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RESEARCH PAPER

Seasonal Changes in Proximate Composition and Mineral-Heavy Metal Content of Pufferfish (*Lagocephalus sceleratus*) from Northeastern Mediterranean Sea

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Abstract

In this study, it was aimed to determine the chemical composition of the pufferfish (*Lagocephalus sceleratus*) caught from Mersin Bay in the Northeastern Mediterranean Sea. For this purpose, the proximate composition, fatty acid profile, mineral and heavy metal levels were investigated. Pufferfish samples were caught using trawl fishing technique over four seasons between December 2012 and October 2013. As a result of the study, the average protein, lipid, water and total mineral substance (TMS) levels for female individuals were determined to be 20.44%, 0.65%, 76.98% and 1.43% respectively; while for male individuals these were determined to be 20.58%, 0.84%, 76.68% and 1.40%. In the fatty acid composition of *L. sceleratus*; total saturated fatty acids (SFA) level was determined to be between 24.78% and 29.75%, total monounsaturated fatty acid (MUFA) level was 15.41% to 23.20%, total polyunsaturated fatty acid (PUFA) level was 39.90% to 45.70%. Macro, trace element and toxic heavy metal levels in pufferfish muscle tissue were determined using ICP-MS. The relationship between the amounts of macro and trace elements in *L. sceleratus* was determined to be K>P>Na>Mg>Ca and Zn>Se>Cu>Ni>Mo>Co for both sexes. In the muscle tissues of pufferfish, Cd levels were found to be over limit levels.

Keywords: Pufferfish, *Lagocephalus sceleratus,* chemical composition, Northeastern Mediterranean.

Introduction

The relationship between food contaminated with natural toxins and toxicity has been determined by humanity over the centuries by trial and error or cause and effect relationships (Iverson & Truelove, 1994). Poisonous fishes were recognised by humanity since ancient times using trial and error, and numerous scientific researches on the subject were performed especially in countries around oceans where poisonous fishes are widespread from the beginning of the 20th century. However, Mediterranean, lacking familiarity with poisonous fishes these countries have, has seen an increased immigration of species known to be toxic with the increasing alien species immigration in recent years (Köşker, Özoğul, Ayas, Durmuş, & Uçar, 2015). Species that immigrate to the Mediterranean have the potential to negatively affect both the ecosystem and local species and fishing, and human health. Alien species, with the increase in their numbers in recent years, are having important ecological and economical effects especially in the Eastern Mediterranean (Galil & Zenetos, 2002). Pufferfish species with their high adaptation capability have significant negative effects both on local species and economically valuable fish species for the fishing industry. In addition, they pose a significant danger to public health due to the toxin they contain. Pufferfish, which has been appearing in print and visual media often in countries around the Mediterranean Sea, are known as fugu, pufferfish, puffers, blowfish, bubble fish, globefish, swellfish, toadfish and balloon fish around the globe (Köşker *et al.,* 2015). Pufferfish belong to two families, Tetraodontidae and Diodontidae. Members of both families are known as pufferfish due to their ability to inflate the front ventral area of their bodies for self defense. The pufferfish that are seen in increasing numbers in Mediterranean Sea shores mostly belong to species from the Tetraodontidae family. The major reason for the interest in fish species belonging to the Tetraodontidae family is the very effective neurotoxin known as TTX that is present in most species belonging to this family (Halstead, 1958; Shipp, 2002; Nelson, 2006; Noguchi & Arakawa, 2008).

L. sceleratus stands out among other Tetraodontidae species with its spreading speed, and negative effects on native species, fishing and public health (Streftaris & Zenetos, 2006; Bilecenoglu, 2010;

Aydın, 2011). *L. sceleratus*, the most prevalent pufferfish species with regards to biomass in Eastern Mediterranean, is a fish species that attracted significant attention from fishermen, people living in coastal areas and even print and visual media in Mediterranean countries due to its ability to reach a length of 110 cm and a weight of 7 kg (Nader, Indary, & Boustany, 2012). In early stages it had even found a place for itself in fish vendors (Aydın, 2011). However, due to the toxin it contains, its fishing and sale was banned by both the Ministry of Food, Agriculture and Livestock in Turkey (Anonymus, 2012); and the European Union (EC, 2004a; EC, 2004b). Pufferfish are subject to attention due to their spreading speed and adaptation capability, and they pose a potential threat to economical species as a top predator.

Pufferfish, known to be toxic for a long time, is still popular in many Far Eastern countries including Japan due to their traditional status despite the risk of death they carry. The number of poisonings and fatalities have decreased due to the precautions taken by governments and the increase in the agriculture of pufferfish. Especially in Japan, the decrease in poisoning cases is due to the place pufferfish has found in the eating habits than any precautions taken. Despite all precautions, poisoning cases continue to occur, even if at low levels (Hwang & Noguchi, 2007).

TTX levels in the meat can be reduced by applying some methods in areas where pufferfish are consumed (Hwang & Noguchi, 2007; Anraku *et al*., 2013). In Taiwan, various treatments are carried out to mix pufferfish and other non-toxic fish to make fish sauce and fish ball. With these procedures (80% of the toxin can be removed from the muscle tissue by treating it with sodium chloride (NaCl, salt) and keeping it at 4°C for 24 hours), the TTX level decrease below the toxic limit (Hwang & Noguchi, 2007). A similar method is a traditional salting and fermentation method applied in Japan. With this method, TTX levels can be reduced to 2-10% (Anraku *et al*., 2013). Pufferfish have been seen intensely on the Mediterranean coast for the last decade and populations are increasing day by day. Despite the short time when *L. sceleratus* has been present in the Mediterranean, there are already studies (Katikou, Georgantelis, Sinouris, Petsi, & Fotaras, 2009; Rodriguez *et al.*, 2012; Kosker *et al.*, 2016; Acar, Ishizaki, & Nagashima, 2017) performed on the TTX content of the fish. Even though the studies show that pufferfish are toxic in general; some samples studied show that muscle tissue is below the threshold for toxicity (Rodriguez *et al*., 2012; Köşker *et al.*, 2016). Although there are more toxicity work in literature, the relationship between pufferfish toxicity and seasons, sexes, individual size and ecological differences can be determined and a better idea about the consumability of this fish can be obtained. This study looks into the effects of sex, season and individual differences on chemical characteristics of pufferfish living in the Mersin Bay.

Materials and Methods

Fish Collection, Measurements and Identification

As there is a ban on fishing *L. sceleratus* species of fish and bringing them to land (Anonymus, 2012), necessary permissions were obtained from local authorities. *L. sceleratus* were caught using trawl fishing, from winter in 2012 to autumn in 2013 for four seasons in Mersin Bay (Figure 1). The samples were caught in December 2012 for winter season, May 2013 for spring season, July 2013 for summer season and October 2013 for autumn season. At the time of catch, fish were immediately stored in ice. After that, no longer than 4 h, they were transferred to the Seafood Processing Technology Laboratory of the Faculty of Fisheries, Çukurova University. Fish were

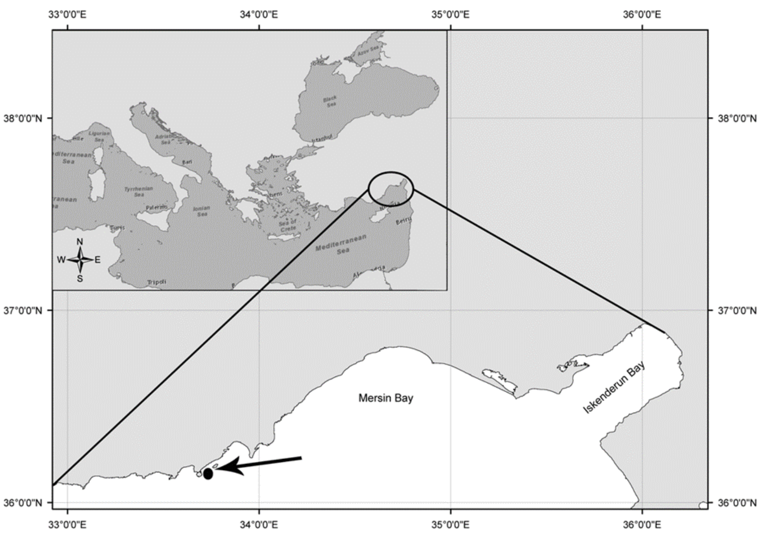

Figure 1. Map of the sampling location. (The marked area is the sampling area).

grouped according to sex and season and their size (cm) and weight (g) were measured. The weight and length of the individual fish ranged from 947 to 4128 g and 43 to 70 cm, respectively (Table 1). Samples were stored in a freezer at -20°C until analysis.

Proximate Analyses

AOAC (1984) method for the TMS (total mineral substance) measurements and water content of pufferfish were used. Total crude protein was measured by Kjeldahl method (AOAC, 1998), and lipid analysis was performed using Bligh and Dyer (1959) method.

Fatty Acids Analyses

In extracted lipids, fatty acid methyl esters were obtained using the Ichibara *et al.* (1996) method. Fatty acid composition was analysed using a Gas Chromatography (GC) Clarus 500 device (Perkin–Elmer, USA), one flame ionization detector (FID) and SGE (60 m x 0.32 mm ID BPX70 x 0.25 µm, USA or Australia) column. Injector and detector temperatures were set as 260°C and 230°C, respectively. During this time, the furnace temperature was kept at 140°C for 8 minutes. After that, it was increased by 4°C per minute until 220°C, and from 220°C to 230°C by increasing the temperature 1°C per minute. It was kept at 230°C for 15 minutes to complete analysis. Sample scale was 1 µl and carrier gas was controlled at 16 ps. For split flow 40, 0 mL/minute (1:40) level was used. Fatty acids were determined using a comparison to the exit times of the FAME mix that contains 37 standard components.

Metal Analyses

The samples (0.1 g dry weight) used for metal analysis were dried at 105°C to reach constant weights, and then concentrated nitric acid (4 mL, Merck, Darmstadt, Germany) and percholoric acid (2 mL, Merck, Darmstadt, Germany) were added to the samples, and they were put on a hot plate set to 150°C until all tissues were dissolved (Canli & Atli, 2003).

Inductively coupled plasma mass spectrometer (ICP-MS, Agilent, 7500ce Model, Japan) was used to determine metals. ICP-MS operating conditions were the following: radio frequency (RF) (W), 1500; plasma gas flow rate (L min-1), 15; auxiliary gas flow rate (L min-1), 1; carrier gas flow rate (L min-1), 1.1; spray chamber T (°C), 2; sample depth (mm), 8.6; sample introduction flow rate (mL min-1), 1; nebuliser pump (rps), 0.1; extract lens (V), 1.5. The levels of macro (Na, Mg, P, K, Ca), trace element (Co, Cu, Zn, Mo, Ni, Se) and, potential toxic metal (Cd, Pb) in samples were detected as µg metal g-1 dry weight. High Purity Multi Standard (Charleston, SC 29423) was used for determination of the metal analyses. Standard solutions for calibleveln curves were prepared by dilutions of the macroand trace elements and potential toxic metals. Solutions have prepared for the toxic metals had a content of lead, cadmium, arsenic and cromium in the range of 1-50 ppb (0.001 to 0.050 mg/L), for the macro and trace elements had a content of copper, iron, and zinc in the range of 1-50 ppm (1 to 50 mg/L).

Metal analysis were conducted with stable samples with fixed weighing (dw). Mathematical transformation of wet weight (ww) was carried out by using the percent dry matter value in order to compare the levels of potential toxic metals with the toxic limits of food codex.

Statistical Analyzes

All experiments were carried out in triplicate and the results were reported as the mean and standard deviation of these measurements. A one-way analysis of variance (ANOVA) was run using the SPSS version 21 software (SPSS, Chicago, Illinois, USA) and the Duncan’s multiple range test comparisons at P value of <0.05 were run to determine significant differences.

Table 1. Seasonally length and weight measurement of pufferfish samples

Season	Sample	Length (cm)	Weight (g)
Winter	♂ 1	67.0	3520.22
	♂ 2	53.6	1879.12
	♀ 1	70.0	4128.17
	♀ 2	52.5	1649.13
Spring	♂ 1	65.4	3380.79
	♂ 2	47.0	1350.64
	♀ 1	59.4	2100.23
	♀ 2	43.0	947.26
Summer	♂ 1	50.5	1647.42
	♂ 2	48.0	1388.42
	♀ 1	68.0	2791.88
	♀ 2	52.5	1795.87
Autumn	♂ 1	58.2	2145.03
	♂ 2	52.7	1754.29
	♀ 1	58.3	2507.25
	♀ 2	57.6	2203.15

♂: male, ♀: female.

Results

Proximate Composition of *Lagocephalus sceleratus*

Data obtained through the chemical composition analysis performed on the muscle tissue of *L. sceleratus* individuals caught in each season from both sexes with two individuals from each sex, were given in Table 2. As a result of the study, the average protein, lipid, water and TMS levels for female individuals were determined to be 20.44%, 0.65%, 76.98% and 1.43% respectively; while for male individuals these were determined to be 20.58%, 0.84%, 76.68% and 1.40%. No statistical changes were observed based on season and sex in TMS levels (P>0.05). While statistical differences were observed based on season in protein levels (P<0.05), no differences were observed based on sex (P>0.05).

In the winter, the highest protein content was found as 22.50% in male individuals. In the summer, autumn and spring, the protein levels were determined as 20.76%; 20.22% and 19.25%, respectively. In female individuals, the highest protein content was determined as 22.87% in the winter while lowest protein level was found as 18.80% in spring.

Differences were observed in protein, lipid and water levels between seasons and sexes (P<0.05), but no difference was observed in TMS levels. It is concluded that these differences are caused by the breeding and feeding cycle of the fish. Aydın, Tufan, Sevgili and Köse (2013), studied the proximate composition of pufferfish caught from Antalya Bay and reported that the protein levels in male individuals are higher in autumn (20.75-21.69%) and spring (19.65-21.10%) and lower in winter (19.98-20.98%) and summer (19.24- 20.88%). In this study, the highest protein level in male individuals was found in winter. Protein content is very important for the positive effect fish has on human health (Sidhu, 2003).

Lipid value of females did not demonstrate any seasonal variations (P>0.05) statistically and was found between 0.66% and 1.07%. The highest lipid value in female individuals was found as 0.87% in spring. In summer, winter and autumn, the lipid levels were measured as 0.71%, 0.58% and 0.44% respectively. In all seasons, no statistically significant differences were determined between lipid contents of male and female individuals (P>0.05). Makoto, Yoshimichi, Fumio, and Shingo (2000) had studied the lipid content of 25 different pufferfish species. The lipid content of *L. sceleratus* was determined as 1.42%. Nurullahoglu and Ulusoy (2013) studied the lipid level of *L. sceleratus* individuals caught in June and July in Antalya Bay and determined it as 1.82%. Aydın *et al.* (2013) again determined the lipid level of fish caught in Antalya Bay all year round as between 0.21-0.33. These researchers have noted that lipid levels varied between seasons. The differences between the results of present study and these studies are thought to arise from the difference in locations the fish are caught. Özogul, Özogul, Çiçek, Polat, and Kuley (2009) determined that the lipid value of *L. agocephalus* was 0.78%. Ghosh, Hazra, Banerjee, and Mukherjee (2005) reported the lipid level of *L. lunaris* was 0.93%. Tao, Wang, Gong, and Liu (2012) studied the proximate composition of aquaculture grown individuals from three pufferfish species (*Fugu obscurus, Fugu flavidus* and *Fugu rupribes*) bred in China; the lipid level in 100 g muscle tissue was reported to be within the range of 0.73-0.83. Koizumi and Hiratsuka (2009) reported lipid levels of individuals belonging to the *Takifugu rubripes*species caught in Japanese waters as 1.01%, 0.87% and 0.92% for winter, summer and autumn respectively. Results acquired from this study are similar to the results of the preset study.

Water contents were determined statistical differences (P<0.05) in the both male and female individuals according to seasons. In males, water levels were determined to be higher in spring and autumn. Water rates were measured as; 75.36%, 78.01%, 75.99% and 77.35% for winter, Spring, summer, and autumn, respectively. In females, the water content was found as 78.65%, 77.52% and 77.49% for summer, autumn and spring respectively, while the lowest water level was found as 74.23% in winter. No statistically significant difference was seen between water contents of male and female pufferfish in any seasons (P>0.05). Aydın et al. (2013) reported

Table 2. Sexual and seasonal proximate composition in the pufferfish muscle

	Sex	Winter $\bar{X} \pm S_X$	Spring $\bar{X} \pm S_X$	Summer $\bar{X} \pm S_X$	Autumn $\bar{X} \pm S_X$
Protein	♂	22.05±0.1^{a,x}	19.25±0.21^{c,x}	20.76±0.38^{b,x}	20.22±0.37^{b,x}
	♀	22.87±0.84^{a,x}	19.69±0.15^{c,x}	18.80±1.59^{c,x}	20.39±0.04^{ab,x}
Lipid	♂	0.66±0.22^{a,x}	0.94±0.09^{a,x}	1.07±0.18^{a,x}	0.69±0.06^{a,x}
	♀	0.58±0.01^{bc,x}	0.87±0.03^{a,x}	0.71±0.07^{b,x}	0.44±0.06^{c,x}
Water	♂	75.36±0.08^{b,x}	78.01±0.59^{a,x}	75.99±0.43^{b,x}	77.35±0.56^{a,x}
	♀	74.23±0.68^{c,x}	77.49±0.28^{ab,x}	78.65±2.23^{a,x}	77.52±0.04^{ab,x}
TMS	♂	1.39±0.04^{a,x}	1.37±0.11^{a,x}	1.38±0.11^{a,x}	1.48±0.08^{a,x}
	♀	1.48±0.11^{a,x}	1.4±0.01^{a,x}	1.36±.011^{a,x}	1.47±0.08^{a,x}

Different letters (a,b, c) in the same row indicate significant difference among seasons (P<0.05). Different letters (x, y) in the same column indicate significant difference between the different genders in the same season (P<0.05). TMS: Total mineral substance. ♂: male, ♀: female. $\bar{X} \pm S_X$: Average±Standard deviation.

that the water content of *L. sceleratus* was 76.32-78.45% for females and between 76.49-78.49% for males. Even though these results are similar to present study for spring, summer and autumn, the water rates measured in this study is lower in winter. In studies performed with different pufferfish species other than *L. sceleratus* the water value were reported as between 76.9-78.0% for three different Fugu species by Tao et al. (2012), and between 79.1-80.6% for *Takifugu rubripes* by Hwang, Chen, Shiau, & Jeng (2000). These water content matches with the results in present study.

TMS analysis has demonstrated that there was not statistical difference in pufferfish depending on both sexes and seasons (P>0.05). Average TMS levels in males and females were measured as 1.43% and %1.4 respectively. TMS levels measured by Aydın *et al.* (2013) in fish caught in Antalya Bay year round are similar to the results acquired in this study. Tao *et al.* (2012), studying the TMS levels in different pufferfish species, found that the TMS levels of three different Fugu species were in the range of 1.47-1.54%. Eswar *et al.* (2014) reported the TMS level of pufferfish belonging to the species *Lagocephalus inermis* as 1.27%. These results match with the results of present study.

Fatty Acid Composition of *Lagocephalus sceleratus*

In the fatty acid composition of samples are given in Table 3. Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) percentages of the total lipid varied between 24.78% and 29.75%, 15.41% and 23.20%, 39.90% and 45.70%, respectively. It was determined that the fatty acids found in highest amounts in male and female were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n9), vaccenic acid (C18:1n7), linoleic acid (C18:2n6), arachidonic acid (C20:4n6), docosadienoic acid (C22:2 cis), eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3).

The SFA with the highest levels measured in the study were stearic acid and palmitic acid. It was determined that the SFA levels in male specimens vary between seasons (P<0.05). Highest SFA level was measured as 29.75% in winter. No statistical differences were observed among spring (28.10%), summer (28.00%), and autumn (28.09%), respectively. SFA levels in female specimens demonstrated statistically significant differences according to seasons (P<0.05). Highest SFA level of female was found as 29.69% in autumn. With regards to SFA levels, only statistically significant difference between male and female individuals caught in the same season was observed in only winter and autumn (P<0.05), and whereas no statistical difference were found in summer and spring (P>0.05). SFA levels for the species *L. sceleratus* was reported by Makoto *et al.* (2000) as 28.80% for fish caught in autumn in Japan; and by Aydın *et al.* (2013) as 24.22-29.45% for fish caught in Antalya Bay. Özogul *et al.* (2009) reported the SFA level for the species *L. lagocephalus* was 33.41%; while Koizumi and Hiratsuka (2009) reported the SFA level for *T. rubripes* caught in Japan was 34.7%.

In the present study, the MUFA levels were examined for male individuals in winter, summer, spring and autumn, they were found 22.86%, 23.20%, 15.41%, and 16.72% (Table 3.), respectively. MUFA levels were found statistical differences according to seasons (P<0.05). Among MUFA, oleic acid, vaccenic acid and palmitoleic acid were predominant fatty acids. In the present study, MUFA levels in the summer and winter were higher than the other seasons. Statistically significant differences were observed between MUFA levels in female fish as well (P<0.05). In winter, summer, autumn and spring, they were found as 22.90%, 19.02%, 18.30% and 17.46% respectively. Makoto *et al.* (2000) reported the MUFA levels of *L. sceleratus* caught in Japan was 18.51% in autumn, and Aydın *et al.* (2013) reported the MUFA level of *L. scelaratus* caught in the Antalya Bay was 7.87-13.94%. The MUFA levels in the studies conducted by Nurullahoglu and Ulusoy (2013) and Makoto et al. (2000) were similar to the date found in the present study. However, the MUFA levels reported by Aydın *et al.* (2013) were lower than the levels found in the present study. Özogul *et al.* (2009) reported a level of 15.89% in pufferfish belonging to the species *Lagocephalus lagocephalus* caught in the Mediterranean; while Koizumi and Hiratsuka (2009) reported a level of 14.7% for pufferfish of the species *T. rubripes* caught in Japan. The difference between present study and the aforementioned studies was thought to arise from species differences. With regards to MUFA levels, no difference was observed between male and female individuals caught in the same season in winter (P>0.05), while statistically significant differences were observed for spring, summer and autumn (P<0.05).

PUFA levels measured in the study are given in Table 2. PUFA levels of male individuals demonstrated statistical differences to seasons, and the highest PUFA levels were detected in spring and autumn as 45.70% and 44.36% respectively. No statistically significant differences were observed in female individuals according to seasons. In these individuals, PUFA levels of female in winter, spring, summer and autumn were found 42.95%, 43.79%, 42.43%, and 41.22%, respectively. Statistically significant differences were observed between PUFA levels of male and female in winter and autumn (P<0.05), while in spring and summer no statistically significant difference was observed (P>0.05). As a result of the study, the PUFA measured at highest levels were linoleic acid, arachidonic acid, docosadienoic acid, EPA and DHA. Similarly, to the results of present study, Aydın et al. (2013) reported PUFA levels within the range of 35.36-42.44% in *L.*

Table 3. Sexual and seasonal fatty acid composition in the pufferfish muscle (%)

Fatty acid	Winter $\bar{X} \pm S_x$	Spring $\bar{X} \pm S_x$	Summer $\bar{X} \pm S_x$	Autumn $\bar{X} \pm S_x$	Sex
Saturated fatty acid (SFA)					
C12	0.20±0.25 ^{a,x}	0.58±0.07^{c,x}	0.13±0.02^{b,x}	0.08±0.01^{c,x}	♂
	0.15±0.01^{a,y}	0.06±0.01^{c,x}	0.08±0.01^{b,y}	0.08±0.03^{c,y}	♀
C14	0.855±0.13^{a,x}	0.38±0.45^{d,x}	0.73±0.08^{b,x}	0.49±0.05^{c,x}	♂
	0.6±0.07^{a,y}	0.47±0.02^{b,y}	0.54±0.05^{a,y}	0.54±0.07^{a,x}	♀
C15	0.12±0.15^{c,x}	0.15±0.1^{b,x}	0.14±0.01^{bc,x}	0.2±0.02^{a,x}	♂
	0.1±0.01^{c,y}	0.14±0.01^{b,x}	0.13±0.01^{b,x}	0.16±0.01^{a,y}	♀
C16	11.5±1.2^{b,x}	13.11±0.26^{a,x}	13.11±0.58^{a,x}	13.07±1.08^{a,x}	♂
	8.33±0.38^{c,y}	12.61±1.05^{b,x}	12.34±0.38^{b,y}	12.11±2.56^{a,y}	♀
C17	0.52±0.07^{c,x}	0.79±0.08^{a,x}	0.55±0.05^{c,x}	0.66±0.08^{b,x}	♂
	0.45±0.05^{c,x}	0.61±0.07^{b,y}	0.61±0.02^{b,y}	0.69±0.05^{a,x}	♀
C18	15.99±1.5^{a,x}	13.24±0.37^{b,x}	12.82±0.88^{b,x}	13.18±1.64^{b,x}	♂
	14.69±1.21^{a,x}	12.35±0.96^{b,x}	12.92±0.98^{b,x}	12.57±0.69^{b,x}	♀
C20	0.54±0.08^{a,x}	0.35±0.05^{b,x}	0.51±0.04^{a,x}	0.41±0.03^{b,x}	♂
	0.47±0.03^{a,x}	0.47±0.06^{a,y}	0.4±0.02^{b,y}	0.4±0.05^{b,x}	♀
∑ SFA	29.75±2.08^{a,x}	28.10±0.51^{b,x}	28.00±1.6^{b,x}	28.09±0.32^{b,x}	♂
	24.78±1.7^{c,y}	26.71±1.76^{b,x}	27.02±0.68^{b,x}	29.69±0.59^{a,y}	♀
Monounsaturated fatty acid (MUFA)					
C16:1	1.72±0.17^{a,x}	1.55±0.11^{b,x}	1.74±0.07^{a,x}	1.67±0.14^{a,b,x}	♂
	1.3±0.13^{c,y}	1.21±0.15^{c,y}	1.59±0.16^{b,y}	1.96±0.11^{a,y}	♀
C17:1	0.29±0.04^{b,x}	0.27±0.03^{b,c,x}	0.25±0.02^{c,x}	0.4±0.03^{a,x}	♂
	0.26±0.17^{c,x}	0.31±0.04^{b,x}	0.28±0.16^{b,c,y}	0.39±0.03^{a,x}	♀
C18:1n9	20.2±1.16^{a,x}	10.15±0.62^{c,x}	16.38±1.30^{b,x}	11.0±0.57^{c,x}	♂
	16.04±0.51^{a,y}	12.36±0.5^{b,y}	12.92±0.82^{b,y}	12.48±0.97^{b,y}	♀
C18:1n7	ND	3.05±0.36^{b,x}	4.24±0.45^{a,x}	3.15±0.56^{b,x}	♂
	4.77±0.31^{a}	3.27±0.27^{c,x}	3.83±0.33^{b,x}	3.01±0.31^{c,x}	♀
C20:1	0.64±0.05^{a,x}	0.38±0.02^{d,x}	0.45±0.05^{c,x}	0.51±0.03^{b,x}	♂
	0.53±0.04^{a,y}	0.32±0.03^{c,y}	0.34±0.03^{c,y}	0.47±0.04^{b,x}	♀
C24:1	ND	ND	0.14±0.01^{a,x}	ND	♂
	ND	ND	0.07±0.01^{a,y}	ND	♀
∑ MUFA	22.86±1.24^{a,x}	15.41±0.62^{b,x}	23.20±1.59^{a,x}	16.72±0.82^{b,x}	♂
	22.90±0.65^{a,x}	17.46±0.57^{a,y}	19.02±0.1^{b,y}	18.30±1.22^{ab,y}	♀
Polyunsaturated fatty acid (MUFA)					
C18:2n6	5.24±0.42^{b,x}	1.95±0.14^{c,x}	5.71±0.49^{a,x}	1.92±0.12^{c,x}	♂
	3.63±0.37^{a,y}	2.18±0.21^{b,y}	2.26±0.23^{b,y}	2.11±0.19^{b,x}	♀
C18:3n6	0.28±0.03^{a,x}	0.10±0.01^{c,x}	0.19±0.01^{b,x}	0.1±0.01^{c,x}	♂
	0.14±0.01^{a,y}	0.10±0.01^{c,x}	0.11±0.01^{c,y}	0.12±0.01^{b,y}	♀
C18:3n3	0.18±0.016^{a,x}	0.19±0.03^{b,x}	0.22±0.03^{b,x}	0.19±0.01^{b,x}	♂
	0.17±0.02^{b,x}	0.25±0.02^{a,y}	0.24±0.02^{a,x}	0.14±0.01^{c,y}	♀
C20:3 n6	0.11±0.02^{b,x}	0.09±0.01^{b,x}	0.15±0.01^{a,x}	0.05±0.03^{c,x}	♂
	0.12±0.01^{a,b,x}	0.11±0.01^{b,y}	0.13±0.01^{a,y}	0.11±0.01^{b,y}	♀
C20:4 n6	7.12±0.31^{c,x}	10.53±0.40^{b,x}	7.72±0.58^{c,x}	13.06±1.3^{a,x}	♂
	8.93±0.57^{b,y}	9.52±0.89^{a,b,y}	8.6±0.73^{b,y}	9.96±0.73^{a,y}	♀
C20:5 n3	1.47±0.17^{b,x}	2.08±0.10^{a,x}	1.51±0.12^{b,x}	1.6±0.52^{b,x}	♂
(EPA)	1.76±0.1^{c,y}	2.11±0.18^{b,x}	2.51±0.24^{a,y}	1.82±0.16^{c,y}	♀
C22:2 cis	2.53±0.28^{d,x}	3.58±0.15^{b,x}	3.14±0.2^{c,x}	4.96±0.43^{a,x}	♂
	2.97±0.28^{c,y}	3.37±0.21^{b,x}	3.03±0.08^{c,x}	4.42±0.19^{a,y}	♀
C22:6 n3	21.96±1.92^{b,x}	27.15±0.9^{a,x}	22.39±1.8^{b,x}	22.47±1.21^{b,x}	♂
(DHA)	25.23±1.7^{a,y}	26.15±2.2^{a,x}	25.55±0.7^{a,y}	24.87±2.09^{b,x}	♀
∑ PUFA	39.90±2.27^{c,x}	45.70±1.10^{a,x}	41.05±1.66^{b,x}	44.36±0.40^{a,x}	♂
	42.95±1.99 ^{a,y}	43.79±3.29 ^{a,x}	42.43±0.56^{a,x}	41.22±2.12^{a,y}	♀
∑ n6	12.75±0.61 ^{c,x}	12.69±0.30 ^{c,x}	13.79±0.38 ^{b,x}	15.14±1.26 ^{a,x}	♂
	12.82±0.49^{a,x}	11.91±0.97^{ab,x}	11.09±0.60^{b,y}	12.30±0.72^{a,y}	♀
∑ n3	23.61±2.06^{b,x}	29.43±0.89^{a,x}	24.13±1.76^{b,x}	24.26±1.23^{b,x}	♂
	27.17±1.73^{a,y}	28.502.27^{a,x}	28.30±0.70^{a,y}	24.49±1.64^{b,x}	♀
n6/n3	0.54±0.06^{b,x}	0.43±0.01^{c,x}	0.57±0.05^{ab,x}	0.63±0.08^{a,x}	♂
	0.47±0.03^{a,y}	0.42±0.02^{b,x}	0.39±0.03^{b,y}	0.50±0.04^{a,y}	♀
DHA/EPA	14.99±1.27^{a,x}	13.08±0.88^{b,x}	14.91±1.98^{a,x}	14.01±0.79^{b,x}	♂
	14.33±1.17^{a,x}	12.45±1.21^{b,x}	10.25±1.16^{c,y}	12.50±1.54^{b,x}	♀

Different letters (a,b, c) in the same row indicate significant difference among seasons ($P\leq0.05$). Different letters (x, y) in the same column indicate significant difference between the different genders in the same season ($P<0.05$). ♂: male, ♀: female. $\bar{X} \pm S_x$: Average±Standard deviation ND: not detected.

sceleratus caught in Antalya Bay. The ratio of n6/n3 ranged from 0.39% to 0.63% and DHA/EPA levels varied between 10.25% and 14.99%.

Element Composition of *L. sceleratus*

Macro (Na, Mg, P, K, Ca) and trace element (Cu, Zn, Mo, Se) levels of the muscle tissue of pufferfish determined using the ICP/MS method were shown in Table 4. The relationship between the amounts of macro elements in *L. sceleratus* over four seasons was determined to be K>P>Na>Mg>Ca for both sexes (Table 4). The order of macro element levels for three pufferfish species (*Fugu obscurus,*

Table 4. Sexual and seasonal macro elements, trace and heavy metal levels in the pufferfish muscle (µg g^{-1})

Metal	Winter	Spring	Summer	Autumn	Sex	Sample
Na	2729.9±460.6^{b,x} (672.65)	2815.9±330.7^{ab,x} (619.22)	4523.3±223.0^{a,x} (1086.04)	4884.6±406.8^{c,x} (1106.36)	♂	1
	3374.5±244.4^{a,x} (831.48)	3858.4±393.0^{b,y} (848.46)	4175.4±596.9^{a,x} (1002.51)	3981.2±662.8^{c,y} (901.74)	♂	2
	2584.4±275.3^{b,x} (666.00)	3609.7±215.3^{ab,x} (812.54)	5591.3±130.5^{a,x} (1193.74)	3200.3±301.6^{c,x} (719.43)	♀	1
	2898.1±100.1^{a,x} (746.84)	4109.4±299.9^{b,y} (925.03)	4857.8±232.6^{a,x} (1037.14)	2898.1±64.1^{c,y} (651.49)	♀	2
Mg	1165.1±139.5^{ab,x} (287.08)	1040.0±143.8^{a,x} (228.70)	1287.9±57.7^{ab,x} (309.22)	1477.5±176.7^{b,x} (334.65)	♂	1
	992.6±60.3^{ab,x} (244.58)	1194.7±240.9^{a,x} (262.71)	1297.4±147.6^{ab,x} (311.51)	1397.9±261.5^{b,x} (316.62)	♂	2
	1101.3±113.3^{a,x} (283.81)	1241.2±79.5^{a,x} (279.39)	1260.5±42.4^{ab,x} (269.12)	1199.7±216.5^{b,y} (269.69)	♀	1
	967.5±32.1^{a,x} (249.32)	1350.3±180.5^{a,x} (303.95)	1362.9±151.3^{ab,x} (290.98)	1091.0±38.0^{b,y} (245.26)	♀	2
P	13084.0±2202.6^{a,x} (3223.90)	12583.0±1718.4^{a,x} (2767.00)	14691.0±556.5^{a,x} (3527.31)	20037.0±3221.0^{b,x} (4538.38)	♂	1
	9889.9±1049.1^{a,x} (2436.87)	12709.0±1649.4^{a,x} (2794.71)	14444.0±2092.3^{a,x} (3468.00)	17178.0±3281.1^{b,x} (3890.82)	♂	2
	12628.0±1555.1^{a,x} (3254.24)	10978.0±567.5^{a,x} (2471.15)	16180.0±2095.1^{a,x} (3454.43)	13572.0±1819.5^{b,x} (3050.99)	♀	1
	9829.4±557.9^{a,x} (2533.04)	18599.0±611.9a,x (4186.63)	16153.0±1275.3^{a,x} (3448.67)	11512.0±359.8^{b,x} (2587.90)	♀	2
K	20577.0±3513.8^{ab,x} (5070.17)	19542.0±2206.8^{a,x} (4297.29)	16722.0±791.9^{ab,x} (4014.95)	20560.0±2559.8^{b,x} (4656.84)	♂	1
	17372.0±1562.9^{ab,x} (4280.46)	19395.0±1139.8^{a,x} (4264.96)	20141.0±3253.7^{ab,x} (4835.85)	22290.0±3737.8^{b,x} (5048.69)	♂	2
	19527.0±2495.4^{ab,x} (3726.34)	14593.0±1325.6^{ab,x} (4010.16)	18301.0±372.9^{a,x} (4334.48)	20383.0±1620.5^{b,x} (4030.21)	♀	1
	14460.0±871.9^{ab,x} (3726.34)	17815.0±1502.9^{ab,x} (4010.16)	20302.0±1641.2^{a,x} (4334.48)	17928.0±515.5^{b,x} (4030.21)	♀	2
Ca	225.8±22.5^{a,x} (55.64)	217.9±9.5^{a,x} (47.92)	636.4.1±100.9^{a,x} (152.80)	1104.5±171.3^{b,x} (250.17)	♂	1
	233.7±12.1^{a,x} (57.58)	222.4±42.7^{a,x} (48.91)	373.4±25.6^{a,x} (89.65)	513.5±32.9^{b,x} (116.31)	♂	2
	196.7±18.9^{a,x} (50.69)	317.9±47.6^{a,x} (71.56)	768.4±177.5^{a,x} (164.05)	608.3±181.8^{b,x} (136.75)	♀	1
	304.5±14.3^{a,x} (78.47)	335.9±50.7^{a,x} (75.61)	550.3±83.9^{a,x} (117.49)	302.0±28.3^{b,x} (67.89)	♀	2
Co	0.50±0.05^{a,x} (0.12)	0.44±0.02^{a,x} (0.10)	0.52±0.03^{a,x} (0.12)	0.69±0.01^{b,y} (0.16)	♂	1
	0.54±0.05^{a,x} (0.13)	0.44±0.06^{a,x} (0.10)	0.49±0.03^{a,x} (0.12)	0.52±0.07^{a,x} (0.12)	♂	2
	0.53±0.01^{a,x} (0.14)	0.54±0.06^{a,x} (0.12)	0.60±0.05^{a,x} (0.13)	0.59±0.04^{a,x} (0.13)	♀	1
	0.51±0.03^{a,x} (0.13)	0.61±0.01^{b,x} (0.14)	0.61±0.00^{b,x} (0.13)	0.46±0.01^{a,x} (0.10)	♀	2
Cu	2.23±0.15^{ab,x} (0.55)	1.69±0.11^{a,x} (0.37)	2.29±0.13^{ab,x} (0.55)	2.47±0.11^{b,x} (0.56)	♂	1
	2.52±0.19^{a,x} (0.62)	1.90±0.19^{a,x} (0.42)	1.89±0.12^{a,x} (0.45)	2.16±0.24^{a,x} (0.49)	♂	2
	2.47±0.46^{a,x} (0.64)	2.65±0.57^{a,x} (0.60)	2.27±0.16^{a,x} (0.48)	2.38±0.26^{a,x} (0.54)	♀	1
	2.05±0.14^{a,x} (0.53)	2.27±0.28^{a,x} (0.51)	2.27±0.05^{a,x} (0.48)	2.28±0.39^{a,x} (0.51)	♀	2
Zn	30.03±0.76^{a,x} (7.40)	26.24±1.70^{a,x} (5.77)	75.73±7.88^{b,z} (18.18)	62.60±5.63^{b,z} (14.18)	♂	1
	32.44±0.96^{ab,x} (7.99)	28.06±2.72^{a,x} (6.17)	36.12±1.12^{b,x} (8.67)	52.76±4.79^{c,z} (11.95)	♂	2
	28.55±1.08^{a,x} (7.36)	54.22±3.56^{b,z} (12.20)	52.90±5.17^{b,y} (11.29)	30.99±0.37^{a,x} (6.97)	♀	1
	64.73±6.23^{b,y} (16.68)	35.84±2.22^{a,y} (8.07)	52.15±8.87^{b,y} (11.13)	47.48±1.87^{b,y} (10.67)	♀	2

Table 4. Contiuned

Metal	Winter	Spring	Summer	Autumn	Sex	Sample
Mo	1.53±0.10^{b,x} (0.38)	1.00±0.04^{a,x} (0.22)	1.49±0.09^{b,x} (0.36)	2.13±0.24^{b,y} (0.48)	♂	1
	1.33±0.07^{a,x} (0.33)	1.03±0.14^{a,x} (0.23)	1.21±0.08^{a,x} (0.29)	1.48±0.22^{a,xy} (0.34)	♂	2
	1.28±0.03^{a,x} (0.33)	1.24±0.17^{a,x} (0.28)	1.84±0.37^{a,x} (0.39)	1.41±0.09^{a,xy} (0.32)	♀	1
	1.23±0.10^{a,x} (0.32)	1.25±0.12^{a,x} (0.28)	1.54±0.06^{a,x} (0.33)	1.15±0.05^{a,x} (0.26)	♀	2
Ni	1.50±0.12^{a,y} (0.37)	1.32±0.30^{a,x} (0.29)	1.76±0.24^{a,x} (0.42)	2.44±0.35^{a,x} (0.55)	♂	1
	1.56±0.13^{a,y} (0.38)	1.27±0.24^{a,x} (0.28)	1.31±0.14^{a,x} (0.31)	1.60±0.18^{a,x} (0.36)	♂	2
	1.91±0.23^{a,y} (0.49)	1.32±0.19^{a,x} (0.30)	1.54±0.18^{a,x} (0.33)	1.48±0.17^{a,x} (0.33)	♀	1
	1.10±0.03^{a,x} (0.28)	1.42±0.15^{a,x} (0.32)	1.64±0.08^{a,x} (0.35)	1.48±0.26^{a,x} (0.33)	♀	2
Se	3.98±0.09^{c,y} (0.98)	2.81±0.08^{a,x} (0.62)	3.47±0.12^{b,y} (0.83)	3.36±0.02^{b,y} (0.76)	♂	1
	3.40±0.14^{a,xy} (0.84)	2.77±0.16^{a,x} (0.61)	3.13±0.03^{a,y} (0.75)	2.82±0.20^{a,x} (0.64)	♂	2
	3.91±0.08^{b,y} (1.01)	3.66±0.14^{ab,y} (0.82)	3.81±0.02^{b,z} (0.81)	3.20±0.24^{a,xy} (0.72)	♀	1
	3.23±0.18^{b,x} (0.83)	3.26±0.16^{b,xy} (0.73)	2.62±0.05^{a,x} (0.56)	3.25±0.09^{b,xy} (0.73)	♀	2
Cd	0.94±0.05^{b,x} (0.23)	0.73±0.03^{a,x} (0.16)	0.83±0.07^{ab,x} (0.20)	0.89±0.03^{ab,x} (0.20)	♂	1
	0.96±0.07^{a,x} (0.24)	0.75±0.09^{a,x} (0.16)	0.80±0.03^{a,x} (0.19)	0.77±0.11^{a,x} (0.17)	♂	2
	0.89±0.02^{a,x} (0.23)	0.91±0.10^{a,x} (0.20)	0.89±0.00^{a,x} (0.19)	0.90±0.06^{a,x} (0.20)	♀	1
	0.88±0.06^{a,x} (0.23)	0.86±0.08^{a,x} (0.19)	0.85±0.03^{a,x} (0.18)	0.75±0.03^{a,x} (0.17)	♀	2
Pb	0.63±0.02^{b,x} (0.16)	0.47±0.00^{a,x} (0.10)	0.68±0.05^{bc,x} (0.16)	0.88±0.12^{c,x} (0.20)	♂	1
	0.61±0.02^{ab,x} (0.15)	0.49±0.08^{a,x} (0.11)	0.62±0.04^{ab,x} (0.15)	0.74±0.03^{b,x} (0.20)	♂	2
	0.59±0.03^{a,x} (0.15)	0.62±0.11^{a,x} (0.14)	0.68±0.04^{a,x} (0.15)	0.91±0.09^{b,x} (0.20)	♀	1
	0.62±0.07^{a,x} (0.16)	0.65±0.11^{a,x} (0.15)	0.76±0.05^{ab,x} (0.16)	0.88±0.09^{b,x} (0.20)	♀	2

Different letters (a,b, c) in the same row indicate significant difference among seasons (P<0.05). Different letters (x, y) in the same column indicate significant difference between the different genders in the same season (P<0.05). ♂: male, ♀: female. $\bar{X} \pm S_X$:Average±Standard deviation. Mathematical transformation of wet weight (ww) was carried out by using the percent dry matter value. Wet weight values are given in parentheses.

Fugu flavidus and *Fugu rupribes*) were reported similarly in a study by Tao *et al*. (2012) as well. In male individuals, the highest macro element levels were found in autumn, while the highest macro element levels of females were found in summer. However, the highest P level in females was measured in spring, and highest K levels were measured in autumn. Maximum K, P, Na, Mg, and Ca levels in both sexes of pufferfish around the year were 22290.00 (µg g^{-1}, dw), 20037.00 (µg g^{-1}, dw), 5591.30 (µg g^{-1}, dw), 1477.50 (µg g^{-1}, dw) and 1104.50 (µg g^{-1}, dw), respectively.

The relationship between the amounts of trace elements in *L. sceleratus* over four seasons was determined to be Zn>Se>Cu>Ni>Mo>Co for both sexes (Table 4). The highest trace element levels in male individuals were found in autumn, while the highest trace element levels differed. Maximum Zn, Se, Cu, Ni, Mo and Co levels in both sexes of pufferfish around the year were 75.73 (µg g^{-1}, dw), 3.98 (µg g^{-1}, dw), 2.65 (µg g^{-1}, dw), 2.44 (µg g^{-1}, dw), 2.13 (µg g^{-1}, dw) and 0.69 (µg g^{-1}, dw), respectively. Macro and trace element levels show that the muscle tissue of pufferfish has nutritionally high quality.

The highest levels of Cu and Zn in pufferfish were determined as 0.64 µg g-1 (ww) and 18.18 µg g-1 (ww), respectively. The limit value for Cu and Zn was reported as 30 µg g-1 (ww) by FAO (1983), and 20 µg g-1 (ww) and 50 µg g-1 (ww) by the Turkish Food Codex (TFC, 2011), respectively. The Cu levels in present study, were found to be below limit which was reported by the FAO (1983) and (TFC, 2011).

Toxic metals investigated over four seasons for the *L. sceleratus* species, Cd and Pb have both chemical and ecological significance. The limit value determined by FAO and EU for Cd is 0.05 µg g^{-1}

(ww). Cd levels in pufferfish were found between 0.16-0.24 µg g-1 (ww) for male and between 0.17-0.23 µg g-1 (ww) for females. Cd levels were observed as higher in winter for both sexes. Cd levels in the muscle tissue of pufferfish belonging to the species *L. sceleratus* determined in present study were determined to be above Turkish Food Codex (TFC, 2011), FAO (1983) and European Commission (EC, 2001) legal limits in all four seasons. Pb levels were found between 0.10-0.20 µg g-1 (ww) for male individuals, and 0.14-0.20 µg g-1 (ww) for female individuals. The limit value determined by FAO and EU for Pb is 0.3 µg g^{-1} (ww). Pb levels were measured as higher in autumn for both sexes. For the Pb level, it was determined to be below Turkish Food Codex (TFC, 2011), FAO (1983) and European Commission (EC, 2001) legal limits in all four seasons. Thiyagarajan, Dhaneesh, Kumar, Kumaresan and Balasubramanian (2012) reported Pb levels in pufferfish belonging to the species *Lagocephalus lunaris* was below legal limits. It is thought that this might be the result of the region, due to high levels of maritime activity and industrial waste dumping in the Mersin Bay.

Conclusions

The chemical composition of pufferfish showed that this species is rich in protein, macro and trace metals. Although this species contains low level of lipid, it had high level of n-3 fatty acids. However, among heavy metals, Cd level was found higher than legal limit. When TTX levels in the meat of this species can be removed by applying some methods in areas where pufferfish are consumed, it is very important fish in terms of nutritional value. Protein content determined in this study show that if the toxin content of the pufferfish can be eliminated, it can be a very valuable fish nutritionally.

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