± 0.00	2.08 ± 0.08^{a}	1.98 ± 0.02^{b}	2.16 ± 0.03^{a}	0.002
16:1 n-9	0.59 ± 0.00	0.46 ± 0.03	0.45 ± 0.01	0.47 ± 0.02	ns
18:1 n-7	7.46 ± 0.24	6.04 ± 0.21	5.94 ± 0.17	6.13 ± 0.11	ns
18:1 n-9	18.9 ± 0.05	15.31 ± 0.16	15.19 ± 0.03	15.35 ± 0.2	ns
20:1 n-9	0.59 ± 0.03	0.53 ± 0.06	0.55 ± 0.07	0.59 ± 0.06	ns
22:1 n-9	0.12 ± 0.01	0.16 ± 0.05	0.16 ± 0.04	0.19 ± 0.09	ns
24:1 n-9	0	0	0	0	ns
Σ monounsaturated	30.9 ± 0.31	24.99 ± 0.39	24.67 ± 0.25	25.27 ± 0.20	ns
16:2 n-6	0.28 ± 0.02	0.09 ± 0.05	0.07 ± 0.02	0.04 ± 0.01	ns
18:2 n-6 (LOA)	6.70 ± 0.04	5.10 ± 0.09^{a}	5.09 ± 0.06^{a}	5.75 ± 0.08^{b}	0.000
18:3 n-6	0.32 ± 0.01	0.29 ± 0.02	0.34 ± 0.05	0.30 ± 0.02	ns
20:2 n-6	0.26 ± 0.01	0.24 ± 0.03	0.24 ± 0.01	0.25 ± 0.02	ns
20:3 n-6 (DGLA)	0.07 ± 0.01	0.11 ± 0.05^{a}	0.10 ± 0.01^{a}	0.17 ± 0.02^{b}	0.002
20:4 n-6 (ARA)	1.35 ± 0.01	1.43 ± 0.03^{a}	1.42 ± 0.04^{a}	2.21 ± 0.05^{b}	0.000
22:4 n-6	0.07 ± 0.04	0.09 ± 0.03	0.07 ± 0.03	0.08 ± 0.01	ns
22:5 n-6 (DPA n-6)	0	1.98 ± 0.10^{a}	2.12 ± 0.06^{a}	3.22 ± 0.08^{b}	0.000
Σ n-6 PUFA	9.05 ± 0.09	9.33 ± 0.09^{a}	9.46 ± 0.04^{a}	12.02 ± 0.11^{b}	0.000
16:3 n-3 (HTA)	0.58 ± 0.02	0.42 ± 0.02	0.42 ± 0.03	0.40 ± 0.02	ns
18:3 n-3 (ALA)	30.58 ± 0.07	22.28 ± 0.24^{b}	21.53 ± 0.16^{a}	22.47 ± 0.34^{b}	0.001
18:4 n-3 (SDA)	4.14 ± 0.09	2.79 ± 0.02^{b}	2.64 ± 0.04^{a}	2.74 ± 0.04^{b}	0.001
20:4 n-3 (ETA)	0.66 ± 0.01	0.61 ± 0.03^{a}	0.58 ± 0.02^{a}	0.71 ± 0.00^{b}	0.000
20:5 n-3 (EPA)	1.70 ± 0.01	2.65 ± 0.04^{a}	2.75 ± 0.05^{b}	3.94 ± 0.03^{c}	0.000
22:5 n-3 (DPA n-3)	0.04 ± 0.05	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}	0.20 ± 0.06^{b}	0.000
22:6 n-3 (DHA)	0.07 ± 0.02	10.43 ± 0.4^{b}	11.42 ± 0.15^{c}	9.09 ± 0.24^{a}	0.000
Σ n-3 PUFA	37.77 ± 0.02	39.25 ± 0.22	39.40 ± 0.12	39.55 ± 0.25	ns
Σ polyunsaturated	46.82 ± 0.04	48.58 ± 0.26^{a}	48.85 ± 0.18^{a}	51.57 ± 0.27^{b}	0.000
n-3/n-6	4.18 ± 0.05	4.21 ± 0.03^{b}	4.16 ± 0.02^{b}	3.29 ± 0.03^{a}	0.000
EPA+DHA	1.77 ± 0.02	13.08 ± 0.40^{a}	14.18 ± 0.13^{b}	13.03 ± 0.26^{a}	0.000
EPA/DHA	-	0.25 ± 0.01^{a}	0.24 ± 0.01^{a}	0.43 ± 0.01^{b}	0.000

Data are given as the mean (n=4) ± SD. Significant differences among treatments were detected $P \leq 0.05$ by using one way ANOVA + Tukey's test. Values from non-enriched Artemia nauplii were not included in the statistic.

2.5. Evaluation of the antioxidant status

The antioxidant status of the larvae was assessed by measuring catalase activity (CAT), protein carbonylation (PC), lipid peroxidation (LPO) and mitochondrial reactive oxygen species production (mtROS). For the analyses of CAT, PC and LPO, three pools of 3 larvae per replicate tank (n = 9 pools per treatment) were sampled at the end of the growth trial (60 dph) and snap-frozen in liquid nitrogen. Samples were homogenised in 500 μl ultra-pure water using an Ultra-Turrax® Homogeniser (IKA®-Werke, Germany). One aliquot containing 4% butylated hydroxytoluene (BHT) in methanol was used for the determination of LPO. The remaining homogenate was diluted (1:1) in 0.2 M K-phosphate buffer, pH 7.4, and centrifuged for 10 min at 10,000 g (4 ºC). The post-mitochondrial supernatant (PMS) was kept in –80 ºC for the analysis of catalase activity (CAT) and protein carbonylation (PC). For mtROS determination 3 larvae per replicate tank (n = 9 pools per treatment) were sampled and snap-frozen in liquid nitrogen. Samples were homogenised in 200 μl ice-cold mitochondria isolation buffer (225 mM mannitol, 75 mM sucrose, 1 mM EGTA and 4 mM HEPES, pH 7.2). Then, the homogenate was centrifuged for 10 min at 600 g and 4 °C. The supernatant was picked off and centrifuged again for 10 min, at 11,000 g and 4 ºC. The pellet was resuspended in buffer containing 250 mM sucrose and 5 mM HEPES (pH 7.2). Catalase activity (CAT) was determined by measuring decomposition of the substrate H_2O_2 at 240 nm (Clairborne, 1985). Protein carbonylation (PC) was measured by the reaction of 2,4-dinitrophenylhydrazine (DNPH) with carbonyl groups, according to the DNPH alkaline method (Mesquita et al., 2014). Endogenous lipid peroxidation (LPO) was determined by measuring thiobarbituric acid-reactive substances (TBARS) (Bird and Draper, 1984). Mitochondrial reactive oxygen species (mtROS) production was assessed by the dihydrodichloro-fluorescein diacetate method, H(2) DCF-DA (van der Toorn et al., 2009). The protein content of PMS (CAT, LPO and PC determinations) and mtROS samples was determined according to the Bradford method (Bradford, 1976) using bovine serum albumin as standard. All biomarkers were determined in 96 well flat bottom microplates using a temperature-controlled microplate reader (Synergy H1, BioTek Instrument, Inc., USA).

2.6. Evaluation of severe skeletal anomalies

A total of 859 European sea bass larvae at 60 dph were stained with alcian blue and Alizarin red according to Pousis et al. (2022). Briefly, the specimens were immersed in a solution of 0.5% KOH and 3% H_2O_2 and subjected to prolonged exposure (5–6 hours) to sunlight. Then all the samples were stained in 200 ml cartilage staining solution (120 ml 95% ethanol, 80 ml glacial acetic acid, 50 mg Alcian blue) and in 200 ml of bone staining solution (60 mg Alizarin red in 200 ml KOH) in the dark for 90 min and 2 hours respectively. After each staining step the samples were washed three times in 0.5% KOH for 5 minutes. Finally, the samples were subjected to the process of diaphanization by consecutive immersion in containing 0.5% KOH and increasing glycerol concentrations (0.5% KOH:glycerol 3:1; 0.5% KOH:glycerolm1:1; 0.5% KOH: glycerol 1:3; 100% glycerol). The duration of the first two steps was about 48 hours while the while the third step was repeated several times until the 0.5% KOH:glycerol 1:3 solution remained colourless after 24 h of immersion.

Double-stained specimens were observed twice by the same operator, under a stereomicroscope (Leica WILD M3C, Wetzlar, DE), in order to reduce possible errors during the analysis of the samples and all the malformed fish were photographed by a digital camera (DFC 420; Leica Microsystems, Cambridge, UK). For each group, severe skeletal anomalies were recorded according to Prestinicola et al. (2013), and the following variables were calculated: 1) relative frequency (%) of individuals with at least one severe anomaly. 2) severe anomalies load (number of severe anomalies/number of individuals with severe anomalies). 3) relative frequencies (%) of severe anomalies in the in the four regions of the vertebral column and in the skull (number of severe anomalies in each region/number of total severe anomalies).

2.7. Statistical analysis

Significant differences in growth performance, fatty acid profile, antioxidant status and frequencies of severe skeletal anomalies between the groups were determined using one-way ANOVA and, whenever significant differences were identified, means were compared by the Post hoc multiple comparisons Tukey's test ($P < 0.05$). Prior to ANOVA, skeletal malformation data were arcsine transform as appropriate for proportion (Sokal and Rohlf, 1969). The assumptions related to data normal distribution and homogeneity of variances were explored through Anderson Darling's test and Levene's test, respectively. In the case data presented a non-normal distribution, significant differences between the groups were determined using Kruskal-Wallis non-parametric test. Analyses were performed using Minitab software (Minitab 19.0.1; Pennsylvania State University, USA). Results are presented as mean ± standard deviation (SD), unless otherwise mentioned.

3. Results

3.1. Growth performance

Wet weight (WW) and dry weight (DW) measured during the trial of European seabass larvae fed *Artemia* metanauplii enriched with the different products are shown in Fig. 1. At the end of the trial no significant differences were detected in specific growth rate (SGR) (7.95 ± 0.31, 7.92 ± 0.14 and 8.33 ± 0.56, respectively in LM1, LM2 and C) and survival rate (65.0 ± 6.9, 68.5 ± 7.1 and 61.5 ± 1.3, respectively in LM1, LM2 and C), among the different groups.

3.2. Fatty acid profile in Artemia metanauplii and larvae

Proximate compositions and fatty acid profiles of *Artemia* metanauplii enriched with the different products are shown in Table 1. No significant differences were observed in the proximate composition of *Artemia* metanauplii (total protein, total lipids, total fatty acids and ash) among treatments ($P > 0.05$). For what concern the differences related to LC-PUFAs, significant differences were observed in the proportions of arachidonic (ARA 20:4 n-6) acid, which was found significantly higher in C compared to LM1 and LM2. Eicosapentaenoic acid (EPA 20:5 n-3) was found significantly higher in C compared to LM1 and LM2 and in LM2 compared to LM1. On the contrary, docosahexaenoic acid (DHA 22:6 n-3) was found significantly higher in LM2 compared to LM1 and C and in LM1 compared to C. According to these, the ratio n-3/n-6 PUFA was significantly higher in *Artemia* metanauplii enriched with LM1 and LM2, the amount of EPA + DHA was significantly higher in LM2, and the ratio EPA/DHA was significantly higher in the control *Artemia* metanauplii.

Fatty acid profiles of European seabass larvae fed *Artemia* enriched with the different products are shown in Table 2. At the end of the trial (60 dph), no significant differences were detected in the total quantity of saturated, mono unsaturated and total PUFA (n-3 + n-6) fatty acids among larvae from the different treatments groups. With regards to the differences related to LC-PUFAs, these follow the same pattern as the *Artemia* metanauplii treatments. Larvae fed *Artemia* metanauplii enriched with C presented significantly higher arachidonic (ARA 20:4 n-6) end eicosapentaenoic (EPA 20:5 n-3 compared to LM1 and LM2. Docosahexaenoic acid (DHA 22:6 n-3) was found significantly higher in LM1 and LM2 compared to C. Concordantly, the ratio n-3/n-6 PUFA was significantly higher in larvae fed *Artemia* metanauplii enriched with LM1 and LM2, the amount of EPA+DHA was significantly higher in LM2 compared to LM1 and C and in LM1 compared to C and the ratio EPA/DHA was significantly lower in LM2 compared to LM1 and C and in LM1 compared to C.

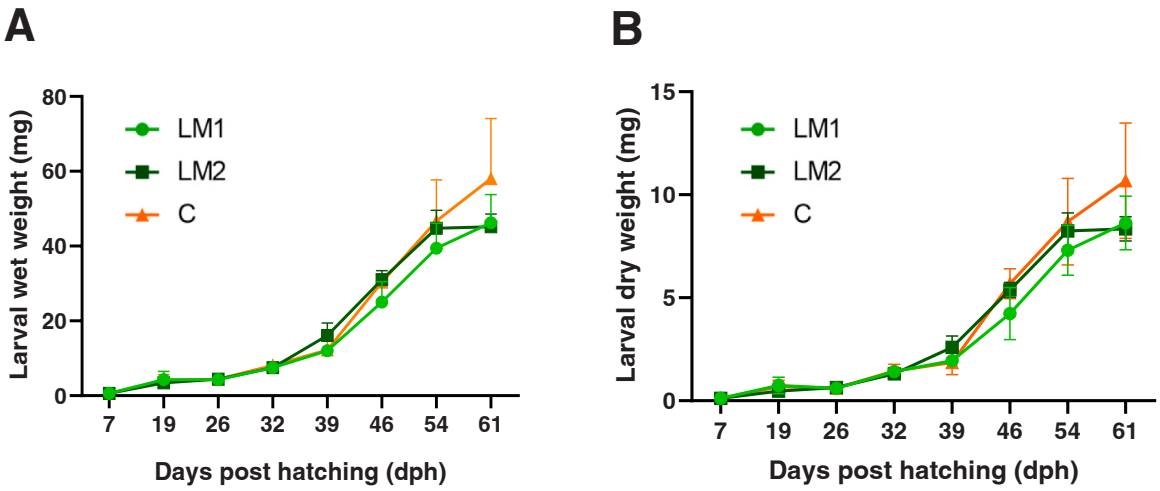

Fig. 1. Larval wet weight (A) and dry weight (B) of European seabass fed *Artemia* metanauplii enriched with the experimental enriching products LM1, LM2 and C from 7 to 60 dph.

Table 2
Fatty acid composition (% of total fatty acids) of European seabass larvae fed *Artemia* metanauplii enriched with the experimental enriching products LM1, LM2 and C at the end of the trial.

	LM1	LM2	C	*P* value
11:0	4.32 ± 0.22	3.79 ± 0.20	4.07 ± 0.27	ns
14:0	1.28 ± 0.03^{c}	1.05 ± 0.07^{b}	0.60 ± 0.06^{a}	0.001
15:0	0.25 ± 0.01^{a}	0.27 ± 0.02^{a}	0.29 ± 0.03^{b}	0.007
16:0	17.25 ± 0.24	17.09 ± 0.47	17.40 ± 0.49	ns
17:0	0.92 ± 0.05	0.91 ± 0.03	0.93 ± 0.02	ns
18:0	10.06 ± 0.09	10.30 ± 0.09	9.86 ± 0.16	ns
20:0	0.27 ± 0.03	0.28 ± 0.03	0.27 ± 0.02	ns
22:0	0.52 ± 0.10	0.47 ± 0.05	0.55 ± 0.03	ns
Σ saturated	34.86 ± 0.33	34.16 ± 0.65	33.98 ± 0.69	ns
14:1 n-5	0.14 ± 0.01	0.14 ± 0.02	0.15 ± 0.02	ns
16:1 n-7	0.84 ± 0.02^{ab}	0.78 ± 0.03^{a}	0.89 ± 0.04^{b}	0.031
16:1 n-9	0.53 ± 0.02	0.52 ± 0.02	0.53 ± 0.02	ns
18:1 n-7	5.12 ± 0.11	5.01 ± 0.10	5.25 ± 0.09	ns
18:1 n-9	12.49 ± 0.11	12.27 ± 0.08	12.28 ± 0.12	ns
20:1 n-9	0.81 ± 0.03	0.80 ± 0.03	0.81 ± 0.04	ns
22:1 n-9	0.23 ± 0.03	0.22 ± 0.02	0.25 ± 0.07	ns
24:1 n-9	0.58 ± 0.02	0.62 ± 0.09	0.56 ± 0.03	ns
Σ monounsaturated	20.75 ± 0.21	20.35 ± 0.16	20.72 ± 0.21	ns
16:2 n-6	0.06 ± 0.04	0.05 ± 0.01	0.06 ± 0.01	ns
18:2 n-6 (LOA)	2.62 ± 0.06^{a}	2.56 ± 0.08^{a}	2.94 ± 0.09^{b}	0.011
18:3 n-6	0.14 ± 0.01	0.16 ± 0.03	0.13 ± 0.02	ns
20:2 n-6	0.55 ± 0.07	0.56 ± 0.06	0.57 ± 0.03	ns
20:3 n-6 (DGLA)	0.14 ± 0.02^{a}	0.15 ± 0.03^{a}	0.19 ± 0.02^{b}	0.001
20:4 n-6 (ARA)	4.84 ± 0.08^{a}	4.86 ± 0.14^{a}	5.74 ± 0.11^{b}	0.000
22:4 n-6	0.21 ± 0.03	0.25 ± 0.12	0.24 ± 0.04	ns
22:5 n-6 (DPA n-6)	2.97 ± 0.07^{a}	3.09 ± 0.17^{a}	3.98 ± 0.12^{b}	0.000
Σ n-6 PUFA	11.54 ± 0.21^{a}	11.67 ± 0.22^{a}	13.85 ± 0.19^{b}	0.000
16:3 n-3 (HTA)	0.35 ± 0.08	0.35 ± 0.09	0.32 ± 0.09	ns
18:3 n-3 (ALA)	6.75 ± 0.15	6.22 ± 0.18	6.98 ± 0.22	ns
18:4 n-3 (SDA)	0.55 ± 0.02	0.53 ± 0.03	0.58 ± 0.04	ns
20:4 n-3 (ETA)	0.52 ± 0.14	0.47 ± 0.07	0.50 ± 0.04	ns
20:5 n-3 (EPA)	5.33 ± 0.08^{a}	5.30 ± 0.15^{a}	5.60 ± 0.13^{b}	0.010
22:5 n-3 (DPA n-3)	0.76 ± 0.03^{a}	0.72 ± 0.05^{a}	0.89 ± 0.06^{b}	0.011
22:6 n-3 (DHA)	17.99 ± 0.29^{b}	19.63 ± 0.62^{b}	15.94 ± 0.50^{a}	0.000
Σ n-3 PUFA	32.24 ± 0.29^{a}	33.21 ± 0.70^{a}	30.81 ± 0.63^{b}	0.004
Σ polyunsaturated	43.78 ± 0.38	44.88 ± 0.76	44.66 ± 0.77	ns
n-3/n-6	2.79 ± 0.06^{b}	2.85 ± 0.06^{b}	2.22 ± 0.03^{a}	0.000
EPA+DHA	23.31 ± 0.3^{b}	24.92 ± 0.72^{c}	21.55 ± 0.61^{a}	0.000
EPA/DHA	0.30 ± 0.01^{b}	0.27 ± 0.01^{a}	0.35 ± 0.01^{c}	0.000

Data are given as the mean (n=3) ± SD. Significant differences among treatments were detected P ≤ 0.05 by using one way ANOVA + Tukey's test.

3.3. Oxidative status

Biomarkers related to oxidative status measured on European seabass larvae fed *Artemia* metanauplii enriched with the different products are shown in Fig. 2. At the end of the trial (60 dph), CAT activity was found significantly lower in larvae fed *Artemia* metanauplii enriched with LM2 compared to C (P = 0.005). No significant differences were detected in mtROS, PC and LPO among experimental groups, although it was observed a trend to higher mtROS and LPO in larvae fed *Artemia* metanauplii enriched with LM2 and LM1, respectively.

3.4. Skeletal anomalies

All larvae with a non-inflated swim bladder (N = 166) were excluded from the analysis and the microscopic analysis to identify skeletal anomalies was performed on the remaining individuals (N = 693). Representative micrographs of the recorded anomalies in the three analysed groups are reported in Fig. 3. No significant differences between the three experimental groups were found in the relative frequency (%) of individuals with at least one severe anomaly (LM1 = 47.5 ± 2.1; LM2 = 34.2 ± 10.7%; C = 37.5 ± 16.4%; P > 0.05) and in the severe anomalies load (LM1 = 2.5 ± 0.1; LM2 = 2.4 ± 0.5; C = 2.7 ± 0.2; P > 0.05) (Table 3). The relative frequencies of severe anomalies observed in each of the four regions of the vertebral column and in the skull are reported in Fig. 4. Most of the severe anomalies affected the caudal vertebral region (LM1 = 58.8 ± 13.3; LM2 = 49.8 ± 10.9; C = 41.0 ± 2.7%), followed by skull anomalies in LM1 (14.7 ± 3.8%) and LM2 (17.4 ± 9.6%), and by anomalies of the cranial region of the vertebral column in C (29.1 ± 5.7%). No statistical differences were observed in the frequency of severe anomalies in any of the examined body regions (P > 0.05 for all comparisons).

4. Discussion

One of the most fascinating aspects of phototrophic production of microalgae and cyanobacteria is the important remediation service offered to the environment (Chauton et al., 2015; Fernández et al., 2020; Mishra et al., 2023). By performing photosynthesis, this microorganisms convert inorganic nutrients, carbon dioxide (CO_2) and light radiation into valuable components, such as oils rich in long chain polyunsaturated fatty acids (LC-PUFAs), while releasing oxygen in the environment. In the case of the present study, the use of geothermic resources provides a constant source of heat and CO_2 contributing to reduce 80% of energy consumption, and at the same time, allowing to

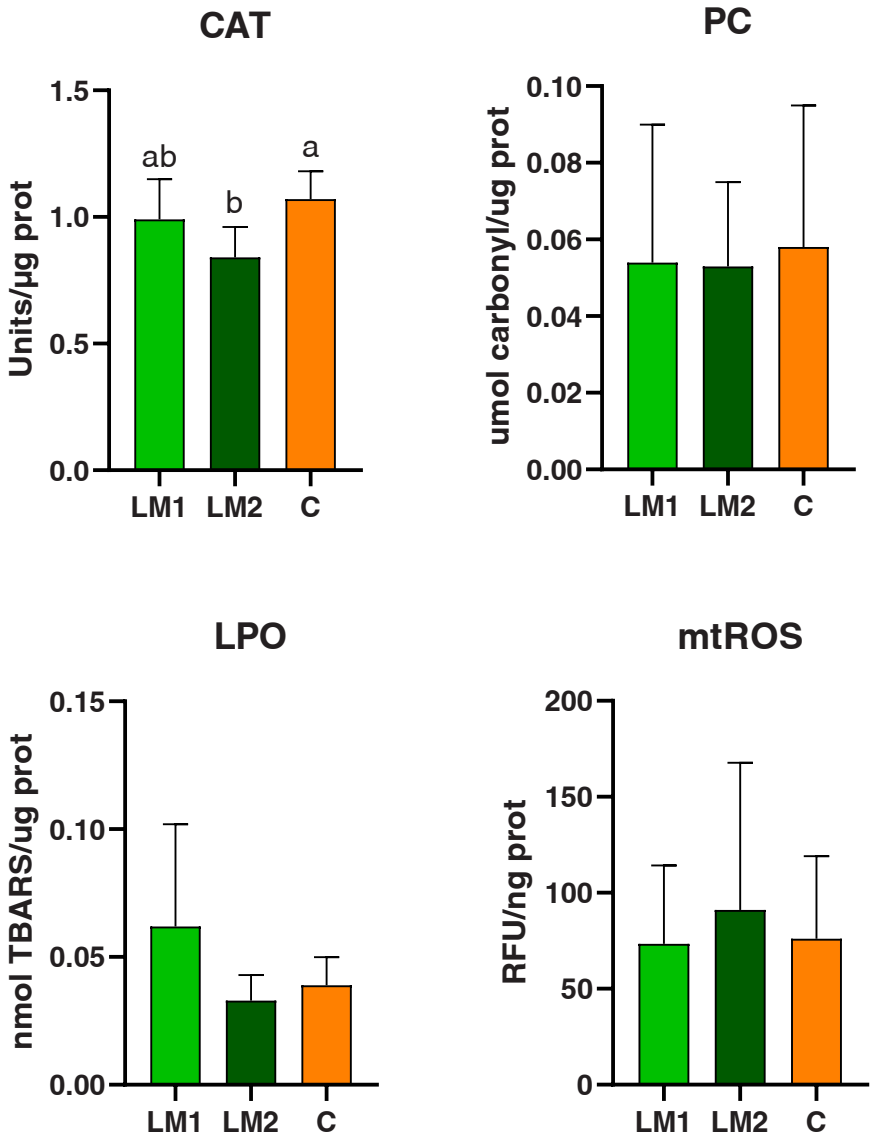

Fig. 2. CAT (catalase activity), mROS (mitochondrial reactive oxygen species), PC (protein carbonylation) and LPO (lipid peroxidation) levels in European seabass fed *Artemia* metanauplii enriched with the experimental enriching products LM1, LM2 and C at 60 dph. Data are given as the mean (n = 3) ± SD. Significant differences among treatments were detected $P \leq 0.05$ by using one way ANOVA + Tukey's test for CAT, mROS and PC and by Kruskal-Wallis non-parametric test for LPO.

obtain a constant product quality all-year round. This is not a minor point because oils derived from wild marine fish suffer from seasonal composition variability which can affect the final fatty acid profile of the enrichment (Gámez-Meza et al., 1999; Zlatanos and Laskaridis, 2007). The present study contributes to expanding the existing literature on successful utilisation of microalgal based products, produced in a perspective of circular nutrients economy (Shah et al., 2018; Tocher et al., 2019; Yarnold et al., 2019; Santigosa et al., 2021; Chen et al., 2021; Harder et al., 2021; Carvalho et al., 2022; Mota et al., 2023). By feeding European seabass larvae with *Artemia* metanauplii enriched with two experimental products (LM1 and LM2) and a commercial control (C), the same results in terms of growth performance, survival rate and skeletal anomalies were obtained among the experimental groups.

In terms of growth performance indicators, these outcomes are in line with previous studies conducted on European seabass larvae reared with a similar protocol (Sfakianakis et al., 2013; Yan et al., 2019; El-Dahhar et al., 2022; El Basuini et al., 2022). Like the majority of marine fish species, European seabass produce altricial larvae, which typically experience high mortality rates during the initial feeding phase. Compared to previous studies conducted by Vanderplancke et al. (2015) and Darias et al. (2010) reporting a survival rate at 45 dph of 45% and 68% respectively, the observed survival rates in the present study, ranging from 61% to 68% at 60 dph, were high in all experimental groups. Larvae mortality occurs both in nature and in captive conditions mostly during the critical phase of yolk sac reabsorption and mouth opening at the beginning of exogenous feeding (Yúfera and Darias, 2007). This is a challenge in fishery biology (Houde, 2008) and the underlying mechanism is not yet completely understood, although feed availability and quality are the main factors explaining mortality in experimental conditions (Cushing, 1973; Malzahn et al., 2022; Benini et al., 2022). Nutritional blunders or deficiency occurring during this delicate phase may impact the correct maturation of the digestive system, the oxidative status and the skeletal ossification of adult fish (Izquierdo et al., 2013; Pham et al., 2023; Wischhusen et al., 2022), therefore it is important to provide larvae with an adequate level of nutrients. Among nutrients, dietary lipids are the main energy source for developing fish larvae. Lipids provide LC-PUFAs, which are needed for

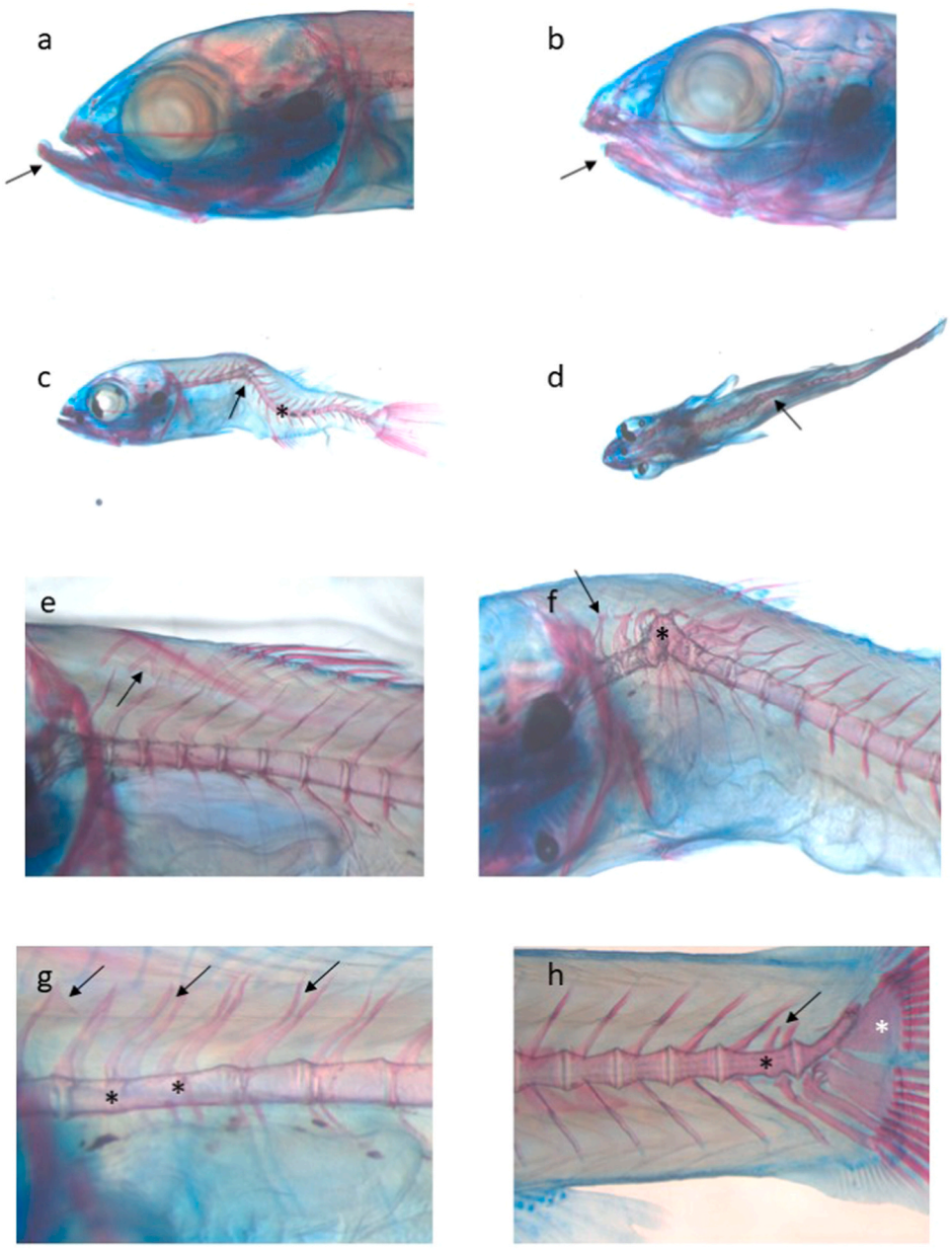

Fig. 3. Skeletal anomalies in European seabass assessed at 60 dph. (a) Anomalous dentary (prognathism) (arrow), (b) Anomalous dentary (lower jaw reduction) (arrow). (c) Pre haemal kyphosis (arrow) haemal lordosis (asterisk). (d) Scoliosis between pre haemal and haemal region (arrow). (e) Anomalous (forked) pre dorsal bone. (f) kyphosis, fused vertebrae and deformed bodies of vertebrae (asterisk) between cranial and pre haemal region; anomalous neural spines are also visible (arrow), (g) fused cranial vertebrae (asterisks); anomalous neural spines are also visible (arrows), (h) fused caudal vertebrae (black asterisk); anomalous neural spine (arrow); fused hypural (white asterisk).

Table 3
Skeletal anomalies of European sea bass larvae fed *Artemia* metanauplii enriched with the experimental enriching products LM1, LM2 and C at the end of the trial.

	LM1	LM2	C	*P* value
Number of examined individuals	195	279	219	ns
Relative frequency of individuals with at least one severe anomaly (%)	47.5 ± 12.1	34.2 ± 10.7	37.5 ± 16.4	ns
Severe anomalies load	2.5 ± 0.1	2.4 ± 0.5	2.7 ± 0.2	ns

Data are mean of three subgroups (tanks) ± SD. No significant differences among LM1, LM2 and C groups were detected (ANOVA, $P > 0.05$).

the synthesis of new cellular structures (Gisbert et al., 2005) and for the primary role on larval survival, growth and immune system modulation (Tocher et al., 2019; Roo et al., 2019; Betancor et al., 2021; Pham et al., 2023).

In this trial, the three enriched *Artemia* metanauplii dietary treatments were isonitrogenous and isolipidic despite some differences in the fatty acids profiles. Due to the importance of bioactive LC-PUFAs such as DHA, EPA and ARA in larval nutrition (Tocher, 2015), this discussion is focused on the implications related to their contents, overlooking other differences observed in less bioactive fatty acids. With regard to the total amount of n-3 PUFAs evaluated in *Artemia* metanauplii in this trial, no difference among the different dietary treatments were observed. However, within n-3 PUFAs, DHA was more abundant in LM1 and LM2 enriched *Artemia* metanauplii while EPA in C. DHA plays an important

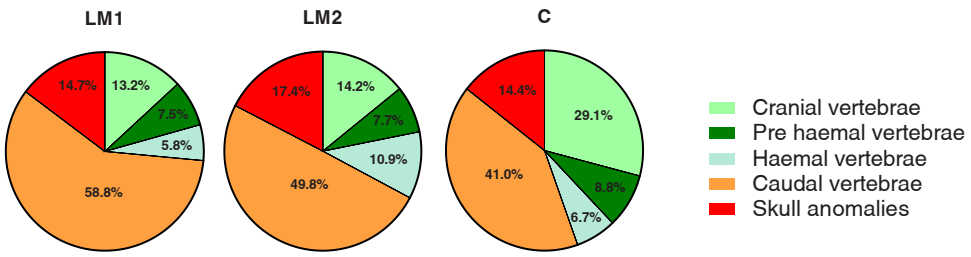

Fig. 4. Relative frequencies (%) of severe anomalies in the four regions of the vertebral column and in the skull of European seabass fed *Artemia* metanauplii enriched with the experimental enriching products LM1, LM2 and C at60 dph. Data are given as the mean (n = 3) ± SD. No significant differences among LM1, LM2 and C were detected $P > 0.05$ by using one way ANOVA.

role in the development of the visual and neural system of marine fish larvae and its deficiency may affect the development of the predatory behaviour resulting in a lower survival rate (Watanabe, 1993; Izquierdo et al., 2013; Roo et al., 2019; Pham et al., 2023). A functional evidence of DHA importance is represented by the very high content of DHA in marine fish eggs (Anderson et al., 1984; Watanabe, 1993; Xu et al., 2017). Still within n-3 PUFA fatty acid family, EPA plays an important role in larvae metabolism, since it regulates cell membranes integrity and function and enhances their fluidity (Izquierdo, 2005; Hashimoto and Hossain, 2018) much more than arachidonic acid (ARA) (Hagve et al., 1998), but less than DHA (Hashimoto et al., 1999). Moreover, it is considered an important regulator of eicosanoids production (Adam et al., 2017; Sissener et al., 2020), being a major substrate for both cyclooxygenases and lipoxygenase enzymes and the main precursor for leukotriene synthesis in many fish species (Izquierdo, 2005; Montero et al., 2015, 2019). Another metabolically active LC-PUFA from the n-6 family is ARA. In this trial, *Artemia* metanauplii enriched with C showed an increased content of ARA. This is not surprising since ARA is highly present in fish-derived products and C is an encapsulated fish-oil based enrichment specifically supplemented with ARA, as stated in the product label. ARA deficiency in aquafeed formulation become more of a problem with the reduction of fish meal and oil (Araújo et al., 2022). ARA is involved in important physiological and immunological functions in marine fish, such as the eicosanoids production, being the main precursors for prostaglandin synthesis (Izquierdo, 2005). In addition, ARA is also involved in the stress response (Martins et al., 2013). The concern over n-6 PUFA derived eicosanoids is due to the dogma of n-6 PUFA derived eicosanoids being pro-inflammatory, as opposed to the n-3 PUFA derived being anti-inflammatory (Hundal et al., 2021; Dong et al., 2023). As a consequence, DHA, EPA and ARA are considered essential fatty acids for the majority of marine fish and are required to be supplied in the diet. Marine species do not have sufficient Δ12 (or n-6) and Δ15 (or n-3) desaturases and elongase activities to produce them from oleic (18:1 n-9) acid precursor (Furuita et al., 1996; Izquierdo, 2005; Tocher et al., 2019), hence their requirements have been extensively investigated in many marine species (Watanabe et al., 1989; Izquierdo, 2005; Izquierdo et al., 2013; Navarro-Guillén et al., 2014; Roo et al., 2019; de Mello et al., 2022). An optimal level is set at 0.6–2.3% for DHA, 0.7–2.3% EPA and up to 1% ARA considered as percentage on dry feed (Izquierdo, 2005), which are met in all experimental groups (data on % of fatty acid/dry feed not shown). What is more difficult is understanding the mechanisms of interaction between the LC-PUFAs. For instance, evidence of competition of LC-PUFAs on the same enzymatic pathways such as lipoxygenases, cyclooxygenases and phospholipases have been reported by several authors (Izquierdo, 2005; Norambuena et al., 2012; Kumar et al., 2016). As a results, when it comes to model the LC-PUFAs requirements, their ratio has to be carefully adjusted. Izquierdo, (2005) and Houston et al. (2017) proposed a ratio of 1:1 (EPA/DHA) with ARA values higher than 0.5% and a total 3% of LC-PUFAs, ideal for gilthead seabream growth. Betancor et al. (2016) found reduced growth when juveniles of gilthead seabream were fed diets with high EPA/DHA ratios, emphasising the importance of DHA. A similar superiority of DHA to EPA has been proposed also by Wu et al. (2002), reporting an increased growth in juveniles grouper (*Epinephelus malabaricus*) with lower ratio of EPA/DHA.

The fatty acid profile of European seabass fingerling at 60 dph reflected the composition of their diets, as observed by several authors (Glencross, 2009; Boglino et al., 2012), with some differences, related to the metabolic transformation occurring in fish, resulting in a compensatory effects between the LC-PUFAs feed input and the final LC-PUFAs output deposited in larvae flesh. For instance, EPA content in *Artemia* metanauplii was 49% and 43% more in C compared to LM1 and LM2, respectively, while in larvae was smoothed over to an only 5% more in C compared to both LM1 and LM2. Likewise, ARA content in *Artemia* metanauplii was 55% more in C compared to both LM1 and LM2 while in larvae 18% more in C compared to both LM1 and LM2. Interestingly the ratio of difference in DHA content remained the same in *Artemia* metanauplii and larvae (approximately 14% and 25% lower in C compared to LM1 and LM2 respectively in both *Artemia* and larvae). Overall data on LC-PUFAs composition of larvae are in line with previous experiments conducted on gilthead seabream (Koven et al., 2001), European seabass (Gisbert et al., 2005), turbot (*Scophthalmus maximus*) and Senegale sole (*Solea senegalensis*) (Boglino et al., 2012), showing enough nutrients stored for the further development in all experimental groups. This statement is confirmed by the absence of differences, among the dietary treatments, in the n-9 PUFAs (particularly 18:1 n-9 and 20:1 n-9) contents in larvae, which are produced by elongation and increased values were proposed as indicator of LC-PUFAs deficiency in gilthead seabream (Rodriguez et al., 1994) and grouper (Wu et al., 2002).

Excessive LC-PUFAs in diets may lead to increased risk of lipid peroxidation (Betancor et al., 2015; Qian et al., 2015; Adam et al., 2017), which is mediated by free radicals and reactive oxygen species (ROS) production (oxidative factors). Nevertheless, a recent study described the antioxidant protection capacity of EPA and DHA against cellular oxidative stress in humans (Aldhafiri, 2022). In line with this, dietary supplementation with a mixture of EPA + DHA has been reported to exert an antioxidant protective role in fish (Kumar et al., 2022). Balanced concentrations of anti- and pro-oxidant factors are continuously generated during regular cellular metabolism, which is known as ROS homeostasis (Lushchak, 2016). Between the metabolic antioxidant factors, the enzyme catalase catalyses the decomposition of hydrogen peroxide to water and oxygen, preventing the cascade of oxidation reactions and closing the lipid peroxidation catalytic cycle (Betancor et al., 2012). However, if ROS generation exceeds the antioxidant protection, the imbalance is called oxidative stress and may lead to reduced larval growth (Betancor et al., 2012; Roo et al., 2019), severe dystrophic lesions in the musculature (Betancor et al., 2012) and higher skeletal anomalies rate (Izquierdo et al., 2010). Lipid peroxidation and its by-products, such as protein carbonylation (Suzuki et al., 2010), have been commonly used as biomarkers of oxidative status in fish larvae (Monroig et al., 2006; Betancor et al., 2012; Guerreiro et al., 2022). In this trial, although not statistically significant, larvae from LM2 treatment group showed a trend to higher mitochondrial ROS production. By

contrast, this group presented statistically lower catalase activity than larvae from C treatment and did not present any tendency to higher oxidative damage (measured as LPO, PC and larval growth performance). From a nutritional point of view, microalgae and cyanobacteria display diverse bioactive compound profiles, being those with antioxidant properties of particular interest. In that sense, both *Nannochloropsis* sp. and *Spirulina* sp. are rich in the pigments β-carotene and zeaxanthin, although, on a dry weight basis, *Spirulina* sp. contains 2.5 and 15-fold higher β-carotene and zeaxanthin than *Nannochloropsis* sp., respectively (Ghaeni et al., 2015; Bernaerts et al., 2020). In addition, *Spirulina* sp. is also rich in phycocyanin, a blue-coloured photosynthetic pigment with free radical scavenging capacity (Fernandes et al., 2023). Altogether, the higher EPA+DHA content found in *Artemia* enriched with LM2 and the higher content in antioxidant compounds of *Spirulina sp.*, results might support a potential preventive effect against oxidative stress of LM2 enrichment (Coulombier et al., 2021; Vignaud et al., 2023). However, further studies are needed to conclusively unravel the antioxidant effects of LM2 blend.

Several skeletal anomalies have been described in marine fish, representing a major problem in aquaculture for the economical, biological and ethical related concerns (Boglione et al., 2013). Anomalies in fish are often associated with a reduced growth and high mortality rate, being a major problem in a hatchery setting due to the derived economical losses. In this trial, the morphological analysis of European seabass did not show an effect of the microalgal enrichments LM1 and LM2 on the total number of severe anomalies and the relative frequencies in the different regions of vertebral column, compared to C. In a wild contest, it is fair to presume that individuals with physical anomalies will have a reduced capacity to swim properly, feed or escape a predator, thus reducing its likelihood to survive. In a survey conducted on wild populations by Boglione et al. (2001) a 4% of wild gilthead seabream presented some severe anomalies. On the contrary, in a hatchery setting, with controlled environmental conditions, food availability and absence of predation, the anomalies rate is expected to be higher. The findings from this study are in line with surveys conducted by Koumoundouros (2010) and Boglione et al. (2013), reporting an incidence of severe anomalies in Mediterranean marine hatcheries of 30–40% of the reared fish. A recent study conducted by (Viegas et al., 2023) on European seabass reported an incidence of severe anomalies of 33–37% at 64 dph. Even though anomalies in fish have been often associated with nutritional causes such as nutrients deficiency (Boglione et al., 2013) or nutrients unbalancing (Izquierdo et al., 2013; Roo et al., 2019), the severe anomalies observed in this trial are more likely to be attributed to general causes such as tank hydrodynamics, temperature and other abiotic and biotic factors.

5. Conclusion

Producing microorganisms such as microalgae and cyanobacteria exploiting industrial geothermal waste outputs could mitigate the overexploitation of marine fish stocks and reduce the costs associate with photobioreactors energy consumption. The results obtained in this study indicated that using blends of so produced products for the enrichments of *Artemia salina* produced equal results in terms of growth performance and larvae anomaly rate compared to a commercial control. Each dietary treatment presented a specific accumulation pattern for ARA, DHA and EPA long chain polyunsaturated fatty acids. Ratio of DHA/EPA of 4:1 as in the case of LM1 and LM2 in the presence of 1.4% of ARA (as a % on total fatty acid) guaranteed equal results to the control which display a ratio of DHA/EPA of 2:1 with 2.2% of ARA. The fatty acids profile of European seabass larvae sampled at 60 dph reflected the composition of the diet, showing no signs of LC-PUFAs deficiency in every dietary treatment. Results on the larvae oxidative status suggested that LM2 enrichment product may exert potential preventive effects against oxidative stress, which could be translated into enhanced fish larvae robustness and, in the long-term, improved health status of the fish. After the radiological survey, European seabass larvae at 60 dph presented a percentage of anomalies ranging from 34% to 47% in all dietary treatments. The relative frequency of anomaly mainly affected caudal vertebrae, followed by skull, cranial vertebrae, pre-haemal vertebrae and haemal vertebrae in all groups. These findings are in line with other research conducted on the species and on other Mediterranean marine fish.

CRediT authorship contribution statement

Chrysovalentinos Pousis: Investigation, Methodology, Writing – review & editing. **Manuel Yúfera:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Isabel Medina:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Maria Jesus González:** Investigation, Methodology. **Pier Paolo Gatta:** Conceptualization, Writing – review & editing. **Andrea Bertini:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Christel Nys:** Conceptualization, Methodology, Writing – review & editing. **Gianluca Ventriglia:** Investigation, Methodology, Writing – review & editing. **Carmen Navarro-Guillén:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Luca Parma:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Elisa Benini:** Methodology, Writing – review & editing. **Alessio Bonaldo:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Arianna Marchi:** Investigation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests Author C.N is employed by VAXA technologies Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

Data will be made available on request.

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