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Fatty acids in female's gonads of the Red Sea fish *Rhabdosargus sarba* during the spawning season

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PEER REVIEW

Peer reviewer

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Comments

This research work in which authors have demonstrated the fatty acids profile in female's gonads of the Red Sea fish, *R. sarba*, during the spawning season. This determination of fatty acid can be used for the fishing time when it is not reproductively active and has high nutritional value in terms of lipid.
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ABSTRACT

Objective: To determine the fatty acids profiles in female fish, *Rhabdosargus sarba* (*R. sarba*) from the Red Sea during the spawning season.
Methods: Monthly individual *R. sarba* were obtained from Bangalah market in Jeddah, Red Sea and transported to the laboratory in ice aquarium. The total length, standard length and weight were measured, fishes were dissected. Ovaries were removed, weighed and 10 mL of concentrated hydrochloric acid were added to 10 g of the ovary in a conical flask and immersed in boiling water until the sample was dissolved and the fat was seen to collect on the surface. The conical was cooled and the fat was extracted by shaking with 30 mL of diethyl ether. The extract was bowled after allowing the layers to separate into a weighed flask. The extraction was repeated three times more and distilled off the solvent then the fat dried at 100 °C, cooled and weighed. Then 50 mg of lipid was put in a tube, 5 mL of methanolic sulphuric acid was added and 2 mL of benzene, the tube well closed and placed in water bath at 90 °C for an hour and a half. After cooling, 8 mL water and 5 mL petroleum were added and shaked strongly and the ethereal layer was separated in a dry tube, evaporated to dryness. The fatty acid methyl esters were analyzed by using a Hewlett Packard (HP 6890) chromatography, a split/splitless injector and flame ionization detector.
Results: In female *R. sarba*, a total of 29 fatty acids were detected in ovaries throughout the spawning season. The main fatty acid group in total lipid was saturated fatty acid (SFA, 28.9%), followed by 23.5% of polyunsaturated fatty acids (PUFA) and 12.9% of monounsaturated fatty acids (MUFA). The dominant SFA were palmitic and stearic, the major MUFA were palmitoleic and oleic, and the major PUFA were C18:2 and C22:2. During spawning stages, there were no significant differences in total SFA, MUFA and PUFA. The highest value of SFA was in late spawning (36.78%). However, the highest value of MUFA and PUFA was in spawning (16.70% and 24.96% respectively). During spawning season there were significant differences in total SFA between March (late spawning stage) and December (nearly ripe stage), $P<0.05$.
Conclusions: From the present study it has been shown that 29 fatty acids were detected in ovaries of *R. sarba* throughout the spawning season. The main fatty acid group in total lipid was SFA followed by PUFA and MUFA. The dominant SFA were palmitic and stearic, the major MUFA were palmitoleic and oleic, and the major PUFA were C18:2 and C22:2. The highest value of SFA was in late spawning. However, the highest value of MUFA and PUFA was in spawning.

KEYWORDS
Sparidae, *Rhabdosargus sarba*, Fish, Fatty acids, Spawning, Gonads, Red Sea

1. Introduction

The Sparidae (sea bream) family is one of the most important fish species in the Red Sea, and has several species that are potentially adaptable to aquaculture[1]. The different compositions of fatty acid (FA) among fish species were reported to be due to the different climatic conditions, diets, ages, spawnings, maturities and types of species[2-4]. Lipids and their constituent FA have a particularly important role in the reproductive parameters

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of fish, such as, egg quality, spawning, hatching rate and survival of larvae[5]. Lipids are utilized as energy sources throughout embryogenesis, and particularly in the later stages of development prior to hatching. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major FAs in the total lipid of eggs of most fish and these FAs markedly influence the reproductive parameters. In addition, arachiodonic (C20:4) as a major FA, stimulates ovarian and testicular steroidogenesis and it is assumed to be involved in embryonic development of the immune system, hatching and early larval performance[6]. Recent studies have clearly shown the importance of polyunsaturated fatty acids (PUFAs) nutritional values for human health[7,8]. The nutritional importance of fish consumption is in great extent associated with the content of PUFAs, especially omega-3 FAs and omega-6 FAs[9]. The aim of the study is to examine the FA profiles in the female *Rhabdosargus sarba* (*R. sarba*) from the Red Sea. It is emphasized that no earlier studies of the FAs composition of this species has occcurred.

2. Materials and methods

2.1. Fish samples

Monthly individual *R. sarba* (L) of fishes were obtained from Bangalah market in Jeddah, Red Sea. The experiments were carried out in different seasons from October 2010 to December 2011. Fishes were transported to the laboratory in ice aquarium, and then the total length, standard length and weight were measured. Fishes were dissected; ovaries were removed, weighed and thoroughly examined.

2.2. Lipid extraction

A total of 10 mL of concentrated hydrachloric acid were added to 10 g of the sample in a conical flask and immersed in boiling water until the sample was dissolved. At this stage the mixture changed to brown or violet in colour and the fat was seen to collect on the surface. The conical was cooled and the fat was extracted by shaking with 30 mL of diethyl ether. The extract was bowled after allowing the layers to separate into a weighed flask. The extraction was repeated three times more and distilled off the solvent then the fat dried at 100 °C, cooled and weighed.

2.3. Methylation of lipid

A total of 50 mg of lipid was put in a tube, 5 mL of methanolic sulphuric acid (1 mL conc sulphuric acid and 100 mL methanol) was added and 2 mL of benzene, the tube well closed and placed in water bath at 90 °C for an hour and a half. After cooling, 8 mL water and 5 mL petroleum were added shacked strongly and the ethereal layer was separated in a dry tube, evaporated to dryness.

2.4. Gas chromatographic conditions

The FA methyl esters were analyzed by using a Hewlett Packard (HP 6890) chromatography, asplit/splitless injector and flame ionization detector.

Data were analysed by using an independent Student *t*-test and One way ANOVA for significant differences. The level of significance used was $P<0.05$.

3. Results

Table 1 showed that there were 29 FAs detected, which include both essential (unsaturated) and saturated FAs. The saturated fatty acids (SFA) form the largest component followed by PUFAs and monounsaturated fatty acids (MUFA), the means were 28.9%, 23.5% and 13%, respectively. It can be seen that the dominant FAs among SFA in females was palmitic acid (C16:0, 14.7%) followed by stearic acid (C18:0, 7.2%). The major MUFA were palmitoleic acid (C16:1, 6.1%) and oleic acid (C18:1, 4%). The dominant among PUFA were linoleic acid (C18:2, 11%).

Table 1

SFA, MUFA and PUFA, FA compositions during the seasonal spawning cycle of *R. sarba*.

SFA	FA composition	MUFA	FA composition	PUFA	FA composition
C6:0	1.3370±0.8060	C14:1	0.506±0.3660	C18:3	2.190±1.3270
C8:0	1.5770±0.4060	C15:1	0.668±0.0841	C18:2	10.947±2.2490
C10:0	0.0510±0.0430	C16:1	6.077±2.6920	C20:5	1.689±0.2239
C11:0		C17:1	0.641±0.5050	C20:4	2.403±0.8060
C12:0	0.0710±0.0490	C18:1	3.991±1.1928	C20:3	0.959±0.3667
C13:0	0.4620±0.8480	C20:1	0.575±0.2620	C20:2	1.595±1.1090
C14:0	0.7110±1.0015	C22:1	0.489±0.6100	C22:2	2.715±1.0360
C15:0	0.3370±0.4430	To	12.95845	C22:6	0.953±1.1240
C16:0	14.6980±1.2990			To	23.45777
C17:0	1.2550±0.1650				
C18:0	7.2139±2.9790				
C20:0	0.2500±0.2940				
C21:0	0.0478±0.0260				
C23:0	0.2670±0.0000				
C24:0	0.6440±0.0000				
To	28.92692				

Table 2 represents FA profile of ovaries samples obtained in four different months (December, January, February and March), which were in spawning period. No significant variations were indentified in total saturated fatty acid (∑SFA), monounsaturated fatty acid (∑MUFA) and polyunsaturated fatty acid (∑PUFA) amongst months, and the highest values were 36.78%, 16.70% and 24.96%, respectively. Twenty nine different FAs were identified in the analyzed *R. sarba* ovaries lipid fraction samples. During the spawning season (December-March), the highest value for ∑SFA was observed in late spawning stage (March) as 36.78% and significantly differ ($P<0.05$) from those (23.71%) in nearly ripe stage (December). Palmitic acid (C16:0) and stearic acid (C18:0) were the major constituents of total saturated FAs. The highest level of these two acids were 16.3% in spawning and 11.7% in late spawning stages, respectively. Variations amongst months were also observed for ∑MUFA ranging from 10.67% to 16.70%. Fluctuation in ∑PUFA of the ovaries samples were observed, ranged between 22.23%-24.95%, in which the highest value was found in spawning stage (February), while the lowest was represented in later spawning (March). Palmitoleic acid (C16:1) was found to be the main MUFA varying between 2.54% and 9.02% with the highest value in spawning stage (February). Oleic acid (C18:1) was the second abundant MUFA, its highest concentration (5.4%) recorded in spawning

stage during February, while ripe stage represented the lowest value (2.7%). The other MUFAs were noticed to be in negligible amount.

Table 2

Fatty acid composition in *R. sarba* ovaries, during the spawning season (2010-2011).

Lipid parameter	Stages			
	Nearly ripe	Ripe	Spawning	Spawning
	December	January	February	March
Total lipid	0.3800	2.3500	0.5000	0.2000
SFA				
C6:0	1.6508	2.0310	1.4927	0.1760
C8:0	0.9940	1.9330	1.6470	1.7340
C10:0	0.0270	0.0190	0.0450	0.1140
C12:0	0.0320	0.0267	0.0980	0.1270
C13:0	0.0369	0.0306	0.0458	1.7350
C14:0	0.1667	0.1509	0.3196	2.2096
C15:0	0.1557	0.0980	0.0930	1.0014
C16:0	13.1820	14.2550	16.2500	15.1049
C17:0	1.4520	1.2738	1.0487	1.2470
C 18:0	5.9290	5.2970	5.9680	11.6590
C20:0	0.0770	0.1270	0.1060	0.6900
C21:0		0.0290		0.0660
C23:0				0.2670
C24:0				0.6440
Total	23.7050*	25.2750	27.1170	36.7790
MUFA				
C14:1	0.1841	0.2732	0.5740	0.9960
C15:1	0.5880	0.7830	0.6720	0.6280
C16:1	5.9470	6.8020	9.0238	2.5370
C17:1	1.3030	0.2680	0.2258	0.7670
C18:1	4.4520	2.7250	5.4350	3.3830
C20:1	0.3850	0.4480	0.5060	0.9610
C22:1	0.1876	0.1070	0.2639	1.4000
Total	13.0470	11.4090	16.7010	10.6740
PUFA				
C18:3	1.9490	3.1205	3.2820	0.4108
C18:2	10.5450	12.8800	12.4300	7.9340
C20:5	1.3645	1.8479	1.8274	1.7167
C20:4	2.8480	2.2380	3.1880	1.3490
C20:3	0.5970	0.7530	1.0590	1.4280
C20:2	1.0550	1.1465	0.9240	3.2537
C22:2	3.6920	1.9540	1.6940	3.5200
C22:6	0.5057	0.1391	0.5508	2.6170
Total	22.5590	24.0810	24.9500	22.2310

Values are significantly different ($P<0.05$).

It can be seen that C18:2 was the main PUFA in *R. sarba* ovary and has the maximum percentage in ripe and spawning stage during January and February (12.9%, 12.4%) respectively. At the end of spawning season (March) the concentration of this acid decreased reaching to the lowest value (7.9%). The second major PUFA were C22:2 and C20:4 and their maximum concentration were 3.7% and 3.2% in nearly ripe and spawning stage (December and February) respectively. Their concentration decreased to the minimum values (1.69%, 1.35%) in spawning stage during February and March respectively. The most abundant FAs in the ovaries samples was palmitic acid (C16:0) ranged from 13.18% to 16.25%.

4. Discussion

In females of *R. sarba*, SFAs of total lipid constituted the majority of the FAs pool, followed by PUFAs and MUFA. The results are in agreement with those reported by Erdem *et al*., for sea bass (*Dicentrarchus labrax*)[10]. The saturated fraction constituted in female 28.93% of the total FAs, with palmitic acid C16:0 as the most important FA followed by stearic acid C18:0. The dominance of palmitic acid in fish lipid has been reported by other authors[11,12]. Similar results of other fish species have also been reported in the literature[13,14].

The relatively higher levels of MUFA in female *R. sarba* are in agreement with the observations of higher levels of monoenoic acid coinciding with the development of *Salmo salar*[15]. As far as MUFA fraction, oleic acid (C18:1) was the basic FA. Similar results were reported for gilthead sea bass and for *Cyprinus carpio*[16,17]. In the present study, linoleic acids (C18:2) was the prominent PUFA, representing 10.9%. This result is in accordance with Bayir *et al*., who reported that C18:2 was the major PUFA for gilthead sea bream and two-banded bream (Sparidae)[18]. Similar findings have been reported in three Tunisian silverside fish[19]. *R. sarba*, as well as other omnivorous species belonging to Sparidae, showed great diversity in the type of food ingested, so as in animal and in plant organisms. These feeding habits might explain the high variability observed in the majority of FA levels from ovaries. Taking into account that the majority of marine organisms are poor in 18:2, the high percentage of this FA in the gonads of *R. sarba* in the present study is remarkable.

Marine fish have low or no capacity to synthesize highly unsaturated fatty acids (HUFAs) from C18 FAs. HUFAs are important components of cell membranes and are thought to play important roles in membrane fluidity, modulation of enzyme activity, neural development and regulation of stress resistance. Especially, (EPA: 20:5n-3) and (DHA: 22:6n-3) were considered as dietary essential FAs for normal growth and survival in most marine fish[20]. In the present study, arachidonic acid (AA) showed lower levels, 2.4%. Marine fish species generally only have PUFA, which originate from marine phytoplankton. Therefore, AA levels were generally low or negligible in total FAs of lipid of marine fish species, the content of AA in marine fishes was lower than freshwater fishes[21]. Therefore, high levels of AA may be useful as a lipid biomarker of herbivorous fishes, which prefer seaweed[22]. Although C20:4 has similar biologically importance with EPA and DHA, it is often neglected in fish because it occurs only at a very low concentration, despite the possessing vital functions as the main precursor of various eicosanoids, which are produced by the ovarian tissue and play important roles in the ovulation process[20], and cholesterol accumulation in tissues[23], which have important roles in a variety of physiological functions including osmoregulation, cardiovascular function and the function of reproductive systems[24]. Prostaglandins plays an important role in fish reproduction[25], with AA being the principle prostaglandins precursor involved in spawning activity of fishes, which including ovulation and sperm production[26], and its lower levels in both sexes were probably due to its mobilization. Evidence for the importance of AA in reproduction was first identified in European sea bass brood stock[27,28]. The DHA content of lipids in tropical and subtropical marine fish species was reported to be lower than those of arctic and subarctic ones[29]. Fishes living in warm-water seas do not appear to require as much DHA in their cell membranes as cold-water species[30]. The lipid content of tropical fish

species is generally influenced by high ambient temperature, and the membrane lipids of these fishes are easily fluidized even if the major FAs in their lipids are composed of saturated and monoenoic FAs. The present study showed that the total SFA in the ovary remained more or less constant between December (pre-spawning) and February (spawning) and then increased in March (36.78%) (post-spawning). This result is in accordance with female gilthed sea bream during their reproductive cycle[31]. It has been assumed that PUFAs such as C20:5 and C22:6 are involved in the physiological reproductive processes of giltheadseabream[32]. In the present study, the highest ovarian level of DHA (2.61%) was in late spawning stage (March) and the lowest one (0.13%) was in ripe stage. These findings are in disagreement with the previous work who reported a decrease in this FAs that observed in spawning stage of *S. aurata*[31]. The high levels of C16:0, C16:1, C18:2, reflected the importance of these FAs in forming an energy reserve and a temporary reservoir of PUFA for future embryonic development. The present result showed that C18:0 was constant in pre-spawning and spawning months. However, the high increase in this FA that observed in March (post-spawning), despite the lipid depletion during gonadal regression, suggested the importance of this FA in the ovary, presumably as an eicosanoid precursor. The high content of C18:2 in the ovary of *R. sarba* was observed in the pre-spawning and spawning months. However, in March high loss of these FA was observed. The higher loss of C18:2 with respect to the remainder of the FAs could be related to the higher levels of this FA in the *R. sarba* eggs[32,33]. In post-spawning month the level of C18:0 was highly increased (11.66%). Similar result has been reported for gilthead seabream[31]. Levels of C16:0, C18:1 and low levels of HUFA were characteristic for neutral lipids of the most marine fish[34]. This is also in concordance with the common opinion that fish species accumulate depot lipids mainly composed of saturated and monoene FAs[35]. The ovary of *R. sarba* contains notable levels of (C20:4 AA) in their spawning season, however, in February, it increased to the highest level (3.2%). It has been reported that C20:4 levels in eggs and new hatched larvae from eight species of marine teleosts have several folds higher than in the ovary of these fish[36]. This indicates that lipid changes in gonad is in relation to sexual maturity and spawning[37].

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Lipids and their constituents FA have a particularly important role in the reproductive parameters of fishes; especially lipids are utilized as energy sources for reproduction. Therefore there is a need of knowing the FAs profiles in the *R. sarba*.

Research frontiers

The present research work aimed to determine the FAs profiles in the female *R. sarba* from the Red Sea, as there is no information on the FAs composition during the spawning season.

Related reports

A total of 29 FAs were detected in ovaries throughout the spawning season. The main FA group in total lipid was SFA followed by PUFAs and MUFAs. The dominant SFA were palmitic and stearic, the major MUFA were palmitoleic and oleic, and the major PUFA were C18:2 and C22:2. The highest value of SFA was in late spawning. However, the highest value of MUFA and PUFA was in spawning.

Innovations and breakthroughs

The Sparidae (sea bream) family contains a number of economically important species. Family Sparidae is one of the most important fish species in the Red Sea. Most seabreams are excellent food fish and are of notable commercial importance. In the present study, authors have determined the FA profiles in the female *R. sarba* from the Red Sea during the spawning season .

Applications

From the literature survey it has been found that the different compositions of FA among fish species were reported to be due to different climatic conditions, diet, age, maturity and type of species. Recent studies have clearly shown the importance of PUFAs nutritional values for human health. The nutritional importance of fish consumption is in great extent associated with the content of PUFAs, especially omega-3 FAs and omega-6 FAs.

Peer review

This research work in which authors have demonstrated the FAs profile in female's gonads of the Red Sea fish, *R. sarba* during the spawning season. This determination of FA can be used for the fishing time when it is not reproductively active and has high nutritional value in terms of lipid.

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