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What are the most effective biotic and abiotic factors affecting fatty acid composition of *Garra rufa* (Heckel, 1843)?

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SUMMARY: Specimens of *Garra rufa* were collected from a warm river and a cool stream in the Bingöl Province, Turkey, once a month over a period of one year. The effects of month, season, gender and location on the fatty acid composition in the muscle and the lipid content were investigated and dietary marker fatty acids were used to obtain dietary preferences in different locations (Ilıcalar, Garip) and periods. Total lipid change was seasonally significant (ANOSIM-R=0.49) at both locations and 18:1ω9, 20:5ω3 and 20:6ω3 were the most abundant dietary fatty acids. Although *G. rufa* are predominantly herbivores, they can also feed omnivorously on mixed diets depending on the presence and absence of their primary diet. The effect of season was significant on fatty acid composition, regardless of the location (P_{perm}=0.001). Significant seasonal changes in all the fatty acid compositions could be attributed to seasonal changes in the abundance and diversity of dietary sources in the environment due to the effect of temperature.

KEYWORDS: *Dietary fatty acids; DHA EPA; Garra rufa; 18:1ω9*

RESUMEN: ***¿Cuáles son los factores bióticos y abióticos más efectivos que afectan a la composición de ácidos grasos de la* Garra rufa *(Heckel, 1843)?.*** Se recolectaron especímenes de *Garra rufa* (pez doctor) de un río cálido y un arroyo frío en la provincia de Bingöl, Turquía, mensualmente durante un año. Se investigaron los efectos del mes, la estación, el género y la ubicación en la composición de ácidos grasos musculares y el contenido de lípidos y se utilizaron los ácidos grasos como marcadores dietéticos para obtener preferencias dietéticas en diferentes lugares (Ilıcalar, Garip) y períodos. El cambio total de lípidos fue estacionalmente significativo (ANOSIM-R=0,49) en ambos lugares y 18:1ω9, 20:5ω3 y 20:6ω3 fueron los ácidos grasos dietéticos más abundantes. Aunque *G. rufa* son predominantemente herbívoros, también pueden alimentarse de forma omnívora con dietas mixtas según la presencia o ausencia de la dieta principal. El efecto de las estaciones fue significativo en la composición de ácidos grasos independientemente de las localizaciones (P_{perm}=0.001). Los cambios estacionales significativos en todas las composiciones de ácidos grasos podrían atribuirse a los cambios en la abundancia y diversidad de las fuentes dietéticas en el medio ambiente debido al efecto de la temperatura.

PALABRAS CLAVE: *Ácidos grasos dietéticos; DHA; EPA; Garra rufa; 18:1ω9*

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1. INTRODUCTION

Garra rufa (Heckel, 1843), one of the smallest members of the Cyprinidae family belongs to the genus *Garra,* which includes about 73 species (Coad, 2010). It is used in ichthyotherapy as an alternative treatment for healing some skin diseases such as psoriasis and eczema. It is therefore called "doctor fish" (Yedier *et al.,* 2016).

Lipids are among the most important energy sources for animals and the fatty acids (FAs) in their structure form the building blocks of cell membranes (Iverson, 2009). Furthermore, they provide the organism with essential fatty acids (EFAs), a key nutrient for proper develeopment (Parrish, 2009). The natural diets of many fish species contain large amounts of long-chain polyunsaturated fatty acids (LC-PUFAs). Unlike terrestrial animals, the lipid composition of aquatic organisms contains high levels of PUFAs, predominantly omega 3 (ω3) FAs (Parrish, 2013). In addition, ω3 FAs play an essential role in the normal development of the embryos and larvae of freshwater fish and in the regular functioning of nervous systems and sensory organs. These processes occur in different ways in different species or subspecies and even in male and female individuals of the same species (Kaushik *et al*., 2006). In addition, the fatty acid composition of fish species varies according to the geographic location, diet, feeding, gender and reproductive cycles. Seasonal variations may also be effective in changing the FAs composition of fish (Kaçar and Başhan, 2015).

Aquatic organisms are dependent on the availability of nutrients, and conducting research on the essential nutrients of these organisms has become important in ecology. Traditionally, understanding the food web is derived from detailed analysis of stomach contents. However, since stomach content analysis only provides a snapshot of an animal's diet, large numbers of samples are required to be analyzed, meaning that sampling can be logistically restrictive or unsustainable. Conversely, the biochemical composition of muscle tissue is the result of long-term feeding histories. Thus, techniques such as stable isotope and fatty acid analysis are increasingly used to reveal complex ecological information (Dalsgaard *et al*., 2003). Stable isotopes also provide a measure of trophic position but can be confounded by differences at the bottom of the food chain. However, fatty acid composition can help identify many unique synthesized structures (Revill *et al*., 2009). Therefore, this study aimed to determine and compare the FAs composition of *G. rufa* in relation to biotic and abiotic factors such as season, gender and different stations of the same region, and to reveal the dietary preferences of *G. rufa* in different periods using dietary marker fatty acids.

2. MATERIALS AND METHODS

2.1. Sampling area and samplings

Samples were taken monthly from two locations, namely Garip and Ilıcalar, on the Garip Stream of the Murat River in the Bingöl Province (Figure 1). The Ilıcalar location (36°59'01.5" N, 40°40"58.9" E) has temperatures above seasonal averages; whereas the Garip location (30°47'10.7"N, 40°32'58.7"E) has colder waters. Water samplings were taken from the same location at both stations between March 2017 and February 2018. Nets with different mesh sizes (12×12 mm, 16×16 mm, 22×22 mm, 32×32 mm) were used for catching the fish. Water samples were taken to determine the level of chlorophyll-*a* (Chl-a). Water temperatures were measured *in situ* at both stations.

2.2. Laboratory studies

The fish samples collected were brought to the laboratory, and at least 3-5 samples for each season during the sampling period were examined. A total of 25 individuals from Garip and 39 individuals from Ilıcalar locations were taken during the sampling period (March 2017-February 2018). Sexually mature fish were used in the analyses. Gender was also determined in the fish samples used for the total lipid and fatty acid analyses. The gender of the fish samples was determined macroscopically from the gonads of the fish samples. The samples to be used in the biochemical analysis were obtained from the edible muscle tissues. Each fish muscle from the non-posterior part was cut into uniform pieces (2.0 × 2 × 1 cm; ~1-2 g) using a scalpel. All the fish muscle samples were stored in a freezer at -80 °C until analysis.

2.2.1. Determination of chlorophyll-a

Chlorophyll-a (chl-a) measurements were made according to the spectrophotometric method (Parsons *et al*., 1984). For the determination of chl-a content, 1

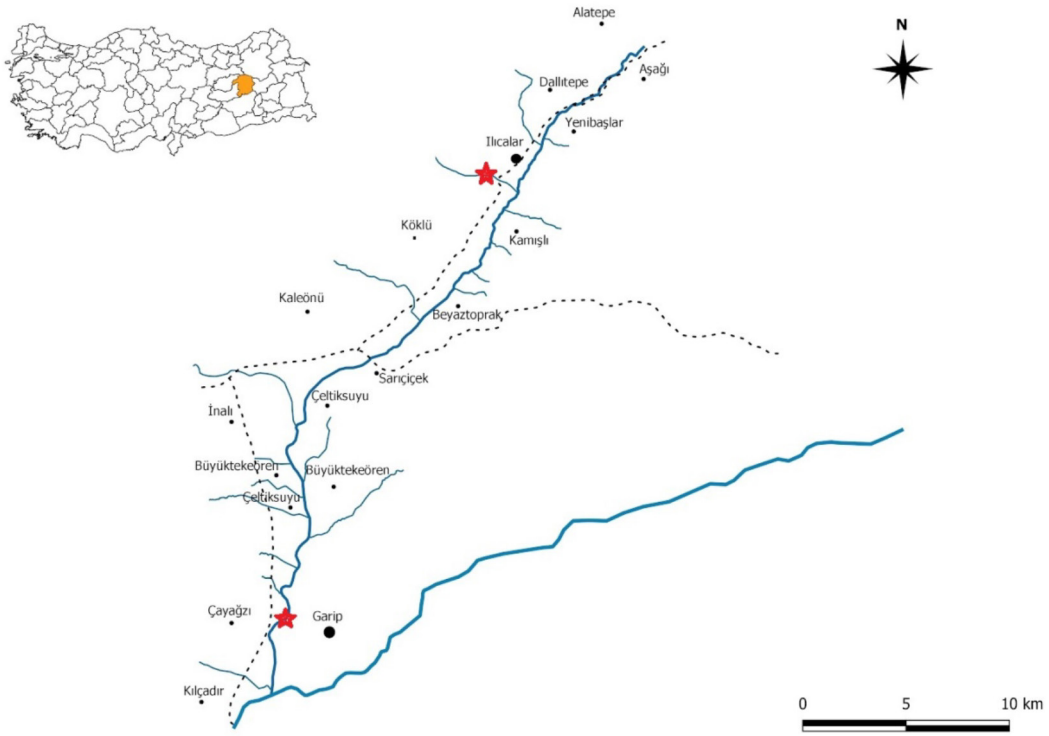

Figure 1. Sampling Area

liter of water was sampled monthly from the specified stations. The sample was then filtered through GFC filters with a pore size of 1 µm. The filter papers were folded and placed in 15 mL centrifuge tubes and 10 mL 90% acetone solution were added to the centrifuge tubes. They were kept in the refrigerator at 4 °C for 24 hours. Then the samples were brought to room temperature and their absorbances were determined at 750, 664, 647, and 630 nm wavelengths by means of a spectrophotometer (SHIMADZU UV 2100).

2.2.2. Lipid extraction and fatty acid derivatization

Lipid extraction was performed on the separated muscle tissue samples. The weight of each sample was determined with a precision of 0.001 mg of wet weight (WW). The wet weight of each sample was about 1-2 g. Hexane/isopropanol (3/2) was used for lipid extraction as suggested by Hara and Radin (1978). For fatty acid transmethylation, 20 g methanolic sulfuric acid were mixed with 1 liter of distilled water to prepare a 2% methanolic sulfuric acid solution. Five mL of this solution were added to a test tube and completely mixed by vortex. The mixture was left to methylate in an oven at 55 °C for 15 hours. At the end of this period, 5 mL of 5% NaCl were added to it and mixed thoroughly. Afterwards, 5 mL of hexane were added to the fatty acid methyl esters formed in the tubes, and the tubes were mixed (Christie, 1992). After waiting three hours at room temperature, the hexane phase formed was taken from the top, 5 mL of 2% $KHCO_3$ solution were added to the tubes and the sample was dried in a nitrogen evaporator (Allsheng WD-12). A weight measurement was taken on a precision scale to determine the dry lipid content after the evaporation process, and the average total lipid content (%) per individual was calculated. Then, the dry lipid layer was hydrated with the addition of 1 mL hexane, and then vortexed. The samples were transferred to 2 mL capped autosampler vials and analyzed in a gas chromatograph/mass spectrometer (GC/MS) system.

2.2.3. GS/MS analysis

The fatty acids were analyzed with a GC/MS system (Agilent 5975 C). A Macherey-Nagel (Germany) capillary column (100 m x 0.25 mm, 0.25 µm) was used for the analysis. The column temperature was kept at 120-220 °C; whereas the injection temperature was 240

°C and the detector temperature was 280 °C throughout the analysis. The column temperature program was set from 120 to 220 °C. The temperature ramp was set at 5 °C / min up to 200 °C and at 4 °C / min from 200 to 220 °C. It was held at 220 °C for 8 min and the total time was 52 min. Helium (0.5 ml/min) was used as carrier gas. The fatty acid methyl esters (FAMEs) of the samples were identified initially based on the retention time of each fatty acid by using the analytical standard of FAMEs (Supelco Component FAME Mix). After analysis, wsearch32 mass spectrometry software (Wsearch 2008; version 1.6 2005, Sidney, Australia) was used to confirm the peak identities of each fatty acid.

2.3. Statistical analysis

Multivariate statistics were used to analyze the differences in total lipid contents and total fatty acid compositions in PRIMER-e 2017. The Bray Curtis similarity coefficient was employed for PERMANOVA and principal coordinates (PCO) and cluster analysis for similarity ranges. In the analyses, the fatty acid data of *G. rufa* were factored by month, season, gender and location (stations). The fatty acids which showed the greatest difference in all samples were investigated in the factor groups. A similarity percentage (SIMPER) analysis (cut-off for low contributions: 70%) was used to identify the fatty acids which contributed the most to the similarities between/within the factor groups. The analysis of similarity (ANOSIM) was performed on the distance matrix using multiple permutations within a significant fixed effect. The ANOSIM-R value indicated the extent to which the groups differed (R > 0.75: well-separated groups, highly different; R=0.50–0.75: separated but overlapping groups, different; R=0.25–0.50: separated but strongly overlapping groups; R<0.25: barely separated groups, similar with some differences) (Pethybridge *et al.*, 2010).

ANOVA test was performed to determine significant ($p < 0.05$) main effects of the factors (station, season, month) and their interactions on FA compositions and total lipid content. Variations among groups were determined by TUKEY HSD test using STATISTICA software (STATISTICA Six Sigma, version 7).

3. RESULTS AND DISCUSSION

3.1. Total lipid content

The values for seasonal and monthly variation in the total lipid amount of *G. rufa* during the sampling period for Garip and Ilıcalar are given in Table 1. The average total lipid content per individual was determined for proportional values (%).

The PERMANOVA results obtained were used to identify similarities in total lipid content, within and among seasons, genders and stations. In the PERMANOVA analysis, the total lipid data were factored by season and gender at the stations. Also, the data were factored by the stations during the sampling period.

According to the ANOVA results, seasonal differences were the most significant between autumn-summer at the Garip station and between autumn-summer and autumn-spring at the Ilıcalar station ($p < 0.05$) (Table 1). In addition, there were no significant differences between gender groups in terms of total lipid content at the Ilıcalar station ($p < 0.05$). Similarly, the ANOSIM-R results showed that there was no separation between gender groups (ANOSIM-R= -0.003) at the Ilıcalar station. However, despite the fact that the seasonal groups were separated, there was strong overlapping between groups (ANOSIM-R=0.49) at the Ilıcalar station. Season and gender groups were barely separated (ANOSIM-R=0.23; ANOSIM-R=0.10, respectively) at the Garip station. Therefore, seasonal difference in the total lipid content was more significant at the Ilıcalar station than at the Garip station.

The lipid content of *G. rufa* varied between 1-6% at the Ilıcalar station and 0.89-5% at the Garip station. The highest value was detected in autumn (5.26, 4.80%, respectively). Olgunoglu *et al*. (2014) determined the highest energy value for *Silurus triostegus* to be in the winter months when the lipid content was at its highest. Considering that the energy levels are related to the lipid content, it can be said that the energy value reached its highest level in the autumn months for *G. rufa*.

It is well known that fish lipids are generally affected by many factors such as age, seasonal change, nutrition, gender, reproductive cycle and geographical location (Všetičková *et al*., 2020). In the present study, adult fish specimens were used during the sampling. Therefore, it is thought that the change in total lipid content was more affected by the temperature changes in both locations. The temperature values throughout the year in the Ilıcalar station (8-26 °C) are always higher than at the Garip station (0.5-21°C) (Table 4). Bauer and Schlott (2009)

Table 1. Average total lipid amount (%) of *G. rufa* at Garip and Ilıcalar stations during the sampling period

	SPRING (n=6)			SUMMER (n=9)			AUTUMN (n=8)			WINTER (n=2)	
	March	**April (n=3)**	**May (n=3)**	**June (n=3)**	**July (n=3)**	**August (n=3)**	**September (n=3)**	**October (n=2)**	**November (n=3)**	**December**	**January** (n=1)**	**February** (n=1)**
GARIP (n=25)	-	2.74±1.81^{ab} 4.17±1.40^{abc}	1.30±0.25^{bcd}	0.56±0.36^{d}	1.56±1.35^{b} 3.22±0.85^{abcd}	0.89±0.44^{cd}	4.35±2.33^{abc}	4.80±1.47^{a} 4.80±0.92^{ab}	5.24±1.05^{a}	-	1.92±0.03^{ab} 1.90	1.94
		SPRING (n=13)			**SUMMER (n=9)**			**AUTUMN (n=10)**			**WINTER (n=7)**	
	March (n=5)	**April (n=5)**	**May (n=3)**	**June (n=3)**	**July (n=3)**	**August (n=3)**	**September (n=3)**	**October (n=4)**	**November (n=3)**	**December (n=4)**	**January (n=3)**	**February**
ILICALAR (n=39)	2.48±1.83	2.93±1.89^{b} 4.25±2.18	1.47±0.71	1.06±0.31	3.03±2.12^{b} 3.06±1.78	4.97±2.63	6.31±0.71	5.26±1.35^{a} 5.04±1.34	4.49±1.52	3.47±0.42	3.64±0.86^{ab} 3.87±1.35	-

Means followed by different letters and letter groups in the same row for months and seasons are significantly different ($p < 0.05$, n=2-5 for months; n=2-13 for seasons) values are means±SD. Means followed by different letters (a, b, c) and letter groups (ab, abc, cd, bd, bd) in the same row for months and seasons are significantly different (ANOVA-TUKEY HSD Test, $p < 0.05$, n=2-3 for months; n=2-9 for seasons) values are means±SD. **: n=1 (Tukey test was not used in the differences of January and February for Garip Station during the sampling period)

found that the average lipid content ranged from 2.7 to 6.9% in fish from three carp farms. However, the authors emphasized that the lipid content in the diet also affected the lipid content in the fish. Similarly, Varga *et al*. (2013) studied carp from different cultures in different parts of Hungary with the same diet, and found that environmental factors significantly (P=0.001) affected their lipid contents.

In a study conducted by Akpinar (1999), in which the fatty acid changes in the lipids of *Cyprinion macrostomus* fed and starved at two different temperatures (24 and 35 °C) in Kangal Fish Hot Spring (Sivas) were investigated, it was determined that food intake was better in fish fed and starved at 24 °C. Some fatty acids which were not in the food were detected in their lipids, and 24 °C was the threshold temperature for this. Also, Akpinar (1999) indicated that high temperature is significantly effective in nutrition and that the food consumed could be used at a minimum level for *Cyprinion macrostomus* in hot spring waters. It is understood that if the temperature is lowered (24 °C), the food is utilized better by the fish and their lipid metabolism becomes accelerated. The Ilıcalar station is a location where water transport is provided to the hot spring area located in the region. However, considering the total lipid values, *G. rufa* had a higher average total lipid content in the fish caught at the Ilıcalar station (4%) than at the Garip station (3%), which has colder waters. However, in the present study, the temperature was below this value (24 °C) at both stations throughout the year. It is thought that the high lipid content of the fish from the Ilıcalar station may have been due to the nutrient content in this location.

When the total lipid content of *G. rufa* was factored by season and gender, regardless of the station during the sampling period, it was observed that the difference was significant only for season (ANOSIM-R=0.26). The pair-wise test results of PERMANOVA revealed that they were significant in terms of lipid content between summer and autumn, and spring and autumn (P_{perm}=0.001). The differences between male and female fish were not significant (P_{perm}=0.12).

Lipids are transported from the muscles to the gonads for the development of the gonads in the reproduction period. The total lipid contents in the muscle are remarkably affected by season, especially during the reproduction period (Všetičková *et al*., 2020).

The spawning period of *G. rufa* females peaks in the middle of spring and decreases slowly from the end of May to November. On the other hand, it peaks in April in *G. rufa* males. This is due to the increase in gonad weight, which indicates that the breeding season declines after April (Abedi *et al*., 2011). The lipid content in the muscles of females decreases to a minimum in the spawning period. However, in males, the lowest lipid content is in the post-spawning period (Všetičková *et al*., 2020). The seasonal ANOSIM-R value was found to be 0.26 for both genders, which means that there was a slight change in the fatty acid composition in male and female individuals. However, there was a significant difference between spring and autumn in females (P_{perm} = 0.001) and between autumn and winter in males (P_{perm} = 0.004, respectively). Thus, the most effective factor on the change in lipid content of *G. rufa* is seasonal changes.

3.2. Fatty acid composition

First, the effects of season and gender on the change in the fatty acid composition of *G. rufa* were investigated and second, their dietary fatty acid composition was determined for different locations (Garip and Ilıcalar stations) and regardless of these locations. Different statistical analysis methods were used, such as ANOSIM-R, P_{perm}, SIMPER, PCO and Tukey tests.

3.2.1. Factors influencing the fatty acid composition of G. rufa according to different locations

The effect of location difference was observed to be significant, albeit only slightly, in the change in the fatty acid composition of *G. rufa* (ANOSIM-R=0.27). The season factor was partially significant in the fatty acid composition of the fish at both stations. However, seasonal variation was more prominent at the Ilıcalar station (ANOSIM-R=0.21) than at the Garip station (ANOSIM-R=0.07). The ANOVA test (TUKEY HSD) results showed that monthly and seasonal changes were significant for total polyunsaturated fatty acids (∑PUFA), 18:0, 22:6ω3 (docosahexaenoic acid, DHA), 20:1ω9 and 20:4ω6 (arachidonic Acid, ARA) at the Ilıcalar station (Tables 2 and 3) and total monounsaturated fatty acids (∑MUFA), 14:0, 20:5ω3 (eicosapentaenoic acid, EPA), 18:1ω9 and 18:0 at the Garip station (Tables 2 and 3). The pair-wise test results, PERMANOVA, revealed that the difference in FA composition was significant between spring and winter at the Ilıcalar station, and between spring and autumn in the Garip station (P_{perm}=0.002). The SIMPER results revealed that the average highest similarity was in winter at the Garip and Ilıcalar stations with close values (88, 89%, respectively). The gender difference in fatty acid composition was not significant at the stations because gender formed barely-separated groups.

Figure 2 shows a two-dimensional configuration plot of the PCO analysis of a resemblance matrix of fatty acids in *G. rufa* collected from different locations (Garip and Ilıcalar). The fish samples from the Ilıcalar station were characterized by 18:1ω9, EPA, DHA and 18:0 fatty acids; whereas those from the Garip station were characterized by 18:1ω9 and 22:1ω9. The stations formed separate but overlapping groups for fatty acid composition (ANOSIM-R=0.27), and differences between the stations were not significant.

The major SFA in all factor groups were 16:0 and 18:0 (Table 2). Similarly, these two fatty acids were reported by Guler *et al*. (2008) as the major fatty acids in *Cyprinus carpio*, ranging from 14.6 to 16.6% in all seasons. Misir *et al.* (2013) reported that 16:0 was the prominent SFA contributing to approximately 60% of ∑SFA, followed by 18:0 for *Chalcalburnus tarichi*. 18:1ω9 was the main MUFA in all muscle tissues of nine freshwater fish species collected from the Tigris River (Turkey). In addition, 18:1ω9 was recorded as the predominant fatty acid in *Cyrinus carpio* for all seasons (15.1–20.3%) (Guler *et al.,* 2008). The SIMPER results also showed that the FAs which contributed the most to the similarity between the Garip and Ilıcalar stations were 18:1ω9 (28%), 16:0 (22%) and DHA (9%), respectively. It was determined that 18:1ω9 was the most significant contributor to FAs for all seasons and genders at both stations (Table 3).

Oleic acid, 18:1ω9 is the subrate for two important desaturases, Δ12 and Δ15, which are only available from primary producers. These enzymes enable the conversion of 18:1ω9 to 18:2ω6 (linoleic acid, LNA) and 18:3ω3 (linolenic acid, LA). Animals obtain these two essential fatty acids from their diet rather than replacing other fatty acids (Dalsgaard *et al*., 2003). It is known that *G. rufa* prefers phy-

Table 2. Fatty acid composition of *G. rufa* at The Garip station during the sampling period (% Total FAME)

Fatty Acids	April (n=3)	May (n=3)	**SPRING (n=6)**	June (n=3)	July (n=3)	August (n=3)	**SUMMER (n=9)**	September (n=3)	October (n=2)	November (n=3)	**AUTUMN (n=8)**	January (n=1)**	February (n=1)**	**WINTER (n=2)**
14:0	1.33±0.54^{a}	1.10±0.17^{a}	**1.21±0.38^{b}**	0.93±0.31^{a}	1.29±0.17^{a}	2.28±0.72^{ab}	**1.50±0.42^{b}**	1.96±0.41^{ab}	2.30±0.74^{ab}	3.40±0.87^{b}	**2.57±0.91^{a}**	1.66	1.18	**1.42±0.34^{ab}**
16:0	16.84±1.27	18.57±1.28	**17.71±1.48**	20.42±1.40	19.26±2.65	20.93±1.56	**20.20±1.84**	13.80±12.07	18.18±0.04	21.54±2.41	**17.80±7.50**	19.70	19.03	**19.37±0.48**
i16:0	0.35±0.09	0.48±0.05	**0.43±0.14**	0.40±0.39	0.28±0.08	0.59±0.36	**0.42±0.34**	0.30±0.12	0.18±0.06	0.22±0.04	**0.24±0.15**	0.25	0.49	**0.37±0.21**
18:0	3.78±0.84^{bc}	5.08±0.44^{ab}	**4.43±0.93^{ab}**	6.58±1.08^{a}	4.05±0.38^{bc}	3.74±0.73^{bc}	**4.79±1.51^{a}**	3.61±0.42^{bc}	2.95±0.18^{bc}	2.92±0.41^{c}	**3.19±0.48^{b}**	3.81	3.32	**3.57±0.35^{ab}**
i17:0	0.81±0.17	0.61±0.53	**0.71±0.37**	0.82±0.12	0.75±0.42	0.65±0.56	**0.74±0.30**	1.00±0.19	0.87±0.19	1.04±0.19	**0.98±0.18**	0.88	0.59	**0.74±0.20**
17:0	1.03±0.22	1.00±0.11	**1.02±0.16**	1.06±0.22	0.79±0.08	0.65±0.56	**0.83±0.35**	1.22±0.43	0.88±0.19	1.10±0.21	**1.09±0.30**	0.97	1.07	**1.02±0.07**
∑SFA	24.69±1.71	27.52±2.21	**26.11±2.29**	31.75±1.18	26.94±2.42	30.35±0.85	**29.66±2.40**	22.76±10.94	26.14±1.16	31.18±2.00	**26.70±7.19**	27.69	26.67	**27.18±0.78**
∑FA*	0.55±0.14	0.68±0.09	**0.60±0.12**	1.54±0.57	0.52±0.22	1.51±0.66	**1.18±0.56**	0.87±0.10	0.78±0.20	0.96±0.25	**0.63±0.20**	0.42	0.99	**0.69±0.29**
16:1ω11	1.41±0.65	1.34±0.10	**1.38±0.42^{ab}**	1.25±0.41	0.79±0.56	1.17±0.68	**1.07±0.53^{b}**	2.16±0.86	1.24±0.57	2.02±0.27	**1.88±0.66^{a}**	0.14	1.70	**0.92±1.11^{ab}**
16:1ω9	5.56±1.35^{ab}	5.55±0.89^{ab}	**5.56±1.02^{ab}**	4.18±1.25^{b}	4.77±0.39^{b}	6.98±1.75^{ab}	**5.31±1.68^{b}**	6.86±1.50^{ab}	6.32±1.99^{ab}	9.55±2.15^{a}	**7.74±2.20^{a}**	9.01	5.76	**7.39±2.30^{ab}**
16:1ω7	0.37±0.06	0.44±0.05	**0.40±0.06**	0.29±0.19	0.52±0.45	0.67±0.70	**0.49±0.45**	0.22±0.20	0.41±0.01	0.52±0.19	**0.38±0.26**	-	1.01	**0.50±0.71**
17:1	0.48±0.38	0.39±0.27	**0.43±0.31**	-	0.92±0.08	0.71±0.64	**0.54±0.53**	0.93±0.36	-	1.49±0.47	**0.91±0.69**	1.02	-	**0.51±0.72**
18:1ω11	0.55±0.22	1.20±1.79	**1.00±0.96**	0.89±0.23	0.44±0.38	0.64±0.52	**0.66±0.40**	0.28±0.25	0.18±0.01	0.19±0.17	**0.22±0.17**	0.68	0.37	**0.52±0.22**
18:1ω9	32.71±2.51^{a}	26.25±6.91^{ab}	**29.48±5.84**	16.29±1.81^{b}	37.51±4.21^{a}	28.67±5.14^{ab}	**27.49±9.86**	33.44±6.65^{a}	35.40±2.35^{a}	32.46±7.04^{a}	**33.57±5.39**	29.80	26.05	**27.93±2.65**
18:1ω7	0.91±0.77	0.41±0.11	**0.66±0.56**	2.34±2.05	0.39±0.15	0.56±0.46	**1.10±1.41**	1.34±1.27	0.41±0.01	0.26±0.23	**0.70±1.28**	-	-	**-**
20:1ω9	0.66±0.39	1.27±0.19	**0.26±0.27^{b}**	-	-	-	**-**	2.15±1.99	2.34±0.18	1.61±0.31	**2.00±1.13^{a}**	-	-	**-**
∑MUFA	43.50±1.43^{a}	36.89±4.89^{ab}	40.22±4.98^{ab}	25.50±2.15^{b}	46.00±4.14^{a}	39.91±4.85^{ab}	37.15±9.69^{b}	48.32±10.51^{a}	46.82±0.62^{a}	48.47±7.81^{a}	47.87±7.04^{a}	41.33	**35.72**	**38.28±3.89^{ab}**
MUFA*	0.85±0.52	0.04±0.02	**1.05±0.64**	0.26±0.24	0.66±0.18	0.51±0.05	**0.47±0.18**	0.94±0.14	0.51±0.14	0.37±0.19	**0.47±0.26**	0.68	0.83	**0.51±0.21**
16:2ω4	1.10±0.91	0.96±0.07	**1.03±0.76**	0.78±0.05	0.50±0.36	1.81±1.03	**0.96±0.85**	1.14±0.71	1.16±0.15	0.54±0.48	**0.92±0.57**	0.81	0.43	**0.62±0.27**
18:2ω6	2.18±0.26	2.32±0.22	**2.25±0.23**	2.11±0.07	1.79±0.03	1.61±1.41	**1.84±0.74**	2.35±1.25	2.54±0.38	1.65±0.62	**2.14±1.13**	1.97	1.87	**1.92±0.07**
18:3ω6	0.77±0.24	0.11±0.09	**0.44±0.17**	0.91±0.58	-	0.71±0.65	**0.54±0.43**	0.81±0.63	0.80±0.02	0.39±0.01	**0.65±0.40**	0.17	0.77	**0.47±0.42**
18:3ω4	0.68±0.28	0.24±0.14	**0.46±0.21**	0.41±0.04	0.41±0.26	0.43±0.33	**0.42±0.21**	0.41±0.01	0.19±0.10	0.73±0.33	**0.47±0.29**	0.38	0.95	**0.67±0.40**
18:3ω3	8.16±0.19	7.76±1.46	**7.96±0.96**	6.32±0.10	8.88±0.65	10.86±2.73	**8.69±2.42**	9.77±1.55	9.06±0.62	5.05±4.65	**7.82±3.50**	7.42	7.26	**7.34±0.11**
20:3ω6	0.54±0.11	0.51±0.14	**0.52±0.11**	1.00±0.61	0.56±0.40	0.40±0.30	**0.66±0.57**	0.41±0.36	0.52±0.18	0.53±0.18	**0.48±0.24**	0.59	0.21	**0.40±0.27**
20:4ω6	1.79±0.31^{bc}	2.83±0.25^{ab}	**2.31±0.62^{ab}**	4.09±0.37^{a}	1.61±0.17^{bc}	1.78±0.39^{bc}	**2.49±1.23^{a}**	1.46±1.05^{bc}	0.87±0.08^{c}	1.04±0.37^{c}	**1.15±0.65^{b}**	2.03	2.70	**2.36±0.47^{ab}**
20:3ω3	0.94±0.05	1.04±0.21	**0.99±0.15**	1.05±0.29	1.17±0.05	1.05±1.01	**1.09±0.53**	0.44±0.38	1.35±0.13	0.90±0.53	**0.84±0.54**	0.78	0.88	**0.83±0.07**
20:4ω3	0.50±0.17	0.35±0.31	**0.43±0.24**	0.43±0.38	0.52±0.46	0.68±0.61	**0.54±0.44**	0.84±0.63	0.63±0.15	0.37±0.25	**0.61±0.53**	0.39	1.03	**0.71±0.45**
20:5ω3	5.74±1.21^{ab}	8.17±2.36^{ab}	**6.96±2.14^{ab}**	10.33±0.22^{b}	4.62±0.14^{a}	5.17±2.30^{a}	**6.71±2.96^{a}**	3.93±1.94^{a}	3.06±0.95^{a}	3.45±1.72^{a}	**3.53±1.48^{b}**	5.61	7.21	**6.41±1.13^{ab}**
21:5ω3	0.64±0.22	0.40±0.35	**0.52±0.29**	0.82±0.37	0.27±0.03	0.38±0.27	**0.49±0.37**	0.30±0.11	0.61±0.44	0.35±0.14	**0.39±0.24**	0.26	0.83	**0.55±0.41**
22:5ω3	0.93±0.19	1.02±0.13	**0.98±0.15**	1.01±0.32	0.56±0.52	0.52±0.46	**0.69±0.45**	0.82±0.32	1.06±0.02	0.55±0.49	**0.78±0.38**	1.15	1.07	**1.11±0.05**
22:6ω3	6.59±1.11^{bc}	8.93±2.00^{ab}	**7.76±1.93**	12.55±0.55^{a}	5.10±0.47^{c}	3.69±0.94^{c}	**7.11±4.17**	4.67±1.77^{c}	3.83±0.75^{c}	4.12±1.31^{c}	**4.25±1.26**	7.84	11.72	**9.78±2.75**
∑PUFA	**31.80±3.14^{abc}**	**35.59±2.98^{ab}**	**33.67±3.43**	**43.13±0.72^{a}**	**26.98±1.54^{bc}**	**29.74±5.44^{abc}**	**33.28±7.83**	**28.92±7.18^{abc}**	**27.04±0.55^{abc}**	**20.35±8.64^{c}**	**25.43±7.15**	**30.98**	**37.61**	**34.52±4.52**
PUFA*	1.23±0.24	0.95±0.50	**1.06±0.51**	1.32±0.64	1.00±0.51	**0.65±0.59**	**1.05±0.86**	1.57±1.12	1.36±0.45	0.68±0.59	**2.03±0.67**	1.58	0.68	**1.37±0.91**
Bacterial	3.50±0.91	3.05±1.21	**3.29±0.99**	3.60±0.28	3.30±0.23	3.87±0.77	**3.59±0.55**	4.57±0.94	2.65±0.58	4.91±1.12	**4.22±1.30**	3.42	3.14	**3.28±0.19**
DHA/EPA	1.16±0.08	1.10±0.06	**1.13±0.07**	1.21±0.03	1.11±0.14	0.77±0.24	**1.03±0.24**	1.26±0.23	1.27±0.15	1.30±0.33	**1.28±0.22**	1.40	1.63	**1.51±0.16**
Zooplankton	0.66±0.49	0.32±0.25	**0.49±0.69**	-	-	-	**-**	2.15±1.99	2.34±0.18	1.61±0.31	**2.00±1.13**	-	-	**-**
Terrestrial	10.34±0.07	10.08±1.68	**10.21±1.07**	8.43±0.13	10.67±0.68	12.47±1.51	**10.53±1.94**	12.12±2.68	11.61±1.00	6.70±5.05	**9.96±4.10**	9.40	9.14	**9.27±0.18**
∑ω3/∑ω6	4.12±0.58	4.57±0.76	**4.35±0.65**	3.92±0.42	5.20±1.16	6.60±3.80	**5.24±2.67**	3.72±0.33	3.93±0.46	3.99±2.36	**3.87±1.29**	4.66	5.42	**5.04±0.53**
16:1ω7/16:0	0.02±0.00	0.02±0.00	**0.02±0.00**	0.01±0.01	0.03±0.02	-	**0.02±0.03**	0.02±0.00	0.02±0	0.03±.0.02	**0.01±0.01**	-	0.05	**0.03±0.04**

Means followed by different letters (a, b, c) and letter groups (ab, abc, bc) in the same row for months and seasons are significantly different (ANOVA-TUKEY HSD Test, $p < 0.05$, n=2-3 for months; n=2-9 for seasons) values are means±SD. **: n=1 (Tukey test was not used in the differences of these months during the sampling period). *: Minor FAs with mean proportion ≤ 0.5 in all the sampling periods, Inluded in this fraction were FAs: ι15:0, 15:0, ι17:0, 19:0, 20:0 from SFA, 14:1, 15:1, 18:1ω6, 18:1ω5, 22:1ω5, 22:1 9, 24:1 from MUFA, 18:2, 18:4ω3, 20:2ω6, 20:2 from PUFA ι: indicates iso-branched FAs.

Table 3. Fatty acid composition of *G. rufa* at the Ilıcalar station during the sampling period (% Total FAME)

Fatty Acids	March (n=5)	April (n=5)	May (n=3)	SPRING (n=13)	June (n=3)	July (n=3)	August (n=3)	SUMMER (n=9)	September (n=3)	October (n=4)	November (n=3)	AUTUMN (n=10)	December (n=4)	January (n=3)	WINTER (n=7)
14:0	2.73±1.96	2.43±1.72	1.09±0.37	**2.23±1.65**	0.83±0.43	2.03±0.64	3.88±3.51	**2.25±2.36**	3.33±0.65	2.46±0.62	4.86±3.50	**3.43±2.03**	2.89±0.41	1.27±0.23	**2.20±0.92**
i16:0	0.31±0.14	0.34±0.10	0.37±0.33	**0.34±0.19^{b}**	0.67±0.16	0.76±0.63	0.67±0.82	**0.70±0.56^{a}**	0.21±0.08	0.16±0.05	0.23±0.08	**0.20±0.07^{b}**	0.16±0.02	0.20±0.08	**0.18±0.06^{b}**
16:0	18.42±3.10	18.39±0.70	17.25±1.39	**18.14±1.99**	18.67±0.84	23.54±3.40	20.16±3.70	**20.79±3.54**	21.85±2.86	18.96±1.43	19.43±3.61	**19.97±2.67**	19.36±2.25	21.33±0.82	**20.20±1.97**
i17:0	0.41±0.38	0.85±0.21	0.87±0.08	**0.69±0.44**	0.83±0.04	0.51±0.37	0.54±0.41	**0.63±0.32**	0.33±0.26	0.79±0.10	1.20±0.50	**0.78±0.46**	0.76±0.06	0.67±0.13	**0.72±0.10**
17:0	1.23±0.48	1.46±0.65	0.92±0.06	**1.25±0.51**	1.07±0.09	0.91±0.38	1.02±0.30	**1.00±0.27**	0.57±0.53	0.84±0.21	1.47±0.74	**0.95±0.58**	0.61±0.44	1.08±0.25	**0.81±0.42**
18:0	3.86±0.94 ^{ab}	4.81±1.59 ^{ab}	4.43±0.69 ^{ab}	**4.36±1.19^{ab}**	6.10±1.25^{a}	5.47±3.04 ^{ab}	2.64±0.37 ^{ab}	**4.74±2.46^{a}**	3.21±0.75 ^{ab}	2.66±0.72^{b}	3.11±0.52^{ab}	**2.96±0.65^{b}**	3.67±0.88^{ab}	4.08±1.02^{ab}	**3.85±0.88^{ab}**
∑FA*	**0.67±0.19**	**0.92±0.10**	**0.86±0.27**	**0.53±0.20**	**0.56±0.40**	**0.64±0.40**	**0.72±0.53**	**0.64±0.39**	**0.81±0.07**	**0.82±0.09**	**1.26±0.59**	**1.23±0.85**	**0.71±0.19**	**0.92±0.72**	**0.90±0.48**
∑SFA	**27.63±5.10**	**29.20±2.85**	**25.79±1.76**	**27.54±3.71**	**28.74±0.30**	**33.,86±0.96**	**29.64±3.70**	**30.74±3.15**	**30.31±3.31**	**26.70±1.81**	**31.56±9.32**	**29.52±5.21**	**28.16±2.01**	**29.55±0.79**	**28.86±1.67**
16:1ω11	1.02±0.78	0.78±0.62	0.90±0.53	**0.90±0.63**	0.76±0.39	0.74±0.53	0.98±0.71	**0.83±0.49**	0.90±0.21	0.93±0.88	1.86±0.60	**1.20±0.93**	1.44±0.78	0.84±0.56	**1.19±0.71**
16:1ω9	7.77±2.41	5.81±2.17	6.14±1.97	**6.64±2.24**	4.83±2.91	7.76±3.36	8.18±1.88	**6.92±3.04**	9.79±1.25	8.38±1.43	7.73±1.22	**8.61±1.45**	7.64±2.05	5.22±0.41	**6.60±1.96**
16:1ω7	1.06±0.79	0.65±0.57	0.29±0.28	**0.72±0.65**	0.25±0.22	0.34±0.32	0.79±0.34	**0.46±0.39**	1.68±0.79	1.38±1.01	1.54±0.60	**1.52±1.41**	1.53±0.77	0.14±0.11	**0.93±0.92**
17:1	1.60±0.37^{a}	1.21±0.90^{ab}	-	**1.08±0.85**	-	0.36±0.31^{ab}	0.87±0.02 ^{ab}	**0.41±0.41**	0.71±0.15 ^{ab}	0.94±0.12 ^{ab}	1.56±0.98 ^{ab}	**1.06±0.59**	0.92±0.42^{a}	0.51±0.42^{ab}	**0.74±0.45**
18:1ω11	0.22±0.20	1.26±1.04	0.78±0.09	**0.75±0.78**	0.98±0.10	0.65±0.53	0.37±0.18	**0.66±0.41**	0.29±0.19	0.30±0.14	0.19±0.10	**0.26±0.16**	0.55±0.53	0.41±0.10	**0.49±0.39**
18:1ω9	26.35±6.45	19.79±11.11	27.87±8.33	**24.18±8.94**	23.54±4.40	26.22±11.8	34.13±8.73	**27.96±9.54**	30.59±4.17	34.32±4.10	23.17±15.43	**29.86±9.31**	35.25±1.73	32.63±7.35	**34.13±4.63**
18:1ω7	0.44±0.36	1.31±1.59	1.52±1.62	**1.02±1.28**	2.06±1.37	2.21±1.64	0.67±0.20	**1.65±1.31**	1.11±0.43	0.95±0.58	0.21±0.16	**0.78±0.58**	1.31±0.44	2.22±1.28	**1.70±0.94**
20:1ω9	2.54±1.19^{a}	1.91±1.10^{ab}	-	**1.71±1.36**	-	-	-	**-**	1.50±1.31^{ab}	0.30±0.21^{b}	2.48±0.57^{a}	**1.31±1.18**	2.39±0.37^{a}	1.94±0.19^{ab}	**2.20±0.37**
MUFA*	**0.54±0.22^{b}**	**0.84±0.69^{b}**	**0.15±0.14^{b}**	**0.57±0.51**	**0.60±0.36^{b}**	**1.19±1.17^{ab}**	**0.13±0.08^{b}**	**0.63±0.98**	**0.55±0.23^{b}**	**0.91±0.79^{b}**	**3.60±2.62^{a}**	**1.36±0.92**	**0.70±0.55^{b}**	**1.54±1.24^{ab}**	**0.61±0.57**
∑ MUFA	**41.54±7.89**	**33.56±11.32**	**37.65±6.08**	**37.58±9.10^{a}**	**32.99±5.45**	**39.47±11.3**	**46.12±6.97**	**39.53±9.73^{ab}**	**47.14±2.47**	**48.41±2.92**	**42.34±7.85**	**45.96±8.97^{ab}**	**51.73±1.21**	**45.45±4.48**	**48.59±4.23^{a}**
16:2ω4	0.39±0.23	0.69±0.50	0.77±0.15	**0.59±0.36**	0.80±0.14	0.72±0.72	0.66±0.11	**0.73±0.16**	0.52±0.50	0.77±0.49	1.17±0.65	**0.82±0.55**	0.89±0.14	0.54±0.31	**0.74±0.45**
16:3ω3	0.81±0.59^{ab}	0.94±0.44^{a}	0.08±0.06^{b}	**0.69±0.56^{a}**	-	-	0.36±0.27^{ab}	**0.12±0.^{27b}**	0.24±0.18^{ab}	0.33±0.28^{ab}	-	**0.19±0.29^{b}**	0.45±0.12^{ab}	0.06±0.04^{b}	**0.29±0.24^{ab}**
18:2a	0.40±0.18	0.26±0.12	0.85±0.71	**0.45±0.39**	0.46±0.07	0.50±0.03	0.39±0.15	**0.45±0.10**	0.60±0.39	0.37±0.09	0.47±0.25	**0.47±0.24**	0.83±0.23	0.46±0.39	**0.67±0.38**
18:2ω6	2.69±0.90	2.85±1.99	1.69±0.30	**2.52±1.36^{a}**	1.73±0.35	2.05±0.43	2.24±0.71	**2.01±0.54^{ab}**	1.87±0.17	1.59±0.31	2.61±1.36	**1.98±0.81^{ab}**	0.81±0.76	1.84±0.10	**1.25±0.88^{b}**
18:3ω6	0.49±0.23	0.60±0.22	0.64±0.25	**0.57±0.22**	0.20±0.17	0.36±0.32	0.57±.29	**0.38±0.34**	0.45±0.05	0.42±0.17	0.43±0.25	**0.43±0.16**	0.25±0.20	0.52±0.36	**0.37±0.31**
18:3ω4	0.51±0.43	0.51±0.45	0.15±0.13	**0.43±0.44**	0.39±0.34	0.24±0.11	0.52±0.35	**0.39±0.29**	0.44±0.25	0.24±0.21	0.58±0.32	**0.41±0.27**	0.59±0.45	0.39±0.18	**0.51±0.42**
18:3ω3	7.36±2.34	7.68±5.13	7.94±0.57	**7.62±3.27^{a}**	6.47±0.56	6.70±0.99	10.20±2.91	**7.79±2.54^{a}**	6.46±0.16	7.95±0.58	8.91±4.97	**7.79±2.58^{a}**	3.64±2.49	3.94±1.14	**3.77±1.89^{b}**
20:2ω6	1.10±0.75^{a}	0.54±0.26^{ab}	0.66±0.21^{ab}	**0.78±0.54^{a}**	0.42±0.36^{ab}	0.15±0.13^{b}	0.26±0.23^{ab}	**0.28±0.25^{b}**	0.22±0.20^{b}	0.37±0.07^{ab}	0.43±0.07^{ab}	**0.34±0.14^{b}**	0.24±0.17^{b}	0.32±0.27^{ab}	**0.28±0.20^{b}**
20:3ω6	0.85±0.41	0.82±0.25	0.72±0.22	**0.81±0.30**	0.88±0.86	0.51±0.14	0.51±0.39	**0.63±0.53**	0.45±0.39	0.69±0.21	0.67±0.26	**0.61±0.28**	0.53±0.28	0.47±0.11	**0.50±0.21**
20:4ω6	1.92±1.00^{ab}	2.98±0.96^{a}	2.41±0.23^{ab}	**2.44±0.94^{a}**	3.24±0.75^{a}	1.79±1.18^{ab}	0.80±0.27^{b}	**1.94±1.31^{ab}**	1.07±0.58^{b}	0.95±0.26^{b}	1.11±0.46^{b}	**1.03±0.39^{b}**	1.17±0.21^{b}	2.02±0.85^{ab}	**1.54±0.68^{ab}**
20:3ω3	1.12±0.72	1.32±0.29	0.84±0.35	**1.13±0.53**	0.94±0.21	0.88±0.39	0.95±0.41	**0.92±0.32**	0.94±0.20	0.96±0.28	0.97±1.09	**0.96±0.55**	0.87±0.22	0.82±0.08	**0.85±0.16**
20:4ω3	0.64±0.29	1.19±0.45	0.94±0.48	**0.92±0.45**	1.22±0.62	0.77±0.49	0.59±0.24	**0.86±0.47**	0.49±0.37	0.58±0.46	0.91±0.77	**0.65±0.60**	0.62±0.45	0.18±0.12	**0.43±0.43**
20:5ω3	4.46±2.76	5.86±2.63	6.98±2.24	**5.58±2.59^{a}**	7.29±1.23	4.30±2.72	2.37±0.98	**4.65±2.64^{ab}**	2.88±0.57	3.15±1.08	2.69±0.19	**2.93±0.72^{b}**	3.55±0.30	4.85±2.04	**4.11±1.38^{ab}**
21:5ω3	0.33±0.14^{b}	0.50±0.14^{ab}	0.64±0.22^{ab}	**0.47±0.20**	1.00±0.60^{a}	0.35±0.26^{ab}	0.15±0.14^{b}	**0.50±0.46**	0.37±0.18^{ab}	0.61±0.23^{ab}	0.26±0.04^{b}	**0.43±0.22**	0.24±0.19^{b}	0.40±0.23^{ab}	**0.31±0.20**
22:5ω3	0.67±0.28	0.93±0.26	0.98±0.28	**0.84±0.29**	1.30±1.13	0.89±0.70	0.56±0.16	**0.92±0.71**	0.59±0.20	1.08±0.03	1.03±0.61	**0.92±0.38**	0.73±0.39	0.98±0.24	**0.84±0.34**
22:6ω3	6.61±3.25^{abc}	9.09±2.5^{abc}	10.14±1.7^{ab}	**8.38±2.89^{a}**	11.32±3.63^{a}	5.74±3.09^{abc}	2.92±1.05^{c}	**6.66±4.93^{ab}**	4.24±0.64^{abc}	4.36±1.40^{bc}	3.57±0.76^{bc}	**4.09±1.00^{b}**	3.85±1.68^{bc}	6.78±3.6^{abc}	**5.11±2.84^{ab}**
PUFA*	**0.48±0.41**	**0.48±0.25**	**0.13±0.13**	**0.65±0.61**	**0.61±0.42**	0.73±0.85	0.19±0.07	0.50±0.58	0.74±0.25	0.47±0.32	0.29±0.26	0.47±0.32	0.85±0.71	0.43±0.22	0.99±0.85
∑PUFA	30.83±7.48^{ab}	37.24±9.53^{a}	36.56±4.9^{ab}	34.87±7.9^{a}	38.27±5.20^{a}	26.67±10.33^{ab}	24.24±5.69^{ab}	29.73±9.62^{ab}	22.55±1.14^{ab}	**24.89±2.2^{ab}**	**26.10±8.73^{ab}**	**24.52±4.20^{b}**	**20.11±1.2^{b}**	**25.00±4.5^{ab}**	**22.56±3.82^{b}**
Bacterial	4.22±1.07^{ab}	5.00±1.60^{ab}	2.85±0.22^{b}	**4.20±1.40**	3.13±0.28^{ab}	3.34±1.63^{ab}	3.82±1.95^{ab}	**3.43±1.40**	2.64±0.65^{b}	3.67±0.33^{ab}	6.37±2.44^{a}	**4.17±1.99**	3.06±0.32^{b}	3.62±0.14^{ab}	**3.30±0.39**
DHA/EPA	1.66±0.41	1.68±0.46	1.51±0.28	**1.63±0.38**	1.56±0.42	1.34±0.25	1.26±0.18	**1.39±0.29**	1.52±0.43	1.53±0.80	1.33±0.27	**1.47±0.53**	1.07±0.37	1.40±0.43	**1.21±0.40**
Zooplankton	2.66±1.24^{ab}	1.91±1.10^{ab}	-	**1.76±1.43**	0.20±0.16^{b}	0.73±0.27^{ab}	-	**0.31±0.30**	1.50±1.31^{ab}	0.37±0.09^{b}	4.65±2.34^{a}	**1.99±1.14**	2.39±0.37^{ab}	2.36±0.77^{ab}	**2.38±0.52**
Terrestrial	10.05±3.17	10.54±7.10	9.63±0.31	**10.14±4.50^{a}**	8.21±0.90	8.75±0.96	12.44±3.60	**9.80±2.95^{ab}**	8.33±0.28	9.53±0.83	11.53±5.25	**9.77±3.30^{b}**	4.45±3.18	5.78±1.22	**5.02±2.46^{a}**
∑ω3/∑ω6	3.14±0.41	3.43±0.57	4.65±0.51	**3.75±0.52**	4.63±0.49	4.07±0.31	4.19±0.59	**4.29±0.45**	4.08±1.22	4.37±0.17	3.07±0.76	**3.84±0.94**	4.94±1.40	3.30±0.54	**4.12±0.97**
16:1ω7/16:0	0.06±0.04	0.04±0.03	0.02±0.02	**0.04±0.03**	0.01±0.01	0.02±0.02	0.04±0.02	**0.02±0.02**	0.08±0.04	0.07±0.05	0.09±0.06	**0.08±0.08**	0.08±0.04	0.01±0.01	**0.05±0.05**

Means followed by different letters (a,b) and letter groups (ab) in the same row are significantly different for months and seasons (ANOVA-TUKEY HSD Test, $p < 0.05$, n=3-5 for months; n=9-13 for seasons) values are means±SD. *: Minor FAs with mean proportion ≤ 0.5 in all the sampling periods, Inluded in this fraction were FAs: ι15:0, 15:0, ι17:0, 19:0, 20:0 from SFA, 14:1, 15:1, 18:1ω6, 18:1ω5, 22:1ω5, 22:1 9, 24:1 from MUFA, 18:2, 18:4ω3, 20:2ω6, 20:2 from PUFA ι: indicates iso-branched FAs.

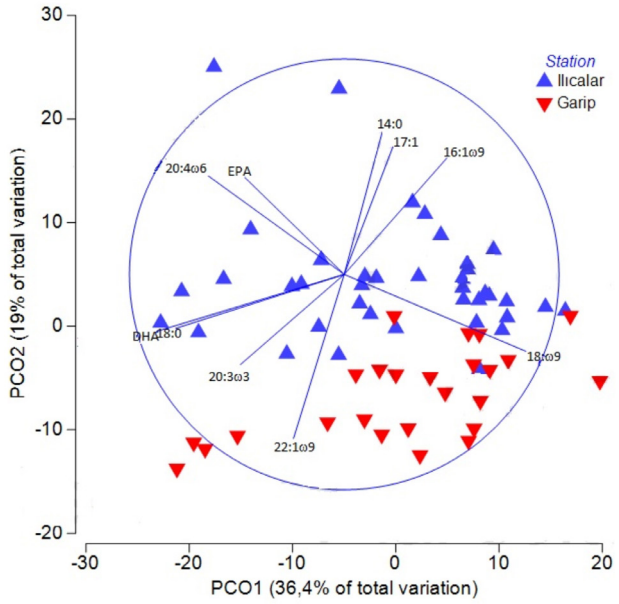

Figure 2. Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids of *G. rufa* at the Garip (n=25) and Ilıcalar (n=39) Stations. The lower tringular matrix was created using by Bray-Curtis similarity coefficients. Pearson correlation > 0.60.

toplankton in its diet especially, Bacillariophyceae members from Chrysophyta (Demirci *et al*., 2016). Yalcin-Ozdilek and Ekmekci (2006) reported that Chrysophyta members were abundant in all seasons in the diet of *G. rufa* in the Asi River, Turkey. Moreover, 18:1ω9 is used as a characteristic fatty acid marker for Cryptophyceae, together with Dinophyceae and Chlorophyta (Dalsgaard *et al.,* 2003). The percentage of abundance of other types of food in the diet of *G. rufa* varies depending on the season (Yalcin-Ozdilek and Ekmekci, 2006).

Guler *et al*. (2008) reported that the PUFA content in *Cyprinus carpio* fillets differed in each season and was 39% in spring, 43% in summer and 36% in autumn. They indicated that DHA was the major PUFA for *Cyrinus carpio* in summer and winter. DHA plays an important role in adaptation processes. When fish are exposed to low water temperatures, PUFA content increases (Lavens *et al*., 1999). Similarly, the highest ∑PUFA content in *G. rufa* (34%; 35%, respectively) was observed to be found in the lowest temperatures detected during the sampling period, which were in winter (8.03 °C) at the Garip station and in spring (12.63 °C) at the Ilıcalar station. The PUFA content was higher in the fish samples from the Garip station (Table 2) than from the Ilıcalar station (Table 3). This is thought to be related to temperature because the temperature of the Ilıcalar station was higher than the Garip station during the sampling period (Table 4).

The fatty acid marker for diatoms (Bacillariophyceae) is EPA and for dinoflagellates (Dinophyceae) is DHA (Viso and Marty, 1993). EPA and DHA contents vary among/within species depending on environmental factors such as diet and habitat, as well as whether the fish are wild or farmed (Tocher, 2010). The fact that 18:1ω9, EPA and DHA are the most abundant fatty acids may indicate that *G. rufa* prefers the members of Cryptophyceae, Dinophyceae, and Chlophyta as food sources in the Murat River. Moreover, the 16:1ω7/16:0 ratio has been used to

Table 4. Chlorophyll-a concentration (µg/L) and water temperature (ºC) at the stations during the sampling period

	SPRING			**SUMMER**				**AUTUMN**		**WINTER**		
	March	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**	**January**	**February**
							GARIP					
Chl-a		**0.10**			**5.81**			**1.98**			**2.55**	
	0.09	0.10	0.10	3.28	4.60	9.54	1.97	3.46	0.52	1.96	3.95	1.75
Temperature		**11.13**			**21.76**			**12.67**			8.03	
	8.20	8.6	16.6	20.12	25.1	20.05	0.50	17.00	20.50	9.60	7.40	7.10
							ILICALAR					
Chl-a		**1.77**			**4.18**			**0.91**			**1.19**	
	0.86	1.03	3.42	1.71	0.41	10.72	0.31	1.02	1.41	1.20	1.15	1.21
Temperature		**12.63**			**23.57**			**21.73**			**16.67**	
	11.00	8.10	18.80	23.7	25.5	21.50	21.50	21.00	22.70	20.5	15.50	14.00

infer a diatom-dominated food chain base (Auel *et al*., 2002). While 16:1ω7 is found in cyanobacteria, dinoflagellates, and a specific isomer, the *trans* one, is found in bacteria, 16:1ω7 is most prevalently associated with diatoms (Parrish, 2013). The 16:1ω7/16:0 ratio varied from 0.02 to 0.08% (summer-autumn) at the Ilıcalar station and 0.01-0.03% (autumn-winter) at the Garip station. These results showed that *G. rufa* at consumed more diatoms the Ilıcalar station than those at the Garip station during the sampling period. However, 18:1ω9, EPA and DHA were the most abundant dietary fatty acids in *G. rufa*. Yalcin-Ozdilek and Ekmekci (2006) showed that *G. rufa*'s main diets primarily comprised diatoms found in the Chrysophyta. However, the fatty acid composition detected in the present study indicated there were more dinoflagellates than diatoms in *G. rufa's* diet. Freshwater fish cannot synthesize certain fatty acids, especially C_{18} PUFA, such as 18:2ω6, 18:3ω6, although they can directly ingest many LC-PUFAs such as ARA, DHA, EPA from their prey (Tocher, 2010). Dietary EPA, DHA, and ARA improve reproductive success and increase the quality of broodstock eggs (Mazorra *et al*., 2003). They are critical to the general health of organisms and most consumers synthesize them inefficiently from their precursors (e.g., 18:3ω3 or ALA and 18:2ω6 or LNA). Therefore, EPA, DHA, and ARA are considered essential dietary FAs in aquatic ecosystems (Dalsgaard *et al*., 2003; Parrish, 2009). 18:3ω3 is also higher in freshwater herbivorous Cypriniformes, including *G. rufa*. 18:3ω3 was one of the highest PUFA at both stations during the sampling period (Tables 2 and 3). It is stated in many studies that terrestrial plants abundantly synthesize 18:3ω3 and 18:2ω6, which are also used as dietary marker in the fatty acid composition of aquatic organisms (Parrish, 2013). ARA was more prominent in the fish from the Ilıcalar station than those from the Garip station for PUFA in the PCO analysis (Figure 2). Freshwater fish have relatively high contents of 18:2ω6 and ARA, which are indicative of freshwater algae and terrestrial dietary sources (Parzanini *et al*., 2020). These fatty acids were present in significant percentages in the fatty acid composition of *G. rufa*. From the fatty acid values, it was deduced that *G. rufa* also preferred diets of terrestrial origin.

C_{13}, C_{15}, C_{16} and C_{17} SFA and MUFA and their isomers, as well as 18:1ω6 characterize bacterial fatty acids (Dalsgaard *et al*., 2003; Parrish, 2013). Therefore, the presence of these fatty acids in fish tissue may indicate a bacterial diet. The results of the present study showed that *G. rufa* fed on bacteria (Tables 2 and 3). However, terrestrial markers outnumbered both bacterial and zooplanktonic markers at both stations during the sampling period (Tables 2 and 3).

The C_{20} and C_{22} group zooplankton fatty acids were in very small pergentages in *G. rufa*, which were found in a smaller amount in the fish samples from the Garip station than in those from the Ilıcalar station. These fatty acids characterize copepod species and are used in the analysis of marine fish to reveal nutritional relationships (Iverson *et al.,* 2009). These fatty acids were observed in *G. rufa* in winter as well as other seasons at the Ilıcalar station, and not in winter at the Garip station. *G. rufa* preferred zooplankton as food in winter months, because the Chl-a content was found to be lower at the Ilıcalar station (1.19 µg/L) than at the Garip station (2.55 µg/L) in winter. The highest Chl-a content was detected in summer at both stations. It was higher at the Garip station (5.81 µg/L) (Table 2) than the Ilıcalar station (4.18 µg/L) in summer (Table 3). In the periods when Chl-a was abundant (Table 4), *G. rufa* tended toward an herbivorous diet, which is their main diet.

The ω3/ω6 ratio is greater in herbivorous freshwater fish (Parzanini *et al*., 2020). The ω3/ω6 ratio varied between 3-5 at the Ilıcalar station (Table 3) and 4-7 at the Garip station (Table 2). It reached the highest value in August at 6.60 at the Garip station (Table 2). The Ilıcalar station included zooplanktonic FAs at higher levels than the Garip station. Phytoplankton were probably more abundant than zooplankton at the Garip station, and *G. rufa* preferred phytoplankton, which is the primary food source in all seasons at the Garip station.

The results of seasonal changes in dietary fatty acid levels showed that the fatty acid composition of *G. rufa* changed according to the change in nutritional content and variety depending on the season. There are already many approaches and studies showing that fatty acids give clues about the food consumed. All these approaches can provide valuable information about consumer nutrients and food ecology in complex aquatic ecosystems. Each of these approaches is evaluated and used in studies

according to the content of the study (Dalsgaard *et al*., 2003). FAs are known indicators of specific food sources, the results can indicate the diet of consumers and are a potentially powerful trophic measures which reflect what is included in an individual's diet over a period of several weeks (Kirsch, 1998). From this point of view, it would not be wrong to say that *G. rufa* predominantly feeds on plants, but can also feed omnivorously. It can be stated that *G. rufa* has a very wide food preference from bacteria to zooplankton. Demirci *et al*. (2016) emphasized similar results in their study on the nutritional characteristics of *G. rufa* from organisms in stomach-intestinal contents. *G. rufa* can feed on various plankton species, although they prefer phytoplankton (Demirci *et al*., 2016). It was also found in the present study that the results of dietary fatty acids indicated the same results.

3.3. Factors affecting the fatty acid composition of *G. rufa*, regardless of location

Figure 3 shows the PCO analysis of seasonal and gender differences in terms of the fatty acid composition, regardless of the stations. The results of the PERMANOVA pair-wise tests revealed that the fatty acid compositions did not differ significantly regardless of the stations during the sampling season. Where several samples represent several seasons at the same time, they were grouped according to their close proximity to the nearest seasons (e.g., warm seasons, cold seasons, hot seasons). In general, spring-summer (hot seasons) and winter-autumn (cold seasons) were located in the same area with 80% similarity. These areas were represented by more samples. However, all seasons were located in the same area with 70% similarity (Figure 3b). The PERMANOVA main test results revealed that the effect of season was significant in the fatty acid composition regardless of the station (P_{perm}=0.001). Almost all seasons were characterized by 18:1ω9 with 80% similarity. The highest difference was detected between autumn and spring (P_{perm}=0.001). However, autumn and winter were characterized by 18:1ω9 with 80% similarity more than the others (Figure 3b).

Figure 3a shows the PCO analysis of the fatty acid composition of the gender groups, regardless of station. The results of the PERMANOVA pair-wise test indicated that the fatty acid composition of females in summer-winter was significantly different from the other seasons (P_{perm}=0.001). Although the difference in the fatty acid composition of males was not as great as in females, the results of spring-winter seasons were different from the other season

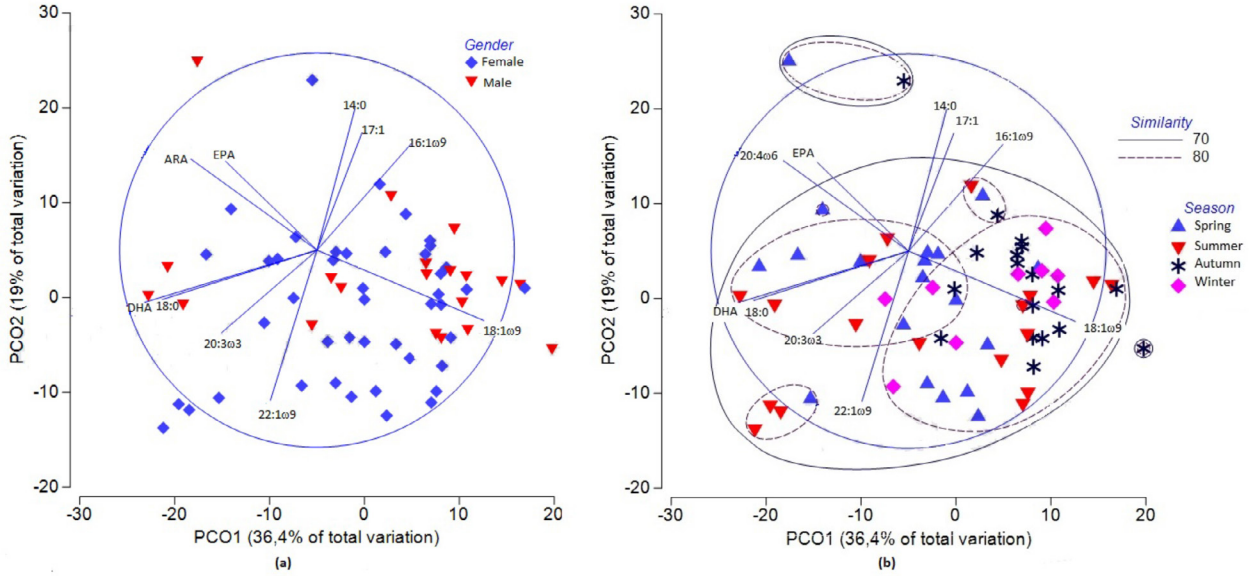

Figure 3. Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in the seasons and genders. The lower tringular matrix was created using by Bray- Curtis similarity coefficients. Pearson correlation > 0.6. (a): Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in the females (n=44) and males (n=20) of *G. rufa*. (b): Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in the seasons regardless of location (n= 19,18,18,9 for spring, summer, autumn and winter, respectively).

(P_{perm}=0.003). The gender variable groups formed barely-separated groups for both males and females (ANOSIM-R=0.13; 0.14, respectively). There was no significant seasonal difference in both genders. Females were characterized by 18:1ω9, DHA, EPA and ARA; whereas males were characterized by DHA, 18:0 and 18:1ω9 (Figure 3a). Şen Özdemir and Caf (2018) found similar results for female seahorses. They reported that female seahorses were characterized by EPA, DHA and ARA in multidimensional scaling results and that 18:1ω9 was not the most significant contributor to the fatty acid composition of both seahorse males and females. The only difference was that in their study, DHA replaced 18:1ω9, contrary to the results obtained in the present study. Such differences in freshwater and marine fish are expected since they have fundamental differences such as different feeding habitat (Parzanini *et al*., 2020). Urquidez-Bejarano *et al.* (2016) reported that 16:0, 18:0, 18:1, ARA and EPA were significantly higher in ripe female gonads than in spent gonads for angelfish. They observed a similar trend in male gonads, and there was no statistically significant difference between genders. Similarly, the difference between males and females in terms of the fatty acid composition of *G. rufa* muscle tissue used in this study were not significant. It is widely known that FAs like EPA, DHA and ARA are involved in numerous physiological processes from growth to reproduction. Therefore, they are vital to consumers including vertebrates like fish species (Paulsen *et al.,* 2014). Also, DHA plays an important role in the female reproductive system. It is transferred from the muscle to the liver and gonads and effects the egg quality and survival of larvae. In addition, a balanced presence of linolenic (18:3ω3) and linoleic (18:2ω3) fatty acids in the feeding of fresh water fish larvae increases the optimal survival rate (Higgs *et al*., 1992).

CONCLUSIONS

This study provides a first comprehensive report on the total lipid content and FA composition of *G. rufa* according to biotic and abiotic factors (season, gender, location) and determines feeding behavior using dietary fatty acids. In particular, the analysis performed revealed fundamental differences and similarities between/within factor groups. Seasonal differences were more prominent than the other factor groups in terms of both total lipid content and fatty acid composition ($p < 0.05$). In addition, annual average total lipid content was higher in females than males during the sampling period. *G. rufa* was characterized by a high MUFA content, mainly 18:1ω9, for all factor groups during the sampling season. *G. rufa* had high percentages of dietary fatty acids such as EPA, DHA, 18:1ω9, ω3/ω6 and ARA fatty acids, thus indicating herbivorous feeding. The study also showed that although *G. rufa* preferred predominantly phytoplankton, it had a very wide range of food preference from bacteria to zooplankton. Although there were no significant differences between locations, both seasonal and gender differences were more prominent in the different locations. According to the dietary fatty acid results, the diet composition, which changes depending on season rather than location, is a major factor in determining the fatty acid composition of *G. rufa*.

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