Seasonal variations in the regiodistribution of oil extracted from small-spotted catshark and bogue

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ABSTRACT

The aim of this work was to seasonally characterize the nutritional quality of oil extracted from small-spotted catshark *(Scyliorhinus canicula)* and bogue *(Boops boops)*. Proximate composition, lipid profile and regiodistribution of the fatty acid in the glycerol backbone were analyzed. Additionally, three nutritional indexes were calculated (atherogenity and thrombogenicity indexes and hypocholesterolaemic/ hypercholesterolaemic ratio). Both species presented PUFA as the predominant fraction, being DHA the most abundant. Healthy values of the aforementioned indexes were maintained throughout the year. Moreover, the relative composition of omega 3 fatty acids in sn-2 position ranged from 47.3 to 66.8 mol%, showing the interest of the employment of these oils as raw source for the production of 2-monoacylglycerols. Regarding the individual behavior of each fatty acids, DHA presented a high tendency to occupy sn-2 bond, whereas EPA presented the opposite behavior.

KEYWORDS Fish discards; Fish oil; Nutraceutical indexes; Omega 3; Regiospecific distribution; Lipid profile

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1. Introduction

Discards are defined as the fraction of the fish catch which is not retained on board but rejected to the sea for any reason. Discards are composed by non-target species (e.g. marine sponges, echinoderms, fish species of low commercial value, seals), juvenile individuals below minimum landing size or target species over fishing quota. The last FAO report[1] estimated a yearly tonnage of discards around 7.3 million tons, representing 8% of worldwide catches. Discarding not only has a negative impact on future fishing productivity, but they also pose a number of environmental problems since they alter marine's trophic chains and contribute to the dissemination of toxic compounds and parasites present in fish viscera[2] .

The discard rate (i.e. ratio of discarded fish related to the total catch) of a given fishery depends on a number of factors such as fishing gear, local markets or fishing regulations, among others. In the case of southwest Mediterranean Sea (Alboran sea), discard rates arise up to 23% for trawling and 10% for purse seine fisheries. This represents an underutilization of fishing stocks, especially in an area where fish catches have been reduced to a half during the past decade[3]. Most of discards in this area comprise non-target species such as bogue or small-spotted catshark, which will be considered in this work.

International organisms have warned against the adverse effects of fishing discards, and their recommendations have so far been incorporated into the fishing regulations of some countries such as Iceland or Norway, which have adopted policies minimizing discards. In the case of the European Union, the new Common Fisheries Policy[4] introduces a progressive discard ban in European fisheries. In application of this policy, all catches from pelagic fisheries, such as mackerel, horse mackerel or sardine, must be brought ashore since the 1st January 2015. As for the rest of species, discard prohibition will come into force from 2017 on, while some specific fisheries such as hake, Norway lobster, common sole or plaice will be exempt of these measures until 2019.

As a consequence of discard bans, a supplementary volume of fish (mainly composed of non-target species) will be landed, which will be difficulty put into marked without an adequate commercial promotion. An alternative solution will be the conversion of these underutilized materials into added-value products of interest in nutraceutical and pharmaceutical applications. For instance, some studies have explored the nutritional properties of the lipid fraction of some Mediterranean discarded species such as bogue or horse mackerel[5]. Fish oils have a high content of polyunsaturated fatty acids (PUFA), which play a beneficial role for the human health[6]. More specifically, eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA C22:6n-3), belonging to the omega-3 family, can prevent cardiovascular diseases due to their anti-thrombotic, anti-arrhythmic and anti-inflammatory activities[6].

A key feature when studying the nutraceutical value of a given compound is its bioavailability (i.e. the fraction of the ingested dose which is absorbed and exerts its biological activity after digestion). Several enzymes are involved in the lipid digestion, being gastric and pancreatic lipase the most important. Both of them are specific towards external carbons (position sn-1 and sn-3) and hydrolyze triacylglycerides (TAG) to 2-monoacylglycerides (2-MAG), (i.e. the fatty acid is bonded in the central position) and free fatty acids. PUFA located in the central bond of the glycerol backbone (sn-2 position) present much better absorption than those released as free fatty acids which precipitate in the intestine. Hence, the human digestibility and metabolism of lipids present different efficiency depending on the position of the fatty acid within the glycerol backbone; being those located in sn-2 position more easily absorbed[7]. Therefore, during last decades, there is a growing interest in the production of structured lipids, where DHA and EPA are located in the sn-2 and short chain fatty acids in sn-1(3).

Due to the regioselectivity of lipases involved in human digestion, the characterization of the relative lipid profile of fatty acids occupying sn-2 bond should be considered as a useful tool aiming at selecting the most appropriate up-grading technique. Those fish oils with high content of PUFA in sn-2 might be considered as a source for the production of 2-MAG which can be enriched in PUFA by physical methods as low temperature fractionation[8]. Monoacylglycerols account around the 75% of the total production of emulsifiers[9], their applications and production technique has been recently reviewed[10]. Moreover, 2-MAG with a high content of PUFA can be esterified aiming to produce structured lipids with a medium chain fatty acids in the sn-1(3) bonds[11]. These structured lipids present a faster absorption than the original oil and their daily intake might result in a less accumulation of fats. Additionally, due to the role that DHA plays on the development of brain and eye of infant[6], they are being employed for the production of ready-to-feed infant formula.

Seasonal variations of the proximal composition and the lipid profile have been widely described for several species (sardine, bogue, horse mackerel, small-spotted catshark, axillary seabream)[5,12,13]. Additionally, the nutritional value of these oils has been evaluated employing indexes as the thrombogenic (TI) or the atherogenicity (AI) ones [14–17]. The regioselectivity of fatty acids in fish oils was firstly described by Brockerhoff et al.[18] in a work which aimed to globally described the lipid of marine sources. During last decades, the regiodistribution of fish oils have been analyzed as an initial characterization of the oils prior to the production of structured lipids[11]. However, no systematically study of the seasonal variations of the regiodistribution of the fatty acids of oils extracted from bogue and small-spotted catshark has been yet described in the literature.

The aim of this study was to evaluate the seasonal variations of the nutraceutical quality of oil extracted from small-spotted catshark *(Scyliorhinus canicula)* and bogue *(Boops boops)*. To this end, proximate composition, lipid profile and fatty acid regiodistribution were analyzed during the year. This characterization is the first approach to the selection of the most adequate technique for the up-grading of these oils.

2.1. Raw materials

Fish samples from small-spotted catshark *(Scyliorhinus canicula)* and bogue *(Boops boops)* were supplied every season by the fishing harbor of Motril (Spain). Both species are discarded in Alboran Sea due to their low commercial value. They were kept in ice during transportation and pressed the same day to avoid microbial spoilage. Three individuals were chosen for the somatometric measurements shown in Table 1.

2.2. Proximate composition and oil extraction

The samples were analyzed for their proximate composition according to the official methods recognized by the A.O.A.C.[19] Fish oil was extracted by hydraulic pressing, according to the method described elsewhere[13]. To this end, two kilograms of whole fish were immersed in a water bath at 40ºC for 30 min. The preheated material was then fed to a hydraulic press (model ESP-K, Sanahuja, Spain), where it was pressed stepwise until attaining a final pressure of 120 bar. The press liquor released from the press chamber was collected and centrifuged at 20,000×g, from which the upper oily phase was recovered. The analysis were done in duplicate.

2.3. Fatty acid profile, lipid composition and desaturase activity

Oil samples were converted into fatty acid methyl esters prior to their analysis. To that end, methylation was conducted following the method described by Rodriguez-Ruiz et al.[20] with minor variations. Firstly, a solution of oil in hexane (1mg/mL) was prepared. An aliquot of 1 mL was extracted and mixed with 1 mL of the freshly prepared transesterification reagent (methanol/acetyl chloride, 20:1, v/v) and 50 µL of standard solution of nonadecanoic acid (Sigma Aldrich) in hexane (2 mg/mL). Then, samples were heated at 90ºC for 1 hour, being shaken every 15 min. After methylation, 1 mL of distilled water was added and the organic phase was manually extracted.

Fatty acid methyl esters were analyzed according to Camacho Paez et al.[21] by means of a chromatograph (Agilent 7890A, Agilent Technologies S.A.) equipped with a capillary column of fused silica Omegawax (0.25 mm × 30 m, 0.25 µm standard film; Supelco, Bellefonte, PA). Results were reported as the average value of three replicates.

The lipid sample was fractionated into monoacylglycerols (MAG), 1,2- or 1,3-diacylglycerols (1,2- or 1,3-DAG) and triacylglycerols (TAG) by thin layer chromatography. To this end, 2 mg of oil were spotted on silica-gel plates (Precoated TLC plates, SIL G-25; Macherey-Nagel, Sigma–Aldrich). A mobile phase consisting of a mixture of chloroform/acetone/methanol (95:4.5:0.5, v/v/v) was employed to separate the different lipid species. After separation, each fraction was recovered and methylated as described before.

2.4. Oxidative and nutritional indices

Fatty acid content was referred to the mass of fish by means of a conversion factor as described by Weihrauch et al[22]. Furthermore, the intrinsic peroxidability index (PI, %) was computed for all samples according to Arakawa and Sangai[23].

Subsequently, the lipid profile was employed to estimate the indices of atherogenicity (AI), thrombogenicty (TI)[16] and the hypocholesterolaemic/ hypercholesterolaemic ratio (HH)[15].

2.5. Determination of the positional distribution of fatty acids in TAGs

An ethanolysis with the lipase Novozym 435 from *Candida Antarctica* was conducted to study the regiodistribution of fatty acids in the TAG, adapted from the method described by Shimada et al [24]. By this approach, all the monoacylglycerols produced are esterified in the second position (2-MAG) so they can be easily separated by thin layer chromatography, as previously described.

The percentage of a given fatty acid in sn-2 position was related to the total content of that fatty acid as follows:

$$\%\text{FAi in sn} - 2\text{ position} = \frac{\text{content of FAi at sn}-2\text{ position}}{3\cdot\text{Total content of FAi in TAG}} \times 100 \qquad (1)$$

The total percentage of each fatty acid located in sn-2 was calculated by multiplying the aforementioned percentage by the global fatty acid percentage, both in molar basis.

2.6. Statistical analysis

Data were presented as an average value ± standard deviation. Additionally, a coefficient of variation, defined as the ratio between standard deviation and mean value, was chosen to evaluate the seasonal variations among each species.

3. Results and discussion

3.1. Proximate composition.

The seasonal proximate composition of both species is shown in Table 2. The ash content remained practically constant along the year with an average value of 3.34±0.55wt%. Similarly, the protein content did not deeply vary throughout the year (average value: 19.34±2.29wt%) being the percentage of small-spotted catshark higher than that of bogue in all seasons (20.6±1.9 and 18.1±2.0wt%, respectively). This difference might be related to the high level of non-protein nitrogen compounds (i.e. ammonia, trimethylamine oxide or urea) which are presented in elasmobranchs species[25]. Protein content was similar to the values previously described in the literature [5,13,17].

Moisture and lipid content showed the highest seasonal variations in the case of bogue (average: 74.9±3.8 and 3.4±2.7wt%, respectively) whereas for small-spotted catshark these values remained relatively constant (average: 75.9±0.4 and 2.0±0.9wt%, respectively) (Table 2). For bogue, the fat content correlated inversely with water content (r^2 = -0.958), trend which has been described for a wide group of fishes [5,13]. Contrarily, small-spotted catshark presented a direct correlation (r^2 = 0.818). Taking into account Ackman's classification for fish species regarding their lipid content, bogue is considered a semi-fatty fish (<8 wt%) while small-spotted catshark belongs to lean fish category[26]. Moreover, these species store lipids in different sites being the liver the main location for the small-spotted catshark[27] and muscles and/or subcutaneous depots in the case of bogue.

The variations of lipid content among species and seasons are related to feed intake, spawning period or migratory habits[18]. It is a common behavior that the minimal content of lipid coincides with the end of the spawning period, because lipids are employed as the main energy source[28]. The reproductive behavior of species located in the Alboran Sea has been studied, being the spawning season of bogue spring while small-spotted catshark has a wider range: from November to July[29]. Bogue has considerable variations of the lipid content thorough the year (Table 2), achieving the maximal and minimal content in autumn (6.0wt%) and spring (1.0wt%) respectively. The maximal content is similar to that reported by Prato and Biandolino[30] and higher than that described by García Moreno et al[13]. In the case of small-spotted catshark, the lipid content remained practically constant along the year with an average value of 1.9±0.2wt% (Table 2), data which agrees with previous works of this group[13].

3.2. Fatty acids profile and nutritional indices.

Fatty acid profile of fish oil depends on a number of factors: reproductive status, age, species, sex or food availability[18]. Among polar fractions, TAG was the only group detected by thin layer chromatography, hence the global lipid profile corresponds uniquely to that fraction.

Table 3 summarizes the fatty acid profile mass distribution during the year. PUFA fraction was the most abundant one (34.4 to 47.1wt%) in both species, followed by saturated fatty acids (20.8 to 31.8wt%) in the case on bogue and by monounsaturated fatty acids (22.1 to 20.5wt%) in the case of small-spotted catshark. Main fatty acids of saturated, monounsaturated and polyunsaturated fatty acids were palmitic (C16:0), oleic (C18:1n-9) and docosahexanoic acid (C22:6n-3) accounting each one more than 60wt% of their respective fraction. EPA was the second most abundant PUFA: representing a 16.9±1.5 and 21.0±1.2wt% of the total PUFA for small-spotted catshark and bogue respectively.

For small-spotted catshark, MUFA showed the highest CV (14.6%) followed by SFA (12.8%) while for bogue the major variations happened in the PUFA fraction (CV 9.7wt%). A negative correlation was found between the percentage of SFA and the fat content for bogue but no correlation was found for small-spotted catshark.

From a nutritional point of view, the proportion n-3/n-6 could be regarded as an index referring the quality of the oil. These groups present opposite behaviors being n-3 anti-inflammatory and anti-aggregatory[31]. EPA and arachidonic acid (C20:4n-6) might compete for some enzymes as cyclooxygenase or lipoxygenase for the production of eicosanoids. Although the recommended n-3:n-6 ratio is 1:2-4, the average real intake in western diet is 1:25[32]. In the studied oils, the omega-3 PUFA content was much higher than the omega-6 PUFA one, resulting in ratios varying from 14.7 to 43.1. Hence, the consumption of these oils could balance the excess of n6 in human diets. Additionally, it has been reported that n-3:n-6 ratios higher than 3.5 might reduce cholesterol levels and improve the plasma lipid profile[33]. The differences observed among species and seasons could be related to the diet habit[18]. Effectively, small-spotted catshark has a diet based mainly on crustaceans, decapods, fishes and mollusks while bogue is herbivorous[29].

In Table 4, it is shown the composition of fatty acids in g/100g fish basis. The influence of seasonality is noticeably higher than in the global profile due to the influence of the fluctuations of the lipid content during the year.

As a result of the high content of PUFA, the current oils are extremely prone to oxidation and, consequently to spoilage. Peroxidability index (PI), is an intrinsic indicator of the tendency of oils to be oxidized. PI values ranged from 221 and 322%, these high values were closely related to the content of PUFA and, more specifically, DHA which was the most unsaturated fatty acid.

Three nutritional indexes (AI, TI and HH ratio) were estimated so as to quantify the quality of the oil. Thrombosis and atherosclerosis are closely related to coronary heart diseases. It has been reported that SFA promote cardiovascular diseases while PUFA and MUFA play a protective role[16]. In this sense, the studied fish oils showed AI and TI minor than 1 (Table 4), and, hence, they can be described as healthy[34]. Averages AI values were 0.32±0.1 and 0.51±0.05 for small-spotted catshark and bogue respectively, the lower values of small-spotted catshark are related to the higher content of DHA. On the other hand, both species presented similar values of TI, being the average value 0.17±0.03. The current data are in the same range as the values estimated for goldfish[14]; moreover the current values were slightly lower than those described for bogue by Šimat[17]. Additionally, AI and TI values were lower than those calculated for lamb, beef, pork or palm oil[16]. HH ratio is a parameter corresponding to the coefficient between the total percentage of hypocholesterolemic and hypercholesterolemic fatty acids. From a nutritional point of view, higher HH values are considered more beneficial for the human health. The values of the hypocholesterolaemic/ hypercholesterolaemic values ranged from 1.78 (bogue, in spring) to 3.43 (small-spotted catshark, in autumn), data which are similar to those reported for black needle or mackerel[35]. The values of these three nutritional indexes show the optimal nutritional quality of oils extracted from bogue and small-spotted catshark.

3.3. Regiospecific distribution of fatty acids in TAGs.

Table 5 shows the mass profile of the 2-MAG produced after the specific alcoholysis. These data were further employed, together with the global profile, for the calculation of the regioselectivity of fatty acids (Eq. 1). In both species, the CV was higher in the case of the sn-2 position than in the global profile (Table 3).

Furthermore, in Table 6 it is shown for the main fatty acids and fractions: (i) the percentage with respect to the total oil content which is located in sn-2 (marked with symbol α) and (ii) the relative composition of the central bond (symbol β). First calculations follow the stoichiometric proportion, being their maximal value 33.3mol%

Due to the specificity of human lipases enzymes, the relative composition of the sn-2 position might be considered as the most effective amount of PUFA which will be properly metabolized and it should be considered when evaluating the nutritional value of these oils.

Small-spotted catshark and bogue contained an average content of PUFA in the central position of 60.7±6.6 and 55.7±7.3mol% respectively. In both cases this value was much higher than the global one (40.8±3.9 and 39.8±3.6mol%). Thus, the total amount of PUFA presented high regiospecifity towards the central position. On the other hand, MUFA presented the opposite behavior, being 1(3)-specific. The regioselectivity of SFA differed between species: in the case of bogue the global and sn-2 relative lipid profile were similar (32.9±2.7 and 30.4±5.0mol% respectively), which implies the absence of regioselectivity. However, as for small-spotted catshark, this fraction presented selectivity towards 1(3) bonds.

DHA showed a high 2-regiospecificity with relative average values of 50.2±8.4 (small-spotted catshark) and 41.4±7.8mol% (bogue). The percentage of the total DHA in the central bond varied from ~50mol% (winter) to ~80mol% (spring). Palmitic was the second most abundant fatty acid of the central position. In the case of bogue, it presented 2-regiospecifity with an average content of 49.9±10.8mol%. However, for small-spotted catshark, this percentage was closed to the stoichiometric one (33.2±8.4mol%), and no specificity to any bond of the glycerol backbone was showed. In the case of EPA and oleic acid, they presented 1(3) specificity, with only a ~15mol% of their global amount situated in sn-2.

These oils presented a high content of PUFA in the central position of the glycerol backbone, being the percentage of DHA greater than 75% during the whole year. Hence, concentrations techniques which preserve the fatty acid esterified in the central position should be employed, as for instance: alcoholysis, hydrolysis or acidolysis. Alcoholysis and hydrolysis could be employed for the production of 2-MAG which might be lately esterified so as to produce structured lipids. Acidolysis, a one-step process, could be considered as one of the simplest techniques for the production of structured lipids; however PUFA located in positions 1(3) are resistant to be displaced by medium-chain fatty acids resulting in a decrease of the yield of the desired structured lipids[36]. Acylmigration is one of the main difficulties of the 2-MAG production; this non-desired process can be minimized by selecting the most suitable solvent, immobilization carrier and enzyme. Munio et al.[11], by combining alcoholysis and esterification, produced 63% of 2-MAG a high yield of recovery (90%). The global production of structured lipids yielded 80% and no acyl-migration was detected. Additionally, 2-MAG were synthesized by enzymatic ethanolysis employing Novozym 435[8]. After solvent fractionation they produced 2-MAG with a purity of 99% and no acyl-migration was detected. As a concentration step, the 2-MAG were crystallized in hexane resulting in an 80% of PUFA, which represented a yield of 50%. Munio et al[11] produced structured lipids from cod liver and tuna oil, whose global DHA content was much lower than the oils studied in this work. Moreover, the proportion of the DHA located in the sn-2 bond was a half less than that obtained in the present study. On the other hand, Wang et al.[8] employed randomized arachidonic acid-rich oil with 46.2% of arachidonic acid and described that the main drawback of their research was the loss of 66.7% of the target PUFA. Based on the results of these studies, it could be a good approach to conduct an alcoholysis of hydrolysis of the small-spotted catshark or bogue oils followed by isolation of MAG and a concentration step. Due to the high relative content of DHA in the central bond which is followed by palmitic and oleic acid, the efficiency and yield of concentration of DHA 2-MAG should be higher than those referred in the literature. However, since the EPA is mainly bonded in 1(3) positions, the remaining free fatty acids or esters might content a considerable percentage of EPA.

The nutritional quality of these oils has been proved not only with nutritional indexes (AI and TI < 1 and HH ratio >1.5) and global profile (PUFA content > 35wt%) but also by measuring the relative composition of the fatty acids esterified in the sn-2 position (PUFA content >47mol%). In the case of small spotted catshark the lipid content did not deeply vary throughout the year and PUFA percentage presented CV of 7.9 (global profile) and 10.8 (sn-2 relative profile). For bogue, the amount of lipid varied more dramatically from 1.0 to 6.1wt%, however, the PUFA content in both, global and relative sn-2, was similar to those obtained for small-spotted catshark (9.7 and 13.1 respectively).

The study of the regiodistribution of the fatty acids might be considered a useful tool prior to the selection of the up-grading technique. Regarding the relative sn-2 profile, these oils might be considered as a raw source for the production of 2-MAG by means of alcoholysis or hydrolysis where the acylmigration should be minimized. Since the DHA is the most abundant PUFA (>80%), physical concentration as low temperature crystallization might be a good technique to produce DHA 2-MAG with high purity.

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Tables caption

Table 1. Seasonal somatometric data of discarded species of the Alboran Sea: means ± standard deviation.

Table 2. Seasonal proximate compositions of discarded species of the Alboran Sea: means ± standard deviation .

Table 3. Seasonal fatty acid profiles (weight %) of oils extracted from discarded species of the Alboran Sea. Data are means of triplicate determinations. SD < 5%.

Table 4. Seasonal fatty acids profiles (g/100g fish), nutritional indexes and oxidative status of oils extracted from discarded species of the Alboran Sea.

Table 5. Seasonal composition of fatty acids in sn-2 position (% of total fatty acid weight) of oils extracted from discarded species of the Alboran Sea. Data are means of triplicate determination being SD <5%.

Table 6. Seasonal composition (%. molar basis) of main fatty acids and fractions in sn-2 position. Relative composition of the fractions in sn-2 position. Data are means of triplicate determination with SD <5%.

Table 1. Seasonal somatometric data of discarded species of the Alboran Sea: means ± standard deviation

Specie	Autumn		Winter		Spring		Summer	
	weight, g	size, cm	weight, g	size, cm	weight, g	size, cm	weight, g	size, cm
Small-spotted catshark	218.4±56.6	38.2±4.1	230.3±39.3	40.1±0.8	281.8±43.8	43.8±1.9	253.4±34.7	41.0±1.0
Bogue	78.7±2.6	15.0±0.1	93.8±4.8	21.3±0.8	84.0±12.4	20.7±1.5	86.2±10.4	21.0±2.0

Table 2. Seasonal proximate compositions of discarded species of the Alboran Sea: means ± standard deviation.

[%]	Small-spotted catshark				Bogue			
	Aut.	Win.	Spr.	Sum.	Aut.	Win.	Spr.	Sum.
Moisture	75.5±0.8	75.5±1.0	76.2±0.7	76.2±0.5	70.4±0.4	73.2±0.6	78.3±0.	77.8±0.7
Ash	2.8±0.3	2.9±0.5	2.7±0.2	3.2±0.6	4.2±0.5	3.5±0.3	4.0±0.3	3.4±0.4
Protein	18.1±0.7	21.5±0.6	20.3±0.5	22.6±0.8	15.8±0.2	20.0±0.6	16.9±0.4	19.5±0.8
Lipid	1.8±0.4	1.8±0.5	2.0±0.3	2.2±0.6	6.1±0.9	5.4±0.7	1.0±0.2	1.1±0.3

Table 3. Seasonal fatty acid profiles (weight %) of oils extracted from discarded species of the Alboran Sea. Data are means of triplicate determinations. SD < 5%.

Fatty acid	Small-spotted catshark						Bogue					
	Au.	Wi.	Sp.	Su.	Average	CV%	Au.	Wi.	Sp.	Su.	Average	CV%
C14:0	1.9	5.0	2.0	1.7	2.7	59.3	5.1	4.9	5.9	5.9	5.5	9.7
C16:0	15.1	17.0	19.1	16.3	16.9	9.9	19.3	16.9	19.5	15.2	17.7	11.6
C16:1n-7	4.7	6.4	5.5	5.2	5.5	13.1	6.6	6.4	6.3	6.7	6.5	2.8
C16:2n-4	0.7	1.0	0.8	0.7	0.8	17.7	1.1	1.0	1.2	1.6	1.2	21.5
C16:3n-4	0.6	0.7	0.0	0.6	0.5	67.4	0.0	0.2	0.0	0.9	0.3	155.3
C16:4n-1	0.0	0.0	0.0	0.5	0.1	200.0	0.0	0.0	0.0	0.3	0.1	200.0
C18:0	3.8	5.4	4.3	3.9	4.4	16.8	5.8	5.3	6.4	5.6	5.8	8.0
C18:1n-7	3.9	2.7	4.3	4.1	3.8	19.2	2.8	2.7	2.5	2.7	2.7	4.7
C18:1n-9	14.6	13.1	18.3	18.5	16.1	16.7	14.4	13.0	14.5	13.1	13.8	5.9
C18:2n-6	1.5	1.5	1.0	1.0	1.3	23.1	1.6	1.5	1.4	1.3	1.5	8.9
C18:3n-3	0.7	1.3	0.0	0.5	0.6	86.0	2.3	1.3	0.9	0.7	1.3	54.8
C18:4n-3	0.2	0.9	0.0	0.5	0.4	97.9	0.0	0.9	1.8	1.6	1.1	75.7
C20:1n-9	2.4	2.3	2.9	2.9	2.6	12.2	1.2	2.3	2.1	2.0	1.9	25.4
C20:3n-6	0.5	1.1	0.0	1.3	0.7	81.5	0.0	0.0	0.0	1.1	0.3	200.0
C20:4n-3	0.6	0.9	0.0	0.7	0.6	70.4	0.0	0.9	0.8	0.7	0.6	68.0
C20:5n-3	7.2	7.8	7.5	6.8	7.3	5.8	8.2	8.4	7.8	8.6	8.3	4.1
C22:1n-9	1.5	0.6	1.3	1.6	1.3	36.1	0.0	0.0	0.8	1.2	0.5	120.0
C22:5n-3	3.3	2.4	2.9	2.8	2.9	13.0	2.4	2.4	2.6	3.3	2.7	16.0
C22:6n-3	31.2	25.6	26.2	27.1	27.5	9.2	25.2	25.5	18.0	20.4	22.3	16.5
Others	5.8	4.3	3.6	3.4	4.3	25.4	3.8	6.4	7.6	6.9	6.2	26.9
SFA	20.8	27.3	25.5	21.8	23.9	12.8	30.3	27.2	31.8	26.7	29.0	8.5
MUFA	27.1	23.8	32.4	32.4	28.9	14.6	25.0	23.0	26.2	25.7	25.0	5.6
PUFA	46.4	44.5	38.5	42.4	43.0	7.9	41.0	43.5	34.4	40.7	39.9	9.7
n-6	2.0	2.5	1.0	2.3	2.0	34.1	1.6	1.5	1.4	2.4	1.7	26.5
n-3	43.1	40.3	36.7	38.3	39.6	7.0	38.2	40.8	31.8	35.4	36.6	10.6
n-3/n-6	21.6	15.8	36.1	16.4	22.5	42.0	23.3	28.1	22.5	14.7	22.2	25.0
EPA+DHA	38.4	33.4	33.8	33.9	34.9	6.8	33.4	33.8	25.8	29.1	30.5	12.5

Table 4. Seasonal fatty acids profiles (g/100g fish), nutritional indexes and oxidative status of oils extracted from discarded species of the Alboran Sea.

	Small-spotted catshark						Bogue					
	Aut.	Win.	Spr.	Sum.	Average	CV.%	Aut.	Win.	Spr.	Sum.	Average	CV.%
SFA	0.46	0.24	0.61	0.36	0.41	37.24	1.67	1.33	0.26	0.24	0.87	83.86
MUFA	0.59	0.21	0.77	0.53	0.53	44.48	1.37	1.13	0.21	0.23	0.74	81.65
PUFA	1.02	0.39	0.91	0.69	0.75	36.59	2.25	2.13	0.28	0.37	1.26	85.86
EPA	0.16	0.07	0.18	0.11	0.13	38.25	0.45	0.41	0.06	0.08	0.25	83.26
DHA	0.68	0.23	0.62	0.44	0.49	41.66	1.39	1.25	0.15	0.18	0.74	90.00
EPA+DHA	0.84	0.30	0.80	0.55	0.62	40.67	1.84	1.66	0.21	0.26	0.99	88.30
PI/100	3.22	2.88	2.75	2.88	2.93	6.86	2.74	2.88	2.21	2.55	2.59	11.06
AI	0.26	0.46	0.31	0.25	0.32	30.42	0.50	0.47	0.58	0.50	0.51	9.34
TI	0.12	0.17	0.15	0.14	0.15	15.62	0.19	0.16	0.23	0.19	0.19	14.61
HH	3.43	2.35	2.65	3.16	2.90	16.86	2.22	2.39	1.78	2.25	2.16	12.23

Table 5. Seasonal composition of fatty acids in sn-2 position (% of total fatty acid weight) of oils extracted from discarded species of the Alboran Sea. Data are means of triplicate determination being SD <5%.

	Small spotted catshark					Bogue			
	Aut.	**Win.**	**Spr.**	**Sum.**	**CV. %**	**Aut.**	**Win.**	**Spr.**	**CV. %**
EPA	3.6	4.2	3.0	2.7	19.6	3.2	3.9	4.5	16.5
DHA	43.1	43.1	63.4	55.8	19.5	48.1	36.9	51.2	16.5
SFA	18.5	20.9	12.9	14.4	22.1	22.7	31.8	22.5	20.7
MUFA	20.1	20.8	16.0	18.9	11.2	10.6	16.0	11.7	22.6
PUFA	56.2	57.5	71.1	65.9	11.3	60.9	50.6	65.8	13.2

Table 6. Seasonal composition (%. molar basis) of main fatty acids and fractions in sn-2 position. Relative composition of the fractions in sn-2 position. Data are means of triplicate determination with SD <5%.

Fatty Acid	Small-spotted catshark						Bogue[1]				
	Aut.	Win.	Spr.	Sum.	Average	CV. %	Aut.	Win.	Spr.	Average	CV.%
C16:0[α]	4.8	6.4	4.4	4.6	5.1	18.1	6.2	7.5	5.9	6.5	13.0
C18:1n-9[α]	3.1	3.2	0.9	4.0	2.8	47.5	1.2	1.9	1.8	1.6	23.2
C20:5n-3[α]	1.1	1.4	1.0	0.9	1.1	19.6	1.1	1.3	1.4	1.3	12.1
C22:6n-3[α]	17.0	13.0	19.7	17.3	16.8	16.6	15.2	10.8	15.3	13.8	18.7
SFA[α]	6.3	8.6	5.2	5.8	6.5	23.0	9.5	12.0	8.9	10.1	16.2
MUFA[α]	6.2	7.8	5.9	6.9	6.7	12.6	4.1	5.6	4.3	4.7	17.5
PUFA[α]	21.0	17.3	22.3	20.7	20.3	10.5	19.8	15.8	20.1	18.6	12.9
Relative percentage at sn-2											
SFA[β]	18.9	25.4	15.5	17.3	19.3	22.4	28.4	36.0	26.7	30.4	16.3
MUFA[β]	18.5	23.1	17.7	20.7	20.0	12.1	12.3	16.7	12.9	14.0	17.1
PUFA[β]	62.6	51.4	66.8	62.0	60.7	10.8	59.3	47.3	60.4	55.7	13.1
DHA[β]	51.1	38.9	59.1	51.8	50.2	16.7	45.7	32.4	46.0	41.4	18.8
DHA + EPA[β]	54.4	43.0	62.1	54.5	53.5	14.7	49.1	36.2	50.3	45.2	17.3

[1] Data of the sn-2 profile are not available for summer.

[α] Data are the product of percentage of fatty acid located in sn-2 position and the percentage of the fatty acid in the total profile divided by 100. Maximal value 33.3%

[β] Relative data are calculated by dividing data calculated in β by 33.33 and multiplying by 100. These data refer to the composition of sn-2 expressed as mole percentage.