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Fatty Acid Distribution in Muscle, Liver, and Gonads of Rays (*Dasyatis marmorata*, *Rhinobatos cemiculus*, and *Rhinoptera marginata*) from the East Tropical Atlantic Ocean

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If a great number of rays are fished in the Tropical East Atlantic Ocean for their caudal fins, only a small amount of ray flesh is processed. Among them, three species of rays, *Dasyatis marmorata*, *Rhinobatos cemiculus*, and *Rhinoptera marginata*, from the Mauritanian coast have been investigated for the fatty acid composition of their lipids. Gas chromatography and gas chromatography−mass spectrometry allowed identification of 50 molecules from muscles, livers, and gonads of these fishes. Principal component analysis, starting from >50 samples, reveals significant differences in various fatty acid distributions, related to the species and sex of the sampled fish. Some of them are preferentially present in one sex or in both species, whereas the occurrence of others characterizes the male and female of one or two species. The results show that rays are potential resources of polyunsaturated fatty acids (PUFA) and should be used in the diet of local populations. The lipidic fractions contained a high amount of PUFA (up to 30% of the total), mainly composed of docosa-4,7,10,13,16,19-hexaenoic acid, eicosa-5,8,11,14-tetraenoic acid, and eicosa-5,8,11,14,17-pentaenoic acid.

KEYWORDS: Rays; *Rhinobatos cemiculus*; *Rhinoptera marginata*; *Dasyatis marmorata*; fishes; lipids; fatty acids; DHA; EPA; multivariate analysis

INTRODUCTION

Great numbers of rays are caught in the Tropical East Atlantic Ocean (TEAO) along the Mauritanian coast. Most are fished for their caudal fins, which are removed, dried, and sold for Asian consumers. Although a small amount of ray flesh is processed, salted and air-dried, and sold for local use, the main part of fished rays is wasted. The lipid content of muscles of rays is low, but high amounts are observed in the liver and gonads (*1*). Lipids of fish are known to contain large proportions of long-chain polyunsaturated fatty acids (PUFA), but the range of these compounds between fish species is subject to various factors such as season, temperature, or food accessibility. Some reviews have been done on the effect of the temperature (*2*) or seasonal variation and distribution of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish lipids (*3*). Seasonal variations in fatty acid composition, particularly in EPA and DHA of sardine (*Sardinops melanosticta*) and mackerel (*Scomber japonicus*), were determined, but the results did not show great differences. Generally, saturated and monoenoic acids began to decrease in winter and were minimum in April, increasing again thereafter, whereas polyenoic acids showed a maximum in April, showing that sardine and mackerel caught in April−June were best for the preparation of oil containing polyunsaturated lipids (*4*). More recently, it was shown that salinity decrease and temperature increase produce an increase of muscular total lipids and of some fatty acids such as C16:0, C18:1, C20:5, and C22:6 (*5*). The effect of habitat on the fatty acid composition of the lipids of bonito (*Euthynnus pelamis*), samples of which were caught at different localities, was investigated, and the authors suggested that the differences among the fatty acid profiles may be due to environmental effects (*6*). Evidence suggests that differences in fatty acid compositions among various fish species may be due to differences in diet or environmental factors such as temperature,

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salinity, and depth at which the fish are caught in the Black Sea and in the Marmara Sea (*7*). The lipid content of the edible parts of some selachian fish (sharks), such as *Rhinobatos rhinobatos* (*8*), showed that PUFA accounted for 22.2−28.0% of total fatty acids ($n-3$, 15.5−25.0%; $n-6$, 1.9−6.4%) and seasonal hepatic lipid accumulation was observed for the sand shark, *Rhinobatos annulatus* (*9*). Some edible fish from the Senegalese coast containing high concentrations of $n-3$ PUFA (*10*) are now commonly consumed by the local population.

Because nutritional intakes of fatty acids for West African populations are mainly composed of saturated fatty acid from ruminants, unsaturated fatty acids of rays could be an attractive means to increase the consumption of PUFA by concerned populations. Defining the way to balance the saturated−unsaturated fatty acid ratio in the diet depends on the knowledge of the lipidic composition of the most frequently collected ray species.

The present paper is concerned with the fatty acid composition of lipids of three organs, muscle, liver, and gonads, of males and females of three rays, that is, *Rhinobatos cemiculus*, *Rhinoptera marginata*, and *Dasyatis marmorata*, which are commonly fished along the Mauritanian coast. Multivariate statistical analyses were applied to 54 samples for species and sex classifications using fatty acids and fatty acid families. Such methods were successfully applied in lipid research (*11*, *12*) and in species and variety differentiations (*13*).

MATERIALS AND METHODS

Species. Fish species determination agrees that of with Maigret and Ly (*14*). One hundred and forty-two specimens of *Rb. cemiculus* (Geoffroy Saint-Hilaire, 1817) (*14*), a guitar-fish from the TEAO, were caught off Nouakchott by local fishermen from November 1996 to February 1997. Specimens were measured (total length, TL) and weighed (eviscerated weight, EW). Male TL ranged from 0.36 to 1.70 m, and female TL ranged from 0.44 to 1.60 m. Male EW ranged from 0.5 to 12.4 kg, and female EW ranged from 0.35 to 10.2 kg. Fifty-five specimens of *Rp. marginata* (Geoffroy Saint-Hilaire, 1817) (*14*) were observed, having EW ranging from 0.4 to 2 kg for males and from 0.7 to 3.7 kg for females. Fifty-two specimens of *D. marmorata* (Steidachner, 1892) (*14*) were sampled; EW of males ranged from 0.5 to 1.2 kg, and EW of females ranged from 0.4 to 2.6 kg.

Lipid Extraction. Samples were kept on ice for <4 h before lipid extraction. For each analysis, ~10 g of muscles, livers, and gonads were homogeneized separately using a Waring Blendor. Lipids were extracted following a modified method of Bligh and Dyer (*15*) by using a mixture of 20 mL of methanol and 10 mL of chloroform during 5 min. After centrifugation, the lower chloroformic phase was kept and dried with sodium sulfate for total lipid content determination.

Fatty Acid Methyl Ester Analyses. Fatty acids were prepared by saponification of total lipids (50 mg) with KOH−ethanol, 2 mol·L^{-1} (1 mL), and acid-catalyzed methylation with methanolic hydrogen chloride as described by Christie (*16*). A Delsi gas chromatograph equipped with a flame ionization detector (FID) and a fused silica capillary column (25 m long, 0.28 mm i.d.) coated with Carbowax 20 M (0.2 μm phase thickness) was used for analyses. Temperatures used were 180 °C (10 min), raised at 2 °C·min^{-1} to 220 °C, for the column and 250 °C for the inlet and detector ovens.

Gas Chromatography—Mass Spectrometry (GC-MS). Identifications of fatty acids were carried out using mass spectrometry of their fatty acid methyl esters (FAME) and their pyrrolidides and compared to previously published results (*10*). *N*-Acyl pyrrolidide derivatives were prepared by direct treatment of FAME [10 μL with pyrrolidine/acetic acid (10:1, v/v; 1 mL)] in a sealed flask during 45 min and purified using silica thin-layer chromatography (TLC) with *n*-hexane/ether (1:2, v/v). Combined GC-MS was performed on a Hewlett-Packard model 5890 gas chromatograph instrument equipped with a mass spectrometer detector Hewlett-Packard model 5989A and a Hewlett-Packard 9000/345 integrator. A DB-1 fused silica capillary column, 30 m × 0.32 mm (i.d.) with a 0.25 μm stationary phase film was used from 170 °C (4 min hold) to 300 °C (3 °C·min^{-1}) for FAME and from 200 °C (4 min hold) to 310 °C (3 °C·min^{-1}) for *N*-acyl pyrrolidide derivatives. Helium was used as carrier gas, ion source temperature was 220 °C, and ionizing voltage was 70 eV.

Statistical Analysis. Principal component analysis (PCA) has been performed by using the data set transformed into centered and reduced variables (standardized PCA). The data sets were first composed by all variables (38 fatty acids) and their relative percentages determined on the Carbowax 20 M column. In a second attempt, for species and sex differentiation, the data set was composed as follows: for each species and for each sex, 3 fishes having approximately the same weight were taken, yielding 54 lipid extracts (18 from muscles, 18 from gonads, and 18 from livers). The seven variables were the sum of linear saturated fatty acids (ΣLS), the sum of branched saturated fatty acids (ΣBS), the sum of monounsaturated fatty acids (ΣMU), the sum of diunsaturated fatty acids (ΣDU), the sum of polyunsaturated fatty acids (ΣPU), the sum of $n-3$ fatty acids (ΣN3), and the sum of $n-6$ fatty acids (ΣN6). Data were processed with STAT-ITCF program version 4 (ITCF, France).

Table 1. Lipid Contents in Muscles, Liver, and Gonads of the Three Ray Species Investigated (Relative Percentage of Whole Fresh Organ)

total lipids (%)	*Rb. cemiculus*[a]		*Rp. marginata*[b]		*D. marmorata*[c]	
	males	females	males	females	males	females
muscle[d]	1.11	1.71	1.42	0.97	1.26	1.16
liver[e]	45.6	35.5	29.0	16.2	50.1	44.8
gonads[f]	5.12	4.66	5.42	4.96	4.20	3.30

[a] Mean of 13 adult rays (whole wt 9–15 kg). [b] Mean of 17 adult rays (whole wt 0.75–3 kg). [c] Mean of 9 adult rays (whole wt 0.50–0.75 kg). [d] Muscles were taken under the skin on the dorsal side. [e] 2.2% of the whole wt for *Rb. c.*; 5.5% of the whole wt for *D. m.*; 2.7% of the whole wt for *Rp. m.* [f] 2.0% of the whole wt for *Rb. c.* and *D. m.*; 1.2% of the whole wt for *Rp. m.*

RESULTS AND DISCUSSION

Lipid Content. Table 1 shows the lipid content of the muscles, livers, and gonads of the three male and female rays investigated. Fishes were collected through three years at the upwelling period (November until February). Lipid content in muscles ranged from 0.97 to 1.7%, in agreement with previously given content by Sebedio (*17*) and Piclet (*18*); the range is between 1 and 5% in other ray species. No significant difference in weighed lipids is observed between males and females for *D. marmorata*. In the case of *Rb. cemiculus*, muscles of females are richer in lipids, that is, 1.71 versus 1.11% (**Table 1**). For *Rp. marginata* a reverse phenomenon is observed (1.42% in males vs 0.97% in females).

The lipid content in livers ranged from 16 to 50%. These results can be compared to those obtained for the shark *Sphyrna lewini* [mean 20.6%, according to Hansel et al. (*19*)] and to results (76−83%) obtained for *Chimaera* and *Hydrolagus* (*20*). In all of these species the lipid content is 10−20% lower for females.

The lipid content in the gonads ranged from 3.3 to 5.4%. Observations realized in other fish give evidence for important variations in the lipid content of gonads. It varies from <1% in *Poecilia reticulata* (*21*) to 19.5% in *Ammodytes lancea* (*22*).

The total lipid fraction was mainly composed of neutral lipids, that is, triglycerides, in muscles and livers. In muscles the polar lipid fractions, mainly composed of phospholipids and glycolipids, represented <6% in *Rb. cemiculus*, 21% in *D. marmorata*, and 37% in *Rp. marginata*. In livers, the average total lipid fraction was <6% for both species. In gonads, polar lipids represented about one-third of the whole lipid fraction.

Table 2. Fatty Acid Composition[a] in Male Muscle[b] of the Rays Investigated (Percent of Total Fatty Acids)

fatty acid[c]	*D. marmorata*	*Rp. marginata*	*Rb. cemiculus*
n-14:0	2.24 ± 0.14	1.54 ± 0.05	5.83 ± 0.21
4,8,12-TM-13:0	0.60 ± 0.08	0.13 ± 0.02	0.43 ± 0.04
i-15:0	0.26 ± 0.02	0.28 ± 0.03	0.31 ± 0.03
n-15:0	0.29 ± 0.02	0.59 ± 0.01	0.34 ± 0.02
16: 3*n*–4	0.56 ± 0.00	0.07 ± 0.01	0.53 ± 0.03
16:1*n*–10	0.34 ± 0.10	0.77 ± 0.05	0.28 ± 0.02
16:1*n*–7	2.85 ± 0.20	3.23 ± 0.18	1.9 ± 0.10
16:1*n*–6	0.53 ± 0.01	tr[d]	0.8 ± 0.02
n-16:0	19.4 ± 1.3	25.9 ± 2.9	21.0 ± 2.0
17:1*n*–11	0.68 ± 0.03	1.02 ± 0.05	0.26 ± 0.02
i-17:0	0.98 ± 0.15	1.39 ± 0.54	0.73 ± 0.08
17:1*n*–8	0.30 ± 0.01	2.60 ± 0.13	0.44 ± 0.02
ai-17:0	0.22 ± 0.02	0.03 ± 0.01	0.41 ± 0.18
n-17:0	0.18 ± 0.01	0.14 ± 0.11	0.39 ± 0.02
18:4*n*–3	0.67 ± 0.03	2.34 ± 0.12	0.68 ± 0.03
18:3*n*–6	0.10 ± 0.01	0.04 ± 0.01	0.75 ± 0.05
18:2*n*–6	1.09 ± 0.45	0.52 ± 0.06	0.40 ± 0.23
18:1*n*–12	1.48 ± 0.04	0.90 ± 0.01	1.81 ± 0.05
18:1*n*–9	8.61 ± 0.50	6.60 ± 0.40	8.00 ± 0.50
18:1*n*–7	3.49 ± 0.20	6.25 ± 0.19	0.54 ± 0.02
n-18:0	9.23 ± 0.61	25.0 ± 1.7	13.5 ± 0.7
20:5*n*–3	4.95 ± 0.13	2.26 ± 0.18	4.73 ± 0.24
20:4*n*–6	5.57 ± 0.16	8.00 ± 0.60	7.32 ± 0.40
7,15–20:2	0.80 ± 0.01	0.95 ± 0.00	0.43 ± 0.03
20:1*n*–10	0.18 ± 0.03	0.10 ± 0.01	0.46 ± 0.02
20:1*n*–9	0.50 ± 0.02	0.33 ± 0.03	0.21 ± 0.01
20:1*n*–7	0.15 ± 0.01	0.66 ± 0.05	0.63 ± 0.06
n-20:0	0.21 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
22:6*n*–3	16.1 ± 1.5	3.74 ± 0.17	18.7 ± 1.8
22:5*n*–3	9.37 ± 0.58	2.36 ± 0.12	3.91 ± 0.16
6,14–22:2	0.19 ± 0.02	0.80 ± 0.04	0.23 ± 0.01
7,13–22:2	3.46 ± 0.14	0.07 ± 0.01	2.28 ± 0.15
7,15–22:2	3.46 ± 0.60	0.73 ± 0.03	0.35 ± 0.02
22:1*n*–15	0.22 ± 0.01	0.08 ± 0.01	0.42 ± 0.02
22:1*n*–14	0.20 ± 0.01	0.13 ± 0.01	0.55 ± 0.03
n-22:0	0.42 ± 0.03	0.15 ± 0.01	0.60 ± 0.03

[a] Relative percentage obtained using CW 20 M results. Mean of three adult rays collected between November and February 1996, 1997, and 1998. [b] Muscles were taken under the skin on the dorsal side. [c] Given following the retention order. [d] tr = trace (<0.01%).

Fatty Acid Composition. Each fatty acid was identified, as methyl ester or pyrrolidide derivative, from its mass spectrum and its GC mobility. *N*-Acyl pyrrolidides are well-known as useful derivatives for fatty acid analysis by GC-MS because they have a more pronounced tendency to retain the positive charge under electron impact and give homologous fragment ions with an interval of 14 atomic mass units (amu) or 12 amu if a double bond is present (*23*). In the analysis given in **Table 2**, only two triunsaturated fatty acids were present in the acid mixture, namely, 16:3(*n*−4) and 18:3(*n*−6). These compounds were readily identified from the GC and MS data. In a preliminary GC analysis (methyl esters) on Carbowax 20 M, the equivalent chain length (ECL) values determined (17.23 for the hexadecatrienoic acid and 18.97 for the octadecatrienoic acid) were in good concordance with those given in the literature (*24*, *25*). The first fatty acid pyrrolidide was eluted from the DB-1 column after the pentadecanoyl pyrrolidide. The molecular ion peak present at *m*/*z* 303 confirmed a 16:3 acid structure.

The second acid pyrrolidide was eluted after the heptadecanoyl pyrrolidide and displayed a molecular ion peak at *m*/*z* 331, thus confirming an 18:3 acid structure.

Mass spectra of both acid pyrrolidides exhibited the same differences of 12 amu between homologous fragment ions at *m*/*z* 154 and 166 (delta 6 unsaturation), *m*/*z* 194 and 206 (delta 9 unsaturation), and *m*/*z* 234 and 246 (delta 12 unsaturation).

Table 3. Sums of the Various Fatty Acid Families in Male and Female Muscle Tissues of the Rays Investigated[a]

fatty	*D. marmorata*		*Rp. marginata*		*Rb. cemiculus*	
acid[b]	male	female	male	female	male	female
ΣBS	2.0 ± 0.2	4.9 ± 0.1	1.8 ± 0.3	2.2 ± 0.0	1.8 ± 0.5	1.4 ± 0.0
ΣLS	32.0 ± 0.9	35.8 ± 0.9	53.4 ± 1.2	43.8 ± 0.8	41.8 ± 2.3	40.1 ± 1.3
ΣMU	19.8 ± 0.3	15.5 ± 0.2	23.4 ± 0.5	23.4 ± 0.4	15.8 ± 0.5	18.4 ± 0.3
ΣDU	9.0 ± 0.3	5.5 ± 0.3	2.4 ± 0.2	3.1 ± 0.6	3.8 ± 0.3	2.8 ± 0.0
ΣPU	36.3 ± 1.1	37.7 ± 1.1	18.8 ± 0.8	27.3 ± 0.1	36.6 ± 1.9	36.7 ± 1.6
Σ*n*–3	31.1 ± 1.3	22.6 ± 0.8	10.7 ± 0.2	19.1 ± 0.4	28.0 ± 1.7	28.1 ± 1.9
Σ*n*–6	7.3 ± 0.6	12.3 ± 0.5	8.6 ± 0.6	8.8 ± 0.8	8.8 ± 0.4	5.9 ± 0.2

[a] Relative percentage of total fatty acids determined on a Carbowax 20 M column. Mean for adult rays caught from November to February during three years. [b] Sum of branched saturated fatty acids (ΣBS), sum of linear saturated fatty acids, (ΣLS), sum of monounsaturated fatty acids (ΣMU), sum of diunsaturated fatty acids (ΣDU), sum of polyunsaturated fatty acids (ΣPU), sum of *n*–3 fatty acids (ΣN3), and sum of *n*–6 fatty acids (ΣN6).

Thus, the methylene-interrupted fatty acids were identified as 6,9,12-hexadecatrienoic acid (16:3*n*−4) and 6,9,12-octadecatrienoic acid (18:3*n*−6). All other unsaturated fatty acids, including the unusual delta 7,13/7,15 and 6,14 structures, were identified in such a way. Experimental results were also compared to recently published work (*10*). Approximately 35−40 fatty acids (FA) were characterized, and **Table 2** gives the FA profiles in the case of male muscles for the rays analyzed. Compounds are listed in increasing order of chromatographic retention times of their FAME on the DB-1 column. As can be seen, the main FA in all samples is palmitic acid (19−26% of total FA) followed by another saturated FA, stearic acid (*n*−18:0, 9−25%). Among the monounsaturated FA, oleic acid is the main one at 6−9%. The *n*−6 FA group is mainly composed of linoleic (18:2*n*−6, 0.4−1.1%) and arachidonic acid (20:4*n*−6, 5.6−8.0%). Docosahexaenoic acid (DHA, 22:6*n*−3) is slightly higher in *D. marmorata* and *Rb. cemiculus* (16 and 19%, respectively) and lower in *Rp. marginata* (3.7%). Two other *n*−3 FA are significantly present: eicosapentaenoic acid (EPA, 20:5*n*−3), ranging from 2 to 5%, and docosapentaenoic acid (22:5*n*−3), which varies from 2 to 9%. The not so common non-methylene-interrupted acids, 7,13−22:2; 7,15−20:2, and 7,15−22:2, were present at relatively high levels accompanied by the quite rare 6,14−22:2.

Multivariate Statistical Analyses. The data set of 54 total lipid fractions was composed of three samples of each part of fish for the three species and for each sex. In the course to follow the change of FA composition between male and female rays species for the three parts of fishes investigated, we have grouped them in various families to reduce the number of variables without neglecting the role of the minor FA. Therefore, seven variables have been considered, including linear saturated (ΣLS), branched saturated (ΣBS), monounsaturated (ΣMU), diunsaturated (ΣDU), and polyunsaturated (ΣPU). We have also considered the variation of two essential FA groups belonging to the *n*−6 family (Σ*n*−6), in particular arachidonic FA, and the *n*−3 family (Σ*n*−3), with EPA and DHA. **Tables 3**−**5** show the FA group profiles for the muscles, gonads, and livers of the rays. The ΣBS composed of 4,8,12-TM-13:0, *i*-15:0, *i*-17:0, and *ai*-17:0 represented in each case 0.8−5.2%. The ΣLS mainly composed of *n*-14:0 to *n*-22:0 represented 26−59%. The ΣMU composed of 13 FA ranged from 15 to 32%. The ΣDU composed of 5 FA ranged from 0.8 to 9%. The ΣPU composed of 9 FA ranged from 19 to 38%. If the Σ*n*−3 PUFA content is relatively high (9−31%), we observed low amounts of *n*−6 PUFA because the Σ*n*−6 is never >17%.

Table 4. Sums of the Various Fatty Acid Families in Male and Female Liver Tissues of the Rays Investigated[a]

fatty acid[b]	*D. marmorata*		*Rp. marginata*		*Rb. cemiculus*	
	male	female	male	female	male	female
ΣBS	1.5 ± 0.0	3.4 ± 0.0	1.4 ± 0.6	3.9 ± 0.0	0.8 ± 0.1	1.1 ± 0.4
ΣLS	26.0 ± 1.1	35.5 ± 2.9	50.4 ± 1.6	41.2 ± 2.2	40.8 ± 1.2	41.2 ± 2.2
ΣMU	32.3 ± 1.3	31.2 ± 0.9	20.7 ± 0.6	15.4 ± 0.4	20.5 ± 0.3	25.4 ± 1.3
ΣDU	6.4 ± 0.8	0.8 ± 0.3	5.1 ± 0.4	2.0 ± 0.5	3.6 ± 0.2	1.7 ± 0.0
ΣPU	33.5 ± 1.8	26.4 ± 2.8	22.2 ± 1.0	24.1 ± 1.0	33.5 ± 0.3	29.9 ± 2.4
Σ*n*–3	23.2 ± 1.5	17.2 ± 2.3	13.6 ± 1.0	18.7 ± 0.2	23.8 ± 0.8	19.3 ± 2.1
Σ*n*–6	11.2 ± 0.6	10.4 ± 0.7	8.8 ± 0.4	7.6 ± 0.5	6.6 ± 0.1	7.6 ± 0.7

[a] Relative percentage of total fatty acids determined on a Carbowax 20 M column. Mean for adult rays caught from November to February during three years. [b] See **Table 3** for fatty acid abbreviations used.

Table 5. Sums of the Various Fatty Acid Families in Male and Female Gonad Tissues of the Rays Investigated[a]

fatty acid[b]	*D. marmorata*		*Rp. marginata*		*Rb. cemiculus*	
	male	female	male	female	male	female
ΣBS	5.2 ± 0.3	4.1 ± 0.0	1.5 ± 0.0	2.9 ± 0.6	1.8 ± 0.1	4.1 ± 0.0
ΣLS	46.0 ± 0.2	46.5 ± 1.4	59.0 ± 0.4	58.4 ± 0.3	35.1 ± 0.8	42.2 ± 1.2
ΣMU	25.3 ± 0.8	27.9 ± 0.5	22.6 ± 0.1	24.6 ± 0.3	25.7 ± 0.6	23.4 ± 1.0
ΣDU	4.1 ± 0.0	6.7 ± 0.5	1.7 ± 0.0	4.4 ± 0.5	6.3 ± 0.1	4.2 ± 0.5
ΣPU	19.3 ± 0.2	17.2 ± 0.6	15.0 ± 0.3	21.1 ± 0.9	30.9 ± 0.5	26.3 ± 0.2
Σ*n*–3	13.5 ± 0.3	10.9 ± 1.3	8.8 ± 0.4	15.0 ± 1.3	22.5 ± 1.3	18.8 ± 0.3
Σ*n*–6	8.6 ± 0.6	7.7 ± 0.4	10.6 ± 0.4	9.6 ± 0.4	11.3 ± 1.2	16.0 ± 0.8

[a] Relative percentage of total fatty acids determined on a Carbowax 20 M column. Mean for adult rays caught from November to February during three years. [b] See **Table 3** for fatty acid abbreviations used.

PCA on reduced and centered variables was realized by diagonalization of the correlation matrix (*3*, *4*), and its main results, for each part of the fishes, are given in **Table 6** for the three main components (axes). Graphic representation of the projection of variables and samples onto the two first principal components is given in **Figures 1**−**3**. As indicated in **Table 6**, the two first components have a variance (eigenvalue) greater than unity, and the three first components account for 97.1% of the total information for the muscles, 90.0% for the gonads, and 91.7% for the livers.

Table 6. Eigenvalues, Percentages of Variance, and Factor Loadings Using Standardized PCA for the Sums of the Various Fatty Acid Families in Male and Female Muscle, Gonad, and Liver Tissues of the Rays Investigated

	muscles			gonads			livers		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
eigenvalue	4.05	1.97	0.78	3.74	1.67	0.89	3.16	2.57	0.69
%[a]	57.9	28.1	11.1	53.4	23.9	12.7	45.1	36.7	9.9
ΣBS[b]	–0.47	–0.83	0.28	–0.11	0.76	0.57	–0.24	–0.73	0.56
ΣLS	0.95	–0.17	–0.21	–0.90	–0.43	–0.03	–0.87	0.50	–0.04
ΣMU	0.82	0.24	0.44	0.23	0.88	–0.32	0.57	–0.74	–0.18
ΣDU	–0.74	0.25	0.60	0.76	–0.24	0.26	0.22	–0.83	–0.40
ΣPU	–0.96	0.11	–0.23	0.95	–0.20	0.11	0.93	0.30	0.19
Σ*n*–3	–0.84	0.49	–0.21	0.92	–0.18	0.24	0.91	0.25	0.28
Σ*n*–6	–0.30	–0.94	–0.06	0.74	0.06	–0.56	0.55	0.64	–0.26

[a] Percentage of information (total variance). [b] See **Table 3** for fatty acid abbreviations used.

In muscles, the first component (57.9%), which is highly correlated positively to linear saturated FA (0.95, **Table 6**) and monounsaturated FA (0.82) and negatively correlated to PUFA and DUFA (−0.96 and −0.74 respectively), leads to a separation of the muscles of the three rays, *D. marmorata* containing less ΣLS (**Table 3**) than the two other species (**Figure 1**). The second component (28.1%), which is highly correlated negatively to Σ*n*−6 PUFA (−0.94) and ΣBSFA (−0.83) and positively with Σ*n*−3 PUFA (0.49), leads to a separation of male from female *D. marmorata*.

In the gonads, the first component (53.4%), which is positively correlated to ΣPUFA (0.95, **Table 6**) and negatively correlated with ΣLSFA (−0.90), leads to a separation of the gonads of *Rb. cemiculus* from the two other ray species (**Figure 2**). Male and female gonads of this last species are well distinguished onto this axis one, considering the change in ΣLSFA (35% for males vs 42% for females) and ΣPUFA (31 vs 26%, respectively). The second component (23.9% of the total information) correlated positively with ΣBSFA (0.76) and

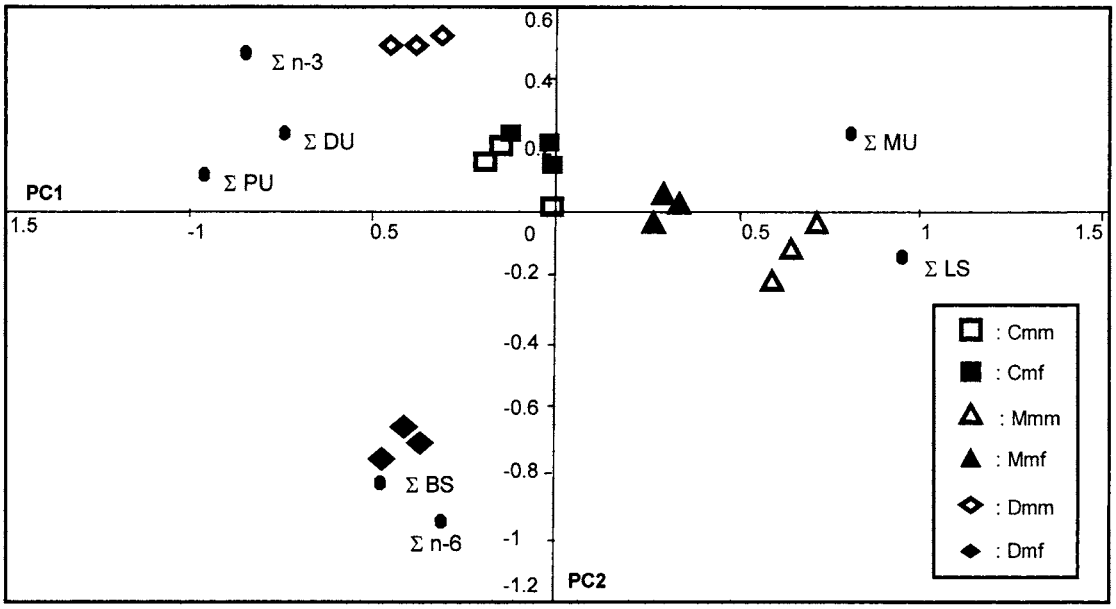

Figure 1. Two-dimensional plot of the fatty acid methyl ester groups from the muscle of the rays investigated in PCA; Cmm, muscle of male *Rb. cemiculus*; Cmf, muscle of female *Rb. cemiculus*; Mmm and Mmf, muscles of male and female *Rp. marginata*; Dmm and Dmf, muscles of male and female *D. marmorata*. For fatty acid methyl ester groups see Materials and Methods.

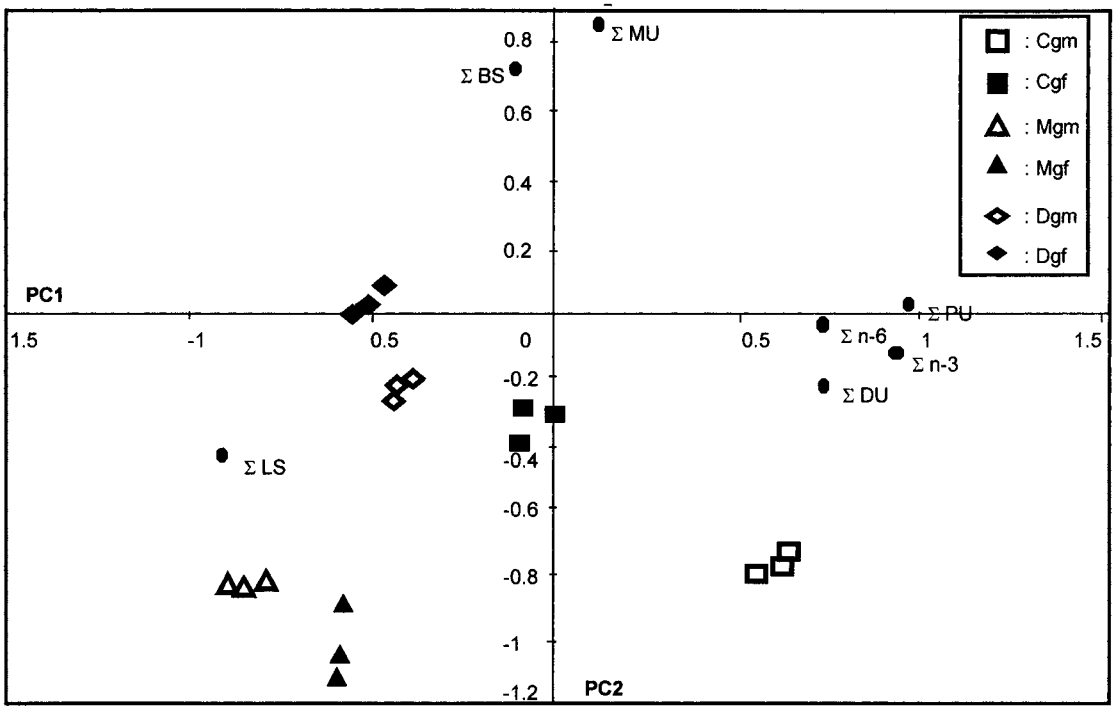

Figure 2. Two-dimensional plot of the fatty acid methyl ester groups from the gonads of the rays investigated in PCA: Cgm, gonads of male *Rb. cemiculus*; Cgf, gonads of female *Rb. cemiculus*; Mgm and Mgf, gonads of male and female *Rp. marginata*; Dgm and Dgf, gonads of male and female *D. marmorata*. For fatty acid methyl ester groups see Materials and Methods.

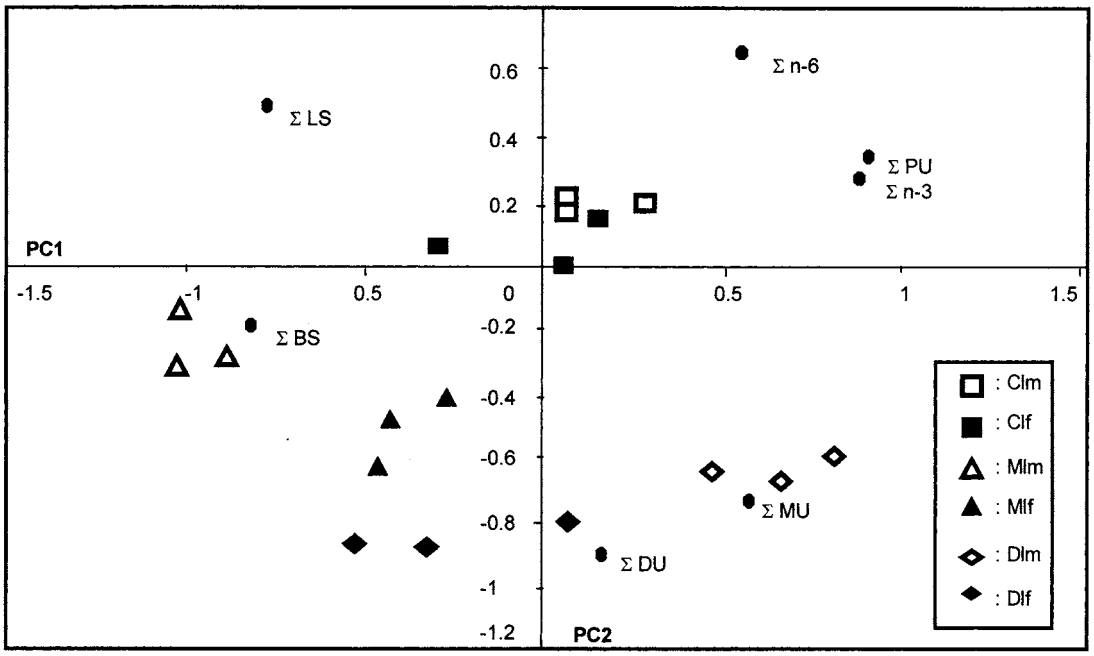

Figure 3. Two-dimensional plot of the fatty acid methyl ester groups from the liver of the rays investigated in PCA: Clm, liver of male *Rb. cemiculus*; Clf: liver of female *Rb. cemiculus*; Mlm and Mlf, livers of male and female *Rp. marginata*; Dlm and Dlf, livers of male and female *D. marmorata*. For fatty acid methyl ester groups see Materials and Methods.

ΣMUFA (0.88), leading to the separation of *D. marmorata* from *Rp. marginata* as the first species presents a higher content than the second species in BSFA, and a lower content in LSFA (see **Table 5**).

In the livers, the first component (45.1% of the total information), which is positively correlated to ΣPUFA (0.93, **Table 6**), in particular with Σ*n*−3 (0.91), and negatively with ΣLSFA (−0.87), leads to a separation of the livers of *Rp. marginata* males from those of *D. marmorata* males (**Figure 3**). Differentiation of *Rb. cemiculus* male and female livers from the other ones was achieved by the second component (36.7% of the total information), which is correlated negatively with ΣBSFA (−0.73), ΣMUFA (−0.74), and ΣDUFA (−0.83).

Conclusion. Although significant differences in lipid contents are observed between species and organs of rays, this work demonstrates that lipids of rays have a fatty acid composition rich in essential fatty acids and should be used in the diet of local populations. The amounts of EPA and DHA are relatively

high in both organs and of the same order as that observed in fish used for oil production. Therefore, such rays, which are now fished for their caudal fins only or for drying only a small amount of flesh, could be used for better development.

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