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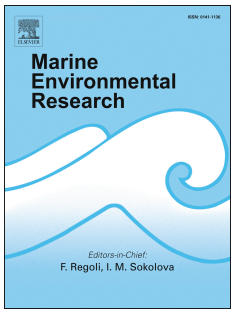

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D. Gonzalez-Silvera, D. Izquierdo-Gomez, P. Sanchez-Jerez, M.T. Elbal, J.A. López-Jiménez, F.J. Martínez-López

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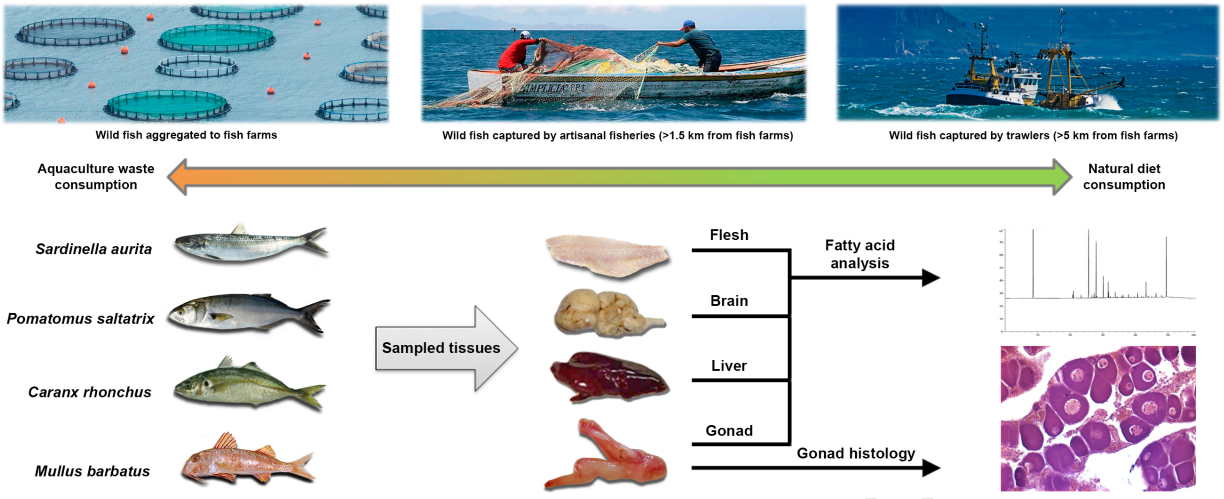

Influence of aquaculture waste on fatty acid profiles and gonad maturation of wild fish aggregations at fish farms.

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ABSTRACT

Wild fish belonging to four species belonging to different trophic groups were captured at three distances from fish farm facilities: long distance (>5 Km), medium distance (1.5 Km) and close to sea-cages. Flesh, brain, liver and gonads were sampled for fatty acid analysis. Fish aggregated near sea-cages showed accumulation of fatty acids of vegetable origin in the studied tissues, due to surplus feed consumption or via predation of fish that consumed the feed. Gonads accumulated vegetable fatty acids in different manner in the different species, and the species least and most influenced by fish-feeds were selected for gonad histological examination. Results showed an acceleration of the final stages of the oocyte development in fish aggregated near fish farms compared to fish captured at long distance. Differences in oocyte development were more acute in the species which incorporated higher quantities of vegetable fatty acids.

Keywords: Fatty acids, trophic transfer, aquaculture, fish, vegetable oils, oocyte development.

1. Introduction

The global aquaculture industry has been growing in importance during the last decades, reaching a production of 80 million tonnes of edible fish in 2016 (FAO 2018). The consumption of fish have increased worldwide due to general raising awareness and knowledge about the healthy properties of fish and shellfish consumption, mainly due to their high content in n-3 long-chain polyunsaturated fatty acids (Simopoulos 1999; Nichols et al. 2010; Tur et al. 2012; Calder 2014; Shahidi and Ambigaipalan 2018). As industry has grown, so too has concern regarding environmental impact, particularly due to the input of high amounts of organic matter in form of lost pellets and faeces into the marine environment. This fact could alter the physicochemical properties of water column and bottom sediments, increasing risk of anoxia due to bacterial activities and thus affecting the local fauna (La Rosa et al. 2001; Maldonado et al. 2005; Sanz-Lázaro and Marín 2011; Fernandez-Gonzalez et al. 2013). At the same time, sea-cage fish farms act as FADs (Fish Aggregation Devices), attracting a large number of different wild fish and macroinvertebrate species which take advantage of the protection provided by the submerged structure, as well as by the supply of large quantities of high energy exceed feed and faeces (Sara et al. 2004; Dempster et al. 2009; Sanchez-Jerez et al. 2011; Black et al. 2012). Aggregated wild fish can consume up to 80 % of fish-feed in form of lost pellets (Vita et al. 2004), contributing to minimise the environmental effect of the discharge of organic matter on the seabed (Katz et al. 2002; Felsing et al. 2005; Fernandez-Jover et al. 2008; Ballester-Moltó et al. 2017). Feeds in aquaculture are currently rich in vegetable oils of different origin and lipid composition, characterized to a large extent by the presence of high levels of short/medium-chain polyunsaturated fatty acids (PUFA), as linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, LNA), besides monounsaturated fatty acids (MUFA) like oleic acid (18:1n-9, OA) and saturated fatty acids (SFA) (Watanabe 2002; White et al. 2019). The fatty acid profile of commercial feeds, rich in n-6 short-chain PUFA, are different from the fatty acid profiles of the natural diets of wild fish, rich in long-chain PUFA mainly corresponding to the n-3 series. It is well known that changes in the n-3/n-6 ratio could lead to alterations of the fish immune system, as well as on reproduction and larvae development (Izquierdo et al. 2001; Simopoulos 2002; Tocher 2003; Kiron 2012; Calder 2013). The amount of n-3 fatty acids, especially eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) in flesh tend to be lower in farmed fish

when compared to their wild counterparts (Sprague et al. 2016). Therefore, it is necessary to control the composition of the artificial diets along the life cycle of cultured fish, in order to obtain enough quantities of DHA and EPA for human consumption via farmed fish. A good example would be the use of finishing diets rich in n-3 fatty acids before sale, resulting in a final product as similar as possible to a wild fish in terms of flesh fatty acid composition (Henriques et al. 2014; Nichols et al. 2014). Unfortunately, it is not possible to exercise that kind of control on aggregated wild fish. They can intake fish-feed rich in n-6 fatty acids of vegetable origin during an indeterminate period of time, and thus modifying the fatty acid profiles of different tissues in a different extent. The egg quality is highly influenced by the dietary lipids, which could be the main factors determining a successful reproduction and survival of the progeny in reared fish (Almansa et al. 1999; Izquierdo et al. 2001). DHA, EPA and arachidonic acid (20:4n-6, ARA) are essential fatty acids for marine fish (Sargent et al. 1999) as they cannot synthesize them from their precursors, LNA and LA. Essential fatty acids are required for the production of vitellogenin, which will be stored in the oocyte in the form of vitello as the only source of nutrients for the embryo (Alvarez-Lajonchère 2006). Wild fish consume the lost pellets for as long as they remain close to the sea-cages, accumulating fatty acids of vegetable origin with the consequent reduction on the percentages of the essential fatty acids ARA, EPA or DHA, which could have an effect on the oocyte development, and this fact may be investigated. The accumulation of fatty acids of vegetable origin may be species-specific, depending on their feeding preferences. Previous works have shown that the uptake of aquaculture wastes by wild fish may be reduced with increasing distance to the fish farms, measured by fatty acid analysis and body condition (Arechavala-Lopez et al. 2011; Dempster et al. 2011; Izquierdo-Gomez et al. 2015; Woodcock et al. 2018;). Nevertheless, the accumulation of fatty acids of vegetable origin used to be only analysed in flesh, due to its importance for human nutrition, whereas less attention has been paid to the impact on other tissues with a more relevant role in the fish physiology.

The aim of this research was to check the possible effect on different tissues (flesh, brain, liver and gonads) of commercial feed consumption in form of lost pellets by sea-cage aggregated wild fish. Four fish species of commercial interest, known to be aggregated near sea-cages, and presenting different feeding behaviour (which may differ in the amount of aquaculture wastes consumed) were captured in the vicinity of

fish farms. Their fatty acid profiles were compared to both, control fish captured off-shore at long distance from the farm, and specimens captured at an intermediate distance to the fish farm facilities. Histological examination of the gonads of two species was also carried out in order to check for differences in oocyte development between groups. The obtained results complement those previously published on muscle by the authors (Izquierdo-Gomez et al. 2015).

2. Material and methods

2.1 Characteristics of the studied species

Because the difficulties of wild fish sampling, just four species were captured in enough number in the different distances studied in this work. The captured species belonged to different trophic niches, and they were red mullet (*Mullus barbatus* Linnaeus 1758, zoobenthic predator), which inhabit the benthic environment, consuming mainly polychaetes, decapods and small crustaceans (Machias and Labropoulou 2002); round sardinella (*Sardinella aurita* Valenciennes 1847, zooplanktivorous), which inhabit the pelagic environment and predating mainly copepods and cladocerans (Morote et al. 2008); false scad (*Caranx rhonchus* Geoffroy Saint-Hilaire 1817, mesopredator), which also inhabit the pelagic environment and predating mainly teleosts, crustaceans, molluscs and annelids (Sley et al. 2008); and bluefish (*Pomatomus saltatrix* Linnaeus 1766, piscivorous), which represents the highest trophic level of this study, teleost being the main prey but also polychaetes, crustaceans and gastropods (Harding and Mann 2001).

2.2 Experimental design

Specimens of *M. barbatus* were sampled from April to September 2011, and *S. aurita*, *C. rhonchus* and *P. saltatrix* specimens were sampled from September to December 2011. Two types of commercial feeds used at the aquaculture facilities were also sampled as well as a sample of fish faeces. The four fish species were divided into three categories, according to increasing distances to the fish farms: group “farm” or F, composed of fish captured in the vicinity of two fish farms sited in the Bay of Santa Pola (Fig. 1), Alicante (South-east of Spain); group “medium distance” or MD, composed of fish captured by artisanal fisheries at a minimum distance of 1.5 km from

the fish farms; and group “long distance” or LD, composed of fish captured off-shore by trawlers at a minimum distance of 5 km from the fish farms.

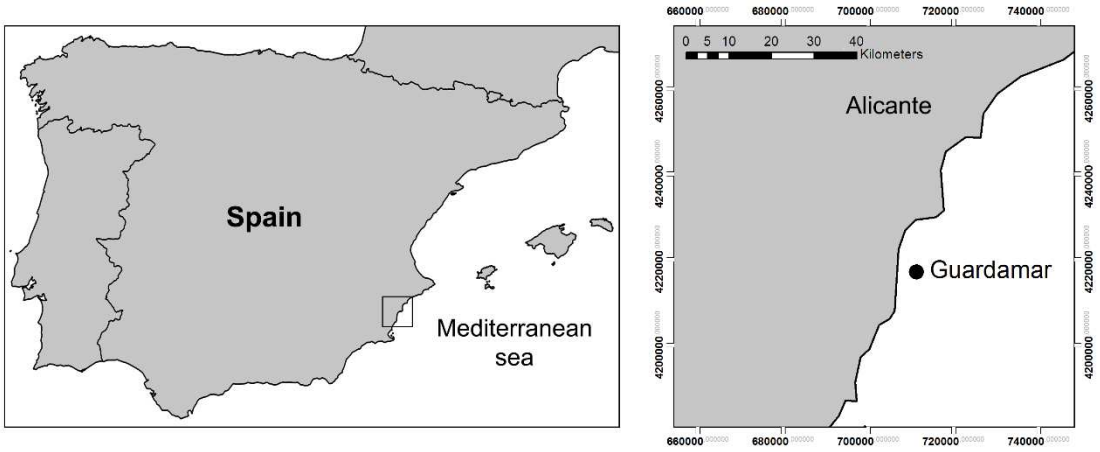

Figure 1. Location of the studied farms in Guardamar, Santa Pola Bay, south-east coast of Spain, in the Western Mediterranean Sea.

2.3 Sample collection

The minimum number of captured specimens by treatment was eight for each species. All samples were kept on ice until delivery to the laboratory. Fish total weight and length (body length from the snout to the place where the main ray of the tail comes out of the body) were recorded and condition factor (CF) was calculated as CF = (weight length-3) × 100. Liver and gonad weights were measured to obtain the hepatosomatic and gonadosomatic indices (ratio of fish liver or gonad weight to body weight) (Table 1). Flesh samples taken from the anterior-dorsal white muscle portion, liver and brain were sampled for fatty acid analysis. Gonad samples were divided in two portions, one ovary for fatty acid analyses and the other for histological examination. Three replicates of each feed were obtained for fatty acid analysis, while just one pool of faeces could be analysed because of the difficulty involved in obtaining a big enough sample. Samples for fatty acid determination were frozen at -80 ºC until analyses.

2.4 Fatty acid analyses

Fatty acids were extracted from 0.3-1.0 g tissue samples by homogenization in 20 ml of chloroform/methanol (2:1 v/v) in an Ultra Turrax tissue disrupter (IKA ULTRA-TURRAX T 25 digital, IKA-WERKE). The total lipids were prepared according to the

method of Folch et al. (1957) and non-lipid impurities were removed by washing with 0.88 % (w/v) KCl. The weight of lipids was determined gravimetrically after evaporation of the solvent and overnight desiccation in vacuum. Fatty acid methyl esters (FAME) were prepared by acid-catalysed transesterification of total lipids according to the method of Christie (2003), and the total lipid samples were transmethylated overnight in 2 ml of 1 % sulphuric acid in methanol (plus 1 ml of toluene to dissolve neutral lipids) at 50 ºC. The methyl esters were extracted twice in 5 ml hexane-diethyl ether (1:1, v/v) after neutralization with 2 ml of 2 % $KHCO_3$, dried under nitrogen and redissolved in 0.1 ml of iso-hexane. Methyl esters were purified by TLC (thin layer chromatography) using iso-hexane:diethyl-ether:acetic acid (90:10:1 v/v/v). FAME were separated and quantified by gas-liquid chromatography using an SP™ 2560 flexible fused silica capillary column (100 m long, internal diameter of 0.25 mm and film thickness of 0.20 mm; SUPELCO) in a Hewlett-Packard 5890 gas chromatograph. The oven temperature of the gas chromatograph was programmed for 5 min at an initial temperature of 140 ºC, and increased at a rate of 3 ºC/min to 230 ºC, further increased at a rate of 2 ºC/min to 240 ºC and then held at that temperature for 12 min. The injector and flame ionization detector were set at 260 ºC. Helium was used as carrier gas at a pressure of 300 kPa, and peaks were identified by comparing their retention times with appropriate FAME standards purchased from the Sigma Chemical Company (St Louis, MO, USA). Individual fatty acid concentrations were expressed as percentages of the total content.

2.5 Gonad histology

M. barbatus and *S. aurita* (6 and 7 replicates respectively) female specimens from treatments F and LD were selected for gonad histology examination, due to the availability of individuals captured at the same time in both, around the sea-cages (group farm) and at long distance from fish farms (group long distance, control), in order to avoid differences in gonad maturation by season. *M. barbatus* specimens were captured on 30th March and *S. aurita* specimens were captured between 7th and 14th October. Gonad samples were processed histologically for the estimation of the oogenesis. Samples from the anterior, central and posterior part of the ovary were fixed in 4 % (v/v) buffered formaldehyde for 24 h. Afterwards, the tissue samples were washed in phosphate buffer and kept in 70 % ethanol. Fixed pieces were processed in an automatic tissue processor (MYR, Spain), and embedded in paraffin wax. For

histological and morphometrical study, dewaxed serial sections (5µm) were rehydrated, routinely stained with haematoxylin-eosin. Samples were analysed in the "Servicio de Análisis de Imagen, University of Murcia" using appropriate equipment for stereological analysis.

2.6 Stereological analysis

Stereological analysis was performed using Visiopharm NewCAST Stereology software, an Olympus BX61 microscope, and a Pixelink (1.9 Mpx) digital camera. Six different fields were randomly selected from each histological slide. Oocyte sizes were determined by the superposition of two dotted grid of different size, and by counting the number of marks in each cell category (Oogonia, oocyte in nucleolus stage, oocyte in transition stage, oocyte in perinuclear stage, oocyte in early vitellogenesis stage, oocyte in late vitellogenesis stage and mature oocyte). Cell volume was determined by the use of nucleators, by pointing the centre of the nucleus and estimating oocyte area by the intersection of six random lines which crosses the oocyte membrane (Bucholtz et al. 2013).

2.7 Statistical analysis

The results are expressed as mean ± standard error. Individual fatty acids data were statistically analysed by two-way analysis of variance (ANOVA) to determine differences between distance treatments. Multidimensional scaling (MDS), SIMPER (similarity percentages) procedure (Warwick et al. 1990; Clarke 1993) and a permutation test (PERMANOVA) (Clarke 1993; Anderson 2004) comprising 4999 permutations were carried out to assess the significance of the overall fatty acid composition among distance treatments for each sampled tissue. SIMPER analyses were performed using the Bray-Curtis dissimilarity index (Bray and Curtis 1957). Statistical analyses were conducted using SPSS Statistical Software System version 15.0 (SPSS Inc., Chicago, IL) and Primer (Plymouth Routines In Multivariate Ecological Research; v.6.1.13) and its complementary statistical package PERMANOVA+ (v.1.0.3). As fatty acid data were percentages, they were transformed with arcosen (x + 1), and all statistical tests were performed with a significance level of $\alpha = 0.05$.

3. Results

3.1 Body condition

All fish specimens from the three distance treatments were adult fish, with no apparent signs of disease or infestation by parasites. Most of the specimens were female. There were some differences in size and weight, hepatosomatic index and the condition factor between distance treatments (Table 1). Even though all specimens were adults, we could not differentiate them by age, and a link between age and the significances found for weight, length, HSI and body condition cannot be discarded.

Table 1. Body condition and number of replicates. Significant differences among distance treatments are shown in bold and italic. Results are expressed as mean ± S.E. HSI: Hepatosomatic index; GSI: Gonadosomatic index; CF: Condition factor. The number of replicates is shown differentiating between females and males.

	S. aurita	*P. saltatrix*	*C. rhonchus*	*M. barbatus*
Standard Length				
A	***23.17 ± 0.81 ab***	***46.44 ± 2.03 a***	***28.44 ± 0.42 a***	***15.82 ± 0.43 a***
B	***21.14 ± 0.54 a***	***33.88 ± 2.55 b***	***27.38 ± 1.54 ab***	***14.67 ± 0.34 a***
C	***23.31 ± 0.41 b***	***29.83 ± 1.66 b***	***23.67 ± 0.26 b***	***13.12 ± 0.38 b***
Weight				
A	***196.30 ± 17.26 a***	***1,437.88 ± 178.32 a***	***397.38 ± 14.42 a***	***75.26 ± 5.60 a***
B	***137.10 ± 11.26 b***	***688.42 ± 111.64 b***	***378.77 ± 63.78 ab***	***60.00 ± 4.07 ab***
C	***165.57 ± 7.79 ab***	***419.22 ± 94.82 b***	***209.05 ± 5.78 b***	***45.32 ± 4.90 b***
HSI				
A	0.62 ± 0.08	1.25 ± 0.23	***1.71 ± 0.10 a***	1.76 ± 0.26
B	0.54 ± 0.09	1.30 ± 0.08	***1.34 ± 0.06 b***	1.44 ± 0.10
C	0.59 ± 0.06	1.15 ± 0.22	***1.04 ± 0.05 c***	1.60 ± 0.33
GSI (♀)				
A	0.57 ± 0.04	0.96 ± 0.40	0.66 ± 0.13	0.97 ± 0.19
B	0.73 ± 0.09	1.01 ± 0.21	0.53 ± 0.05	1.34 ± 0.21
C	0.60 ± 0.03	0.64 ± 0.15	0.55 ± 0.12	1.14 ± 0.29
CF				
A	***1.56 ± 0.06 a***	1.40 ± 0.05	***1.72 ± 0.02 a***	1.86 ± 0.04
B	***1.44 ± 0.09 ab***	1.49 ± 0.08	***1.67 ± 0.03 ab***	1.85 ± 0.03
C	***1.30 ± 0.03 b***	1.45 ± 0.03	***1.58 ± 0.03 b***	1.90 ± 0.05
Number of replicates				
A	10 (7♀ & 3♂)	8 (5♀ & 3♂)	8 (7♀ & 1♂)	13 (13♀ & 0♂)
B	10 (10♀ & 0♂)	19 (18♀ & 1♂)	13 (12♀ & 1♂)	20 (16♀ & 4♂)
C	14 (11♀ & 3♂)	9 (9♀ & 0♂)	10 (7♀ & 3♂)	19 (17♀ & 2♂)

3.2 Fatty acid profile of fish-feeds and faeces

SFA and MUFA were present in high percentages in both types of feeds (55–59 %). PUFA content was approximately 41 % in feed A and 45 % in feed B, where OA was the major fatty acid in feed A (19 %) and 16:0 and LA were the major fatty acids in feed

B (18 % and 15 % respectively). The LNA content was 0.27 % and 2 % and the DHA content was 12 % and 9 % in A and B feeds respectively (Supplementary material, Table S1).

The sample of faeces showed a high content of SFA (32 %) and MUFA (48 %), OA present in a 28 % and a PUFA percentage of 19% of the total lipid sample, LA being the major PUFA (9 %). Low values were obtained for ARA (0.34 %), LNA (0.32 %), EPA (2.76 %) and DHA (3.36 %).

3.3 Fatty acid profile of the different tissues

Significant differences in individual main fatty acids among the three distance treatments are shown in Tables 2, 3, 4 and 5 for *S. aurita*, *P. saltatrix*, *C. rhonchus* and *M. barbatus* respectively. In general, when significant differences occur, they follow the same trend in the four tissues studied in all species: the group F showed high levels of OA, total MUFA, LA, total n-6 content and LNA, and low levels of total SFA, ARA, EPA, DHA, total n-3 content and n-3/n-6 ratio, in comparison with group MD, group LD, or both. There were some exceptions, like EPA in brain of *C. rhonchus*, which percentage was higher in fish from group F than fish from group LD; total MUFA in brain and gonad of *M. barbatus*, which showed the lowest levels in fish from group F; and ARA in brain of *M. barbatus*, showing higher percentages in group F compared to MD and LD.

PERMANOVA pointed to significant differences of the complete fatty acid profile, generally between group F and group MD, or group F and group LD, while *M. barbatus* showed significant differences among the three distance treatments (Table 6). SIMPER analysis showed that the fatty acids with more influence on the dissimilarities among distance treatments were OA, LA and DHA (Supplementary material, Table S2). Samples in the MDS plot are identified by increasing distance to the fish farm (groups F, MD and LD respectively) (Figure 2), where samples showing similar fatty acid profiles are placed closer.

Table 2. Fatty acids, n-3/n-6 index, sum of LA and LNA (commercial feed consumption index) and total lipid percentages of flesh (data from Izquierdo-Gomez et al. 2015), brain, liver and gonad samples of *S. aurita* (mean values ± S.E.). Significant differences among distance treatments are represented in bold and italic.

	Flesh			Brain			Liver			Gonad		
	Group F	Group MD	Group LD	Group F	Group MD	Group LD	Group F	Group MD	Group LD	Group F	Group MD	Group LD
SFA[1]	***30.62 ± 0.87 a***	***34.31 ± 1.12 a***	***38.04 ± 0.99 b***	28.83 ± 1.17	25.14 ± 1.75	26.87 ± 1.22	***36.87 ± 1.25 a***	***51.41 ± 3.04 b***	***50.82 ± 1.77 b***	***31.65 ± 0.71 a***	***49.00 ± 2.49 b***	***55.72 ± 3.56 b***
OA	***22.08 ± 0.48 a***	***8.84 ± 2.41 b***	***4.97 ± 0.46 b***	34.97 ± 2.44	35.07 ± 1.33	35.34 ± 0.62	***27.11 ± 1.79 a***	***11.43 ± 2.42 b***	***9.51 ± 1.17 b***	***21.25 ± 0.65 a***	***10.51 ± 0.96 b***	***9.20 ± 0.42 b***
MUFA[2]	***32.04 ± 0.85 a***	***14.34 ± 3.45 b***	***8.88 ± 0.87 b***	47.94 ± 3.26	48.72 ± 1.53	50.26 ± 0.95	***35.72 ± 1.49 a***	***18.85 ± 3.06 b***	***15.95 ± 1.60 b***	***31.22 ± 1.03 a***	***19.60 ± 1.09 b***	***17.08 ± 0.55 b***
LA	***16.58 ± 1.60 a***	***5.52 ± 2.10 b***	***1.79 ± 0.14 b***	***2.79 ± 0.65 a***	***1.16 ± 0.45 ab***	***0.20 ± 0.04 b***	***8.23 ± 1.13 a***	***2.94 ± 0.89 b***	***1.81 ± 0.10 b***	***14.63 ± 1.37 a***	***2.92 ± 0.50 b***	***1.96 ± 0.13 b***
ARA	***0.62 ± 0.03 a***	***1.50 ± 0.17 b***	***1.64 ± 0.07 b***	2.59 ± 0.17	2.96 ± 0.13	2.62 ± 0.23	***0.87 ± 0.12 a***	***2.24 ± 0.30 b***	***2.63 ± 0.31 b***	***0.94 ± 0.11 a***	***2.65 ± 0.54 b***	***3.41 ± 0.59 b***
n-6 PUFA[3]	***18.93 ± 1.65 a***	***8.36 ± 1.99 b***	***4.33 ± 0.14 b***	***6.33 ± 0.67 a***	***5.36 ± 0.53 ab***	***4.27 ± 0.38 b***	***11.17 ± 1.28 a***	***6.14 ± 0.75 b***	***5.58 ± 0.40 b***	***17.24 ± 1.41 a***	***6.41 ± 0.80 b***	***6.48 ± 0.78 b***
LNA	***2.11 ± 0.09 a***	***1.78 ± 0.58 ab***	***0.80 ± 0.21 b***	0.41 ± 0.15	1.07 ± 0.64	0.69 ± 0.45	***0.92 ± 0.15 a***	***0.58 ± 0.12 ab***	***0.45 ± 0.05 b***	***1.90 ± 0.09 a***	***0.64 ± 0.11 b***	***0.38 ± 0.04 c***
EPA	5.82 ± 0.50	5.41 ± 0.41	5.13 ± 0.31	2.31 ± 0.47	2.25 ± 0.28	1.77 ± 0.27	***4.17 ± 0.49 a***	***5.28 ± 0.38 ab***	***6.04 ± 0.51 b***	6.53 ± 0.47	6.15 ± 0.93	4.69 ± 0.67
DHA	***8.58 ± 0.83 a***	***34.49 ± 5.47 b***	***41.75 ± 1.82 b***	13.35 ± 1.56	16.91 ± 1.31	15.70 ± 1.44	***9.44 ± 0.94 a***	***16.50 ± 1.56 b***	***19.73 ± 1.65 b***	9.50 ± 1.15	16.80 ± 2.65	14.42 ± 2.40
n-3 PUFA[4]	***18.41 ± 1.24 a***	***43.00 ± 5.31 b***	***48.75 ± 1.66 b***	16.90 ± 2.12	20.77 ± 1.75	18.60 ± 1.38	***16.24 ± 1.64 a***	***23.60 ± 1.67 b***	***27.64 ± 2.18 b***	19.89 ± 1.48	24.99 ± 3.39	20.73 ± 3.19
Total PUFA	***37.34 ± 1.57 a***	***51.36 ± 3.69 b***	***53.08 ± 1.68 b***	23.23 ± 2.63	26.13 ± 2.00	22.87 ± 1.49	27.41 ± 2.54	29.74 ± 1.55	33.23 ± 2.50	37.13 ± 1.63	31.39 ± 3.20	27.20 ± 3.82
n-3/n-6	***1.09 ± 0.18 a***	***8.03 ± 1.68 b***	***11.40 ± 0.54 b***	2.70 ± 0.17	4.06 ± 0.39	4.40 ± 0.36	***1.60 ± 0.23 a***	***4.12 ± 0.45 b***	***5.03 ± 0.26 b***	***1.25 ± 0.15 a***	***4.31 ± 0.77 b***	***3.11 ± 0.38 b***
∑(LA; LNA)	***18.69 ± 1.68 a***	***7.30 ± 2.17 b***	***2.58 ± 0.18 b***	3.20 ± 0.70	2.22 ± 0.78	1.16 ± 0.45	***9.15 ± 1.27 a***	***3.51 ± 0.99 b***	***2.26 ± 0.13 b***	***16.53 ± 1.45 a***	***3.56 ± 0.50 b***	***2.35 ± 0.14 b***
TL	***9.90 ± 1.55 a***	***2.70 ± 1.38 b***	***0.99 ± 0.14 b***	13.89 ± 0.65	15.18 ± 0.69	13.63 ± 0.72	***14.52 ± 2.34 a***	***4.51 ± 1.03 b***	***5.96 ± 1.23 b***	***11.25 ± 2.69 a***	***2.34 ± 0.82 b***	***2.66 ± 0.64 b***

1: includes 14:0, 15:0, 16:0, 18:0, 20:0, and 22:0
2: includes 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9
3: includes 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6 and 22:4n-6
4: includes 18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3

Table 3. Fatty acids, n-3/n-6 index, sum of LA and LNA (commercial feed consumption index) and total lipid percentages of flesh (data from Izquierdo-Gomez et al. 2015), brain, liver and gonad samples of *P. saltatrix* (mean values ± S.E.). Significant differences among distance treatments are represented in bold and italic.

	Flesh			Brain			Liver			Gonad		
	Group F	Group MD	Group LD	Group F	Group MD	Group LD	Group F	Group MD	Group LD	Group F	Group MD	Group LD
SFA[1]	***31.58 ± 0.76 a***	***32.98 ± 0.62 a***	***36.34 ± 1.19 b***	***32.06 ± 0.85 a***	***35.12 ± 0.87 ab***	***36.41 ± 0.92 b***	35.62 ± 1.42	38.81 ± 1.65	40.20 ± 1.88	35.81 ± 1.74	44.13 ± 2.50	42.26 ± 2.49
OA	19.27 ± 1.67	18.93 ± 1.93	19.29 ± 1.87	***30.26 ± 1.33 a***	***27.67 ± 0.74 ab***	***25.69 ± 0.44 b***	26.62 ± 1.58	24.43 ± 2.40	27.79 ± 1.69	18.12 ± 0.71	15.52 ± 0.87	15.22 ± 1.01
MUFA[2]	27.77 ± 2.41	26.52 ± 2.48	27.59 ± 2.62	***41.73 ± 1.52 a***	***37.48 ± 0.85 a***	***34.92 ± 0.25 b***	38.25 ± 1.44	35.42 ± 2.87	40.13 ± 1.52	27.49 ± 1.00	24.49 ± 1.27	23.66 ± 1.55
LA	***7.25 ± 1.87 a***	***3.29 ± 1.14 ab***	***0.83 ± 0.12 b***	***3.38 ± 1.17 a***	***1.52 ± 0.33 a***	***0.49 ± 0.12 b***	***6.01 ± 1.29 a***	***2.92 ± 0.92 ab***	***0.97 ± 0.17 b***	***6.01 ± 1.38 a***	***2.61 ± 0.58 ab***	***1.70 ± 0.25 b***
ARA	2.46 ± 0.43	2.75 ± 0.21	3.04 ± 0.23	2.24 ± 0.16	2.47 ± 0.14	2.88 ± 0.23	1.73 ± 0.39	2.35 ± 0.39	2.47 ± 0.40	5.18 ± 0.91	5.53 ± 0.66	7.54 ± 1.06
n-6 PUFA[3]	***11.15 ± 1.70 a***	***7.21 ± 1.09 ab***	***5.00 ± 0.17 b***	6.83 ± 1.24	4.77 ± 0.38	4.22 ± 0.28	***9.81 ± 1.32 a***	***6.65 ± 1.02 ab***	***4.63 ± 0.81 b***	12.70 ± 1.06	9.24 ± 0.89	10.58 ± 1.17
LNA	***1.35 ± 0.19 a***	***0.72 ± 0.13 b***	***0.73 ± 0.22 ab***	0.38 ± 0.15	0.15 ± 0.04	0.13 ± 0.10	0.62 ± 0.12	0.35 ± 0.08	0.48 ± 0.18	***0.65 ± 0.14 a***	***0.24 ± 0.06 b***	***0.36 ± 0.08 ab***
EPA	4.74 ± 0.35	4.03 ± 0.21	4.09 ± 0.27	3.33 ± 0.13	3.01 ± 0.15	3.14 ± 0.23	2.35 ± 0.12	2.19 ± 0.19	2.04 ± 0.34	4.46 ± 0.34	3.54 ± 0.35	4.04 ± 0.48
DHA	20.98 ± 2.86	26.89 ± 2.75	24.17 ± 2.68	***14.45 ± 0.91 a***	***18.40 ± 0.92 b***	***19.93 ± 0.75 b***	10.95 ± 1.14	14.85 ± 1.90	10.51 ± 1.24	16.73 ± 2.11	16.81 ± 2.24	16.99 ± 2.33
n-3 PUFA[4]	29.50 ± 2.85	33.29 ± 2.86	31.08 ± 2.75	***19.39 ± 0.68 a***	***22.63 ± 0.98 b***	***24.44 ± 0.73 b***	16.32 ± 0.97	19.13 ± 2.08	15.04 ± 1.91	24.00 ± 2.37	22.14 ± 2.63	23.49 ± 2.85
Total PUFA	40.65 ± 2.32	40.49 ± 2.77	36.08 ± 2.83	26.21 ± 1.40	27.40 ± 1.05	28.66 ± 0.87	26.13 ± 1.16	25.78 ± 2.49	19.67 ± 2.64	36.71 ± 2.24	31.38 ± 3.19	34.07 ± 3.74
n-3/n-6	3.39 ± 0.80	5.93 ± 0.72	6.21 ± 0.48	***3.46 ± 0.53 a***	***5.25 ± 0.41 b***	***5.91 ± 0.31 b***	2.09 ± 0.51	3.61 ± 0.44	3.50 ± 0.29	2.02 ± 0.32	2.26 ± 0.28	2.27 ± 0.21
∑(LA; LNA)	***8.60 ± 1.96 a***	***4.01 ± 1.23 b***	***1.56 ± 0.21 b***	***3.76 ± 1.31 a***	***1.67 ± 0.37 a***	***0.62 ± 0.22 b***	***6.63 ± 1.40 a***	***3.27 ± 0.99 ab***	***1.45 ± 0.35 b***	***6.66 ± 1.51 a***	***3.22 ± 0.63 b***	***2.11 ± 0.29 b***
TL	1.97 ± 0.64	2.06 ± 0.62	1.69 ± 0.48	12.10 ± 1.34	9.54 ± 1.74	9.16 ± 0.45	10.19 ± 2.12	7.84 ± 1.07	6.61 ± 0.57	3.21 ± 0.26	2.84 ± 0.51	1.86 ± 0.14

1: includes 14:0, 15:0, 16:0, 18:0, 20:0, and 22:0
2: includes 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9
3: includes 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6 and 22:4n-6
4: includes 18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3

Table 4. Fatty acids, n-3/n-6 index, sum of LA and LNA (commercial feed consumption index) and total lipid percentages of flesh (data from Izquierdo-Gomez et al. 2015), brain, liver and gonad samples of *C. rhonchus* (mean values ± S.E.). Significant differences among distance treatments are represented in bold and italic.

	Flesh			Brain			Liver			Gonad		
	Group F	Group MD	Group LD	Group F	Group MD	Group LD	Group F	Group MD	Group LD	Group F	Group MD	Group LD
SFA[1]	***29.01 ± 0.75 a***	***35.84 ± 0.99 b***	***35.04 ± 1.13 b***	***28.11 ± 1.00 a***	***30.76 ± 0.83 ab***	***33.30 ± 1.46 b***	***32.18 ± 0.78 a***	***39.88 ± 1.31 b***	***43.73 ± 1.56 b***	***34.77 ± 1.65 a***	***43.45 ± 1.73 b***	***51.17 ± 3.32 b***
OA	***23.46 ± 0.45 a***	***16.00 ± 1.65 b***	***11.96 ± 0.89 b***	30.40 ± 0.56	27.67 ± 0.47	28.93 ± 1.07	27.64 ± 0.92	26.57 ± 2.13	26.09 ± 2.21	***22.69 ± 0.69 a***	***19.23 ± 0.97 b***	***15.71 ± 0.68 c***
MUFA[2]	***35.03 ± 0.77 a***	***23.25 ± 2.28 b***	***19.55 ± 1.69 b***	43.25 ± 1.04	40.03 ± 0.55	41.25 ± 1.42	39.99 ± 0.97	37.33 ± 2.57	37.71 ± 2.80	***35.10 ± 1.09 a***	***29.13 ± 1.15 b***	***25.69 ± 1.11 b***
LA	***11.92 ± 0.89 a***	***2.38 ± 0.75 b***	***1.24 ± 0.10 b***	***3.41 ± 0.55 a***	***0.63 ± 0.11 b***	***0.55 ± 0.07 b***	***10.63 ± 0.83 a***	***2.33 ± 0.74 b***	***0.79 ± 0.15 b***	***10.27 ± 0.73 a***	***3.02 ± 0.82 b***	***1.61 ± 0.39 b***
ARA	***0.89 ± 0.11 a***	***2.46 ± 0.27 b***	***2.92 ± 0.27 b***	***2.24 ± 0.18 a***	***2.92 ± 0.16 b***	***2.46 ± 0.13 ab***	***0.80 ± 0.07 a***	***2.17 ± 0.33 b***	***1.96 ± 0.30 b***	***0.92 ± 0.09 a***	***2.93 ± 0.48 b***	***3.33 ± 0.71 b***
n-6 PUFA[3]	***14.34 ± 0.83 a***	***5.91 ± 0.74 b***	***5.16 ± 0.27 b***	***6.60 ± 0.48 a***	***4.41 ± 0.16 b***	***3.77 ± 0.07 c***	***12.91 ± 0.89 a***	***5.65 ± 0.87 b***	***3.62 ± 0.50 b***	***12.51 ± 0.77 a***	***7.23 ± 0.91 b***	***6.11 ± 0.79 b***
LNA	***1.58 ± 0.12 a***	***0.43 ± 0.11 b***	***0.33 ± 0.06 b***	0.13 ± 0.05	0.00 ± 0.00	0.06 ± 0.04	***1.20 ± 0.08 a***	***0.32 ± 0.08 b***	***0.09 ± 0.04 c***	***1.49 ± 0.15 a***	***0.53 ± 0.11 b***	***0.25 ± 0.05 b***
EPA	5.84 ± 0.37	4.43 ± 0.32	6.21 ± 0.81	***3.71 ± 0.24 a***	***3.20 ± 0.21 ab***	***2.91 ± 0.16 b***	4.16 ± 0.32	3.15 ± 0.35	2.93 ± 0.31	5.80 ± 0.97	4.35 ± 0.41	3.73 ± 0.48
DHA	***11.29 ± 1.37 a***	***28.41 ± 2.97 b***	***31.54 ± 3.45 b***	16.72 ± 0.78	20.23 ± 0.66	17.40 ± 1.08	***7.03 ± 0.76 a***	***11.99 ± 1.53 b***	***10.37 ± 1.27 ab***	***7.90 ± 0.83 a***	***13.48 ± 0.96 b***	***11.66 ± 1.88 ab***
n-3 PUFA[4]	***21.62 ± 1.11 a***	***35.00 ± 2.76 b***	***40.25 ± 2.64 b***	22.03 ± 1.01	24.80 ± 0.68	21.69 ± 1.16	15.03 ± 0.95	17.14 ± 1.82	14.94 ± 1.67	17.62 ± 1.98	20.18 ± 1.10	17.03 ± 2.43
Total PUFA	35.95 ± 0.87	40.91 ± 2.83	45.41 ± 2.77	***28.63 ± 1.12 ab***	***29.20 ± 0.77 a***	***25.46 ± 1.19 b***	***27.92 ± 1.44 a***	***22.79 ± 2.29 ab***	***18.56 ± 2.13 b***	30.13 ± 2.40	27.42 ± 1.74	23.14 ± 3.04
n-3/n-6	***1.59 ± 0.21 a***	***6.61 ± 0.63 b***	***7.85 ± 0.51 b***	***3.40 ± 0.27 a***	***5.66 ± 0.19 b***	***5.75 ± 0.29 b***	***1.19 ± 0.09 a***	***3.64 ± 0.45 b***	***4.26 ± 0.25 b***	***1.42 ± 0.16 a***	***3.25 ± 0.40 b***	***2.81 ± 0.37 b***
∑(LA; LNA)	***13.50 ± 0.99 a***	***2.81 ± 0.85 b***	***1.57 ± 0.14 b***	***3.54 ± 0.59 a***	***0.63 ± 0.11 b***	***0.60 ± 0.10 b***	***11.83 ± 0.91 a***	***2.66 ± 0.81 b***	***0.88 ± 0.18 b***	***11.76 ± 0.85 a***	***3.55 ± 0.92 b***	***1.86 ± 0.43 b***
TL	***6.35 ± 1.21 a***	***2.26 ± 0.41 b***	***1.98 ± 0.66 b***	12.85 ± 0.94	11.81 ± 0.49	11.00 ± 0.33	***17.01 ± 1.45 a***	***9.52 ± 1.59 b***	***9.79 ± 1.56 ab***	***11.68 ± 1.80 a***	***4.83 ± 1.06 b***	***2.88 ± 0.65 b***

1: includes 14:0, 15:0, 16:0, 18:0, 20:0, and 22:0
2: includes 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9
3: includes 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6 and 22:4n-6
4: includes 18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3

Table 5. Fatty acids, n-3/n-6 index, sum of LA and LNA (commercial feed consumption index) and total lipid percentages of flesh (data from Izquierdo-Gomez et al. 2015), brain, liver and gonad samples of *M. barbatus* (mean values ± S.E.). Significant differences among distance treatments are represented in bold and italic.

	Flesh			Brain			Liver			Gonad		
	Group F	Group MD	Group LD	Group F	Group MD	Group LD	Group F	Group MD	Group LD	Group F	Group MD	Group LD
SFA[1]	36.19 ± 0.80	36.10 ± 0.80	36.38 ± 0.62	32.27 ± 0.86	31.98 ± 0.95	34.98 ± 1.02	***36.66 ± 0.84 a***	***37.27 ± 1.62 a***	***44.42 ± 2.09 b***	34.17 ± 3.32	33.29 ± 1.42	41.31 ± 3.05
OA	***17.12 ± 0.87 a***	***14.51 ± 1.03 a***	***8.99 ± 0.83 b***	21.50 ± 0.63	24.22 ± 0.64	23.06 ± 1.02	23.53 ± 1.31	19.90 ± 1.46	17.34 ± 1.65	8.53 ± 0.55	8.73 ± 0.77	11.19 ± 1.16
MUFA[2]	***31.23 ± 1.34 a***	***29.95 ± 1.49 a***	***18.39 ± 1.57 b***	***32.69 ± 0.87 a***	***36.96 ± 0.67 b***	***34.34 ± 0.89 ab***	***42.02 ± 1.28 a***	***37.69 ± 2.11 a***	***29.60 ± 2.14 b***	***15.81 ± 1.08 a***	***19.87 ± 0.94 ab***	***21.27 ± 1.83 b***
LA	***4.71 ± 0.81 a***	***1.14 ± 0.09 b***	***0.96 ± 0.04 b***	***1.12 ± 0.18 a***	***0.31 ± 0.07 b***	***0.59 ± 0.08 a***	***2.89 ± 0.43 a***	***0.67 ± 0.08 b***	***0.64 ± 0.05 b***	***2.74 ± 0.28 a***	***0.78 ± 0.11 b***	***1.05 ± 0.10 b***
ARA	***4.04 ± 0.30 a***	***3.62 ± 0.49 a***	***6.52 ± 0.66 b***	***5.36 ± 0.25 a***	***4.25 ± 0.18 b***	***3.89 ± 0.17 b***	2.56 ± 0.23	2.81 ± 0.43	3.71 ± 0.47	10.00 ± 0.75	6.88 ± 0.68	6.82 ± 1.11
n-6 PUFA[3]	***12.17 ± 0.91 a***	***6.76 ± 0.63 b***	***9.04 ± 0.67 c***	***7.57 ± 0.48 a***	***5.24 ± 0.22 b***	***5.65 ± 0.31 b***	***7.45 ± 0.69 a***	***4.46 ± 0.55 b***	***5.56 ± 0.60 ab***	***15.51 ± 1.10 a***	***9.67 ± 0.58 b***	***9.44 ± 1.18 b***
LNA	***0.86 ± 0.16 a***	***0.42 ± 0.06 a***	***0.24 ± 0.02 b***	0.34 ± 0.22	0.04 ± 0.02	0.14 ± 0.06	***0.35 ± 0.07 a***	***0.15 ± 0.03 b***	***0.11 ± 0.05 b***	0.20 ± 0.05	0.28 ± 0.13	0.30 ± 0.13
EPA	***7.89 ± 0.38 a***	***9.89 ± 0.47 b***	***11.57 ± 0.36 c***	3.71 ± 0.27	4.48 ± 0.35	4.69 ± 0.37	5.10 ± 0.43	5.64 ± 0.50	6.13 ± 0.88	9.76 ± 0.63	10.38 ± 0.49	8.49 ± 1.24
DHA	***9.00 ± 1.33 a***	***14.40 ± 0.96 b***	***21.76 ± 1.51 c***	22.20 ± 1.06	20.15 ± 0.78	18.76 ± 1.48	6.53 ± 0.67	13.23 ± 2.05	12.19 ± 1.73	21.33 ± 1.41	24.26 ± 2.00	16.83 ± 2.33
n-3 PUFA[4]	***20.42 ± 1.60 a***	***27.19 ± 1.24 b***	***36.19 ± 1.70 c***	27.48 ± 0.96	25.83 ± 0.99	25.03 ± 1.43	13.87 ± 1.15	20.57 ± 2.63	20.42 ± 2.75	34.52 ± 1.80	37.13 ± 2.28	27.98 ± 3.63
Total PUFA	***32.59 ± 1.74 a***	***33.95 ± 1.35 a***	***45.23 ± 1.94 b***	35.05 ± 1.26	31.07 ± 1.13	30.68 ± 1.27	21.32 ± 1.48	25.04 ± 2.97	25.98 ± 3.15	50.02 ± 2.69	46.80 ± 2.23	37.42 ± 4.27
n-3/n-6	***1.78 ± 0.22 a***	***4.50 ± 0.41 b***	***4.43 ± 0.41 b***	***3.75 ± 0.20 a***	***4.99 ± 0.18 b***	***4.81 ± 0.47 ab***	***1.94 ± 0.17 a***	***4.91 ± 0.46 b***	***3.75 ± 0.46 b***	***2.28 ± 0.13 a***	***4.03 ± 0.31 b***	***3.16 ± 0.45 ab***
∑(LA; LNA)	***5.57 ± 0.88 a***	***1.56 ± 0.10 b***	***1.20 ± 0.05 c***	***1.46 ± 0.25 a***	***0.34 ± 0.08 b***	***0.74 ± 0.13 ab***	***3.24 ± 0.50 a***	***0.82 ± 0.10 b***	***0.75 ± 0.08 b***	***2.94 ± 0.31 a***	***1.06 ± 0.18 b***	***1.35 ± 0.21 b***
TL	***4.13 ± 0.76 a***	***3.44 ± 0.48 a***	***0.92 ± 0.13 b***	10.00 ± 0.27	10.06 ± 0.39	9.24 ± 0.33	***11.80 ± 0.96 a***	***7.79 ± 0.84 b***	***6.02 ± 0.81 b***	2.24 ± 0.60	2.89 ± 0.43	4.74 ± 1.01

1: includes 14:0, 15:0, 16:0, 18:0, 20:0, and 22:0
2: includes 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9
3: includes 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6 and 22:4n-6
4: includes 18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3

Table 6. PERMANOVA results for each species and the four tissues studied. Significant P-values are shown in both bold and italic.

		Source	df	SS	MS	Pseudo-F	P(perm)	F - MD	F - LD	MD - LD
S. aurita	Flesh	*Fatty acid profile*	2	3638.9	1819.4	26.41	***0.0002***	***0.0002***	***0.0002***	0.054
		Res	30	2066.8	68,893					
		Total	32	5705.7						
	Brain	*Fatty acid profile*	2	659.6	329.8	17,622	0.0872	-	-	-
		Res	30	5614.6	187.15					
		Total	32	6274.2						
	Liver	*Fatty acid profile*	2	2139.6	1069.8	14,395	***0.0002***	***0.0004***	***0.0002***	0.4822
		Res	27	2006.6	74,318					
		Total	29	4146.2						
	Gonad	*Fatty acid profile*	2	2866.3	1433.1	18,809	***0.0002***	***0.0002***	***0.0002***	0.1306
		Res	28	2133.5	76,196					
		Total	30	4999.7						
P. saltatrix	Flesh	*Fatty acid profile*	2	382.53	191.27	18,369	0.09	-	-	-
		Res	32	3332	104.12					
		Total	34	3714.5						
	Brain	*Fatty acid profile*	2	278.77	139.39	3,125	***0.0122***	0.0636	***0.015***	0.1128
		Res	31	1382.7	44,603					
		Total	33	1661.5						
	Liver	*Fatty acid profile*	2	352.18	176.09	19,112	0.084	-	-	-
		Res	33	3040.4	92,134					
		Total	35	3392.6						
	Gonad	*Fatty acid profile*	2	447.27	223.63	20,433	0.0582	-	-	-
		Res	33	3611.8	109.45					
		Total	35	4059						
C. rhonchus	Flesh	*Fatty acid profile*	2	1638.6	819.31	15,575	***0.0002***	***0.0002***	***0.0002***	0.4288
		Res	23	1209.9	52,604					
		Total	25	2848.5						
	Brain	*Fatty acid profile*	2	276.61	138.31	36,121	***0.0002***	***0.0006***	***0.0044***	0.1158
		Res	25	957.25	38.29					
		Total	27	1233.9						
	Liver	*Fatty acid profile*	2	1172.1	586.07	10,183	***0.0002***	***0.0002***	***0.0002***	0.2414
		Res	27	1553.9	57,553					
		Total	29	2726.1						
	Gonad	*Fatty acid profile*	2	890.31	445.16	48,574	***0.0022***	***0.0002***	***0.0292***	0.0676
		Res	34	3115.9	91,645					
		Total	36	4006.3						
M. barbatus	Flesh	*Fatty acid profile*	2	1565.2	782.6	16,581	***0.0002***	***0.0002***	***0.0002***	***0.0002***
		Res	42	1982.4	47.2					
		Total	44	3547.6						
	Brain	*Fatty acid profile*	2	404.14	202.07	35,278	***0.0268***	***0.0032***	***0.003***	***0.0342***
		Res	45	2577.6	57.28					
		Total	47	2981.8						
	Liver	*Fatty acid profile*	2	1016.5	508.23	54,061	***0.0002***	***0.004***	***0.0002***	***0.0114***
		Res	40	3760.4	94.01					
		Total	42	4776.9						
	Gonad	*Fatty acid profile*	2	1068.1	534.06	43,085	***0.0006***	***0.0002***	***0.0068***	***0.0166***
		Res	36	4462.4	123.96					
		Total	38	5530.5						

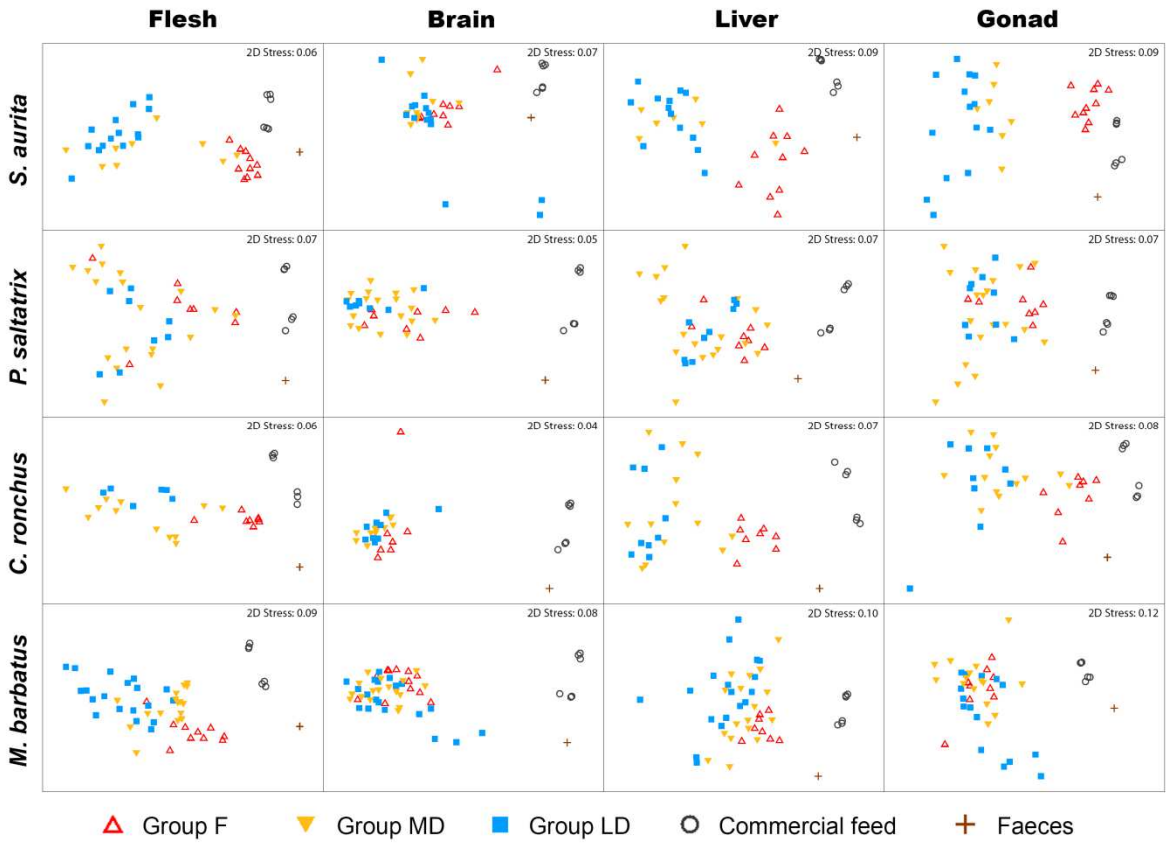

Figure 2. MDS plots of the fatty acid percentages from the different tissues studied in each species. Data from both types of fish-feed and faeces are also included, in order to check aquaculture waste influence in wild fish fatty acid profiles.

3.4 Gonad histology

Two species were selected for gonad study according to their gonad fatty acid content: *M. barbatus*, which showed minimal variations in gonad fatty acids among distance treatments; and *S. aurita*, which showed a high number of significant differences among distance treatments in fatty acids. The histological and morphometrical analysis of the ovaries of *M. barbatus* and *S. aurita* revealed the presence of oocytes in different maturation stages, which indicates that these species present an asynchronous ovary. The usual maturation stages of teleost were observed: oogonia, oocyte in chromatin nucleolus stage, oocyte in perinucleolar stage, oocyte in early vitellogenic stage, and oocyte in late vitellogenic stage, as well as mature oocytes (Figure 3). *M. barbatus* oocytes in late vitellogenic stage were only found occasionally in fish from group F, and *S. aurita* late vitellogenic oocytes and mature oocytes were also only found in group F.

The percentage of oocytes which have started the oogenesis (oocytes in early vitellogenic stage) was significantly higher in *S. aurita* from group F, and a similar tendency was found in *M. barbatus* from group F. The oocytes in perinuclear stage were significantly lower, in gonads of both species captured close to the fish farm (group F) compared with control fish (group LD) (Figure 4A and 5A). There were no volume differences at any stages of development (Figure 4B and 5B) for both species. The studied histological sections did not show abnormalities, degradation or malformations in the gonads.

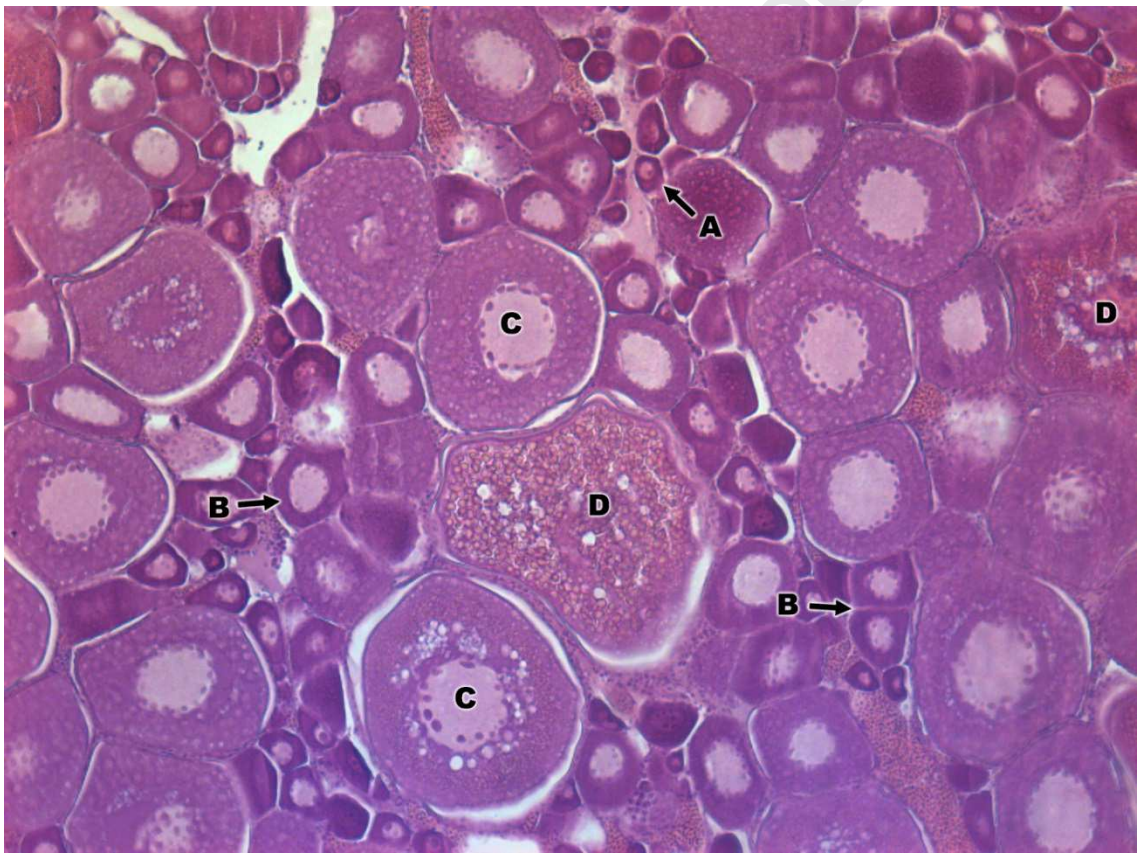

Figure 3. Haematoxylin-eosin light micrograph of ovary section in *M. barbatus* (100x), showing different oocyte development stages.

A- Oocyte in chromatin nucleolus stage.
B- Oocyte in perinuclear stage.
C- Oocyte in early vitellogenic stage.
D- Oocyte in late vitellogenic stage.

4. Discussion

This work demonstrates, by using fatty acids as biomarkers of waste feed consumption, that farm-associated wild fish have an altered fatty acid profile in several tissues, and this effect attenuates with increasing distance to the aquaculture facilities. Aquaculture sites attract and concentrate large quantities of wild fish from contiguous areas (Dempster et al. 2009; Callier et al. 2017), being the main attracting factor the availability of high energy feed in form of lost pellets that directly feeds the aggregated wild fish (Tuya et al. 2006; Fernandez-Jover et al. 2008). Predators are also attracted to fish farms, where some species modify their feeding behaviour consuming lost pellets, while other species are attracted by the high density of potential prey in the area, as it is the case of *P. saltatrix* (Fernandez-Jover et al. 2008; Sanchez-Jerez et al. 2008; Izquierdo-Gomez et al. 2015). The levels of particulate organic matter also increase in the vicinity of sea cages (Sara et al. 2004; White et al. 2017), and it is used as a trophic resource by planktivorous species like *S. aurita* (Sanchez-Jerez et al. 2011) as well as macroinvertebrate species (Gonzalez-Silvera et al. 2015).

The input of organic matter from fish farms to the marine environment is mainly due to lost pellets and faeces. Previous research showed that not only pellets but also faeces could be used as a trophic resource by aggregated fauna (Johansson et al. 1998; Madin et al. 2009; Gonzalez-Silvera et al. 2015), so even with the lack of enough number of replicates, faeces data were used in this work together with aquafeeds data to perform the MDS plots. MUFA represented the major component of the faeces total lipids (48.01 %) while PUFA levels were low (19.65 %). These results agree with those found by Van Biesen and Parrish (2005), being the high proportion of MUFA due to the former's poor digestibility by fish.

Benthic (*M. barbatus*) and pelagic (*S. aurita* and *C. rhonchus*) fish species showed a similar response to the fish farm influence in terms of individual fatty acid percentages. The fatty acid profile of flesh and liver reflected the fatty acid composition of the commercial diets, showing higher percentages of OA and LA and lower percentages of DHA in group F compared to groups MD and LD. OA and LA were the major fatty acids in fish-feeds and faeces, and were also pointed by SIMPER as the fatty acids with more influence on the differences observed in this work. Therefore, they are good candidates as tracers of aquaculture waste consumption in the studied area, especially LA which was the only fatty acid present at significant higher levels in group F

compared with fish captured at longer distances from the fish farm, in the four analysed tissues from the four studied species. These results about the suitability of the fatty acid listed above confirm those previously found by other authors in flesh of farm-associated wild fish species (Fernandez-Jover et al. 2011) and by our research group in golden mullet under laboratory conditions (Gonzalez-Silvera et al. 2016). Significant differences in individual fatty acid percentages were found between fish captured in the vicinity of the fish farm and fish captured by trawlers at long distance from the fish farm, which are supposed to have had limited or null contact with aquaculture facilities.

There are several studies, which similarly to our results, highlight the differences found in flesh and liver fatty acids between wild and reared fish. Alasalvar et al. (2002) and Fuentes et al. (2010) reported differences in flesh fatty acids between wild and reared sea bass (*Dicentrarchus labrax*), the last showing higher levels of OA and LA and lower levels of ARA, EPA and DHA. Arechavala-Lopez et al. (2011) found differences in flesh fatty acid profile of bogue (*Boops boops*) aggregated near sea-cages and bogue caught by fishermen using trammel nets in comparison with individuals caught by trawlers at long distance. Bogue specimens captured by trammel nets presented a similar fatty acid profile to fish farm associated bogue, and both showed presence of feed pellets in their gut content, while bogue captured by trawlers at long distance from farms consumed natural trophic items. Changes in fatty acids followed the same trend than in our results, where bogue captured around the fish farm presented higher levels of LA, LNA and OA and lower levels of ARA and DHA, although EPA levels where higher in bogue captured at farms and by trammel nets. Ramírez et al. (2013) obtained similar results, in bogue captured within a radius of 3 km from a fish farm. Fernandez-Jover et al. (2007) found that wild horse mackerel (*Trachurus mediterraneus*) were aggregated around fish farms throughout the year, and their flesh showed a different fatty acid profile than individuals captured at control sites. High percentages of LA, LNA and OA, and lower percentages of ARA and DHA were found in aggregated specimens, compared to control counterparts. Similar results were found in other marine fish species, like gilthead sea bream (*Sparus aurata*) (Grigorakis et al. 2002), aggregated golden mullet (*Liza aurata*) and saddled sea bream (*Oblada melanura*) (Fernandez-Jover et al. 2009), and also in Atlantic cod (*Gadus morhua*) and saithe (*Pollachius virens*) (Fernandez-Jover et al. 2011) which was also reported to have

differences in fillet taste (Skog et al. 2003). Few studies reported the opposite result, as the work of Rueda et al. (2001), who found higher levels of LA, EPA and DHA in flesh of reared sharpsnout sea bream (*Diplodus puntazzo*), in comparison with flesh from wild specimens; and the work of Mnari et al. (2007), who also found higher values of DHA and EPA in reared compared to wild *S. aurata*. In any case, LA levels in those studies were higher in cultured fish.

Irrespectively of their habitat and feeding regimes, the four studied species showed differences in their fatty acid profile, where fish captured around the sea cages had a fatty acid profile more similar to fish-feed and faeces, as showed by the MDS plots. The predator *P. saltatrix* has been previously reported to feed on species aggregated near fish farms, and even on reared fish (Sanchez-Jerez et al. 2008; Arechavala-Lopez et al. 2014) instead of lost pellets. The fatty acids analysis of brain, liver and gonads of this species reasserted the results previously published by Izquierdo-Gomez et al. (2015) based only in flesh fatty acid composition. The accumulation of LA in the four studied tissues of *P. saltatrix* demonstrates the transfer of fatty acids of vegetable origin through different trophic levels. Potential preys aggregated near fish farms, as *S. aurita*, may have been consuming lost pellets and therefore, present a modified fatty acid profile with high inclusion of LA. *P. saltatrix* prey on those wild fish in the vicinity of fish farms, and LA is assimilated and accumulated in flesh, liver, brain and gonads, the latter also showing higher levels of LNA compared to controls. The obtained results demonstrate the capacity of fatty acids of vegetable origin to accumulate along the food chain, and the consequences for human consumption would be a higher intake of short chain n-6 fatty acids at the expense of essential long-chain polyunsaturated fatty acids from the n-3 family (EPA and DHA) and n-6 (ARA). Fish consumption is the main source of EPA and DHA for human nutrition, and depletion of these fatty acids in aggregated wild fish may affect the recommendations of human fish consumption to achieve an optimal health status.

We have demonstrated that the impact of aquaculture waste consumption differs from one species to another, probably depending on their preferred habitat and feeding behaviour, and the effect on fatty acid percentages attenuates with increasing distances to the fish farms. Migration to remote areas may revert these changes, but our group has already demonstrated that only 2 weeks of commercial feed consumption can be enough to modify the fatty acid profile of flesh in *Liza aurata*, together with a modulation of the

immune responses (Gonzalez-Silvera et al. 2017). We also demonstrated in other study that a shift from a commercial diet to a natural diet consumption for a minimum of two months is not a guaranty of recovering a natural fatty acid profile, as LA levels will remain at high percentages (Gonzalez-Silvera et al. 2016). Those results lead us to hypothesize that the fatty acid profile will remain modified, with high percentages of LA, even in the case of migration to areas none influenced by aquaculture.

Due to aquafeeds composition, wild fish aggregated near fish farms use to show higher fat content and hepatosomatic index than controls (Dempster et al. 2009; Arechavala-Lopez et al. 2011), which, together with a decrease in the n-3/n-6 ratio, represent an important physiological change. In any case, it has been reported that increases in the fat content and condition factor result in an increase in fecundity and hatching success, although egg quality and larvae survival rate could be affected by the low levels of DHA and n-3 fatty acids in general, provided by artificial diets (Fernández-Palacios et al. 1995; Adams 1999; Almansa et al. 1999; Izquierdo et al. 2001; White et al. 2016). During the vitellogenesis, fish need high fat and protein feeds in order to produce vitellogenin, a phospholipoprotein precursor of lipovitellin and phosphovitellin which are stored in the oocytes in the form of vitello. The amount and quality of the vitello are key factors for a successful reproduction, as the vitello is the unique food source for the embryo and the first larvae stages (Alvarez- Lajonchère 2006).

Hauville et al. (2015) suggested that, despite the high fat levels found in reared common snook (*Centropomus undecimalis*), cholesterol and ARA levels were lower than their wild counterparts, which would have a negative effect in reproductive success and gametogenesis, as ARA has been reported to have an important role in gonadal maturation (Pérez et al. 2007; Norberg et al. 2017). Nevertheless, Cejas et al. (2003) reported no differences in gonad total lipids between reared and wild white sea bream (*Diplodus sargus*), and unlike our results in *M. barbatus*, but similarly to the results on the other three species, cultured fish showed lower gonad ARA levels than wild fish.

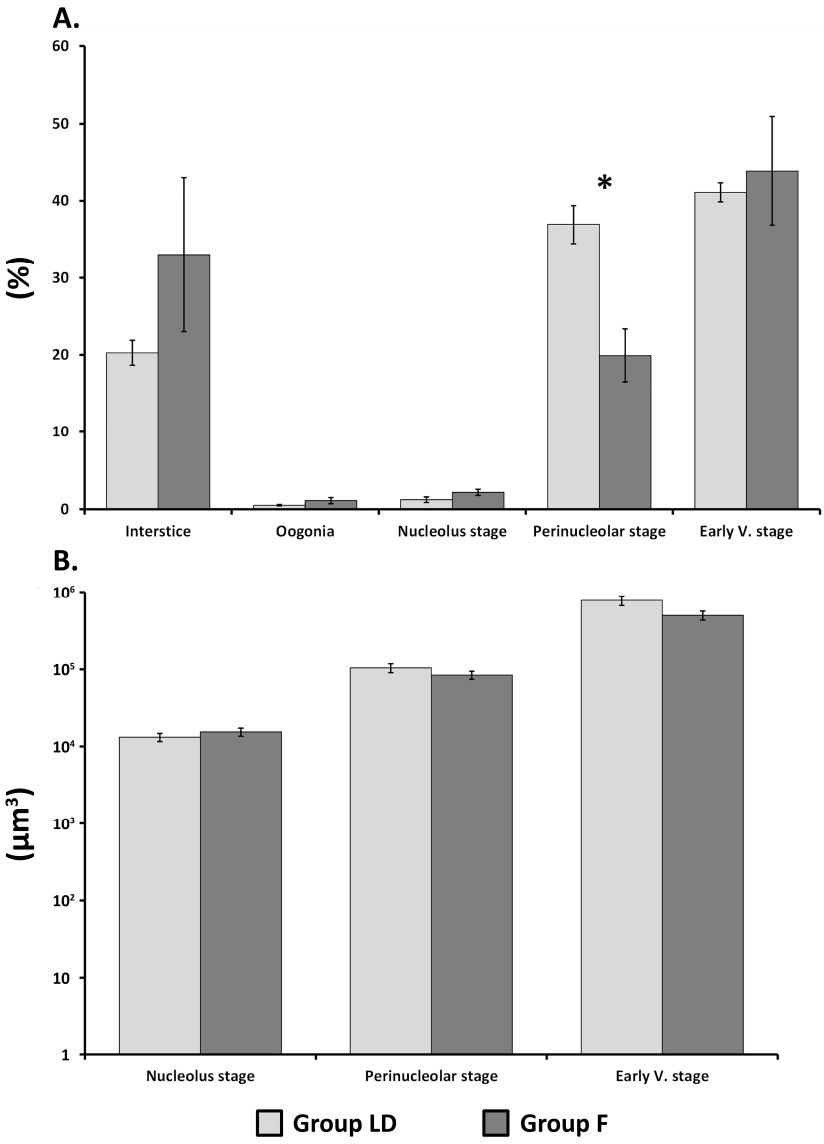

Figure 4. Mean proportions (A) and volumes (B) of the different oocyte development stages on *M. barbatus* for groups F and LD. Volumes are represented in logarithmic scale. The "*" indicates significant differences between distance treatments.

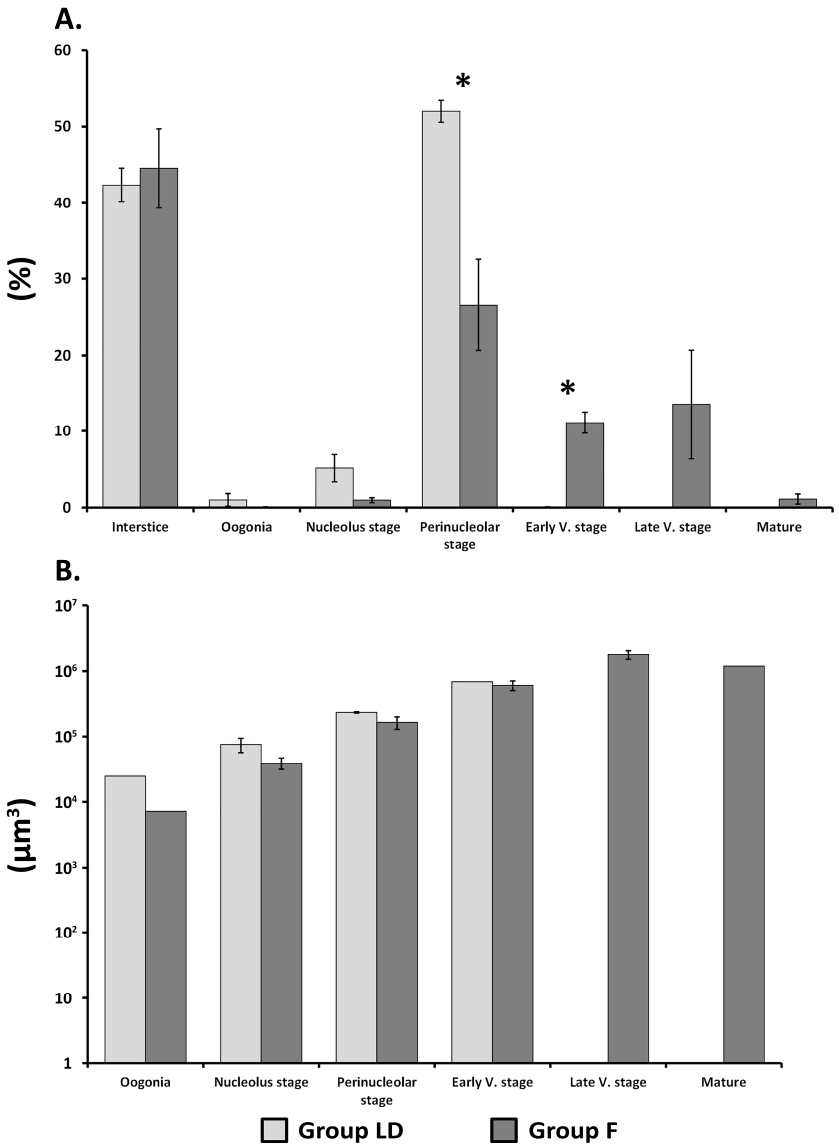

Figure 5. Mean proportions (A) and volumes (B) of the different oocyte development stages on *S. aurita* for groups F and LD. Volumes are represented in logarithmic scale. The "*" indicates significant differences between distance treatments.

Our results showed, in *S. aurita* and *M. barbatus* specimens, higher total lipid percentage and lower n-3/n-6 ratio in liver, which is supposed to have a direct influence on eggs production (Marshall et al. 1999; Salze et al. 2005). The gonad fatty acid profile was clearly influenced by the composition of aquafeeds in *S. aurita* specimens, whereas there was just little influence in gonads of *M. barbatus* specimens. Therefore, these two

species were selected for gonad histological examinations, as examples of high and low aquafeed impact, with the aim of checking for differences in oocyte maturation between aggregated fish (group F) and non-influenced fish (group LD). The gonad of the two studied species showed higher percentages of LA, lower percentages of DHA and lower n-3/n-6 ratio in aggregated fish compared with LD fish. In addition, *S. aurita* specimens also showed higher percentages of OA and lower percentages of ARA in group F compared to group LD. Fish from group F in both species showed oocytes in perinuclear stage in higher number than group LD, and oocytes in early vitellogenic stage were found in lower number in aggregated *S. aurita* specimens compared to non-aggregated specimens. The proportions of the oocyte stages in gonads of *S. aurita* were different between LD and F groups, the last showing presence of late vitellogenic and mature oocytes that were not found in LD specimens. Therefore, it is likely that *M. barbatus* associated to fish farms, ingesting aquaculture wastes, have a slightly faster development of the oogenesis, while *S. aurita* specimens captured in the vicinity of fish farms and reflecting the fatty acid composition of fish-feeds showed an accelerated development compared to controls, and it may be caused by the higher ingest of fat and/or vegetable fatty acids in the diet. The cell volumes of the different stages were the same in both distance treatments, which is indicative of a lack of differences in the amount of vitello accumulated. In any case, fatty acids and stereological results in *M. barbatus* specimens did not shed light on the possibility of negative effects derived of such shift in diet, while other species more influenced by the consumption of lost pellets such as *S. aurita* should be studied in greater depth. Other factors may be considered in future studies, such as the influence of the altered fatty acid profiles on the immune system, or the ecological impact derived from behavioural modifications due to the access to large quantities of waste feed.

5. Conclusion

Different species of wild fish aggregated near fish farms, with different feeding behaviour, showed alterations of their fatty acid profile in different degrees in the four tissues studied, pelagic species showing the most modified profiles in flesh and liver. We confirmed a trophic transfer of fatty acids of vegetable origin, mainly LA, from surplus feeds to aggregated wild fish, and from those to predators in the vicinity of fish

farms. These effects attenuate at a distance of more than 1.5 km from fish farm, and totally disappear in fish captured at long distances (minimum of 5 km from farms). We encouraged the use of fish caught as far away as possible from fish farms in studies which require obtaining natural fatty acid profiles as controls, in order to avoid the possibility of aquaculture influence.

Fatty acid transfer to gonads may be well regulated in *M. barbatus*, as just few differences were found in farm-associated fish compared to controls. *S. aurita* aggregated specimens showed huge accumulation of vegetable fatty acids in gonads, which may be related to an accelerated oocyte development compared to non-aggregated fish. We can therefore conclude that uncontrolled consumption of surplus feed rich in vegetable fatty acids can produce modifications in the development of the gonads, the extent of these depending on the feeding behaviour of the species studied.

Supplementary material

The fatty acid profile of fish feeds, the complete fatty acid profile for each tissue and species, a table with dates of capture, and the results of the SIMPER analysis can be found in the supplementary material to this work.

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References

Adams SM. 1999. Ecological role of lipids in the health and success of fish populations. pp 132-160. In: Lipids in freshwater ecosystems. Arts MT, Wainman BC (Eds). Springer-Verlag, New York

659 **Alasalvar C, Taylor KDA, Zubcov E, Shahidi F, Alexis M**. 2002. Differentiation of
660 cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and
661 trace mineral composition. Food Chemistry 79: 145-150. doi: 10.1016/S0308-
662 8146(02)00122-X

663 **Almansa E, Pérez MJ, Cejas JR, Badía P. Villamandos JE, Lorenzo A**. 1999.
664 Influence of broodstock gilthead seabream (*Sparus aurata* L.) dietary fatty acids on egg
665 quality and egg fatty acid composition throughout the spawning season. Aquaculture
666 170:323-336. doi: 10.1016/S0044-8486(98)00415-3

667 **Alvarez-Lajonchère L**. 2006. Nutrición de reproductores en peces marinos. pp 1-19.
668 In: Avances en nutrición acuícola VIII. VIII Simposium Internacional de Nutrición
669 Acuícola. 15-17 Noviembre. Cruz Suárez LE, Rique Marie D, Tapia Salazar M et al.
670 (Eds). Universidad Autónoma de Nuevo León, Monterrey, México.

671 **Anderson MJ**. 2004. A FORTRAN computer program for permutational multivariate
672 analysis of variance (for any two-factor ANOVA design) using permutation tests.
673 Department of Statistics, University of Auckland, New Zealand

674 **Arechavala-Lopez P, Sanchez-Jerez P, Bayle-Sempere J, Fernandez-Jover D,**
675 **Martinez-Rubio L, Lopez-Jimenez JA, Martinez-Lopez FJ**. 2011. Direct interaction
676 between wild fish aggregations at fish farms and fisheries activity at fishing grounds: a
677 case study with *Boops boops*. Aquaculture Research 42: 996-1010. doi: 10.1111/j.1365-
678 2109.2010.02683.x

679 **Arechavala-Lopez P, Izquierdo-Gomez D, Uglem I, Sanchez-Jerez P**. 2014.
680 Aggregations of bluefish *Pomatomus saltatrix* (L.) at Mediterranean coastal fish farms:
681 seasonal presence, daily patterns and influence of farming activity. Environmental
682 Biology of Fishes 98: 499-510. doi: 10.1007/s10641-014-0280-5

683 **Ballester-Moltó M, Sanchez-Jerez P, Aguado-Gimenez F.** 2017. Consumption of
684 particulate wastes derived from cage fish farming by aggregated wild fish. An
685 experimental approach. Marine Environmental Research 130: 166-173. doi:
686 0.1016/j.marenvres.2017.07.014

687 **Black KD, Calder LA, Nikell TD, Sayer MDJ, Orr H, Brand T, Cook EJ, Magill**
688 **SH, Katz T, Eden N, Jones KJ, Tsapakis M, Angel D**. 2012. Chlorophyll, lipid
689 profiles and bioturbation in sediments around a fish cage farm in the Gulf of Eilat,
690 Israel. Aquaculture 356-357: 317-327. doi: 10.1016/j.aquaculture.2012.04.049

691 **Bray JR, Curtis JT**. 1957. An ordination of the upland forest communities of Southern
692 Wisconsin. Ecological Monographs 27: 325-349. doi: 10.2307/1942268

693 **Bucholtz RH, Tomkiewicz J, Nyengaard JR, Andersen JB**. 2013. Oogenesis,
694 fecundity and condition of Baltic herring (*Clupea harengus* L.): A stereological study.
695 Fisheries Research 145: 100-113. doi: 10.1016/j.fishres.2013.02.003

696 **Calder PC**. 2013. n-3 fatty acids, inflammation and immunity: new mechanisms to
697 explain old actions. Proceedings of the Nutrition Society 72: 326-335. doi:
698 10.1017/S0029665113001031

699 **Calder PC**. 2014. Very long chain omega-3 (n-3) fatty acids and human health.
700 European Journal of Lipid Science and Technology 116: 1280-1300. doi:
701 10.1002/ejlt.201400025

702 **Callier MD, Byron CJ, Bengston DA, Cranford PJ, Cross SF, Focken U, Jansen**
703 **HM, Kamermans P, Kiessling A, Landry T, O'Beirn F, Petersson E, Rheault RB,**
704 **Strand Ø, Sundell K, Svåsand T, Wikfors GH, McKindsey CW**. 2017. Attraction
705 and repulsion of mobile wild organisms to finfish and shellfish aquaculture: a review.
706 Reviews in Aquaculture 0: 1-26. doi: 10.1111/raq.12208

707 **Cejas JR, Almansa E, Villamandos JE, Badía P, Bolaños A, Lorenzo A**. 2003. Lipid
708 and fatty acid compositions of ovaries from wild fish and ovaries and eggs from captive
709 fish of white sea bream (*Diplodus sargus*). Aquaculture 216: 299-313. doi:
710 10.1016/S0044-8486(02)00525-2

711 **Christie WW.** 2003. Lipid analysis. 3rd Edition. The Oily Press, Bridgewater, UK. pp.
712 205–224

Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117-143. doi: 10.1111/j.1442-9993.1993.tb00438.x

Dempster T, Uglem I, Sanchez-Jerez P, Fernandez-Jover D, Bayle-Sempere J, Nilsen R, Bjørn PA. 2009. Coastal salmon farms attract large and persistent aggregations of wild fish: an ecosystem effect. Marine Ecology Progress Series 385: 1-14. doi: 10.3354/meps08050

Dempster T, Sanchez-Jerez P, Fernandez-Jover D, Bayle-Sempere J, Nilsen R, Bjørn PA, Uglem I. 2011. Proxy measures of fitness suggest coastal fish farms can act as population sources and not ecological traps for wild gadoid fish. PLoS ONE: 6(1): e15646. doi:10.1371/journal.pone.0015646

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.

Felsing M, Glencross B, Telfer T. 2005. Preliminary study on the effects of exclusion of wild fauna from aquaculture cages in a shallow marine environment. Aquaculture 243: 159-174. doi: 10.1016/j.aquaculture.2004.09.033

Fernandez-Gonzalez V, Aguado-Giménez F, Gairin JI, Sanchez-Jerez P. 2013. Exploring patterns of variation in amphipod assemblages at multiple spacial scales: natural variability versus coastal aquaculture effect. Aquaculture Environments Interactions 3: 93-105. doi: 10.3354/aei00054

Fernandez-Jover D, Lopez-Jimenez JA, Sanchez-Jerez P, Bayle-Sempere J, Gimenez-Casalduero F, Martinez-Lopez FJ, Dempster T. 2007. Changes in body condition and fatty acid composition of wild Mediterranean horse mackerel (*Trachurus mediterraneus*, Steindachner, 1868) associated with sea cage fish farms. Marine Environmental Research 63: 1-18. doi: 10.1016/j.marenvres.2006.05.002

Fernandez-Jover D, Sanchez-Jerez P, Bayle-Sempere JT, Valle C, Dempster T. 2008. Seasonal patterns and diets of wild fish assemblages associated with Mediterranean coastal fish farms. ICES Journal of Marine Science 65: 1153-1160. doi: 10.1093/icesjms/fsn091

Fernandez-Jover D, Sanchez-Jerez P, Bayle-Sempere JT, Arechavala-Lopez P, Martinez-Rubio L, Lopez Jimenez JA, Martinez Lopez FJ. 2009. Coastal fish farms are settlement sites for juvenile fish. Marine Environmental Research 68: 89-96. doi: 10.1016/j.marenvres.2009.04.006

Fernandez-Jover D, Martinez-Rubio L, Sanchez-Jerez P, Bayle-Sempere JT, Lopez Jimenez JA, Martínez Lopez FJ, Bjørn PA, Uglem I, Dempster T. 2011. Waste feed from coastal fish farms: A trophic subsidy with compositional side-effects for wild gadoids. Estuarine, Coastal and Shelf Science 91: 559-568. doi: 10.1016/j.ecss.2010.12.009

Fernández-Palacios H, Izquierdo MS, Robaina L, Valencia A, Salhi M, Vergara JM. 1995. Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). Aquaculture 132: 325-337. doi: 10.1016/0044-8486(94)00345-O

Folch J, Lee M, Stanley GA. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509

Fuentes A. Fernández-Segovia I, Serra JA, Barat JM. 2010. Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. Food Chemistry 119: 1514-1518. doi: 10.1016/j.foodchem.2009.09.036

Gonzalez-Silvera D, Izquierdo-Gomez D, Fernandez-Gonzalez V, Martínez-López FJ, López-Jiménez JA, Sanchez-Jerez P. 2015. Mediterranean fouling communities assimilate the organic matter derived from coastal fish farms as a new trophic resource. Marine Pollution Bulletin 91: 45-53. doi: 10.1016/j.marpolbul.2014.12.029

Gonzalez-Silvera D, Martinez-Rubio L, Abad Mateo ME, Rabadan-Ros R, López Jiménez JA, Martínez López FJ. 2016. Assessing feeding history and health status through analysis of fatty acids and fat content in golden mullet *Liza aurata*. ICES Journal of Marine Science 73(10): 2632-2643. doi: 10.1093/icesjms/fsw092

Gonzalez-Silvera D, Guardiola FA, Cordero H, Cuesta A, Esteban MA, Martínez López FJ, López Jiménez JA. 2017. The short-term effects of farmed fish food

consumed by wild fish congregating outside the farms. Marine Pollution Bulletin 114: 689-698. doi: 10.1016/j.marpolbul.2016.10.055

Grigorakis K, Alexis MN, Taylor A, Hole M. 2002. Comparison of wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. International Journal of Food Science and Technology 37:477-484. doi: 10.1046/j.1365-2621.2002.00604.x

Harding JM, Mann R. 2001. Diet and habitat use by bluefish, *Pomatomus saltatrix*, in a Chesapeake Bay estuary. Environmental Biology of Fishes 60: 401-409. doi: 10.1023/A:1011071127930

Hauville MR, Rhody NR, Resley MJ, Bell JG, Main KL, Migaud H. 2015. Comparative study of lipids and fatty acids in the liver, muscle and eggs of wild and captive common snook broodstock. Aquaculture 446: 227-235. doi: 10.1016/j.aquaculture.2015.04.026

Henriques J, Dick JR, Tocher DR, Bell JG. 2014. Nutritional quality of salmon products available from major retailers in the UK: content and composition of n-3 long chain PUFA. British Journal of Nutrition 112: 964-975. doi: 10.1017/S0007114514001603

Izquierdo-Gomez D, Gonzalez-Silvera D, Arechavala-Lopez P, López-Jiménez JA, Bayle-Sempere JT, Sanchez-Perez P. 2015. Exportation of excess feed from Mediterranean fish farms to local fisheries through different targeted fish species. ICES Journal of Marine Science 72(3): 930-938 doi:10.1093/icesjms/fsu179

Izquierdo MS, Fernández-Palacios H, Tacon AGJ. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197: 25-42. doi: 10.1016/S0044-8486(01)00581-6

Johansson T, Håkanson L, Borum K, Persson J. 1998. Direct flows of phosphorus and suspended matter from a fish farm to wild fish in Lake Southern Bullaren, Sweden. Aquacultural Engineering 17: 111-137. doi: 10.1016/S0144-8609(98)00010-7

Katz T, Herut B, Genin A, Angel DL. 2002. Grey mullets ameliorate organically enriched sediments below a fish farm in the oligotrophic Gulf of Aqaba (Red Sea). Marine Ecology Progress Series 234:205-214. doi: 10.3354/meps234205

Kiron V. 2012. Fish immune system and its nutritional modulation for preventive health care. Animal Feed Science and Technology 173: 111-133. doi: 10.1016/j.anifeedsci.2011.12.015

La Rosa T, Mirto S, Mazzola A, Danovaro R. 2001. Differential responses of benthic microbes and meiofauna to fish-farm disturbance in coastal sediments. Environmental Pollution 112: 427-434. doi: 10.1016/S0269-7491(00)00141-X

Machias A, Labropoulou M. 2002. Intra-specific variation in resource use by red mullet, *Mullus barbatus*. Estuarine, Coastal and Shelf- Science 55: 565-578. doi: 10.1006/ecss.2001.0924

Madin J, Chong VC, Basri B. 2009. Development and short-term dynamics of macrofouling assemblages on fish-cage nettings in a tropical estuary. Estuarine, Coastal and Shelf Science 83: 19-29. doi: 10.1016/j.ecss.2009.03.012

Maldonado M, Carmona MC, Echeverría Y, Riesgo A. 2005. The environmental impact of Mediterranean cage fish farms at semi-exposed locations: does it need a re-assessment? Helgoland Marine Research 59: 121-135. doi: 10.1007/s10152-004-0211-5

Marshall CT, Yaragina NA, Lambert Y, Kjesbu OS. 1999. Total lipid energy as a proxy for total egg production by fish stocks. Nature 402: 288-290. doi: 10.1038/46272

Mnari A, Bouhlel I, Chraief I, Hammami M, Romdhane MS, El Cafsi M, Chaouch A. 2007. Fatty acids in muscles and liver of Tunisian wild and farmed gilthead sea bream, *Sparus aurata*. Food Chemistry 100: 1393-1397. doi: 10.1016/j.foodchem.2005.11.030

Morote E, Olivar MP, Villate F, Uriarte I. 2008. Diet of round sardinella, *Sardinella aurita*, larvae in relation to plankton availability in the NW Mediterranean. Journal of Plankton Research 30(7): 807-816. doi: 10.1093/plankt/fbn039

824 **Nichols PD, Petrie J, Singh S**. 2010. Long-chain omega-3 oils – An update on
825 sustainable resources. Nutrients 2(6): 572-585. doi: 10.3390/nu2060572

826 **Nichols PD, Glencross B, Petrie JR, Singh SP**. 2014. Readily available sources of
827 long-chain omega-3 oils: Is farmed Australian seafood a better source of the good oil
828 than wild caught seafood? Nutrients 6: 1063-1079. doi: 10.3390/nu6031063

829 **Norberg B, Kleppe L, Andersson E, Thorsen A, Rosenlund G, Hamre K**. 2017.
830 Effects of dietary arachidonic acid on the reproductive physiology of female Atlantic
831 cod (*Gadus morhua* L.). General and Comparative Endocrinology 250: 21-35. doi:
832 10.1016/j.ygcen.2017.05.020

833 **Pérez MJ, Rodríguez C, Cejas JR, Martín MV, Jerez S, Lorenzo A**. 2007. Lipid and
834 fatty acid content in wild white seabream (*Diplodus sargus*) broodstock at different
835 stages of the reproductive cycle. Comparative Biochemistry and Physiology, Part B
836 146: 187-196. doi: 10.1016/j.cbpb.2006.10.097

837 **Ramírez B, Montero D, Izquierdo M, Haroun R**. 2013. Aquafeed imprint on bogue
838 (*Boops boops*) populations and the value of fatty acids as indicators of aquaculture-
839 ecosystem interaction: are we using them properly? Aquaculture 414-415: 294-302. doi:
840 10.1016/j.aquaculture.2013.08.010

841 **Rueda FM, Hernández MD, Egea MA, Aguado F, García B, Martínez FJ**. 2001.
842 Differences in tissue fatty acid composition between reared and wild sharpsnout sea
843 bream, *Diplodus puntazzo* (Cetti, 1777). British Journal of Nutrition 86: 617-622. doi:
844 10.1079/BJN2001438

845 **Salze G, Tocher DR, Roy WJ, Robertson DA**. 2005. Egg quality determinants in cod
846 (*Gadus morhua* L.): egg performace and lipids in eggs from farmed and wild
847 broodstock. Aquaculture Research 36: 1488-1499. doi: 10.1111/j.1365-
848 2109.2005.01367.x

849 **Sanchez-Jerez P, Fernandez-Jover D, Bayle-Sempere J, Valle C, Dempster T, Tuya**
850 **F, Juanes F**. 2008. Interactions between bluefish *Pomatomus saltatrix* (L.) and coastal
851 sea-cage farms in the Mediterranean Sea. Aquaculture 282: 61-67. doi:
852 10.1016/j.aquaculture.2008.06.025

Sanchez-Jerez P, Fernandez-Jover D, Uglem I, Arechavala-Lopez P, Dempster T, Bayle-Sempere J, Valle-Perez C, Izquierdo D, Bjørn P, Nilsen R. 2011. Coastal fish farms as fish aggregations devices (FADs). p.187-208. In: Artificial reefs in fisheries management. S.A. Bortone, F. Pereira-Brandini, G. Fabi, S. Otake (Eds.) CRC Press. Taylor & Francis Group. Florida U.S. 350p.

Sanz-Lázaro C, Marín A. 2011. Diversity patterns of benthic macrofauna caused by marine fish farming. Diversity 3: 176-199. doi: 10.3390/d3020176

Sara G, Scilipoti D, Mazzola A, Modica A. 2004. Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): A multiple stable isotope study (d13C and d15N). Aquaculture 234: 199-213. doi: 10.1016/j.aquaculture.2003.11.020

Sargent J, McEvoy L, Estevez A, Bell G, Bell M, Henderson J, Tocher D. 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179: 217-229. doi: 10.1016/S0044-8486(99)00191-X

Shahidi F, Ambigaipalan P. 2018. Omega-3 polyunsaturated fatty acids and their health benefits. Annual Review of Food Science and Technology 9: 345-381. doi: 10.1146/annurev-food-111317-095850

Simopoulos AP. 1999. Essential fatty acids in health and chronic disease. The American Journal of Clinical Nutrition 70: 560s-569s. doi: 10.1093/ajcn/70.3.560s

Simopoulos AP. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy 56: 365-379. doi: 10.1016/S0753-3322(02)00253-6

Skog TE, Hylland K, Torstensen BE, Berntssen MHG. 2003. Salmon farming affects the fatty acid composition and taste of wild saithe *Pollachius virens* L. Aquaculture Research 34: 999-1007. doi: 10.1046/j.1365-2109.2003.00901.x

Sley A, Jarboui O, Ghorbel M, Bouain A. 2008. Diet composition and food habits of *Caranx rhonchus* (Carangidae) from the Gulf of Gabes (central Mediterranean). Journal

of the Marine Biological Association of the United Kingdom. 88(4): 831-836. doi: 10.1017/S0025315408001379

Sprague M, Dick JR, Tocher DR. 2016. Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006-2015. Scientific Reports 6: 21892. doi: 10.1038/srep21892

Tocher DR. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11(2): 107-184. doi: 10.1080/713610925

Tur JA, Bibiloni MM, Sureda A, Pons A. 2012. Dietary sources of omega 3 fatty acids: public health risks and benefits. British Journal of Nutrition 107: s23-s52. doi: 10.1017/S0007114512001456

Tuya F, Sanchez-Jerez P, Dempster T, Boyra A, Haroun R. 2006. Changes in demersal wild fish aggregations beneath a sea-cage fish farm after the cessation of farming. Journal of Fish Biology 69: 682-697. doi: 10.1111/j.1095-8649.2006.01139.x

Van Biesen G, Parrish CC. 2005. Long-chain monounsaturated fatty acids as biomarkers for the dispersal of organic waste from a fish enclosure. Marine Environmental Research 60(3): 375-388. doi: 10.1016/j.marenvres.2004.12.003

Vita R, Marin A, Madrid JA, Jiménez-Brinquis B, Cesar A, Marin-Guirao L. 2004. Effects of wild fishes on waste exportation from a Mediterranean fish farm. Marine Ecology Progress Series 277: 253-261. doi: 10.3354/meps277253

Warwick RM, Platt HM, Clarke KR, Agard J, Gobin J. 1990. Analysis of macrobenthic and meiobenthic community structure in relationship to pollution and disturbance in Hamilton Harbour, Bermuda. Journal of Experimental Marine Biology and Ecology 138: 119-142. doi: 10.1016/0022-0981(90)90180-K

Watanabe T. 2002. Strategies for further development of aquatic feeds. Fisheries Science 68: 242-252. doi: 10.1046/j.1444-2906.2002.00418.x

White CA, Dworjanyn SA, Nichols PD, Mos B, Dempster T. 2016. Future aquafeeds may compromise reproductive fitness in a marine invertebrate. Marine Environmental Research 122: 67-75. doi: 10.1016/j.marenvres.2016.09.008.

908 **White CA, Nichols PD, Ross DJ, Dempster T**. 2017. Dispersal and assimilation of an
909 aquaculture waste subsidy in a low productivity coastal environment. Marine Pollution
910 Bulletin 120: 309-321. doi: 10.1016/j.marpolbul.2017.05.042

911 **White CA, Woodcock SH, Bannister RJ, Nichols PD**. 2019. Terrestrial fatty acids as
912 tracers of finfish aquaculture waste in the marine environment. Reviews in Aquaculture
913 11: 133-148. doi: 10.1111/raq.12230

914 **Woodcock SH, Strohmeier T, Strand Ø, Olsen SA, Bannister RJ**. 2018. Mobile
915 epibenthic fauna consume organic waste from coastal fin-fish aquaculture. Marine
916 Environmental Research 137: 16-23. doi: 10.1016/j.marenvres.2018.02.017

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Highlights

Sea-cage aggregated wild fish consume lost pellets rich in terrestrial fatty acids.

Fatty acid profiles of four fish of different trophic level were analysed.

Flesh, brain, liver and gonad fatty acids reflect the composition of surplus feed.

Gonad development is accelerated in fish species aggregated at farms.

Consumption of lost pellets modulates fatty acid composition and gonad maturation.

Declaration of interests

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