The effect of spawning season on fatty acid composition of tigertooth croaker, *Otolithes ruber* from Abadan and Khoramshahr areas (Iran)

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Introduction

Fatty acids especially Docosahexaenoic (DHA) and Eicosapentaenoic acid (EPA) as more prevalent among the essential fatty acids are very important for normal physiology of marine fishes and also very beneficial to human health in particular for reducing risk of heart diseases, growth of fetal nerve tissue and infant brain and visual function (Colquhoun *et al.,* 2008; Khoddami *et al.,* 2009; Oksuz *et al.,* 2011; Khoddami *et al.,* 2012). Few studies have been conducted on fatty acid content and composition of fish carcass during the spawning season. Tziask *et al.,* (2007) showed that the fat content of Mediterranean horse mackerel, *Trachurns mediterraneus* carcass reaches its lowest level during the spawning season. Also, the lipid content of two fish species *Sardinella longicep* and *Sardinella fimbriata* was found to be higher in fish sampled before sexual maturation compared to those sampled at the peak of sexual maturation (Som and Radhakrishnan, 2013). However, there was no significant difference in lipid content of mackerel, *Rastrelliger kanagurta* before sexual maturation and at the peak of sexual maturation (Ganga, 2010). Also, There were significant differences ($P<0.05$) in the levels of SFA,MUFA and PUFA in terms of species, season and muscle types of Mediterranean Octopuses (Ayas, 2012).

Tigertooth croaker, *Otolithes ruber* is a commercially and economically valuable fish species inhabiting the Persian Gulf and Oman Sea basin. Tigertooth croaker, *O. ruber* is commercially and economically one of the most important species of Sciaenidae family inhabiting the

Persian Gulf and Oman Sea basin (Taghavi Motlagh *et al.*, 2004).

Material and methods

A total of 45 Tigertooth croaker specimens were randomly sampled from Abadan and Khorramshahr ports, landing, in three sexual stages including before sexual maturation (n=15), peak of sexual maturation (n=15) and after spawning (n=15) during January 2013 to July 2014. The samples were transferred on ice powder to the laboratory of Ahwaz University. Immediately after delivery to the lab, they were prepared for bioassay. The standard length, width, weight and gonad weight were measured and then fish were dissected to determine the stage of sexual maturation. The gonadosomatic index (GSI) was calculated by dividing gonad weight by the total body weight as recommended by Fennessy (2000) and Funamoto (2004). 5-step key was used to determine the sexual maturation stage (Biswas, 1993) and inclusion of characteristics in forms. The separation of flesh and skin was carried out precisely as well. To analyze the fatty acid composition in muscle, three samples of fillet were randomly prepared from fish of each step. 15 fillets were divided into three groups of 5 and each 5 fillet samples were mixed, homogenized and then one sample was prepared from each group of 5. Until analysis of fatty acids, the prepared samples were stored at -80°C (Abi-Ayad *et al.*, 2000).

Assessment of fatty acids was done using gas chromatography Agilnet-6890 as follows:

Wet samples were weighed precisely (to an accuracy of 1 g) and after homogenization, they were transferred to the test tube with thread and the fat extraction was done by the Folch method using a mixture of chloroform + methanol. To accelerate the extraction, after closing the tube tubes, they were vigorously stirred and placed in an ultrasound for 10 min, and then the mixture was centrifuged and the solvent containing fat was transferred into a tube whose weight had already been measured, and the above step was repeated twice. Then, chloroform and methanol were vapored using nitrogen gas and the weight of pipe containing fat was measured and afterward, the fat percentage was calculated from their difference (Folch *et al*, 1957).

In all fatty acids, saturated, mono-unsaturated and poly unsaturated fatty acids of *O. ruber* fillets, the ratio of n_3 to n_6, and the ratio of unsaturated to saturated fatty acids were calculated.

Henderson and Tocher (1987) have introduced the ratio of n_3 to n_6 in freshwater fish from 0.5 to 3.8 and in saltwater fish from 4.7 to 14.4.

The ratio of unsaturated fatty acids to saturated ones in edible fats is important, if the ratio is more than 0.35, it is useful for human nutrition (Kminkova *et al.*, 2001). One-way analysis of variance (ANOVA) was employed to compare the means of fatty acids at 95% confidence level. When significant F-ratios were calculated by ANOVA, the Duncan test was applied to identify which groups were different.

Results and discussion

The lipid content of muscle tissue did not show significant differences in various stages of the spawning season (p>0.05). However, the highest values were observed at the peak of sexual maturation. PUFA levels at pre-sexual maturation and at the peak of sexual maturation were higher than the SFA and MUFA (Table 1, p<0.05). After spawning, PUFA values were less than SFA and at all three steps of maturation, the amount of MUFA was less than other PUFA and SFA (Table 1, p<0.05). Palmitic acid was the most saturated fatty acid in all three stages of the spawning season (Table 1).

Tabel 1: Fatty acid composition (weight % of total fatty acids) during spawning season (n=45).

Fatty acid	Before sexual maturation (%)	Peak of sexual maturation (%)	After spawning (%)
C12:0	0.11^{a} ±0.42	0.05^{a}± 0.36	-
C14:0	0.33^{a}±2.78	0.16^{b}±3.51	0.15^{a}±2.87
C16:0	0.29^{a}±23.39	0.11^{a}±23.61	0.30^{b}±26.47
C18:0	0.16^{a}±6.94	0.36^{a}±6.64	0.21^{b}± 7.85
C20:0	0.06^{a}±0.28	0.13^{a}±0.36	0.06^{a}±0.10
C22:0	0.16^{a}±0.48	0.24^{a}±0.58	0.07^{a}±0.13
C24:0	0.07^{a}±0.38	0.06^{a}±0.53	0.07^{b}±2.87
Total SFA	1.22^{a}±34.67	1.25^{a}±35.59	1.52 ^{b}±40.14
C14:1n-5	0.11^{ab}±0.28	0.03^{a}±0.08	0.05^{b}±0.79
C16:1n-7	0.48^{a}±7.73	0.05^{a}±6.93	0.76^{a}±6.99
C18:1n-9	0.36^{a}±12.59	0.18^{a}±12.54	0.46^{a}±12.53
Total MUFA	0.30^{a}±20.6	0.18^{b}±19.55	0.25^{a}±20.31
C18:2n-6	0.13^{a}±1.42	0.08^{a}±1.87	0.12^{b}±0.85
C18:3n-3	0.10^{a}±1.12	0.02^{a}±1.14	0.08^{a}±0.94
C18:3n-6	0.08^{a}±0.86	0.07^{b}±0.46	0.07^{b}±0.47
C18:4n-3	0.19^{a}±0.66	0.21^{a}±0.75	0.007^{b}±0.02
C20:3n-6	0.17^{a}±0.48	0.14^{a}±0.93	0.01^{b}±0.09
C20:3n-3	0.16^{a}±4.12	0.04^{a}±0.23	0.07^{a}±0.33
C20:4n-6	0.10^{a}±0.37	0.24^{b}±2.87	0.08^{c}±3.92
C20:5n-3	0.38^{a}±5.62	0.10^{b}±7.99	0.36^{c}±4.29
C22:5n-6	0.10^{a}±0.75	0.12^{a}±0.40	0.18^{b}±2.82
C22:5n-3	0.31^{a}±1.68	0.18^{a}±1.34	0.06^{b}±0.38
C22:6n-3	0.41^{a}±18.48	0.48^{b}±20.41	0.17^{c}±12.09
Total PUFA	1.27 ^{a}± 35.56	1.29^{b}±38.39	0.89^{c}±25.64
n3∑	1.07 ^{a}± 31.48	0.11^{a}±31.86	0.23^{b}±18.05
n6∑	0.18^{a} ±3.88	0.35^{a}±6.53	0.48^{a}±8.15
n3/n6	0.52 ^{a}± 8.11	0.39 ^{b}± 2.21	0.20^{c}±2.21
USFA/SFA	0.12^{a}±1.61	0.11 ^{a}± 1.62	0.02^{b}±1.14
∑USFA	3.02 ^{a}± 56.16	3.12^{b}±57.94	2.86^{c}±45.95

Different letters represent significant difference. Data were represented as means±SD.

In this study, the amount of Palmitic acid after spawning (26.47±0.3 %) increased compared to that at the pre-sexual maturation (23.61±0.11%) and the peak of sexual maturation (23.39±0.29%) (Table 1) (p<0.05). Among mono unsaturated fatty acids, Oleic acid showed the highest level (12.59 -12.53 %) (Table 1) (p<0.05). The prevalent fatty acid was DHA and its levels at the peak of sexual maturation (20.41± 0.48 %) were more than that before sexual maturation (18.48±0.41 %) and after spawning (12.09) (Table 1) (p<0.05).

The highest and lowest n3/n6 ratio were 8.11 and 2.21 found before sexual maturation and after spawning, respectively (Table 1, p<0.05).

The ratio USFA/SFA was more than one at all stages of the spawning season with maximum and minimum ratios at the peak of sexual maturation (1.62) and after the spawning season (1.41), respectively (Table 1). Sathivel *et al.* (2002) reported that fish diet is the most important factor affecting the composition of fatty acids. During fish growth, fat is stored in the body and used during the spawning season as energizing materials of breeding (Solberg *et al*., 2006). For instance, in *Trachurus meditteraneu*, body fat stores are reduced during the spawning season (Tzikas *et al.*, 2007). (Chitra Som and Radhakrishnan (2013) observed higher lipid content before sexual maturation compared to that at the peak of sexual maturation in *S. longiceps* and *S. fimbriata*. Also, the highest and lowest lipid content of *O. ruber* was observed at the peak of sexual maturation and after spawning, respectively (Papahn and Ronaq, 2002) as found in our study. Our results were similar to the findings of Hardy and Keay (1972) who reported that the increases in body fat of *Cornish marcel* in relation to reduction of water temperature in winter (i.e. before sexual maturation) from 30-31°C to 25-26°C. Similar results were obtained for other Sardine fishes (Games-Mezaa *et al.,* 1999). In addition to temperature, the accumulation of food in the winter may be another reason for increases in body fat stores. Fish can consume it in large quantities and thus receive more fatty acid and PUFA in winter (before sexual maturation) following that (Som and Radhakrishnan, 2013). Palmitic acid as metabolic energy is very important during fish growth and development (Henderson *et al.*, 1984). According to Table 1, the saturated fatty acids showed highest levels in all three stages of the spawning season of Tigertooth croaker compared to other fatty acids. The palmitic acid comprises the most saturated fatty acid in Atlantic herring, *Clupea harengus pallasi* and Baltic Herring, *Clupea harengus membras and Indian* mackerel, *R. kanagurta,* Rainbow Trout (*Oncorhynchus mykiss*). The amount of palmitic acid in the Baltic herring reduced at spawning time which apparently was directly in relation to the palmitic acid content of the planktonic lipid (Huynh *et al.,* 2007; Ganga *et al.,* 2010, Oraei *et al.,*2011).

In most fish, oleic acid was reported as the most mono unsaturated fatty acid. In Atlantic herring, oleic acid as most mono unsaturated fatty acid showed a decreasing trend during breeding season (Huynh *et al.,* 2007) in order to provide energy for the metabolism and gonadal development (Henderson *et al.,* 1984). This result was in consistent with the results of this study. This situation was also reported in the Baltic herring where feeding was stopped during migration and Oleic acid used for spawning (Linko *et al.,* 1985). In *S. longiceps* and *S. fimbriata*, oleic acid had the highest levels among the mono unsaturated fatty acids (Chitra Som and Radhakrishnan) (Radhakrishnan and Som, 2013). According to our results, DHA and EPA had higher levels among PUFAs as reported for Caspian kutum (Tocher and Horvie, 1988), *S. fimbriata* and *S. longiceps* (Som and Radhakrishnan, 2013). DHA is important in the lipid membrane composition (Tocher and Horvie, 1988) and its energetic role is not significant. It seems that Oleic acid has a key role in metabolism during the spawning season as less oleic acid and more DHA was observed in *Clepea herengus pallasi* during the spawning season (peak of sexual maturation) (Huynh *et al.,* 2007). The presence of DHA and PUFA fatty acids in aquatic animals is due to their accumulation in the food chains. These fatty acids are made by a variety of seaweeds and then consumed by planktons and other small sea organisms (micro-planktons and zooplankton) and finally incorporated in fish which feed on these sea organisms (Holub, 1992). DHA and EPA are involved in the reproductive process and their presence in diet increases reproduction efficiency, fertilization, and egg quality. In Indian mackerel, DHA as the main PUFA was the highest before the sexual maturation stage (Ganga *et al.,* 2010) which was contrary to the result of our study. However, the results of Saito *et al.* (1997) on Skipjack**,** *Bonito tuna* were similar to our results where higher levels of DHA were observed at the peak of sexual maturation. In Skipjack, DHA is more used at the peak of sexual maturation in the formation of eggs in the female gonads (Henderson *et al.,* 1984; Wiegand and *Idler,* 1985). Ratio of n_3 to n_6 in this study reduced from 8.11 during before sexual maturation to 4.87 at the peak of sexual maturation and to 2.21 after spawning. Increasing of this fatty acid in the diet reduces plasma lipid, incidences of cancer, and heart disease and shock syndrome (Bell *et al.,* 1991; Gershanovich *et al.,* 1991). In our study, the ratio of unsaturated fatty acids to saturated ones was more than one in all maturation stages which represents the good quality of muscle fat in *O. ruber*. Similar results were found in Pacific herring (Huynh *et al.,* 2007). According to Panetsos (1978), fish with more than 8%, 3.8%-8% and less than 3% fat are considered as fatty fish, average fat and low fat fish, respectively. Based on this classification, *O. ruber* with 4.32% fat before sexual maturation, 4.45% fat at the peak of sexual maturation and 4.30% fat after spawning could be considered as fish with average fat. Tigertooth croaker with fat content of 4-4.5% could be considered as fatty and low fat fish when compared with skip-jack (11.4% fat) and also *Thunnus tonggol* (5% fat) and bluefin tuna (8.9%) (FAO, 1989).

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