DIVERSIFY

New species for EU aquaculture

Deliverable Report

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Description This report will include both proximate composition and analytic fatty acid profiles of fish receiving different dietary treatments. The effect of dietary treatment on end product quality will be assessed within this frame, by correlations between individual quality attributes and the dietary history (e.g. dietary fat and protein levels, fat sources, etc.) or other rearing parameters (e.g. rearing system, temperature, or density…). The dietary or the environmental parameters that will be correlated to the end product quality for each species will largely depend on the GWP 5 (Grow out husbandry) and the choices of parameters that will be made within this package. A technical quality evaluation of row material from selected species will be also evaluated in relation to farming history. A specific dossier per product, including (a) nutritional value in terms of protein, fat and w-3 contents and (b) sensory characteristics, regarding fish origin, rearing conditions and feeding regime will be presented.

Deviations: Deliverable 28.7 is delivered with a 5-month delay, which was necessary in order to complete some new pending sensorial (IRTA) and nutritional (ULL) analyses, especially those corresponding to the last samples of pikeperch and grey mullet fattening (WP22 and WP23) sent by P9 and P18, respectively. The delay was also necessary to allow the use of relevant and closely related information that came out in months 54-55 in the immediately preceding deliverables, D28.5 and D28.6, which was a prerequisite for the completion of D28.7. The deviation did not create any further delay or affect the completion of other Tasks in the DOW.

Table of Contents

1. Introduction **3**
1.1. General introduction 3
1.2. Overall objective 5
2. Materials and methods **6**
2.1. Fish origin/ rearing/ sampling 6
2.2. Somatometric measurements 7
2.3. Proximate and fatty acid composition analysis 8
2.4. Sensory analysis 10
2.5. Statistics-correlations 10
3. Results and Discussion **11**
3.1. **Greater amberjack**
Somatometric – technical indexes 111
Proximate and fatty acid composition 224
Sensory analysis .... **Error! Bookmark not defined.**19
3.2. **Grey Mullet**
Somatometry – technical indexes 22
Proximate and fatty acid composition 23
Sensory analysis 27
3.3. **Pikeperch**
Proximate and fatty acid composition 2229
Sensory analysis .... **Error! Bookmark not defined.**3
3.4. **Comparisons among the four species**
Somatometry – technical indexes 35
Proximate and fatty acid composition 36
4. Conclusions .... Error! Bookmark not defined.**0**
5. Sources **411**

1. Introduction

1.1. General introduction

As stated in the DOW, the European aquaculture sector is well situated to be among world leaders in the efficient and sustainable production of safe seafood of the highest quality and nutritional value, considering consumer preferences (EATIP, 2012). In this sense, the emphasis of DIVERSIFY´s Socioeconomic WPs has been given to 4 selected species including meagre (*Argyrosomus regius*) and greater amberjack (*Seriola dumerili*) for their particular interest in the Mediterranean or warm-water cage culture industry, grey mullet (*Mugil cephalus*) addressed through intensive-tank rearing systems or pond/extensive and integrated cultures, and the pikeperch (*Sander lucioperca*) as a freshwater species mainly reared in recirculation aquaculture systems (RAS). As shown in previous deliverables of this GWP, these emerging species can be either marketed as a whole large size fish but also processed easily into a range of products including fillets among many others, to provide the consumer with both a wider diversity of fish species and new processed nutritional and sustainable products.

Fish has long been recognized as a valuable source of high-quality protein in the human diet. In recent years, fish lipids have also assumed great nutritional significance owing to their protective role against the development of many different health disorders (Zárate *et al.*, 2017). Fish consist of between 60 - 85% water, while proteins account for around 20%, fat between 0.1 and 10% and inorganic matter, including minerals, less than 2%. In some cases, a very small percentage of carbohydrates is also present. Fish is easily digested due to its little collagen content and constitutes a healthy food suitable for all ages, because it consists of all essential amino acids, minerals and vitamins that the body cannot synthesize. Fish is also providing a reduced percentage of a very high-quality fat (CECOPESCA, 2012).

Fish are the primary dietary source of long chain n-3 (omega-3; w3) polyunsaturated fatty acids (LC-PUFA), particularly, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), that exert a range of health benefits through all life stages by their molecular, cellular and physiological actions. Farmed fish were traditionally fed a diet with high levels of marine ingredients, such as fish oil and fishmeal, derived from pelagic fisheries, which kept these ideal components also in the final fish product. However, the continued pressures on wild fish stocks as aquaculture production grows, along with greater competition for LC-PUFA sources from the nutraceutical and pharmaceutical industries have resulted in changes in aquaculture feed formulations. Terrestrial ingredients such as plant sources of meal and fats, mainly of oilseed origin, are increasingly used in consolidated species feeds without detriment to fish health or growth. Like humans, marine species of fish are inefficient at converting the shorter-chain fatty acid, α -linolenic acid (ALA; 18:3n-3), into EPA and DHA, and must therefore obtain the n-3 LC-PUFA through the diet. Nevertheless, the fatty acid profiles of vegetable oils differ from those of fish oil, being richer in n-6 and n-3 18C PUFA (18:2n-6 and 18:3n-3) and devoid of n-3 LC-PUFA, resulting in changes of the fatty acid composition of farmed fish (Sprague *et al*., 2016). Consequently, without a rational use of these terrestrial ingredients, and a sufficient knowledge of new targeted species for aquaculture diversification, the nutritional benefit of their commercial products to the consumer can be lowered, as well as the fish growth and wellbeing.

Nowadays, due to its higher content of total fat, farmed consolidated fish species generally contain higher levels of total EPA, and DHA than most wild-caught fish of the same species (Nichols *et al*., 2014) and all terrestrial livestock (Sprague *et al.,* 2016). However, farmed fish also tends to contain significantly higher levels of saturated fatty acids and 18:2n-6 (Hossain, 2011; Nichols *et al*., 2014). The fish fillet fatty acid nutritional and healthy quality can be also assessed calculating different fatty acid health indicator indexes (Ulbricht *et al*., 1991; Abrami *et al*., 1992; Senso *et al*., 2007; Pérez *et al*., 2014), and all these aspects will be evaluated in the new fish batches presented in this Deliverable.

Adequate rearing conditions are also very important to ensure animal well-being. Temperature, type of rearing system, tank water volume and stocking density among other factors, are known to affect fish physiology and welfare and as a consequence, the performance and quality of cultured finfish (Mylonas *et al*., 2010, Rodríguez-Barreto *et al*., 2015; Timalsina *et al*., 2017). Under stressful conditions, each teleost species has a particular response to a given stressor that may also vary considerably depending on the intensity and duration of the stressor (Schreck, 2010). Therefore, culture conditions can also have a direct effect on the pattern of utilization and mobilization of energy reserves (Portz *et al*., 2006) and as a result of this, to the fish final nutritional value.

Finally, as the diets of farmed fish are being changed to lower production costs, and to fit sustainability criteria, differences in the flavor profiles of fish fillets have been noted in some studies (Noor *et al*., 2011) but not in others (González *et al*., 2006). Similarly, changing rearing conditions including temperature, stocking densities etc., can also affect the flesh firmness and texture and all these aspects should also be evaluated in the new emerging species. Sensory properties are important drivers of food selection and consumption, so the changes introduced in animal diets should not alter in a considerable way the main sensory characteristics of the raw material. As stated by Ares *et al.*, (2010) and Verbeke (2006), consumers do not seem to be ready to compromise the sensory features of their food products for potential benefits to their health. In fact, an important percentage of consumers prefer to diminish the intake of certain products or even avoid them rather than consume a supposedly healthy and tasteless version (Guerrero et al., 2011). According to the review performed by Gatlin et al., (2007), flavour, colour, odour and/or texture are significantly affected by diet in about half the studies examined. Given the physiological, nutritional, environmental and compositional differences among aquacultured finfish, conclusions reached about the product quality of one species cannot be automatically applied to another. Generally speaking, different flavour attributes, such as nutty, sweet, fishy, painty or grassy, have been used to describe the effects of dietary ingredients on the specific flavour characteristics of fish from different dietary treatments (Gatlin et al., 2007). However, in most cases these differences cannot be perceived by regular consumers. Although numerous studies have reported that dietary lipid sources can affect some of the principal characteristics of the final eating qualities of farmed fish, such as sensory and organoleptic characteristics, texture, storage stability, flavour volatile compounds and pigmentation, the results are often contradictory and are not yet fully understood (Turchini et al., 2009).

In collaboration with a number of SMEs, DIVERSIFY´s partners have already built up a strong knowledge to overcome the initially documented bottlenecks in the aquaculture production of the selected species. However, the effort must continue to change consumer beliefs regarding the quality of farmed versus wild fish. The growing aquaculture sector must face emerging biological, economic and social challenges that may influence the ability to maintain ethically sound, productive and environmentally friendly production of healthy and nutritious fish products (Hixson, 2014; Føre *et al*., 2018). In general, farmed fish is perceived to be less affected by marine pollution, heavy metals and parasites. On the contrary, still the wild fish is considered to have healthier and more natural feeding and as a consequence, beliefs related to quality are in favor of wild fish (Claret *et al*., 2014). Two important aspects that affect quality are the above mentioned nutritional and technical features of the fish. The former refers to the nutritional value of the food, including the fillet proximate composition and total content of ω3 polyunsaturated fatty acids (ω3 LC-PUFA) (due to their numerous health benefits) (Vanhonacker *et al.*, 2013). The technical quality refers to the processing losses and the edible output expressed as somatometric indexes and technical yields (dressing and filleting yield), which is mainly an economic aspect and it is of interest for both processors and consumers. Furthermore, the organoleptic quality of the fish, i.e. the human sensory impression, is among the capital factors for purchasing this product (Grigorakis, 2007).

All these aspects have been evaluated in deliverables 28.3, 28.5 and 28.6, for a few batches of meagre, greater amberjack, pikeperch and mullet specimens attained during 2014 and 2015, from very different origins (both farmed and wild) and an uncomplete or null knowledge of biological conditions and dietary composition. The present deliverable adds the quality evaluation of some new fish batches produced within DIVERSIFY´s grow-out tasks including WP21 (greater amberjack), WP22 (pikeperch), and WP23 (mullet), and its relationship with the evaluation performed to previous batches. Since many controversial issues in aquaculture regarding food safety, nutrition, and sustainability are directly related to the nutrition and feeds for farmed fish, the proximate and fatty acid composition of the extruded diets together with the main rearing history factors are also given and correlated with the technical and sensory quality of the fillets as well as their nutritional value expressed in absolute terms per 100 g serving portions.

1.2. Overall objective

Sub-task 28.3.2 (ULL) attempts to correlate some technical quality features including nutritional value and sensory characteristics (ULL, HCMR, IRTA, CTAQUA) of greater amberjack, grey mullet, pikeperch and meagre with their previous nutritional - rearing history, in order to provide fish farmers and other potential chain partners a strong input for value positioning statement and communication claims of the four selected species. By relating new determinations made in the framework of WP21, WP22 and WP23, with the previous analyses (see D28.3, D28.5 and D28.6), it is intended to show how diet and other fish rearing conditions may influence the nutritional quality of the final product. Furthermore, it is also shown that DIVERSIFY´s partners possess the knowledge and technology necessary to produce a fish product capable of meeting the nutritional, safety and environmental sustainability expectations of the international market, without losing the differential sensory characteristics of each of the 4 species selected in this work package.

2. Materials and methods

2.1. Fish origin/ rearing/ sampling

All new batches of fish specifically evaluated for the present Deliverable were from a reared origin (see Table 2.1, in blue).

The new **greater amberjack** specimens (Batches 3 and 4) from the Atlantic Ocean were obtained from two different rearing trials performed within Task 21.3 "Development of appropriate husbandry practice", and specifically in Action 21.3.2 (IEO/ULL) "Definition of optimal stocking density", at the P8. IEO facilities in Tenerife (Spain). In Batch 3, fish of 5 g average weight were assayed at three different stocking densities (9 groups) in 500 l tanks, from September 2015 to January 2016 (20.7-25.4 ºC). Batch 4 was sampled from 4 different stocking densities (12 groups), where 150 g-fish were stocked in 4000 l tanks for a period of 4 months (February-July 2017; 19.0-22.9 ºC). The animals were fed the same turbot commercial extruded diet (Table 2.3) manufactured by P20. SARS (Skretting, Norway), by only varying the pellet size accordingly to the fish age (Skretting R3-R5/R5-R7 for Batch 3 and Skretting R5-R7 for Batch 4). The fish reached a similar final size in both trials (Table 2.1).

Two new **grey mullet** batches (Batches 2 and 3) were attained as follows (Table 2.1):

Batch 2 was sampled on December 2016 from a trial performed within WP23 (Grow out husbandry - grey mullet) and specifically within Task 23.4 "Compare the effect of feeding an improved grey mullet diet on the grow-out in monoculture of wild juveniles" led by CTAQUA (P18) in PISTRESA farm (Cádiz, Spain). The wild caught grey mullet juveniles had been stocked in earthen ponds (1100 m^2) and natural temperature, and fed an experimental grey mullet extruded feed as described in Task 23.2 (P4 IOLR/P31 IRIDA; Table 2.3), although natural feeding was also available in the ponds.

Grey mullet Batch 3, sampled in December 2017 from PIMSA farm (Cádiz, Spain), was also produced in earthen ponds under natural temperature, in polyculture regimen with European seabass (*Dicentrachus labrax*). The mullets were fed a carp extruded diet (SORGAL, Table 2.3), although some natural feeding was also available in the ponds. Four specimens were kindly provided to DIVERSIFY by the company at the end of the trial, and subjected to the evaluation of somatometric measurements and nutritional and sensorial analysis.

Fish of the new **pikeperch** Batch 2 (WP22, Grow out husbandry – pikeperch) were sampled from an experiment which ended the 31st of January 2018, date at which P9. UL had done a growth control. Individual final weights were between 300-500 g, for 32-34 cm total length specimens. The fish were from a Czech Republic genetic pool supplied by the P29. ASIALOR on 22th September 2017 (initial mean body weight = 102.4 g). They received an extruded diet from Le Gouessant (sturgeon grower) (Tables 2.1 and 2.3).

Finally, no new batches of meagre were available within DIVERSIFY´s GWP5 for the present Deliverable. Therefore, comparisons made in the final chapter are based on previous information already given for this species in Deliverables 28.3, 28.5 and 28.6 (Tables 2.1.1, 2.3.1 and 3.4.1).

Table 2.1.1. Origin, season of sampling and size information of fish used in Task 28.3

Species	Batch	Season	N	Origin-farming	Feed	Fish size	WP
Greater amberjack (*Seriola dumerili*)	1	Feb 2015	10	Farm (Corfu S.A.), NW Greece. Floating sea cages	Commercial extruded feed	1-1.5 kg	
	2	Apr 2015	8	Farm (Argosaronikos S.A.), Attiki, Greece. Floating sea cages	Commercial extruded feed	15-20 kg	
	3	Jan 2016	49	IEO Canarias, Tenerife, Spain (P15). Concrete ponds, FT rearing system	Commercial extruded feed (P20)	400-650 g	21
	4	Jul 2017	24	IEO Canarias, Tenerife, Spain (P15). Concrete ponds, FT rearing system	Commercial extruded feed (P20)	300-600 g	21
Grey mullet (*Mugil cephalus*)	1	Feb 2015	10	Wild fish. Bay of Cadiz, Spain. Earthen ponds	Natural feeding	500 g-1 kg	
	2	Dec 2016	6	P18/PISTRESA Cadiz, Spain. Earthen ponds	Natural feeding & experimental extruded feed	150-300 g	23
	3	Dec 2017	4	PIMSA, Cadiz, Spain. Earthen ponds	Natural feeding & commercial extruded feed	600-800 g	23
Pikeperch (*Sander lucioperca*)	1	Jul 2014	10	France. Fresh water intensive farm	Commercial extruded feed	1-2 kg	
	2	Jan 2018	4	Farm (P9/ P29 ASIALOR), NW France. Land based ponds, RAS	Commercial extruded feed (Le Gouessant)	300-500 g	22
Meagre (*Argyrosomus regius*)	1	Nov 2014	10	Farm (Andromeda Group), Burriana, Spain. Floating sea cages	Commercial extruded feed	1.5-2 kg	

Data in blue have been specifically obtained for the present Deliverable

2.2. Somatometric measurements

Fish total weight was taken in sampled individuals (not available for pikeperch-Batch 2). As described in Deliverable 28.3 fish were subsequently gutted and gutted body weighed, visceral, gonad and liver weights were also determined. When possible, visceral fat was separated from the rest of the viscera and weighed. Fish were subsequently filleted and fillets were also weighed. The following somatometric indexes were calculated individually:

Condition index (CI) = [100× body weight (g) / body length^3 (cm^3)],

Dressing yield (DY) = [100× (gutted body weight / body weight)],

Filleting yield (FY) = [100 × (fillet weight / body weight)],

Hepatosomatic index (HSI) = [100 × (liver weight / body weight)],

Gonadosomatic index (GSI) = [100 × (gonad weight / body weight)] and

Viscerosomatic index (VSI) = [100 × (total viscera weight / body weight)].

Visceral fat index (VFI) = [100 × (visceral fat weight / body weight)].

Apart from the somatometric measurements described above, one fillet from each fish was sampled, packed and stored at -20 °C until sensory analysis (IRTA), while the second fillet was used for biochemical composition determinations together with samples of the extruded diets (ULL).

2.3. Proximate and fatty acid composition analysis

Proximate composition analysis (protein, fat, moisture and ash) of fish fillet and extruded diets (Table 2.3.1) were conducted following the AOAC (2005) methods. Specifically, moisture was calculated gravimetrically after complete drying of fish tissue and total inorganic content (ash %) after total burn of organic matter. Total protein content was determined by the Kjeldahl method, calculated as % Nitrogen x 6.25. Crude fat was determined after chloroform/methanol (2:1; v:v) cold extraction (Folch *et al.*, 1957).

A total lipid fraction (1-2 mg) from each sample was subjected to direct acid-catalysed trans methylation during 16 h at 50 °C to obtain fatty acid methyl esters (FAME). FAME were purified by TLC (Christie, 2003) with hexane/diethyl ether/acetic acid (90:10:1 by volume) and then separated and analysed using a TRACE-GC Ultra gas chromatograph (Thermo-Fisher Scientific Inc., Waltham, Massachusetts, USA) equipped with an on-column injector, a flame ionization detector (240 °C) and a fused silica capillary column (Supelcowax™ 10; Sigma- Aldrich Co., St. Louis, Missouri, USA). The column temperature was programmed with four different ramps of temperature for an increase from 50 to 230 °C. FAME were identified by comparison with retention times of a standard FAME mixture consisting of C4-C24 (Supelco 18,919-1AMP), PUFA No. 3 from menhaden oil (Supelco 47085-U) and a commercial cod roe FAME. When necessary, identification of individual FAME was confirmed by GC–MS chromatography (DSQ II, Thermo Fisher Scientific Inc. Waltham, Massachusetts, USA).

The two *Indexes of lipid quality* most frequently used to assess fillet nutritional value in terms of fatty acid composition (Senso *et al*., 2007) were also determined:

(1). *Index of atherogenicity (IA):* indicating the relationship between the sum of main saturates and that of main unsaturated fatty acids, the former being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory systems), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro and macrocoronary diseases) (Ulbricht *et al*., 1991; Pérez *et al*., 2014).

$$\text{IA} = \frac{[(12{:}0 + (4 \text{ x } 14{:}0) + 16{:}0)]}{[\sum \text{MUFAs} + \text{n-6 PUFA} + \text{n-3 PUFA}]}$$

(2). *Index of thrombogenicity (IT):* showing the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenetic (saturated) and the anti-thrombogenetic fatty acids (MUFA, n-6 PUFA and n-3 PUFA) (Ulbricht *et al*., 1991; Pérez *et al*., 2014).

$$\text{IT} = \frac{(14{:}0 + 16{:}0 + 18{:}0)}{[(0.5 \text{ x } \sum \text{MUFAs} + 0.5 \text{ x n-6 PUFA} + 3 \text{ x n-3 PUFA}) + (\text{n-3 PUFA/n-6 PUFA})]}$$

Table 2.3.1. Proximate composition (% wet matter) and main fatty acid profile (% total fatty acids) of diets used to feed the different fish batches.

	Greater amberjack	Grey mullet		Pikeperch	Meagre
Batch	1, 3 & 4	2	3	2	1
Moisture	5.95 ± 0.07	6.47 ± 0.02	6.67 ± 0.06	7.62 ± 0.07	9.70
Fat	18.78 ± 0.02	15.09 ± 0.68	8.79 ± 0.42	10.25 ± 0.04	17.16
Protein	50.14 ± 0.01	35.10 ± 2.46	35.80 ± 1.95	44.09 ± 0.00	43.51
Ash	7.61 ± 0.10	7.01 ± 0.07	9.02 ± 0.20	9.62 ± 0.03	9.31
16:0	13.89 ± 0.21	11.99 ± 0.05	18.56 ± 0.05	18.51 ± 0.15	14.83
18:0	3.07 ± 0.07	2.50 ± 0.04	5.38 ± 0.09	3.70 ± 0.03	3.63
Total SFA	22.91 ± 0.44	19.16 ± 0.22	27.05 ± 0.01	30.44 ± 0.37	23.99
16:1[1]	5.16 ± 0.09	4.19 ± 0.05	3.75 ± 0.17	7.20 ± 0.06	3.33
18:1[2]	27.64 ± 0.79	32.89 ± 0.32	32.61 ± 0.16	17.54 ± 0.10	27.45
Total MUFA	40.39 ± 1.01	47.02 ± 0.29	39.94 ± 0.12	28.71 ± 0.08	32.99
18:2n-6	11.28 ± 0.07	15.57 ± 0.00	22.50 ± 0.02	8.34 ± 0.11	21.61
20:4n-6	0.52 ± 0.01	0.37 ± 0.01	0.54 ± 0.01	0.67 ± 0.00	0.60
Total n-6 PUFA	12.36 ± 0.08	16.62 ± 0.01	23.50 ± 0.18	9.01 ± 0.11	23.40
18:3n-3	3.44 ± 0.07	3.35 ± 0.02	2.47 ± 0.13	1.70 ± 0.02	3.42
20:5n-3	6.61 ± 0.35	3.45 ± 0.06	1.52 ± 0.03	10.04 ± 0.02	5.29
22:6n-3	8.17 ± 0.43	4.98 ± 0.09	3.06 ± 0.10	8.57 ± 0.02	8.36
Total n-3 PUFA	22.26 ± 0.93	15.02 ± 0.16	8.37 ± 0.37	24.55 ± 0.06	18.45
DHA/EPA	1.24 ± 0.00	1.45 ± 0.00	2.02 ± 0.03	0.85 ± 0.00	0.79
ARA/EPA	0.08 ± 0.00	0.11 ± 0.00	0.35 ± 0.01	0.07 ± 0.00	0.11
n-3/n-6	1.80 ± 0.06	0.90 ± 0.01	0.36 ± 0.01	2.73 ± 0.03	0.79

Data are means ± SD (n=2, except for meagre where n=1)

2.4. Sensory analysis

Sensory analysis

The new bathes of fillets from greater amberjack, grey mullet and pikeperch were sensory characterized (IRTA) in four modalities: odour, appearance, flavour and texture, as described in Deliverable 28.3.

To make this characterization, a list of sensory attributes was previously generated to assess and score the fish samples keeping those able to discriminate among the samples. Panellists were specifically trained to be familiarized with the descriptors and their intensity scales. In all cases, the scales developed and described in Deliverable 28.3 were also used for the new batches. Eight panellists with previous experience in the sensory profiling of food products were recruited for this training before evaluating the samples.

Sample's analysis was performed in one session for pikeperch samples, three sessions for grey mullet and three additional sessions for greater amberjack. Samples from all the different batches per species were tasted in each session. Panellists assessed samples in the subsequent order: odour modality first then appearance followed by flavour and finally texture.

In each tasting session, the order of sample presentation and the first order and carry-over effects (Macfie *et al*., 1989) were blocked. In all cases, the evaluation of species was carried out in isolated sensory testing booths (ISO, 2007). All assessors were provided with mineral water to cleanse their palates between samples.

Samples were cooked in a convection oven at 115ºC for 20 minutes in individual transparent glass jars designed to make samples easy to visualize. Jar lids were used to keep the samples' odour from disappearing (Model B-250, Juvasa, Spain). Jars were then placed inside electrical heaters at 60ºC to keep them warm while being tasted.

2.5. Statistics- correlations

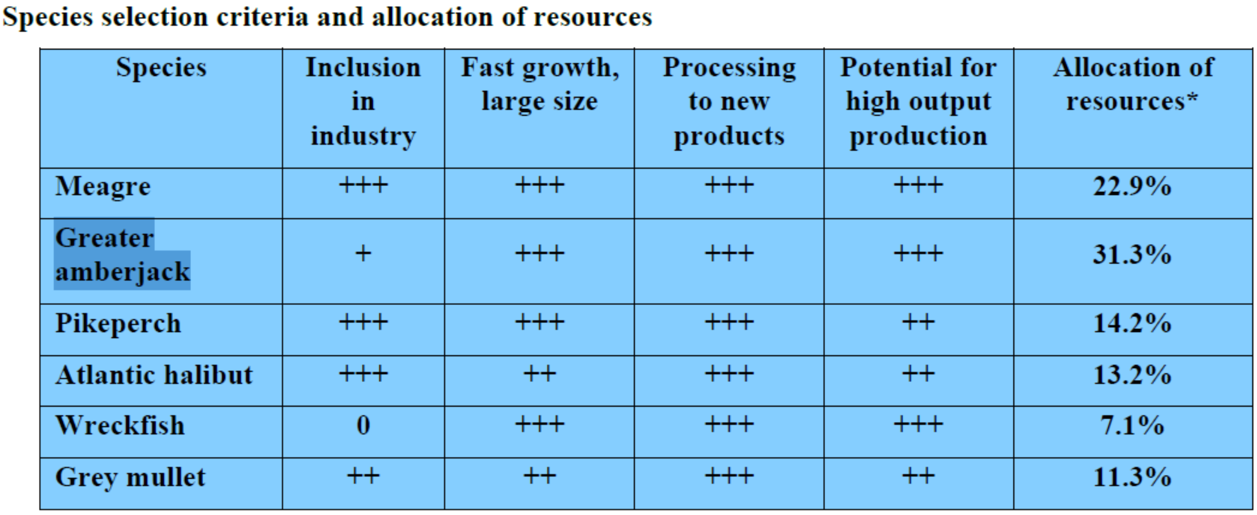
Results are presented as means ± SD for a variable number of samples (n). For all statistical tests, $P<0.05$ was considered as significantly different. Prior to analysis, data were checked for normal distribution as well as for homogeneity of the variances with the Levene test (Zar, 1999). Arcsine square root transformation was applied to all data expressed as percentage. Differences in somatometric measures and proximate and fatty acid composition were analysed by a one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test (Zar, 1999) or through a Student's t-test where appropriate. When normal distribution and/or homogeneity of the variances were not achieved, data were subjected to the Kruskal-Wallis test, followed by a Games-Howell non-parametric multiple comparison test (Zar, 1999). All statistical analysis was performed using the IBM SPSS package 21.0 (IBM Corp., NY, USA).

Sensory data was submitted to a three-way ANOVA for grey mullet and greater amberjack, thus including fish treatment (density or batch), tasters and sessions as fixed factors. In the case of pikeperch only mean values were obtained (all the samples belonged to the same fed treatment).

3. Results and Discussion

3.1. Greater amberjack

This is a cosmopolitan carnivorous species of great interest to the aquaculture sector due to its excellent flesh quality, worldwide market availability and high consumer acceptability (Nakada, 2000). As proven within DIVERSIFY´s frame, its rapid growth (*i.e.*, short time to market size) and large size makes this species very suitable for aquaculture diversification and development of value-added products. It is a large fish with high flesh quality and market value. In addition to its economic potential in the EU market, cultured greater amberjack has a significant potential for exports, as it is distributed worldwide and congener species are produced commercially elsewhere. This cultured fish has proven its potential in other markets. Therefore, a consumer-oriented market introduction of cultured amberjack has been one of the objectives of Socioeconomics WPs. Also, market development is necessary for growth with preservation of the added value and price, once production increases.



Species selection criteria and allocation of resources

Species	Inclusion in industry	Fast growth, large size	Processing to new products	Potential for high output production	Allocation of resources*
Meagre	+++	+++	+++	+++	**22.9%**
Greater amberjack	+	+++	+++	+++	**31.3%**
Pikeperch	+++	+++	+++	++	**14.2%**
Atlantic halibut	+++	++	+++	++	**13.2%**
Wreckfish	0	+++	+++	+++	**7.1%**
Grey mullet	++	++	+++	++	**11.3%**

Somatometry – technical indexes

As shown in Tables 3.1.1 and 3.1.2 the somatometric indexes and technical yields of the new two batches of greater amberjack, confirmed some of the features described in the DOW to justify the selection and effort allocated to this species (see table above). Independently of the stocking densities assayed, in only 5 months the animals reached more than 500 g (Table 3.1.1), displaying high condition indexes and dressing and filleting yields under intensive rearing conditions. A similar situation and highly consistent values, was also found in the second trial performed with 150 g fish reared at 4 stocking densities during only 4 months (Table 3.1.2).

Table 3.1.1. Somatometric indexes and technical yields of greater amberjack (*Seriola dumerili*) from Batch 3 reared from 5 g at three different culture densities (LD, low density; MD, medium density; HD, high density).

	LD	MD	HD
Body weight (g)	544.3 ± 69.8	535.9 ±126.4	534.2 ± 97.5
CI	2.18 ± 0.12	2.21 ± 0.16	2.12 ± 0.11
Dressing yield (%)	93.54 ± 0.58	94.09 ± 0.17	92.61 ± 0.65
Filleting yield (%)	54.73 ± 3.39	55.12 ± 2.93	54.66 ± 1.54
HSI	0.79 ± 0.12 ab	0.72 ± 0.04 a	0.95 ± 0.14 b
VSI	5.47 ± 0.43 a	4.86 ± 0.18 a	6.31 ± 0.55 b
VFI	0.44 ± 0.13	0.32 ± 0.14	0.48 ± 0.15

Data are means ± SD (LD, n=21; MD, n=15 and HD, n=13 for body weight and CI; LD, n=6; MD and HD, n=4 for technical yields). Different letters within a row indicate significant differences among treatments ($P<0.05$). CI: condition index; HSI: hepatosomatic index; VSI: viscerosomatic index; VFI: visceral fat index.

Table 3.1.2. Somatometric indexes and technical yields of greater amberjack (*Seriola dumerili*) from Batch 4 reared from 150 g at four different culture densities (LD, low density; MLD, medium-low density; MHD, medium-high density; HD, high density).

	LD	MLD	MHD	HD
Body weight (g)	387.5 ± 92.6	452.5 ±119.8	408.0 ± 99.3	478.0 ±153.1
CI	1.91 ± 0.07	1.83 ± 0.14	1.98 ± 0.22	1.99 ± 0.22
Dressing yield (%)	94.59 ± 0.69	94.94 ± 0.38	93.97 ± 1.00	94.30 ± 0.60
Filleting yield (%)	47.86 ± 5.96	46.29 ± 3.12	47.72 ± 2.49	47.19 ± 0.74
HSI	0.78 ± 0.21	0.92 ± 0.25	1.02 ± 0.18	0.99 ± 0.11
VSI	4.05 ± 0.88	3.87 ± 0.40	4.75 ± 0.80	4.57 ± 0.60

Data are means ± SD (LD, n=4; MLD, MHD and HD, n=6). CI: condition index; HSI: hepatosomatic index; VSI: viscerosomatic index.

Optimum stocking densities in a flow-through aquaculture system are necessary in terms of maintaining a positive correlation between density and growth rate. It is necessary to find balance between the maximum profit and the minimum incidence of physiological and behavioral disorders (Ayyat *et al.*, 2011). Since availability of oxygen and fish movement capacity is affected by the increasing stocking density, this rearing parameter can affect the performance and quality of the reared populations and the final products (Timalsina *et al.*, 2017). However, the present results support the hypothesis that under the assayed conditions, greater amberjack juveniles have mechanisms to cope with the stress and metabolic changes associated to higher density conditions with no detrimental effects on growth performance and filleting yield, and that they are able to adapt to the increasing stocking densities up to 7.5 kg m^{-3} without showing an immunosuppressive state typically observed in chronically stressed fish. The findings of the current study have practical significance for establishing greater amberjack commercial rearing practices.

Preliminary observations also reported that high stocking density has no marked effects on growth and food utilization of young pikeperch, and that pikeperch juveniles can be kept at high densities ranging between 30–60 kg m^{-3} without any increase in physiological stress response (Dalsgaard *et al.*, 2013). However, another study reported that high density can increase the susceptibility to diseases for pikeperch juveniles (Jensen *et al.,* 2011). Deliverable 22.1 "Effects of multiple variables on stress, immune response and growth performances and recommendations of optimal conditions for pikeperch grow out" also supports that this species has not a good welfare at high stocking densities.

In the recently delivered D23.2 "Stocking protocols for pond monoculture grow out of F1 and wild caught grey mullet", the implicated Diversify partners compared the effect of feeding an improved grey mullet diet on the grow-out in monoculture of F1 juveniles stocked at two different densities in cement and earthen ponds. In contrast with greater amberjack, the results consistently show that increasing the fish stocking density in mullet can lead to decreased growth in an increasing segment of the population resulting in larger numbers of smaller fish. Omnivores and herbivores specimens are constantly grazing resulting in continuous consumption. Carnivores, in contrast, consume prey in discreet meals. As feed also represents a significant cost in the production of grey mullet, the effect of increased ration size, use of extruded or not pelleted diets as well as the number of meals per day (simulate continuous feeding) should be considered.

The previous studies highlight that to attain a final good quality and profitable product from these new emerging species further on growing studies are needed.

Table 3.1.3 shows the average somatic indexes obtained for both the DIVERSIFY´s WP21 new batches and previous batches described in earlier Deliverables (D28.3; Table 2.1) and produced out of DIVERSIFY GWP5.

Table 3.1.3. Somatic indexes and technical yields of greater amberjack (*Seriola dumerili*)

	Batch 1	Batch 2	Batch 3	Batch 4
Body weight (kg)	1.19 ± 0.19 b	13.00 ± 1.63 c	0.54 ± 0.01 a	0.43 ± 0.06 a
CI	1.90 ± 0.09 b	1.51 ± 0.07 a	2.17 ± 0.13 c	1.95 ± 0.19 b
Dressing yield (%)	92.79 ± 0.79 a	94.73 ± 0.38 b	93.43 ± 0.76 a	94.38 ± 0.77 b
Filleting yield (%)	50.49 ± 2.90 b	-----	54.82 ± 2.65 c	47.16 ± 3.77 a
HSI	1.61 ± 0.24 c	1.14 ± 0.26 b	0.82 ± 0.14 a	0.94 ± 0.19 ab
VSI	5.60 ± 0.71 c	2.84 ± 0.33 a	5.54 ± 0.69 c	4.37 ± 0.74 b
VFI	0.39 ± 0.21	-----	0.42 ± 0.15	-----

Data are means ± SD (Batch 1, n=10, Batch 2, n=8, Batch 3, n=49 for body weight and CI, and n=14 for technical yields; Batch 4, n=24). Different letters within a row indicate significant differences among treatments ($P<0.05$). CI: condition index; HSI: hepatosomatic index; VSI: viscerosomatic index; VFI: visceral fat index.

The condition index (CI) is indicative of the feeding condition of the fish and has been shown to increase in well-fed fish, like in the intensive farming-originated fish in comparison with extensively farmed and wild fish (Floss *et al.*, 2002; Grigorakis, 2007; Piccolo *et al.*, 2007; Martelli *et al.*, 2013).

The dressing yield is also important because it determines the yield for the fish when sold as gutted, without further processing, which is one custom way of commercialization in EU for fresh. The filleting yield is an important parameter especially for species such as the greater amberjack in which filleting is among their custom processing, because it describes their actual edible gain. As shown in the table, the dressing yields for all batches are quite similar exceeding 90%.

Consistently with previous Deliverables our present data confirm the good CI and dressing yields indexes of the selected species and show that filleting yields are also high for greater amberjack, already exceeding 50% for a fish size of around 0.5 kg. The present results indicating that greater amberjack intensively produced within DIVERSIFY can be economically advantageous in this aspect.

Proximate and fatty acid composition

As the greater amberjack is a highly carnivorous species, high protein requirements are expected for optimum growth. However, unsustainability of fish meal and fish oil has encouraged the use of alternative plant proteins and fats in commercially available diets for consolidated species, including those used for the present WP21 trials, which was a commercial diet manufactured by Skretting for turbot (*Scophthalmus maximus*) on growing (see Table 2.3.1). In this sense, the lack of a commercial diet specifically designed to cover greater amberjack requirements may greatly affect its growth performance and fillet quality. Assessing available commercial on growing diets with a cost-effective alternative protein/fat sources in novel species and its effect at different rearing conditions (e.g. stocking densities) is of particular interest nowadays. The ingredient choice must be based not only on nutrient level, digestibility, and cost, but also upon other criteria such as sustainability and environmental impact of production, and the resultant fish-in fish-out ratio (FIFO) (Jackson, 2009; Kaushik & Troell, 2010).

In the present study, the effect of several stocking densities in two different fish size classes of Atlantic greater amberjack individuals fed a partially plant based extruded feed, was also evaluated in terms of the fillets proximate and fatty acid composition. This nutritional composition is further compared with previous batches of Mediterranean specimens reared in cages with the same (Batch 1) or a different diet (Vitalis Cal, Skretting; Batch 2), or even with greater amberjack wild specimens.

As shown in Tables 3.1.4 and 3.1.5, the stocking density did not affect the proximate composition of the fillets, with both batches displaying a very similar average composition independently of their initial size. Consistently with literature, the fillet protein content of all fish groups was quite similar, nearing a 20% of total fillet constituents (Thakur *et al.*, 2009). In fact, fillet protein is generally believed to be around this stable value in fish and, except for broodstock, not to be greatly influenced by external parameters such as the stocking density (Love, 1992; Grigorakis, 2010; Timalsina *et al.*, 2017). Also consistent are the values attained for the ash total contents which, as described in literature, are below 2%.

The observed fillet composition distinguishes the reared greater amberjack for its high fat fillet contents, with values near to 3 g per 100 g fillet which are very similar to that previously reported for Batch 1 in 1-1.5 kg cage cultured-fish (3.87 ± 0.93). However, these values contrast with that of a recent study (0.91 ± 0.28 g of total fat per 100 g) in market available fillets of wild individuals (Cladis *et al.*, 2014). The fat content can be much higher specially for reared broodstock animals as those from Batch 2 (12.3 ± 0.11) (D28.3), which additionally undertake important seasonal variations as also described in wild specimens (Thakur *et al.*, 2009; Grigorakis, 2010; Rodríguez-Barreto *et al.*, 2012; Zupa *et al.*, 2017). Although seasonality of fat depot in fillets has been mentioned for wild fish of various species (Gökçe *et al.*, 2004; Özyurt *et al.*, 2005; Özyurt & Polat, 2006; Ersoy *et al.*, 2008; Cardinal *et al.*, 2011; Özogul *et al.*, 2011), there is also a widespread belief that fats depots increase with size (Grigorakis, 2010), as confirmed through DIVERSIFY´s analyzed batches of greater amberjack.

Table 3.1.4. Proximate composition of fillets of greater amberjack (*Seriola dumerili*) from Batch 3 reared at three different culture densities (LD, low density; MD, medium density; HD, high density)

	LD	MD	HD	**Average**
Moisture (%)	74.05 ± 0.73	74.07 ± 1.92	75.09 ± 0.57	**74.80 ± 1.29**
Fat (% ww)	3.16 ± 0.95	2.28 ± 0.69	2.14 ± 0.48	**2.63 ± 0.91**
Protein (% ww)	21.28 ± 1.30	20.74 ± 0.42	21.24 ± 0.70	**21.05 ±0.52**
Ash (% ww)	1.47 ± 0.04	1.53 ± 0.29	1.45 ± 0.08	**1.48 ± 0.16**

Data are means ± SD (n=4).

Table 3.1.5. Proximate composition of fillets of greater amberjack (*Seriola dumerili*) from Batch 4 reared at four different culture densities (LD, low density; MLD, medium-low density; MHD, medium-high density; HD, high density)

	LD	MLD	MHD	HD	**Average**
Moisture (%)	75.73 ± 1.18	74.98 ± 0.37	74.51 ± 2.53	75.06 ± 0.91	**75.31 ± 1.05**
Fat (% ww)	2.89 ± 1.30	3.39 ± 0.93	3.32 ± 0.81	2.90 ± 1.11	**3.07 ± 0.82**
Protein (% ww)	19.70 ± 0.98	20.04 ± 0.55	19.85 ± 0.96	21.07 ± 1.09	**19.99 ± 0.66**
Ash (% ww)	1.68 ± 0.15	1.54 ± 0.23	1.52 ± 0.28	1.55 ± 0.33	**1.51 ± 0.21**

Data are means ± SD (n=4).

The fatty acid profile and fatty acid indexes of greater amberjack juveniles (Batches 3 and 4), are given in Tables 3.1.6 and 3.1.7, respectively. To make this information more understandable we must highlight that the concept "Total FA" used at the beginning of each table is the quantity of total fatty acids present in the total fats of each fillet. For example, if the average content of fat in LD fish-fillets from Batch 3 was 3.16 g per 100 g portion (Table 3.1.4), 2.69 g correspond to the fat that was recovered as FA (Table 3.1.6), being the rest cholesterol or other lipid-origin hydro carbonated chains. This value allows making relevant calculations in absolute terms, to those who wish to know the equivalence in grams/milligrams of a specific fatty acid or group of fatty acids. However, in these tables, the fatty acid profile is given as % of total fatty acids to also highlight the relative proportions of the most abundant and physiological important fatty acids, and their relationship with the changing rearing conditions.

In the last chapter of the present deliverable, the absolute contents of each fatty acid and fatty acid group (saturates, monoenes, or n-6 and n-3 PUFA) per 100 g of fillet is also given and compared among species in correlation with the corresponding ingested extruded diets (Tables 3.4.1 and 2.3.1, respectively).

Depletion of lipid reserves or some specific fatty acids has been observed in several species kept under high stocking densities (Montero *et al.*, 1999, 2001; Papoutsoglou *et al.*, 2006; Karakatsouli *et al.*, 2007). However, as shown in Tables 3.1.4 and 3.1.5 and as above mentioned, not significant differences were found in terms of fat content of fillets from fish reared up to 7.4 kg m^{-3}, and only monounsaturated fatty acids (MUFA), particularly oleic acid (18:1n-9), seemed to display this response for depletion of energy stores under the higher stocking densities assayed, and only when the fish initial size was about 5 g (Table 3.1.6). Homeostatic-catabolic mechanisms for the initially bigger sized fish seemed to more efficient to face this rearing stressor (Table 3.1.7).

Aside from MUFA, no other significant differences were found for any fatty acid among treatments, with a very consistent general fatty acid profile being also found between Batches 3 and 4. In this sense, the most prominent group of FAs was MUFA with oleic acid as the most abundant FA (26-32%). Total saturates (SFA) and specifically 16:0 was also high (13-15%) in greater amberjack flesh. Among the n-6 PUFA and consistently with bibliography concerning the use of plant oil-substituted commercial diets (see Table 2.3.1) for this and other carnivorous fish species, linoleic acid; 18:2n-6, accounted for 16-18% of total fatty acids, even a higher proportion than those of EPA and DHA together (15-17%) (Rodríguez-Barreto *et al.*, 2012, 2014, 2015; Nichols *et al.*, 2014; Zupa *et al.*, 2017).

Table 3.1.6. Total fatty acid content (g 100 g fillet^{-1}) and main fatty acid composition (% total fatty acids) and indexes of fillets of greater amberjack (*Seriola dumerili*) from Batch 3 reared at three different culture densities (LD, low density; MD, medium density; HD, high density)

	LD	MD	HD
Total FA	2.69 ± 0.73	1.78 ± 0.57	1.57 ± 0.36
16:0	13.60 ± 1.67	14.49 ± 0.48	14.92 ± 0.45
18:0	3.74 ± 0.40 a	4.65 ± 0.54 b	4.76 ± 0.32 b
Total SFA	21.24 ± 2.07	23.20 ± 0.86	23.65 ± 0.65
16:1[1]	4.83 ± 0.23 b	4.10 ± 0.38 a	4.16 ± 0.34 a
18:1[2]	29.13 ± 0.55 b	26.32 ± 2.01 a	27.07 ± 1.16 ab
Total MUFA	38.12 ± 0.78 b	34.26 ± 2.36 a	35.28 ± 1.81 ab
18:2n-6	18.08 ± 0.68	16.61 ± 1.22	16.38 ± 0.43
20:4n-6	0.67 ± 0.06	0.92 ± 0.20	0.82 ± 0.14
Total n-6 PUFA	19.33 ± 0.66	18.26 ± 0.89	17.95 ± 0.24
18:3n-3	3.36 ± 0.22	3.02 ± 0.32	2.98 ± 0.20
20:5n-3	4.81 ± 0.20	5.00 ± 0.19	4.77 ± 0.26
22:6n-3	8.28 ± 0.78	11.39 ± 2.37	10.09 ± 2.35
Total n-3 PUFA	20.02 ± 0.79	23.72 ± 2.33	22.31 ± 2.45
DHA/EPA	1.72 ± 0.17	2.16 ± 0.39	2.29 ± 0.40
ARA/EPA	0.14 ± 0.01	0.17 ± 0.03	0.17 ± 0.02
n-3/n-6	1.04 ± 0.02 a	1.31 ± 0.19 b	1.24 ± 0.15 b
IA	0.36 ± 0.04	0.37 ± 0.01	0.39 ± 0.02
IT	0.22 ± 0.03	0.22 ± 0.01	0.23 ± 0.02

Data are means ± SD (n=4). Different letters within a row indicate significant differences among treatments ($P<0.05$). [1] mainly n-7 isomer; [2] mainly n-9 isomer; DHA, docosahexaenoic acid, 22:6n-3; EPA, eicosapentaenoic acid, 20:5n-3; ARA, arachidonic acid, 20:4n-6. IA, Index of atherogenicity; IT, Index of thrombogenicity.

Table 3.1.7. Total fatty acid content (g 100 g fillet^{-1}) and main fatty acid composition (% total fatty acids) and indexes of fillets of greater amberjack (*Seriola dumerili*) from Batch 4 reared at four different culture densities (LD, low density; MLD, medium-low density; MHD, medium-high density; HD, high density)

	LD	MLD	MHD	HD
Total FA	2.69 ± 1.06	3.40 ± 0.66	2.72 ± 0.73	2.33 ± 0.97
16:0	14.19 ± 0.63	14.10 ± 0.34	14.03 ± 0.47	14.51 ± 0.52
18:0	4.11 ± 0.66	3.69 ± 0.17	3.99 ± 0.26	4.12 ± 0.34
Total SFA	22.35 ± 1.54	21.78 ± 0.35	22.29 ± 0.44	22.89 ± 0.98
16:1[1]	4.80 ± 0.69	5.01 ± 0.22	4.80 ± 0.24	4.81 ± 0.27
18:1[2]	30.28 ± 1.78	31.48 ± 0.53	31.01 ± 1.73	30.62 ± 0.97
Total MUFA	40.60 ± 2.40	42.18 ± 0.75	41.59 ± 2.50	40.83 ± 1.16
18:2n-6	12.25 ± 0.19	12.17 ± 0.43	12.08 ± 0.74	11.95 ± 0.55
20:4n-6	0.68 ± 0.18	0.55 ± 0.04	0.63 ± 0.06	0.67 ± 0.09
Total n-6 PUFA	13.67 ± 0.28	13.50 ± 0.34	13.45 ± 0.72	13.57 ± 0.63
18:3n-3	3.07 ± 0.63	3.44 ± 0.17	3.01 ± 0.22	3.06 ± 0.22
20:5n-3	4.52 ± 0.28	4.61 ± 0.14	4.38 ± 0.42	4.55 ± 0.19
22:6n-3	10.52 ± 2.45	9.27 ± 0.79	10.38 ± 1.91	10.02 ± 1.18
Total n-3 PUFA	21.86 ± 1.30	21.29 ± 0.79	21.61 ± 2.36	21.28 ± 0.61
DHA/EPA	2.36 ± 0.71	2.01 ± 0.17	2.37 ± 0.36	2.21 ± 0.33
ARA/EPA	0.15 ± 0.05	0.12 ± 0.01	0.14 ± 0.01	0.15 ± 0.02
n-3/n-6	1.60 ± 0.07	1.58 ± 0.08	1.61 ± 0.20	1.57 ± 0.10
IA	0.38 ± 0.01	0.38 ± 0.00	0.38 ± 0.00	0.39 ± 0.01
IT	0.22 ± 0.01	0.22 ± 0.02	0.22 ± 0.01	0.23 ± 0.01

Data are means ± SD (n=4). [1] mainly n-7 isomer; [2] mainly n-9 isomer; DHA, docosahexaenoic acid, 22:6n-3; EPA, eicosapentaenoic acid, 20:5n-3; ARA, arachidonic acid, 20:4n-6. IA, Index of atherogenicity; IT, Index of thrombogenicity.

In spite of these findings, the fillets DHA/EPA ratios were of around 2/1 regarded as good ones in terms of health and nutrition of fish and human wellbeing, as also were the low IA and IT indexes due to the flesh high levels of MUFA and PUFA compared to SFA (Senso *et al.*, 2007; Pérez *et al.*, 2014). However, the percentage in which the main n-3 LC-PUFA (EPA plus DHA) appear in greater amberjack muscle, with respect to the totality of the lipids, can be greatly improved by limiting the inclusion of plant-origin oils in extruded diets, to better resembling the healthy and nutritious properties of wild greater amberjack. More specifically, recent studies performed to compare the FA profile of wild versus reared greater amberjack and *S. lalandi* tissues show that the total SFA, MUFA and PUFA were statistically similar between the two groups; however the farmed fish had significantly higher contents of 18:2n-6 and ω6/ω3ratios and wild specimens had significantly higher and lower DHA and EPA proportions, respectively, than the farmed fish (Rodríguez-Barreto *et al.*, 2012, 2015; O´Neill *et al.*, 2015; Zupa *et al.*, 2017). 18:2n-6 is already a particularly abundant FA in western population's diets (cereals, dairy and meat products, etc.) and as the main precursor of the pro-inflammatory ARA (20:4n-6) it should be advisable to balance its presence in reared fish flesh. Recent findings reviewed by Zárate *et al.* (2017), highlight that increments in omega-3 LC-PUFAs consumption and an omega-6/omega-3 ratio of 2-4:1, is associated with a reduced risk of a wide range of inflammation disorders, including autoimmune diseases.

Table 3.1.8. Proximal composition (% fresh weight), main fatty acid content (mg 100 g fillet^{-1}) and health lipid indexes of greater amberjack (*Seriola dumerili*) fillets cultured under different rearing conditions

	Batch 1	Batch 3	Batch 4
Moisture	70.76 ± 0.94 a	74.80 ± 1.29 b	75.31 ± 1.05 b
Fat	**3.92 ± 1.01 b**	**2.63 ± 0.91 a**	**3.07 ± 0.82 a**
Protein	**23.09 ± 0.78 c**	**21.05 ± 0.52 b**	**19.99 ± 0.66 a**
Ash	**1.51 ± 0.09**	**1.48 ± 0.16**	**1.51 ± 0.21**
Total SFA	**432.7 ± 32.2 a**	**450.9 ±141.3 a**	**608.0 ±171.9 b**
Total MUFA	**909.8 ± 36.7 a**	**736.1 ±304.7 a**	**1140.3 ±373.8 b**
18:2n-6	**308.2 ± 13.1**	**349.1 ±145.7**	**333.7 ±105.8**
20:4n-6	13.8 ± 1.0 a	15.2 ± 3.0 ab	17.0 ± 4.2 b
Total n-6 PUFA	345.1 ± 16.0	377.4 ±150.7	373.2 ±114.3
18:3n-3	13.4 ± 1.1 a	64.7 ± 28.9 b	88.7 ± 33.7 b
20:5n-3	67.9 ± 6.3 a	99.1 ± 34.8 b	125.6 ± 41.4 b
22:6n-3	178.0 ± 15.7 a	192.9 ± 36.1 a	269.2 ± 57.4 b
Total n-3 PUFA	285.2 ± 20.9 a	431.5 ±125.2 b	589.6 ±167.6 c
EPA+DHA	**245.9 ± 19.2 a**	**292.0 ± 69.9 a**	**394.7 ± 97.9 b**
n-3/n-6	0.83 ± 0.04 a	1.20 ± 0.18 b	1.60 ± 0.11 c
IA	0.35 ± 0.02 a	0.37 ± 0.03 ab	0.38 ± 0.01 b
IT	0.26 ± 0.02 b	0.22 ± 0.02 a	0.22 ± 0.01 a

Data are means ± SD (Batch 1, n=10; Batch 3, n=12; Batch 4, n=16). Different letters within a row indicate significant differences among treatments (P<0.05). IA, Index of atherogenicity; IT, Index of thrombogenicity.

Finally, and in order to give fish farmers and other potential chain partners insights in the nutritional values of the generated products, Table 3.1.8 provides the nutritional information per 100 g fillet of greater amberjack from the new two Atlantic batches and also from that of the Mediterranean Batch 1 of 1-2 kg specimens produced with the same commercial diet out of DIVERSIFY project, and already evaluated in Deliverable 28.5. It is considered that, at least the nutritional information highlighted in bold, should be given on the food packages labelling. This information is also valuable for further optimization of the products' generation processes (*e.g.* potential changes of feed formulation and grow out conditions) to better balance their nutritional-healthy and environmentally friendly qualities.

Although a more fine-tuning is still needed to particular groups of age and physiological status, 450-500 mg of EPA + DHA per day is suggested for general health condition of the adult population. This could be met with a weekly ingestion of 2-3 portions of oily fish. Increased amounts of up to 1 to 3–4 g/day of omega-3 LC-PUFAs should be ingested during pregnancy and lactation or to prevent most cardiovascular, neurodegenerative and pro-inflammatory disorders (Zárate *et al.*, 2017). Therefore and even for the unspecific turbot diet, an average consumer portion (200 g) of greater amberjack reared within the frame of DIVERSIFY project provides EPA + DHA concentrations that meet the daily needs recommended by many National Departments of Health (Zárate *et al.*, 2017).

Sensory analysis

Table 3.1.9 shows the mean values obtained for each sensory descriptor and culture density in Batch 3.

Table 3.1.9. Means values for the sensory parameters (O, Odour; F, Flavour; T, Texture) of greater amberjack (*Seriola dumerili*) from Batch 3 reared from 5 g at three different culture densities (LD, low density; MD, medium density; HD, high density).

Sensory descriptor	LD	MD	HD
O_Sardine	4.6	4.3	5.3
O_Ammonia	2.1	2.2	3.4
O_Earthy	1.2 a	1.7 ab	2.0 b
O_Butter	1.3	1.3	0.9
O_Sea food	0.1 ab	0.5 b	0.0 a
O_Acid	1.0	0.9	1.2
O_Boiled vegetables	0.5	0.3	0.5
Colour white to brown	4.1 a	5.1 ab	5.9 b
Colour uniformity	5.7	5.4	4.7
White spots	1.8 ab	2.1 b	1.0 a
Laminar structure	2.5	2.7	2.9
Exudates quantity	4.0 b	4.1 b	2.6 a
Exudates turbidity	2.2	2.0	2.1
Fat droplets	1.4	1.2	1.8
Exudate particles	2.9 b	3.0 b	2.1 a
Exudate proteins	1.6	1.5	1.1
Black lines in the flesh	2.3	1.4	2.3
Brightness	5.0	5.0	4.4
F_Sweet	0.8	0.6	0.9
F_Acid	1.9	2.2	1.8
F_Bitter	4.5	4.8	5.7
F_Earthy	1.5	1.7	1.5
F_Sardine	5.6	5.3	5.5
F_Butter	1.0	0.5	0.5
F_Sea food	0.0	0.1	0.1
F_Boiled vegetables	0.8	1.3	1.0
T_Firmness	7.3	6.8	6.5
T_Crumbliness	5.0	4.5	5.4
T_Juiciness	2.8	2.7	3.1
T_Chewiness	6.5 ab	6.9 b	5.6 a
T_Pastiness	4.1	4.0	4.3
T_Teeth adherence	2.7 a	3.0 ab	3.3 b

Different letters within a row indicate significant differences among densities (P<0.05).

Statistical differences were observed in only eight descriptors. Most of these differences, although significant (P<0.05), are small and irrelevant. In any case it is worth to mention the existence of a tendency to have a loss of quality when increasing the culture density (higher ammonia and earthy odours, less colour uniformity, higher laminar structure, higher bitterness, lower firmness and higher teeth adherence).

Table 3.1.10 shows the mean values obtained for each sensory descriptor and culture density in Batch 4.

Table 3.1.10. Means values for the sensory parameters (O, Odour; F, Flavour; T, Texture) of greater amberjack (*Seriola dumerili*) from Batch 4 reared from 150 g at four different culture densities (LD, low density; MLD, medium-low density; MHD, medium-high density; HD, high density).

Sensory descriptor	LD	MLD	MHD	HD
O_Sardine	3.0	2.0	2.9	2.4
O_Ammonia	2.0	1.7	1.8	1.9
O_Earthy	1.5	1.3	1.7	1.2
O_Butter	2.2	1.7	1.8	2.5
O_Sea food	0.0	0.4	0.2	0.2
O_Acid	1.2	1.1	0.9	0.9
O_Boiled vegetables	0.4	0.0	0.3	0.5
Colour white to brown	2.7	4.7	4.3	4.3
Colour uniformity	5.3	5.2	4.7	5.5
White spots	4.7	4.4	5.2	5.6
Laminar structure	2.8	2.3	2.8	2.6
Exudates quantity	6.2 b	4.8 a	6.9 b	6.0 b
Exudates turbidity	3.1	2.2	3.0	2.7
Fat droplets	1.9	0.9	2.1	1.7
Exudate particles	3.5	2.8	4.0	3.8
Exudate proteins	3.4	2.7	3.3	3.6
Black lines in the flesh	0.5	0.1	0.7	0.5
Brightness	4.1	3.3	4.3	4.9
F_Sweet	0.9	1.7	1.3	1.0
F_Acid	2.4	2.0	2.0	2.2
F_Bitter	3.5	3.7	3.5	3.0
F_Earthy	1.2	0.9	1.2	1.5
F_Sardine	3.0	2.6	3.0	3.0
F_Butter	1.9	2.1	1.5	1.3
F_Sea food	0.6 b	0.2 a	0.2 a	0.3 ab
F_Boiled vegetables	0.6	0.0	0.4	0.6
T_Firmness	6.8	7.0	6.3	6.1
T_Crumbliness	5.0	4.7	4.6	5.1
T_Juiciness	3.6	3.0	3.5	3.4
T_Chewiness	6.3	6.1	6.2	6.4
T_Pastiness	5.4	5.9	5.6	5.3
T_Teeth adherence	4.0	4.2	4.3	4.3

Different letters within a row indicate significant differences among densities ($P<0.05$).

Statistical differences were observed in only two descriptors. These differences, although significant ($P<0.05$), are small and irrelevant. In this case no effect of culture density was observed on the sensory quality of the fillets.

In the case of sensory data, the comparison with previous batches from other DIVERSIFY deliverables cannot be made since the training process, tasters and reference scale values were not exactly the same. In previous batches references were developed and adjusted for all the species to assess, in the present results this process was adjusted for each species independently in order to improve the discriminant ability of the panel. In addition, no distinction between batches 1 and 2 for the sensory characterization was made in the work described in deliverable D28.3.

3.2. Grey Mullet

The grey mullet (*Mugil cephalus*), an euryhaline herbivore fish suitable for warm-water pond, extensive and integrated culture, is also detritivore in the wild, a reason why it has been stocked in fish ponds to improve sediment quality and avoid oxygen depletion (Milstein *et al.*, 1991). Therefore, it is considered an excellent candidate for the enhancement of aquaculture in polyculture earthen ponds, coastal lagoons, "valli" and deserted Salinas that exist throughout the EU Mediterranean countries. A market for grey mullet is also well established, though a niche one, in the Mediterranean.

Given their large size and fast growth, they provide for high dress-out and fillet yield, and suitability for product diversification and development of value-added products. Among these products, the salted and dried roe (bottarga) from gravid females, is considered a highly prized delicacy in the southern Mediterranean and an added value product from the culture of this species (Mylonas *et al.,* 2017). A highly nutritious value product (>100 € kg^{-1}), rich in w-3 LC-PUFA and carotenoids, whose market is expanding around the Mediterranean.

Hatchery produced juvenile females have been grown to 1.9 kg in 2 years on a fishmeal-containing pelleted feed (P4. IOLR), but the development of fishmeal-free on growing feeds to reduce the cost of fish production, and a more sustainable and environmentally friendly practice, has been also an objective within DIVERSIFY partners. At present, one of the major challenges for the aquaculture industry is the environmental sustainability of the activity. New sustainable and environmentally friendly ingredients for aqua feeds that do not compromise the nutritional benefits of fish consumption for humans are needed. As fish oil production is currently declining, other sources of w-3 LC-PUFAs are currently also being evaluated. Micro and macroalgae, some of them with valuable nutritional FA and antioxidants profiles, are already under industrial exploitation for direct human consumption or as fish feed ingredients after optimization of culture and growth medium conditions (Zárate *et al.*, 2016). In this way, an herbivorous fish such as the grey mullet would be more acceptable to an increasingly aware consumer public that demands sustainability and lower environmental impacts.

Somatometry – technical indexes

Batch 2 was produced in PISTRESA farm (Cádiz, Spain), in the frame of CTAQUA´s WP23 tasks, in order to analyse the effect of improved algae-containing diets on the grow-out of grey mullet in earthen ponds (1100 m^2) monoculture, and under natural temperature. The fish were fed a grey mullet experimental extruded feed (see Task 23.2; P4 IOLR/P31 IRIDA) (Tables 2.1.1 and 2.3.1), although natural feeding was also available in the ponds.

Batch 3 consisted of bigger fish, but the environmental previous rearing history was similar to that of Batch 2, with the sampling time also being December and the fish reared in earthen ponds under natural temperature in Cádiz (Spain). However, the dietary composition was different with mullets being fed a carp commercial extruded diet (SORGAL, Portugal) (Table 2.3.1) in PIMSA farm, with natural feeding also available in the ponds.

Fish from Batch 1 was already evaluated in Deliverables 28.3 and 28.5, consisting of wild caught mullets maintained in earthen ponds of the same area in Cádiz, under natural temperature and feeding. This is why it has been considered of interest to be included in the new tables for comparisons between reared and wild specimens (Tables 3.2.1, 3.2.5).

Table 3.2.1. Average somatic indexes and technical yields of new batches of grey mullet (*Mugil cephalus*) reared in earthen ponds with different extruded diets and natural temperature in Cádiz (Spain) compared to wild specimens (Batch 1) reared under similar environmental conditions

	Batch 1	Batch 2	Batch 3
Body weight (g)	748.9 ±154.3 b	200.3 ± 69.9 a	732.1 ± 79.3 b
CI	1.09 ± 0.06 b	0.96 ± 0.08 a	1.26 ± 0.13 b
Dressing yield (%)	87.77 ± 2.11 a	90.43 ± 1.47 b	86.87 ± 2.72 a
Filleting yield (%)	36.20 ± 3.00 a	46.84 ± 1.17 b	55.07 ± 0.78 c
HSI	1.46 ± 0.22 b	0.84 ± 0.17 a	1.91 ± 0.59 b
VSI	10.31 ± 2.08 b	4.12 ± 0.51 a	11.51 ± 1.77 b
VFI	------	------	1.21 ± 0.71

Data are means ± SD (Batch 1, n=10; Batch 2, n=6; Batch 3, n=4). Different letters within a row indicate significant differences among treatments (P<0.05). CI: condition index; HSI: hepatosomatic index; VSI: viscerosomatic index; VFI: visceral fat index.

As shown in Table 3.2.1, somatometric measurements were significantly different for the 2 reared batches of fish, probably due to the different size class. In spite of this, filleting yield of the reared fish were good and higher than that of the wild fish, with the dressing yields for all batches also being high and close to 90%.

From the condition index (CI), it can be presumed a better condition of fish from Batch 1 of wild fish and Batch 3 compared to those of Batch 2 (Table 3.2.1) (Flos *et al.*, 2002; Grigorakis, 2007; Piccolo *et al.*, 2007; Martelli *et al.*, 2013).

Proximate and fatty acid composition

Table 3.2.2. Proximate composition of fillets of grey mullet (*Mugil cephalus*) reared in earthen ponds with different extruded diets and natural temperature in Cádiz (Spain)

	Batch 2	Batch 3
Moisture (%)	77.98 ± 0.70	74.76 ± 0.40 *
Fat (% ww)	0.86 ± 0.17	3.23 ± 0.71 *
Protein (% ww)	19.33 ± 0.80	20.54 ± 0.81 *
Ash (% ww)	1.21 ± 0.08	1.01 ± 0.02 *

Data are means ± SD (Batch 2, n=6; Batch 3, n=4). * Indicate significant differences among groups (P<0.05).

The proximate composition of the fillets was also significantly different among the two groups of reared fish (Table 3.2.2) although, as in greater amberjack and many other fish species, protein contents were of around 20%.

Considering that the proximate composition of the two extruded diets mainly differed in the total fat content and that it was clearly higher for Batch 2 (15.09%) than for the Bath 3 (8.79%) (Table 2.3.1), it is surprising that the Batch 2 fish flesh reflects exactly the opposite trend. In fact, lipid contents of Batch 3 fillets were more than 3 times that of the IOLR/IRIDA formula-fed fish, this being consistent with the former displaying higher HIS and VSI (Table 3.2.1). It is well documented that aquafeeds provide high contents of total fats (Table 2.3.1), compared to what the species naturally consume in the wild. The consumption of these fast-growing high-energy diets results in an elevation of total fat levels in flesh and other tissues (Rodríguez *et al.*, 2004; Rodríguez-Barreto *et al.*, 2015) as that one displayed by the SORGAL fed fish (Batch 3; Table 3.2.2).

According to the fat contents of Batch 1-wild mullets (Deliverable 28.3; Table 3.2.4), the DIVERSIFY´s Batch 2, better resembles a wild specimen fat content (0.58 ± 0.01) than a reared one. Additionally, a grey mullet is supposed to contain high fat contents in fillet, and has been characterized as a medium- to high-fat species (El-Sebaiy *et al.*, 1987; Özogul & Özogul, 2007; Özogul *et al.*, 2009; Kumaran *et al.*, 2012), but this does not seem to be confirmed in the wild fish caught in Cádiz area. A recent study to evaluate fatty acid profiles of 77 commercially available finfish fillets in the United States, also confirmed this higher fat contents expected for wild specimens (2.75 ± 2.07 g per 100 g) (Cladis *et al.*, 2014).

In spite of the improved and more sustainable formula produced by IOLR/IRIDA for Batch 2, the fish seemed not to easily get use to this experimental diet, completing their daily feeding intake with the natural food items available in the earthen ponds (CTAQUA, personal communication). Therefore, the above-mentioned difficulties to accept the experimental diet, together with the partial naturally feeding, may explain the lower flesh fat contents and HSI and VSI found in these individuals.

Sustainability of aquaculture extruded diets has obliged feed manufactures to use alternative sources of plant meals and oils in the last few years, a fact which can be chemo-metrically followed not only by the higher fish fat contents but also through their fatty acid profiles. The relative contents of n-3 PUFA in farmed fish is generally lower than that in wild fish because commercial feeds usually contain high proportions of lipids from vegetable sources that are richer in SFA, MUFA and n-6 PUFA, but poorer in n-3 PUFA (Grigorakis, 2007). Therefore, changes in lipid quality and quantity will be expected with the replacement of marine oils by namely vegetable oils, which will particularly increase the levels of linoleic acid, 18:.2n-6 in farmed fish profiles (Rodríguez *et al.*, 2004; Grigorakis 2007; O´Neill *et al.*, 2015; Chaguri *et al.*, 2017).

As shown in Table 2.3.1, the assayed extruded diets contributed with high contents of MUFA and SFA, followed by n-6 and then n-3 PUFA. Compared to the Batch 2-experimental diet, Batch 3 from SORGAL provided higher contents of SFA and 18:2n-6 and lower of EPA and DHA, denoting a higher degree of dietary substitution of marine origin ingredients, which was only clearly evident in fillets from Batch 3 (Table 3.2.3). Batch 3 fish displayed high fat contents with low proportions of arachidonic acid (20:4n-6, ARA) and high proportions of 18:2n-6 among n-6 PUFA. In contrast, both eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) were clearly reduced whereas MUFA increased (Table 3.2.3).

It should be expected for Batch 2 fillets a much higher total fat content according to the diet and a similar trend for their fatty acid profiles than that described for Batch 3 (Tables 2.3.1 and 3.2.3). However, the PISTRESA fish displayed a low flesh fat content, richer in ARA, EPA and DHA and also much lower contents of MUFA and 18:2n-6 (Table 3.2.3).

Table 3.2.3. Total fatty acid content (g 100 g fillet^{-1}) and main fatty acid composition (% total fatty acids) of fillets of grey mullet (*Mugil cephalus*) reared in earthen ponds with different extruded diets and natural temperature in Cádiz (Spain)

	Batch 2	Batch 3
Total FA	0.52 ± 0.11	2.44 ± 0.49 *
16:0	21.16 ± 1.01	21.94 ± 1.16
18:0	5.95 ± 0.42	3.43 ± 0.47 *
Total SFA	33.96 ± 1.37	28.85 ± 0.84 *
16:1[1]	4.97 ± 1.03	9.92 ± 1.72 *
18:1[2]	7.13 ± 0.80	28.69 ± 2.40 *
Total MUFA	14.91 ± 1.62	40.91 ± 3.87 *
18:2n-6	2.00 ± 0.24	14.45 ± 0.56 *
20:4n-6	4.22 ± 0.95	0.73 ± 0.04 *
Total n-6 PUFA	8.73 ± 2.00	17.01 ± 0.50 *
18:3n-3	3.31 ± 1.40	1.30 ± 0.01 *
20:5n-3	8.80 ± 1.40	0.88 ± 0.10 *
22:6n-3	22.17 ± 2.29	4.48 ± 0.18 *
Total n-3 PUFA	40.82 ± 1.20	6.70 ± 0.29 *
DHA/EPA	2.58 ± 0.54	5.24 ± 0.53 *
ARA/EPA	0.48 ± 0.08	0.82 ± 0.12 *
n-3/n-6	4.90 ± 1.22	0.40 ± 0.01 *
IA	0.55 ± 0.05	0.53 ± 0.04
IT	0.21 ± 0.01	0.56 ± 0.02 *

Data are means ± SD (Batch 2, n=6; Batch 3, n=4). [1] mainly n-7 isomer; [2] mainly n-9 isomer; DHA, docosahexaenoic acid, 22:6n-3; EPA, eicosapentaenoic acid, 20:5n-3; ARA, arachidonic acid, 20:4n-6. IA, Index of atherogenicity; IT, Index of thrombogenicity. * Indicate significant differences among groups (P<0.05).

The similarity of fish from Batch 2 with wild individuals (Batch 1) is presented in Table 3.2.4, where the absolute contents of each fatty acid are given in mg per 100 g of fillet. These results seem to confirm that PISTRESA fish were partially eating the naturally abundant food and not exclusively the experimental extruded diet. Commonly, wild and farmed fish have different diets that lead to clearly distinctive biochemical, sensorial, and physical characteristics, including their fatty acids (Grigorakis, 2007), but PISTRESA fish display a fat profile in between both situations, with a very healthy and more balanced lipid profile than fish fed the SORGAL diet. For instance, the fillets from PISTRESA are of low fat content and poorer in 18:2n-6 than Batch 3 fish, presenting a total content of n-3 LC PUFA (EPA+DHA) twice than that of wild fish.

Kumaran *et al.* (2012) reported a total lipid content of 2.42±0.21% in wild mullets caught in the Indian coast, with a FA profile also rich in ARA (3.53±0.09), and particularly EPA (7.98±0.17) but much poorer in DHA (3.97±0.06) than the wild fish evaluated in the present GWP or that reported by Cladis *et al.*

(2014) from wild mullets caught at the USA coasts, also confirming the regional and seasonal variations of wild fish nutritional composition according to their natural feeding regimes and environmental factors (Tufan *et al.,* 2018).

Table 3.2.4 Proximal composition (% fresh weight), main fatty acid content (mg 100 g fillet^{-1}) and health lipid indexes of fillets of grey mullet (*Mugil cephalus*) reared in earthen ponds with different extruded diets and natural temperature in Cádiz (Spain), compared to wild specimens (Batch 1) reared under similar environmental conditions

	Batch 1	Batch 2	Batch 3
Moisture	**76.53 ± 1.07 b**	**77.98 ± 0.70 c**	**74.76 ± 0.40 a**
Fat	**0.58 ± 0.02 a**	**0.86 ± 0.17 b**	**3.23 ± 0.71 c**
Protein	**21.37 ± 0.72 b**	**19.33 ± 0.80 a**	**20.54 ± 0.81 ab**
Ash	**1.27 ± 0.16 b**	**1.21 ± 0.08 b**	**1.01 ± 0.02 a**
Total SFA	97.7 ±17.2 a	180.8 ±43.4 b	707.2 ±160.5 c
Total MUFA	49.2 ± 2.6 a	79.9 ±23.4 b	1003.3 ±249.3 c
18:2n-6	**7.0 ± 4.9 a**	**10.8 ± 3.2 a**	**353.7 ± 76.4 b**
20:4n-6	27.2 ± 2.4	21.7 ± 3.5	22.2 ± 8.6
Total n-6 PUFA	40.0 ± 4.7 a	45.0 ± 8.6 a	414.8 ± 79.3 b
18:3n-3	1.1 ± 0.3 a	18.4 ±10.9 ab	31.7 ± 6.5 b
20:5n-3	**42.6 ± 6.7 b**	**46.1 ±10.3 b**	**28.3 ± 13.2 a**
22:6n-3	**58.0 ± 0.9 a**	**116.1 ±21.4 ab**	**147.8 ± 73.3 b**
Total n-3 PUFA	115.7 ± 9.7	215.9 ±45.5	215.6 ± 98.9
EPA+DHA	**100.6 ± 7.1**	**162.2 ± 65.9**	**176.1 ± 86.4**
n-3/n-6	2.94 ± 0.55 b	4.90 ± 1.22 c	0.40 ± 0.01 a
IA	0.50 ± 0.10	0.55 ± 0.05	0.53 ± 0.04
IT	0.23 ± 0.03 a	0.21 ± 0.01 a	0.56 ± 0.02 b

Data are means ± SD (Batch 1, n= 3; Batch 2, n=6; Batch 3, n=4). Different letters within a row indicate significant differences among treatments (P<0.05). IA, Index of atherogenicity; IT, Index of thrombogenicity.

Fillets analyzed from wild grey mullet specimens available at the USA market, contained 2.75±2.07 g of total fat per 100 g fillet with the following composition of FAs expressed in mg per 100 g: 707±652 (n-3); 143±91 (n-6); 812±793 (SFA); 386±395 (MUFA) and 905±801 (PUFA) of which 61±64 was LNA (18:2n-6); 19±21 ARA (20:4n-6); 56±10 EPA (20:5n-3); and 263±231 DHA (22:6n-3) (Cladis *et al.*, 2014). Compared to the grey mullets from Batch 3, which also supplied a total fat content close to 3 g per 100 g fillet, the USA wild specimens display a healthier profile suitable to cover the international daily recommendations of w-3 LC-PUFA intake without supplying excessive proportions of 18:2n-6.

To attain a final product of these characteristics, after on growing of any of the selected species, should be a clear objective to continue the collaboration among all DIVERSIFYs partners, including nutritionists and aquaculture research specialists, sensory evaluators, farmers, feed manufacturers and marketing experts. This has been the main focus and the objective of Deliverable 23.1 "Cost-effective weaning strategies for wild-caught grey mullet grow out and their effect on growth and health status", where results indicated that weaning diets for wild grey mullet harvested for restocking aquaculture ponds and on-growing purposed may be formulated with a high level of fishmeal replacement by alternative plant protein sources, but ensuring a good supply of fish oil to ensure the good final profile of w-3 LC-PUFA. Diets with a 50 and 75% of fish meal replacement by plant protein sources (corn gluten, wheat gluten and soy protein concentrate) were 15.5 and 23.6% cheaper than the FM diet, which is of special relevance considering that feed costs account for over 50 percent of the production costs in aquaculture facilities.

There is increasing interest in the culture of the omnivorous grey mullet as a high quality source of protein and as a species that requires little or no dietary fishmeal (FM), in addition, a selection of mullet products that has been sensory-tested in five countries among regular fish consumers including: 1) fresh fillet with healthy seasoning, 2) thin smoked fillet and 3) fish fillets in olive oil prepared by the DIVERSIFY partner P18. CTAQUA showed that the tested products from grey mullet were well accepted (Deliverable D29.4). Furthermore, since products with a lower degree of processing generated higher expected scores and higher acceptability in the blind test, further insights are necessary to perform mullet grow out trials with Eco sustainable diets able to provide optimum quality fillets in terms of both sensorial and proximate and fatty acid attributes.

Sensorial analysis

Table 3.2.5 shows the mean values obtained for each sensory descriptor of grey mullet specimens reared in earthen ponds with different extruded diets and natural temperature (Batches 2 and 3).

In this case and as observed in the chemical parameters, important differences in the sensory parameters were detected. Thus, there were statistical differences in 60% of the descriptors evaluated (21 out of 35). The differences observed between batches, both in the amount of total fat and in its composition, can explain an important part of the results shown. Batch 2, with a considerably lower fat content (0.86% vs 3.23%) was the one that presented fewer fat droplets in the exudate, lower brightness and a firmer and less juicy texture. Batch 3, with a higher fat content, had a higher sweet taste, which has been associated with the presence of fat in other species such as pork meat. Adhesion between the teeth was also higher for Batch 3, probably also influenced by the higher total fat content (Rincón et al., 2016), as well as butter flavours. However, the parameters associated with the degree of fat oxidation such as sardine odour and flavour were much higher in Batch 2, which also affected the coloration of the fillet (browner colour) and the greater presence of bitter compounds. According to Lazo et al. (2017), there seems to be a marked relationship between the bitter taste and the compounds derived from oxidation in this species, a fact which could be related to a much higher proportion of highly unsaturated w3 fatty acids (almost 41%) compared to saturates or MUFA in the fillets of this batch.

Finally, it is noteworthy the presence of earthy odours and flavours detected especially in Batch 3. Since both batches were reared in the same conditions (earthen ponds), the differences observed could be due to a greater accumulation of the compounds responsible for these sensory descriptors in the fat.

As commented above for greater amberjack, the comparison with previous batches from other DIVERSIFY deliverables cannot be made for the sensory data.

Table 3.2.5. Mean values for each sensory descriptor of fillets of grey mullet (*Mugil cephalus*) reared in earthen ponds with different extruded diets and natural temperature in Cádiz (Spain).

Sensory descriptor	Batch 2 (PISTRESA)	Batch 3 (PIMSA)
O_Sardine	5.2	1.4 *
O_Ammonia	1.6	1.7
O_Earthy	2.0	4.2 *
O_Butter	2.0	2.4
O_Sea food	0.3	0.3
O_Acid	0.5	0.8
O_Boiled vegetables	0.2	0.3
O_Cheese	0.0	0.0
O_Rancid	0.1	0.0
Colour white to brown	5.9	3.4 *
Colour uniformity	4.8	5.0
White spots	2.6	5.6 *
Laminar structure	2.9	2.7
Exudates quantity	2.9	5.6 *
Exudates turbidity	3.4	3.6
Fat droplets	0.8	2.7 *
Exudate particles	4.1	4.9
Exudate proteins	2.2	3.6 *
Black lines in the flesh	1.4	0.4 *
Brightness	4.0	4.6 *
Yellowness	2.2	0.9 *
F_Sweet	1.1	1.6 *
F_Acid	0.5	0.7
F_Bitter	3.2	2.4 *
F_Earthy	3.3	6.1 *
F_Sardine	5.4	2.2 *
F_Butter	1.1	2.1 *
F_Sea food	0.2	0.1
F_Boiled vegetables	0.1	0.3 *
T_Firmness	5.3	4.3 *
T_Crumbliness	5.5	6.0 *
T_Juiciness	3.4	5.0 *
T_Chewiness	4.8	4.8
T_Pastiness	3.8	4.8 *
T_Teeth adherence	3.0	4.1 *

* Indicate significant differences among groups ($P<0.05$).

3.3. Pikeperch

Pikeperch (*Sander lucioperca*) demand has been strengthened by the strong decline of wild catches (FAO, 2009). Pikeperch aquaculture has gained commercial interest in recent years and bio-economic feasibility of the intensive rearing of the species has been demonstrated (Steenfeldt & Lund, 2008; Steenfeldt *et al.,* 2010). Farming methods in Europe are in transition from traditional more extensive freshwater pond farming to intensive closed recirculation aquaculture systems (RAS) (Dalsgaard *et al*., 2013; Kestemont *et al*., 2015). Over the last decade, several new farms have been built in Europe to produce pikeperch using (RAS) (Fontaine *et al.,* 2012). These RAS also allow high densities of 80-100 kg m^{-3} to produce a final product with a neutral taste, lending itself to different forms of preparation, and fillets without bones (unlike carp), which competes on the same market segment. At present, pikeperch is sold either as whole fish at a weight of 600-3000 g or as fillets of 100-800 g to markets in Europe (mainly Western, Eastern and Northern areas) and North-America, showing strong demand. The market value is high at 8-11 € kg^{-1} at farm gate, whole fish. To keep up the high market value, product development and market development is also necessary for coordinated growth. Therefore, potential markets and consumer segments have been identified by Diversify Socioeconomic partners to maintain or increase the added value of this species.

A major bottleneck for further expansion of pikeperch culture today includes a high mortality and impairment in growth rate during on growing, with high sensitivity to stressors, handling and husbandry practices that result in high and sudden mortalities. Characterization of stress sensitivity is an important factor for optimizing the commercial production of a new species in aquaculture. In this sense, a multifactorial experiment including 16 factors-modalities was conducted in order to select the most suitable husbandry and environmental conditions for improving the performance and welfare status of pikeperch juveniles reared under intensive culture (see D22.1 and D22.2). Indeed, it has been reported that reduction of stress responsiveness may be an important part of domestication, because of the positive selection of stress-resistant fish with an improvement of fitness along generations (Douxfils *et al.,* 2011, 2012). Compared to salmonids, the first observations showed high levels of cortisolemia in pikeperch (88-122 ng/ml), confirming its high sensitivity to captive environmental conditions (Fontaine *et al.,* 2015), and light intensity and the type of feed clearly appeared as directive factors for pikeperch culture, with a strong effect of the feed type observed also on growth parameters (Baekelandt *et al*., 2018) and, as a consequence, in the final product quality.

Fish species of non-marine origin, i.e. the grey mullet and the pikeperch, has been characterized within this GWP by the presence of "earthy" odour and flavour, and to be better used for the design of products that come with dressing's spices or sauces that can cover these earthy characters that are mostly unwanted. Thus, ideas like 14 and 21 (fresh products with spices or marinates) or 25 and 13 (frozen products with marinates) of deliverable D28.2 seem to be ideal for these species, although the adequate selection of food ingredients during grow out can greatly help to improve the nutritional quality and final sensorial acceptance of these species. The new batch (Batch 2) of pikeperch fillets produced in WP22 was evaluated for these quality factors and compared with Batch 1 (Table 2.1.1) previously evaluated in D28.3 and 28.5.

Proximate and fatty acid composition

Although fed artificial feed is known to vary composition from that of fish living under natural conditions, with differences involving primarily the lipid content and the fatty acid profiles (Jobling M., 2001), so far, effects of diet on the pikeperch body composition have not been widely studied (Jankowska *et al*., 2003; Kowalska *et al.*, 2011).

The meat of wild percid fish is usually rich in HUFA (Jankowska *et al*., 2003; Kowalska *et al*., 2011; Cladis *et al*., 2014). The lipids in pikeperch fillets are mainly phospholipids that are rich in EPA and DHA (Jankowska *et al*., 2003), although the total content of fat greatly vary accordingly to the species and to the fat content of their natural diet.

Jankoswka *et al.* (2003) reported values (%) of 18.01±0.25 and 0.96±0.07 for contents of protein and fat, respectively, in wild fish and of 18.81±0.20 and 2.87±0.15 for the artificial fed fish, when using a diet (TROUVIT, Nutreco Aquaculture, Holland) containing 46% crude protein and 11% raw fat.

Similarly, Kowalska *et al* (2011) reported more recently, fillet values of 20.28±3.6 and 0.87±0.1 for protein and fat for RAS reared pikeperch. These animals had been fed a diet containing 45% of protein and 10% of fat. The main sources of protein in this base feed (Aller-Aqua, Golub-Dobrzyń, Poland) are fish and soy meals. And the final lipid quantity was attained from 60 g/kg feed, derived mainly from fish meal with the addition of fish oil and soy up to 10% total fat.

As previously described, the new batch of 300-500 g pikeperch individuals (Batch 2) from WP22, Grow out husbandry-pikeperch, was sampled from an experiment which ended the 31 January 2018, date at which UL (P9) had done a growth control (Tables 2.1.1). The fish received an extruded diet from Le Gouessant (sturgeon grower) which provided 44.09±0.00% and 10.25±0.04 % of proteins and lipids, respectively, and with balanced profiles of w6/w3 FAs and DHA+EPA (Table 2.3.1).

Table 3.3.1. Proximate composition of fillets of pikeperch (*Sander lucioperca*) fed a commercial diet

	Batch 2
Moisture (%)	79.00 ± 0.75
Fat (% ww)	0.93 ± 0.23
Protein (% ww)	18.80 ± 1.01
Ash (% ww)	1.21 ± 0.19

Data are means ± SD (n=4)

As shown in Table 3.3.1 the total content of proteins from DIVERSIFY´s Batch 2 fillets is quite similar to those previously reported for either wild or reared specimens (Jankowska *et al.*, 2003), whereas its low fat content is more similar to that reported for the wild specimens or to the most recent results reported for RAS reared pikeperch individuals either fed 6, 10 or 18% total fat content extruded diets with an increasingly addition of vegetable origin oils (Kowalska *et al.*, 2011). These total contents of protein and fat are generally considered as healthy and nutritional for the fish whitefish.

Interestingly, when a detailed analysis is made of the profile of the main fatty acids of the meat in relation to the diet of wild perch individuals (1 batch) (Jankowska *et al.*, 2003) and cultivated ones (3 batches) (Kowalska *et al.*, 2011), including that of present Batch 2, we can confirm an important fact already highlighted for this species. Regardless of the dietary changes generated in the EPA and DHA profiles, the pikeperch flesh always tends to increase the dietary proportions of DHA with DHA/EPA ratios ranging between 2.5 and 5.2. Similar results were obtained by Xu *et al.* (2001) who analysed dietary effects on fatty acid composition in muscles and liver of European perch; and found high accumulation of DHA, compared to the feed used, and that DHA contents in muscle lipids were similar in all the groups examined. This has led several authors to conclude that pikeperch is highly capable of transforming native forms of 18n-3 into long-chain acids, as a result of which the meat has a high DHA content (Xu *et al.*, 2001; Jankowska *et al.*, 2003). However, more recent findings show that diets low in LC-PUFAs, especially DHA, may not affect growth of pike perch, but provoke increased mortality; shock syndromes; short and long-term stress sensitivity; and deficiency in neural development during early ontogeny, that may affect behavior and learning (Lund & Steenfeldt, 2011; Lund *et al.*, 2014). In fact, DIVERSIFY´s partners have confirmed the high requirement of DHA but a low elongation/desaturation capacity in pikeperch in contrast to many other omnivorous or carnivorous freshwater fish species Lund *et al.* (2018).

Also interesting is the fact that when the levels of dietary 18:2n-6 range between 6.6 and 24%, the content of this fatty acid in the fillets corresponds to values of 6.1 and 10.8% of total fatty acids, respectively, with no apparent detrimental effects, denoting that in contrast to the above DIVERSIFY´s evaluated species, this freshwater species do not trend to incorporate in the flesh an excess of 18:2n-6 from a vegetable origin.

For instance, as shown in Table 2.3.1 the extruded diet from Le Gouessant (France) supplied 8.34% of 18:2n-6, 10.53% of EPA and 8.57% of DHA, whereas the flesh displayed proportions of 6.98% of 18:2n-6, 7.85% of EPA and 23.27% of DHA, and DHA/EPA ratios of 2.95 (Table 3.3.2).

Table 3.3.2. Total fatty acid content (g 100 g fillet^{-1}) and main fatty acid composition (% total fatty acids) of fillets of pikeperch (*Sander lucioperca*) fed a commercial diet.

	Batch 2
Total FA	0.60 ± 0.17
16:0	19.35 ± 1.12
18:0	4.32 ± 0.79
Total SFA	27.69 ± 1.53
16:1[1]	5.20 ± 1.16
18:1[2]	17.43 ± 2.91
Total MUFA	24.86 ± 4.30
18:2n-6	6.98 ± 1.47
20:4n-6	1.50 ± 0.30
Total n-6 PUFA	9.06 ± 1.20
18:3n-3	1.18 ± 0.32
20:5n-3	7.85 ± 0.49
22:6n-3	23.27 ± 4.51
Total n-3 PUFA	35.35 ± 4.31
DHA/EPA	2.95 ± 0.49
ARA/EPA	0.19 ± 0.03
n-3/n-6	4.02 ± 0.99
IA	0.47 ± 0.01
IT	0.21 ± 0.01

[1] mainly n-7 isomer; [2] mainly n-9 isomer; DHA, docosahexaenoic acid, 22:6n-3; EPA, eicosapentaenoic acid, 20:5n-3; ARA, arachidonic acid, 20:4n-6. IA, Index of atherogenicity; IT, Index of thrombogenicity.

These very relevant findings denote that the combined quantity of n-3 HUFA, and particularly DHA in pikeperch fillets is high for fish fed low to medium fat diets, even when the degree of substitution of marine origin oil by plant-based ingredients is quantitatively prominent. In addition, Kowalska *et al.* (2011) demonstrate that the application of low-fat feed is beneficial in the rearing of pikeperch which displays better slaughter yield (skinned fillet) and advantageous fatty acid profiles (n-3/n-6 ratio), indicating that at an advanced grow out stage, the pikeperch might be also a good candidate for an economically and environmentally sustainable feeding without substantially decreasing the sensorial and nutritional value of the final product.

Finally, as shown in Table 3.3.3, both European reared pikeperch Batches (1&2) confirm the nutritious and healthy profiles of final products. The evaluated fillets not only display a high protein and low-fat content, but also low IA and IT indexes, a moderate level of SFA and a high absolute amount of EPA+DHA for a 100g portion of less than 1g of total fat content. DHA content is particularly prominent and very convenient to target the nutrition of pregnant and breastfeeding mothers, and population groups at risk in the suffering of neurodegenerative diseases (Zárate *et al.*, 2017).

Table 3.3.3. Proximal composition (% fresh weight), main fatty acid content (mg 100 g fillet^{-1}) and health lipid indexes of pikeperch (*Sander lucioperca*) fillets cultured under different rearing conditions

	Batch 1	Batch 2
Moisture	76.67 ± 0.67	79.00 ± 0.75 *
Fat	0.48 ± 0.23	0.93 ± 0.23 *
Protein	21.24 ± 0.54	18.80 ± 1.01 *
Ash	1.29 ± 0.09	1.21 ± 0.19
Total SFA	141.2 ± 15.2	165.3 ± 38.7
Total MUFA	59.5 ± 15.3	156.0 ± 65.3 *
18:2n-6	17.3 ± 4.1	44.2 ± 19.9 *
20:4n-6	8.6 ± 1.0	8.7 ± 1.4
Total n-6 PUFA	25.9 ± 4.7	56.4 ± 21.7 *
18:3n-3	2.8 ± 1.3	7.6 ± 3.8 *
20:5n-3	33.1 ± 4.0	47.2 ± 12.3 *
22:6n-3	164.1 ± 17.0	134.6 ± 20.4 *
Total n-3 PUFA	200.0 ± 20.5	208.1 ± 40.8
EPA+DHA	197.2 ± 20.2	178.8 ± 43.3
n-3/n-6	7.86 ± 0.94	4.02 ± 0.99 *
IA	0.48 ± 0.04	0.47 ± 0.01
IT	0.19 ± 0.02	0.21 ± 0.01 *

Data are means ± SD (Batch 1, n=10; Batch 2, n=8). * Indicate significant differences among groups (P<0.05). IA, Index of atherogenicity; IT, Index of thrombogenicity.

Sensorial analysis

Results from the sensory analysis of the pikeperch samples are shown in table 3.3.4.

Table 3.3.4. Mean values and standard deviation for the different sensory descriptors of the pikeperch (*Sander lucioperca*) fillets from batch 2.

Sensory descriptor	Mean value	Standard deviation
O_Sardine	0.3	0.24
O_Ammonia	1.8	0.31
O_Earthy	3.7	0.70
O_Butter	2.9	0.23
O_Acid	0.3	0.11
O_Boiled vegetables	0.8	0.46
O_Toasted	0.1	0.19
O_Flower	0.5	0.28
Colour white to brown	2.5	0.15
Colour uniformity	7.2	0.35
White spots	2.9	0.60
Laminar structure	2.0	0.16
Exudates quantity	2.3	0.95
Exudates turbidity	3.5	1.74
Fat droplets	0.1	0.06
Exudate particles	4.1	1.50
Exudate proteins	3.0	0.99
Black lines in the flesh	3.1	0.57
Brightness	4.3	0.21
F_Sweet	1.7	0.51
F_Acid	0.7	0.33
F_Bitter	2.1	0.61
F_Earthy	5.3	0.85
F_Sardine	0.6	0.25
F_Butter	2.0	0.39
F_Boiled vegetables	0.7	0.13
T_Firmness	5.8	0.14
T_Crumbliness	5.2	0.40
T_Juiciness	3.2	0.31
T_Chewiness	5.1	0.22
T_Pastiness	3.8	0.37
T_Teeth adherence	3.0	0.62

The pikeperch samples are characterized by having bright fillets with an homogeneous and white color, which is usually highly valued by fish consumers. Its flavor is quite neutral and its texture is consistent, crumbly and slightly juicy, which makes this species an ideal product for those looking for a fish with delicate sensory properties. The main problem associated with this species, as already seen in Batch 1 analyzed and described in deliverable D28.3 and the paper from Lazo *et al.* (2017), is the presence of a clear earthy odour and an even more accentuated earthy flavour. It is therefore evident that the improvement of this species necessarily involves purging of animals in clean fresh water before slaughter them to reduce the effect of their farming in recirculation systems (RAS).

As commented above for greater amberjack and grey mullet, the comparisons with previous batches from other DIVERSIFY deliverables cannot be made for the sensory data.

3.4 Comparisons made among the four species

Somatometry – technical indexes

As stated in D28.3, data present in literature on the technical yields of the studied species are very limited. Therefore, Table 3.4.1 provides new data of somatometric and technical indexes for DIVERSIFY´s new batches of greater amberjack and grey mullet, and makes a comparison with those previously evaluated in Deliverable 28.3 for pikeperch and meagre.

Morphometric-based condition indices are widely used to assess proximate body composition and collaterally, feeding and living conditions of fish. The condition index is a way to measure the overall health status of a fish by comparing its weight with that of other fish of the same kind and of similar length, although its relation to life history traits and seasonality have not been fully explored yet. As previously mentioned, only intra-species comparisons of condition index (CI) are actually meaningful, since allometry differences between species mostly affect the potential differences found. In spite of these considerations, the highest condition index was always found for greater amberjack, independently of the interspecific differences on the fish size.

Remarkably, the dressing yields for all selected species are quite similar, exceeding 90%. This is an important measurement because it determines the yield for the fish when sold as gutted, without further processing, which is one custom way of commercialization in EU for fresh fish (Grigorakis, 2017).

The filleting yield is also a relevant parameter, especially for species in which filleting is among their custom processing, because it describes their actual edible gain (Grigorakis, 2017). Lack of information on yield of the filleting process of highly appreciated species on the market triggers interest in current research aiming at the analysis of the influence of body weight on the processing yield, and on the chemical composition of the fillets.

Deliverables 28.3 and 28.7 add some new data to the actually limited literature mainly focused to meagre and pikeperch (D 28.3; Table 3.9). Our present results confirm that filleting yield is subjected to the fish species and that the highest filleting yields were obtained for greater amberjack, with values of 50% even for a fish size of around 0.5 kg. Compared to Deliverable 28.3, a better filleting yield, also close to 50%, was found for the new Diversify´s produced Batch 2 of grey mullet. For pikeperch and meagre, the only data available were those previously shown in D28.3 with the filleting yield slightly lower to 40%. The present results confirming that amberjack is the most advantageous species in this aspect.

Table 3.4.1. Average somatic indexes and technical yields from greater amberjack (*Seriola dumerili*), grey mullet (*Mugil cephalus*), pikeperch (*Sander lucioperca*) and meagre (*Argyrosomus regius*) produced within Diversify´s Grow out GWP

	Greater amberjack	Grey mullet	Pikeperch	Meagre
Batch	**3 & 4**	**2**	**1**	**1**
Body weight (g)	452.8 ±104.9 b	200.3 ± 69.9 a	1096.6 ±217.2 c	1834.6 ±140.4 d
CI	2.02 ± 0.19 c	0.96 ± 0.08 a	0.71 ± 0.13 b	0.87 ± 0.05 a
Dressing yield (%)	94.0 ± 0.9 bc	95.1 ± 0.5 c	93.5 ± 1.3 b	91.1 ± 0.6 a
Filleting yield (%)	50.0 ± 5.0 b	46.8 ± 1.2 b	36.2 ± 4.4 a	38.4 ± 2.4 a
HSI	0.90 ± 0.18	0.84 ± 0.17	0.81 ± 0.18	0.87 ± 0.05
VSI	4.80 ± 0.90 a	4.12 ± 0.51 a	5.73 ± 1.30 ab	5.75 ± 0.44 b

Data are means ± SD (n=38 for greater amberjack; n=6 for grey mullet; n=10 for pikeperch and meagre). CI: condition index; HSI: hepatosomatic index; VSI: viscerosomatic index.

Proximate and fatty acid composition

Certain issues related to fish nutrition and rearing practices have become controversial because they impact the environment and/or affect the final product for consumption. Some of these issues include: feed and nutrient efficiency, overfeeding and waste, unsustainable feed ingredients, fish health issues, biotechnology and human health concerns. Ultimately, each of these issues can affect the final product for human consumption, either nutritionally, environmentally or economically. Achieving a balance between efficient and safe food production with environmental sustainability will be a great challenge for the industry (Hixson, 2014).

We have reviewed here several issues of fish nutrition and rearing history in the farmed selected species, relating to providing quality food products while maintaining environmental sustainability. A great variety of diets already adapted for consolidated species and/or with a medium to high degree of substitution of marine origin ingredients have been tested for grow out by DIVERSIFY´s partners and confirm that each species has a different metabolic pattern in terms of dietary modulation of flesh nutritional quality. According to these results it is evident that more efforts should be oriented towards an improvement of nutritional image of farmed fish than towards an enhancement of their sensory properties (Claret *et al.,* 2014, 2016).

Information on fish chemical composition is highly relevant for the standardization of food products based on nutritional criteria. It provides elements for decisions on nutritional characteristics and on the follow-up of industrial processes or research by changes in the chemical components (Rodrigues de Souza *et al*., 2015). Over the past few decades, the availability of aquaculture-sourced seafood has greatly risen and now accounts for more than the half of all fish available in commercial markets (Bostock *et al*., 2010; FAO, 2016; APROMAR, 2017). The diet of farmed fish is controlled by the farmer, who also depends upon the ingredient-selection decisions made by feed manufacturers. Given the myriad of possible health benefits associated with EPA and DHA and other fish essential nutrients (Swanson *et al*., 2012; Sprague *et al*., 2016; Zárate *et al*., 2017) and the potential differences expected between farmed and wild-caught fish, it is imperative that more information is made available to consumers through labelling, concerning aquaculture seafood nutritional composition.

In this sense, data for fillet composition of wild and reared specimens of the 4 selected new candidate species, and its relationship with the rearing history have been added to the present deliverable. The new data from GWP5; greater amberjack (IEO, Batches 3 and 4), grey mullet (CTAQUA, Batch 2) and pikeperch (UL/ASIALOR, Batch 2) and the corresponding extruded diets are summarized for comparisons in Tables 3.4.2., and 2.3.1, respectively. Data from meagre given in D28.3 have been also used here (Table 3.4.2), by adding the composition of the corresponding diet (Table 2.3.1).

The following values are the protein and fat contents corresponding to the 4 extruded diets (Table 2.3.1).

	Greater amberjack	Grey mullet	Pikeperch	Meagre
Protein	50.14 ± 0.01	35.10 ± 2.46	44.09 ± 0.00	43.51
Fat	18.78 ± 0.02	15.09 ± 0.68	10.25 ± 0.04	17.16

In spite of the differences in the dietary formulation for the protein supplementation levels or origin (fish or plant origin meal), the fillet protein of all fish species remained stable near 20% of total fillet constituents, as widely stated in literature. This has been confirmed for the selected species in both the herein results (Table 3.4.2) and also in those of fish evaluated for D28.3 (Table 3.2). These results seem to indicate that as long as the fish is provided with sufficient amount of protein, the quality of the final product in terms of protein is guaranteed even when certain degree of substitution of fish meal by plant or algae meal is performed.

Aside from this general assertion, the observed fillet composition for the studied species, distinguishes the greater amberjack and meagre for their higher contents of protein, followed by the grey mullet and finally, the pikeperch.

Table 3.4.2. Comparison between proximal composition (% fresh weight), main fatty acid content (mg 100 g fillet^{-1}) and health lipid indexes of fillets from greater amberjack (*Seriola dumerili*), grey mullet (*Mugil cephalus*), pikeperch (*Sander lucioperca*) and meagre (*Argyrosomus regius*).

	Greater amberjack	Grey mullet	Pikeperch	Meagre
Batch	**3 & 4**	**2**	**2**	**1**
Moisture	75.62 ± 1.40 a	77.98 ± 0.70 b	79.00 ± 0.75 b	77.18 ± 0.29 ab
Fat	3.07 ± 1.02 b	0.86 ± 0.17 a	0.93 ± 0.23 a	0.53 ± 0.36 a
Protein	20.75 ± 1.06 b	19.33 ± 0.80 ab	18.80 ± 1.01 a	20.66 ± 0.20 b
Ash	1.56 ± 0.22 b	1.21 ± 0.08 a	1.21 ± 0.19 a	1.35 ± 0.01 a
Total SFA	545.4 ±114.3 b	180.8 ± 43.4 a	165.3 ± 38.7 a	137.9 ± 11.3 a
Total MUFA	978.5 ±282.4 c	79.9 ± 23.4 a	156.0 ± 65.3 ab	145.9 ± 8.3 b
18:2n-6	**337.0 ± 76.9 d**	**10.8 ± 3.2 a**	**44.2 ± 19.9 b**	**105.2 ± 5.0 c**
20:4n-6	16.3 ± 1.8 b	21.7 ± 3.5 b	8.7 ± 1.4 a	7.0 ± 1.5 a
Total n-6 PUFA	372.8 ± 78.8 c	45.0 ± 8.6 a	56.4 ± 21.7 a	135.5 ± 15.9 b
18:3n-3	79.0 ± 21.8 b	18.4 ± 10.9 a	7.6 ± 3.8 a	6.2 ± 0.1 a
20:5n-3	115.0 ± 24.6 c	46.1 ± 10.3 b	47.2 ± 12.3 b	15.4 ± 1.4 a
22:6n-3	240.7 ± 47.8 c	116.1 ± 21.4 b	134.6 ± 20.4 b	65.5 ± 15.3 a
Total n-3 PUFA	528.3 ±114.2 c	215.9 ± 45.5 b	208.1 ± 40.8 b	92.8 ± 17.0 a
EPA+DHA	**335.7 ± 70.2 c**	**162.2 ± 65.9 b**	**178.8 ± 43.3 b**	**80.9 ± 16.7 a**
n-3/n-6	1.45 ± 0.22 b	4.90 ± 1.22 c	4.02 ± 0.99 c	0.68 ± 0.04 a
IA	0.38 ± 0.01 a	0.55 ± 0.05 c	0.47 ± 0.01 c	0.42 ± 0.01 b
IT	0.22 ± 0.01 b	0.21 ± 0.01 ab	0.21 ± 0.01 a	0.30 ± 0.01 c

Data are means ± SD (greater amberjack, n=30; pikeperch, n=8; grey mullet, n=6; meagre, n=3). IA, Index of atherogenicity; IT, Index of thrombogenicity

Also accordingly to DIVERSIFY´s previous results and to the literature (Thakur *et al.*, 2009; Rodríguez-Barreto *et al.*, 2012, 2015; Lazo *et al.*, 2017), greater amberjack is distinguished by the profoundly and statistically higher lipid contents (Table 3.4.2), with a significant increase of fillet fat with fish size as already mentioned in previous sections and in D28.3, as occurs in wild (Rodríguez-Barreto *et al.*, 2012, 2015; Zupa *et al.*, 2017). All other species exhibit fillet fat of <1%. As has been largely confirmed in the literature, meagre (Poli *et al.*, 2003; Hernández *et al.*, 2009; Grigorakis *et al.*, 2011; Giogios *et al.*, 2013; Martelli *et al.*, 2013) and pikeperch (Jankowska *et al.*, 2003; Kowalska *et al.*, 2011), are indeed low-fat species and thus, similar fat patterns will be expected throughout the year. However, the grey mullet is supposed to contain higher fillet fat, being described as a medium- to high-fat species (Özogul & Özogul, 2007; Özogul *et al.*, 2009). Seasonality in fillet fat depots has been mentioned for wild fish of various species (Ersoy *et al.*, 2008; Cardinal *et al.*, 2011; Özogul *et al.*, 2011; Tufan *et al.*, 2018). The new two batches evaluated here were sampled in December, as that of wild fish from Batch 1 (D28.3). More individuals sampled at different seasons, would perhaps clarify this consistently low-fat contents of winter-sampled grey mullet. It is evident from these results that this low-fat presence in the flesh of the three species occurs independently of the dietary fat content, and that greater amberjack fat deposition capacity is five to six times that of the meagre, which is also being fed a high fat dietary content (Table 2.3.1). This means that fat flesh quality in greater amberjack is also much more susceptible of dietary modulation. In fact, this high capacity for fat deposition is confirmed by the absolute amounts of FAs present in greater amberjack per 100 g fillet portions compared to the other three species (Table 3.4.2).

As already mentioned, and in contrast to the final flesh protein contents, changes in dietary marine ingredients directly modify tissue fatty acid composition (Torstensen *et al.*, 2000; Pérez *et al.*, 2014), which may alter not only the new selected species´s metabolism, health and performance, but also, reduce their nutritional health promoting benefits. A great advantage of aquaculture products is their constant nutritional profile compared to wild fish which greatly varies their biochemical composition according to seasons and habitat (Tufan, 2018). For this reason, the use of good and balanced ingredients to ensure a stable and high quality and sustainable final product must be a priority.

Fishmeal and fish oils from forage fisheries are still the benchmark in terms of nutritional quality for farmed species. If these fisheries are responsibly managed and traceability is ensured, their use in aquafeeds can be maintained. Given the continued global growth of aquaculture, it would be necessary to use these valuable raw materials in the most economical possible way, reserving their use for particular stages of farming such as spawning, larval and juvenile stages or as finishing feed (IUCN, 2017). On a technical and economic level, many vegetable materials are sustainable alternatives to the marine proteins and lipids needed to feed farmed fish and this is now the ‘norm’ in fish feed manufacture. There are protein-rich seeds and meals with high essential amino acid contents and incoming new algae products that according to our results might be suitable for the selected species.

Vegetable oils can also replace fish oils, provided that essential fatty acids (EFA) and fish oil supplements are added to the formulated feeds for some fish species, or at certain stages, however it is still needed to perform more grow out trials with the four selected species to establish a good range of fish oil substitution to ensure the final quality of the product.

To summarize and simplify the understanding of the most relevant findings in terms of fatty acid composition of the fillets, the following numbers have been extracted from dietary composition of greater amberjack, grey mullet, pikeperch and meagre, respectively (Table 2.3.1).

	Greater amberjack	Grey mullet	Pikeperch	Meagre
Total SFA	22.91 ± 0.44	19.16 ± 0.22	30.44 ± 0.37	23.99
18:2n-6	11.28 ± 0.07	15.57 ± 0.00	8.34 ± 0.11	21.61
20:5n-3	6.61 ± 0.35	3.45 ± 0.06	10.04 ± 0.02	5.29
22:6n-3	8.17 ± 0.43	4.98 ± 0.09	8.57 ± 0.02	8.36

Pikeperch received the highest proportions of both saturates and EPA+DHA, followed by greater amberjack and meagre, which received similar contents and then, grey mullet. In terms of the inclusion of vegetable sources, 18:2n-6 levels denote meagre receiving the most “sustainable feeding”. As a result, and considering the total amount of fat, the following numbers are the resultant contents of these fatty acids per 100 g of serving portion of fillet (Table 3.4.2):

	Greater amberjack	Grey mullet	Pikeperch	Meagre
Total SFA	545.4 ±114.3 b	180.8 ± 43.4 a	165.3 ± 38.7 a	137.9 ± 11.3 a
18:2n-6	337.0 ± 76.9 d	10.8 ± 3.2 a	44.2 ± 19.9 b	105.2 ± 5.0 c
EPA+DHA	335.7 ± 70.2 c	162.2 ± 65.9 b	178.8 ± 43.3 b	80.9 ± 16.7 a

Each species has a completely different dynamic of fatty acid incorporation and as a result, greater amberjack is the healthiest one in terms of total contents of EPA+DHA to supply international intake recommendations. However, with the diets assayed, its fillets are also supplying higher quantities of saturates and 18:2n-6 which is not an objective from a nutritional value point of view. Grey mullet and pikeperch are in this sense more balanced even if a higher portion is needed to fulfill the recommended intake of w3 LC-PUFA.

In recent years, consumers are seeking healthier foods and increasingly discerning regarding food they will consume, however, consumers often do not find the information they seek. Thus, the fish authenticity and correct labelling is an important requirement to ensure quality, provide adequate security controls and develop effective regulations. Food traceability includes food components identification to verify the compliance with labeling to prevent fraud. Fish industry must be providing information in the label about species, origin, age, and production systems (Chaguri *et al.*, 2017) but also a basic information of the food ingredients and main proximate and fatty acid nutritious composition.

Regulation 1924/2006 regarding information to consumers, establishes that in order to achieve a high level of health protection for consumers and to guarantee their right to information, it should be ensured that consumers are informed appropriately as regards the food they buy and consume. EU food laws provide a basis for consumers to make informed choices in relation to food they eat and to prevent any practices that may mislead them. Other voluntary information concerns to the Nutritional & Health claims; provisions on nutrition and health claims in order to ensure the effective functioning of the internal market whilst providing a high level of consumer protection. This applies to nutrition and health claims made in commercial communications, whether in the labelling, presentation or advertising of foods to be delivered as such to the final consumer. Nutrition claim states that a food has particular beneficial nutritional properties and health claim states that a relationship exists between a food and health, both aspects that are present in the intended DIVERSIFYs products.

DIVERSIFY’s partners have the capacity and the required knowledge to fulfil all these requisites. To attain a sustainable and properly labelled final product, of high sensorial and nutritional qualities in any of the selected species, is a clear objective that encourage to continue the collaboration among all DIVERSIFY’s partners, including nutritionists and any other aquaculture specialist, sensory evaluators, farmers, feed producers and marketing experts.

4. Conclusions

With certain uncertainties due to the limited number of specimens from some of the selected species, or limited number of different rearing histories, some interesting aspects have been confirmed and some new suggestions can be also drawn upon the results presented here.

The four fish species were studied for their fillet composition, technical yields and fillet sensory properties. It can be concluded that:

1) The dressing yields for all species are quite similar, slightly exceeding 90%.
2) Greater amberjack showed high fillet fat reaching 3-4% in 0.5-1.5 kg fish, whereas all other fish exhibited very low fillet fat, not exceeding 1%.
3) Filleting yields and protein contents did not seem to be influenced significantly by fish size or rearing and dietary histories at the grow out stage. All species showed similar and typical fillet protein content close to 20%.
4) Greater amberjack displayed the highest filleting yields and final contents of protein, fat and especially EPA+DHA. However, further grow out trials are advisable in order to establish the best extruded diet to balance the final levels of saturated and 18:2n-6 fatty acids. Due to its vulnerability to fat oxidation, it is suggested that commercial sizes should be 1-2 kg with a relatively lower fillet fat content than bigger fish.
5) Meagre filleting yield and protein content were quite attractive. Its total fat contents did not seem to be influenced highly by the dietary or growing history, displaying low contents of fat even in the wild, an attractive feature for low fat dietary regimes. However, the degree substitution of dietary marine origin ingredients by terrestrial vegetables components should be taken with more caution and highly controlled in this species, which displayed high contents of 18:2n-6 and lower comparable amounts of EPA+DHA.
6) Non exclusively-marine fish species, *i.e.* the grey mullet and the pikeperch, display good filleting yields as well as nutritious high protein and healthy low-fat contents.
7) Grey mullet is confirmed as the best candidate for marine ingredients substitution either by terrestrial or marine origin vegetable sources. Independently of the rearing history, their fillets display a comparable high amount of EPA+DHA, low contents of 18:2n-6 and a good balance of saturated fatty acids, all of them attractive nutritional and health promoting qualities.
8) Pikeperch fillet was also attractive in terms of protein and fatty acid profiles. The carnivorous nature of the species might not allow such a high degree of dietary inclusion of vegetable components, but still its high dynamic to retain DHA in the flesh and its low fat content makes it a highly attractive and health-promoting fish.
9) In the case of greater amberjack, the different tested culture densities seem to have a negligible effect on the sensory quality of the final product.
10) The diet had an important effect on the sensory characteristics of the grey mullet, especially in aspects related to the fillet fat content and its oxidative stability.
11) Pikeperch had neutral sensory characteristics, which makes it an ideal fish for those people looking for mild flavours.
12) In the case of the pikeperch it is important to control the ante-mortem treatment in order to guarantee the absence of sensory defects (earthy flavour).

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