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Physicochemical Composition and Selected Quality Characteristics of the New Product: Ready to Eat Shrimp

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Abstract

In this study, it was aimed to develop an alternative product for seafood consumption by applying the smoking and marinating process on shrimp. Physicochemical, microbiological, and sensory analyzes were performed on a certain day of each month to determine the quality and shelf life of the obtained smoked product. Firstly, hot smoking process was applied and then the marination process was applied on shrimp used in the study. According to the results of microbiological analysis, while the number of TMAB was detected as 1.54 LogCFU/g in fresh shrimp, the number of microorganisms was determined lower than 1 LogCFU/g in the smoked and marinated product. It was determined that the products which were kept in refrigerator conditions for 10 months started to lose quality in the 8th month of storage and the shelf life of the product was determined as 7 months for the consumer.

Keywords: Pink deep-water shrimp (*Parapenaeus longirostris*), hot smoking, marinate, fatty acids, shelf life.

Yeni bir Ürünün Fizikokimyasal Kompozisyonu ve Bazı Kalite Parametreleri: Tüketime Hazır Karides

Özet

Bu çalışmada, karides etine dumanlama ve marinasyon işlemi uygulanarak su ürünleri tüketimine alternatif bir ürün geliştirilmeye çalışılmıştır. Elde edilen dumanlanmış marine ürünün buzdolabı koşullarındaki kalitesini ve raf ömrünü tespit etmek amacıyla her ayın belirli bir gününde fiziksel, kimyasal, mikrobiyolojik ve duyusal analizler yapılmıştır. Çalışma kapsamında karidesler önce dumanlanmış ardından marine edilmiştir. Mikrobiyolojik analiz sonuçlarına, göre taze karideste tespit edilen TMAB sayısı 1,54 LogKOB/g iken yapılan marinasyon+dumanlama işlemi ile bu değer 1LogKOB/g altına düşmüştür. Dumanlanmış marine karideste depolama süresince mikroorganizma yükü 1LogKOB/g'ın altındadır. Buzdolabı koşullarında 10 ay muhafaza edilen ürünlerin, depolamanın 8. ayında kalitesini kaybetmeye başladığı belirlenmiş, tüketici için belirlenen raf ömrü ise 7 ay olarak tespit edilmiştir.

Anahtar kelimeler: Pembe derin su karidesi (*Parapenaeus longirostris*), sıcak dumanlama, marinat, yağ asitleri, raf ömrü.

INTRODUCTION

Seafood is a valuable human food that is used especially in meeting the needs of protein, minerals, and essential fatty acids and in changing healthy eating habits. Today, with the developing technology, like other foodstuffs, seafood is processed in a wide variety of forms, making it ready for consumption. For people to have adequate and balanced nutrition, they need to increase their food resources or make more use of existing food resources. The demand for seafood, which constitutes an important food group in this field, is increasing day by day.

The purpose of the smoke technology, which is one of the oldest protection methods, which is widely used in the world, economically important and known, is to improve the sensory properties of the product by taking advantage of the aroma and color given by the smoke, to extend the shelf life of the product by benefiting from the effects of heating and water loss and smoke components (antimicrobial, antioxidant) (Gülyavuz and Ünlüsayın, 1999). Another of the oldest known methods of conservation is marination. The purpose of this technology is enzymatic maturation of fish in acid and salt solution without heat treatment. The product obtained by adding sugar, spices, brine, sauce, or vegetables to add different flavors is a preservation method created by placing it in glass bottles or plastic containers (McLay, 1972). The raw material is made edible with the marinating process and

semi-canned products can be created by reducing the losses caused by cooking (Kılınç and Çaklı, 2004a; Björkoth, 2005).

Shrimp, valuable seafood rich in protein, is easy to digest due to the low amount of connective tissue. Pink deep-water shrimp have a great economic value all over the world. It has a good market, frozen or canned. It has a great economic value in our country as it is the most caught shrimp in Turkey. Today, new products are developed by applying different methods to extend the shelf life of the foods. When the shelf life of the food is increased, the producer is provided with a longer time to market the product and the consumer to consume it and the product becomes more economical (Morrais and Kai 1981; Mermelstein, 1998). Cold marinated (Cadun et al., 2005; Kalıştır, 2008; Cadun et al., 2008), baked and marinated (Bilgin et al., 2006; Erdem and Bilgin, 2004) and freeze (Bingöl et al., 2013) of different shrimp species; are found in the literature. Although there is some seafood (Dalgıç, 2000; Ozogul et al., 2010; Balıkçı, 2009, Karslı, 2013; Keskin, 2019) where both smoking and marination are applied together, no such study has been observed in shrimp.

In this study, it was aimed to determine the shelf life as a result of the evaluation of chemical, microbiological and sensory quality criteria, as well as to create a new product for the food sector, by changing the composition of the nutrient composition and the chemical, microbiological and sensory quality criteria during the storage of marinates obtained from smoked shrimp (*Parapenaeus longirostris*, Lucas 1846).

MATERIALS and METHODS

Materials

As research material, a total of 15 kg of pink deep-water shrimp (*Parapenaeus longirostris* Lucas 1846), with an average length of 11.67±0.19 cm and an average weight of 7.37 ± 0.36 g, were caught from the Istanbul-Tuzla fishermen's shelter. The caught shrimp were immersed in boiling water for 1 minute as soon as the ship arrived at the port and cooled in the air stream. The cooled shrimp were placed in a single row in locked bags, with an average of 500 g, and one layer of the product was placed (four layers in total including shrimp and sea ice) in a Styrofoam box and brought to the laboratory under a cold chain.

Smoking process of shrimp

Shrimp meat was salted for 10 minutes in salt brine, shrimp: brine ratio was 1:2, for 15 minutes and filtered. Then it was lightly lubricated with sunflower oil, lined up on the oven wires, and subjected to smoke treatment. Shrimp were pre-drying at 30 °C for 20 min., then kept at 60 °C for 10 min. were smoked. After this process, shrimp were removed from the oven and rested at room temperature before marinating (Figure 1).

Figure 1. Smoking process of shrimp (a: pickling in brine, b: draining, c: smoking, d: resting)

Marination process of smoking shrimp

The rested shrimp meat was placed in marinate brine (1 % alcohol vinegar, 2.2 % salt, and ‰ 0.4 citric acids) (shrimp: brine ratio, 1:9) and matured under refrigerator conditions for 2 days (Figure 2a).

Packaging process of marinated smoked shrimp

Marinated smoked shrimp were leaked in a strainer for 2 hours. At the end of this process, its average weight was 130g smoked marinated shrimp meat was placed in the packages and it was filled with sunflower oil and closed without air bubbles. The products were stored monthly at +4 °C and analyzed monthly (Figure 2 b-f).

Figure 2. Packaging of marinated shrimp (a: marination, b: draining, c: adding sunflower oil, d-f: packaging and storage)

Methods

During the study (10 months), chemical, microbiological and sensory analyzes were performed monthly and 2 repetitions were performed in 2 parallel, and the shelf life of the products was tried to be determined in refrigerator conditions.

Proximate Composition Analyses of Shrimp

Crude protein and crude fat analysis were performed according to the Kjeldahl method (AOAC, 1980) and Soxhlet method (AOAC, 2005). Dry matter and crude ash analyzes were done according to AOAC (1995). After calculating the carbohydrate value of shrimp, the energy value was calculated according to the Atwater method (Falch et al., 2010).

Fatty acids composition was performed according to the IID-19 method by IUPAC (1979) on Thermo Scientific Trace 1310.

Physicochemical Analysis

Total Volatile Basic Nitrogen (TVB-N) amount of fresh samples and products obtained was determined by the Lucke-Geidel method, modified by Antonopoulos (Ludorf and Meyer, 1973). To

detect fatty acid oxidation, Erkan et al. (2011) TBARs analysis modified and applied was used. The amount of total salt and total acid (in terms of acetic acid) of shrimp were calculated according to Varlık et al. (2007). Shrimp meat was diluted with pure water at a ratio of 1:1 and pH measurement was made with the portable pH-meter probe of WTW Multi 340i model (Curran et al., 1980). Water activity measurements were determined according to AOAC (1980) using the Novasina LabSwift water activity measurement device. Konica Minolta /CR-A 33a color measuring device was used for color measurements (Osaka, Japan). Values of L*, a*, and b* were measured according to the International Commission on Illumination (CIE, 1976).

Microbiological Analyses

The microbiological analysis in the study were Total mesophilic aerobe bacteria (TMAB), total psychrophilic aerobe bacteria (TPB), total yeast-mold (TYM), and total coliform bacteria (TC) count. The outer surface of the packages was wiped with 70 % ethyl alcohol and then opened with the help of a sterile knife. 10 grams of fish samples with sterile spatula were taken into sterile stomacher bags and homogenized in the stomacher by adding 90 ml of peptone solution beside the flame (Sivertsvik et al., 2003). Dilutions of 10^{-1}-10^{-6}; prepared using 1ml homogenate and 9 ml 0.85 % NaCl solution. All of the analyzes were performed as 2 repeats and 2 parallel. Plate Count Agar (PCA, Merck no: 105463.0500) was used for TMAB and TPB. Petri dishes were incubated for 2 days at 37 °C for TMAB and 10 days at 7 ^{o}C for TPB. Potatoes Dextrose Agar (PDA, Merck no: 1.10130) was used for TYM analysis. It was left at 3-day incubation at 28 °C. Violet Red Bile Agar (VRBA, Merck no: 1.01406) was used for TC count and incubated at 37 °C for 24 hours (Halkman 2005).

Sensorial Analysis

For sensory analysis, 5 experienced panelists (academics of the Seafood Processing Technology department) were selected and a form was given to the panelists to evaluate the products. Sensory evaluation form Varlık (1993) and modified from the table for marinated products used by Schormüller (1968). The products were rated between 0 and 5 in terms of color, odor, flavor, texture, and general taste (0-1: Inexpensive, 1-2: Bad, 2-3: Not bad, 3-4: Good, 4-5: Very good), products below 2 points are considered as inexhaustible.

Statistical Evaluation

The average values and standard deviations of the results obtained in the research were made using Microsoft Office Excel 2018 package program and statistical evaluations using one-way analysis of variance and Tukey test with the help of Minitab 17 package program (Sümbüloğlu and Sümbüloğlu, 2000).

RESULTS and DISCUSSION

Average Weight, Length, and Meat Yield of Shrimp

The average weight and length of shrimp used in the study were 7.37±7.30.36 and 11.67±0.19, respectively. It was determined that the meat yield of the shrimp was 35 %, and the meat yield of the product was 26.56%. Total weight loss was determined as 73.44 % until the final product was obtained. The reason for the high loss; it is thought that the scalding process used in killing shrimp and the loss of head and shell extraction due to the small shrimp were affected. Diler and Ataş (2003) reported the meat yield of shrimp (*P. semisulcatus*) as about 1.36 %, while Zamorano et al. (2009) stated that shrimp lose 50 % weight after cleaning. Similarly, Çankırılıgil and Berik (2017) determined that the meat yield of deepwater pink shrimp was 48.46 %. The meat yield of the species used in our study was lower compared to the shrimp species in the literature; it may be due to less length and weight, and hence greater shell weight. Also, meat yield of shellfish products; may vary depending on size, species, sex, hunting area, nutritional status, and the structure of the shell and head (Venugopal and Gopakumar, 2017). Our study also shows that differences in processing methods significantly affect meat yield. Indeed, as a result of heat treatment during smoking, the moisture content of shrimp meat decreases, which reduces efficiency. Also, the amount of salt used in brine causes shrimp meat to lose weight.

Proximate Composition Results of Shrimp

In Table 1, the proximate composition analysis results of fresh shrimp, after smoking, marination, the beginning of storage (1st day), and end of storage period (10th month) samples are given.

Table 1. The proximate composition analysis results and energy values of shrimp.

	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Ash (%)	Energy (Kcal/100g)
Fresh shrimp	78.08±0.43[a]	18.82±0.27[b]	0.23±0.02[e]	1.52±0.02[b]	82.78±1.70[d]
Smoked shrimp	59.62±0.21[e]	20.90±0.05[a]	2.67±0.07[c]	4.32±0.28[a]	157.55±0.39[b]
Marinated shrimp	70.84±0.60[b]	19.00±0.10[b]	0.83±0.07[d]	1.04±0.02[b]	116.64±2.83[c]
Packed shrimp (1th day)	66.24±0.13[c]	18.56±0.19[b]	4.17±0.09[b]	1.07±0.06[b]	151.62±0.79[b]
Packed shrimp (10th monhts)	61.56±0.41[d]	17.42±0.10[c]	8.55±0.14[a]	1.44±0.07[b]	190.76±2.33[a]

ab ↓: Difference between groups is important in the same column ($p<0.05$)

In the present study, moisture, crude protein, crude oil, and a crude ash content of fresh shrimp were as follows; 78.08±0.43 %, 18.82±0.27 %, 0.23 ± 0.02 %, and 1.52 %. The moisture, crude protein, crude oil, and crude ash contents of shrimp were investigated by different researchers. These values vary according to the shrimp type; for moisture; 75.40 % in *P. semisulcatus*, 72.90 % in *P. japonicas,* and 70.95 % in P. *monodon* (Diler and Ataş, 1999), for crude protein; 17 % in *P. borealis* and *P. jordani* (Oner and Yıldırım, 2018), 20.13 % in *P. semisulcatus* (King et al., 1990), for crude fat; 0.35 % in *P. longirostris* (Cadun, 2002) and for crude ash; 1.60 % (Yanar, 2003) in *M. monoceros* and *P. semisulcatus*. Hacıoğlu (2010), moisture, crude protein, crude oil, and crude ash amounts of pink deep-water shrimp were 76.72 %, 10.86 %, 2.14 %, and 8.13 %, respectively.

After the smoking process, the moisture content of shrimp meat decreased to 59.62±0.21 % ($p<0.05$) with the effect of heat treatment, and the amount of crude protein increased by 20.90±0.05 % ($p<0.05$). The smoking process also caused an increase in the crude fat and crude ash content of raw shrimp ($p <0.05$). With the marination after the smoking process, the moisture content of the product increased ($p <0.05$), and a decrease in crude protein, crude fat, crude ash content were observed ($p <0.05$). Shrimp that had been smoked and then marinated were packaged with the addition of oil. On the first day of this process, the crude protein content of the final product was statistically similar ($p> 0.05$) to the marinated shrimp. At the end of the ten-month storage period, a decrease in the crude protein content ($p <0.05$) and an increase in the crude fat content ($p> 0.05$) was observed.

Cadun et al. (2005) determined the moisture, crude protein, crude fat and crude ash amount of raw and marinated pink deep-water shrimp as 85.49 %, 11 %, 0.35 %, 2.43 % and 75.48 %, 20.4 %, 0.54 %, 2.78 %, respectively. Similar to the present study, the process of marination caused water loss in the product. In another marinate study made from *M. stebbingi*, it determined the moisture, crude protein, crude fat, and crude ash contents of fresh shrimp as 81.41 %, 16.29 %, 1.1 %, 0.65 %, respectively. These values after marination were 75.24 %, 20.77 %, 1.32 %, 2.98 % respectively (Kalıştır, 2008). In a study in which smoking, and marination processes were applied separately and in combination, the researchers reported that the content of the proximate composition increased with the procedures applied as in the present study (Karslı, 2013).

While the energy content of raw shrimp was 87.78 Kcal/100g, a calorie increase was detected after smoking. Due to the loss of water in the product; especially the increase in crude fat and carbohydrate content was the reason for the increase in calorie value. Also, energy reduction was observed with the removal of crude fat and water-soluble carbohydrates from shrimp tissue by marinating (116.64 Kcal/100g). The oil used as a filling during storage also caused an increase in calories.

Fatty acid composition results

Fatty acid contents of fresh, smoked, marinated, and packaged shrimp are given in Table 2. The total saturated fatty acid (Σ SFA), total monounsaturated fatty acid (Σ MUFA), and total polyunsaturated fatty acids (Σ PUFA) values of fresh shrimp were found as 25.48±0.25, 30.923±0.18, 43.57±0.41, respectively. Emami (2014) reported the rates of SFA, MUFA, and PUFA of *P. vannamei* and *P. semisulcatus* as 37.26 %, 24.9 %, 37.84 % and 49.12 %, 33.76 %, 16.9 %, respectively. Turan et al. (2011) reported the rate of SFA in brown shrimp as 33.04 % and PUFA content as 29 %. Ouraji (2011) stated the SFA values of natural and culture samples in Indian shrimp as 32.88 % and 33.79 %, respectively. The ΣMUFA value of the same kind of shrimp in the present study was 26.09 % (Oksuz et al., 2009), this difference in fatty acid ratios may be due to the caught area, seasonal conditions, and other environmental factors.

The amounts of ΣSFA, ΣMUFA, and ΣPUFA of the smoked shrimp were determined as 19.97±0.14, 35.81±0.45, 44.20±0.57 %, respectively. In this process step, as in raw shrimp, the dominant SFA were palmitic acid and stearic acid. However, an increase in the content of behenic acid was observed with the smoking process ($p<0.05$).

The third processing step applied to the product before storage was marination. Shrimp after marinating; the amounts of ΣSFA, ΣMUFA, and ΣPUFA were 23.07±0.11, 28.89±0.41, and 48.04±0.28 %, respectively. In this step, an increase in stearic acid content was observed compared to smokes ($p <0.05$), but this value was found similar to raw shrimp ($p> 0.05$).

The amounts of ΣSFA, ΣMUFA, and ΣPUFA were determined as 22.83±0.62, 32.47±0.29, 44.70±0.40 %, respectively, on the first day of storage in the packaged product. The ΣSFA amount of the packaged product was not different from the marinated product ($p> 0.05$). However, the contents of ΣMUFA and ΣPUFA were different ($p <0.05$). The oleic acid content of the product increased with the addition of oil ($p <0.05$). The fatty acid content of the sunflower oil used is highly oleic and linoleic acid (38.78 % oleic acid, 49.99 % linoleic acid). Therefore, both the oleic and linoleic acid content of the packaged product increased ($p<0.05$). The addition of sunflower oil proportionally affected the EPA and DHA content of the product, and the EPA+DHA content caused approximately a half decrease compared to the previous process step.

Table 2. The fatty acid composition of shrimp.

	Fresh shrimp	Smoked shrimp	Marinated shrimp	Packed shrimp (1th day)	Packed shrimp (10th months)
C4:0	0.01±0,01^{a}	-	0.01±0.01^{a}	-	0.02±0.01^{a}
C6:0	-	-	0.01±0.01^{a}	-	0.01±0.00^{a}
C8:0	-	0,02±0.00^{c}	0.01±0.00^{d}	0.04±0.00^{b}	0.06±0.00^{a}
C12:0	0.02±0.00^{a}	0.01±0.00^{b}	0.02±0.01^{ab}	0.01±0.00^{b}	0.01±0.00^{b}
C13:0	0.01±0.00^{a}	-	0.01±0.00^{a}	-	-
C14:0	1.29±0.02^{a}	0.58±0.05^{c}	0.90±0.01^{b}	0.48±0.02^{cd}	0.44±0.02^{d}
C15:0	1.27±0.02^{a}	0.46±0.04^{c}	0.96±0.00^{b}	0.39±0.00^{c}	0.24±0.01^{d}
C16:0	11.22±0.04^{a}	10.20±0.23^{b}	9.49±0.10^{bc}	9.01±0.23^{c}	11.35±0.16^{a}
C17:0	1.53±0.03^{a}	0.53±0.02^{c}	1.24±0.02^{b}	0.48±0.02^{c}	0.29±0.03^{d}
C18:0	9.20±0.09^{a}	5.00±0.40^{b}	8.79±0.05^{a}	9.89±0.37^{a}	4.42±0.28^{b}
C20:0	0.23±0.01^{a}	0.18±0.02^{ab}	0.18±0.00^{ab}	0.09±0.03^{c}	0.13±0.00^{bc}
C21:0	0.11±0.02^{a}	0.08±0.02^{ab}	0.08±0.00^{ab}	0.04±0.01^{b}	0.04±0.01^{b}
C22:0	0.38±0.03^{e}	2.63±0.04^{b}	1.25±0.03^{d}	2.23±0.07^{c}	3.34±0.04^{a}
C23:0	0.05±0.00^{a}	0.01±0.00^{c}	0.03±0.00^{b}	0.01±0.00^{c}	-
C24:0	0.14±0.01^{a}	0.28±0.25^{a}	0.12±0.01^{a}	0.15±0.03^{a}	0.04±0.04^{a}
Σ SFA	**25.48±0.25^{a}**	**19.97±0.14^{c}**	**23.07±0.11^{b}**	**22.83±0.62^{b}**	**20.39±0.42^{c}**
C14:1	0.39±0.01^{a}	0.13±0.01^{c}	0.29±0.01^{b}	0.11±0.00^{c}	0.06±0.00^{d}
C15:1 cis10	0.55±0.02^{a}	0.18±0.01^{c}	0.44±0.01^{b}	0.16±0.01^{c}	0.09±0.00^{d}
C16:1	4.82±0.02^{a}	2.14±0.12^{c}	3.51±0.04^{b}	1.76±0.02^{d}	1.50±0.06^{d}
C17:1 cis10	1.98±0.02^{a}	0.75±0.03^{c}	1.56±0.01^{b}	0.64±0.02^{d}	0.42±0.01^{e}
C18:1n9c	13.50±0.10^{e}	27.39±0.24^{b}	16.51±0.03^{d}	25.51±0.06^{c}	36.61±0.78^{a}
C18:1n9t	5.48±0.04^{a}	2.87±0.15^{bc}	3.57±0.42^{b}	2.66±0.33^{bc}	1.56±0.57^{c}
C20:1 cis 11	1.81±0.05^{a}	1.32±0.10^{b}	1.33±0.02^{b}	1.04±0.02^{c}	1.32±0.03^{b}
C22:1n9	1.04±0.05^{a}	0.36±0.06^{c}	0.71±0.01^{b}	0.22±0.02^{cd}	0.11±0.01^{d}
C24:1	1.35±0.01^{a}	0.67±0.02^{c}	0.99±0.01^{b}	0.39±0.03^{d}	0.61±0.13^{cd}
Σ MUFA	**30.923±0.18^{cd}**	**35.81±0.45^{b}**	**28.89±0.41^{d}**	**32.47±0.29^{c}**	**42.27±0.88^{a}**
C18:2n6c	0.34±0.01^{d}	25.62±0.83^{b}	17.54±0.00^{c}	29.05±0.51^{a}	26.97±0.40^{ab}
C18:2n6t	0.55±0.03^{a}	0.31±0.08^{ab}	0.33±0.03^{ab}	0.24±0.08^{b}	0.13±0.00^{b}
C18:3n3	0.41±0.02^{e}	1.27±0.05^{b}	0.74±0.02^{d}	1.10±0.04^{c}	1.73±0.01^{a}
C18:3n6	1.21±0.04^{a}	0.78±0.11^{b}	0.67±0.01^{b}	0.50±0.02^{b}	0.41±0.16^{b}
C20:2 cis11.14	1.57±0.03^{a}	0.64±0.04^{c}	1.27±0.02^{b}	0.57±0.11^{c}	0.54±0.03^{c}
C20:3n3 cis11.14.17	0.86±0.07^{a}	0.26±0.09^{bc}	0.36±0.00^{b}	0.14±0.02^{bc}	0.07±0.00^{c}
C20:3n6 cis8.11.14	2.50±0.04^{a}	0.87±0.08^{c}	1.48±0.01^{b}	0.65±0.02^{d}	0.40±0.02^{e}
C20:4n6	7.44±0.03^{a}	3.13±0.07^{c}	5.25±0.02^{b}	2.45±0.08^{d}	1.39±0.08^{e}
C20:5n3cis5.8.11.14.17	13.94±0.39^{a}	5.60±0.11^{c}	10.45±0.30^{b}	5.26±0.49^{c}	2.80±0.17^{d}
C22:2 cis13.16	0.15±0.02^{d}	0.27±0.01^{ab}	0.19±0.01^{cd}	0.25±0.02^{bc}	0.32±0.01^{a}
C22:6n3 cis4.10.13.16.19	14.61±0.22^{a}	5.45±0.04^{c}	9.78±0.03^{b}	4.49±0.24^{d}	2.55±0.14^{e}
Σ PUFA	**43.57±0.41^{b}**	**44.20±0.57^{b}**	**48.04±0.28^{a}**	**44.70±0.40^{b}**	**37.31±0.47^{c}**
TOTAL	**99.98±0.01**	**99.98±0.00**	**99.10±0.03**	**100.01±0.00**	**99.97±0.00**
ω 3	**29.82±0.51^{a}**	**12.57±0.11^{c}**	**21.33±0.31^{b}**	**10.99±0.70^{c}**	**7.16±0.32^{d}**
ω 6	**12.04±0.06^{d}**	**30.71±0.66^{b}**	**25.26±0.02^{c}**	**32.89±0.45^{a}**	**29.29±0.35^{b}**
ω3/ω6	**2.48±0.06^{a}**	**0.41±0.01^{c}**	**0.84±0.01^{b}**	**0.34±0.03^{cd}**	**0.24±0.01^{d}**
PUFA/SFA	**1.71±0.03^{d}**	**2.21±0.04^{a}**	**2.08±0.00^{ab}**	**1.96±0.07^{bc}**	**1.83±0.02^{cd}**
EPA+DHA	**28.55±0.49^{a}**	**11.04±0.09^{c}**	**20.23±0.33^{b}**	**9.76±0.73^{c}**	**5.35±0.31^{d}**

ab→: Difference between groups is important in the same column ($p<0.05$)

At the end of storage (10th month), ΣSFA, ΣMUFA, and ΣPUFA amounts of the product were determined as 20.39±0.42, 42.27±0.88, 37.31±0.47 %, respectively. At the end of the 10-month storage period, the SFA content of the final product decreased and it was found statistically different from the first day of the packaged product ($p> 0.05$). In a similar study conducted on marinated anchovy, it was determined that the SFA content of the samples increased during storage, and the PUFA content decreased as in our study, and it was stated that this was caused by oxidation in fatty acids during storage (Özden, 2005).

Omega 3 fatty acids, which are very important in terms of health, were detected at high rates in shrimp. The total ω3 content of fresh shrimp decreased with the applied smoking process, increased with marination, but decreased again with the added sunflower oil. In our study, the ω3 content of fresh shrimp was found to be 29.82 %. Similarly, Beydoun et al. (2007) reported ω3 content of raw shrimp as 35 mg/100g. The amount of ω6 in fresh shrimp was found as 12.04±0.06 and the processing

methods applied to the product and the addition of sunflower oil increased this value. The amount of ω6 increased with the effect of processing methods decreased the ratio of ω3/ω6 from 1.71 to 0.24 at the end of the storage period. Both the effect of processing methods and increased storage time led to a decrease in this rate. The desired rate for ω6/ω3 intake is between 1/1 and 4/1 (Simopoulos, 2002). When the findings obtained from the study were evaluated, it was found that the ratio of ω6 / ω3 in raw shrimp was quite low, the smoking and marination process increased this rate, but the differences in the process applied statistically were not significant ($p > 0.05$).

The PUFA / SFA ratio was determined to be 1.71±0.03 in fresh shrimp. This value had reached the level of 2.21±0.04 with the smoking process and it had been determined that the highest amount among the groups was in this group. The PUFA / SFA ratio decreased after this processing step until the end of the storage and reached 1.83±0.02. The optimum PUFA / SFA ratio is specified by HMSO (1994) as 0.45. It was determined that the PUFA/SFA ratio was above the optimum value in all groups. Ozogul et al. (2010) found the PUFA/SFA ratio of the hot smoked anchovy marinate produced by using similar processing methods as 1.32 at the beginning of the trial and stated that there was no significant difference with the beginning and at the end of the storage period. In the present study, a decrease in this rate was found and it is thought that this difference is due to the combination of sunflower oil added to the package.

The fresh shrimp contained 0.23 g/100g of crude oil, so the EPA+DHA content of 200 g fresh deep-water pink shrimp meat was 0.06 g, which was low compared to most fish meat. Lee et al. (2003) reported that blue crab (natural) and shrimp (natural) of shellfish contain <200 mg EPA+DHA, and mussels and oysters contain 500-100 mg EPA+DHA. The present study was similar to this literature.

Physicochemical analyses results

Physicochemical analyses results of fresh, smoked, and marinated shrimp

TVB-N, TBARs, salt, total acid, pH values of fresh shrimp, smoked shrimp, and marinated shrimp are given in Table 3.

Table 3. TVB-N, TBARs, salt, total acid, pH values of fresh, smoked, and marinated shrimp

	TVB-N (mg/100g)	TBARs (mgMDA/kg)	Salt (%)	Total acid (%)	pH	Aw
Fresh shrimp	7.96±0.32^{c}	0.26±0.01^{c}	2.33±0.02^{b}	0.17±0.01^{c}	7.16±0.06^{a}	0.97±0.00^{a}
Smoked shrimp	11.18±0.27^{a}	1.12±0.01^{a}	3.12±0.00^{a}	0.27±0.01^{b}	6.41±0.01^{b}	0.95±0.00^{b}
Marinated shrimp	9.92±0.15^{b}	0.62±0.01^{b}	1.15±0.01^{c}	1.04±0.01^{a}	2.56±0.06^{c}	0.95±0.00^{b}

ab ↓: Difference between groups is important in the same column ($p<0.05$)

In the study, the TVB-N value of fresh shrimp was determined as 7.96 mg/100g. Some researchers reported TVB-N values of different types of shrimp as 1.02 mg/100g in *P. adspersus* (Erdem and Bilgin, 2004), 8.87mg /100g in *C. crangon* (Bilgin and Erdem, 2006), 8.24 mg /100g in *P. semisulcatus* (Oner and Yıldırım, 2018). TVB-N is one of the most used chemical methods in determining the freshness of seafood (Varlık et al. 1993). It is known that this value affects factors such as the variety of seafood, fishing season, degree of maturity, sex, and age. The TVB-N value of the shrimp was increased by the smoking process (11.18±0.27 mg/100g) and it was determined as 9.92±0.15 mg/100g by decreasing in the marination ($p<0.05$). Similarly, with our study, it was reported by Kılınç and Çaklı (2004b) that the marination applied to the sardine caused a decrease in TVB-N value and that this is due to the dissolution of some of the TVB-N components in a salt-water solution.

The primary analysis used in the determination of oxidation of fatty acids in seafood is TBARs. The TBARs content of raw shrimp was determined as 0.26 ± 0.01 mgMDA/kg, increased in smoke and reached 1.12 ± 0.01 mgMDA/kg and decreased to 0.62 ± 0.01 mgMDA/kg by marination ($p<0.05$).

Salt used in the marinating process affected the flavor, ripening, and texture of meat, flavor formation, and shelf life of the product. The recommended brine salt ratio for lean raw materials is 6-8% (Varlık et al., 1993). As a result of the sensory data obtained from the preliminary studies, it was determined that the use of less amount of salt than the stated ratios would be more suitable for deepwater pink shrimp. In this study, the salt ratio of brine was 1.05%. The salt content of fresh

shrimp was at the rate of 2-3 % salt, which was defined as full salt, and the salt content of the end product was below 1.5 %, which is called light saline (Varlık et al., 2004).

While the average amount of total acid before storage was 0.17±0.01 in fresh shrimp, it increased slightly due to water loss after smoking and reached 0.27±0.01, and it was found to be 1.04 ± 0.01 by increasing with the effect of acid used in brine after marinating. A statistically significant difference was found between the groups ($p < 0.05$). Different researchers emphasize that the acid concentration to be used in marination should be 2-7 %, at least 4% for complete ripening and 1-2% in the final product (Kılınç and Çaklı, 2004a; Özden and Varlık, 2004). The acid concentration of the brine used in the present study was 2.4%, the acid concentration of the final product was 1.04%. Similar to the present study, Keskin et al. (2018) reported that approximately 50 % of the amount of total acid in the brine passes into fish meat and a balance occurs between fish and brine.

The pH value before storage was determined as 7.16±0.06 in fresh shrimp and 2.56 ± 0.06 in marinated shrimp and a statistical difference was observed between the groups ($p < 0.05$). Reported that the pH value of fresh shrimp was between 7-7.64. It has been reported by different researchers that the smoking process decreases the pH value (Kaya 2006; Günlü, 2007; Özoğul et al., 2010; Tosun and Özden, 2014).

The water activity (aw) in fresh shrimp was 0.97± 0.00, decreased due to the processes performed before storage and statistically, a significant difference was found between fresh shrimp and marinated shrimp groups ($p < 0.05$). In salted products, the water activity value is low, so these products are more durable (Çaklı and Kışla 2003).

Physicochemical analyses results of packed shrimp

The TVB-N values of the packed shrimp showed a time-dependent change during storage (Table 4). TVB-N values of the packaged product were below the limit values. TVB-N was determined as 10.26±0.25 mg/100g at the end of the storage. TVB-N analysis does not give direct results in marinating products, the results are far below the limit values and the changes are not stable (Varlık et al. 1993). It can be said that the use of TVB-N analysis as a parameter of deterioration is not suitable for smoked shrimp marinades.

Table 4. TVB-N, TBARs, Salt, Total acid, pH, and aw values of packed shrimp during storage.

		TVB-*N* (mg/100g)	TBARs (mg MDA/kg)	Salt (%)	Total acid (%)	pH	aw
	1st Day	9.72±0.05^{de}	0.32±0.01^{e}	1.36±0.01^{e}	0.85±0.01^{e}	2.87±0.01^{f}	0.96±0.00^{a}
Months	***1***	12.37±0.09^{ab}	0.45±0.01^{ab}	1.46±0.05^{e}	1.23±0.00^{a}	2.71±0.01^{g}	0.95±0.00^{ab}
	2	13.50±0.28^{a}	0.39±0.01^{bcd}	1.43±0.01^{e}	0.87±0.00^{de}	3.11±0.01^{e}	0.94±0.00^{bc}
	3	11.50±0.34^{bc}	0.46±0.02^{a}	1.4±0.06^{e}	0.96±0.01^{cd}	3.32±0.02^{d}	0.93±0.00^{cd}
	4	13.25±0.40^{a}	0.42±0.00^{abc}	1.45±0.04^{e}	1.12±0.00^{b}	3.28±0.01^{d}	0.93±0.00^{def}
	5	7.90±0.17^{f}	0.32±0.00^{e}	1.51±0.04^{e}	0.98±0.02^{c}	3.42±0.01^{c}	0.94±0.00^{bc}
	6	9.99±0.41^{de}	0.34±0.03^{de}	1.11±0.01^{f}	0.86±0.04^{e}	3.51±0.01^{b}	0.92±0.00^{ef}
	7	9.23±0.27^{ef}	0.35±0.00^{cde}	2.32±0.04^{d}	0.73±0.02^{f}	3.53±0.01^{b}	0.93±0.00^{cde}
	8	10.97±0.25^{bcd}	0.38±0.03^{cde}	2.51±0.03^{c}	0.58±0.02^{g}	3.65±0.01^{a}	0.94±0.00^{cd}
	9	10.56±0.43^{cde}	0.37±0.01^{cde}	2.74±0.01^{b}	0.56±0.02^{g}	3.62±0.01^{a}	0.93±0.00^{def}
	10	10.26±0.25^{cde}	0.42±0.02^{abc}	3.22±0.05^{a}	0.55±0.04^{g}	3.67±0.01^{a}	0.92±0.00^{f}

ab ↓: Difference between groups is important in the same column ($p<0.05$)

At the beginning of storage, TBARs value was determined as 0.32 mgMDA/kg, fluctuated during storage, and did not exceed 0.46 mgMDA/kg. A TBA value of less than 3 indicates that the product is in a "very good" condition in terms of oxidation (Varlık et al. 1993). Karslı (2013) reported that the amount of TBA between 0.52-1.05 (mg MDA/kg) during storage in a smoked marinated cockle. Kalıştır (2008) detected 0.66 mg/kg in the fresh sample of the marinated shrimp (*M. stebbingi*), while this value increased during the storage in the refrigerator and reported that it was 4.05 mgMDA/ kg at the end of storage. Cadun et al. (2008) obtained marinate from deep pink water shrimp and TBA value of fresh shrimp was 0.26 mg MDA/kg, this value increased to 0.9 mgMDA/kg after marination, and they ended the study because they exceeded the consumable limit value on 75th day after storage.

While the salt content of the packed shrimp smoked during the storage period was not statistically different until the 6th month ($p > 0.05$), it increased until the end of the trial after the 6th month ($p < 0.05$).

During the storage period, the amount of vinegar fluctuated up to the 4th month and continued to decrease until the end of the trial after the 4th month. There was no statistical difference between the groups at the beginning of the trial (1st day), 2nd, and 6th months ($p > 0.05$), and no statistical differences were observed after the 8th month.

During the storage period, the amount of total acid fluctuated up to the 4th month and continued to decrease until the end of the storage. There was no statistical difference between the groups at the beginning of the storage (1st day), 2nd, and 6th months ($p > 0.05$), and no statistical difference was observed after the 8th month.

Average pH values fluctuated between 2.70 and 3.70 during storage. The maximum pH value was measured at 10 months and this value was statistically not different between the 8th and 9th months ($p > 0.05$). The initial pH value increased over time due to the sunflower oil added to the product.

Water that fumes away by smoking caused aw drop and no change was observed by marination. The aw value fluctuated during storage. Similar results were also identified by Šimat et al. (2011), Karslı (2013), Kocatepe et al. (2019).

Color analysis results

Color analysis results of fresh, smoked, and marinated shrimp

The L * (brightness) value of fresh shrimp was determined as 77.92 and this value decreased with the effect of smoking (Figure 3). The composition of the smoke may have adversely affected the brightness of the product. After marinating, the brightness of the product increased and it was found statistically similar to raw shrimp ($p > 0.05$). It is known that the marinating effect increases the brightness of the product. Marine products are requested by consumers to be bright. It can be said that marinating reduces the negative effect of hot smoke on brightness and a more attractive product was developed for the consumer. In the fresh product, a (+) redness value of 3.72 was found and this value increased with the effect of smoking, but the effect of the marination process was found insignificant ($p > 0.05$). After the smoking process, the b (+) yellow value of the raw shrimp increased, but the yellowness of the color decreased after the smoked marination ($p < 0.05$). Yellowness decreased due to the lightening properties of the acid during the marination process.

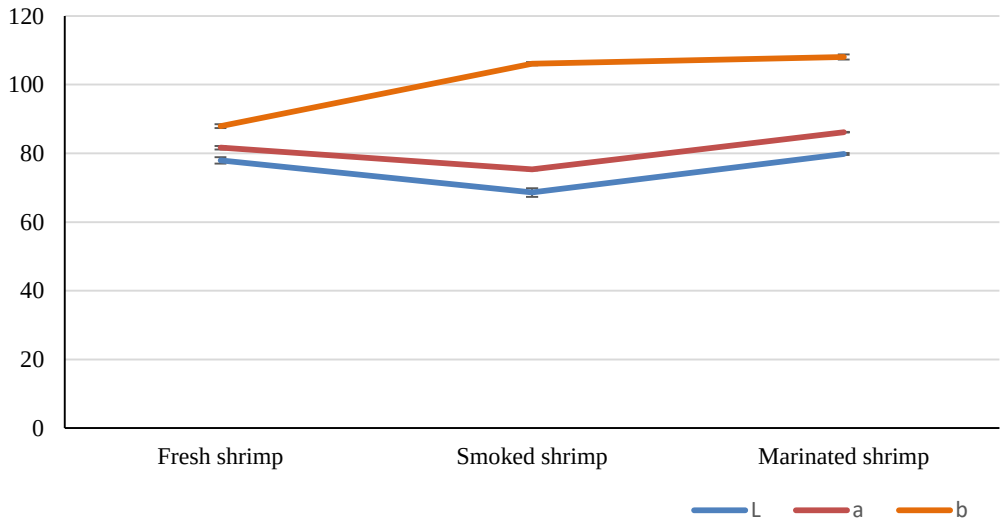

Figure 3. Color analysis results of fresh, smoked, and marinated shrimp

Color analysis results of packed shrimp

The brightness of marinated shrimp fluctuated during storage and all values were detected lower than the first day of marination. The brightness value decreased by 11.6 % in the 6th month of storage compared to the previous month ($p < 0.05$). A (+) red value of the final product fluctuated during

storage. B (+) yellow value increased after the 1st day of marination (Figure 4). Karslı (2013) reported that the brightness value of the smoked clam marinades decreased during storage.

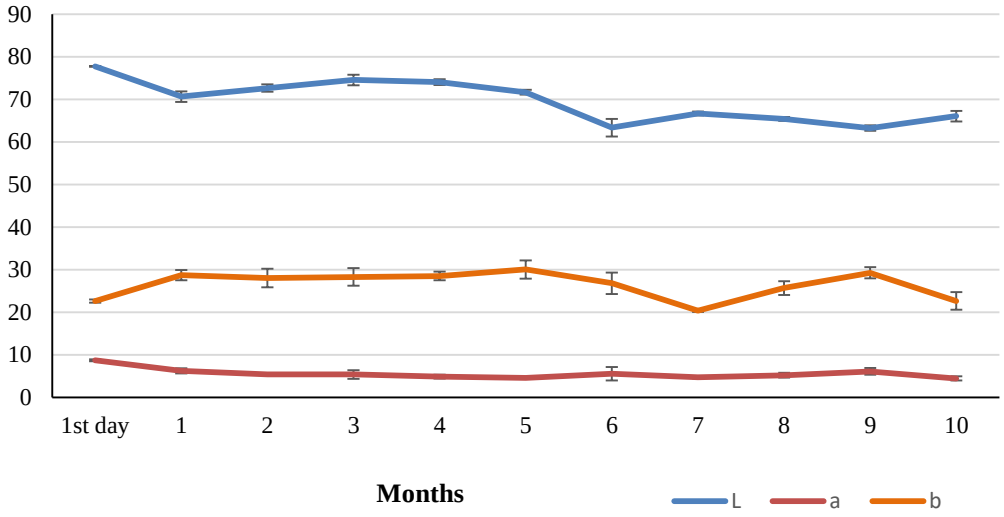

Figure 4. Color analysis results of packed shrimp

Microbiological analysis results

In the study, all groups were examined in terms of Total Mesophilic Aerobe Bacteria (TMAB), Total Aerobe Mesophyll Bacteria (TPB), Total Yeast-Mold (TYM), and Total Coliform Bacteria (TC) count given in Table 5.

Table 5. Microbiological analysis results of shrimp (Log CFU/g)

	TMAB	TPB	TYM	TC
Fresh shrimp	1.54±0.06 ^{a}	1.92±0.02 ^{a}	<1 ^{b}	<1 ^{a}
Smoked shrimp	1.45±0.15 ^{a}	1.59±0.11 ^{a}	1.39±0.09 ^{a}	<1 ^{a}
Marinated shrimp	<1 ^{b}	<1 ^{b}	<1 ^{b}	<1 ^{a}

ab ↓: Difference between groups is important in the same column ($p<0.05$)

The number of bacteria of fresh shrimp TMAB, TPB, TYM, and TC was 1.54, 1.92, <1, and <1 LogCFU/g respectively. Oner and Yıldırım, (2018), TMAB and TC counts of *P. semisulcatus* were 3.84 and <2.0 LogCFU/g. However, Diler and Ataş (2013) reported that the number of TMAB and TC were $5.8x10^4$ and $1.9x10^2$ CFU/g in the same shrimp. Patir et al. (2009) reported the TC count of raw shrimp meat as 2.53 LogCFUg-1 and the TYM content as 1.78 LogCFU/g. After removing the shells of the deepwater pink shrimp used in our study, the meat ready for processing was analyzed microbiologically and the microbiological load was found to be quite low from this literature. These data show that the shrimp used in the present study were exposed to cross-contamination at very low levels until processing. With the effect of heat treatment and antimicrobials in the smoke, TMAB and TPB count decreased, but they were found statistically insignificant ($p> 0.05$). However, it has been observed that the smoking process increases the total number of yeast molds of shrimp. This number fell below 1 LogCFU/g again by marinating.

Inal (1992) reported that the number of TC should not be more than 2 Log CFU/g in fresh shrimp meat. Coliform group bacteria are used as an indicator of fecal contamination. The Japan Food Sanitation Law stated that Coliform should be 0 tolerant in frozen foods including cooked shrimps (Department of Fisheries, 2004).

The counts of microorganisms (TMAB, TPB, TYM, TC) determined in packed shrimp has remained below the detectable limit value (<1 LogCFU/g) during the storage period, with the protective effect of both hot smoking and marination.

Sensory analysis results

After the smoked and marinated shrimp meat was packed, they were analyzed monthly starting from the first day; the product was evaluated by scoring between 0 and 5 in terms of color, odor, flavor, texture, and general taste (0-1: Inexpensive, 1-2: Bad, 2-3: Not bad, 3-4: Good, 4-5: Very good). The product that is under 2 points is considered as non-consumable. Sensory analysis results did not fall below 4 points in terms of smell and there was no statistical difference from day 1 to month 7 ($p > 0.05$). When it was evaluated as flavor, it decreased to 1.2 ± 0.12 at the end of the storage period and reached not consumable value in the 9th month (Figure 5).

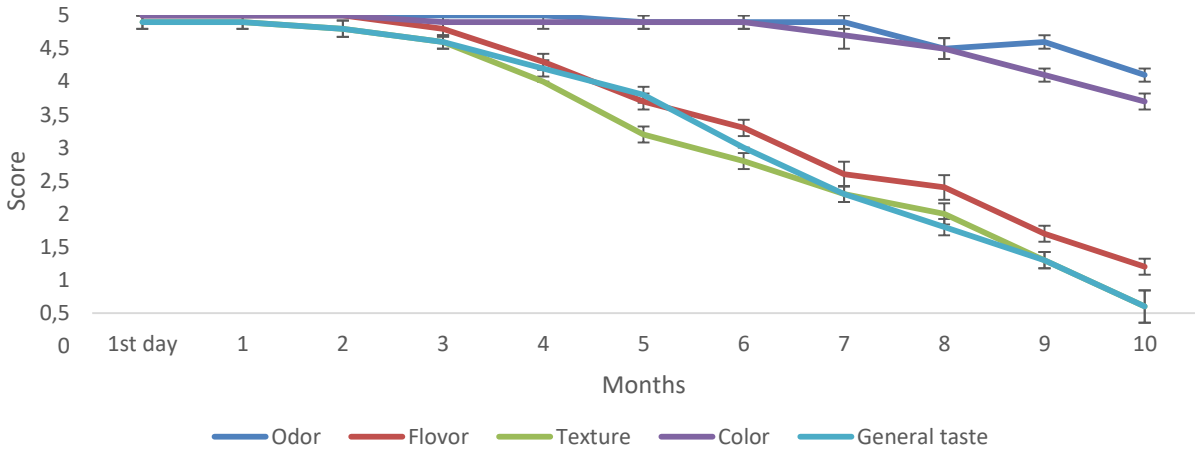

Figure 5. Sensory analysis results of shrimp

CONCLUSION

In this study, a very delicious and aromatic product had been obtained by smoking and marinating process, and it had been concluded that this product had high nutritional value and that smoking, and marination affect the shelf life of the product positively. Sensory analysis results were evaluated very precisely in this study and the shelf life of smoked deepwater pink shrimp marinate was determined as 7 months.

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Reproductive Biology of Abu Mullet, *Planiliza abu* (Heckel, 1843), in Karun River, Southwestern Iran

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Research Article

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Abstract

Some aspects of the reproductive biology of *Planiliza abu* (Mugilidae) were investigated by collecting of 428 specimens caught with trawl by local fishermen from Karun River, Ahwaz, from November 2016 to September 2017. The total length for males ranged between 10-17.1 (13.6±1.26 SD) cm and for females, between 10.5-17.3 (13.44±1.35) cm. The age range of fish in both sexes was between 0^{+}-7^{+} years and the most abundant age group was the 4^{+}. The sex ratio in the studied fish was equal (P>0.05). The mean egg diameter was significantly different (P<0.05) in different months and the individual size ranged between 0.12 and 0.57 mm. The absolute fecundity ranged from 3600 to 48600 eggs and the relative fecundity from 120 to 900 eggs per 1 g of body weight. The highest hepatosomatic index in females was in February during yolk vitellogenesis (1.89). Based on gonadosomatic index, egg diameter, and monthly distribution of ovarian maturation stages, the spawning season of this fish is long, but peaks in February (GSI 12.35) and March (GSI 12.18). This species appears to have a group synchrony reproductive pattern.

Keywords: Mugilidae, Gonadosomatic Index, Spawning period, Fecundity, Egg diameter

Ebu Kefalinin Üreme Biyolojisi, Planiliza abu (Heckel, 1843), Karun Nehri, Güneybatı İran

Planiliza abu'nun (Mugilidae) üreme biyolojisi, Kasım 2016'dan Eylül 2017'ye kadar Ahvaz, Karun Nehri'nde yerel balıkçılar tarafından trolle yakalanan 428 örnek üzerinde araştırıldı. Erkeklerin toplam uzunluğu 10-17.1 (13.6 ± 1.26 SS) cm ve dişiler 10.5-17.3 (13.44 ± 1.35) cm. Her iki cinsiyette de balıkların yaş aralığı 0 + -7 + yaş arasında ve en bol olan yaş grubu 4+ ve cinsiyet oranı eşitti (P> 0.05). Ortalama yumurta çapı aylara göre önemli ölçüde farklıydı (P <0.05) ve bireysel boyut 0.12 ile 0.57 mm arasında değişiyordu. Mutlak verimlilik 3600 ila 48600 yumurta ve nispi verimlilik vücut ağırlığının 1 g'ı başına 120 ila 900 yumurta arasında değişiyordu. Dişilerde en yüksek hepatosomatik indeks Şubat ayında yumurta sarısı vitellogenez sırasında görüldü (1.89). Gonadosomatik indeks, yumurta çapı ve yumurtalık olgunlaşma aşamalarının aylık dağılımına göre, balığın yumurtlama mevsimi uzun ve Şubat (GSI 12.35) ve Mart'ta (GSI 12.18) pik yapar. Bu türün grup eşzamanlı üreme modeline sahip olduğu görülmektedir.

Anahtar Kelimeler: Mugilidae, Gonadosomatik İndeks, Yumurtlama dönemi, Verimlilik, Yumurta çapı

INTRODUCTION

Iran's freshwater fishes consist of approximately 300 species in 109 genera, 30 families, 24 orders, and three classes distributed throughout the basins (Keivany et al., 2016; Esmaeili et al., 2017; Esmaeili et al., 2018; Keivany and Esmaeili, 2019). The mullets are found in temperate to tropical coastal waters, readily entering estuaries, and even reside in freshwaters. This family is an important element of the aquatic ecosystems and many species are of commercial or other significance (Coad, 2017). This family consists of 17 genera with 72 species, amongst which seven are found in Iranian waters (Esmaeili et al., 2018; Keivany and Esmaeili, 2019).

Planiliza abu inhabits Asian countries such as Iraq, Syria, Pakistan, Iran, and Turkey (Turan et al., 2004; Coad, 2020) and was reported from Iranian tributaries of the Tigris River basin including Karun River. It appears in fish markets as a regular food fish in Ahvaz, Khuzestan, and is an important food

fish in southern Iraq (Coad, 2020). Knowledge of the reproductive cycle and factors affecting it are important issues in fish and fishery biology (Tomkiewicz et al., 2003; Asadollah et al., 2017; Keivany et al., 2017a). It has three key components including sexual maturity, reproductive period, and fecundity, which are important demographic characteristics, essential for understanding a species life history (Mousavi-Sabet et al., 2017; Ghafori et al., 2019). There are some works on the biology of this fish in nearby countries (Mhaisen and Al-Jaffery, 1989; Doğu et al., 2013; Mohamed, 2014; Ay and Özcan, 2016; Birecikligil et al., 2017), but almost none in Iran. The main object of this study was to provide data on the reproductive biology of *P. abu*, including sex ratio, gonadosomatic index, fecundity, oocyte diameter, and spawning season which are necessary for conducting conservation and management programs in Karun River.

MATERIALS and METHODS

Some 428 specimens of *Planiliza abu* (Mugilidae) caught with trawl by local fishermen from Karun River, Ahvaz, from November 2016 to September 2017, were sampled. All the collected specimens were fixed and preserved in 10% formaldehyde solution and transferred to the ichthyology laboratory. For each specimen, total length (TL) and standard length (SL) were measured using digital calipers with a precision of 0.01mm and body weight (W) was taken on a digital balance to the nearest 0.01g. Age determination was carried out through scales from between the lateral line and the dorsal fin, 5-10 scales washed with warm water and the age was determined under the microscope by counting the annual growth circles (Hsu and Tzeng, 2009; Ellender et al., 2012). The sex ratio deviation from 1:1 was tested statistically by chi-squared analysis (Sokal and Rohlf, 1973). Gonadosomatic Index (GSI) was used to investigate the monthly changes in gonads to estimate the spawning season of this species: (GSI= Wg/W×100); (Wg) is the ovaries weight and (W) the body weight (Biswas, 1993). Also, the monthly Hepatosomatic Index (HSI) was calculated using the formula: HSI = [WL / W] × 100, where WL is the liver weight and (W) the body weight. To calculate absolute fecundity, total eggs were counted in 20 ovaries by gravimetric method. While relative fecundity was determined as the proportion of absolute fecundity to the eviscerated body weight. To calculate absolute fecundity, ovaries in stages IV or V were used. Egg diameter was measured with a scaled ocular micrometer. After identifying the various stages of sexual maturation, diameter of sex cells was measured during different growth phases under magnifications of 40× by a scaled ocular micrometer. The stage of gonad maturity was determined visually following Brown-Peterson et al. (2011) which is widely used in different works (e.g., Abaszadeh et al., 2013; Hamzeh et al., 2017; Siami et al. 2017; Keivany et al., 2018a; Ghafouri et al., 2019). Reproductive seasonality was determined by examination of the monthly changes in the gonadosomatic index. The obtained data were analyzed in SPSS 16.0 and Microsoft Excel 2016 software packages. One-way ANOVA followed by Duncan post-hoc was used to analyze differences in means of GSI% and egg diameter of fish at 95% confidence level.

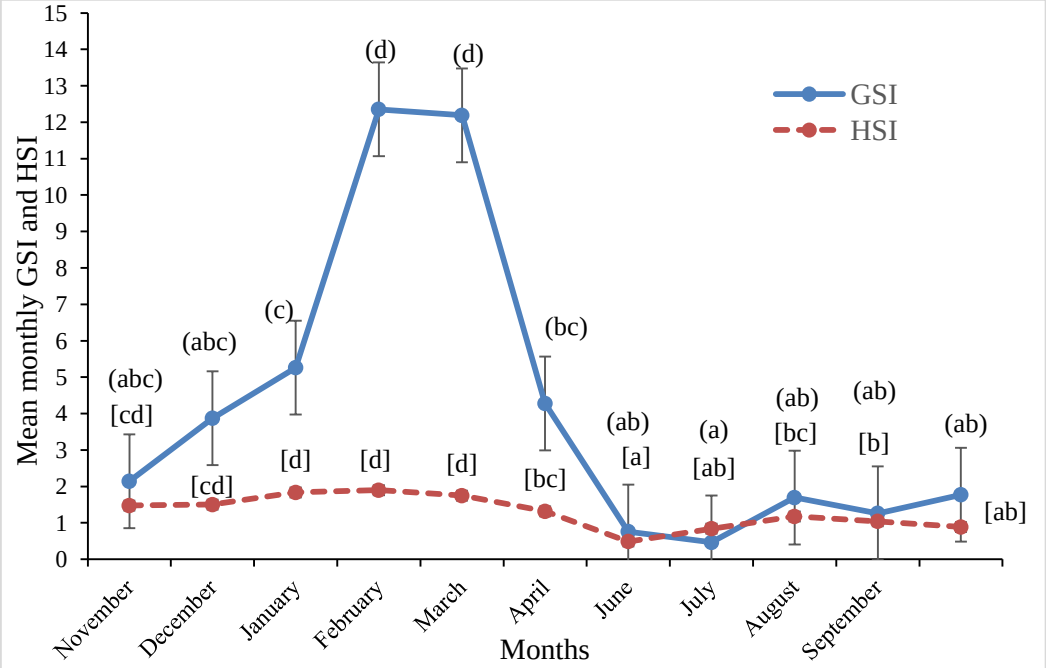
RESULTS

The length and weight of males ranged from 10.00 to 17.10 (13.16±1.26) cm and 12.92 to 71.28 (30.51±8.64) g and those of females from 10.50 to 17.30 (13.44±1.35 SD) cm and 16.23 to 69.89 (33.00±9.41) g, respectively (Table 1). The age of this species ranged from 0^+ to 7^+ years. The dominant age group was the 4^+ years for both males and females (Table 2). Amongst the 428 collected specimens, 221 were females and 207 were males, giving an overall sex ratio of about 1M:1F (X^2= 0.45; P>0.05). Assessment of the spawning period of *P. abu* in Karun River was based on the GSI, analysis of seasonal development in mean egg diameter, and direct observation of the gonads. Significant differences were found in the males and females GSI. Also, significant differences were found in females egg diameters ($p < 0.05$) in different months. The highest GSI value for both sexes occurred from February to March. The GSI increased gradually in November and reached the maximum value in February and decreased gradually from April to June (Figure 1). The highest HSI value for males was in July and for females in January to February. Significant differences were found in HSI in different months (P<0.05). The egg diameter ranged from 0.12 to 0.57 mm and the mean values were significantly different during the year (P< 0.05). The highest mean egg diameter (0.48±0.04 mm) was observed in February and the lowest one in August (0.19±0.04 mm) (Figure 2). The mean of the absolute fecundity of 20 females determined during the spawning period was 3600-

48600 (22400±11000) eggs and relative fecundity was 120-900 (500±210) eggs/g body weight. Fecundity-total length, fecundity-weight and fecundity-ovary weight relationships in *P. abu* in Karun River was as $F=3.66L^{3.19}$ ($r^2=0.16$), $F=12.29W^{1.95}$ ($r^2=0.43$) and $F= 1230.10X^{1.26}$ ($r^2=0.84$), respectively (Figures 3, 4).

Table 1. Mean egg diameter variations in Planiliza abu in Karun River from November 2016 to September 2017

Months	Mean TL±SD (mm)	Mean TW±SD (g)	Egg diameter range	Mean egg diameter ±SD
November 2016	14.32±0.96	37.24±8.62	0.14-0.45	0.27±0.08
December	13.53±0.71	29.66±3.89	0.29-0.40	0.35±0.03
January	13.33±0.89	30.84±5.63	0.25-0.48	0.39±0.06
February	15.24±1.04	46.27±7.10	0.42-0.57	0.48±0.04
March	14.10±0.98	38.11±7.49	0.34-0.48	0.43±0.04
April	12.44±0.83	26.43±5.92	0.22-0.41	0.32±0.07
May	11.95±1.06	22.54±6.05	0.25-0.38	0.30±0.04
June	11.93±0.55	24.76±2.78	0.20-0.40	0.26±0.06
July	11.78±0.89	22.65±5.09	0.13-0.27	0.21±0.05
August	12.55±0.93	27.64±6.76	0.12-0.22	0.19±0.04
September 2017	13.52±0.73	34.60±6.11	0.16-0.34	0.23±0.04



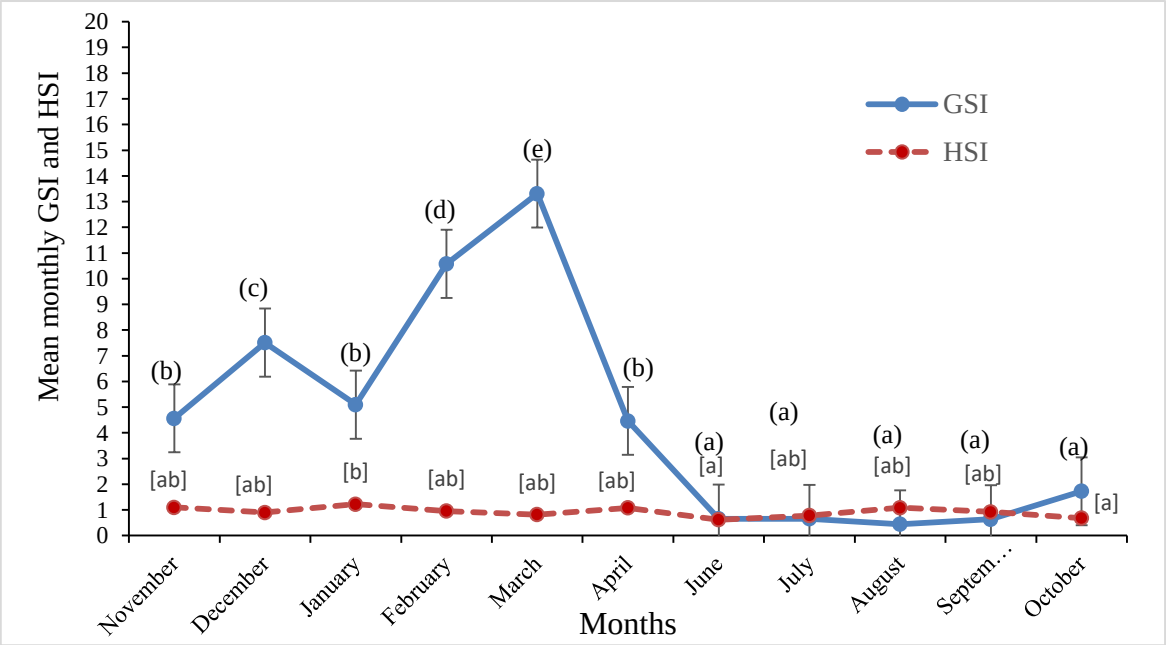


Figure 1. GSI and HSI variations in females (Top) and males (Bottom) of Planiliza abu in Karun River from November 2016 to September 2017

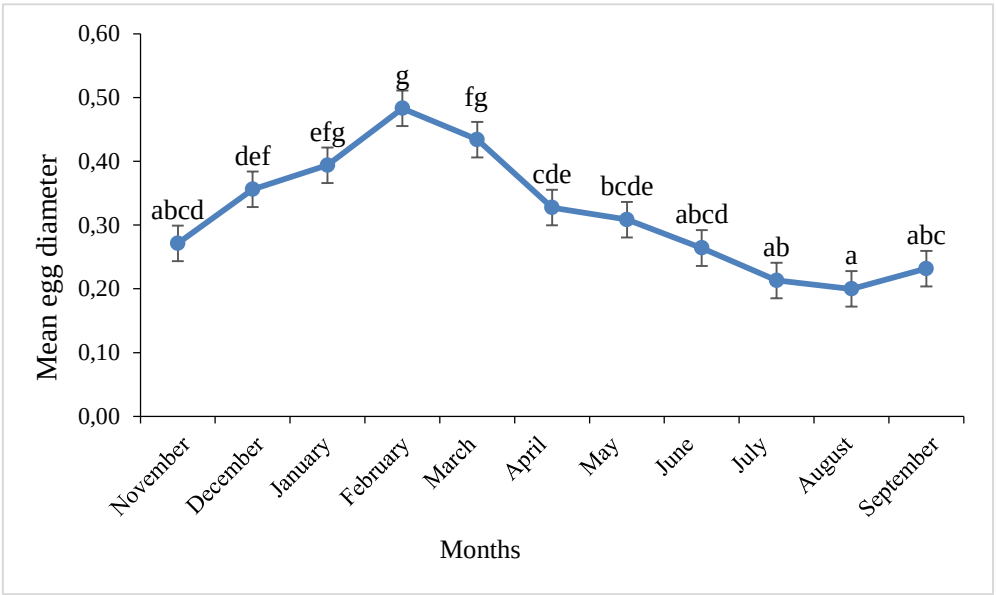
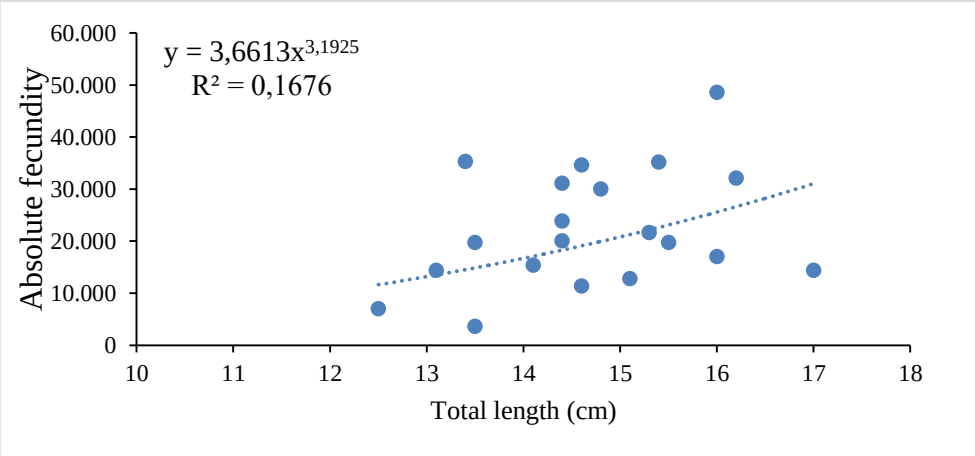


Figure 2. Variation of mean egg diameter (mm) in female Planiliza abu in Karun River in different months

Table 2. Number and sex ratios of Planiliza abu specimens in different age groups

Age group	Sex	Number	M:F ratio	P=0.05
0^+	M	0	0	-
	F	1		
1^+	M	4	4	P>0.05
	F	1		
2^+	M	29	1.52	P>0.05
	F	19		
3^+	M	52	1.01	P>0.05
	F	51		
4^+	M	65	0.89	P>0.05
	F	73		
5^+	M	36	0.76	P>0.05
	F	47		
6^+	M	16	0.72	P>0.05
	F	22		
7^+	M	5	0.62	P>0.05
	F	8		



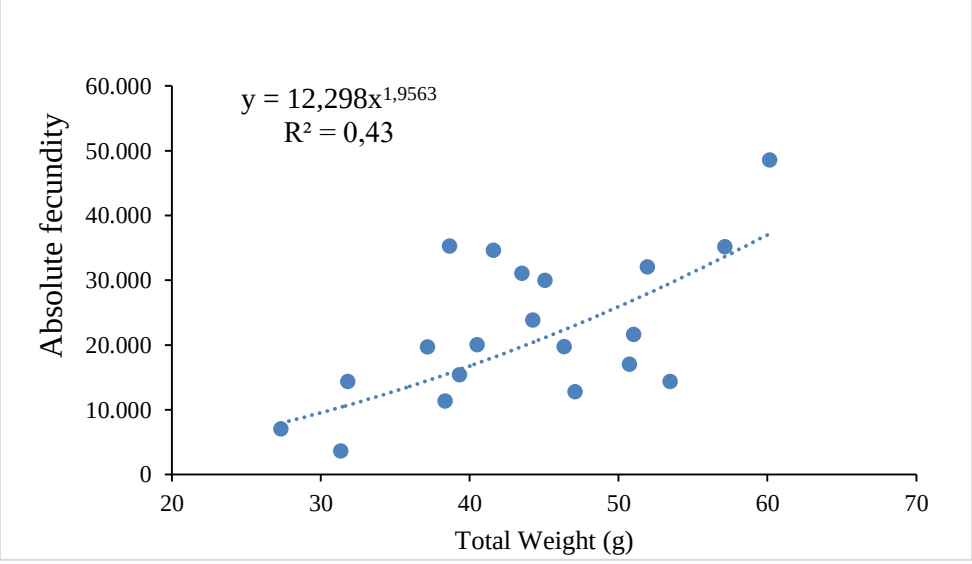


Figure 3. Fecundity-total length, fecundity-weight relationship in Planiliza abu in Karun River from November 2016 to September 2017

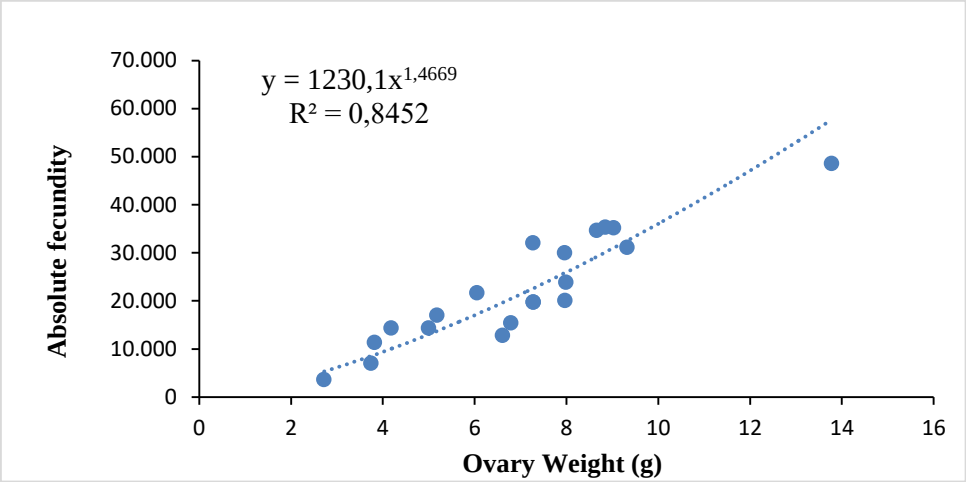


Figure 4. Fecundity-ovary weight relationship in Planiliza abu in Karun River from November 2016 to September 2017

DISCUSSION

The length of the females was higher than that of the males as seen in many other fishes (Asadollah et al., 2017; Keivany et al., 2017b, 2018b). In this study, the maximum observed total length and weight of *P. abu* was 17.3 cm and 71.28 g, respectively. In other studies, the maximum reported fork length and weight is 22.1 cm and 136.6 g in Atatürk Dam Lake (Doğu et al., 2013), 22 cm and 66.1 g in East Hammar marsh of Iraq (Mohamed, 2014) and 18.5 cm and 66.40 g in the Orontes River, Turkey (Ay and Özcan, 2016), 65.0 cm (Garbin et al., 2014) in southeastern coastal regions of Brazil and 23.1 cm and 166.1 g in Ceyhan River Basin of Turkey (Birecikligil et al., 2017). In general, the differences in the length of the fish could be due to differences in the fishing methods and seasons, different geographic areas, biological and ecological conditions, or inter-species differences.

The study of reproductive biology is an effective method for recognizing the stocks and life cycle of fishes (Abedi et al., 2011). The sex ratio in this study was equal (1M:1F), however, it differed in different seasons and length classes. In other studies, the sex ratio of *P. abu* was reported as 1M:2.7F in Khozestan Province, Iran (Chelemal et al., 2009), as equal in Atatürk Dam Lake (Doğu et al., 2013) and as 1M:1.3F in the Orontes River, Turkey (Ay and Özcan, 2016). These differences are most probably related to sexual differences in growth rate, natural mortality rate, and energetic cost of reproduction (Stergiou et al., 1996). Also, it is suggested that dominance of one sex relative to the other can be due to different behaviors leading to an easier catch of one sex and differences in mortality of the sexes (Keivany et al., 2017a).

Gonadosomatic (GSI) and hepatosomatic (HIS) indices are among the biological indicators used to determine the fish spawning season (Ghanbahadur et al., 2013). The HSI for females is more important because of the vitellogenesis performed for eggs in the liver. Typically, in many fish species, at the peak of spawning, the GSI is in the highest value and the HSI in the lowest (Keivany and Soofiani, 2004). The lowest value of HSI in *P. abu* was in May and the highest in February. The monthly variation in HSI was lower in males than in females. In the present study, the HSI and GSI showed a similar trend, probably indicating the continued feeding of the fish (Dopeikar et al., 2015). The monthly variation in GSI was highly associated with the seasonality of the maturity stages assigned macroscopically. An increase in the GSI of females was observed from January which peaked in February and March and decreased from April to May. In the case of males, the spawning season lasted from February to March and peaked in March. Şahinöz et al. (2011), in Turkey's Atatürk Dam Lake, found the highest GSI of female *P. abu* in April and that of males in May.

The mean maximum egg diameter was significantly different during the year. The size of the egg increased during the spawning season. The highest mean egg diameter was observed in February. In the study of the reproductive biology of *P. abu* in Turkey, the mature egg diameter was in the range of 0.31 to 0.63 with a mean of 0.44 mm (Ay and Özcan, 2016). Egg diameter variations in fish are probably one of the important strategies in determining reproductive activities (Tomasini et al., 1996). The maximum absolute fecundity of *P. abu* was 48000 oocytes and that of the relative fecundity was

910 Oocytes per gram of body weight. Fecundity is the most common criterion for determining the potential of reproduction in fishes (Nichol and Acuna, 2001). In the study of Ay and Özcan (2016), *P. abu* fecundity was calculated for 51 adult females in the spawning season, and the absolute fecundity ranged between 12000-25000 eggs with an average of 14400±1600 eggs. Several factors such as size and age of females, life history strategy, food supply, and water temperature affect the fecundity (Thrope et al., 1984). The GSI peak, the maximum diameter of the eggs, and the highest number of hydrated oocytes were observed in January-March, therefore the spawning peak of *P. abu* occurred in February-March. Macroscopic development of the testes followed a similar trend to those of the ovaries. It could be concluded that *P. abu* is a group synchronous multiple batch spawner with a long period of reproduction which lasts from January to March and peaks in February-March.

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Faunal Diversity and Distribution of Hygrobatidae Koch, 1842 Species (Acari: Hydrachnidia) of Turkey and Three Neighboring Countries

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Abstract

This study is aimed to determine the distributions, habitats, and endemism rates of Hygrobatidae species of Turkey and three neighboring countries. Similarity calculations were made between Turkey and three neighboring countries using the Bray-Curtis index. As a result, it has been determined that Turkey has a high of similarity with Greece and Bulgaria and a low index of similarity with Iran. Additionally, a new record, *Atractides* (*Tympanomegapus*) *lacustris* (Lundblad, 1925), has been given for the fauna of Turkey. The number of Hygrobatidae species has been reached 55 with *A. lacustris* and Hygrobatidae species have been recorded from 21 provinces in Turkey.

Keywords: Biodiversity, Hydrachnidia, Hygrobatidae, taxonomy, Turkey.

Türkiye ve Üç Komşu Ülkenin Hygrobatidae Koch, 1842 (Hydrachnidia: Acari) Türlerinin Faunal Çeşitliliği ve Dağılımı

Özet

Bu çalışmada, Türkiye ve üç komşu ülkenin Hygrobatidae türlerinin dağılımı, habitat tercihleri ve endemizm oranlarının belirlenmesi amaçlanmıştır. Türkiye ve üç komşu ülke arasındaki benzerlik hesapları Bray-Curtis indeksi kullanılarak yapılmıştır. Sonuç olarak Türkiye'nin Yunanistan ve Bulgaristan ile Hygrobatidae tür benzerliğinin yüksek, İran ile düşük olduğu belirlenmiştir. Ek olarak, Türkiye faunası için yeni kayıt *Atractides (Tympanomegapus) lacustris* (Lundblad, 1925) verilmiştir. Türkiye'de 21 ilden kaydedilmiş olan Hygrobatidae türlerinin sayısı *A. lacustris* ile 55'e ulaşmıştır.

Anahtar kelimeler: Biyolojik çeşitlilik, Hydrachnidia, Hygrobatidae, taksonomi, Türkiye.

INTRODUCTION

Water mites, also known as Hydracarina, Hydrachnidia, or Hydrachnellae, are one of the polyphilic groups in Acari subclass. The term Hydracarina was firstly coined. However, this nomenclature also includes Halacaridae. But the morphologies and life cycles of Halacaridae members are different from Hydrachnidia (Bartsch et al., 2007).

Up to date, 400 genera and more than 6000 species of water mites belonging to 57 families have been identified and 1642 species have been reported from the Palearctic region including Turkey (Bartsch et al., 2007; Di Sabatino et al., 2008). The latest water mite checklist has been-published by Erman et al., (2019). It has been recorded that 335 species and 62 genera belonging to 25 families from Turkey. In terms of the highest number of species, Arrenuridae ranks first with 58 species, and Hygrobatidae ranks second with 54 species.

Hygrobatidae species have a wide range of morphological adaptations and types of habitat preference. This family consists of five genera: *Atractides*, *Hygrobates*, *Iranobates*, *Mesobates*, and *Mixobates*. The genus *Atractides* has the highest number of species. Members of this genus probably have an ancestor in common with members of other genera with similarly modified I-L. The genus *Atractides* is probably of paraphyletic origin. The task of the characteristic I-L has not yet been clear (Pešić, et al., 2011; Gerecke et al., 2016). *Atractides* species were found only in clean water with well-

conserved substrates and in general, inhabit running water. They are probably sensitive to many anthropogenic factors (Gerecke, 2003). In the genus *Hygrobates*, the absence of modified I-L-5 is a plesiomorphy, but an importend requisite for defining this genus. In the genus *Mixobates*, the presence of a pair of distinctly shaped distoventral setae on I-L-5 indicates a relation with *Atractides*-like genera rather than with *Hygrobates* (Gerecke et al., 2016).

Our purpose in this study is to revise the species belonging to the family Hygrobatidae in Turkey and three neighboring countries and compare similarities between them. It also aims to give information on the endemism rate, habitats, and distributions of Hygrobatidae species in Turkey. Additionally, a new record, *Atractides* (*Tympanomegapus*) *lacustris* (Lundblad, 1925), was given for the fauna of Turkey.

MATERIALS and METHODS

Water mites were collected by hand-netting, sorted on the spot from the living material, preserved in Koenike's fluid, and dissected for slide-mounting in Hoyer's fluid. All measurements were given in μm. See Gerecke (2003) for a detailed description and discussion of the characteristics of the genus *Atractides* and a detailed methodological introduction. In this study, the Bray-Curtis index was calculated using the Past Program. The following abbreviations are used: Cx–I = first coxae, L = length, I–L–6 = Leg 1, sixth segment, P–1 = palp, first segment, S–1 = large proximal ventral seta at I–L–5, S–2 = large distal ventral seta at I–L–5, Vgl = ventroglandulare, W= width.

RESULT

Family Hygrobatidae Koch, 1842

Distribution of Hygrobatidae Species in Turkey

Atractides (39), *Hygrobates* (14), and *Mixobates* (2) species were recorded from 21 provinces of Turkey. While Erzurum province ranks first with 15 species, followed by Malatya with 12 species and Rize with 9 species and Afyonkarahisar and Antalya with 8 species. *Atractides nodipalpis* Thor, 1899 and *Atractides robustus* (Sokolow, 1940), are the most common species recorded from six provinces (Table 2). Six species and one subspecies of Hygrobatidae are endemic to Turkey. *Hygrobates* (*Hygrobates*) *turcicus* Pešić & Esen, 2017 was recorded from four provinces in Turkey. The other endemic species are only known from the type localities (Table 1). The endemism rate is found as 12.7.

Table1. Hygrobatidae species endemic to Turkey

ENDEMIC SPECIES	PROVINCES
Atractides (*Atractides*) *anatolicus* Pešić, Erman & Esen, 2010	Bingöl
Atractides (*Atractides*) *martini* Pešić, Erman & Esen, 2010	Erzurum
Atractides (*Atractides*) *oezkani* Pešić & Erman, 2006	Malatya
Atractides (*Atractides*) *reinhardi* Gülle, Gülle & Boyacı, 2015	Burdur
Hygrobates (*Hygrobates*) *anatolicus* Esen & Pešić 2013	Kahramanmaraş
Hygrobates (*Hygrobates*) *turcicus* Pešić & Esen 2017	Bayburt, Bingöl, Rize, Antalya
Mixobates (*Mixobates*) *brachypalpis ozkani* Pešić & Turan, 2006	Rize

Table 2. The distribution of Hygrobatidae species according to the provinces in Turkey

SPECIES	Adıyaman	A. karahisar	Antalya	Artvin	Bayburt	Bingöl	Burdur	Elazığ	Erzincan	Erzurum	Isparta	K. maraş	Kayseri	Konya	Malatya	Mersin	Rize	Siirt	Sivas	Trabzon	Van
Atractides (*Atractides*) *allgaier*			X														X				
Atractides (Atractides) anatolicus						X															
Atractides (Atractides) anellatus														X							
Atractides (Atractides) arcuatus														X							
Atractides (Atractides) dentipalpis											X										
Atractides (*Atractides*) *distans*			X																		
Atractides (*Atractides*) *fissus*														X							
Atractides(*Atractides*) *fluviatilis*															X						
Atractides (*Atractides*) *fonticolus*						X				X					X		X				
Atractides (*Atractides*) *gibberipalpis*										X					X						
Atractides (Atractides) glandulosus									X												
Atractides (Atractides) gomerae	X																				
Atractides (*Atractides*) *graecus*						X															
Atractides (*Atractides*) *inflatipalpis*										X											
Atractides (*Atractides*) *inflatipes*							X														
Atractides (*Atractides*) *inflatus*			X											X		X					
Atractides (*Atractides*) *lunipes*															X						
Atractides (Atractides) martini										X											
Atractides (*Atractides*) *nahavandii*									X												
Atractides (Atractides) nikooae																		X			
Atractides (*Atractides*) *nodipalpis*		X						X		X			X		X				X		
Atractides (Atractides) nodipalpoides														X							
Atractides (Atractides) oezkani															X						
Atractides (*Atractides*) *ovalis*										X											X
Atractides (*Atractides*) *panniculatus*		X						X		X					X						X
Atractides (*Atractides*) *pennatus*		X																			
Atractides (*Atractides*) *protendens*									X												
Atractides (Atractides) reinhardi							X														
Atractides (*Atractides*) *remotus*										X											
Atractides (Atractides) rivalis															X						
Atractides (*Atractides*) *robustus*			X							X					X	X	X			X	
Atractides (*Atractides*) *spinipes*										X											
Atractides (Atractides) turcicus																	X				
Atractides (*Polymegapus*) *orghidani*						X															
Atractides (Polymegapus) persicus											X										
Atractides (*Polymegapus*) *polyporus*							X														
Atractides (*Tympanomegapus*) *acutirostris*															X						
Atractides (*Tympanomegapus*) *lacustris*			X																		
Atractides (*Tympanomegapus) longirostris*			X			X															
Hygrobates (Hygrobates) anatolicus												X									
Hygrobates (*Hygrobates*) *angustipalpis*																		X			
Hygrobates (*Hygrobates*) *bucharicus*										X											
Hygrobates (*Hygrobates*) *calliger*								X								X	X				
Hygrobates (*Hygrobates*) *fluviatilis*				X				X		X			X				X				
Hygrobates (*Hygrobates*) *longipalpis*		X								X			X								
Hygrobates (*Hygrobates*) *longiporus*		X								X				X			X				
Hygrobates (*Hygrobates*) *nigromaculatus*		X						X		X					X						
Hygrobates (Hygrobates) persicus					X																
Hygrobates (*Hygrobates*) *porrectus*		X																			
Hygrobates (*Hygrobates*) *trigonicus*								X													
Hygrobates (Hygrobates) turcicus			X		X	X											X				
Hygrobates (*Dekabates*) *quanaticola*		X						X					X								
Hygrobates (*Rivobates*) *diversiporus*															X						
Mixobates (*Mixobates*) *incurvatus*			X																		
Mixobates (*Mixobates*) *brachypalpis ozkani*																	X				

Habitats of Hygrobatidae species determined from Turkey.

When we examined the habitat distributions of Hygrobatidae species in Turkey, streams ranked first with 62% (38 species), followed by springs with 22% (13 species), lentic and slow-flowing waters with 13% (8 species), and hyporheic with 3% (2 species) (Table 3, Figure 1). Our results are consistent with previous studies (Gerecke 2003; Gerecke et al., 2016).

Table 3. The habitats of Hygrobatidae species determined from Turkey.

Species	Streams	Springs	Lentic and slow-flowing waters	Hyporheic
Atractides (*Atractides*) *allgaier*	X	X		
Atractides (Atractides) anatolicus	X			
Atractides (Atractides) anellatus	X			
Atractides (Atractides) arcuatus	X			
Atractides (Atractides) dentipalpis	X			
Atractides (*Atractides*) *distans*	X			
Atractides (*Atractides*) *fissus*	X			
Atractides (*Atractides*) *fluviatilis*	X			
Atractides (*Atractides*) *fonticolus*		X		
Atractides (*Atractides*) *gibberipalpis*	X			
Atractides (Atractides) glandulosus	X			
Atractides (Atractides) gomerae	X			
Atractides (*Atractides*) *graecus*		X		
Atractides (*Atractides*) *inflatipalpis*	X			
Atractides (*Atractides*) *inflatipes*	X			
Atractides (*Atractides*) *inflatus*	X			
Atractides (*Atractides*) *lunipes*	X			
Atractides (Atractides) martini	X			
Atractides (*Atractides*) *nahavandii*	X	X		
Atractides (Atractides) nikooae	X			
Atractides (*Atractides*) *nodipalpis*	X			
Atractides (Atractides) nodipalpoides	X			
Atractides (Atractides) oezkani	X			
Atractides (*Atractides*) *ovalis*			X	
Atractides (*Atractides*) *panniculatus*		X		
Atractides (*Atractides*) *pennatus*		X		
Atractides (*Atractides*) *protendens*		X		
Atractides (Atractides) reinhardi		X		
Atractides (*Atractides*) *remotus*	X			X
Atractides (Atractides) rivalis	X	X		
Atractides (*Atractides*) *robustus*	X			
Atractides (*Atractides*) *spinipes*	X			
Atractides (Atractides) turcicus	X			
Atractides (*Polymegapus*) *orghidani*	X			X
Atractides (Polymegapus) persicus		X		
Atractides (*Polymegapus*) *polyporus*		X		
Atractides (*Tympanomegapus*) *acutirostris*	X			
Atractides (*Tympanomegapus*) *lacustris*			X	
Atractides (*Tympanomegapus) longirostris*	X			
Hygrobates (Hygrobates) anatolicus	X			
Hygrobates (*Hygrobates*) *angustipalpis*			X	
Hygrobates (*Hygrobates*) *bucharicus*			X	
Hygrobates (*Hygrobates*) *calliger*	X			
Hygrobates (*Hygrobates*) *fluviatilis*	X	X		
Hygrobates (*Hygrobates*) *longipalpis*			X	
Hygrobates (*Hygrobates*) *longiporus*			X	
Hygrobates (*Hygrobates*) *nigromaculatus*			X	
Hygrobates (Hygrobates) persicus	X			
Hygrobates (*Hygrobates*) *porrectus*	X			
Hygrobates (*Hygrobates*) *trigonicus*	X			
Hygrobates (Hygrobates) turcicus	X			
Hygrobates (*Dekabates*) *quanaticola*		X		
Hygrobates (*Rivobates*) *diversiporus*	X			
Mixobates (Mixobates) incurvatus			X	
Mixobates (Mixobates) brachypalpis ozkani	X			

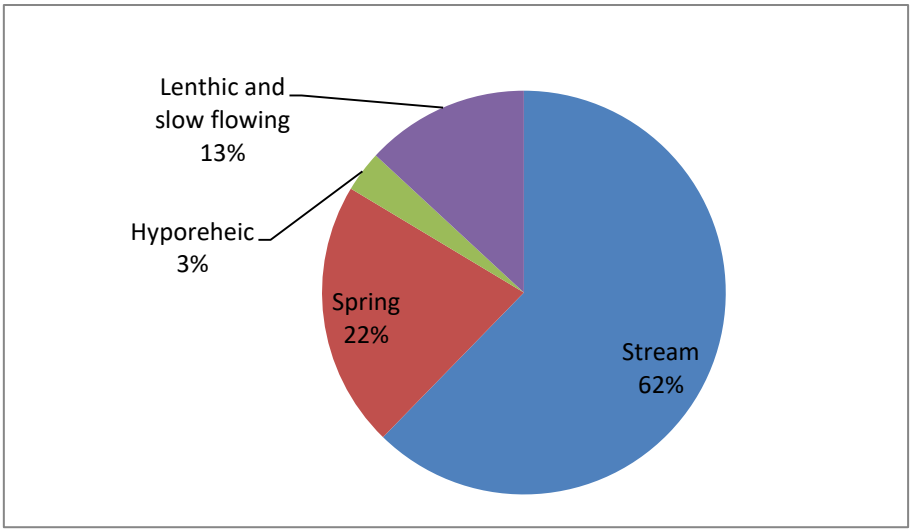

Figure 1. Percentages of habitat distribution of Hygrobatidae in Turkey

A comparison of Turkey with three neighboring countries in terms of the similarity of Hygrobatidae species

Checklists of the Hygrobatidae species recorded from Turkey, Bulgaria, Greece, and Iran have been revised and similarity calculations have been made. Turkey has 55 Hygrobatidae species, Bulgaria, Greece, Iran, 35, 25, and 37 respectively. Turkey has to same 18 species with Bulgaria, 20 with Greece, and 17 with Iran (Table 4). Similarity calculations between Turkey and three neighboring countries were made using the Bray-Curtis index. As a result, it was determined that Turkey had a high rate of similarity with Bulgaria and Greece while having a low rate of similarity with Iran. Similarities between Bulgaria and Greece were also found to be higher (Figure 2). Common species, such as *Atractides* (*Atractides*) *gibberipalpis* Piersig, 1898, *Atractides* (*Atractides*) *nodipalpis* Thor, 1899, *Atractides* (*Atractides*) *robustus* (Sokolow, 1940), *Atractides* (*Tympanomegapus*) *acutirostris* (Motaş & C. Angelier, 1927), *Hygrobates* (*Hygrobates*) *calliger* Piersig, 1896, *Hygrobates* (*Hygrobates*) *fluviatilis* (Ström, 1768), *Hygrobates* (*Hygrobates*) *longipalpis* (Hermann, 1804), *Hygrobates* (*Hygrobates*) *longiporus* Thor, 1898, *Hygrobates* (*Hygrobates*) *trigonicus* Koenike, 1895, have been recorded from all mentioned countries.

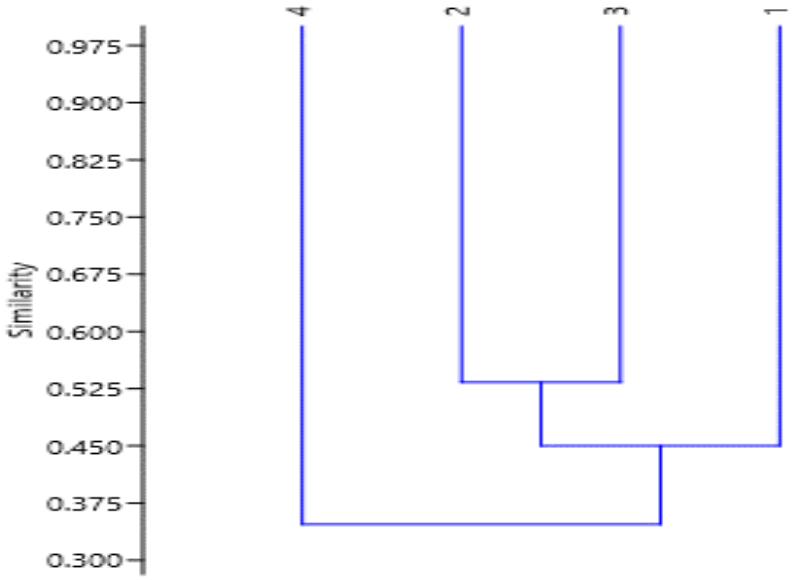

Figure 2. Similarity diagram of Hygrobatidae species of Turkey and three neighboring countries (1. Turkey, 2. Bulgaria, 3. Greece, 4. Iran)

Table 4. The Hygrobatidae species recorded from Turkey and neighboring countries. (Turkey: Erman et al., 2010, 2019; Iran: Pešić and Saboori 2007; Pešić et al., 2014; Greece and Bulgaria: Pešić et al., 2010, 2018)

SPECIES	Turkey	Bulgaria	Greece	Iran
Atractides (*Atractides*) *allgaier* Gerecke, 2003	X		X	
Atractides (Atractides) anatolicus Pešić, Erman & Esen, 2010	X			
Atractides (Atractides) anellatus Lundblad, 1956	X			
Atractides (Atractides) arcuatus Thor, 1904	X			
Atractides (*Atractides*) *asticae* Petrova, 1968		X		
Atractides (Atractides) balneatoris Pešić & Saboori, 2007				X
Atractides (Atractides) biscutatus Cook, 1967				X
Atractides (*Atractides*) *caspicus* Pešić, Dinipour, Vafaei & Saboori, 2007				X
Atractides (*Atractides*) *cisternarum* (K. Viets, 1935)		X		
Atractides (*Atractides*) *denticulatus* (Walter, 1947)		X		X
Atractides (Atractides) dentipalpis (Walter, 1935)	X			
Atractides (*Atractides*) *distans* (K. Viets, 1914)	X	X	X	
Atractides (Atractides) elburzensis Pešić, Smit & Saboori, 2014				X
Atractides (*Atractides*) *fissus* (Walter, 1927)	X			
Atractides fluviatilis (Szalay, 1929)	X	X		X
Atractides (*Atractides*) *fonticolus* (K. Viets, 1920)	X	X	X	
Atractides (*Atractides*) *gereckei* Pešić, 2004				X
Atractides (*Atractides*) *gibberipalpis* Piersig, 1898	X	X	X	X
Atractides (Atractides) glandulosus (Walter, 1918)	X			
Atractides (Atractides) gomerae Lundblad, 1962	X			
Atractides (*Atractides*) *gorgani* Pešić, Jabaleh & Saboori, 2009				X
Atractides (*Atractides*) *graecus* K. Viets, 1950	X	X	X	
Atractides (Atractides) hormozganus Pešić, Smit & Saboori, 2012				X
Atractides (*Atractides*) *hyrcaniensis* Pešić, Jabaleh & Saboori, 2009				X
Atractides (*Atractides*) *inflatipalpis* K. Viets, 1950	X	X	X	
Atractides (*Atractides*) *inflatipes* Lundblad, 1956	X			
Atractides (*Atractides*) *inflatus* (Walter, 1925)	X		X	X
Atractides (*Atractides*) *iranicus* Pešić & Asadi, 2002				X
Atractides (*Atractides*) *kermanensis* Pešić, 2005				X
Atractides (*Atractides*) *latipalpis* (Motaş & Tanasachi, 1946)		X		
Atractides (*Atractides*) *latipes* (Szalay, 1935)				
Atractides (*Atractides*) *longiporus* Petrova, 1968		X		
Atractides (*Atractides*) *lunipes* Lundblad, 1956	X		X	X
Atractides (*Atractides*) *markaziensis* Pešić, 2004				X
Atractides (Atractides) martini Pešić, Erman & Esen	X			
Atractides (*Atractides*) *mirkopesici* Pešić, 2004				X
Atractides (*Atractides*) *mossahebii* Pešić, 2004				X
Atractides (*Atractides*) *nahavandii* Schwoerbel & Sepasgozarian, 1976	X			X
Atractides (Atractides) nikooae Pešić, 2004	X			X
Atractides (*Atractides*) *nodipalpis* Thor, 1899	X	X	X	X
Atractides (Atractides) nodipalpoides Aşçı, Boyacı & Özkan 2011	X			
Atractides (*Atractides*) *oblongus* (Walter, 1944)		X	X	
Atractides (Atractides) oezkani Pesic & Erman, 2006	X			
Atractides (*Atractides*) *ovalis* Koenike, 1883	X			
Atractides (*Atractides*) *panniculatus* (K. Viets, 1925)	X		X	
Atractides (*Atractides*) *pennatus* (K. Viets, 1920)	X			
Atractides (*Atractides*) *phreaticus* (Motaş & Tanasachi, 1948)		X		
Atractides (*Atractides*) *protendens* K.O. Viets, 1955	X		X	
Atractides (*Atractides*) *pumilus* (Szalay, 1946)		X		
Atractides (*Atractides*) *pygmaeus* (Motaş & Tanasachi, 1948)		X		
Atractides (Atractides) reinhardi Gülle, Gülle & Boyacı 2015	X			
Atractides (*Atractides*) *remotus* Szalay, 1953	X	X		
Atractides (Atractides) rivalis Lundblad, 1956	X			
Atractides (*Atractides*) *robustus* (Sokolow, 1940)	X	X	X	X
Atractides (*Atractides*) *sokolowi* (Motaş & Tanasachi, 1948)		X		
Atractides (*Atractides*) *spinipes* Koch, 1837	X	X		
Atractides (*Atractides*) *subterraneus* (K. Viets, 1932)		X		
Atractides (*Atractides*) *tener* Thor, 1899		X	X	
Atractides (Atractides) turcicus Aşcı, 2009	X			
Atractides (*Atractides*) *walteri* (K. Viets, 1925)		X		

Atractides (*Polymegapus*) *orghidani* Motaş & Tanasachi, 1960	X		X	
Atractides (Polymegapus) persicus Pešić & Asadi, 2010	X			X
Atractides (*Polymegapus*) *polyporus* (K. Viets, 1922)	X	X		X
Atractides (*Tympanomegapus*) *acutirostris* (Motaş & C. Angelier, 1927)	X	X	X	X
Atractides (*Tympanomegapus*) *lacustris* (Lundblad, 1925)			X	
Atractides (*Tympanomegapus) longirostris* (Walter, 1925)	X			
Atractides (*Tympanomegapus*) *omanensis* Smit & Pešić, 2010				X
Atractides (*Tympanomegapus*) *pavesii* Maglio, 1905				
Hygrobates (Hygrobates) anatolicus Esen & Pešić 2013	X			
Hygrobates (*Hygrobates*) *angustipalpis* K.O. Viets, 1982	X			X
Hygrobates (*Hygrobates*) *bucharicus* Sokolow, 1928	X			X
Hygrobates (*Hygrobates*) *calliger* Piersig, 1896	X	X	X	X
Hygrobates (*Rivobates*) *diversiporus* Sokolow, 1927	X			
Hygrobates (*Hygrobates*) *fluviatilis* (Ström, 1768)	X	X	X	X
Hygrobates (*Hygrobates*) *foreli* (Lebert, 1874)		X	X	
Hygrobates (*Hygrobates*) *hamatus* K. Viets, 1935				X
Hygrobates (*Hygrobates*) *longipalpis* (Hermann, 1804)	X	X	X	
Hygrobates (*Hygrobates*) *longiporus* Thor, 1898	X	X	X	X
Hygrobates (Hygrobates) marezaensis Pešić & Dabert, 2017				
Hygrobates (*Hygrobates*) *nigromaculatus* Lebert, 1879	X			X
Hygrobates (Hygrobates) persicus Pešić & Asadi 2017	X			
Hygrobates (*Hygrobates*) *porrectus* Koenike, 1908	X	X		
Hygrobates (*Hygrobates*) *properus* Láska, 1954		X		
Hygrobates (*Dekabates*) *quanaticola* Schwoerbel & Sepasgozarian, 1976	X			X
Hygrobates (*Hygrobates*) *setosus* Besseling, 1942				X
Hygrobates (*Hygrobates*) *trigonicus* Koenike, 1895	X	X	X	X
Hygrobates (Hygrobates) turcicus Pešić & Esen 2017	X			
Hygrobates (*Rivobates*) *diversiporus* Sokolow, 1927		X		
Hygrobates (*Rivobates*) *norvegicus* (Thor, 1897)		X		
Hygrobates (Rivobates) zawali Pešić, 2015				
Mesobates forcipatus Thor, 1901			X	
Mixobates (Mixobates) incurvatus (Láska, 1954)	X		X	
Mixobates (Mixobates) brachypalpis ozkani Pešić & Turan, 2006	X			
Iranobates hesabii Pešić, Smit & Asadi, 2011				X

Atractides **(***Tympanomegapus***)** *lacustris (Lundblad, 1925)*

Female. Idiosoma L/W 796/550 and integument striated (Figure 3A). P-1 is about the length of P-2. P-4 has the shape of a bulging cylinder and is long. The sword seta on P-4 is closer to the distoventral seta (Figure 3C).

Dorsal L of palp segments are L 54-75-75-118-33=355, Cx-I-IV L 79-80-88-200, respectively. I-L-5, L/W 165/36, I-L-6, L/W 124/32 (Fig. 3B). S-1 and S-2 on I-L-5 short and with narrow interspace. Leg segments L (in respective order I-IV): 53-60-95-136-165-124 = 633, 51-65-98-151-112-101 = 578, 52-53-100-158-151-136 = 650, 99-102-198-197-224-200 = 1020.

Vgl-1 fused to Vgl-2. The genital plate is in the form of beans. Genital plate L/W 51/24 (Figure 3A).

Studied Material and Habitat: Slow flowing stream, reed area, 36° 85,006' N, 31° 29,024' E, 10.04.2010, 1♀, Sarısu, Manavgat, Antalya.

Distribution. Sweden, Ireland, Netherlands, France, Germany, Switzerland, Russia (Gerecke et al., 2016).

New records for the fauna of Turkey.

DISCUSSION

Atractides lacustris, which is found in stagnant or slow-flowing waters, does not show sexual dimorphism in palp. It is separated from similar species; P-1 approximately P-2 in length, P-4 resembles a bulging cylinder, I-L-5 narrow and short, S-1 and S-2 on I-L-5 short and with narrow interspace and Vgl-1-2 are fused (Gerecke, 2003; Gerecke et al., 2016).

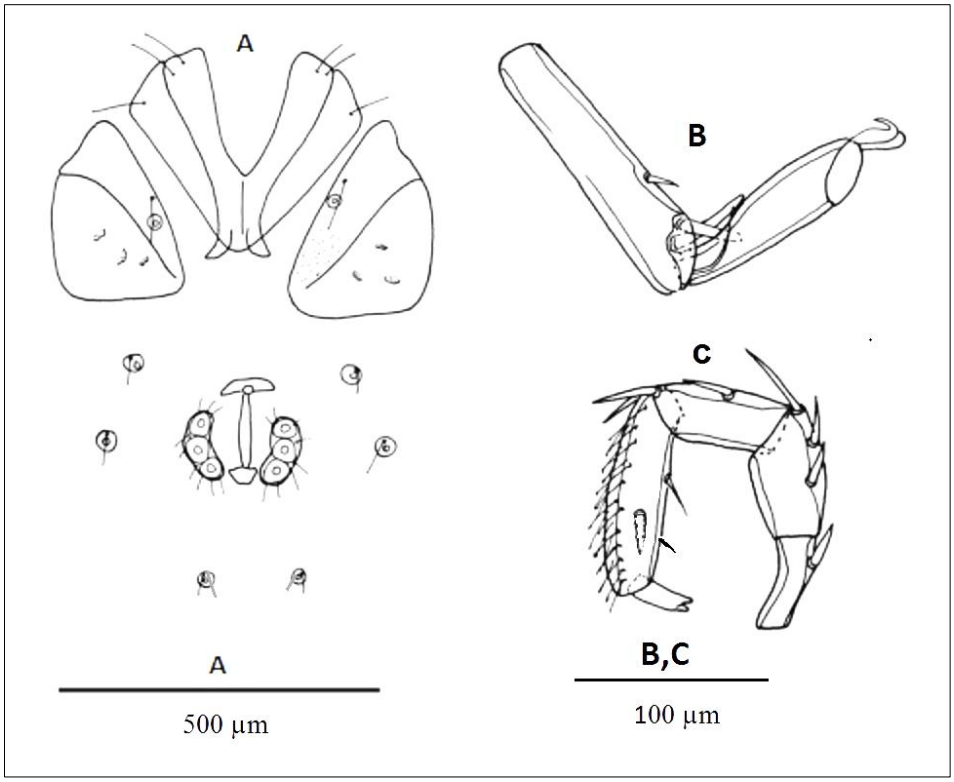

Figure 3. Atractides (*Tympanomegapus*) *lacustris A) Idiosoma ventral, B) I-L-5-6, C) Palp*

CONCLUSION

Erman et al., (2019) has recently reported that Hygrobatidae was the second family in water mite species number found in Turkey. The number of Hygrobatide species reached to 55 with *A. lacustris*. The Hygrobatidae species were recorded from 21 provinces in Turkey. The province with the highest number of records was Erzurum (15), followed by Malatya (12) and Rize (9). Six species and one subspecies of Hygrobatidae are endemic to Turkey. The endemism rate is found as 12.7.

The Hygrobatidae species preferred stream habitats with 62% (38 species), followed by spring habitats with 22% (13 species), lentic and slow-flowing waters with 13% (8 species), and hyporheic with 3% (2 species). Our results are consistent with previous studies (Gerecke, 2003; Gerecke et al., 2016).

Bray-Curtis similarity index was calculated between Turkey and three neighboring countries. Turkey had a high rate of similarity with Bulgaria and Greece while having a low rate of similarity with Iran. Similarities between Bulgaria and Greece were also found to be high.

Aşçı et al., (2011) reported *Atractides (Atractides) arcuatus* as a new record for the fauna of Turkey. Due to insufficient drawing and description and being a little-known species, we suspect in the diagnosis of this species.

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Ilıman Bir Türkiye Nehir Havzasında Dere Su Kalitesinin Çok Değişkenli Analiz ve Biyolojik Yaklaşımlarla Değerlendirilmesi

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Özet

Bentik algler indikatör türler içerir. Özellikle lotik ekosistemlerin ekolojik yapısının belirlenmesinde çok iyi bir göstergedir. Günümüzde insan faaliyetleri sular üzerinde birçok baskı oluşturmuştur, bunlardan besin zenginleştirmesi ekolojik durumu değiştiren en önemli su kalitesi sorunudur. Bu çalışmada Doğu Karadeniz Havzası içinde yer alan Elekçi Deresi'nin fiziko-kimyasal ve biyolojik (bentik diyatomeler) özelliklerinin ve su kalitesinin değerlendirilmesi amaçlanmıştır. Bir yıl boyunca aylık olarak üç farklı lokalitede örnekleme yapılmıştır. Yirmi farklı su parametresinin analizi zamansal ve mekânsal olarak değerlendirilmiştir. Yerüstü Su Kalitesi Yönetmeliği'ne göre akarsuyun su kalite sınıfı belirlenmiştir. Çok değişkenli istatistiksel analizler, su kalite indeksi (WQI), çeşitlilik indeksleri, klorofil-*a*, Palmer'ın pollusyon indeksi ve baskın cins skorları kullanılarak suyun trofik düzeyi ve su kalitesi değerlendirilmiştir. Yıllık ortalama değerlere göre, Elekçi Deresi amonyum azotu ve toplam fosfor parametreleri bakımından II. sınıf, serbest klor hariç (IV. sınıf) diğer su kalite parametreleri bakımından I. sınıftır. Elekçi Deresi'ndeki WQI değeri su kalite özelliğinin "mükemmel" olduğunu göstermiştir. Faktör analizinde, birinci faktöre göre nütrient elementleri su kalitesindeki değişikliklerden sorumludur. Diğer faktörlerdeki bileşenler jeolojik yapıdan ve klimatik faktörlerden oluşmaktadır.

Elekçi Deresi'nde toplam 93 bentik diyatome taksonu tanımlanmıştır. *Navicula*, *Nitzschia*, *Cymbella* ve *Gomphonema* en fazla kaydedilen taksonlardır. *Cocconeis placentula* var. *euglypta*, *Gomphonema truncatum*, *Navicula lanceolata*, *N. tripunctata* ve *Nitzschia palea* tüm istasyonlarda her mevsim bulunmuştur. Shannon ve Simpson çeşitlilik indeksi analiz sonuçlarına göre Elekçi Deresi "zayıf" düzeyde çeşitliliğe sahiptir, kirlilik düzeyi ise "orta"dır. Nemli subtropikal iklim bölgesinde bulunan Elekçi Deresi düzensiz rejimli bir akarsudur. Çevresel ve klimatik faktörler derenin diyatome komünitesini doğrudan etkilemiştir. Pollusyon indeksi sonucu çok hafif organik kirlilik tespit edilmiştir. Klorofil-*a* konsantrasyonuna göre derede ötrofikasyon riski yoktur. Dominant alg taksonlarının skor değerleri Elekçi Deresi'nin trofik seviyesinin "mezotrofik/mezo-ötrofik", su kalitesinin "orta/orta-kirli" olduğunu göstermiştir. Genel sonuçlar, Elekçi Deresi'nin akış yönünde antropojenik faaliyetlerle ilişkilendirilebilen çeşitli kaynaklardan gelen kirleticilerle kontamine olduğunu ve diyatome çeşitliliğinin bu durumdan etkilendiğini göstermiştir.

Anahtar kelimeler: Biyoindikatörler, çok değişkenli analiz, diyatomeler, su kalitesi, trofik durum.

Assessment of Stream Water Quality in a Temperate Turkey River Basin by Multivariate Analysis and Biological Approaches

Abstract

Benthic algae include indicator species. Particularly, it is a very good indicator to determine the ecologic structure of lotic ecosystems. Nowadays, human activities have produced multiple pressures on waters, of them nutrient enrichment is the most important water quality problem that changed the ecological status. In this research, it is aimed to evaluate the physicochemical and biologic (benthic diatoms) properties and water quality of Elekçi Stream located in the Eastern Black Sea Basin. Throughout the year, samples from three different localities were collected monthly. Analysis of twenty water parameters were evaluated spatially and temporally. According to the Surface Water Quality Regulation, the water quality class of the stream has been determined. Trophic level and water quality of the stream were evaluated by using multivariate statistical analysis, water quality index (WQI), diversity indices, chlorophyll-*a*, Palmer's index of pollution, and dominant genus scores. According to annual average values, Elekçi Stream is class II as to ammonium nitrogen and total phosphorus parameters. Also, it has class I in terms of other water quality parameters, excluding free chlorine (class IV). WQI value in the Elekçi Stream showed that the water quality feature is "excellent". In factor analysis, the first factor showed that nutrient elements are responsible for changes in water quality. Components in other factors consist of geological structure and climate factors.

Total 93 benthic diatom taxa have been defined in Elekçi Stream. *Navicula*, *Nitzschia*, *Cymbella*, and *Gomphonema* genera are the most recorded taxa. *Cocconeis placentula* var. *euglypta*, *Gomphonema truncatum*, *Navicula lanceolata*, *N. tripunctata*, and *Nitzschia palea* were recorded in all stations every season. According to the analysis results of Shannon and Simpson diversity indices, Elekçi Stream has a "poor" level of diversity, and the pollution level is "moderate". Elekçi Stream, located in the humid subtropical climate zone, is an irregularly flowed stream. Environmental and climatic factors directly affected the diatom community of the stream. As a result of the pollution index, very light organic pollution was detected. The stream does not carry eutrophication risk in terms of chlorophyll-*a* concentration. The scores of the dominant algal taxa showed that the trophic level of Elekçi Stream is "mesotrophic/meso-eutrophic" and the water quality is "moderate/moderately polluted". General results showed Elekçi Stream is contaminated with pollutants coming from various sources that can be associated with anthropogenic activities downstream, and diversity of diatoms are affected by this sitıation.

Keywords: Bioindicators, multivariate analysis, diatoms, water quality, trophic state.

GİRİŞ

Sucul ekosistemlerin primer üreticileri olan algler, aynı zamanda su ortamında meydana gelen fiziksel ve/veya kimyasal değişimlere diğer sucul canlılara oranla çok daha kısa sürede cevap verdikleri için, su kalitesinin belirlenmesi ve izlenmesinde indikatör olarak kullanılırlar. Bu nedenle biyolojik kalite elemanı olarak biyoindikatör türler içeren bentik alglerin kullanımı oldukça yaygındır. Alglerin yoğunluğu ve çeşitliliği sucul biotanın verimliliği hakkında bilgi verirken, çevre şartlarına bağlı olarak değişim göstermesi, özellikle indikatör türler, organik kirliliği, ötrofikasyonu ve su kalitesini gösterir (Palmer, 1977). Özellikle diyatomeler gibi biyoindikatör türler içeren alg grubu suyun ekolojik durumunun değerlendirilmesinde önemli bir rol oynar. Sucul ekosistemlerin önemli bir bileşeni olan diyatomeler çevresel özellikler ile yüksek korelasyon içerisinde bulunurlar (Hill vd., 2001). Bu nedenle, diyatomeler su kalitesinin iyi göstergeleri olarak kabul edilmiştir (Stevenson, 2014). Aynı zamanda, diyatomeler pH, tuzluluk, besin kullanılabilirliği, organik ve inorganik kirlilik gibi çevresel parametrelere karşı yüksek hassasiyet ve dar tür toleransı olan biyolojik göstergeler olarak iyi bilinir (Sládeček, 1986; Van Dam vd., 1994; Whitton ve Kelly, 1995; Vasiljević vd., 2014; 2017; Lobo vd., 2016; Marcel vd., 2017; Salmaso vd., 2019). Yine, optimum büyüme koşulları bilgisi, su kalitesini değerlendirmek için kullanılır (Virtanen ve Soininen, 2016). Diyatomeler şehirleşme ve nüfus artışına paralel olarak artan kirliliğe karşı da duyarlı indikatörlerdir (Sonneman vd., 2001; Chessman ve Townsend, 2010; Lavoie vd., 2014; Stevenson, 2014). Bundan dolayı, akarsuların biyolojik açıdan trofik durumunun belirlenmesinde diyatomeler indikatör organizma grubu olarak yaygın olarak kullanılmaktadır (Solak ve Àcs, 2011; Taş ve Yılmaz, 2015; Bere, 2016; Jakovljević vd., 2016; Virtanen ve Soininen, 2016; Tan vd., 2017; Atıcı vd., 2018; Taş vd., 2019; Tokatlı vd., 2019; Tokatlı vd., 2020).

Bütün bu özellikleriyle, diyatomelerin lotik ekosistemlerin ekolojik yapısının belirlenmesinde çok iyi bir gösterge olduğu, özellikle taş, kaya gibi substratumlarda baskın olarak bulunduğu, hayat döngülerinin kısa olması, suyun fiziksel ve kimyasal değişikliklerine tepki vermesi ve her örneklemde toplanıp izlenebilirliği nedeniyle araştırmacılara havzalar arasında zamansal-mekânsal karşılaştırma imkânı sağladığı görülmektedir. Diyatomelerin doğal ve/veya antropojenik kökenli kirlilik hakkında bilgi veren türler içermesi, su kalitesinin biyolojik yaklaşımla değerlendirilmesinde en önemli biyoindikatör/biyomonitör olarak kullanılma sebebidir.

Akarsuların doğal yapısının bozularak su kalitesinin her geçen gün kötüleşmesi dünya genelinde ana çevresel sorunlardan biridir. Kirlenme genellikle noktasal ve yayılı kaynaklardan (hızlı ve düzensiz kentleşme, nüfus artışı, sanayileşme, tarımda aşırı endüstriyel gübreler ve pestisitler gibi kimyasalların kullanımı, evsel atıklar/atık sular, vahşi katı atık depolama alanları, akarsu yataklarından kum-çakıl çıkarma, enerji üretimi amacıyla çok sayıda küçük barajlar yapımı, maden işletmeleri gibi faaliyetler) antropojenik kökenli olarak meydana gelmektedir (Dalu ve Froneman, 2016; Dalu vd., 2017; Nhiwatiwa vd., 2017; Mutlu, 2019; Taş vd., 2019; Ustaoğlu ve Islam, 2020; Ustaoğlu vd., 2020a). Dolayısıyla, su ortamlarının modern zamanlarda bozulmaya başladığı bir gerçektir. Yine, son yıllarda küresel iklim değişikliği dünyanın en önemli çevre sorunu olarak görülmektedir ve subtropikal kuşaktaki sucul ekosistemlerin hidrolojik döngüsünü olumsuz etkilemektedir. Türkiye, iklim değişikliğine karşı en savunmasız alanlardan biridir. Sıcaklıklardaki yükselme nedeniyle buharlaşmanın artması ve yağışlardaki düşüş lotik ve lentik sistemlerde tatlısu rezervlerinin azalması, sulak alanların ve bazı sığ göllerin kuruması bunu göstermektedir. Türkiye akarsularındaki su miktarı, küresel ısınmanın etkisiyle azalmakta ve bu durum su havzasında kirliliğin artmasından dolayı daha da

ciddi boyutlara ulaşmaktadır (Taş ve Kolören, 2017; Tokatlı, 2020). Akarsulardan kaynaklanan kirlenmenin, ötrofikasyona karşı çok hassas olan tüm Karadeniz Bölgesi için endişe verici olduğu bildirilmektedir (Jin vd., 2013).

Türkiye'nin kuzeyinde yer alan 22 numaralı Doğu Karadeniz Havzası içinde zengin akarsu sistemleri bulunur. Bu sular hem yerüstü suyu olarak doğrudan hem de alüvyal dolgularda akiferleri oluşturarak kentlerin içme-kullanma suyu, tarımsal sulama, doğal ve kültür balıkçılığı, enerji üretimi gibi çok çeşitli amaçlar için kullanılmaktadır. Ancak, aşağı akış yönünde akarsular üzerinde noktasal ve yayılı kaynaklardan gelen kirlilik baskısı vardır. Bu baskı sadece insan kaynaklı değildir; erozyon, yağış, akıntı gibi toprak ve hidrolojik faktörlerden kaynaklı doğal mekanizmalar da akarsu ekosistemini değiştirebilmektedir. Özellikle her mevsim yağış alan, fakat etkili bahar yağışları olan Karadeniz Bölgesi gibi ılıman bölgelerde, düzensiz rejimli akarsularda klimatik ve hidrolojik faktörler etkili olmaktadır.

Doğu Karadeniz Bölgesi'nde yer alan akarsuların kaynak bölgeleri yüksek biyolojik çeşitlilik içerdiği için bu alanların korunması oldukça önemlidir. Fakat yukarı havzalarda da çeşitli amaçlarla arazi kullanımlarının neden olduğu bozulmalar görülmektedir (Kazancı, 2016). Dolayısıyla, su kalitesinin düşük olması ve sudaki biyolojik çeşitliliğin azalması zamanımızın en büyük çevresel sorunları arasındadır (Virtanen ve Soininen, 2016). Oysa akarsu habitatları biyolojik çeşitlilik açısından önemli ve benzersiz ekosistemlerdir. Bu nedenle, su kalitesinin izlenmesi ve su kaynaklarının korunması oldukça önemlidir (Başören ve Kazancı, 2016). Sucul ekosistemlerin izlenmesinde ve su kalitesinin değerlendirilmesinde fizikokimyasal değişkenler ile birlikte biyolojik yöntemlerin bütüncül olarak incelenmesi daha iyi sonuçlar elde etmemizi sağlar (Kazancı vd., 1997; Taş vd., 2019). Böylece akarsu akışı boyunca değişim ve suyun ekolojik durumu tespit edilebilir.

Bu çalışmada, Doğu Karadeniz Havzası içinde yer alan ve Ordu ilinin en büyük akarsularından biri olan Elekçi Deresi'nin yukarı havzadan aşağı havzaya doğru yüzey suyu kalitesini ve ekolojik durumunu tespit etmek amaçlanmıştır. Bunun için fizikokimyasal yöntemler ile bentik diyatom komünitesi birlikte değerlendirilmiştir. Elde edilen sonuçlara çok değişkenli istatistiksel analizler uygulanmıştır. İndikatör diyatomelerin tespiti ve su analiz sonuçlarının ilgili yönetmelik ve referanslarla karşılaştırılması neticesinde Elekçi Deresi'nin trofik seviyesi ve su kalitesi belirlenmiştir.

MATERYAL ve YÖNTEM

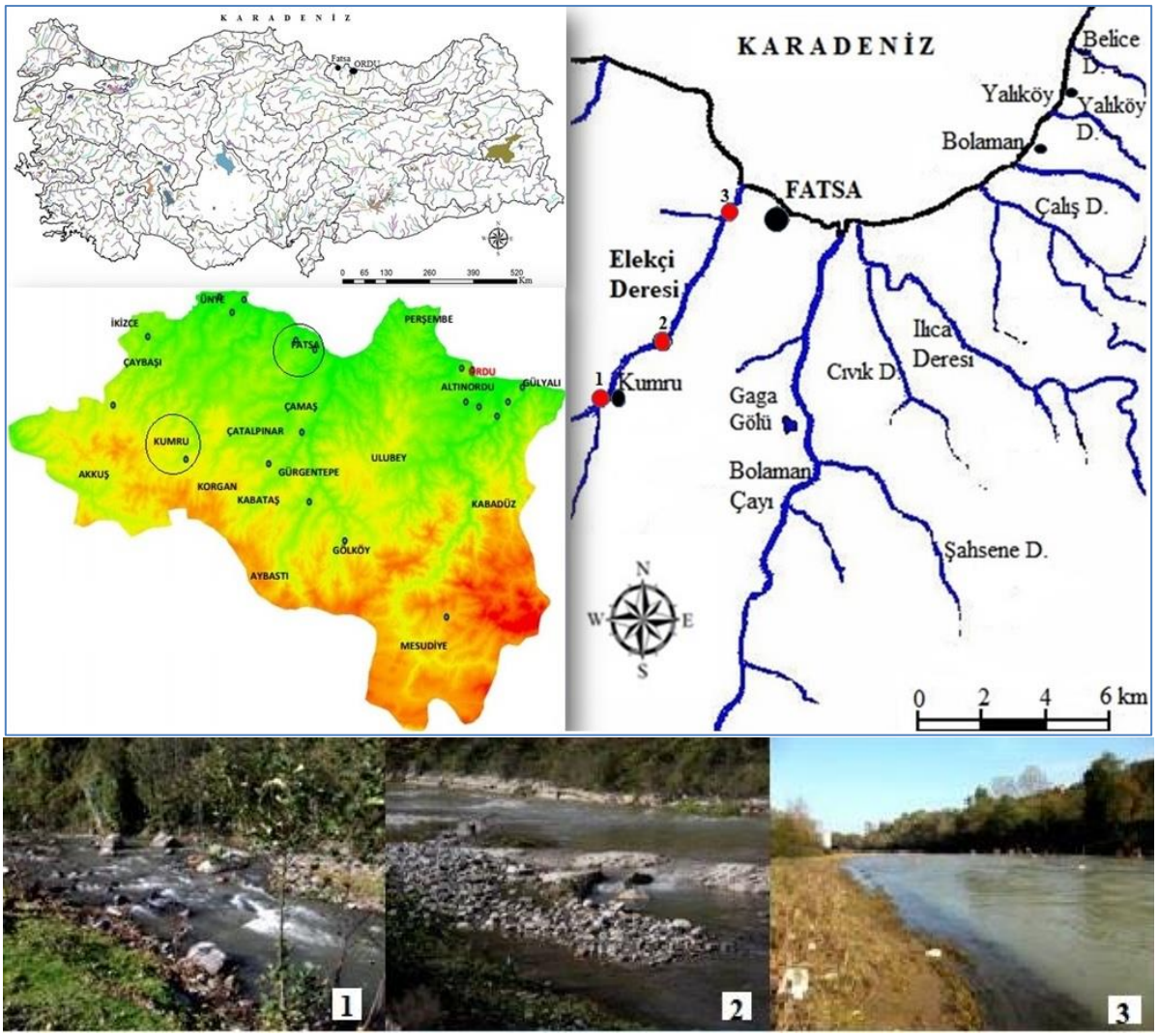
Çalışma havzası ve örnekleme istasyonları

Elekçi Deresi (Ordu, Fatsa), Doğu Karadeniz Havzası'nın Ordu-Giresun Alt Havzası içinde yer alır. Bolaman Çayı Havzası'nın hemen batısında bulunan Elekçi Deresi (Şekil 1), Melet Irmağı, Bolaman Çayı ve Turnasuyu Çayı'ndan sonra, Ordu ilindeki büyük akarsulardan biridir. Kaynağını Fatsa ilçe merkezinin 45 km güneyinde bulunan yaylalardan alan Elekçi Deresi, kıyıdan 35 km güneybatıda kalan Kumru ilçesinin tam ortasından geçerek güneybatı-kuzeydoğu doğrultusunda akıp sularını Karadeniz'e ulaştırır. Canik Dağları'nın vadileri arasında yer alan Kumru ilçesi dağlık ve engebeli olduğu için havzada mevcut olan irili ufaklı birçok su kaynağı birleşerek Elekçi Deresi'ni oluşturur. Bölge yağışlı ve ılıman bir iklimin etkisi altında olduğu için su kaynaklarında her mevsim su bulunur. Fatsa'da yıllık ortalama yağış 1048,3 mm, Kumru'da 897,1 mm'dir. Elekçi Deresi'nin yağış alanı 443 km^2, kolektör uzunluğu 51,5 km, memba ile mansap arasındaki kot farkı 1592 m'dir. Yıllık ortalama debisi 5,46 m^3/sn, yıllık toplam akım değeri ise 172 milyon m^3'tür. Maksimum debi Mart ayındadır, fakat Aralık, Nisan, Mayıs ve Haziran aylarında da yüksek debiler görülmektedir. Elekçi Deresi'nde en yüksek su seviyeleri kış sonu başlayıp bahar ayları boyunca devam eder. Çoğu zaman 120 cm'ye kadar ölçülen maksimum seviyeler sıcaklığın en yüksek olduğu Temmuz ve Ağustos aylarında azalarak 60 cm ve altına iner. Elekçi Deresi taşkın zararına neden olabilecek potansiyele sahiptir. Özellikle kış mevsimi sonu ve yaz başlarında bölgede etkili olan sağanak yağışlar ve kar erimeleri sonucu akarsuların debileri dikkate değer bir şekilde yükselmekte; bu durum 10 ile 15 yıllık periyotlarda önemli sel ve taşkın olaylarının görülme sıklığını artırmaktadır (Özlü, 2012). Bu nedenle akarsu yatağında özellikle aşağı havzada ıslah çalışmaları yapılmıştır.

Elekçi Deresi ve havza içindeki diğer akarsular Üst Kretase volkanik serisine dahil tüf, konglomera ve dasif gibi kayaçlar üzerinde akış gösterirler. Elekçi Deresi'nin her iki sahilinde, taşkın alanı içerisinde kumlu, killi, çakıl şeklinde alüvyon ve kolüvyal birikintiler görülür (Özlü, 2012).

Elekçi Deresi'nin fizikokimyasal özelliklerini ve bentik diyatomelerini incelemek için akarsuyun yukarı (istasyon 1, 40°52'15''N – 37°14'44''E, 485 m), orta (istasyon 2, 40°53'37''N – 37°18'48'',

317 m) ve aşağı (istasyon 3, 41°01’23’’N – 37°28’50’’ E, 14 m) bölgesinden üç istasyon belirlenmiştir (Şekil 1). Örneklemeler Aralık 2011 ile Kasım 2012 tarihleri arasında periyodik olarak her ay yapılmıştır. Akarsuyun yukarı bölgesi dik vadi içinden akar, çevresinde fundalık-ormanlık alanlar ile fındık bahçeleri bulunur. Orta bölge Kumru ilçesinin mansabındadır (~10 km), noktasal ve yayılı kaynaklı (evsel atık ve atıksu, tarımsal, az miktarda endüstriyel) kirlenme baskısı altındadır. İstasyonun üstünde vahşi çöp depolama alanı bulunmaktadır. Aşağı havzadan seçilen 3. istasyon Fatsa ilçe merkezi sınırlarında yer alır. Beş yıldızlı otelin mansabında yer alır. Evsel, tarımsal ve endüstriyel kirlenme baskısı altındadır.



Şekil 1. Elekçi Deresi’nde yüzey suyu ve bentik alg örneklemesi için seçilen örnekleme yerleri ile çalışma alanının haritası.

Fiziksel, kimyasal ve biyolojik parametreler

Su sıcaklığı, pH, çözünmüş oksijen (ÇO), oksijen doygunluğu, elektriksel iletkenlik (EC) ve toplam çözünmüş madde miktarı (TDS) üç istasyonda Hach Lange HQ 40d multiparametre cihazı ile yerinde ölçülmüştür. Azotlu bileşiklerden amonyum azotu (NH_4-N), nitrit azotu (NO_2-N), nitrat azotu (NO_3-N), toplam fosfat (PO_4-P), sülfat (SO_4), serbest klor (Cl_2), kalsiyum (Ca), magnezyum (Mg) ve toplam sertlik (TH) analizleri yukarı havza ile aşağı havzayı karşılaştırmak için 1. ve 3. istasyonlardan alınan sularda yapılmıştır. Analizler Hach test kitleri kullanılarak Hach prosedürüne uygun olarak yapılmış ve spektrofotometrik sonuçlar Hach DR 2800 UV-Vis spektrofotometre ile ölçülmüştür. Askıda katı madde (AKM) tayini standart yöntemlere göre yapılmıştır (APHA, 1998). Laboratuvar analizlerinin tümü Ordu Üniversitesi Hidrobiyoloji Araştırma Laboratuvarı’nda yapılmıştır. Elekçi Deresi’nde

ölçülen su kalite parametrelerine ait analiz sonuçları ortalama değerler dikkate alınarak Yerüstü Su Kalitesi Yönetmeliği (YSKY, 2012)'ne göre değerlendirilmiştir.

Biyolojik parametrelerden epilitik diyatomelerin örneklemesi için üç istasyondan her ay taş örnekleri toplanıp üzerindeki film tabakası kazınıp yıkanarak ayrılmış ve %4'lük formaldehit ile fikse edilmiştir. Laboratuvarda geçici ve kalıcı preparatlar hazırlanarak diyatome türleri incelenmiştir. Daimi preparat hazırlamak için toplanan diyatome örnekleri önce asit karışımında yıkanmış (%98 H_2SO_4 ve %35 HNO_3), ardından organik materyallerinden uzaklaştırılan diyatome kabuklarından entellan ile kalıcı prepartlar hazırlanarak (Round, 1993) 400x ve 1000x büyütmeli binoküler mikroskoplarda (Nikon E100, Leica DM500) ilgili literatürlerden yararlanarak (Cox, 1996; Hartley, 1996; Krammer ve Lange-Bertalot, 1986; Krammer ve Lange-Bertalot, 1988; Krammer ve Lange-Bertalot, 1991a; Krammer ve Lange-Bertalot, 1991b) tanımlanmıştır.

Diyatomelerin nispi bolluklarını hesaplamak için, örnekleme yapılan her ayda, her bir istasyon için üç daimi preparat hazırlanmış, her preparatın orta kısmından belirlenen hayali bir hat üzerindeki 100 adet diyatome kabuğu, rastgele olmak üzere, tür düzeyinde belirlenerek kaydedilmiştir. Üç tekrar sonucunda bulunan 300 kabuk içindeki her bir türün sayısı belirlendikten sonra üçe bölünerek % baskınlık oranları belirlenmiştir. Elekçi Deresi'nde bulunan diyatome türlerinin sıklık analizi de hesaplanmış ve beş kategoride değerlendirilmiştir (Kocataş, 2006).

Nispi bolluğu ≥ % 50 olan diyatomeler dikkate alınarak akarsuyun organik kirliliği ve trofik yapısı Palmer (1969)'a göre, dominant cinsler dikkate alınarak su kalitesi ve trofik seviye durumu ise Peerapornpisal vd. (2007)'ne göre belirlenmiştir.

Bir diğer biyolojik parametre olan fotosentetik pigment analizi (klorofil-*a, -b, -c*) % 90'lık aseton ile ekstraksiyondan sonra spektrofotometrik olarak (Shimadzu UV-1800) yapılmıştır (Strickland ve Parsons, 1972).

Su kalite indeksi (WQI)

Su kalitesi indeksi (WQI), suyun içilebilirliğinin değerlendirilmesinde kullanılan önemli bir araçtır. İlk olarak ABD'de Horton (1965) tarafından geliştirilen WQI; Avrupa, Afrika ve Asya ülkelerinde yaygın olarak kullanılmaktadır. Farklı kalite parametrelerinin değerlendirilmesine dayanan ve çeşitli parametrelerin içme suyunun genel kalitesi üzerindeki etkisini gösteren su derecelendirme ölçeğidir. Temel amacı, fazla sayıda olan su kalitesi verisini anlaşılır ve bilgilendirici bir şekilde azaltmaktır. Bu çalışmada WQI; pH, EC, Cl_2, SO_4, TH, Mg, Ca, NO_2-N, NO_3-N, Fe, TDS parametreleri kullanılarak hesaplanmıştır (Tablo 1). Parametrelerin seçiminde analizi yapılan parametrelerin WHO tarafından tavsiye edilen limit değerlerinin olup olmaması dikkate alınmıştır. WQI aşağıdaki formülleri verilen dört aşamada hesaplanmıştır. İlk aşamada su kalitesini etkilemedeki göreceli önemlerine göre, çalışılan her parametreye 1 ile 5 arasında ağırlık değeri (AW) atanmıştır ve aşağıdaki formül kullanılarak göreceli ağırlık (RW) hesaplanmıştır.

$$RW = \frac{AW}{\sum_{i=1}^{n} AW} \quad (1)$$

İkinci aşamada, analiz sonucunda ölçülen parametrelerin (C_i), Dünya Sağlık Örgütü (WHO, 2011) tarafından izin verilen içme suyu değerlerine (S_i) bölünüp 100 ile çarpılmasından kalite derecelendirilmesi (Qi) hesaplanmıştır.

$$Q_i = \left(\frac{C_i}{S_i}\right) \times 100 \quad (2)$$

WQI hesaplamak için alt-indeksler (SI) hesaplanmış ve elde edilen bu indeksler toplanarak WQI hesaplanmıştır.

$$SI_i = RW \times Q_i \quad (3)$$

$$WQI = \sum_{i=1}^{n} SI_i \quad (4)$$

WQI değerleri beş kategoride (0–25 mükemmel su, 26–50 iyi su, 51–75 kötü su, 76–100 çok kötü su ve > 100 uygun olmayan su) sınıflandırılmıştır (Yadav, 2010).

Tablo 1. Parametrelerin bağıl ağırlığı

Parametreler	WHO (2011)	Atanan ağırlık (AW)	Nispi ağırlık (RW)
pH	7,5	4	0,100
EC (µS/cm)	1500	4	0,100
Cl_2 (µg/L)	5	4	0,100
SO_4 (mg/L)	250	5	0,125
TH (mg/L $CaCO_3$)	100	1	0,025
Mg (mg/L)	50	2	0,050
Ca (mg/L)	75	2	0,050
NO_2-N (mg/L)	0,15	5	0,125
NO_3-N (mg/L)	11,3	5	0,125
Fe (µg/L)	300	4	0,100
TDS (mg/L)	600	4	0,100
		40	1,000

İstatistiksel analizler

Çok değişkenli istatistiksel analizler, su kalitesi verilerinin değerlendirilmesinde son yıllarda yaygın olarak kullanılmaktadır (Köse vd., 2014; Köse vd., 2018). Çalışmamızda verilerin tanımlayıcı istatistikleri (mevsimsel ortalama, standart sapma, minimum ve maksimum değerleri), mevsimsel değerler arasında anlamlı bir farkın olup olmadığını belirlemek için tek yönlü varyans analizi (ANOVA) yapılmıştır. Fizikokimyasal parametreler arasındaki ilişkinin yönünü ve miktarını belirlemek için Pearson korelasyon analizi uygulanmıştır. Araştırmada veri setini ingirgemek, değişkenler arasındaki ilişkileri belirlemek ve su kalitesi üzerine muhtemel çevresel baskıları belirlemek için temel bileşenler analizi (PCA/TBA) yapılmıştır. Mevsimsel benzerlikleri belirlemek için de kümeleme (cluster) analizinden faydalanılmıştır. Verilerin değerlendirilmesinde SPSS 22 istatistiki paket programından yararlanılmış ve sonuçlar, faktöriyel düzeyinde varyans analizi testi ile $p < 0,05$ önem düzeyinde test edilmiştir. Cluster analizi için ise Past 3.x istatistik paket programı kullanılmıştır.

Epilitik diyatomelerin her ay için tür sayısı ve her bir türün birey sayısı dikkate alınarak elde edilen verilerinden Shannon çeşitlilik ve düzenlilik indeksleri ile Simpson çeşitlilik indeksi analizleri yapılmıştır. Lokalite ve aylık örneklemler arasındaki benzerliği belirlemek için Bray-Curtis cluster analizinden faydalanılmıştır. Bu analizlerde BioDiversity Professional Version 2.0 yazılımı kullanılmıştır.

BULGULAR ve TARTIŞMA

Çevresel parametreler ve su kalite kriterleri

Elekçi Deresi'nde yapılan çalışmada suyun fiziksel ve kimyasal parametreleri ile biyolojik parametrelerden klorofil-*a* değerinin mevsimsel ortalamaları ile yıllık minimum, maksimum ve ortalama±standart sapma (SD, σ) değerleri Tablo 2'de verilmiştir.

Elekçi Deresi'nin yüzey suları Yerüstü Su Kalitesi Yönetmeliği (YSKY, 2012)'ne göre değerlendirildiğinde; su kalitesi fiziksel parametreler bakımından (sıcaklık, bulanıklık, AKM, TDS, EC) I. sınıf, yani yüksek kaliteli su özelliği taşımaktadır ve bu durum yönetmeliğimize göre "çok iyi" su durumunu ifade eder. Kimyasal parametrelerden NH_4-N (II. sınıf), TP (II. sınıf) ve serbest Cl_2 (IV. sınıf) parametreleri dışındaki diğer parametreler I. sınıf su kalitesi değerlerini göstermektedir (Tablo 2). Evsel veya endüstriyel atık suların akarsuya deşarjı sonucu sulardaki amonyum miktarı artmaktadır (Egemen ve Sunlu, 1996). Fosfor da tarımsal, evsel, endüstriyel ve doğal kayaçlardan yerüstü sularına katılmakta ve çoğu zaman sekonder kirlenmeye yani ötrofikasyona yol açmaktadır. Bu nedenle fosfatlı bileşikler akarsularda kirliliğin belirlenmesinde indikatör parametrelerdendir. Elekçi Deresi'nde ortofosfat değeri (o-PO_4-P) ortalama değer olarak yukarı havzada (1. istasyon) ve aşağı havzada (2. istasyon) sırasıyla 0,35 mg/L ve 0,70 mg/L olarak ölçülmüştür. Yukarı havza suyu bu parametre bakımından III. sınıf (0,16 – 0,65 mg/L), aşağı havza ise IV. sınıf (> 0,65 mg/L) özellik taşımaktadır.

TP (PO_4-P) içeriği bakımından değerlendirildiğinde yukarı havza II. sınıf (0,115 mg/L), aşağı havza II. sınıf (0,246 mg/L) su kalitesine sahiptir.

Tablo 2. Elekçi Deresi'nde ölçülen çevresel değişkenlerin mevsimsel değerleri, yıllık ortalama değer dikkate alınarak ulusal yönetmeliğimize göre su kalite sınıfının değerlendirilmesi.

Parametreler	Kış	İlkbahar	Yaz	Sonbahar	Min	Max	Ortalama±SD	YSKY
Su sıc. (°C)	7,29^{a}	13,09^{b}	22,84^{c}	16,57^{b}	5,40	26,30	14,95±6,74	I
pH	7,00	7,17	6,96	7,31	6,65	7,94	7,11±0,32	I
EC (µS/cm)	229,99^{ab}	176,28^{a}	274,97^{b}	288,08^{b}	67,20	364,00	242,33±84,66	I
TDS (mg/L)	110,32^{ab}	85,07^{a}	132,42^{ab}	140,89^{b}	31,90	178,70	117,18±42,27	I
AKM (mg/L)	44,23^{a}	10,60^{b}	15,83^{b}	7,23^{b}	1,70	118,40	19,48±28,95	I
ÇO (mg/L)	10,40^{a}	8,84^{b}	8,45^{b}	8,99^{b}	7,35	11,20	9,17±0,94	I
O_2 doygunluğu (%)	93,63^{a}	93,98^{a}	105,58^{b}	100,19^{ab}	86,40	118,20	98,34±7,72	I
Türbidite (NTU)	72,92^{a}	65,38^{a}	61,87^{a}	18,64^{b}	2,11	195,00	54,70±51,86	-
NH_3 (mg/L)	0,56^{a}	0,44^{a}	0,42^{a}	0,15^{b}	0,00	1,28	0,39±0,36	-
NH_4-N (mg/L)	0,45^{a}	0,36^{a}	0,35^{a}	0,12^{b}	0,00	1,046	0,32±0,29	II
NO_2-N (mg/L)	0,009^{a}	0,008^{a}	0,008^{a}	0,015^{b}	0,00	0,050	0,010±,011	I
NO_3-N (mg/L)	0,60^{a}	0,65^{a}	0,48^{a}	0,83^{b}	0,00	1,60	0,64±0,39	I
TP (mg/L)	0,24^{a}	0,16^{a}	0,21^{a}	0,07^{b}	0,00	0,53	0,17±0,14	II
SO_4 (mg/L)	8,83^{a}	7,17^{a}	12,00^{b}	17,83^{b}	3,00	32,00	11,46±7,44	I
Fe (mg/L)	0,39^{a}	0,35^{a}	0,40^{a}	0,07^{b}	0,01	1,53	0,30±0,34	I
TH (mg/L)	61,13	56,40	64,17	71,10	22,90	120,00	63,20±24,91	-
Ca (mg/L)	41,60	35,38	41,35	43,72	16,40	69,00	40,51±14,02	-
Mg (mg/L)	1,21^{a}	2,94^{ab}	2,67^{ab}	3,54^{b}	0,00	10,00	2,59±3,21	-
Cl_2 (mg/L)	0,11^{a}	0,07^{a}	0,10^{a}	0,04^{b}	0,00	0,47	0,08±0,10	IV
Kl-*a* (µg/L)	0,005^{a}	0,015^{b}	0,008^{a}	0,006^{a}	0,003	0,029	0,008±0,005	✓

Aynı satırdaki farklı harfler, mevsimsel farkın önemli olduğunu ifade eder ($p<0,05$).

Yönetmeliğimize göre (YSKY, 2012), sınıf I–II sular "iyi" su durumunu (az kirlenmiş su) gösterirken, sınıf II–III "orta" su durumunu (kirlenmiş su) ifade etmektedir. Elekçi Deresi'nde kaydedilen yüksek fosfor içeriği özellikle aşağı havzada ötrofikasyon riski oluşturabilir. Dodds vd. (1998) akarsuları TP derişimine göre, 25 µg/L'den küçük olanları oligotrofik–mezotrofik, 25–75 µg/L olanları mezotrofik–ötrofik sınırında sınıflandırmaktadır. Elekçi Deresi'nde yıllık ortalama TP derişimi 170 µg/L (0,17 mg/L)'dir. Bu durumda özellikle aşağı havzada kaydedilen yüksek TP konsantrasyonuna göre Elekçi Deresi ötrofik–hipertrofik sınırında gruplandırılabilir.

Akarsuda yüksek konsantrasyonlarda fosfatlı ve azotlu bileşiklerin bulunmasının en önemli nedeni tarımsal alanlarda kullanılan gübrelerdir. Elekçi Deresi havzası içinde çoğunlukla fındık tarımı yapılmakta ve verimi artırmak için kimyasal gübreler yaygın olarak kullanılmakadır. Azot ve fosfor içeriğine sahip bu gübreler toprağa gömülmediği için bölge arazisinin eğimli yapısı ve her mevsim yağışlı olması sebebiyle yüzeysel akışlarla akarsuya taşınmakta ve suyun nütrient içeriğini yükseltmektedir. Aynı zamanda, Elekçi Deresi aşağı akış yönünde Kumru ilçe merkezinden, ilçenin hemen mansabındaki vahşi deponi alanından, birçok yan kollarla bağlantılı belde ve dağınık yerleşim alanlarından geçerken bünyesine evsel atıksular katılmakta ve aşağı havzada akarsuyun kirlilik yükü oldukça artmaktadır. Elekçi Deresi Fatsa ilçe merkezinden geçerken bütün kirlilik yükünü Karadeniz'e deşarj etmektedir. Benzer durum Karadeniz Bölgesi'ndeki birçok akarsularımızda da gözlenmektedir (Taş vd., 2011; Taş vd. 2015; Tepe ve Aydın, 2017; Taş vd., 2019; Ustaoğlu vd., 2017; Ustaoğlu ve Tepe, 2019; Ustaoğlu ve Islam, 2020; Ustaoğlu vd., 2020a; Ustaoğlu vd., 2020b).

Aşağı havzada sudaki nütrient kirliliğiyle birlikte yüksek bulanıklık da dikkat çekmiştir. Organik veya inorganik maddelerden veya ikisinin kombinasyonundan kaynaklanabilen türbidite, yaklaşık 4,0 NTU'nun üzerinde çıplak gözle fark edilebilir (WHO, 2011). Araştırma alanımızda türbidite yukarı,

orta ve aşağı istasyonlarda artarak sırasıyla 39,31 NTU, 43,32 NTU ve 81,47 NTU olarak ölçülmüştür. Tablo 3'te fizikokimyasal parametreler arasındaki ilişkinin yönünü ve miktarını belirlemek için yaptığımız Pearson korelasyon analizi sonuçlarında da görüldüğü gibi, türbiditenin amonyak, amonyum, demir ve serbest klor ile çok yüksek ilişkisi olduğu görülmektedir ($p<0,01$). Organik kirliliğin göstergesi olan inorganik kirlilik parametrelerinden azotlu bileşiklerin de kendi arasında (amonyak ve amonyum) çok yüksek korelasyonu söz konusudur ($r=1,0$; $p<0,01$). Türbidite ile TP arasında da ilişki vardır. Sudaki fosforun artışı algal üretimi hızlandırır, dolayısıyla sudaki süspanse madde yoğunluğu artar. Korelasyon matriksinde bu iki parametre arasında yüksek ilişki söz konusudur ($r=0,624$; $p<0,01$).

Katılar suda çözelti veya süspansiyon halinde bulunur. Sudaki toplam çözünmüş katı (TDS) ve askıda katı madde (AKM/TSS) toplamı toplam katı madde değerini verir (Alley, 2007). Tatlı suların TDS derişimi 0–1000 mg/L aralığındadır (Uslu ve Türkman, 1987). Elekçi Deresi'nde toplam katı madde miktarı oldukça az olup yıllık ortalama TDS derişimi 117 mg/L'dir. Aynı ildeki Melet Irmağı'nda yıllık ortalama TDS değerinin 161 mg/L (Ustaoğlu vd., 2017), Turnasuyu Çayı'nda 66 mg/L (Ustaoğlu vd., 2020a) olduğu bildirilmiştir. TDS parametresi ile doğrudan ilişkili olan suyun elektriksel iletkenliği (EC) sudaki iyon konsantrasyonuna bağlıdır ve aralarında pozitif ilişki vardır. Pearson korelasyon analizi sonucunda da (Tablo 3) görüldüğü gibi, TDS ve EC arasında pozitif yönde çok yüksek bir ilişki vardır ($r=0,996$; $p<0,01$). Suda EC parametresinin analizi, suyun sulama ve yangınla mücadele için uygunluğunu belirlemek için de oldukça önemlidir (Omer, 2019). Elekçi Deresi'nde yıllık ortalama EC değeri 242,3 µS/cm ölçülmüştür. Bu parametreye göre akarsuyun tuzluluk sınıfı C1 "az tuzlu" (<250 µS/cm), su kalite sınıfı I. sınıf (<400 µS/cm) yani "çok iyi" sudur.

Elekçi Deresi suyu tatlı su özelliğinde, nötr pH değerinde ve yumuşak bir sudur. Yıllık ortalama sertlik değeri 0–75 mg/L $CaCO_3$ aralığında ölçülmüştür (63,20 mg/L $CaCO_3$). Ca ve Mg iyonları su sertliğini (TH) ile doğrudan etkiler. Korelasyon matriksi incelendiğinde (Tablo 3) TH ile Ca arasında çok yüksek, TH ile Mg arasında yüksek ilişki olduğu görülmektedir ($p<0,01$). HCO_3^- bakımından zengin sularda Ca konsantrasyonu Mg konsantrasyonundan fazladır (Wetzel, 1983). Elekçi Deresi'nde yıllık ortalama Ca 40,51 mg/L, Mg 2,59 mg/L olarak ölçülmüştür. Suyun pH değeri de bu literatürü desteklemektedir. Ordu ilinde yapılan benzer çalışmalarda Melet Irmağı nötr çevresinde hafif alkali (ort. pH 7,96) ve orta sertlikte (97 mg/L $CaCO_3$) iken (Ustaoğlu vd., 2017), Turnasuyu Çayı alkali karakterde (ort. pH 8,60) oldukça yumuşak (44,77 mg/L $CaCO_3$) su özelliğine sahiptir (Ustaoğlu vd., 2020a).

Çalışmamızda veri setini azaltarak çevresel değişkenleri sınıflandırıp su kalitesi üzerine muhtemel çevresel baskıları tahmin etmek için çok değişkenli istatistiksel analizlerden biri olan temel bileşenler analizi (PCA/TBA) yapılmış ve yeni faktörler oluşturulmuştur. Temel bileşenler analizi, çalışma alanındaki başlıca potansiyel kirlilik kaynaklarını ayırt etmek ve doğrulamak için kullanılmakta ve pollutantlar gruplandırılmaktadır (Tablo 4). Bu analiz gözlemlenemeyen gizli kirlilik kaynaklarının tanımlanabilmesine imkan sağlar (Su vd., 2011). Faktör yükleri ise 0,30–0,50 zayıf, 0,50–0,75 orta (ılımlı), >0,75 kuvvetli bir ilişki olduğunu gösterir (Wang vd., 2013). Verilerimizin analizi sonucunda, toplam varyansın % 77,28'ini açıklayan özdeğeri 1'den büyük dört faktör oluşmuştur. Bu faktörlerin varyansları sırasıyla ~% 28, % 23, % 16 ve % 12'dir. Birinci faktör ile ikinci faktörün, üçüncü faktör ile dördüncü faktörün % varyans değerleri birbirine yakındır. Genel olarak, dört faktörden birinin diğerine baskın olmadığı tespit edilmiştir. Toplam varyansın en yüksek değerini alan birinci faktörde NH_4-N, NH_3-N, türbidite, Fe, Cl_2 ve TP parametrelerinin hepsi kuvvetli pozitif yük oluşturmuştur (Tablo 4). Nütrient parametrelerini içeren birinci faktörün, çoğunlukla kimyasal gübrelerden kaynaklı tarımsal kirlilik etmenlerini temsil ettiği sonucuna varılmıştır. İkinci faktörde TH, Ca ve Mg kuvvetli pozitif yük, NO_3-N, EC ve TDS orta (ılımlı) pozitif yük oluşturmuştur. Bu parametrelere göre ikinci faktör çoğunlukla havzanın jeolojik yapısından kaynaklı noktasal olmayan inorganik kirliliği temsil eder. Üçüncü faktörde sıcaklık kuvvetli pozitif, çözünmüş oksijen kuvvetli negatif, oksijen doygunluğu ise orta şiddette pozitif yüklüdür. Bu faktöre göre Elekçi Deresi üzerinde klimatolojik faktörlerin etkili olduğu söylenebilir. Dördüncü faktörü oluşturan kl-*a* orta şiddette negatif yüklü, NO_2-N ve SO_4 ise orta şiddette pozitif yüklüdür. Bu faktöre göre Elekçi Deresi su kalitesi üzerinde evsel atıksuların, tarım arazilerinden gelen yüzeysel akış, erozyon ve drenaj sularının etkili olduğunu söyleyebiliriz.

Birinci faktör içinde yer alan ve kuvvetli pozitif yük oluşturan azotlu ve fosforlu nütrient parametrelerinin Elekçi Deresi'nde tarımsal ve evsel kaynaklı atıkların akarsuyun su kalitesi üzerinde

büyük etkisinin olduğunu göstermektedir. Bölgede bu pollutantlar daha çok yayılı kaynaklardan gelmektedir. Turnasuyu Çayı'nda da bu nütrient parametreleri birinci faktör içinde yer almıştır (Ustaoğlu vd., 2020a). Çalışma alanımızdaki bu inorganik besi elementlerinin konsantrasyonu bölgedeki diğer akarsulardan çok yüksek değerlerde ölçülmemiştir. Örneğin Giresun ilinde Batlama Çayı (0,019–0,689 mg/L) ve Pazarsuyu Deresi'nde (0,02–0,52 mg/L) TP değerlerinin çalışma alanımızdan daha yüksek konsantrasyonlarda olduğu bildirilmiştir. Bu akarsularda azotlu bileşikler de yüksektir; NO_3-N değeri Pazarsuyu Deresi'nde 0,23 ila 2,96 mg/L aralığında ölçülmüş, ortalama değer ise 1,17 mg/L'dir (Tepe ve Aydın, 2017; Ustaoğlu ve Tepe, 2019).

Faktör analizi kullanılarak su kalitesinin değerlendirildiği bir başka çalışmada, Marmara Bölgesi'ndeki Mustafakemalpaşa Çayı'nın su kalitesi üzerine madencilik faaliyetleri ile havzanın jeolojik yapısının etkili olduğu görülmüştür (Dalkıran vd., 2020). Bu akarsuyun havzası incelendiğinde, maden kaynakları yönünden zengin ve içinde maden işletmelerinin olduğu görülmektedir. Dolayısıyla birinci ve ikinci faktörlerde madenlerle ilgili metaller kuvvetli faktör yükü oluşturmuştur.

Çalışma alanımızda Elekçi Deresi havzası içinde daha çok tarım yapıldığı ve akarsu yerleşim yerlerinin merkezinden geçtiği için, çıkan faktör yükleri kirletici kaynakları net şekilde göstermektedir. Ancak ilerleyen süreçte bu kirlilik yükleri değişebilir. Özellikle Elekçi Deresi'nin alt havzası içinde faaliyete geçen altın madeni işletmesinin akarsu üzerine etkisi izlenmesi gereken çok önemli bir lokalitedir. Bu işletmeden kaynaklı herhangi bir sızıntı sadece su kalitesini değil sucul biyotayı ve halk sağlığını da olumsuz etkileyecektir.

Çevresel parametrelerin veri setine kümeleme (cluster) analizi uygulanmış ve mevsimlerin iki kümede toplandığı görünmüştür (Şekil 2). Yaz ve sonbahar verileri % 92 benzerlik seviyesinde bir kümeyi, ilkbahar ve kış verileri % 88 benzerlik seviyesinde ikinci kümeyi oluşturmuştur. Benzerlik oranı en az olan (% 79) mevsimler ise ilkbahar ve sonbahardır. Ilıman iklim özelliği gösteren Karadeniz Bölgesi'nin sahil kuşağında son yıllarda yaz sezonu sonbahar ortalarına kadar devam etmekte, kış sezonu da ilkbaharda kendini hissettirmektedir. Bu iklimsel faktörler cluster analizinde kendini gösterirken aynı zamanda temel bileşenler analizinde de 3. faktör içinde su sıcaklığı kuvvetli pozitif yük taşıyarak su kalitesi üzerinde etkili olduğunu göstermiştir. Dolayısıyla Elekçi Deresi su kalitesi üzerinde tarımsal, evsel ve klimatolojik faktörlerin etkli olduğunu söyleyebiliriz.

Tablo 3. Elekçi Deresi çevresel parametrelerinin korelasyon matrisi

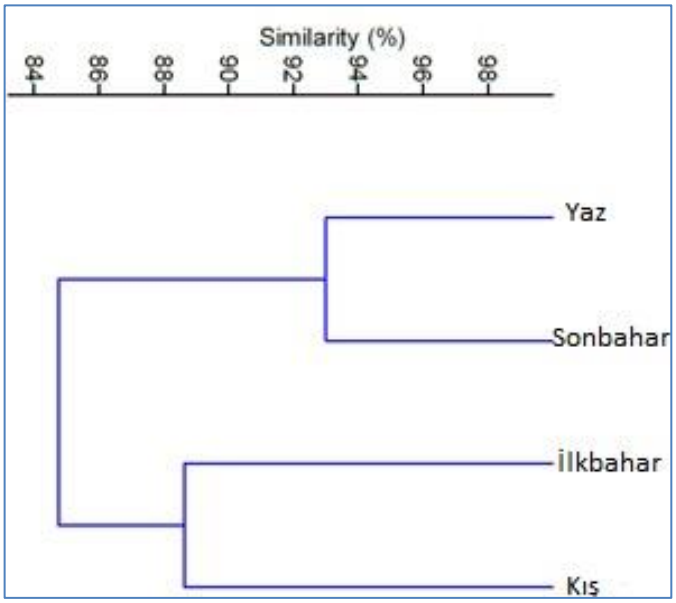
	TDS	Sıc.	EC	pH	ÇO	O_2 (%)	Turb.	NO_2-N	NO_3-N	SO_4	Fe	NH_3	NH_4	TH	Ca	Mg	Cl_2	TP	AKM	Kl-a
TDS	1																			
Sıc.	,412*	1																		
EC	,996**	,408*	1																	
pH	-,057	-,017	-,075	1																
ÇO	-,014	-,688**	-,009	-,057	1															
O_2 (%)	,427**	,795**	,425**	-,084	-,159	1														
Turb.	,043	-,194	,054	,154	-,029	-,406*	1													
NO_2-N	,172	,144	,200	,174	-,102	,138	-,279	1												
NO_3-N	,178	-,089	,187	-,038	,005	-,205	-,292	,231	1											
SO_4	,673**	,272	,684**	,068	-,221	,167	-,225	,557**	,281	1										
Fe	-,374	-,157	-,364	,415*	-,052	-,401	,948**	-,315	-,358	-,255	1									
NH_3	-,259	-,305	-,240	,251	,109	-,508*	,818**	,008	-,340	-,058	,798**	1								
NH_4	-,255	-,303	-,236	,250	,108	-,506*	,817**	,009	-,339	-,056	,797**	1,000**	1							
TH	,639**	,234	,648**	-,222	-,210	,017	-,063	,389	,507*	,454*	-,216	,023	,028	1						
Ca	,590**	,180	,599**	-,199	-,144	,012	-,033	,368	,460*	,373	-,186	,035	,040	,975**	1					
Mg	,558**	,280	,565**	-,311	-,301	-,016	-,068	,226	,503*	,460*	-,184	,016	,019	,790**	,640**	1				
Cl_2	-,221	-,111	-,215	,422*	-,103	-,324	,801**	-,109	-,286	-,176	,789**	,578**	,579**	-,031	,061	-,241	1			
TP	-,032	-,055	-,014	-,074	-,086	-,331	,624**	,054	,041	,055	,545**	,679**	,681**	,370	,421*	,169	,441*	1		
AKM	-,083	-,275	-,091	-,102	,267	-,216	,148	,070	-,029	-,136	,079	,120	,121	,117	,231	-,203	,393	,278	1	
Kl-a	-,208	-,132	-,228	,019	-,138	-,324	,360	-,272	-,191	-,249	,184	,205	,201	-,195	-,209	-,076	,058	-,002	-,204	1

* Korelasyon 0,05 düzeyinde anlamlı, ** Korelasyon 0,01 düzeyinde anlamlı

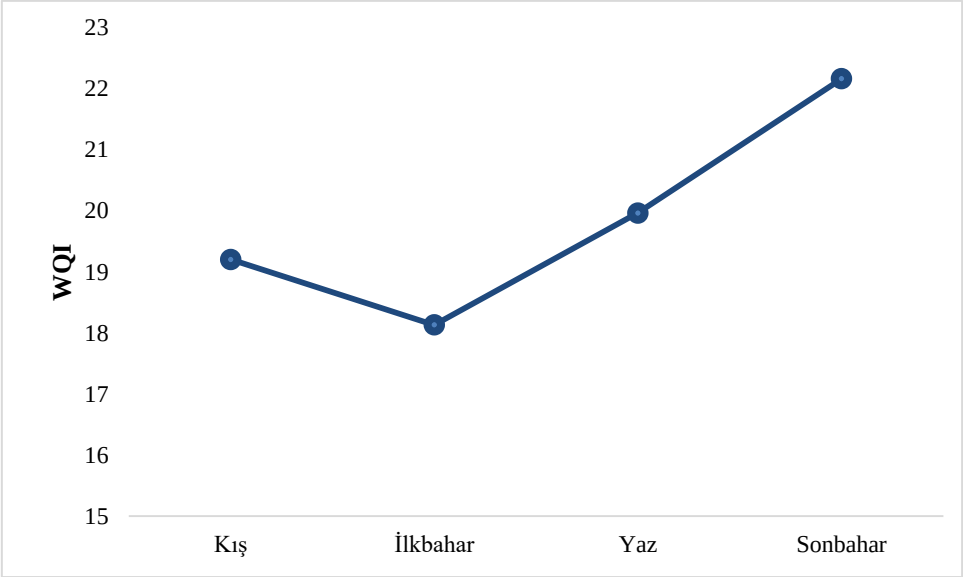
Tablo 4. Döndürülmüş faktör matriksi sonuçları

	1. Faktör	2. Faktör	3. Faktör	4. Faktör
NH_4	**0,930**	-0,010	-0,199	0,018
NH_3	**0,929**	-0,014	-0,201	0,015
Türbidite	**0,919**	-0,052	0,005	-0,327
Fe	**0,893**	-0,229	-0,004	-0,224
Cl_2	**0,789**	-0,142	0,044	-0,059
TP	**0,753**	0,332	-0,070	0,039
TH	0,078	**0,922**	0,110	0,226
Mg	-0,018	**0,861**	0,161	0,006
Ca	0,117	**0,856**	0,069	0,251
NO_3-N	-0,347	**0,688**	-0,313	-0,062
EC	-0,168	**0,595**	0,468	0,418
TDS	-0,184	**0,589**	0,478	0,393
Sıcaklık	-0,126	0,122	**0,941**	0,066
DO	-0,080	-0,212	**-0,812**	0,239
O_2 (%)	-0,409	-0,171	**0,723**	0,359
Kl-*a*	0,145	-0,010	0,018	**-0,718**
NO_2-N	-0,032	0,269	-0,028	**0,641**
SO_4	-0,041	0,478	0,228	**0,583**
Özdeğer	6,17	4,20	2,24	1,30
Varyans (%)	27,64	22,63	15,53	11,48
Kümülatif %	27,64	50,27	65,80	77,28

Suyun içilebilirliğinin değerlendirilmesinde kullandığımız su kalitesi indeksi (WQI), su kalitesi verilerinin kamuya, politika yapıcılara ve yöneticilere çok daha basit bir şekilde sunulmasında oldukça etkilidir. Bu indeks, dünyanın çeşitli ülkelerinde ve yurdumuzda su kalitesini değerlendirmek için yaygın olarak kullanılmaya başlamıştır (Varol ve Davraz, 2015; La Mora-Orozco vd., 2017; Khalid, 2019; Kükrer ve Mutlu, 2019; Omwene vd., 2019; Ustaoğlu vd., 2020a). Elekçi Deresi'nin WQI değerleri mevsimsel olarak hafif dalgalı bir seyir izlemiştir (Şekil 3). Kış, ilkbahar, yaz ve sonbahar mevsimlerinde WQI değeri sırasıyla 19,2, 18,3, 19,96 ve 22,16 olarak hesaplanmıştır. Ortalama WQI değeri 19,86'dır. Elde edilen WQI değerine göre Elekçi Deresi tüm mevsimlerde "mükemmel" su kalitesi özelliği taşımaktadır (Yadav vd., 2010).



Şekil 2. Çevresel analizlerin mevsimsel dendrogramı



Şekil 3. Su kalitesi indeksinin mevsimsel değişimi

Biyolojik parametreler

Lentik sistemlerin trofik durumları trofik indeksler veya indikatör parametreler kullanılarak sınıflandırılırken, lotik sistemelere böyle bir trofik sınıflandırma yapılmadığını görüyoruz. Genelde akarsular oligotrofik veya ötrofik olarak sınıflandırılır (Kelly ve Whitton, 1995). Trofik durumun belirlenmesinde indikatör biyolojik parametrelerden klorofil-*a* (kl-*a*) pigmenti bütün alglerde bulunduğu için, sucul ekosistemin alg yoğunluğu hakkında bilgi verir (Taş vd., 2011). Dolayısıyla, sestonik kl-*a* konsantrasyonu primer üretimin indikatörüdür. Dodds vd. (1998), süspanse kl-*a* değerinin <10 µg/L olduğu akarsuların trofik durumunu "oligotrofik" olarak tanımlar. Elekçi Deresi'nde yıllık ortalama kl-*a* konsantrasyonu 0,008 µg/L'dir. Ölçülen bu değer akarsuyun verimliliğinin oldukça düşük olduğunu göstermektedir. Çalışma alanında ölçülen en yüksek kl-*a* değeri ilkbahar ortasında aşağı havzada kaydedilmiştir (0,0292 µg/L). Kl-*a* dışında diğer fotosentetik pigmentlerden kl-*b* ve kl-*c* maksimum derişimleri de yine ilkbahar ortasında aşağı havzada kaydedilmiştir (0,0196 µg/L, kl-*b*; 0,0428 µg/L, kl-*c*). Kl-*a* değeri Elekçi Deresi'nde ötrofikasyon riskinin olmadığını göstermektedir (YSKY, 2012).

Sestonik kl-*a* büyük ve yavaş hareket eden akarsularda özellikle alt havzalarda yüksek derişimlerde kaydedilirken yukarı havzalarda daha düşük derişimlerde ölçülür. Çünkü alt havzada fitoplankton çoğalma fırsatı yakalar. Ancak hem eğimin fazla olduğu hem de düzensiz rejimli olan Karadeniz Bölgesi akarsuları yağışlı sezonlarda oldukça hızlı akar. Bu nedenle özellikle yukarı havzalarda fotosentetik pigment konsantrasyonu az kaydedilir. Ordu ilinde 43 akarsuda yapılan pigment analizinde süspanse kl-*a* konsantrasyonu 0,051 ila 3,86 µg/L aralığında ölçülmüştür (Taş vd., 2011). İlin en büyük akarsuyu Melet Irmağı'nda kl-*a* derişimi 3,78 µg/L (Ustaoğlu vd., 2017), Turnasuyu Çayı'nda 0,488 µg/L olarak ölçülmüştür (Taş vd., 2019). Bu verilere göre, bölge akarsuları ile karşılaştırıldığında Elekçi Deresi'nin planktonik primer üretiminin daha az olduğu söylenebilir.

Biyolojik parametrelerden sucul ekosistemin primer üreticileri olan alglere bakıldığında; Elekçi Deresi epilitik alg komünitesinde 5 farklı filuma ait 105 takson tespit edilmiştir. Diyatomeler (Bacillariophyta) tanımlanan 93 takson ile dominant alg grubudur. Bunun dışında epilitik florada klorofitlerden 5, karofitlerden 4, siyanobakterilerden 2 ve öglenoidlerden 1 takson tanımlanmıştır (Yılmaz ve Taş, 2013; Yılmaz ve Taş, 2016). Tablo 5'te Elekçi Deresi'nde epilitik florada kaydedilen diyatome taksonları listelenmiştir.

Tablo 5. Elekçi Deresi epilitik diyatome komünitesindeki taksonların listesi

1	*Achnanthidium minutissimum* (Kützing) Czarnecki	48	*Navicula atomus* var. *permitis* (Hustedt) Lange-Bertalot
2	*Amphora ovalis* (Kützing) Kützing	49	*Navicula capitoradiata* Germain
3	*Caloneis silicula* (Ehrenberg) Cleve	50	*Navicula cincta* (Ehrenberg) Ralfs
4	*Cocconeis pediculus* Ehrenberg	51	*Navicula cryptocephala* Kützing
5	*Cocconeis placentula* var. *euglypta*	52	*Navicula cuspidata* (Kützing) Kützing
6	*Cyclotella kuetzingiana* Thwaites	53	*Navicula gregaria* Donkin
7	*Cymatopleura elliptica* (Brébisson) W.Smith	54	*Navicula hungarica* Grunow
8	*Cymatopleura solea* (Brébisson) W.Smith	55	*Navicula lanceolata* (C.Agardh) Kützing
9	*Cymatopleura solea* var. *apiculata* (W.Smith) Ralfs	56	*Navicula lenzii* Krasske
10	*Cymbella affinis*	57	*Navicula menisculus* Schumann
11	*Cymbella caespitosa*	58	*Navicula minima* Grunow
12	*Cymbella cistula*	59	*Navicula protracta* (Grunow) Cleve
13	*Cymbella cistula* var. *maculata*	60	*Navicula radiosa* Kützing
14	*Cymbella cymbiformis*	61	*Navicula resecta* J.R.Carter
15	*Cymbella cymbiformis var. nonpunctata*	62	*Navicula salinarum* Grunow
16	*Cymbella helvetica*	63	*Navicula similis* Krasske
17	*Cymbella minuta* var. *semicircularis* (Lagerstedt) Foged	64	*Navicula slesvicensis* Grunow
18	*Cymbella silesiaca* Bleisch	65	*Navicula tripunctata* (O.F.Müller) Bory de Saint-Vincent
19	*Cymbella sinuata* W.Gregory	66	*Navicula trivialis* Lange-Bertalot
20	*Cymbella tumida* (Brébisson) van Heurck	67	*Navicula tuscula* (Ehrenberg) Grunow
21	*Diatoma hiemalis* var. *quadratum* (Kützing) R.Ross	68	*Neidium globiceps* (Cleve-Euler) Cleve-Euler
22	*Diatoma mesodon* (Ehrenberg) Kützing	69	*Nitzschia acicularis* (Kützing) W.Smith
23	*Diatoma moniliforme* (Kützing) D.M.Williams	70	*Nitzschia bacillum* Hustedt
24	*Diatoma vulgaris* Bory	71	*Nitzschia clausii* Hantzsch
25	*Didymosphenia geminata* (Lyngbye) M.Schmidt	72	*Nitzschia constricta* (Kützing) Ralfs
26	*Diploneis elliptica* (Kützing) Cleve	73	*Nitzschia fonticola* (Grunow) Grunow
27	*Encyonema minutum* (Hilse) D.G.Mann	74	*Nitzschia frustulum* (Kützing) Grunow
28	*Encyonema prostratum* (Berkeley) Kützing	75	*Nitzschia gracilis* Hantzsch
29	*Epithemia turgida* (Ehrenberg) Kützing	76	*Nitzschia lorenziana* Grunow
30	*Eunotia acus* Metzeltin & Lange-Bertalot	77	*Nitzschia microcephala* Grunow
31	*Eunotia implicata* Nörpel, Lange-Bertalot & Alles	78	*Nitzschia palea* (Kützing) W.Smith

32	*Fragilaria vaucheriae* (Kützing) J.B.Petersen	79	*Nitzschia paleacea* (Grunow) Grunow
33	*Gomphonema angustum* C.Agardh	80	*Nitzschia sigma* (Kützing) W.Smith
34	*Gomphonema apicatum* Ehrenberg	81	*Nitzschia sigmoidea* (Nitzsch) W.Smith
35	*Gomphonema augur* Ehrenberg	82	*Pinnularia borealis* Ehrenberg
36	*Gomphonema clavatum* Ehrenberg	83	*Pinnularia brebissonii* (Kützing) Rabenhorst
37	*Gomphonema minutum* (C.Agardh) C.Agardh	84	*Pinnularia rupestris* Hantzsch
38	*Gomphonema olivaceum* var. *olivaceoides* (Hustedt) Lange-Bertalot	85	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot
39	*Gomphonema parvulum* (Kützing) Kützing	86	*Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot
40	*Gomphonema truncatum* Ehrenberg	87	*Surirella amphioxys* W.Smith
41	*Gyrosigma acuminatum* (Kützing) Rabenhorst	88	*Surirella angusta* Kützing
42	*Gyrosigma attenuatum* (Kützing) Rabenhorst	89	*Surirella brebissonii* var. *kuetzingii* Krammer & Lange-Bertalot
43	*Gyrosigma parkeri* (M.B.Harrison) Boyer	90	*Surirella minuta* Brébisson
44	*Hannaea arcus* (Ehrenberg) R.M.Patrick	91	*Surirella tenera* var. *nervosa* A.Schmidt
45	*Hantzschia amphioxys* (Ehrenberg) Grunow	92	*Ulnaria biceps* (Kützing) Compère
46	*Melosira varians* C.Agardh	93	*Ulnaria ulna* (Nitzsch) Compère
47	*Meridion circulare* (Greville) C.Agardh		

Bentik alglerden 93 diyatome türü 10 farklı takım içinde yer almaktadır. Takımların sıralaması en fazla takson içerenden aza doğru; Naviculales (29 takson) > Cymbellales (23 takson) > Bacillariales (14 takson) > Fragilariales (9 takson) > Surirellales (8 takson) > Achnanthales (4 takson) > Eunotiales (2 takson) > Thalassiophysales (2 takson) > Melosirales (1 takson) > Rhopalodiales (1 takson) şeklindedir. Tür sayısı açısından en zengin gruba 20 takson ile Naviculales ordosundan *Navicula* sahiptir. Bundan sonra, *Nitzschia* 13, *Cymbella* 11, *Gomphonema* 8 ve *Surirella* 5 tür ile en çok tür içeren taksonlardır. Türkiye tatlısu algleri içinde 631 tane diyatome türü kaydedilmiştir (Solak vd., 2012). Yapılan araştırmalarda en yaygın ve bol olarak kaydedilen diyatomelerin *Navicula, Nitzschia, Surirella* ve *Cymbella* cinslerine ait olduğu bildirilmiştir. Karadeniz Bölgesi'nin sahil akarsularında yapılan bentik alg çeşitliliği çalışmalarında diyatomeler dominant alg grubudur (Taş vd., 2019), ancak çok zengin bir çeşitlilik söz konusu değildir (Şahin, 2003; Taş ve Yılmaz, 2015; Maraşlıoğlu vd., 2017; Temizel vd., 2017).

Elekçi Deresi'nde epilitik diyatome florasında nispi bolluk hesaplamaları sonucunda 7 türün baskın olduğu belirlenmiştir. Tüm istasyonlardaki ortalama nispi bolluk sonuçlarına göre, *Cocconeis placentula* var. *euglypta* epilitik florada hâkim takson olarak belirlenmiştir (% 13). Bu türü sırasıyla; *Navicula tripunctata* (%10), *Gomphonema truncatum* (% 7), *N. lanceolata* (% 7), *N. salinarum* (% 7), *C. pediculus* (% 6) ve *N. menisculus* (% 6) izlemiştir. *C. pediculus* ve *C. placentula* var. *euglypta* özellikle sıcaklık ile pozitif ilişki göstermiştir. *G. truncatum* serbest klor ve turbidite ile yüksek pozitif korelasyona sahiptir. *N. mensiculus* ise nitrit ile pozitif ilişkilidir.

C. placentula türünün nispeten organik olarak az kirlenmiş sularda yaygın olduğu ve yüksek elektriksel iletkenliğe toleranslı olduğu belirtilmiştir (Tuchman ve Blinn, 1979). Bu tür organik kirliliğe hassas olarak sınıflandırılırken (Lange- Bertalot, 1979; Szczepocka ve Szulc, 2009), düşük ila orta derecede kirliliğe toleranslı olduğu (Kelly vd., 2001; Bere, 2016) ve ileri derecede ötrofik sularda da iyi geliştiği bildirilmiştir (Kwandrans vd., 1998). *C. placentula* var. *euglypta* aşağı havzaya doğru kirlilikle beraber artış göstermiş ve 3. istasyonda "devamlı mevcut" tür olarak kaydedilmiştir. Aynı cinsin farklı türü olan *C. pediculus*, yüksek elektriksel iletkenliğe sahip hafif tuzlu (acısu) sularda yaygındır (Cox, 1996). Elekçi Deresi'nde kaydedilen elektriksel iletkenlik değer aralığı 67,2–357 µS/cm'dir. Aşağı akış yönünde kirlilikle doğru orantılı olarak artış gösteren ve alt istasyonda "devamlı mevcut" türlerden biri olan *G. truncatum* yüksek elektriksel iletkenliğe sahip sularda sıkça rastlanan diğer bir yaygın taksondur. Fakat bu türün β-mezosaprobikten daha kötü şartlarda gözlenmediği bildirilmiştir (Cox, 1996). *Navicula* tatlı sularda en yaygın bulunan cinstir. Çalışma alanımızda *N. lanceolata, N. menisculus, N. salinarum* ve *N. tripunctata* taksonları da 3. istasyonda "devamlı mevcut" türler içinde yer almıştır. *N. salinarum* özellikle sıcaklığın arttığı, su akışının azaldığı yaz

sonundan sonbahar ortalarına kadar belirgin bir artış göstermiştir. Bu tür mineral içeriği zengin sularda yayılış gösteren kozmopolit bir türdür (Krammer ve Lange-Bertalot, 1986). Nather Khan (1990) *Navicula* türlerinin fakültatif veya kayıtsız türler içinde yer aldığını, hem organik madde bakımından zengin hem de organik madde bakımından fakir ortamlarda yaygın ve bol olarak bulunabileceğini bildirmiştir.

Elekçi Deresi'nde üst istasyonda "bazen mevcut", orta istasyonda "ekseriya mevcut", aşağı istasyonda ise "devamlı mevcut" türlerden olan *Cymbella affinis* ve *Achnanthidium minutissimum* taksonlarının su kalitesinin sınıf I-II arasında olduğu (az kirlenmiş) sularda bulunduğu yani organik kirliliğe hassas olduğu bildirilmiştir (Nather Khan, 1990; Kelly, 1998; Kwandrans vd., 1998; Solak, 2011). *A. minutissimum* taksonunun baskınlığı da aşağı doğru artmış ve 3. istasyonda "devamlı mevcut" türler arasında kaydedilmiştir. Bu türün atık sulara ve β-α-mezosaprobik şartlara duyarlı, çok sık rastlanan yaygın bir tür olduğu, kalite sınıfı bakımından ise farklı ekolojik şartlara sahip sularda gelişebildiği ve bulunabildiği bildirilmiştir (Cox, 1996).

Aşağı havzaya doğru orantısal bir artış gösteren ve özellikle alt istasyonda baskın türler içinde yer alan bir diğer takson *Ulnaria ulna* organik kirliliğe toleranslıdır (Palmer, 1969), β-α-mezosaprobik zonlarda yaygındır (Watanabe vd., 1988) ve oligo-ötrofentik trofik durumu gösterir (Van Dam vd., 1994). Yine, araştırma alanımızda özellikle Ağustos, Eylül ve Ekim aylarında baskın türler arasında yer alan *Nitzschia palea* kirlilikle doğru orantılı bir biçimde artış göstermiştir. Bu tür çok geniş yayılım gösteren kozmopolit bir taksondur (Cox, 1996). Organik kirliliğin göstergesidir (Palmer, 1969), polisaprobik ve hiperötrofentik koşulların indikatörlerindendir (Van Dam vd., 1994). Kirliliğe toleranslı indikatör diyatom türlerinin aşağı havzaya doğru bulunma sıklığındaki artış alt havzalarda kirliliğin arttığını göstermektedir.

Shannon tür çeşitliliği indeksi, bir örnekte bulunan tüm türleri ve bu türlere ait bireylerin dağılımlarını gösterir. Shannon indeks değeri 0–5 arasında değişmektedir. Bu indeks değeri, bir komünitede taksonların sayısı ve dağılımı arttıkça artar. Yani, indeks değerinin yüksek olması tür çeşitliliğinin yüksek, türlerin dağılımlarının dengeli ve kirlilik düzeyinin daha az olduğunu ifade eder (Shannon ve Weaver, 1949). Shannon indeks değerlerine göre suların kirlilik düzeyi hakkında yorum yapılabilir. Shannon indeks değeri 0–1,0 aralığında kaydedildiğinde sistemde çeşitlilik düzeyi için çok zayıf, kirlilik düzeyi için ağır kirli yorumlaması yapılırken, 3,0–4,5 aralığındaki çeşitlilik indeksi çok iyi çeşitlilik düzeyini ve çok hafif kirlilik düzeyini belirtir (Wilhm ve Dorris, 1968). Pielou düzenlilik indeksi ise, dominansinin türlere göre dağılımını gösteren bir indekstir, çalışma alanındaki türlerin birey sayılarına göre dağılımlarının düzenliliğini ve homojenliğini belirlemek için kullanılır. Her tür eşit sayıda birey ile temsil ediliyorsa bu indeks 1'e eşit olur. Düzenlilik indeks değeri 0–1 arasında limitlidir. Değerin 1'e yaklaşması dağılımın düzenli olduğunu göstermektedir (Pielou, 1960).

Elekçi Deresi epilitik diyatomelerinin kantitatif analizleri sonucu hesaplanan Shannon çeşitlilik indeks değerleri üst havzada (1. istasyon) 0,95 (Haziran) ile 1,257 (Mart) arasında değişmiştir (Tablo 6). Düzenlilik indeks değeri ise en yüksek Mart ayında (0,828), en düşük Haziran ayında (0,698) hesaplanmıştır. Orta havzada (2. istasyon) Shannon çeşitlilik indeksi 1,428 (Ekim) ile 0,938 (Ağustos) arasında değişmiştir. Düzenlilik indeksi en yüksek Kasım ayında (0,919), en düşük Ağustos ayında (0,671) hesaplanmıştır. Alt havzada (3. istasyon) Shannon indeksi 1,438 (Ekim) ile 1,108 (Mart) arasında değişmiştir. Düzenlilik indeksi en yüksek Temmuz ayında (0,847), en düşük Nisan ayında (0,737) hesaplanmıştır. Tüm istasyonlardaki Shannon indeksi değerleri ile düzenlilik değerleri benzer bir mevsimsel değişim göstermiştir. Çeşitlilik indeks değerleri 1'e yakın ve 1'den yüksektir. Analiz sonucuna göre Elekçi Deresi'nin çeşitlilik düzeyi "zayıf", kirlilik düzeyi ise "orta" olarak yorumlanabilir (Wilhm ve Dorris, 1968). Düzenlilik indeks değerleri ise 1'e yakındır. Bu sonuç türlerin dağılımının düzenli olduğunu göstermektedir.

Tür çeşitliliği tür zenginliği ve düzenliliği ile doğru orantılıdır. Simpson çeşitlilik indeksi, hem zenginliği hem de düzenliliği dikkate alan bir çeşitlilik ölçüsüdür. Ortam çeşitliliğini gösterir, ortam çeşitliliği ile ters orantılıdır ve dominansiyi ortaya çıkarır. Simpson çeşitlilik indeksi 0–1 arasında limitlidir (Simpson, 1949). Simpson indeksinin sonuçları Shannon indeksi ile tutarlıdır (Tablo 6). Akarsuyun üst havzasında yüksek indeks değerlerinin kaydedildiği Mart ve Ağustos aylarında *C. affinis, G. truncatum, N. lanceolata* ve *N. tripunctata* dominant türlerdir. Orta havzada yüksek değerlerin hesaplandığı Ekim ve Kasım aylarındaki dominant taksonlar *C. placentula* var. *euglypta, Fragilaria vaucheriae* ve *N. tripunctata* türleridir. Alt havzada ise Ağustos, Eylül ve Ekim aylarında

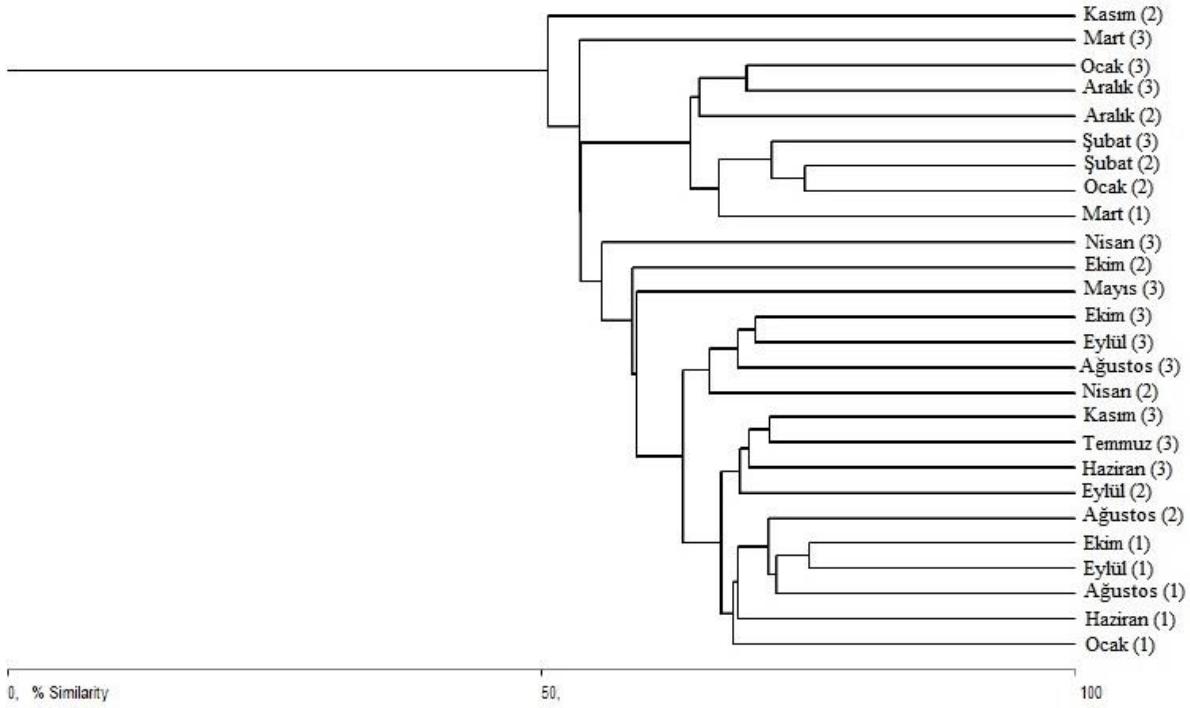
yüksek indeks değeri hesaplanmıştır. Bu aylarda epilitik diyatome komünitesinde *C. affinis, N. menisculus, N. salinarum, N. palea* ve *U. ulna* taksonları baskın ve yaygın olarak bulunmuştur.

Elekçi Deresi'nin epilitik diyatome florasında nispi bolluk hesaplamalarına göre 15 taksonun baskınlığı söz konusudur: *A. minutissimum, C. pediculus, C. placentula* var. *euglypta, C. affinis, Encyonema minutum, G. parvulum, G. truncatum, Melosira varians, N. tripunctata, N. menisculus, N. lanceolata, N. salinarum, N. palea, Surirella brebissonii* var. *kuetzingii, U. ulna*. Genellikle yukarı havzada trofik ağırlığı düşük taksonların, aşağı havzada ise trofik ağırlığı yüksek olan taksonların (Çelekli vd., 2019) mevcudiyeti dikkat çekmektedir.

Tablo 6. Epilitik diyatomelerin çeşitlilik ve düzenlilik indeks değerleri

Aylar	İstasyon	Shannon İndeksi (H')	Pielou İndeksi (J')	Simpson İndeksi (1-D)
Aralık	2	1,22	0,79	0,91
	3	1,91	0,81	0,92
Ocak	1	1,11	0,77	0,87
	2	1,12	0,80	0,89
	3	1,25	0,84	0,93
Şubat	2	1,06	0,74	0,87
	3	1,13	0,76	0,89
Mart	1	1,26	0,83	0,93
	3	1,11	0,76	0,89
Nisan	2	1,25	0,82	0,93
	3	1,11	0,74	0,89
Mayıs	3	1,26	0,82	0,93
Haziran	1	0,95	0,69	0,80
	3	1,30	0,81	0,94
Temmuz	3	1,29	0,85	0,94
Ağustos	1	1,15	0,77	0,89
	2	0,94	0,67	0,79
	3	1,33	0,82	0,94
Eylül	1	1,03	0,73	0,83
	2	1,22	0,76	0,91
	3	1,40	0,83	0,95
Ekim	1	1,12	0,76	0,88
	2	1,43	0,84	0,95
	3	1,44	0,84	0,95
Kasım	2	1,32	0,92	0,95
	3	1,19	0,84	0,92

Diyatomelerin nispi bolluk dağılımlarına göre, araştırma lokaliteleri arasındaki benzerliklerin belirlenmesi için kümeleme/cluster analizi yapılmıştır. Analiz sonucunda, % 50'lik benzerlik seviyesinde tek küme oluştuğu görülmüştür (Şekil 4). Bir yıllık araştırma boyunca aylık olarak yapılan değerlendirmede en yüksek benzerlik (% 75) Eylül ve Ekim aylarında 1. istasyonda kaydedilmiştir. Bu aylarda *A. minutissimum, C. placentula* var. *euglypta, G. parvulum, N. tripunctata* ve *N. palea* türleri dominansi gösteren taksonlardır. Daha sonraki en yüksek benzerlik 2. istasyonda % 74,65'lik benzerlik seviyesinde Ocak ve Şubat ayları arasında görülmektedir. *G. parvulum, G. turncatum, N. lanceolata* ve *N. tripunctata* bu aylarda baskın ve yoğunlukları benzer taksonlardır. Tüm istasyonlar arasında yapılan bu analizde *C. placentula* var. *euglypta* dominant, *N. tripunctata* subdominant tür olarak kaydedilmiştir.



Şekil 4. Diyatome nispi bolluğuna göre zamansal ve mekânsal kümelenmeyi gösteren dendrogram

Elekçi Deresi'nde tespit edilen bentik alg türlerinin indikatör özellikleri ve Palmer (1969)'ın kirlilik indeksine göre akarsuyun ekolojik durumu değerlendirilmiştir. Kirlilik indeksinde, yıl boyunca nispi bollukları % 50'nin üzerinde kaydedilen türler değerlendirmeye alınmıştır. Zamansal ve mekânsal incelemeler ve kirlilik indeksi sonuçları Elekçi Deresi'nde organik kirlilik olmadığını göstermiştir. Yukarı ve orta havzalarda eğimin ve akımın fazla olması taşlık dere yatağında alglerin yıkanmasına neden olmaktadır. Akarsuyun alt havzasında organik kirliliğin göstergesi olan taksonlar (örneğin, *Achnanthidium, Cocconeis, Cymbella, Gomphonema, Navicula, Surirella, Oscillatoria* ve *Euglena* gibi) birçok ayda gözlenmesine rağmen, bu cinslere ait türler yıl boyunca sayıca baskın duruma geçmemişlerdir (Yılmaz ve Taş, 2013; Yılmaz ve Taş, 2016).

Dominant cinslere göre (Peerapornpisal vd., 2007) Elekçi Deresi'nin su kalitesi ve trofik yapısı değerlendirildiğinde; genel olarak akarsuda iki basamaktan oluşan bir trofik düzey tespit edilmiştir: mezotrofik (3.6-5.5) ve mezo-ötrofik (5.6-7.5). Bu sonuçlar akarsuyun su kalitesinin "orta" ve "orta kirli" olduğunu göstermektedir (Peerapornpisal vd., 2007). Akarsuyun yukarı istasyonlarında genellikle mezotrofik olarak belirlenen trofik durum, özellikle yaz sonlarında (Ağustos ve Eylül) yağışın ve debinin azalması, akarsu boyunca kirlilik baskısının etkisiyle mezo-ötrofik seviyeye ilerlemiştir. Trofik durumun belirlenmesinde; *Cocconeis, Cymbella, Gomphonema, Navicula, Nitzschia, Surirella* ve *Synedra* taksonları gösterge olmuştur. Aşağı istasyonda gösterge taksonlardan *Navicula, Gomphonema* ve *Surirella* cinslerinin baskınlığı özellikle kış aylarında dikkat çekmiştir. Yapılan değerlendirmeler neticesinde 3. istasyonda trofik seviyenin "mezo-ötrofik", su kalitesinin ise "orta kirli" olduğu belirlenmiştir. Genel olarak, Elekçi Deresi su kalitesinin kaynaktan mansaba doğru kirlendiği, trofik yapısının da mezotrofik seviyeden mezo-ötrofiğe doğru değiştiği tespit edilmiştir. Elekçi Deresi ile aynı bölgede Bolaman Çayı Havzası içinde yer alan Ilıca Deresi'nde yapılan limnolojik araştırmada, dominant cinslere göre akarsuyun trofik seviyenin yukarıdan aşağıya doğru mezotrofikten mezo-ötrofik doğru değiştiği bildirilmiştir (Taş ve Çetin, 2016). Ilıca Deresi'ndeki trofik durum ve su kalitesi, özellikle yaz aylarındaki trofik seviyenin yükselmesi çalışma alanımızla benzerlik göstermektedir. Yine, Elekçi Deresi ile aynı alt havza içinde yer alan Turnasuyu Çayı (Taş vd., 2019; Ustaoğlu vd., 2020a), Akçaova Deresi (Taş vd., 2017) ve Melet Irmağı (Özoktay ve Taş, 2013; Taş ve Kurt, 2014; Taş vd., 2015; Ustaoğlu vd., 2017) gibi akarsuların mansap istasyonlarının yukarı havzalardan taşınan noktasal ve/veya yayılı kaynaklardan gelen çeşitli kirlilik faktörleri nedeniyle trofik seviyenin üst havzalardan daha yüksek olduğu, su kalitesinin ise çok temiz su özelliliği taşımadığı bildirilmektedir. Batı Karadeniz Bölgesi'nde Zerveli Deresi'nde de kaynaktan

uzaklaştıkça su kalitesinin düştüğü ve WQI değerinin mükemmel ile çok zayıf arasında değiştiği bildirilmiştir (Mutlu, 2019). Karadeniz Bölgesi'ndeki akarsuların çoğunun taşıdığı bu kirlilik yükünün Karadeniz'e deşarjı, ötrofik olan Güney Karadeniz kıyılarının trofik seviyesinin daha da yükselmesine neden olacaktır. Nitekim Karadeniz akarsularında yapılan çalışmalarda, denize deşarj bölgelerindeki suların fosfor içeriğinin yüksek olduğu ve ötrofikasyonun anahtar elementlerinden olan fosfor parametresi bakımından su kalite sınıflarının sınıf II ila sınıf IV arasında değiştiği bildirilmektedir (Taş ve Kolören, 2017; Mutlu ve Verep, 2018). Aynı zamanda, akarsuların taşıdığı ağır metal kirliliği de hem Karadeniz suları hem de insan sağlığı açısından bir risk oluşturmaktadır. Karadeniz Bölgesi akarsularının farklı metaller bakımından farklı yükler taşıdığı, Ordu'daki Akçaova ve Çalışlar derelerinde mevcut ağır metal kirliliğinin az olduğu, zenginleştirme faktörünün ise orta derecede olduğu bildirilmektedir (Ustaoğlu ve Islam, 2020; Ustaoğlu vd., 2020b).

SONUÇ ve ÖNERİLER

Elekçi Deresi'nde bir hidrolojik yıl boyunca yapılan araştırmada, fizikokimyasal analiz sonuçlarına göre su kalitesinin serbest klor hariç genel olarak "iyi" su durumuna sahip olduğu (sınıf I-II) yani "az kirlenmiş su" olduğu tespit edilmiştir. Su kalitesinin sınıflandırılmasında sınıf II değerini gösteren parametrelerden ammonyum azotu ve toplam fosforun bölgedeki fındık üretiminin yapıldığı tarım alanlarında yaygın olarak kullanılan gübrelerden kaynaklandığı düşünülmektedir. Nitekim faktör analizinde birinci faktörde yüksek korelasyon değerlerini gösteren nütrient parametreleri tarımsal orijinlidir. Noktasal ve/veya yayılı kaynaklardan gelen bu antropojenik faktörler suyun hem trofik seviyesini yükseltmekte hem de su kalitesini olumsuz yönde etkilemektedir. Faktör analizinde diğer bileşenlerin jeolojik yapıdan ve klimatik faktörlerden oluştuğu görülmüştür. Bu durum subtropikal kuşakta yer alan akarsulardaki çevresel faktörlerle benzerlik göstermektedir. Su sıcaklığı ile epilitik algler arasında doğrusal yönde yüksek korelasyon tespit edilmiştir. Yine, hidrolojik faktörlere bağlı olarak debinin azaldığı sezonlarda bentik diyatomelerin çeşitliliğinde ve yoğunluğunda artışlar kaydedilmiştir. Shannon indeksi sonuçlarına göre Elekçi Deresi "zayıf" düzeyde çeşitlilik gösterirken, kirlilik düzeyi "orta"dır. WQI sonuçlarına göre ise Elekçi Deresi'nin su kalite özelliği "mükemmel"dir.

Akarsuda akış boyunca yukarıdan aşağıya doğru antropojenik faaliyetlerden kaynaklı, özellikle evsel ve tarımsal kirlenme baskısı indikatör türlerle de tespit edilmiştir. Kirlilik faktörleri suyun fiziksel, kimyasal ve biyolojik özelliklerinin zamansal ve mekânsal olarak değişmesine yol açmış, yaz aylarında yağışların ve su debisinin azalmasıyla organik kirliliğe toleranslı türlerin çeşitliliğinde ve yoğunluğunda artışlar kaydedilmiştir. Ancak, aşırı baskınlık söz konusu değildir. Biyolojik parametrelere göre akarsu değerlendirildiğinde; fotosentetik pigment konsantrasyonunun akarsuda ötrofikasyon riski oluşturmayacağını ve "oligotrofik" olduğunu göstermiştir. Baskın ve yaygın olarak tespit edilen diyatomeler genelde kirliliğe karşı hassas türlerdir. Çoğunluğu akarsularda kozmopolit olan taksonlardır. Dominant taksonlar trofik seviyenin "mezotrofik" ile "mezo-ötrofik", su kalitesinin ise "orta" ile "orta kirli" olduğunu göstermiştir.

Sonuç olarak, ılıman ve yağışlı iklime sahip Ordu-Giresun Alt Havzası'ndaki akarsular iklimsel baskılardan daha çok antropojenik baskıya maruz kalmaktadır. Bölgede birinci çevresel sorun evsel katı atıklar, ikinci sorun su kirliliğidir. Aşağı havzalardaki dolayısıyla Karadeniz üzerindeki karasal orijinli kirlilik baskısını azaltmak için su havzalarında gerekli tedbirler alınmalıdır. Havza bazında koruma statüsünün uygulanması ve izleme çalışmalarıyla temiz tatlısu havzaları korunabilir. Tarımsal faaliyetlerde kullanılan ve toprakta biriken gübre ve pestisitlerin, yüzey akışları ile akarsuya doğrudan karışmasını önlemek amacıyla akarsu ve tarım arazileri arasında 5-10 m'lik doğal koruma zonları oluşturulmalı ve su bitkilerince zenginleştirilmelidir. Riparian alanların fonksiyonları dikkate alınarak, özellikle aşağı havzalarda hidrofitler kontrollü olarak korunmalıdır. Akarsuya yakın konumda bulunan tesislerde arıtma sistemleri aktif çalışmalı, evsel atıkların dereye deşarjı önlenmelidir. Nüfusun artması ve kişi başına düşen tatlısu miktarının her geçen gün azaldığı günümüzde, Türkiye'nin kuzeyinde yer alan Karadeniz Bölgesi'ndeki akarsu havzaları korunarak değerlendirilmelidir.

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In vitro Antiparasitic Activity of Ginger (*Zingiber officinale*) Bulb and Pomegranate (*Punica granatum*) Peel Against Monogenean Fish Parasite., *Dactylogyrus* sp.

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Research Article

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Abstract

The Monogenean parasite, *Dactylogyrus* sp., is being considered as one of the most dangerous pathogens in freshwater fish with a high infestation in common carp (*Cyprinus carpio*). The treatment of parasites including Monogeneans is an important part of fish health maintenance in the overall cycle of aquaculture. Novel applications of natural plant products to eradicate the parasites have taken high attention in aquaculture since they are reported to have less adverse impacts on the environment and fish in comparison to other chemical treatments. In the present study, *in vitro* antiparasitic effects of the ginger (*Zingiber officinale*) bulb and pomegranate (*Punica granatum*) peel against *Dactylogyrus* sp. were investigated by using *in vitro* tests. Individuals of the parasite were exposed to different concentrations of ginger (10, 50, 100, and 250 mg/ml) and pomegranate peel (50, 100, 250, and 500 mg/ml) for a specified period. *In vitro* cumulative mortality values reached 100% in 5 minutes after exposure to ginger at the concentration of 250 mg/ml in 9 minutes after exposure to 100 mg/ml and 50 mg/ml. Cumulative mortality was 58% in 9 minutes after exposure to ginger at the concentration of 10 mg/ml. In *in vitro* pomegranate tests, cumulative mortality values were assessed 100% in 3 minutes after exposure to pomegranate peel at the concentration of 500 mg/ml and in 6 minutes after exposure to 250 mg/ml and 100 mg/ml. Cumulative mortality was 66% in 6 minutes after exposure to pomegranate peel at the concentration of 50 mg/ml. *In vitro* results revealed that ginger and pomegranate peel solutions had antiparasitic effects on *Dactylogyrus* sp. to some degree, depending on solution concentration and exposure time. The efficacy of the ginger and pomegranate peel solutions against Monogeneans should be confirmed in fish through *in vivo* tests.

Keywords: Antiparasitic activity, *Dactylogyrus* sp., ginger, pomegranate peel, *Cyprinus carpio*.

Zencefil (*Zingiber officinale*) ve Nar Kabuğunun (*Punica granatum*) Monogenean Parazitlerden *Dactylogyrus* sp.'e Karşı Antiparazitik Aktivitesinin *In vitro* Olarak Belirlenmesi

Özet

Monogenean parazitlerden *Dactylogyrus* sp., sazanlarda (*Cyprinus carpio*) yaygın olarak bulunan en tehlikeli patojenlerden biri olarak kabul edilir. Genel olarak, Monogenean parazitlerin tedavisi, su ürünleri yetiştiriciliğinde balık sağlığının muhafazası bakımından önemli bir sorun teşkil eder. Bitkisel ürünlerin paraziter hastalıklarda tedavi edici uygulamaları su ürünleri yetiştiriciliğinde kimyasal tedavilere kıyasla çevre ve balık sağlığı üzerinde daha az olumsuz etkiye göstermesi nedeniyle büyük ilgi görmektedir. Bu çalışmada zencefil (*Zingiber officinale*) ve nar kabuğunun (*Punica granatum*) *Dactylogyrus* sp.'e karşı antiparaziter etkileri incelenmiştir. *Dactylogyrus* sp. parazitleri, farklı sürelerde, farklı konsantrasyonlarda zencefil (10, 50, 100 ve 250 mg/ml) ve nar kabuğu içeren solusyaonlara (50, 100, 250 ve 500 mg/ml) maruz bırakılmıştır. *In vitro* testlerde kümulatif mortalite değerleri, zencefilde 100 mg/ml ve 50 mg/ml'ye maruz bırakıldıktan sonra 9 dakika içinde, 250 mg/ml konsantrasyonuna maruz bırakıldıktan sonra ise 5 dakika içinde % 100'e ulaşmıştır. Kümulatif mortalite oranı, zencefilin 9 dakika süreyle 10 mg/ml konsantrasyonuna maruz bırakılan *Dactylogyrus* sp. için % 58 olarak bulunmuştur. *In vitro* nar kabuğu testlerinde, kümulatif mortalite değerleri 500 mg /ml konsantrasyonuna maruz kaldıktan sonra 3 dakika içinde ve 250 mg/ml ve 100 mg/ml'ye maruz bırakıldıktan 6 dakika içinde %100'e ulaşmıştır. Nar kabuğu solusyonuna 6 dakika süreyle 50 mg/ml konsantrasyonda maruz bırakılan *Dactylogyrus* sp.de kümulatif mortalite oranı % 66 olarak saptanmıştır. *In vitro* testlerle elde edilen sonuçlar, zencefil ve nar kabuğu çözeltilerinin *Dactylogyrus* sp.'e karşı antiparazitik etkisinin zamana ve konsantrasyona bağlı olduğunu göstermiştir. Ancak, balıklarda zencefil ve nar kabuğu çözeltilerinin Monogenean parazitlere karşı antiparazitik etkisi *in vivo* testlerle desteklenmelidir.

Anahtar Kelimeler: Antiparazitik aktivite, *Dactylogyrus* sp., zencefil, nar kabuğu, *Cyprinus carpio*.

INTRODUCTION

Dactylogyrus with more than 900 species is one of the most dangerous sources of parasitic infections of freshwater fish in aquaculture in the world which can cause high morbidity and mortality in many species of freshwater fish. The parasite, *Dactylogyrus* sp., mainly exists on the gills (Jain, 1959) and inhabits the host causing gill hyperplasia, swelling, reduction in surface area for respiration, and excess mucus production, the latter potentially affording the parasite some protection from chemical treatments. There is extensive literature on the increase of occurrence and severeness of the gill *Dactylogyrus* infestations of fish in recent years (Topić et al., 2001; Kır and Özan, 2007). Aggressive compounds and conventional antiparasitics against Monogeneans such as *Dactylogyrus* sp. are applied in aquaculture for removing the parasite from the gills. Formalin is one of the most commonly used chemicals although their use has not been recommended because of their severe side effects (Diggles et al., 1993; Pavanelli et al., 2002). Some other used chemicals against Monogeneans are mebendazole, toltrazuril, praziquantel, and chelidonine (Schmahl and Mehlhorn, 1985; Schmahl et al., 1988; Treves-Brown, 1999; Yao et al., 2011). The risks of chemical use for eliminating the parasites include anthelmintic resistance, risk of residue, environmental contamination, and toxicity to hosts. Thus, the undesired effects of these chemicals have highlighted the need for other novel alternative control methods for protecting fish from parasitic pathogens (Goven et al., 1980; Klinger and Floyd, 2002).

The use of medicinal plants to eradicate the parasites has been tested due to their advantages. Park et al. (2011) and Hao et al. (2012) have mentioned that phytochemicals are a realizable alternative to conventional synthetic pesticides or drugs due to their lower environmental toxicity. The use of phytochemicals as anthelmintic drugs for fish health may help to support sustainable and environmentally acceptable treatment applications. There are previous studies reviewing investigations that have examined the antiparasitic effects of plants on fish parasites (Wink, 2012; Ramudu and Dash, 2013; Reverter et al., 2014; Syahidah et al., 2015; Lieke et al., 2019). In recent years, many researchers have focused on the use of herbal plants against parasitic species in aquaculture (Chitmanat et al., 2005; Puk and Guz, 2014; Williams et al., 2016; Trasvina-Moreno et al., 2017; Yildiz et al., 2019). The various parts of ginger and pomegranate are considered as medicinal plants that can be applied to tackle aquaculture diseases. El-Sayed and El-Saka (2015) have stated that *Z. officinale* has significant anthelmintic activity against *Toxocara canis, Angiostrongylus cantonensis, Dirofilaria immitis, Hymenolepis nana, Schistosoma mansoni, Anisakis simplex* both *in vitro* and *in vivo*, and antiprotozoal activity against *Giardia lamblia, Blastocystis, Trypanosoma brucei* and *Toxoplasma gondii* species. Efficacy of ginger solution against different parasites in fish was studied using both oral and bath treatments (Abo-Esa, 2008; Khalil and Houseiny, 2013; Levy et al., 2015; Fu et al., 2019). In comparison, less literature is observed on the use of pomegranate solution against parasitic infections in fish. To our knowledge, the antiparasitic activity of ginger and pomegranate solutions on the eradication of *Dactylogyrus* sp. was not investigated hitherto. Therefore, in this study, we tested *in vitro* exposure of *Dactylogyrus* sp. to ginger and pomegranate solutions to evaluate their antiparasitic potential.

MATERIALS and METHODS

Fish and parasites

Carp (*Cyprinus carpio*) were obtained from the aquaponic system (co-production of carp and mint (*Mentha* spp)) in Ankara University, Department of Fisheries and Aquaculture. Scrapings from heavily parasitized fish gills were examined under the microscope. The fish length and weight were 11.66 ± 1.15 cm and weight 24.33 ± 4.93 g, respectively. The fish stocking density in fiberglass fish tanks (80x60x50 cm) was 35 kg/m^3. The water in the tanks was at temperature 20-22 °C, dissolved oxygen 5.50- 5.97 mg/L, and pH 6.97-7. Fish were fed with the commercial rainbow trout feed with 45% raw protein at 2% total body weight ratio.

The parasites on the gills were identified as *Dactylogyrus* (Malmberg, 1970; Bruno et al., 2006). Parasite samples on the slides were counted under the microscope for *in vitro* parasite survival tests.

Fish management and experimental protocols (reference number of 2019-7-72) were approved by the Ankara University, Ethics Committee. No fish were killed during the experiments.

Solution preparation

The ginger (*Z. officinale*) and pomegranate (*P. granatum*) were obtained from the local market, Ankara, Turkey. Ginger or pomegranate peel was well crushed and the stock solution of each was prepared by diluting 10 gr of each in 20 ml distilled water. The stock solution was diluted with distilled water to adjust the necessary concentration. The tested ginger concentrations were 10, 50, 100 & 250 mg/ml and pomegranate peel concentrations were 50, 100, 250 & 500 mg/ml.

***In vitro* assay**

During the *in vitro* tests, ginger or pomegranate peel solution was tested by directly pouring on the alive parasite on the slides. The movement and contraction of parasites were continuously observed under the microscope. Ginger solution concentrations of 10, 50, 100 & 250 mg/ml and pomegranate peel solution concentrations of 50, 100, 250 & 500 mg/ml were tested. The parasite behavior was observed and recorded for 10 minutes. Three replicates, each containing 4 parasites, were used per each concentration (Hutson et al., 2018).

Statistics

Statistical analysis was done by using one-way ANOVA. Differences were considered significant at $p < 0.05$.

RESULTS

***In vitro* parasite survival**

In vitro survival of *Dactylogyrus* sp. varied by concentrations of ginger or pomegranate peel solutions and exposure time (F (critical value 2.90) = 7.60) for ginger experiment and (F (critical value 2.68) = 6.72) for pomegranate experiment ($p < 0.05$). The cumulative mortality values of *Dactylogyrus* sp. exposed to ginger and pomegranate peel solutions were shown in Figure 1 and Figure 2, respectively.

Antiparasitic effects of ginger solutions on *Dactylogyrus* sp. were concentration and exposure time-dependent. Cumulative mortality values reached 100% in 5 minutes after exposure to ginger solution of 250 mg/ml, 100% in 9 minutes after exposure to 100 mg/ml and 50 mg/ml ginger solutions. After exposure to a ginger solution of 10 mg/ml concentration for 9 minutes, cumulative mortality was assessed by 58%.

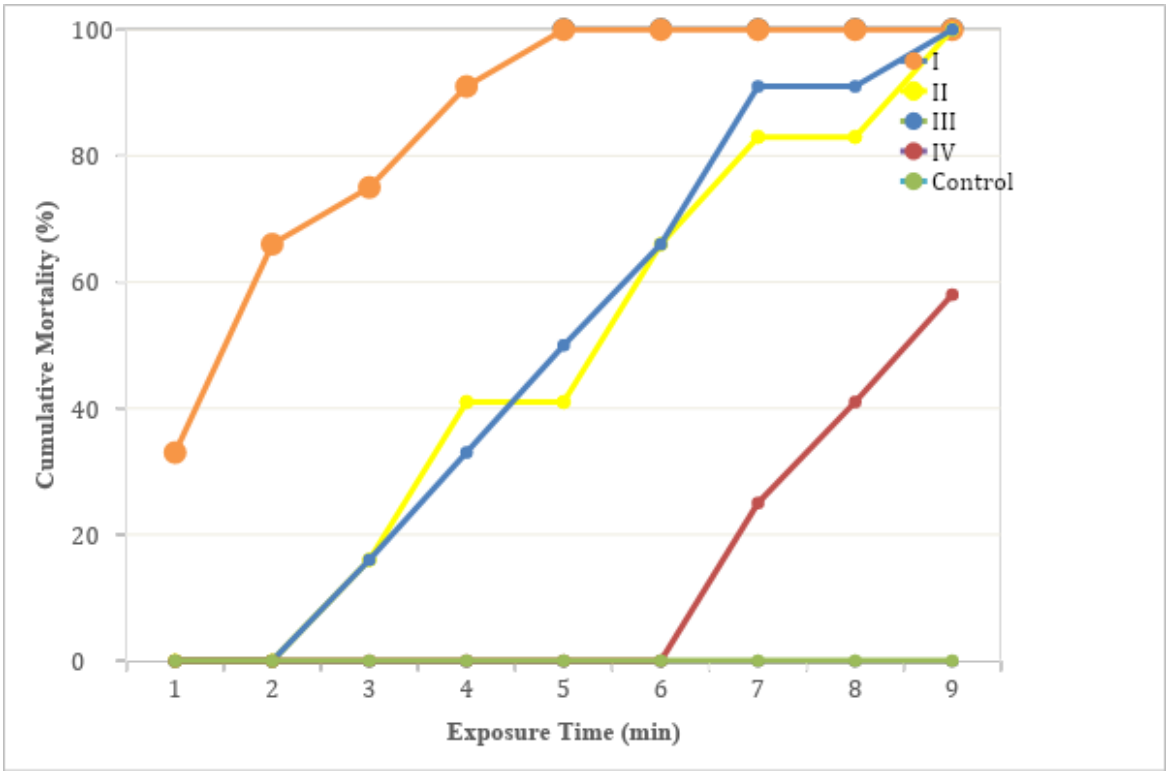


Figure 1. Cumulative mortality values of *Dactylogyrus* sp exposed to ginger (*Z. officinale*) solution at concentrations (mg/mL) of I-250; II-100; III-50; IV-10). Control: no solution exposure.

Antiparasitic effects of pomegranate peel solutions on *Dactylogyrus* sp. were also concentration and exposure time-dependent (Figure 2). Cumulative mortality values reached 100% in 3 minutes after exposure to pomegranate peel solution of 500 mg/ml, 100% in 6 minutes after exposure to 250 mg/ml, and 100 mg/ml pomegranate peel solutions. Cumulative mortality was 66% in 6 minutes after exposure to pomegranate peel at the concentration of 50 mg/ml.

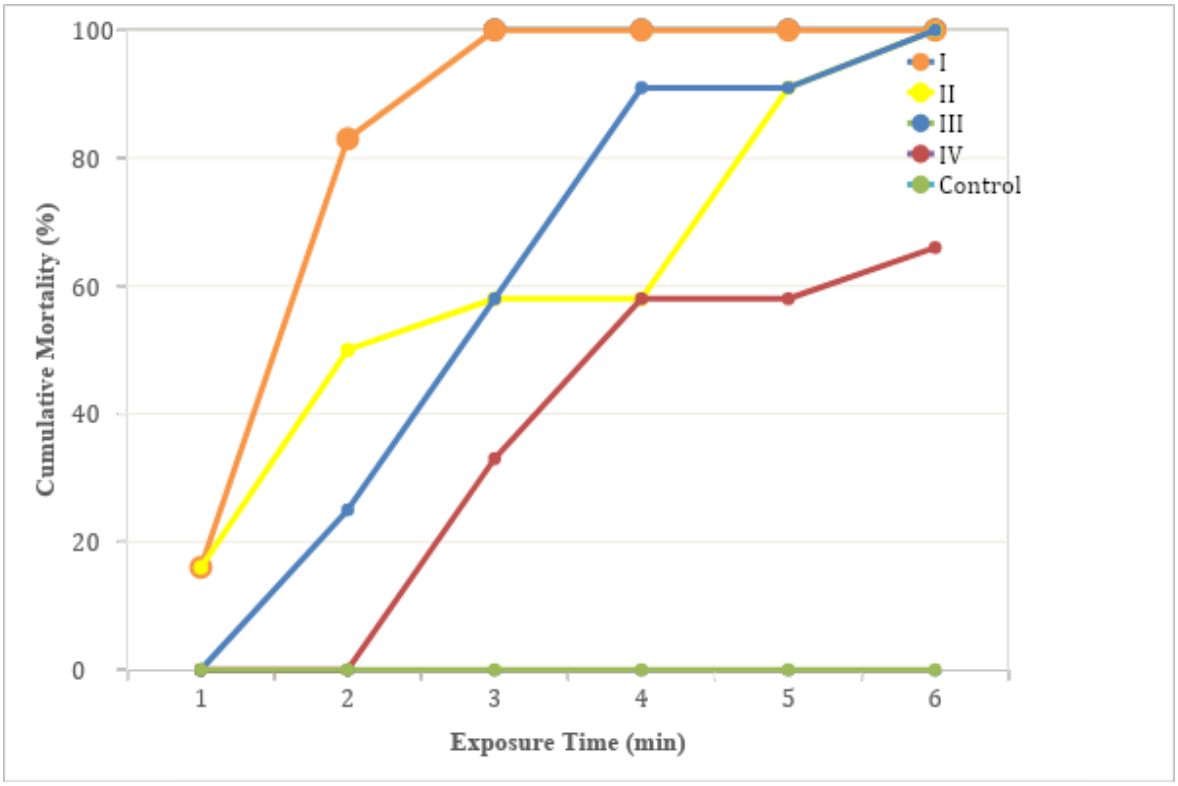
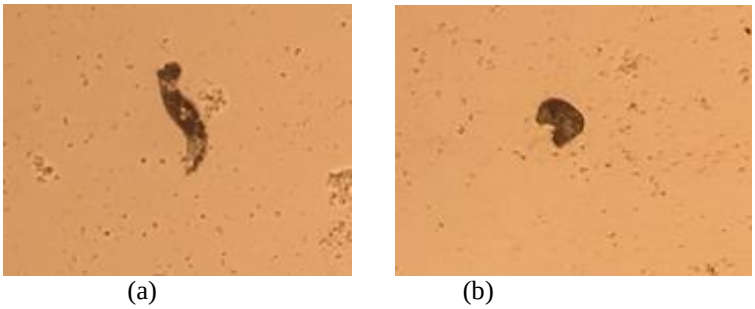


Figure 2. Cumulative mortality of *Dactylogyrus* sp. exposed to pomegranate (*P. granatum*) solution at concentrations (mg/ml) of I-500, II-250, III-100, IV-50. Control: no solution exposure.

The contact of *Dactylogyrus* sp. with the solutions of ginger or pomegranate peel resulted in these in sequence: 1) increase in mobility of the parasite 2) stretching of the parasite 3) contraction of the parasite and 4) shrinking to the smallest size before death (Figure 1).



(a) (b)

Figure 3. Parasite behavior after contact with the ginger (*Z. officinale*) (a) *Dactylogyrus* sp. before exposure (alive); (b) Contraction of *Dactylogyrus* sp. after exposure to ginger solutions

DISCUSSION

Parasitic fish diseases constitute an important problem in aquaculture. It is noted that the sustainability of the aquaculture industry may be compromised by parasitic fish diseases (Soler-Jiménez et al., 2017; Soares et al., 2017a, b). With the development of aquaculture, the number of fish infected by Monogenean parasites is being increased (Topić et al., 2001; Kır and Özan, 2007). Currently, parasitic diseases observed in aquaculture production are generally treated with chemicals such as formalin, acetic acid, potassium permanganate, praziquantel, etc. However, the use of such

substances can lead to problems such as the development of resistant organisms, the accumulation of chemical residues, and damage to the environment and eventually to human beings (Ling et al., 2015; Hashimoto et al., 2016; Soares et al., 2016; Soares et al., 2017a, b). Therefore, the use of chemicals to control fish diseases has been limited in many countries (Ling et al., 2015). Furthermore, it seems that current antiparasitic treatments can be costly. Seeking environmentally friendly, cost-effective, and effective medicines have brought up the use of plant-based substances in fish diseases (Yavuzcan Yildiz and Bekcan, 2020).

In our study, *in vitro* anthelmintic activity of ginger (*Z. officinale*) bulb and pomegranate (*P.granatum*) peel against the Monogenean parasite, *Dactylogyrus* sp., recovered from common carp (*Cyprinus carpio*) were examined. The efficiency of ginger against some Monogeneans has been studied, however, its potential use against *Dactylogyrus* sp. has not been studied before. In the case of pomegranate peel, to our knowledge, no research has been done on its efficiency against Monogenean infections in fish. Our study provides new knowledge on the lethal effects of pomegranate peel on *Dactylogyrus* sp.

During the *in vitro* tests, ginger and pomegranate peel were observed to have a dose-dependent antiparasitic effect against *Dactylogyrus* sp. The time and concentration-dependent activity of ginger against the *Dactylogyrus* sp. (Monogenea) were similar to previous researches on ginger with different parasite species by Trasvina Moreno et al. (2017) and Levy et al. (2015). In our tests with ginger or pomegranate peel, the time to death of *Dactylogyrus* sp. *were* from 1 min to 9 min. In a similar study of Levy et al. (2015) survival period of *Gyrodactylus* sp. (Monogenea) ranged between 1 min and 90 min following exposure to 200, 150, 100, and 75 ppt ginger concentrations. The differences in survival time can be attributed to the differences in the resistance of parasite species against the various antiparasitic compounds. There is no previous research on the anthelmintic effects of ginger on *Dactylogyrus* sp, nevertheless, some other plant extracts have been screened to detect their potential use against *Dactylogyrus* sp. *In vitro* tests conducted by Zoral et al. (2017) using *Rosmarinus officinalis* against *Dactylogyrus minutus* (Monogenea) recovered from *Cyprinus carpio* showed that the survival time ranged from 1 min to 71 min after exposure to the concentrations of 200, 150, and 100 g/l. The findings on the antiparasitic activity of ginger against *Quadriacanthus* sp. (Monogenea) recovered from *Clarias gariepinus (*Khalil and El-Hosseiny 2013) and *Neobenedenia* sp., (Monogenean) (Trasvina Moreno et al. 2017) agree with our results. In the study of Trasvina Moreno et al. (2017), the antiparasitic potential of water-ethanol extracts of garlic *(Allium sativum),* ginger *(Zingiber officinale),* basil *(Ocimum basilicum),* bitter chaparro *(Castela tortuosa),* onion *(Allium cepa),* and papaya *(Carica papaya)* against *Neobenedenia* sp. parasites were examined, reporting that ginger solution had the most toxic effect on the parasites among the all herbal solutions tested. Thus, it is revealed that the antiparasitic potential of ginger against *Dactylogyrus is apparent.*

The peel and seeds of pomegranate (*P. granatum*) have various therapeutic applications such as antibacterial, antifungal, antioxidant, antitumor, antiviral, antimalarial, and antimutagenic effects (Yones et al. 2016). Research has not been found in the literature on pomegranate peel efficiency against Monogenean infections in fish. However, the antiparasitic activity of pomegranate extracts to *Schistosoma mansoni* (Trematoda) has been studied by Fahmy et al. (2009). In their study, the mortality value of the adult worms was observed to reach 100% after 24 h exposure for all tested leaves and peels methanol extract at the concentrations of 100 µg/ml, 300 µg/ml and 500 µg/ml. At the highest concentration of 500 µg/ml, 100% mortality occurred within 10-12 hours. The efficiency depended on the duration of exposure and the concentration, which is in agreement with our test results. The dose-dependent antiparasitic activity of pomegranate extracts against *S.mansoni* was shown by the study of Yones et al. (2016) as well. Abdel-Hafezz (2016) researched rats infected by *Blastocystis* spp. (Protozoan) and the feeding trials with *P. granatum* showed a decrease in the intensity of parasites. In line with our study, they also stated that pomegranate peel can be used as an alternative antiparasitic.

In terms of parasite behavior after contact with the test solutions, typically, *Dactylogyrus* sp. was observed to increase mobility at first, then stretching, contraction, shrinking, and finally stop moving Previous studies on the antiparasitic effect of ginger on different Monogeneans did not indicate an increase in mobility after the parasite contact with the ginger solution. Unlike our observations, it was recorded that relaxation of parasite at first, then contraction, slowing down, and eventually lack of motion of parasite before death (Khalil and El-Hosseiny 2013; Levy et al., 2015; Trasvina Moreno et

al., 2017). The research by Yones et al. (2016) on Trematoda indicated that applying pomegranate peel solution resulted in the light contractions as soon as contact with the related test solution, then motility reduction and paralysis before parasites death, which are similar to the observations in our study concerning parasite response to test solutions. Besides in the study of Yones et al. (2016), the pomegranate was stated as a promising candidate as a new antiparasitic agent.

CONCLUSION

Antiparasitic effects of ginger (*Z. officinale*) bulb and pomegranate (*P. granatum*) peel solutions on *Dactylogyrus* sp. recovered from common carp (*Cyprinus carpio*) were tested in this study. These two plant solutions showed dose and concentration-dependent antiparasitic activity and displayed to have a promising alternative treatment potential against Monogenean infections of fish in aquaculture. However, further evaluations should be done to understand the active constituents available in the solutions eradicating parasites and the mechanism of actions associated with their efficiency in fish. The ginger and pomegranate peel solutions need to be evaluated for their toxicity and side effects on fish, showing the requirement of detailed *in vivo* studies.

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Food Composition of the Whiting, *Merlangius merlangus* in the South-eastern Coast of the Black Sea

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Research Article

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Abstract

In this study, the food composition of whiting, *Merlangius merlangus*, from the South-eastern coasts of the Black Sea was seasonally investigated. With this aim, stomach contents of 762 specimens, the total length of 12.7-18.6 cm, and the body weight of 17-56.5 g, collected between September 2016 and August 2017 were analyzed. It was determined that 75.7% (577 stomachs) of the stomachs examined were empty. The percentage of empty stomachs didn't show seasonal variation with maximal occurrence in autumn (76.9%) and minimal in winter (74.5%). The prey items identified macroscopically in the stomachs were horse mackerel, *Trachurus mediterraneus*, anchovy, *Engraulis encrasicolus*, whiting, *Merlangius merlangus*, sprat, *Sprattus sprattus*, goby, *Gobius* sp. from fish species and Gastrapod from Mollusca. Horse mackerel was the most important ingested prey (IRI%=43.2), followed by anchovy (IRI%=32.0) and whiting (IRI%=19.3). In the diet of the whiting, the sprat and the goby were less important than other fish species. The most important food was horse mackerel in the spring and summer seasons, while it was anchovy in the autumn and winter seasons. Cannibalism was the highest (F%=25) in the spring period and followed by summer (F%=23.8), autumn (F%=21.6) and winter (F%=16.2), respectively. It was determined that whiting consumed individuals of their species as food, up to 37.6% of their length and 11.2% of their weight.

Keywords: South-eastern Black Sea, Whiting, *Merlangius merlangus*, Food composition, Cannibalism.

Karadeniz'in Güney-doğu Kıyılarında Mezgit Balığının, *Merlangius merlangus* Besin Kompozisyonu

Özet

Bu çalışmada, Karadeniz'in güney-doğu kıyılarındaki mezgit, *Merlangius merlangus* balıklarının besinleri ve besleme alışkanlıkları mevsimsel olarak araştırılmıştır. Bu amaçla, Eylül 2016-Ağustos 2017 arasında avlanan ve boyları 12,7-18,6 cm, ağırlıkları 17-56,5 g arasında değişen 762 bireyin mide içeriği analiz edilmiştir. İncelenen midelerin %75,7'sinin (577 mide) boş olduğu belirlenmiştir. En fazla boş mideye sonbaharda (%76,9), en az boş mideye ise kış (%74,5) mevsiminde rastlanmıştır. Mezgit balığı midelerinde makroskopik olarak yapılan incelemelerde besin olarak balık türlerinden istavrit, *Trachurus mediterraneus*, hamsi, *Engraulis encrasicolus*, mezgit, *Merlangius merlangus*, çaça, *Sprattus sprattus* ve kaya balığı, *Gobius* sp. ile mollusklardan Gastrapoda tespit edilmiştir. En önemli besinin istavrit (%IRI=43,2) olduğu, onu hamsi (%IRI=32,0) ve kendi türünün (mezgit) (%IRI=19,3) izlediği saptanmıştır. Mezgit balığı diyetinde çaça ve kaya balığının önemi diğer balık türlerine göre daha düşük bulunmuştur. İlkbahar ve yaz mevsimlerinde en önemli besin istavrit iken, sonbahar ve kış mevsimlerinde hamsidir. Kanibalizm ilkbaharda en yüksek (%F=25) iken, bunu sırasıyla yaz (%F=23,8), sonbahar (%F=21,6) ve kış (%F=16,2) mevsimleri izlemiştir. Mezgit balıklarının kendi türüne ait boylarının %37,6'sına, ağırlıklarının %11,2'sine kadar olan bireyleri besin olarak tükettikleri belirlenmiştir.

Anahtar kelimeler: Güney-doğu Karadeniz, Mezgit, *Merlangius merlangus*, Besin kompozisyonu, Kanibalizm

INTRODUCTION

Studies on feeding habits of marine fish, such as predator-prey relationships are useful to assess the role of marine fish in the ecosystem (Bachok et al., 2004). However, data on food composition are useful for developing trophic models as a tool for understanding the complexity of coastal ecosystems (Lopez-Peralta and Arcila, 2002; Stergiou and Karpouzi, 2002). Diet analysis is also necessary for exploring the trophic overlap within and between species and determining the intensity of the inter-and intraspecific interactions in marine fish communities (Morte et al., 2001)

The whiting is distributed from Norway and Iceland to the Mediterranean and into the Adriatic, the Aegean, the Azov, and the Black Seas (Milić and Kraljević, 2011). This species, which is found intensely on the shores of the Atlantic Ocean, is very rare in the northern coasts of the Mediterranean and more intensely in the northern coasts of the Black Sea. Whiting, *Merlangius merlangus* is the most important fish species for small-scale fisheries on the Turkish coast of the Black Sea.

There are few studies on the diet of whiting in the Black Sea (İşmen, 1995; Banaru and Harmelin-Vivien, 2009; Samsun et al., 2011; Mazlum and Bilgin, 2014; Şensurat-Genç et al., 2019), although it is one of the most important target species. Spatial and temporal monitoring of changes in the trophic levels of the fishes are closely related to their vitality or sustainability. Trophic relationships can be disrupted by pollution, anthropogenic effects, and interspecific competition (Şensurat-Genç et al., 2019). Şensurat-Genç et al. (2019) stated that whiting mainly consumes small fish and crustaceans in the Black Sea. However, in recent years fishing pressure on small-bodied fish species such as red mullet, *Mullus barbatus*, horse mackerel, *Trachurus mediterraneus* and sprat, *Sprattus sprattus* has increased. For this reason, the populations of these fish species, which are the important foods of whiting, have decreased. Cannibalism increases in the population of whiting which feeds almost only on small fish, if there is not enough food in the environment (Bromley et al., 1997). It is inevitable to be affect the whiting diet from this decrease. Therefore, it is beneficial to investigate the biological and ecological characteristics of fish species that may occur due to environmental factors. This study aimed to investigate the food variety and cannibalistic behavior characteristics of the whiting living in the South-eastern Black Sea.

MATERIALS AND METHODS

Sampling

Whiting samples were collected monthly from gillnet fisheries in Ordu coasts and from gillnet fisheries and bottom trawl fisheries in Samsun coasts, between September 2016 and August 2017 (Figure 1).

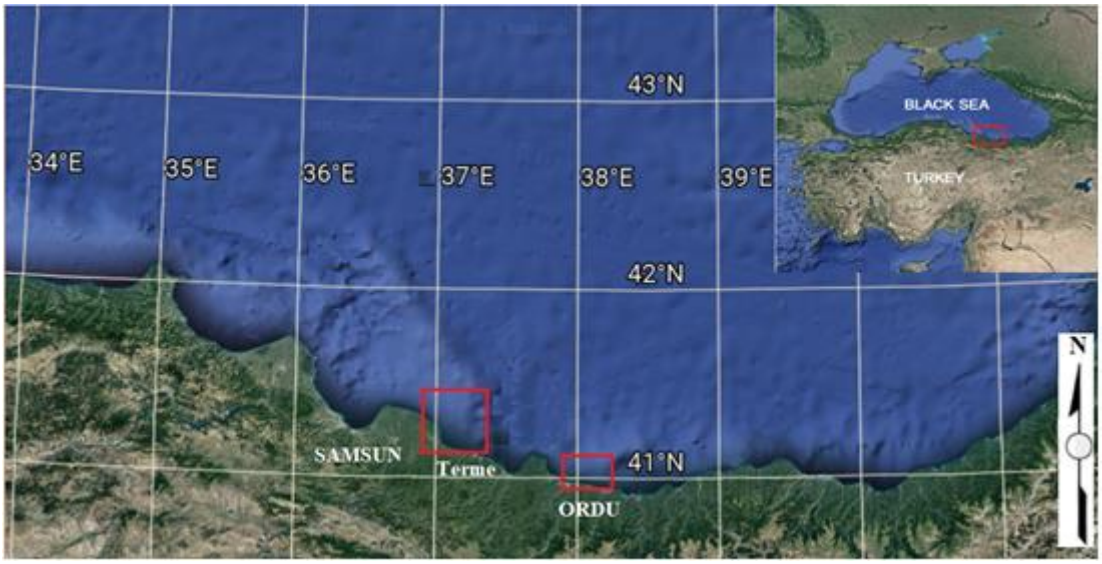

Figure 1. Study areas

After the fishing operation, the whiting samples were randomly taken immediately after the gillnet boats and bottom trawl vessels entered fishing ports. Total length to the nearest cm and body weight to the nearest gram were recorded from fresh fish. Then the stomachs were removed immediately from all fish and preserved in 4% formaldehyde solution for later analysis. In the laboratory, only macroscopic food items in the stomachs were identified to the lowest possible taxonomic level after which they were counted and weighed after removal of surface water using blotting paper (Hyslop, 1980).

Stomach data analysis

Macro-food-containing stomachs were categorized as "full" and not containing "empty". The importance of the different prey types was evaluated calculating the percentage frequency of occurrence (F%) = (Number of stomachs containing prey *i*/total number of non-empty stomachs)* 100, Percentage numerical abundance (N%)=(Number of prey *i*/Total number of prey items in all stomachs)*100 and Percentage gravimetric composition (W%)=(Wet weight of prey *i*/Total weight of all preys)*100 (Hyslop, 1980). The index of relative importance (IRI) of prey type *i* as given by Cortés (1997) is derived as follows: IRI=F%*(W%+N%). Also, the percentage of relative importance index: IRI%=(IRI/ΣIRI) *100 was determined.

Cannibalism

Proportionally relationships between predator and prey whiting in length and weight were determined.

Statistical analysis

Proportional food overlap among between seasons was calculated using Schoener's dietary overlap index (C) (Schoener, 1979): $C_{xy}=1-0.5*\sum|P_{xi}-P_{yi}|$, where P_{xi} and P_{yi} are the proportion of prey *i* (based on IRI%) found in the diet of groups *x* and *y*. This index ranges from 0 (no prey overlap) to 1 (all food items in equal proportions). Schoener's index values above 0.6 are usually considered to indicate significant overlap (Wallace, 1981).

Percentage of relative importance index (IRI%) by weight of each prey category was computed for each individual. IRI% for all prey types was then square root transformed to reduce the importance of the most abundant prey. Bray-Curtis similarity was used to compare the differences among seasons. To test the relationship between lengths of the predator and prey whiting, Pearson correlation analysis was used for parametric data and Spearman correlation analysis was used non-parametric. Statistical analyses were carried out using the PRIMER 6.1.18 statistical package and SPSS programs.

RESULTS

A total of 762 stomach samples (380 samples from Ordu and 382 samples from Samsun) were collected during the study. Of the total number of stomachs examined (n=762), 577 were empty (75.7%). As seen in Table 1, seasonal index of the empty stomachs varied slightly over the yea**r.**

Table 1. Seasonal distributions of full and empty stomachs collected from the south-eastern coast of the Black Sea.

	Full		**Empty**	
Season	N	N%	N	N%
Spring	46	24.2	144	75.8
Summer	36	24.3	112	75.7
Autumn	49	23.1	163	76.9
Winter	54	25.5	158	74.5
Total	185	24.3	577	75.7

A total of 6 prey types (macro-organisms) were found in the stomachs of whiting collected from the south-eastern coast of the Black Sea. The percentage frequency of occurrence (F%), percentage abundance (N%), the percentage by weight (W%), and index of relative importance (IRI%) for prey types of whiting are given in Table 2. According to IRI% values, horse mackerel (43.2%), anchovy (32.0%), and whiting (19.3%) were the main prey groups of whiting in the south-eastern coast of the Black Sea.

Table 2. Food composition of *M. merlangus* in the south-eastern coast of the Black Sea expressed as F% - frequency of occurrence; N% - Numerical abundance; W% - weight percentage; IRI% - index of the relative importance

Prey	N%	F%	W%	IRI	IRI%
Fish species					
T. mediterraneus	32.3	32.3	34.8	2164.4	43.2
E. encrasicolus	29.1	29.1	25.9	1603.4	32.0
M. merlangus	21.3	21.3	24.2	965.9	19.3
S. sprattus	11.0	11.0	10.4	236.2	4.7
Gobius spp.	3.9	3.9	4.4	32.8	0.7
Mollusc					
Gastropod	2.4	2.4	0.4	6.4	0.1

It is understood that whiting consumed mostly horse mackerel in the spring season in the south-eastern coast of the Black Sea (Table 3). The second most important prey type was whiting in this season. The importance of other food types was slight. In the summer season, a food composition similar to the spring season was determined (Figure 2). However, the importance of anchovy and sprat increased in this season. Contrary, the importance of horse mackerel decreased in the summer season. Anchovy was the most important prey type in autumn and winter. This food type was followed by whiting, horse mackerel, and sprat in the autumn season and by horse mackerel, whiting, and sprat in the winter season, respectively. Other types of food had little importance in autumn and winter.

Table 3. Seasonal food composition of *M. merlangus* in the south-eastern coast of the Black Sea expressed as F% - frequency of occurrence; N% - Numerical abundance; W% - weight percentage; IRI% - index of the relative importance.

	Spring					**Summer**				
Prey	N%	F%	W%	IRI	IRI%	N%	F%	W%	IRI	IRI%
Fish species										
T. mediterraneus	53.1	53.1	57.1	5854.2	78.7	38.1	38.1	41.7	3041.4	57.5
E. encrasicolus	6.3	6.3	3.3	59.9	0.8	19.0	19.0	18.1	707.4	13.4
M. merlangus	25.0	25.0	30.1	1376.7	18.5	23.8	23.8	21.4	1077.2	20.4
S. sprattus	6.3	6.3	5.3	72.0	1.0	14.3	14.3	15.7	429.0	8.1
Gobius spp.	6.3	6.3	3.9	63.5	0.9	4.8	4.8	3.0	36.9	0.7
Mollusc										
Gastropod	3.1	3.1	0.3	10.9	0.1	0.0	0.0	0.0	0.0	0.0
	Autumn					**Winter**				
Prey	N%	F%	W%	IRI	IRI%	N%	F%	W%	IRI	IRI%
Fish species										
T. mediterraneus	18.9	18.9	20.4	743.8	13.3	24.3	24.3	21.2	1108.5	20.6
E. encrasicolus	43.2	43.2	35.4	3402.2	60.7	40.5	40.5	43.8	3420.4	63.7
M. merlangus	21.6	21.6	25.9	1028.0	18.3	16.2	16.2	18.2	557.9	10.4
S. sprattus	13.5	13.5	17.6	420.9	7.5	10.8	10.8	7.5	197.6	3.7
Gobius spp.	0.0	0.0	0.0	0.0	0.0	5.4	5.4	8.9	77.5	1.4
Mollusc										
Gastropod	2.7	2.7	0.6	9.0	0.2	2.7	2.7	0.3	8.2	0.2

However, it can be said from Schoener Overlap Index values (C>0.8) that there is a significant similarity between seasons in terms of the food sources of whiting the south-eastern coast of the Black Sea.

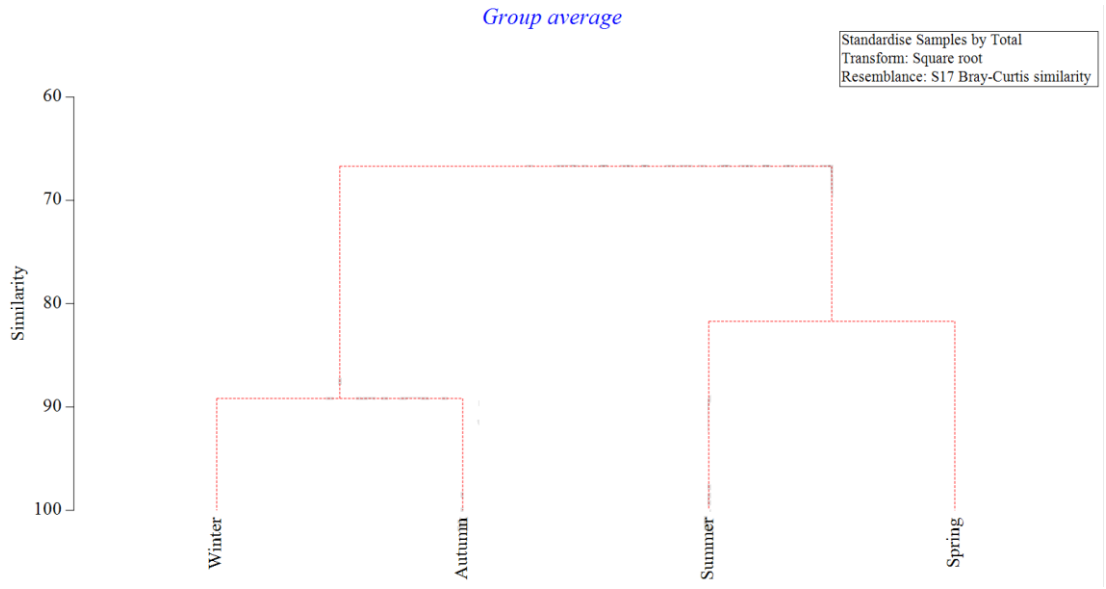

Figure 2. Similarity dendogram based on cluster analysis plot ordination of seasonal food composition collected from the south-eastern coast of the Black Sea.

Cannibalism

During the study, a total of 27 whiting foods were identified in the stomachs of whiting samples collected from Ordu and Samsun coasts. Only one whiting was observed in each stomach. Spearman correlation analysis results showed that there was no significant relationship between the lengths (r=0.072; P=0.720) and the weights (r=0.244; P=0.229) of predator and prey whiting. Cannibalism was the highest (F%=25) in the spring period. Mean length and weight values of predator and prey whiting were given in Table 4 and Figure 3. It was determined that whiting consumed up to 37.6% of their maximum length and up to 11.2% of their maximum weight.

Table 4. The mean, minimum and maximum lengths and weights of predator and prey whiting collected from the south-eastern coast of the Black Sea.

		Predator	**Prey**
	N	27	27
Total length (cm)	Mean	14.9	3.9
	Std. Error of Mean	0.3	0.3
	Minimum	12.7	1.7
	Maximum	18.6	7.0
Weight (g)	Mean	27.5	3.0
	Std. Error of Mean	2.1	0.2
	Minimum	17.0	1.33
	Maximum	56.5	6.32

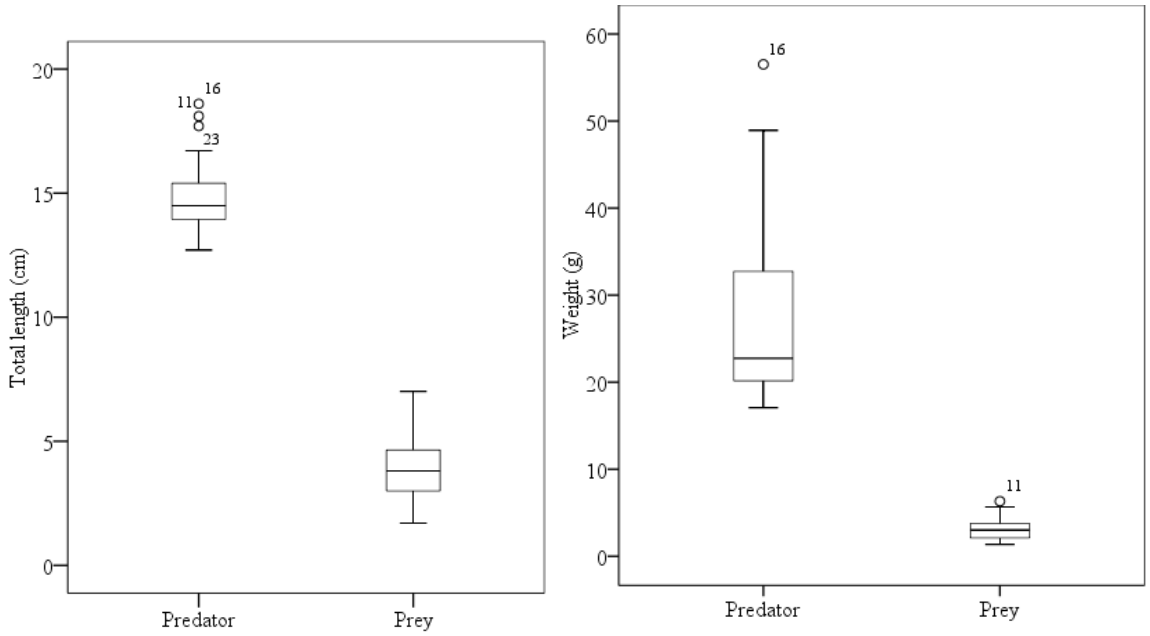

Figure 3. Boxplot graphs for lengths and weights of predator and prey whiting which consumed their own species or consumed by own species the south-eastern coasts of the Black Sea.

DISCUSSION

In terms of macro-organism content, 75.7% of stomachs examined were empty. The proportion of empty stomachs was found as 57% in the middle Black Sea between 2001 and 2003 (Samsun et al., 2011), 23.9% in an area of the south-eastern Black Sea near our sampling sites (Şensurat-Genç et al., 2019), approximately 50% in the North Sea (Hislop et al., 1991), 37.2% in 1991, 40.3% in 1992 and 1993 in the eastern Irish marine ecosystem (Seyhan, 1994). Another study reported that 50% of the stomachs examined were empty in all seasons and approximately 20% were completely full in all seasons in Rize offshore of the South-eastern Black Sea in 2004-2005 (Mazlum ve Bilgin, 2014). In the western Baltic Sea, the rate of empty stomachs was determined as 34% by Ross et al. (2018). According to that study, the rate of empty stomachs was high, possibly because whiting followed a different feeding strategy or it was fed less but larger foods. McDermott and Fives (1995) stated that only 2 of the 40-whiting had empty stomachs in the western Irish Sea. In general, it is understood that the rate of the empty stomach in our study is higher than the rates reported in previous studies conducted in the Black Sea and some other seas. There could be many reasons for this. Probably the most important factor is to consider only macro-organisms in our study. Secondly, there may be a decrease in the small, bodied fish populations that the whiting consumes as food. Our research has revealed that the whiting in the south-eastern coasts of the Black Sea is fed almost exclusively with small individuals of its own species (whiting) and other small-bodied fish species (horse mackerel, anchovy, sprat, and rockfish). Especially the decrease of anchovy in the last 25 years and sprat in the last 10 years (Balık, 2019) has negatively affected the feeding of whiting in the south-eastern Black Sea.

According to İşmen (1995), the whiting in the Black Sea mostly feeds on fish, crustaceans, and polychaetes. However, Samsun et al. (2011) reported in the central Black Sea coast of Turkey that anchovy was the dominant fish species in the diet of this species in the early 2000s. In the study carried out by Mazlum and Bilgin (2014) in the Rize offshore of the South-eastern Black Sea between 2004 and 2005, was reported that this fish species is generally fed with three fish species (anchovy, whiting, and sprat). It is reported by Bradova and Prodanov (2003) that the whiting feeds mainly on sprat but during the winter months, it consumes also anchovy especially along the Anatolian coast of Turkey and the Caucasian coast in front of Georgia. In the north-western Black Sea, the whiting diet was mainly composed of S. sprattus, polychaetes (Melinna palmata and Nereis spp.), shrimps (C. crangon), and amphipods (Ampelisca diadema). Off the Danube Delta of Romania, whiting preyed mainly on polychaetes in spring and autumn seasons, while in the south of the delta, it consumed a high quantity of S. sprattus in spring. Rare prey of whiting included bivalves, cumaceans, and mysids

in both seasons (Banaru and Harmelin-Vivien, 2009). In terms of foods of whiting, it is understood that the results of our study were similar with the results of studies conducted previously in Turkey's Black Sea coast.

The most important difference understood from these comparisons is that the importance of anchovy in the diet of whiting has decreased in recent years. This is an expected result. Because, annual the catch of anchovy in Turkey was 280.000 tons in 2000 and 229.000 tons in 2010, while it decreased to 96.5 tons in 2018 (TOB, 2020). The decrease in the catch indicates that the anchovy population on Turkish coasts of the Black Sea has decreased significantly in recent years.

In studies conducted in habitats other than the Black Sea, slightly different results are reported. For example, Hislop et al. (1991) reported that at least 85% of whiting foods consisted of fish and Crustacea in the North Sea. All of the foods were commercially important fish species (Gadus morhua, Melanogram musaeglefinus, M. merlangus, Trisopterusesmarkii, S. sprattus, Clupea harengus and Ammodyte smarinus). McDermott and Fives (1995) reported that whiting in the western Irish Sea was composed of copepods, fish, decapod and mysids. In the study conducted by Staniland (1995) on the northeastern shores of the Shetland Islands in 1991, it was found that crustaceans were dominant in the diet of small whiting and fish were dominant in the diet of whiting larger than 15 cm. It was reported that the most consumed fish species was sand eels, but large individuals also feed on gadoids and clupeids. From these results, it is understood that the main food of whiting is related to the size of the fish and seasonal abundance of food. With the increase in the size of the whiting, the importance of prey fish increased, while the importance of crustaceans and polychaetes decreased.

Seasonally, the whiting in the south-eastern Black Sea was fed mostly with horse mackerel in spring and summer, and anchovy in autumn and winter. The second most important food was its own species in the spring, summer, and autumn seasons, and the horse mackerel in the winter. The rate of cannibalism was almost equal from spring to autumn. It was lower in winter than in other seasons Samsun et al. (2011) was found that the cannibalism rate was higher in the spring and summer seasons than in the autumn and winter seasons on Turkish middle Black Sea coast. In the study conducted by Mazlum and Bilgin (2014) in Rize coasts of the south-eastern Black Sea, it is reported that sprat fish constitute the most important food sources in spring, whiting in summer, and anchovy in the autumn and winter seasons. According to Bromley et al. (1997), they nearly feed only from fish, the whiting has a multistage ovulation period during the year, the fry that comes towards the ovulation, is the nutrition sources for older fry. Food requirements of whiting vary significantly depending on the water temperature. According to Özdemir (1983), whiting needs less food in winter. Larger et al. (1988) states that during the periods when the water temperature rises, fish receive more food due to the increase in digestive enzymes.

The results of this study showed that the feeding regime of whiting depends on the abundance of other foods in the same environment. With the decrease of other foods, the tendency towards cannibalism increases. According to Hislop et al. (1991), whiting is one of the most important carnivorous fish species in the North Sea. Ross et al. (2018) report that this species is the main piscivorous species in the western Baltic Sea. As stated by many authors above, the whiting is a carnivorous species. While cannibalism is frequently a response to food or density, other factors may also be important; in many species, several such factors are known to be involved (Fox, 1975). Starvation may increase cannibalistic tendencies, but it is not essential for initiating this behavior. Many animals will cannibalize as soon as all other food items are removed, but they may also respond simply to a reduction in the relative availability of alternatives (Fox, 1975). Our study results agree with this information.

Also, it was determined that whiting consumed individuals of their own species as food, up to 37.6% of their length and 11.2% of their weight. As a result, it is understood that cannibalism among whiting will increase if the small-bodied fish species decreases further.

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Distributions and Length-Weight Relationships of Some Lessepsian Cardinalfishes (Apogonid species) in the Northeastern Mediterranean (Antalya, Turkey)

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Research Article

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Abstract

The distributions and length-weight relationships of three cardinalfishes, namely Broadbanded cardinalfish *Ostorhinchus fasciatus* (White, 1790), Spotfin cardinalfish *Jaydia queketti* (Gilchrist, 1903), and Smith's cardinalfish *Jaydia smithi* Kotthaus, 1970 caught as discards in bottom trawl fisheries in the Northeastern Mediterranean (Gulf of Antalya and Finike Bay) were investigated. A total of 607 specimens were sampled with 108 trawl hauls carried out in 3 stations at a depth of 20-200 m seasonally. *Ostorhinchus fasciatus* was the most abundant species with 552 specimens, contributing 90.9% of the total sampling. Total amounts of *Jaydia smithi* and *Jaydia queketti* species had fewer sample numbers like 31 (5.1%) and 24 (4%), respectively. All three species were sampled the most in the summer season. The length-weight relationships were significant ($p > 0.001$), with values of r^2 ranging from 0.90 to 0.94. The b values ranged from 3.09 to 3.20 and positive allometric growth was observed in three apogonid species.

Keywords: trawl fishery, length-weight relationships, Apogonidae, cardinalfishes.

Bazı Lesepsiyen Kardinal Balıklarının (Apogonid türler) Kuzeydoğu Akdeniz'deki (Antalya, Türkiye) Dağılımları ve Boy-Ağırlık İlişkileri

Özet

Kuzeydoğu Akdeniz (Antalya Körfezi ve Finike Körfezi) trol balıkçılığında ıskarta türler olarak yakalanan Kardinal balıklarından *Ostorhinchus fasciatus* (White, 1790), *Jaydia queketti* (Gilchrist, 1903) ve *Jaydia smithi* Kotthaus, 1970 dağılımı ve boy-ağırlık ilişkileri araştırılmıştır. Mevsimsel olarak üç istasyonda, 20-200 m derinlik aralığında gerçekleştirilen 108 trol çekimi sonunda toplam 607 birey örneklenmiştir. Toplam apogonid örneklemesinin %90,9'unu oluşturan *Ostorhinchus fasciatus*, 552 birey ile en çok yakalanan tür olmuştur. Daha az yakalanan *Jaydia smithi* ve *Jaydia queketti* türlerinin örnek sayıları sırasıyla 31 (%5,1) ve 24 (%4) olarak kaydedilmiştir. Her üç tür de en fazla yaz mevsiminde örneklenmiştir. Tanımlayıcılık katsayısı (r^2) değeri 0,90 ile 0,94 arasında değişmiş olup, boy-ağırlık ilişkileri istatistiki açıdan önemli bulunmuştur ($p > 0,001$). Hesaplanan b değerleri 3,09 ile 3,20 arasında gözlenmiştir. Her üç apogonid türünde de pozitif allometrik büyüme görülmüştür.

Anahtar kelimeler: trol balıkçılığı, boy-ağırlık ilişkisi, Apogonidae, kardinal balıkları.

INTRODUCTION

Apogonidae family, commonly known as cardinalfishes, constitute the greatest number of alien species after Tetradontids in Turkish waters (Turan et al., 2016). Five apogonid species except for the native species, *Apogon imberbis* (Linnaeus, 1758), have been reported in Turkish marine waters to date. These are; *Apogonichthyoides pharaonis* (Bellotti, 1874) reported by Gucu et al. (1994); Oz et al. (2007); De Moe et al. (2018), *Jaydia queketti* (Gilchrist, 1903) reported by Eryilmaz and Dalyan (2006); Erguden et al. (2009); Gokoglu et al. (2011); Filiz et al. (2012); Akyol and Unal (2015); Yapici et al. (2015); De Moe et al. (2018), *Jaydia smithi* Kotthaus, 1970 reported by Goren et al. (2009a); Gokoglu et al. (2010); Erguden et al. (2015); De Moe et al. (2018) *Ostorhinchus fasciatus* (White, 1790) reported by Akamca et al. (2010); Turan et al. (2010); Gokoglu et al. (2012); Erguden et

al. (2015); De Moe et al. 2018) and *Cheilodipterus novemstriatus* (Rüppell, 1838) reported by Irmak and Engin (2015); Turan et al. (2015).

In this study, the distribution of three apogonid species (*Ostorhinchus fasciatus*, *Jaydia queketti* and *Jaydia smithi*) in the Gulf of Antalya and Finike Bay were investigated considering seasons and depths. Also, the length-weight relationships of these three species, for the first time in *O. fasciatus* and *J. queketti* in the northeastern Mediterranean, were determined and compared with other studies performed in the Mediterranean Sea.

MATERIALS and METHODS

This study was conducted in the Gulf of Antalya and Finike Bay located in the Northeastern Mediterranean Sea (Figure 1). Totally 108 trawl samples were carried out during the daytime in the three stations (Table 1). Trawling had been banned since 2004 in Station A which was located in the most eastern part of the study. Station B was the main commercial trawl area in the Gulf of Antalya and Station C, located in the most western part of the study, was another commercial trawl area in the region.

The trawl hauls were carried out varying from 20 to 200 m at three depth contours (20-50 m, 51-100 m, and 101-200 m) with the research vessel "R/V Akdeniz Su" from December 2011 to November 2012. The towing duration was about one hour for each haul. The average towing speed was 2.5 knots, and the cod-end mesh size was 22 mm (knot to knot). The apogonid species were identified (Gon and Randall, 2003) on each haul and the total length (TL), body weight (W) of each individual measured nearest 0.01 g and 0.1 cm, regardless of sex.

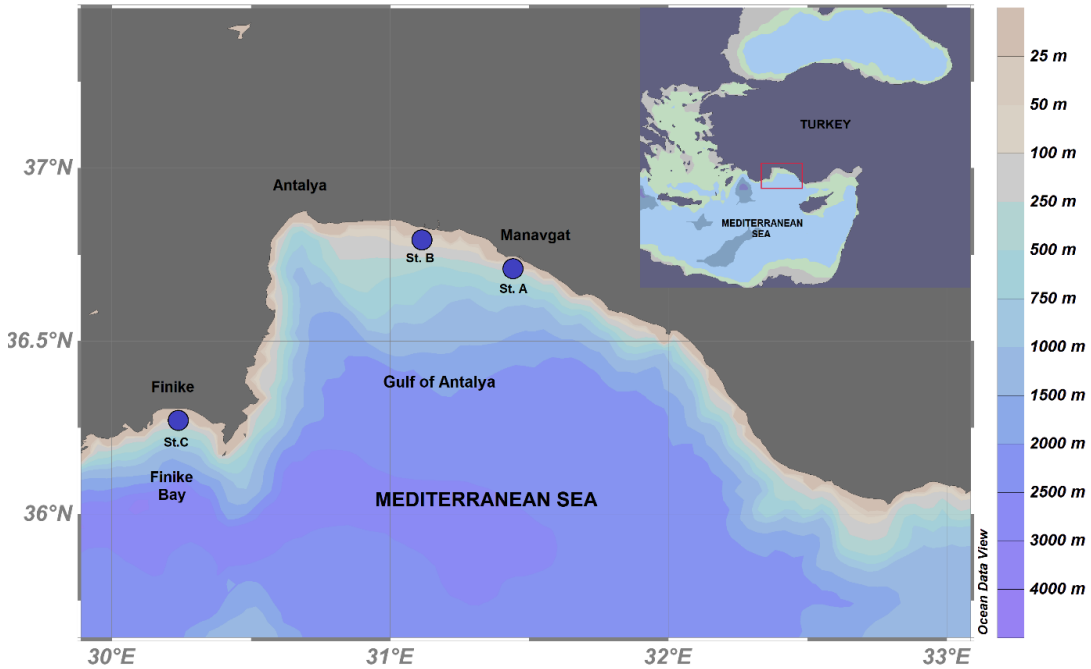

Figure 1. Map of the study area

Table 1. Tow coordinates of the three stations

Stations	No. of hauls	Depth range (m)	Tow co-ordinates
St. A	36	20-200	36°44'N 31°27'E - 36°43'N 31°24'E
St. B	36	20-200	36°47'N 31°07'E - 36°45'N 31°09'E
St. C	36	20-200	36°16'N 30°19'E - 36°15'N 30°13'E

The length-weight relationships were estimated with allometric model $W=aL^b$, where W is the weight (g), and L is the total length (cm) (Froese, 2006). The frequency of occurrence was computed as percentage of positive trawl hauls (presence of at least 1 specimen). Statistical differences between the seasonal catching amounts of the species were tested by one-way ANOVA ($\alpha = 0.05$) using SPSS v. 23.0.

RESULTS and DISCUSSION

A total of 108 trawl hauls were performed at a depth of 20-200 m. *Ostorhinchus fasciatus* was the most abundant species in the catches with 552 individuals, contributing 90.9 % of the total cardinalfish sampling. Similarly, in the study conducted by Erguden et al. (2015) in the eastern Mediterranean between September 2011 and July 2012, *O. fasciatus* was the most caught Apogonid species with 66%. The distribution of apogonids and biology of *O. fasciatus* were investigated in Taiwan and reported that *O. fasciatus* (86.6%) was the most dominant species at each station of the study (Wu, 2009).

Ostorhinchus fasciatus, first recorded in the Gulf of Antalya in December 2011 (Gokoglu et al., 2012), was caught at all the stations during each season in this study. The species was most sampled with 313 (56.7%) individuals in the summer season. This value was followed by 193 (35.0%) in autumn, 30 (5.4%) in spring and 16 (2.9%) in winter sampling. The total numbers of *J. smithi* and *J. queketti* species were 31 (5.1 %) and 24 (4.0 %), respectively (Figure 2).

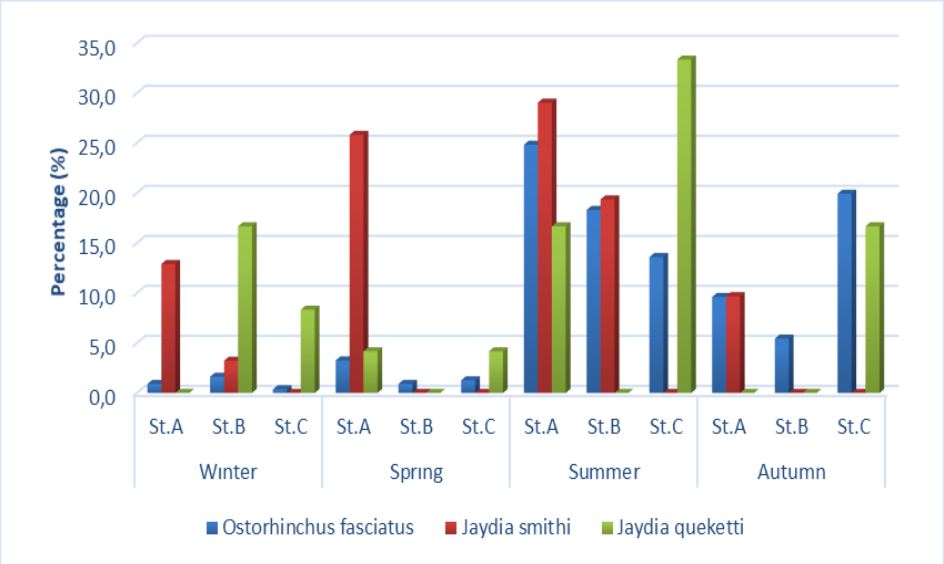

Figure 2. Percentage distribution of *Ostorhinchus fasciatus*, *Jaydia queketti* and *Jaydia smithi* according to stations and seasons.

There was no statistical difference between the catching amounts of *J. smithi* and *J. queketti* species in all stations seasonally. However, the difference between the number of *O. fasciatus* caught in different seasons was statistically significant ($p<0.05$).

Ostorhinchus fasciatus, has been reported to have 2-128 m distribution area (Fraser, 2005) and is generally present at a depth range of 15-40 m in the Mediterranean Sea (Allen and Erdmann, 2012; Goren et al., 2009b). The species was observed at depth of 30-50 m on the Mediterranean coasts of Turkey (Akamca et al., 2010; Turan et al., 2010; Gokoglu et al., 2012). Also, Bilecenoglu et al. (2013) reported a single individual in the Aegean Sea.

Ostorhinchus fasciatus were caught only at a depth between 20 and 50 m in this study. The frequency of occurrence for St. A, St. B and St. C were estimated as 100%, 75%, and 75% respectively.

The minimum size of mature female *O. fasciatus* was 4.64 cm TL, and the size at maturity (L50) was estimated 7.54 cm TL by Wu (2009) and the maximum length for this species was reported as 12.6 cm (Erguden et al., 2015). Total length of *O. fasciatus* ranged from 3.8 cm to 10.1 cm in this study (Figure 3).

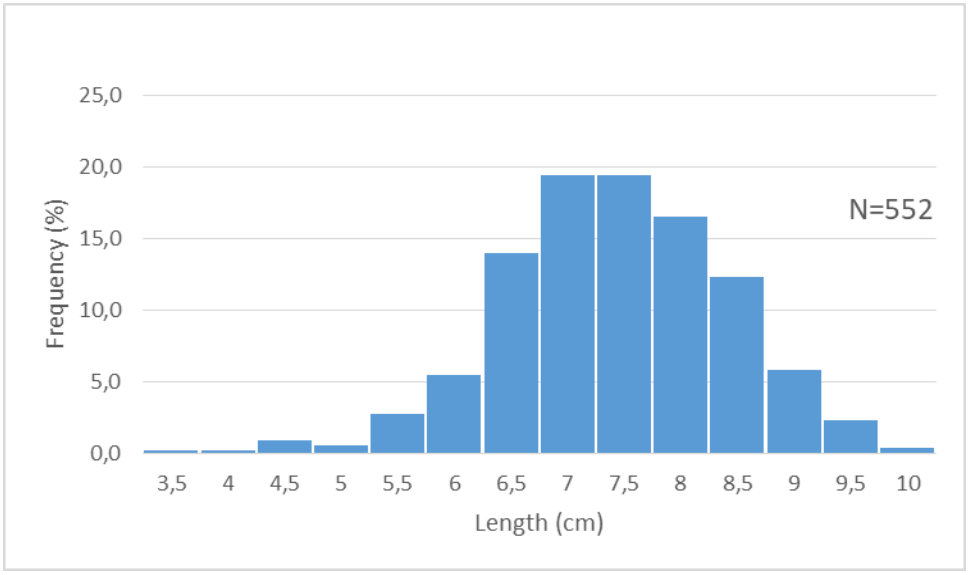

Figure 3. The length-frequency distribution of *Ostorhinchus fasciatus*

Jaydia smithi, which was observed in the Mediterranean Sea at depths of 20-50 m (Goren et al., 2009a; Golani et al., 2008; Gokoglu et al., 2010), was sampled between 20-100 m depth in this study. The frequency of occurrence of this species for trawl hauls at depths of 20-50 m and 51-100 m were 22.22% and 8.33%, respectively.

Jaydia queketti, which extends its distribution to the Aegean Sea and sampled at a depth of 40 m (Akyol and Unal, 2015) and 52 m (Filiz et al., 2012), has been reported at 50 m (Eryilmaz and Dalyan, 2006) and 140-150 m depth (Gokoglu et al., 2011) in the Mediterranean Sea. This species was generally seen at a depth of 51-100 m and the frequency of occurrence of this depth was calculated as 22.22% in this study. *Jaydia queketti* was sampled in only one trawl haul at each depth contours of 20-50 m and 101-200 m.

Length-weight relationships of *O. fasciatus* and *J. queketti* were determined the first time in the Gulf of Antalya and Finike Bay (Northeastern Mediterranean) (Figure 4).

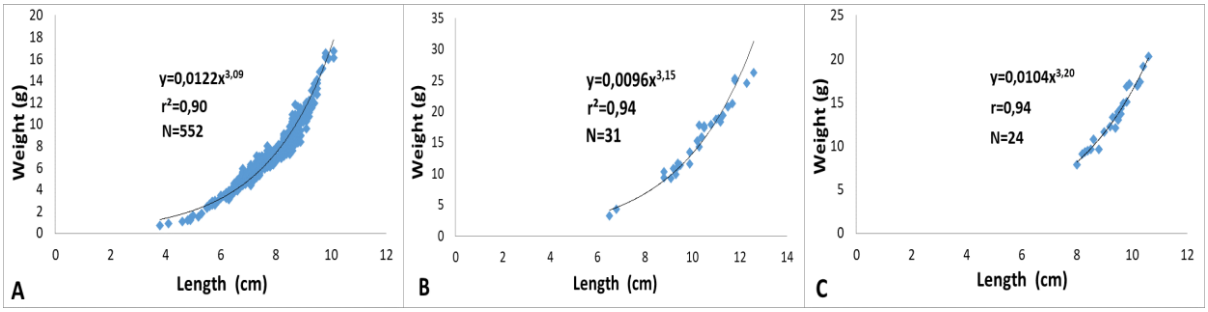

Figure 4. The length-weight relationship of *Ostorhinchus fasciatus* (A), *Jaydia smithi* (B) and *Jaydia queketti* (C)

All relationships were significant ($p > 0.001$) with r^2 values, 0.90 (*O. fasciatus*) and 0.94 (*J. smithi* and *J. queketti*). The r^2 value of *O. fasciatus* is comparably lower than other studies. The estimation of length-weight relationship parameters may be affected by many factors, such as season, sex and maturity of a species (Karachle and Stergiou, 2008). In this study, fish sampling was performed seasonally regardless of sex and maturity. The b values ranged from 3.05 to 3.20 and positive allometric growth was observed in three Apogonidae species. In other studies, conducted in the Mediterranean, the b values varied between 2.95 and 3.61, and negative allometry was reported only for *J. smithi* (Edilist, 2014) (Table 2).

Table 2. Descriptive statistics and length-weight relationships for three Apogonidae species in the Northeastern Mediterranean

Species / Location	N	L_{min}	L_{max}	*a*	*b*	SE_b	r^2	Reference
Ostorhinchus fasciatus								
Israeli continental shelf	42	5.5	9.5	0.0140	3.16	0.009	0.94	Edelist, 2014
Iskenderun Bay, Turkey	230	4.8	12.6	0.0133	3.12	0.038	0.97	Erguden et al., 2015
NE Mediterranean Sea, Turkey	552	3.8	10.1	0.0122	3.09	0.003	0.90	Present study
Jaydia queketti								
Iskenderun Bay, Turkey	48	7.1	12.3	0.0157	3.06	0.100	0.95	Erguden et al., 2009
SE Aegean Sea, Turkey	11	10.7	11.4	0.0869	3.61	0.018	0.92	Yapici et al., 2015
NE Mediterranean Sea, Turkey	31	8.0	10.6	0.0104	3.20	0.005	0.94	Present study
Jaydia smithi								
Gulf of Antalya	32	7.8	11.3	0.0133	3.06	0.093	0.97	Gokoglu et al., 2010
Israeli continental shelf	145	3.4	14.5	0.0161	2.95	0.007	0.98	Edelist, 2014
Iskenderun Bay, Turkey	116	6.3	14.0	0.0044	3.50	0.047	0.98	Erguden et al., 2015
NE Mediterranean Sea, Turkey	26	6.5	12.6	0.0096	3.15	0.006	0.94	Present study

CONCLUSION

This study provides the distribution of three cardinalfishes species, *O. fasciatus*, *J. queketti* and *J. smithi* seasonally and the first information on length-weight relationships for *O. fasciatus* and *J. queketti* from the northeastern Mediterranean coast of Turkey, Gulf of Antalya, and Finike Bay. *Ostorhinchus fasciatus* was the most abundant species and sampled up to 50 m depth. All three Apogonidae species were sampled most during the summer season. It was reported that apogonid fishes that act with parental protection instinct contribute to the survival of young individuals (Moyle and Marchetti, 2006). This advantageous situation can increase the chance of the species to exist among local fish communities (Irmak and Engin, 2015). Cardinalfishes (Apogonid species), although not having economic value and do not threaten human life but needs to be monitored like other lessepsian fish species for sustainable environment and ecological balance.

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First Record of *Metacyclops subdolus* Kiefer, 1938 (Copepoda, Cyclopidae) from Turkey

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Research Article

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Abstract

Copepod samples were collected from the lake in Gilindire Cave in Aydıncık District (İçel, Turkey). A total of 3 taxa including 1 rotifer, 1 copepod, and 1 amphipod were found. *Metacyclops subdolus* was reported from Turkish inland waters for the first time.

Keywords: *Metacyclops subdolus*, Zooplankton, Copepoda, Gilindire Cave, underground lake

Türkiye'den *Metacyclops subdolus* Kiefer, 1938 (Copepoda, Cyclopidae)'un İlk Kaydı

Özet

Kopepod örnekleri Aydıncık İlçesi (İçel, Türkiye) Gilindire Mağarası'ndaki gölden toplanmıştır. 1 rotifer, 1 kopepod ve 1 amfipod olmak üzere toplam 3 takson bulundu. *Metacyclops subdolus* ilk kez Türkiye iç sularından rapor edilmiştir.

Anahtar kelimeler: *Metacyclops subdolus*, Zooplankton, Kopepoda, Gilindire Mağarası, yeraltı gölü

INTRODUCTION

Gilindire Cave which is located in the southern part of Turkey (Mersin) and was discovered by a shepherd in 1999. The lake in the cave has brackish waters at the first 10 meters depth, and at the later depths it has saltwater characteristics and the average water temperature is about 25°C and absolute humidity is 80% (Nazik et al., 2001). This subterranean ecosystem, characterized by chemoautotrophic resources, was described and named as "Ophel" by Por (2007). Since the entrance of the cave is flat and narrow, there is no direct air movement from outside. Gilindire Cave, which is a multi-period and multi-origin cave in terms of geomorphological formation and development, is a very important natural formation that developed into the literature in the national sense in the science of speleology by developing in the Cambrian dolomite and limestone lithology (Nazik et al., 2001).

Some zooplanktonic organisms, such as copepods, may be acceptable markers of natural surroundings heterogeneity. Zooplankton has significant roles in groundwater food webs and ecosystems, but their natural capacities in groundwater are not sufficiently known (Gibert and Deharveng, 2002). Cyclopoid copepods compose an important part of the groundwater biodiversity in karstic habitats, and they live in planktonic or epibenthic and hyperbenthic environments in the underground source (Dussart and Defaye, 2006).

The genus *Metacyclops* Kiefer, 1927 contains 67 nominal species and subspecies (Dussart and Defaye 2006), and three of them are listed on the IUCN Red List, *M. campestris* from Brazil, *M. gasparoi* from Italy, and *M. postojnae* from Slovenia (Walter, 2018).

The genus *Metacyclops* is widespread in tropical and temperate regions, most of them have been recorded in different groundwater habitats, such as wells, caves, anchialine habitats (Pesce, 2015). However, despite its potentially rich fauna, only six species have been recorded from Turkey so far: *M. amoenus* Mann, 1940, *M. gracilis* (Lilljeborg, 1853), *M. minitus* (Claus, 1863), *M. planus* (Gurney, 1909), *M. stammeri* Kiefer, 1938 and *M. grandispinifer* (Lindberg, 1940) (Ustaoğlu, 2004; Ustaoğlu 2015).

M. subdolus has a European Mediterranean distribution (no record from North Africa); it has first been reported by Kiefer (1938) from southern Italy (La Zinzulusa, Abysso caves), then from Sardinia (Lindberg, 1956), Italy (Pesce et al., 1978; Pesce, 1985), Greece (Peloponnesos, Attica, Crete: Pesce, 1978; Pesce and Maggi, 1981, 1983), Mallorca (Can Pastilla: Lescher-Moutoué, 1981), Isreal (Dimentman and Por, 1991; Defaye and Por, 2010; Spring and Cave), and northern Negev (Defaye and Dussart, 1995).

A new species of *M. subdolous* was reported in a limited number of countries and regions, and for the first time the report has been in Turkey. Some supplementary drawings and descriptions from Gilindire specimens are provided as a basis for future comparison.

MATERIALS and METHODS

Gilindire cave is located in Aydıncık district of Içel province, at a longitude of 33° 24' 11.04"east, and a latitude of 36° 07' 58.08" north (Figure 1). The cave consists of three separate sections that are interconnected but occur at different periods. The cave, whose width reaches 100 m in places and the ceiling height reaches 18 m and extends between + 22 / -28 meters according to the entrance, is divided into many halls and rooms by large and thick dripstones. There is a large lake in the last part of Gilindire Cave. The length of the lake is approximately 140 m, its width is18-30 m, and the ceiling height is 35-40 m, the water level is 46 meters from the entrance of the cave and it is the same as the sea level (Nazik et al., 2001; Özşahin and Kaymaz, 2014) (Figure 2).

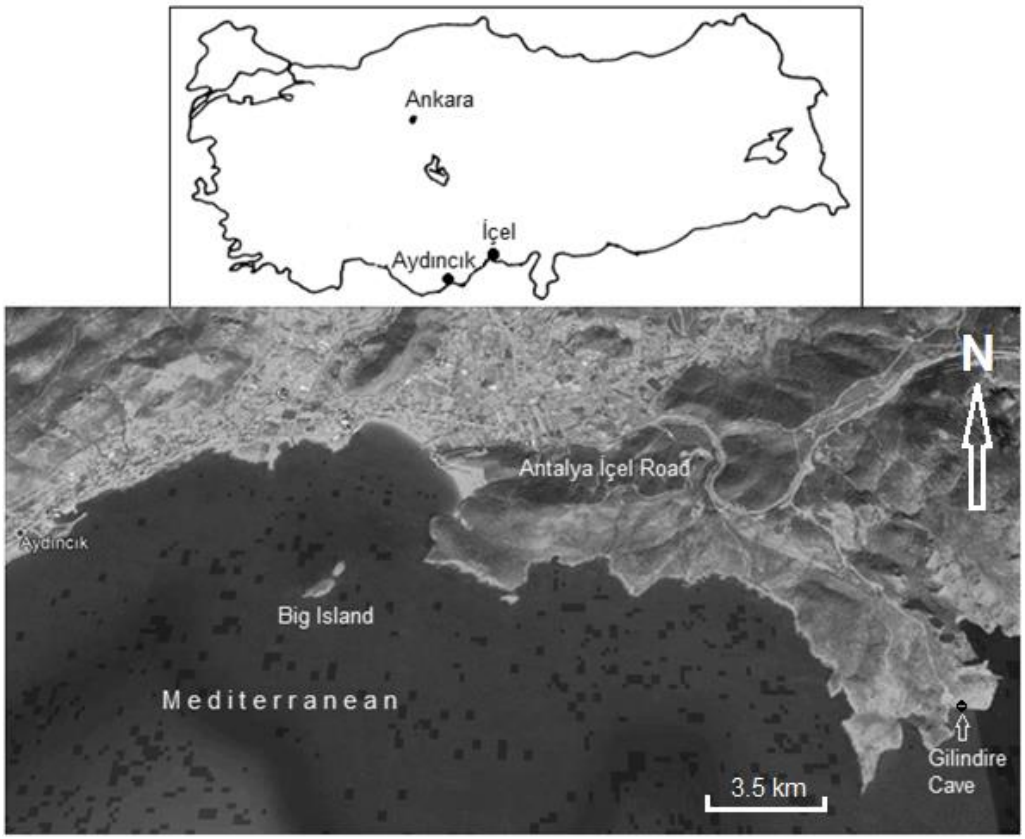

Figure 1. The location of Gilindire Cave.

It was determined that the first 10 meters of the lake water have the feature of mixing water and when it goes down from this depth, it gains seawater feature completely. It was reported that the water of the lake is largely seawater, and the groundwater leaking from the rain and reaching the lake is mostly mixed with seawater at the upper elevations of the lake (Nazik et al., 2001; Özşahin and Kaymaz, 2014).

In the observations made by scuba diving in the underground lake, no significant groundwater flow was detected in any direction, especially from the land direction towards the sea. Also, it was

determined that the water level did not change in the section between the lake and the sea, where the horizontal distance is about 250 m. On the other hand, in the region where the average precipitation is low, the waters leaking from the surface continue to form sediments in the Vadose section (Nazik et al., 2001; Özşahin and Kaymaz, 2014).

Copepod specimens were collected in May, June, and November 2015 by throwing the plankton net (60 µm mesh size) from the lakeshore to the interior (about 10-12 meters), pulling it from the surface to the shore, and also collected at different depth. This process was done at least 10 times. Samples were replaced into a glass jar and fixed with 4% buffered formaldehyde. Specimens were examined in a mixture of distilled water and glycerol. Drawings and measurements were made by using an Olympus microscope with a drawing-tube an ocular micrometer. Copepod body lengths were measured at 10x magnification with micrometric ocular attached to the ocular. The species were identified according to Dussart (1967), Damian-Georgescu (1970), Kiefer (1978), Pesce (1978), Defaye and Por (2010).

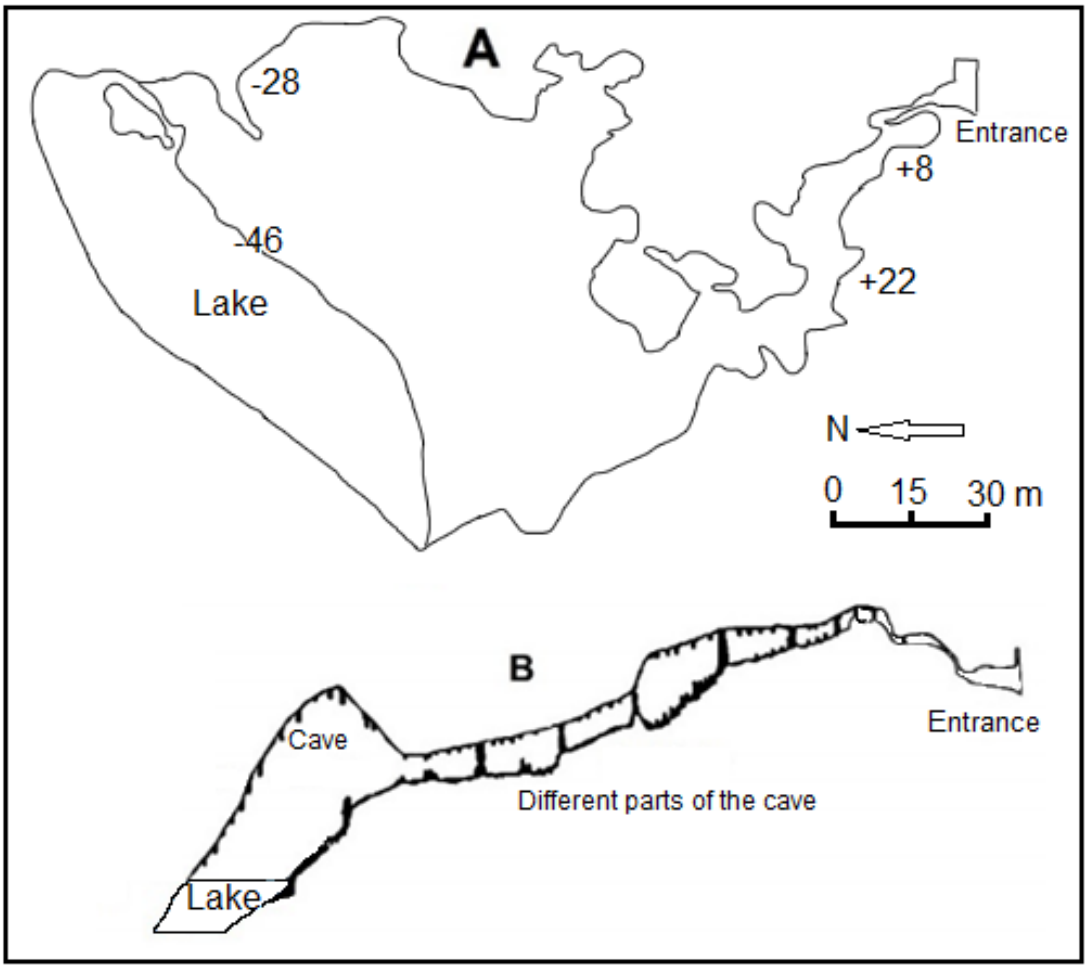

Figure 2. Plan of the Gilindire Cave (A top view, B side view) (redrawn from Nazik et al. 2001)

RESULTS

The water quality criteria (temperature, pH, electrical conductivity, salinity, and dissolved oxygen) measured by depths are given in Table. According to Nazik et al. (2001), while there was no change in temperature and pH depending on the depth, there was a significant increase in electrical conductivity and salinity, and a significant decrease in dissolved oxygen was reported.

Table. Physical-chemical properties of Aynalı Lake in Gilindire Cave (quotation from Nazik et al., 2001).

Depth (m)	Temp (°C)	pH	EC (µS/cm)	Salinity (ppt)	DO (% sat)	DO (mg/L)
1.2	21.65	7.29	4334	2.4	86.6	6.81
2.6	21.65	7.25	5106	2.8	84.1	6.60
3.9	21.65	7.27	6397	3.5	79.7	6.22
5.6	21.64	7.26	9002	5.1	75.9	5.87
6.8	21.64	7.27	11131	6.3	74.4	5.71
8.3	21.64	7.29	13426	7.7	71.5	5.44
9.9	21.64	7.30	17012	10.0	69.6	5.22
11.3	21.64	7.31	20691	12.3	61.5	4.55
13.1	21.65	7.30	25844	15.7	53.7	3.89
14.2	21.65	7.31	30923	19.2	46.2	3.28
16.0	21.65	7.30	36708	23.2	43.6	3.02
17.7	21.65	7.32	41700	26.8	46.2	3.13
20.0	21.65	7.33	44831	29.0	48.9	3.27
22.1	21.65	7.34	46796	30.5	47.5	3.15
23.8	21.66	7.35	47899	31.3	44.2	2.92
25.3	21.66	7.35	48294	31.6	42.1	2.77
27.0	21.67	7.33	48419	31.7	43.5	2.87

In this study, 1 rotifer (*Philodina* sp.), 1 copepod (*Metacyclops subdolus*), and 1 amphipod (under review) were identified in the cave.

Taxonomic account:
Order Cyclopoida Burmeister, 1835
Family Cyclopidae Rafinesque, 1815
Subfamily Cyclopinae Rafinesque, 1815
Genus *Metacyclops* Kiefer, 1927
Metacyclops subdolus Kiefer, 1938 (Figures 3-5)

Redescription of the female (Figures 3-4). Nineteen specimens were measured and body lengths ranged between 0.586 mm and 0.778 mm, except for caudal seta, and the mean was 0.682 mm. Body about slender, widest at the posterior part of the cephalothorax in dorsal view (Figure 3A). Cephalothorax nearly 1.8 times as long as the following pedigerous somites. Prosome/urosome ratio 1.7; body length/width ratio 2.5; cephalothorax/genital double-somite width ratio is about 2.9. Somite bearing the P5 slightly wider than genital double-somite and a serial endowed with lateral hairs.

Figure 3. *Metacyclops subdolus* Female. A) Habitus, dorsal; B) Caudal rami and anal somite, ventral; C) Antennule; D) Antenna; E) P5 and P6, lateral; F) Genital field, ventral. Scale bars: A 250 μm; B, C, D 100 μm; E, F 50 μm.

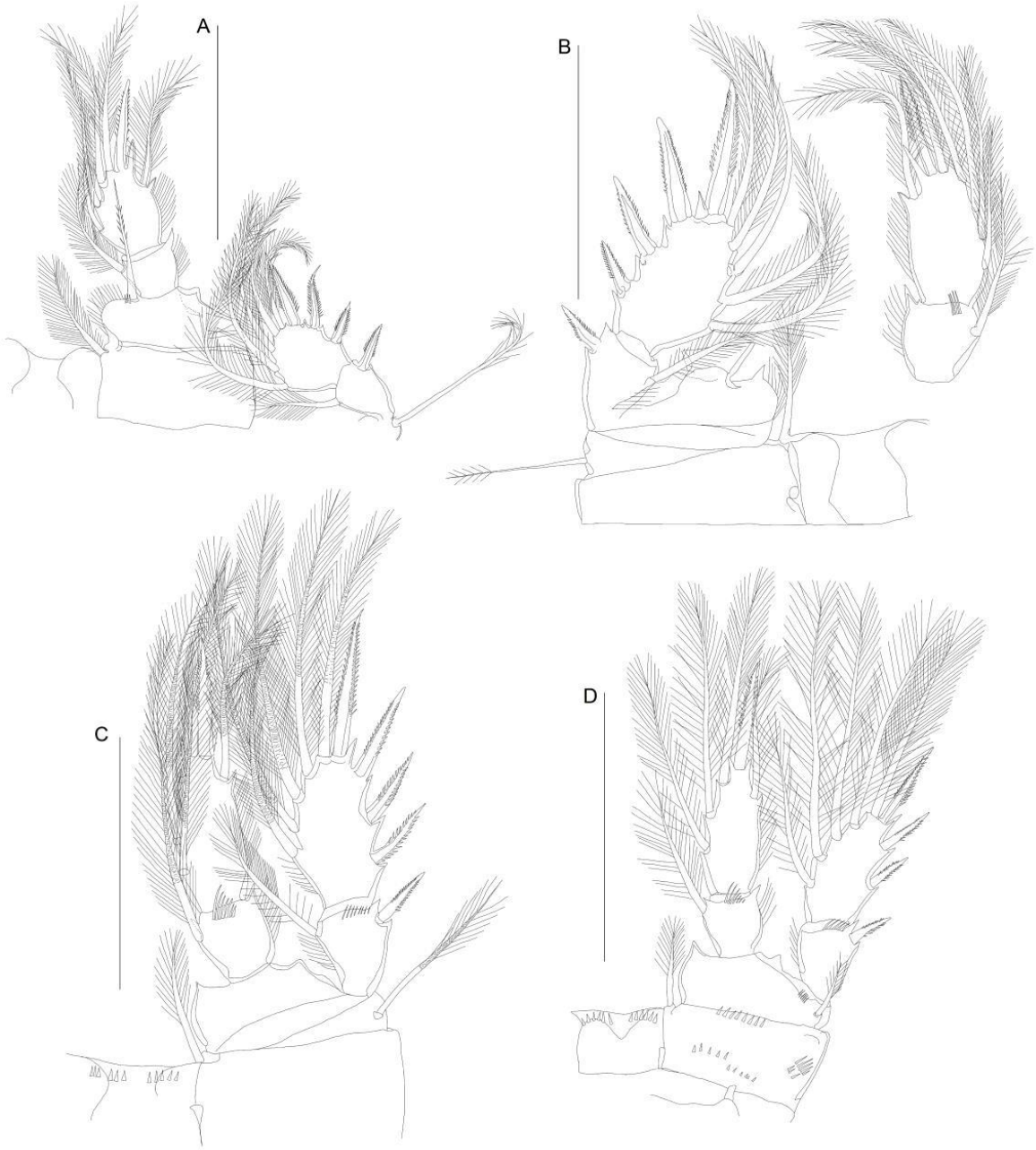

Figure 4. *Metacyclops subdolus* female swimming legs, A) P1, anterior; B) P2, anterior; C) P3, anterior; D) P4, posterior. Scale bars: 100 μm (A-D).

Genital double-somite (Figures 3A, F), slightly longer than wide (about 1 to 1.2 times as long as wide), other abdominal segments wider than long. In ventral view, the seminal receptacle consisting of two parts, with the anterior part compressed, and a small, bulbous posterior part (Figure 3F), and a common structure for *Metacyclops*. While copulatory pore is located at about 0.40 of the somite length from the anterior margin, gonopores are located in lateral position, protected by the reduced P6. The following two urosomites (Figure 3A) are almost the same length, 0.4 and 0.3 times as long as the genital somite, and slightly narrowing towards the anal somite, respectively (Figure 3B). Anal somite is slightly shorter than the preceding somite, with a smooth margin, convex, and bearing a short anal operculum, located in the anterior half of the somite. Without ornamentation on the distal margin of any somite, only 10-13 small ventral spinules on the distal margin of the anal somite, at the base of each of the furcal rami. Furcal rami (Figures 3A, B) 2.2 times wider. Furcal setae: the median external (lateral) seta inserted about 70% from the base of the furcal ramus; the outermost terminal seta shorter than the innermost and the dorsal seta; external median terminal seta is 2.31 as long as the dorsal seta,

internal median terminal seta 3.8 times as long as furcal rami. All furcal setae plumose. Antennule 11-segmented (Figure 3C), short, barely reaching posterior margin of cephalothorax, setation from proximal to distalmost segments (number of setae in parentheses; a, aesthetasc; s, spine): 1(8), 2(4), 3(6), 4(2), 5(1 + s), 6(2), 7(3), 8(2 + a), 9(2), 10(2 + a), and 11(7 + 1a). Segment 1 is ornamented with spinules (Figure 3C). Segment 3 shows suture of residues of two original segments, so probably consisting of two fused segments. In the 5th segment, one of the two setae spiniform and short. The antenna (Figure 3D) consists of 4-part basipodite and 3-endopod. Basipodite bearing 2 internal setae inserted antero-distally (Figure 3D), and externally the long exopodal seta inserted distally. Ornamentation is visible on the basipodite as two lateral groups of 5 spinules on the external frontal edge. Endopodite bearing is divided into three segments 1, 9, and 7 setae, respectively. All three ornamented with a row of hairs or spines on the outer margin, discontinuous on the distal segment.

Swimming legs P1-P4 (Figures 4A-D), both rami 2-segmented. Setal formula (setal arrangement of exp-2 of P1-P4) 5,5,5,5. Spine formula (arrangement of spines on exp-2 of P1-P4) 3,4,4,3. The spine and setal formula are as follows (spines in Roman numerals, setae in Arabic numerals; legend: outer/inner spine or seta; outer/terminal/inner).

	Coxopodite	**Basipodite**	**Exopodite**	**Endopodite**
P1	0-1	1-I	I-1; III, 2, 3	I-1; 1, 1-I, 3
P2	0-1	1-0	I-1; III, I-1, 4	0-1; 1, I-1, 4
P3	0-1	1-0	I-1; III, I-1, 4	0-1; 1, I-1, 4
P4	0-1	1-0	I-0; II, I-1, 4	0-1; 1, I, 3

Ornamentation of setae and spinules are shown in the figures. According to this P1 (Figure 4A), intercoxal sclerite with paired lateral lobes produced posteriorly, both the frontal and caudal surfaces smooth without transverse spinule or setule row. No spinules on Coxa smooth; 1 plumose seta located in the inner distal. Basis armed with 1 pinnate seta distomedially, its tip almost reaches the top of enp-2; lateral seta well developed, plumose. Inner seta present on both enp-1 and exp-1; enp-2 bearing 4 plumose setae along its inner margin, not modified to pinnate or spinous form.

P2-P3 (Figures 4B, C), paired lateral lobes of intercoxal sclerite; lateral seta on basis short, plumose distally; inner seta on both enp-1 and exp-1; enp-2 bearing 5 plumose setae along its inner margin, not modified to pinnate or spinous form. The distal margin of intercoxal sclerite of P3 with transverse spinule.

P4 (Figure 4D), intercoxal sclerite with paired lateral lobes poorly produced posteriorly with 5-6 spinules on inner distal margin. Coxa with transverse row of 8-9 sharp spinules along the posterior margin; 1 inner distal seta well developed, plumose. Basis, lateral seta too short, plumose. Enp-1 with 1 inner distal seta; exp-1 lacking inner seta. P4 enp-2 extends 2.05 times as long as wide; slightly shorter than enp-2 (about 0.84 times as long as enp-2) apically armed with single spine; the outer distal seta crosses tip of apical spine.

P5 (Figure 3E), protopodal segment completely incorporated into fifth pedigerous somite, with outer basal seta, situated rather dorsolaterally and flanking 5-6 spinules at its base; free exopodal segment very small, subapically bearing 1 spine inner sand 1 outer apical plumose seta; spine slightly shorter than exopod; outer apical seta about 5.6 times longer than the inner spine. No colour observed. Egg-sacs paired, each bearing 6 medium size-eggs. P6 (Figure 3E), bearing two slender and short (slightly longer in the males) setae subequal in size.

Male (Figures 5A-C). Fourteen specimens were examined and body lengths excluding caudal seta ranged between 0.556 mm and 0.626 mm, with an average of 0.592 mm. Anterior part of the body has the same shape as that of a female. Urosome of 5 somites, a row hair laterally, as in female; the genital somite the largest, twice as long as wide (Figure 5A); the three following urosomites, it slightly narrows towards the anal somite. Anal somite in the same width as the preceding somite (Figure 5B). Anal operculum (Figure 5A) as in female, short, with distal margin convex and no ornamentation on sinus. A few spinules at the base of the furcal rami go laterally from the middle of the ramus. Furcal rami 2.15 times as long as broad. A spine is present at the insertion of the outermost terminal seta. No ornamentation at the posterior margin of any somite.

Antennule (Figure 5C), 16-segment, relatively longer than in female; geniculate between segments 7 and 9, and between segments 14 and 15; segments 14, 15 and 16 are elongated; Setal formula: 1(8),

2(4), 3(2), 4(2), 5(1), 6(2), 7(1), 8(2), 9(2), 10(2), 11(2), 12(1), 13(0), 14(0), 15(1), 16(10), most setae smooth. The aesthetascs present on segments 1(3), 4(1), and 9(1) are all long. First segment with a proximal, oblique row of 6 spinules. Antenna without significant differences from that of the female. Segmentation of swimming legs P1-P4 identical to that in the female, without sexual dimorphism. P5 similar to the female, with apical external seta half as long as genital somite. P6 (Figure 5B) is composed of two short elements located close to each other at the outer corner, on a small cuticular plate.

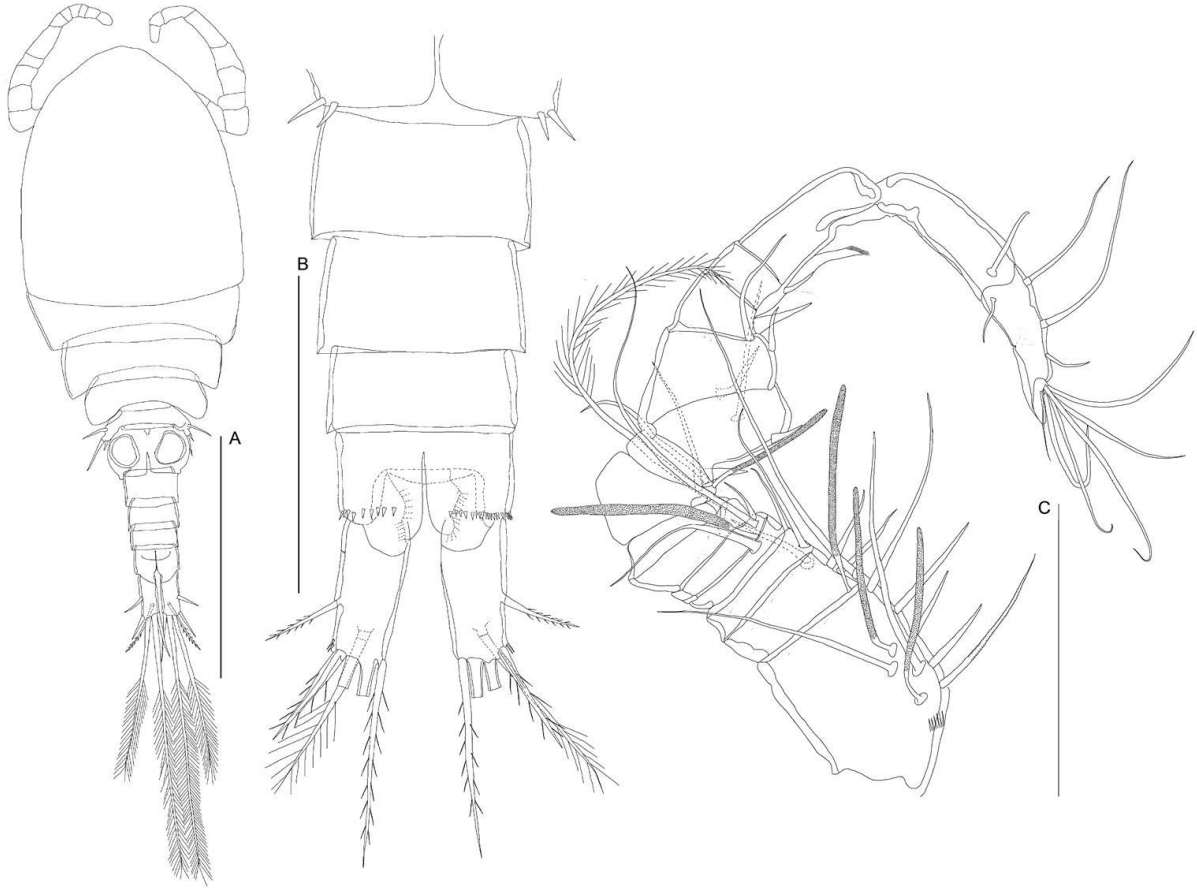

Figure 5. *Metacyclops subdolus* Male. A) Habitus, dorsal; B) P6, caudal rami and anal somite, ventral; C) Antennule. Scale bars: A 250 μm, B, C 100 μm.

DISCUSSION

The genus *Metacyclops* is widespread in tropical and temperate regions, the most productive in the regions of European, African and South American, poorly known for North America and Australia. At present the genus includes 67 valid species and subspecies, most of which are recorded in different groundwater habitats, such as wells, caves, anchialine habitats (Defaye and Por, 2010).

Current taxonomic studies (Herbst 1988, Reid 1987, 1991, Fiers 2001, Karanovic 2004a) recognize four groups based on the spine formula of the terminal exopodite segment of the 1-4 legs (designated as spine formula of legs 1-4). The first group with 3443 spine formula contains 52 of the 62 species of the genus (The group including *M. subdolus*). The second (3442) and third (3433) groups each contain one species, *M. mortoni* Pesce, De Laurentiis and Humphreys, 1996 and *M. cushae* Reid, 1991, respectively. The fourth group among the *Metacyclops* species is the trispinosus-group, which has 3333 spine formula (Karanovic, 2004b; Mercado-Salas et al, 2013).

The genus *Metacyclops* is widely distributed around the world and has been found in diverse (various) biotopes, both epigean and hypogean. In epigean biotopes, many widespread (common) species are frequently encountered, such as the type-species, *M. gracilis* (Lilljeborg, 1853), as well as *M. minutus* (Claus, 1863), *M. planus* (Gurney, 1909), and *M. mendocinus* (Wierzejski, 1892). In

hypogean biotopes, we can mention, for example, *M. subdolus* Kiefer, 1938, recorded from groundwaters of peri-Mediterranean countries (Defaye and Por, 2010).

Metacyclops is a very ancient genus and has probably colonized fresh waters very early, before the break-up of Pangaea (Boxshall and Jaume, 2000), many species of the genus are endemic, as the species recently described from Western Australian groundwaters (Karanovic, 2004a, 2004b). After the colonization of hypogean fresh waters occurred, it led to the diversification of more or less closely related taxa on different plates and continents. Further examinations in underground waters and a total correction of the *Metacyclops* genus will be important to comprehend the connections between the species of these commonly conveyed variety (Defaye and Por, 2010).

The occurrence of the species is certainly related to the characteristics of the biotope. The lake of Gilindire Cave has dual source of water: fresh groundwater mixed saltwater. The *M. subdolus* population in the lake consisted of a large number of adults, copepodite and nauplii. The reason for the abundance of *M. subdolus* is thought to be the absence of another creature feeding on it.

M. subdolus prefers sulfidic and slightly brackish groundwater (Defaye and Por, 2010), and has a European Mediterranean distribution (no record from North Africa); it has first been reported by Kiefer (1938) from southern Italy (La Zinzulusa, Abysso caves), then from Sardinia (Lindberg, 1956), Italy (Pesce et al., 1978; Pesce, 1985), Greece (Peloponnesos, Attica, Crete: Pesce, 1978; Pesce and Maggi, 1981, 1983), Mallorca (Can Pastilla: Lescher-Moutoue, 1981). *M. subdolus* has already been identified from Israel by Dimentman and Por (1991) from slightly brackish springs near the Dead Sea, the northern Negev by Defaye and Dussart (1995) and finally by Ayyalon Cave (Defaye and Por, 2010).

The waters where *M. subdolus* has been found with light brackish water until now are spring water, caves and wells. Therefore it confirms that *M. subdolus* is a type of groundwater species that prefers slight brackish waters.

Minor differences were detected in some characters of *M. subdolus* in Gilindire cave. İn the previous definitions, intercoxal scleritis of P1-P4 ornamented with 2 rows of spinules on ventral margin but in the present, intercoxal scleritis of third and fourth legs ornamented with a row of spinules on ventral margin, first and second smooth. Two-row spinule present ventral margin of P4 coxa but in previous, coxa smooth. P5: the base of the seta inserted on the somite has a row of spinules.

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Effect of Dietary Sumac (*Rhus coriaria* L.) Supplementation on Non-Specific Immune Response and Hematology of Rainbow Trout (*Oncorhynchus mykiss*), Resistance Against *Vibrio anguillarum*

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Research Article

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Abstract

This study aimed to investigate the effects of sumac (*Rhus coriaria* L.) fruit powder in different concentrations as feed additives on non-specific immune response, hematology, and the disease resistance in rainbow trout (*Oncorhynchus mykiss*). Fish (35.67±0.88 g) were fed with experimental diets (1.0, 3.0, 5.0 and 10.0 g kg^{-1}) at four different concentration. There were no significant differences between groups fed with sumac fruit powder and control group by the mean of red blood cell count (RBC), white blood cell count (WBC), differential leukocytes count (monocyte, lymphocyte, and neutrophile), hematocrit (Hct), hemoglobin (Hb), cell hemoglobin (MCH pg), cell hemoglobin concentration (MCHC %), cell hemoglobin volume (MCV μm^3) and plasma lysozyme, Immunoglobulin M (IgM), total protein level on the 8 weeks. After 8 weeks of feeding, fish were challenged with *Vibrio anguillarum* and cumulative mortality was recorded over 21 days. Dietary administration of 1.0, 3.0, and 5.0 g kg^{-1} sumac fruit powder significantly increased fish survival rate ($p<0.05$). The 10.0 g kg^{-1} diet received fish showed no mortality post challenged with *V. anguillarum*. These results showed that the sumac fruit powder improved disease resistance when added to the rainbow trout diet.

Keywords: Hematology, non-specific immune response, *Oncorhynchus mykiss*, *Rhus coriaria* L., *Vibrio anguillarum*.

Yeme İlave Edilen Sumak (*Rhus coriaria* L.)' ın Gökkuşağı Alabalıkları (*Oncorhynchus mykiss*)' nın Spesifik Olmayan Bağışıklık Tepkisi, Hematoloji ve *Vibrio anguillarum*' a Karşı Direnç Üzerine Etkisi

Özet

Bu çalışmanın amacı, yem katkı maddesi olarak farklı düzeylerde kullanılan sumak (*Rhus coriaria* L.) meyvesi tozunun gökkuşağı alabalığında (*Oncorhynchus mykiss*) spesifik olmayan bağışıklık yanıtı, hematoloji ve hastalık direnci üzerindeki etkilerini araştırmaktır. Balıklar (35,67±0,88 g) dört farklı konsantrasyonda (1,0, 3,0, 5,0 ve 10,0 g/kg) sumak ilaveli yemlerle beslenmişlerdir. Sumak meyve tozu ilaveli yemle beslenen gruplar ve kontrol grubu arasında kırmızı kan hücresi sayısı (RBC), beyaz kan hücresi sayısı (WBC), diferansiyel lökosit sayısı (monosit, lenfosit ve nötrofil), hematokrit (Hct), hemoglobin (Hb), ortalama hücre hemoglobin değeri (MCH pg), hücre hemoglobin konsantrasyonu (% MCHC), hücre hemoglobin hacmi (MCV µm3) ve plazma lizozim, immunoglobulin M (IgM) ve toplam protein düzeyinde 60. günde önemli bir farklılık görülmemiştir. 8 haftalık besleme sonrasında balıklara *Vibrio anguillarum* ile deneysel enfeksiyon uygulaması yapılmış ve 21 gün boyunca kümülatif mortalite kaydedilmiştir. Yeme 1,0, 3,0 ve 5,0 g/kg oranında ilave edilen sumak meyve tozu balık yaşam oranını önemli ölçüde artırmış olduğu tespit edilmiştir ($p<0.05$). 10,0 g/kg oranında *V. anguillarum* ile deneysel enfeksiyon sonrasında mortalite görülmemiştir. Bu sonuçlar gökkuşağı alabalığı yemine eklenen sumak meyve tozunun hastalık direncini artırdığını göstermektedir.

Anahtar kelimeler: Hematoloji, non-spesifik bağışıklık tepkisi, *Oncorhynchus mykiss*, *Rhus coriaria* L., *Vibrio anguillarum*.

INTRODUCTION

Infectious disease is the main problem for the development and sustainability of aquaculture. One of the most important bacterial diseases that causes mortality in rainbow trout is vibriosis and *Vibrio anguillarum* is the most common pathogen of disease. Recently, there has been increased interest in

the possibility of using medicinal herbs as disease resistance of cultured fish (Terzioğlu and Diler, 2016; Diler et al., 2017a; Diler et al., 2017b; Uluköy et al., 2018). Sumac (*Rhus coriaria* L.) is a plant species in the Anacardiaceae family that is used as a spice and a native medicine and grows wild in the region extending from Canary Island over the Mediterranean coastline. It has a long history of use by indigenous people for medicinal and other applications. *Rhus coriaria* has been reported to possess antibacterial (Iauk et al., 1998; Ali-Shtayeh et al., 2013; Kossah et al., 2013; Al-Boushi et al., 2014), antifungal (Onkar et al., 2011), antioxidant Aliakbarlu et al., 2014), anti-inflammatory (Panico et al., 2009). Sumac fruits contain phenolic acids, flavonols, hydrolysable tannins, anthocyanin, and organic acids (Mavlyanov et al., 1997). A large number of active metabolites have been reported in sumac including gallic acid, quercetin, vanillic acid (Abu-Reidah et al., 2014; Al-Boushi et al., 2014). *R. coriaria* extract had a strong *in vitro* antibacterial activity against tested bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Shigella* spp., *Staphylococcus aureus*, *Pseudomonas aerugenisis*, *Salmonella enteric* and *Bacillus cereus* (Gabr et al., 2014; Mahdavi et al., 2018). Currently, *in vivo* studies linking the antimicrobial effect of sumac (*R. coriaria* L.) in animal production are scarce. In a study, sumac fruit powder was determined to increase growth performance and not effected immune response of broiler chicks (Toghyani and Faghan, 2017). The determination of health status of fish, the blood parameters are useful tool (Campbell, 2004). Some authors reported which blood parameters such as hematocrit, hemoglobin and total erythrocyte count below the normal ranges are all signs of anaemia. Anaemia is caused that reduced tolerance to secondary stressors and fish are sensitive to certain secondary pathogens (Gatlin, 2007; Rios et al., 2005). This study aimed to determine the effects of sumac fruit powder (SFP) on hematology, non-specific immune response, and to disease resistance against *Vibrio anguillarum* in rainbow trout (*Oncorhynchus mykiss*, Walbaum).

MATERIALS and METHODS

Experimental design

This study was carried out in the commercial trout farming in Aksu-Isparta. The fish for the experiment was obtained from a commercial rainbow trout farm in Aksu (Isparta, Turkey). Fish were stocked into fiberglass tanks and an adaptation period of 20 days was applied prior to the trial. A total of 450 fish (35.67±0.88 g) were randomly allotted as 30 fish in each tank (5 groups with 3 triplicate). During the feeding experiment, the average water temperature was 10.2±0.5ºC, the dissolved oxygen was 8.9±0.2 mg L^{-1} and the pH was 7.2±0.3. Sumac fruit powder (SFP) was purchased from a local market in Isparta province. Different concentration (0, 1.0, 3.0, 5.0 and 10.0 g kg^{-1}) of the SFP were added into experimental diet. Five diets were formulated (40.26 g kg^{-1} crude protein and 16.72 g kg^{-1} crude lipid) based on the study of New (1987) (Table 1). Fish fed twice daily for a total of 8 weeks.

Table 1. Formulation of experimental diets and proximate analysis

Ingredients (g kg $^{-1}$)	Groups				
	Control (0 g kg^{-1})	**Sumac (1 g kg^{-1})**	**Sumac (3 g kg^{-1})**	**Sumac (5 g kg^{-1})**	**Sumac (10 g kg^{-1})**
Fish meal	31.00	31.00	31.00	31.00	31.00
Soybean meal	44.00	44.00	44.00	44.00	44.00
Corn Starch	1.00	0.90	0.70	0.50	0.00
Wheat flour	11.00	11.00	11.00	11.00	11.00
Soybean oil	11.70	11.70	11.70	11.70	11.70
Vit-Min*	1.00	1.00	1.00	1.00	1.00
Pellet binder	0.30	0.30	0.30	0.30	0.30
Sumac fruit powder	0.00	0.10	0.30	0.50	1.00
Chemical composition					
Dry matter	92.23	92.24	92.25	92.27	92.30
Crude protein	40.26	40.27	40.27	40.28	40.29
Total lipid	16.72	16.72	16.71	16.71	16.70
Crude fiber	2.81	2.81	2.80	2.80	2.80
Crude ash	10.64	10.64	10.65	10.65	10.66
Energy (kcal/kg)	4067	4064	4059	4053	4040

*Vitamin premix contained the following per kilogram; 4 000 000 IU vitamin A, vitamin D3 480 000 IU, 2400 mg vitamin E, 2400 mg vitamin K3, 4000 mg vitamin B1, 6 000 mg vitamin B2, 4 000 mg Niacin, 10 000 mg Cal.D. Pantothenate, 4 000 vitamin B6, 10 mg vitamin B_{12}, 100 mg D-Biotin, 1200 mg folic acit, 40 000 mg vitamin C, 60 000 mg inositol.
*Mineral premix contained the following per kilogram; 23 750 mg manganese, 75 000 mg zinco, copper 5 000 mg, cobalt 2 000 mg, iodine 2750 mg, selenium 100 mg, magnesium 200 000 mg.

Analysis of phenolic constituents

The procedure for the phenolic contents has been described by Capanio et al. (1999). High-performance liquid chromatography was used. Detection and quantification was carried out with a SLC-10Avp system controller (Shimadzu, Japan) SIL-10 AD vp Autosampler, LC-10 AD vp pump, DGU-14a degasser, CTO-10 A vp column heater and diode array detector set at 278 nm. AnAgilent Eclipse XDB C-18 column (250x4.6mm,5mikrometre) was used. The flow rate was 0.8 mL/min, injection volume was 10 microlitre and the column temperature was set at 30°C. Methanol and 3% acetic acid were used for mobile phases. The data were integrated and analyzed using the Shimadzu Class-VP (Chromatography Laboratory Automated Software System (Tokyo, Japan). Plant samples, standard solutions, and mobile phases were filtered using a 0.45 micrometer pore size membrane filter (Vivascience AG, Hannover, Germany). The amount of phenolic contents in the plant sample was calculated as g kg^{-1} herb using external calibration curves, constructed for each pure phenolic standart. All determinations were carried out in triplicate and the results were presented as mean ± standard error.

Blood collection

Blood samples of randomly selected five fish were collected. Fish were anesthetized with clove oil and then blood was taken from the caudal vein by using hypodermal heparinized syringe (1 mL). The 200 µL blood volume was transferred to ethylenediaminetatraacetic acid (EDTA) tubes for hematological analysis. The rest of the blood sample (600 µL) was put in plastic tubes for biochemistry and immunological analysis. These blood samples were coagulated, the tubes were centrifuged at 5000 x g for 10 min at 4°C for serum separation, which was stored below -20°C.

Hematological analysis

Red blood cells (RBC, 10^6 mm^3), hematocrit (Hct, %) and hemoglobin (Hb, g dL^{-1}) were determined by using the method by Blaxhall and Daisley (1973). RBC was counted with a Thoma hemocytometer using Dacie' s diluting fluid. Hct was determined using a capillary hematocrit tube. Hb concentration was determined by the cyanomethahaenoglobin method in the spectrophotometer (540 nm). The hematological findings of mean cell haemoglobin concentration (MCHC: g dL^{-1}), mean cell haemoglobin (MCH: pg), and mean cell volume (MCV: fl) were calculated using the total RBC count, Hb concentration, and Ht. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following formula.

(1) MCV (μm^3) = [(Hct, %) x 10] / (RBC, x 10^6 per mm^3),
(2) MCH (pg) = [(Hb, g/dl) x 10] / (RBC, x10^6 per mm^3),
(3) MCHC (%) = [(Hb, g/dl)x100] / (Hct, %)

Immunological analysis

Lysozyme activity

The lysoplate assay was performed as described by Ellis (1996) with a final concentration of 0.12% *M. lysodeikticus* cells in 100 mL of 0.5% agarose in 66 mM sodium phosphate, pH 6.2. The zones of lysis were measured with a microcaliper after 20 h of incubation at 36°C. The results for standards were plotted on semilogarithmic graph paper and sample values extrapolated from this standard curve.

Immunoglobulin M

Immunoglobulin M (IgM) level in fish serum samples was assayed by enzyme-linked immunosorbent assay (ELISA) using a fish Immunoglobulin M (IgM) ELISA Kit (Cusabio Biotech Co. Ltd., CSB-E12045Fh, MD, USA). The manufacturer's instructions were followed. The absorbance of samples was read at a wavelength of 630 nm with a Bio-Tek FLX 800 plate reader.

Biochemical analysis

Biochemical findings in serum including total protein (TPROT), albumin (ALB), globulin (GLO) were determined using bioanalytic test kits (Bioanalytic Diagnostic Industry, Co) and measured in a spectrophotometer (PG Instruments, UK).

***V. anguillarum* challenge test**

Experimental groups were included dietary administration of the SFP (1.0, 3.0, 5.0, 10.0 g kg^{-1}) and control group in which diet had no sumac feed supplementation. After 8 weeks of feeding, a challenge test was performed on each group with *Vibrio anguillarum* which was isolated before in our preliminary study. The LD_{50} value of the *V. anguillarum* pathogen was calculated by determining the cumulative mortality rates post-injection at different doses. The challenge was performed in thriplicate. Total 30 fish in each group (10 fish/replicate) at the end of the trial were transferred to challenge tanks. Fish were inoculated with 0.1 mL suspension (containing 2.10^5 CFU/mL (LD_{50} dose) of *V. anguillarum* by intraperitoneal injection in each fish. Mortalities were recorded daily for up to 21 days. The collected all dead fish were examined bacteriologically to determine the presence of the pathogen. The relative percent survival (RPS) was calculated according to Amend (1981).

RPS= (1- % mortality in experiment group % mortality in control) x 100.

Statistic

In this study, the variation analyses were carried out with Duncan multiple comparison tests, and the differences between groups were carried out by SPSS 18 statistics program to evaluate the relationships between the data were obtained from the test groups. Statistical significance was established at $p<0.05$.

RESULTS

Phenolic constituents

The results of the chemical analysis of sumac were presented in Table 2. Major components of sumac were determined as gallic acid and quercetin.

Table 2. Phenolic content (g kg^{-1}) of sumac

Component	
Gallic acid	5206.2
Quercetin	1106.4
Protocatechic acid	391.5
Catechin	80.7
Syringic acid	24.3
Epicatechin	*nd
p-coumaric acid	*nd
Ferulic acid	*nd
Hesperidin	*nd
Cinnamic acid	*nd

Hematological, biochemical and immunological analysis

No significant differences were observed between treatment groups with SFP and control group in terms of haematological indices, differential leukocytes count, hematocrit (Hct), hemoglobin (Hb), and all the values of red blood cell findings at the end of the study (Table 3).

Table 3. The values of hematological parameters in rainbow trout fed with different levels of SFP at 8th weeks

		Groups				
		Control	**1.0 g kg^{-1}**	**3.0 g kg^{-1}**	**5.0 g kg^{-1}**	**10.0 g kg^{-1}**
Haematological indices	**WBC (10^3/µ)**	401.10 ±10.46	365.80 ±102.95	436.40 ±7.49	346.90 ±65.76	406.60 ±29.98
	RBC (10^6/µ)	0.86 ±0.11	0.88 ±0.09	1.02 ±0.02	0.87 ±0.03	0.95 ±0.04
Differential leukocytes count	**LY (%)**	82.30 ±2.54	80.65 ±9.97	76.25 ±0.49	75.70 ±13.15	78.55 ±0.35
	MO (%)	14.50 ±4.24	14.65 ±6.29	10.80 ±3.25	15.30 ±3.11	18.05 ±1.20
	NE (%)	2.75 ±1.76	4.40 ±3.25	11.70 ±1.27	8.30 ±9.61	2.80 ±0.98
	LY (10^3/µ)	330.45 ±18,87^{a}	289.75 ±46.45^{ab}	332.45 ±3.32^{a}	258.45 ±4.45^{b}	319.40 ±21.92^{ab}
	MO (10^3/µ)	57.80 ±15.41	56.80 ±38.18	47.25 ±15.06	54.05 ±20.71	73.60 ±10.32
	NE (10^3/µ)	11.00 ±7.35	17.75 ±16.33	51.15 ±4.59	31.80 ±38.74	11.25 ±3.04
	HCT (%)	51.66 ±2.88	53.66 ±7.76	50.00 ±5.56	50.66 ±1.52	48.66 ±4.16
	Hb (g/dL)	10.15 ±1.20	7.65 ±4.03	11.50 ±0.14	8.40 ±1.55	11.35 ±0.35
The values of red blood cell findings	**MCV (µm^3)**	613.06 ±39.54	590.17 ±58.54	496.34 ±90.02	577.12 ±0.92	501.75 ±33.22
	MCH (pg)	118.12 ±1.56	84.53 ±36.76	112.76 ±1.74	96.43 ±21.67	118.91 ±2.46
	MCHC (%)	19.30 ±0.98^{ab}	14.08 ±4.83^{b}	23.06 ±3.83^{ab}	16.71 ±3.78^{ab}	23.76 ±2.06^{a}

*Data are presented as the means ±SEM (n=3) values within the same row having different superscipts are significantly different (p<0.05).
*Hb, haemoglobin, Hct, hematocrit, RBC, red blood cells, MCV, meancell hemoglobin volume MCH meancell haemoglobin, MCHC, meancell haemoglobin concentration. Data represent as mean ±SE. Within a row, means with differing letters are significantly different (p<0.05).

At the end of the trial, biochemical and immunologic values in serum samples showed no significant result in experimental groups and control group (Table 4).

Table 4. Biochemical and immunological analysis of rainbow trout fed with different levels sumac powder for 8 weeks.

		Groups				
		Control	**1.0 g kg^{-1}**	**3.0 g kg^{-1}**	**5.0 g kg^{-1}**	**10.0 g kg^{-1}**
Serum biochemical values	**T.PRO. g/dL**	3.65±0.43	3.19±0.13	3.89±0.16	3.72±0.37	3.84±0.07
	GLOB g/dL	2.21±0.24	1.99±0.12	2.35±0.05	2.26±0.26	2.30±0.12
	ALB g/Dl	1.44±0.19^{ab}	1.20±0.01^{b}	1.54±0.10^{a}	1.46±0.10^{ab}	1.53±0.04^{a}
Serum immunologic values	**IgM (µg/ml)**	62.33±15.14	55.00±15.00	65.00±17.43	67.00±9.84	67.66±11.01
	Lysozyme (mg/ml)	0.005±0.00	0.006±0.00	0.006±0.00	0.006±0.00	0.007±0.00

*Values are mean ±SEM (n=6) Within a row, means with differing letters are significantly different (p<0.05).

Challenge results indicated that the fish fed with SFP supplemented diets had determined better survival rates against *V. anguillarum*. All four treated groups (1.0, 3.0, 5.0 and 10.0 g kg^{-1}) showed reduced mortality compare to the control. The fish received 10.0 g kg^{-1} SFP supplemented diet

performed best result that did not seen mortality. Sumac supplemented diets caused higher relative percentage survival (RPS) than control group (Table 5).

Table 5. The mortality rate of fish fed with diets containing different concentrations of SFP for 8 week and challenged with *V. anguillarum* pathogen.

Groups	Mortality (%)	RPS
Control	56.66±0.00[a]	-
1.0 g kg^{-1}	33.42±0.13[b]	41.08±0.12[d]
3.0 g kg^{-1}	7.31±0.60[c]	87.41±0.58[c]
5.0 g kg^{-1}	5.65±0.14[d]	90.10±0.14[b]
10.0 g kg^{-1}	0.00±0.00[e]	100.00±0.00[a]

*Values are mean ±SEM (n=3) Within a column, means with differing letters are significantly different ($p<0.05$).

DISCUSSION

The use of plant products as health promoters is a very topical concept in aquaculture (Citarasu, 2010; Yılmaz et al., 2013; Ahmadifar et al., 2014; Gormez and Diler, 2014; Metin et al., 2015; Diler et al., 2017b). In some studies, the *in vitro* antibacterial assays carried out on sumac (*R. coriaria* L.) used either ethanol or water extracts (Nimri et al., 1999; Nasar-Abbas and Halkman, 2004; Candan and Sökmen, 2004; Gulmez et al., 2006; Akrayi et al., 2016). The water and hydro-methanol extracts obtained from the fruits of sumac were found to have a great inhibitory activity against bacterial species (Nasar-Abbas and Halkman, 2004).

In this study, fish fed with the sumac-supplemented diet challenged with *V. anguillarum* had better survival rates against *V. anguillarum*. All four experimental groups (1.0, 3.0, 5.0, and 10.0 g kg^{-1}) reduced mortality compared to the control. The 10.0 g kg^{-1} diet showed no mortality. The fish fed with a sumac supplemented diet showed high relative percentage survival (RPS). There was an inverse relationship between the mortality rate and the amount of plant extract in the diet. As the amount of plant extract increased in the diet, the rate of mortality decreased. Similar results have been reported by Gharaei et al. (2020) that sumac increased resistance to the *Yersinia ruckeri* in rainbow trout. In another study performed by Diler et al. (2015) that *Artemisia vulgaris* provided resistance to challenge with pathogenic bacteria, *Vibrio anguillarum* in rainbow trout. Nya and Austin (2011) dietary garlic application reduced the mortality rate of *A. hydrophila* infection compared to control in rainbow trout. Further, Feng et al. (2020) reported that dietary administration of *Rehmannia glutinosa* polysaccharide significantly reduced fish mortality.

The knowledge of the mechanism for the antimicrobial activity of spices and herbs is very limited. In the present study, gallic acid was determined as a major component of phenolics of sumac (Al-Boushi et al., 2014). Phenolics have antibacterial effects against bacteria due to their toxicity and effects on bacterial enzymes (Cowan, 1999). Also, Gabr et al. (2014) analyzed sumac extracts by GC-MS methods. They found that phenols (41.8%), glycosides (19.4%), alkaloids (17.5%) and terpenoids (11.3%) were major components. The abundance of polyphenols may explain the mechanisms for the antimicrobial activity of sumac (Abu-Reidah et al., 2014). Therefore, in the present study, the percentage mortality was significantly decreased in fish fed sumac powder supplemented diet challenged with *Vibrio anguillarum*.

The blood parameters have been used as a diagnostic parameter for the investigation of disease and physiological disorders (Fazio, 1999). In this study, haematological parameters (RBC, WBC), differential leukocytes count (LY, MO, NE), Hct, Hb, the mean values of cell hemoglobin (MCH pg), cell hemoglobin concentration (MCHC %), and cell hemoglobin volume (MCV μm^3) were no adversely affected in fish feeding with sumac powder when compared control group. These observations are in agreement with the obtained results of other researchers, who reported that rainbow trout treated with *Origanum vulgare* extract and carvacrol powder were no significant differences in RBC, Hb, MCV, MCH, MCHC parameters (Ahmadifar et al., 2011; Haghighi and Rohani, 2015; Yılmaz et al., 2015). In contrast, Gharaei et al. (2020) observed that WBC and RBC, lymphocyte, monocyte, and neutrophil value was significantly increased in fish fed a sumac supplemented diet.

Serum proteins are various humoral elements of the non-specific immune system, measurable total protein albumin and globulin levels suggest that high concentrations are likely to be a result of the enhancement of the non-specific immune response of fish. In this study, total protein (TP), globulin

(GL), and albümin (ALB) values had no significant differences on the 8th week. Similar results was reported in *Dicentrarchus labrax* fed carvacrol supplemented diet after 4 and 8 weeks (Volpatti et al., 2014) and in nile tilapia fed the chinese herbs supplemented diet (Ardo et al., 2008). In contrast, the use *Laurus nobils*, *Cotinus coggyria* and *Origanum vulgare* enhanced the non-specific immune parameters in rainbow trout (Bilen ve Bulut, 2010; Bilen et al., 2011; Haghighi and Rohani, 2015).

Immunoglobulin M (IgM) is the major component of humoral immune system. Also, lysozyme is a humoral component of the non-specific defense mechanism which can prevent the growth of bacteria by splitting β-1,4 glycosidic bonds in the peptidoglycan of bacterial cell walls, resulting in bacteriolysis (Ellis, 1999). However, this study was found no effect on plasma lysozyme and Immunoglobulin M (IgM) level at the 8th week. This result is opposed to Gharaei et al. (2020) that reported significant stimulation in lysozyme activity of rainbow trout with sumac diet. Similarly, Feng et al. (2020) reported that the lysozyme activity increased *Rehmannia glutinosa* polysaccharide in common carp.

CONCLUSION

In conclusion, administration of *Rhus coriaria* (sumac) fruit powder as a dietary supplement reduced the mortality rate against *Vibrio anguillarum* infection. This is the first study to provide data that the sumac powder evaluated against *V. anguillarum* possess *in vivo* potential antibacterial activity. *Rhus coriaria* can be utilized as a health promoter in rainbow culture.

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Ecological Requirements of Larval Simuliidae (Insecta, Diptera) Species of some Streams in Camili Valley (Artvin, Turkey)

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Research Article

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Abstract

This study was carried out in July 2016 in Camili (Macahel) which is Turkey's first and only biosphere reserve area. This study aimed to investigate the Simuliidae species of some streams in Camili Valley, to identify the "reference site" which are important for the Water Framework Directive (WFD), to determine ecological characteristics of the studied sites according to System A and System B Classification of Water Framework Directive (WFD), to explore the relationship between the recorded species and some environmental variables of streams by using CCA technique. For this purpose, 257 individuals of Simuliidae were sampled from nine studied sites. Physicochemical variables (dissolved oxygen, pH, water temperature, electrical conductivity, velocity) were measured at each studied site. In Camili Valley the following species were collected: *Simulium (Nevermannia) angustitarse, Simulium (Nevermannia) cryophilum, Simulium (Nevermannia) vernum, Simulium (Simulium) bezzii, Simulium (Simulium) tuberosum, Simulium (Simulium) variegatum*. Because anthropogenic stresses were insignificant in the research area, studied sites and Simuliidae fauna of the area were not affected negatively. Also, six of the studied sites have reference habitat conditions.

Keywords: Canonical Correspondence Analysis, Camili (Macahel), physicochemical variables, reference site, Simuliidae.

Camili Vadisi'ndeki (Artvin, Türkiye) bazı akarsuların larval Simuliidae (Insecta, Diptera) türlerinin ekolojik gereksinimleri

Özet

Bu çalışma, Temmuz 2016 tarihinde Türkiye'nin ilk ve tek biyosfer rezerv alanı olan Camili (Macahel) bölgesinde gerçekleştirilmiştir. Çalışma ile Camili Vadisi'nde bulunan bazı akarsulardaki Simuliidae türlerinin ve Su Çerçeve Direktifi (SÇD) için önem taşıyan "referans istasyon" ların belirlenmesi, Su Çerçeve Direktifi (SÇD)'nin Sistem A ve B sınıflandırmasına göre çalışılan istasyonların ekolojik karakterlerinin tespit edilmesi, CCA analizi kullanılarak bazı çevresel değişkenler ile kaydedilen türler arasındaki ilişkinin ortaya çıkarılması amaçlanmıştır. Bu amaçla, dokuz örnekleme noktasından Simuliidae familyasına ait 257 birey toplanmıştır. Her örnekleme alanında fizikokimyasal değişkenler (su sıcaklığı, pH, elektriksel iletkenlik, çözünmüş oksijen, akıntı hızı) ölçülmüştür. Teşhisler sonucunda *Simulium (Nevermannia) angustitarse, Simulium (Nevermannia) cryophilum, Simulium (Nevermannia) vernum, Simulium (Simulium) bezzii, Simulium (Simulium) tuberosum, Simulium (Simulium) variegatum* türleri tespit edilmiştir. Çalışma alanında antropojenik baskı önemsiz düzeyde olduğu için çalışılan istasyonlar ve Simuliidae faunası olumsuz etkilenmemiştir. Ayrıca, istasyonlardan altı tanesi referans istasyon özelliği göstermektedir.

Anahtar Kelimeler: Camili (Macahel), fizikokimyasal değişkenler, Kanonik Uyum Analizi, referans istasyon, Simuliidae.

INTRODUCTION

The family Simuliidae has spread on all continents except Antarctica. According to the most recent edition of the inventory of world Simuliidae (Adler, 2020), Simuliidae fauna comprises 2348 species (2331 living and 17 fossils). Larvae of Simuliidae, an important member of aquatic invertebrate communities, play a significant role in aquatic food webs (Crosskey, 1990). Filter-feeding larvae feed on dissolved organic matter (DOM) and suspended particles (Wotton, 2009; Ciborowski et al., 2017).

Simuliidae species can be used as a bioindicator for indicating habitat degradation of streams (Feld et al., 2002). Many environmental factors such as dissolved oxygen, pH, electrical conductivity, water temperature, substrate structure influence the distribution of blackflies (Ross and Merritt, 1978;

Lautenschlager and Kiel, 2005). Some Simuliidae species are very sensitive to environmental changes, while some species are tolerant to changes in habitats (Seitz, 1992; Feld et al., 2002; Kazancı, 2006).

The European Water Framework Directive (WFD), which is the most comprehensive water legislation of the European Union (EU), aims to protect the ecological status of aquatic ecosystems, to prevent their degradation, to restore all water bodies, and to conserve water resources in Europe (Council of European Communities, 2000). Biomonitoring of the ecological quality of aquatic ecosystems is very important and necessary for WFD studies. Benthic macroinvertebrates are widely used as bioindicators for monitoring habitat quality (Rosenberg and Resh, 1983; De Pauw et al., 2006). Another purpose of WFD is to determine which water bodies could be classified as "reference conditions". The WFD defines the reference conditions as the conditions that prevail in the absence or near absence of human disturbance or alteration (Council of European Communities, 2000).

The number of WFD studies using macroinvertebrates has increased in last 10 years in Turkey (Kazancı and Ertunç, 2010; Kazancı et al., 2010a; Kazancı et al., 2010b; Duran and Akyıldız, 2011; Ekingen and Kazancı, 2012; Kazancı et al., 2013a; Kazancı et al., 2013b; Zeybek et al., 2014; Kazancı et al., 2015; Türkmen and Kazancı, 2015; Arslan et al., 2016a; Arslan et al., 2016b; Başören and Kazancı, 2016; Bolat et al., 2016; Türkmen and Kazancı, 2016; Gültekin et al., 2017; Kazancı et al., 2017; Zeybek, 2017; Akay and Dalkıran, 2019; Gültekin, 2019).

Eastern Black Sea Region, known as a sub-ecoregion of the Caucasus Biodiversity Hotspot, which is one of the 25 World Biodiversity Hotspot regions (Myers et al., 2000; Kazancı et al., 2011). The Caucasus is one of the Worldwide Fund for Nature's (WWF) Global 200 Ecoregions, identified as globally outstanding for biodiversity. Camili (Macahel) is surrounded by Karçal Mountains on three sides. This area was declared by United Nations Educational, Scientific, and Cultural Organization to be the first and only "Biosphere Reserve Area" in Turkey (UNESCO, 2005).

This study aimed to investigate the Simuliidae species of some streams in Camili Valley, to identify the "reference site" which are important for the Water Framework Directive (WFD), to determine ecological characteristics of the studied sites according to System A and System B Classification of WFD, to explore the relationship between the recorded species and some environmental variables of streams by using CCA technique.

MATERIAL AND METHODS

Camili (Macahel), which is in Borçka district of Artvin province in northeastern Turkey, is located in a valley on the slopes of Karçal Mountains (Figure 1). Since this area has not been much impacted by anthropogenic activities, the streams, especially at higher altitudes, are slightly polluted or unpolluted.

Nine (9) sites were sampled from running waters which are located in Camili in July 2016. In each site, water temperature, pH, electrical conductivity, dissolved oxygen concentration, and velocity were measured in the field by using a YSI 556 multi-probe system and Hydro Bios current meter RHCM. The water quality classes of the studied sites were evaluated by using the Surface Water Quality Regulation Annex-5 (Anonymous, 2015).

Larvae and pupae of Simuliidae were collected by a standard D-shaped pond net and by hand. Samples were preserved in 80% ethyl alcohol. Leica MZ75 stereomicroscope and Olympus CX21FS1 binocular microscope were used for identifications. Simuliidae species were identified according to Rubtsov, 1990; Crosskey, 2002; Jedlicka et al., 2004; Lechthaler and Car, 2005; Crosskey and Zwick 2007.

To investigate the relationships between Simuliidae species and the environmental variables, canonical correspondence analysis (CCA) was performed using the ECOM 2.1.3.137 version (Henderson and Seaby, 2007) software programs.

Some geological characteristics required for System A and System B of WFD and physical variables of the studied sites were recorded for the definition of the stream types.

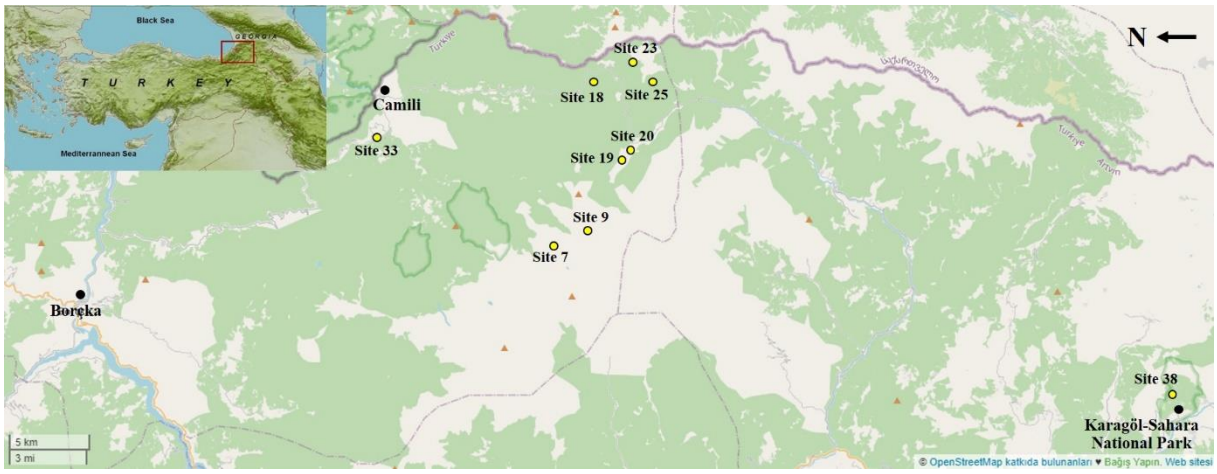


Figure 1. Studied area and collecting sites of Camili Valley.

RESULTS

A total of 257 blackfly larvae and pupae were collected, and six species were identified from nine studied sites. Identified species were *Simulium (Nevermannia) angustitarse*, *Simulium (Nevermannia) cryophilum*, *Simulium (Nevermannia) vernum*, *Simulium (Simulium) bezzii*, *Simulium (Simulium) tuberosum*, *Simulium (Simulium) variegatum* (Table 1). According to this table, the highest species number (4) was found in Site 9 and 20 while the lowest species number (1) was found in Site 25 and 33.

Table 1. Simuliidae species list of collecting sites.

Species/Sites	7	9	18	19	20	23	25	33	38
S. (N.) angustitarse					*	*			*
S. (N.) cryophilum		*			*	*			
S. (N.) vernum			*		*				
S. (S.) bezzii		*		*					
S. (S.) tuberosum	*	*	*	*	*				*
S. (S.) variegatum	*	*	*	*			*	*	*

The results of the physicochemical variables (water temperature, pH, electrical conductivity, dissolved oxygen concentration, velocity) were given in Figure 2.

The water temperature values recorded were between 7.03 and 18.15 °C. The dissolved oxygen values recorded were between 8.5 and 11.8 mg/l. The pH values recorded were 2.84 and 6.53. The electrical conductivity values recorded were between 29 and 85 µS/cm. The velocity values recorded were between 0.33 and 2.33 m/s.

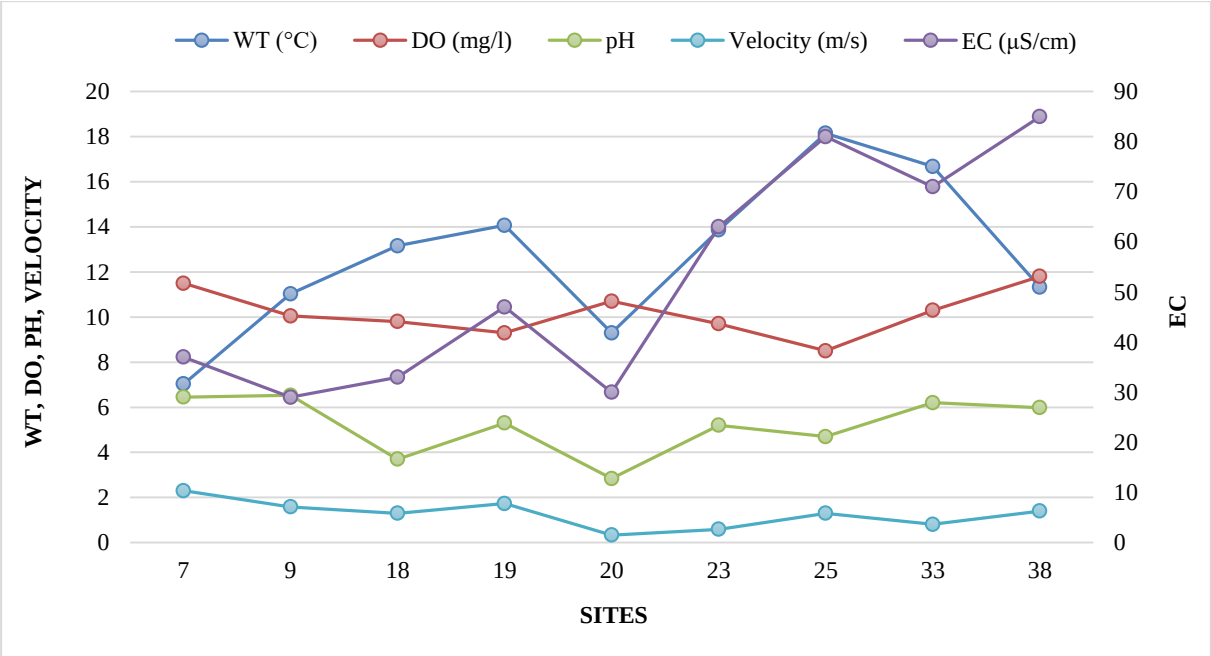


Figure 2. Values of water temperature (°C), dissolved oxygen (mg/L), pH, electrical conductivity (µS/cm), and velocity (m/s)

The nine sites and six species detected in these sites were shown in the CCA diagram (Figure 3). The order of significance of environmental variables was found to be, from most significant to least significant: velocity, electrical conductivity, water temperature, dissolved oxygen concentration, and pH.

Some physical and geological characteristics of studied sites required for System A and System B of WFD were given in Table 3.

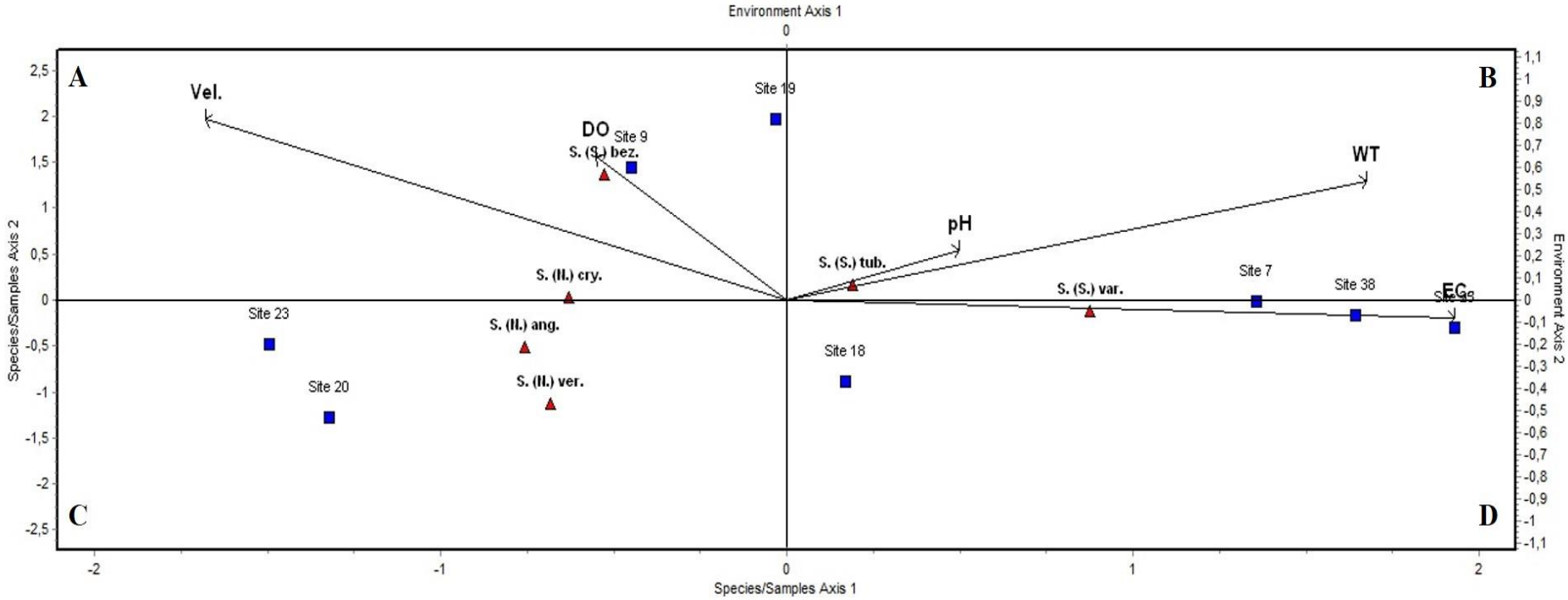
Table 2. Water quality classes of the studied sites according to the physicochemical variables (Anonymous, 2015)

Sites	Site name	WT (°C)	DO (mg/l)	pH	EC (µS/cm)	Final Water Quality Class
7	Lekoban Plateau-1	I	I	I	I	III
9	Lekoban Plateau-2	I	I	I	I	I
18	Merata Plateau-1	I	I	IV	I	IV
19	Merata Plateau-2	I	I	IV	I	IV
20	Merata Plateau-3	I	I	IV	I	IV
23	Çurupira Plateau-1	I	I	IV	I	IV
25	Çurupira Plateau-3	I	I	IV	I	IV
33	Gohinav Stream	I	I	III	I	III
38	Karagöl-1	I	I	IV	I	IV

(WT: temperature, DO: dissolved oxygen, EC: electrical conductivity)

Table 3. Geological and physical characteristics of sampling sites according to System A and System B Classification of WFD

	Site 7	**Site 9**	**Site 18**	**Site 19**	**Site 20**	**Site 23**	**Site 25**	**Site 33**	**Site 38**
Ecoregion (System A)	Y	Y	Y	Y	Y	Y	Y	Y	Y
Altitude (System A)	High (>800m)	High (>800m)	High (>800m)	High (>800m)	High (>800m)	High (>800m)	High (>800m)	Medium (200m-800m)	High (>800m)
Altitude (System B)	2386m	2388	2092	2171	2190	2190m	1963m	563m	1642m
Catchment Area (System A)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)
Geology (System A and System B)	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous
Latitude (System B)	41° 24' 0.84" N	41° 24' 0.61" N	41° 29' 0.90" N	41° 26' 5.41" N	41° 26' 4.94" N	41° 30' 0.28" N	41° 29' 6.59" N	41° 27' 5.4" N	41° 18' 34" N
Longitude (System B)	42° 02' 2.87" E	42° 03' 3.85" E	42° 03' 9.81" E	42° 04' 5.53" E	42° 04' 6.90" E	42° 05' 2.79" E	42° 06' 1.28" E	41° 53' 54.32" E	41° 28' 56" E
Substratum	30% rock, 50% stone, 15% gravel, 5% sand	40% rock, 40% stone, 15% gravel, 5% sand	40% rock, 40% stone, 15% gravel, 5% sand	20% rock, 50% stone, 25% gravel, 5% sand	20% rock, 50% stone, 20% gravel, 10% sand	20% rock, 50% stone, 20% gravel, 10% sand	40% rock, 40% stone, 15% gravel, 5% sand	30% rock, 40% stone, 20% gravel, 10% sand	20% rock, 35% stone, 30% gravel, 15% sand
Stream Zone	Hypocrenon	Hypocrenon	Hypocrenon	Epirhithron	Epirhithron	Epirhithron	Epirhithron	Metarhithron	Epirhithron
Riparian vegetation	0%	10%	10%	10%	0%	40%	10%	100%	50%
Stream width in dry period	1.5m	1.5m	2m	4m	1m	2m	2m	9m	4m
Stream width in wet period	2.5m	3.5m	1m	15m	1m	7m	8m	15m	8m



Abbreviations: *S. angustitarse* – *S. ang; S. cryophilum* – *S. cry; S. vernum* – *S. ver; S. bezzi* – *S. bez; S. tuberosum* – *S. tub; S. variegatum* – *S. var*; velocity – Vel; dissolved oxygen – DO; water temperature – WT; electrical conductivity – EC.

Figure 3. CCA diagram with environmental variables and Simuliidae species (▲: Species, ■: Sites)

DISCUSSION AND CONCLUSION

Investigation of the Simuliidae fauna of Turkey is necessary and important for biological monitoring studies and WFD research. In this study, the Simuliidae species composition of nine sites in Camili Valley were determined, information on their habitat preferences was given, and some physicochemical variables of sites were measured. The pH values were low in almost all studied sites because of snowmelt and rainfall runoff in the research area. These events during spring and early summer, cause a short-term decline in alkalinity. This process is called episodic acidification and was reported by Kazancı (2009) for the first time in the Eastern Black Sea Region and the Yeşilırmak River Basin.

In almost all studied sites, pH value was associated with Class III and Class IV water quality while other physicochemical variables were associated with Class I water quality. Blackflies may be more tolerant to decreases in pH value than another group of freshwater macroinvertebrates (Bernard et al., 1990; Chmielewski and Hall, 1992). In other words, many blackfly species will be able to cope with the short-term decline in alkalinity. Therefore, Simuliidae species were sampled in our studied sites, even under low pH conditions caused by episodic acidification.

Six of the studied sites (Site 7, 9, 18, 19, 20, and 23) have reference habitat conditions. There are no anthropogenic impacts or habitat degradation in the study area and all sites have high water quality according to physicochemical variables (except pH values due to episodic acidification). Site 25 and 33 were located near the villages. Site 38 was located in Karagöl-Sahara National Park and samples were collected from inlet stream. Also, there was a regulator near Site 38. Therefore, these sites have not to reference habitat conditions.

The relationship between six Simuliidae species and some physicochemical variables (water temperature, dissolved oxygen, pH, electrical conductivity, and velocity) of streams were shown in the CCA diagram (Figure 3). The determinant environmental variables in quadrant A were velocity and dissolved oxygen. According to the CCA diagram, *S. (S.) bezzii* was positively correlated with these two variables. *S. (S.) bezzii* usually lives in fast-flowing unimpaired running waters, but it also can live in degraded eutrophic waters (Seitz, 1994; Lechthaler and Car, 2005). This species prefers oligosaprobic and betamesosaprobic habitats (CSN 75 7716, 1998). There is no information concerning stream zonation preferences in the literature. Başören and Kazancı (2017) recorded this species from crenon, epirhithron, and metarhithron zone of the streams in the Eastern Black Sea Region. Site 9 and 19 were placed in quadrant A. *S. (S.) bezzii* was recorded only from these sites. Site 9 was located in hypocrenon zone of the mountain stream in Lekoban Plateau and has Class I water quality. Site 19 was located in epirhithron zone of the mountain stream in Merata Plateau and has Class IV water quality because of pH value.

The determinant environmental variables in quadrant B were pH and water temperature. *S. (S.) tuberosum* was placed in this quadrant but it was close to the center of the CCA diagram because of a weak correlation with the environmental variables. This species was the second common species after *S. (S.) variegatum* in this study. It was recorded from six sites (Site 7, 9, 18, 19, 20, and 38). *S. (S.) tuberosum* was previously recorded by Kazancı and Ertunç (2008) in Artvin province. It inhabits mostly medium sized upland streams with fast-flowing, but it can be found in wide streams with rich vegetation and slow current (Zahar, 1951; Bass, 1998). This species primarily prefers oligosaprobic and betamesosaprobic habitats, but it also occurs in xenosaprobic habitats. It has wide habitat preferences range from epirhithron to epipotamon, but *S. (S.) tuberosum* prefers mainly metarhithron and hyporhithron of streams (Lechthaler et al., 2017). Site 7 was placed in between quadrant B and D. In this site, only *S. (S.) tuberosum* and *S. (S.) variegatum* were recorded. Therefore, they were closely located in the CCA diagram. Site 7 was located in hypocrenon zone of the mountain stream in Lekoban Plateau and has Class I water quality. *S. (S.) tuberosum* was collected from the other two sites (Site 9 and 18) in hypocrenon zone of streams. That is, it can be said that this species can also prefer this region of streams.

There was no determinant environmental variable in quadrant C. *S. (S.) angustitarse* and *S. (S.) vernum* were placed in this quadrant. They were negatively correlated with water temperature and pH. *S. (S.) angustitarse* has a widespread distribution through entire Europe. It is a stenothermal species and predominates in clean and cold waters near the source of rivers (Lechthaler and Car, 2005). Therefore, they were placed opposite the representing water temperature variable. However, this species can survive in organically polluted water (Rubtsov, 1990). It mainly prefers betamesosaprobic

habitats, but it also occurs in oligosaprobic and alphamesosaprobic habitats. The stream zonation preferences of this species are epirhithron, metarhithron and hyporhithron mainly, but it occurs also hypocrenon zone of streams (Lechthaler et al., 2017).

S. (S.) vernum can live in a wide range of running waters from small mountain streams to large rivers (Scheder and Waringer, 2002). It is generally found in oligosaprobic and betamesosaprobic habitats, but it can be inhabited in xenosaprobic and alphamesosaprobic habitats. The stream zonation preferences of this species are epirhithron and metarhithron mainly, but it occurs also hypocrenon and hyporhithron zone of streams. (Lechthaler et al., 2017). *S. (S.) vernum* was recorded from Site 18 and 20 with the lowest pH values (3.7 and 2.84 respectively) in this study. Similarly, this species was found by Başören (2015) at three different sites with low pH value (4 and 5.5) in Aksu Stream (Giresun, Turkey). For this reason, it is expected that it was placed opposite the arrow representing pH variable. Site 20 and 23 were placed in quadrant C. *S. (S.) angustitarse*, *S. (S.) vernum* and *S. (N.) cryophilum* were recorded mostly from these sites. *S. (N.) cryophilum* was placed in between quadrant A and C. This species is one of the most common blackfly species in undisturbed mountain brooks (Scheder and Waringer, 2002; Crosskey and Howard, 2004). It prefers also cold and fast-flowing running waters (Kazancı, 2006). Therefore, *S. (N.) cryophilum* was placed opposite the representing water temperature variable and close the representing velocity variable. This species is generally found in oligosaprobic and betamesosaprobic habitats, but it can be inhabited in xenosaprobic and alphamesosaprobic habitats. The stream zonation preference of this species is epirhithron mainly, but it occurs also hypocrenon and metarhithron zone of streams (Lechthaler et al., 2017). Site 20 and 23 that were placed in quadrant C, were located in epirhithron zone of the mountain streams in Merata and Çurupira Plateau respectively. These studied sites have Class IV water quality because of pH value.

The determinant environmental variable in quadrant D was electrical conductivity. *S. (S.) variegatum* was placed in this quadrant. According to the CCA diagram, *S. (S.) variegatum* was positively correlated with this variable. This species was the most abundant (52%) in this study. It is widespread from Europe to the Caucasus (Crosskey and Howard, 2004). It mostly lives in mountain streams and small rivers with high dissolved oxygen concentrations (Kiel, 2001; Scheder and Waringer, 2002). *S. (S.) variegatum* is generally found in oligosaprobic and betamesosaprobic habitats, but it can be inhabited in alphamesosaprobic habitats. Epirhithron, metarhithron, and hiporhithron are stream zonation preferences of this species (Lechthaler et al., 2017). In Başören and Kazancı (2017), this species was recorded mainly from epirhithron zone of the streams in the Eastern Black Sea Region. Additionally, this species was also found in crenon zone. Site 18, 25, 33, and 38 were placed in quadrant D. Sites 25 and 33 do not appear clearly in the CCA diagram because they overlap. The reason for this situation is that only *S. (S.) variegatum* was recorded from both sites. Since Site 25, 33, and 38 have the highest EC values (81, 71, 85 µS/cm respectively), they are expected to be positioned next to the arrow representing EC. *S. (S.) variegatum* has the highest number of individuals (111 individuals) in Site 38. Site 18 was in hypocrenon zone of the mountain stream in Merata Plateau and has Class IV water quality. Site 25 was in epirhithron zone of the mountain stream in Çurupira Plateau and has Class IV water quality. Site 33 was in metarhithron zone of the Gohinav streams and has Class III water quality. Site 38 was situated in Karagöl-Sahara National Park and there was a regulator near the sampling site. This site also has Class IV water quality. Site 25 and 33, which were located near the villages, and Site 38 have not to reference habitat conditions. The reason why these four studied sites have Class IV water quality is the pH value.

Camili (Macahel) declared as the first and only biosphere reserve area of Turkey is one of the biologically richest regions. Anthropogenic impacts on this area are insignificant and six of the studied sites also showed reference habitat conditions. However, biological degradation, tourism activities, hydroelectric power plant construction and road construction have recently threatened aquatic habitats in this region. Because our studied sites were generally in isolated areas, Simuliidae fauna was not affected by these negative impacts. All sites are suitable for the survival of Simuliidae species. In this study, *S. (N.) angustitarse*, *S. (N.) cryophilum*, *S. (N.) vernum*, *S. (S.) bezzii*, *S. (S.) tuberosum*, and *S. (S.) variegatum* were recorded from Camili Valley. However, it can be expected that much more Simuliidae species will be found because this region is rich in biodiversity.

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Effect of Mediterranean Mussels *(Mytilus galloprovincialis)* From Polluted Areas on Hepatotoicity in Rats by Immunuhistochemical Method

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Abstract

Mussels *(Mytilus galloprovincialis*) are aquatic organisms that can accumulate all the factors that pollute the fresh and saltwater environment. Although the Dardanelles has been exposed to environmental pollution for many years, it is also an important region for crustaceans. We aimed to investigate the histopathological changes in the liver parenchyma which is an important gland of the digestive system by feeding these mussels to rats. Twenty-four male Wistar albino were used in the study. The first group (control): fed with standard rat feed, the second group (experiment 1): 4/5 mussel + 1/5 standard rat feed daily; third group (experiment 2): 4/5 mussel + 1/5 standard rat feed every other day, the fourth group (experiment 3): groups were formed with 4/5 mussel + 1/5 standard rat feed every three days. All liver tissue samples taken from the experimental and control groups were stained with hematoxylin-eosin, and immuohitochemically staining inflammatory marker TNF-α and NF-κB after routine histopathological follow-up and analyzed with a light microscope image analysis system. It was observed that mononuclear cells caused inflammation of portal areas, increased sinusoidal dilatation and congestion and degeneration due to vacuolization in hepatocytes in the liver parenchyma of mussel-fed rats. Besides, immunohistochemical staining, TNF-α, and NF-κB immunoreactivity were observed in the liver cells of especially in the second group of rats. As a result, it has been shown that the consumption of mussels obtained and marketed without considering environmental pollution may trigger important digestive system organs of liver diseases.

Keywords: Immunohistochemistry, inflammation, liver, hepatocyte degeneration, mussels.

Kirlenmiş Alanlardan Toplanan Akdeniz Midyesi'nin Ratlarda İmmünohistokimyasal Metotla Hepatotoksik Etkisi

Özet

Midye (*Mytilus galloprovincialis*), tatlı ve tuzlu su ortamını kirleten tüm faktörleri biriktirebilen sucul organizmalardır. Çanakkale Boğazı uzun yıllar boyunca çevre kirliliğine maruz kalsada, kabuklular için de önemli bir bölgedir. Bu midyelerin sindirim sisteminin önemli bir bezi olan karaciğer parankimindeki histopatolojik etkilerini araştırmayı amaçladık. Çalışmada 24 adet erkek Wistar albino türü rat kullanıldı. Birinci grup (kontrol): standart sıçan yemi ile beslendi, ikinci grup (deney 1): hergün 4/5 midye + 1/5 standart sıçan yemi; üçüncü grup (deney 2): 4/5 midye + 1/5 standart sıçan yemi 2 günde bir, dördüncü grup (deney 3): gruplar 4/5 midye + 1/5 standart sıçan yemi ile her üç günde bir oluşturuldu. Deney ve kontrol gruplarından alınan tüm karaciğer doku örnekleri, histopatolojik takipten sonra rutin hematoksilin-eozin ve enflamatuar belirteç olan TNF-α ve NF-κB ile immünohistokimyasal boyama yapıldı ve ışık mikroskobu görüntü analiz sistemi ile analiz edildi. Mononükleer hücrelerin portal alanlarda yangıya sebep olduğu, karaciğer parankimindeki hepatositlerde vakuolizasyonun dejenerasyon sonucu meydana geldiği, santral ven ve sinüzoidal dilatasyon ve konjesyonun olduğu gözlemlendi. Ek olarak, özellikle ikinci sıçan grubunun karaciğer hücrelerinde immünohistokimyasal boyama, TNF-α ve NF-κB şiddetli immünoreaktivitesi gözlendi. Sonuç olarak, çevre kirliliği dikkate alınmadan elde edilen ve pazarlanan midye tüketiminin sindirim sisteminin önemli bir organı olan karaciğer hastalıklarını tetikleyebileceği gösterilmiştir.

Anahtar kelimeler: İmmünohistokimya, inflamasyon, karaciğer, hepatosit hasarı, midye

INTRODUCTION

The relationship between healthy food and quality of life is gaining importance day by day. Accordingly, it increases consumers' orientation towards natural and delicious products that are not contaminated with drugs or chemicals. Despite all these positive thoughts, the increasing population every day and the increase in food consumption parallel to this increase in the orientation towards alternative food sources. Consumption in fish and shellfish occasionally bursts. Especially in coastal areas, there are seashells that have become food culture. Creatures such as mussels, oysters, and sea urchins are among the more preferred products as nutrients. Mussels are organisms that feed on organic and inorganic substances in the water. Mussels can also filter toxic substances during water filtration. Because of these properties, they can store harmful substances in water in their bodies al. (Figueras et al., 2019). The mussels are fed with microorganisms that secrete neurotoxins, especially in the summer months. Also, they filter the water and accumulate heavy metals, pesticides, and bacteria in the tissues. As a result of the pollution of seawater with environmental factors, toxin levels increase in the body of these creatures (Voudanta et al., 2016). Dardanelles is the marine area where the mussel population is the most and it is very prone to contamination in terms of location. The seas have indeed been polluted for years with domestic waste, pesticides, and industrial waste (Yigit et al., 2018).

Most of the liver studies have been conducted to examine regeneration as a result of either experimental or chemical damage (Palmes & Spiegel, 2004). TNF-α plays a role in the pathophysiology of TNF-α, viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease and, ischemia-reperfusion injury in the liver. Studies have shown that when the liver enters the degenerative process, TNF-α induces increased synthesis in hepatocytes and Kupffer cells in the cytoplasms (Yang & Seki, 2015) When oxidative stress in tissues and associated damage to cells increases, activation of the TNF-α receptor is followed by activation of the nuclear factor kappa B (NF-κB). Thus, NF-κB goes to the nucleus, and then NF-κB activates genes that try to block TNF-induced apoptosis. In resting cells, NF-κB is an inactive form in the cytoplasm (Karin, 2006; Schattenberg & Schuchmann, 2009).

Heavy metals are defined as metallic elements with a relatively high density than water (Fergusson, 1990). Heavy metals contain metalloids and heavy metals such as arsenic can have toxic effects even at low doses (Pourret & Hursthouse, 2019). In recent years, there has been an increasing ecological and global public health concern associated with environmental pollution by these metals. Besides, human exposure has increased significantly as their use has increased exponentially in some industrial, agricultural, domestic and technological applications (Tchounwou et al., 2012). In biological systems, it has been reported that heavy metals affect cellular organelles and components such as cell membranes, mitochondria, lysosomes, endoplasmic reticulum, nuclei, and enzymes involved in metabolism, detoxification, and damage repair (Wang & Shi, 2001). Metal ions have been found to interact with cell components such as DNA and nuclear proteins and cause conformational changes that can lead to DNA damage and cell cycle modulation, carcinogenesis, or apoptosis (Chang et al., 1996; Hubbard, 2005). Various forms of heavy metals, pesticides, viral and bacterial organisms that crustaceans filter and accumulate in their tissues also pass to mammals that consume these creatures (Gorinstein et al., 2008). As a result, it will inevitably produce important histopathological results in the liver. In this study, we aimed to draw attention to the consumption of seafood consumption in a healthier and more reliable environment by showing the changes in the liver tissue with histochemistry and immunohistochemical techniques by giving them to the rats with an experimental nutrition model in vivo.

MATREIALS and METHODS

The mussels used in the study were removed by diving from various locations determined in the Dardanelles of April- May 2019. Mussels were selected from the same region and those close to the same size. Mussel muscle tissue was assumed to be contaminated based on analysis results. The mussels were boiled in the shell and after the water was taken in the oven, they were turned into o pellet and fed to the subjects as feed. In this study, 24 male Wistar albino rats (250-300 g in weight) were used. All rats were housed in a 12-hour light and 12-hour dark environment with an average temperature of 22 ± 1°C, humidity 55 ± 5, ventilation and air conditioning system. Rats were given as

much water as they could drink. Standard rat food and mussel were given according to 15% of the weight of each rat in feeding planning (Gezen, 2018).

Experimental groups

The first group (Control, n = 6); Standard rat food,

The second group (Experiment 1, n = 6); 4/5 mussels + 1/5 standard rat food daily,

The third group (Experiment 2, n = 6); 4/5 mussels + 1/5 standard rat food every two days; other day standard rat food,

The fourth group (Experiment 3, n = 6); 4/5 mussels + 1/5 standard rat food every three days; the other two days are standard rat food; It was fed for four weeks.

Ethics Statement

A total of 24 male Wistar albino rats were used in the study. The study protocol was approved by the Canakkale Onsekiz Mart University Ethics Committee for Animal Research (Protocol number: 2020/04-07).

Histopathological Examination

30 days after the start of the study, rats were removed for this study after rats were sacrificed under ketas (150 mg/kg; i.p) and alfazyne 2% (25 mg/kg; i.p) anesthesia. Liver tissue samples from all groups were detected in a 10% neutral buffered formaldehyde solution (Bio Optica) for 24 hours. Tissue samples were passed through graded alcohols and their juices were removed. Then, the tissues passed through xylene were made transparent and the alcohols of the tissues were removed. Paraffin was allowed to enter into the tissue samples passed through xylene + paraffin and paraffin stages in a 60°C oven. Tissue samples removed from paraffin were blocked using a tissue embedding device. Tissue samples taken to the blocks were cut 4-5 microns thick in microtome for routine histopathological staining and taken into a water bath. The tissue samples opened here were taken on a normal slide and Hematoxylin-Eosin staining was applied.

Immunohistochemically Examination

Immunohistochemically reactions were performed according to the ABC technique described. Following this step, the sections were incubated with a polyclonal nuclear factor Kappa-B (NF-κB p50, Abcam), tumor necrosis factor (TNF-α, Abcam), then the sections were incubated with biotinylated anti-mouse Immunoglobulin-G (DAKO LSAB 2 Kit, Invitrogen). Following this step, the sections were incubated with the ABC complex (DAKO LSAB 2 Kit). For background staining, Mayer's Hematoxylin (Gürpınar et al., 2012; Öztürk et al., 2019).

Evaluation of tissue samples and statistics

During the evaluation of the results, the immunoreactivity was evaluated with the H-score method, calculating the ratio of immunopositivity cells to all cells in the selected fields. Immunoreactive cell count was performed by a blinded observer and graded as follows: 0 denoted no staining; 1 denoted weakly; 2 denoted moderate; 3 denoted strong staining in a specified field. The respective score was then calculated using the following formula: H-score = (% stained cells at 0) x 0 + (%stained cells at 1+) x 1 + (%stained cells at 2+) x 2 + (%stained cells at 3+) x3. The H-score value varies from 0 to 300. SPSS 19 version applied for statistical evaluation of the results obtained with this formula. To determine the differences NF-κB and TNF-α immunoreactivities between groups, the Kruskal –Wallis Test, which is one of the nonparametric tests, will be used. $P<0.05$ Difference between the groups will be considered significant.

RESULT and DISCUSSION

Histophatological Findings

Control Group: Hepatocellular damage, biliary tract damage, vascular damage, and sinusoid cell damage, and no histopathological findings of tumor cases were observed when staining liver tissue samples from rats fed with standard rat food every day with Hematoxylin and Eosin (Figure 1). Remark cord structure, sinusoid, and central vein, and portal vein, portal artery, and bile ducts located in the portal area were observed to have normal histological structure.

Experimental groups: The following histopathology table occurred in all experimental groups, respectively.

a- Hepatocellular Damage; Vacuolar degeneration, focal necrosis, inflammatory cells including neutrophils and eosinophils in the lobule and portal area were detected. Vacuolar degeneration in most of the hepatocytes manifested by diffuse swelling, pale staining of the cytoplasm, and the appearance

of the cytoplasmic residues around the nucleus. In some hepatocytes, the picnotic nucleus was observed, lobular inflammation in small foci, periductal inflammation with portal inflammation (Figure 1, Table 1).

Table 1. Histopathological evaluation of liver tissue samples.

	Groups			
Parameters	**Control**	**Experiment 1 (Every day giving mussel)**	**Experiment 2 (Two day giving mussel)**	**Experiment 3 (Three day giving mussel)**
Congestion	-	++++	++	+
Dilatation	+	++++	+++	++
Inflammation	-	+++	++	+

b- Vascular Damage; Central, portal, and sinusoidal dilatation, congestion in portal veins, central veins and sinusoid were observed. These histophatological changes occurred more severely in the first group of mussels given daily. In other groups, liver damage decreased due to mussel consumption (Figure 1).

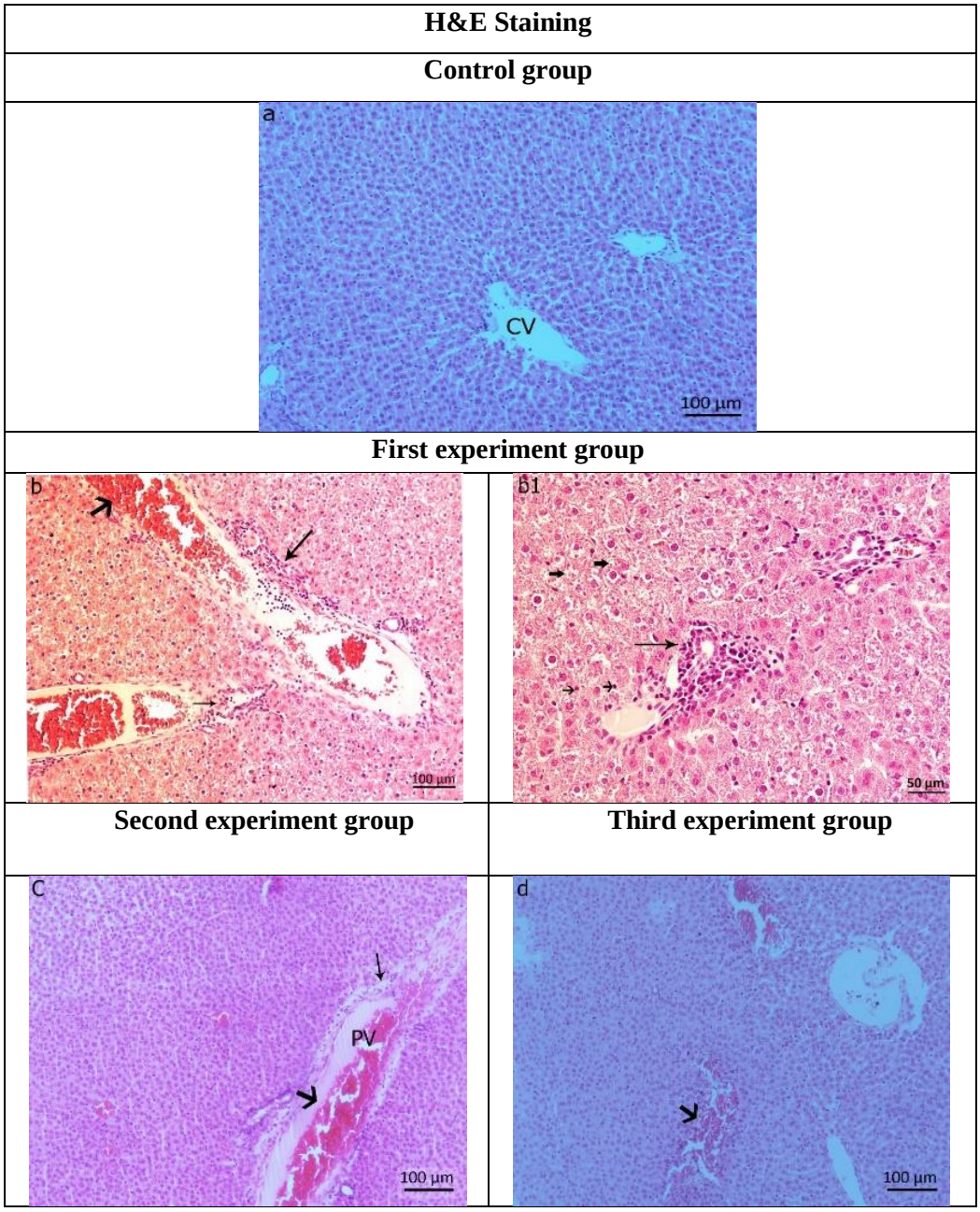


Figure 1. Control and experiment groups of liver tissue, H&E (CV: Central vein, long arrow: inflammation, short arrow: congestion, thick arrow: vacuolar degeneration).

NF- κB and TNF-α findings

Immunohistochemical staining results obtained from our study, we observed that NF-κB and TNF-α expression showed higher immunoreactivity in the liver due to the increase in the first experiment group, and staining was largely in the cell cytoplasm. The apoptotic mechanism was found to be very high in the liver tissues, especially in the first experimental group. Immunohistochemical staining with NF-κB, positive immunoreactivity was observed according to the amount of mussel given (Figure 2 and Figure 3). In the first experiment group, high reactivity was observed around the central vein. In the second and third groups, it was observed that the immunoreactivity around the central vein was moderate. A statistically significant difference was observed between the control and the first

experimental group of the subjects ($p<0.0001$). There was a weak significant difference between the control and third experiment group ($p<0.05$) (Table 2).

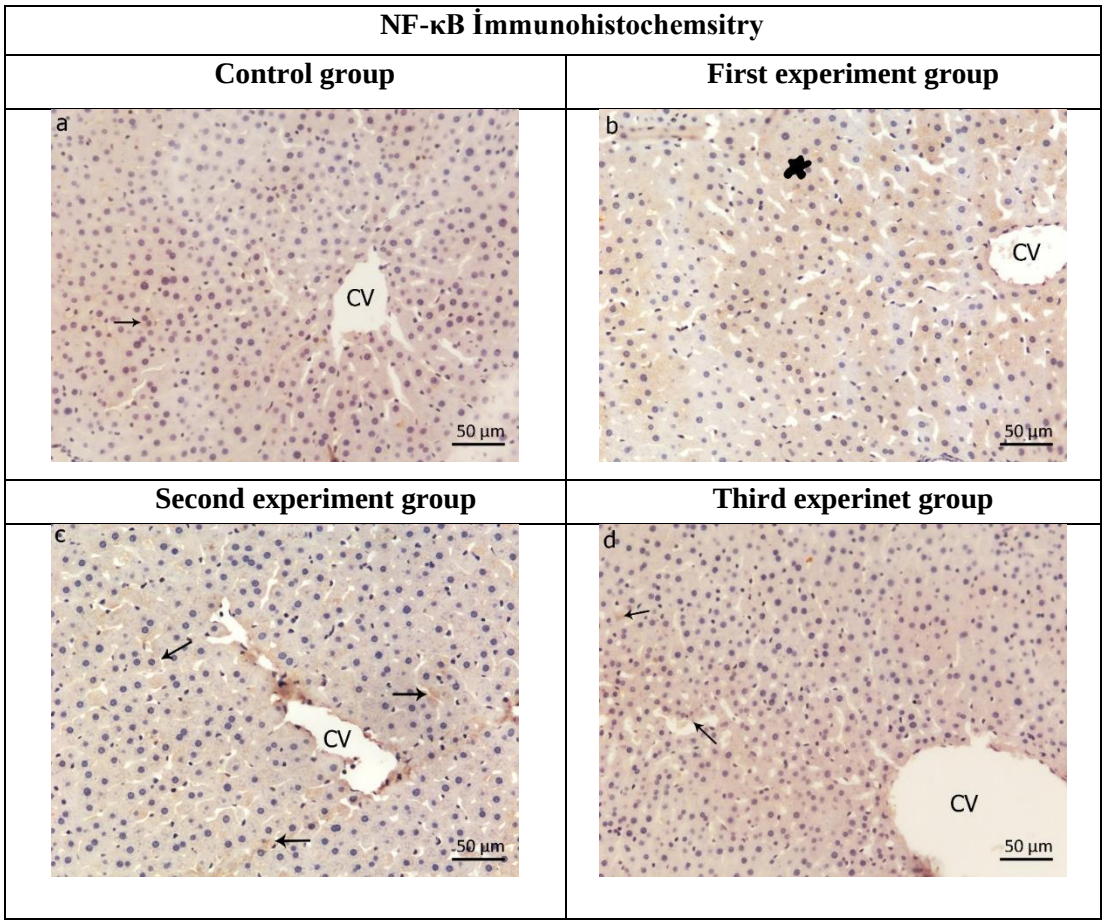


Figure 2. The immunohistochemical distribution of NF-κB in the control and experiment group of liver tissue (star and arrow: immunreactivity, CV: Central vein).

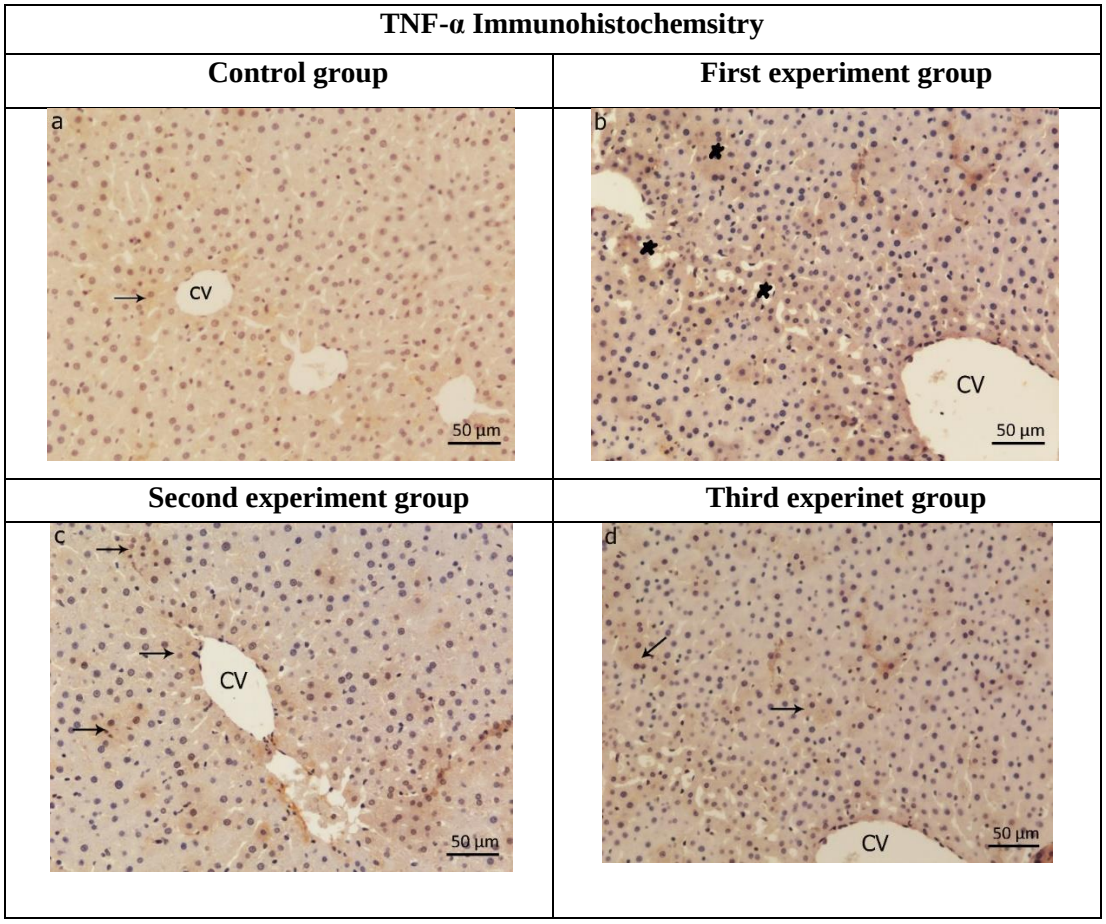
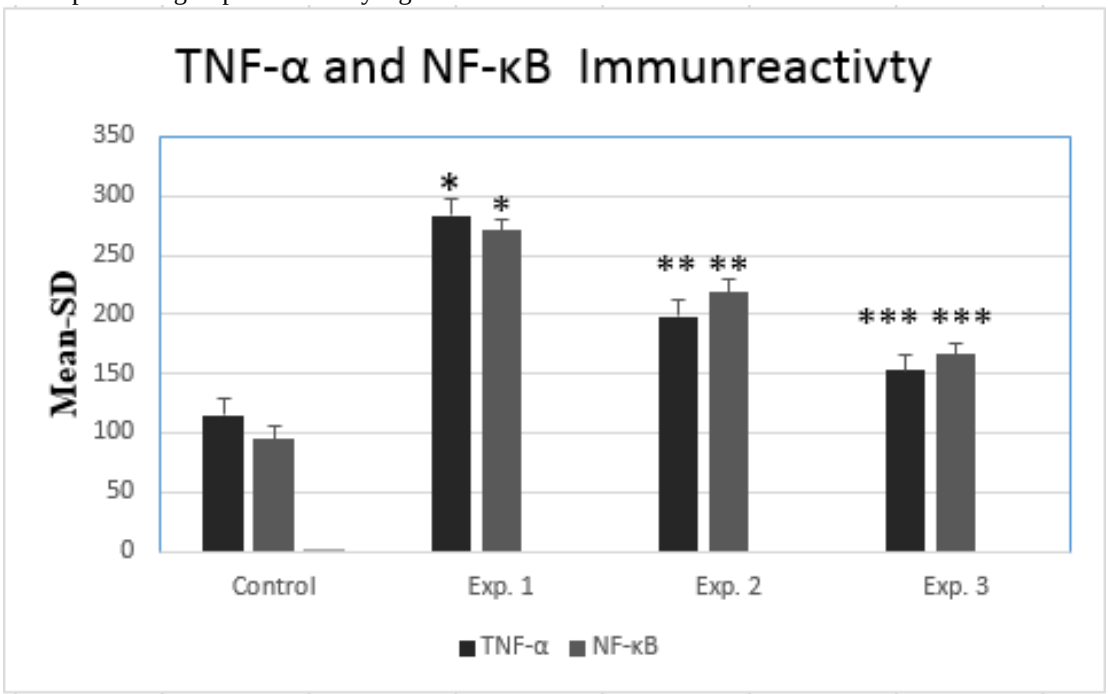


Figure 3. The immunohistochemical distribution of TNF-α in the control and experiment group of liver tissue (star and arrow: immunreactivity, CV: Central vein).

Table 2. TNF-α and NF-κB Immunoreactivty of control and all experiment groups. *p<0.0001 compared to the control group, **p<0.001 compared to the control group, ***p<0.05 compared to the control group. All experiment groups statistically significant differences were determined.



Heavy Metal Analysis Results

According to the research findings, considering the Cd, Pb, Cu, and Zn data in M. galloprovincialis, the places where the heavy metal concentration is the highest are Çamburnu, Yenikordon, and Çardak stations, respectively (Table 3).

Table 3. Heavy metal concentrations of *M. galloprovincialis* muscle tissue (µg/g dry weight.)

	Heavy metals			
Region	***Cd***	***Pb***	***Cu***	***Zn***
Çamburnu	1.22	0,56	1.35	18.74
Yenikordon	1.10	0,45	1.10	16.86
Çardak	0.84	0,42	0.72	16.24
Ortalama	1.05	0.47	1.05	17.28

There were differences and similarities between the groups in terms of histopathological damage in the liver tissues of the rats, which were collected with mussels collected from the Dardanelles. In our study, we researched Mediterranean mussels from shellfish, which have a high potential to cause liver damage to dietary habits. When mussels, which are both commercial and highly nutritious, are obtained from environmentally polluted environments, they can cause permanent damage to many organs, especially the digestive system (Gorinstein et al., 2008). Mussel dishes, which have become food culture especially in coastal areas and consumed frequently, can also cause permanent damage in one of the most important organs of our body, such as the liver. It supports this hypothesis in our research findings. The size of histopathological effects of mussels, which are frequently consumed and collected from unhealthy environments (exposed to industrial wastes), on the liver was found to be considerably large. Studies have reported that toxic substances such as heavy metals accumulate in fish and mussels due to marine pollution (Kayhan et al. 2006). In the researches, the presence of many heavy metals in single and bivalve seafood grown in the Dardanelles was determined (Ustunada et al., 2011). In our country, the annual pollution rate is quite high compared to the regions due to the transit ship passes of the Dardanelles and the Bosphorus. Again in the heavy metal detection studies carried out in the Mediterranean, trace elements such as cadmium, iron, and copper were found to accumulate in the bivalve (Göksu et al., 2005). Our findings show that, in the light of the data obtained from these studies, mussels can accumulate heavy metal and other pollutants in their tissues and create cytotoxic effects in humans and other organisms fed with them.

Long-term to heavy metals such as mercury (Hg), lead (Pb), chromium (Cr), cadmium (Cd), arsenic (As), copper (Cu), vanadium (V), nickel (Ni), and zinc (Zn) It has been reported that exposure may cause certain cancers in humans with chronic inflammation, cardiac, pulmonary and neurological effects (Mantovani et al., 2008; Nieboer et al., 2013). In our study, the detection of prominent foci of inflammation in the portal areas of the liver and parenchymal tissue was found similar to the findings of inflammation caused by the toxic effects of these investigators in people exposed to heavy metal. However, in our study, no findings related to liver cancer were encountered. Literature information has shown that heavy metals can cause cancer (Sá et al., 2016), but our study suggests that there is no cancer in the liver and that mussels are fed to rats for a short time, such as 30 days. Rats fed with mussels for a longer period may think that cancer may occur in the liver tissue.

Toxic liver diseases caused by drugs and chemicals mainly affect hepatocytes, bile ducts, vascular system, sinusoidal cells, and Kupffer cells, causing various morphological changes in the liver. Changing rates of hepatocyte damage, necrosis, lobular and portal inflammation are observed in hepatocellular damage. In its mildest form, balloon degeneration and apoptotic bodies, as well as focal necrosis foci, and inflammatory cells, including neutrophils and eosinophils, are observed in hepatocytes (Boone et al., 2005; David & Hamilton, 2010; Mantovani et al., 2008). In our study, balloon degeneration, portal and lobular fissures in hepatocytes are similar to the literature information seen in toxic liver cases. We detected necrosis, inflammation in the lobule and portal area. The fact that balloon degeneration is much more common in the group that feeds on mussels every day suggests that continuous mussel consumption causes more hepatocellular damage. In toxic hepatitis, cytotoxic hepatocellular damage can be seen as well as the cholestatic type (Bioulac-Sage & Balabaud, 2009; Lucena et al., 2008). Acute hepatocellular damage with cytotoxic effect may also be in mixed

form, where both cholestatic and cytotoxic damage can coexist. Cholestatic changes are accompanied by mild balloon degeneration, necrosis, and apoptosis (Schattenberg & Schuchmann, 2009). Our balloon degeneration and necrosis findings in our study suggest that mussels show cytotoxic effects in mixed form. We think that the source of histopathological damage occurring in the liver is due to the transition of heavy metals to mussels and toxic effects on the liver.

It has been shown that in liver injuries triggered by hemorrhagic shock, the protein supplements obtained from the crustaceans decrease the damage, and the level of inflammatory marker TNF α (Lee et al., 2012). However, it was shown that the severity of inflammation increased due to liver damage caused by shellfish collected and consumed without considering environmental pollution. Studies for its protective effects have also been shown in primary liver culture. When the protein products obtained from the crustaceans are given to the cells that are induced with carbon tetrachloride, it has been shown that the antioxidant level increases and the damage in the cells decreases (Chi et al., 2010). In another study, it was reported that pacific oyster extract decreased liver fibrosis, and inflammatory markers such as TGF-beta and NF-KB decreased (Zhou et al., 2015). In our study, it was determined that mussels caused degeneration of hepatocellular structure in the liver and that TNF-α immunoreactivity increased as the dose increased. TNF-α, a cytokine secreted for conservation purposes, shows that the liver's damaged structure can be cleaned by apoptosis and inflammatory events and re-trigger regeneration. When oxidative stress in tissues and associated damage to cells increases, activation of the TNF-alpha receptor is followed by activation of the nuclear factor kappa B (NF-kB). Thus, NF-κB goes to the nucleus, and then NF-κB activates genes that try to block TNF-induced apoptosis. In resting cells, NF-B is an inactive form in the cytoplasm (Karin, 2006; Schattenberg & Schuchmann, 2009). Our findings show that in previous studies, we observed that the expression of TNF-α and NF-κB increased in hepatocyte cytoplasms parallel to its regulatory role in liver tissue. As the hepatotoxic effect increased as consumption scallop increased, TNF-α and NF-κB expression was observed to be at the highest level in the first experimental group.

CONCLUSION

Our study results have confirmed the parameter results, which are encountered in many regions and countries, depending on the amount of consumption and environmental pollution. Heavy metal accumulation in mussels collected from the Dardanelles was observed to cause inflammation and degeneration in the liver. While searching for an alternative food source, environmental factors should not be ignored and it should be paid attention to the consumption of clean and healthy products since it causes tissue damage in many systems especially in the digestive system. Besides, before the consumption of mussels, especially heavy metal and other environmental pollution analysis should be done. Our people should be made aware of how much heavy metals they take with food accumulate in their organs and should be protected from possible liver diseases.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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Effects of Dietary Natural Zeolite (Clinoptilolite) on Growth and Some Blood Parameters of Rainbow Trout (*Onchorynchus mykiss*, Walbaum 1792)

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Research Article

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Abstract

This study was performed to evaluate the effects of adding different levels of natural zeolite (clinoptilolite) to juvenile rainbow trout (*Onchorynchus mykiss*) diets about growth performance and some biochemical blood parameters. The experimental system was designed as a semi-recirculating aquaculture system and 20 rainbow trouts which weigh 7.91±0.03 g distributed randomly for each tank (300 L). Fish were fed to apparent satiation with diets containing 45% crude protein and 14% crude fat for 70 days which included no zeolite (Control), 0.5% zeolite (Z05), 1% zeolite (Z1), 2.5% zeolite (Z25) in triplicate. Finally, blood samples were collected from all fish tanks to analyze some biochemical blood parameters like cholesterol, triglycerides, LDL, HDL, and VLDL. At the end of the experiment, Z25 group showed negative results in final body weight, weight gain, and specific growth rate ($p<0.05$). Although Z05 group showed no significant difference, it showed the best results in final body weight, weight gain, specific growth rate, feed intake, feed conversion rate, and protein efficiency. In terms of blood parameters, all the groups had similar values with no significance ($p>0.05$) compared to the control group. As a result, there was a tendency towards decreased growth and feed utilization due to the addition of zeolite higher than 1% in diets. It could be concluded that limited usage of clinoptilolite in rainbow trout diets might have beneficial effects on growth parameters.

Keywords: Zeolite, trout, growth, blood parameters.

Diyetteki Doğal Zeolitin (Klinoptilolit) Gökkuşağı Alabalığının (*Onchorynchus mykiss*, Walbaum 1792) Büyüme ve Bazı Kan Parametreleri Üzerindeki Etkileri

Bu çalışma, genç gökkuşağı alabalığı (*Onchorynchus mykiss*) diyetlerine farklı seviyelerde doğal zeolit (klinoptilolit) eklenmesinin büyüme performansı ve bazı biyokimyasal kan parametreleri üzerindeki etkilerini değerlendirmek amacıyla yapılmıştır. Deney sistemi, yarı devridaim yapan bir kültür balıkçılığı sistemi olarak tasarlanmış ve her bir tanka (300 L) 7.91±0.03 g ağırlığında 20 gökkuşağı alabalığı rastgele dağıtılmıştır. tasarlanmıştır. Balıklar, %45 ham protein ve %14 ham yağ kompozisyonlu zeolit içermeyen (Kontrol),% 0.5 zeolit (Z05),% 1 zeolit (Z1),% 2.5 zeolit (Z25) içeren diyetlerle 70 gün boyunca doyana kadar beslenmiştir. Son olarak, kolesterol, trigliseritler, LDL, HDL ve VLDL gibi bazı biyokimyasal kan parametrelerini analiz etmek için tüm balık tanklarından kan örnekleri alınmıştır. Deney sonunda Z25 grubu son vücut ağırlığı, ağırlık artışı ve spesifik büyüme oranında negatif sonuçlar göstermiştir ($p <0.05$). Z05 grubu, anlamlı bir fark olmamasına rağmen son vücut ağırlığı, ağırlık artışı, spesifik büyüme oranı, yem alımı, yemden yararlanma oranı ve protein veriminde en iyi sonuçları göstermiştir. Kan parametreleri açısından tüm gruplar, kontrol grubuna göre anlamlı olmayan benzer değerlere sahiptir ($p> 0.05$). Sonuç olarak, zeolitin diyetlere %1'den fazla eklenmesi nedeniyle büyüme ve yemden yararlanmada azalma eğilimigörülmüştür. Gökkuşağı alabalığı diyetlerinde sınırlı klinoptilolit kullanımının büyüme parametreleri üzerinde faydalı etkileri olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Zeolit, alabalık, büyüme, kan parametreleri.

INTRODUCTION

Farmed rainbow trout (*Oncorhynchus mykiss*) production had increased by 19% from 2008 to 2017 (FAO, 2017). Most of the problems like environmental impact and sustainability in aquaculture are directly related to the origin of the diets which were used for carnivorous feeding (Tacon, 1997; Naylor et al., 2000). Most of the salmonid diets were used to be manufactured from animal originated

raw materials where plant-based raw materials and feed additives were only used as supplementary ingredients. To decrease the environmental impact and economical concerns, recent trends about fish feed manufacturing are to reduce protein cost, waste products, and marine originated raw materials (Papatryphon, 2004). Balanced diets having high digestibility rates had an important role in reducing the nitrogenous wastes which were excreted by the fish. Zeolites can also help to reduce ammonia excretion rates as well as using highly digestible raw materials. Zeolites were described as aluminosilicates of the alkaline and alkaline earth cations having an infinite, open and three-dimensional structure, capable of exchanging water and cations in the crystal structure without changing the crystal structure and containing water molecules (Mumpton and Fishman, 1977). Their general application areas were construction, water, wastewater treatment, adsorption, catalysis, removal of nuclear wastes, and agriculture. Animal nutrition and welfare, horticulture, removal of animal wastes, and aquaculture were some of its usage areas in agriculture. Zeolites were mainly used for; removal of ammonia from hatcheries, aquariums, transportation media, and feed additives for fish feed (Bernal et al., 1993; Mumpton, 1999) in aquaculture. Despite having this much broad application and there were many examples of dietary usage in terrestrial animals (Valpotić et al., 2017), there were limited experiments in aquaculture (De Silva and Anderson, 1995). Few trials were conducted on sea bass *Dicentrarchus labrax*, (Dias et al., 1998), sea bream *Sparus aurata*, (Kanyilmaz et al., 2015), rainbow trout *Onchorynchus mykiss*, (Obradovic et al., 2006; Demir and Aybal, 2004; Eya et al., 2008; Danabas, 2009; 2011; Alinezhad et al., 2017), coho salmon *Onchorhyncus kisutch*, (Edsall and Smith, 1989), snakehead murrel *Channa striata*, (Jawahar et al., 2016) and tilapia *Oreochromis niloticus, Tilapia zillii*, (Tore, 2006; Yildirim et al., 2009; Zahran et al., 2020), common carp *Cyprinus carpio*, (Kanyilmaz, 2008) and crayfish *Astacus leptodactylus*, (Aksu, 2016). The working mechanism of zeolites regarding growth is reported as selective adsorption of ions (Ames 1967; Mercer et al., 1970), changing feces profile and extending the passage time from the digestive tract (Lanari et al., 1996; Dias et al., 1998). Also, blood in terms of blood parameters, it was remarked (Ly et al., 2007; Kanyilmaz and Tekellioglu, 2016) that serum cholesterol and triglycerides values were affected by absorption of the short-chain fatty acids by zeolites. This study aimed to evaluate any effects of dietary natural zeolite (clinoptilolite) on rainbow trout diets on growth and some blood biochemical parameters.

MATERIAL AND METHOD

Experimental Design and Rearing

Rainbow trouts (*Onchornhychus mykiss*, n=360) obtained from a local commercial trout farm in Canakkale - Turkey were transported to the Faculty of Fisheries at Canakkale Onsekiz Mart University Research Unit. Before the start of the feeding trial, fish were fed a commercial diet (45% protein, 12% fat) for acclimatization. After the conditioning period, triplicate groups of 20 fish, which were initially averaging 7.91±0.03 g were stocked in cylindrical tanks (twelve tanks of 300 L), each filled with dechlorinated tap water. The experiment was carried out with 240 fish selected from 360, divided into 12 tanks (four groups and triplicate tanks). The water flow was 3 L/min, and all tanks were aerated by air stones. The semi-recirculating system used in the experiment consisted of eighteen 300 L tanks, a 300 L sump, 200 L sand filter, and a 300 L biofilter containing bio balls. The total volume of the system was 4400 L. One thousand liters of makeup water was exchanged daily corresponding to about 22,7% of the total volume. During the growth experiment, dissolved oxygen and water temperature daily and nitrite, nitrate and total ammonia) weekly monitored daily and all water quality parameters were in the optimal ranges. Through the experiment 70 days of feeding was and on every 15 days, the fish weighed. Twenty fish were randomly distributed to the experimental tanks from the stock tank and there were 12 tanks. To prevent weight and size inequality every fish was measured individually. In final weighing standard error was calculated. Total of four diets were prepared and given to the experimental groups three times a day to apparent satiation. Feed intake behavior and appetite were observed to prevent overfeeding. The amount of the feed was recorded every day to calculate feed intake.

Zeolite

Natural zeolite (clinoptilolite) used in the experiment was from Gordes Region in Manisa, Turkey. Chemical composition of the zeolite was given in Table.1.

Table.1. Chemical composition of the natural zeolite (Clinoptilolite) used in the experiment

Ingredients*	%
SiO_2	71.0
CaO	3.4
Fe_2O_3	1.7
Al_2O_3	11.8
K_2O	2.4
MgO	1.4
Na_2O	0.4
TiO_2	0.1

*Mineralogic – petrographic report; was issued at İstanbul Technical University Mining Faculty to the samples which were obtained from Kalabak Damlari (Gordes – Manisa, Turkey) region.

Experimental Diets

The experimental diets were manufactured to contain 45% crude protein (CP) and 14% crude fat (CF). They were formulated to be isonitrogenic and isocaloric. The formulation of the experimental diets was given in Table.2. The wheat meal rate in the control group was decreased to add zeolite to the diet groups. At a rate of 0.5% (Z05), 1% (Z1), and 2.5% (Z25) zeolite were added to the other three experimental groups. The pre-weighed dry ingredients were carefully mixed using a laboratory food mixer, with separate addition of the fish oil and vitamin/mineral premix. The mixture was primed with water to yield a suitable mash. Moist diets were made into pellets of 2 mm diameter and dried at 40 °C in a fan-assisted drying cabinet. Formulation and nutritional composition of the experimental diets were given in Table. 2 and Table. 3.

Table.1. Formulation of the experimental diets

Ingredients (%)	Groups			
	Control	Z05	Z1	Z25
Fish Meal[1]	50	50	50	50
Wheat Meal[2]	14	13.5	13	11.5
Soy Meal[3]	24.5	24.5	24.5	24.5
Fish Oil[4]	10	10	10	10
Zeolite (clinoptilolite)[5]	0	0.5	1	2.5
Vitamin – mineral[6]	1.5	1.5	1.5	1.5

[1] Anchovy Meal, Can Kardesler Balık Unu Sirketi, Samsun - Turkey
[2] Kepez Un, Canakkale - Turkey
[3] Abalioglu Yem ve Gida Sanayi, Denizli - Turkey
[4] Anchovy Fish Oil, Can Kardesler Balık Unu Sirketi, Samsun - Turkey
[5] Clinoptilolite, Rota Madencilik, Manisa - Turkey
[6] Vit – Min. Vitamin per g: vitamin A: 342 IU; vitamin D3: 329 IU; vitamin E: 0.0274 IU; vitamin K3: 5.48 mg; vitamin B1: 2.05 mg; vitamin B2: 3.42 mg; vitamin B3: 20.5 mg; vitamin B5: 5.48 mg; vitamin B6: 2.05 mg; vitamin B12: 2.74 mg; vitamin C: 24.0 mg, Mineral per g: biotin: 0.411 mg; folic acit: 0.685 mg; Zn: 12.3 mg; Mn: 4.80 mg; Cu: 1.64 mg; I: 0.274 mg; Se: 0.0274 mg; Ca: 125 mg; K: 189 mg, Kartal Kimya San. Ve Tic., Kocaeli -Turkey.

Table 3. Nutritional composition of the experimental diets

Parameter	Control	Z05	Z1	Z25
Crude Protein	44.96	44.90	44.84	44.86
Crude Fat	14.06	14.05	14.04	14.01
Crude Ash	8.1	8.6	9	9.6
Moisture	10.2	10.3	10.1	9.9

Sampling, Weighing, and Measurements

The weighing was done via a 0.1 g precision scale (Scaltec, Germany). At the beginning of the experiment, no anesthetics were used due to the small size of the fish but MS_{222} was used at a rate of 50 mg/L at the end of the experiment. Through the weight measurement, all the fish were extracted from the tank and weighted individually.

Nutritional Analysis of Experimental Feed

Sample diets were left to dry out in a drying oven for 24 at 105 ^{o}C until their weight was stabilized. A food processor was used for grinding the feed samples into powder form. Moisture, crude protein, crude fat, crude ash analysis of feed samples were done according to AOAC (2000) method.

Moisture Analysis

Five grams of samples were taken from the experimental diets. They were put into the previously tared aluminum holders and left to dry out in a drying oven for 24 at 105 ^{o}C until their weight was stabilized. Moisture percentage was calculated according to the formula below.

$$Moisture\ (\%) = (\text{Dry Sample} + \text{Tare (g)} - \text{Initial Sample Weight (g)}) / (\text{Initial Sample Weight (g)}) \times 100$$

Crude Protein Analysis

Kjeldahl Method was preferred for protein analysis in experimental diets. Approximately 0.5 g of samples were used for analysis. The first catalyst tablet and 15 ml sulphuric acid (H_2SO_4) was added to the glass digestion tubes. Protein digestion was carried out at the Gerhard Kjaldelterm digestion block. Samples were incinerated at 250 ^{o}C for 30 minutes initially, following 75 minutes at 380 ^{o}C. Samples were taken to the Gerhardt Vapodest 3S distillation unit, neutralized with 40% NaOH solution, and diluted with distilled water. After distillation, inorganic ammonia was collected in 25 ml orthoboric acid solution containing BDH "4.5 " indicator. Samples were then titrated with 0.1 mol hydrochloric (HCl). The protein percentage was (P,%) calculated according to the formula below.

$$\text{Crude Protein (\%)} = [(B - S)\text{x N x } 1.4007 \text{ x F}] / W$$

P: percentage of crude protein
F: protein factor (6.25)
B: ml, NaOH back titration of blank
S: ml, NaOH back titration of a sample
N: normality of NaOH
W: g, the weight of the sample
14.007: Molecular mass of Nitrogen

Crude Fat Analysis

The soxhlet extraction method was used for crude fat analysis. 3 g of dry matter was weighed and placed in the separation section of the instrument. The sample was then siphoned with 130 ml of petroleum ether for 40 minutes to collect the petroleum ether in the volumetric flask. Circulation was continued for 70 minutes after siphoning. After the end of the circulation process, siphoning was applied again and the remaining solution was removed by evaporation in the volumetric flask. The fat content of the dry matter was calculated by the following formula.

$$\text{Crude Fat (\%)} = \text{Fat Accumulated (g)} / \text{Sample Weight (g)} \times 100$$

Crude Ash Analysis

The dry matter was calculated by taking 0.5 g of sample and weighing by putting it into pre-tared porcelain crucibles. The crucibles were then fired in the incinerator at 525^{o}C for 12 hours. The ash content of the samples according to the weight change of the containers was calculated according to the following formula.

$$\text{Crude Ash (\%)} = \text{Weight change in porcelain container (g)} / \text{Sample Weight (g)} \times 100$$

Data Analysis

Growth indices were calculated according to the formula given below (Khodazanary et al., 2013; Jawahar et al., 2016).

$$\text{Initial Body Weight (IBW)} = \text{Initial Weight (g)} / \text{n Fish}$$

$$\text{Final Body Weight (FBW)} = \text{Final Weight (g)} / \text{n Fish}$$

$$\text{Weight Gain (WG)} = \text{Final Weight (g)} - \text{Initial Weight (g)}$$

$$\text{Weight gain Percent (WG\%)} = [\text{Final Weight (g)} - \text{Initial Weight (g)}] / \text{Initial Weight (g)} \times 100$$

$$\text{Sp. Growth Rate (SGR)} = [\text{Ln (Final Avr. Weight (g))} - \text{Ln (Initial Avr. Weight (g))}] / \text{Day} \times 100$$

$$\text{Feed intake (g/fish/day)} = \text{Feed Consumption (g)} / \text{Day}$$

$$\text{Feed Intake (average)} = \text{Feed Consumption (g)} / \text{No of Fish}$$

$$\text{Feed Conversion Rate (FCR)} = \text{Feed consumed (g)} / \text{Weight Gain (g)}$$

$$\text{Protein Efficiency Rate (PER)} = \text{Weight Gain (g)} / \text{Protein Consumption (g)} \times 100$$

Blood Sampling

All the fish were fasted for a day before blood sampling. Fish were anesthetized via 50 mg/L diluted MS_{222} and fixed with a dry towel to remove water, mucus from the body of the fishes. Approximately 1.5 – 2 ml of blood was collected from each fish to vacuette (Greiner Bio-One) gel containers via a single cut to the caudal vena. Immediately after the procedure samples were rushed to the laboratory and zanalyzed via auto analyzer (Olympus Optical Corp., Shizuoka-ken) (Kandemir et al., 2010).

Statistical Analysis

All the results were first subjected to analysis of variance (ANOVA) and later differences were evaluated by "Duncan's Multiple Range Test" was carried out in a 95% confidence interval.

RESULTS

Growth Trial

All the experimental groups showed no significant difference compared to the control group except Z25 group. Adding 2.5% zeolite had a statistically significant ($p<0.05$) negative effect on FBW, %WG, WG, and SGR. However, Z05 and Z1 groups showed a significant difference between each other on FCR also, the same observed between Z05 and Z25 ($p<0.05$). Growth and feed utilization values were presented in Table.4.

Table 4. Growth Parameters and Feed Utilisation Values

Parameters	Control	Z05	Z1	Z25
IBW (g)	7.94±0.01^{a}	7.93±0.06^{a}	7.89±0.03^{a}	7.86±0.04^{a}
FBW (g)	75.53±2.43^{a}	81.06±2.69^{a}	74.13±5.61^{a}	62.36±0.31^{b}
WG (g)	67.59±2.44^{a}	73.13±2.68^{a}	66.24±5.64^{a}	54.50±0.30^{b}
% WG	850.55±31,20^{a}	921.96±34.11^{a}	839.50±74.54^{a}	692.97±4.83^{b}
SGR	3.00±0.04^{a}	3.09±0.04^{a}	2.98±0.10^{a}	2.76±0,00^{b}
FI $^{g/fish/day}$	0.86±0.05^{a}	0.87±0.03^{a}	0.86±0.08^{a}	0.75±0.02^{a}
Av. FI $^{d/n}$	65.09±3.76^{a}	65.49±2.73^{a}	65.13±6.37^{a}	56.68±1.51^{a}
FCR	0.96±0.005^{abc}	0.89±0.009^{c}	0.98±0,01^{ab}	1.04±0.01^{a}
PER	2.26±0.01^{a}	2.48±0.02^{a}	2.26±0.03^{a}	2.13±0.03^{a}

The values of the experiment groups with different exponential letters in the same row are different from each other ($p<0.05$) *± SE: Standart Error

Blood Parameters

At the last step of the experiment, some biochemical parameters were studied. Biochemical blood parameters were presented in Table. 5. Comparing with the control group there were no significant differences were observed among cholesterol (CHO), high-density lipoporteins (HDL), low-density lipoproteins (LDL), and blood urea nitrogen (BUN) ($p>0.05$). However, Z1 and Z25 showed a significant difference between them regarding triglycerides and very low-density lipoproteins (VLDL) ($p<0.05$).

Table 5. Biochemical blood parameters at the end of the trial

mg/dl	Control	Z05	Z1	Z25
Triglycerides	211.00±57.51^{ab}	161.00±20.30^{ab}	234.67±16.50^{a}	136.67±34.59^{b}
Cholesterol	186.33±70.30^{a}	143.33±12.01^{a}	180.00±6.08^{a}	133.67±24.50^{a}
HDL	68.00±30.64^{a}	49.33±3.79^{a}	58.33±1.53^{a}	52.67±8.50^{a}
LDL	76.00±28.79^{a}	61.67±4.51^{a}	74.67±3.21^{a}	53.67±9.61^{a}
VLDL	42.33±11.50^{ab}	32.33±4.16^{ab}	47.00±3.60^{a}	27.33±6.65^{b}
BUN	3.80±0.52^{a}	3.96±0.85^{a}	4.03±0.87^{a}	2.10±0.70^{a}

The values of the experiment groups with different exponential letters in the same row are different from each other ($p<0.05$) *± SE: Standart Error

DISCUSSION

It was reported that due to their probable role in efficient use of nutrients (Olver, 1989) and/or detoxifying role, incorporation of natural zeolites to animal diets resulted in increased growth and development of living organisms (Harvey et al., 1993; Parlat et al., 1999; Ortatatli and Oguz, 2001; Rizzi et al., 2003). There were mixed results reported in terms of dietary usage of zeolites on growth

parameters of different species in aquaculture. Reinitz (1984) reported that adding %5, 10%, and 15% bentonite to rainbow trout diets harm the weight gain of the animals. Leonard (1979) stated that there was no significant difference between the live weights of the trout fed with 2% clinoptilolite feed (Pond and Mumpton, 1984). Edsall and Smith (1989) reported that adding 5% and 10% of clinoptilolite to the diets of coho salmon did not cause any effect on growth rate. Dias et al. (1998) investigated the effect of different feed additives (silica, cellulose, and natural zeolite) on protein digestibility, growth, FI, and feed transition time in seabass by adding 10% and 20% natural zeolite to feeds and reported that there was no effect between SGR, FCR, FBW values. In a study carried out by Demir and Aybal (2004) where the change in growth parameters of different rates (1-2-3-4-5-6%) of clinoptilolite added to rainbow trout diets evaluated, it was reported that there was no difference between the control group and the experimental groups in terms of FBW and FCR. Although there was no statistical significance, there were fluctuations between values that did not follow any trend. In this experiment, although it was not significant, a trend in growth parameters could be easily seen in parallel to the quantity of the zeolite added to the diet. Alinezhad et al. (2017) reported that in rainbow trout juveniles, adding different rates of nano-structured zeolite (0.5-1%) and aflatoxin B1 (5 mg) combination had no significant differences in growth parameters like FW, WG, BWI, SGR, FCR and condition factor. This experiment showed parallelism with the aforementioned trials. However, Lanari et al. (1996) reported that adding zeolite to rainbow trout diets at a rate of 2.5% and 5% increased weight gain and feed efficiency. Obradovic et al. (2006) conducted a trial with rainbow trout reported that adding 1% zeolite (Minazel) to pellets and certain rates of zeolite (Ambizel-V) in the test ponds had positive effects on growth parameters. The positive effect seen as a result of 1% zeolite addition was similar to the positive effect seen in the Z05 group in this study. It might be interpreted as different rates of zeolite addition cause similar effects, depending on the different starting weight of the fish, the production system, and the type of zeolite used. Eya et al. (2008) reported that adding two different zeolites (bentonite and mordenite) varieties at the rates of 2.5- 5% and 10 to rainbow trout diets had a positive effect on many growth parameters. Adding 2.5% mordenite and 5% bentonite to feeds had a positive effect on WG, SGR, FCR. These values were the values that cause the maximum positive effect, and other bentonites and mordenite ratios have been reported to cause a positive effect compared to the control group in certain amounts. Although this study (Eya et al., 2008) showed the positive effects of zeolites on growth and feed conversion parameters, the results of the Z25 group which were observed in the current study did not coincide with the negative effect on growth and feed conversion. In another study, it was reported that (Kanyilmaz, 2008), adding natural zeolite to carp feeds at different rates (1-2-3-4%) did not affect parameters such as FI, FCR, SGR, PER. However, in the current study adding 2.5% zeolite to the diet had a significant negative effect on WG, FCR, and SGR. From this aspect, the two studies did not overlap in terms of the results seen by adding high levels of zeolite to the feed. The use of 1% zeolite, although it was not statistically significant, had a very low negative impact on parameters such as FCR, SGR, PER, FI. The study carried out in this respect shows parallelism with the Z1 group in the current study. Danabas (2009) reported that the addition of 1% clinoptilolite into rainbow trout diets showed remarkable effects on the growth and feed utilization values. In terms of FBW, groups fed with feeds containing 1% and 2% zeolite showed the best growth. This study was in line with the work done by us in terms of the positive contribution of zeolite to growth. It was reported by the researcher that FBW values in fish fed with 3% zeolite added diets were similar to the control group. However, in this study, it was concluded that the Z25 group showed the lowest growth. Kanyilmaz et al. (2015) reported that the addition of zeolites at a rate of 1-2-3-4% to gilthead seabream diets had a significant increasing effect on FBW, SGR, FCE, and SGR. Researchers found that around 2.7% inclusion rate was optimal for seabream juveniles however, in this experiment 2.5% inclusion rate had a significant limiting effect on rainbow trout juveniles. A trial was done by Jawahar et al. (2016) showed that the incorporation of different rates (2-4-6%) of zeolite had positive effects on FBW, SGR, FCR, PER, and average daily WG of snakehead murrell against *Aphanomyces invadans*. Danabas (2009) stressed that beneficial effects of natural zeolites could be originated from the resistance of zeolite minerals to liquids (Mumpton, 1999; Ivkovic et al., 2004) which played an important role in the digestion of undigested nutrients through the intestines (Mumpton and Fishman, 1977; Dias et al., 1998; Meisinger et al., 2001). Moreover, it has been suggested that during the movement of the zeolite in the digestive system, it may increase the protein efficiency by causing better synthesis by binding the ammonia in the environment and releasing the

nitrogen within the protein synthesis (Ayvaz, 2004; Hargreaves and Tucker 2004; Ivkovic et al., 2004; Danabas, 2009). However, in this study, the fact that there was no change in the PER values of all groups did not suggest an improvement due to protein metabolism. Due to the natural structural feature of clinoptilolite, the selectivity to the ions such as Cs, Rb, K, NH_4, Ba, Sr, Na, Ca, Fe, Al, Mg, Li (Ames, 1967; Mercer et al., 1970) might cause lack of mineral substances in animals and thought to had negative effects on growth and weight gain. The use of non-digestible silicate materials (zeolites, kaolin, bentonite) as feed additives had changed the feces profile in some fish species such as trout and sea bass (Lanari et al., 1996; Dias et al., 1998), and it had been reported to extend the passage time from the digestive tract. Moreover, it was stated by the researchers that the pH of the stomach might affect these properties and each fish species might react differently to such feed additives (Dias et al., 2010). Different results in the conducted studies suggested that many variables might have a direct effect on the results of the study. Considering that even the properties such as the type, geographic location, and particle size of the zeolite used (Mumpton and Fishman 1977; Willis et al., 1982) might affect the results of the studies, other variables like the age, weight, fish species, grow-out systems, the nutritional content of the rations, nutritional needs of the species and the ambient conditions (temperature, pH, etc.), it can easily be said that having diverse results was very predictable.

More research was required on biochemical blood values in fish to obtain normal values related to fish biology and clinical pathology (Manera and Britti, 2006; Fazio et al., 2013; 2016). Difficulties considering determining reference values of blood parameters in rainbow trouts were reported by many researchers (Roscoe Miller et al., 1983; Yilmaz and Ergun, 2018). Plasma lipoproteins were involved in transporting insoluble lipids from their locations (eg: muscle, liver) to the areas where they will be used (Henderson and Torcher, 1987). According to Hoar et al. (1992), lipoproteins in fish were defined as chylomicrons, VLDL, LDL, and HDL, similar to humans. It was reported that in the blood VLDL; in terms of triglycerides, LDL; in terms of cholesterol, and finally HDL; had been reported to be rich in cholesterol and phospholipids (Mckay et al., 1985). Plasma lipoprotein values differ very much in terms of fish species at feeding and breeding periods. HDL values dominate total lipoproteins in bony fish and cover more than 50% of total lipoproteins. These values vary both between separate species and between the same species (Hoar et al., 1992). HDL values were reported as 238 mg/dl in young red salmon and 3300 mg/dl in pink salmon before ovulation (Babin and Vernier, 1989). High HDL values were thought to be related to high cholesterol (Hoar et al., 1992). Moreover, while rainbow trout fasted for 8 weeks, VLDL and LDL values decreased by 67% - 47% respectively, while HDL values were not affected (Black and Skinner, 1986).

The reference blood values of fish were still unclear, there was a very limited number of trials regarding the effects of dietary zeolite inclusion to the fish diets. Khodanazary et al. (2012) reported that the addition of %2.5 zeolite had no effects on serum cholesterol levels in common carp which was parallel with this experiment. Kanyilmaz and Tekellioglu (2016) stated that different rates of dietary zeolite inclusion had a significant effect on serum cholesterol and triglycerides but no effect on BUN levels of gilthead seabream. The researchers (Kanyilmaz and Tekellioglu, 2016) also stressed that the reason for the alterations in serum cholesterol and triglycerides might be originated from absorption of short-chain fatty acids by zeolites (Ly et al., 2007). Jawahar et al. (2016) reported that different rates of dietary zeolite level elevated triglyceride and cholesterol compared to the control group in snakehead murrel. All the researchers strongly stressed that the mechanisms behind the possible effects of dietary zeolite to blood chemistry values were still unknown (Khodanazary et al., 2013; Jawahar et al., 2016; Kanyilmaz and Tekellioglu, 2016). The current experiment showed that triglyceride and VLDL levels were highest with the addition of 1% zeolite and the lowest with the addition of 2.5% compared to the control group. It was observed that there was a significant difference only between these two groups in terms of triglyceride and VLDL values and there was no difference among the other groups. In line with these results, it could be said that adding zeolite up to 2.5% did not change the triglyceride and VLDL levels in rainbow trouts. However, considering the lowest feed utilization of the Z25 group among the experimental groups and the lowest VLDL and triglyceride values could be accepted to be in parallel as an indication that some blood parameters will be negatively affected by the increasing rates of zeolite. Moreover, VLDL, one of the lipoproteins found in blood serum, was synthesized directly from triglyceride (Ando and Mori, 1993).

In the light of the final growth and feed utilization values of the study, considering that the worst growth and feed utilization rate was seen in the Z25 group, it was concluded that rainbow trouts

cannot fully benefit from the feed they received. It could be concluded that the high rate (2.5%) of the zeolite's negative effect on growth is also in line with the growth results when viewed in blood values.

CONCLUSION

The researches about natural zeolites, especially clinoptilolite, were focused on their use as animal feed additives. This research aimed to evaluate the effects of using different levels of natural zeolite (clinoptilolite) in rainbow diets. If growth and feed utilization values were compared with similar studies conducted by other researchers, some studies showed parallelism in some aspects and incompatibility with others. This could be originated from many factors such as; initial weights, species of the animals, different rations, the amount, and the origin of the natural zeolite used. Also due to the biological features of the fish, the presence of a wide range of reference blood values in the natural and controlled environment caused the study results related to the blood values to be limited. More in-depth studies should be carried out to investigate the real effects and mechanisms of dietary zeolites in fish species.

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Japon Balığı (*Carassius auratus*) Gonad ve Viseral Organları Üzerine Bisfenol S'nin Toksik Etkileri

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Özet

Bisfenol A (BPA), polikarbonat plastiklerin ve epoksi reçinelerinin üretiminde kullanılan çevresel bir kirleticidir. Bununla birlikte bisfenol S (BPS), son zamanlarda BPA ürünlerine alternatif olarak kullanılmaya başlanan bir bisfenol analoğudur. Bu çalışmada farklı BPS konsantrasyonlarına (0, 100 ve 500 µg/L) 21 gün maruz bırakılan japon balıklarının *(Carassius auratus)* karaciğer, böbrek, gonad ve solungaç dokularındaki bir dizi etki histopatolojik olarak belirlenmiştir. Solungaçlarda BPS'nin hiperemi, ödem, epitel hücrelerinde deskuamasyon ve nekroza neden olduğu dikkat çekmiştir. Böbreklerde nekroz ve melanomakrofaj infiltrasyonları sıklıkla gözlenmiştir. Karaciğerde BPS'nin hiperemi ve inflamatuar hücre infiltrasyonlarına neden olduğu saptanmıştır. Bu çalışma, BPS'nin *Carassius auratus*'un çeşitli viseral organlarında dejeneratif değişikliklere neden olduğu ve histopatolojik değişikliklerin şiddetinin doza bağlı olduğunu ortaya koymuştur.

Anahtar Kelimeler: Endokrin bozucu, mikroplastik, histopatoloji, ksenoöstrojen

Toxic Effects of Bisphenol S on the Gonad and Visceral Organs of Goldfish (*Carassius auratus*)

Abstract

Bisphenol-A (BPA) is an environmental contaminant used in the manufacturing of polycarbonate plastics and epoxy resins. Whereas bisphenol S (BPS) is a bisphenol analogue which is recently used to replace BPA products. We demonstrated the effects of BPS on liver, kidney, gonad, and gills in goldfish (*Carassius auratus*) by a histopathological examination. Fishes treated with different concentrations (0, 100 and 500 µg/L) of BPS were tested for a duration of 21-days. In gills, BPS caused hyperemia, edema, epithelial desquamation and necrosis. Kidney lesions included necrosis and melanomacrophage infiltrations. BPS stress caused hyperemia and inflammatory cell infiltrations in livers. The present study revealed that BPS causes degenerative changes in various visceral organs of *Carassius auratus* and severity of histopathological changes were dose related.

Keywords: Endocrine disruptor, microplastic, histopathology, xenoestrogen

GİRİŞ

Dünya, kısıtlı su kaynaklarına ilaveten çevresel kirliliğin orantısız etkileriyle mücadele etmektedir. Endüstrileşmenin artmasıyla göl ve nehir gibi sucul ekosistemlere deşarj edilen çevresel kirleticiler, canlılar için önemli sağlık problemlerine neden olmaktadır. Çevresel kirleticilerin, ökaryotik organizmalarda endokrin fonksiyonların bozulmasına neden olduğu bilinmektedir. Endokrin bozucular; endojen hormonlarını taklit edip hormon sentezini ve hormonal fonksiyonları engelleme, depolanan hormonları serbest bırakma, salgı ve taşınım mekanizmalarını durdurma, doğal hormonları devre dışı bırakma, hormon reseptörlerini antagonize ya da agonize etme gibi etkiler ile vücudun hormonal sistemine etki etmektedir (Cek ve Sarıhan, 2010; Schönfelder vd., 2002). Endokrin bozucular olarak tanımlanan molekül grubu; bisfenol A (BPA), bisfenol S (BPS), 4-nonilfenol (NF), oktilfenol, vinklozolin ve dietilstilbestrol (DES) olarak kullanılan sentetik kimyasalları içermektedir (Molina vd., 2018). BPA; gıda ve içecek ambalajları, yapıştırıcılar, elektronik parçalar gibi endüstriyel

ürünlerin üretiminde kullanılmakta ve toplam üretim kapasitesiyle dünyanın en çok üretim hacmine sahip olan kimyasallardan biri haline gelmiştir (Wright-Walters, 2011; Liao vd., 2012a; Li vd., 2010; Flint vd., 2012).

Dünyada BPA ile ilgili kaygıların artması ve bazı ülkelerde yasaklanması endüstriyel olarak üretilen ürünlerde BPA'nın yapısal bir analoğu olan BPS'nin kullanımını artırmaya başlamıştır. Bununla birlikte, yapılan çalışmalar BPS'nin BPA ile benzer endokrin bozucu etkilere sahip olduğunu ve ortamdaki varlığının benzer şekilde ekosistemler ve insan sağlığı üzerine önemli riskler oluşturabileceğini göstermektedir (Qiu vd., 2018). Düşük doz BPS maruziyetinin zebra balığının embriyonik evresinde beyin gelişimine etki ettiği, balık larvalarında erken hipotalamik nörojenezi ve eşlik eden hiperaktif davranışı arttırdığını bildirmişlerdir (Grignard vd., 2012; Qui vd., 2016).

Bisfenol grubu bileşiklerin farklı canlı türleri için dokular üzerindeki histopatolojik etkileri ile ilgili çeşitli araştırmalar yapılmıştır. Yapılan bir çalışmada sıçanlara içme suyu ile verilen BPA'nın üreme, testis patolojisi ve spermatogenezis üzerinde olumsuzluklar meydana getirdiği belirlenmiştir (Buckiova vd., 2001). Bir başka çalışmada, 1 mg/L BPA'ya maruz bırakılmış tilapia (*Oreochromis mossambicus*) balıklarının karaciğerlerindeki parankimatöz dokuda lezyon, ödem bulgular, vakuolizasyon, belirgin dejenerasyon ve sinüzoidlerin daralması gibi dejenerasyonlar görülmüştür. Ayrıca hepatositlerde şişme ve nekrozun şekillendiği bildirilmiştir (Vasu vd., 2019). Yapılan bir diğer araştırmada, 2 farklı konsantrasyonda (1 ve 50 µg/kg vücut ağırlığı/gün) BPS'ye maruz bırakılan sıçanların spermatogenezisi üzerinde kısıtlayıcı bir etkiye sebep olduğu belirlenmiştir (Ullah vd., 2016).

Bisfenol bileşiklerinin çevrede ve iç sularda bulunduğu ve besin zinciri içinde insanlara ulaşmasının olağan olduğu bilinmektedir. Japonya' da yapılan araştırmalarda BPS'nin; sedimentte bisfenol grubu bileşiklerden en fazla bulunan kimyasal olduğu bildirilmiştir (Liao vd., 2012b). Yüzeysel ve atık sularda en yüksek BPS konsantrasyonu 3 µg/L olarak rapor edilmiştir (Kienhuis ve Geerdink, 2000). BPS, ileride yaratabileceği problemler sebebiyle incelenmesi yararlı olacak maddeler listesine alınmıştır (OEHHA 2012). Bu nedenle de bu maddelerin canlılarda birikiminin değerlendirilmesi ve deney hayvanlarında çeşitli dozlarda deneysel çalışmalar yapılarak etkilerinin belirlenmesi önem taşımaktadır. Balık dokularının histopatolojisi, çevresel stres etkilerinin değerlendirilmesini sağlayan güvenilir bir izleme aracı olup suda yaşayan organizmadaki insan kaynaklı stresörlerin neden olduğu sağlık bozukluğunun da en güvenilir göstergelerinden biridir.

BPS'nin sucul ortamdaki canlılar üzerinde olumsuz etkisine dair bilgi eksikliği dikkate alınarak, bu çalışmada söz konusu kimyasalın kademeli konsantrasyonlarına (100 ve 500 µg/L) maruz bırakılan japon balıklarındaki (*Carassius auratus*) karaciğer, böbrek, solungaç ve gonad dokularının histopatolojik bulguları incelenmiştir.

MATERYAL VE YÖNTEM

Etken Maddenin Hazırlanması

Çalışmada seçilen tüm kimyasallar (saflık ≥ %99) Sigma–Aldrich (St. Louis, MO, USA)'dan temin edilmiştir. BPS, hassas terazi ile tartılmış ve metanol:su (%10 (h/h)) ikili karışımlarında stok çözelti hazırlanmış, 4 °C'de cam şişede muhafaza edilmiştir. Stok solüsyonları akvaryum suyu ile seyreltilerek istenilen konsantrasyonlar elde edilmiştir (Chen vd., 2017).

Deney Gruplarının Oluşturulması ve Deneysel Uygulamalar

Bu çalışmada akvaryum balıkları satış ofisinden 180 adet japon balığı (Carassius auratus) (ortalama 3 aylık, 5-10 g ağırlığında, 5,5-7 cm uzunluğunda) temin edilmiştir. Araştırmada balıklar içerisinde 100 L su bulunan cam akvaryumlara her bir gruptan 3 paralel ve her paralelde 20 balık olacak şekilde dağıtılmış ve 2 hafta süreyle adaptasyona tabi tutulmuştur. Adaptasyon sonrasında balıklar 100 ve 500 µg/L olmak üzere 2 farklı konsantrasyonda BPS'ye 21 gün boyunca maruz bırakılarak 12 saat aydınlık:12 saat karanlık fotoperiyot uygulaması yapılmıştır (Lindholst vd., 2001). Maruziyet için hazırlanan çözeltiler daha önceki bir çalışmadan uyarlanmıştır (Wang vd., 2019). Deneme süresince su sıcaklığı 24±2 °C olarak ayarlanmış ve japon balıkları için kullanılan pelet yemle günde iki kez doyuncaya kadar beslenmiştir. Tanklardaki yem artıkları su kalitesinin bozulmaması için sifon yardımıyla ortamdan uzaklaştırılmıştır.

Histopatolojik İncelemeler

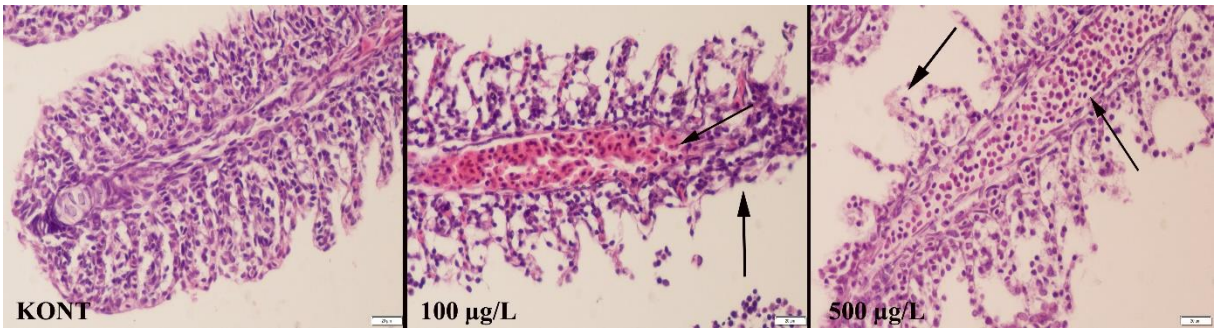
Araştırma süresi sonunda her gruptan 9 adet balık fenoksietanol (0,1-0,5 ml/L) ile anestezi edildikten sonra disekte edilmiş ve karaciğer, böbrek, solungaç ve gonad dokuları alınarak %10'luk

formaldehit solüsyonu içerisinde tespit edilmiştir. Ardından doku örnekleri Leica ASP300S otomatik doku takip cihazı (Leica Mikrosistem, Nussloch, Almanya) tarafından rutin doku takip işleminden geçirilerek parafine gömülmüştür. Tam otomatik mikrotom ile 5 µm'lik kesitler alınmıştır (Leica RM 2155, Leica Mikrosistem, Nussloch, Almanya). Daha sonra alınan kesitler hematoksilen ve eozin (HE) ile boyanmış ve ışık mikroskobu (Olympus CX41, Olympus Corporation, Tokyo, Japonya) altında incelenmiştir. Morfometrik değerlendirme ve mikrofotografi işlemleri; Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) kullanılarak yapılmıştır (Bancroft ve Stevens, 1977).

BULGULAR

Solungaçlar

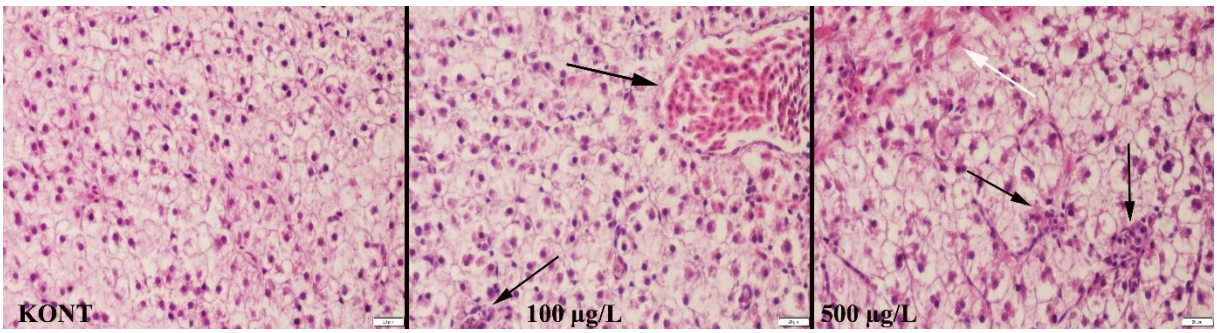
Solungaçların histopatolojik değerlendirilmesi sonucunda kontrol grubunda normal doku yapısı gözlenmiştir. Bununla birlikte, BPS uygulaması şiddetli hiperemi, ödem ve epitel hücrelerinde deskuamasyon ve nekroza neden olmuştur. Söz konusu bulgular 500 µg/L uygulanan grupta 100 µg/L' ye maruz kalan gruptan daha fazla gözlenmiştir (Şekil 1).



Şekil 1. Kontrol, 100 µg/L ve 500 µg/L BPS konsantrasyonlarının solungaç dokularındaki histopatolojik bulgular: Kontrol grubunda normal doku histolojisi, BPS'ye maruz bırakılan balıkların solungaçlarında belirgin hiperemi (ince oklar) ve solungaç lamellerinde deskuamasyon ve nekrozlar (kalın oklar), HE, Barlar = 20 µm.

Karaciğer

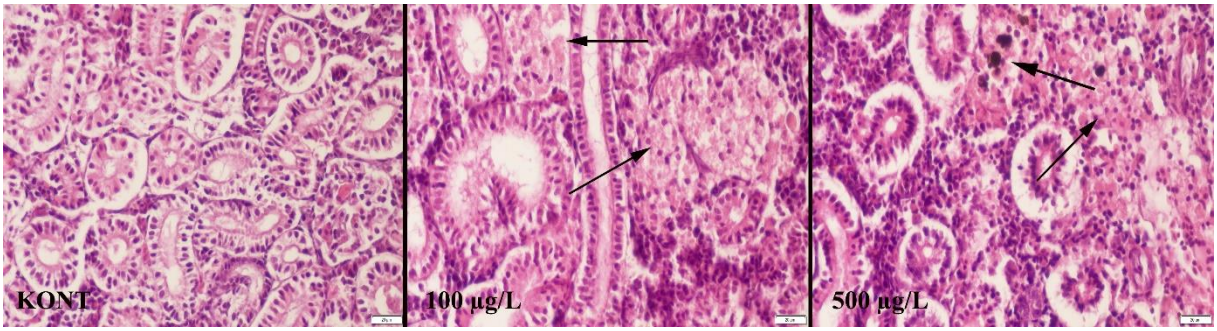
Karaciğerin mikroskobik incelenmesi sonucunda kontrol grubunda normal doku histolojisi görülürken BPS uygulanan her iki grupta da hiperemi, küçük kanamalar, hepatositlerde dejenerasyonlar ve inflamatuar hücre infiltrasyonları görülmüştür (Şekil 2).



Şekil 2. Kontrol, 100 µg/L ve 500 µg/L BPS konsantrasyonlarının karaciğer dokularındaki histopatolojik bulgular: Kontrol grubunda normal doku histolojisi, BPS uygulanan gruplarda belirgin hiperemi (kalın ok), inflamatuar hücre infiltrasyonları (ince oklar) ve küçük kanama alanları (beyaz ok) , HE, Barlar = 20 µm.

Böbrek

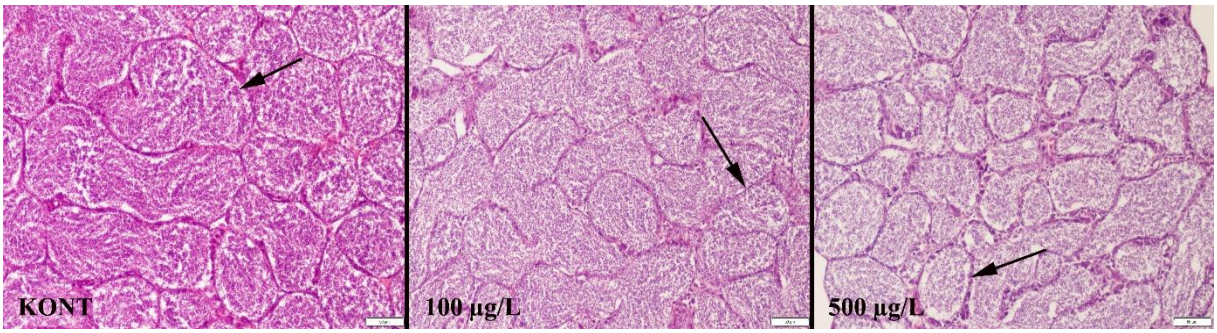
Böbrek dokusu için yapılan incelemelerde kontrol grubunda, normal böbrek histolojisi gözlenirken, BPS'ye maruz bırakılan düşük konsantrasyon grubunda nekroz, yüksek konsantrasyonda ise nekroz ve melanomakrofaj infiltrasyonu dikkati çekmiştir (Şekil 3).



Şekil 3. Kontrol, 100 µg/L ve 500 µg/L BPS konsantrasyonlarının böbrek dokularındaki histopatolojik bulgular: kontrol grubunda normal böbrek histolojisi, BPS'ye maruz kalan gruplarda nekroz (ince oklar), melanomakrofaj infiltrasyonları (kalın oklar), HE, Barlar = 20µm.

Testisler

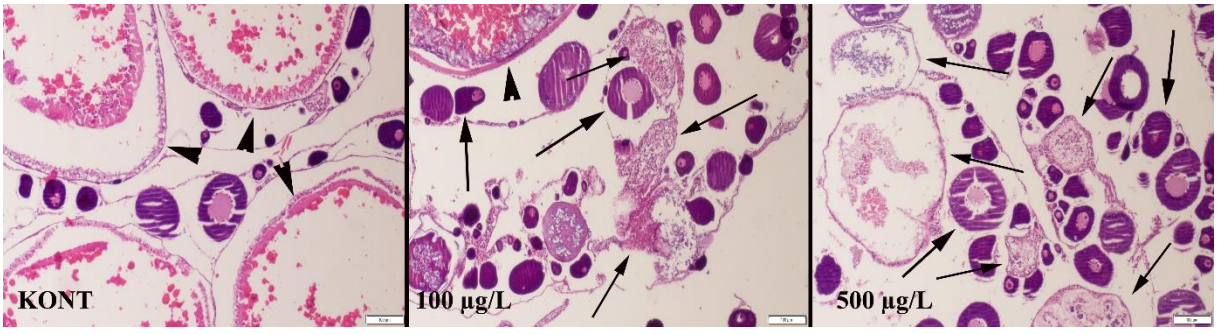
Testislerin histopatolojik incelemesi sonucunda, BPS maruziyeti olan gruplardaki spermatidlerde kontrol grubuna kıyasla belirgin bir azalma şekillendiği ve testis fonksiyonunun bozulduğu saptanmıştır (Şekil 4).



Şekil 4. Kontrol grubunda normal testiküler histoloji, BPS maruziyetli gruplarda seminifer tubuller içerisindeki spermatidlerde belirgin azalma (oklar), HE, Barlar=50µm.

Ovaryumlar

Ovaryumların histopatolojik incelemesi sonucunda, kontrol grubunda mikroskobik olarak normal histoloji gözlenmiştir. BPS uygulaması, olgun oosit sayısında belirgin azalmaya ve dejeneratif veya akretik oosit sayısında belirgin artışa neden olduğu ve ovaryum fonksiyonlarında bozulmaya sebep olduğu saptanmıştır (Şekil 5).



Şekil 5. Kontrol grubunda çok sayıda olgun oositler (ok başları), BPS uygulanan grupta olgunlaşmış oositslerde belirgin azalma (ok başı) ile olgunlaşmamış oositlerde (kalın oklar) ve dejeneratif veya atretik oositlerde (ince oklar) belirgin artış, HE, Barlar = 100µm.

TARTIŞMA VE SONUÇ

Yirminci yüzyıl başlarında cam ve çeliğin yerine geçebilecek bir malzeme olarak üretilen BPA, yapılan çalışmalarda etki mekanizmasından, östrojenik hasara; immunotoksik etkilerden, doku hasarına kadar pek çok alanda üzerinde çalışılmış bir kimyasal maddedir (Qiu vd., 2016; Lindholst vd., 2001; Vasu vd., 2019). Faheem vd., (2016) tarafından yapılan bir çalışmada *Catla catla* balıkları 15 gün boyunca çeşitli konsantrasyonlarda (1, 2, 3 ve 4 mg/L) BPA'ya maruz bırakılmıştır. Solungaç dokularındaki inceleme sonucunda, mukoza hücrelerinde hiperplazi, sekonder lamellalarda dejenerasyon ve nekroz gözlendiği bildirilmiştir. Yapılan bir başka çalışmada *Oreochromis mossambicus* balıkları 1 mg/L konsantrasyonunda BPA'ya farklı temas süreleriyle (10 ve 20 gün) maruz bırakılmıştır (Chitra ve Sajitha, 2014). Balıkların solungaç dokularındaki lamellalarda birleşme ve hipertrofi gözlenmiştir. Elshaer vd. (2013)'ne göre *Gambusia affinis ve Poecilia reticulata* balıkları 15 ve 30 gün süreyle 50 mg/L konsantrasyonunda BPA'ya maruz bırakılmış ve solungaç dokularının histapatolojik değişimleri incelenmiştir. Sekonder lamellaların epitelyumunda dejenerasyon ya da deskuamasyon ve nekrozis, lamellalarda birleşme, ödem ve kopma, epitel hücrelerde ise hiperplazi görülmüştür. Mevcut çalışmada, farklı konsantrasyonlarda BPS'ye maruz bırakılan japon balıklarının solungaçları incelenmiş, BPS'nin solungaçlarda dejeneratif değişikliklere neden olduğu gözlenmiştir. BPS'ye maruz kalan balıkların solungaç anomalileri hiperemi, ödem, deskuamasyon ve nekroz içermektedir. Ayrıca çalışmada 100 ve 500 µg/L BPS konsantrasyonları karşılaştırıldığında artan konsantrasyon miktarı ile histopatolojik değişimlerin arttığı belirlenmiştir.

Balıklarda karaciğer, ksenobiyotiklerin detoksifikasyonu için temel organdır. Karaciğer histolojisinin incelenmesi herhangi bir kirleticinin balıklar üzerindeki etkisini değerlendirmek için etkili bir yöntemdir. Ksenoöstrojenler üzerine yapılan bir çalışmada tilapia (*Oreochromis mossambicus)* balıkları 28 gün boyunca BPA'ya maruz bırakılmış ve 7, 14, 21 ve 28. günlerde kas, karaciğer, beyin ve böbrek dokuları incelenmiştir. BPA'ya maruz kalan balıkların karaciğerlerinde parankimatöz dokuda lezyonlar, ödem, vakuolizasyon, belirgin dejenerasyon ve sinüzoidlerde daralma gibi lezyonlar bildirilmiştir (Vasu vd., 2019). Başka bir çalışmada ise *Catla catla* balıklarında BPA maruziyetinin karaciğerde, vena sentraliste tromboz, iltihaplanma, ödem, dejenerasyon ve hepatositlerin nekrozuna neden olduğu bildirilmiştir (Faheem vd., 2016). Bu çalışmada, farklı BPS konsantrasyonlarına maruz kalan japon balıklarının karaciğeri incelenmiş, hiperemi, dejenerasyon ve inflamatuar hücre infiltrasyonları gözlenmiştir.

Böbrekler balıklarda hematopoezis ve osmoregülasyondan sorumlu organlardır. Solunum ile kullanılan kanın büyük bir kısmı böbreklere gittiği için çevresel kirliliğin böbrek dokusu üzerindeki etkisi balık sağlığı ve refahı için önem teşkil etmektedir. Faheem vd., (2016)'ya göre subletal konsantrasyonda (1-4 mg/L) 15 gün boyunca BPA'ya maruz kalan *Catla catla* balıklarının böbrek dokusundaki histolojik değişikliklerin nekroz, glomerulusta hipertrofi,böbrek tübüllerinde dejenerasyon ve glomerulus atrofisi olarak bildirilmiştir. Bu çalışmada da benzer şekilde böbrek dokuları etkilenmiş ve farklı BPS konsantrasyonlarına maruz kalan japon balıklarının böbrek dokularında belirgin nekroz ve melanomakrofaj infiltrasyonu gözlenmiştir.

Balıkların üreme sağlığı ve gelişim aşamalarında gonad histolojisi önemli bir parametredir. Bu çalışmada gonad dokusu; testisler ve ovaryumlar olarak ayrı ayrı incelenmiştir. Testislerdeki spermatidlerde belirgin azalma; ovaryumlarda ise olgun oositlerde azalma ve dejeneratif veya atretik oosit sayısında artma BPS konsantrasyonlarının kademeli artışı ile daha da belirginleşmiştir. Yapılan benzer bir çalışmada sazan *(Cyprinus carpio)* balıkları 14 gün boyunca farklı BPA (1, 10, 100 ve 1000 µg/L) konsantrasyonlarına maruz bırakılmış ve gonadların histolojik değişimleri incelenmiştir. Erkek sazanlarda BPA, 1 µg/L'den başlayarak testis yapısında ciddi değişikliklere neden olmuştur. Ayrıca, bazı örneklerde tipik lobüler yapı kaybolmuş ve sıklıkla lümen içinde dejener serbest spermatozoa ile birbirine karışan spermatojenik kistlerle karşılaşılmıştır. Dişi sazanlarda; oosit atrezisi, 1 µg/L BPA konsantrasyonunda gözlenmeye başlanmıştır ve en yüksek konsantrasyon olan 1000 µg/L'de dişilerin yaklaşık %57,1'inde gözlenmiştir (Mandich vd., 2007). Bir başka çalışmada BPS'nin üreme potansiyeli ve endokrin sistem üzerindeki etkileri zebra balığında (*Danio rerio*) incelenmiştir. Çalışmada olgun erkek ve dişi zebra balıkları iki hafta boyunca temiz suda beslenmiş, fertilizasyonlarının ardından oluşan embriyolar iki hafta sonra alınmış, 75 gün boyunca kademeli olarak (0; 0,1; 1; 10 ve 100 µg/L) BPS'ye maruz bırakılmışlardır. Maruziyet süresinin sonunda dişi ve erkek balıklar ayrılarak on dört gün boyunca temiz suya bırakılmıştır. Çalışma sonunda, düşük BPS konsantrasyonlarına maruz bırakılan zebra balığının gonad büyümesinde,

yumurta üretimi ve sperm sayılarındaki azalmalar endokrin sistemin farklı bölümleri üzerinde olumsuz etkileri olduğu belirlenmiştir (Naderi vd., 2014).

Endokrin bozucu kimyasallar günlük yaşantımızda neredeyse her alanda, fark ettiğimiz veya etmediğimiz birçok şekilde bize ulaşıp etki gösterebilen, dünya genelinde büyük ölçüde üretilen ve üretilmesine de devam edileceği düşünülen kimyasallardır. Kimyasallara maruziyet böylece kaçınılmaz iken; maruziyetin sonucundaki etkilerinin incelenmesi de bir o kadar önemli ve gerekli hale gelmiştir. BPS ve metabolitlerinin; BPA'ya alternatif olarak kullanıldığı malzemelere uygulanmasına artan ilgi dikkate alındığında; akut toksisite, genotoksisite ve östrojenik aktivite açısından daha fazla çalışma yapılması ve yapısal olarak aydınlatılması gerekmektedir.

BPS'nin balık dokuları üzerindeki histopatolojik etkileri hakkında literatürde bir bilgi eksikliği mevcuttur. Bu nedenle, bu çalışmada elde edilen sonuçlar, endokrin bozucu kimyasal olan BPS'nin japon balıkları üzerindeki ilk bulguları olup ileride yapılacak çalışmalar için BPS'nin ayrıntılı etkilerinin açıklanmasında faydalı olacaktır.

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Meriç Delta Balıklarında Toksik Metal Birikimlerinin Değerlendirmesi: Muhtemel İnsan Sağlığı Riskleri

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Özet

Meriç Deltası Türkiye'nin Trakya bölgesinde yer alan dünya çapında öneme sahip sulak alanlardan biridir. Delta içinde yer alan Gala ve Sığırcı gölleri, biyo-çeşitlilik bakımından Türkiye'nin önemli göllerindendir. Bu çalışmada Sığırcı ve Gala göllerinden elde edilen altı balık türünün *(Carassius gibelio, Carassius carassius, Scardinius erythrophthalmus, Cyprinus carpio, Sander lucioperca* ve *Perca fluviatilis*) kaslarındaki toksik ve esansiyel element (Li, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sb, Ba, Tl, ve Pb) konsantrasyonları tespit edilerek, bu elementlerin tüketimi ile ilişkili potansiyel insan sağlığı riskleri, yetişkinlerde "tahmini günlük tüketim (EDI)" dikkate alınarak değerlendirildi. Ayrıca, genel popülasyon için balık tüketim sıklığı dikkate alınarak, "hedef tehlike katsayısı (THQ)", "tehlike indeksi (HI)" ve "kanserojen riski (CR)" değerleri belirlendi. Bu sonuçlara göre, tüm balık türlerin yenilebilir kaslarında Li, B, Cr, Co, Se, Tl, ve Pb için EDI değerleri genel olarak izin verilen günlük doz limitini aşmıştır. Ancak 1'in altında tespit edilen THQ ve HI değerleri kanserojen sağlık riskinin olmadığını göstermiştir. Ayrıca, As, Cr, Ni ve Pb için CR değerleri kabul edilebilir sınırlardadır. Sonuçlar, Sığırcı ve Gala göllerindeki balıkların kaslarındaki toksik element konsantrasyonlarının tüketiciler için önemli bir sağlık riski oluşturmadığını ortaya koymuştur.

Anahtar Kelimeler: Gala ve Sığırcı Gölleri, Balıklar, Toksik Metaller, Sağlık Riski Değerlendirmesi

Evaluation of Toxic Metal Accumulations in Meriç Delta Fish: Possible Risks to Human Health

Abstract

Meriç Delta is in the Thrace region of Turkey and it is one of the wetlands of worldwide importance. Sığırcı and Gala lakes are in the delta and they are two of Turkey's most important wetland in terms of biodiversity. In this study, toxic and essential element concentrations(Li, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sb, Ba, Tl, and Pb) in the muscles of six fish species (*Carassius gibelio, Carassius carassius, Scardinius erythrophthalmus, Cyprinus carpio, Sander lucioperca,* and *Perca fluviatilis*) obtained from Sığırcı and Gala lakes were determined and potential human health risks associated with the consumption of these elements were evaluated by calculating "estimated daily intake (EDI) in adults. In addition, considering the frequency of fish consumption for the general population, "target hazard quotient (THQ)", "hazard index (HI)" and "carcinogen risk (CR)" values were determined. According to these results, EDI values for Li, B, Cr, Co, Se, Tl, and Pb in all edible muscles of all fish species generally exceeded the allowable daily dose limit. However, THQ and HI values below 1 showed that there was no carcinogenic health risk. Also, CR values for As, Cr, Ni and Pb are within acceptable limits. The results revealed that the toxic element concentrations in the muscles of the fish in Sığırcı and Gala lakes do not pose a significant health risk for consumers.

Keywords: Gala and Cattle Lakes, Fish, Toxic Metals, Health Risk Assessment

GİRİŞ

Son yıllarda, hızlı endüstrileşme, ekonomilerin yüksek kalkınma hızı, kentleşme, nüfus artışı ve tarımsal faaliyetler nedeniyle oluşan çevre kirliliği ve bununla ilişkili riskler, ciddi bir sorun haline gelmiştir (Çiçek vd., 2013; Köse vd., 2018; Ustaoğlu ve Islam, 2020). Ekosistemler, birçok kirleticinin baskısına maruz kalmıştır. Özellikle eser elementler (EE), toksisite, biyoakümülasyon ve biyomagnifikasyon özelliklerinden dolayı su ekosistemlerini (su, sediment, biyota) en çok kirleten kontaminantlar olarak öne çıkmıştır (Türkmen vd., 2008; 2012; Paramasivam vd., 2015; Ustaoğlu vd.,

2020a). EE; esansiyel (Zn, Cu, Fe, Co vb) ve esansiyel olmayan (Pb, Cd, As, Hg vb.) olarak sınıflandırılır. Her iki TE grubu da yüksek konsantrasyonlarda toksisiteye sebep olabilir. Sucul ortamlardaki EE'ler hem antropojenik faaliyetlerden (Örneğin sanayi ve endüstriyel atık suları, madencilik ve arıtma, tarımsal drenaj, evsel atıklar ve atmosferik birikim) hem de doğal kaynaklardan (genellikle toprak ve kayaların ayrışması, volkanik patlamalar, orman yangınları erozyon) kaynaklanır (Sundaray vd., 2011; Tokatlı, 2019; Ustaoğlu vd., 2020b).

İnsanların, EE'lere maruz kalmasının başlıca yollarında biriside gıda tüketimi ile olmaktadır. EE'ler, ortamda düşük konsantrasyonlarda olsalar bile, besin zincirindeki biyolojik birikim nedeniyle insanlar için sağlık riski oluşturabilir. Bu yüzden diyet yoluyla EE alınması risk değerlendirmesinde önemli bir konudur (Türkmen vd., 2010; Ahmed vd., 2015). Örneğin balıklar içerdikleri değerli proteinler, mikro elementler, vitaminler, omega-3 yağ asitleri kolayca sindirilen ve emilen doymamış yağ asitleri bakımından zengindir. Bu nedenle dünya çapında insan diyetinin çok önemli bir kısmını oluşturlar (Li vd., 2020). Ayrıca su ortamlarının önemli bir biyoindikatörü olarakta kabul edilirler. Ancak balıkların yaşadıkları ortamdan kolayca etkilendiği, çevrelerindeki su, sediment ve besinlerdeki ağır metal gibi zararlı kontaminantları biriktirdiği de bilinen bir gerçektir (Islam vd., 2015; Yin vd., 2020). Bu sebeple yüksek besin değerine rağmen, kontamine olmuş balıkların tüketimi dünya çapında ciddi bir halk sağlığı tehlikesi oluşturma potansiyeline sahiptir (Alamdar et al., 2017). Son yıllarda EE tarafından kontamine olmuş balıkların tüketimi ve risk değerlendirmesi ile ilgili pek çok araştırma yürütülmüştür (Fang vd., 2017; Feng vd., 2020; Kosker, 2020; Maurya vd., 2019; Miao vd., 2020; Varol vd., 2020; Varol ve Sünbül, 2020, 2018, 2018; Yin vd., 2020; Zerizghi vd., 2020).

Göller su ürünlerinin ana kaynaklarından biri olmakla birlikte, çevresindeki insani aktivitelere karşıda bir o kadar hassastır. Göllerin hızlı endüstriyel ve tarımsal büyüme ile ağır metaller tarafından kirlendiği ve insan sağlığı için potansiyel bir tehdit oluşturduğu bildirilmiştir (Ali vd., 2019).

Meriç Deltası, Türkiye'nin Trakya Bölgesi'ndeki Meriç Nehri'nin ağzında yaklaşık 45.000 ha alanı içermektedir. Özellikle kuş varlığı açısından Türkiye'nin en zengin su yaşam alanlarından biridir. Uluslararası kriterlere göre "A Sınıfı Sulak Alan" olarak sınıflandırılmıştır (aynı anda 25.000'den fazla su kuşunu barındırabileceği anlamına gelir). Uluslararası öneme sahip bu ekosistem, tarımsal, evsel ve endüstriyel uygulamalar yoluyla yoğun bir kirliliğe maruz kalmaktadır. Meriç Nehri çevresinde yapılan çeltik tarımı ve Ergene Nehri Havzası çevresinde gerçekleştirilen endüstriyel faaliyetler, sistemi etkileyen ana kirlilik faktörleri olarak kabul edilmektedir. Türkiye'nin toplam pirinç üretiminin yaklaşık% 25'i bu havzadan sağlanmaktadır. Ergene Nehri de Türkiye'nin en kirli nehir ekosistemlerinden biri olarak biliniyor. 2005 yılında Gala Gölü "Milli Park" olarak ilan edilmiş ve Avrupa ile Afrika arasında göç eden birçok kuş türü için konaklama yeri sağlamaktadır. Sığırcı Gölü de yerel balıkçılık için özel bir öneme sahiptir ve Meriç Deltası'nda yer almaktadır. Bu iki önemli göl, çeltik tarlalarının sulanması için de kullanılmaktadır ve tarım alanlarının drenaj suları, drenaj kanalları yoluyla tekrar bunlara deşarj edilmektedir. Ayrıca, bölgede yapılan sosyal araştırmalar, yerel halkın bu önemli su sistemleri konusunda oldukça duyarsız olduğunu göstermektedir. Bu durum deltanın her geçen gün daha da kirlenmesine ve ötrofik bir karakter sergilemesine neden olmaktadır (Tokatlı vd., 2014; Tokatlı ve Gürbüz, 2014; Tokatlı ve Baştatlı, 2016; Tokatlı, 2017; 2018; 2019).

Bu çalışmanın amacı, Gala ve Sığırcı göllerindeki altı balık türünün yenilebilir dokusu olan kaslarındaki EE (Li, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sb, Ba, Tl ve Pb) konsantrasyonlarını belirleyerek bu balıkların yetişkinler tarafından tüketilmesi durumunda oluşabilecek potansiyel sağlık risklerini değerlendirmektir.

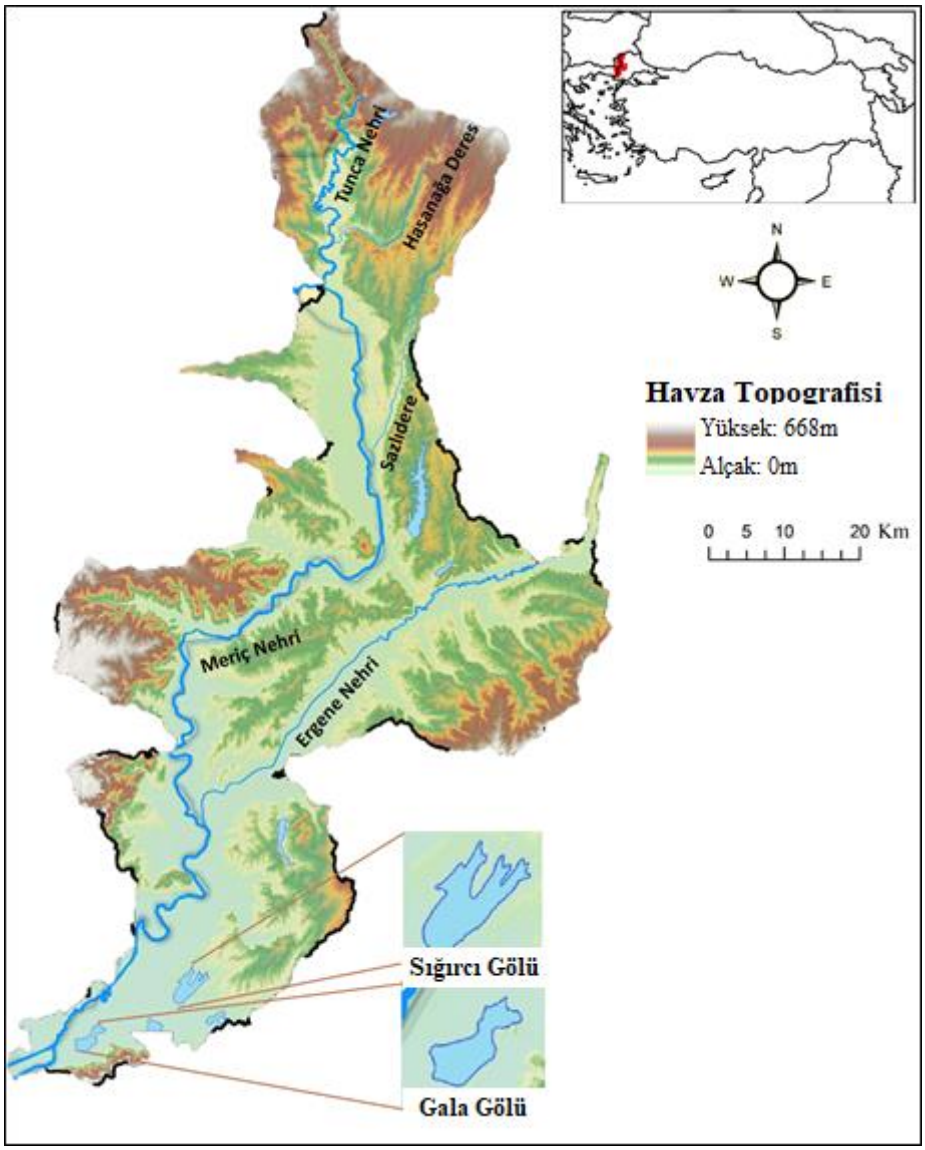
MATERYAL VE METOT

Çalışma Alanı ve Örekelerin Toplanması

Meriç Nehir havzasını topografik haritası ve çalışma alanı Şekil 1'de verilmiştir. Balık örnekleri [*Carassius gibelio* (Bloch, 1782), *Carassius carassius* (Linnaeus, 1758)] ve Sığırcı [*Scardinius erythrophthalmus* (Linnaeus, 1758), *Cyprinus carpio* Linnaeus, 1758, *Sander lucioperca* (Linnaeus, 1758), *Perca fluviatilis* Linnaeus, 1758], 2016 yılının yaz mevsiminde, serpme ağlar kullanılarak temin edilmiştir. Balıklara ait bazı metrik karakterler Tablo 1'de verilmiştir.

Tablo 1. Meriç Nehri Deltası'ndan yakalanan balıkların bazı metrik özellikleri

Lokalite	Balık Türü	Ağırlık (gr ± SD)	Boy (mm ± SD)		
			Standart	Çatal	Total
Gala Lake	*C, gibelio* (n = 4)	552,5 ± 9,8	243,7 ± 14,2	270,2 ± 15	284,7 ± 18,1
	C, carassius (n = 5)	1166 ± 105	315 ± 16,9	342,2 ± 15,6	385,8 ± 11,1
Sığırcı Lake	*S, erythroph,* (n = 5)	261,6 ± 5,2	241,4 ± 12,1	264,8 ± 12,6	287 ± 13,5
	C, carpio (n = 2)	1115 ± 49,4	306,5 ± 9,1	332 ± 11,3	369,5 ± 7,7
	S, lucioperca (n = 1)	430 ± 0	333 ± 0	362 ± 0	375 ± 0
	P, fluviatilis (n = 3)	189,3 ± 14	198,3 ± 22,5	216,6 ± 16	228,3 ± 20,2



Şekil 1. Meriç Havzası ve incelenen göller

Kimyasal Analizler

Balık örneklerinin kas dokuları çıkarıldı ve her numuneden 0.50 gr alınarak CEM Mars Xpress 5 mikrodalga sindirim reaktörlerine yerleştirildi. Reaktörlere sırasıyla 1:3 oranlı HC1:$HN0_3$ asitleri eklenmiştir. Numuneler, otuz dakika boyunca 200 °C'de mineralize edildi. Daha sonra, hacimleri 100 ml'ye ultra saf su ile tamamlandı. Balık dokularındaki element seviyeleri, "Agilent 7700 xx" markalı Endüktif Olarak Çiftleşmiş Plazma - Kütle Spektrometresi (ICP-MS) cihazı kullanılarak belirlenmiştir (ASTM, 1985; USEPA, 1998; 2001).

Sağlık Riski Değerlendirmesi

Balıklarda bulunan EE'lerin yetişkin insanlardaki sağlık risklerini değerlendirmek için "Edible Daily Intake (EDI)", "Target Hazard Coefficient (THQ)", "Hazard Index (HI)" ve "Cancer Risk (CR)" hesaplanmıştır.

Edible Daily Intake (EDI)

EE'lerin yetişkinler için EDI değeri (EDI= mg/kg vücut ağılığı/gün) aşağıdaki formüle göre hesaplandı (Javed ve Usmani 2016):

$$EDI = \frac{C_{element} \times D_{food\ intake}}{BW}$$

$C_{element}$: Balık kasındaki metal konsantrasyonu (mg/kg),

$D_{food\ intake}$: Günlük ortalama balık tüketimi (g / kişi / gün) (Türkiye'de 15.069 gr / kişi / gün) (GDFA, 2018),

BW: Ortalama vücut ağırlığıdır (yetişkinler için 70 kg).

Bu çalışmada hesaplanan EDI değerleri, USEPA (2019) tarafından önerilen oral referans doz (RfD_o) değerleriyle karşılaştırıldı.

Target Hazard Coefficient (THQ)

THQ, kirletici maruziyetine bağlı olarak risk seviyesinin (kanserojen olmayan) bir tahminidir. Her ağır metalin THQ'sunu belirlemek için kullanılan yöntem, aşağıdaki formülle hesaplanmıştır (Chien vd., 2002):

$$THQ = \frac{EF \times ED \times FIR \times C}{RfD_o \times BW \times AT} \times 10^{-3}$$

EF: Maruz kalma sıklığı (365 gün / yıl),

ED: Maruz kalma süresi (ortalama yaşam süresi: 70 yıl),

FIR: Gıda alım oranı (20 gr / gün),

C: Balık kas dokusunda ortalama ağır metal konsantrasyonu (mg / kg),

RfDo: Oral referans doz (mg / kg / day),

BW: Ortalama vücut ağırlığı (ortalama 70 kg),

AT: Kanserojen olmayanlar için ortalama maruz kalma süresi (365 gün / yıl x maruziyet yılı sayısı)

Eğer THQ değerleri 1'i aşarsa, karsinojenik olmayan olumsuz sağlık etkilerinin ortaya çıkma potansiyeli olabilir. Eğer değerler 1'den düşükse, karsinojenik olmayan sağlık etkileri beklenmez (Mehmood et al., 2020; Çulha et al., 2016).

Hazard Index (HI)

HI, gözlemlenen tüm metaller için THQ'nun toplamıdır (HI<1 ise güvenli, HI> 1 ise tehlikeli) (Varol ve diğerleri, 2019).

$$HI = \sum_{i=1}^{n} THQ_i$$

Cancer Risk (CR)

Cr, Ni, Pb ve As ömür boyu maruz kalma neticesinde oluşabilecek hedef kanser riski (CR) aşağıdaki denkleme göre hesaplanmıştır (Javed ve Usmani 2016):

$$CR = \frac{EF \times ED \times FIR \times C \times CSF}{BW \times AT} \times 10^{-3}$$

CSF: Entegre Risk Bilgi Sisteminden kanserojen eğim faktörüdür (Cr: 0,5; Ni: 1,7; Pb: 0,0085; As: 1,5 mg/kg/gün).

Hesaplanan CR katsayılarının değerlendirilmesi şu şekilde yapılmaktadır; $CR<10^{-6}$: ihmal edilebilir; $10^{-4} > CR > 10^{-6}$: kabul edilebilir; $CR>10^{-4}$: kabul edilemez (USEPA, 2019).

SONUÇLAR VE TARTIŞMA

Son yıllarda sanayileşme ve tarımsal faaliyetlerin artması nedeniyle Sığırcı ve Gala Göllerinin su kalitesi her geçen gün kötüleşmektedir. Bu nedenle göl sularındaki EE'ler, balıkların su ve sedimentlerle etkileşimi sonucu balık dokularında birikmektedir. Balığa dayalı bir beslenme şeklinin pek çok faydaları vardır, ancak toksik metaller, pestisit ve diğer toksinler ile kontamine olmuş balıklar tüketicilerin sağlığını tehlikeye atabilir. Carassius gibelio, Carassius carassius, Scardinius erythrophthalmus, Cyprinus carpio, Sander lucioperca, ve Perca fluviatilis gibi türler Gala ve Sığırcı gölleri yakınlarındaki insanlar tarafından yaygın olarak tüketilmektedir. Balık türleri için hesaplanan EDI değerleri Tablo 2'de listelendi. Türler arasında tespit edilen EDI değerleri arasındaki bu farklılıklar çoğunlukla balık türlerinin yaşam ortamları, besin tercihleri, metabolizmaları ve fizyolojileri ile ilişkilidir (Roleda et al., 2019; Tepe, 2009). EDI değerleri, oral referans doz (RfD) değerleri ile karşılaştırıldı. Sonuçlar, Li, B, Cr, Co, Se, Tl ve Pb için EDI değerlerinin tüm balık türlerinde genellikle izin verilen günlük dozdan daha yüksek olduğunu göstermiştir. Geri kalan metallerde (Al, V, Mn, Fe, Ni, Cu, Zn, As, Sr, Ag, Cd, Sb ve Ba) ise EDI < RfD olarak hesaplanmıştır. İnsan nüfusu aynı anda birden fazla EE'ye maruz kalmaktadır. Bu nedenle son yıllarda EE'lerin insan sağlığı üzerindeki interaktif etkileri üzerine çok sayıda çalışma yapılmıştır. Örneğin As ve Pb arasında sinerjik bir etkinin olduğu tespit edilmiştir ve çocuklarda As ve Pb toksisitesinin birinci hedefinin merkezi sinir sistemi olduğunu ve zihinsel engelli olma olasılığını arttırdığı bildirilmiştir (Freire vd., 2018).

Tablo 2. Tespit edilen EDI değerleri

		EDI (mg/kg BW/gün)					
	RfD (mg/kg/gün)	**1**	**2**	**3**	**4**	**5**	**6**
Li	2.00E-03	5.18E-02	4.92E-02	5.18E-02	5.52E-02	5.30E-02	5.13E-02
B	2.00E-01	1.12E+00	1.08E+00	1.12E+00	1.11E+00	1.11E+00	1.10E+00
Al	1.00E+00	4.51E-01	3.70E-01	3.70E-01	3.26E-01	3.35E-01	9.31E-01
V	5.00E-03	5.36E-03	1.31E-03	2.19E-03	9.66E-04	7.17E-04	1.62E-03
Cr	3.00E-03	5.36E-03	3.77E-03	4.41E-03	2.18E-02	5.46E-03	5.27E-03
Mn	1.40E-01	1.51E-02	3.64E-02	2.09E-02	1.17E-02	9.34E-03	2.37E-02
Fe	7.00E-01	6.13E-01	5.33E-01	5.17E-01	7.90E-01	4.90E-01	8.64E-01
Co	3.00E-04	5.43E-04	4.91E-04	4.37E-04	4.37E-04	3.97E-04	4.17E-04
Ni	2.00E-02	1.01E-02	1.22E-02	1.28E-02	7.58E-03	9.37E-03	6.87E-03
Cu	4.00E-02	1.39E-02	1.19E-02	1.13E-02	1.99E-02	1.64E-02	1.08E-02
Zn	3.00E-01	2.68E-01	1.06E-01	1.35E-01	1.19E-01	1.83E-01	1.16E-01
As	3.00E-04	5.12E-04	2.45E-04	4.23E-04	3.62E-04	3.16E-04	2.86E-04
Se	5.00E-03	6.95E-03	8.79E-03	7.47E-03	5.61E-03	8.21E-03	7.45E-03
Sr	6.00E-01	3.15E-02	5.38E-02	8.20E-02	5.38E-03	5.93E-03	5.75E-03
Ag	5.00E-03	1.27E-02	8.64E-03	8.31E-03	1.00E-02	8.52E-03	8.35E-03
Cd	1.00E-03	1.21E-03	8.74E-04	8.51E-04	9.20E-04	9.63E-04	1.04E-03
Sb	4.00E-04	6.10E-03	5.86E-04	4.74E-04	4.43E-04	5.29E-04	5.34E-04
Ba	2.00E-01	8.70E-03	1.40E-02	2.02E-02	4.05E-03	4.23E-03	6.57E-03
Tl	1.00E-05	1.26E-03	2.37E-04	2.31E-04	1.80E-04	1.97E-04	2.03E-04
Pb	3.00E-03	1.46E-02	1.18E-02	1.22E-02	1.27E-02	1.51E-02	1.60E-02

1=*C. gibelio*, 2=*C. carassius*, 3=*S. erythrophthalmus*, 4=*C.carpio*, 5=*S. Luciope*, 6= *P.fluviatilis*

Altı balık türünün tüketimi yoluyla EE'ler için tahmin edilen THQ değerleri Tablo 3'te sunulmuştur. Gala ve Sığırcı göllerindeki her balık türündeki EE'lerin THQ değerleri 1'i geçmemiştir. Ayrıca her balık türü için metallerin toplam THQ değerleri de (HI) 1'den düşüktür. THQ ve HI sonuçlarına göre, tüm balık türlerinde bireysel veya kombine elementlerin alımından, tüketiciler için herhangi bir sağlık etkisi beklenmemektedir. Bu risk tahmini yöntemi son zamanlarda birçok araştırmacı tarafından kullanılmış, geçerli ve yararlı olduğu gösterilmiştir.

As, Ni, Cr ve Pb için tahmin edilen CR değerleri Tablo 3'te sunulmuştur. Tüm balık türlerinde As ve Pb için CR değerleri 10^{-6} dan küçük (ihmal edilebilir), Cr ve Ni değerleri ise 10^{-4} – 10^{-6} (kabul edilebilir) aralığındadır. Bu nedenle Sığırcı ve Gala göllerindeki altı balık türünün tüketilmesiyle As, Pb, Ni ve Cr'ye maruz kalma durumunda tüketiciler için kayda değer bir kanserojen riski yoktur.

Tablo 3. THQ, HI, ve CR değerleri

	THQ						CR					
	1	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Li	2.59E-02	2.46E-02	2.59E-02	2.76E-02	2.65E-02	2.56E-02						
B	5.58E-03	5.40E-03	5.59E-03	5.54E-03	5.57E-03	5.52E-03						
Al	4.51E-04	3.70E-04	3.70E-04	3.26E-04	3.35E-04	9.31E-04						
V	1.07E-03	2.63E-04	4.38E-04	1.93E-04	1.43E-04	3.24E-04						
Cr	1.79E-03	1.26E-03	1.47E-03	7.26E-03	1.82E-03	1.76E-03	2.68E-06	1.89E-06	2.21E-06	1.09E-05	2.73E-06	2.64E-06
Mn	1.08E-04	2.60E-04	1.49E-04	8.39E-05	6.67E-05	1.69E-04						
Fe	8.75E-04	7.62E-04	7.39E-04	1.13E-03	7.00E-04	1.23E-03						
Co	1.81E-03	1.64E-03	1.46E-03	1.46E-03	1.32E-03	1.39E-03						
Ni	5.05E-04	6.11E-04	6.40E-04	3.79E-04	4.69E-04	3.43E-04	1.72E-05	2.08E-05	2.18E-05	1.29E-05	1.59E-05	1.17E-05
Cu	3.46E-04	2.98E-04	2.83E-04	4.97E-04	4.11E-04	2.71E-04						
Zn	8.93E-04	3.54E-04	4.52E-04	3.95E-04	6.11E-04	3.86E-04						
As	1.71E-03	8.17E-04	1.41E-03	1.21E-03	1.05E-03	9.54E-04	7.68E-07	3.68E-07	6.34E-07	5.43E-07	4.74E-07	4.29E-07
Se	1.39E-03	1.76E-03	1.49E-03	1.12E-03	1.64E-03	1.49E-03						
Sr	5.25E-05	8.97E-05	1.37E-04	8.97E-06	9.88E-06	9.58E-06						
Ag	2.54E-03	1.73E-03	1.66E-03	2.01E-03	1.70E-03	1.67E-03						
Cd	1.21E-03	8.74E-04	8.51E-04	9.20E-04	9.63E-04	1.04E-03						
Sb	1.53E-02	1.46E-03	1.19E-03	1.11E-03	1.32E-03	1.34E-03						
Ba	4.35E-05	6.98E-05	1.01E-04	2.03E-05	2.11E-05	3.29E-05						
Tl	1.26E-01	2.37E-02	2.31E-02	1.80E-02	1.97E-02	2.03E-02						
Pb	4.87E-03	3.94E-03	4.08E-03	4.24E-03	5.02E-03	5.35E-03	1.24E-07	1.00E-07	1.04E-07	1.08E-07	1.28E-07	1.36E-07
HI	1,93E-01	7,03E-02	7,15E-02	7,35E-02	6,94E-02	7,01E-02						

1 = *C. gibelio*, 2 = *C. carassius*, 3 = *S. erythrophthalmus*, 4 = *C. carpio*, 5 = *S. luciope*, 6 = *P. fluviatilis*

Arsenik, balık dokusunda hem organik hem de inorganik formlarda bulunur ve inorganik As insanlar için en toksik olan formdur (Jara ve Winter, 2014). Bu nedenle As maruziyetinden kaynaklanan insan sağlığı risklerini tahmin etmek için kullanılan EDI, RfD ve CSF değerlerinin hesaplanmasında inorganik As kullanılması uygundur. Ancak bu çalışmada balık örneklerinde toplam (inorganik + organik) As ölçülmüştür. Balık tüketmenin insan sağlığı risklerini tahmin etmek için balıklarda inorganik As yüzdesi hakkında bir varsayım yapılmalıdır. Yapılan araştırmalarda çoğu balıklarda inorganik As yüzdesinin % 0 ila % 9,5 arasında değiştiği bildirmiştir (Varol ve Sünbül, 2020). Çalışmamızda daha koruyucu olmak için toplam arseniğin %10'unun inorganik As olduğu varsayılmıştır.

Varol ve Sünbül (2018), mevcut çalışmamızda elde edilen veriler ile benzer şekilde, Türkiye'nin en büyük 2. baraj gölündeki beş balıkta THQ ve HI değerlerinin insan sağlığı için risk oluşturmayacak seviyede olduğunu (1 den küçük) ve iAs için kanser riski (CR) eşik değerini (10^{-6}) aşmadığını bildirmişlerdir. Başka bir çalışmada Garnero ve ark. (2020), mevcut çalışmamızda elde edilen verilerden farklı olarak, Río Tercero Barajında (Arjantin) altı balık türündeki iAs konsantrasyonlarının izin verilen maksimum günlük konsantrasyonları aştığını, THQ ve total THQ (HI) değerlerinin 1'in birkaç katı olduğunu, bildirmişlerdir. Ayrıca, arseniğin neden olduğu kanser riskinin kabul edilebilir değerden (10-4) fazla olduğunu, dolayısıyla bu rezervuardaki incelenen balık türlerin besin olarak tüketiminin, insanlar için toksikolojik bir riskinin olabileceğini tespit etmişlerdir.

SONUÇ

Bu çalışma ile Meriç - Ergene havzasının önemli sulak alanlarından Sığırcı ve Gala göllerindeki altı balık türünün (Carassius gibelio, Carassius carassius, Scardinius erythrophthalmus, Cyprinus carpio, Sander lucioperca, ve Perca fluviatilis), insanlar tarafından besin olarak tüketilen kas dokularındaki eser element (Li, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sb, Ba, Tl ve Pb) konsantrasyonları kullanılarak, balık tüketiminden kaynaklanabilecek insan sağlığı için bazı risk değerlendirmeleri yapılmıştır. Bu risk değerlendirmelerine (EDI, THQ, HI ve CR) dayanarak, TE maruz kalmanın Gala ve Sığırcı Göllerindeki balıklarla beslenen insanlar için şimdilik önemli bir sağlık riski oluşturmadığı tespit edildi. Bu çalışmanın sonuçları, balık tüketiminin olası olumsuz etkileri konusunda uyaran, ileri çalışmalar için bir başlangıç noktasıdır. Bu şekilde, bu bilgiler su ortamlarını ve onlarla etkileşime giren insanları korumak için politikaların geliştirilmesine veri sağlayabilir.

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Farklı Baharatlar Kullanılarak Üretilen Kalamar Esaslı Atıştırmalıkların Duyusal Değerlendirmesi

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Özet

Türkiye'de satışa sunulan çok sayıda atıştırmalık ürün olmasına karşın, bu ürünlerin çoğunun beslenmeye katkılarının olmadığı literatürde belirtilmektedir. Buna karşın, atıştırmalıkların beslenmeye katkı sağlaması, insan sağlığı açısından yararlı olan su ürünlerinden elde edilmesi, Türk damak tadına uygun olarak üretilmesi, endüstriye kazandırılarak tüketiminin arttırılması gerekmektedir. Dünyada ise su ürünleri içerikli atıştırmalıklar satışa sunulup tüketilmesine rağmen, ülkemizde endüstriyel anlamda su ürünlerinden atıştırmalık üretimi ve satışı bulunmamaktadır. Bu amaçla çalışmada sağlık açısından faydalı kalamar atıştırmalıklarının üretilmesi ve üretilen bu atıştırmalıkların duyusal açıdan değerlendirilmesi hedeflenmiştir. Çalışmada kalamarlardan 12 farklı baharat (toz kırmızıbiber, kimyon, kekik, yenibahar, çörek otu, susam, karabiber, zerdeçal, zencefil, soğan tozu, sarımsak tozu, tarçın) kullanılarak tüketime hazır baharat kaplı kalamar atıştırmalıkları elde edilmiştir. Her bir grup galeta unu+baharat karışımı ile ayrı ayrı kaplandıktan sonra 200 dereceye ayarlanmış olan fırında 20 dakika pişirme işlemi uygulanmıştır. Pişirme işlemi sonrasında atıştırmalıklar 30 adet paneliste sunularak duyusal açıdan değerlendirilmesi istenmiştir. Yapılan çalışmanın istatistiki değerlendirilmesi sonucunda panelistler tarafından genel kabul edilebilirlik açısından en beğenilen baharat kaplı kalamar atıştırmalıklarının soğan tozu, sarımsak tozu ve kekik kullanılarak hazırlanmış olan atıştırmalıkların olduğu, en beğenilmeyen grupların ise tarçınlı, yenibaharlı ve karabiber içerikli baharat kaplı kalamar atıştırmalıklarının olduğu belirtilmiştir. Sonuç olarak; bu tip tüketime hazır halde farklı formülasyonlarda baharat kaplı kalamar atıştırmalıklarına yönelik çalışmaların yapılması, en beğenilen formülasyonların belirlenmesi, farklı formülasyonlarda baharat kaplı kalamar atıştırmalıklarının üretilmesi ve marketlerde satışa sunulması arzu edilmektedir.

Anahtar kelimeler: Kalamar, atıştırmalık, baharat, duyusal analiz, su ürünleri

Sensory Evaluation of Squid Based Snacks Produced by Using Different Spices

Abstract

Although there are many snack products available for sale in Turkey, it is stated in the literature that most of these products do not contribute to nutrition. On the other hand, snacks should contribute to nutrition, be obtained from water products that are beneficial for human health, be produced according to Turkish taste, and be added to the industry and increase their consumption. In the world, even though fishery snacks are offered for sale and consumed, there is no industrial snack production and sale of fishery products in our country. For this purpose, the study aims to produce health-beneficial squid snacks and to evaluate these snacks from a sensory point of view. In the study, 12 different spices (red pepper, cumin, thyme, allspice, black seed, sesame, black pepper, turmeric, ginger, onion powder, garlic powder, cinnamon) were used to obtain ready-to-eat squid snacks from squid. After each group of breadcrumbs+ seasoning mixture is covered separately, 20 minutes of the baking process is applied in the oven, which is set to 200 degrees. After the cooking process, the snacks were presented to 30 panelists and asked to be evaluated from a sensory point of view. Statistical evaluation all the general acceptability of the study by the panelists as a result of the most popular squid snacks that are prepared using onion powder, garlic powder, and oregano are the most unpopular groups in cinnamon, and spicy black pepper squid snacks set out for new content. As a result, it is desirable to carry out studies on squid snacks in different formulations, determine the most popular formulations, produce squid snacks in different formulations, and offer them for sale in markets.

Keywords: Squid, snack, spice, sensory analysis, seafood

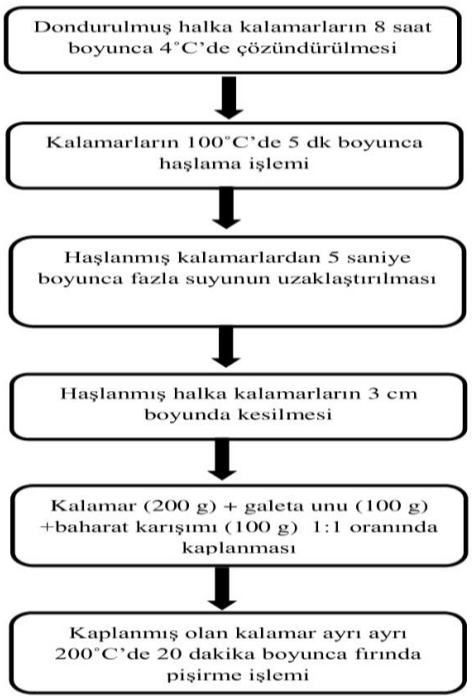
GİRİŞ

Atıştırmalıkların her toplumun beslenmesinde önemli yer tuttuğu belirtilmektedir (Değerli ve El, 2019). Atıştırmalık veya snack kelime anlamı olarak hafif hızlı yemek olarak tanımlanmaktadır. Bazıları ise sadece bir ısırımlık veya lokmalık ürünler olarak tarif etmektedir. Bu ürünler tatlı veya tuzlu çerezler, krakerler, halkalar veya çubuklar şeklinde olabilmektedir. Bu ürünlerin düzenli tüketilen gıdalardan farklı olarak çoğu insan tarafından besinsel içerik açısından değil de sadece memnuniyet vermesi nedeniyle tüketildiği belirtilmektedir (Matz, 1991). Tüketicilerin ilgi çekici duyusal ve dokusal özelliklere sahip uygun atıştırmalıkları talep etmesi nedeniyle, yemeğe hazır bu ürünlerin tüketiminin önümüzdeki yıllarda hızla büyüyeceği tahmin edilmektedir (Brennan vd., 2013). Atıştırmalıklar patates, mısır ve diğer tahıl ürünlerinden elde edilen cipsler, et bazlı, patlamış mısır bazlı, tahıl patlağı ve patlamış mısır gibi şişirilmiş, patates cipsi görünümlü, fırınlanmış, fındık bazlı atıştırmalıklar gibi çeşitli şekillerde üretilebilmektedir (Matz, 1984). Tahıl ürünlerine dayalı atıştırmalıkların çoğu düşük protein içeriğine ve yüksek kaloriye sahip ürünler olarak marketlerde bulunmaktadır. Bu sebeplerden dolayı yüksek protein içeriğine sahip balık içerikli atıştırmalıkların geliştirildiği belirtilmiştir (Solanki vd., 2020). Deniz ürünleri atıştırmalıklarının protein kaynağı yanı sıra omega-3 yağ asitleri, vitaminler ve temel mineraller açısından da zengin olduğu bildirilmiştir. Bu tür atıştırmalıkların geliştirilmesinin sadece sağlık açısından besleyici gıdaların üretimini sağlamakla kalmayacağı aynı zamanda balık tüketimini de teşvik edeceği vurgulanmaktadır (Shaviklo, 2017).

Dünyada ve ülkemizde özellikle son yıllarda yemeğe hazır halde olan atıştırmalıkların üretimi üzerine yapılmış çeşitli çalışmalar bulunmaktadır. Mısır, soya fasulyesi, ekmek ağacı (*Treculia africana*) atıştırmalıkları (Nwabueze vd., 2008), buğday cipsi (Göncü, 2011), nohut cipsi (Sancak ve Turan, 2011), zenginleştirilmiş gluten cipsi (Ertop vd., 2016), krakerler (Venkatachalam ve Nagarajan, 2017), mantarın cips üretiminde kullanımı (Doğan vd., 2017), chia tohumu kullanılarak zenginleştirilen galetalar (Özgören vd., 2018), lüpen katkılı cips üretimi (Çoban, 2018), probiyotik atıştırmalıkların elma ve *Lactobacillus rhamnosus* GG (LGG) türü mikroorganizma kullanılarak elde edilmesi (Saydam vd., 2019), tatlı ve tuzlu peynir cipsleri (Uğur, 2019), patates cipsi (Kvak vd., 2019), glütensiz cipsler (Yüksel vd., 2019), yemeğe hazır atıştırmalık ve bisküvi (Yilma ve Admassu, 2019), adaçayı tohumu unu ile zenginleştirilmiş mısır cipsi (Yüksel ve Baltacı, 2019) çalışmaları yapılmıştır. Ayrıca balıketi/su ürünleri kullanılarak veya içeriğinde balıketi/su ürünleri bulunan atıştırmalıkların üretimi üzerine de çeşitli çalışmalar bulunmaktadır. Balık etinden yapılan krakerler (King 2002), surimi tozu kullanılarak elde edilen balık cipsleri (Duman vd., 2012), kalamar cipsi (Kılınç ve Sürengil, 2012), balık cipsi (Dinçer vd. 2013; Kuzgun, 2017a), balıklı bisküvi, kraker ve kurabiyeler (Kılınç ve Besler, 2015), balık krakeri (Neiva vd., 2011; İzci ve Bilgin, 2015), karides tozu ile zenginleştirilmiş cipsler ve krakerler (Ikasari ve Hastarini, 2016; Akonor vd., 2017), karides-mısır atıştırmalıkları (Topuz vd., 2017), tilapia balığı kullanılarak yapılan atıştırmalıklar (Cortez Netto vd., 2014), spirulina ile zenginleştirilmiş atıştırmalıklar (Lucas vd., 2018), balık fileto protein konsantresi ilave edilerek hazırlanmış bisküvi (Abraha vd., 2018), buğday, patates ve mısır unları ve toz halde balık eti kullanılarak zenginleştirilmiş balık cipsleri (Büyüksaraç, 2018) yapılan çalışmalara örnek olarak verilebilir. Zhao vd. (2017) tarafından son yıllarda atıştırmalıkların tüketiminin özellikle deniz ürünleri kullanılarak üretilen atıştırmalıkların tüketiminin arttığı bildirilmiştir. Bu nedenle kurutulmuş kalamar içeren yeni ürünlerin geliştirildiği ve sıcak hava kurutma yöntemi ile deniz ürünleri atıştırmalıklarının elde edildiği belirtilmiştir. Ayrıca Kuzgun (2017b) tarafından az yağlı, düşük kalorili ve yüksek besin değeri olan balık atıştırmalıklarının duyusal kabul edilebilirliğinin geliştirilmesinin, balık tüketimini arttırmasının yanı sıra insanların diyet kalitesini arttırmak içinde önemli bir strateji olabileceği vurgulanmaktadır. Dünyada su ürünleri içerikli atıştırmalıklar satışa sunulup tüketilmesine rağmen, ülkemizde endüstriyel anlamda su ürünlerinden atıştırmalık üretimi ve satışı bulunmamaktadır. Türkiye'de patates cipsleri oldukça popüler bir atıştırmalık olup her yaş grubundaki insanlar tarafından sevilerek tüketilen atıştırmalıklardır. Buna karşın, gıda endüstrisinde üretilen atıştırmalıkların yüksek enerji ve düşük besin içerikleri nedeniyle beslenmeye katkıları olmadığı bildirilmektedir (Değerli ve El, 2019). Bu nedenle atıştırmalıkların beslenmeye katkı sağlaması, insan sağlığı açısından yararlı olan kalamardan elde edilmesi, Türk damak tadına uygun olarak üretilmesi, endüstriye kazandırılarak tüketiminin arttırılması gerekmektedir. Bu amaçla çalışmada sağlık açısından faydalı baharat kaplı kalamar atıştırmalıklarının üretilmesi ve üretilen bu atıştırmalıkların duyusal açıdan değerlendirilmesi hedeflenmiştir.

MATERYAL VE YÖNTEM

Çalışmada ham materyal olarak marketten satın alınan 2.4 kg dondurulmuş halka kalamar (*Illex argentinus*) kullanılmıştır. Dondurulmuş kalamarlar 8 saat önceden buzdolabında (4˚C) bekletilerek çözündürülmüştür. Çözündürülmüş kalamarlara kaynamış su (100˚C) içerisinde 5 dk haşlama işlemi uygulanmıştır. Haşlanmış olan halka kalamarlarda bulunan fazla su ise süzgeç yardımı ile 5 saniye boyunca uzaklaştırılmıştır. Daha sonra halka kalamarlar yaklaşık 3 cm boyunda kesilmiştir. Kesim işleminden sonra şerit halindeki kalamarlar marketten satın alınan galeta unu ve baharat karışımı kullanılarak kaplanmıştır. Kaplama işlemi naylon plastik poşetler içerisinde kalamar + galeta unu +baharat karışımı 1:1 oranında (200 g kalamar +100 g galeta+ 100 g baharat karışımı) olacak şekilde yerleştirilmiştir. Kalamar şeritlerinin tamamen galeta unu (Bağdat baharat galeta unu, Türkiye) ve baharat (Bağdat baharat, Türkiye) karışımı ile kaplanması için plastik poşetlere konularak 1 dakika (60 defa) çalkalanmıştır. Bu işlemler her bir farklı baharat grubu için ayrı ayrı gerçekleştirilmiştir. Çalışmada kalamarların kaplanması için 12 farklı baharat toz kırmızı biber (*Capsicum annuum L.*), kimyon (*Cuminum cyminum L.*), kekik (*Thymus vulgaris L.*), yenibahar (*Pimenta dioica L.*), çörek otu (*Nigella sativa L.*), susam (*Sesamum indicum L.*), karabiber (*Piper nigrum L.*), zerdeçal (*Curcuma longa*), zencefil (*Zingiber officinalis*), soğan tozu (*Allium cepa L.*), sarımsak tozu (*Allium sativum L.*), tarçın (*Cinnamomum verum*) kullanılarak 12 farklı örnek oluşturulmuştur. Her bir grup galeta unu+ baharat karışımı ile ayrı ayrı kaplandıktan sonra baharat kaplı kalamar atıştırmalıkları fırında (Beko, Türkiye) 200˚C'de 20 dakika pişirme işleminden sonra oda sıcaklığında bekletilerek soğutulduktan sonra panelistlerin değerlendirmesi istenmiştir. Aşağıda üretimi gerçekleştirilen baharat kaplı kalamar atıştırmalıkların işlem basamakları Şekil 1 de verilmiştir.



Şekil 1. Baharat Kaplı Kalamar Atıştırmalıkları Üretim İşlem Basamakları

Çalışmada farklı baharatlar kullanılarak üretilen baharat kaplı kalamar atıştırmalıkları Şekil 2'de verilmiştir.

Şekil 2. Farklı baharatlar kullanılarak üretilen baharat kaplı kalamar atıştırmalıkları

Duyusal kalite değerlendirmesi

Beslenme ve gastronomide gıdaların kalite kontrollerinde duyusal analizlerin oldukça önemli olduğu belirtilmektedir. Gıdaların kalite karakterlerinin koklama, dokunma, tatma veya görme duyularının tepkilerini ölçen bir analiz yöntemi olduğu vurgulanmaktadır. Ayrıca duyusal değerlendirmede tat ve aromanın en önemli özellikler olduğu bildirilmektedir (Batu, 2017). Çalışmada baharat kaplı kalamar atıştırmalıklarının duyusal kalitelerinin belirlenmesi amacıyla farklı özellikleri içeren iki farklı form kullanılmıştır. Baharat kaplı kalamar atıştırmalıklarının duyusal değerlendirmesi için iki farklı form rastgele örnekleme yöntemi ile Ege Üniversitesi Su Ürünleri Fakültesinde görev alan ve eğitim gören su ürünleri konusunda deneyimli 30 paneliste sunulmuştur. Panelistlerin her değerlendirdikleri örnek sonrasında ağızlarını su ile çalkalaması sağlanarak ardından diğer bir örneği değerlendirmesi istenmiştir. Böylece örneklerin ağızda bıraktıkları lezzetlerin birbirine karışımı önlenmiştir. Baharat kaplı kalamar atıştırmalıklarının panelistler tarafından duyusal kalitesinin belirlenmesi oda sıcaklığında (25˚C) gerçekleştirilmiştir. Çalışmada baharat kaplı kalamar atıştırmalıklarının panelistler tarafından duyusal kalitelerinin belirlenmesi amacıyla kullanılan iki formda da 9 puanlık hedonik skala (1- aşırı kötü, 2- çok kötü, 3-orta derecede kötü, 4-kötü, 5-kararsızım, 6-iyi, 7-orta derecede iyi, 8-çok iyi ve 9-aşırı iyi) kullanılmıştır. Baharat kaplı kalamar atıştırmalıklarının (görünüm, renk, koku, tat, doku (gevreklik) ve genel kabul edilebilirliğini) 6 farklı özelliğini değerlendirilmesi için Bandre vd. (2018) tarafından belirlenmiş parametrelerin bulunduğu duyusal form kullanılmıştır. Panelistlere uygulanan ikinci formda ise (renk yoğunluğu, renk homojenliği, sosun uyumu, sertlik, çıtırlık, gevreklik, ağızda dağılma ve tat yoğunluğunu) içeren 8 farklı özelliğin değerlendirilmesi istenmiştir. Panelistlere sunulan ikinci formda ise Albert vd. (2009) tarafından belirlenen parametrelerin bulunduğu duyusal form revize edilerek kullanılmıştır.

İstatistiksel değerlendirme

Bu çalışmada tüm istatistiki değerlendirme SPSS 25.0 paket programı kullanılarak yapılmıştır. 1. duyusal formda yer alan 6 özellik (görünüm, renk, koku, tat, doku ve genel kabul edilebilirlik) ile 2. duyusal formda yer alan 8 özelliğin (renk yoğunluğu, renk homojenliği, sosun uyumu, sertlik, çıtırlık, gevreklik, ağızda dağılma, tat yoğunluğu) her biri için 12 farklı baharat kullanılarak elde edilen atıştırmalıkların her birine panelistlerin verdikleri puanlara göre tablolar oluşturulmuştur. Böylece hangi atıştırmalıkta hangi özelliğe kaç panelistin kaç puan verdiği belirlenmiştir. Her bir atıştırmalık için her bir özelliğe göre verilen toplam puanlar tablolarda yer almaktadır.

1. duyusal (Bandre vd., 2018) formda yer alan 6 özellik (görünüm, renk, koku, tat, doku ve genel kabul edilebilirlik) için 12 örneğin her birinde k bağımlı örneklem için Friedman testi yapılmıştır. Friedman S testi parametrik iki yönlü varyans analizi yönteminin parametrik olmayan alternatifidir. Bu testin temel özelliği normallik ve homojen varyans varsayımı gerektirmemesi ve ölçüm değerlerine atanan büyüklük sıra sayılarına dayanmasıdır (Gamgam ve Altunkaynak, 2012). Örnek bazında farklılığın hangi özellikten kaynaklandığını belirlemek amacıyla da iki bağımlı örnek işaret testi yapılmıştır. İşaret testi, kitlelerin dağılımlarının normal olası durumunda kullanılan parametrik t testinin parametrik olmayan karşılığıdır. Benzer şekilde formda yer alan 6 özellik (görünüm, renk,

koku, tat, doku ve genel kabul edilebilirlik) parametresinin her biri örnek bazında değerlendirilmesi amacıyla k bağımlı örneklem Friedman testi yapılmıştır. Farklılığın hangi örneklerden kaynaklandığını belirlemek amacıyla da iki bağımlı örneklem işaret testi yapılmıştır. Test sonuçları 0.05 anlamlılık seviyesinde değerlendirilmiştir. Her test için ayrıntılı değerler bulgular bölümünde belirtilmiştir.

Yine 2. duyusal formda yer alan 8 özelliğin (renk yoğunluğu, renk homojenliği, sosun uyumu, sertlik, çıtırlık, gevreklik, ağızda dağılma, tat yoğunluğu) atıştırmalıklarda kullanılan 12 baharatta etkilerinin farklı olup olmadığının belirlenmesi amacıyla k bağımlı örneklem için Friedman testi yapılmıştır. Farklılığın hangi özellikten kaynaklandığını belirlemek amacıyla da iki bağımlı örneklem için işaret testi kullanılmıştır. Formda yer alan 8 özelliğin her birinin örnek bazında değerlendirilmesi amacıyla k bağımlı örneklem Friedman testi yapılmıştır. Farklılığın hangi örnekten kaynaklandığını belirlemek amacıyla da iki bağımlı örneklem işaret testi kullanılmıştır.

BULGULAR

Baharat kaplı kalamar atıştırmalıklarına uygulanan 1.duyusal değerlendirme sonuçları Tablo 1'de verilmiştir. Duyusal değerlendirmede panelistlere göre en beğenilen grupların sırasıyla görünüm açısından zerdeçal, sarımsak tozu ve toz kırmızı biber kaplı kalamar atıştırmalıkları, renk açısından sırasıyla, sarımsak tozu, zerdeçal ve kekik kaplı kalamar atıştırmalıkları, koku açısından sırasıyla; kekik, sarımsak tozu ve soğan tozu kaplı kalamar atıştırmalıkları olduğu belirlenmiştir. Tat ve doku açısından değerlendirildiğinde ise sırasıyla; sarımsak tozu, soğan tozu ve kekik kaplı kalamar atıştırmalıkları ve genel kabul edilebilirlik açsından ise soğan tozu, sarımsak tozu ve kekik kaplı kalamar atıştırmalıkların en beğenilen gruplar olduğu belirlenmiştir. Panelistlere göre en beğenilmeyen grupların sırasıyla görünüm, renk, koku ve genel kabul edilebilirlik açısından yenibahar kaplı kalamar atıştırmalığı, tat için karabiber kaplı kalamar atıştırmalığı, doku için tarçın ve yenibahar kaplı kalamar atıştırmalıkları olduğu belirtilmiştir.

Tablo 1. Baharat kaplı kalamar atıştırmalıklarının1.duyusal analiz değerlendirme sonuçları.

Örnek	Görünüm	Renk	Koku	Tat	Doku	Genel Kabul Edilebilirlik
Toz kırmızı b.	7,06 ±1,38^{a1}	6,9±1,51^{a1}	6,23±1,54^{a2}	5,5±1,83^{a3}	4,9±1,86^{a3}	6,0±1,41^{a}
Kimyon	6,03±1,58^{a1}	5,96±1,73^{a1}	5,83±1,74^{a1}	5,2±1,90^{a1}	4,56±1,92^{a1}	5,4±1,71^{a}
Kekik	6,9±1,37^{b1}	7,0±1,01^{b1}	7,33±1,18^{b1}	6,9±1,56^{b1}	5,93±1,83^{b1}	6,83±1,17^{b}
Yenibahar	3,96±2,25^{b1}	4,36±2,24^{c1}	4,86±1,53^{c1}	4,36±1,94^{c1}	4,36±1,75^{c1}	4,53±1,83^{c}
Çörekotu	6,56±1,52^{c1}	6,1±1,56^{d2}	6,76±1,04^{d3}	5,83±1,53^{d4}	5,3±1,66^{d4}	5,93±1,66^{d}
Susam	5,8±1,91^{d1}	5,86±1,65^{d1}	5,83±1,44^{e1}	5,7±1,64^{d1}	5,06±1,61^{d1}	5,8±1,52^{d}
Karabiber	4,8±1,95^{e1}	4,53±2,01^{d1}	5,2±1,90^{e1}	3,98±1,90^{e1}	4,43±1,94^{d1}	4,36±1,83^{d}
Zerdeçal	7,4±1,13^{f1}	7,46±0,97^{e1}	6,0±1,82^{e2}	5,26±1,95^{e3}	5,03±2,11^{d3}	5,76±1,63^{e}
Zencefil	6,3±1,42^{g1}	5,8±1,71^{f2}	5,63±1,54^{e2}	5,16±1,88^{e3}	5,3±1,90^{d3}	5,53±1,91^{e}
Soğan Tozu	6,76±1,45^{g1}	6,53±1,50^{f1}	7,03±1,27^{f1}	7,36±1,74^{f1}	5,93±1,74^{d2}	7,1±1,21^{f}
Sarımsak Tozu	7,2±1,54^{g1}	7,6±1,64^{f1}	7,23±1,52^{f1}	7,16±1,66^{f1}	6,13±2,13^{d1}	7,03±1,50^{f}
Tarçın	5,43±2,06^{h1}	5,2±1,85^{g1}	5,23±1,77^{g1}	4,83±1,86^{g1}	4,36±1,99^{e1}	4,7±1,89^{g}

Sonuçlar n:30, x_{ort} ± standart sapma olarak gösterilmiştir. Aynı sütundaki harfler gruplar arası istatiksel farkı, aynı satırdaki rakamlar her bir baharat için grup içi farkı göstermektedir (p<0,05).

Buna göre 1. duyusal formda (Bandre vd., 2018) 12 farklı baharat kullanılarak üretilen baharat kaplı kalamar atıştırmalıkları arasındaki istatistiksel anlam farkları değerlendirilmiştir. Testin sonucunda toz kırmızı biberli atıştırmalık için görünüm ile koku, tat ve doku arasındaki farklar anlamlıdır (p-değeri<0.01). Yine renk ile koku (p-değeri=0.023), tat ve doku arasındaki farklar anlamlıdır (p-değeri<0.01). Koku ile tat (p-değeri=0.035) ve doku (p-değeri<0.01) arasındaki farklar da anlamlıdır. Kimyonlu atıştırmalık için görünüm ile tat (p-değeri=0.043) ve doku (p-değeri=0.015) arasındaki farklar anlamlıdır. Doku ile renk (p-değeri<0.01) ve koku (p-değeri=0.023) arasındaki farklar anlamlıdır. Kekikli atıştırmalık için görünüm ile doku (p-değeri=0.019), doku ile renk (p-değeri=0.041) ve doku ile koku (p-değeri<0.01) arasındaki farklar anlamlıdır. Yenibaharlı atıştırmalık

için özellikler arasındaki farklar anlamlı görünmemektedir (p-değeri=0.058). Çörekotlu atıştırmalık için görünüm ile renk, tat ve doku arasındaki farklar anlamlıdır (p-değeri<0.01). Yine renk ile koku (p-değeri=0.027) ve doku arasındaki farklar anlamlıdır (p-değeri<0.01). Koku ile tat ve doku arasındaki farklar da anlamlıdır (p-değeri<0.01). Susamlı atıştırmalık için sadece doku ile koku arasındaki fark anlamlıdır (p-değeri<0.01). Karabiberli atıştırmalık için özellikler arasındaki farklar anlamlı görünmemektedir (p-değeri=0.08). Zerdeçallı atıştırmalık için görünüm ile koku, tat ve doku arasındaki farklar anlamlıdır (p-değeri<0.01). Yine renk ile koku, tat ve doku arasındaki farklar anlamlıdır (p-değeri<0.01). Koku ile tat (p-değeri=0.013) ve doku (p-değeri=0.031) arasındaki farklar da anlamlıdır. Zencefilli atıştırmalık için görünüm ile renk (p-değeri=0.012), koku (p-değeri=0.031), tat (p-değeri<0.01) ve doku (p-değeri=0.017) arasındaki farklar anlamlıdır. Koku ile tat arasındaki fark da anlamlıdır (p-değeri=0.049). Soğan tozlu atıştırmalık için tat ile renk ve doku ile koku arasındaki farklar anlamlıdır (p-değeri<0.01). Sarımsak tozlu atıştırmalık için doku ile görünüm, doku ile renk ve ve doku ile koku arasındaki farklar anlamlıdır (p-değeri<0.01). Tarçınlı atıştırmalık için doku ile renk ve doku ile koku arasındaki farklar anlamlıdır (p-değeri<0.01). Kullanılan 1. duyusal form (Bandre vd., 2018) için elde edilen istatistiksel frekans tablosuna ait değerler Tablo 2'te verilmiştir.

Tablo 2. 1. duyusal analiz sonuçlarının değerlendirilmesine ilişkin istatiksel frekans tablosu.

Puanlama	Görünüm	Renk	Koku	Tat	Doku	Genel kabul edilebilirlik
(1)Aşırı kötü	6 (%1,7)	7 (%1,9)	5 (%1,4)	8 (%2,2)	10 (%2,8)	4 (%1,1)
(2)Çok kötü	13 (%3,6)	11 (%3,1)	8 (%2,2)	19 (%5,3)	34 (%9,4)	18 (%5,0)
(3)Orta derece kötü	15 (%4,2)	16 (%4,4)	15 (%4,2)	38(%10,6)	33 (%9,2)	21 (%5,8)
(4)Kötü	37 (%10,3)	44(%12,2)	24 (%6,7)	37(%10,3)	53(%14,7)	44 (%12,2)
(5)Kararsızım	41 (%11,4)	44(%12,2)	66(%18,3)	57(%15,8)	71(%19,7)	60 (%16,7)
(6)İyi	70 (%19,4)	67(%18,6)	92(%25,6)	81(%22,5)	70(%19,4)	85 (%23,6)
(7)Orta derece iyi	86 (%23,9)	87(%24,2)	77(%21,4)	47(%13,1)	47(%13,1)	57 (%15,8)
(8)Çok iyi	53 (%14,7)	56(%15,6)	44(%12,2)	47(%13,1)	31 (%8,6)	57 (%15,8)
(9)Aşırı iyi	39 (%10,8)	28 (%7,8)	29 (%8,1)	26 (%7,2)	11 (%3,1)	14 (%3,9)

Verilen sayılar 12 örnek için puan veren toplam panelist sayısını ifade etmektedir. %'lik ise panelist sayılarının yüzdesini ifade etmektedir.

İstatistiksel frekans tablosuna (Tablo 2) göre görünüm için 12 örneğin aşırı iyi olduğunu belirten toplam panelist sayısı 39'dur. Renk için 28, koku için 29, tat için 26, doku için 11 iken genel kabul edilebilirlik için 12 örneğin aşırı iyi olduğunu belirten toplam panelist sayısı ise 14'tür.

Tablo 3. Baharat kaplı kalamar atıştırmalıklarının 1. duyusal analiz sonuçlarının puan ve panelist sayılarına göre değerlendirilmesi.

Baharatlar	Görünüm	Renk	Koku	Tat	Doku	Genel kabul edilebilirlik
Toz kırmızı b.	13 panelist, 7 puan	10 panelist, 7 puan	10 panelist, 7 puan	7 panelist, 6 puan	6'şar panelist, 5 ve 7 puan	9 panelist, 6 puan
Kimyon	11 panelist, 7 puan	10 panelist, 7 puan	10 panelist, 7 puan	9 panelist, 6 puan	7'şer panelist, 3 ve 6 puan	9 panelist, 6 puan
Kekik	11 panelist, 7 puan	14 panelist, 7 puan	14 panelist, 7 puan	8 panelist, 6 puan	8 panelist, 6 puan	10 panelist, 6 puan
Yenibahar	7'şer panelist, 2 ve 6 puan	8 panelist, 4 puan	8 panelist, 4 puan	6'şar panelist, 3,4 ve 6 puan	10 panelist, 5 puan	12 panelist, 5puan
Çörekotu	8 panelist, 8 puan	9 panelist, 7 puan	9 panelist, 7 puan	8'er panelist, 5 ve 6 puan	9 panelist, 5 puan	10 panelist, 6 puan
Susam	10 panelist, 6 puan	9 panelist, 7 puan	9 panelist, 7 puan	8'er panelist, 5 ve 6 puan	8 panelist, 6 puan	12 panelist, 6 puan
Karabiber	7 panelist, 4 puan	9 panelist, 4 puan	9 panelist, 4 puan	9 panelist, 3 puan	8 panelist, 4 puan	7 panelist, 4 puan
Zerdeçal	9 panelist, 7 puan	12 panelist, 8 puan	12 panelist, 8 puan	9 panelist, 6 puan	7 panelist, 6 puan	10 panelist, 6 puan
Zencefil	9 panelist, 7 puan	7'şer panelist, 5 ve 7 puan	7'şer panelist, 5 ve 7 puan	7 panelist, 5 puan	7 panelist, 7 puan	8 panelist, 5 puan
Soğan Tozu	9 panelist, 6 puan	9 panelist, 8 puan	9 panelist, 8 puan	10 panelist, 9 puan	7 panelist, 7 puan	8'er panelist, 7 ve 8 puan
Sarımsak Tozu	8 panelist, 8 puan	7'şer panelist, 8 ve 9 puan	7'şer panelist, 8 ve 9 puan	8 panelist, 9 puan	5'er panelist, 6, 7 ve 9 puan	7 panelist, 8 puan
Tarçın	7 panelist, 5 puan	7 panelist, 5 puan	7 panelist, 5 puan	7 panelist, 5 puan	7 panelist, 4 puan	11 panelist, 4 puan

Baharat kaplı kalamar atıştırmalıklarının değerlendirilmesi sırasında panelistler tarafından en çok verilen puanlar verilmiştir.

Görünüm için toz kırmızı biber kaplı kalamar atıştırmalıklarına 7 puan veren en fazla 13 panelist, renk ve koku için kekik kaplı kalamar atıştırmalıklarına 7 puan veren en fazla 14 panelist, tat için soğan tozu kaplı kalamar atıştırmalıklarına 9 puan veren 10 panelist bulunmaktadır. Doku için yenibahar kaplı kalamar atıştırmalıklarına 5 puan veren 10 panelist bulunurken genel kabul edilebilirlik için yenibahar kaplı kalamar atıştırmalıklarına 5 puan veren 12 panelist ve susam kaplı kalamar atıştırmalıklarına 6 puan veren 12 panelist bulunmaktadır (Tablo 3). Baharat kaplı kalamar atıştırmalıklarına uygulanan 2.duyusal değerlendirme sonuçları Tablo 4'de verilmiştir.

Tablo 4. Baharat kaplı kalamar atıştırmalıklarının 2.duyusal analiz değerlendirme sonuçları.

Örnek	Renk Yoğunluğu	Renk Homojenliği	Sosun Uyumu	Sertlik	Çıtırlık	Gevreklik	Ağızda Dağılma	Tat Yoğunluğu
Toz kırmızı b.	$7,36\pm1,27^{a1}$	$6,96\pm1,45^{a1}$	$5,63\pm1,99^{a2}$	$4,83\pm1,98^{a2}$	$3,86\pm2,21^{a3}$	$4,36\pm1,47^{a3}$	$4,36\pm1,92^{a3}$	$5,26\pm1,98^{a4}$
Kimyon	$6,5\pm1,07^{b1}$	$6,43\pm1,33^{a1}$	$4,9\pm1,71^{a2}$	$5,3\pm1,71^{a2}$	$3,96\pm1,85^{a3}$	$4,3\pm1,80^{a3}$	$4,5\pm2,16^{a3}$	$5,26\pm1,84^{a4}$
Kekik	$6,83\pm1,58^{b1}$	$5,9\pm1,73^{a2}$	$6,83\pm1,72^{b3}$	$6,5\pm1,50^{b3}$	$5,6\pm2,22^{b4}$	$5,76\pm1,88^{b4}$	$5,23\pm1,92^{a4}$	$6,83\pm1,40^{b5}$
Yenibahar	$5,43\pm2,46^{b1}$	$5,66\pm2,94^{a1}$	$4,06\pm1,98^{c2}$	$4,23\pm2,18^{c2}$	$4,0\pm1,86^{c2}$	$3,96\pm1,43^{c2}$	$3,96\pm1,59^{a2}$	$4,56\pm1,63^{c2}$
Çörekotu	$5,73\pm1,91^{b1}$	$5,56\pm1,91^{a1}$	$5,43\pm1,48^{d1}$	$5,26\pm1,89^{c1}$	$4,26\pm2,25^{c2}$	$4,96\pm2,12^{d3}$	$4,6\pm2,06^{a3}$	$5,0\pm1,58^{c3}$
Susam	$5,1\pm1,88^{b1}$	$5,8\pm1,75^{a2}$	$5,13\pm1,69^{d2}$	$4,9\pm1,92^{c2}$	$4,13\pm1,68^{c3}$	$4,23\pm1,91^{d3}$	$4,7\pm2,23^{a3}$	$4,83\pm1,51^{c3}$
Karabiber	$6,33\pm1,12^{c1}$	$6,13\pm1,85^{a1}$	$4,33\pm2,28^{d2}$	$4,4\pm2,37^{c2}$	$4,3\pm2,14^{c2}$	$4,16\pm2,18^{d2}$	$4,13\pm2,21^{a2}$	$5,26\pm2,23^{c2}$
Zerdeçal	$7,96\pm0,96^{d1}$	$7,9\pm0,99^{b1}$	$5,13\pm2,03^{d2}$	$4,76\pm1,91^{c2}$	$4,23\pm2,18^{c2}$	$4,53\pm1,99^{d2}$	$5,16\pm2,10^{b2}$	$5,56\pm1,87^{c2}$
Zencefil	$5,93\pm1,66^{e1}$	$5,96\pm1,42^{c1}$	$4,6\pm1,65^{d2}$	$4,5\pm2,15^{c2}$	$3,9\pm2,01^{c2}$	$4,3\pm2,09^{d2}$	$5,63\pm2,04^{b2}$	$5,66\pm1,71^{c2}$
Soğan Tozu	$6,73\pm1,59^{e1}$	$6,2\pm1,71^{c1}$	$7,16\pm1,34^{e2}$	$5,7\pm2,17^{d3}$	$5,13\pm2,11^{d4}$	$5,33\pm1,95^{e4}$	$5,8\pm2,02^{c4}$	$7,56\pm1,07^{d4}$
Sarımsak Tozu	$7,03\pm1,99^{e1}$	$6,73\pm1,74^{c1}$	$7,23\pm1,55^{e1}$	$5,9\pm2,09^{d2}$	$5,53\pm2,24^{d2}$	$5,76\pm2,22^{e2}$	$5,8\pm2,25^{c2}$	$7,46\pm1,36^{d2}$
Tarçın	$6,5\pm2,01^{e1}$	$6,6\pm1,92^{c1}$	$4,73\pm2,18^{e2}$	$3,9\pm2,20^{d2}$	$3,63\pm2,22^{d2}$	$3,66\pm2,22^{e2}$	$3,96\pm2,27^{c2}$	$5,7\pm1,98^{d2}$

Sonuçlar n:30, x_{ort} ± standart sapma olarak gösterilmiştir. Aynı sütundaki harfler gruplar arası istatiksel farkı, aynı satırdaki rakamlar her bir baharat için grup içi farkı göstermektedir ($p<0,05$).

Buna göre 2. duyusal formda (Albert vd., 2009)12 farklı baharat kullanılarak üretilen baharat kaplı kalamar atıştırmalıklarının tümü için özelliklerin etkileri arasındaki farkın anlamlı olduğu saptanmıştır (p-değeri<0.01). Renk yoğunluğu içinde kullanılan 12 baharatın etkilerinin farklı olduğu bulgulanmıştır (p-değeri<0.01). Farklılığın hangi örnekten kaynaklandığını belirlemek amacıyla yapılan iki bağımlı örneklem için işaret testine göre; kırmızı toz biberli atıştırmalık ile kimyonlu, yenibaharlı (p-değeri=0.015), çörekotlu, susamlı, karabiberli (p-değeri=0.023), zerdeçallı (p-değeri=0.012), zencefilli, tarçınlı (p-değeri=0.035) kalamar atıştırmalıkları arasında fark olduğu belirlenmiştir (p-değeri<0.01). Kimyonlu kalamar atıştırmalıkları ile susamlı, zerdeçallı kalamar atıştırmalıkları arasında da fark bulgulanmıştır (p-değeri<0.01). Kimyonlu atıştırmalık ile susamlı (p-değeri<0.01) ve zerdeçallı (p-değeri=0.011) kalamar atıştırmalıkları arasında da fark olduğu saptanmıştır. Yenibahar kaplı kalamar atıştırmalıklar ile zerdeçallı, soğan tozlu (p-değeri=0.014), sarımsak tozlu, tarçınlı (p-değeri=0.043) atıştırmalık arasında farkın olduğu belirlenmiştir (p-değeri<0.01). Çörekotu kaplı kalamar atıştırmalık ile zerdeçallı ve sarımsak tozlu kaplı kalamar atıştırmalıkları arasında fark belirlenmiştir (p-değeri<0.01). Susam kaplı kalamar atıştırmalıkları ile karabiberli (p-değeri=0.035), zerdeçallı, zencefilli (p-değeri=0.031), soğan tozlu, sarımsak tozlu, tarçınlı (p-değeri=0.011) kalamar atıştırmalıkları arasında da fark saptanmıştır (p-değeri<0.01). Karabiber kaplı kalamar atıştırmalığı ile zerdeçal kaplı kalamar atıştırmalık arasındaki farkın anlamlı (p-değeri<0.01), zerdeçal kaplı kalamar atıştırmalığı ile zencefil, soğan tozu, tarçın kaplı kalamar atıştırmalıkları arasında farkın da anlamlı olduğu belirlenmiştir (p-değeri<0.01). Zencefil kaplı kalamar atıştırmalığı ile sarımsak tozu kaplı kalamar atıştırmalığı arasındaki farkta anlamlı bulunmuştur (p-değeri=0.043). Duyusal değerlendirme sonuçlarına göre (Tablo 5) en beğenilen grupların renk yoğunluğu ve renk homojenliği açısından sırasıyla zerdeçal, toz kırmızı biber ve sarımsak tozu kaplı kalamar atıştırmalıkları, sos uyumu açısından sırasıyla sarımsak tozu, soğan tozu ve kekik kaplı kalamar atıştırmalıkları, sertlik ve çıtırlık açısından kekik, sarımsak tozu ve soğan tozu kaplı kalamar atıştırmalıkları olduğu belirlenmiştir. Gevreklik açısından en beğenilen grupların kekik ve sarımsak tozu kaplı kalamar atıştırmalıkları, ağızda dağılma ve tat yoğunluğu açısından en beğenilen grupların soğan ve sarımsak tozu a kaplı kalamar tıştırmalıkları olduğu belirlenmiştir. Renk yoğunluğu, renk homojenliği, sos uyumu, açısından en beğenilmeyen gruplar sırasıyla; susam, çörekotu, yenibahar, sertlik, çıtırlık ve gevreklik tarçın, ağızda dağılma açısından tarçın ve yenibahar, tat yoğunluğu açısından ise yenibahar kaplı kalamar atıştırmalıkları olduğu belirlenmiştir.

Kullanılan 2. duyusal form (Albert vd., 2009) için elde edilen istatistiksel frekans tablosuna ait değerler Tablo 5'te verilmiştir.

Tablo 5. 2. duyusal analiz sonuçlarının değerlendirilmesine ilişkin istatiksel frekans tablosu.

Puanlama	Renk yoğunluğu	Renk homojenliği	Sos uyumu	Sertlik	Çıtırlık	Gevreklik	Ağızda dağılma	Tat yoğunluğu
(1)Aşırı kötü	4 (%1,1)	2 (%0,6)	19 (%5,3)	24 (%6,7)	53(%14,7)	18 (%5,0)	28 (%7,8)	3 (%0,8)
(2)Çok kötü	12 (%3,3)	12 (%3,3)	20 (%5,6)	23 (%6,4)	26 (%7,2)	60(%16,7)	34 (%9,4)	23 (%6,4)
(3)Orta kötü	11 (%3,1)	16 (%4,4)	30 (%33)	43(%11,9)	44(%12,2)	25 (%6,9)	46(%12,8)	26 (%7,2)
(4)Kötü	30 (%8,3)	26 (%7,2)	33 (%9,2)	59(%16,4)	55(%15,3)	68(%18,9)	51(%14,2)	36(%10,0)
(5)Kararsızım	47(%13,1)	45 (%12,5)	65(%18,1)	53(%14,7)	68(%18,9)	66(%18,3)	72(%20,0)	61(%16,9)
(6)İyi	58(%16,1)	75 (%20,8)	82(%22,8)	58(%16,1)	53(%14,7)	56(%15,6)	49(%13,6)	85(%23,6)
(7)Orta iyi	73 (20,3)	84 (%23,3)	52(%14,4)	59(%16,4)	33 (%9,2)	34 (%9,4)	39(%10,8)	50(%13,9)
(8)Çok iyi	76 (21,1)	65 (%18,1)	37(%10,3)	24 (%6,7)	21 (%5,8)	25 (%6,9)	28 (%7,8)	50(%13,9)
(9)Aşırı iyi	49(%13,6)	35 (%9,7)	22 (%6,1)	17 (%4,7)	7 (%1,9)	8 (%2,2)	13 (%3,6)	26 (%7,2)

Verilen sayılar 12 örnek için puan veren toplam panelist sayısını ifade etmektedir. %'lik ise panelist sayılarının yüzdesini ifade etmektedir.

İstatistiksel frekans tablosuna (Tablo 5) göre renk yoğunluğu için 12 örneğin aşırı iyi olduğunu belirten toplam panelist sayısı 49'dur. Renk homojenliği için 35, sos uyumu için 22, sertlik için 17, çıtırlık için 7, gevreklik için 8, ağızda dağılma için 26 iken tat yoğunluğu için 12 örneğin aşırı iyi olduğunu belirten toplam panelist sayısı ise 26'dır.

Tablo 6. Baharat kaplı kalamar atıştırmalıklarının 2. duyusal analiz sonuçlarının puan ve panelist sayılarına göre değerlendirilmesi.

Baharatlar	Renk Yoğunluğu	Renk Homojenliği	Sosun Uyumu	Sertlik	Çıtırlık	Gevreklik	Ağızda Dağılma	Tat Yoğunluğu
Toz kırmızı biber	9 panelist, 8 puan	8'er panelist, 7 ve 8 puan	12 panelist, 6 puan	6 panelist, 5 puan	6'şar panelist, 1 ve 6 puan	12 panelist, 5 puan	7 panelist, 5 puan	7 panelist, 6 puan
Kimyon	8 panelist, 5 puan	11 panelist, 7 puan	8 panelist, 6 puan	9'ar panelist, 4 ve 7 puan	7 panelist, 6 puan	9 panelist, 6 puan	5'er panelist, 3, 4, 6 ve 7 puan	7 panelist, 7 puan
Kekik	10 panelist, 8 puan	7 panelist, 6 puan	7 panelist, 7 puan	9 panelist, 6 puan	7 panelist, 7 puan	9 panelist, 7 puan	10 panelist, 5 puan	9 panelist, 6 puan
Yenibahar	7 panelist, 5 puan	6 panelist, 6 puan	9 panelist, 5 puan	5'er panelist, 3 ve 4 puan	7 panelist, 5 puan	8 panelist, 4 puan	7'şer panelist, 3 ve 4 puan	10 panelist, 5 puan
Çörekotu	8 panelist, 7 puan	7'şer panelist, 6 ve 7 puan	10 panelist, 6 puan	8 panelist, 6 puan	8 panelist, 5 puan	6'şar panelist, 5 ve 6 puan	7 panelist, 5 puan	9 panelist, 5 puan
Susam	8 panelist, 4 puan	9 panelist, 7 puan	10 panelist, 6 puan	11 panelist, 6 puan	8 panelist, 4 puan	8 panelist, 4 puan	6 panelist, 3 puan	8 panelist, 5 puan
Karabiber	8 panelist, 6 puan	9 panelist, 7 puan	5'er panelist, 2 ve 5 puan	4'er panelist, 1,2,3,4,5,7 ve 8 puan	6'şar panelist, 4 ve 5 puan	8 panelist, 2 puan	6 panelist, 3 puan	7 panelist, 6 puan
Zerdeçal	12 panelist, 8 puan	10'ar panelist, 8 ve 9 puan	8 panelist, 6 puan	8 panelist, 4 puan	6 panelist, 6 puan	7'şer panelist, 2 ve 4 puan	8 panelist, 5 puan	10 panelist, 6 puan
Zencefil	8 panelist, 7 puan	8 panelist, 6 puan	8 panelist, 3 puan	7 panelist, 4 puan	10 panelist, 5 puan	6 panelist, 4 puan	6 panelist, 5 puan	10 panelist, 6 puan
Soğan Tozu	7'şer panelist, 7 ve 8 puan	8 panelist, 5 puan	9 panelist, 8 puan	6 panelist, 5 puan	7'şer panelist, 4 ve 6 puan	7'şer panelist, 4 ve 6 puan	6 panelist, 5 puan	12 panelist, 8 puan
Sarımsak Tozu	9 panelist, 8 puan	8 panelist, 8 puan	10 panelist, 8 puan	7 panelist, 7 puan	8 panelist, 7 puan	6'şar panelist, 7 ve 8 puan	8'er panelist, 5 ve 8 puan	12 panelist, 8 puan
Tarçın	8 panelist, 7 puan	9 panelist, 7 puan	9 panelist, 5 puan	5'er panelist, 1 ve 2 puan	8 panelist, 7 puan	7 panelist, 1 puan	6'şar panelist, 1 ve 6 puan	10 panelist, 6 puan

Baharat kaplı kalamar atıştırmalıklarının değerlendirilmesi sırasında panelistler tarafından en çok verilen puanlar verilmiştir.

Renk yoğunluğu için kekik kaplı kalamar atıştırmalıklarına 8 puan veren en fazla 10 panelist, renk homojenliği için kimyon kaplı kalamar atıştırmalıklarına 7 puan veren en fazla 11 panelist, sosun uyumu için toz kırmızı biber kaplı kalamar atıştırmalıklarına 6 puan veren en fazla 12 panelist, sertlik için susam kaplı kalamar atıştırmalıklarına 6 puan veren en fazla 11 panelist bulunmaktadır (Tablo 6).

Duyusal analiz formunda yer alan çıtırlık için zencefil kaplı kalamar atıştırmalıklarına 5 puan veren en fazla 10 panelist, gevrek için toz kırmızı biber kaplı kalamar atıştırmalıklarına 5 puan veren en fazla 12 panelist, ağızda dağılma için kekik kaplı kalamar atıştırmalıklarına 5 puan veren en fazla 10 panelist bulunurken, tat yoğunluğu için soğan tozu ve sarımsak tozu kaplı kalamar atıştırmalıklarına 8 puan veren en fazla 12 panelist bulunmaktadır (Tablo 6).

TARTIŞMA VE SONUÇ

Farklı formülasyonda hazırlanan baharat kaplı atıştırmalıklar üzerine çeşitli çalışmalar bulunmaktadır. Yapılan bir çalışmada büyük göz balığı (*Brachydeuterus auritus*) kullanılarak yapılan balık krakerlerinde duyusal açıdan en çok beğenilen formülasyonların %50 balık/%50 nişasta ve %40 balık/%60 nişasta kombinasyonların kullanıldığı krakerlerin olduğu belirtilmiştir (King, 2002). Gümüş balığı (*Atherina boyeri,* RISSO 1810) kullanılarak balık cipsi üretimi ve bazı kalite değişimlerinin belirlenmesi üzerine yapılan bir çalışmada panelistlerin duyusal analiz sonuçlarına göre balık cipslerinin çok beğenildiği bildirilmiştir. Balık cipslerinin -18°C'de 6 ay dondurarak depolama sonunda duyusal, kimyasal ve mikrobiyolojik açıdan tüketilebilir olduğu belirtilmiştir (İzci vd., 2011). Kömür balığı *(Pollachius virens*) surimisinden yapılan farklı oranlarda %3, %5, %7 ve %9 balık protein tozu ile güçlendirilmiş mısır aperatifin hazırlandığı çalışmada ise % 9 balık protein tozu içeren atıştırmalıkların, koku, doku, lezzet ve genel kabul edilebilirlik açısından diğer oranda balık protein tozu %3, %5 ve %7 içeren atıştırmalıklardan önemli ölçüde daha düşük beğeniye sahip olduğu bildirilmiştir (Shaviklo vd., 2011). Sis balığı (*Aspius vorax*) kullanılarak manuel yöntemle surimi elde edilen çalışmada surimi kurutularak toz haline getirilmiş ve farklı oranlarda surimi tozu kullanılarak elde edilen balık cipsilerin kalitesi incelenmiştir. Ürüne surimi tozu katılmasıyla renk, koku, gevreklik ve lezzet bakımından kontrol grubuna göre beğeninin azaldığı ve surimi tozu konsantrasyonu artıkça surimi tozundan gelen aromanın, koku ve lezzet üzerinde olumsuz etki yaptığı bildirilmiştir (Duman vd., 2012). Cortez Netto vd., (2014) tarafından yapılmış bir çalışmada kıyılmış tilapia balığı kullanılarak yapılan atıştırmalıklarda (%20-%40) oranlarında balık içeren atıştırmalıkların duyusal açıdan kabul edilebilir oldukları belirtilmiştir. Ayrıca atıştırmalıkların belirtilen oranlarda balıketi içermesinin atıştırmalıkların besinsel değerini arttırdığı ve fizikokimyasal özelliklerini ise etkilemediği vurgulanmıştır. Pianjing vd., (2016) tarafından ticari Tayland atıştırmalıklarının protein değerinin arttırılması amacıyla tilapia *(Oreochromis niloticus*) balığı kullanılmıştır. Yapılan bu çalışmada tilapia balığı ilave edilmiş atıştırmalıkların genel kabul edilebilirlik değerinin 5.87' den 7.73' e değişim gösterdiği ve bu değerlerin orijinal ticari ürünlerin genel kabul edilebilirlik değerlerinden daha yüksek olduğu belirtilmiştir. Ikasari ve Hastarini, (2016) tarafından lindur meyvesi unu ve patates unu kullanılarak karides kabuğu tozu ile zenginleştirilerek cips üretiminin gerçekleştirildiği çalışmada bütün panelistlerce çoğunlukla karides kabuk tozu ilave edilmeksizin lindur meyvesi unu ve patates unu (40:60) oranlarında kullanıldığında en çok tercih edilen cipsler olduğu bildirilmiştir. Topuz vd. (2017) tarafından mısır unu içerikli atıştırmalıkların duyusal özelliklerini arttırmak ve besinsel değerlerini yükseltmek amacıyla karides eti kullanılan çalışmada mısır unu ile birleştirilen karides etinin istenen tat ve lezzete sahip olması nedeniyle yeni karides-mısır atıştırmalıklarının üretiminde başarılı olduğu belirtilmiştir. Büyüksaraç (2018) tarafından yapılan çalışmada buğday, patates ve mısır unları kullanılarak ve %5, %10 ve % 20 oranlarında toz halde balıketi kullanılarak zenginleştirilmiş balık ciplerinin üretimi gerçekleştirirmiştir. Çalışmada yapılan duyusal analiz sonuçlarına göre %10'un üzerinde toz balıketi kullanımının balık cipsi üretiminde uygun olmadığı sonucuna varılmıştır. Spirulina kullanılarak yapılan atıştırmalıklarda %2.6 konsantrasyonda kullanılan spirulina içerikli atıştırmalıkların besinsel içerik ve duyusal kabul edilebilirlik açısından kullanılabileceği belirtilmiştir (Lucas vd., 2018). Costa vd. (2019) tarafından yapılan diğer bir çalışmada atıştırmalıkların formülasyonuna % 9,65 oranında karides tozu ilave edildiğinde atıştırmalıklarda en ideal karides tadının elde edildiği vurgulanmıştır.

Yukarıda belirtilen çalışmalarda farklı formülasyonda hazırlanan baharat kaplı kalamar atıştırmalıkların duyusal açıdan panelistlere göre en çok beğenilen formülasyonları belirtilmiştir. Yapılan bu çalışmada da farklı baharatların kullanımıyla hazırlanan farklı formülasyonlarda baharat

kaplı kalamar atıştırmalıkları duyusal açıdan en çok beğenilen formülasyonun saptanması amacıyla panelistlere farklı özellikleri içeren iki ayrı duyusal form uygulanmıştır. Çalışma kapsamında elde edilen verilerle yapılan analizler somucunda 1. formda yer alan 6 özellik ile 2. duyusal formda yer alan 8 özelliğin her biri için 12 farklı formülasyondan elde edilen atıştırmalıklara panelistlerin verdikleri puanlar arasında istatistiksel olarak anlamlı farklar olduğu görülmüştür. 1 formda yer alan 5 özelliğe 12 formülasyon için verilen toplam puanlann görünüm için %80'i, renk için %78'i, koku için %86'sı, tat için %72'si, doku için %64'ü 5 ve üstünde puan almıştır. Yine 1. formda yer alan genel kabul edilebilirlik parametresi toplamda %76 oranıyla 5 ve üstünde puan almıştır. 2 formda yer alan 8 özelliğe 12 formülasyon için verilen toplam puanların renk yoğunluğu için %84'ü, renk homojenliği için %84'ü, sos uyumu için %72'si, sertlik için %59'u, çıtırlık için %51'i, gevreklik için %52'si, ağızda dağılma için %56'sı, tat yoğunluğu için %76'sı 5 ve üstünde puan almıştır. Yapılan çalışma sonucunda panelistlere uygulanan her iki duyusal analiz formuna göre en beğenilen baharat kaplı kalamar atıştırmalıkların soğan tozu, sarımsak tozu kekik kullanılarak hazırlanmış olan atıştırmalıkların olduğu belirlenmiştir. Panelistlere uygulanan her iki duyusal analiz formuna göre de en beğenilmeyen grupların tarçınlı, yenibahar ve karabiber içerikli baharat kaplı kalamar atıştırmalıklarının olduğu saptanmıştır.

Çalışmada farklı baharatlar kullanılarak yemeğe hazır baharat kaplı kalamar atıştırmalıklar üretilmiştir. Bu atıştırmalıklar panelistler tarafından duyusal açıdan değerlendirildiğinde ise görünüm, renk, koku, tat, doku ve genel kabul edilebilirlik, renk yoğunluğu, renk homojenliği, sosun uyumu, sertlik, çıtırlık, gevreklik, ağızda dağılma, tat yoğunluğu özelliklerinin her birine göre farklı sonuçların elde edildiği bulgulanmıştır. Yapılan duyusal değerlendirme sonucunda panelistlere göre genel kabul edilebilirlik açısından en beğenilen baharat kaplı kalamar atıştırmalıklar soğan tozu, sarımsak tozu ve kekik kullanılarak hazırlanmış olan atıştırmalıkların olduğu belirlenmiştir. Panelistlere göre genel kabul edilebilirlik açısından en beğenilmeyen grupların ise tarçınlı, yenibahar ve karabiber içerikli baharat kaplı kalamar atıştırmalıkları olduğu saptanmıştır. Sonuç olarak; bu tip tüketime hazır halde farklı formülasyonlarda kalamar atıştırmalıklarına yönelik çalışmaların yapılması, en beğenilen formülasyonların belirlenmesi, farklı formülasyonlarda kalamar atıştırmalıklarının üretilmesi ve marketlerde satışa sunulması arzu edilmektedir.

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Sample Article

A Taxonomic Study on Zooplankton Fauna of Kiğı Dam Lake (Bingöl-Turkey)

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Abstract

The present study was conducted to determine zooplankton fauna of Kiğı Dam Lake during September 2012 and August 2013 seasonally. The zooplankton samples were collected by using plankton net with the mesh size of 55µm horizontally and preserved in 4% formaldehyde. Total 22 taxa (16 Rotifera, 4 Cladocera, and 2 Copepoda) were identified in Kiğı Dam Lake.

Keywords: Kiğı Dam Lake, species distribution, zooplankton.

INTRODUCTION

The majority of zooplankton (Copepoda, Cladocera and Rotifera) transform the phytoplankton to animal protein (Cirik and Gökpınar, 1993), and they play a significant role in food chain. It was reported that some species are the indicators of water quality, and eutrophication due to their sensitivity to environmental changes and therefore zooplankton studies on lakes have acquired significant importance (Berzins and Pejler, 1987; Mikschi, 1989).

Many studies were carried on zooplankton in Turkey (Özdemir and Şen, 1994; Göksu et al., 1997, 2005; Saler and Şen, 2002; Bozkurt and Sagat, 2008; Bulut and Saler, 2013a, 2013b; 2014a, 2014b; Saler et al., 2015a, 2015b). No previous research about zooplankton of Kiğı Dam Lake has been recorded. In this study zooplankton species and their seasonal variations of Kiğı Dam Lake have been investigated.

MATERIAL and METHODS

Kiğı Dam Lake was built on Peri Stream between 1997 and 2003. The maximum water capacity is 507.55 hm3 and has surface area 8.35 km2 and maximum depth of 168 m (Şimşek, 2016) (Figure.1). The species were identified according to Edmondson (1959), Flössner (1972), Ruttner-Kolisko (1974), Kiefer (1978), Koste (1978), Negrea (1983), Segers (1995), and Einsle (1996).

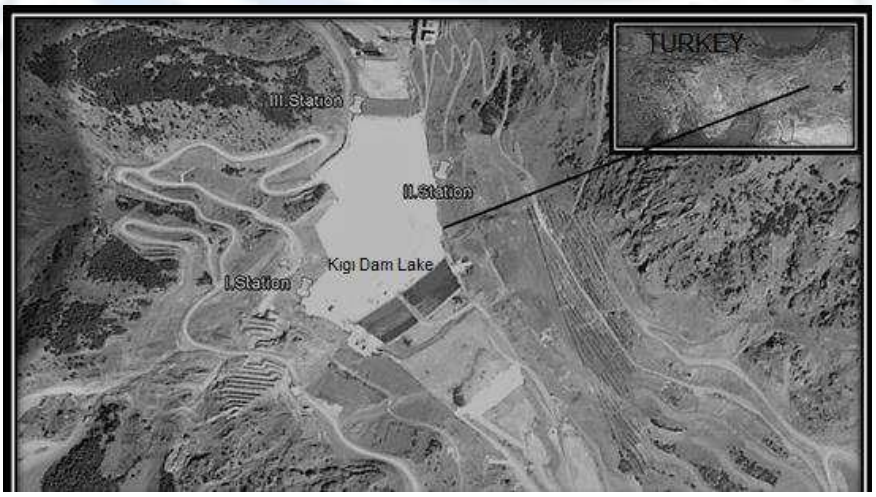

Figure1. Stations of Kiğı Dam Lake

RESULTS

A total of 22 taxa consisting of 16 Rotifera, 4 Cladocera and 2 Copepoda species were identified in the Dam Lake (Table 2).

The lowest numbers of taxa were recorded in winter at first stations (4 species). Some water quality parameters (pH, dissolved oxygen, and surface water temperature) were measured at study field (Table 3).

Table 3. Seasonal changes of water quality parameters in Kiğı Dam Lake

	Autumn	**Winter**	**Spring**	**Summer**
Water temperature (℃)	16	7.2	17.2	22.5
pH	7.0	6.8	6.9	7.3
D.O (mgL-1)	6.2	7.3	6.0	5.2

DISCUSSION

Zooplankton is known as the indicator of trophic status of aquatic habitats. They are also used to signify the water quality in freshwater systems. *K. cochlearis* and *P. dolichoptera* from Rotifera are indicators of productive habitats, while *N. acuminata* and *N. squamula* are indicators of cold waters (Kolisko, 1974). In Kiğı Dam Lake *K. cochlearis*, *P. dolichoptera* and *N. squamula* were observed.

In Murat River (Bulut and Saler, 2014a), Kalecik Dam Lake (Bulut and Saler, 2013b), Peri Stream (Saler et al., 2011), that were located in the same region with Kiğı Dam Lake, rotifers were recorded as dominant species as to number of individuals and abundance, followed by Cladocera and Copepoda species.

ACKNOWLEDGEMENT

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Ö R N E K MAKALE

Eğirdir Gölü'nden Tatlısu Istakozu (*Astacus leptodactylus* Eschscholtz, 1823)'nun Sindirim Enzim Aktivitelerinin Mevsim, Büyüklük ve Cinsiyete Bağlı Olarak Değişimi

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Özet

Bu çalışma, Eğirdir Gölü'nde yaşayan *Astacus leptodactylus* türünde mide sindirim enzim aktiviteleri üzerine (proteaz α-amilaz ve lipaz) mevsim ve eşeyin etkisini araştırmak amacıyla oluşturulmuştur. Bu amaçla kerevitler 1 yılda 4 mevsim olarak Eğirdir Gölü'nden avlandı. İlkbahar mevsiminde,kerevitlerin pinterlere girmemesi nedeniyle örnekleme yapılamadı. Bu nedenle, sonuçlar üç mevsim (sonbahar, kış, yaz) ve eşeyler üzerinden faktöriyel düzeyinde varyans analizi ile değerlendirildi. Bulgular, eşey ve mevsim faktörlerinin kerevitlerin midesindeki proteaz ve lipaz aktivitesini önemli düzeyde etkilediğini ($p<0,05$), α-amilaz aktivitesinde ise istatistiksel olarak önemli düzeyde etkili olmadığını gösterdi ($p>0,05$). Bulgular ayrıca, kerevitlerin,proteaz enzim aktivitesinin sonbahar ve kışın, lipaz enzim aktivitesinin ise kışın eşeyler arası önemli değişim gösterdiğini ortaya çıkardı ($p<0,05$).

Anahtar kelimeler: Astacus leptodactylus, sindirim enzim aktivitesi, lipaz, α-amilaz, proteaz.

Variation of Digestive Enzyme Activities Depending on Season, Size and Sex of Freshwater Crayfish (*Astacus leptodactylus* Eschscholtz, 1823) from Lake Eğirdir

Abstract

This study was conducted to search the effect of sex and season on digestive enzyme activities (protease, α-amylase, lipase) in Astacus leptodactylus species that lives in Egirdir Lake.The freshwater crayfish were caught as four seasons in a year from Egirdir Lake. The sampling was not possible for crayfish since they were not entered into trap in spring season. Therefore, the results were evaluated over three seasons and sex by factorial variance analysis. The results showed that sex and season affected to protease and lipase activities ($p<0.05$), whereas they did not affect statistically α-amylase activity in crayfish stomach ($p>0.05$). Results also indicated that protease enzyme activity in crayfish showed significant changes in autumn and winter while lipase enzyme activity showed changes in winter between sexs ($p<0.05$).

Keywords: Astacus leptodactylus, digesitve enzyme activity, α-amylase, lipase, protease.

GİRİŞ

Astacus leptodactylus, dünyada Türk ıstakozu (kerevit) olarak da bilinen (Köksal,1988) ve ülkemiz içsularında doğal olarak bulunan bir decapoda (on ayaklı) türüdür. Astacus ekonomik değeri yüksek kabuklu türlerinden biridir (Bolat, 2001). Kerevit 1986 yılı öncesi özellikle Eğirdir Gölü balıkçılarının başlıca gelir kaynağı iken bu türün daha sonra gerek aşırı avcılık ve gerekse hastalık nedeniyle popülasyonu azalmıştır (Köksal, 1988; Ackefors, 2000; Bolat, 2001; Harlıoğlu ve Aksu 2002; Harlıoğlu ve Mişe 2007; Bilgin vd., 2008).

MATERYAL ve YÖNTEM

Bu çalışmada, Ekim 2014 ile Haziran 2015 tarihleri arasında Eğirdir Gölü'nde kerevit (10-15 cm) avcılığı gerçekleştirilmiştir. Avcılıkta kerevit pinterleri kullanılmış, örneklemeler mevsimsel olarak yapılmıştır. İlkbahar mevsiminde, kerevitlerin pinterlere girmemesi nedeniyle örnekleme yapılamamıştır.

Enzim aktivite analizler

Bu çalışmada; α-amilaz, proteaz ve lipaz olmak üzere üç çeşit sindirim enzim aktivitesi araştırılmıştır.Tatlısu istakozundaki α-amilaz enzim aktivitesi, Metais ve Bieth (1968)'e göre yapılmıştır.

İstatistiksel analizler

Verilerin değerlendirilmesinde SPSS 23 istatistiki paket programından yararlanılmış ve sonuçlar, faktöriyel düzeyinde varyans analizi testi ile $P<0,05$ önem düzeyinde test edilmiştir. Faktörlerin seviye ortalamalarının arasındaki farklılıkların belirlenmesinde Tukey testi kullanılmıştır.

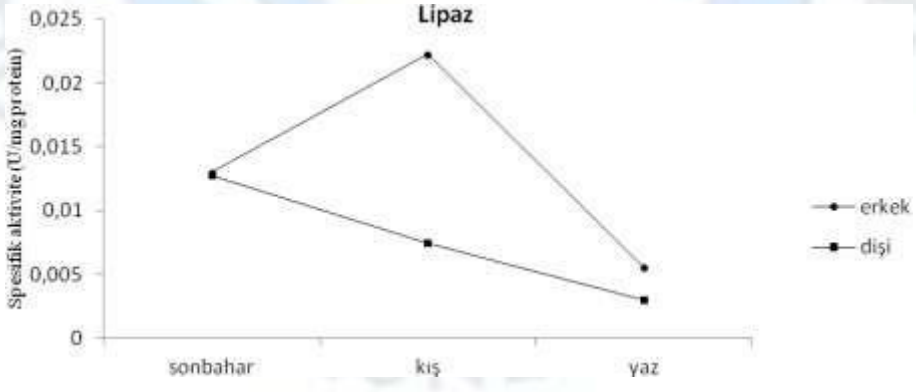
BULGULAR

Eşey ve mevsimsel farklılıkların kerevit midesindeki α-amilaz enzim aktivitesi üzerinde önemli bir etkisinin olmadığı tespit edilmiştir ($p>0,05$) (Tablo 1).

Tablo1. Kerevit midesindeki α-amilaz aktivitesinin eşey ve mevsime göre değişimi,(Ort.± S.H.) (U/mgprotein)

Eşey/Mevsim	Sonbahar	Kış	Yaz
Dişi	$^{a}0,013\pm 0,0011^{a}$	$^{b}0,007 \pm 0,0006^{b}$	$^{b}0,003 \pm 0,0010^{a}$
Erkek	$^{a}0,013 \pm 0,0035^{a}$	$^{b}0,022 \pm 0,0026a$	$^{c}0,006\pm 0,0006^{a}$

Lipaz aktivitesi erkek kerevitlerde sonbahar mevsiminden kış mevsimine kadar artan ve kış mevsiminden yaz mevsimine kadar azalan bir grafik oluşturmuş, dişi bireylerde ise sonbahar mevsiminden yaz mevsimine kadar azalan bir grafik oluşmuştur (Şekil 3).



Şekil 3. Kerevit midesinde farklı mevsimlerdeki lipaz aktivitesi (U/mg protein)

TARTIŞMA ve SONUÇ

Mevcut sindirim enzimlerinin profili ve faaliyetleri başta olmak üzere sindirim sisteminde ortaya çıkan fizyolojik süreçler, türlerin geniş besin çeşitliliğinden faydalanmasını etkilemektedir. Farklı kabuklu türleri, farklı beslenme alışkanlıklarına ve yaşam alanlarını yansıtan bir dizi sindirim enzime sahiptir (Anonim, 2017; Coccia vd., 2011)…….

Ayrıca, maksimum enzim aktivitesini belirleyebilmek için; farklı pH, sıcaklık ve reaksiyon sürelerinde denemeler yapılması da önemli taşımaktadır……

TEŞEKKÜR

Bu çalışma, yüksek lisans tezinden özetlenmiştir. (veya varsa proje desteği yazılmalı)

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