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ORIGINAL PAPER

Muscle Lipids and Fatty Acid Profiles of the Sea Catfish (*Arius madagascariensis*) in Madagascar Inland Waters

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Abstract Muscle lipids and fatty acids (FA) of catfish *Arius madagascariensis* were determined in catfish caught in the Betsiboka River, Madagascar, during a 5-month sampling period. Total lipids from muscle were extracted and quantified. Fatty acids were identified by means of gas chromatography–mass spectrometry of FA methyl esters and FA pyrrolidides, leading to the identification of 42 FA. Lipid content was relatively high in our fish sample and ranged from 4.3 to 6.6% of wet muscle. Three FA dominated the FA composition: palmitic acid (C16:0, 22.9–32.6%), oleic acid (C18:1n-9, 11.3–13.4%) and stearic acid (C18:0, 10.8–12.0%). A number of polyunsaturated FA (PUFA) were present in appreciable amounts, including arachidonic acid (C20:4n-6, 4.7–7.6%), docosahexaenoic acid (C22:4n-6, 3.0–8.1%), eicosapentaenoic acid (C20:5n-3, 0.6–1.0%), n-3 docosapentaenoic acid (C22:5n-3, 1.1–1.6%), n-6 docosatetraenoic acid (C22:4n-6, 0.7–1.2%) and n-6 docosapentaenoic acid (22:5n-6, 0.9–1.8%). The sum of the n-6 PUFA and n-3 PUFA was 11.3–18.8 and 7.5–13.4%, respectively. These results indicate that *A. madagascariensis*, an abundant freshwater fish in Madagascar rivers, may be good source of dietary PUFA.

Keywords Arachidonic acid · Ariidae · *Arius madagascariensis* · Docosahexaenoic acid · Eicosapentaenoic acid · Fatty acid · Lipid · Madagascar · PUFA

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Introduction

Inland fish are one of the main sources of high-quality animal protein in the continental highlands of Madagascar, particularly in rural regions. In the major fishing areas, fishing has taken on a commercial structure in which fresh fish are collected and transported by road to Antananarivo, the main market. The sea catfish from Madagascar (*Arius madagascariensis* Vaillant (Fam. Ariidae; local name "Gogo") is a marine fish that is abundant mainly on the west and east coasts of Madagascar, near estuaries. This species has been reported, mostly on the west coast, to swim up the rivers and stay almost all year in freshwater bodies [1]. It can be caught in the northwest part of the island during the austral winter to spring seasons, and accounts for 20–30 tons per year. Although there have been many reports on the lipids of the various genera and species of freshwater catfish, only a few references have been made to the sea catfish. The biochemical and pharmacological properties and the lipid composition of epidermal secretions from various *Arius* catfish of the Arabian Gulf have been investigated [2, 3]. However, there has been very little mention of the fatty acid (FA) composition of catfish muscle lipid, with the report by Sinclair et al. [4] being the

only publication on FA composition in *Arius* catfish (in this case, *Arius* catfish from the northern coast of Australia).

n-3 polyunsaturated FA (n-3 PUFA) in the diet has been recognized to have important beneficial properties for the prevention of human coronary artery disease [5]. Although most people assume that all fish are rich sources of n-3 PUFA, researchers have shown that freshwater fish generally contain lower proportions n-3 PUFA than marine fish [6, 7]. Furthermore, since fish need PUFA to provide tolerance to low water temperature [8], low amounts should be expected in fish native to warmer waters, such as in tropical areas as Madagascar. *Arius madagascariensis* is originally from the sea, but it has established itself in freshwater bodies and may have a FA composition that is linked to their diet. If the PUFA content is relatively high, the consumption of such freshwater fish, therefore, should contribute significantly to the amount of n-3 PUFA in the diet of the inland population.

The objective of this study was to follow the change in muscle lipid content of *A. madagascariensis* during a 5-month sampling period and to determine the FA composition.

Experimental Procedures

Sampling

Three fish were caught each month for a period of 5 months (July to November) in the Betsiboka River; sampling was carried out on the same day of the month and at the same location (under the bridge road from Betongoa to Ambato Boeni; (16°28′21.4″S, 42′39.1″E; elevation 100 m a.s.l.; northwest region of Madagascar). For all samplings, the fish were 20–30 cm long and weighed 150–300 g (roughly 1 year old). Individual fish were dissected, and portions of muscle tissue below the dorsal fin (skin on) were kept in ice for less than 4 h prior to lipid extraction. The sex of the sampled fish was not determined.

Lipid Extraction

Approximately 10 g of muscle from one fish was homogenized using a Warring Blender. Lipids were extracted using the Bligh and Dyer method [9]. Following centrifugation, the lower chloroform phase was kept, dried with sodium sulfate and evaporated. The remaining oil was used for total lipid content and FA determinations.

Preparation and Gas Chromatography Analysis of FA Methyl Esters

Fatty acids were prepared by saponification of the oil (50 mg) with KOH-ethanol, 2 *M* (1 mL) and acid-catalyzed methylation with HCl in MeOH, as described by Christie [10]. A gas chromatograph (GC; Delsi Instruments, Suresnes, France) equipped with a flame ionization detector (FID) and a fused silica capillary column (25 m × 0.25 mm i.d.) coated with Carbowax–20 M (phase thickness 0.2 μm) was used for the analyses. The oven program temperature consisted of an 180°C for 10 min with 2°C increases per minute up to 220°C. The injector and detector were both set at 250°C. Fatty acid methyl esters (FAME; Sigma, France) were used as quantitative external standards. Small standard deviations (SD) were obtained, which is comparable to the level of repeatability recommended [11].

Gas Chromatography–Mass Spectrometry

Fatty acids were identified by mass spectrometry (MS) of the respective FAME and pyrrolidide and compared to previously published results [12, 13]. The GC/MS data for the pyrrolidide derivatives were obtained to confirm the structures and to determine double bond positions. *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 for 45 min at 80°C and purified on silica thin-layer chromatographs (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 a mass spectrometer detector (model 5989A; Hewlett-Packard, Palo Alto, CA) and a Hewlett-Packard 9000/345 integrator. A DB1 fused silica capillary column [length 30 m, i.d. 0.32 mm; with a 0.25-μm stationary phase film thickness] was used along with a temperature program of 170°C (4-min hold) to 300°C (3°C per minute) for FAME separation and from 200°C (4-min hold) to 310°C (3°C per minute) for *N*-acyl pyrrolidide separation. Helium was used as the carrier gas. The ion source temperature of 220°C, and an ionizing voltage 70 eV was used in the mass detection of the FAME and *N*-acyl pyrrolidide derivatives.

Results and Discussion

Our analysis of the lipids extracted from *A. madagascariensis* muscle and subsequent determination of FA led to the identification of 42 FA. Table 1 gives the change (mean and standard deviation) in FA and lipid content that occurred during the 5-month sampling period (July–November; austral winter and spring authorized fishing seasons).

Lipid Content

Lipid content of the wet muscle of *A. madagascariensis* ranged from 4.3 to 6.6% over the 5-month sampling period. Lawan et al. [14] reported that a carnivorous fish, such as

Table 1 Fatty acid composition and lipid content of *Arius madagascariensis* muscle

Month	July		August		September		October		November	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fatty acid										
12:0	0.26	0.04	0.27	0.05	0.35	0.06	0.21	0.03	0.38	0.04
13:0	0.21	0.05	0.18	0.01	0.21	0.03	0.15	0.01	0.20	0.02
I-14:0[a]	0.19	0.07	0.22	0.03	0.13	0.01	0.19	0.06	0.12	0.03
14:0	2.43	0.12	2.38	0.17	2.55	0.15	2.33	0.21	2.70	0.18
14:1n-7	0.22	0.02	0.20	0.02	0.26	0.02	0.19	0.02	0.17	0.02
I-15:0	0.24	0.04	0.28	0.05	0.15	0.03	0.27	0.04	0.11	0.01
ai-15:0[a]	1.19	0.22	0.87	0.09	0.96	0.21	0.68	0.09	0.47	0.05
15:0	1.56	0.19	0.79	0.10	0.97	0.18	0.67	0.05	0.54	0.07
15:1n-8	0.18	0.05	0.23	0.03	0.12	0.03	0.15	0.01	0.16	0.02
I-16:0	0.45	0.06	0.22	0.04	0.34	0.09	0.26	0.07	0.10	0.01
ai-16:0	0.32	0.03	0.42	0.11	0.50	0.11	0.36	0.05	0.67	0.11
16:0	32.6	2.62	30.2	1.89	27.8	2.34	25.1	3.20	22.9	1.17
16:1n-7	10.0	0.86	10.0	0.73	8.74	1.14	7.24	1.05	6.11	1.06
I-17:0	0.83	0.11	0.71	0.18	0.83	0.07	0.78	0.08	0.69	0.13
ai-17:0	0.90	0.07	0.64	0.10	0.55	0.12	0.41	0.12	0.44	0.66
16:2n-4	0.46	0.10	0.34	0.09	0.28	0.03	0.20	0.05	0.12	0.02
17:0	1.32	0.34	0.97	0.21	1.17	0.10	1.46	0.34	1.28	0.23
17:1n-8	0.82	0.20	0.50	0.16	0.77	0.07	0.81	0.07	0.68	0.13
I-18:0	0.11	0.01	0.16	0.03	0.28	0.04	0.33	0.08	0.44	0.04
ai-18:0	0.15	0.02	0.16	0.02	0.36	0.07	0.41	0.12	0.63	0.06
17:2	0.59	0.07	0.36	0.08	0.21	0.01	0.19	0.02	0.31	0.03
18:0	10.8	0.21	10.2	0.34	11.4	0.39	11.1	0.25	11.9	0.17
18:1n-9	11.3	0.31	11.7	0.30	12.5	0.56	12.2	0.44	13.4	0.38
18:1n-7	2.48	0.18	3.09	0.21	2.92	0.37	3.39	0.30	3.25	0.27
18:2n-6	2.58	0.75	3.19	0.76	4.47	0.88	5.87	1.06	6.27	0.58
18:2n-4	0.16	0.02	0.16	0.02	0.27	0.02	0.21	0.02	0.26	0.03
18:3n-6	0.65	0.13	0.57	0.10	0.44	0.07	0.48	0.06	0.43	0.06
19:1n-10	0.27	0.05	0.15	0.03	0.31	0.03	0.26	0.08	0.41	0.05
18:3n-3	1.82	0.40	3.84	0.66	2.25	0.60	3.30	0.24	2.49	0.36
18:4n-3	0.20	0.07	0.34	0.03	0.22	0.03	0.29	0.05	0.19	0.02
20:0	0.71	0.12	0.42	0.07	0.55	0.10	0.41	0.07	0.51	0.07
20:1	1.06	0.14	0.65	0.10	0.74	0.09	0.59	0.12	0.53	0.04
20:2n-6	0.55	0.11	0.46	0.08	0.44	0.06	0.39	0.04	0.33	0.04
20:3n-6	0.87	0.10	0.81	0.13	0.97	0.05	1.07	0.17	1.28	0.13
20:4n-6	5.14	0.50	4.70	0.53	5.50	0.73	6.41	0.55	7.55	0.47
20:3n-3	0.17	0.02	0.25	0.03	0.22	0.04	0.28	0.02	0.21	0.04
20:4n-3	0.22	0.04	0.27	0.01	0.21	0.01	0.19	0.04	0.24	0.02
20:5n-3	0.98	0.07	0.84	0.05	0.74	0.12	0.63	0.17	0.59	0.06
22:4n-6	0.65	0.06	0.90	0.10	1.20	0.09	1.31	0.10	1.20	0.15
22:5n-6	0.89	0.10	1.14	0.07	1.01	0.08	1.43	0.05	1.75	0.21
22:5n-3	1.13	0.08	1.30	0.10	1.44	0.05	1.39	0.09	1.62	0.17
22:6n-3	3.02	0.41	4.89	0.69	5.43	0.71	6.27	0.86	8.06	0.38
Lipid %	4.2	0.11	4.3	0.22	5.6	0.05	5.8	0.37	6.6	0.19
Sum of n-6 PUFA	11.33		11.77		13.83		16.96		18.81	
Sum of n-3 PUFA	7.54		11.73		10.51		12.35		13.39	
n-3/n-6 ratio[b]	0.67		1.00		0.76		0.73		0.71	

Fatty acid composition is expressed as a percentage (w/w) of total FA content. Lipid content is expressed as a percentage (w/w) of muscle tissue

[a] I, Iso; ai, anteiso

[b] Sum of n-3 PUFA/sum of n-6 PUFA

Channa striata found in Thailand, was considered to be a fatty fish. *Arius madagascariensis* is also considered to be a fatty fish [1], and we have observed a characteristic deposit of fat tissues around viscera of aged specimens. In fact, *A. madagascariensis* is most likely one of the fattiest fish among those regularly caught from Malagasy freshwater bodies. In comparison, other local fish, such as common carp (*Cyprinus carpio*) or Nile tilapia (*Oreochromis niloticus*), are not considered fatty because they contain less than 2% lipids in their muscle [15, 16]. The amount of lipids increased steadily during the 5-month sampling period, which corresponds to the earlier spawning period for *A. madagascariensis*, i.e. the hot season [1]. During this period, dietary and novel-synthesized lipids are mostly used for egg maturation, which requires significant amounts of FA [17].

Fatty Acid Profile

The three major FA of the 42 identified were C16:0 (palmitic acid, 23–33%), C18:1n-9 (oleic acid, 11–13%) and C18:0 (stearic acid, 11–12%). Figure 1 shows the change in saturated and monounsaturated FA (MUFA) in *A. madagascariensis* muscle during the 5-month sampling period. Saturated FA, predominantly in the form of C16:0, C18:0 and C14:0 represented 45.8% of all FA in July) and 37.6% in November. The amount of C16:0 decreased significantly from 32.6 to 22.9% in this period, and it was not balanced by the slight increase in C18:0 during the same time interval (10.8 to 12.0%). The MUFA consisted mainly of oleic (C18:1n-9) and palmitoleic (C16:1n-7) acids. Similar to the palmitic acid, the content of palmitoleic acid decreased from 10.0 to 6.1% during the sampling period and oleic acid increased slightly from 11.3 to 13.4%. The total amount of MUFA remained relatively constant—23.8% in July and 22.7% in November (Fig. 2). Arachidonic acid (C20:4n-6, AA), the main FA of this series, increased from 4.7 up to 7.6% and linoleic acid (C18:2n-6) increased from 2.6 to 6.3%. Other predominant n-6 PUFA seemed to follow this trend, such as n-6 docosatetraenoic acid (C22:4n-6, 0.7–1.2%) and n-6 docosapentaenoic acid (C22:5n-6, 0.9–1.8%). Docosahexaenoic acid (DHA, 22:6n-3) was the major n-3 PUFA (Fig. 3) and showed an increase from 3.0 to 8.1% over the sampling period. Low levels of eicosapentaenoic acid (EPA, C20:5n-3, 0.6–1.0%) and n-3 docosapentaenoic acid (C22:5n-3, 1.1–1.6%) were observed.

These data show that the pattern of FA in *A. madagascariensis* is similar to that of freshwater fish, with saturated FA (SFA) and MUFA being the most prevalent FA and n-6 PUFA being the main PUFA. High levels of SFA and MUFA are regularly reported in freshwater fish of both temperate [18] and tropical waters [7]. Chatakondi et al. [19] found common carp to have linoleic acid at a level of 11.5/100 g muscle, while Ackman et al. [20] reported about 40–50% SFA and 24–39% MUFA in Indian carp. Although Sinclair et al. [4] suggested that marine fish from the southern hemisphere would have different patterns of n-6 PUFA, most published studies report higher levels of n-6 than n-3 PUFA. In the case of *A. madagascariensis*, the sum of the n-6 and n-3 PUFA were 11.3–18.8% and 7.5–13.4%, respectively, giving a n-6/n-3 ratio of between 0.67 and 0.76 (Table 1)—with the exception of the August data when we found a ratio of 1.00, which was due to an exceptionally high amount of linolenic acid (C18:3n-3). The very low amount of EPA, far lower than that generally reported in freshwater fish, was also a

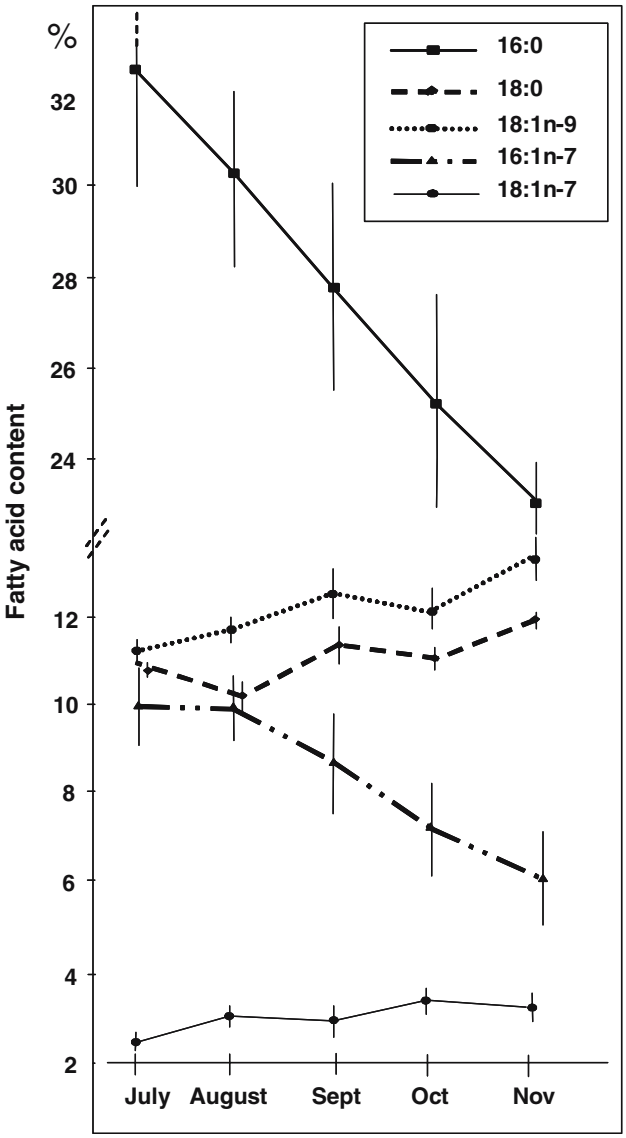

Fig. 1 Changes in the levels of the main saturated and monounsaturated fatty acids (FA) of *Arius madagascariensis* muscle during the 5-month sampling period

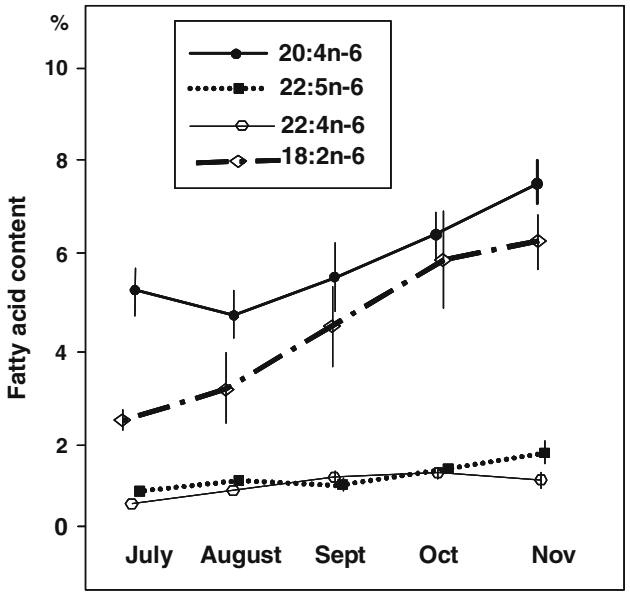

Fig. 2 Changes in the levels of n-6 polyunsaturated FA (PUFA) content of *A. madagascariensis* muscle during the sampling period

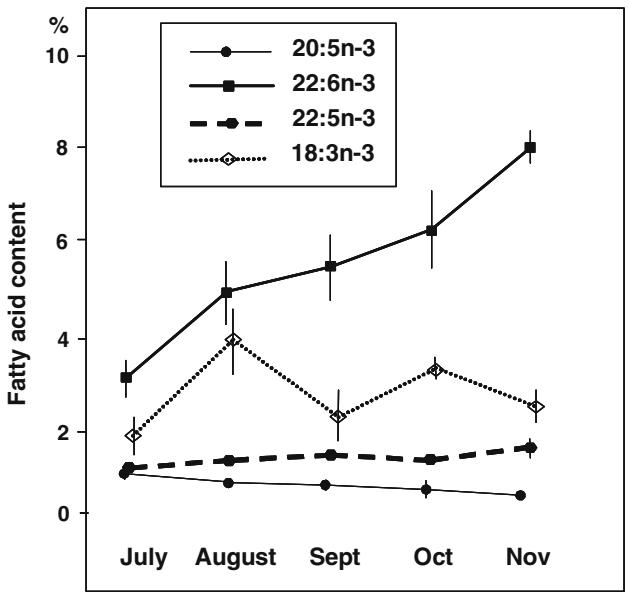

Fig. 3 Changes in the levels of n-3 PUFA of *A. madagascariensis* muscle

specific characteristic of the *A. madagascariensis* muscle lipids.

The FA profile of *A. madagascariensis* generally resembles that of other freshwater fish in that it contains high amounts of SFA and MUFA and higher levels of n-6 PUFA than n-3 PUFA. The changes in lipid content and FA profile during the 5-month sampling period indicates that this fish, which was caught in freshwater, shows the generally accepted patterns, i.e. an increase in lipid and PUFA content during the pre-awning period. The occurrence of high amounts of PUFA indicates that *A. madagascariensis*, an abundant freshwater fish in Madagascar rivers, is a relatively good source of dietary PUFA.

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