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Effects of graded levels of a blend of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass on growth and muscle tissue composition of European sea bass (*Dicentrarchus labrax*) fed diets low in fish meal and oil

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ABSTRACT

The aim of this study was to evaluate nutrient digestibility, growth response, carcass and fillet yields, muscle tissue composition and skin colour appearance of European sea bass (*D. labrax*) fed graded levels of a mixture of *Tisochrysis lutea* and *Tetraselmis suecica* freeze-dried biomass in partial substitution for fish meal and oil in diets containing substantial levels of vegetable oils and protein-rich derivatives. Five complete diets were formulated to be grossly isoproteic and isolipidic and prepared by including a blend of the two freeze-dried microalgae biomass in a 2:1 weight ratio, to replace approximately 15, 30 and 45% fish meal protein and 12, 24 and 36% fish lipid of a positive control diet. A negative control complete feed high in soybean meal was also prepared. Each diet was offered in triplicate, during 105 days until visual satiety of fish (204 ± 12.7 g) kept in a semi-closed recirculating marine water system ensuring optimal water quality to E. sea bass.

The results of the study have shown that replacing about 45% crude protein and 36% lipid from fish meal and lipid by a mixture of *Tisochrysis lutea* and *Tetraselmis suecica* dried biomass, did not adversely affect growth performance and feed conversion efficiency of European sea bass. A slight decline in dry matter, protein and energy digestibility occurred in response to graded levels of dietary microalgae biomass, which was compensated by increased feed intake. Moreover, the diet including the dried microalgae resulted in a higher nutritive value than that of the negative high-soybean meal control feed. No major changes were observed in biometry traits and slaughter yield while the nutritional properties of the edible muscle tissue were little affected in terms of n-3 PUFA. The presence of the dried microalgae in the diet resulted in a greenish pigmentation of the skin, with a slight tendency towards redness and diminished lightness and hue. Overall the results obtained here reveal a potential improvement in sustainability in terms of reduced reliance on halieutic feed resources in case of the microalgae-containing diets. Hence the sustainable use of the dried mixture of the 2 marine microalgae biomass as feed ingredients in diets for the E. sea bass seems to be mainly limited by the low availability and unaffordable market price which are both expected to improve over the next 10 years.

1. Introduction

Among feed resources quoted as possible candidate ingredients for more sustainable aquafeeds, single cell microorganisms have recently deserved renewed attention. Whole-cell dried microalgae biomass in particular, due to a minimal overall environmental impact compared to most conventional feed/food commodities, have been proposed as raw materials in partial substitution for fish-derivatives in fish diets, even if scarce availability and high market price still set a limit to their use in commercial aquafeeds (Muller-Feuga, 2004; Shields and Lupatsch, 2012; Zmora and Richmond, 2004). According to Draaisma et al. (2013) the feed-mill industry requires large quantities of biomass produced at low cost (< 1 € kg^{-1}) whereas independent economic estimates and analysis indicated the production costs of 1 kg of dried weight microalgae biomass to lie in a range of 4.14 to 5.96 € (Norsker et al., 2011), or 3.2 to 12.4 € (Tredici et al., 2016), depending on the species, cultivation system, geographic location and scale of production. Based on the potential improvements in productivity, it is assumed

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that over the next 5–10 years a sustainable reduction of microalgae production costs will be achieved.

As sources of nutrients, dry microalgae biomass are characterised by medium to high crude protein levels, ranging from 28 to 71% DM, depending on the species and culture/harvesting conditions, with an amino acid profile comparable with, or even better than that of conventional plant crops or vegetable feeds rich in protein (Becker, 2007). Cell wall structure and composition have been claimed to adversely affect bioavailability of nutrients supplied by certain microalgae species but, at the moment, this general assumption remains speculative in fish, where information on digestibility of different dried microalgae biomass or diets including them at substantial inclusion levels is still very limited (Burr et al., 2011; SØrensen et al., 2016; Tibaldi et al., 2015; Walker and Berlinski, 2011). Despite this, previous studies have shown dried biomass of a number of different microalgae species to be a valuable supplementary protein source or partial substitute for fish meal protein in the diet of various omnivorous and carnivorous fish species at the juvenile stage (Badawy et al., 2008; Belay et al., 1996; Hussein et al., 2013; Kiron et al., 2012, 2016; Nandeesha et al., 2001; Olvera-Novoa et al., 1998; Tulli et al., 2012; Vizcaìno et al., 2014, 2016; Walker and Berlinsky, 2011).

Certain marine microalgae are also rich in lipid and n-3 long chain polyunsaturated fatty acids (n-3 (LC)PUFAs) and dry preparations of docosahexaenoic acid (DHA)-rich *Schizochytrium* spp., *Crypthecodinium chonii, Nannochloropsis* sp., *Pavlova viridis* have proven successful as partial replacers for fish oil in starter diets for the juvenile stages of certain fish species (Atalah et al., 2007; Carter et al., 2003; Haas et al., 2015; Miller et al., 2007; Sarker et al., 2016b).

In this context, the dry biomass of *Tisochrysis lutea,* formerly *Isochrysis galbana* (T-iso), has been poorly studied so far, but it deserves attention as it combines medium-high levels of protein with high lipid and DHA contents (Ben-Amotz et al., 1987; Sánchez et al., 2000). Furthermore, *Tisochrysis lutea* being small in size (4–6 μm) and lacking a structured cell wall (naked) is expected to be readily digested (Vizcaìno et al., 2016). All these attributes make it a potential candidate ingredient for diets low in fish meal and fish oil for the E. sea bass which, besides requiring high protein diets, is notably incapable of significant n-3-(LC)PUFAs biosynthesis (Mourente et al., 2005) and where a huge substitution of fish oil by vegetable counterparts has been shown to be detrimental in terms of healthy attributes of the flesh due to a marked reduction of the n-3(LC)PUFA content (Montero et al., 2005; Mourente and Bell, 2006). Encouraging results in this direction have been recently observed in this marine fish species (Tibaldi et al., 2015), in that the use of dried *Tisochrysis lutea* biomass to replace up to 20% crude protein from fish meal and up to 36% fish lipid in a diet with reduced level of fish oil, did not adversely affect feed intake or growth performance. However, this resulted in a slight decline of energy digestibility and n-3(LC)PUFA content in muscle tissue.

Tetraselmis suecica (Prasinophyceae, Chlorophyta) is also of particular interest being medium-high in protein and relatively easily mass-cultivable at low cost, when produced in a new generation of photobioreactors (Tredici et al., 2015). *Tetraselmis* has a pretty thin cell wall and is widely used in hatcheries for feeding juvenile bivalve molluscs, penaeid shrimp larvae and rotifers as a source of nutrients and n-3(LC) PUFA being relatively rich in eicosapentaenoic acid (EPA) (Brown et al., 1997). When E. sea bass were fed to satiety complete diets where *T. suecica* was up to 16% by weight, a linear decline in nutrient digestibility was observed althought without adversely affecting the growth performance of the fish (Tulli et al., 2012).

It should be noted that, in most of the studies carried out so far to evaluate fish response to variable levels of different microalgae dried biomass in feeds, the test diets were compared to control preparations largely based on fish meal and oil as major protein and lipid sources. This could have led to underestimate the nutritive value of microalgae when compared with that of protein-rich plant and oil sources which are currently increasingly being used as major alternatives to fish meal and oils in commercial aquafeeds.

Besides the nutritive value to fish, microalgae are also a source of pigments and their inclusion in the diet has been shown to affect the colour appearance of a range of fish species including marine ones (Chatzifotis et al., 2011; Ribeiro et al., 2017; Tulli et al., 2012; Tibaldi et al., 2015; Walker and Berlinski, 2011). This is particularly important since colour and visual appearance are known to influence market value, flavour perception and acceptability in market size specimens of fish food products (Spence et al., 2010; Vasconcellos et al., 2013).

On this basis, the aim of this study was to evaluate growth response, dressing out yield, muscle tissue composition and skin colour appearance of E. sea bass (*D. labrax*) fed graded levels of a mixture of freeze-dried *T Isochrysis lutea* and *Tetraselmis suecica* in partial substitution for fish meal and oil in diets containing substantial amounts of vegetable oils and protein-rich plant derivatives.

2. Materials and method

2.1. Test ingredients and diets

The chemical composition and fatty acid profile of the freeze-dried *T. lutea* and *T. suecica* are shown in Tables 1 and 2, respectively.

Five diets were formulated to be grossly isoproteic (49.3% DM) and isolipidic (18.5% DM). The ingredient and proximate composition of the diets and fatty acid profile are shown in Tables 3 and 4, respectively. A positive control diet (C +) was prepared in order to have

Table 1
Chemical composition of *T. lutea* and *T. suecica* freeze-dried biomass (data expressed on dry matter basis).

	T. lutea	*T. suecica*
Proximate composition (%)		
Water	10.0	5.9
Crude protein	46.3	48.7
Total lipid	26.0	8.0
Ash	11.3	17.5
Phosphorous (g/kg)	0.8	1.1
ß-carotene (mg/kg)	761.7	267
Essential AA (%)		
Arginine	2.52	2.05
Histidine	0.91	0.72
Isoleucine	1.76	1.41
Leucine	3.92	3.27
Lysine	2.46	2.27
Methionine + cysteine	1.41	1.66
Phenylalanine + tyrosine	3.75	3.98
Threonine	2.38	1.81
Tryptophan	0.56	0.30
Valine	2.37	1.87
Non essential AA (%)		
Alanine	3.17	2.86
Aspartic acid	4.19	3.44
Glutamic acid	4.58	5.06
Glycine	2.64	2.52
Proline	2.36	1.80
Serine	2.17	1.67
Fatty acid composition[a]%		
SFA	5.66	1.03
MUFA	3.89	1.07
n-6 PUFA	1.98	2.41
n-3 PUFA	5.52	0.16
20:5n-3	0.19	0.26
22:6n-3	1.81	–
n-3/n-6	2.79	0.07

[a] The following fatty acids were considered in the respective composite fractions but are not shown in the table: 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 18:0, 20:0; 14:1n-5; 16:1n-7, 16:1n-9, 17:1, 18:1n-9, 18:1n-7, 20:1n-9, 20:1n-11, 22:1n-9, 22:1n-11; 16:2n-4, 18:2n-6, 18:3n-6, 20:4n-6, 22:5n-6; 16:3n-4, 18:3n-3; 16:4n-1, 18:4n-1, 18:4n-3, 20:4n-3, 22:4n-6, 21:5n-3; 22:5n-3.

Table 2
Fatty acid profile of freeze-dried microalgae *T. lutea* and *T. suecica* (% FAMES).

	T. lutea	*T. suecica*
12:0	2.1	0.1
14:0	10.5	1.2
16:0	6.8	17.6
18:0	1.1	0.2
20:0	1.6	–
SFA[a]	32.3	21.9
16:1 n-7 + 9	6.3	5.8
18:1 n-9	9.4	11.4
18:1 n-7	2.1	1.4
20:1 n-9	0.5	1.3
MUFA[a]	22.6	22.9
18:2 n-6	5.8	3
18:3 n-6	1.6	0.1
20:4 n-6	0.5	0.3
22:5 n-6	2.1	–
n-6 PUFA[a]	11.5	3.4
16:4 n-3	–	15.5
18:3 n-3	7.4	22.5
18:4 n-3	10.4	7.2
20:4 n-3	0.3	0.3
20:5 n-3	1.1	5.6
22:6 n-3	10.5	–
n-3 PUFA[a]	32.0	51.6

[a] The following fatty acids were included in the respective composite fractions but are not shown in the table: 10:0, 11:0, 13:0, 15:0; 14:1n-5; 17:1, 20:1n-11, 22:1n-9, 22:1n-11; 16:2n-4; 16:3n-4, 16:4n-1, 18:4n-1, 22:4n-6, 21:5n-3; 22:5n-3.

approximately a 50:50 ratios between fish and vegetable derived protein and between fish and vegetable derived lipid. Three diets identified as Isotetra 10, 20 and 30, were prepared by including a blend of *T. lutea* and *T. suecica* dried biomass in a 2:1 ratio to replace approximately 15, 30 and 45% of fish meal protein and 12, 24 and 36% of fish lipid from the C + diet. Also a negative control (C −) was prepared to have about 30:70 ratio between fish and vegetable derived protein and 50:50 ratio between fish and vegetable derived lipid. Diets Isotetra 30 and C– were supplemented with L-methionine to keep their sulfur amino acid levels at/or slightly above the requirement of the E. sea bass (Tulli et al., 2010). All ingredients were ground (0.5 mm sieve), mixed, and pelletized through a 4.5 mm die in the pilot plant of the Department of Agrifood, Environment and Animal Science of the University of Udine. The diets were stored at − 20 °C until used.

2.2. Growth trial

One hundred and eighty fish (mean body weight 204 ± 12.7 g) were randomly divided among 15 groups, each consisting of 12 specimens, kept in 300-L fiberglass tanks in a marine semi-closed recirculating aquaculture system (daily replacement rate, 3% in volume with mechanically filtered and UV treated sea water) ensuring optimal water condition for sea bass (temperature 22.8 ± 0.5 °C, salinity 25.8 ± 1.3%, dissolved oxygen 6.8 ± 0.43 mg/L, pH 8 ± 0.13, total ammonia nitrogen 0.04 ± 0.02 mg/L, nitrite-nitrogen 0.2 ± 0.06 mg/L). After stocking, fish were fed diet C + and adapted over 2 weeks to the experimental conditions. After that, dietary treatments were randomly assigned in triplicate to the 15 groups. Fish were fed the experimental diets over 105 days by hand-fed, six days a week in two daily meals (8:00 am and 4:00 pm), until the first feed item was refused. The fish handling procedures and sampling methods used in the trial followed the guidelines of the E. Union directive 2010/63/EU on the protection of animals used for scientific purposes.

At the end of the growth trial, fish were group-weighed after 24 h fasting period, under moderate anaesthesia (25 ppm of Finquel®, Argent Laboratories, Redmont-VI, USA) and final biomass, absolute feed intake (AFI), relative feed intake (RFI), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER), were calculated per each tank.

Three fish per tank were randomly selected, euthanized with an overdose of the same anaesthetic (700 ppm), subjected to individual biometry measurements (total length, body weight) and dissected in order to evaluate major organ weight and slaughter yield. The following parameters were calculated: fillet yield with or without skin, carcass yield, condition factor (CF), hepatosomatic index (HSI), mesenteric fat index (MFI). The muscle mass of the skinned left fillets obtained so far was minced and kept frozen (− 20 °C) to be analysed for proximate and fatty acid analysis. Other 4 fish per tank (12 fish per dietary treatment) were euthanized with an overdose of anaesthetic and immediately subjected to skin colour analysis.

Table 3
Ingredient and proximate composition of the test diets.

	Diets				
	C +	Isotetra 10	Isotetra 20	Isotetra 30	C −
Ingredient composition (g/kg)					
Chile prime fish meal[a]	275	232	190	150	150
Fish solubles (CPSP 90)[b]	50	50	50	50	50
Wheat gluten meal (80.7% CP[c])	200	200	200	200	200
Dehulled soybean meal (48%CP)	150	150	150	150	350
Gelatinized wheat starch	130	121	108	90	40
Cod liver oil	56	51	48	45	62
Palm oil	60	57	55	50	62
Soy lecithin (69.8% CL[c])	25	25	25	25	25
Freeze-dried *Tisochrysis lutea*	0	40	80	120	0
Freeze-dried *Tetraselmis suecica*	0	20	40	60	0
L-Methionine	0	0	0	6	7
Mineral supplement[d]	4	4	4	4	4
Vitamin supplement[e]	5	5	5	5	5
Na lignosulfite	30	30	30	30	30
Celite	15	15	15	15	15
Proximate composition (%)					
Dry matter	93.8	93.8	93.8	93.8	93.1
Crude protein	45.9	46.1	46.7	46.1	46.8
Total lipid	17.4	17.3	17.5	17.4	17.2
Ash	11.0	9.3	9.8	12.7	11.6
FM[3] protein replaced by MA[c] protein (%)	0	14	28	43	0
Fish protein (% dietary crude protein)	50	43	37	31	31
Veg protein (% dietary crude protein)	50	51	52	52	69
MA[c] protein (% dietary crude protein)	0	6	11	17	0
Fish lipid replaced by microalgae lipid (%)	0	12	24	36	0
Fish lipid (% dietary lipid)	51	46	41	38	48
Veg lipid (% dietary lipid)	49	48	46	43	52
MA[c] lipid (% dietary lipid)	0	6	13	19	0
LC-n-3 PUFA (EPA + DHA)	1.74	1.69	1.67	1.65	1.71

[a] Crude protein 66.7%, crude lipid 10.3%.
[b] Crude protein 89%, crude lipid 9.6%.
[c] CP, crude protein; CL, crude lipid; FM, fish meal; MA, microalgae.
[d] Mineral supplement composition (g/100 g mixture): $CaHPO_4*2H_2O$, 78.9; MgO, 2.725 g; KCl, 0.005; NaCl, 17.65; $FeCO_3$, 0.335; $ZnSO_4*H_2O$, 0.197; $MnSO_4*H_2O$, 0.094; $CuSO_4*5H_2O$, 0.027; Na_2SeO_3, 0.067.
[e] Vitamin supplement composition (% mixture): Thiamine HCL, 0.16; Riboflavin, 0.39; Pyridoxine HCl, 0.21; Cyanocobalamine, 0.21; Niacin, 2.12; Calcium pantotenate, 0.63; Folic acid, 0.10; Biotin, 1.05; Myoinositol, 3.15; stay C® DSM, 4.51; Choline chloride 83.99; α-tocoferol, 3.15; menadione, 0.24; Vit A (2500 IU/kg diet) 0.026; Vit D3 (2400 IU/kg diet) 0.05.

Table 4
Fatty acid profile[1] of the test diets.

Fatty acids %	C +	Isotetra 10	Isotetra 20	Isotetra 30	C −
16:0	21.4	25.0	23.6	25.3	23.4
18:0	3.6	3.5	3.4	3.2	3.5
SFA	33.0	37.5	36.7	37.7	34.6
18:1n-9 cis	22.0	18.2	17.3	17.9	23.3
18:1-n7	2.7	2.4	2.4	2.3	2.6
20:1n-9	1.8	1.7	1.7	1.4	1.7
MUFA	33.0	29.3	28.7	28.0	33.5
18:2n-6 cis	11.6	11.1	10.7	12.0	13.0
20:2-n6	0.5	0.3	0.3	0.3	0.4
20:3-n6	0.3	0.3	0.3	0.3	0.3
20:4-n6	0.7	0.7	0.7	0.6	0.6
n-6PUFA	13.5	12.9	13.0	13.8	14.8
18:3n-3	2.4	2.0	2.5	3.1	2.6
18:4n-3	1.2	1.9	2.5	2.9	1.2
20:3-n3	0.3	0.3	0.3	0.3	0.3
20:4-n3	0.6	0.5	0.5	0.4	0.6
20:5n-3	5.2	5.4	5.4	4.6	4.5
22:6n-3	6.7	6.5	6.1	5.5	4.7
n-3PUFA	16.7	16.7	18.3	16.7	14.0
n-3/n-6	1.2	1.3	1.4	1.2	0.9

[1] The following fatty acids were considered in the respective composite fractions but are not shown in the table: 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 20:0; 14:1n-5; 16:1n-7, 16:1n-9, 17:1, 20:1n-9, 20:1n-11, 22:1n-9, 22:1n-11; 16:2n-4, 18:3n-6, 20:4n-6, 22:5n-6; 16:3n-4, 18:3n-3; 16:4n-1, 18:4n-1, 22:4n-6, 21:5n-3; 22:5n-3.

2.3. Feed digestibility

The apparent digestibility of the test diets was measured at the same time of the growth trial adopting the indirect method and using the 3-tank unit system and settling columns as developed by the University of Guelph (Guelph CYAQ-2; Cho, 1992) which was connected to the partially-recirculating water system described above. Each 60-L vessel of a 3-tank unit was stocked with 10 sea bass (average body weight 131 ± 7 g; 3.9–4.0 kg biomass per unit). Acid-insoluble ash (AIA) was used as an external indigestible marker (Celite®, Prolabo, France, containing > 95% w/w of acid-insoluble ash) added in equal amounts (15 g/kg) to each diet before final mixture and dry pelleting. The digestibility measurements of each diet were performed in duplicate according to procedures previously described by Tibaldi et al. (2006, 2015). Fish were left to adapt to the culture conditions over 30 days before starting measurements and adapted over 3 weeks to a diet prior to faeces collection. The apparent digestibility coefficients (ADCs) of DM, crude protein, and gross energy were calculated according to Maynard and Loosly (Cho, 1992): ADC (%) = {[(% nutrient in the diet / % marker in the diet) − (% nutrient in faeces / % marker in faeces)] / (% nutrient in the diet / % marker in diet) × 100.

2.4. Skin colour measurements

Individual skin colour measurements were performed by a CM 2600d Chroma Meter (Minolta, UK). Skin colour readings were carried out at three dorsal (post-cranial, medial, caudal) and two ventral (post-cranial, medial) locations. Data were expressed using the L* a* b* system, representing lightness, red/greenness and yellow/blueness, respectively (CIE, 1976). In addition, the values of "Chroma" = $(a^{*2} + b^{*2})^{1/2}$ as a measure of colour saturation and "Hue" = arctan (b*/a*) were calculated.

In order to estimate perceptible colour differences (ΔE*) of fish fed microalgae-containing diets against those fed the positive control, the CIE76 formula was applied:

$$\Delta E^* = \sqrt{(L^*_c - L^*_i)^2 + (a^*_c - a^*_i)^2 + (b^*_c - b^*_i)^2}$$

For dorsal and ventral zones, average values of colour coordinates were used for ΔE* calculation (c = positive control; i = microalgae –containing diets.) According to Mahy et al. (1994), distances between colours were considered as being indicative of a "just noticeable perceptual difference" when ΔE* ≈ 2.3, or a "clear perceptual difference" when ΔE* > 2.3.

2.5. Analytical methods

The dried microalgae biomass, test diets, faeces, and fillet muscle tissue were analysed for dry matter and crude protein according to AOAC (1998). Microalgae biomass were also analysed for β carotene and total phosphorous content (AOAC, 1998). The acid-insoluble ash (AIA) content of the test diets and faeces was determined according to the method CEE-EU (G.U. European Community n. L.155/21, 12.7.71) and the gross energy content was measured by an adiabatic bomb calorimeter (IKA C7000, Werke GmbH and Co., Staufen, Germany). The amino acid analyses of the microalgae dried biomass were performed as described by Tibaldi et al. (2015). Acid hydrolysis with HCl 6 M at 115–120 °C for 22–24 h was used for all amino acids except cysteine (Cys) and methionine (Met), for which performic acid oxidation preceeded acid hydrolysis and tryptophan that was determined after lithium hydroxide (4M) hydrolysis.

The total lipid fraction of the freeze-dried microalgae biomass, test diets and fillet muscle tissue, was extracted with chloroform-methanol (2:1 v:v) mixture (Folch et al., 1957). The fatty acid methyl esters were obtained according to Morrison and Smith (1964), and their quantitative composition was determined by Varian gas chromatograph 430-GC under the condition previously described (Tulli et al., 2012). The fatty acid methyl esters were identified by comparison to reference standards (Supelco, Bellefonte, PA, USA) and an internal standard (C23:0) was used to obtain their quantification.

2.6. Calculations

In Table 3 the % replacement of FM protein and fish lipid of the C + diet by protein and lipid from microalgae were calculated as follows:

$$\text{FM protein replacement (\%)} = 100 \times (\%CP_{T.lutea} \times T.\ lutea_{(j)g/kg} + \%CP_{T.suec} \times T.\ suec_{(j)g/kg}/(\%CP_{FM} \times FM_{control+g/kg})$$

$$\text{Fish lipid replacement (\%)} = 100 \times (\%CL_{T.lutea} \times T.\ lutea_{(j)g/kg} + \%CL_{T.suec} \times T.\ suec_{(j)\,g/kg}/(\%CL_{FM} \times FM_{control+g/kg} + \%CL_{CPSP} \times CPSP_{g/kg} + \text{Cod liver oil}_{g/kg})$$

In addition the contribution of fish protein/lipid (FP, FL), vegetable protein/lipid (VP,VL), microalgae protein/lipid (MP, ML) as % of dietary crude protein/lipid (DCP, $DCL)_i$ were calculated as follows:

$$FP_i = 100 \times (\%CP_{FM} \times FM_{(j)\,g/kg} + \%CP_{CPSP90} \times CPSP90_{g/kg})/DCP_{i\,g/kg}$$

$$VP_i = 100 \times (\%CP_{WG} \times WG_{g/kg} + \%CP_{SBM} \times SBM_{g/kg})/DCP_{i\,g/kg}$$

$$MP_i = 100 \times (\%CP_{T.lutea} \times T.\ lutea_{(j)g/kg} + \%CP_{T.suec} \times T.\ suec_{(j)\,g/kg})/DCP_{i\,g/kg}$$

$$FP_i = 100 \times (\%CP_{FM} \times FM_{(j)\,g/kg} + \%CP_{CPSP90} \times CPSP90_{g/kg})/DCP_{i\,g/kg}$$

$$FL_i = 100 \times (\%CL_{FM} \times FM_{(j)\,g/kg} + \%CL_{CPSP90} \times CPSP90_{g/kg} + CL_{FO} \times FO_{(j)\,g/kg})/DCL_{i\,g/kg}$$

$$VL_i = 100 \times (\%CL_{palm\ oil} \times Palm\ oil_{(j)\ g/kg} + \%CL_{Soy-lecith} \times Soy - lecithin_{g/kg} + \%CL_{WG} \times WG_{g/kg} + \%CLSBM \times SBM_{(j)g/kg}\ CLWG + SBM_{FO} \times FO_{(j)\ g/kg})/DCL_{i\ g/kg}$$

$$ML_i = 100 \times (\%CL_{T.lutea} \times T.\ lutea_{(j)g/kg} + \%CL_{T.suec} \times T.\ suec_{(j)\ g/kg}) /DCL_{i\ g/kg}$$

where i = diets and j = level of specific ingredient in the diet

In all the above calculation, results were rounded to the next 1%

At the end of the growth trial the following variables were calculated per tank-group:

AFI: absolute feed intake (g/fish/day) = feed intake/n. of fish/days

RFI: relative feed intake (g/kg ABW/day) = feed intake per tank /[(Initial biomass + final body mass)/2] /days

SGR: specific growth rate = 100 × [(ln final body weight − ln initial body weight)/days].

FCR: feed conversion ratio = feed intake per tank /biomass gainweight gain.

PER: weight gain/crude protein intake.

Based on individual biometry and carcass dissection measurements the following parameters were calculated:

Condition factor (FC): body weight/standard length3) × 100.

Carcass yield (%): (gutted body weight/body weight) × 100.

Fillet yield (%): (fillet with skin weight/body weight) × 100.

Fillet without skin yield (%): (fillet without skin weight/body weight) × 100.

HSI, hepatosomatic index(%): (liver weight/body weight) × 100.

MFI, mesenteric fat index(%): (mesenteric fat weight/body weight) × 100.

2.7. Statistical analysis

Data were subjected to one-way ANOVA and, if adequate, means were compared using the Duncan's test, set for $P < 0.05$. The linear regression analysis was used to fit the relationship between nutrient digestibility and dietary level of dried microalgae biomass and between dietary and muscle fatty acid composition. All analyses were carried out using the SPSS-PC release 17.0 (SPSS Inc., Chicago, IL,USA).

3. Results

Fish promptly accepted all the diets and no mortality occurred throughout both digestibility and growth trials.

The ADC of dry matter (DM), crude protein (CP) and gross energy (GE) of the test diets are summarised in Table 5. In all diets the DM ADC was significantly reduced when compared to C+ ($P < 0.05$), while diet Isotetra 10 was similar to C+ in CP and GE ADC values ($P > 0.05$). Diets Isotetra 20 and 30 resulted in similar low values of CP and GE ADC when compared to C– ($P > 0.05$). A linear tendency towards declining ADC values ($r = -0.96/-0.99$, $P < 0.01$) was noted in response to increasing levels of dry microalgae mixture in the diet.

Feed intake, growth performance, feed conversion and protein efficiency ratios of sea bass fed the test diets over 105 days are shown in Table 6.

Table 5
Apparent digestibility coefficients (ADCs) of dry matter, crude protein and gross energy of the test diets (n = 2).

ADC (%)[1]	Diets					
	C+	Isotetra 10	Isotetra 20	Isotetra 30	C−	s.e.m.
DM[2]	79.1[a]	77.8[b]	75.7[b]	71.1[c]	71.2[c]	1.86
CP[3]	92.6[a]	91.0[ab]	88.9[b]	87.4[bc]	86.7[bc]	1.19
GE[4]	88.6[a]	86.8[ab]	86.1[bc]	83.8[c]	82.0[c]	1.34

Row means not sharing common superscript letters are significantly different (a, b, c: $P < 0.05$).

[1] ADC(%) = {[(% nutrient in the diet / % marker in the diet) − (% nutrient in faeces / % marker in faeces)] / (% nutrient in the diet / % marker in diet)} × 100.

[2] DM: dry matter.

[3] CP: crude protein.

[4] GE: gross energy.

Table 6
Growth performance, feed intake, specific growth rate, feed conversion rate and protein efficiency ratio of E. sea bass fed the test diets over 105 days.

	Diets					
	C+	Isotetra 10	Isotetra 20	Isotetra 30	C−	s.e.m.
IBW (g)	204.3	203.9	203.9	204.3	204.5	0.78
FBW (g)	410.4[a]	427.3[a]	421.8[a]	419.5[a]	387.7[b]	14.04
AFI[1]	3.23[bc]	3.39[ab]	3.43[ab]	3.44[a]	3.12[c]	0.118
RFI[2]	10.52[b]	10.77[ab]	11.00[ab]	11.13[a]	10.61[b]	0.093
SGR[3]	0.66[a]	0.70[a]	0.69[a]	0.68[a]	0.61[b]	0.032
FCR[4]	1.75[b]	1.66[b]	1.67[b]	1.69[b]	1.89[a]	0.089
PER[5]	1.27[a]	1.35[a]	1.34[a]	1.31[a]	1.13[b]	0.076

Row means not sharing common superscript letters are significantly different, a, b, c: $P < 0.05$).

[1] AFI: absolute feed intake, g/fish/day.

[2] RFI: relative feed intake, g/kg ABW/day.

[3] SGR: 100 × [(ln final body weight − ln initial body weight) / days].

[4] FCR: feed intake / weight gain.

[5] PER: weight gain / crude protein intake.

Fish fed the C– diet resulted in the lowest final weight as well as in the worst growth rate, feed conversion and protein efficiency ratios when compared to the other dietary groups ($P < 0.05$). This was first a consequence of a lower absolute feed intake which was significantly reduced when compared to that observed in fish fed all the microalgae-including diets ($P < 0.05$). Relative to the positive control treatment, a slight increase in absolute and relative feed intake was observed in fish fed increasing levels of dietary microalgae biomass with a significantly higher value only in the case of fish given diet Isotetra 30 (+6% vs. C+, $P < 0.05$). The observed differences in feed intake were not mirrored in the growth performance as all microalgae-containing diets resulted in individual body weight, SGR, FCR and PER which were similar to those exhibited by fish fed the positive control diet ($P > 0.05$).

Biometry and dressing out yields are shown in Table 7. No major differences among dietary treatments were observed in total length, whole body weight and CF ($P > 0.05$). All dietary treatments also resulted in similar dressing out carcass yield, HSI and MFI ($P > 0.05$). Only the fillet yield in fish fed diet Isotetra 30 was significantly reduced when compared to that of fish fed the positive control feed ($P < 0.05$).

The effect of the dietary treatment on fillet muscle water, crude protein, total lipid and ash contents (% of the wet weight) of E. sea bass at the end of the trial are shown in Table 8. There were changes in muscle composition due to the diet. Fish fed the negative control diet resulted in the highest water and the lowest lipid contents relative to those found in positive controls ($P < 0.05$). There was a trend towards

Table 7
Biometry and dressing out yield of E. sea bass fed the test diets over 105 days (n = 9).

	Diets					
	C +	Isotetra 10	Isotetra 20	Isotetra 30	C −	s.e.m.
Total length (cm)	31.8	32.2	32.5	32.2	31.8	0.19
Whole body weight (g)	402.5	419.8	409.2	403.0	398.2	7.51
Condition factor (CF)[1]	1.2	1.3	1.2	1.2	1.2	0.02
Carcass yield%[2]	87.5	87.1	88.1	87.5	87.1	0.27
Fillets yield %[3]	45.8^{a}	44.1^{ab}	43.9^{ab}	43.0^{b}	44.6^{ab}	0.34
HSI[5]	1.9	1.8	1.5	1.6	1.8	0.08
MFI[6]	15.5	16.1	16.7	16.3	16.4	1.91

Row means not sharing common superscript letters are significantly different, a, b: P < 0.05).

Table 8
Fillet muscle proximate analysis (% of the wet weight), fatty acid profile[1] (% total fatty acids) and content of E. sea bass fed the test diets over 105 days (n = 9).

	Diets					
	C +	Isotetra 10	Isotetra 20	Isotetra 30	C −	s.e.m.
Water	70.5^{b}	70.4^{b}	71.4^{ab}	71.2^{ab}	72.2^{a}	0.20
Lipids	7.1^{a}	7.1^{a}	6.2^{ab}	5.9^{ab}	5.5^{b}	0.20
Proteins	20.4^{b}	20.5^{ab}	20.7^{ab}	20.8^{a}	20.4^{b}	0.07
Ash	1.2	1.3	1.3	1.3	1.2	0.01
Fatty acids profile						
16:0	20.5	21.7	20.6	21.4	20.1	1.27
18:0	3.9	3.9	3.8	3.9	3.9	0.18
SFA	31.6^{bc}	33.3^{a}	32.4^{ab}	32.7^{ab}	31.1^{c}	1.40
18:1n-9 cis	22.7^{a}	21.2^{b}	20.2 ^{b}	21.1 ^{b}	22.8 ^{a}	1.45
18:1n-7	2.8	2.6	2.4	2.5	2.7	0.42
20:1n-9	1.4	1.3	1.5	1.4	1.6	0.28
MUFA	33.5^{a}	32.4^{ab}	31.0^{c}	31.7^{bc}	33.5^{a}	1.58
18:2n-6 cis	11.1^{bc}	10.6^{c}	11.2^{bc}	11.6^{ab}	12.1^{a}	0.76
20:2n-6	0.6	0.5	0.6	0.6	0.7	0.09
20:4n-6	0.7	0.8	0.9	0.8	0.9	0.14
n-6PUFA	13.6^{bc}	13.1^{c}	13.8^{b}	14.0^{b}	14.6^{a}	0.71
18:3n-3	2.1^{b}	1.7^{b}	2.3^{ab}	2.6^{a}	2.1^{b}	0.53
18:4n-3	0.8^{c}	1.2^{b}	1.5^{b}	2.0^{a}	1.0^{c}	0.53
20:5n-3	5.4	5.6	5.9	5.4	5.7	1.97
22:6n-3	8.0	7.9	8.0	7.8	7.8	0.93
n-3PUFA	18.3	18.3	18.0	18.6	17.8	2.00
n-3/n-6	1.3^{a}	1.4^{a}	1.3^{a}	1.3^{a}	1.2^{b}	0.15
Fatty acid content g/100 g						
20:5n-3	0.31	0.33	0.31	0.27	0.25	0.068
22:6n-3	0.48^{a}	0.47^{a}	0.46^{a}	0.40^{b}	0.35^{b}	0.080
Total n-3PUFA	1.08^{a}	1.07^{a}	1.02^{a}	0.92^{ab}	0.79^{b}	0.187

Row means not sharing common superscript letters are significantly different, a, b: P < 0.05).

[1] The following fatty acids were considered in the respective composite fractions but are not shown in the table: 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 20:0; 14:1n-5; 16:1n-7, 16:1n-9, 17:1, 20:1n-11, 22:1n-9, 22:1n-11; 16:2n-4, 18:3n-6, 22:5n-6; 16:3n-4; 16:4n-1, 18:4n-1, 20:4n-3, 22:4n-6, 21:5n-3; 22:5n-3.

reduced muscle lipid as the level of marine microalgae biomass was increased in the diet with fish fed diet Isotetra 10 exhibiting similar values compared to positive controls (P > 0.05) but significantly lower than those of negative ones (P < 0.05). Fish fed diets Isotetra 20 and 30 showed intermediate values between diets C + and C– (P > 0.05). The ash content of muscle did not change due to the dietary treatment while the crude protein level was somehow affected in fish fed diet Isotetra30 resulting in the highest protein level relative to both control treatments (P < 0.05). Although statistically significant, the magnitude of the differences in protein content appeared minor indeed for being considered as relevant in terms of fillet nutritional value.

The fatty acid profile of the lipid fraction of sea bass fillet muscle (Table 8) was substantially affected by the dietary treatment and most changes were mirroring parallel changes in the dietary fatty acid profile. As a result, positive linear relationships between muscle and dietary SFA, MUFA, n-6 PUFA and n-3 PUFA were obtained with "r" values ranging from 0.84 to 0.95 (P < 0.05). Muscle lipid of fish fed the positive and negative control diets had similar SFA profile. A slightly elevated incidence of this lipid fraction was noted with all the microalgae-containing diets, but only fish fed diet Isotetra 10 resulted in a significantly higher SFA incidence relative to both control diets. Conversely, the proportion of 18:1n-9 and total MUFA, were equally elevated in muscle lipid of fish fed both control diets relative to those of sea bass given the microalgae-containing diets, particularly in the case of those fed diets Isotetra 20 and 30. The linoleate and total n-6 PUFA showed a tendency towards increasing incidence in muscle lipid as the level of microalgae biomass mixture was increased in the diet. No substantial diet-induced changes were noted in the proportion of arachidonate while the highest and the lowest incidences of n-6 PUFA were found in muscle lipid of fish given the negative control diet and diet Isotetra 10, respectively (P < 0.05). Replacing increasing levels of fish and conventional vegetable lipid sources with those supplied by the microalgae mixture, resulted in a stepwise increase in the incidence of linolenate (P < 0.05), without differences among diets in the incidence of EPA, DHA and total n-3 PUFA in the lipid fraction of the flesh.

However, the DHA content in fillet muscle was reduced in fish fed the highest level of microalgae (0.40 vs. 0.48 g/100 g, P < 0.05) while no changes (P > 0.05) were observed in the EPA and total n-3 PUFA content of the flesh as the level of microalgae was increased in the diet. Fish fed diet C^{+} or all preparations including the marine microalgae mixture, resulted in higher n-3/n-6 PUFA ratio relative to diet C^{-} (P < 0.05).

Feeding the test diets over 105 days resulted in some changes in instrumental colour parameters of the skin. As shown in Table 9, the b* component and Chroma were not affected by dietary treatments in either skin locations. Fish fed the two control diets resulted in very similar skin colour parameters irrespective of the body position (P > 0.05). Relative to controls there was a significant increase in the values of the parameter a* towards redness in both dorsal and ventral regions of the skin in fish fed diets including the microalgae mixture. This was coupled with diminished lightness (L*) and also Hue, the former only in ventral position (P < 0.05). Overall, a calculated "clear perceptual difference" appeared in the dorsal skin colour of fish fed all

Table 9
Dorsal and ventral skin colour parameters of E. sea bass fed the test diets over 105 days (n = 12).

	Diets					s.e.m.
	C +	Isotetra10	Isotetra20	Isotetra30	C −	
Dorsal[1]						
L*	49.8^{ab}	52.4^{a}	44.2^{b}	47.2^{ab}	44.0^{b}	7.65
a*	0.90^{b}	2.13^{a}	1.83^{a}	2.00^{a}	0.58^{b}	0.509
b*	14.61	14.07	15.42	14.19	13.16	2.799
Chroma	14.64	14.23	15.54	14.35	13.18	2.808
Hue	86.5^{a}	81.4^{b}	83.0^{b}	81.8^{b}	87.3^{a}	1.86
Ventral[1]						
L*	82.4^{a}	81.2^{ab}	81.2^{ab}	77.7^{b}	83.1^{a}	4.12
a*	-1.76^{c}	− 0.77^{b}	− 0.28^{ab}	0.17^{a}	− 1.54^{c}	0.869
b*	12.00	11.89	12.68	13.44	12.42	3.164
Chroma	12.19	11.99	12.76	13.47	12.54	3.117
Hue	99.5^{a}	94.9^{bc}	93.0^{cd}	89.7^{d}	97.9^{ab}	4.94

Row means not sharing common superscript letters are significantly different, a, b: P < 0.05).

[1] Values for the different positions in the same region were pooled because not significantly different.

microalgae-containing diets relative to those given diet C + and in the ventral skin colour between treatment C + and Isotetra30 (ΔE* between 2.85 and 5.73), while comparing treatments C + with Isotetra10 or Isotetra20 resulted in just a "slight perceptual difference" in the ventral skin colour (ΔE* ≈ 1.75).

4. Discussion

In this study for the first time a definite mixture of dried marine microalgae biomass has been assayed as partial substitute for fish protein and lipid in diets for the E. sea bass already including substantial amounts of protein-rich plant derivatives and vegetable oils. The results obtained so far have shown that, even at the highest level of inclusion investigated (i.e. 180 g/kg in the present diet) a mixture of *T. lutea* and *T. suecica* gave rise to growth response, feed and protein conversion efficiency that were similar to those of fish fed a positive control diet including a larger proportion of fish derivatives. Such results agree or are even better than the outcome of previous experiments on the same fish species. In fact, in the present study, the use of a blend of *T. lutea* and *T. suecica,* allowed a dietary inclusion level higher than the one observed when the two microalgae were used as single ingredients (Tibaldi et al., 2015; Tulli et al., 2012). It should be noted that this occurred despite a tendency towards reduced nutrient and energy apparent digestibility as the level of microalgae biomass was increased in the diet which was apparently compensated by a slight but significant increase in feed intake. Lower organic matter, crude protein and gross energy apparent digestibility were also noted by Tulli et al. (2012) in juvenile E. sea bass given diets where *T. suecica* alone was used to replace graded levels of fish meal and trimmings. This was only partly the case when E. sea bass were fed diets with increasing levels of *T. lutea* as partial substitute for fish meal and oil, in that a slight decline occurred in bioavailability of lipid and gross energy whereas dry matter and crude protein digestibility were barely affected by graded levels of microalgae inclusion (Tibaldi et al., 2015).

Very few investigations on gross nutrient and energy bioavailability of dried microalgae biomass have been carried out in monogastric animals (Becker, 2007; Skrede et al., 2011) and even more so in fish. In salmonids and cod the apparent digestibility of crude protein and gross energy of a dry biomass of *Spirulina* sp. or *Pheodactylum tricornutum* were shown to fall in the range of 82–92% comparing favourably with those of commonly used plant protein derivatives (Burr et al., 2011; Reitan et al., 2013). In Nile tilapia, crude protein and gross energy ADCs of *Spirulina*, *Chlorella* and *Schizochytrium* have been shown to vary in a similar range of values, 80–86.1% and 83.9–86.5%, respectively (Sarker et al., 2016a). Crude protein apparent digestibility values in the range of 87–88% have been reported by Kousoulaki et al. (2015) in Atlantic salmon fed diets including up to 15% *Schizochytrium* dry biomass. In the latter fish species, similarly to what was observed in our study, also SØrensen et al. (2016) found a linear reduction in ADC of dry matter, lipid and crude protein as the level of the marine microalgae *P. tricornutum* dry biomass was increased in the diet from 0 up to 12%. As with other single cell organisms, cell wall thickness and composition have been claimed to adversely affect bioavailability of nutrients supplied by microalgae, by resisting enzymatic digestion (Norambuena et al., 2015; SØrensen et al., 2016). To what extent this assumption could apply to microalgae species which lack a definite cell wall like *Tisochrysis* spp. remains questionable. Therefore, it seems that slightly declining values of energy and crude protein apparent digestibility with diets supplying graded levels of the dried microalgae mix observed in the present study, were to some extent reflecting the reduced digestibility of *Tetraselmis,* likely due to its cell wall relatively thin (Domozych et al., 2012).

Even higher levels of replacement of fish derivatives for marine microalgae biomass than those tested in the present study have been successful in different fish species. This was the case of *S. almeriensis* and *I. galbana* sp. (T-ISO) in gilthead sea bream juveniles (Vizcaìno et al., 2014; Palmegiano et al., 2009), *Nanofrustulum* sp. in and Atlantic salmon and common carp (Kiron et al., 2012) and *Pavlova viridis* in juveniles E. sea bass (Haas et al., 2015). On the other hand, impaired growth, as a consequence of lower feed consumption, was observed in goldfish fry fed a diet containing just 5% by weight of a dried biomass of *I. galbana* (Coutinho et al., 2006) and in Atlantic cod (*Gadus morua*) juveniles given a diet suppling 140 g/kg of a blend of dried marine microalgae including a substantial proportion of *Isochrysis* sp. (Walker and Berlinsky, 2011). Hence, based on available literature, it is likely that variable response in feed consumption and growth performance depend on the fish species and size, the level of inclusion, the nutritional value and eventually the palatability of the microalgae biomass which are known to be affected by microalgae species as well as the cultivating, harvesting, processing and drying conditions (Brown et al., 1997; Reitan et al., 1994; Sánchez et al., 2000).

The diverse composition of the control or basal diet utilized in different experiments makes it even more difficult to compare the results of previous studies on the effects of the inclusion of microalgae in the diet. In most of the experiments carried out so far, control preparations were largely based on fish meal and oil as major protein and lipid sources. This could have led to underestimating the nutritive value of microalgae when compared with that of plant protein and oil sources as conventional dietary alternatives to fish derivatives. Under this perspective the results of the present experiment have shown that the diet highest in microalgae resulted in improved growth and nutritive value than the negative control diet, which contained the same level of fish meal but was higher in soybean meal and fish oil. The growth response of E. sea bass fed the negative control diet in this study confirms previous findings on the same fish species where high levels of dehulled, roasted and solvent-extracted soybean meal in the diet resulted in impaired growth and feed efficiency (Oliva-Teles et al., 1998, Tibaldi et al., 1998, Tibaldi et al., 2006).

In the present experiment, dietary algae inclusion did not affect slaughter yield and major visceral organ weight indices compared to both control diets. Also Tibaldi et al. (2015) did not find changes in major biometry traits and HSI in E. sea bass fed diets including graded levels of *T. lutea*. Similarly, unchanged HSI values were observed by Palmegiano et al. (2009), in gilthead sea bream juveniles fed diets including up to 70% by weight of *T. lutea* and by Vizcaìno et al. (2014), in the same species when given diets containing up to 26% dry matter basis of *S. almeriensis* meal as a substitute for FM. In the latter study, only the highest inclusion level (i.e. 39%) caused a significant decrease of HSI. Conversely, Tulli et al. (2012) reported that graded level of *T. suecica* in the diet of juvenile E. sea bass resulted in decreased liver weight compared to a control diet. Lower values of HSI in fish fed diets including microalgae biomass was considered to be somehow related to a reduced deposition of fat in liver as a consequence of lower digestible lipid/energy intake with diets including the dry microalgae biomass and/or to a lipotropic action of certain unknown bioactive compounds supplied by microalgae (Tulli et al., 2012).

There is evidence from various animal models, including fish, that certain dietary ingredients like soybean derivatives and different protein sources (animal vs. vegetable) could affect levels and partitioning patterns of fat deposition in different organs and tissue according to various mechanisms (Iritani et al., 1986, 1996; Dias et al., 2005; Messina et al., 2013). The lipid content of fish liver was not analysed in this study and no diet-induced changes occurred in mesenteric fat depot of E. sea bass. Conversely, muscle adiposity was progressively reduced in fish fed graded levels of the microalgae mixture relative to positive control fish. This lipid lowering effect in fish given the diet including the highest level of marine microalgae was similar in magnitude to that observed in fish given the negative control feed high in soybean meal. Also Kiron et al. (2012) observed lowered lipid content in muscle of Atlantic salmon (*S. salar*), fed a diet including a mixture of *Nanofrustulum* sp. and *Tetraselmis* sp. when compared to a control diet. A lipid lowering effect of a *Chlorella* extract, due to the activation of the

lipid metabolism, was found in muscle of ayu (*Plecoglossus altivelis*) by Nematipour et al. (1987, 1990). Similarly, lipid mobilization and reduced lipid accumulation was shown to be induced in red sea bream by dietary *Spirulina* supplementation (Nakagawa et al., 2000). Conversely, no major changes in muscle lipid relative to control fish due to graded dietary levels of *T Isochrysis lutea* were observed by Tibaldi et al. (2015) in E. sea bass. Also Vizcaìno et al. (2014) noted that sea bream flesh proximate composition did not show major changes when fish were fed on *S. almeriensis* supplemented diets.

Further studies are needed to establish if and to what extent diminished muscle adiposity in fish fed diets including various species and levels of microalgae biomass depends on specific bioactive compounds acting on lipid metabolism eventually supplied by the microalgae themselves. It could be the case of fucoxanthin, a major carothenoid in *Tisochrysis* (Brown et al., 1997; Kim et al., 2012) that has recently been associated with reduced whole body adiposity in gilthead sea bream fed diets including 2.5% of dried biomass of the fucoxanthin-rich microalgae *P. tricornutum* (Ribeiro et al., 2017). Fucoxanthine is known to reduce accumulation of white adipose tissue in rodends as a consequence of depressed activity of lipogenic enzymes and increased fatty acid oxidation (Peng et al., 2011; Ha and Kim, 2013; Maeda, 2015).

In this study, apart from lipid and conversely water content, the fillet muscle protein and ash contents were little affected by dietary treatments.

The microalgae mixture used in the current experiment had relatively high protein and lipid levels and was an important source of n-3(LC)PUFAs. The incorporation of these polyunsaturated fatty acids into the flesh is of primary importance in order for farmed fish to impart the human health benefits that have been ascribed to fish consumption (Mozaffarian and Rimm, 2009; Brasky et al., 2012). In the present study, as extensively noted in cultured fish species, changes in dietary fatty acid composition were mirrored in the fatty acid profile and composition of the edible muscle tissue of the E. sea bass. A stepwise increase in the incidence of 16:0, total SFA and 18:1n-9 in the fish flesh were expected due to the relative abundance of the same fatty acids in the corresponding diets. Differently to what has been observed by Tibaldi et al. (2015) in E. sea bass given diets including graded levels of *T. lutea*, in the present trial the proportion of nutritionally crucial n-3(LC)PUFAs such as EPA and DHA were not lowered in the flesh of fish due to dietary microalgae inclusion. These results confirm that n-3(LC) PUFAs supplied by the microalgae were actually deposited in the flesh of the E. sea bass which is notably incapable of a significant biosynthesis of n-3(LC)PUFA from dietary linolenate (Mourente et al., 2005). In terms of health attributes of the edible portion it is worth to mention that, compared to fish fed the positive control feed and despite a concurrent reduction of muscle lipid, the DHA content in fillet muscle was slightly reduced only in fish fed the highest level of microalgae (0.40 vs. 0.48 g/100 g, $P < 0.05$) while no changes ($P > 0.05$) were observed in the EPA and total n-3 PUFA content of the flesh as the level of microalgae was increased in the diet.

Besides the nutritional properties, microalgae are also sources of pigments that could affect skin and muscle colour appearance. This is particularly important in certain fish species at market size in that colour and visual appearance are known to influence market value, flavour perception and acceptability of fish food products (Spence et al., 2010; Vasconcellos et al., 2013). Previous studies with fish showed that various pigments in microalgae can result in enhanced pigmentation of the skin or flesh or both, (Belay et al., 1996; Chatzifotis et al., 2011; Choubert et al., 2006; Ribeiro et al., 2017; Tulli et al., 2012; Walker and Berlinski, 2011). This was also evident in the present study where the skin pigmentation of E. sea bass fed the microalgae mixture appeared basically greenish with a slight tendency towards redness and slightly reduced lightness and Hue, the latter in the ventral region of fish fed the microalgae mixture relative to those measured in fish fed both control diets. Although minor in magnitude such changes were mostly independent from the microalgae inclusion level, resulting in a “clear perceptual difference” in the dorsal skin colour of fish fed all microalgae-containing diets relative to those given the positive control feed. In a recent study in which the E. seabass were fed *Tisochrysis*-rich diets (Tibaldi et al., 2015), skin lightness (L*) was not affected by dietary microalgae inclusion while, contrary to the observation in the present study, there was a tendency towards increase of greenness (a*) in the dorsal skin of the fish fed the diet including the highest level of microalgae, which was coupled with increased hue values and slightly different colour saturation (chroma). An enhanced greenish skin pigmentation was also described in E. seabass juveniles fed diets including *T. suecica* (Tulli et al., 2012). Hence combining the two microalgae dry biomass apparently resulted in a skin colour pattern tendency that was slightly different relative to that potentially perceived when single microalgae were included in the diet. This warrants further investigation on the mechanisms of tissue colour/pigment saturation to establish a possible role of different combinations of carotenoids/xanthophyll supplied by the two microalgae in affecting the actual skin colour pattern and perception in fish.

In conclusion, the results of the present study have shown that replacing 43% crude protein and 36% lipid from fish derivatives, with protein and lipid supplied by a mixture of dried *Tisochrysis lutea* and *Tetraselmis suecica* biomass in diets already containing substantial levels of protein and oil from vegetable sources, did not adversely affect growth performance and feed conversion efficiency of adult E. sea bass despite a parallel slight decline in protein and energy digestibility which was compensated by increased feed intake. Moreover, the mixture of dried microalgae resulted in a higher nutritive value than that of a commonly used vegetable protein ingredient like the toasted dehulled soybean meal. No major changes induced by replacing fish derivatives with the dried microalgae biomass were observed in biometry traits and slaughter yield while the nutritional properties of the edible muscle tissue were just barely affected in terms of n-3 PUFA.

Irrespective of the investigated inclusion level the presence of the dried microalgae mixture in the diet resulted in a grey-greenish pigmentation of the skin, with a slight tendency towards redness and diminished lightness and hue. It is currently unpredictable to what extent these minor changes in skin colour pattern of sea bass could impact consumer appeal since, to our knowledge, there is no market reference for the skin appearance established in this species.

Overall the results obtained here also reveal a potential marked improvement in sustainability in terms of reduced reliance on alieutic feed resources in case of the microalgae-containing diets with a Fish In – Fish Out index, as outlined by Jackson (2009) of 1.43 with diet isotetra30 vs. 2.30 and 1.71 for the positive and negative controls, respectively. Therefore, the sustainable use of the dried mixture of the two marine microalgae biomass in sea bass diet is not conditioned by low nutritional value but by the current low availability and high market price which are both expected to become less limiting over the next 10 years (Draaisma et al., 2013; Norsker et al., 2011; Tredici et al., 2016).

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References

AOAC, 1998. Official methods of analysis of the association of Official analytical chemists, 14th ed. A.A.V.V., Washington DC (1198 pp.).

Atalah, E., Hernández Cruza, C.M., Izquierdo, M.S., Rosenlund, G., Caballero, M.J., Valencia, A., Robaina, L., 2007. Two microalgae *Crypthecodinium cohnii* and *Phaeodactylum tricornutum* as alternative source of essential fatty acids in starter feeds for seabream (*Sparus aurata*). Aquaculture 270, 178–185.

Badawy, T.M., Ibrahim, E.M., Zeinhom, M.M., 2008. Partial replacement of fish meal with dried microalgae (*Chlorella* spp) in Nile tilapia (*Oreochromis niloticus*) diets. In: Elghobashi, H., Fitzsimmons, K., Diab, A.S. (Eds.), From the Pharaos to the Future: Proceeding of the 8th International Symposium on Tilapia in Aquaculture. Egypt Ministry of Agriculture, Cairo, pp. 801–810.

Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25, 207–210.

Belay, A., Kato, T., Ota, Y., 1996. Spirulina (*Arthrospira*): potential application as an animal feed supplement. J. Appl. Phycol. 8, 303–311.

Ben-Amotz, A., Fishler, R., Schneller, A., 1987. Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Mar. Biol. 95, 31–36.

Brasky, T.M., Crowe, F.L., Kristal, A.R., 2012. N-3 fatty acids and prostate cancer risk. Br. J. Nutr. 108 (9), 1721.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315–331.

Burr, G.S., Barrows, F.T., Gaylord, G., Wolters, W.R., 2011. Apparent digestibility of macronutrients and phosphorus in plant-derived ingredients for Atlantic salmon, *Salmo salar* and Arctic charr, *Salvelinus alpinus*. Aquac. Nutr. 17, 570–577.

Carter, C.G., Bransden, M.P., Lewis, T.E., Nichols, P.D., 2003. Potential of *Thraustochytrids* to partially replace fish oil in Atlantic salmon feeds. Mar. Biotechnol. 5, 480–492.

Chatzifotis, S., Vaz Juan, I., Kyriazi, P., Divanach, P., Pavlidis, M., 2011. Dietary carotenoids and skin melanin content influence the coloration of farmed red porgy (*Pagrus pagrus*). Aquac. Nutr. 17 (2), e90–e100. http://dx.doi.org/10.1111/j.1365-2095.2009.00738.x.

Cho, C.Y., 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquaculture 100, 107–123.

Choubert, G., Mendes-Pinto, M.M., Morais, R., 2006. Pigmenting efficacy of astaxanthin fed to rainbow trout *Oncorhynchus mykiss*: effect of dietary astaxanthin and lipid sources. Aquaculture 257, 429–436.

CIE (Commission International de l'Eclairage), 1976. Colorimetry. vol. 15 Bureau Central de la CIE, Vienna. Austria (345 pp.).

Coutinho, P., Rema, P., Otero, A., Pereira, O., Fabregas, J., 2006. Use of biomass of the marine microalga *Isochrysis galbana* in the nutrition of goldfish (*Carassius auratus*) larvae as source of protein and vitamins. Aquac. Res. 37, 793–798.

Dias, J., Alvarez, M.J., Arzel, J., Corraze, G., Diez, A., Bautista, J.M., Kaushik, S.J., 2005. Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 142, 19–31.

Domozych, D.S., Ciancia, M., Fangel, J.U., Mikkelsen, M.D., Ulvskov, P., Willats, W.G.T., 2012. The cell walls of green algae: a journey through evolution and diversity. Front. Plant Sci. 1–7.

Draaisma, R.B., Wijffels, R.H., Slegers, P.M., Brentner, L.B., Roy, A., Barbosa, M.J., 2013. Food commodities from microalgae. Curr. Opin. Biotechnol. 24, 169–177.

Folch, J., Lees, M., Sloane-Stanley, H.S., 1957. A simple method for the isolation and purification of the total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Ha, A.W., Kim, W.K., 2013. The effect of fucoxanthin rich power on the lipid metabolism in rats with a high fat diet. Nutr. Res. Pract. 7 (4), 287–293.

Haas, S., Bauer, J.L., Adakli, A., Meyer, S., Lippemeier, S., Schwarz, K., Schulz, C., 2015. Marine microalgae *Pavlova viridis* and *Nannochloropsis sp.* as n3-PUFA source in diets for juveniles European sea bass (*Dicentrarchus labrax*). J. Appl. Phycol. 28 (2), 1011–1021.

Hussein, E., Dabrowski, K., El-Saidy, D., Lee, B., 2013. Enhancing the growth of Nile tilapia larvae/juveniles by replacing plant (gluten) protein with algae protein. Aquac. Res. 44 (6), 937–949.

Iritani, N., Nagashima, K., Fukuda, H., Akihiko, K., Tanaka, T., 1986. Effects of dietary proteins on lipogenic enzymes in rat liver. J. Nutr. 116, 190–197.

Iritani, N., Hosomi, H., Fukuda, H., Tada, K., Ikeda, H., 1996. Soybean protein suppressed hepatic lipogenic enzyme gene expression in wistar fatty rats. J. Nutr. 126, 380–388.

Jackson, A., 2009. Fish in Fish out Ratios Explained. 34. Aquaculture Europe, pp. 5–10.

Kim, S.M., Kang, S.W., Kwon, O.N., Chung, D., Pan, C.H., 2012. Fucoxanthin as a major carotenoid in *Isochrysis* aff. *galbana*: characterization of extraction for commercial application. J. Korean Soc. Appl. Biol. Chem. 55, 477. http://dx.doi.org/10.1007/s13765-012-2108-3.

Kiron, V., Phromkunthong, W., Huntley, M., Archibald, I., De Scheemaker, G., 2012. Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. Aquac. Nutr. 18, 521–531.

Kiron, V., Sørensen, M., Huntley, M., Vasanth, G.K., Gong, Y., Dahle, D., Palihawadana, A.M., 2016. Defatted biomass of the microalga *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic salmon. Front. Mar. Sci. 3, 67.

Kousoulaki, K., Østbye, T.K.K., Krasnov, A., Torgersen, J.S., Mørkøre, T., Sweetman, J., 2015. Metabolism, health and fillet nutritional quality in Atlantic salmon (*Salmo salar*) fed diets containing n-3-rich microalgae. J. Nutr. Sci. 4, e24. https://www.ncbi.nlm.nih.gov/pubmed/26495116.

Maeda, H., 2015. Nutraceutical effects of fucoxanthin for obesity and diabetes therapy: a review. J. Oleo Sci. 64 (2), 125–132.

Mahy, M., Van Eycken, L., Oosterlinck, A., 1994. Evaluation of uniform color spaces developed after the adoption of CIELAB and CIELUV. Color. Res. Appl. 19 (2), 105–121. http://dx.doi.org/10.1111/j.1520-6378.1994.tb00070.x.

Messina, M., Piccolo, G., Tulli, F., Messina, C.M., Cardinaletti, G., Tibaldi, E., 2013. Lipid composition and metabolism of European sea bass (*Dicentrarchus labrax* L.) fed diets containing wheat gluten and legume meals as substitutes for fish meal. Aquaculture 376-379, 6–14.

Miller, M.R., Nichols, P.D., Carter, C.G., 2007. Replacement of fish oil with *thraustochytrid Schizochytrium* sp. L oil in Atlantic salmon parr (*Salmo salar* L) diets. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 148, 382–392.

Montero, D., Robaina, L., Caballero, M.J., Gines, R., Izquierdo, M.S., 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time-course study on the effect of a re-feeding period with a 100% fish oil diet. Aquaculture 248, 121–134.

Morrison, W.R., Smith, L.M., 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5, 600–608.

Mourente, G., Bell, J.G., 2006. Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): effects on flesh fatty acid composition, plasma prostaglandins E-2 and F-2 alpha, immune function and effectiveness of a fish oil finishing diet. Aquac. Nutr. 11, 25–40.

Mourente, G., Dick, J.R., Bell, J.G., Tocher, D.R., 2005. Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and β-oxidation of [1-14C]18:3n − 3 (LNA) and [1-14C]20:5n − 3 (EPA) in hepatocytes and enterocytes of European sea bass (*Dicentrarchus labrax* L.). Aquaculture 248, 173–186.

Mozaffarian, D., Rimm, E., 2009. Fish intake, contaminants, and human health: evaluating the risk and the benefits. J. Am. Med. Assoc. 296, 1885–1889.

Muller-Feuga, A., 2004. Microalgae for aquaculture. The current global situation and future trends. In: Richmond, Amos (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Publishing, pp. 352–364.

Nakagawa, H.E., Mustafa, M.D.G.H., Takii, K.E., Umino, T.E., Kumai, H.I., 2000. Effect of dietary catechin and spirulina on vitamin C metabolism in red sea bream. Fish. Sci. 66 (2), 321–326.

Nandeesha, M.C., Gangadhara, B., Manissery, J.K., Venkataraman, L.V., 2001. Growth performance of two Indian major carps. catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels of *Spirulina platensis*. Bioresour. Technol. 80 (2), 117–120.

Nematipour, G.F., Nakagawa, H., Nanba, K., Kasahara, S., 1987. Effect of chlorella-extract supplementation to diet on lipid-accumulation of ayu. Nippon Suisan Gakkaishi 53 (9), 1687–1692.

Nematipour, G.F., Nakagawa, H., Ohya, S., 1990. Effect of chlorella-extract supplementation to diet on in vitro lipolysis in ayu. Nippon Suisan Gakkaishi 56 (5), 777–782.

Norambuena, F., Hermon, K., Skrzypczyk, V., Emery, J.A., Sharon, Y., Beard, A., Turchini, G.M., 2015. Algae in fish feed: performances and fatty acid metabolism in juvenile Atlantic salmon. PLoS One 10, e0124042. http://dx.doi.org/10.1371/journal.pone.0124042.

Norsker, N.H., Barbosa, M.J., Vermuë, M.H., Wijffels, R.H., 2011. Microalgal production — a close look at the economics. Biotechnol. Adv. 29, 24–27.

Oliva-Teles, A., Pereira, J.P., Gouveia, A., Gomes, E., 1998. Utilization of diets supplemented with microbial phytases by sea bass (*D. labrax*) juveniles. Aquat. Living Resour. 11, 255–259.

Olvera-Novoa, M.A., Dominguez-Cen, I.J., Olivera Castillo, L., Martinez-Palacios, C.A., 1998. Effects of the use of the microalga *Spirulina maxima* as fishmeal replacements in diets for tilapia *Oreochromis mossambicus* (Peters), fry. Aquac. Res. 29, 709–715.

Palmegiano, G.B., Gai, F., Gasco, L., Lembo, G., Spedicato, M.T., Trotta, P., Zoccarato, I., 2009. Partial replacement of fish meal by T-ISO in gilthead sea bream (*Sparus aurata*) juveniles diets. Ital. J. Anim. Sci. 8 (Suppl. 2), 869–871.

Peng, J., Yuan, J.-P., Wu, C.F., Wang, J.H., 2011. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. Mar. Drugs 9 (10), 1806.

Reitan, K.I., Rainuzzo, J.R., Olsen, Y., 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. J. Phycol. 30, 972–979.

Reitan, K.I., Eriksen, T., Berge, G.M., Ruyter, B., Sørensen, M., Galloway, T.F., Kjørsvik, E., 2013. Nutrient digestibility and effect on gut morphology of diets with increasing content of dried microalgae *Phaeodactylum tricornutum* in Atlantic cod and Atlantic salmon. In: Lecture presented at the 3rd Danish Macro Algae Conference, Grenaa, Denmark, 9th–10th October, . http://www.algecenterdanmark.dk/conferences/.

Ribeiro, A.R., Gonçalves, A.C., Barbeiro, M.I., Bandarra, N., Nunes, M.L., Carvalho, M.L., Silva, J., Narvalho, J., Dinis, M.T., Tomé, S., Dias, J., 2017. *Phaeodactylum tricornutum* in finishing diets for gilthead seabream: effects on skin pigmentation, sensory properties and nutritional value. J. Appl. Phycol. http://dx.doi.org/10.1007/s10811-017-1125-3.

Sánchez, S., Martinez, E.M., Espinol, F., 2000. Biomass production and biochemical variability of the marine microalga *Isochrysis galbana* in relation to culture medium. Biochem. Eng. J. 6, 13–18.

Sarker, P., Gamble, M., Kelson, S., Kapuscinski, A., 2016a. Nile tilapia (*Oreochromis niloticus*) show high digestibility of lipid and fatty acids from marine *Schizochytrium* sp. and of protein and essential amino acids from freshwater *Spirulina* sp. feed ingredients. Aquac. Nutr. 22, 109–119.

Sarker, P.K., Kapuscinski, A.R., Lanois, A.J., Livesey, E.D., Bernhard, K.P., Coley, M.L., 2016b. Towards sustainable aquafeeds: complete substitution of fish oil with marine microalga *Schizochytrium* sp. improves growth and fatty acid deposition in juvenile Nile tilapia (*Oreochromis niloticus*). PLoS One 11, e0156684.

Shields, R.J., Lupatsch, I., 2012. Algae for aquaculture and animal feeds. J. Anim. Sci. 21, 23–37.

Skrede, A., Mydland, L.T., Ahlstrom, O., Reitan, K.I., Gilserød, H.R., Øverland, M., 2011. Evaluation of microalgae as sources of digestible nutrients for monogastric animals. J. Anim. Feed Sci. 20 (1), 131–142.

SØrensen, M., Berge, G.M., Reitan, K.I., Ruyter, B., 2016. Microalga *Phaeodactylum tricornutum* in feed of Atlantic salmon (*Salmo salar*)- effect on nutrient digestibility and utilization of feed. Aquaculture 460, 116–123.

Spence, C., Levitan, C.A., Shankar, M.U., Zampini, M., 2010. Does food colour influence taste and flavour perception in humans? Chemosens. Percept. 68–84. http://dx.doi.org/10.1007/s12078-010-9067-z.

Tibaldi, E., Tulli, F., Dias Freire, R., 1998. Fonti proteiche alternative alla farina di pesce nell'alimentazione di giovanili di spigola (*D. labrax)*. Biol. Mar. Mediterr. 5, 2034–2043.

Tibaldi, E., Hakim, Y., Uni, Z., Tulli, F., de Francesco, M., Luzzana, U., Harpaz, S., 2006. Effects of the partial substitution of dietary fishmeal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). Aquaculture 261, 182–193.

Tibaldi, E., Chini Zittelli, G., Parisi, G., Bruno, M., Giorgi, G., Tulli, F., Venturini, S., Tredici, M.R., Poli, B.M., 2015. Growth performance and quality traits of European Sea bass (*D. labrax)* fed diets including increasing levels of freeze-dried *Isochrysis* sp. (T-ISO) biomass as a source of protein and n-3 long chain PUFA in partial substitution of fish derivates. Aquaculture 440, 60–68.

Tredici, M.R., Bassi, N., Prussi, M., Biondi, N., Rodolfi, L., Chini Zittelli, G., Sampietro, G., 2015. Energy balance of algal biomass production in a 1-ha "Green Wall Panel"plant: how to produce algal biomass in a closed reactor achieving a high Net Energy Ratio. Appl. Energy 154, 1103–1111.

Tredici, M.R., Rodolfi, L., Biondi, N., Bassi, N., Sampietro, G., 2016. Techno-economic analysis of microalgal biomass production in a 1-ha Green Wall Panel (GWP (R)) plant. Algal Res. 19, 253–263.

Tulli, F., Messina, M., Calligaris, M., Tibaldi, E., 2010. Response of European sea bass (*Dicentrarchus labrax*) to graded levels of methionine (total sulfur amino acids) in soya protein-based semi-purified diets. Br. J. Nutr. 104, 664–673.

Tulli, F., Chini Zittelli, G., Giorgi, G., Poli, B.M., Tibaldi, E., Tredici, M.R., 2012. Effects of the inclusion of dried *Tetraselmis suecica* on growth, feed utilization and fillet composition of European sea bass juveniles fed organic diets. J. Aquat. Food Prod. Technol. 21, 1–11.

Vasconcellos, J.P., Vasconcellos, S.A., Pinheiro, S.R., de Oliveira, T.H.N., Ribeiro, N.A.S., Martins, C.N., Porfírio, B.A., Sanches, S.A., de Souza, O.B., Telles, E.O., Balian, S.C., 2013. Individual determinants of fish choosing in open-air street markets from Santo André, SP/Brazil. Appetite 68, 105–111. http://dx.doi.org/10.1016/j.appet.2013.04.019.

Vizcaìno, A.J., Lòpez, G., Sàez, M.I., Jiménez, J.A., Barros, A., Hidalgo, L., Camacho-Rodrìguez, J., Martìnez, T.F., Ceròn-Garcìa, M.C., Alarcòn, F.J., 2014. Effects of the microalga *Scenedesmus almeriensis* as fishmeal alternative in diets for gilthead sea bream. *Sparus aurata* juveniles. Aquaculture 431, 34–43.

Vizcaìno, J.A., Mendes, I.S., Varela, L.J., Ruiz-Jarabo, I., Rico, R., Figueroa, F.L., Abdala, R., Moriñigo, M.A., Mancera, J.M., Alarcón, F.J., 2016. Growth, tissue metabolites and digestive functionality in *Sparus aurata* juveniles fed different levels of macroalgae, *Gracilaria cornea* and *Ulva rigida*. Aquac. Res. 47 (10), 3224–3238.

Walker, A.B., Berlinski, D.L., 2011. Effects of partial replacement of fish meal protein by microalgae on growth, feed intake, and body composition of Atlantic cod. North Am. J. 73 (1), 76–83.

Zmora, O., Richmond, A., 2004. Microalgae for aquaculture. In: Richmond, Amos (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Publishing Ltd, Oxford, UK, pp. 365–379(DOI: 20.1002/9780470995280.ch20).