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Research Paper

Study on the sex-specific seasonality of fatty acid profiles in golden mahseer (*Tor putitora*) collected from a lacustrine ecosystem of Indian Himalaya

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ABSTRACT

A sex-specific seasonal fatty acid profile study was conducted in sexually matured male and female golden mahseer (*Tor putitora*). For this study, five males and females were collected from Bhimtal lake, Uttarakhand, India, during summer, monsoon, pre-winter, and winter. Biochemical (proximate) and fatty acid compositions were analyzed in homogenized muscle samples. Among biochemical composition, only moisture and lipid contents were found to fluctuate, but inversely, with seasons in both the sexes. A substantial sex-specific change in major fatty acid classes, namely Sc-FAs, n-3 and n-6 PUFAs were found, explicitly driven by reproductive status, in the latter half of spawning season, i.e., in monsoon. This seasonality was more pronounced in females than males, in terms of diminishing Sc-FAs, n-6 and n-3 PUFAs. Both males and females appeared to be the best in fatty acids in winter followed by pre-winter. However, in sex-wise comparison in each season, males appeared to be the best compared to females in summer, better in monsoon, but both were equally good in pre-winter and winter.

Key words: Fatty acid profile, golden mahseer, seasonal, sex-specific

Preference for fish over other animal proteins is a trend among health-conscious people of this generation (Joshi *et al*., 2017). People opt for fish over other animal proteins because it has the best inherent nutrient quality, mainly due to its superior oil and fatty acids (FAs). Among different sources of edible oils, fish oil is best because it contains abundant long-chain n-3 polyunsaturated FAs (n-3 LC-PUFAs) such as eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) (Lund, 2013). Due to its richness in n-3 LC-PUFAs, it is considered as the panacea against many serious ailments, namely cardiovascular diseases (CVDs), rheumatoid arthritis, asthma, diabetes, cancer, Alzheimer's disease, schizophrenia, attention-deficit hyperactivity disorder (ADHD), dyslexia, personality disorder, depression, bipolar disorder, etc. (Riediger *et al.*, 2009). Although it contains high levels of alpha-linolenic acid (ALA; C18:3n-3; Gogus and Smith, 2010), even linseed oil cannot substitute fish oil because humans cannot convert ALA to EPA and DHA as efficiently as fish does (Tocher, 2010).

Due to these healthful properties of fish oil, more and more indigenous food fishes from different corners of the world are being evaluated for oil and FA compositions; and as a result, a database on the functionality of these fishes are being generated for past some decades (Ackman, 1967, 1990; Grigorakis, 2007; Huynh and Kitts, 2009; Murillo, Rao and Durant, 2014; Osman, Suriah and Law, 2001; Özogul and Özogul, 2007; Rahman, Huah, Hassan and Daudb, 1995). Such information is also being generated for many indigenous food fishes from the Indian subcontinent in the past few years (Ackman *et al.*,, 2002; Bogard *et al*., 2015; Joshi *et al*., 2017; Mohanty *et al*., 2016; Sarma *et al*., 2019; Sharma *et al*., 2010). However, these reports are based on one-time (or single) sampling only, wherein vital FA influencing factors like season, sex, and reproductive status of male and female fishes are not discussed deliberately.

The oil and FA composition of fishes vary from species to species depending on their food and feeding habits (Gomes *et al*., 2016). Even within the same species, they change with change in season; especially, with the seasonal shift in food, environmental factors, reproductive status of respective sexes, etc. (Almansa *et al*., 2001; Grigorakis, 2007; Kaçar and Başhan, 2015). Considering these factors, many researchers have evaluated and reported the seasonal changes in FA profiles of fishes from both freshwater and marine ecosystems. Such marine fishes include: *Trachurus trachurus* (Bandarra *et al*., 2001), *Engraulis encrasicholus*, *Sardina pilchardus*, *Sprattus sprattus*, *Trachurus trachurus* (Pirini *et al*., 2010), *Solea solea* (Gökçe *et al*., 2004), *S. pilchardus*, *E. encrasicholus*, *Spicara smaris* (Zlatanos and Laskaridis, 2007). Similarly, freshwater fishes include *Sander lucioperca* (Guler *et al*., 2007), *Carassius auratus* (Dal Bosco *et al*., 2012), *Colossoma* *macropomum*, *Leporinus friderici*, *Prochilodus nigricans*, *Brachyplatystoma filamentosum*, *Brachyplatystoma flavicans* (Petenuci *et al*., 2016) and *Thymallus arcticus* (Sushchik *et al*., 2007). However, in these reports, sex-dependent seasonality, a vital FA influencing factor, is ignored. Since, depending on sex, some FAs from muscle get partitioned to gonad for spawning preparation (Johnson *et al*., 2017). Only a few have reported the sex-specific seasonality in detail, considering these factors. Kacar *et al.* (2016) have presented such phenomenon in detail in males and females *Silurus triostegus* separately.

Golden mahseer is among many native food-grade Indian Himalayan cyprinids (IHCs), which are reported to be superior in FAs composition (Joshi *et al*., 2017; Mohanty *et al*., 2016). It is one of the important fish of the Himalaya, known for its excellent food and game value (Nautiyal, 2014; Sharma *et al*., 2016). It is popular among all available fishes in the Himalayan aquatic ecosystem, starting from Afghanistan in the west to Myanmar in the east (Nautiyal, 2014; Sharma *et al*., 2016). This species is considered highly delicious throughout these regions, and it is rarely available in the market because of high demand; and consequently, it fetches a good price as well. Therefore, high-demand and good-price-driven overexploitation issues are linked with this species (Nautiyal, 2014). Its nutritional and functional value, especially oil and FA composition, has already been reported by Mohanty *et al.* (2016). However, in this report, detailed sex-specific FA seasonality is missing. Therefore, the present study was designed to evaluate the sex and season-specific FA profiles of males and females separately, with the following questions.

Do FA compositions of male/female golden mahseer remain the same throughout the year; if not, then in which season, males have the best FA profile? Similarly, in which season, females have the best? And also, in each season, which sex has the best FA composition?

MATERIALS AND METHODS

Sample collection and preparation:

Mature male and females golden mahseer (n=10 to 12 in each sampling) were collected from Bhimtal lake (Bhimtal, Uttarakhand, India) in different seasons, i.e., summer (May-June, 2016), monsoon (July- August, 2016), pre-winter (October-November, 2016) and winter (February, 2016). For this, a gill net was used at night, and the fishes were collected at dawn. Water temperature was also recorded at the time of fish samplings. The collected fish and plankton samples were transferred to the “Nutrient Profiling and Environmental Fish Biology Laboratory” of ICAR-DCFR, Bhimtal, Uttarakhand, India, within an hour, in an icebox containing crushed ice. After the arrival at the laboratory, fish were killed by a sharp blow on the head, then biometrics (standard length and weight) of individual fish samples were recorded. The individual fish samples were then subjected to beheading, degutting, muscle extraction (from the edible parts of the body). The extracted muscle tissue was then mixed and minced, and finally, aliquots of muscle samples were packed in zip-lock bags. They were stored at -20°C for total lipid extraction.

Proximate composition and total lipid extraction and FA analysis:

The proximate composition of the collected samples was analyzed following the method described in AOAC (1995). The moisture was determined by drying a known amount of sample in an oven at 105°C until constant weights. Ash, crude fat, and protein content were estimated, respectively, using muffle furnace (600°C for 6 h), Soxhlet extraction system and Kjeldahl apparatus.

Total lipid from fish muscle and plankton sample was extracted using 2:1 (v/v) chloroform/methanol mixture as described by Folch *et al*. (1957). Extracted lipid samples were esterified using boron-trifluoride methanol (BF3-methanol, Merck, Germany) and recovered in hexane as described in Joshi *et al*. (2017). Before analysis, the derived FA methyl ester (FAME) samples were dissolved in 0.5 ml of hexane (CDH, Pvt. Ltd. New Delhi, India), and mixed adequately with a vortex mixer. Exactly 2µl of each of this final extract was injected into a gas chromatograph (GC; Ceres 800 plus, Chemito Instruments Pvt. Ltd., Mumbai, India) equipped with a BP20 column (SGE Analytical Science; dimensions-30mX0.25mm; 0.25 µm film thickness of stationary phase-polyethylene glycol) and flame ionization detector (FID). The operating conditions were as follows, injector and detector temperatures set at 250^{0}C and 280^{0}C, respectively. The oven temperature was initially set at 60^{0}C, and from there, it was raised to 260^{0}C @ 3^{0}C/min, and held for 5 min (for better peak separation). With a flow rate of 0.75ml/min, nitrogen gas was used as a mobile phase, while the detector gases used were hydrogen and air. The FAs were identified by comparing their retention times to FA methyl ester standards (37 component FAME mixture, C4-C24, SUPELCO Analytical, Bellefonte, PA, USA). Further confirmation of different FAs was done by spiking the samples with the standards. According to their peak areas, the composition of FAs was expressed in the relative percentage of the total FAs identified.

Statistical analysis:

Two-way analysis of variance (2-way ANOVA, against seasons and sexes) was conducted on total lipid and FA data of fishes for detecting the sex-linked seasonal shift in FA profile, using SPSS (SPSS, 16.0, SPSS, Inc., Chicago, IL, USA). The sex-specific seasonality of most FAs was significant in 2-way ANOVA; therefore, male and female data were separately analyzed in one-way analysis of variance (1-way ANOVA) against seasons. In addition, the total lipid and FAs of planktons were analyzed in 1-way ANOVA against seasons, using SPSS. Followed by each 1-way ANOVA, Tukey multiple comparison tests were used for comparing means against seasons.

RESULTS AND DISCUSSION

Seasonal change in proximate composition of male and female golden mahseers is presented in Table 1. Protein and ash content was similar in both sexes through all seasons. However, lipid and moisture were different seasonally. Both sexes exhibited a similar seasonal pattern of lipid, but opposite to moisture content; high lipid in winter (10.49% in males and 7.47% in females) and low in all other seasons. The opposite was the case for the moisture content. High lipid content in winter, in both sexes, indicates post-spawning recovery and reproductive preparation for the subsequent spawning. According to Nautiyal (2014), golden mahseer has an extended natural spawning season starting from summer (starting from May) to late monsoon (until the end of September). Similar to the present study's finding, low lipid content during the reproductively active season is also reported in *S. solea* (Osman *et al*., 2001) and *T. trachuru* (Bandarra *et al*., 2001).

Table 1: Seasonal changes in proximate composition (g/100 g on a wet weight basis) from muscles tissue of golden mahseer (*Tor putitora*)

Sex	Parameters	Summer	Monsoon	Pre-winter	Winter
Males	Moisture	79.94±2.37^{b}	81.29±2.71^{b}	79.50±5.94^{b}	70.25±1.33^{a}
	Lipid	2.31±0.86^{a}	1.86±1.19^{a}	2.12±1.53^{a}	10.49±7.31^{b}
	Protein	17.50±2.96	17.71±2.80	18.68±3.33	20.02±4.65
	Ash	0.21±0.06	0.18±0.04	0.25±0.03	0.24±0.04
Females	Moisture	80.40±4.07^{ab}	80.37±1.35^{b}	78.35±2.11^{ab}	76.12±4.03^{a}
	Lipid	2.64±0.90^{a}	2.31±1.39^{a}	2.14±0.58^{a}	7.47±6.13^{b}
	Protein	17.08±3.80	18.45±3.04	19.40±1.80	16.63±7.60
	Ash	0.18±0.04	0.19±0.05	0.22±0.02	0.21±0.06

Results are expressed in means ± standard deviation. Different superscript letters (a and b) along the row indicate statistical ($p<0.05$; 1-way ANOVA followed by Tukey's multiple comparison test) difference.

The total lipid content of male and female golden mahseer, and the plankton samples collected in four different seasons is presented in Tables 2, 3 and 4, respectively (along with respective FA profiles). Both sexes exhibited a similar seasonal pattern of lipid; high in winter (10.49% in males and 7.47% in females) and equally low in all other seasons. Sex-specific difference was not found in the case of total lipid, as the 2-way ANOVA was not significant (season: F=10.42, p=0.000; sex: F=0.35, p=0.561; interaction: F=0.61, p=0.611). High lipid content in winter, in both sexes, indicates post-spawning recovery and reproductive preparation for the next spawning. However, in planktons, the highest lipid content was found in monsoon (26.32%) followed by summer (18.47%), the natural spawning season of golden mahseer (Nautiyal, 2014). Despite high lipid contribution through natural food (supplied by planktons) in breeding seasons, both sexes of this species exhibited low lipid content in summer, monsoon, and pre-winter. However, in winter, the lipid was restored, possibly due to active feeding and nutritional recovery from pre-winter to winter. This mismatching dynamics of the lipid content of fish and planktons revealed the fact that the influence of the potential reproductive partitioning is stronger than that of food.

Seasonal FA profiles of male and female golden mahseers is presented in Table 2 and 3. The statistical test revealed that, almost all the major FAs classes, namely sum of MUFAs, n-6, n-3, and also the ratio of sum of n-3 and n-6 (i.e., n-3/n-6 ratio) were found to differ ($p<0.05$) seasonally. More interestingly, the 2-way ANOVA was significant for n-6 (season: F=11.54, p=0.000; sex: F=18.03, p= 0.000; interaction: F=19.56, p=0.000) and n-3 FAs (season: F=8.27, p=0.001; sex: F=13.98, p= 0.001; interaction: F=5.14, p=0.007) by season, sex and interaction (both way). The sum of MUFAs was found to differ only seasonally (season: F=6.42, p=0.003; sex: F=1.09, p= 0.308; interaction: F=2.05, p=0.136), but not by sex and interaction.

The sum of SFAs was found to be numerically different through season and by sex (particularly in monsoon); however, statistically it was not significant (season: F=1.34, p=0.286; sex: F=3.70, p= 0.067; interaction: F=1.72, p=0.190). Such stability of SFAs through different seasons is reported in many marine (Bandarra *et al*., 2001; Sushchik, Gladyshev and Kalachova, 2007; Zlatanos and Laskaridis, 2007) and freshwater fishes (Luzia, Sampaio, Castellucci, and Torres, 2003). Contrarily, Kaçar *et al.* (2016) have reported significant ($p<0.05$) seasonal and sex-specific SFAs dynamics in *S. triostegus*. In plankton samples, the sum of

SFAs was high in winter (34.73%), intermediate in summer and pre-winter, and low in monsoon (24.97%). This seasonal pattern of SFAs in planktons did not reflect in fish muscle; although, it is known that dietary FA regulates the FA compositions of fish muscle (Tocher, 2010).

Table 2: Seasonal changes in fatty acid profiles (relative percentage), from muscles tissues of male golden mahseer (*Tor putitora*)

Fatty acids	**Seasons**			
	Summer	**Monsoon**	**Pre-winter**	**Winter**
C14:0	0.35±0.19^{a}	0.93±0.31^{a}	3.45±1.53^{b}	0.13±0.06^{a}
C16:0	**16.75±3.69^{a}**	**8.77±4.15^{a}**	**10.5±4.44^{a}**	**29.8±5.20^{b}**
C17:0	0.38±0.19*	3.30±0.56*	1.38±0.21*	nd
C18:0	**18.55±13.17***	**5.03±1.24**	**9.65±2.86**	**4.05±0.07**
C20:0	0.73±0.57^{a}	1.90±0.87^{ab}*	2.20±0.53^{b}	2.30±0.20^{b}*
C22:0	1.72±1.47^{ab}	3.90±2.52^{ab}*	4.38±0.40^{b}*	0.77±0.25^{a}
C24:0	0.30±0.00^{a}	1.17±0.74^{ab}*	4.28±1.56^{b}	0.13±0.06^{a}
∑Sc-SFAs	**1.45±1.18^{a}***	**8.90±1.85^{b}***	**4.43±2.34^{a}**	**0.67±0.25^{a}**
∑SFAs	**40.00±10.00**	**33.90±3.97**	**39.70±4.44**	**36.50±5.94**
C14:1	1.38±0.28*	1.50±0.17	0.95±0.42	1.33±0.25
C15:1	0.43±0.15^{a}	1.87±0.67^{b}*	0.40±0.17^{a}	0.10±0.00^{a}
C16:1	4.00±1.72	2.90±0.79	2.78±1.91	3.33±0.25
C17:1	0.98±0.78	0.73±0.12	1.10±0.50	1.4±0.30
C18:1	**28.23±4.72^{b}**	**16.87±6.09^{a}**	**16.45±2.41^{a}**	**29.63±2.36^{b}**
C20:1	0.43±0.17	0.47±0.21	1.73±1.15	1.67±0.40
C22:1	0.52 ±0.31	1.23±0.80	1.00±0.16	1.37±0.67
∑m-MUFAs	0.30 ±0.22^{a}	1.27±0.80^{b}	nd	0.30 ±0.10^{a}
∑MUFAs	**36.25±6.78^{ab}***	**26.83±6.20^{a}**	**24.30±2.07^{a}**	**39.10±3.70^{b}**
C18:2n-6	2.03±1.16	1.47±0.90	nd	1.20±0.17
C18:3n-6	1.15±.76^{a}*	1.70±0.96^{ab}	3.25±1.00^{b}	0.40±0.00^{a}
C20:2n-6	2.83±2.45	0.15±.07	nd	0.23±0.11
C20:3n-6	0.40±0.14	nd	0.33±0.17	0.47±0.21
C20:4n-6	**0.10±0.00^{a}**	**16.47±5.97^{c}***	**8.28±2.96^{b}**	**0.20±0.10^{a}**
∑n-6 PUFAs	**6.50±2.06^{ab}**	**19.73±4.57^{c}***	**11.85±4.06^{b}**	**2.37±0.29^{a}**
C18:3n-3	0.23±0.15^{a}	1.93±0.81^{b}*	0.65±0.17^{a}	0.57±0.35^{a}
C20:3n-3	3.40±0.90^{b}	1.10±0.90^{a}	2.18±0.38^{ab}	0.67±0.15^{a}
C20:4n-3	0.23±0.26^{a}	6.13±0.98^{b}*	1.45±0.87^{a}	0.35±0.07^{a}
C20:5n-3	**1.05±0.51^{a}**	**7.60±0.85^{b}**	**2.70±1.50^{a}**	**12.53±1.35^{c}**
C22:6n-3	**12.48±7.17^{ab}***	**3.27±1.16^{a}**	**17.80±3.18^{b}**	**8.80±2.33^{ab}**
∑n-3 PUFAs	**17.38±7.41**	**20.03±1.45***	**24.10±1.57**	**22.80±3.10**

Results are expressed in means ± standard deviation of fatty acids (expressed in area percentage of each chromatogram of identified fatty acids among all detected ones). Different superscript letters (a, b and c) along the row indicate statistical ($p<0.05$; 1-way ANOVA followed by Tukey multiple comparison test) difference. Asterisk (*) indicates significantly ($p<0.05$; independent t-test) high in males compared to females, within the season. Sc-FAs include C4:0, C6:0, C8:0, C10:0, C11:0, C12:0: and C13:0; m- MUFAs include C18:1n-7, C20:1n-7 and C24:1n-9.

Total MUFAs, in both males and females, were found to be highest in winter (39.1% in males and 48.17% in females), and lowest in pre-winter (24.3% in males and 24.30% in females). In addition to the pre-winter, the lowest total MUFAs in males was recorded in monsoon as well. Similarly, in planktons, the sum of MUFAs was high in winter (37.5%) and low in all other seasons (23.23-26.6%). Thus, both fishes and planktons were found to exhibit the phenomenon of low-temperature-induced FA remodeling, an adaptation mechanism to low temperature through the FA desaturation process. High MUFAs content in winter compared to other seasons is also reported in *C. auratus* (Dal Bosco *et al.*, 2012) and *S. lucioperca* (Guler *et al.*, 2007).

Table 3: Seasonal changes in fatty acid profiles (relative percentage), from muscles tissues of female golden mahseer (*Tor putitora*)

Fatty acids	**Seasons**			
	Summer	**Monsoon**	**Pre-winter**	**Winter**
C14:0	0.15±0.10^{a}	0.46±0.38^{a}	4.52±2.08^{b}	0.10±0.06^{a}
C16:0	**29.15±1.68^{b}**	**20.16±6.72^{b}**	**15.72±3.90^{ab}**	**10.23±5.09^{a}**
C17:0	0.25±0.10	0.22±0.08	0.36±0.05	0.27±0.06
C18:0	**10.40±2.18^{a}**	**30.58±15.16^{b}***	**15.86±1.79^{ab}**	**16.77±2.04^{ab}**
C20:0	1.28±0.19^{ab}	0.44±0.27^{a}	2.54±0.84^{b}	1.87±1.57^{ab}
C22:0	3.20±0.72^{c}	0.86±0.60^{ab}	0.32±0.16^{a}	1.90±1.15^{bc}
C24:0	0.87±0.12^{a}	0.20±0.10^{a}	3.34±1.51^{b}	0.40±0.30^{a}
∑Sc-SFAs	**1.20±0.18^{a}**	**0.54±0.26^{a}**	**3.98±1.93^{b}**	**0.60±0.20^{a}**
∑SFAs	**45.48±4.12**	**53.46±19.23**	**46.64±5.00**	**32.10±7.25**
C14:1	0.80±0.54^{ab}	1.67±0.53^{b}	0.52±0.32^{a}	1.33±0.25^{ab}
C15:1	0.53±0.05^{bc}	0.25±0.13^{ab}	0.48±0.13^{b}	0.10±0.00^{a}
C16:1	5.38±3.47	2.66±0.89	1.84±1.11	2.00±1.65
C17:1	1.40±0.74^{b}	0.23±0.15^{a}	1.54±0.44^{b}	1.07±0.85^{ab}
C18:1	**19.68±2.24^{ab}**	**32.58±14.74^{ab}**	**14.00±4.09^{a}**	**39.70±16.68^{b}***
C20:1	0.27±0.15^{a}	0.30±0.20^{a}	4.80±0.74^{c}	2.33±1.75^{b}
C22:1	0.47±0.12^{a}	0.64±0.30^{ab}	0.54±0.15^{a}	1.37± 0.67^{b}
∑m-MUFAs	0.60±0.08^{b}	0.72±0.51^{b}	nd	0.30±0.10^{a}
∑MUFAs	**28.93±5.18^{ab}**	**38.62±14.89^{ab}**	**23.72±4.23^{a}**	**48.17±13.35^{b}**
C18:2n-6	4.48±0.79^{b}	1.40±0.28^{a}	nd	1.37±.38^{a}
C18:3n-6	0.18±0.09^{a}	0.72±0.64^{a}	5.92±0.69^{b}	0.37±0.12^{a}
C20:2n-6	2.18±0.50	0.20±0.10	nd	0.30±0.14
C20:3n-6	0.50 ±0.00*	1.02±0.65	0.36±0.05	0.93±0.75
C20:4n-6	**0.10±0.00**	**0.28±0.15**	**1.82±0.86**	**2.37±3.06***
∑n-6 PUFAs	**7.40±1.16^{ab}**	**3.34±1.80^{a}**	**8.10±1.35^{b}**	**5.23 ±3.91^{ab}**
C18:3n-3	0.23±0.05^{a}	0.26±0.15^{a}	0.50±0.23^{ab}	0.97±0.42^{b}
C20:3n-3	6.90±1.40^{b}	0.46±0.38^{a}	0.30±0.19^{a}	0.67±0.35^{a}
C20:4n-3	0.35±0.26^{a}*	0.40±0.26^{a}	3.66±0.59^{c}	1.80±1.04^{b}
C20:5n-3	**0.50±0.32^{a}**	**0.72±0.33^{ab}**	**3.96±1.57^{c}**	**3.03±1.90^{bc}**
C22:6n-3	**10.30±1.85^{b}**	**2.78 ±2.29^{a}**	**13.52±3.92^{b}**	**9.07±2.25^{b}**
∑n-3 PUFAs	**18.22±3.70^{b}**	**4.54±3.10^{a}**	**21.94±5.33^{b}**	**15.53±5.15^{b}**

Results are expressed in means ± standard deviation of fatty acids (expressed in area percentage of each chromatogram of identified fatty acids among all detected ones). Different superscript letters (a, b and c) along the row indicate statistical ($p<0.05$; 1-way ANOVA followed by Tukey multiple comparison test) difference. Asterisk (*) indicates significantly ($p<0.05$; independent t-test) high in females compared to males, within the season. Sc-FAs include C4:0, C6:0, C8:0, C10:0, C11:0, C12:0: and C13:0; m- MUFAs include C18:1n-7, C20:1n-7 and C24:1n-9.

In general, freshwater fish, compared to marine counterparts, contains more n-6 LC-PUFAs, including all members of the C18 and C20 n-6 family (Ackman 1967; Özogul and Özogul, 2007; Rahman *et al.*, 1995). Similarly, Joshi *et al.* (2017) also reported the prevalence of more n-6 PUFAs over n-3, in IHCs belonging to the *Schizothorax* genera, namely *S. niger*, *S. curvifrons*, *S. esocinus*, *S. plagiostomus*, and *S. progastus*. Contrarily, in the present study, the sum of n-3 FAs was consistently higher than n-6 throughout all seasons in both males and females. A similar finding is also reported by Mohanty *et al.* (2016) in closely related species such as *S. richardsonii* and *Neolissochilus hexagonolepis*; but their analysis was based on one-time sampling (not seasonal) where males and females were pooled together without considering their reproductive status and influence of seasons.

Table 4: Seasonal changes in fatty acid profiles and total lipid of planktons collected (randomly), at three different sites of lakes, during the time of fish collection

Fatty acids	Summer	Monsoon	Pre-winter	Winter
C14:0	0.60±0.30^{ab}	0.23±0.25^{a}	1.80±0.60^{c}	1.30±0.30^{bc}
C15:0	0.40±0.10^{a}	1.80±0.50^{b}	2.93±0.70^{b}	2.20±0.40^{b}
C16:0	7.87±0.31^{a}	7.00±2.00^{a}	7.30±0.46^{a}	8.20±0.60^{a}
C17:0	0.87±0.38^{a}	1.77±0.40^{ab}	2.03±0.50^{ab}	2.50±0.50^{b}
C18:0	5.07±1.55^{a}	4.17±0.31^{a}	4.44±0.70^{a}	5.30±0.50^{a}
C20:0	2.90±0.80^{ab}	1.57±0.25^{a}	2.30±0.30^{ab}	4.00±1.00^{b}
C22:0	4.60±0.60^{c}	2.53±0.40^{ab}	1.80±0.60^{a}	3.43±0.64^{bc}
C24:0	1.00±0.26^{a}	1.37±0.31^{a}	2.77±0.55 ^{b}	3.70±0.70^{b}
∑Sc-FAs	2.80±0.36^{ab}	4.53±0.80^{c}	1.70±0.50^{a}	4.10±0.60^{bc}
∑SFAs	**26.10±2.62^{ab}**	**24.97±4.10^{a}**	**27.07±4.64^{ab}**	**34.73±3.17^{b}**
C14:1	1.60±0.20^{ab}	1.20±0.40^{a}	.80±0.40^{a}	2.60±0.60^{b}
C15:1	0.53±0.31^{a}	4.40±0.60^{b}	.60±0.40^{a}	0.40±0.20^{a}
C16:1	5.17±1.56^{bc}	0.33±0.15^{a}	3.30±0.50^{b}	6.10±0.80^{c}
C17:1	2.53±0.75^{b}	3.80±0.80^{b}	2.70±0.70^{b}	0.50±0.00^{a}
C18:1	11.87±0.40^{b}	6.87±0.85^{a}	9.37±0.75^{ab}	18.30±1.65^{c}
C20:1	0.40±0.10^{a}	3.30±0.60^{b}	5.37±0.81^{c}	5.80±0.80^{c}
C22:1	0.43±0.12^{a}	4.10±0.60^{b}	1.00±0.20^{a}	3.50±0.70^{b}
∑m-MUFAs	1.40±0.36^{b}	2.60±0.60^{c}	0.10±0.00^{a}	0.30±0.20^{a}
∑MUFAs	**23.93±1.36^{a}**	**26.60±4.35^{a}**	**23.23±3.75^{a}**	**37.50±4.95^{a}**
C18:2n-6	2.87±0.40^{a}	13.63±1.00^{c}	10.10±1.20^{b}	1.60±0.60^{a}
C18:3n-6	0.57±0.15^{ab}	1.20±0.40^{b}	0.40±0.20^{a}	0.70±0.30^{ab}
C22:2n-6	1.23±0.21^{a}	1.00±0.50^{a}	1.80±0.50^{a}	0.90±0.60^{a}
C20:3n-6	0.63±0.25^{ab}	1.70±0.70^{b}	3.30±0.40^{c}	0.50±0.30^{a}
C20:4n-6	0.57±0.23^{a}	1.50±0.50^{a}	5.47±0.75^{b}	1.70±0.70^{a}
∑ n-6 PUFAs	**5.87±0.29^{a}**	**19.03±3.10^{b}**	**21.07±2.65^{b}**	**5.40±2.50^{a}**
C18:3n-3	0.73±0.21^{a}	1.23±0.56^{ab}	2.30±0.60^{b}	0.93±0.55^{a}
C20:3n-3	13.66±2.52^{b}	0.66±0.31^{a}	1.70±0.70^{a}	0.70±0.50^{a}
C20:4n-3	0.56±0.25^{a}	2.77±0.45^{b}	2.97±1.04^{b}	1.40±0.40^{ab}
C20:5n-3	2.53±0.45^{a}	10.03±1.10^{c}	6.40±0.40^{b}	2.80±0.70^{a}
C22:6n-3	19.13±2.01^{c}	2.30±0.60^{a}	16.40±0.60^{bc}	13.53±2.01^{b}
∑ n-3 PUFAs	**36.63±5.43^{c}**	**17.00±2.82^{a}**	**29.77±3.31^{bc}**	**19.37±4.15^{ab}**
Total lipid	**18.47±5.71^{bc}**	**26.32±1.94^{c}**	**11.90±1.77^{ab}**	**9.53±2.08^{a}**

Results expressed in means±standard deviation (with n = 3-5) of fatty acids (expressed in area % of each identified chromatogram of fatty acids among all detected ones). Different superscript letters (a, b and c) indicate statistical ($p<0.05$; 1-way ANOVA followed by Tukey multiple comparison test) difference in means.

In the present study, the sum of n-3 and n-6 FAs fluctuated seasonally, but differently in males and females. In females, the fluctuation was more pronounced, possibly linked to their seasonal gonadal status. Because the sum of both n-6 (3.34%) and n-3 (4.54%) FAs was the lowest in the later half of spawning season, i.e., in monsoon compared to all other seasons. Following this season, these FAs were found to recover quickly in pre-winter. Whereas in males, the lowest value of total n-6 was found in winter (2.37%); following this season, the value increased gradually through summer and peaked in monsoon (39.1%). The pattern of total n-6 FAs from plankton was found to be more or less coinciding with those of males; therefore, the plausible reason behind the unique trend of total n-6 FAs in males could be the dietary response. On the other hand, the total n-3 FA was consistently high throughout all seasons.

The seasonal changes in FA composition due to gonadal cycling is reported in many species, such as *C. auratus* (Dal Bosco *et al.*, 2012), *T. arcticus* (Sushchik *et al.*, 2007), *S. solea* (Gökçe *et al.*, 2004), and *Silurus asotus*, *Clarias macrocephalus*, *C. galipinus* (Shirai *et al.*, 2002a). However, in most of these reports, sex-specific reduction of PUFAs (both n-3 and n-3), similar to the case of females in the present study, is missing. FAs, particularly n-3 and n-6 PUFAs, partition towards the ovary during vitellogenesis from the muscular reserve to ensure better embryonic development and yolk-sac larvae (Johnson *et al.*, 2017). Moreover, golden mahseer displays extended yolk-sac larval phase, i.e., up to 10-12 days post-hatching (Sharma *et al.*, 2016);

hence, the nutrient makeup of yolk of this fish species is expected to be enriched with LC-PUFAs. On the other hand, food-induced seasonality of FA profile, similar to the males in this study, is reported in *S. melanostictus* (Shirai *et al.*, 2002b). Kaçar *et al.* (2016) reported a different trend of PUFAs, i.e., low during pre-and post-spawning and high in peak spawning season in female *S. triostegus*.

The sex-specific seasonality pattern of n-3 and n-6 metabolites appear to be influenced by diet and reproductive status, but differently in males and females. In females, C20:4n-6 had a clear trend of abundance starting from low in summer to high in winter, and intermediates in monsoon and pre-winter. In summer, and in both sex, intermediate metabolites of n-6 elongation and desaturation pathways, like C18:2n-6 and C20:2n-6, were more prominent C20:4n-6. Again in pre-winter, C18:3n-6 was more pronounced in both males and females. This clearly indicates the presence of active seasonal elongation and desaturation mechanism in golden mahseer. A similar mechanism of elongation and desaturation is reported in many fishes. However, the efficiency of this bioconversion of short-chain PUFAs to LC-PUFAs varies among freshwater and marine species, and it is believed to be more effective in freshwater groups (Tocher, 2010). An elevated level of C20:4n-6 in males was found in monsoon (16.47%) followed by pre-winter (8.28%). This pattern appears to have been influenced by plankton FA composition through elongation and desaturation; because, in planktons, the precursor FAs (C18:2n-6 and C20:3n-6) were prevalent in these two seasons.

Similarly, metabolites of n-3 FAs also were found differently abundant in all four seasons, depending on sex. In females, C20:3n-3 and C22:6n-3 in summer, and C20:4n-3, C20:5n-3 and C20:6n-3 in both pre-winter and winter were prominent. This appeared to be influenced by the planktonic n-3 FA family, except in monsoon, because in monsoon, dietary influence appeared minimal compared to the impact of reproductive partitioning. Other than monsoon, in all other seasons, trends of n-3 FAs of planktons more or less matched with those of females from respective seasons. In males, C20:3n-3 and C22:6n-3 in summer, C20:4n-3 and C20:6n-3 in monsoon, C22:6n-3 in pre-winter, and C20:5n-3 and C22:6n-3 in winter were prominent. The reason for this dynamic prevalence of n-3 family metabolites could be the influence of plankton FAs composition.

Sc-FAs such as C4:0, C6:0, C8:0, C10:0, C11:0 and C12:0 were detected in both males and females. However, their occurrence was not uniform throughout all seasons and in both the sexes. Therefore, their content is summed up under the category of total Sc-FAs. These FAs from goat and cow milk are deliberately studied for their importance in human health (Haenlein, 2004). These FAs are easily and rapidly digestible, and play a vital role in regulating cholesterol metabolism; these have also been used in treating childhood epilepsy, cystic fibrosis, gallstones, etc. (Haenlein, 2004). In males, compared to females, the total Sc-FAs was high throughout all seasons. A significant difference between the males and females was seen during the monsoon. In males, the level of this FA group was comparatively high (8.9%) in this season, compared to all other three; following this, a second high was seen in pre-winter (4.43%). The peak of Sc-FAs in males, in monsoon, coincides with that of planktons (4.53%), reflecting dietary influence. Low levels of Sc-FAs in monsoon only among females, despite available food, could be due to the diversion of this nutrient for ovarian development.

Different FA classes have different degrees of significance in human nutrition and physiology. Sc-FAs are healthful FAs, as mentioned above. SFAs are mainly used for energy, but a higher level of these FAs in the diet is detrimental because they promote low-density lipoprotein (LDL)-cholesterol (Tvrzicka, Kremmyda, Stankova, and Zak, 2011). MUFAs, on the other hand, help to raise the level of high-density lipoprotein (LDL)-cholesterol (Tvrzicka *et al*., 2011). LC-PUFAs like n-3 and n-6 LC PUFAs are the precursors of eicosanoids, the inflammatory responses compounds; the products of n-3 LC-PUFAs are more beneficial than those of n-6 LC-PUFAs (Riediger *et al*., 2009). Further, n-3 LC-PUFAs are a panacea against several fatal diseases (Riediger *et al*., 2009).

Based on this information of the physiological significance of different FAs (mentioned above) and summarised information in Figures 1 and 2, the seasonal nutritional quality of male and female golden mahseer can be compared. If the males alone are compared against all four seasons, they appeared to be the best in winter, followed by pre-winter and monsoon, and comparatively poor in summer. Similarly, if females alone are compared in all four seasons, they appeared the best in winter and relatively poor in monsoon, and intermediate in pre-winter and summer. The FAs contribution from planktons seemed to be the best in summer, followed by pre-winter. From Figure 1, it appeared that the planktonic influence did not match fully with either sex. In females, it was partially similar in pre-winter and summer. While in males, it was partly similar in pre-winter and monsoon. The disparity might have come from reproductive status and other unknown factors. On the other way round, when we compare males and females in each season based on Figure 2, males appeared to be superior to females in summer, followed by monsoon and winter. In pre-winter, both males and females seemed equally good.

In conclusion, the FA composition of male and female golden mahseer changed differently with changes in seasons. Seasonality was more distinct in females in terms of n-3 and n-6 PUFAs, followed by MUFAs. During the later half of the breeding season, i.e., in monsoon, both n-3 and n-6 FAs were significantly drained in females, compared to males. Changes in SFAs and MUFAs were appeared to be under dietary or other unknown influence. When functional values of males and females were separately compared against all four

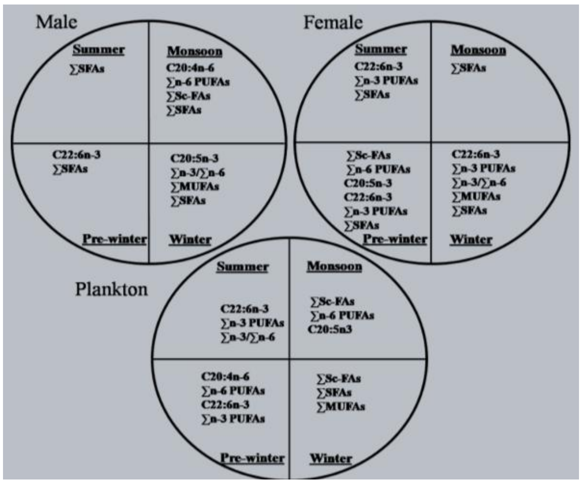

Fig. 1: Seasonal dominance pattern of important (C20:4n-6, C20:5n-3 and C22:6n-3) and major fatty acid groups (sum of Sc-FAs, SFAs, MUFAs, n-6 and n-3 PUFAs) in male and female golden mahseer, and plankton from Bhimtal lake

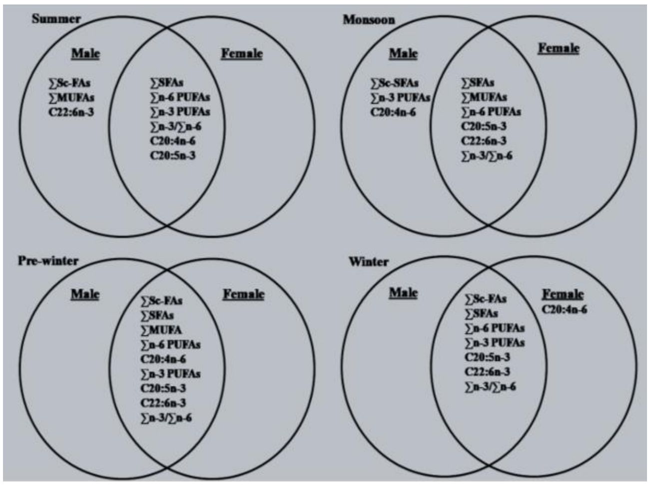

Fig. 2: Seasonal nutritional comparison among male and female golden mahseer based on dominance pattern of important (C20:4n-6, C20:5n-3 and C22:6n-3) and major fatty acid groups (sum of Sc-FAs, SFAs, MUFAs, n-6 and n-3 PUFAs)

seasons, both males and females appeared the best in winter followed by pre-winter. However, when males were compared with females in each season, they appeared superior to females in summer, followed by monsoon and winter, but both sexes seemed equally good in pre-winter. This information will help to provide nutritional guidance to the consumer.

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REFERENCES

Ackman, R.G. 1967. Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. *Comparative Biochemistry and Physiology*, **22:** 907–922.

Ackman, R.G. 1990. Seafood lipids and fatty acids. *Food Reviews International*, **6**: 617–646.

Ackman, R.G., Mcleod, C., Rakshit, S., and Misra, K.K. 2002. Lipids and fatty acids of five freshwater food fishes of India. *Journal of Food Lipids*, **9**: 127–145.

Almansa, E., Martín, M.V., Cejas, J.R., Badía, P., Jerez, S. and Lorenzo, A. 2001. Lipid and fatty acid composition of female gilthead seabream during their reproductive cycle: Effects of a diet lacking n-3 HUFA. *Journal of Fish Biology*, **59**: 267–286.

Bandarra, N.M., Batista, I., Nunes, M.L and Empis, J.M. 2001. Seasonal variation in the chemical composition of horse-mackerel (*Trachurus trachurus*). *European Food Research and Technology*, **212**: 535–539.

Bogard, J.R., Thilsted, S.H., Marks, G.C., Wahab, M.A., Hossain, M.A.R., Jakobsen, J.and Stangoulis, J. 2015. Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. *Journal of Food Composition and Analysis*, **42**: 120–133.

Dal Bosco, A., Mugnai, C., Mourvaki, E and Castellini, C. 2012. Seasonal changes in the fillet fatty acid profile and nutritional characteristics of wild Trasimeno Lake goldfish (*Carassius auratus* L.). *Food Chemistry*, **132**: 830–834.

Folch, J., Lees, M., and Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, **226**: 497–509.

Gogus, U and Smith, C. 2010. N-3 omega fatty acids: a review of current knowledge. *International Journal of Food Science and Technology*, **45**: 417–436.

Gökçe, M. A., Taşbozan, O., Çelik, M.and Tabakoglu, Ş. S. 2004. Seasonal variations in proximate and fatty acid compositions of female common sole (*Solea solea*). *Food Chemistry*, **88:** 419–423.

Gomes, A.D., Tolussi, C.E., Boëchat, I.G., Pompêo, M.L.M., Cortez, M.P.T., Honji, R.M. and Moreira, R.G.

2016. Fatty acid composition of tropical fish depends on reservoir trophic status and fish feeding habit. *Lipids*, **51**: 1193–1206.

Grigorakis, K. 2007. Compositional and organoleptic quality of farmed and wild gilthead sea bream (Sparus aurata) and sea bass (*Dicentrarchus labrax*) and factors affecting it: A review. *Aquaculture*, **272**: 55–75.

Guler, G.O., Aktumsek, A., Citil, O.B., Arslan, A., and Torlak, E. 2007. Seasonal variations on total fatty acid composition of fillets of zander (*Sander lucioperca*) in Beysehir Lake (Turkey). *Food Chemistry*, **103**: 1241–1246.

Haenlein, G.F. W. 2004. Goat milk in human nutrition. *Small Ruminant Research*, **51**: 155–163.

Huynh, M.D. and Kitts, D.D. 2009. Evaluating nutritional quality of pacific fish species from fatty acid signatures. *Food Chemistry*, **114**: 912–918.

Johnson, R.B., Kroeger, E.L., Reichert, W.L., Carter, C.S. and Rust, M.B. 2017. Uptake and selective partitioning of dietary lipids to ovarian and muscle tissue of maturing female coho salmon, *Oncorhynchus kisutch*, during secondary oocyte growth. *Comparative Biochemistry and Physiology, Part B 208–209*, 7–18.

Joshi, V., Akhtar, M.S., Sharma, P., Singh Kushwaha, S., Baruah, D., Ciji, A., Sarma, D. 2017. Himalayan Fish Manifest Higher Potential of Quality Nutrients for Human Health. *Journal of Aquatic Food Product Technology*, **26**: 843–855.

Kaçar, S. and Başhan, M. 2015. Seasonal variations in the fatty acid composition of phospholipid and triacylglycerol in gonad and liver of mastacembelus simack. *JAOCS, Journal of the American Oil Chemists' Society*, **92**:1313–1320.

Kaçar, S., Başhan, M. and Oymak, S. A. 2016. Effect of seasonal variation on lipid and fatty acid profile in muscle tissue of male and female *Silurus triostegus*. *Journal of Food Science and Technology*, **53**: 2913–2922.

Lund, E.K. 2013. Health benefits of seafood; Is it just the fatty acids? *Food Chemistry*, **140**: 413–420.

Luzia, L.A., Sampaio, G.R., Castellucci, C.M.N., and Torres, E.A.F.S.2003. The influence of season on the lipid profiles of five commercially important species of Brazilian fish. *Food Chemistry*, **83**: 93–97.

Mohanty, B.P., Ganguly, S., Mahanty, A., Sankar, T.V., Anandan, R., Chakraborty, K., Sridhar, N. 2016. DHA and EPA content and fatty scid profile of 39 food fishes from India. *BioMed Research International*, 1–14.

Murillo, E., Rao, K.S. and Durant, A.A. 2014. The lipid content and fatty acid composition of four eastern central Pacific native fish species. *Journal of Food Composition and Analysis*, **33**: 1–5.

Nautiyal, P. 2014. Erratum: Review of the art and science of Indian mahseer (Game Fish) from nineteenth to twentieth century: Road to extinction or conservation? *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, **84**: 215–236.

Osman, H., Suriah, A.R. and Law, E.C. 2001. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. *Food Chemistry*, **73**: 55–60.

Özogul, Y. and Özogul, F. 2007. Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black Seas. *Food Chemistry*, **100**: 1634–1638.

Petenuci, M.E., Rocha, I. do N.A., de Sousa, S.C., Schneider, V.V. A., da Costa, L.A.M. A. and Visentainer, J. V. 2016. Seasonal variations in lipid content, fatty acid composition and nutritional profiles of five freshwater fish from the amazon basin. *JAOCS, Journal of the American Oil Chemists' Society*, **93**: 1373–1381.

Pirini, M., Testi, S., Ventrella, V., Pagliarani, A. and Badiani, A. 2010. Blue-back fish: Fatty acid profile in selected seasons and retention upon baking. *Food Chemistry*, **123**: 306–314.

Rahman, S.A., Huah, T.S., Hassan, O. and Daudb, N.M. 1995. Fatty acid composition of some Malaysian freshwater fish. *Food Chemistry*, **54**: 45–49.

Riediger, N.D., Othman, R.A., Suh, M. and Moghadasian, M. H. 2009. A systemic review of the roles of n-3 fatty acids in health and disease. *Journal of the American Dietetic Association*, **109**: 668–679.

Sarma, D., Joshi, V., Akhtar, M. S., Ciji, A., Sharma, P., Kushwaha, S. S., Das, P., Singh, A. K. 2019. Nutrient composition of six small indigenous fish from NEH region and their contribution potential to human nutrition. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, **89**: 475–482.

Sharma, P., Kumar, V., Sinha, A. K., Ranjan, J., Kithsiri, H. M. P., and Venkateshwarlu, G. 2010. Comparative fatty acid profiles of wild and farmed tropical freshwater fish rohu (Labeo rohita). *Fish Physiology and Biochemistry*, **36**: 411–417.

Sharma, P., Akhtar, M. S., Singh, A. K., Das, P. and Sarma, D. 2016. Histomorphological changes in digestive tract of golden mahseer (*Tor putitora*) during different developmental stages. *Fish Physiology and Biochemistry*, **42**: 1681–1698.

Shirai, N., Suzuki, H., Tokairin, S., Ehara, H. and Wada, S. 2002a. Dietary and seasonal effects on the dorsal meat lipid composition of Japanese (*Silurus asotus*) and Thai catfish (*Clarias macrocephalus* and hybrid

Clarias macrocephalus and *Clarias galipinus*). *Comparative Biochemistry and Physiology Part A*, **132**: 609–619.

Shirai, Nobuya, Terayama, M. and Takeda, H. 2002b. Effect of season on the fatty acid composition and free amino acid content of the sardine *Sardinops melanostictus*. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, **131**: 387–393.

Sushchik, N.N., Gladyshev, M.I. and Kalachova, G.S. 2007. Seasonal dynamics of fatty acid content of a common food fish from the Yenisei river, Siberian grayling, *Thymallus arcticus*. *Food Chemistry*, **104**: 1353–1358.

Tocher, D.R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research*, **41**: 717–732.

Tvrzicka, E., Kremmyda, L. S., Stankova, B. and Zak, A. 2011. Fatty acids as biocompounds: Their role in human metabolism, health and disease - a review. part 1: Classification, dietary sources and biological functions. *Biomedical Papers*, **155**: 117–130.

Zlatanos, S. and Laskaridis, K. 2007. Seasonal variation in the fatty acid composition of three mediterranean fish - sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). *Food Chemistry*, **103**: 725–728.