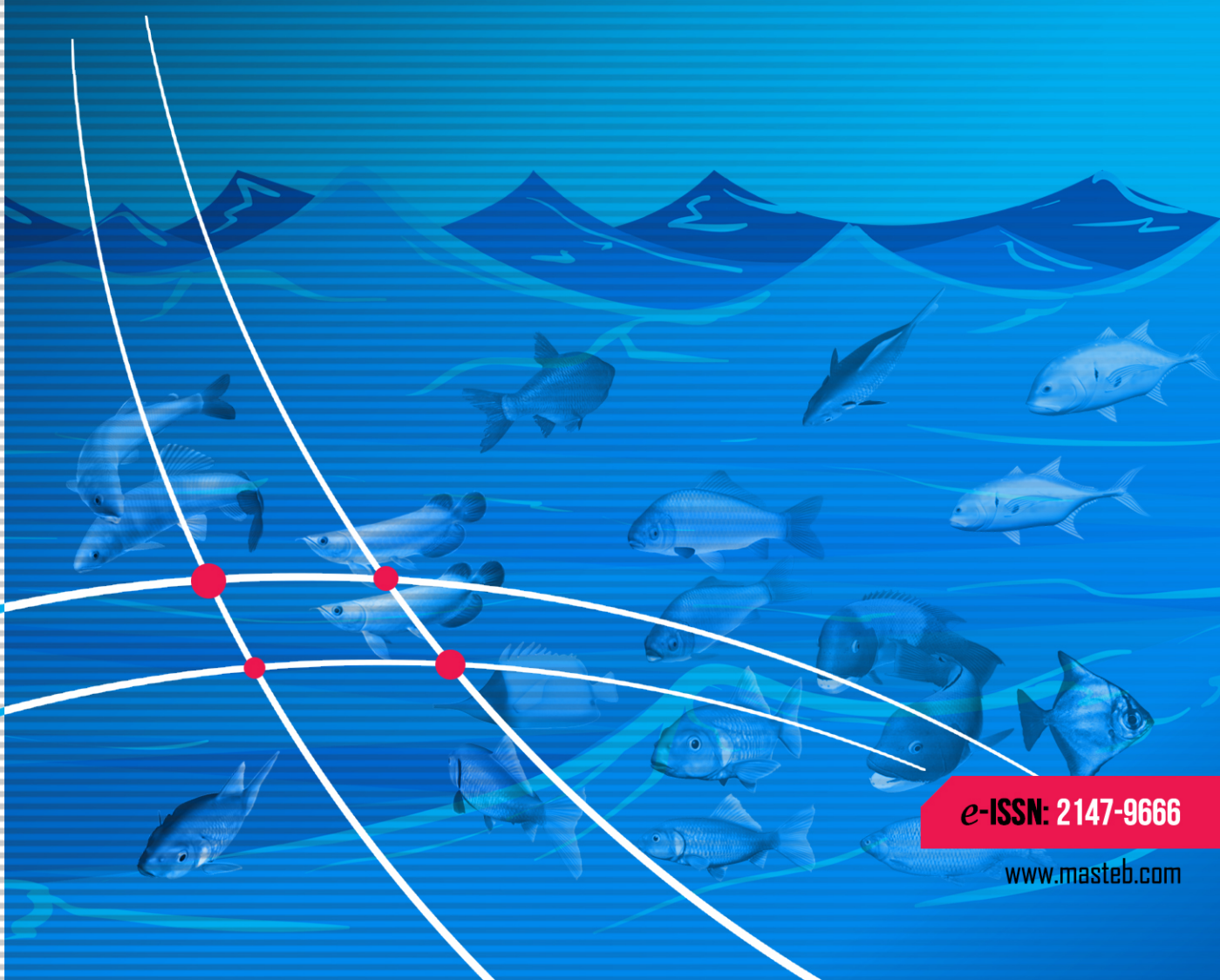


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MARINE SCIENCE AND TECHNOLOGY BULLETIN

RESEARCH ARTICLE

Isolation and partial characterization of serum immunoglobuline in two species of sturgeons, Siberian sturgeon (*Acipenser baerii*) and Stellate sturgeon (*Acipenser stellatus*).

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ABSTRACT

The immunoglobulins (Igs) were isolated and purified respectively from serum of Siberian sturgeon (*Acipenser baerii*) and Stellate sturgeon (*Acipenser stellatus*) by using 50% saturated ammonium sulfate precipitation. Analysis of the purified Ig of the two species of fishes on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions revealed two bands, heavy chains and light chains respectively. The molecular weight of the heavy chains of two fishes was about 70 kD and the molecular weight of the light chains was 27kD for Siberian sturgeon and 27.5 kD for Stellate sturgeon. The results suggested that the isolated and purified serum Immunoglobuline in two species of sturgeons, Siberian sturgeon (*A. baerii*) and Stellate sturgeon (*A. stellatus*) can be used to produce polyclonal and monoclonal antibodies against sturgeon Ig for developing diagnostic tests against a wide variety of pathogens.

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Introduction

Immunoglobulins are important components in immune response of fish. The major immunoglobulin class in fish blood serum is IgM molecule consisting of light (L) and heavy (H) chains (Koumans-van Diepen et al., 1995). It has a

molecular weight (MW) between 6.00, and 8.00, kD, depending on the species of the fish (Ellis, 1982). The characteristics of the fish immunoglobulins have been investigated in a few species including *Labeo calbasu*, *Dicentrarchus labrax*, *Salmo salar*, *Oreochromis niloticus*, *Thunnus maccoyii*, *Lates calcarifer*, *Cyprinus carpio* (Behera et al., 2009; Vesely et al., 2006; Bourmaud et al., 1995; Magnadottir et al., 1996; Al-Harbi et al., 2000; Watts et al., 2001; Crosbie and Nowak, 2002). There are little studies on the serum immunoglobulins of chondroesti sturgeons. Study

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on immunoglobulin will be useful in developing immunodiagnostic methods against a wide variety of pathogens in aquaculture industry. The aim of this study was to isolate and partially characterize serum Igs from two important species of sturgeons, Siberian sturgeon (*A. baerii*) and Stellate sturgeon (*A. stellatus*) in order to develop polyclonal and monoclonal antibodies against sturgeon Ig.

Material and Methods

Fish

Eight healthy *A. stellatus* and *A. baerii* weighing about (5.47±0.34 kg, 5.27 kg±0.87 respectively) were collected from a fish farm in Kamfiruz city, Fars province, Iran. Blood samples were collected from the caudal vein and allowed to clot overnight at 4°C. The sera were collected and stored at -20°C until required for the assay.

Isolation and Purification of Fish Igs

Serum immunoglobulins (Igs) were precipitated with 50% saturated ammonium sulphate (SAS) for 6 hours at 4°C. The precipitate was centrifuged at 9000g for 15 min at 4°C. Then, the precipitate was suspended in 1 ml of PBS (1x) and dialyzed for 24 hours at 4°C. Ammonium sulphate was removed by dialysis against phosphate buffer saline (PBS pH 7.2). The purified Ig was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Purified Ig was characterized by polyacrylamide gel electrophoresis under reducing conditions to determine purity and molecular weight of reduced Ig molecules. Electrophoresis was performed using a discontinuous buffer system at 3% stacking gel, pH 6.8 and 10% separating gel, pH 8.8 as described by Laemmli (1970). Samples were mixed with sample buffer (Tris HCL, pH 6.8; 10% SDS; Glycerol; 0.1% Bromophenol blue; 2-mercaptoethanol and distilled water) and allowed to boiled for 5 minutes at 100°C before application. After electrophoresis for about 2 hours at a constant voltage of 100v, the gel was stained with Coomassie Brilliant blue and destained with 10% acetic acid- 40% methanol solution until the gel became clear. Relative molecular weights of bands were calculated using Protein marker (Cinnagen [SL7012] prestained protein ladder).

Results

SDS-PAGE analysis from the purified serum Igs in two species, the Siberian sturgeon and Stellate sturgeon, resulted in two distinct bands at approximately 70 and 27-27.5kDa (Figure 1). These could be identified as the H and L chain of Ig, respectively. The presumed light chain of Stellate sturgeon Ig showed a slightly higher MW i.e. 27.5kDa, while Siberian sturgeon was similar i.e. 27kDa.

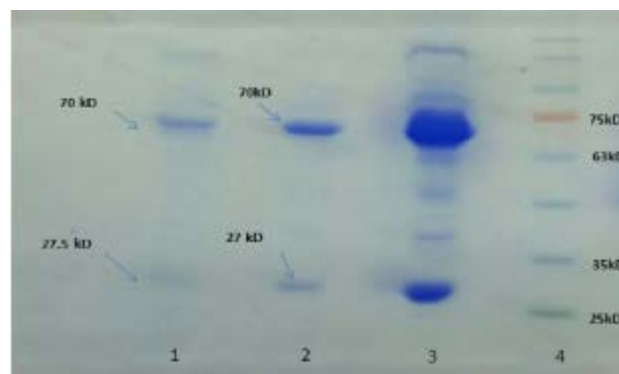


Figure 1. SDS-PAGE under reducing conditions for estimation of molecular weight of Ig of the sturgeons. Lanes (1-2): Purified Igs from *Acipenser stellatus* and *Acipenser baerii* respectively, Lane (3): Unpurified Ig from *Acipenser baerii*, Lane (4): Marker (Cinnagen [SL7012] prestained protein ladder).

Discussion

Research on fish immunoglobulins is important for developing immunological reagents and in assessing health status. Immunoglobulin had been isolated from a variety of fish species such as European eel, Atlantic salmon, Asian sea bass, Japanese eel, Indian major carp, and Asiatic cat fish (Håvarstein et al., 1988; Buchmann et al., 1992; Uchida et al., 2000; Swain et al., 2004; Bag et al., 2009; Choudhury and Pani Prasad, 2011).

In the present study, immunoglobulin was purified from two important species of sturgeons, Siberian sturgeon (*A. baerii*) and Stellate sturgeon (*A. stellatus*) serum. The immunoglobulin was found with heavy and light chains of 70 and 27-27.5kDa, respectively. Pilström and Petersson (1991) found the heavy and light chains molecular weight of cod (*G. morhua*) Ig to be 81 kDa and 27.5 kDa, respectively. Kofod et al. (1994) reported one heavy chain of 79 kDa and two light chains of 27 and 29 kDa for turbot (*S. maximus*) Ig. Adkison et al. (1996) analyzed purified immunoglobulins from white sturgeon (*A. transmontanus*) serum. The MW of the heavy and light chains of the purified Igs determined by SDS-PAGE 73 and 27-30 kDa, respectively. Palenzuela et al. (1996) showed that European sea bass (*D. labrax*) Ig contains one heavy chain of 78 kDa and two light chains of 27.5 and 28.5 kDa.

Conclusion

The present results will be helpful for developing immunodiagnostic kits in aquaculture industry. The purified Ig of the Siberian sturgeon (*A. baerii*) and Stellate sturgeon (*A. stellatus*) can be used to raise polyclonal antisera and monoclonal antibodies which have applications in diagnosis of fish diseases.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

The structural and economic analysis of the rainbow trout farming: Case of Erzurum province.

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ABSTRACT

Within the scope of the research, a total of 14 enterprises were studied and it was determined that 85.7% of these enterprises were producing in the ponds on land, 7.1% in net cages and 7.1% in both ponds and net cages. When examined according to their legal structures, it is seen that 85.7% of the enterprises are private companies, 7.1% are limited liability companies and 7.1% are joint stock companies. In the analysis of the workforce in the enterprises, it was found that the ratio of the family labour force is 2.0 Man Power Unit (MPU), the male labour force is the highest as 1.07, and the female labour force is highest as 0.27 at the age group of 15-49. It was determined that the active capital amounts (17,095,548 TRY) of the cage breeding enterprises are much higher than the active capital amounts (453,482 TRY) of the pond enterprises. Operating expenses are calculated as 77,041 TRY in enterprises having ponds, and 749,578 TRY in cage enterprises and 1,087,900 TRY in both pool and cage enterprises. The biggest share of operating costs is constituted by the feed price. The profitability ratios in the enterprises were determined as 66.86% for pond enterprises, 94.73% for cage enterprises, and 91.59% for both pool and cage enterprises.

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Introduction

Fish is an indispensable source of the human nutrition due to the essential amino acids, vitamins and minerals that it contains. However, because of its relatively low cost and easy availability, fish is a food with significant superiority

over many animal products (Ezihe et al., 2014, Karakaya and Kırıcı, 2016).

Nowadays, considering the economic conditions, aquaculture is an important sector in terms of the solution of nutritional problems and its place in balanced nutrition. The fish consumption in the world has been increasing in parallel with population growth, urbanization, widespread adoption of western type eating habits, and the rise in the per capita income (Akanbi, 2015). The need for animal protein has been increasing for the sufficient and balanced nutrition, as a

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result of the rapid growth of world population day by day. Production of aquaculture is a very important source in eliminating this necessity due to inadequate land resources in meeting animal protein (Elbek, 1981). Aquaculture has reduced the pressure on natural stocks that emerged because of hunting, and it has avoided negative balance changes.

The aquaculture sector has undergone an astonishing development in the last 50 years, with the training activities and rapid technology transfer. Aquaculture has been identified as the fastest growing food industry in the world by Food and Agriculture Organization (FAO). The production of aquatic products in the world was under 1 million tons in the 1950s, reaching 7 million tons in the 1980s, exceeding 70 million tons in 2015 (Anonymous, 2017a).

Aquaculture, which has an important place in the agricultural sector, has a significant socio-economic place as a business line as well as being a valuable food source. Aquaculture provides significant added value to Turkey's economy by providing raw materials to the industrial sector, creating employment, contributing to rural development and food production. For example, trout farming has become an area of economic activity that provides substantial economic input and employment in Turkey. Entrepreneurs have created integrated systems consisting of production, processing and marketing under free market economy conditions (Doğan & Yıldız, 2008).

In the recent years, aquaculture in Turkey has gained momentum in parallel with the developing technology and economic growth. As a result of the excessive amount of hunting and reduction of fish population, the importance of breeding has been increasing day by day. The aquaculture studies first started in the inner waters, then left their place in marine environments, and with the identification and implementation of economic methods of breeding, the studies that were at the level of being initiatives have turned into efforts of a sectoral structure. Although it was directed towards carp breeding, which is easier to breed, initially, the breeding of trout, sea bream and sea bass species, which have high economic value, have been more prevalent today (Sayılı et al., 1999).

According to the latest statistics of TURKSTAT, a total of 588 thousand tons of aquatic products were produced in Turkey in 2016, consisting of 335 thousand tons of hunting and 253 thousand tons of aquaculture. In 2016, the production through hunting has decreased by 22.27% compared to the previous year, while aquaculture production has increased by 5.42%. Approximately 56.97% of total aquaculture production in 2016 was obtained through hunting and 43.03% by aquaculture (Anonymous, 2017b).

This study was carried out to investigate the economic situation of the trout farming enterprises in Erzurum province. In the study, socio-economic structures and economic performances of the enterprises were examined. We believe that the results of this research will provide important findings for the policymakers and related stakeholders.

Material and Methods

Material

The research aimed to reveal the structural conditions of the trout farming enterprises in Erzurum province and analyse them from the economic perspective. For this reason, research material mainly consists of the data obtained from the surveys of the trout farms that exist in this province.

In the direction of the aim of the study, the enterprises engaged in trout breeding in Erzurum province were included in the research. For this purpose, firstly, support was obtained from Erzurum Provincial Food, Agriculture and Animal Husbandry Directorate to reach the number of the trout farming enterprises. In the Regulation of Aquaculture, the definition given in Article 4 and the categories related to aquaculture in Articles 6 and 9 are explicitly mentioned as aquaculture pool and cage cultivation (Anonymous, 2018a). In the light of the written and verbal information provided by this institution, it was determined that there were 14 trout production facilities that were active in the whole research area and all of these facilities were included in the survey in accordance with the complete sample method.

In the study, survey method was used as the data collection tool. The questionnaires used in the study were designed by the researcher, within the scope of two main objectives. The aim of the first part of the surveys (structural analysis of enterprises) was to determine the structural characteristics (production mode, production quantity, marketing method, type of operation, labour quality, etc.) of the enterprises that cultivating trout. The purpose of the second part of the surveys (economic analysis of the enterprises) was to determine the economic characteristics (such as active capital structures, profitability, labour productivity, etc.) of trout farming enterprises.

With the data obtained through the questionnaires, the production values and costs of the year 2017 were calculated. Although the method used in this study did not resemble with the doctoral dissertation of the first author entitled "*Comparative Structural and Economic Analysis of Trout Establishments in Eastern Anatolia and Mediterranean Regions*", the questionnaire was reworked and new results were obtained with current data.

Method

The service capacity of the population in the enterprises was determined by calculating in man power unit (MPU). The units used by Açıl and Demirci (1984) were utilized in converting the data to MPU (Table 1).

Active capital consists of farms (land, land arrangement, building and pond, cage, breeding fish) and enterprises (instrument and machinery, fish, material, money), while the passive capital is composed of debts and capital stock.

Table 1. Coefficients used in the conversion to man power unit

Age Groups	Male	Female
0 - 6	-	-
7 - 14	0.50	0.50
15 - 49	1.00	0.75
50 +	0.75	0.50

Source: Açı and Demirci (1984)

The capital structure of the investigated enterprises has been determined by considering the following criteria (Sayılı et al., 1999):

- Land capital covers the area where ponds are used only for fish production. For the land capital, the purchase-sale value that is valid in the research area is taken as the basis,
- For the land arrangement capital, the evaluation is made considering the cost for the new ones, and the ageing degree depending on the reconstruction cost for the old ones,
- For building, pond and cage capital, local construction prices and unit price lists are used. In this valuation, the wear condition in use is taken into account,
- Instrument-machinery capital is taken as the value of purchase for the new instruments, and for the old ones, it is valued over purchase-sale value according to the level of their usability,
- The prices declared by the farmers and the sale price on the market are taken into consideration for the fish capital. The depreciation rate is also considered for the broodstock fish,
- Purchase-sale value is taken as basis for material capital,
- The statement of the enterprise owners is taken as basis for the money amount, receivables and debts.

In the study, capital structures and annual operating results (operating and production costs, gross profit, net income, net profit, profitability, business productivity) of the enterprises surveyed are set forth. The determination of the annual operating results in agricultural enterprises is usually made according to the Laur accounting method. According to Laur, the gross product is the quantity and value expression of an increase in the parts of the capital by means of newly produced goods, goods exchanges and revaluation during an operation year as a result of the economic activity in an agricultural enterprise, considered as an economic whole (Akil and Demirci, 1984).

The net return is the value of the capital invested in the business or the profit of the active capital. The net return, which is the result of the capital used in the business, is obtained by subtracting the calculated costs from the gross revenue, excluding the interest of the active capital. The net

profit was obtained by subtracting the production costs from the gross profit (Karagölge, 2001, Peker and Kan, 2010).

Operating costs refer to the total expenses incurred outside the interest or interest coverage of the active capital so that the gross receipts can be obtained. Operational costs are divided into two categories: variable costs and fixed costs (Erkuş et al., 1995). Production costs were found by adding active capital interest value to operating costs value (Koç, 2007). In the analysis of production cost, labour costs spent on aquaculture activity, material costs, shipping costs, product prices and market prices related to other goods and services used were evaluated (Özkan and Yılmaz, 1999). 3% of the variable costs are included in the calculation of the general overhead cost included in the operating costs (Kıral et al., 1999). The interest rate on operating capital is the variable cost, which is the opportunity cost of the capital invested in the production activity. For the year 2017, when the research was carried out, the interest rates for the operating capital are taken as the remaining 5% excluding the subsidy from the 10% current interest rate applied to the fishery loans by T.C. Ziraat Bank. Rates of depreciation of fixed capital elements of the examined companies were used as 3.5% for land improvement capital, 11% for building-pond and cage capital, 16.5% for breeding fish capital and 11% for instrument-machinery capital (Anonymous, 2018b).

Profitability is a measure, where the profit is provided from the capital used in the business. The profitability ratio in the enterprises is calculated by using the formula of Karagölge (2001):

$$\text{Profitability Factor} = \frac{\text{Net Return}}{\text{Gross Product}} \times 100$$

The value obtained by dividing the production to the labour force used in that production is called the labour productivity (Çetin and Bilgüven, 1991).

Results and Discussion

Structural Features of the Investigated Businesses

Among the trout farms investigated in the province of Erzurum, 85.7% (12) of them are producing in ponds, 7.1% (1) in net cages and 7.1% (1) in both ponds and net cages. It has been determined that 76.9% of the businesses that produce trout on the pond bring water using PVC pipes, 15.4% using concrete canals and 7.7% using PVC pipes + concrete canals. In addition, it has been determined that a very large majority (92.3%) of these pond enterprises give the water to the ponds separately.

It was observed that 85.7% of the enterprises are in the valley, 7.1% are in the open area and 7.1% are in the mountainside according to the type of the land they were built. When the road conditions of the enterprises are examined, it is seen that 57.1% is stabilized and 42.9% is asphalt. Regarding their legal structure, 85.7% of the enterprises are private ownership, 7.1% of them are limited companies and 7.1% are joint-stock companies. While 85.7% of the farming enterprises are producing in their own

property, 14.3% of them are producing on the land that they have rented.

When the enterprises are examined according to their production status, 85.7% (12) of them carry out complete production, meaningly collecting eggs from their own breeding fish and feeding them until the marketing phase, while 14.3% (2) of them produce edible fish, meaningly purchase juveniles from other enterprises and feed them. When analyzed according to marketing channels used in sales, it was found that 64.3% of businesses use both retail and wholesale, 14.3% use both retail and restaurant, 7.1% only use retail and 14.3% use all three sales channels.

In terms of production conditions, it was determined that 85.7% of the enterprises are small (0-49 tons/year) and 14.3% are medium-sized enterprises (50-499 tons/year). It was found that 57.1% of the enterprises wanted to increase their existing production capacities if the conditions were appropriate over time and it was determined that 100% of the trout operations operating on the sea were not members of any union, but it was also found that they believe that collecting of all the enterprises under a union or cooperative will be useful. 57.1% of the producing enterprises did not use business or investment loans, while 42.9% benefited from business or investment credits.

Workforce Existence

Due to the specific nature of agriculture, the farmer's family has a very important place in agricultural production. For this reason, the examination of the population structures of the enterprises is of great importance in terms of revealing the social structures of the agricultural enterprises and making the economic analyses (Karakayacı and Oğuz, 2006).

Table 2. The amount of family and stranger workforce by age groups in the enterprises studied (MPU)

Age Group	Gender	Family	Neighbour
7 - 14 Ages	Male	-	-
	Female	-	-
15 - 49 Ages	Male	1.07	0.93
	Female	0.27	-
50+ Ages	Male	0.59	-
	Female	0.07	-
Total	Male	1.66	0.93
	Female	0.34	-
Grand Total		2.00	0.93

In the analysis of the labour force in the examined enterprises, it was found that the family labour force ratio is 2.0 MPU, the highest male labour force is in the 15-49 age group with 1.07 MPU and the highest female labour force is in the 15-49 age group with 0.27 MPU. It was found that 67% of the population in the surveyed enterprises is composed of 15-49 age group. This high ratio indicates that the workforce potential of the surveyed enterprises is good. In the results obtained for the labour force of strangers in the enterprises, it was determined that the ratio of stranger labourer utilization was 0.93 MPU (Table 2).

Capital Structure of Investigated Enterprises

The active capital in the researched enterprises consists of the farms and the operating capital. In the province of Erzurum there are pond, cage, and pond and cage breeding enterprises.

As seen in Table 3, average of total active capital enterprises; 453,482 TRY for pool operators, 17,095,548 TRY for cage operators and 15,657,588 TRY for both pool and cage operators. The largest share in the active capital is the fish. The ratio of fish capital to active capital is 51.10% for pond enterprises, 83.20% for cage businesses and 82.60% for pond and cage businesses.

The amount of active capital per 'kg', is determined as 47.90 TRY in the average of the pond enterprises, 62.63 TRY in the average of the cage enterprises and 62.17 TRY in the average of the pond and cage enterprises. The active capital per fish was determined as 10.18 TRY for pond enterprises, 15.54 TRY for cage establishments and 15.66 TRY for pond and cage establishments.

The passive capital of enterprises consists of the sum of debts and equity capital. On average, the amount of debt in establishments was determined as 4,783 TRY in pond enterprises and 24,304 TRY in pond and cage establishments. Equity capital is determined as 448,699 TRY in the average of pond enterprises, 17,095,548 TRY in the average of cage establishments and 15,633,284 TRY in the average of pond and cage establishments.

The share of debt in the passive capital is 1.05% in pond enterprises and 0.16% in pond and cage enterprises. Equity constitutes 98.95% of passive capital in pond enterprises, 100% in cage enterprises and 99.84% in pond and cage enterprises. The debt and equity capital of the investigated enterprises are shown in Table 3.

Annual Activity Results of Investigated Enterprises

Operating and production costs for the enterprises investigated are given in Table 4. The calculation of the sum of the operating costs in the fisheries breeding enterprises is calculated by subtracting the revolving fund interest from the production costs. In the enterprises that carry out aquaculture, the sum of operating expenses is calculated as 77,041 TRY in pond enterprises, 749,578 TRY in cage enterprises and 1,087,900 TRY in pond and cage enterprises on average.

On the average, the total production costs are 80,350 TRY for pond enterprises, 784,066 TRY for cage enterprises and 1,138,907 TRY for pond and cage enterprises.

The largest share of production costs is the feed cost, which is 43.35% in the pond, 59.23% in the cage, and 66.07% in the pond and cage enterprises.

In the examined enterprises, the gross profit was calculated as 232,485 TRY in pond enterprises, 14,231,250 TRY in cage establishments and 12,937,500 TRY in pond and

Table 3. Capital structure of trout enterprises investigated in Erzurum province

Capital Components	Pond Averages		Cage Averages		Pond and Cage Averages		
	TRY(₺)	%	TRY(₺)	%	TRY(₺)	%	
Farm Capital							
<i>Land Capital</i>	8,552	1.9	0	0.0	0	0.0	
<i>Land Adjustment Capital</i>	12,257	2.7	16,044	0.1	24,979	0.2	
<i>Building and Pond Capital</i>	136,189	30.0	178,267	1.0	277,543	1.8	
<i>Cage Capital</i>	0	0.0	400,000	2.3	251,816	1.6	
<i>Total</i>	156,998	34.7	594,311	3.9	554,338	3.9	
Operating Capital							
<i>Machine-Equipment Capital</i>	14,466	3.2	2,100	0.0	16,100	0.1	
<i>Fish Capital</i>	231,954	51.1	14,231,250	83.2	12,937,500	82.6	
<i>Material Capital</i>	8,708	1.9	116,100	0.7	188,125	1.2	
<i>Breeding Fish Stock</i>	6,032	1.3	17,100	0.1	20,900	0.1	
<i>Money</i>	35,324	7.8	2,134,688	12.5	1,940,625	12.4	
<i>Total</i>	296,484	65.3	16,501,238	96.1	16,003,250	96.1	
<i>Active Total</i>	453,482		17,095,548		15,657,588		
Passive Capital	<i>Debts</i>	4,783	1.05	0	0	24,304	0.16
	<i>Equity Capital</i>	448,699	98.95	17,095,548	100	15,633,284	99.84
	<i>Passive Total</i>	453,482		17,095,548		15,657,588	

Table 4. Production costs of enterprises investigated in Erzurum

Cost Elements	Pond Averages		Cage Averages		Pond + Cage Averages	
	TRY(₺)	%	TRY(₺)	%	TRY(₺)	%
<i>Feed Costs</i>	34,832	43.35	464,400	59.23	752,500	66.07
<i>Labour Costs</i>	25,785	32.09	77,749	9.92	118,018	10.36
<i>Egg and Juvenile Cost</i>	1,440	1.79	19,000	2.42	2,660	0.23
<i>Heating-Lighting</i>	1,023	1.27	24,300	3.10	24,300	2.13
<i>Chemical and Disinfectant Value</i>	18	0.02	3,200	0.41	2,080	0.18
<i>Maintenance-Repair Costs</i>	5	0.01	0	0.00	4,800	0.42
<i>Shipping Costs</i>	1,733	2.16	3,100	0.40	24,800	2.18
<i>Tax Cost</i>	1,333	1.66	98,000	12.50	91,000	7.99
<i>Total Costs</i>	66,169	82.35	689,749	87.97	1,020,158	89.57
<i>Interest Rate of Operating Capital (0.05%)</i>	3,308	4.12	34,487	4.40	51,008	4.48
Total Variable Costs	69,478	86.47	724,236	92.37	1,071,116	94.05
<i>General Administration Expenses (3%)</i>	2,084	2.59	21,727	2.77	32,135	2.82
<i>Production Areas' Rental and Water Price</i>	315	0.39	11,330	1.45	8,305	0.73
<i>Land arrangement. depreciation</i>	429	0.53	562	0.07	874	0.08
<i>Building. Pond and Cage Depreciation</i>	5,448	6.78	23,131	2.95	21,174	1.86
<i>Machine-Equipment Depreciation</i>	1,591	1.98	231	0.03	1,771	0.16
<i>Breeder Fish Amortization</i>	1,005	1.25	2,849	0.36	3,482	0.31
Total Fixed Costs	10,872	13.53	59,829	7.63	67,742	5.95
Total Operating Costs	77,041	95.88	749,578	95.60	1,087,900	95.52
Production Costs	80,350	100	784,066	100	1,138,907	100

cage enterprises on average. On average, the ratio of gross revenue to active capital was 0.51 for pond businesses, 0.83 for cage businesses, and 0.83 for pond and cage businesses. Pond + cage enterprises are found to be in the most advantageous position due to the gross amount falling to the unit number of fish with 12.94 TRY, while there are pond operations with 5.22 TRY in the worst case position. When they are examined in terms of the gross amount falling to the unit amount of 'kg', it is seen that the most advantageous case is the pond + cage enterprise with 51.75 TRY, similar to the case of unit fish, and pond enterprise with 24.56 TRY in the worst case.

The average of the enterprises surveyed with net returns was calculated as 155,444 TRY in pond enterprises, 13,481,672 TRY in cage enterprises and 11,849,600 TRY in both pond and cage establishments. On the other hand, the ratio of net return to active capital was 0.34 for the pond, 0.79 for the cage, 0.76 for pond and cage, respectively. With regards to the net return per fish, the most advantageous case is the cage enterprise with 12.26 TRY and the worst case is the pond enterprises with 3.49 TRY. When they are examined with respect to the net return per "kg", it is seen that the most advantageous case is the cage business with 49.02 TRY while the worst case is the pond business with 16.42 TRY. The gross profit and net return figures of the enterprises are given in Table 5.

Table 5. Gross profit and net return values of the enterprises investigated in Erzurum province

	Pond Averages (TRY)	Cage Averages (TRY)	Pond + Cage Averages (TRY)
Gross Profit (1)	232,485	14,231,250	12,937,500
GP / Active Capital	0.51	0.83	0.83
Operating Costs (2)	77,041	749,578	1,087,900
Net Return (1-2)	155,444	13,481,672	11,849,600
NR / Active Capital	0.34	0.79	0.76

For the enterprises surveyed within the scope of the research, the value of net profit is calculated as 152,135 TRY for pond enterprises, 13,447,184 TRY for cage establishments and 11,798,593 TRY for pond and cage establishments. On average, the net profit value of the unit 'kg' is determined as 16.07 TRY for pond operators, 48.90 TRY for cage operators and 47.19 TRY for both pond and cage operators. When the value of the net profit per unit of fish is examined, it is determined as 3.41 TRY for pond and 12.22 TRY for cage and 11.80 TRY for both pond and cage. The net profit figures for the businesses are shown in Table 6.

The profitability ratios in the examined enterprises are given in Table 7. The profitability ratios were calculated as 66.86% for pond enterprises, 94.73% for cage enterprises and 91.59% for pond and cage establishments, respectively. In terms of profitability, the cage establishments are in the

most advantageous state.

Table 6. Net profit figures of the studied enterprises

	Pond Averages (TRY)	Cage Averages (TRY)	Pond + Cage Averages (TRY)
Gross Profit (1)	232,485	14,231,250	12,937,500
Production Costs (2)	80,350	784,066	1,138,907
Net Profit (1-2)	152,135	13,447,184	11,798,593

Table 7. The profitability ratios of the studied enterprises in the province of Erzurum

	Pond Averages (TRY)	Cage Averages (TRY)	Pond + Cage Averages (TRY)
Net Return (1)	155,444	13,481,672	11,849,600
Gross Profit (2)	232,485	14,231,250	12,937,500
Profitability (%) (1/2)	66.86	94.73	91.59

Total production in the provinces surveyed in Erzurum province and the amount of manpower consumed to realize this production are shown in Table 8 in days. It was determined that the maximum production amount for a daily operation was obtained in cage establishments with 251.14 kg/day, whereas the pond enterprises was at a disadvantageous state with 11.41 kg/day.

Table 8. Workforce productivity in the surveyed enterprises

Factors of Productivity	Pond	Cage	Pond + Cage
Annual Production Amount (kg/year) (1)	9,468	275,000	250,000
Working Duration (Days) (2) (Table 1)	830	1,095	2,188
Labour Productivity (kg/day) (1/2)	11.41	251.14	114.26

Conclusion

In this study, the structural and economic analysis of trout operations in Erzurum province was made. Within the scope of the research, a total of 14 enterprises were examined. Businesses were examined in detail regarding their breeding types, production situations, types of feeding fishes, marketing channels used in sales, water supply type, maintenance periods of ponds and cages, credit utilization situations, capacity increase situations, views on producer organizations, association memberships and family and the amount of stranger labour force.

Within the framework of economic analyzes, mainly active and passive capital structures have been analyzed in detail. In this context, in terms of active capital, it has been evaluated on the basis of farmland capital, land capital, land

regulatory capital, building and pond capital, cage capital, breeding fish capital, farmland, instrument and machinery capital, fish capital, material capital and money capital. In the evaluations made in terms of passive capital, debts and equity were examined.

The study also includes a detailed analysis of operating and production costs, gross profit, net profit, profitability and business productivity.

As is known, the production enterprises are producing for a full period of the year. This leads to the conclusion that the employer needs labour throughout the year. In the analysis of the labour force in the enterprises, it is determined that the ratio of the family labour force is 2.0, the male labour force is in the 15-49 age group with 1.07 and the female labour force is in the 15-49 age group with 0.27. It can be considered that the fact that the female labour force in all enterprises, in general, is much smaller than the male labour force is due to the fact that the physical labour portion of the work is much more intense. In the results obtained about the foreign labour force in the enterprises, the ratio of foreign labour force utilization was found to be 0.93. As a result of this situation, it has been observed that the operations of Erzurum province have a low use of foreign labour force due to the fact that the family labour force is sufficient for production because it operates as a small size family business.

As a result of the structural analysis, it is seen that a very important part of these enterprises is the "private operation" when the legal structure of the enterprises in the field is evaluated. This is thought to be due to the fact that the businesses are small family businesses. The same situation has also been observed as a result of examining the ownership of the enterprises. The vast majority of businesses are seen to perform their activities in their own properties. Since these enterprises are "private entities", they are seen as "own property" status. A large part of the existing enterprises is small-scale enterprises.

The trout farms were seen to be a large aquaculture farmer. At the same time, it has been determined that businesses are doing "complex" production, that is, they take eggs from their own breeding fish and feed them from the offspring to the marketing stage. In the question of which establishments have provided their puppies, it has been found that businesses operating on the ground are assuring the puppies from their own bodies.

When the maintenance periods of the ponds and cages in the enterprises are examined, it is seen that the enterprises clean the ponds and the cages periodically once a week or every two weeks.

It is understood that when companies look at producers' organizations, they are warmly welcomed by producer organizations. As a result of this positive outlook, it is thought that businesses in the field are expecting that they will benefit from the promotion of their products from the organizations, marketing and government incentives.

Nevertheless, when it was examined whether or not businesses on the field were members of any association, it

was observed that none of the enterprises in the province of Erzurum was members of a union or co-operative. Although the producers are warm to the organization, it is thought that the reason for not becoming active in the association is due to inadequate or ineffective organizations in the region.

When the active capital amounts of the enterprises are examined, it is seen that the active capital amounts of the enterprises which are growing in the cage in Erzurum province are much higher than the active capital amounts of the pond enterprises and the enterprises which cultivate both ponds and cages.

In the study, it is seen that the costs vary considerably when fixed and variable costs of the enterprises are examined. The main reason for this is that the items that make up the variable costs are the main expense items of the enterprises. Another remarkable fact is that the sum of both variable and fixed costs of pond + cage facilities is much higher than that of pond enterprises and cage enterprises, which is due to the fact that the production quantities of pond + cage facilities are much higher than the production quantities of pond enterprises and cage enterprises.

When the enterprises are analysed in terms of gross receipts, it is seen that the highest gross value of the reindeer is in the cage enterprises. This is thought to be due to the fact that the production volume of the enterprises is much higher than the production volume of other enterprises.

Since the net returns values of the enterprises are technically calculated on the gross revenues values, it is seen that the results obtained when the net revenues of the enterprises are examined are likewise very similar to the results obtained in the examination of the gross revenues. When businesses are assessed in terms of net profit situations, it appears that the net profit ratios of the cage enterprises are similar to the above cases.

When the profitability ratios of the enterprises are examined, it is seen that the highest profitability is in the cage enterprises and the lowest profitability is in the pond enterprises. When the enterprises are evaluated in line with the values of the business productivity, it is seen that the highest production amount is obtained in the cage business.

Conflict of Interest

The authors declare that there is no conflict of interest.

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MARINE SCIENCE AND TECHNOLOGY BULLETIN

RESEARCH ARTICLE

Effect of *Tilia tomentosa* methanolic extract on growth performance, digestive enzyme activity, immune response and haematological indices of common carp (*Cyprinus carpio*).

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ABSTRACT

This study was conducted to determine the effect of dietary supplementation with *Tilia tomentosa* on the growth performance, digestive enzyme activity, haematological indices and nonspecific immune indices of juvenile common carp (*Cyprinus carpio*). Fish with an average weight of 4.35 ± 0.16 g were fed a diet supplemented with an aqueous methanolic extract of *T. tomentosa* at a dose of 0% (control), 0.01%, 0.05% or 0.1% for 45 days. The final weight, weight gain and specific growth rate were observed to be significantly higher for the 0.01% and 0.1% groups compared with the control group ($P < 0.05$). The feed conversion ratio was significantly decreased in the 0.05% and 0.1% groups compared with the control ($P < 0.05$). The activities of various digestive enzymes (amylase, lipase and trypsin) were also measured and no significant differences were observed compared to the control ($P > 0.05$). The mean cell volume of the 0.01% group was significantly increased compared to the control ($P < 0.05$) and increased lysozyme activity was observed in the 0.05% and 0.1% groups. Respiratory burst activity was significantly increased ($P < 0.05$) on days 15 and 30 for the 0.1% and 0.05% groups, respectively. No differences were observed for myeloperoxidase activity among the four groups. These results suggest that aqueous methanolic extract of *T. tomentosa* has a growth-promoting and immunostimulatory effect on common carp.

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Introduction

Aquaculture is the most important sector in the world.

Also it considered as the most growing food produce industry with an average growth rate more than 7.7% per year through the last decades, the majority of aquaculture production are come from Asia (Gjedrem et al., 2012). Approximately 600 aquatic species are raised in captivity in around 190 countries for produce in fish culture system of varying input intensity

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and technological sophistication (Aklakur et al., 2015).

During last decades there has been a continuous growth of aquaculture industries all over the world and such intensive production would experience disease problems. Infectious diseases that occur as sporadic events in wild fish populations may cause high mortalities when appearing in intensive fish farming (Gudding et al., 1999). Enhancing the immune system of cultured fish appears to be the most promising method for preventing disease. Fish typically depend on nonspecific immune mechanisms to a much greater extent than most animals (Chakrabarti and Vasudeva, 2006).

Medicinal plants contain phytochemicals, which exhibit biological activities such as the prevention of chronic degenerative diseases (Fukumoto and Mazza, 2000). Herbal medicines have been used in aquaculture to promote growth, improve the immune system, and stimulate the appetite and fight against microbes and other stressors, due to the presence of various active compounds like flavanoids, alkaloids, phenolics, pigments, steroids, terpenoids and essential oils (Citarasu, 2010). Recently, immunostimulants of herbal origin have been shown to boost the disease resistance of fish towards a number of diseases by improving their nonspecific and specific defence mechanisms (Harikrishnan et al. 2011). Indeed, many herbs have been reported to enhance the immune response of fish (Khondoker et al., 2016).

The *Tilia tomentosa* extracts contained flavonols (quercetin, kaempferol, apigenin derivatives) as principal components with the exception of a single commercial extract with hydroxycinnamic acids as the most abundant metabolites (İleri et al., 2015). *T. tomentosa* is used as a medicinal plant in Turkish folk medicine (Baser et al., 2005). The present study was carried out to determine whether the *T. tomentosa* extract influences the digestive enzyme activity, growth, haematological parameters, or activity of the nonspecific immune response of the common carp (*Cyprinus carpio*).

Material and Methods

Preparation of *T. tomentosa* Extract

The plants were collected from the Kastamonu province in the north of Turkey and extracted using a methanol extraction method (Pakravan et al., 2012) with some modifications (Bilen et al., 2016) as follows: All ripe parts of the fruit except the seeds were ground in a mechanical grinder to a fine powder. Every 50 g of the ground plant were mixed with 1 L of 40% methanol (Sigma-Aldrich) and the mixture was allowed to stand at room temperature for three days with brief shaking once a day to mix. The extract was then filtered through filter paper (Whatman filter paper No. 1) and the filtrate was collected and evaporated in a rotary evaporator at 55-65 °C to remove the alcohol from the fruit extract. The final crude product was dissolved in distilled water and kept in a flask at 4 °C for later experiments.

Experimental Design

Common carp (*C. carpio*) with an average body weight of

4.35 ± 0.16 g were obtained from a commercial fish farm in Antalya, Turkey. The fish were transported to the Faculty of Fisheries and Aquaculture, Kastamonu University, Turkey. The 480 carp were randomly divided into four groups of three replicates each (i.e. four groups of three aquariums, 12 aquariums in total) with 40 fish per aquarium. The fish were acclimatised for two weeks before the experiment started. During acclimation, the fish were fed with a commercial diet twice a day. During the experimental period, the fish were fed the commercial diet supplemented with *T. tomentosa* extract at the following percentages (w/w): 0%, 0.01%, 0.05%, or 0.1%. The fish were fed by hand with the experimental diet for 45 days to satiation twice a day (at 9 am and 4 pm). The fish were maintained under a natural photoperiod (12-h dark/12-h light). The water quality parameters were checked daily and were within the accepted margins throughout the experiment (dissolved oxygen, 6.8-7.2 mg/L; pH, 7.7-8.5; water temperature, 25-28 °C).

Sample Collection

Every 15 days of the feeding trial (i.e. on days 15, 30 and 45), three fish per aquarium (a total of nine carp from each experimental group) were randomly chosen, anaesthetised by phenoxyethanol at 0.01 mL/L and individually weighed and sampled. The kidney tissues were collected and transferred individually to 1.5 mL RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) for direct assay of the immunological parameters. Blood was collected on day 45 of the feeding trials from the caudal vein using heparinised syringes in EDTA-tubes, and later used for the direct assay of the haematological parameters. Samples of the intestine were also collected on day 45 of the feeding trials. These samples were cleaned to remove waste and all visible fat and then stored at -80 °C for the digestive enzyme assay.

Growth Performance Parameters

The weight of each fish was individually measured at the beginning and end of the study. The growth performance was calculated according to the following equations (Tekinay and Davies, 2001):

$$WG(\%) = 100 \times \left[\frac{(\text{Final Fish Weight} - \text{Initial Fish Weight})}{\text{Initial Fish Weight}} \right]$$

$$SGR (\text{weight } \% d^{-1}) = 100 \times \left[\frac{\ln(\text{Final Fish Weight}) - \ln(\text{Initial Fish Weight})}{\text{Experimental Days}} \right]$$

$$FCR = \frac{\text{Feed Intake (g)}}{WG (g)}$$

$$SR(\%) = 100 \times \left[\frac{\text{Final Number of Fish}}{\text{Initial Number of Fish}} \right]$$

In these formulae, WG indicates weight gain, SGR indicates specific growth rate, FCR indicates feed conversion ratio, and SR indicates survival rate.

Digestive Enzyme Activity

The intestine samples were homogenised with a Potter Elvehjem homogeniser in cold double-distilled water (0.1 g/mL) and centrifuged at 9,000 rpm for 20 min at 4 °C. The

supernatant was removed and stored at $-80\text{ }^{\circ}\text{C}$ to test for digestive enzyme activity as follows. The amylase activity was determined using 2% starch (Sigma-Aldrich) as a substrate according to the Worthington (1991) method. The lipase activity was determined using hydrolysis of 4-nitrophenyl myristate (Sigma-Aldrich) according to the method described by Gawlicka et al. (2000). The trypsin activity was determined using the method of Erlanger et al. (1961) and benzoyl-DL-arginine p-nitroanilide (Sigma-Aldrich) as the substrate. The protein concentrations were evaluated with Bradford (1976) method.

Haematological Profiles

White blood cell counts ($\text{WBC} \times 10^7\text{ mm}^{-3}$), red blood cell counts ($\text{RBC} \times 10^6\text{ mm}^{-3}$), haemoglobin levels (Hb, g/dL) and haematocrit measurements (Hct, %) were measured according to the methods described by Blaxhall and Daisley (1973). Blood indices included the mean cell volume (MCV, fL), mean cell Hb (MCH, pg) and mean cell Hb concentration (MCHC, %), which were calculated according to the formulae of Lewis et al. (2001).

Nonspecific Immune Parameters

Head kidney cells were isolated from euthanised *C. carpio* according to the method of Kono et al. (2012) with slight modifications as follows. Briefly, the head kidney tissue was carefully removed and gently pushed through a 100- μm nylon mesh (John Stanier & Co., Whitefield, Manchester, UK) into RPMI-1640 medium (Invitrogen) supplemented with 5% foetal bovine serum (Invitrogen) and a 1% solution of 10,000 g/mL streptomycin plus 10,000 U/mL penicillin (Invitrogen). This mixture was then pushed through a 40- μm nylon mesh cell strainer (Becton, Dickinson & Co., Franklin Lakes, NJ, USA). The final homogenate was placed in a 3-mL Falcon tube. Head kidney cell suspensions were pelleted at 1,800 rpm for 3 min at $4\text{ }^{\circ}\text{C}$. After centrifugation, the supernatant was collected to measure myeloperoxidase activity using 3,3',5,5'-tetramethyl benzidine hydrochloride (Sigma-Aldrich) as the substrate (Sahoo et al., 2005). The lysozyme activity was measured using a lyophilised *Micrococcus lysodeikticus* bacterial cell solution (Sigma-Aldrich) as the substrate (Bilen et al., 2014). Each pellet was resuspended in 1 mL of the same medium to directly assay nitroblue tetrazolium (Sigma-Aldrich) reduction, according to the method described by Biswas et al. (2013).

Statistical Analysis

The results were analysed with SPSS software. One-way ANOVA and Duncan's multiple range test were used to determine the significant differences between groups. All results were expressed as the mean \pm SE and $P < 0.05$ was considered statistically significant.

Results

Growth

The final weight (FW), FCR, SGR, WG and SR of common carp fed on the experimental diets for 45 days were determined and presented in Table 1.

Table 1. Growth indices of *Cyprinus carpio* supplemented with a methanolic extract of *T. tomentosa* for 45 days.

Groups	IW (g)	FW (g)	WG (%)	FCR	SGR (%/day)
Control	4.09 \pm 0.10 ^a	6.88 \pm 0.08 ^a	168.07 \pm 5.06 ^a	1.68 \pm 0.01 ^a	1.15 \pm 0.04 ^a
0.01%	4.06 \pm 0.05 ^a	7.71 \pm 0.09 ^b	189.87 \pm 6.28 ^b	1.78 \pm 0.02 ^a	1.42 \pm 0.02 ^b
0.05%	4.23 \pm 0.06 ^a	7.25 \pm 0.07 ^a	171.23 \pm 4.57 ^c	1.24 \pm 0.01 ^b	1.20 \pm 0.02 ^a
0.10%	4.12 \pm 0.14 ^a	7.56 \pm 0.21 ^b	183.22 \pm 6.19 ^d	1.56 \pm 0.01 ^c	1.34 \pm 0.03 ^b

Note: Values represent the mean \pm SE; different superscript letters in a column indicate significant differences between groups ($P < .05$). IW: initial weight, FW: final weight, WG: weight gain, FCR: feed conversion ratio, SGR: specific growth rate, SR: survival rate.

Significant increases ($P < 0.05$) in FW, WG and SGR were observed for fish supplemented with 0.01% and 0.1% extract compared to the control. In addition, the FCR was significantly ($P < 0.05$) lower in the 0.05% and 0.1% supplemented groups compared with the control group. No significant differences were observed between the FW, FCR, SGR and SR of the other treated groups compared with the control group.

Digestive Enzymes

The digestive enzyme activity levels for each experimental group are shown in Table 2. No significant differences in the activity of amylase, lipase or trypsin were observed for fish supplemented with 0.01%, 0.05%, or 0.1% *T. tomentosa* extract compared with the control group.

Table 2. Digestive enzyme activity in the intestines of *Cyprinus carpio* supplemented with a methanolic extract of *T. tomentosa* for 45 days.

Groups	Enzymes		
	Amylase	Lipase	Trypsin
Control	1.84 \pm 0.54	0.009 ^a \pm 0.003	0.11 \pm 0.01
0.01%	1.58 \pm 0.51	0.009 \pm 0.001	0.08 \pm 0.01
0.05%	3.95 \pm 1.26	0.004 \pm 0.002	0.12 \pm 0.01
0.10%	3.96 \pm 1.58	0.006 \pm 0.015	0.08 \pm 0.05

Note: Values represent the mean \pm SE.

Haematological Profiles

Fish supplemented with *T. tomentosa* extract at different concentrations had the same haematological parameters as

the control group throughout the experimental periods (Table 3), with the exception of the MCV of the 0.01% group on day 30 of the experiment ($P < 0.05$) and the MCHC of the control on day 30 of the experiment (highest recorded result compared with the other groups; $P < 0.05$).

Nonspecific Immune Parameters

The immunostimulatory effects of the *T. tomentosa* extract are shown in Figures 1, 2 and 3. The lysozyme activity of fish supplemented with different concentrations of *T. tomentosa* extract was increased on day 30 of the experiment; this increase was found to be significant ($P < 0.05$) only for the 0.1% supplementation compared with the control (Figure 1). On the other hand, the lysozyme activity decreased significantly on day 45 of the experiment for the 0.01% supplementation compared with the control ($P < 0.05$). The lysozyme activity levels of the other supplementation groups did not change significantly over time.

The myeloperoxidase activity of the treated fish did not significantly differ from that of the control fish during the

study period (Figure 2).

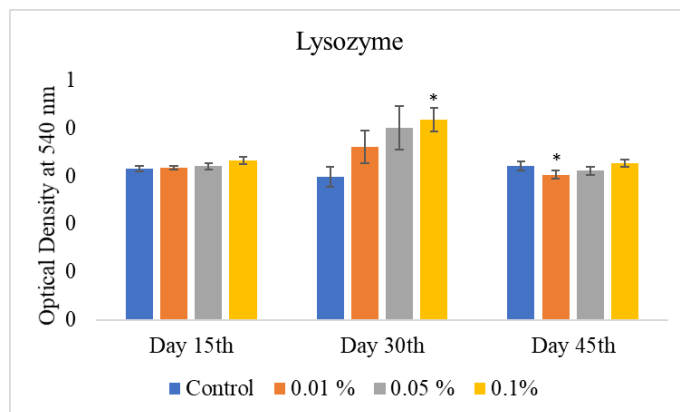


Figure 1. Lysozyme activity in kidney leucocytes of *Cyprinus carpio* fed with different experimental diets for 45 days. Values are expressed as the mean \pm SE. Different symbols above the bars indicate significant differences between groups ($P < 0.05$).

Table 3. Haematological profiles of *Cyprinus carpio* supplemented with a methanolic extract of *T. tomentosa* for 45 days.

Groups	WBC ($\times 10^7$ cells mm^{-3})	RBC ($\times 10^6$ cells mm^{-3})	Hb (g dl^{-1})	Hct (%)	MCV (fl)	MCH (pg)	MCHC (%)	
15 th day	Control	24.47 \pm 0.18	1.98 \pm 0.04	8.22 \pm 0.07	27.82 \pm 0.84	141.11 \pm 7.12	41.57 \pm 1.05	296.47 \pm 7.63
	0.01%	25.63 \pm 1.26	1.86 \pm 0.09	7.77 \pm 0.25	27.68 \pm 0.55	148.92 \pm 3.73	41.76 \pm 0.78	281.13 \pm 4.32
	0.05%	22.57 \pm 1.48	1.97 \pm 0.06	8.07 \pm 0.10	27.88 \pm 0.53	142.44 \pm 6.20	41.14 \pm 0.89	289.85 \pm 8.25
	0.10%	26.03 \pm 0.97	1.98 \pm 0.05	8.27 \pm 0.11	28.09 \pm 0.87	143.29 \pm 8.22	42.02 \pm 1.08	295.31 \pm 10.34
30 th day	Control	22.61 \pm 1.67	1.97 \pm 0.08	8.37 \pm 0.17	25.99 \pm 0.47	132.72 \pm 2.71	42.69 \pm 0.89	321.95 \pm 2.56
	0.01%	26.84 \pm 0.91	1.76 \pm 0.09	7.72 \pm 0.15	26.72 \pm 0.33	153.71 \pm 6.98*	44.23 \pm 1.42	289.10 \pm 4.66
	0.05%	26.34 \pm 1.00	1.81 \pm 0.03	7.68 \pm 0.36	25.59 \pm 0.73	141.85 \pm 2.26	42.51 \pm 1.21	300.12 \pm 7.38
	0.10%	26.35 \pm 0.45	1.93 \pm 0.07	8.08 \pm 0.10	26.84 \pm 0.09	140.89 \pm 5.95	42.12 \pm 1.32	301.50 \pm 4.48
45 th day	Control	23.76 \pm 1.85	1.90 \pm 0.09	7.31 \pm 0.33	25.59 \pm 0.62	134.97 \pm 4.07	38.45 \pm 0.55	285.78 \pm 7.19
	0.10%	24.16 \pm 1.73	2.02 \pm 0.05	7.81 \pm 0.19	26.35 \pm 0.64	131.08 \pm 4.68	38.70 \pm 1.29	269.27 \pm 1.26
	0.50%	26.74 \pm 1.13	1.97 \pm 0.07	7.80 \pm 0.10	26.18 \pm 0.27	133.49 \pm 5.52	39.65 \pm 1.08	298.42 \pm 6.54
	0.01%	27.41 \pm 0.15	1.92 \pm 0.06	7.70 \pm 0.23	26.87 \pm 0.48	140.42 \pm 4.23	40.14 \pm 0.30	286.51 \pm 7.17

The myeloperoxidase activity of the treated fish did not significantly differ from that of the control fish during the study period (Figure 2).

The respiratory burst level of the 1% group was significantly lower ($P < 0.05$) than that of the control on day 15 of the experiment (Figure 3). No significant differences in the respiratory burst level were observed among the other

treated groups on day 15 compared to the control group. On day 30 of the experiment, significantly higher levels of respiratory burst were observed for the 0.05% and 0.1% groups, while a significantly lower level was observed for the 0.01% group compared with the control ($P < 0.05$). The same pattern was exhibited on day 45 of the experiment ($P < 0.05$).

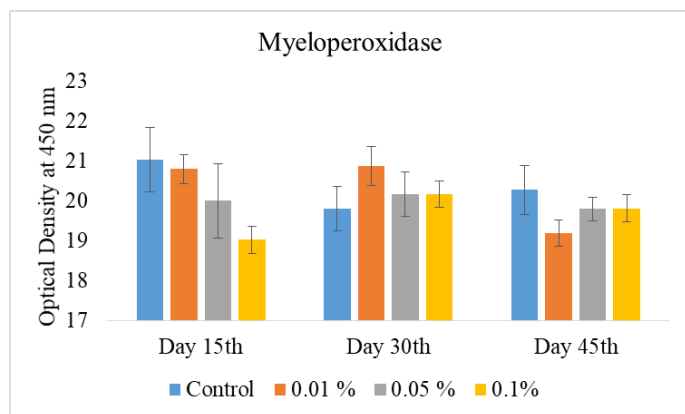


Figure 2. Myeloperoxidase activity in kidney leucocytes of *Cyprinus carpio* fed with different experimental diets for 45 days. Values are expressed as the mean \pm SE.

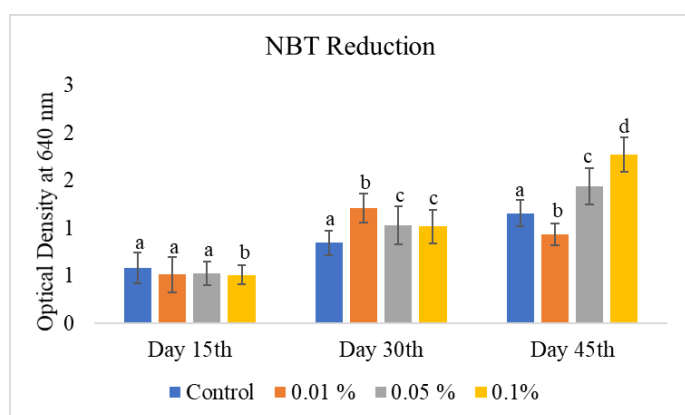


Figure 3. Respiratory burst level in kidney leucocytes of *Cyprinus carpio* fed with different experimental diets for 45 days. Values are expressed as the mean \pm SE. Different letters above the bars indicate significant differences between groups ($P < 0.05$).

Discussion

In the present study, *T. tomentosa* was shown to promote the growth of common carp based on observations of increased WG, SGR and efficiency of feed conversion. These results agree with those of previous studies demonstrating that medicinal plants promote the growth of various aquatic animals (Kour et al., 2004; Kaleeswaran et al., 2011; Ojha et al., 2014). The WG was significantly improved when Japanese flounder (*Paralichthys olivaceus*) were supplemented with an herbal mixture to 500 mg/kg (Seung-Cheol et al., 2007). Most probably fat was used for energy, and protein was used for growth in the herbal-supplemented diet (Yilmaz et al., 2012). Nile tilapia fingerlings fed with a basal diet containing 0, 0.5, 1 and 1.5 g/100 g fenugreek (*Trigonella foenum-graecum*) seed meal for three months (Mostafa et al. 2009) and they found that the use of 1 g/100 g fenugreek seed meal improved the fish growth performance. However, although some herbs have positive effects on the fish growth (Xie et al., 2008; Mahdavi et al., 2013), other herbal supplements have not been observed to have any effects. For example, Farahi et al. (2012) found that dietary supplementation with

Aloe vera did not promote the growth performance of rainbow trout (*Oncorhynchus mykiss*). Yilmaz et al. (2012) reported that the WG, FCR and SGR of sea bass (*Dicentrarchus labrax*) were unaffected by 1000 mg/kg dietary rosemary (*Rosmarinus officinalis*) and fenugreek. Also, the growth rate of koi carp (*Cyprinus carpio*) was unaffected by dietary supplementation with tetra (*Cotinus coggygria*) (Bilen et al., 2013).

Digestive enzymes play a significant role in the hydrolysis of proteins, lipids and carbohydrates, thereby assisting with the assimilation of nutrients. These nutrients are transported into the tissues and incorporated into cellular materials or used as an energy source for growth and reproduction (Furne et al., 2005). The digestion of foods begins with the digestive enzymes in the stomach and continues in the intestine with the digestive enzymes secreted by the pancreas, such as trypsin, chymotrypsin, amylase and lipase (Cockson and Bourne, 1972; Moriarty, 1973; Fang and Chiou, 1989).

Our study did not find any significant differences in the amylase, lipase and trypsin activity levels in any of the experimental groups compared with the control group. This result is in line with a study done by Iqbal et al. (2016), who found no significant differences in the digestive enzyme activity and haematology of juvenile *Labeo rohita* following supplementation with different plant and animal origin feeds (fishmeal). In contrast, a study by Kawai and Ikeda (1973) reported an increase in amylase activity in *O. mykiss* when fed a diet containing increased amounts of plant protein.

Many fish physiologists have concentrated on haematological studies as this has proved a valuable diagnostic approach for evaluating fish quality (Kori-Siakpre et al., 2005; Oluyemi et al., 2008; Patra et al., 2014). Variations in the haematological parameters of fish are due to environmental stress (Hickey, 1982), malnutrition (Casillas and Smith, 1977), gender (Siddique and Naseem, 1979; Collazos et al., 1998), fish size (Garcia et al., 1992), seasonal differences and breeding efficiency (Cech and Wohlschlang, 1981). The blood characteristics of fish are therefore an effective and sensitive index for monitoring physiological and pathological changes (Iwama et al., 1976; Chakrabarti and Banerjee, 1988; Orun et al., 2003; Patra et al., 2014). The present study indicated no significant effect on the blood parameters of *C. carpio* supplemented with 0.01%, 0.05%, or 0.1% *T. tomentosa* extract over the study period. This result suggested that the extract tested here did not stress the fish physiologically.

Lysozyme is an important enzyme in the humeral nonspecific defence mechanism that provides defence against microbial invasion (Evelyn, 2002). The bactericidal action of this enzyme involves the hydrolysis of the peptidoglycan layers of the bacterial cell wall, which lyses the cells and prevents colonisation by microorganisms (Saurabh and Sahoo, 2008). Lysozyme also induces antibacterial activity in the presence of a complement (Harikrishnan et al., 2011). The present study recorded significantly increased lysozyme activity in the 1% supplemented group compared with the control. Increasing lysozyme activity is in agreement with several reports on herbal immunostimulants (Rao et al., 2006;

Choi et al., 2008; Bilen et al., 2011). Similar results were observed for common carp when fed a diet supplemented with methanolic extracts of *Cotinus coggygria* (Bilen et al., 2014) or various Chinese herbal extracts (Jian and Wu, 2004).

Neutrophils contain myeloperoxidase in their cytoplasmic granules (Rodriguez et al., 2003). Myeloperoxidase is an important enzyme with microbiocidal properties as it utilises a reactive oxygen species (H₂O₂) to produce hypochlorous acid (Dalmo et al., 1997). This process is believed to be key in killing microorganisms (Johnston, 1978). Although some studies have shown increased myeloperoxidase activity in fish following supplementation with, for example, quercetin or black cumin seed oil (Awad et al., 2013; Alexander et al., 2010; Bilen et al. 2013), our study showed no significant effect on myeloperoxidase activity following supplementation with *T. tomentosa* extract at different concentrations.

Phagocytosis and the respiratory burst response by phagocytes in blood and tissues present a major antibacterial defense mechanism in fish (Secombes, 1996). Respiratory burst activity measured by nitroblue tetrazolium (NBT) is one of the most important bactericidal mechanisms in fish (Secombes and Fletcher, 1992). In this study, the respiratory burst levels of common carp were significantly decreased on days 15, 30 and 45 of supplementation with 0.1%, 0.05% and 0.01% *T. tomentosa* extract, respectively, compared with the control group. For the groups supplemented with 0.05% and 0.1% extract, on days 30 and 45 of the experiment we found a significant increase in this parameter compared with the control. This was similar to results observed by Bilen et al. (2011) when *O. mykiss* was supplemented with *Cotinus coggygria* leaves. Harikrishnan et al. (2010) reported a significant increase in respiratory bursts for olive flounder supplemented with three different Korean plants. Haghighi and Rohani (2013) reported a significantly higher respiratory burst level in rainbow trout fed a commercial diet containing *Zingiber officinale*. Our results are also in agreement with several studies on the use of dietary immunostimulants in various fish species (Yin et al., 2009; Bilen and Bulut, 2010).

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Conflict of Interest

The authors declare that there is no conflict of interest.

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MARINE SCIENCE AND TECHNOLOGY BULLETIN

RESEARCH ARTICLE

Investigation of Beyler Dam Lake water quality in terms of some physical and chemical parameters.

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ABSTRACT

This study was carried out for a period of 12 months from September 2016 to August 2017 at 4 stations determined in Beyler Dam Lake in Kastamonu, Turkey. In this study, some parameters such as temperature, pH, conductivity, dissolved oxygen, ammonium, nitrate, nitrite, phosphate, chemical oxygen demand (COD), biological oxygen demand (BOD) were investigated. Temperature, pH, conductivity, turbidity and the amount of dissolved oxygen of the water samples were measured by multi parameter measurement device in the field during sampling. Chemical parameters such as ammonium, nitrate, nitrite, phosphate, BOD and COD were determined by spectrophotometric methods in laboratory conditions. Study indicates such results as; the temperature was between 0.70 and 23.50 °C and the mean temperature was 11 °C, pH was between 7.46 and 9.50 and average pH was 8.34, amount of dissolved oxygen varied from 7.48 to 12.16 mg/l and the average dissolved oxygen amount was 9.73 mg/l, conductivity was between 102.50 and 240 µS cm⁻¹ and the average conductivity was 157.68 µS cm⁻¹, turbidity changed from 1.23 to 5.57 FNU/NTU and the average turbidity was 3.0 FNU/NTU, nitrate varied from 0.003 to 1.0 mg/l and the average nitrate was 0.35 mg/l, nitrite was between 0.001 and 0.007 mg/l and the average nitrite was 0.003 mg/l, ammonium changed from 0.009 and 0.130 mg/l and the average ammonium was 0.052 mg/l, phosphate was between 0.24 and 5.21 mg/l and the average phosphate was 2.541 mg/l, COD which is an important water quality parameters, was detected as the lowest 0.04 mg/l and the highest 28.1 mg/l and the average was found 9.45 mg/l, BOD was detected between 2.50 and 6.0 mg/l and the average BOD was calculated 4.17 mg/l. The results of the study revealed that there is no significant difference between the measured parameters according to the Surface Water Quality Regulation (SWQR) in Turkey. Although Beyler Dam Lake was evaluated as Class I for most parameter values, in total it can be classified as Class II according to water quality classification.

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Introduction

Currently, environmental problems are the most important threats to the ecological balance. Environmental

pollution emerged for the first time with the beginning of urban life and increased in line with industrial development. In particular, in the second half of the 20th century, the environmental pollution increased because of the accelerated growth rate of population, causing more pollution of living resources. Consequently, ecosystem degradation has become a considerably serious issue. As a matter of fact, when water

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environment, which is a part of the ecosystem, is used as a receiver and remover for used water and other wastes, it becomes the most intensively contaminated part of the ecosystem compared to air and soil. These polluting elements disrupt the natural balance and can be classified as follows: organic wastes, industrial wastes, petroleum derivatives, artificial agricultural fertilizers, detergents, radioactive materials, pesticides, inorganic salts, artificial organic chemicals, and waste heat. According to this classification, heavy metals fall under industrial wastes and some pesticides and they reach a level that can threaten the ecological balance (Kaya et al., 1998; Yarsan et al., 2000).

Different factors contribute to pollution, and the levels of quality parameters vary according to the areas in which they are used. Based on their sources, contaminants can exhibit a physical, chemical, or biological character. In this context, many parameters, including temperature, pH, oxygen level, electrical conductivity (EC), turbidity, nitrate content, nitrite content, phosphate content, biological oxygen demand (BOD), and chemical oxygen demand (COD) can be considered as the basic criteria for determining the water quality and pollution level, particularly in surface waters. These criteria are considerably important in terms of the lifecycle of the biodiversity of a water resource and are directly related to human health due to their use as drinking and irrigation water. An increase in the number of pollution elements and their dynamic structure in accordance with the changes in the characteristics of these elements require repetition of studies on ecosystems at periodic intervals. The determination of water quality parameters of water resources is important as they serve as a guide for the decision-making mechanisms and effective water management.

In line with the above aspects, some physicochemical water quality parameters of the Beyler Dam Lake, which is located within the boundaries of Devrekâni District in Kastamonu Province with a significant biodiversity, were monitored for a year and an attempt was made to determine the pollution level and the physicochemical quality of the dam lake.

Material and Methods

Beyler Dam Lake supplies irrigation water to the area of 5137 ha field for agricultural usage (Uğış et al., 2016). Therefore, it is an important water resource for the agricultural activities and production. In this study, sampling stations were chosen to demonstrate whole characteristics of the lake and samples were collected from 4 stations that illustrated in Figure 1. The physical structure of the dam lake, the area where it is fed, and the places where fishery facilities are located were considered while determining the location of the stations.

To investigate the physicochemical properties of the content of the lake water, water samples were collected for 12 months and stored in dark polyethylene bottles. First, the pre-washed bottles were rinsed with distilled water. Then,

the closed bottles were immersed in the lake at a depth of 1m and opened and closed again to expel the surface.

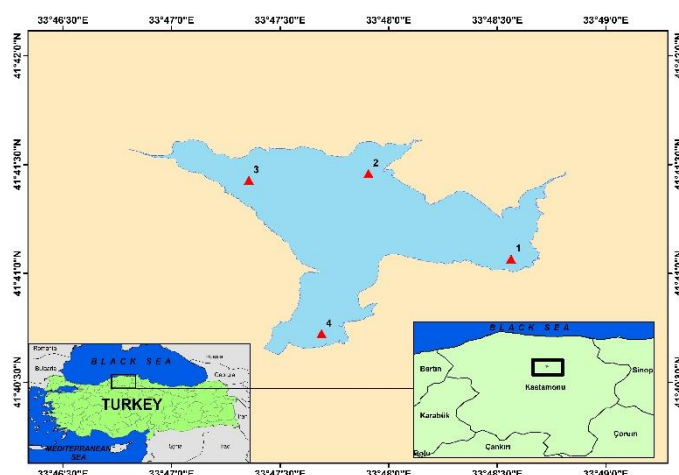


Figure 1. The location of sampling stations

Table 1. Quality classes of the intra-continental surface water sources according to the surface water quality regulation.

Water Quality Parameters	Water Quality Classes			
	I	II	III	IV
Temperature ($^{\circ}\text{C}$)	≤ 25	≤ 25	≤ 30	> 30
pH	6-9	6-9	6-9	6-9
Conductivity ($\mu\text{S}/\text{cm}$)	< 400	1000	3000	> 3000
Dissolved oxygen ($\text{mg O}_2/\text{L}$)	> 8	6	3	< 3
Chemical oxygen demand (COD) (mg/L)	< 25	50	70	> 70
Biological oxygen demand (BOD) (mg/L)	< 4	8	20	> 20
Ammonium nitrogen ($\text{mg NH}_4^+-\text{N}/\text{L}$)	< 0.2	1	2	> 2
Nitrite nitrogen ($\text{mg NO}_2--\text{N}/\text{L}$)	< 0.01	0.06	0.12	> 0.3
Nitrate nitrogen ($\text{mg NO}_3--\text{N}/\text{L}$)	< 3	10	20	> 20
Total phosphorus ($\text{mg P}/\text{L}$)	< 0.03	0.03-0.16	0.16-0.65	> 0.65

The parameters temperature, dissolved oxygen, pH level, and EC were recorded with the Hach Lange brand HQ40D model digital multicenter simultaneously with water sampling. Turbidity was also recorded momentarily with the WTW brand Turb[®] 430 turbidity meter during the collection of water samples. The parameters nitrate content, nitrite content, phosphate content, ammonium content, BOD, and COD were determined using a spectrophotometric method, and the same samples were analyzed at the same day using the Hach Lange UV-VIS spectrophotometer and Hach Lange LT200 the thermo reactors.

Water quality classes and interpretation of the measured water parameters were revised in 2012 based on the "Quality Criteria According to Classes in terms of General Chemical and Physico-chemical Parameters of Intra-continental Surface Water Resources" specified in the "Surface Water Quality Regulation" published in the official gazette in Turkey

(Anonymous, 2015; 2016).

Results and Discussion

Water temperature is one of the most important physical properties of the water ecosystems. It depends on the sun to a large extent as is the case for the pieces of land. Therefore, it changes according to the seasons, various hours of the day, air temperature, precipitation, its geographical position, and water depth. The water temperature directly and indirectly affects the water biology. Furthermore, water temperature is a decisive factor in terms of the water quality parameters because it indirectly affects some other water quality parameters (Sönmez et al., 2008).

In the study, the data for the water temperature is consistent with the data reported in the literature; however, it shows a variation over time, possibly because of the continuous fluctuations of the lake water in certain seasons, the lack of depth, the amount of precipitation, and the climatic conditions of the region. The overall average temperature was determined as 12.72°C, and the water quality of the dam lake was evaluated as Class I according to the Surface Water Quality Regulation. The changes in the temperature values are shown in Figure 2.

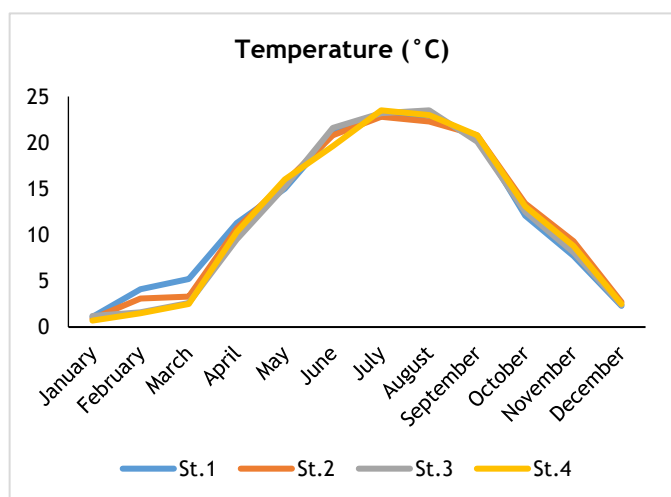


Figure 2. Monthly variation of temperature

The pH level is a measure of the acidity and alkalinity of a solution. It usually corresponds to the concentration of hydrogen ions and greatly affects the aquatic flora and fauna. The pH level in the range 6-8.5 usually constitutes the ideal living environment for many aquatic plants and animals. In particular, in the lakes, the value of the pH level affects the ratio of ammonium-ammonium dioxide. For this reason, the pH level is a considerably important water quality parameter. There is an increase in the pH level in the summer months. The value of the pH level largely depends on the amount of carbon dioxide in water and is inversely proportional to it. When the amount of carbon dioxide decreases, the pH level increases, whereas when the amount of carbon dioxide increases, the pH level decreases. Therefore, in summer, carbon dioxide is less soluble in water; thus, the pH level is higher. However, it is lower in winter (Sönmez et al., 2008). In terms of the pH level, the dam lake was rated as Class I

according to the Surface Water Quality Regulation. The monthly changes in the pH level at each station are shown in Figure 3.

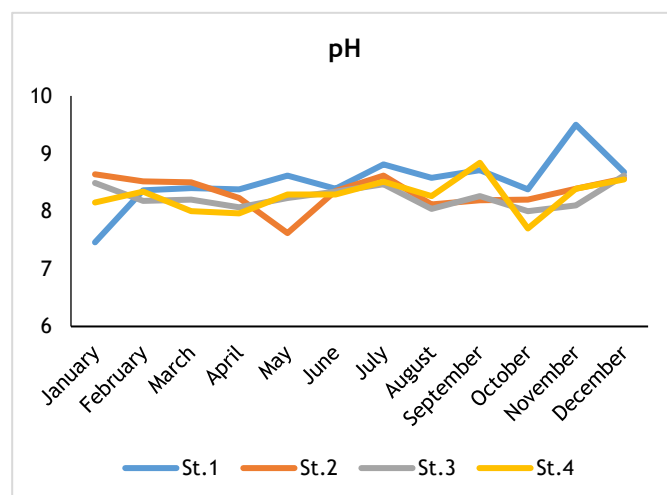


Figure 3. Monthly changes of the pH level

Dissolved oxygen is one of the most important parameters in the water quality assessment and reflects common biological and physical processes in the water. It is an important element for the life of water and the physical environment characteristics (Egemen, 2006). Additionally, it is of great importance to all living organisms and is regarded as the only parameter that can reveal the ecology of the whole water body. Eutrophic water bodies have a large dissolved oxygen range, while oligotrophic water bodies have a narrow dissolved oxygen range (Rucinski et al., 2010).

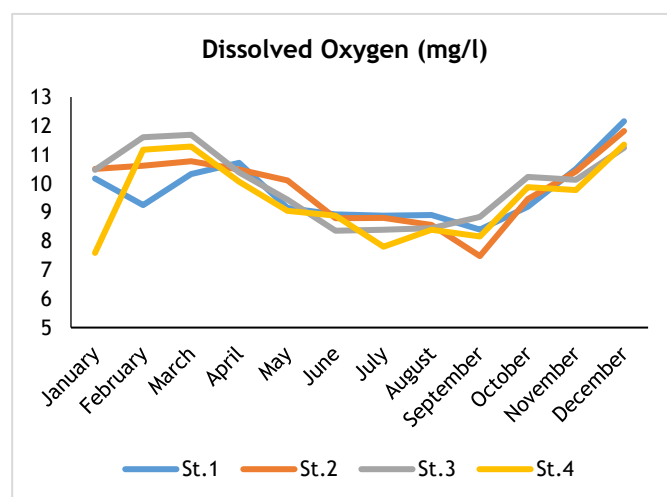


Figure 4. Monthly changes of dissolved oxygen (mg/l)

Ultimately, determining the level of dissolved oxygen is important in the quality assessment of all water resources. The lowest temperature value at the Beyler Dam, which was examined in terms of the dissolved oxygen level, was measured at the first station in September (7.48 mg/l), while the highest level of oxygen was detected in January (12.16 mg/l). In terms of the oxygen level, the dam lake water was evaluated as Class I. The results related to dissolved oxygen were consistent with those of research conducted by (Verpe et al., 2002, 2005; Tepe, 2009; Taş et al., 2010; Ates et al.

2016). Temporal distribution of dissolved oxygen is shown in Figure 4.

The main nitrogen compounds found in water according to the decreasing oxidation level were nitrate nitrogen ($\text{NO}_3\text{-N}$), nitrite nitrogen ($\text{NO}_2\text{-N}$), ammonia nitrogen ($\text{NH}_3\text{-N}$), and organic nitrogen (Org-N). The water quality can be determined by measuring these nitrogenous compounds. Nitrite is the intermediate product of the nitrogen cycle, does not accumulate in the environment, and quickly converts into nitrate. Nitrite also contributes to the development of plankton, such as nitrate. Taş (2011) and Nisbet and Verneaux (1970) reported that water pollution begins when the amount of nitrite in water exceeds 1 mg/l. Nitrogen compounds are important for determining the pollution level of water. In our study, the ammonium, nitrate, and nitrite content averages were determined as 0.051, 0.43, and 0.01 mg/l, respectively. Although there was an increase in some seasons within the year, in general terms, the parameter values were observed to be well below the reference values. In terms of all three parameters, the dam lake water was ranked as Class I. The monthly changes in the amounts of nitrate, nitrite, and ammonium at each station are shown in Figure 5.

The amount of phosphate in the dam reservoir varied between 0.235 and 5.21 mg/l, and the average was determined to be 2.31 mg/l. Phosphorus is the most basic element in eutrophication that occurs in the water environment (Harper, 1992). It has been reported that in most lakes, the average total phosphorus content varies between 0.010 and 0.030 mg/l. Tanyolaç (2004) and Nisbet and Verneaux (1970) suggested that when phosphate content is 0.115-0.30 mg/l in water, productivity is high, but when this value is higher than 0.30 mg/l, water is considered to have polluted. According to (Thomann and Mueller, 1987), the lake is oligotrophic when the total amount of phosphorus is less than 10 $\mu\text{g/l}$, mesotrophic when this amount is 10-20 $\mu\text{g/l}$, and eutrophic when this amount is greater than 20 $\mu\text{g/l}$. The amount of phosphate obtained in the study was above the limits specified in the Surface Waters Quality Regulation; therefore, the lake water has Class IV water quality. Monthly changes of phosphate content are shown in Figure 6.

The turbidity data obtained in this study varied between 1.23 and 15.5 NTU. In a study conducted by (Ates et al., 2016) at the Germeçtepe Dam, turbidity varied between 0.74 and 8.17 NTU. In a similar study conducted by (Alp et al. 2010) at the lakes of the Atatürk Dam, Birecik Dam, Karkamış Dam, and Hacı Hidir Dam located in the Southeastern Anatolian Region, turbidity varied between 5 and 20 NTU. They also reported that there was a high correlation between turbidity-TSS levels and sodium, potassium, and sulfate levels in particular, which tend to increase in seasons when the precipitation increases. In a study conducted by (Bayram and Kenanoglu, 2016) at the Borçka Dam, turbidity was reported to be 46 NTU. Our data are consistent with those reported in the literature and below the results reported in the literature in general. The increases in the transition seasons were evaluated as the addition of materials into the water through the rainfall. Although no category related to turbidity is

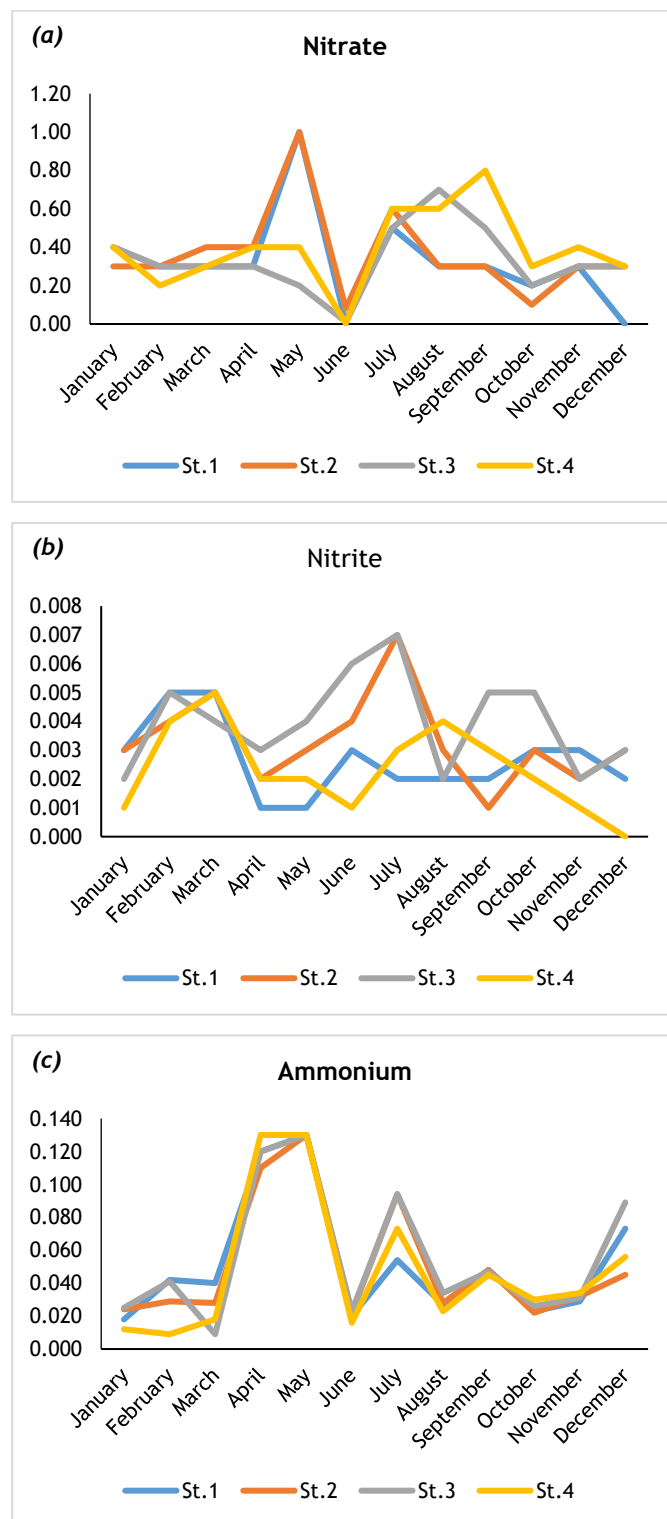


Figure 5. Monthly changes of the nitrate (a), nitrite (b), and ammonium (c) contents (mg/l)

mentioned in the Surface Waters Quality Regulation, it was found to be within the tolerable limits according to the WHO (2008) classification and high according to the US Environmental Protection Agency classification and the European Union (Anonymous, 1998) classification. The monthly changes in turbidity at each station are shown in Figure 7.

Based on our study data, we determined that the general electrical conductivity of the water in the Beyler Dam Lake

was between 102.50-240 μScm^{-1} and the water quality of this lake was evaluated as Class I according to the Surface Waters Quality Regulation. It was reported that the minimum and maximum EC values for the Ataturk Dam, Birecik Dam, Karkamış Dam, and Hacı Hıdır Dam were determined as 295-4345 μScm^{-1} , 314-447 μScm^{-1} , 310-479 μScm^{-1} , and 254-400 μScm^{-1} , respectively (Alp et al., 2010). The EC values measured for the Caspian Lake had a general average value of 2260 $\mu\text{m Hos/cm}$, and the water quality of this lake was evaluated as Class IV, indicating that the water of this lake was not suitable for irrigation (Ünlü et al., 2008). The conductivity in Eğrigöl was reported to vary between 210 and 291 μScm^{-1} and exhibited a homogeneous distribution in the lake (Başaran-Kaymakçı and Egemen, 2006). It was observed that the results of our study were below the general results reported in the literature. Temporal distribution of conductivity is shown in Figure 8.

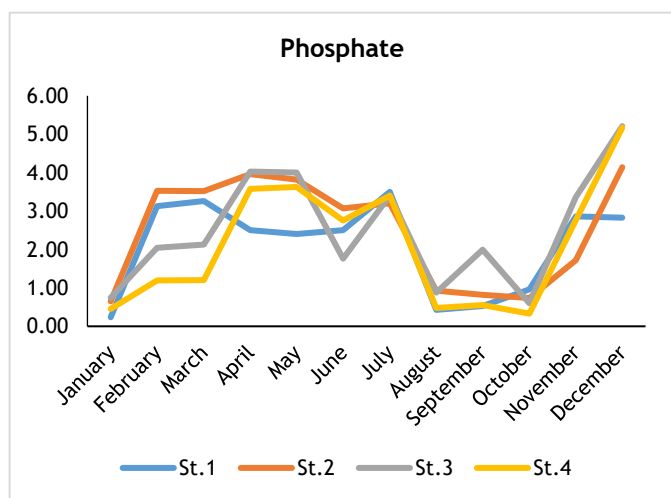


Figure 6. Monthly changes of phosphate content (mg/l)

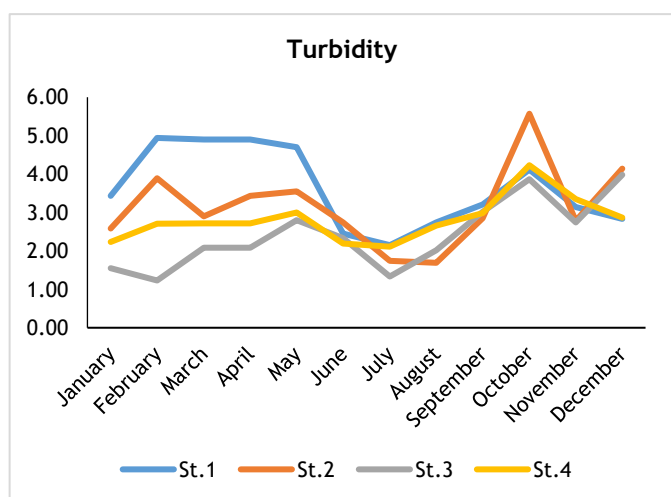


Figure 7. Monthly changes of turbidity (FNU/NTU)

The minimum and maximum COD values for the Beyler Dam were 0.1 and 28.1 mg/l, respectively. The dam reservoir water was evaluated as Class I in terms of the COD value, with an average value of 9.45 mg/l. In another study conducted to determine the water quality of the Uluabat Lake, the COD level value was found to be 49.33 mg/l in the summer, 56 mg/l in spring, 37.33 mg/l in winter, and 53.13

mg/l in autumn (İleri et al., 2014). In a study conducted at the Uluabat Lake, the annual average of the COD value was reported to be 35.74 mg/l (Elmacı et al., 2010). In various other studies, the COD value was reported as 16.32 and 20.23 mg/l for the Görentaş Lake (Tepe et al., 2004), 7.80-42.19 mg/l for the Karagöl Lake (Mutlu et al., 2013), and 18-41 mg/l for the Reyhanlı Lake (Tepe, 2009). The data obtained in this study are consistent with those reported in the literature. The COD, which tended to increase in summer, exhibited a stable course in other months. COD is an important parameter that exhibits the extent of organic pollution in water. Due to an increase in the microbial activity in spring and summer, the rate of degeneration of organic substances increases, thereby increasing the COD value (İleri et al., 2014). The COD data are shown in Figure 9.

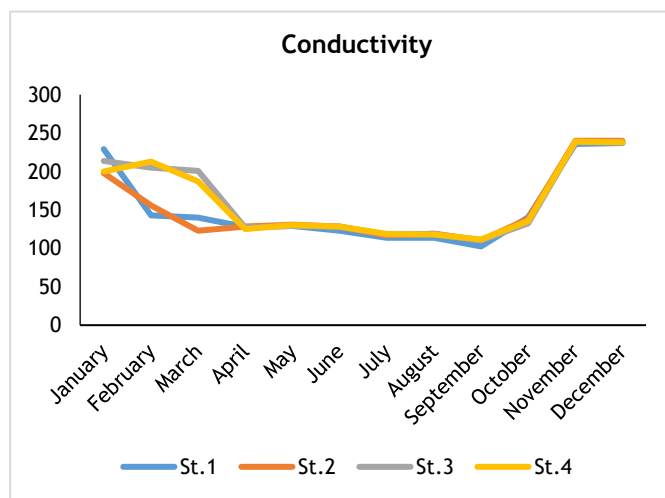


Figure 8. Monthly changes of conductivity ($\mu\text{S cm}^{-1}$)

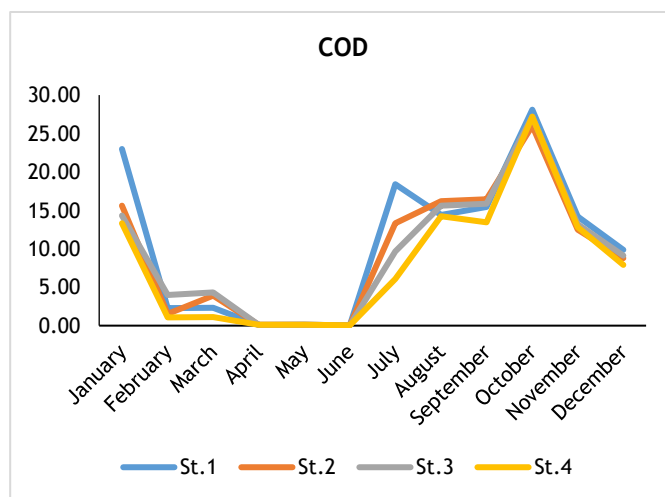


Figure 9. Monthly changes of chemical oxygen demand (mg/l)

BOD is an important parameter and is considered as a general measure of the organic pollution effect in the receiving waters. According to our BOD measurements results, the BOD value for the Beyler Dam varied between 2.50 and 6 mg/l. The dam reservoir water was evaluated as Class I in terms of BOD, with an average value of 4.17 mg/l. In a study conducted to determine the water quality of the Caspian Lake, the BOD value was reported to be 8.9 mg/l

(Ünlü et al., 2008). In another study conducted at the Germeçtepe Dam, the average BOD value was reported to be 21.26 mg/l (Ates et al., 2016). In the studies conducted at the Atatürk Dam, Birecik Dam, Karkamış Dam, and Hacı Hıdır Dam to determine their water quality, the BOD values varied in the ranges of 1-2.4 mg/l, 0.6-1.8 mg/l, 1.1-2.7 mg/l, and 2.3-6.8 mg/l, respectively (Alp et al., 2010). The data obtained in this study were consistent with those reported in the literature, and it was interpreted that there was no organic pollution load at a level that could cause pollution. The monthly changes in BOD at each station are shown in Figure 10.



Figure 10. Monthly changes of biological oxygen demand (mg/l)

Conclusion

Our study was conducted at the Beyler Dam Lake, the lake water of which was found to belong to the Class I water quality for the most parameters. But on the whole, it can be classified as Class II due to exception of phosphate content parameter, for which the lake water was evaluated as Class IV according to SWQR in Turkey. Phosphorus consistence in natural waters depends on morphometry of the basin, chemical context of the region's geological structure, organic matter that enters into the water, domestic flow (especially if it is detergent) and organic metabolism in the water (Taş, 2011). Since there is not any industrial activity around the lake, the source of phosphorus surplus may be domestic wastes or agricultural activities.

When the overall water quality of the dam lake was assessed, there usually was no difference between the stations. This was attributed to the fact that the area of the lake was small and the distance between the stations was less. The occurrence of seasonal parameter variations from time to time was explained by the fact that the lake volume is small and there is no strong source to feed the lake.

Nevertheless, analyzing the overall water quality of the lake, we found that the lake is not under intense pollution pressure and that it remains considerably virgin. This virginity is primarily attributed to the fact that the lake has an almost closed structure, so the weak water supply does not result in

much pollution. In contrast, there are no settlements in the vicinity of the dam lake and the amount of agricultural land is limited. It is also close to other pollutants due to road conditions. The lake water was considered to be a convenient environment for the biological life it hosts.

Acknowledgement

This study has been summarized from Khalifa Moftah Abdelali's PhD thesis study at Kastamonu University Institute of Natural and Applied Sciences Aquaculture Department.

Conflict of Interest

The authors declare that there is no conflict of interest.

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MARINE SCIENCE AND TECHNOLOGY BULLETIN

RESEARCH ARTICLE

A study on fatty acid profile and some mineral contents of mantis shrimp (*Erugosquilla massavensis* Kossmann, 1880) from Northeastern Mediterranean Sea (Turkey).

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ABSTRACT

This study was carried out to detect the percentage content of fatty acids (FA) and some minerals (Ca, Mg, K, Na, Zn, Fe) of 40 mantis shrimp (*Erugosquilla massavensis*) obtained from Northeastern Mediterranean Sea, Turkey. The protein and fat contents were identified as 13.10±0.1% and 2.06±0.5, respectively. Distribution of fatty acids in samples was SFA > MUFA > PUFA. The order of average mineral concentrations found in samples was Mg>K>Na>Ca>Zn>Fe. The results showed that the ratio of PUFA/SFA (0.29) of mantis shrimp was not within the range reported as good (0.45) for human diets.

Keywords:

Erugosquilla massavensis
Mantis shrimp
Fatty acids
Minerals

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Introduction

Mantis shrimp, *E. massavensis* is a potentially edible benthic crustacean that have a small yet growing economic important in the markets (rare on the markets of Cyprus, Israel and Turkey) and it is also an important resource for the Mediterranean demersal fisheries in Spain, Italy, Egypt, and

Morocco (Rossetti et al., 2005; Fahmy and Hamdi, 2011, Salam and Hamdi 2015; Fard et al., 2016; Sealifebase, 2018). In Asia, the importance in mantis shrimp as a fishery resource has long been recognized (Zamri et al., 2016). Therefore this crustacean is a favorite seafood in Japan, China, Malaysia, Indonesia, Hong Kong, and Taiwan and has been commercially exploited by small bottom-trawlers and gill nets (Fard et al., 2016).

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E. massavensis originating from Persian Gulf and Red Sea migrated through Suez Canal into the Mediterranean Sea where it was firstly recorded off the Mediterranean coast of Egypt (Amor et al., 2015). *E. massavensis* is distributed along the entire Mediterranean coast of Turkey, with a westernmost limit extending as far as Fethiye (Türeli et al., 2017). The species more recently was recorded from the eastern region of the Libyan coast and then from Tunisian waters. *E. massavensis* is now widely distributed along the Levantine coasts; the south, eastern and western Aegean Sea; the Marmara Sea; westwards toward Egypt; and the central Mediterranean. (Foka et al., 2017). Although it has been reported from the varying depths between 150-200 m in southern Aegean coast of Turkey (Özcan et al., 2008), it is regularly caught between 20 and 80 m above the muddy, sandy, argillaceous funds and of gravel (Rossetti et al., 2005; Gökoglu et al., 2008; Sealifebase, 2018).

Recently, many studies have been done on the nutritional value of seafood which has low saturated fat, high omega-3 polyunsaturated fatty acids (PUFA), and high-quality protein, amino acids and minerals (Gökoglu and Yerlikaya, 2003; Celik et al., 2004; Olgunoglu et al., 2011; Ayas and Ozogul, 2012; Olgunoglu and Olgunoglu, 2017, Göçer et al., 2018). Especially polyunsaturated fatty acids (PUFAs) have been recognized to have special pharmacological and physiological effects on human health. They are beneficial for the reduction of coronary artery disease (Cherif et al., 2008). The minerals in diets also participate in several biochemical reactions and serve as components of bones, soft tissues and co-factors and co-activators of various enzymes important in human nutrition (Soundarapandian et al., 2014). Therefore, many authors have recently investigated the mineral and the fatty acid (FA) profiles of different crustacean species in various parts of the World (Oksuz et al., 2009; Tag El-Din et al., 2009; Sağlık and Imre, 1997; Ouraji et al., 2011; Turan et al., 2011; Yanar et al., 2011; Fatima et al., 2013). However, the studies on fatty acid profiles in mantis shrimp (*E. massavensis*) is very limited. Most studies on mantis shrimp focus on the biology, fishery, and population structures (Fard et al., 2016). Literature reviews have also showed that there is no enough information on investigation of the mineral contents of mantis shrimp was available. Therefore, the current study is carried out to evaluate the nutritional value and the fatty acid profiles of mantis shrimp (*E. massavensis*) caught in the Northeastern Mediterranean Sea, Turkey.

Material and Methods

Collection and Preparation of Samples

Mantis shrimp were captured along the coast of Mediterranean Sea (Turkey) by using fishing nets in June 2017 (Figure 1). Immediately, after capturing, mantis shrimp were stored in a plastic container over a layer of ice in a cooler and transferred to the laboratory. After removing the heads, shells and intestines, the meat of mantis shrimps are kept at -18°C until chemical analysis. The total number of samples was 40.

Chemical Analysis

The crude protein analysis of mantis shrimp samples was carried out according to the Kjeldahl Method and the fat was determined according to the Acid Hydrolysis Soxhlet System (AOAC, 1995). Inductively coupled plasma-optical emission spectrometry (Perkin Elmer-NexION 350X) was used to determine phosphorus (K), magnesium (Mg), sodium (Na), calcium (Ca), zinc (Zn) and iron (Fe) in the samples. The analyses were performed at least in triplicate and the concentrations were expressed as mg/100g wet weight. IUPAC (1979) Methods II. D. 19 was used to prepare the methyl esters of fatty acids of mantis shrimp samples. To determine the fatty acid composition of samples, analyses were done by using a Perkin Elmer Autosystem XL Gas Chromatography and Flame Ionization Detector (FID) equipment and a Supelco 2330 fused silica capillary column (30 m \times 0.25 mm \times 0.20 μm film thickness).

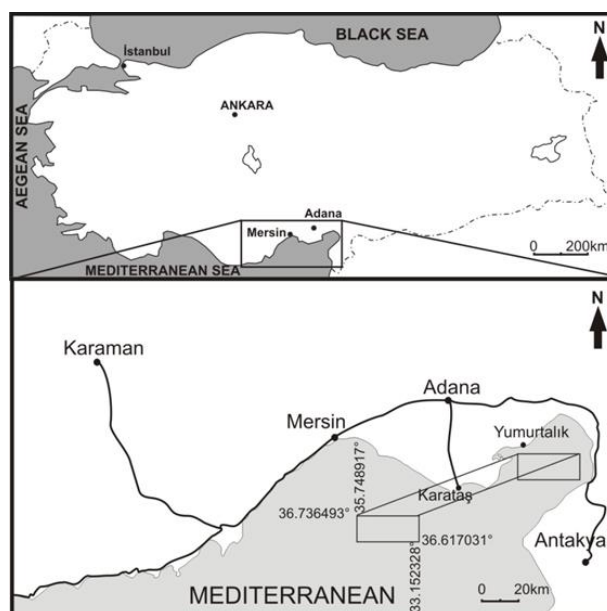


Figure 1. Sampling area in the Northeastern Mediterranean Sea

Results

There are limited studies in relating to fatty acids and macro minerals of mantis shrimp, which were investigated in this study. Table 1 shows, protein and fat contents of *E. massavensis* examined in the study. The fat and protein content of aquatic organisms is a crucial factor to evaluate the nutritional condition (Mahaliyana et al., 2015). According to the results of our study mantis shrimp consists of $13.10 \pm 0.1\%$ protein and $2.06 \pm 0.5\%$ fat, respectively.

Table 1. The quantity of the crude protein and fat in *E. massavensis*

Parameters	<i>Erugosquilla massavensis</i>
Protein	$13.10 \pm 0.1\%$
Fat	$2.06 \pm 0.5\%$

The fatty acid profile in the fat of analyzed mantis shrimp was shown in Table 2.

Table 2. The quantity of the crude protein and fat in *E. massavensis*

Fatty Acids	<i>Erugosquilla massavensis</i> (%)
C4:0	0.40±0.02
C6:0	0.22
C10:0	0.13±0.03
C11:0	0.75±0.01
C12:0	0.30±0.01
C13:0	0.16
C14:0	3.57±0.02
C15:0	1.10±0.01
C16:0	21.59±0.13
C17:0	0.93±0.02
C18:0	15.21±0.01
C20:0	0.26±0.01
C21:0	0.47±0.43
C22:0	0.18±0.02
C23:0	11.83±0.02
C24:0	0.28±0.02
ΣSFA	57.38
C14:1	0.36±0.03
C15:1	0.51±0.04
C16:1	18.09±0.12
C17:1	0.11±0.05
C18:1n-9	3.94±0.01
C22:1n-9	0.46±0.55
C24:1	1.20±0.14
ΣMUFA	24.67
C18:2n-6	0.93±0.14
C18:3n-6	0.62±0.03
C20:2	1.63±0.16
C20:3n-6	3.73±0.12
C20:4n-6	0.29
C20:5n-3 (EPA)	0.61±0.33
C22:2	0.12±0.00
C22:6n-3 (DHA)	8.66±0.53
ΣPUFA	16.59
Σn3	9.27
Σn6	5.57
PUFA/SFA	0.29

The fatty acid content was 57.38% saturated (SFAs), 24.67% monounsaturated (MUFAs), and 16.59% polyunsaturated acids (PUFAs). Distribution of fatty acids in *E. massavensis* were seen as SFA > MUFA > PUFA. The major saturated fatty acids (SFAs) of mantis shrimp in this study were C16:0 (palmitic acid, 21.59±0.13%) and C18:0 (stearic acid, 15.21±0.01%). The most abundant monounsaturated fatty acids (MUFA) is oleic acid (C18:1n-9) with a level of 3.94±0.01%. The highest PUFAs was docosahexaenoic acid (DHA, C22:6n-3), contributing approximately 52% of the total PUFA content.

The concentration levels of six minerals (Ca, Mg, K, Na, Zn, Fe) of *E. massavensis* are shown in Table 3. The order of average mineral concentrations found in *E. massavensis*

samples were Mg>K>Na>Ca>Zn>Fe (Table 3).

Table 3. The mineral contents (mg/100g) in *Erugosquilla massavensis*

Parameters	<i>E. massavensis</i>
Calcium (Ca)	90.07±1.22
Magnesium (Mg)	431.43±10.13
Potassium (K)	272.40±6.22
Sodium (Na)	90.43±2.13
Zinc (Zn)	3.36±0.03
Iron (Fe)	0.24±0.07

Discussion

Seafood helps human beings to maintain good health by providing all essential nutrients consuming a variety of foods in balanced proportions, and will prevent deficiency diseases and chronic diet-related disorders (Rexi et al., 2015). The nutritional composition of marine organisms may change greatly from one species to another species depending on collection method, handling procedures, age, sex, environment and season with protein levels ranging from 16 - 21% and lipids 0.1 - 25% (Ozer, 2004; Lilly et al., 2017). The protein and fat content of *E. massavensis* is close to what has been previously reported for other different marine organisms.

The results on fatty acids profile obtained in our study are agreement with studies reported by several authors on fatty acids found in various species and subspecies of sea and freshwater shrimps (Oksuz et al., 2009; Tag El-Din et al., 2009; Saglik and Imre, 1997; Ouraji et al., 2011; Turan et al., 2011; Yanar et al., 2011; Fatima et al., 2013). However, in a similar study on mantis shrimp (*E. massavensis*), different percentage compositions of fatty acids were also reported by Ayas and Ozogul (2012). In their study, SFA, MUFA and PUFA rates in *E. massavensis* were reported as 33.82%, 23.84% and 35.44% respectively. The results obtained in this study showed differences with the findings of the mentioned researchers. These differences may be explained by geographical variation, seasonal conditions and different types of diet and feeding system in mantis shrimp. Fatty acid content could be also influenced by maturity period, size and age of shrimp. In the present study, PUFA/SFA ratio was of 0.29 for the mantis shrimp, which was lower than the minimum suggested (0.45) for a human healthy diet (Mendoza et al., 2014). In our study, in contrast to previous reports for some marine organisms, the examined *E. massavensis* demonstrated a lower percentage of EPA and DHA. The difference could be attributed to locations of sampling and the kind of solvents used for lipid extraction (Ridzwan et al., 2014).

Calcium (Ca) and Magnesium (Mg) are major component of bones therefore they are important for bone formation. Small fish is known to be a good source of these minerals Potassium (K) and sodium (Na) are important for muscle contractions, transmission of impulses in the nerves and sugar metabolism. Zinc (Zn) is a component of many metalloenzymes, important for gene expression and cellular growth. Iron (Fe) is mostly

important for transporting oxygen around the body (Mogobe et al., 2015).

Palani et al. (2014) pointed out that the average Ca contents in the fish species range from 64 to 1887 mg/100g. Bernard and Bolatito (2016) reported that mineral level of Mg, Ca, Na, K, Zn, Fe as 174.8 mg/100g, 134.8 mg/100g, 199.2 mg/100g, 52.45 mg/100g, 42.1 mg/100g, 28.05 mg/100g for *Penaeus notialis* and as 128.8 mg/100g, 142.2 mg/100g, 117.3 mg/100g, 89.1 mg/100g, 45.55 mg/100g, 41.25 mg/100g for *Penaeus monodon* respectively. The values of various minerals in *Parapenaeus longirostris* obtained from Marmara Sea were found to be in the range of 79.04-101.61 mg/100g for Ca, 49.57-65.02 mg/100g for Mg, 281.37-331.78 mg/100g for Na, 370.22-447.96 for K, 1.109-1.770 for Zn and 6.176-8.843 mg/100g for Fe (Ozden, 2010).

Conclusion

The result of the present study demonstrated that the PUFA/SFA of mantis shrimp (*E. massavensis*) from Mediterranean Sea was not within the range reported as good for human diets. This study also showed that this species has low protein, fat and mineral contents except Mg, K and Ca when compared with other economical shrimp species.

Conflict of Interest

The authors declare that there is no conflict of interest.

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MARINE SCIENCE AND TECHNOLOGY BULLETIN

RESEARCH ARTICLE

The effects of season on the metal levels of tissues of some lessepsian species caught from the Northeastern Mediterranean Sea.

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ABSTRACT

The concentrations of iron, copper, zinc, chromium, arsenic, cadmium and lead were determined by inductively coupled plasma mass spectrometer (ICP-MS) in the muscle, gill, brain and liver tissues of lessepsian fish species sampled from Taşucu region located on the south of Turkish coastal waters in the Mediterranean Sea for all seasons. While iron showed the highest levels, cadmium showed the lowest levels in the examined tissues of all fish species (*Pelates quadrilineatus*, *Upeneus moluccensis*, *Nemipterus randalli*, *Saurida lessepsianus*). Metal levels showed tissue-dependent changes in the species studied. Cadmium was detected only in the liver tissue in all fish, in addition to a few other tissues (*U. moluccensis*'s brain tissues in summer season and *P. quadrilineatus*'s gill tissues in winter season). The maximum accumulation of Fe except for *N. randalli* and *S. lessepsianus* was detected in liver tissue. The maximum accumulation of Cu in all species was detected in liver tissue. The maximum accumulation of Zn except for *S. lessepsianus* was also detected in liver tissue. The highest accumulations of As and Cr in other fish species except for *N. randalli* were also detected in liver tissue. Except for As accumulation in *N. randalli*, the least accumulation for metals in all species was determined in muscle tissue. According to the seasons, there was no statistically significant relation between metal accumulations. Metal concentrations in edible parts of fish species were 17.26-108.22 µg g⁻¹ dw for iron, 0.54-3.65 µg g⁻¹ dw for copper, 11.50-31.17 µg g⁻¹ dw for zinc, 0.32-1.09 µg g⁻¹ dw for chromium, 4.32-69.44 µg g⁻¹ dw for arsenic, below limit (not detectable) for cadmium, N.D.-1.12 µg g⁻¹ dw for lead. In this study, for all metals except arsenic there is no health risk through an exposure of consumption of certain fish. Additionally, the results obtained for the elements in analyzed fish species were within acceptable limits for human consumption.

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Introduction

Lakes, rivers, seas and oceans have been ignored by people for a long time and used as waste zones which are thought to have unlimited capacity. The widespread discharge of industrial, agricultural and domestic wastewater to coastal waters is becoming widespread in many parts of the world. As

a result, pollution has increased rapidly in the water environments of coastal and inland areas and affects the living things in this environment negatively.

In recent years, fish consumption in the world has increased in parallel to its nutritive and therapeutic properties (Storelli, 2008). Besides being an important protein source, fish has rich nutritional content such as basic minerals,

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vitamins and unsaturated fatty acids (Medeiros et al., 2012). The American Heart Association recommended eating fish at least twice per week in order to reach the daily intake of omega-3 fatty acids (Kris-Etherton et al., 2002). However, despite their known benefits, fish are susceptible to metal contamination and when the metal accumulation in the food chain is taken into consideration, fish species can become a serious risk for human health, especially the most consumable fish species (Castro-Gonzalez et al., 2008). Therefore, it is important to investigate the amount of metal accumulation of different fish species in order to provide data on how heavy metals will reach human beings by consuming fish (Visnjic-Jeftic et al., 2010; Kalyoncu and Arslan, 2012).

There are many studies on the target organs of heavy metals in fish used as bioindicators in the aquatic ecosystem (Kalay et al., 1999; Atli and Canli, 2003; Karayakar et al., 2010, 2017; Kulcu et al., 2014). Fish can accumulate high amounts of metal by absorbing metals from the gill surface, kidney, liver and intestinal system (Annabi et al., 2013). Fish can also accumulate heavy metals that are absorbed directly from the water environment or food chain at higher levels than the environmental concentration (Bervoets and Blust, 2003). The accumulation of heavy metals by organisms can be passive or selective. Differences in the accumulation of heavy metals among the living beings are due to assimilation, egestion, or differences in both (Egila and Daniel, 2011).

As the metal accumulation in fish changes according to the metal it is exposed to, it varies according to many factors such as species, tissue (Petrovic et al., 2013), gender, age, size, breeding cycle, swimming style, feeding behavior and living environment of fish (Canli and Atli, 2003; Zao et al., 2010). Mersin Bay, which is located in the Eastern Mediterranean coast of Turkey, has high potential for pollution due to heavily used in agricultural activities fertilizers and pesticides, domestic waste in the region, wastes from the chrome, plastics, fertilizers, glass and port industries (Kalay et al., 2004) and due to the increased population (Karayakar et al., 2010).

With the opening of the Suez Canal and the establishment of the Aswan Dam, no geographical obstacles remain between the Red Sea and the Mediterranean Sea. Indo-Pacific species have begun to migrate to the Eastern Mediterranean in terms of ecological parameters such as salinity and temperature, similar to those of the Red Sea. These species are able to find suitable areas in the Mediterranean Sea due to their feeding habits, habitat and their depth of distribution. That's why, their transition from the Red Sea to the Mediterranean has been accelerated (Golani, 2002).

The most important lessepsian species for the fishing industry of the Northeastern Mediterranean Sea are the Randall's threadfin bream (*N. randalli* Russell, 1986), the brushtooth lizardfish (*S. lessepsianus* Russell, Golani and Tikochinski, 2015), the goldband goatfish (*U. moluccensis* Bleeker, 1855) and the fourlined terapon (*P. quadrilineatus* Bloch, 1790). The aim of this study is to reveal the seasonal changes in the levels of metal accumulation in the tissues (muscle, gill, brain, liver) of some important Lessepsian fish species; *N. randalli* (Russell, 1986), *S. lessepsianus* (Russell, 2015), *U. moluccensis* (Bleeker, 1885), *P. quadrilineatus*

(Blotch, 1790) in the Northeast Mediterranean Sea.

Material and Methods

Materials

Samples were caught by trawl net from Taşucu region located on the south Turkish coastal waters of the Mediterranean Sea for all seasons. In every season, 40 specimens in each species were taken (except for *N. randalli* and *S. lessepsianus* in the summer season, for *P. quadrilineatus* except for spring season) and placed in polystyrene boxes with ice and brought to the laboratory within 1 hour and kept at -18°C until analysis. The size and weight of all samples were measured and given in Table 1. The tissues from 40 individuals of each species were taken out. All samples of the homogenates were analyzed on triplicate.

Table 1. The mean total lengths and weights of the samples

Season	Species	Lengths (cm)		Weights (g)	
		$\bar{X}\pm S_{\bar{x}}$	Min-Max.	$\bar{X}\pm S_{\bar{x}}$	Min-Max.
Spring	NR	16.03±0.28 ^x	15-17.3	61.38±2.31 ^a	53-71
	SL	-	-	-	-
	UM	10.56±0.33 ^x	8-12	9.78±0.41 ^a	8-27
	PQ	13.00±0.41 ^x	11-15	42.44±4.00 ^a	25-63
Summer	NR	15.66±0.56 ^x	13-17.3	55.75±5.29 ^a	37-65
	SL	-	-	-	-
	UM	12.22±0.14 ^y	11-13.2	28.78±1.14 ^c	22-35
	PQ	14.01±0.33 ^x	11.6-15.8	48.69±3.01 ^b	36-59
Autumn	NR	19.00±0.23 ^y	17.5-19.5	87.00±2.28 ^b	75-91
	SL	19.74±1.30 ^x	14-24	67.44±12.49 ^a	36-99
	UM	10,82±0,11 ^x	10-12	15.93±0.56 ^b	11-21
	PQ	14.00±0.20 ^x	33-47	40.31±1.56 ^a	12.7-14.5
Winter	NR	-	-	-	-
	SL	19.48±1.01 ^x	13-22	64.72±10.07 ^a	28-78
	UM	11.50±0.29 ^y	10-15	25.07±1.23 ^c	17-42
	PQ	13.48±0.12 ^x	13-14,5	41.13±1.20 ^a	36-52

Note: Superscript letters (a, b, c, d) in the same rows for each season indicate significant differences ($p<0.05$). Superscript letters (x, y, z, q) in the same columns for each tissue in the same species indicate significant differences ($p<0.05$). $\bar{X}\pm S_{\bar{x}}$: mean±standard error, NR: *N. randalli*, SL: *S. lessepsianus*, UM: *U. moluccensis*, PL: *P. quadrilineatus*.

Metal Analysis

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 perchloric 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 and Atli, 2003).

Inductively coupled plasma mass spectrometer (ICP-MS, Agilent, 7500ce Model, Japan) was used to determine metals. ICP-MS operating conditions were as 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 trace element (Fe, Cu, Zn) and toxic metal (Cd, Pb, As, Cr) 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 calibration curves were prepared by dilutions of the macro, trace elements and toxic metals. Solutions that have been prepared for the toxic metals had a content of lead, cadmium, arsenic and chromium in the range of 1-50 ppb (0.001 to 0.050 mg L⁻¹), for the trace elements had a content of copper, iron, and zinc in the range of 1-50 ppm (1 to 50 mg L⁻¹).

Statistical Analysis

One-way analysis of variance (ANOVA) was run using the SPSS version 22 software. Duncan's multiple range test comparisons at 0.05 significance level were used to evaluate the effects of season on the metal levels of the tissues (muscle, gill, brain and liver) of species.

Results

The highest accumulation of Fe in *N. randalli* is in the gills (1355.6 µg g⁻¹ dw) in the autumn season and the lowest accumulation is in the muscle (16.6 µg g⁻¹ dw) in the spring season. Similarly, the highest Fe accumulation in *S. lessepsianus* is in the gills (1332.55 µg g⁻¹ dw) in autumn season and the lowest Fe accumulation is in muscle tissue in spring season (17.26 µg g⁻¹ dw). The lowest accumulation of Fe in *U.*

moluccensis is in muscle tissue in spring season (41.64 µg g⁻¹ dw), the highest Fe accumulation is in liver tissue in autumn season (1912.31 µg g⁻¹ dw). The lowest accumulation of Fe in *P. quadrilineatus* is in muscle tissue in winter season (50.27 µg g⁻¹ dw), the highest Fe accumulation is in liver tissue in winter season (927.95 µg g⁻¹ dw) (Table 2).

According to the seasons, copper accumulates in the tissues: The highest accumulation in *N. randalli* is in the liver (122.56 µg g⁻¹ dw) in spring season and the lowest accumulation is in the muscle (0.88 µg g⁻¹ dw) in winter season. The highest accumulation in *S. lessepsianus* is in the liver (32.37 µg g⁻¹ dw) in winter season and the lowest accumulation is in the muscle (0.54 µg g⁻¹ dw) in winter season. The highest accumulation in *U. moluccensis* is in the liver (132.06 µg g⁻¹ dw) in winter season and the lowest accumulation is in the muscle (1.33 µg g⁻¹ dw) in spring season. The highest accumulation in *P. quadrilineatus* is in the liver (33.72 µg g⁻¹ dw) in winter season and the lowest accumulation is in the muscle (0.75 µg g⁻¹ dw) in autumn season (Table 3).

The highest accumulation of Zn in *N. randalli* is in the liver (554.4 µg g⁻¹ dw) in spring season and the lowest accumulation is in the muscle (11.50 µg g⁻¹ dw) in autumn season. The highest Zn accumulation in *S. lessepsianus* is in the brain tissue (135.08 µg g⁻¹ dw) in winter season and the lowest Zn accumulation is in muscle tissue in the same season (14.48 µg g⁻¹ dw). The lowest accumulation of Zn in *U. moluccensis* is in muscle tissue in spring season (16.13 µg g⁻¹ dw), the highest Zn accumulation is in liver tissue in summer season (640.55 µg g⁻¹ dw). The lowest accumulation of Zn in *P. quadrilineatus* is in muscle tissue in autumn season (15.98 µg g⁻¹ dw), the highest Zn accumulation is in liver tissue in winter season (153.25 µg g⁻¹ dw) (Table 4).

Table 2. The effects of season on iron levels of some tissues of the lessepsian species ($\bar{X} \pm S_{\bar{x}}$, µg g⁻¹)

Species	Season	Muscle	Gill	Brain	Liver
NR	Spring	16.60±1.63 ^{a,x}	387.99±68.86 ^{c,x}	142.7±23.66 ^{b,x}	616.33±73.22 ^{c,x}
	Summer	-	-	-	-
	Autumn	38.89±14.41 ^{a,x}	1355.6±235.26 ^{b,y}	112.9±22.35 ^{a,x}	929.46±180.82 ^{b,x}
	Winter	19.82±3.38 ^{a,x}	462.67±107.99 ^{c,x}	162.71±39.61 ^{b,x}	426.43±71.47 ^{c,x}
SL	Spring	17.26±2.33 ^{a,x}	598.58±130.63 ^{b,x}	221.29±88.37 ^{b,x}	425.69±78.97 ^{b,x}
	Summer	-	-	-	-
	Autumn	41.92±11.71 ^{a,x}	1332.55±79.11 ^{c,y}	384.19±102.59 ^{b,x}	301.01±20.83 ^{b,x}
	Winter	17.59±4.27 ^{a,x}	956.95±127.23 ^{c,xy}	244.89±50.59 ^{b,x}	863.95±50.84 ^{c,y}
UM	Spring	41.64±4.46 ^{a,x}	274.1±38.55 ^{b,x}	256.07±87.98 ^{b,x}	1841.91±214.88 ^{c,y}
	Summer	50.35±8.10 ^{a,xy}	494.44±115.39 ^{b,xy}	293.49±76.45 ^{b,x}	540.36±94.63 ^{b,x}
	Autumn	62.74±5.19 ^{a,xy}	486.58±36.94 ^{c,y}	193.54±17.61 ^{b,x}	1912.31±371.25 ^{d,y}
	Winter	67.09±4.94 ^{a,y}	868.42±207.88 ^{c,y}	216.43±30.90 ^{b,x}	1058.7±179.81 ^{c,xy}
PQ	Spring	-	-	-	-
	Summer	108.22±33.51 ^{a,x}	211.93±26.42 ^{ab,x}	142.21±24.29 ^{a,x}	394.02±86.23 ^{b,x}
	Autumn	52.39±11.63 ^{a,x}	372.05±36.45 ^{b,y}	240.62±46.09 ^{b,x}	920.68±117.05 ^{c,y}
	Winter	50.27±6.26 ^{a,x}	522.19±60.33 ^{c,y}	240.51±17.21 ^{b,x}	927.95±180.66 ^{c,y}

Note: Superscript letters (a, b, c, d) in the same rows for each season indicate significant differences (p<0.05). Superscript letters (x, y, z, q) in the same columns for each tissue in the same species indicate significant differences (p<0.05). $\bar{X} \pm S_{\bar{x}}$: mean±standard error, NR: *N. randalli*, SL: *S. lessepsianus*, UM: *U. moluccensis*, PQ: *P. quadrilineatus*.

Table 3. The effects of season on copper levels of some tissues of the lessepsian species ($\bar{X} \pm S_{\bar{x}}$, $\mu\text{g g}^{-1}$)

Species	Season	Muscle	Gill	Brain	Liver
NR	Spring	0.93±0.29 ^{a,x}	4.29±0.38 ^{b,y}	9.15±2.73 ^{b,x}	122.56±28.49 ^{c,z}
	Summer	-	-	-	-
	Autumn	0.92±0.18 ^{a,x}	2.07±0.42 ^{a,x}	6.91±1.56 ^{b,x}	12.08±1.57 ^{b,x}
	Winter	0.88±0.08 ^{a,x}	4.05±0.60 ^{b,xy}	13.62±2.78 ^{c,x}	35.78±6.24 ^{d,y}
SL	Spring	0.78±0.12 ^{a,x}	2.52±0.25 ^{b,x}	4.83±1.28 ^{b,x}	22.42±5.10 ^{c,x}
	Summer	-	-	-	-
	Autumn	3.65±1.82 ^{a,x}	2.93±0.75 ^{a,x}	15.79±8.85 ^{a,x}	23.34±9.66 ^{a,x}
	Winter	0.54±0.08 ^{a,x}	2.43±0.20 ^{b,x}	5.55±0.53 ^{c,x}	32.37±3.60 ^{d,x}
UM	Spring	1.33±0.17 ^{a,x}	3.60±0.40 ^{b,x}	5.56±0.93 ^{c,x}	44.30±13.22 ^{d,x}
	Summer	1.53±0.05 ^{a,x}	3.50±0.17 ^{b,x}	14.68±2.72 ^{c,y}	42.39±8.87 ^{d,x}
	Autumn	2.42±0.15 ^{a,y}	5.36±0.74 ^{b,x}	15.13±3.24 ^{c,y}	36.50±9.54 ^{c,x}
	Winter	2.21±0.19 ^{a,y}	3.04±0.90 ^{a,x}	13.05±2.79 ^{b,xy}	132.06±20.10 ^{c,y}
PQ	Spring	-	-	-	-
	Summer	1.79±0.45 ^{a,xy}	1.71±0.22 ^{a,x}	2.48±0.74 ^{a,x}	9.00±1.70 ^{b,y}
	Autumn	0.75±0.13 ^{a,x}	1.36±0.19 ^{ab,x}	2.65±0.62 ^{b,x}	1.05±0.04 ^{a,x}
	Winter	1.95±0.26 ^{a,y}	7.52±2.01 ^{b,y}	8.30±1.09 ^{b,y}	33.72±7.82 ^{c,z}

Note: Superscript letters (a, b, c, d) in the same rows for each season indicate significant differences ($p < 0.05$). Superscript letters (x, y, z, q) in the same columns for each tissue in the same species indicate significant differences ($p < 0.05$). $\bar{X} \pm S_{\bar{x}}$: mean±standard error, NR: *N. randalli*, SL: *S. lessepsianus*, UM: *U. moluccensis*, PQ: *P. quadrilineatus*.

Table 4. The effects of season on zinc levels of some tissues of the lessepsian species ($\bar{X} \pm S_{\bar{x}}$, $\mu\text{g g}^{-1}$)

Species	Season	Muscle	Gill	Brain	Liver
NR	Spring	16.19±2.12 ^{a,x}	62.08±2.43 ^{b,y}	59.77±10.86 ^{b,x}	554.4±42.32 ^{c,z}
	Summer	-	-	-	-
	Autumn	11.50±0.65 ^{a,x}	30.07±7.81 ^{b,x}	38.76±6.68 ^{b,x}	94.00±9.68 ^{c,x}
	Winter	13.02±0.58 ^{a,x}	52.56±4.57 ^{b,y}	90.41±29.78 ^{b,x}	211.93±16.99 ^{c,y}
SL	Spring	17.60±0.94 ^{a,x}	109.07±17.95 ^{b,x}	89.29±10.46 ^{b,x}	79.84±16.55 ^{b,x}
	Summer	-	-	-	-
	Autumn	20.16±2.69 ^{a,x}	91.78±10.68 ^{b,x}	84.15±21.47 ^{b,x}	96.76±13.72 ^{b,x}
	Winter	14.48±1.45 ^{a,x}	92.40±13.34 ^{b,x}	135.08±24.8 ^{b,x}	94.25±31.45 ^{b,x}
UM	Spring	18.13±0.57 ^{a,x}	102.38±5.00 ^{b,y}	103.66±41.31 ^{b,x}	417.52±66.35 ^{c,x}
	Summer	25.57±3.55 ^{a,x}	118.07±10.11 ^{c,y}	70.97±4.36 ^{b,x}	640.55±57.39 ^{d,x}
	Autumn	19.33±1.90 ^{a,x}	105.7±3.05 ^{c,y}	63.84±12.72 ^{b,x}	239.59±42.83 ^{d,x}
	Winter	20.92±1.33 ^{a,x}	60.41±11.41 ^{b,x}	46.12±6.01 ^{b,x}	587.64±154.13 ^{d,x}
PQ	Spring	-	-	-	-
	Summer	31.17±8.78 ^{a,x}	58.48±15.73 ^{a,x}	44.12±7.79 ^{a,x}	65.2±8.47 ^{a,x}
	Autumn	15.98±1.14 ^{a,x}	52.87±3.25 ^{b,x}	53.02±13.27 ^{b,x}	136.69±8.30 ^{c,y}
	Winter	17.07±1.17 ^{a,x}	93.48±19.22 ^{bc,x}	59.25±12.26 ^{b,x}	153.25±15.16 ^{c,y}

Note: Superscript letters (a, b, c, d) in the same rows for each season indicate significant differences ($p < 0.05$). Superscript letters (x, y, z, q) in the same columns for each tissue in the same species indicate significant differences ($p < 0.05$). $\bar{X} \pm S_{\bar{x}}$: mean±standard error, NR: *N. randalli*, SL: *S. lessepsianus*, UM: *U. moluccensis*, PQ: *P. quadrilineatus*.

The lowest accumulation of Zn in *U. moluccensis* is in muscle tissue in spring season (16.13 $\mu\text{g g}^{-1}$ dw), the highest Zn accumulation is in liver tissue in summer season (640.55 $\mu\text{g g}^{-1}$ dw). The lowest accumulation of Zn in *P. quadrilineatus* is in muscle tissue in autumn season (15.98 $\mu\text{g g}^{-1}$ dw), the highest Zn accumulation is in liver tissue in winter season (153.25 $\mu\text{g g}^{-1}$ dw) (Table 4).

According to the seasons, chromium accumulates in the tissues: The highest accumulation in *N. randalli* is in the brain (3.37 $\mu\text{g g}^{-1}$ dw) in spring season and the lowest accumulation is in the muscle (0.32 $\mu\text{g g}^{-1}$ dw) in winter season. The highest accumulation in *S. lessepsianus* is in the brain tissue (8.59 $\mu\text{g g}^{-1}$ dw) in autumn season and the lowest accumulation is in the muscle (0.33 $\mu\text{g g}^{-1}$ dw) in winter season. The highest accumulation in *U. moluccensis* is in the gill (9.97 $\mu\text{g g}^{-1}$ dw) in

summer season and the lowest accumulation is in the muscle (0.55 µg g⁻¹ dw) in winter season. The highest accumulation in *P. quadrilineatus* is in the liver (3.92 µg g⁻¹ dw) in summer season and the lowest accumulation is in the muscle tissue (0.53 µg g⁻¹ dw) in summer season (Table 5).

The highest accumulation of As in *N. randalli* is in the liver (74.43 µg g⁻¹ dw) in winter season and the lowest accumulation is in the brain tissue (19.16 µg g⁻¹ dw) in autumn season. The highest As accumulation in *S. lessepsianus* is in the liver tissue

(96.76 µg g⁻¹ dw) in autumn season and the lowest accumulation of As is in brain tissue in spring season (13.37 µg g⁻¹ dw). The lowest accumulation of As in *U. moluccensis* is in gill tissue in summer season (13.26 µg g⁻¹ dw), the highest As accumulation is in the liver tissue in winter season (165.54 µg g⁻¹ dw). The lowest accumulation of As in *P. quadrilineatus* is in muscle tissue in summer season (4.32 µg g⁻¹ dw), the highest As accumulation is in liver tissue in winter season (24.52 µg g⁻¹ dw) (Table 6).

Table 5. The effects of season on chromium levels of some tissues of the lessepsian species ($\bar{X} \pm S_{\bar{x}}$, µg g⁻¹)

Species	Season	Muscle	Gill	Brain	Liver
NR	Spring	0.42 ± 0.03 ^{a,x}	1.67 ± 0.27 ^{b,x}	3.37 ± 1.14 ^{b,x}	1.07 ± 0.24 ^{ab,x}
	Summer	-	-	-	-
	Autumn	0.71 ± 0.19 ^{a,x}	3.21 ± 0.92 ^{b,x}	2.32 ± 1.01 ^{ab,x}	1.49 ± 0.34 ^{a,x}
	Winter	0.32 ± 0.02 ^{a,x}	1.75 ± 0.36 ^{b,x}	1.48 ± 0.27 ^{b,x}	0.68 ± 0.11 ^{a,x}
SL	Spring	0.40 ± 0.05 ^{a,x}	2.76 ± 0.95 ^{bc,x}	5.35 ± 1.25 ^{c,xy}	1.32 ± 0.39 ^{b,x}
	Summer	-	-	-	-
	Autumn	0.94 ± 0.17 ^{a,y}	1.42 ± 0.33 ^{a,x}	8.59 ± 0.57 ^{b,y}	1.02 ± 0.16 ^{a,x}
	Winter	0.33 ± 0.03 ^{a,x}	3.22 ± 0.32 ^{b,x}	2.69 ± 0.73 ^{b,x}	0.56 ± 0.21 ^{a,x}
UM	Spring	0.91 ± 0.16 ^{a,x}	0.83 ± 0.14 ^{a,x}	2.66 ± 0.64 ^{b,x}	3.14 ± 0.22 ^{b,xy}
	Summer	1.09 ± 0.43 ^{a,x}	9.97 ± 1.05 ^{c,z}	2.84 ± 0.47 ^{b,x}	0.99 ± 0.17 ^{a,x}
	Autumn	0.94 ± 0.09 ^{a,x}	1.86 ± 0.13 ^{b,y}	2.70 ± 0.47 ^{b,x}	4.62 ± 0.90 ^{b,y}
	Winter	0.55 ± 0.02 ^{a,x}	3.15 ± 0.78 ^{b,y}	1.80 ± 0.40 ^{b,x}	1.88 ± 0.41 ^{b,x}
PQ	Spring	-	-	-	-
	Summer	0.53 ± 0.10 ^{a,x}	0.59 ± 0.06 ^{a,x}	0.86 ± 0.18 ^a	3.92 ± 0.98 ^b
	Autumn	0.75 ± 0.13 ^{a,x}	1.36 ± 0.19 ^{ab,xy}	2.65 ± 0.62 ^b	1.05 ± 0.04 ^a
	Winter	0.53 ± 0.05 ^{a,x}	1.48 ± 0.37 ^{a,y}	2.93 ± 1.29 ^a	0.72 ± 0.06 ^a

Note: Superscript letters (a, b, c, d) in the same rows for each season indicate significant differences (p<0.05). Superscript letters (x, y, z, q) in the same columns for each tissue in the same species indicate significant differences (p<0.05). $\bar{X} \pm S_{\bar{x}}$: mean ± standard error, NR: *N. randalli*, SL: *S. lessepsianus*, UM: *U. moluccensis*, PQ: *P. quadrilineatus*.

Table 6. The effects of season on arsenic levels of some tissues of the lessepsian species ($\bar{X} \pm S_{\bar{x}}$, µg g⁻¹)

Species	Season	Muscle	Gill	Brain	Liver
NR	Spring	55.57 ± 4.27 ^{a,y}	50.48 ± 9.19 ^{a,x}	34.21 ± 11.43 ^{a,x}	51.44 ± 11.42 ^{a,x}
	Summer	-	-	-	-
	Autumn	24.66 ± 6.22 ^{ab,x}	27.43 ± 13.76 ^{ab,x}	19.16 ± 4.10 ^{a,x}	64.11 ± 14.65 ^{b,x}
	Winter	69.44 ± 12.07 ^{a,y}	61.61 ± 8.26 ^{a,x}	49.04 ± 13.28 ^{a,x}	74.43 ± 11.7 ^{a,x}
SL	Spring	23.83 ± 4.28 ^{a,x}	17.05 ± 1.65 ^{a,x}	13.37 ± 1.37 ^{a,x}	16.36 ± 3.22 ^{a,x}
	Summer	-	-	-	-
	Autumn	20.16 ± 2.69 ^{a,x}	91.78 ± 10.68 ^{b,y}	84.15 ± 21.47 ^{b,y}	96.76 ± 33.72 ^{b,y}
	Winter	25.58 ± 7.92 ^{a,x}	19.96 ± 6.65 ^{a,x}	13.55 ± 2.75 ^{a,x}	19.13 ± 5.47 ^{a,x}
UM	Spring	32.53 ± 6.28 ^{a,x}	51.6 ± 8.81 ^{ab,y}	37.13 ± 6.64 ^{a,y}	85.21 ± 9.79 ^{b,xy}
	Summer	24.3 ± 3.89 ^{a,x}	13.26 ± 1.49 ^{a,x}	16.15 ± 2.82 ^{a,x}	20.31 ± 2.60 ^{a,x}
	Autumn	28.99 ± 1.95 ^{a,x}	41.4 ± 3.26 ^{ab,y}	31.88 ± 2.73 ^{a,y}	54.79 ± 5.72 ^{b,xy}
	Winter	38.59 ± 9.59 ^{a,x}	14.15 ± 4.00 ^{a,x}	30.35 ± 3.61 ^{a,y}	165.54 ± 35.89 ^{b,y}
PQ	Spring	-	-	-	-
	Summer	4.32 ± 0.67 ^{a,x}	5.28 ± 1.57 ^{a,x}	5.20 ± 0.80 ^{a,x}	6.20 ± 0.82 ^{a,x}
	Autumn	4.99 ± 0.67 ^{a,x}	4.80 ± 0.34 ^{a,x}	4.72 ± 0.50 ^{a,x}	13.17 ± 1.64 ^{b,y}
	Winter	5.88 ± 0.53 ^{a,x}	9.43 ± 3.25 ^{a,x}	5.17 ± 0.74 ^{a,x}	24.52 ± 2.49 ^{b,z}

Note: Superscript letters (a, b, c, d) in the same rows for each season indicate significant differences (p<0.05). Superscript letters (x, y, z, q) in the same columns for each tissue in the same species indicate significant differences (p<0.05). $\bar{X} \pm S_{\bar{x}}$: mean ± standard error, NR: *N. randalli*, SL: *S. lessepsianus*, UM: *U. moluccensis*, PQ: *P. quadrilineatus*

Cadmium has been determined in the liver tissues of fish for all seasons between 0.7-5.51 $\mu\text{g g}^{-1}$ dw and has also been determined in the gill tissue (1.21 $\mu\text{g g}^{-1}$ dw) of *P. quadrilineatus* in winter season and brain tissue (0.99 $\mu\text{g g}^{-1}$ dw) of *U. moluccensis* in summer season. The level of cadmium for the other tissues is below the limits that can be determined

(Table 7).

According to the seasons, lead accumulates in the tissues: The highest accumulation in *N. randalli* is in the gill tissue (1.88 $\mu\text{g g}^{-1}$ dw) in autumn season and the lowest accumulation is in the muscle (under the detection limit) in autumn season.

Table 7. The effects of season on cadmium levels of some tissues of the lessepsian species ($\bar{X}\pm S_{\bar{x}}$, $\mu\text{g g}^{-1}$)

Species	Season	Muscle	Gill	Brain	Liver
NR	Spring	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	1.08±0.23 ^{b,x}
	Summer	-	-	-	-
	Autumn	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	1.16±0.06 ^{b,x}
	Winter	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	1.40±0.30 ^{b,x}
SL	Spring	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}
	Summer	-	-	-	-
	Autumn	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	1.11±0.05 ^{b,z}
	Winter	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	0.45±0.09 ^{b,y}
UM	Spring	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	2.23±0.70 ^{b,xy}
	Summer	N.D. ^{a,x}	N.D. ^{a,x}	0.99±0.04 ^{a,y}	0.82±0.23 ^{b,x}
	Autumn	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	2.22±0.40 ^{b,xy}
	Winter	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	5.51±1.19 ^{b,y}
PQ	Spring	-	-	-	-
	Summer	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	0.83±0.02 ^{b,x}
	Autumn	N.D. ^{a,x}	N.D. ^{a,x}	N.D. ^{a,x}	0.70±0.09 ^{b,x}
	Winter	N.D. ^{a,x}	1.21±0.21 ^{b,y}	N.D. ^{a,x}	1.14±0.25 ^{b,x}

Note: Superscript letters (a, b, c, d) in the same rows for each season indicate significant differences ($p<0.05$). Superscript letters (x, y, z, q) in the same columns for each tissue in the same species indicate significant differences ($p<0.05$). $\bar{X}\pm S_{\bar{x}}$: mean±standard error, NR: *N. randalli*, SL: *S. lessepsianus*, UM: *U. moluccensis*, PQ: *P. quadrilineatus*, N.D.: not detected.

Table 8. The effects of season on lead levels of some tissues of the lessepsian species ($\bar{X}\pm S_{\bar{x}}$, $\mu\text{g g}^{-1}$)

Species	Season	Muscle	Gill	Brain	Liver
NR	Spring	0.35±0.01 ^{a,z}	0.86±0.10 ^{b,x}	1.85±0.01 ^{c,q}	0.87±0.19 ^{b,x}
	Summer	-	-	-	-
	Autumn	N.D. ^{a,x}	1.88±0.34 ^{d,x}	0.81±0.04 ^{c,y}	0.57±0.02 ^{b,x}
	Winter	0.14±0.02 ^{a,y}	1.02±0.12 ^{bc,x}	1.49±0.09 ^{c,z}	0.87±0.03 ^{b,x}
SL	Spring	N.D. ^{a,x}	1.09±0.33 ^{b,x}	N.D. ^{a,x}	0.80±0.26 ^{b,y}
	Summer	-	-	-	-
	Autumn	N.D. ^{a,x}	0.79±0.05 ^{c,x}	2.36±0.16 ^{d,z}	0.38±0.03 ^{b,y}
	Winter	N.D. ^{a,x}	1.15±0.15 ^{c,x}	1.49±0.15 ^{c,y}	0.19±0.04 ^{b,x}
UM	Spring	0.52±0.02 ^{a,y}	0.90±0.11 ^{b,x}	1.98±0.15 ^{c,q}	1.25±0.13 ^{b,y}
	Summer	1.12±0.12 ^{b,z}	1.79±0.09 ^{c,y}	0.52±0.03 ^{a,y}	0.61±0.07 ^{a,x}
	Autumn	N.D. ^{a,x}	0.88±0.14 ^{b,x}	1.17±0.07 ^{b,z}	1.10±0.17 ^{b,xy}
	Winter	N.D. ^{a,x}	2.38±0.21 ^{c,y}	N.D. ^{a,x}	1.25±0.11 ^{b,y}
PQ	Spring	-	-	-	-
	Summer	0.77±0.20 ^{a,y}	1.46±0.24 ^{a,x}	0.84±0.27 ^{a,y}	1.39±0.40 ^{a,x}
	Autumn	N.D. ^{a,x}	0.87±0.09 ^{b,x}	N.D. ^{a,x}	0.73±0.05 ^{b,x}
	Winter	N.D. ^{a,x}	0.69±0.13 ^{b,x}	1.10±0.10 ^{b,y}	0.74±0.03 ^{b,x}

Note: Superscript letters (a, b, c, d) in the same rows for each season indicate significant differences ($p<0.05$). Superscript letters (x, y, z, q) in the same columns for each tissue in the same species indicate significant differences ($p<0.05$). $\bar{X}\pm S_{\bar{x}}$: mean±standard error, NR: *N. randalli*, SL: *S. lessepsianus*, UM: *U. moluccensis*, PQ: *P. quadrilineatus*, N.D.: not detected.

The highest accumulation in *S. lessepsianus* is in the brain tissue ($2.36 \mu\text{g g}^{-1} \text{dw}$) in autumn season and the lowest accumulation is in the muscle (under the detection limit) in all season. The highest accumulation in *U. moluccensis* is in the gill ($2.38 \mu\text{g g}^{-1} \text{dw}$) in winter season and the lowest accumulations is in the muscle (under the detection limit) in autumn and winter seasons and in the brain tissue (under the detection limit) in winter season. The highest accumulation in *P. quadrilineatus* is in the gill ($1.46 \mu\text{g g}^{-1} \text{dw}$) in summer season and the lowest accumulations is in the muscle and brain tissues (under the detection limit) in autumn and in the muscle tissue (under the detection limit) in winter season (Table 8).

Discussion

Fish are under the influence of many pollutants in the aquatic ecosystem. The different concentration of contaminants in their tissues can be attributed to the expression of metalloproteins at different ratios in their different tissues (Spanopoulos-Zarco et al., 2017). Three of the seven analyzed metals (Cu, Zn, Cd) were found in liver tissues with the highest accumulation for all seasons and in all species. Liver plays an important role in contaminant storage, redistribution, detoxification or transformation and also acts as an active site of pathological effects induced by contaminants (Evans et al., 1993). As far as relative abundances of analyzed elements are concerned, the sequence of average concentrations in all tissues of fish from all seasons were $\text{Fe} > \text{Zn} > \text{As} > \text{Cu} > \text{Cr} > \text{Pb} > \text{Cd}$.

In fish, metal accumulation differs according to the type of metal (Tchounwou et al., 2012). Cadmium was detected only in the liver tissue in all fish, in addition to a few other tissues (*P. quadrilineatus*'s gill tissues in winter season and *U. moluccensis*'s brain tissues in summer season). The maximum accumulation of Fe except for *N. randalli* and *S. lessepsianus* was detected in liver tissue. The maximum accumulation of Cu in all species was detected in liver tissue. The maximum accumulation of Zn except for *S. lessepsianus* was also detected in liver tissue. The highest accumulations of As and Cr in other fish species except for the *N. randalli* were also detected in liver tissue. Except for As accumulation in *N. randalli*, the least accumulation for metals in all species was determined in muscle tissue. This difference between tissue and organs in terms of metal concentration may be due to ecological needs, swimming behaviors and metabolic activities among different fish species as well as their different structures and functions. Differences in metal concentrations in tissues may be an outcome of inducing capacity for metal binding proteins such as metallothioneins (Heath, 1995; Atli and Canli, 2003).

While low levels of metals such as Cu, Fe and Zn are necessary for enzymatic activity and for various biological processes in fish, their high levels may become toxic (Al-Weher, 2008; Ozden et al., 2010). Essential metals cause toxic effects on fish, such as the alteration of physiological activities and biochemical parameters in their blood (Nemesok, 1988). Kulcu et al. (2014) reported that Fe, Cu and Zn levels of muscles of *N. randalli*, *S. lessepsianus* and *U. moluccensis* from the Northeastern Mediterranean Sea were 85.52, 0.24, 16.81;

78.12, 0.34, 17.59; 83.7, 1.37, 24.57 $\mu\text{g g}^{-1} \text{dw}$, respectively. The findings of the researchers are similar to our findings.

Gills are the first target organs followed by the liver and muscle for iron toxicity (Jahan et al., 2015). The basic biological function of iron is to transport oxygen from the respiratory organs to the cells and the carbon dioxide from cells to the respiratory organs. Adsorption of metals to the gill surface can have a significant effect on the total metal level of the gill tissue (Canli and Furness, 1993). According to our results, the most iron accumulation for *N. randalli* and *S. lessepsianus* was in the gills, the liver tissue was in the second rank. While the maximum accumulation for *U. moluccensis* and *P. quadrilineatus* was in the liver, it was followed by the gill tissue.

Fish are under the influence of many biotic and abiotic factors in the aquatic environment. Heavy metal concentrations in fish tissues show seasonal changes depending on seasonal changes in water parameters (water temperature, pH, salinity) and changes in food consumption habits of fish (Regoli and Orlando, 1994; Foster et al., 2000). This study aimed to reveal the relation between heavy metal concentrations depending on seasons and species in particular. The results show that there is no statistically significant relationship between metal accumulation and seasonality.

The accumulation of metal in fish varies depending on the species of fish (Canli and Atli, 2003; Erdem et al., 2004; Dural et al., 2007; Karayakar et al. 2010, 2017). In this study, statistically significant differences were found in the metal concentrations of each tissue from different fish species. It was found that all of the metals showed the highest accumulation in *U. moluccensis* tissues in all seasons. The lowest accumulation was found in *P. quadrilineatus* for Fe, As, Pb, Cd metals, and *S. lessepsianus* for Zn and Cu metals. This difference can stem from many different biological needs, such as feeding habits and habitats (Kalay et al., 1999; Yılmaz, 2003).

Fish muscle tissue is the most important target tissue for metal accumulation, and also the most analyzed tissue of the reasons why fish muscle tissue constitute the main edible part of the fish (Kumar et al., 2011). Significant differences were found among the tissues in studies carried out by various researchers on various fish species; accumulation was found to be lowest in muscle tissue (Kalay et al., 1999; Dural et al., 2007; Karayakar et al., 2010). In this study, the lowest metal accumulation was found in muscle tissue in all samples except for As accumulation in *S. lessepsianus*.

Lead is a non-essential element that increases mucus formation in fish when taken at high doses and causes lack of survival, growth rates, development and metabolism (Burger et al., 2002). Kalay et al. (1999) determined Pb level in *Mullus barbatus*, *Caranx crysos* and *Mugi1 cephalus* muscle tissues collected in Mersin Bay as 4.43–9.11 $\mu\text{g g}^{-1}$. Kulcu et al. (2014) determined Pb level in muscle tissues of *N. randalli*, *S. lessepsianus*, *U. moluccensis* collected from the Northeastern Mediterranean Sea as 6.20, 5.44, 5.63 ($\mu\text{g g}^{-1} \text{dw}$), respectively. Our findings for Pb metal were found to be in the range of N.D.-1.12 $\mu\text{g g}^{-1} \text{dw}$ and were also found to be lower than findings obtained in other studies in the region.

Chromium, which has widespread use in the industry, is considered a heavy metal and pollutant, as well as a biologically available form, a microelement that plays an important role in glucose metabolism. Chromium was detected in almost all the samples, with the highest concentration ($1.09 \mu\text{g g}^{-1}$ dw). In muscle of *U. moluccensis* the values were within the limits of $12\text{--}13 \text{ mg kg}^{-1}$ (USFDA, 1993).

Cd is a serious contaminant and highly toxic element, which is transported in the air. Mormede and Davies (2001) suggested that liver was the target organ showing the detoxification and accumulation role of liver. According to our findings, cadmium was detected only in the liver tissue in all fish ($0.7\text{--}5.51 \mu\text{g g}^{-1}$ dw), in addition to a few other tissues (*P. quaderilineatus*'s gill tissues in winter season and *U. moluccensis*'s brain tissues in summer season).

Arsenic is biologically available to aquatic organisms living in contaminated habitats (Eisler, 1988). The exposure of fish to arsenic, as other nonessential metals, is conditioned by the concentration of this element in the surrounding water (Pagenkopf, 1983). Kalantzi et al. (2017) found As values for sardine and anchovy in the Greek coastline as 8.6 to 58.8 mg kg^{-1} dw. Similar to these findings, the amount of As in our study was found to be $4.32\text{--}69.44 \mu\text{g g}^{-1}$ dw. The Joint FAO/WHO Expert Committee (1983) imposed a limit of 0.1 mg kg^{-1} , ww for total arsenic in food (Muñoz et al., 2000; De Gieter et al., 2002). In all species except *P. quadrilineatus*, the amount of As was found to be very high.

The accumulation of As and Fe metals is higher than the other studies. The most important minerals containing arsenic are as follow: As_2S_3 (orpiment), AsS (realgar), FeAsS, FeAs₂, NiAs, CoAsS, $\text{Cu}_{12}\text{As}_4\text{S}_{13}$ and Cu_3AsS_4 (Matschullat, 2000; Bissen and Frimmel, 2003). The coexistence of these two heavy metals in the aquatic environment supports our findings.

Conclusion

Heavy metals (Fe, Cu, Zn, Cr, As, Cd and Pb) accumulations were investigated according to the tissues (muscle, gill, brain, liver) in the 4 important lessepsian fish species from Mersin Bay. The highest level in these 7 metals was determined for Fe, and the lowest accumulation for Cd. Nonetheless, these values were in the range stated in the literature.

Metal concentrations in edible parts of fish species were $17.26\text{--}108.22 \mu\text{g g}^{-1}$ dw for iron, $0.54\text{--}3.65 \mu\text{g g}^{-1}$ dw for copper, $11.50\text{--}31.17 \mu\text{g g}^{-1}$ dw for zinc, $0.32\text{--}1.09 \mu\text{g g}^{-1}$ dw for chromium, $4.32\text{--}69.44 \mu\text{g g}^{-1}$ dw for arsenic, below limit (not detectable) for cadmium, N.D.- $1.12 \mu\text{g g}^{-1}$ dw for lead. In this study, for all metals except arsenic there is no health risk through an exposure of consumption of certain fish. In all species except *P. quadrilineatus*, the amount of As was found to be very high. It is thought that this is due to the uncontrolled use of pesticides and fertilizers because of the agricultural area of the region. In this study, for all metals except arsenic there is no health risk through an exposure of consumption of certain fish. Additionally, the results obtained for the elements in analyzed fish species were within acceptable limits for human consumption.

Conflict of Interest

The authors declare that there is no conflict of interest.

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MARINE SCIENCE AND TECHNOLOGY BULLETIN

REVIEW

The importance of aquatic recreation areas in urban living and their contribution on health.

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ABSTRACT

The rapid increase in population, the dizzying developments in science and technology, cause human life more intense and stressful. Daily activities have begun to force and to tire people up more. Besides, adverse environmental effects also affect the physical and psychological health of person. A very important part of the population lives in big buildings in cities and this lifestyle people removes especially children from natural life. For today's people, especially the leisure time outside the working time, full, happy, satisfying, rest at the same time, having fun and learning activities is important in reducing stress. The meaning of recreation is the vitality and renewal of human beings both in terms of bodily and spiritual. One of the recreational activities known since the first ages is the close monitoring of aquatic life. Aquatic life presents a relaxing visuality and information about itself to human beings. It is obvious that aquariums will be a versatile decoration tool for living spaces as well as restful effects when considering the above reasons and will also affect the quality of life positively by reducing stress. The lack of nature and nature history museums in our cities may be seen as a suitable motivation for the dissemination of the aquatic life-based recreation of the decrease in the possibility of healthy contacts that should be found between man and nature. Aquariums that will be installed in shopping centers, schools, and other living areas in contemporary cities today, especially in residential buildings, will allow people to contact nature from another perspective. The recreational areas where the aquatic life is observed will give people the opportunity to relax in the natural life.

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Introduction

The experience and relationship between human and aquatic environments is extremely complicated and is entirely emotionally charged. The meaning of recreation is the vitality and renewal of human beings both in terms of bodily and spiritual. There is a great deal of literature showing that discover the natural environment and connect with nature is

generally useful to health and wellbeing. In addition, the majority of reported benefits relate to mental health and wellbeing. Other results include factors such as stress reduction, improving concentration skills, alleviating depression and improving self-esteem (Maller et al., 2006; Prosser et al., 2008). Many people prefer natural environments where they can relax and recover psychologically when they have stress

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and mental fatigue. Documents that indicate that human, animal, and nature interactions people feel better about themselves, that they are happy, and that they have health-enhancing effects, have become the focus of many researches (Filan and Llewellyn-Jones, 2006). Nature and animals have been used since ancient Greeks to improve the emotional and functional status of people (Riede, 1987). One of the recreational activities known since the first ages is the close monitoring of aquatic life. City aquariums exhibit wildlife and have a special link with nature. They offer an extraordinary opportunity to show the miracle of life to many cities inhabitants. Also city aquariums can contribute to the preservation of education and living spaces (Miller et al., 2004). In some studies, positive effects of fish aquariums on human health have been identified (Cole and Gawlinski, 2000). Fish aquariums in the home or in the therapeutic environment are a common way of introduce of animals or "nature". Participants who had the opportunity to observe a fish aquarium under laboratory conditions experienced significant reductions in their blood pressure (Edwards and Beck, 2002; Barker et al., 2003a).

In some other researches, many animal species such as turtles, chicks, rabbits, birds, pets, fishes (aquariums), dogs and horses have been used for therapy (Macauley, 2006). There are also studies to reduce the level of anxiety and stress in therapies made by interacting with different animals (Wilson, 1991; Barker and Dawson, 1998). However, the use of different animal species in therapy is a limiting factor for allergy patients. Fishes and aquariums would be good choices for people those allergic to most animals (Barba, 1995).

Current research on the potential for stress reduction of aquariums also reports that the biological diversity of the aquarium has a significant impact on the physiological and psychological response of humans (Cracknell et al., 2012). Monitoring of aquatic environments potentially provides a learning-centered experience. It also provides a learning environment to explore, examine, choose, establish personal connections, develop and control your own understanding (Paris, 1997; Adelman et al., 2000).

Monitoring aquatic life presents a relaxing visuality and information about itself to human beings. Monitoring aquatic life also affect the quality of life positively by reducing stress. It is purposed that people' to look at nature from a different perspective through the aquatic recreation areas that will be installed especially in houses, shopping centers, schools and other living areas in modern cities.

Aquatic Environment and Well-Being

It is obvious that aquariums will be a versatile decoration tool for living spaces also it will also affect the quality of life positively by reducing stress. Because of the strong positive responses to the water, the design of blue areas is very important for positive or valuable total quality and good ecosystems. Aquatic natural landscape has three essential components. These are water, vegetation and fish varieties (Steinwender et al., 2008). Aquatic environmental enrichment provides resources that facilitate psychological well-being by facilitating typical behaviours of species or abruptly removing abnormal behaviour (Mellen and Sevenich MacPhee, 2001). A

peaceful sense of place about the blue area, can positively affect to wellbeing and other emotional feelings. Aquatic area is recognized as a mysterious mirror by presenting a picture that is not as clear as normal mirror (Burmil et al., 1999; Nasar and Li, 2004; Wyles et al., 2013). Aquatic areas, on people provide perception, emotional, restoration and recreational benefits, as well as direct health benefits. Nevertheless, scenery ecology and landscape are an important feature in terms of emotional connection (Purcell et al., 2001; Berto, 2005; Han, 2007; Kweon et al., 2008). Watching to the blue aquatic environment may have calming and stress-relieving effects on humans. Also, it is reported that such benefits may be higher in areas with more species diversity. Longer monitoring of the aquatic area with increased biological diversity results in a further increase in the personally reported mental wellbeing with decline in heart rate (Cracknell et al., 2016). The aquatic environments or blue areas have positive effects on human health, it is indicated that those living in such environments tend to be healthier and longer life (De Vries et al., 2003). People who watched aquariums in their free time were compared to people who visited museums or art galleries. According to these study, people that watched the aquatic environments in the aquariums during their leisure time have found to aquariums more enjoyable, they also found significant differences in their perceptions about themselves (Packer and Ballantyne, 2010). Katcher et al. (1984) have informed that watching in an aquarium to fishes can reduce anxiety in medical and dental waiting rooms. Other than this, Riddick (1985) examined the effects of introducing new hobby fish aquariums on the elderly. He reported that compared to the other two groups that did not receive this intervention, the aquarium group was significantly positive changes that overall leisure satisfaction, relaxation states and diastolic blood pressure.

According to Dogu et al. (2011), aquariums have included methods and practices to have ensure that experience for guests' pleasure as long as the animals are safe and comfortable. They create an area where guests can touch and interact with real aquatic animals. Furthermore, aquariums are both used as a form of recreation and training for children and families, besides it is an important method of increasing awareness of information about aquatic species. Hence, there is a lot of evidence for the benefits that humans monitoring of aquatic environment, but more research is needed on this subject.

Discussion

It is quite important that people, especially children, have contact with nature for mental, emotional and social well-being. Contacting with nature, may have many benefits including self-respect, better perception and increased self-confidence. Spending more time in activities that involve connecting with nature in the school can be a very important tool to support the development of children's mental, emotional and social well-being (Maller, 2009). Recently, humans beginning to appreciate benefits that come from the nature and biodiversity on human health. Sandifer et al. (2015) assessed the relationship between human health and nature and biodiversity and produced a comprehensive list of reported

health effects. They found strong evidences linking biodiversity with production of ecosystem services between exposure to nature and human health. Health and well-being can be defined in terms of perception, preference, landscape design, emotion, restoration and recreation, which are directly related to the aquatic recreation areas. Aquatic life inspires and motivates people in cities to establish a more harmonious and sustainable relationship with the natural World (Rabb and Saunders, 2005). In addition, the direct health benefits of aquatic recreation areas have been noted in many studies. Völker and Kistemann (2011) should support researchers and practitioners analysing health impacts in the blue space.

In relation to the subject Churchill et al. (1999), Friedmann and Son (2009) have identified that animal-based therapy increases emotional well-being, reduces loneliness, and reduces anxiety and physical stress reactions. Also, Tsai et al. (2010) examined that Animal Assisted Treatment (AAT) the effects on cardiovascular response, situational anxiety and medical fear of hospitalized children. They reported that AAT can reduce physiological stimulation in hospitalized children and may help them cope better at the hospital for this reason. A study of brain disease, followed a more specific approach and explored whether the presence of fish aquariums affected the feeding of Alzheimer's patients (AD). They studied 62 individuals with AD who lived in a private nursing home. Baseline nutritional data were obtained after a two-week treatment period in which aquariums were watched. Then they collect data every day for 6 weeks. They said that, according to the results, with AD persons' nutritional intake increased markedly when aquariums were introduced. Weight also increased significantly continued to increase during the six-week observation period. The authors have found that the presence of fish aquariums enriches the habitats and thus improves the mood of the residents and increases their food supply (Edwards and Beck, 2002). One of the first uses of fish aquariums for older adults was a study administered in communal subsidized housing. People who accept the same services but who had an aquarium and did not have an aquarium were compared. In their leisure time, residents with a fish aquarium were found to increase their overall satisfaction levels and were more comfortable (Wells, 2009).

Barker et al. (2003b) investigated the pre-treatment effect of the aquarium in anxiety, fear, frustration and depression patients treated with electroconvulsive therapy. Forty-two patients who underwent to aquarium and non-aquarium rooms were directed to ECT. Depression, anxiety, fear and frustration, heart rate and blood pressure measurements of the patients were done. They found that patients who passed through the room with the aquarium had 12% less anxiety. Kidd and Kidd (1999) found that 70% of aquarium owners identified their fish as relaxing and stress decreasing. In this field, it is suggested that existing researches indeed make it available in health care centers because of the potentially relaxing and calming properties of fish aquariums. DeSchraver and Riddick (1990) have investigated the effect of watching the aquarium on the stress of elderly. A placebo videotape was shown to members of the control group while members of the experiment group were watching a fish aquarium or an aquatic environment video cassette. Three separate treatment

sessions of eight minutes were organized repeatedly one week. They said that aquarium observers tend to have a slower pulse rate and a decrease in muscle tension and an increase in skin temperature.

Conclusion

The lack of nature and nature history museums in our cities may be seen as a suitable motivation for the dissemination of the aquatic life-based recreation of the decrease in the possibility of healthy contacts that should be found between man and nature. Aquariums that will be installed in shopping centers, schools, and other living areas in contemporary cities today, especially in residential buildings, will allow people to contact nature from another perspective. The recreational areas where the aquatic life is observed will give people the opportunity to relax in the natural life.

Conflict of Interest

The authors declare that there is no conflict of interest.

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