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Influence of season on the lipid content and fatty acid profiles of three tilapia species (*Oreochromis niloticus*, *O. macrochir* and *Tilapia rendalli*) from Madagascar

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Abstract

Lipids extracted from three tilapia species (*Oreochromis niloticus*, *O. macrochir* and *Tilapia rendalli*) of mean weight 100–250 g collected in Itasy lake of Madagascar highlands during three seasons, were analyzed for their fatty acid compositions. The muscle fishes contained less than 1.4% lipid by weight. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS), allowed identification of more than 40 fatty acids and 30 of them were quantified in muscle tissue lipids of fishes. Among them, those occurring in the highest proportions were palmitic, stearic, oleic, palmitoleic and linoleic acids. The main polyunsaturated fatty acids were arachidonic acid, eicosa-5,8,11,14,17-pentaenoic acid (EPA) and docosa-4,7,10,13,16,19-hexaenoic acid (DHA). The relative amount of these acids changed significantly between species and season of collect, in particular DHA which decreased from 11.4% to 6.0% for *O. macrochir* during the spring to autumn period. For *O. niloticus* and *T. rendalli*, DHA decreased from 9.8% to 4.9% and to 10.1% to 4.4%, respectively during the same period. Therefore the $\sum n-3/\sum n-6$ ratios vary, for each species according to season of collect, being lower in autumn (0.5–0.6) and raising up to 0.7–1.6 in winter. Multivariate statistical analyses, starting from the fatty acid composition, lipid percentage and biometric ratio (weight/size) data of 113 samples, reveals significative differences between species and season of collect. Furthermore differences in fatty acid profiles are higher within season than species. The results show that wild tilapias from tropical lakes of the Madagascar highlands possess beneficial properties and therefore contribute significantly of the $n-3$ fatty acids intake of the local population diet.

Keywords: Tilapias; *Oreochromis niloticus*; *O. macrochir*; *Tilapia rendalli*; Fishes; Lipids; Fatty acids; DHA; EPA; Season influence; Multivariate analysis

1. Introduction

The occurrence of $n-3$ polyunsaturated fatty acids ($n3$ PUFA) has been recognized as important beneficial properties for the prevention of human coronary artery desease (Mensik & Kathan, 1990). Researchers have shown that freshwater fish generally contain lower proportions $n3$ PUFA than marine fish (Rahman, Huah, Hassan, & Daud, 1995; Vlieg & Body, 1988). Futhermore since fish need PUFAs to provide tolerance to low water temperature (Bolgova, Bogdan, & Ripatti,

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1983), low amounts should be expected in warmer waters like in tropical areas such as Madagascar. In this country, although the coast population consume seawater fish, population of the highlands, which represent the majority of the overall population, consume traditionally proteins and lipids coming mainly from pork and beef (*Bos indicus*, Gaydou, Iatrides, Ramananarivo, Artaud, & Peiffer, 1984). The consumption of freshwater fish contributes significantly to the amount of *n*3 PUFA in the diet of the highlands population. Among the different freshwater fishes found on local markets, various tilapia species are available. In the case of tilapias, some studies have been done on FA composition of different part of this fish and FA composition of wild and cultured tilapias have been compared (Al-Shagrawi, Al-Sheddy, & Hewedy, 1998; Xie, Yang, Wang, & Yao, 2001). Zenebe, Ahlgren, and Boberg (1998) have shown that freshwater fish such as tilapia from tropical lakes of Ethiopian Rift Valley, have a PUFA content comparable to temperate freshwater fish such as sheepshead, *Aplodinotus grunniens* or tullibee, *Coregonus artedii* (Ackman, 1967). Futhermore Luzia, Sampaio, Castellucci, and Torres (2003) have studied the influence of two seasons (summer and winter) on the total lipid, FA and cholesterol contents in five popular fish species, including tilapia.

Since the fishery ministery in Madagascar encourages the freshwater aquaculture among the riceculturists of the highlands, a program to evaluate the PUFA composition between the widespread tilapia species has been undertaken.

The present paper is concerned with the muscle lipid content and the FA composition of three wild tilapias, i. e., *Oreochromis niloticus*, *O. macrochir* and *Tilapia rendalli*, which are commonly found in the tropical lakes and ricefields of the Madagascar highlands.

2. Materials and methods

2.1. Species

Among the various freshwater fishes found in the Highlands of Madagascar, and consumed by local population, we investigated three tilapia species, representing a large volume of sales according to the Agricultural Ministery yearly Report of Madagascar. Tilapia species identification agrees with Arnoult (1959), Kiener (1963) and Moreau (1970). *Oreochromis niloticus*, local name ''barahoa'' has been introduced in the highlands of Madagascar in 1956 and is now largely found on every market. *Oreochromis macrochir* local name ''malemiloha'' has been introduced in various lakes of the highlands of Madagascar and in particular in Itasy lake in 1951. Now it is found mainly in other large lakes such as Alaotra Lake. *Tilapia rendalli* was introduced at the same period and is found in most of lakes and ricefields.

Fishes investigated were caught in Itasy Lake (150 km west from Antananarivo, altitude, 1100 m) during three seasons: winter (15 of July), spring (end of October) and summer (March), in 1997, 1998. Specimens were measured and weighed. The number of specimens investigated by species and season is given in Table 1. Fishes ranged from 0.16 to 0.30 m, and weighed from 100 to 250 g. The biometric index (BI, g weight divided by cm size) is given in Table 2.

2.2. Lipid extraction

Individual fishes were dissected and portions of muscle tissue below the dorsal fin, devoid of skin and bone were kept in ice for less than 4 h before lipid extraction. For each analysis, about 10 g of white muscle were homogeneized separately using a warring blender. Lipids were extracted following a modified from Bligh and Dyer method (1959) by using a mixture of 20 ml methanol and 10 ml chloroform during 5 min. After centrifugation, the lower chloroformic phase was kept

Table 1
Number of tilapia species investigated during the three seasons

Season[a]	*T. rendalli*	*O. niloticus*	*O. macrochir*	$\sum$
Winter	8	10	14	32
Spring	13	12	15	40
Autumn	14	13	14	41
$\sum$	35	35	43	113

[a] Winter (july); spring (october); autumn (march).

Table 2
Biometric index and lipid values determined for the tilapia species investigated

Season	*T. rendalli*		*O. niloticus*		*O. macrochir*	
	BI[a]	Lipid[b]	BI[a]	Lipid[b]	BI[a]	Lipid[b]
Winter	2.33 ± 0.08	0.86 ± 0.15	2.45 ± 0.12	0.83 ± 0.17	2.30 ± 0.32	1.37 ± 0.28
Spring	2.92 ± 0.32	1.29 ± 0.32	2.74 ± 0.14	0.99 ± 0.29	2.47 ± 0.32	1.29 ± 0.24
Autumn	2.31 ± 0.16	0.94 ± 0.08	2.39 ± 0.14	1.08 ± 0.13	2.49 ± 0.32	1.18 ± 0.10

[a] Biometric index (g weight divided by cm size).
[b] % Wet weight basis.

and dried with sodium sulphate for total lipid content determination.

2.3. 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 (1982). 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 20M (0.2 μm phase thickness) was used for analyses. Temperatures used were 180 °C (10 min), then until 220 °C, 2 °C min^{-1} for column and 250 °C for inlet and detector ovens. Available fatty acid methyl esters (FAME) were used for quantitative external standards.

2.4. Gas chromatography–mass spectrometry

Identifications of fatty acids were carried out using mass spectrometry of their FAME and their pyrrolidides and compared to previously published results (Njinkoué, Barnathan, Miralles, Gaydou, & Samb, 2002; Ould El Kebir, Barnathan, Siau, Miralles, & Gaydou, 2003). *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 were performed on a Hewlett–Packard Model 5890 gas chromatograph instrument equipped with Mass Spectrometer detector Hewlett–Packard Model 5989A and Hewlett–Packard 9000/345 integrator. A DB1 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 220 °C, ionizing voltage 70 eV.

2.5. Statistical analysis

Multivariate statistical analyses were applied for species and season classifications using in particular fatty acids. Such methods were successfully applied in lipid research (De Silva, Gunasekera, & Austin, 1997; Ould El Kebir et al., 2003; Ratovohery, Lozano, & Gaydou, 1988) and in species and variety differentiations (Mouly, Arzouyan, Gaydou, & Estienne, 1994). Principal component analysis (PCA) and Factorial Discriminant Analysis (FDA) have been performed by using the data set (composed of 113 fishes, Table 1) transformed into centered and reduced variables (standardized PCA). The data set was first composed by all variables: relative percentages of 48 fatty acids determined on the Carbowax 20M column, the biometric index (BI) and the lipid values (%LIP, percentage of lipids in white muscle). In a second attempt, for species and season differentiations the data set was composed of 32 variables (30 fatty acids, the BI and the %LIP), taking into account high correlation coefficients between variables. Data were processed with STAT-ITCF program version 4 (ITCF, France).

3. Results and discussion

3.1. Lipid content

The lipid content, in fishes, change according to species, diet, geographical origin and season. In a same species, sex maturity and age contribute to significant differences. Table 2 shows the lipid content of the dorsal muscles of the three wild tilapia species investigated from the Itasy lake of Madagascar highlands. Fishes were collected through three seasons, winter, spring

Table 3
Mean weight percentage of main fatty acids in *Oreochromis niloticus* during the three seasons investigated

Fatty acid	Season		
	Winter mean	Spring mean	Autumn mean
12:0	0.33 ± 0.19	0.58 ± 0.16	0.16 ± 0.07
13:0	0.35 ± 0.18	0.40 ± 0.08	0.20 ± 0.08
14:0	2.57 ± 0.94	2.14 ± 0.16	2.19 ± 0.58
14:1*n*7	0.31 0.18	0.32 ± 0.08	0.27 ± 0.07
ai-15:0	0.34 ± 0.14	0.39 ± 0.08	0.49 ± 0.14
15:0	1.78 ± 0.44	2.64 ± 0.24	1.74 ± 0.32
15:1*n*6	0.56 ± 0.18	1.08 ± 0.13	0.36 ± 0.11
i-16:0	0.43 ± 0.12	1.02 ± 0.18	0.44 ± 0.10
ai-16:0	0.85 ± 0.15	0.29 ± 0.08	0.79 ± 0.18
16:0	*17.4* ± 1.25	*15.5* ± 0.47	*20.3* ± 1.60
16:1*n*7	*8.32* ± 0.94	*6.96* ± 0.23	*9.93* ± 2.14
i-17:0	1.39 ± 0.28	1.37 ± 0.14	1.44 ± 0.29
17:0	2.18 ± 0.41	2.64 ± 0.22	2.07 ± 0.47
i-18:0	0.34 ± 0.10	0.77 ± 0.08	0.27 ± 0.17
ai-18:0	1.01 ± 0.39	0.81 ± 0.08	0.71 ± 0.22
17:2	0.10 ± 0.19	0.02 ± 0.04	nd[b]
18:0	*6.59* ± 0.47	*8.48* ± 0.29	*5.60* ± 1.07
18:1(*n*9 + *n*7)[a]	*11.8* ± 1.70	*10.6* ± 0.54	*13.8* ± 1.87
18:2*n*6	*4.48* ± 0.75	*3.48* ± 0.29	*7.88* ± 1.80
18:2*n*4	0.20 ± 0.10	0.22 ± 0.06	0.20 ± 0.04
18:4*n*3	0.43 ± 0.24	0.15 ± 0.06	0.53 ± 0.12
20:0	0.27 ± 0.09	0.26 ± 0.06	0.24 ± 0.06
20:1(*n*9 + *n*7)[a]	0.88 ± 0.37	0.76 ± 0.18	0.75 ± 0.28
20:2*n*6	0.64 ± 0.18	0.39 ± 0.06	0.48 ± 0.08
20:3*n*6	0.58 ± 0.09	0.73 ± 0.18	0.55 ± 0.07
20:4*n*6	6.08 ± 0.74	6.15 ± 0.45	5.19 ± 1.20
20:3*n*3	0.65 ± 0.29	0.40 ± 0.05	0.64 ± 0.09
20:5*n*3	1.72 ± 0.41	1.65 ± 0.07	1.20 ± 0.31
22:5*n*6	*2.70* ± 0.59	*2.57* ± 0.15	*1.55* ± 0.41
22:6*n*3	*7.72* ± 1.28	*9.83* ± 0.31	*4.95* ± 1.53

[a] Together for multivariate analyses, since in some GC chromatograms these isomers were not well resolved.
[b] Not detected.

and autumn. Lipid content ranged from 0.87% to 1.4% in white muscle (Table 2), showing that tilapias belong to lean fish according to Bennion (1980). Our results are in agreement with previously given content by Justi, Hayashi, Visentainer, de Souza, and Matsushita (2003), Al-Shagrawi et al. (1998) and Luzia et al. (2003) since the range is comprised between 1% and 1.9% in other tilapias. The mean total lipid of the edible portion from wild tilapia is significantly lower compared to that found in cultured tilapia (1.18 vs 2.62) according to Al-Shagrawi et al. (1998). No higher difference in lipid weight is observed between seasons for *O. macrochir* (1.2–1.4%). In the case of *T. rendalli* and *O. niloticus*, lower lipid content are observed in winter (0.83–0.86%) and higher content in spring for *T. rendalli* (1.3%) and in autumn for *O. niloticus* (1.1%).

3.2. Fatty acid composition

Each FA was identified, as methyl ester (FAME) 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 since they have a more pronounced tendency to retain the positive charge under electron impact and give homologous fragment ions with an interval of 14 amu (atomic mass units), or 12 amu if a double bond is present (Andersson, 1978). Experimental results were also compared to recently published work (Njinkoué et al., 2002; Ould El Kebir et al., 2003). Tables 3–5 give the mean weight percentage of 30 FA, for each tilapia species and during the three seasons. Table 6 gives the mean of these FA averaged for all tilapia species during the three seasons and Table 7 results considering seasons together for each tilapia species. Among them, those occurring in the highest proportions were palmitic acid (16:0; 15–21%), stearic acid (18:0; 5–8%), oleic + vaccenic acids (18:1*n*9 + 18:1*n*7; 6–14%), palmitoleic acid (16:1*n*7; 6–14%), linoleic acid (18:2*n*6; 2–8%), arachidonic acid (20:4*n*6; 4–8%), eicosa-5,8,11,14-tetraenoic acid (20:4*n*6, 3.7–8.2%), eicosa-5,8,11,14,17-pentaenoic acid (EPA, 20:5*n*3; 1–3%) and docosa-4,7,10,13,16,19-hexaenoic acid (DHA, 22:6*n*3; 4–11%). These results are in agreement with previous published data on FA

Table 4
Mean weight percentage of main fatty acids in *Oreochromis macrochir* during the three seasons investigated

Fatty acid	Season		
	Winter mean	Spring mean	Autumn mean
12:0	0.17 ± 0.07	0.72 ± 0.26	0.24 ± 0.13
13:0	0.20 ± 0.09	0.51 ± 0.18	0.25 ± 0.15
14:0	*5.61* ± 0.62	*2.19* ± 0.71	*2.04* ± 0.75
14:1*n*7	0.10 ± 0.03	0.25 ± 0.07	0.24 ± 0.22
ai-15:0	0.36 ± 0.11	0.60 ± 0.27	0.44 ± 0.18
15:0	0.85 ± 0.23	2.55 ± 0.97	1.51 ± 0.55
15:1*n*6	0.27 ± 0.14	0.70 ± 0.31	0.56 ± 0.23
i-16:0	0.19 ± 0.10	0.99 ± 0.37	0.35 ± 0.15
ai-16:0	0.61 ± 0.18	0.56 ± 0.32	1.62 ± 0.44
16:0	*18.4* ± 0.94	*20.0* ± 1.38	*20.8* ± 1.42
16:1*n*7	*13.8* ± 1.17	*6.54* ± 1.46	*8.26* ± 4.28
i-17:0	1.32 ± 0.30	1.01 ± 0.16	1.08 ± 0.35
17:0	1.26 ± 0.22	2.35 ± 0.44	1.92 ± 0.28
i-18:0	0.22 ± 0.08	0.30 ± 0.08	0.31 ± 0.11
ai-18:0	0.43 ± 0.13	0.39 ± 0.11	0.78 ± 0.29
17:2	0.05 ± 0.02	0.27 ± 0.10	0.61 ± 0.30
18:0	*5.02* ± 0.44	*7.87* ± 1.18	*6.84* ± 1.24
18:1(*n*9 + *n*7)[a]	*6.32* ± 0.76	*9.32* ± 3.58	*13.7* ± 1.03
18:2*n*6	*1.90* ± 0.33	*2.68* ± 0.87	*5.53* ± 1.78
18:2*n*4	0.35 ± 0.06	0.18 ± 0.12	0.15 ± 0.04
18:4*n*3	0.62 ± 0.04	0.30 ± 0.12	0.40 ± 0.20
20:0	0.16 ± 0.07	0.21 ± 0.03	0.26 ± 0.11
20:1(*n*9 + *n*7)[a]	0.57 ± 0.24	0.40 ± 0.17	0.53 ± 0.24
20:2*n*6	0.41 ± 0.16	0.42 ± 0.08	0.49 ± 0.19
20:3*n*6	0.62 ± 0.05	0.62 ± 0.09	0.62 ± 0.20
20:4*n*6	*3.68* ± 0.61	*8.14* ± 1.04	*7.26* ± 2.32
20:3*n*3	0.37 ± 0.03	0.57 ± 0.14	0.46 ± 0.23
20:5*n*3	*3.51* ± 0.36	*1.78* ± 0.28	*1.46* ± 1.54
22:5*n*6	*2.10* ± 0.52	*4.57* ± 0.82	*2.15* ± 0.83
22:6*n*3	*9.88* ± 2.14	*11.4* ± 2.18	*6.00* ± 2.28

[a] Together for multivariate analyses, since in some GC chromatograms these isomers were not well resolved.

Table 5
Mean weight percentage of main fatty acids in *Tilapia rendalli* during the three seasons investigated

Fatty acid	Season		
	Winter mean	Spring mean	Autumn mean
12:0	0.33 ± 0.32	0.45 ± 0.12	0.38 ± 0.06
13:0	0.19 ± 0.11	0.32 ± 0.12	0.38 ± 0.06
14:0	*1.96* ± 0.67	*3.97* ± 0.48	*3.52* ± 0.34
14:1*n*7	0.15 ± 0.07	0.36 ± 0.15	0.26 ± 0.06
ai-15:0	0.18 ± 0.10	0.45 ± 0.21	0.26 ± 0.07
15:0	1.19 ± 0.34	1.37 ± 0.35	1.25 ± 0.13
15:1*n*8	0.29 ± 0.19	0.43 ± 0.25	0.79 ± 0.13
i-16:0	0.19 ± 0.05	0.34 ± 0.12	0.33 ± 0.06
ai-16:0	0.93 ± 0.17	0.47 ± 0.16	1.60 ± 0.17
16:0	*18.5* ± 0.79	*19.2* ± 0.72	*21.3* ± 0.65
16:1*n*7	*6.37* ± 1.69	*10.7* ± 0.86	*9.31* ± 0.34
i-17:0	0.69 ± 0.10	0.83 ± 0.14	0.72 ± 0.08
17:0	1.74 ± 0.14	1.38 ± 0.32	1.57 ± 0.12
i-18:0	0.19 ± 0.08	0.65 ± 0.16	1.27 ± 0.04
ai-18:0	0.90 ± 0.33	0.41 ± 0.19	0.91 ± 0.07
17:2	0.06 ± 0.12	0.00	0.70 ± 0.04
18:0	*7.15* ± 0.64	*8.10* ± 0.68	*5.67* ± 0.46
18:1(*n*9 + *n*7)[a]	*11.4* ± 2.11	*13.6* ± 1.42	*13.9* ± 0.56
18:2*n*6	*6.43* ± 2.17	*2.80* ± 0.58	*6.34* ± 0.36
18:2*n*4	0.09 ± 0.06	0.19 ± 0.09	0.19 ± 0.04
18:4*n*3	0.26 ± 0.08	0.55 ± 0.11	0.48 ± 0.05
20:0	0.34 ± 0.04	0.27 ± 0.10	0.20 ± 0.03
20:1(*n*9 + *n*7)[a]	0.69 ± 0.20	0.35 ± 0.13	0.38 ± 0.04
20:2*n*6	0.65 ± 0.18	0.53 ± 0.28	0.38 ± 0.04
20:3*n*6	0.65 ± 0.11	0.80 ± 0.15	0.50 ± 0.06
20:4*n*6	*5.57* ± 1.11	*5.39* ± 0.44	*4.78* ± 0.37
20:3*n*3	1.19 ± 0.58	0.37 ± 0.09	0.53 ± 0.06
20:5*n*3	2.35 ± 0.60	2.84 ± 0.40	2.33 ± 0.27
22:5*n*6	1.71 ± 0.48	1.19 ± 0.13	1.03 ± 0.13
22:6*n*3	*9.28* ± 1.56	*10.1* ± 1.35	*4.41* ± 0.26

[a] Together for multivariate analyses, since in some GC chromatograms these isomers were not well resolved.

Table 6
Mean weight percentage of main fatty acids for the three tilapia species during the three seasons investigated

Fatty acid	Season		
	Winter mean	Spring mean	Autumn mean
12:0	0.27 ± 0.22	0.55 ± 0.25	0.26 ± 0.13
13:0	0.25 ± 0.16	0.41 ± 0.15	0.25 ± 0.11
14:0	3.48 ± 1.78	2.73 ± 1.00	2.59 ± 0.89
14:1*n*7	0.19 ± 0.15	0.30 ± 0.11	0.26 ± 0.15
ai-15:0	0.30 ± 0.14	0.48 ± 0.22	0.39 ± 0.17
15:0	*1.28* ± 0.53	*2.19* ± 0.85	*1.49* ± 0.42
15:1*n*6	0.38 ± 0.21	0.72 ± 0.35	0.57 ± 0.24
i-16:0	0.28 ± 0.15	0.78 ± 0.40	0.37 ± 0.12
ai-16:0	0.79 ± 0.22	0.44 ± 0.24	1.35 ± 0.48
16:0	*18.0* ± 1.14	*18.4* ± 2.16	*20.8* ± 1.34
16:1*n*7	9.72 ± 3.39	8.00 ± 2.11	9.14 ± 2.87
i-17:0	1.17 ± 0.39	1.05 ± 0.26	1.07 ± 0.39
17:0	1.72 ± 0.48	2.62 ± 0.25	1.85 ± 0.37
i-18:0	0.25 ± 0.11	0.55 ± 0.23	0.28 ± 0.12
ai-18:0	0.77 ± 0.40	0.52 ± 0.23	0.80 ± 0.23
17:2	0.05 ± 0.14	0.10 ± 0.14	0.45 ± 0.36
18:0	*6.19* ± 1.04	*8.12* ± 0.87	*6.05* ± 1.14
18:1(*n*9 + *n*7)[a]	*9.73* ± 3.00	*11.1* ± 2.97	*13.8* ± 1.26
18:2*n*6	*4.12* ± 2.22	*2.95* ± 0.73	*6.55* ± 1.76
18:2*n*4	0.22 ± 0.13	0.19 ± 0.09	0.18 ± 0.04
18:4*n*3	0.45 ± 0.21	0.33 ± 0.19	0.47 ± 0.15
20:0	0.25 ± 0.10	0.24 ± 0.07	0.24 ± 0.08
20:1(*n*9 + *n*7)[a]	0.72 ± 0.32	0.49 ± 0.23	0.55 ± 0.26
20:2*n*6	0.56 ± 0.21	0.44 ± 0.18	0.45 ± 0.13
20:3*n*6	0.62 ± 0.09	0.79 ± 0.16	0.56 ± 0.14
20:4*n*6	5.07 ± 1.34	6.64 ± 1.39	5.76 ± 1.88
20:3*n*3	0.72 ± 0.50	0.45 ± 0.13	0.53 ± 0.16
20:5*n*3	2.54 ± 0.89	2.08 ± 0.59	1.67 ± 0.62
22:5*n*6	2.20 ± 0.67	2.87 ± 1.51	1.58 ± 0.71
22:6*n*3	*8.94* ± 1.95	*10.5* ± 1.70	*5.12* ± 1.73

[a] Together for multivariate analyses, since in some GC chromatograms these isomers were not well resolved.

Table 7
Mean weight percentage of main fatty acids during the three seasons investigated for each tilapia species

Fatty acid	Species		
	T. rendalli mean[b]	*O. niloticus* mean[b]	*O. macrochir* mean[b]
12:0	0.39 ± 0.14	0.35 ± 0.14	0.38 ± 0.16
13:0	0.27 ± 0.09	0.31 ± 0.11	0.32 ± 0.14
14:0	3.31 ± 0.47	2.28 ± 0.54	3.25 ± 0.69
14:1*n*7	0.27 ± 0.10	0.30 ± 0.10	0.20 ± 0.11
ai-15:0	0.31 ± 0.13	0.41 ± 0.12	0.47 ± 0.19
15:0	1.28 ± 0.26	2.06 ± 0.33	1.66 ± 0.59
15:1*n*6	0.54 ± 0.19	0.66 ± 0.14	0.51 ± 0.23
i-16:0	0.30 ± 0.08	0.64 ± 0.13	0.52 ± 0.21
ai-16:0	1.03 ± 0.17	0.64 ± 0.14	0.92 ± 0.31
16:0	*19.9* ± 0.71	*17.8* ± 1.11	*19.8* ± 1.25
16:1*n*7	9.14 ± 0.84	8.45 ± 1.14	9.46 ± 2.28
i-17:0	0.75 ± 0.11	1.40 ± 0.24	1.13 ± 0.27
17:0	1.54 ± 0.20	2.30 ± 0.37	1.86 ± 0.32
i-18:0	0.39 ± 0.09	0.46 ± 0.12	0.28 ± 0.09
ai-18:0	0.72 ± 0.17	0.83 ± 0.22	0.53 ± 0.18
17:2	0.29 ± 0.04	0.04 ± 0.07	0.31 ± 0.14
18:0	6.91 ± 0.58	6.87 ± 0.63	6.61 ± 0.96
18:1(*n*9 + *n*7)[a]	*13.2* ± 1.23	*12.1* ± 1.37	*9.77* ± 1.83
18:2*n*6	*5.05* ± 0.86	*5.40* ± 0.98	*3.35* ± 0.97
18:2*n*4	0.17 ± 0.06	0.21 ± 0.06	0.23 ± 0.07
18:4*n*3	0.46 ± 0.08	0.37 ± 0.13	0.44 ± 0.15
20:0	0.26 ± 0.06	0.26 ± 0.07	0.21 ± 0.07
20:1(*n*9 + *n*7)[a]	0.44 ± 0.11	0.79 ± 0.27	0.50 ± 0.22
20:2*n*6	0.50 ± 0.14	0.49 ± 0.10	0.44 ± 0.14
20:3*n*6	0.65 ± 0.10	0.62 ± 0.11	0.62 ± 0.11
20:4*n*6	*5.19* ± 0.57	*5.77* ± 0.81	*6.40* ± 1.32
20:3*n*3	0.62 ± 0.19	0.56 ± 0.13	0.47 ± 0.13
20:5*n*3	2.52 ± 0.39	1.50 ± 0.26	2.24 ± 0.39
22:5*n*6	1.24 ± 0.21	2.23 ± 0.37	2.98 ± 1.05
22:6*n*3	7.64 ± 0.96	7.08 ± 1.04	9.15 ± 2.20

[a] Together for multivariate analyses, since in some GC chromatograms these isomers were not well resolved.
[b] See Table 1 for number of fishes investigated.

of tilapia (Corser, Ferrari, De Martinez, Salas, & Cagnasso, 2000; Xie et al., 2001). We can observe that the relative amount of these acids change significantly between species and season of collect, in particular DHA which decreased from 11.4% to 6.0% for *O. macrochir* during the spring to autumn period. For *O. niloticus* and *T. rendalli*, DHA decreased from 9.8% to 4.9% and to 10.1% to 4.4%, respectively, during the same period.

Our results show that the $\sum n3/\sum n6$ ratios vary, for each species according to season an species, being lower in autumn (0.5–0.6) and raising up to 0.7–1.6 in winter (Table 8). The average of all species ratio is near 1 in winter and decreased until 0.5 in autumn. This is explained by a decreasing value of the $\sum n3$ PUFA total value from 12.0% to 7.3% for *O. niloticus*, 13.9% to 7.7% for *T. rendalli* and 14.4% to 8.3% for *O. macrochir*, and an increasing $\sum n6$ PUFA total value from 13.3% to 15.6%, 10.7% to 13.0% and 8.7% to 16.0%, respectively. Ackman (1967) have shown that the total linolenic to total linoleic types of acids was lower in the freshwater fish oils in comparison with marine oils suggesting a basic difference in dietary availability of these acids. Similar

Table 8
Fatty acid $\sum n3/\sum n6$ ratio in the muscle of tilapia species investigated during three seasons

Season[a]	Winter	Spring	Autumn	All seasons
O. niloticus	0.73	0.90	0.47	0.66
O. macrochir	1.65	0.86	0.52	0.89
T. rendall	0.87	1.29	0.59	0.89
All species	1.01	0.98	0.52	

[a] Winter (july); spring (october); autumn (march).

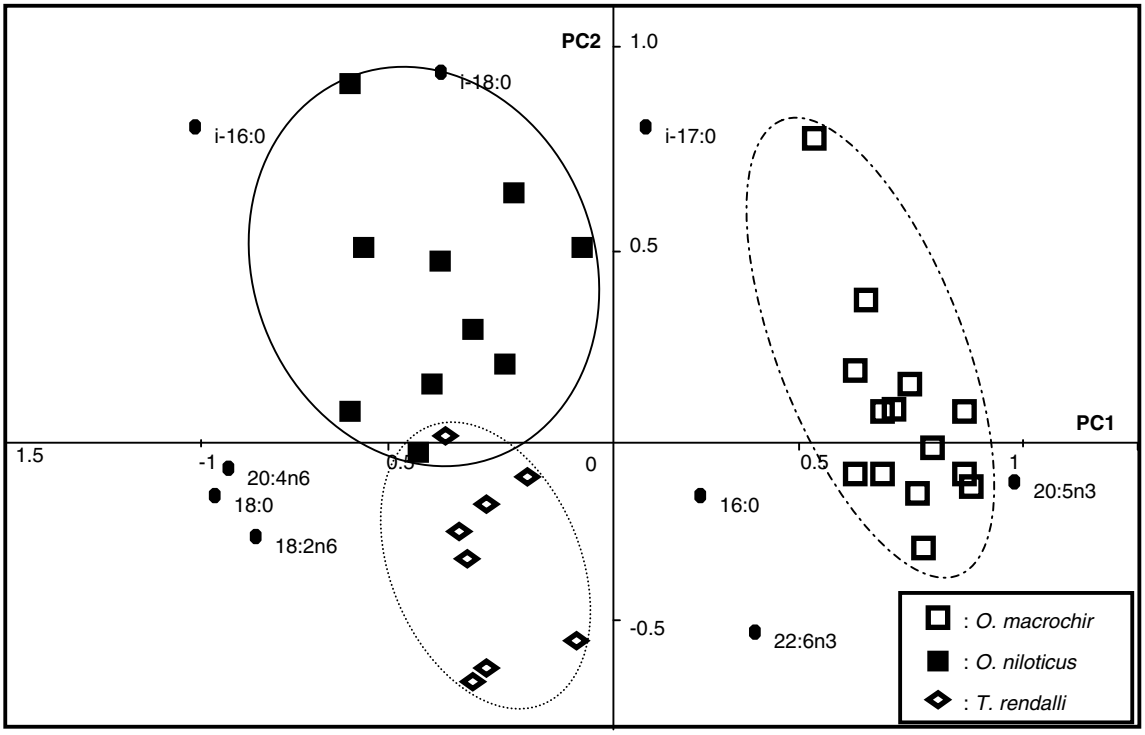

Fig. 1. Two-dimensional plot of the fatty acid methyl ester profiles of muscle from tilapia species investigated in PCA for the winter collect. The amount of variance relative to each axis and the fatty acid methyl esters providing the major contribution is given.

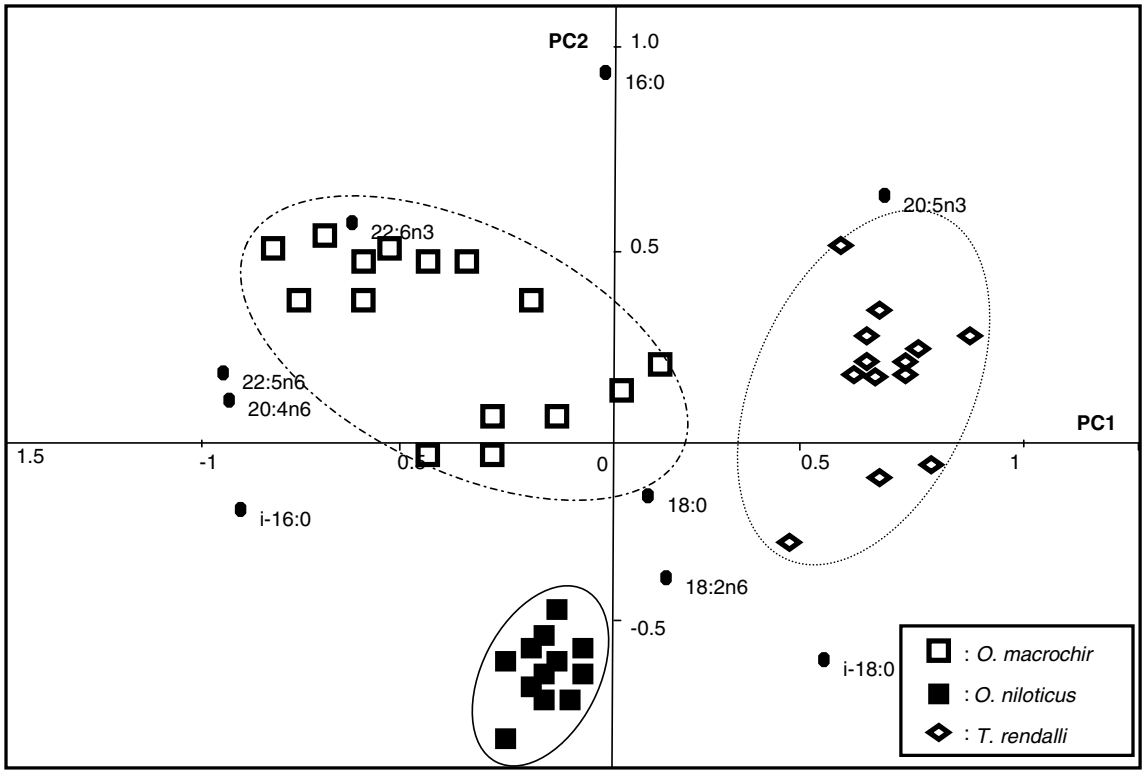

Fig. 2. Two-dimensional plot of the fatty acid methyl ester profiles of muscle from tilapia species investigated in PCA for the spring collect. The amount of variance relative to each axis and the fatty acid methyl esters providing the major contribution is given.

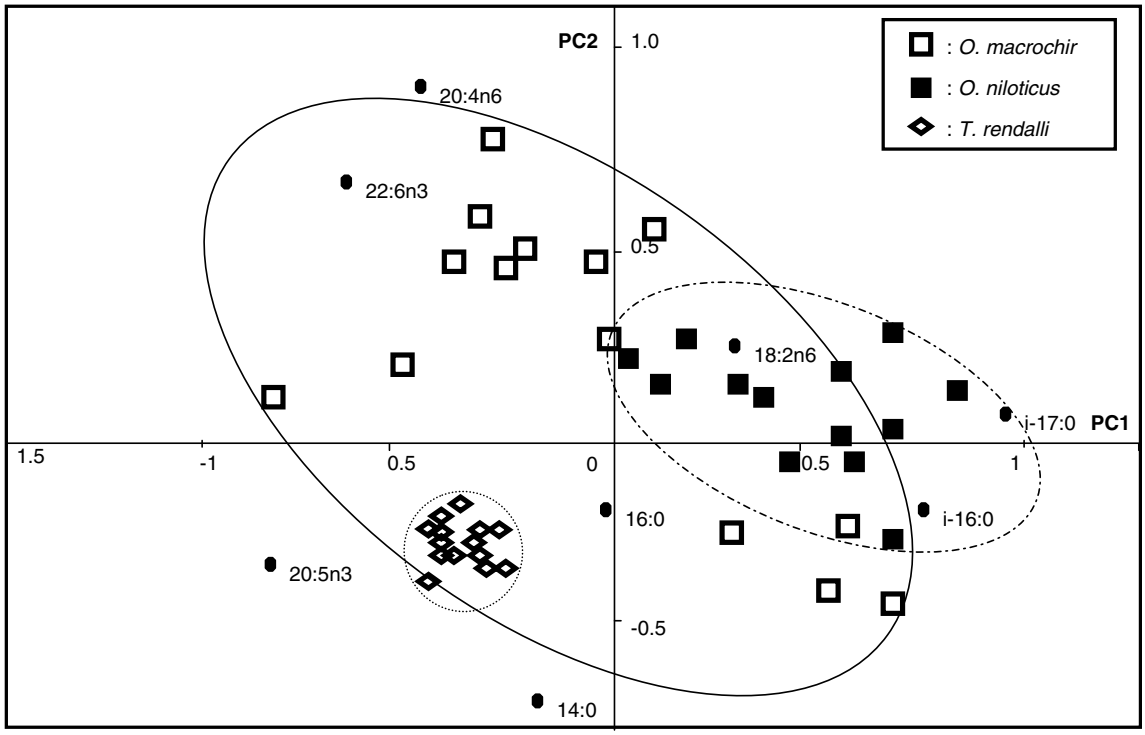

Fig. 3. Two-dimensional plot of the fatty acid methyl ester profiles of muscle from tilapia species investigated in PCA for the autumn collect. The amount of variance relative to each axis and the fatty acid methyl esters providing the major contribution is given.

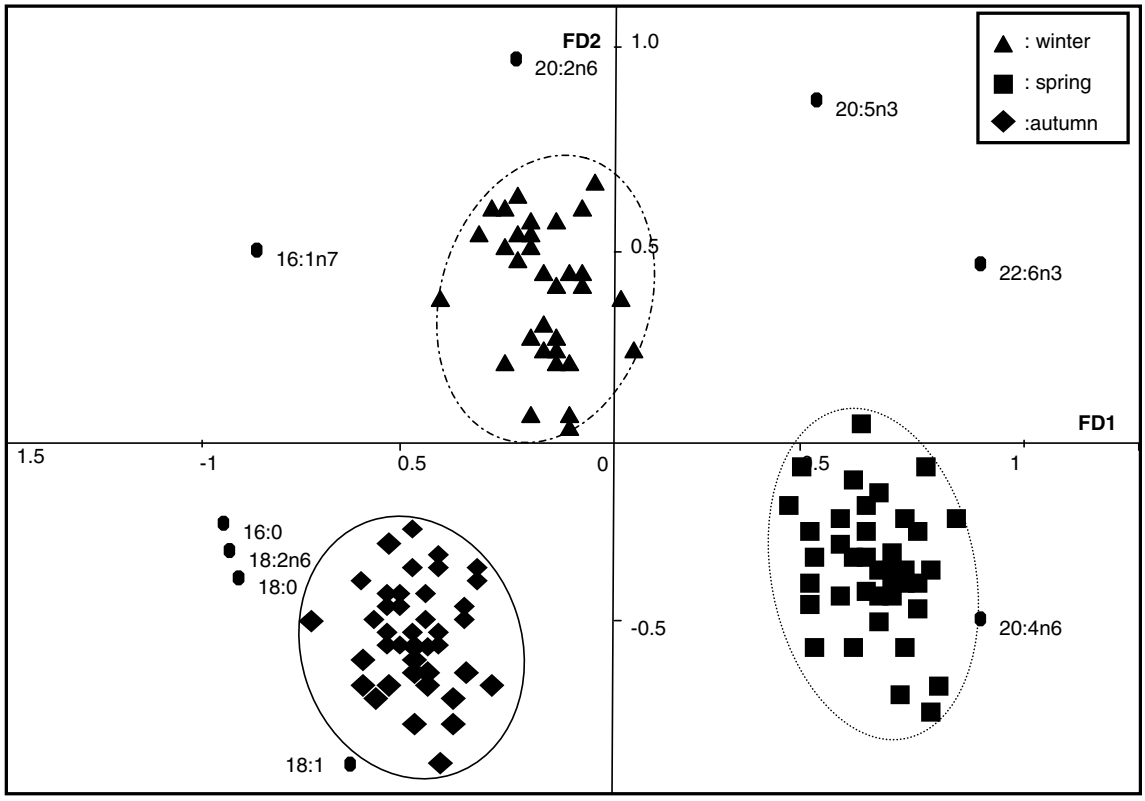

Fig. 4. Two-dimensional plot of the fatty acid methyl ester profiles of muscle from all tilapia species investigated in FDA using the three season collects as discriminant factor. The amount of variance relative to each axis and the fatty acid methyl esters providing the major contribution is given.

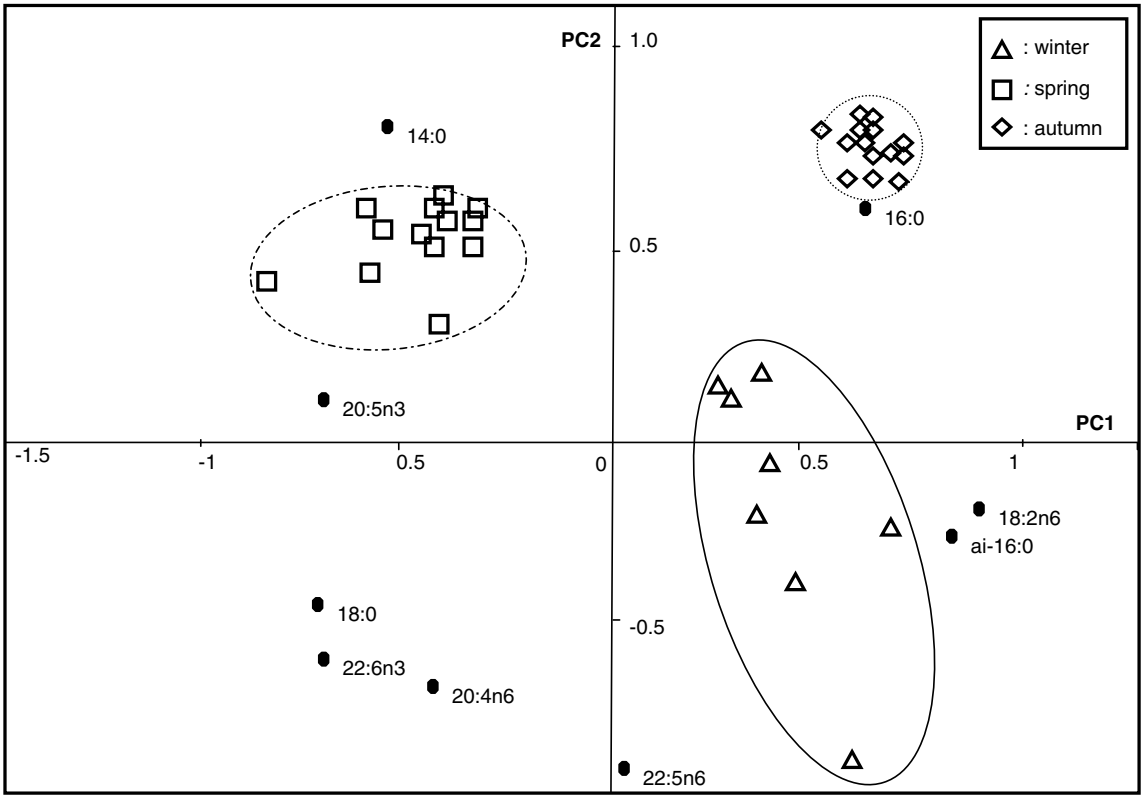

Fig. 5. Two-dimensional plot of the fatty acid methyl ester profiles of muscle from the three season collects investigated in PCA for *T. rendalli*. The amount of variance relative to each axis and the fatty acid methyl esters providing the major contribution is given.

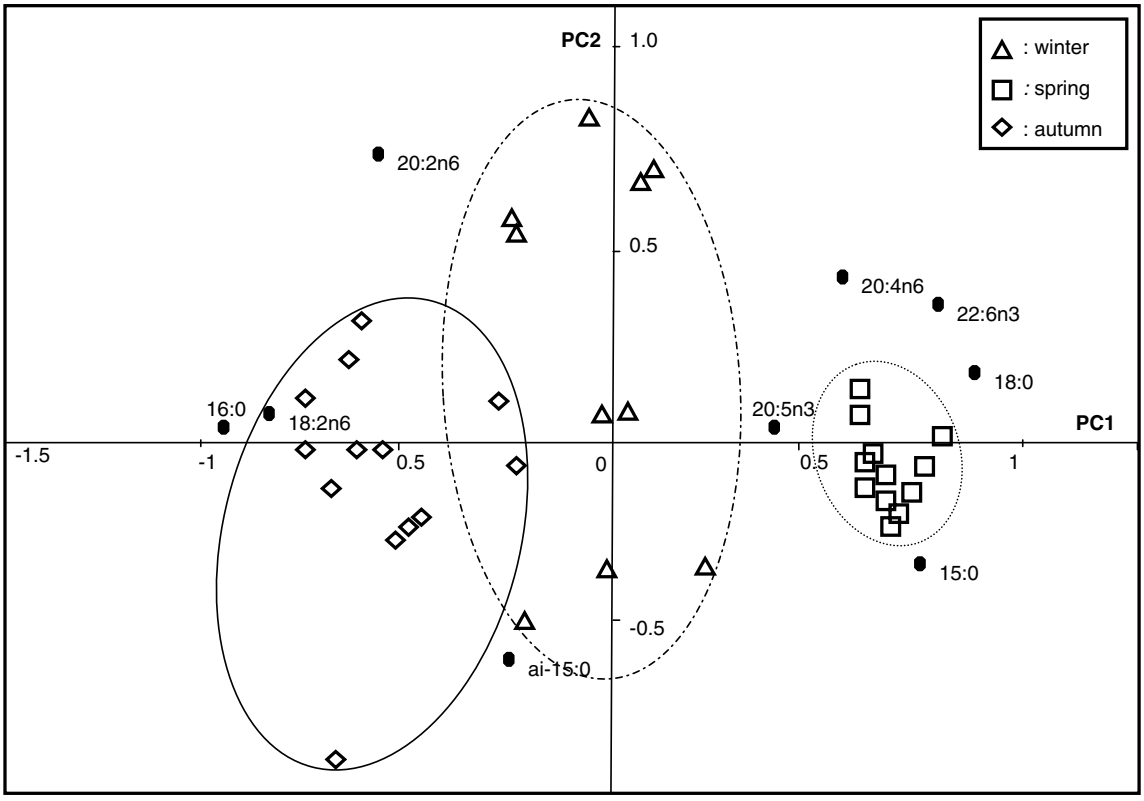

Fig. 6. Two-dimensional plot of the fatty acid methyl ester profiles of muscle from the three season collects investigated in PCA for *O. niloticus*. The amount of variance relative to each axis and the fatty acid methyl esters providing the major contribution is given.

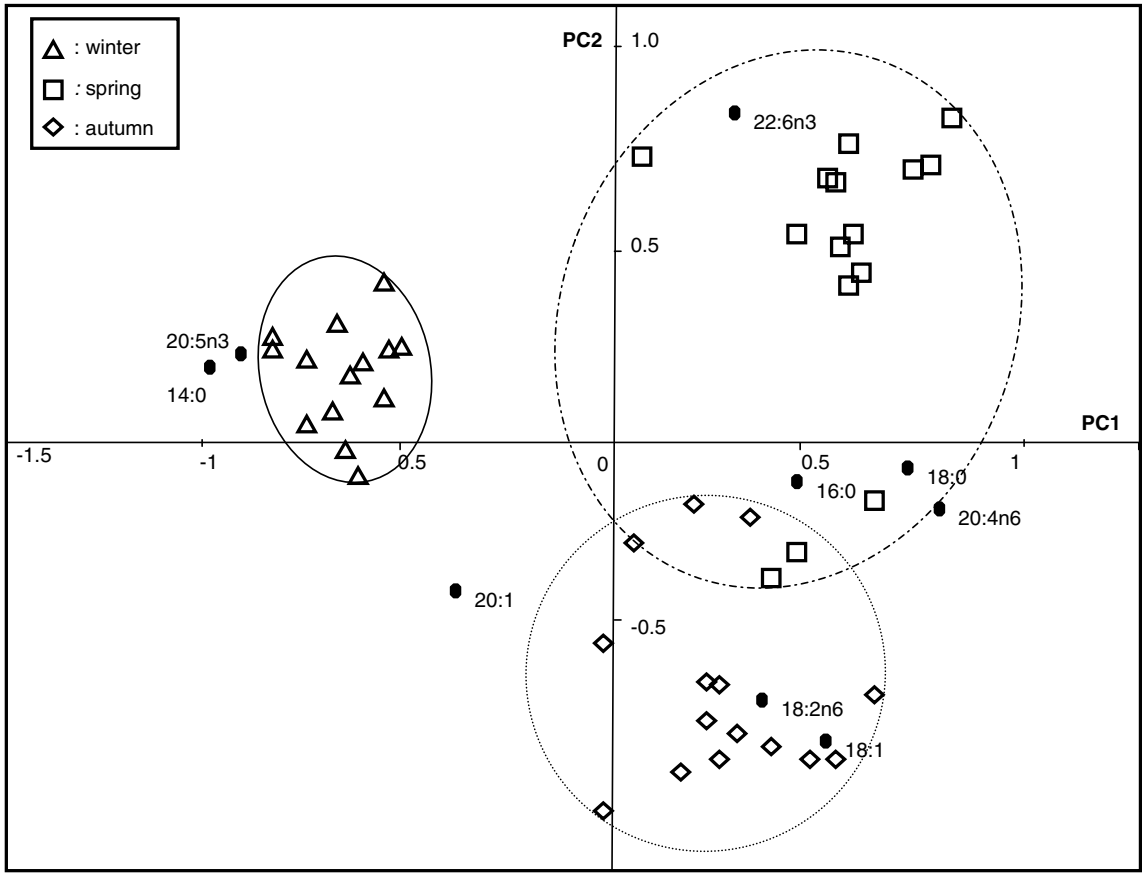

Fig. 7. Two-dimensional plot of the fatty acid methyl ester profiles of muscle from the three season collects investigated in PCA for *O. macrochir*. The amount of variance relative to each axis and the fatty acid methyl esters providing the major contribution is given.

results were observed by Bézard et al. (1994) between sea fish and freshwater fish, when they compared examples of *n*6 and *n*3 essential fatty acid concentration in lipids of some animals. Zenebe et al. (1998) observed that the $\sum n3/\sum n6$ ratio varied considerably (1.1–7.6) for freshwater fish of commercial importance, including *O. niloticus*.

Our results are in agreement with those of Luzia et al. (2003) who observed in tilapias, the lowest EPA + DHA contents in summer, showing a season dependence of these acids. A $\sum n3/\sum n6$ ratio of 0.93–0.94 was observed in the muscle of male and female hybrid red tilapia, *O. mossambicus* x *O. niloticus* reared in seawater by De Silva et al. (1997). This ratio raised until 1.05 for red tilapia reared in freshwater. A higher ratio was found by Rahman et al. (1995) for tilapia, but the species and the season of collect were not given.

3.3. Multivariate statistical analyses

Among the 42 FA characterized, some of them, highly correlated with other FA in preliminary multivariate statistical analyses, were deleted from the data set. They are *i*-14:0, *i*-15:0, *ai*-17:0, 16:2*n*4, 17:1*n*8, 18:3*n*6, 18:3*n*3 (α-linolenic), 20:4*n*3 and 22:4*n*6. Acids 18:1*n*9 + 18:1*n*7 and 20:1*n*9 + 20:1*n*7 were taken together for multivariate analyses, since in some GC chromatograms, these isomers were not well resolved. Finally, the data set used was composed of 113 lipid fractions of tilapia muscle (Table 1) using 32 variables (30 fatty acids, the biometric index, BI and the percentage of lipid in the white muscle, %LIP). To compare the influence of season on fatty acid profiles versus species we first investigate species differentiation for each collect season.

A graphic representation of the projection of variables and samples onto the two first principal components is given in Fig. 1, using Principal component analysis (PCA). Axis 1, which represents 27.9% of the total information is positively loaded with EPA (20:5n3) and negatively loaded with arachidonic acid (20:4n6), stearic and linoleic acids. Differentiation of *O. macrochir* species from the two other ones in winter (32 samples) is done on this axis. On axis 2 (21.1 % of the total information), which is positively loaded with some iso fatty acids (*i*-16:0, *i*-17:0, *i*-18:0) and negatively with DHA (22:6*n*3), *O. niloticus* is partially differentiated from *T. rendalli*. During the spring

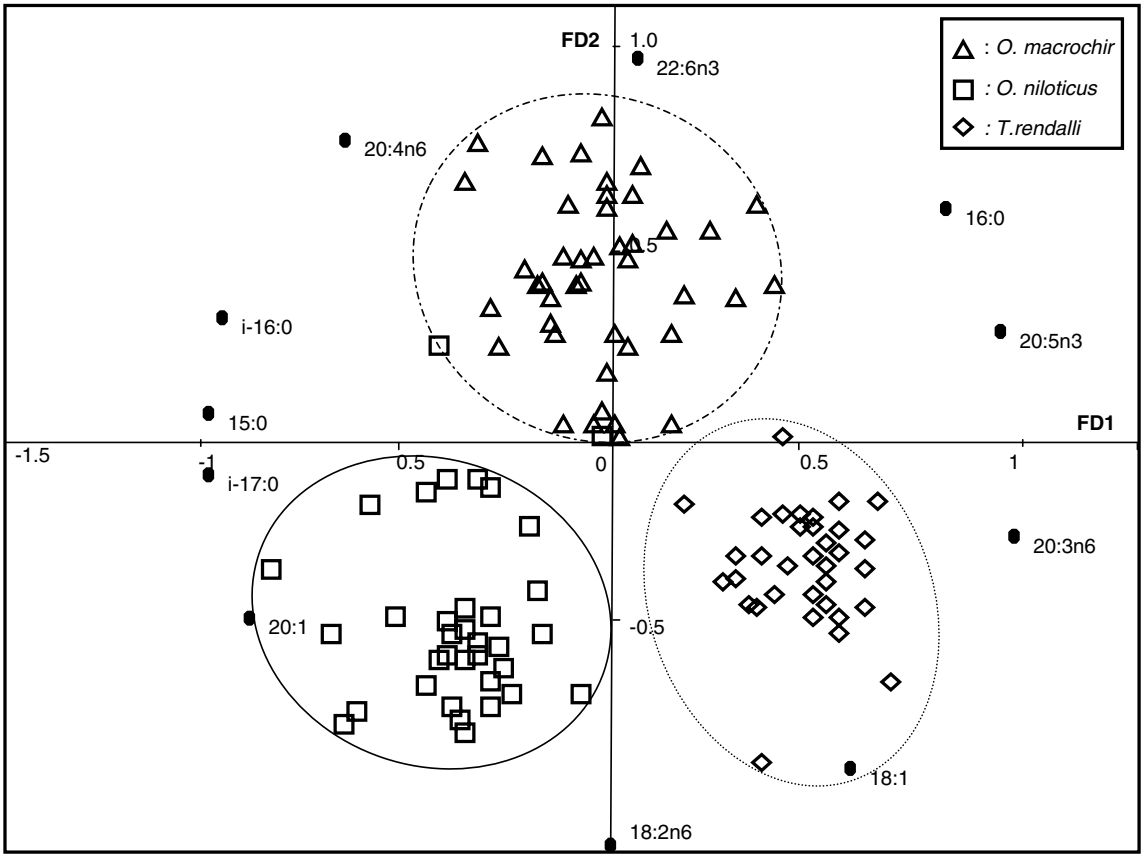

Fig. 8. Two-dimensional plot of the fatty acid methyl ester profiles of muscle from all season collects investigated in FDA using tilapia species as discriminant factor. The amount of variance relative to each axis and the fatty acid methyl esters providing the major contribution is given.

collect (40 samples), the first component (26.6% of the total information) leads to the separation of *T. rendalli* samples, showing that this species (Fig. 2) is richer in EPA (20:5*n*3) compared to the two others and with lower content in arachidonic acid (20:4*n*6) and docosapentaenoic acid (22:5*n*6). *O. niloticus* samples are differentiated from *O. macrochir* fishes by axis 2 (19.8% of the total information), showing that DHA (22:6*n*3) content is higher in the second species and that *O. niloticus* fishes are characterized by higher content in linoleic and *i*-18:0 acids. The autumn collect (41 samples) was characterized by a wide range of fatty acid content for *O. macrochir* fishes, as shown in Fig. 3, *O. niloticus* and *T. rendalli* samples showing a lesser variation. Axis 1 (23.9% of the total information) which is highly correlated positively to some branched saturated FA (*i*-16:0 and *i*-17:0), and negatively correlated to EPA (20:5*n*3), and slightly negatively correlated to DHA (22:6*n*3) and arachidonic acid (20:4*n*6), leads to a separation of *O. niloticus* from *T. rendalli*. Attempt to differentiate all the 113 fish samples according to collect season using PCA was not achieved showing therefore the relative little changes in FA amounts. Factorial Discriminant Analysis (FDA), using season as discriminant factor was successful with only 15 FA to obtain a correct classification as shown in Fig. 4. Among the FA playing a main role on axis 1, EPA (20:5*n*3), DHA (22:6*n*3) and arachidonic acid (20:4*n*6) contribute to characterize fishes collected in spring. Autumn fishes are richer linear saturated FA such as palmitic and stearic acids and oleic and linoleic acids. Fishes collected in winter are characterized by higher content in 20:2*n*6, 16:1*n*7 and EPA and lower amounts in arachidonic acid (20:4*n*6) and saturated FA than in autumn.

To further investigate the change in FA profiles within season, additional PCA were carried out separately on each species. In the case of *O. rendalli*, response of this species to season influence is strong as shown in Fig. 5. Axis 1 (30.6% of the total information) negatively correlated to EPA (20:5*n*3) and DHA (22:6*n*3), and positively to palmitic acid (16:0) separates the spring group of fishes from the two other ones. Axis 2 (22.7% of the total information) positively correlated to linear saturated FA (14:0 and 16:0) and negatively to DHA (22:6*n*3), arachidonic acid (20:4*n*6) and 22:5*n*6, separates the winter group from the autumn ones. For *O. niloticus*, season differentiation occurred mainly on axis 1 (29.1% of the total information) as shown in Fig. 6. The spring group

is highly correlated with stearic acid, DHA (22:6*n*3), arachidonic acid (20:4*n*6), and is negatively correlated with palmitic and linoleic acids. Autumn and winter groups remained partially overlapped. Axis 2 (21.7% of the total information) partially correlated to 20:2*n*6 and negatively to *ai*-15:0, did not succeeded in the total separation of autumn and winter groups. *O. macrochir* species gave a good separated winter group (Fig. 7), on axis 1 (29.7% of the total information). The main FA positively loaded on this axis are arachidonic acid (20:4*n*6) and on the negative part, EPA (20:5*n*3) and myristic acid (14:0). Axis 2 (19.4% of the total information) positively loaded with DHA (22:6*n*3), and negatively with oleic and linoleic acids, separated partially the spring and autumn groups. Attempt to differentiate tilapia species during seasons in PCA with the 113 samples was not achieved. FDA, using species as discriminant factor, indicates a lower species effect versus the season collect effect. As shown in Fig. 8, two fish samples belonging to *O. niloticus* species were classified within the *O. macrochir* fishes even using all variables. These results show therefore a higher change in FA profiles within season than within species.

4. Conclusion

Though significant differences in lipid content and fatty acid composition are observed between species and season of collect, this work demonstrates that tropical freshwater wild fish belonging to the herbivore *Oreochromis* and *Tilapia* genera are comparable to temperate freshwater fish as sources of essential polyunsaturated fatty acids. Therefore these fish species contribute significantly to the *n*3 fatty acids intake of the highland population of Madagascar.

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