



# Interactions of season, sex and size on nutrient composition of freshwater crayfish (*Astacus leptodactylus* Eschscholtz, 1823) from Lake Eğirdir

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## Abstract

Effects of interaction of season, sex and size on nutrient composition and fatty acid profiles of freshwater crayfish (*Astacus leptodactylus*) caught from the Eğirdir Lake were investigated on a factorial level. In the factorial analysis; 4 levels of season factors (autumn, winter, spring, summer), 2 levels of sex factor (male, female) and 1 level size factor were available. According to the results of biochemical analysis, effects of seasons were determined on crude protein and moisture levels. Crude ash levels changed depending on sex and season. There were no interactions of these three factors on the crude fat levels. The season and sex affected on LA,  $\alpha$ -LNA and  $\Sigma$ HUFA levels. ARA, EPA, DHA,  $\Sigma$ SFA and  $\Sigma$ PUFA levels were affected from seasonal changes. Interaction of season/sex affected to  $\Sigma$ MUFA levels. The crude protein content, DHA, EPA and n-3 contents in tail muscle of *A. leptodactylus* increased by feeding in summer while these contents decreased by gonadal development in autumn. Therefore, in terms of nutritional protein contents, EPA, DHA and n-3 of this species were higher in summer. EPA, n-3 and DHA contents are important for *A. leptodactylus*.

**Keywords:** *Astacus leptodactylus*; biochemical composition; fatty acid; season; sex.

**Practical Application:** nutritional quality as seasonal of *Astacus leptodactylus*.

## 1 Introduction

*Astacus leptodactylus* is naturally and widely distributed in lakes, ponds and rivers throughout Turkey (Harlioğlu & Harlioğlu, 2004). In recent years, there has been a gradual decrease in the production of crayfish in Turkey from 5000 tons in 1984 to 532 tons in 2015 (Turkish Statistical Institute, 2017). The greatest cause of this decline is crayfish plague fungus (*Aphanomyces astaci*) seen after 1985 (Baran et al., 1987; Rahe & Soylu, 1989; Harlioğlu, 2008). There is no crayfish culture in Turkey and all production is obtained from wild harvests (Harlioğlu et al., 2012). Therefore, culture of crayfish is needed to replenish native stocks of *A. leptodactylus*.

Feeding habits of *A. leptodactylus* compose bivalves (*Dreissena polymorpha*), gastropods (*Greacoanatolica lacustriturca*), crayfish eggs (Acar Kurt, 2016), Nematoda (*Capillaria* sp.) and sometimes fish *Aphanius anatoliae*, aquatic plant *Ceratophyllum demersum*, *Elodea canadensis*, and *Epithemia* sp., *Cocconeis* sp., *Amphora* sp., *Synedra* sp., *Diatoma* sp., *Pinnularia* sp., *Cymbella* sp., *Navicula* sp., *Fragilaria* sp., *Gyrosigma* sp (Uysal, 2011).

Crayfish meat is juicy, low-fat, low calories, and rich in protein (Koniczny et al., 2004). Regarding the biochemistry of crustaceans, it has been stated that environmental factors such as habitat, food availability, and seasonality can modify their metabolism (Schirf et al., 1987; Kucharski & Silva, 1991; Oliveira et al., 2003; Moreno-Reyes et al., 2015) and their chemical composition (Rosa & Nunes, 2003).

The knowledge of nutritional requirements in decapods is crucial to successful culture (Moreno-Reyes et al., 2015). According to Dempson et al. (2004), the proximate body composition (moisture, lipids, protein and ash) is a good indicator of the nutritional status of an organism. There is not a study about interactions of season, sex and size on nutrient composition of crayfish. This investigation provides basic information about nutritional requirements of the species in recovering natural populations.

## 2 Materials and methods

### 2.1 Sample collection

Crayfish were supplied by the Eğirdir Lake, Isparta, Turkey. They were collected by trapping from their natural habitat at 4 seasons and size range (7.1-9.9 cm). Total of 335 crayfish were caught in autumn (October), winter (January), spring (April) and summer (July) (2014-2015). The crayfish were separated by sex and weighed on an electronic scale (0.001 g) in the laboratory (Table 1). Total length was measured from the rostrum to the end of the telson. Crayfish were anaesthetized by placing them at freezer ( $-80 \pm 1^\circ\text{C}$ ) for 30 min. The abdominal muscle were removed and stored in a freezer at  $-80^\circ\text{C}$  until they were used for the biochemical analyses.

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## 2.2 Proximate composition

Moisture contents were detected with an automatic moisture analyzer (AND MX-50). The crude protein contents according to Kjeldahl method (Nx6,25) (Association of Official Analytical Chemists, 2000), crude lipid contents by Bligh & Dyer (1959)'s method and crude ash contents according to AOAC (Association of Official Analytical Chemists, 2006) were done.

## 2.3 Fatty acid analysis

The operating conditions of the GC-MS (GC Clarus 500 with autosampler (Perkin Elmer, USA): Column was SGE (60 m x 0.25 mm ID. BPX5. 0.25 µm USA), the oven temperature was maintained at 60 °C for 10 min and increased to 220 °C at a rate of 4 °C/min. The oven temperature was maintained at 220 °C for 10 min, then increased to 250 °C at a rate of 4 °C/min and maintained at 250 °C for 10 min, the Carrier gas was Helium (1.5 mL/min), and Injector temperature was 240 °C, Split ratio was 1:100, Mass spectra was 70 eV, and Mass range was 35-425 m/z. Fatty acids were identified by comparing the retention times of FAME (catalogue number 18919; Supelco) with the standard 37- component FAME mixture.

## 2.4 Statistical analysis

The data obtained were analyzed in terms of specifications elaborated by analysis of factorial design ANOVA using the SPSS 13.0 computer program (SPSS Inc., Chicago, USA) techniques. In the factorial analysis; 4 levels of season factors (autumn, winter,

spring, and summer), sex factor (male, female) and size factor (7.1-9.9 cm) were available. Tukey test was used to determine the differences between the average levels of the factor.

## 3 Results

### 3.1 Proximate compositions

Binary or triple interaction was not obtained when crude protein, crude ash and moisture analysis results were evaluated in tail meat of *A. leptodactylus*. Alone the season significantly affect these nutrient compositions ( $p < 0.05$ ). The highest protein contents were found in spring and summer ( $p < 0.05$ ). The highest ash content was determined in the autumn while the lowest ash content was found in the winter ( $p < 0.05$ ). The highest moisture contents were determined in the spring and the winter while the lowest moisture content was found in the summer ( $p < 0.05$ ) (Table 2). Any an interaction was not found for crude fat contents of tail meat of *A. leptodactylus*. Crude fat means in tail meat of *A. leptodactylus* were given in the Table 3. Also, the sex factor was a significant affect over crude ash contents ( $p < 0.05$ ) (Table 4).

### 3.2 Fatty acids

Effect of season was determined on some fatty acids in tail meat of *A. leptodactylus*. The highest SFA, linoleic and linolenic acid contents were found in the autumn. The highest ARA was in the winter-spring, EPA in the summer, DHA in

**Table 1.** Mean length and weight of samples.

	Mean length (S.E)	Mean weight (S.E)	Sex	Number
Autumn (October)	8.2 ± 0.1	14.3 ± 2.6	Male	30
			Female	30
Winter (January)	7.9 ± 0.1	13.9 ± 1.2	Male	51
			Female	50
Spring (April)	8.0 ± 0.1	13.4 ± 3.2	Male	24
			Female	43
Summer (July)	9.1 ± 0.0	15.7 ± 2.6	Male	54
			Female	53
Total				335

**Table 2.** Seasonal variation of some nutrient composition in tail meat of *A. leptodactylus* (mean ± S.E.).

Proximate Composition (%)	Seasons			
	Autumn (October)	Winter (January)	Spring (April)	Summer (July)
Crude protein	15.2 ± 0.3 <sup>b</sup>	14.1 ± 0.2 <sup>b</sup>	15.8 ± 0.3 <sup>a</sup>	15.9 ± 0.3 <sup>a</sup>
Crude ash	1.7 ± 0.1 <sup>a</sup>	1.3 ± 0.0 <sup>d</sup>	1.4 ± 0.0 <sup>c</sup>	1.5 ± 0.0 <sup>b</sup>
Moisture	81.6 ± 0.4 <sup>b</sup>	82.4 ± 0.3 <sup>a</sup>	82.1 ± 0.5 <sup>a</sup>	80.9 ± 0.2 <sup>c</sup>

Means within a row having different superscripts are significantly different ( $P < 0.05$ ).

**Table 3.** Crude fat means in tail meat of *A. leptodactylus* (mean ± S.E.).

Crude lipid (%)	Seasons			
	Autumn (October)	Winter (January)	Spring (April)	Summer (July)
Female (♀)	1.3 ± 0.3	0.7 ± 0.0	1.1 ± 0.0	0.89 ± 0.1
Male (♂)	0.8 ± 0.1	0.7 ± 0.0	0.9 ± 0.1	1.09 ± 0.1

spring-summer and PUFA in the winter ( $p < 0.05$ ). The lowest  $\Sigma$  HUFA content was obtained in the autumn ( $p < 0.05$ ) and the other seasons were similar to each other ( $p > 0.05$ ) (Table 5). In addition, it was found that the sex affected to  $\Sigma$  HUFA, linoleic and linolenic acid contents ( $p < 0.05$ ). The linoleic and linolenic acid contents were higher in the female than the male while  $\Sigma$  HUFA content was higher in male than female ( $p < 0.05$ ) (Table 6).  $\Sigma$ MUFA contents in the tail meat of *A. leptodactylus* showed season and sex interaction. The lowest  $\Sigma$ MUFA content was defined in females in the autumn ( $p < 0.05$ ) while  $\Sigma$ MUFA contents were similar to in the others seasons ( $p > 0.05$ ). The lowest  $\Sigma$ MUFA content

was defined in the autumn in females, in the winter in males ( $p < 0.05$ ) while  $\Sigma$ MUFA contents in females and males were similar in the others seasons ( $p > 0.05$ ) (Table 7).

## 4 Discussion

### 4.1 Crude protein contents

In this study, effect of season only was determined on crude protein contents in tail meat of *A. leptodactylus*. The highest crude protein content (15.90%) was detected in summer samples and similar result was obtained in spring, as well. The lowest protein content was detected in the winter and similar result was recorded in the autumn (Table 2). Similarly to the present study, Berber et al. (2014) reported that the highest protein contents were assigned in both sexes in tail meat of *A. leptodactylus* in summer. But, Berber et al. (2014) did not research differences between sexes. Silva-Castiglioni et al. (2007) indicated that total muscle proteins were high during summer in both sexes of *Parastacus varicosus* and there was no difference between the sexes during the year. Inanlı & Çoban (2007) and Bilgin et al., (2008) did not determine difference between sexes in terms of

**Table 4.** Effect of sex on crude ash in tail meat of *A. leptodactylus* (mean  $\pm$  S.E.).

Sex	Crude ash (%)
Female (♀)	1.4 $\pm$ 0.0 <sup>b</sup>
Male (♂)	1.5 $\pm$ 0.3 <sup>a</sup>

Means within a column having different superscripts are significantly different ( $P < 0.05$ ).

**Table 5.** Effect of season on some fatty acids in tail meat of *A. leptodactylus* (mean  $\pm$  S.E.).

Fatty acid (%)	Seasons			
	Autumn (October)	Winter (January)	Spring (April)	Summer (July)
$\Sigma$ SFA	22.1 $\pm$ 0.3 <sup>a</sup>	17.6 $\pm$ 0.2 <sup>c</sup>	17.3 $\pm$ 0.3 <sup>c</sup>	20.1 $\pm$ 0.2 <sup>b</sup>
C18:2n6 (Linoleic, LOA)	3.9 $\pm$ 0.1 <sup>a</sup>	3.3 $\pm$ 0.1 <sup>b</sup>	3.2 $\pm$ 0.1 <sup>b</sup>	3.2 $\pm$ 0.1 <sup>b</sup>
C18:3n3 (Linolenic, $\alpha$ -LNA)	1.1 $\pm$ 0.0 <sup>a</sup>	0.8 $\pm$ 0.0 <sup>b</sup>	0.7 $\pm$ 0.0 <sup>b</sup>	0.6 $\pm$ 0.0 <sup>c</sup>
C20:4n6 (ARA)	11.0 $\pm$ 0.2 <sup>b</sup>	13.4 $\pm$ 0.3 <sup>a</sup>	12.5 $\pm$ 0.3 <sup>a</sup>	9.7 $\pm$ 0.3 <sup>c</sup>
C20:5n3 (EPA)	10.1 $\pm$ 0.3 <sup>c</sup>	12.5 $\pm$ 0.2 <sup>b</sup>	12.8 $\pm$ 0.1 <sup>b</sup>	14.3 $\pm$ 0.2 <sup>a</sup>
C22:6n3 (DHA)	4.4 $\pm$ 0.1 <sup>b</sup>	4.9 $\pm$ 0.1 <sup>ab</sup>	5.3 $\pm$ 0.2 <sup>a</sup>	5.4 $\pm$ 0.2 <sup>a</sup>
$\Sigma$ PUFA	30.6 $\pm$ 0.4 <sup>c</sup>	34.9 $\pm$ 0.4 <sup>a</sup>	34.6 $\pm$ 0.3 <sup>ab</sup>	33.3 $\pm$ 0.5 <sup>b</sup>
$\Sigma$ n-3	15.7 $\pm$ 0.4 <sup>c</sup>	18.2 $\pm$ 0.3 <sup>b</sup>	18.9 $\pm$ 0.2 <sup>b</sup>	20.2 $\pm$ 0.3 <sup>a</sup>
$\Sigma$ n-6	14.9 $\pm$ 0.2 <sup>b</sup>	16.7 $\pm$ 0.3 <sup>a</sup>	15.7 $\pm$ 0.2 <sup>b</sup>	12.9 $\pm$ 0.3 <sup>c</sup>
n-3/n-6	1.1 $\pm$ 0.0 <sup>c</sup>	1.1 $\pm$ 0.0 <sup>c</sup>	1.2 $\pm$ 0.0 <sup>b</sup>	1.6 $\pm$ 0.0 <sup>a</sup>
$\Sigma$ HUFA	25.6 $\pm$ 0.4 <sup>b</sup>	30.8 $\pm$ 0.4 <sup>a</sup>	30.6 $\pm$ 0.3 <sup>a</sup>	29.4 $\pm$ 0.5 <sup>a</sup>
Total fatty acid	71.5 $\pm$ 0.4 <sup>b</sup>	70.8 $\pm$ 0.2 <sup>b</sup>	71.1 $\pm$ 0.5 <sup>b</sup>	73.7 $\pm$ 0.4 <sup>a</sup>

Means within a row having different superscripts are significantly different ( $P < 0.05$ ).

**Table 6.** Effect of sex on some fatty acids in the tail of *A. leptodactylus* (mean  $\pm$  S.E.).

Fatty acid (%)	Female (♀)	Male (♂)
C18:2n6 (Linoleic)	3.5 $\pm$ 0.1 <sup>a</sup>	3.2 $\pm$ 0.1 <sup>b</sup>
C18:3n3 (Linolenic)	0.8 $\pm$ 0.1 <sup>a</sup>	0.8 $\pm$ 0.1 <sup>b</sup>
$\Sigma$ HUFA	28.7 $\pm$ 0.7 <sup>b</sup>	29.5 $\pm$ 0.7 <sup>a</sup>

Means within a row having different superscripts are significantly different ( $P < 0.05$ ).

**Table 7.** Variation of total  $\Sigma$  MUFA contents in the tail of *A. leptodactylus* according to season and sex interaction (mean  $\pm$  S.E.).

$\Sigma$ MUFA (%)	Seasons			
	Autumn (October)	Winter (January)	Spring (April)	Summer (July)
Female (♀)	17.1 $\pm$ 0.2 <sup>Ba</sup>	18.44 $\pm$ 0.2 <sup>ABa</sup>	19.65 $\pm$ 0.3 <sup>Aa</sup>	19.6 $\pm$ 0.5 <sup>Aa</sup>
Male (♂)	18.4 $\pm$ 0.2 <sup>Aa</sup>	16.99 $\pm$ 0.5 <sup>Bb</sup>	17.73 $\pm$ 0.2 <sup>ABb</sup>	18.5 $\pm$ 0.3 <sup>Ab</sup>

The big letters in the line, different among seasons in same sex; small letters in column, different among sexes in season.

protein contents in tail meat of *A. leptodactylus*. The protein contents in tail meat of *A. leptodactylus* were determined as 15.98% male and 16.39% female in (Inanlı & Çoban 2007), 14.61% in (Harlioğlu et al., 2012), 16.32% in (Çoban et al., 2012), in 15.23-16.66% in (Duman et al., 2012), male (15.68%) and female (17.59%) in (Berber et al., 2014), 15.41% in (Oksuz & Mazlum 2016). The reason of differences in protein contents in these studies can be habitat different, environmental factors and nutrient availability (Winberg, 1956). *A. leptodactylus* feeds as active from spring to early autumn (Alpbaz, 1993) therefore the protein contents in their muscles are high in summer (nutrient-rich in high temperature). The protein content in their muscles loses during starvation period in winter (Dall, 1981; Rosa & Nunes 2003). *A. leptodactylus* completes its gonad development in the autumn and mates in winter (Alpbaz, 1993). For this reason, the protein, fat and energy are transferred to the gonads when crayfish reaches maturity (Güner & Mazlum, 2010). Also, Abdu et al. (2002) determined that there is an increase in the protein composition in the gonad as a result of transfer of energy from hepatopancreas.

#### 4.2 Crude lipid contents

In the present study, no interaction was observed in crude lipid contents of tail meat of *A. leptodactylus* (Table 3). Similarly to the present study, Inanlı & Çoban (2007) and Silva-Castiglioni et al. (2007) did not determine difference between sexes. Also, Buckup et al. (2008) did not found seasonal differences in lipids contents in muscle tissue of *P. defossus*. Contrastly, Berber et al. (2014) determined seasonal differences in tail meat lipid contents of *A. leptodactylus*. Silva-Castiglioni et al. (2007) indicated the highest lipid contents were during winter in both sexes of *P. varicosus*. Buckup et al. (2008) determined differences between sexes in terms of total lipids content.

There are different reports in terms of crude lipid contents tail meat of *A. leptodactylus*; 0.57% (Harlioğlu et al., 2012), 1.25% (Oksuz & Mazlum 2016). Bilgin et al. (2008) reported the highest lipid content was 1.91% and the lowest lipid content was 1.09% in different size female crayfish. These differences may be caused by environmental factors, age, species and nutrient in environment.

#### 4.3 Fatty acid contents

##### $\Sigma$ MUFA contents

In the present study, season/sex interaction effected to  $\Sigma$ MUFA. The high  $\Sigma$ MUFA contents in female were found in the spring (19.65%) and summer (19.64%), the lowest  $\Sigma$ MUFA content was in the autumn (17.99%). High  $\Sigma$ MUFA contents in male were found in the autumn (18.35%) and summer (18.46%) and the lowest  $\Sigma$ MUFA content was found in the winter (16.99%). Also,  $\Sigma$ MUFA contents in males were found lower than female in winter, spring and summer (Table 7). Similarly, Stanek et al. (2013) indicated that  $\Sigma$ MUFA contents were higher in female (29.26%) than male (26.61%) in tail muscle of *O. limosus* from Lake Goplo (Polond). Berber et al. (2014) reported that  $\Sigma$ MUFA reached maximum levels in April and May while stayed as normal in the other months. In contrast, Stanek et al. (2011) reported that

$\Sigma$  MUFA contents were similar between spring and summer in tail muscle of *O. limosus* from Lake Goplo (Polond). The reason of this difference may be that two season only were researched this study. Harlioğlu et al. (2012) detected  $\Sigma$ MUFA content as 28.17% in tail muscle of *A. leptodactylus*. Oksuz & Mazlum (2016) indicated as 24.60%  $\Sigma$ MUFA content in tail muscle of *A. leptodactylus*. Contrary to our results,  $\Sigma$ MUFA contents were high in previous studies.

##### $\Sigma$ PUFA, $\Sigma$ SFA, ARA, EPA and DHA contents

In this study, only seasonal differences were determined for  $\Sigma$ SFA,  $\Sigma$ PUFA, ARA, EPA and DHA. The highest  $\Sigma$ PUFA content (34.91%) was in the winter while the lowest content (30.63%) was in autumn. Also,  $\Sigma$ PUFA contents were higher than  $\Sigma$ MUFA,  $\Sigma$ SFA and  $\Sigma$ HUFA. Similarly the present study, Berber et al. (2014) defined that  $\Sigma$ PUFA content in the muscle was higher than  $\Sigma$ HUFA and  $\Sigma$ SFA but  $\Sigma$ PUFA content detected higher in female than that of male in tail meat of *A. leptodactylus* (Table 5). Similarly, Stanek et al. (2013) indicated no differences between sexes in  $\Sigma$ PUFA contents, however  $\Sigma$ PUFA contents were found similar between spring and summer. Harlioğlu et al. (2012) and Oksuz & Mazlum (2016) determined higher  $\Sigma$ PUFA (45.97%, 53.52% respectively) than  $\Sigma$ SFA and  $\Sigma$ MUFA. Also, they detected higher  $\Sigma$ PUFA contents than that of the present study in tail muscle of *A. leptodactylus*.

The highest  $\Sigma$ SFA content (22.14%) was found in the autumn in this study (Table 5). Unlike for the present study, Stanek et al. (2011) indicated that there were no difference in SFA contents between spring and summer and SFA contents were difference in between female (22.27%) and male (23.56%) in tail muscle of *O. limosus*. The cause of this situation may be species and habitat differences. Similarly, Oksuz & Mazlum (2016) determined SFA content as 21.32% in tail muscle *A. leptodactylus* caught from Egirdir Lake. Harlioğlu et al. (2012) reported SFA content as 25.56% in tail muscle of *A. leptodactylus* caught from Lake Keban Dam.

In the present study, the highest ARA content (13.42%) was found in the winter and the lowest content (9.70%) was determined in the summer. The highest EPA content (14.27%) was found in the summer and the lowest level (10.11%) was determined in the autumn (Table 5). The high DHA's were observed in the spring (5.34%) and summer (5.40%). The lowest DHA content was determined in the autumn (% 4.43) (Table 5). Similarly, Bottino et al. (1980) reported that ARA content in shrimps decrease in the summer when the water temperature rises. Berber et al. (2014) determined seasonal differences in EPA contents in the female, in DHA contents in the male. Berber et al. (2014) signed that the highest EPA content in the female in November, the lowest in June, the highest DHA content in the male in January –March, the lowest contents DHA content in November-May. They explained this situation with reproductive and gonadal development period of the female, indicated that reproductive was affected directly by DHA content. Whereas, in the present study, both of EPA and DHA contents was low in tail meat of both of male and female in reproductive season. Unlike the present study, Stanek et al. (2011) did not determine differences in point of EPA, ARA, in tail muscle of *O. limosus*

between spring and summer. Bilgin et al. (2008) did not observe differences sexes in DHA contents while Stanek et al. (2013) observed differences sexes.

#### LOA and $\alpha$ -LNA contents

In current study, differences among seasonal and sex were determined on C18:2n6 linoleic acid and C18:3n3 linolenic acid. The highest linoleic acid content (3.87%) and linolenic acid content (1.14%) were found in the autumn and linoleic acid contents were higher than linolenic acid contents. Also, linoleic and linolenic acid contents were higher in female than male. Similarly, Castell (1983) and Chanmugam et al. (1983) reported that linoleic acid contents were higher than linolenic acid contents in freshwater crustaceans. On the contrary, Berber et al. (2014) did not determine differences among seasons in linoleic acid and linolenic acid contents. Stanek et al. (2013) and Bilgin et al. (2008) were not observed differences sexes (Table 6).

## 5 Conclusion

The protein content in tail muscle of *A. leptodactylus* decreases by gonadal development in autumn but increases by feeding in summer. This decreasing continues in food deprivation (winter). However, the lipid contents didn't show seasonal difference. It may be that this species do not prefer fatty foods. DHA, EPA and n-3 contents in tail muscle of *A. leptodactylus* increase by feeding in summer while these contents decrease by gonadal development in autumn. Therefore, EPA, n-3 and DHA contents are important for *A. leptodactylus*. According to results of study, the female brood stocks in culture are feed diets with high protein, EPA, DHA and n-3 contents. In addition, nutritional contents in terms of protein, EPA, DHA and n-3 of this species are higher in summer.

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