Ege Journal of Fisheries and Aquatic Sciences

www.egejfas.org

E-ISSN 2418-3140

EgeJFAS

Su Ürünleri Dergisi

Volume 38 Number 4 2021

Ege University Faculty of Fisheries

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Su Ürünleri Dergisi

Ege Journal of Fisheries and Aquatic Sciences

Volume 38 Number 4 **ISSN 1300-1590 / E-ISSN 2418-3140**

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Su Ürünleri Dergisi yılda dört sayı olarak yayınlanır. Ege Journal of Fisheries and Aquatic Sciences is published in four issues annually.

T.C. Kültür ve Turizm Bakanlığı Sertifika No: 18679
Ministry of Culture and Tourism Sertificate No:18679

Yayımlanma Tarihi **Publishing Date**
15 Aralık December 15th, 2021

İletişim **Contact**
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Ege Journal of Fisheries and Aquatic Sciences, 38(4), 399-409 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.01

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Evaluation of water quality of Çoruh River Basin (Turkey) using some biotic indices

Çoruh Nehir Havzası (Türkiye) su kalitesi'nin bazı biyotik indeksler kullanılarak belirlenmesi

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Received date: 28.12.2020 *Accepted date: 30.07.2021*

How to cite this paper:
Baytaşoğlu, H. & Gözler, A.M. (2021). Evaluation of water quality of Çoruh River Basin (Turkey) using some biotic indices. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 399-409. DOI: 10.12714/egejfas.38.4.01

Abstract: In this study, it was aimed to evaluate the water quality of the Çoruh Basin using BMWP (Biological Monitoring Working Party) index, ASPT (Average Score Per Taxon) index, Shannon-Wiener Diversity Index, EPT (Ephemeroptera Plecoptera Trichoptera taxa), EP (Ephemeroptera Plecoptera taxa) index scores and benthic macroinvertebrates. The benthic macroinvertebrates were collected from 54 stations at the Çoruh basin between 2014-2016 years. Standard hand net (D-frame net) and Ekman-Birge grab were used as sampling tools. As a result of the diagnoses, a total of 7246 individuals belonging to Insecta, Crustacea, Mollusca, Oligochaeta and Plathyhelminthes were obtained. It was determined that the most dominant group was Insecta and the rarest group was Plathyhelminthes. It was observed that the BMWP score ranged between 5 and 94, and the lowest and highest number of families detected in the stations were 1 and 18, respectively. It was determined that the Shannon Wiener diversity index value was between 0.54-2.20, therefore the basin streams generally showed moderate pollution. The results of BMWP index show that the basin streams had mostly show 3rd and 4th class water quality and also biodiversity decreases with the deterioration of the riverbed or exposure to pollution.

Keywords: Biotic indexes, Çoruh River, macroinvertebrates, water quality

Öz: Bu çalışmada BMWP (Biyolojik İzleme Çalışma Grubu) indeksi, ASPT (Her Taksonun Ortalama Değeri) indeksi, Shannon-Wiener Çeşitlilik İndeksi, EPT (Ephemeroptera Plecoptera Trichoptera taxa), EP (Ephemeroptera Plecoptera taxa) indeks skorları ve bentik makroomurgasızlar kullanılarak Çoruh Havzası'nın su kalitesinin değerlendirilmesi amaçlanmıştır. Bentik makroomurgasızlar 2014-2016 yılları arasında havza genelinde seçilen 54 istasyondan örneklenmiştir. Örnekleme aleti olarak standart el kepçesi (D-şekilli kepçe) ve Ekman sediment kepçesi kullanılmıştır. Yapılan teşhisler sonucu Insecta, Crustacea, Mollusca, Oligochaeta and Plathyhelminthes'e ait toplam 7246 birey elde edilmiştir. En baskın grubun Insecta, en nadir rastlanan grubun Plathyhelminthes olduğu tespit edilmiştir. BMWP skorunun 5-94 arasında değiştiği, istasyonlarda tespit edilen en düşük ve en yüksek familya sayılarının sırasıyla 1ve 18 olduğu görülmüştür. Shannon Wiener çeşitlilik indeks değerinin 0.54-2.20 arasında olduğu, dolayısıyla havza akarsularının genellikle orta derecede kirlenme gösterdiği belirlenmiştir. BMWP indeks sonuçlarına göre havza akarsularının çoğunlukla 3. ve 4. sınıf su özelliği gösterdiği, akarsu yatağının bozulması veya kirliliğe maruz kalması ile biyoçeşitliliğin azaldığı tespit edilmiştir.

Anahtar kelimeler: Biyotik indeks, Çoruh Nehri, makroomurgasızlar, su kalitesi

INTRODUCTION

Rivers cover 2% of the surface fresh water on the earth and contributed to the water cycle such as seas, oceans and lakes. Water pollution in river systems increases in parallel with population and industrialization. It is seen that the factors causing pollution are generally domestic wastes from settlements in the basin, substances such as fertilizers and pesticides mixed from agricultural lands and pollutants from enterprises (Gümrükçüoğlu and Baştürk, 2007). Disturbances in water quality, contamination of any pollutant into the water, and habitat degradation cause damage to living groups (Wimbaningrum et al., 2016). Chemical parameters were used for a long time to determine water quality. However, in the following years, researchers evaluated different organisms as biological quality components and proved their usage in determining the water quality of aquatic communities such as phytoplankton, phytobenthos, macrophytes, macroinvertebrates and fish. Among these groups, macroinvertebrates give different responses to organic pollutants and toxic substances, so they are the one of the most important groups in river.

In the Water Framework Directive (WFD, 2000) adopted by the member states of the European Union, the macro-invertebrates can be used as bioindicator organisms due to their response to pollution. The fact that these groups are found almost everywhere, relatively easy sampling and obtaining sufficient number have enabled them to be used for biomonitoring purposes (Kazancı *et al.,* 1997; Kazancı *et al.,* 2010a; Zeybek and Kalyoncu, 2012). The use of bioindicators to determine water quality in surface waters dates back to the 1800s. After this date, many researchers have used different mathematical methods to evaluate water quality using these

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organisms. Due to different current conditions, geographical distribution, and biodiversity differences, countries have developed and used different indices. In Turkey, the biotic index studies began with a work in Sakarya and Seyhan catchment areas by Government Water Works in 1992 (DSI, 1992). On this field, the studies have importantly accelerated since 1992 (Kazancı and Dügel, 2000; Kazancı *et al*., 2003; Duran *et al*., 2003; Balık *et al*. 2006; Sukatar *et al*., 2006; Kazancı and Dügel, 2008; Kazancı *et al*., 2008; *Kazancı et al*., 2009; Kazancı, 2009; Kazancı *et al*., 2010b; Türkmen and Kazancı, 2010a; Türkmen and Kazancı, 2010b; Yıldız *et al*., 2010; Türkmen and Kazancı, 2011; Topkara *et al*., 2011; Zeybek *et al*., 2014; Yıldız *et al.* 2015; Yorulmaz *et al*., 2015; Başören and Kazancı, 2016; Zeybek, 2017; Özbek *et al.* 2019; Tüzün Tereshenko, 2019; Koşal Şahin and Zeybek, 2019). One of these indices used in monitoring studies is the BMWP (Biological Monitoring Working Party) index that was established in 1976 to determine the biological quality of water by family identification of aquatic invertebrates collected from rivers in the UK and Scotland.

The aim of this study is to get an overall view of the benthic macro-invertebrate composition along the Çoruh River Drainage and to assess the water quality assessment of Çoruh River by using various metrics (benthic macro-invertebrate based biotic indices, biodiversity indices, EP and EPT).

MATERIAL AND METHODS

Study area

The Çoruh River originates from the west of the Mescit Mountains at an altitude of 3000 m, within the boundaries of Erzurum province. It turns eastward along with the tributaries that are involved in the Bayburt plain and continues to flow along a tectonic line. Together with Tortum and Oltu Streams, it passes through the Yusufeli district and continues to flow towards the north. It leaves from Muratlı Town (Artvin Province) in Turkey and enters the borders of Georgia. It flows into the Black Sea by the delta formed by alluviums it carries from Batumi, the capital of Ajara, which is the semi-autonomous province of Georgia. A large part of the drainage area (91%) is located within the borders of Turkey, and the rest (9%) is located within the borders of Georgia (*Akpınar et al.,* 2009; Baytaşoğlu and Gözler, 2018). The total length is 466 km. In this study, a total of 54 stations (Table 1) were selected on the Çoruh River from the source to the drainage in our country. The map of the sampling stations is given in Figure 1. QGIS geographic information system was used in the map.

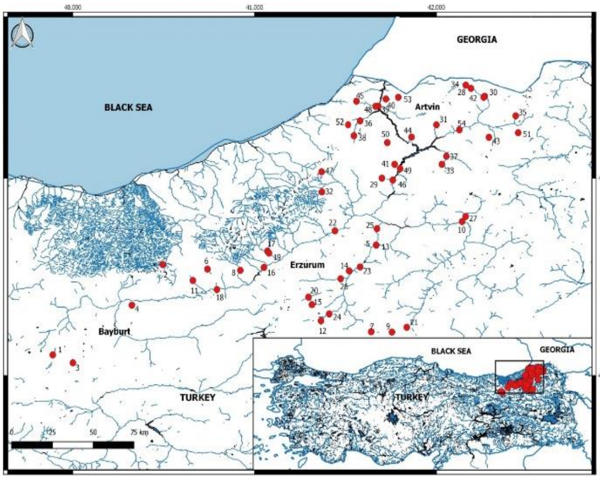

Figure 1. The sampling stations on Çoruh River and its tributaries

Table 1. Some information belonging to stations

Station No	Stations Name	Province/Town	Coordinates	Sampling Instruments	Sampling Date	Substrat
1	Stream Catıksu	Aydıntepe/Bayburt	40. 476 N 39. 983 E	D-Frame Net	20.09.2014 13.5.2015	Stony
2	Stream Yoncalı	Demirözü/Bayburt	40.499 N 40.566 E	D-Frame Net	15.5.2016 17.8.2016	Stony, Vegetated
3	Güvercindere Irrigation Channel	Demirözü/Bayburt	40.132 N 38.896 E	D-Frame Net	21.09.2014 13.5.2015	Concrete structure
4	Stream Aydıncık	Merkez/Bayburt	40.356 N 40.324 E	D-Frame Net	21.09.2014 13.5.2015	Stony, Vegetated
5	Tortum Waterfall (Lower)	Tortum/Erzurum	40.660 N 41.668 E	D-Frame Net	24.09.2014 14.5.2015	Stony, Vegetated
6	Stream Anur	İspir/Erzurum	40.539 N 40.740 E	D-Frame Net	22.09.2014 15.5.2015	Stony, Vegetated
7	Stream Başkale (Hamidiye)	Tortum/Erzurum	40.221 N 41.640 E	D-Frame Net	23.09.2014 14.5.2015	Stony
8	Stream Capan	İspir Erzurum	40.532 N 40.921	D-Frame Net	15.5.2016 17.8.2016	Stony
9	Stream Demirci (Caylıca)	Tortum/Erzurum	40.219 N 41.755 E	D-Frame Net	25.09.2014 14.5.2015	Stony
10	Stream Alabalık	Olur/Erzurum	40.779 N 42.141 E	D-Frame Net	23.9.2014 15.5.2015	Stony, Vegetated

Table 1. Continued

11	Stream Karataş	İspir/Erzurum	40.481 N 40.660 E	D-Frame Net	15.5.2016 17.8.2016	Stony
12	Stream Yağcılar	Tortum/Erzurum	40.277 N 41.365 E	D-Frame Net	23.9.2014 14.5.2015	Stony, Vegetated
13	Tortum Waterfall (Upper)	Tortum/Erzurum	40.661 N 41.668 E	Ekman Grab, D-Frame Net	24.09.2014 14.5.2015	Stony, Vegetated, Muddy
14	Stream Dikyar	Tortum/Erzurum	40.530 N 41.520 E	D-Frame Net	16.5.2016 15.5.2015	Stony, Vegetated
15	Stream Doruklu	Tortum/Erzurum	40.358 N 41.314 E	D-Frame Net	24.09.2014 15.5.2015	Stony, Vegetated
16	Stream Yedigöze (Çoruh River)	İspir/ Erzurum	40.547 N 41.051 E	D-Frame Net	25.09.2014 16.5.2015	Stony
17	Stream Catakkaya (upper)	Ispir/Erzurum	40.630N 41.070 E	D-Frame Net	24.09.2014 16.5.2015	Stony
18	Stream Anur 2	İspir/Erzurum	40.435 N 40.792 E	D-Frame Net	22.09.2014 16.5.2015	Stony, Muddy
19	Stream Catakkaya (Lower)	Ispir/Erzurum	40.62 N 41.079 E	D-Frame Net	24.09.2014 16.5.2015	Stony
20	Stream Kaleboynu	Tortum/Erzurum	40.3970 N 41.2960 E	D-Frame Net	23.9.2014 15.5.2015	Stony, Vegetated
21	Stream Baskale (Mercimekli)	Tortum/Erzurum	40.244 N 41.837 E	D-Frame Net	23.9.2014 15.5.2015	Stony
22	Stream Kılıckaya Village	İspir/Erzurum	40.7332 N 41.4417 E	D-Frame Net	16.5.2016 17.8.2016	Stony
23	Stream Sapaca	Uzundere/Erzurum	40.55 N 41.58 E	D-Frame Net	17.5.2016 17.8.2016	Stony
24	Alapınar Fountain	Tortum/Erzurum	40.312 N 41.410 E	D-Frame Net	24.09.2014 15.5.2015	Concrete structure
25	Stream Morkaya	Tortum/Erzurum	40.744 N 41.673 E	Ekman Grab, D-Frame Net	17.5.2016 15.5.2015	Stony, Vegetated, Muddy
26	Stream Uzunkavak	Tortum/Erzurum	40.490 N 41.473 E	Ekman Grab, D-Frame Net	17.5.2016 17.8.2016	Stony, Muddy
27	Stream Olurdere	Olur/Erzurum	40.8058 N 42.1608 E	D-Frame Net	23.9.2014 15.5.2015	Stony
28	Stream Mansuret	Şavşat/Artvin	41.455 N 42.190 E	D-Frame Net	27.9.2014 17.5.2015	Stony
29	Stream Narlık	Yusufeli/Artvin	41.00 N 41.70 E	D-Frame Net	26.9.2014 19.5.2015	Stony
30	Stream Balıklı	Şavşat/Artvin	41.4150 N 42.266 E	D-Frame Net	27.9.2014 17.5.2015	Stony, Vegetated
31	Stream Ortaköy	Şavşat/Artvin	41.27 N 42.00 E	D-Frame Net	27.9.2014 17.5.2015	Stony, Vegetated
32	Stream Altıparmak	Yusufeli/Artvin	40.93 N 41.37 E	D-Frame Net	26.9.2014 19.5.2015	Stony
33	Stream Torbalı	Ardanuç/Artvin	41.07 N 42.03 E	D-Frame Net	26.9.2014 17.5.2015	Stony
34	Stream Göknar	Şavşat/Artvin	41.4712 N 42.1618 E	D-Frame Net	27.9.2014 18.5.2015	Vegetated
35	Stream Savsat (Veliköy)	Şavşat/Artvin	41.316 N 42.436 E	D-Frame Net	27.9.2014 18.5.2015	Stony, Vegetated
36	Stream Basköy	Murgul/Artvin	41.29 N 41.58 E	D-Frame Net	29.9.2014 20.5.2015	Stony
37	Stream Eksinar	Ardanuç/Artvin	41.1109 N 42.055 E	D-Frame Net	28.9.2014 20.5.2015	Stony

Table 1. Continued

38	Stream Damar	Murgul/Artvin	41.215 N 41.546 E	D-Frame Net	29.9.2014 20.5.2015	Stony Muddy
39	Stream Deviskel	Borçka/Artvin	41.365 N 41.680 E	D-Frame Net	29.9.2014 20.5.2015	Stony Muddy
40	Stream Aralık	Borçka/Artvin	41.401 N 41.722 E	D-Frame Net	29.9.2014 20.5.2015	Stony, Vegetated
41	Stream Sarıbudak	Artvin	41.070 N 41.77 E	D-Frame Net	29.9.2014 20.5.2015	Stony
42	Stream Balıklı 2	Şavşat/Artvin	41.41 N 42.26 E	D-Frame Net	29.9.2014 18.5.2015	Stony, Vegetated
43	Stream Arpalı Village	Şavşat/Artvin	41.207 N 42.289 E	D-Frame Net	29.9.2014 18.5.2015	Stony
44	Stream Seyitler	Artvin	41.208 N 41.863 E	D-Frame Net	28.9.2014 20.5.2015	Stony, Vegetated
45	Stream Cifteköprü	Borçka/Artvin	41.39 N 41.56 E	D-Frame Net	27.9.2014 20.5.2015	Stony, Vegetated
46	Stream Kirazalan	Yusufeli/Artvin	40.99 N 41.76 E	D-Frame Net	28.9.2014 19.5.2015	Stony
47	Cıro Waterfall	Yusufeli/Artvin	41.032 N 41.368 E	D-Frame Net	28.9.2014 19.5.2015	Stony
48	Stream Cuhala	Cankurtaran/Artvin	41.3640 N 41.6655 E	D-Frame Net	27.9.2014 20.5.2015	Stony
49	Stream Hızarlı	Artvin	40.86 N 39.66 E	D-Frame Net	27.9.2014 20.5.2015	Stony
50	Stream Bashatıla	Borçka/Artvin	41.18 N 41.73 E	D-Frame Net	28.9.2014 20.5.2015	Stony, Vegetated
51	Stream Ballı	Şavşat/Artvin	41.23 N 42.45 E	D-Frame Net	28.9.2014 18.5.2015	Stony
52	Stream Kokolet 2	Murgul/Artvin	41.304 N 41.631 E	D-Frame Net	27.9.2014 18.5.2015	Stony
53	Stream Ogül	Şavşat/Artvin	41.41 N 41.79 E	D-Frame Net	27.9.2014 18.5.2015	Vegetated, Muddy
54	Stream Sungu	Şavşat/Artvin	41.245 N 42.126 E	D-Frame Net	27.9.2014 18.5.2015	Stony, Vegetated

Indices for determination of biological water quality

For the determination of water quality by biological methods, BMWP index, ASPT index, EP and EPT taxa values, Shannon-Wiener diversity index were used. The BMWP and ASPT indexes is based on the sensitivity of invertebrates for pollution. The score is between 1 and 10. It is calculated according to the values of the families in the samples. As the total value approaches 100, the pollution rate decreases (Kazancı *et al*., 2010c). ASPT gives the average tolerance values of all taxa in the community. The ASPT value of taxa is found by dividing the BMWP scor by the total number of families at the sampling point. According to the ASPT index, values less than 4 indicate extremely dirty, values between 4-5 indicate moderately polluted, values between 5-6 indicate doubtful waters that are not certain to be of good quality, and values above 6 indicate clean waters (Armitage *et al*., 1983). The Shannon-Wiener index is also used to interpret water quality. In the Shannon-Wiener index, which is between 0 and 5, the low score indicates low water quality and the high score indicates high water quality (Shannon ve Wiener 1963, Jorgensen *et al*., 2005.)

RESULTS AND DISCUSSION

Sampling studies were carried out at the selected 54 stations where the Turkish side of the Çoruh River basin. Date, station name, station no, province, coordinates, substrate and sampling instrument information belonging to stations are shown in Table 1. As a result of sampling studies and diagnoses in Çoruh River and its tributaries, a total of 7246 individuals were sampled; of them 5283 individuals belonging to Insecta, 1442 individuals to Crustacea, 176 individuals to Mollusca, 208 individuals to Oligochaeta and 137 individuals to Plathyhelminthes. Insecta was the most dominant group among the taxa and Platyhelminthes was the rarest one. In the Insecta group, Ephemeroptera individuals were the most common with 40% and Odonata individuals were the least encountered with 1% (Figure 2).

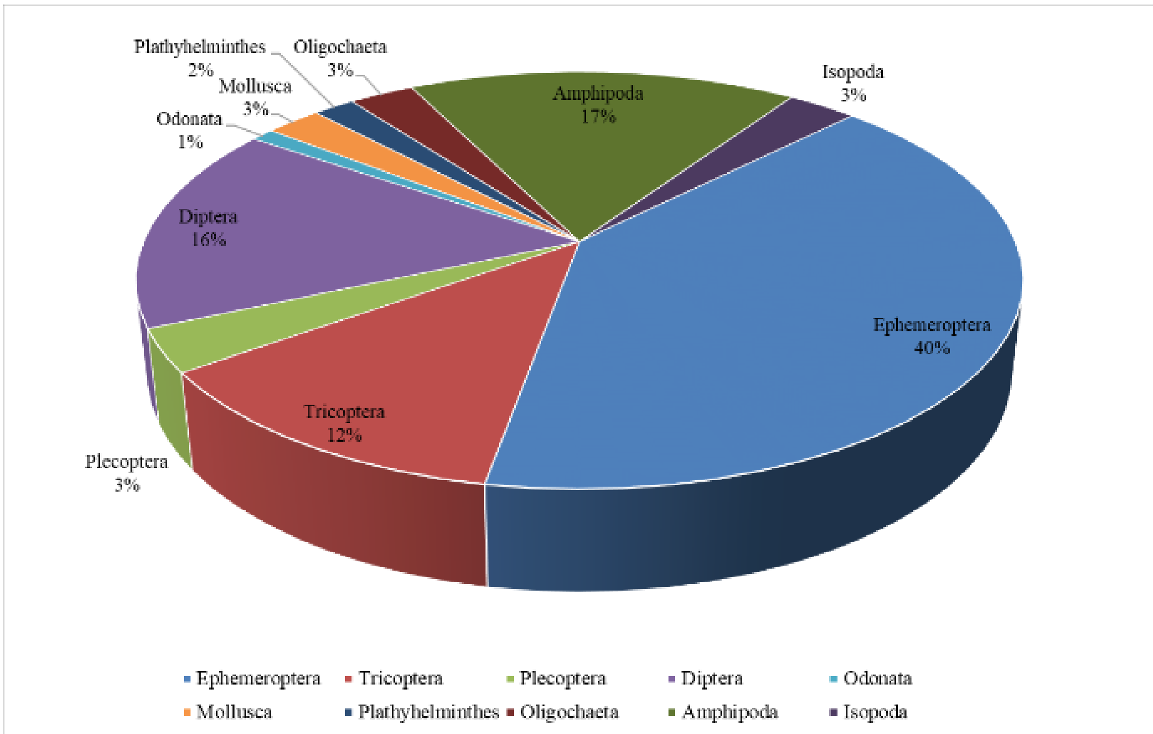

Figure 2. Rational distributions of the determined groups

The taxa observed at the stations were given in Table 2. It has been observed that the stations with high family numbers are far from the settlements and there are no factors that could destroy the streambed in the nearby location.

The highest number of family was found at St -36 (18 families) and the lowest number of family at St -23(1 families) (Table 3).

It is estimated that the stations with the lowest family numbers are selected from the near point to the main body of the Çoruh River (St-22, St-23), so the diversity is affected by both the flow velocity and the pollution load carried along the stream bed. St-24 station is a concrete structure built for animals to drink water. Although there are living groups transported here by various means, the high level of exposure to daily humanitarian activities caused the diversity to be very low. Although Ephemeroptera, Trichoptera and Plecoptera are good indicators for uncontaminated waters, some families like Baetidae, Caenidae and Hydropsychidae are tolerant to organic pollution and deterioration in the physicochemical properties of streams (Minaya *et al*., 2013; Kaboré *et al*., 2016). During the study, Ephemeroptera, Tricoptera and Plecoptera families were sampled both from the upper zones with the least pollution effect and from the locations where wastewater mixtures and structures such as touristic facilities, dams and HEPPs are located. It has been reported that the families Gyrinidae, Dytiscidae, Hydrophilidae and Notonectidae have a high capacity to reflect the ecological and geographical changes that occur throughout the year (Mauricio da Rocha *et al*, 2010). In this study, Dytiscidae family was identified from the stations that shows 3rd and 4th class water quality characteristics and Gyrinidae family was also identified at the stations showing 2nd and 3rd class water quality characteristics.

In the evaluation of the data in this study, the scores of BMWP used to determine water quality with biological data. According to the BMWP score system, the highest scores were 94 (St -36 and St -30) and 85 (St -35), and the lowest BMWP scores were 5 (St -23), 7 (St -22) and 11 (St -24). In studies conducted on different river systems, researchers reported that in locations with better water quality, the diversity of the benthic macroinvertebrates is high, and the diversity decreases as the pollution increases (Duran *et. al*. 2003, Kalyoncu ve Zeybek, 2011). It has been observed that the stations with low biodiversity in the Çoruh River are selected from the main body with high flow or the fast flowing tributaries, the points where the pollution is concentrated, had a channel modification and the wastes are directly mixed. The stations having 2nd class water quality characteristics were S-9, St-29, St-30 and St-32. St-9 is the closest station to the source of the river and the natural habitat was not disturbed at the St -29, St -30 and St-32 was selected from the areas officially declared protected areas. Stations with 3rd and 4th class water quality were deformed due to the construction of hydroelectric power plants (St-16, St-18), and mining activities (St -38). In addition, the stations where touristic activities (St-

44) are intense and streams flowing close to agricultural areas were also 3rd or 4th water quality.

In this study, the ASPT index gave similar results to the BMWP score at many of the stations. However, there were differences between the results to the indices at some of the stations. Although some stations show polluted water characteristics according to the BMWP score, they showed clean water characteristics according to the ASPT index (Table 3).

Shannon-Wiener diversity index is the most widely used diversity index in determining habitat quality using invertebrates. The Shannon index increases as the number and distribution of taxa within a community increases. (Shannon-Wiever, 1949). According to Wilhm and Dorris (1968), if the Shannon–Weaner index value ranges from >3 it indicates clean water, 1–3 indicates moderate pollution, <1 indicates heavy pollution. In this context, the streams of the Çoruh basin have moderate pollution (between 1-3). Shannon index range from 2.20 to 0.54 in the Çoruh River basin. Shannon index value was calculated at the highest St-10 and the lowest at St-23 (Table 3). It was observed that as the BMWP score decreased, Shannon index values decreased. In these calculations made at the stations, it was determined that the data of the two indexes fit together.

EPT taxa values give an idea about the water quality of the sampling area due to their sensitivity to water pollution. In this study, the highest EPT value was recorded at St-36, St-30 and St-32 stations. On the other hand, the lowest EPT value was recorded at St-24 and St-22 stations. St-36 station passes through the settlements and the river bed is partially exposed to trash. Therefore, sampling was made from the upper zone of the river as much as possible. St-32 station was selected from the area known as Altıparmak Mountains Nature Park. Since this area contains endemic species, so it is protected by the local administration. At the St-36 and St-32 stations EP taxa value was calculated as the highest, whereas it was the lowest at St-16, St-22, St-23, St-24 and St-25 stations. Similarly, BMWP, EP and EPT values were used to evaluate the Aksu stream in the Eastern Black sea basin by Kazancı *et al.* (2010a) , and they stated that urbanization, tourism, agricultural activities and the destruction of the river bed changed the community structure of the benthic macroinvertebrates. In this study, we can say that similar reasons may have effective role on the streams of the Çoruh basin.

Ephemerellidae and Caenidae families belonging to Ephemeroptera are pollution tolerant families (Yaman, 2019). In this study, Ephemerellidae family was found in stations with 2nd and 3rd class water characteristics and Caenidae family was found in stations with 3rd and 4th class water characteristics, according to BMWP index.

Habitat quality assessment of Altındere Valley was made by using biotic indices and physicochemical parameters by Türkmen and Kazancı, (2016). The families of Heptageniidae, Leptolepiidae, Leuctridae, Nemouridae have been determined as an indicator group for uncontaminated waters. (Türkmen and Kazancı, 2016). In this study, according to BMWP scores, Nemouridae was detected at stations that were slightly polluted, and other families were detected at polluted station.

Studies on determining the water quality by using invertebrate fauna and indices in the Çoruh River Basin, which is a transboundary streams, is very limited. *Kazancı et al.*, (2015) reported 31 taxa from 5 stations on the river. The taxa determined in the present study are different from those reported by Kazancı *et al.* (2015) because of the numbers and locations of the stations. Kazancı *et al.,* 2015 reported that the main source of pollution of the Çoruh Basin is the interference of urban wastewater into the river system, depending on land usage. In this study, construction of hydroelectric power plants, domestic wastewater and river beds affects the biodiversity in the basin. The two studies are similar in terms of some results obtained.

"Reference condition" is represented unimpaired (or minimally impaired) point in terms of biological, chemical and morphological characters of rivers. In this study, St-36 and St-30 had unimpaired properties in terms of BMWP, EP and EPT taxa. Thus these stations can be selected as reference stations.

According to BMWP score and number of EPT-Taxa, Kazancı et al 2015 accepted the first station as a reference habitat in Çoruh River. In this study, St-36 and St-30 had unimpaired properties in terms of BMWP, EP and EPT taxa. Thus, these stations can be selected as reference stations.

Table 2. The detected families at the stations.

															ARTHROPODA																		MOLLUSCA				PLATYHELMINTHES	ANNELIDA
	Heptageniidae	Baetidae	Ephemerellidae	Leptolepiidae	Caenidae	Polycentropodidae	Beraeidae	Hydropsychidae	Brachycentridae	Rhyacophilidae	Hydroptilidae	Glossosomatidae	Perlidae	Leuctridae	Perlodidae	Taeniopterygidae	Nemouridae	Tipulidae	Tabanidae	Chironomidae	Stratiomyidae	Aeshnidae	Gomphidae	Corduliidae	Gyrinidae	Dytiscidae	Elminthidae	Gammaridae	Asellidae	Acroloxidae	planorbidae	Pysidae	Sphaeriidae	Lymnaeidae	valvatidae	Bithyniidae		
1	*	*						*				*	*		*			*			*					*						*	*				*	*
2		*							*												*					*		*					*		*	*		*
3		*																										*	*				*				*	*
4												*	*															*	*								*	
5		*						*		*					*			*	*	*	*	*				*		*	*		*	*	*	*				*
6	*	*	*					*	*				*		*			*		*								*			*			*		*		
7		*		*				*		*		*						*		*						*											*	*
8	*	*						*					*	*				*	*	*							*											
9	*	*		*				*	*	*						*	*	*		*																	*	*
10		*						*	*				*					*		*						*			*			*					*	*
11	*	*		*					*																													*
12		*																*		*									*				*				*	*
13	*	*						*												*						*		*	*		*							
14		*						*								*				*																		*
15		*			*			*	*				*					*		*	*					*		*	*	*	*	*	*	*				*
16								*										*						*							*				*			
17	*			*									*	*				*		*													*					
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Table 2. Continued

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27		*																		*		*						*									*	
28	*	*						*	*			*	*					*	*	*	*	*						*	*			*						
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32	*	*						*	*				*		*	*	*	*		*																	*	
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35	*	*	*					*	*									*		*	*	*			*			*	*			*	*				*	*
36	*	*	*	*	*			*	*	*		*	*		*			*		*			*							*	*	*					*	
37		*			*			*			*							*		*		*																
38	*	*						*					*																									
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42	*	*						*	*								*	*			*																*	*
43	*	*						*	*	*			*		*			*	*	*								*										*
44	*	*	*					*										*		*								*	*									*
45	*	*	*					*	*	*				*						*								*										
46		*						*	*	*										*											*							
47		*						*					*				*	*	*	*		*			*												*	
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54	*	*							*														*															

Table 3. Evaluation of stations according to the index values

Stations	Number of family	BMWP score	class	ASPT	Shannon-Wiener Index value (Diversity)	EP- Taxa	EPT Taxa
1	13	61	3	4.7	2.09	4	6
2	9	33	4	3.7	1.4	1	2
3	6	19	4	3.1	1.4	1	1
4	5	32	4	6.4	1.36	1	2
5	17	73	2	4.2	1.76	2	4
6	13	78	2	6	2.13	5	7
7	10	52	3	5.2	1.05	2	5
8	9	55	3	6.1	2.06	4	4
9	12	76	2	6.3	1.54	5	7
10	11	56	3	5.09	2.2	2	4
11	5	35	4	7	1.39	3	4
12	7	26	4	3.7	1.42	1	1
13	8	38	4	4.75	1.96	2	3
14	5	22	4	4.4	1.46	2	3
15	17	65	3	3.82	1.92	3	5
16	5	24	4	4.8	1.37	0	1
17	7	47	3	6.7	1.48	4	4
18	5	31	4	6.2	1.27	1	1
19	7	51	3	7.2	1.33	4	6
20	10	59	3	5.9	1.48	4	5
21	7	39	4	5.5	1.64	2	3
22	2	7	5	3.5	0.6	0	0
23	1	5	5	5	0.54	0	1
24	3	11	4	3.6	1.1	0	0
25	4	12	4	3	0.74	10	1
26	4	29	4	7.25	1	2	4
27	5	25	4	5	1.15	1	1
28	14	72	2	5.14	1.91	3	6
29	13	84	2	6.46	2.18	4	7
30	14	94	2	6.71	1.97	5	8
31	7	51	3	7.2	1.52	3	5
32	11	78	2	7.09	1.68	6	8
33	5	27	4	5.4	1.23	1	3
34	13	68	3	5.2	1.89	3	6
35	16	85	2	5.3	2.03	4	6
36	18	94	2	5.2	1.94	6	10
37	7	37	4	5.2	1.39	2	4
38	4	29	4	7.25	1.09	3	4
39	4	26	4	6.5	0.79	3	3
40	9	56	3	6.2	1.77	3	4
41	7	48	3	6.8	1.29	4	5
42	9	55	3	6.1	1.72	3	5
43	12	64	3	5.3	1.81	4	7
44	9	46	3	5.1	1.84	3	4
45	9	58	3	6.4	1.8	4	7
46	6	31	4	5.1	1.36	1	4
47	10	50	3	5	1.72	3	3
48	7	55	3	7.8	1.5	4	6
49	4	15	4	3.75	0.63	1	1
50	7	39	4	5.57	1.82	2	3
51	9	44	3	4.9	1.33	2	4
52	8	30	4	3.75	0.86	3	3
53	5	36	4	7.2	1.03	2	3
54	4	32	4	8	0.72	2	3

CONCLUSION

Çoruh River and its tributaries are located at the intersection of two different features as geological and climatic. Due to its high flow rate, it is the focal point of hydroelectric power plants and dams, as well as for agricultural activities and recreational purposes. The presence of biodiversity hotspot points and bird migration routes increases the importance of the Çoruh Basin. In this study, Çoruh River, which is the fastest flowing stream of Turkey, was evaluated by using macroinvertebrates according to BMWP, Shannon-Wiener, Margalef and Simpson indices. According to BMWP score values, 9 of the stations are II. Quality, 19 stations III. Quality, 24 stations IV. Quality and 2 stations were determined to be in V. water quality class. According to these findings, it is predicted that the freshwater resources of the Çoruh Basin are under the influence of human activities and if the habitat destruction and pollutants continue or increase, the macroinvertebrate diversity will decrease.

ACKNOWLEDGEMENT

I would like to thank Salih Kumru and Diana (from Manchester) who helped to prepare the article according to the English spelling rules.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 411-416 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.02

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

A preliminary study of the effects of cold, frozen, or room temperature storage of commercial feeds on growth performance and feed consumption of juvenile rainbow trout (*Oncorhynchus mykiss*)

Soğuk, dondurulmuş veya ortam koşullarında depolanan yemlerin gökkuşağı alabalığı (*Oncorhynchus mykiss*) yavrularında büyüme performansı ve yem tüketimi üzerindeki etkileri hakkında bir ön çalışma

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Received date: 19.01.2021 *Accepted date: 14.08.2021*

How to cite this paper:
Khan, U. & Seyhan, K. (2021). A preliminary study of the effects of cold, frozen, or room temperature storage of commercial feeds on growth performance and feed consumption of juvenile rainbow trout (*Oncorhynchus mykiss*). *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 411-416.
DOI: 10.12714/egejfas.38.4.02

Abstract: The storage of commercial feeds in a cool and dry place is a prerequisite management strategy to minimize the deterioration of commercial feeds. This study investigated the effects of feed storage conditions on feed intake and growth performances of juvenile rainbow trout (*Oncorhynchus mykiss*). A total of 240 juvenile rainbow trout, weighing 8.7–10.5 g, were randomly distributed into four groups. Each group was further subdivided into three replicates with 20 fish in each. The experimental fish were fed to apparent satiation twice daily over a 35-days period using four commercial feeds previously kept at different storage conditions, *i.e.*, room temperature storage (20.8°C), cold storage (10°C), or frozen storages (-1.1°C and -15°C). The feed utilization was assessed by relative feed intake (RFI%) and feed conversion rate (FCR), while growth performance was evaluated by the thermal-unit growth coefficient (TGC) and specific growth rate (SGR). The preliminary findings suggest that the food storage conditions did not significantly affect feed intake and growth performance in juvenile rainbow trout. The findings provide practical information for fish farmers in the management of feed storage, which covers a remarkable proportion of the total production costs in aquaculture facilities.

Keywords: Aquafeeds, storage conditions, relative feed intake, growth performance, *Oncorhynchus mykiss*

Öz: Ticari yemlerin serin ve kuru bir yerde depolanması, yemin bozulma hızının en aza indirilmesi için ön koşul olan bir yönetimdir. Bu çalışmada, oda sıcaklığında (20.8°C), soğuk ortamda (10°C) ve dondurulmuş koşullarda (-1.1°C ve -15°C) muhafaza edilen yemlerle günde 2 kez doygunluk derecesinde 35 gün süreyle beslenen gökkuşağı alabalığı (*Oncorhynchus mykiss*) yavrularında büyüme performansı yem tüketim oranları üzerindeki etkileri incelenmiştir. 8,7-10,5 g ağırlığında toplam 240 adet yavru gökkuşağı alabalığı rastgele seçilerek dört gruba ayrılmıştır. Her bir grup ayrıca her birinde 20 balık bulunan üç tekrar olacak şekilde ayrılmıştır. Yem tüketimi, yüzde yem tüketim oranı (% RFI) ve yem dönüşüm oranı (FCR) ile değerlendirilirken, büyüme performanı, spesifik büyüme oranı (SGR) ve termal büyüme katsayısı (TGC) ile değerlendirilmiştir. Elde edilen veriler, yem depolama koşullarının gökkuşağı alabalığının yem tüketimi ve büyüme performansları üzerinde önemli derecede etkilemediği kaydedilmiştir. Bu çalışmada elde edilen bulgular, balık çiftliklerinde toplam üretim maliyetinin önemli bir kısmını kapsayan ticari yemlerin depolama yönetiminde üreticiler için pratik bilgiler ortaya koymaktadır.

Anahtar kelimeler: Balık yemi, depolama şartları, yüzde yem tüketimi, büyüme performansı, *Oncorhynchus mykiss*

INTRODUCTION

The aquaculture sector plays a vital role in contributing to poverty alleviation, economic development, and food security, representing ~82 million tonnes of aquatic animal production in 2018 and is projected to increase to 109 million tonnes by 2030 (FAO, 2020). Aquafeeds are one of the most important and relatively expensive aquaculture components, representing about 40%-90% of operating expenditures of fish farms (Rana *et al*., 2009; Pinfold, 2013; El-Sayed *et al*., 2015). The prices for different feed raw materials used in aquafeeds have risen considerably over the last decade: fishmeal, soybean meal, corn, and wheat increased by 55 to 225% (Rana *et al*., 2009; Wong *et al*., 2016; Naylor *et al*., 2021). Consequently, aquafeeds need to be carefully considered and managed for profitable production (FAO, 2020).

The aquafeed production reached 44 million tonnes in 2019 worldwide (Alltech, 2020) and is projected to increase to 82 million tonnes by 2023 (CISION, 2018). Turkey's aquafeeds production rose from 0.59 million tonnes in 2019 to 0.63 million tonnes in 2020 and is projected to increase to 0.90 million tonnes by 2023 (Atalay and Maltaş, 2020; TUYEM, 2021).

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The quality of aquafeeds can be subject to rapid deterioration developing molds if not stored properly (Robb *et al*., 2013). The lousy storage condition can initiate a repaid oxidization of aquafeeds, increasing the number of peroxides that lead to rancidity in the aquafeeds (Kop *et al*., 2019). The use of oxidized aquafeed results in retarded growth, poor feed efficiency, lethargy, and anemia, with dark body coloring in farmed fish (Tacon, 1992; Kop *et al*., 2019). Several biological risk factors have also been identified in the aquaculture industry due to poor storage conditions, such as the nesting of rodents, microorganisms, and human endoparasites, including genetically modified ones in areas where aquafeeds and fish products are stored (Fırat *et al*., 2020). In addition, aquafeeds are highly susceptible to insect infestations during their storage at ambient temperature, causing quantity and quality losses with the passing of time (Smith, 1980; Solomon *et al*., 2016). Insects attacking stored aquafeeds show a rapidly increasing population at temperatures between 25°C to 32°C (Smith, 1980; Fields *et al*., 2012). On the other hand, these insects stop feeding, develop slower, and eventually die at a temperature below 13°C (Fields *et al*., 2012). The storage of aquafeeds at low temperatures can also prolong the shelf life of aquafeeds since low temperatures reduce many of the chemical reactions that occur in feed (Singh and Desrosier, 2018).

Consequently, the storage of aquafeeds at a temperature below 13° C will probably restrain insect infestation and retard the rate of various chemical reactions that can damage the quantity and quality of aquafeeds during their storage. However, the water content of aquafeeds may form ice crystals at low temperatures, which may influence the digestive process (dos Santos, 1990; Hui *et al*., 2006) retarding farmed fish growth. The effects of aquafeeds storage conditions (frozen and non-frozen) on the digestive process have been investigated by Khan and Seyhan (2019) for farmed brook trout. They found no significant effects of storage conditions on the gastric evacuation rates of farmed brook trout. However, data on aquafeed storage conditions' impact on fish growth lack in the published literature. This study thus aimed at filling this knowledge gap by examining the growth performance and feed utilization of juvenile rainbow trout (*Oncorhynchus mykiss*) fed aquafeeds (hereafter "commercial feeds") kept at different storage conditions (ambient, cold, frozen) have been evaluated.

MATERIALS AND METHODS

A total of 240 juvenile rainbow trout, weighing 8.7–10.5 g, were obtained from the Sürmene Faculty of Marine Sciences in Trabzon. They were randomly distributed into four groups. Each group was further subdivided into three replicates with 20 fish in each. They were stocked in 12 experimental tanks (~100 L). Each experimental tank received a constant supply of fresh water at a flow rate of 2.5 l/min from a brook, and a continuous air bubbling in each tank ensured oxygen saturation. The experiments were conducted in an indoor environment under ambient water temperatures.

The experimental feeds were stored in two refrigerators equipped with varying ranges of temperature. They were fully adequate for obtaining desired temperatures. One of the refrigerators had two sections with two standard temperature ranges: 10°C and -1.0°C, while the other was a deep freezer with a single standard temperature of -15.0°C. A refrigerator thermometer (TFA 14.4000, TFA Dostmann, Germany) was used to reaffirm the temperatures of the above-mentioned refrigerators. Experimental feeds were first placed in airtight bottles and then stored in the refrigerators for 1 week.

Fish were fed by hand twice a day (08:00 h and 16:00 h) to apparent satiation throughout the feeding period that lasted for 35 days. The fish were fed with one of the four different experimental feeds previously kept at different storage conditions, *viz*., room temperature storage (20.8 ± 0.4°C), cold storage (10°C), and frozen storage (-1.1°C and -15°C). The feeds stored at cold and frozen conditions were fed immediately to experimental fish without thawing to defrost.

The uneaten feed (pellets) was carefully siphoned using a water hose without disturbing the experimental fish. The uneaten feed was then dried in an oven (Ecocell Drying Oven, MMM Medcenter, Germany) at 60°C until a constant weight was reached. The uneaten feeds ' mass was subtracted from the feed offered to calculate the satiation meal for fish in a tank. The commercial feeds used in this study were acquired from Skretting Aquaculture (www.skretting.com) packed in food-grade oxygen barrier polyethylene/aluminum bags. Their chemical compositions are listed in Table 1. The water temperature and dissolved oxygen of experimental tanks were measured using a HACH portable multi-meter (HQ40D) twice a day.

Weekly measurements of the total biomass (g) of replicate groups for each tank were performed at 04:00 to 05:00 h to prevent feeding cessation after weighing. Fish from each replica was carefully transferred into a bucket filled with water (known amount of water in kg) to take measurements.

Table 1. Main ingredients and chemical composition of commercial feeds* used in the experiment

Nutrient value (%)	
Crude protein	44.0
Crude fat	21.0
Crude fiber	3.9
Crude ash	9.0
Macro Elements (%)	
Calcium	1.8
Phosphorus	1.0
Sodium	0.2
Trace Elements (mg/kg)	
Iron	80
Copper	7
Zinc	110

[*] Skretting Aquaculture (Nutreco Company, Turkey)

Growth measurements

The weekly feed consumption was divided by total fish biomass in each tank at the end of the week and then multiplied by 100 to express in percentage to evaluate changes in feed intake (relative feed intake, RFI%) over the growth period. The values of RFI% were plotted against time, and their relationship was described by an exponential function that provided the best fit to the data than the linear function.

The feed conversion rate (FCR), specific growth rate (SGR), and thermal-unit growth coefficient (TGC) were estimated using the following equations (Korkut *et al.*, 2007; Karabulut *et al.*, 2011)

$$\mathrm{FCR} = \frac{\text{Feed eaten by fish}}{\text{Weight gained by fish}}$$

$$\mathrm{SGR} = \frac{\ln(W_f) - \ln(W_i)}{t}$$

$$\mathrm{TGC} = \frac{W_f^{1/3} - W_i^{1/3}}{\text{temp.}\,(°\,\mathrm{C}) \times t}$$

where W_f is the final total biomass weight of all fish in each tank, W_i is the initial total biomass of all fish in each tank weight, t is time (days) between W_f and W_i.

Growth pattern modeling

The relationship between fish weight and time was described by the linear function: $W = a + \rho t$, and exponential function: $W = ae^{\rho t}$, where W is weight, t is time, a is the intercept, and ρ is the regression slope, which can be considered as a growth rate parameter.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test significant differences between all groups. The analysis of covariance (ANCOVA) was used to compare the regression slope values ρ estimated from different groups to test significance differences. All statistical analyses were carried out using SigmaPlot version 14, from Systat Software, Inc., San Jose California USA, www.sigmaplot.com.

RESULTS AND DISCUSSION

The water temperature ranged from 15.1 to 16.2° C (mean ± S.D., 15.4 ± 0.4), and oxygen saturation was 90.53% (± 4.45). No mortality was observed, and all fish actively fed throughout the feeding experiment.

The RFI% decreased with fish growth (Figure 1), which was comparable to the findings of Craig *et al*. (2017). Smaller fish generally require more protein than larger fish and consume a larger amount of meals related to their body size than larger fish (Craig *et al*., 2017). Consequently, feed intake per body weight often decreases over time as fish grow. In this study, the RFI% of juvenile rainbow trout showed no significant differences between all experimental groups (One-way ANOVA, $F_{12,3}$= 0.229, P= 0.874). The relationship between RFI% and time could thus be described by $\mathrm{FI\%} = 33.17e^{-0.022t}$; R^2 = 0.98, where t is time for combined data. This parameterized exponential function was used to provide a curved line for Figure 1. The RFI% data from different experimental groups are used together in Figure 1.

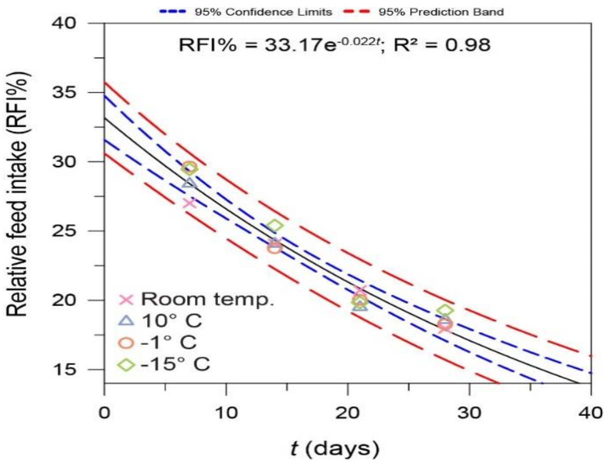

Figure 1. Mean relative feed intake in juvenile rainbow trout (*Oncorhynchus mykiss*) fed commercial feeds stored at different temperatures.

The results of FCR, SGR, and TGC (mean ± S.D.) are presented in Table 2. The feed intake and growth performance of juvenile rainbow trout fed either cold, frozen, or non-frozen feeds showed no significant difference between treatments. Likewise, based on weekly data, feed intake and growth performance of different experimental groups also did not reveal any significant difference.

Table 2. Summary statistics for growth and feed intake in juvenile rainbow trout (*Oncorhynchus mykiss*) fed commercial feeds stored at different temperatures.

	STORAGE CONDITIONS					
	Room temperature	Cold condition	Frozen condition		one-way ANOVA	
Parameter*	20.8 (± 0.4)°C	10°C	-1.0°C	-15.0°C	$F_{3,8}$	P
TGC	0.005 ± 0.000	0.004 ± 0.000	0.004 ± 0.000	0.005 ± 0.000	1.207	0.368
SGR	5.983 ± 0.044	6.056 ± 0.030	5.972 ± 0.095	5.969 ± 0.051	1.422	0.306
FCR	1.083 ± 0.026	1.020 ± 0.018	1.013 ± 0.022	1.071 ± 0.068	2.446	0.139
ρ	10.239 ± 1.549	10.182 ± 0.912	9.393 ± 2.529	9.958 ± 1.803	0.834**	0.48

* Thermal-unit growth coefficient, TGC); standard growth rate, SGR; feed conversion rate, FCR; regression slopes of the linear function, ρ ** $F_{3,68}$, ***simple linear function

The linear function best described the relationship between fish weight gain and time since it consistently provided a higher value of the coefficient of determination, R^2, than the exponential function. A positive regression slope value was obtained for each group, indicating increased body weight over time (Figure 2). Based on regression slope values, the growth rates in juvenile rainbow trout fed either cold stored, frozen, or non-frozen feeds did not differ significantly (One-way ANOVA, $F_{3,68} = 0.834$, $P = 0.480$). The ANCOVA reaffirmed these results and revealed homogeneous slopes suggesting that juvenile rainbow trout from different groups had similar growth rates (ANCOVA, $F_{(3,64)} = 0.834$, $P = 0.480$). Several authors have used the ANCOVA to validate the homogeneity of regression slopes estimated for different groups (Gillanders, 1997; Basusta and Khan, 2021). The relationship between fish weight gain and time has been determined using the linear function, exponential function, asymptotic function, and von Bertalanffy growth function (Hopkins, 1992; Hamre *et al.*, 2014).

Fish growth generally has two phases: an initial exponential phase that changes linearly as fish reaches its maximum asymptotic weight (Soderberg, 2017). Such a relationship can appropriately be described by von Bertalanffy growth function (Katsanevakis, 2006; Lugert *et al.*, 2016). However, this study found a linear growth rate in juvenile rainbow trout, which is consistent with previous studies' results (Yakupitiyage *et al.*, 1991; Lugert *et al.*, 2016; Khan, 2019). Consequently, the growth in fish during their initial stage might follow a linear growth trend, followed by an exponential phase that would eventually lead to a relatively linear as a fish achieve a maximum asymptotic weight.

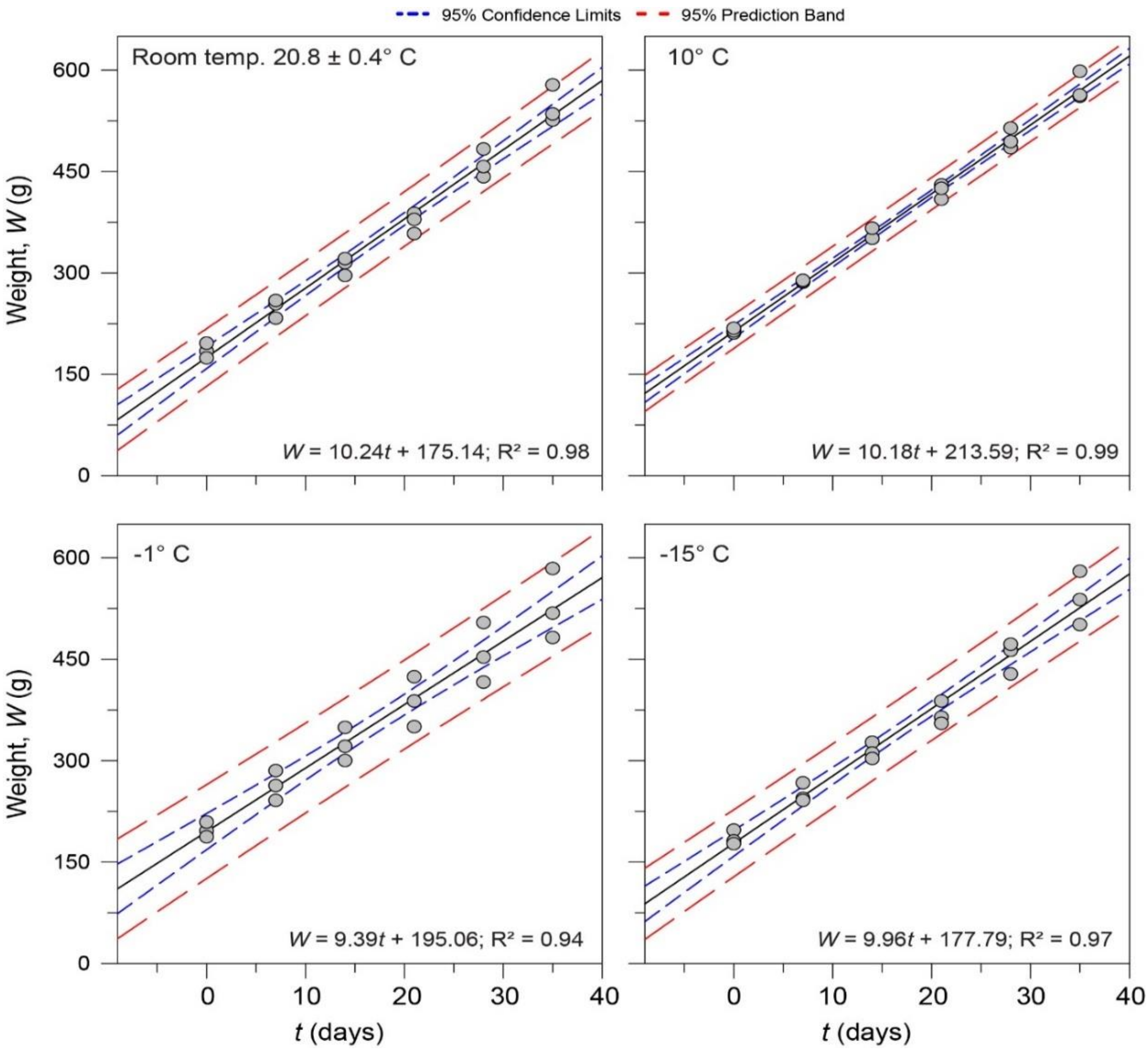

Figure 2. Growth rate of juvenile rainbow trout (*Oncorhynchus mykiss*) fed commercial feeds stored at different temperatures.

The water content of commercial feeds could form ice crystals due to the freezing process, which may affect different biochemical and physicochemical processes and may influence the digestive process in the stomach of fish (dos Santos, 1990; Hui *et al*., 2006). Andersen (2012) fed Atlantic cod *Gadus morhua* with the previously frozen (−20° C) and fresh *Ammodytes tobianus* to examine the possible influence of non-frozen and frozen meals on the gastric evacuation in fish. The author found that both previously frozen and freshly killed *A. tobianus* evacuate at a similar rate from the stomach of *G. morhua*. Similarly, brook trout (*Salvelinus fontinalis*) were fed non-frozen and frozen (-15.0° C) feeds; the trout evacuated both meal types with a similar rate of gastric evacuation, indicative of no effect of feed storage on digestive processes (Khan and Seyhan, 2019).

CONCLUSION

The preliminary findings suggest that the growth and feed consumption of juvenile rainbow trout were not affected by the storage conditions of the feed at either cold, frozen, or room-temperature conditions. Generally, the fish feeds are stored at room temperature for future use. Kop *et al.* (2019) found that the number of peroxides in feed (lipid) increases with increasing temperature, demonstrating a short shelf life for feed stored at room temperature than samples stored at 4°C. These findings may provide helpful information for fish farmers in managing feed storage of commercial feeds with prolonging shelf life, which is known to cover a significant amount of the total production costs in the aquaculture industry. This preliminary study included juvenile fish; consequently, subsequent research should include larger size fish coupled with the examination of feed quality properties in order to draw a broader conclusion.

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Ege Journal of Fisheries and Aquatic Sciences, 38(4), 417-426 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.03

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Ege Denizi'nde gırgır ağları ile yakalanan bazı pelajik türlere ait av miktarlarının tarihsel analizi

An analysis of historical landing data of some pelagic species caught by purse seines in the Aegean Sea

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Received date: 14.04.2021 *Accepted date: 25.08.2021*

How to cite this paper:
Tosunoğlu, Z. & Ceyhan, T. (2021). An analysis of historical landing data of some pelagic species caught by purse seines in the Aegean Sea. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 417-426. DOI: 10.12714/egejfas.38.4.03

Öz: Hamsi (*Engraulis encrasicolus* Linnaeus, 1758) hariç tutulduğunda, Türkiye'deki pelajik balıkların nerdeyse yarısı Ege Denizi'nde avlanan gırgır tekneleri ile yakalanmaktadır. Çalışma bu nedenle 1973 ve 2019 yılları arasında Ege bölgesinde avcılık yapan gırgırlarda yakalanan bazı önemli pelajik türlerin av miktarlarını incelemeyi amaçlamıştır. 1990'lı yılların ortaları, sardalyanın (*Sardina pilchardus* Walbaum, 1792) Ege Denizi ve Türkiye denizlerinde en çok yakalandığı yıllar olup, bu miktar 2000'li yılların ortalarında 10000 tonlar seviyesine gerilemiş ve günümüze kadar bu düzeyde seyretmiştir. Sardalya 2011 yılına kadar Ege'de en fazla yakalanan tür iken bu durum son yıllarda hamsi lehine değişmiştir. Türkiye'de uskumru *(Scomber scombrus* Linnaeus, 1758), kolyoz (*Scomber japonicus* Houttuyn, 1782) ve kupez (*Boops boops* Linnaeus, 1758) gibi türlerin neredeyse tamamı Ege Denizi'nden yakalanmaktadır. Çalışmamızda yukarıda belirtilen türlerin tarihsel süreçteki av miktarları, avcılığı düzenlemek için çıkarılmış olan sirküler ve tebliğlerin yanı sıra uygulamaya sokulan diğer düzenlemelerle ilişkilendirilmeye çalışılmıştır. Ayrıca, bundan sonraki süreçte söz konusu türlerin sürdürülebilir kullanımının önündeki sorunlar ortaya konarak bu sorunlara yönelik çözüm önerileri getirilmeye çalışılmıştır.

Anahtar kelimeler: Sirküler, tebliğ, sürdürülebilirlik, bölgesel balıkçılık

Abstract: The fishing activity with purse seines in the Aegean Sea accounts for almost half of the pelagic fish excluding anchovy (*Engraulis encrasicolus* Linnaeus, 1758) in Turkey. In this study, we aimed to analyse the catch amounts of some important pelagic species caught by Aegean purse seiners between 1973 and 2019. While mid-1990s are the most captured years for the European pilchard (*Sardina pilchardus* Walbaum, 1792) in Aegean Sea and also Turkish Seas, this amount has decreased at the level of 10000 mt in the mid-2000s and remained similar amounts up to now. Though European pilchard was the most caught species in the Aegean until 2011, this has changed in favour of anchovy in the last decade. Recently, almost all pelagic species such as mackerel (*Scomber scombrus* Linnaeus, 1758), chub mackerel (*Scomber japonicus* Houttuyn, 1782) and bogue (*Boops boops* Linnaeus, 1758) have been caught from the Aegean Sea in Turkey. In the study, the production of these species in the historical period was tried to be associated with the regulations implemented with circulars and notifications, and the problems preventing the sustainable use of these species that were caught by purse seiners were determined and solutions were proposed for the future.

Keywords: Circular, notification, sustainability, regional fisheries

GİRİŞ

Gırgır, dünyadaki en üretken av aracı olup son elli yıldır tek başına, küresel avın yaklaşık 1/3'ünü yakalamıştır (Watson vd., 2006). Norveç, yıllık 500.000 ton ringa (*Clupea harengus* Linnaeus, 1758) ile 180.000 ton uskumruyu *(Scomber scombrus* Linnaeus, 1758) bu av aracı ile avlamaktadır (Breen vd., 2012; Marçalo vd., 2019). Bu durum gırgır balıkçılığını miktar açısından en fazla balık avlayan ve dolayısı ile en karlı avcılık konumuna getirmiştir. Bazen tek bir avda 1.000 tonun üzerinde balık yakalanmakta ve bunun ticari değeri de yaklaşık 1 milyon Euro'yu aşmaktadır. Türkiye'de başta hamsi (*Engraulis encrasicolus* Linnaeus, 1758) olmak üzere sardalya (*Sardina pilchardus* Walbaum, 1792), istavrit (*Trachurus trachurus* Linnaeus, 1758) gibi birçok pelajik balık türünün neredeyse tamamı gırgır ağları ile avlanmakta ve bu miktarın da avcılık yolu ile üretilen su ürünlerinin yarısından fazlası olduğu TUIK istatistiklerinde rapor edilmektedir.

Beş yıllık kalkınma planlarında, 1990 öncesinde avcılık üretimini artırmaya yönelik planlamalar yapılırken, 2000'li yıllardan sonra sürdürülebilir avcılık en önemli konu olmuştur (Anonim, 2018). Su ürünleri kaynaklarının sürdürülebilir kullanımı için, mevcut kaynakların nitelikleri ve bu kaynakların kullanılma (hatta sömürülme) durumunun sürekli izlenmesi, yeterli ve güncel verilerin toplanması, bilimsel yöntemlerle değerlendirme yapılarak geleceğe yönelik kararlar alınması ve uygulanması gerektiği son kalkınma planında da yer almaktadır (Anonim, 2018). Bu çalışmanın da kalkınma planında belirtilen amaçlara hizmet ettiği düşünülmektedir.

Türkiye'de gırgır balıkçılığında elde edilen ürün miktarı diğer balıkçılıklar ile kıyaslandığında, oldukça yüksek olsa da bu balıkçılık üzerine yapılan bilimsel çalışma sayısı (%2) oldukça azdır (Özbilgin vd., 2015). Türkiye genelinde av

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araçları ve balıkçılığı üzerine yapılan 610 çalışmanın 22 adedi çevirme av araçları ve yöntemi ile yapılmış olup bunlardan sadece 12'si gırgırı kapsamaktadır. Bu çalışmalar da gırgır ağlarının teknik özellikleri (Tokaç, 1985; Hoşsucu vd., 1995; Karakulak vd., 2002; Doyuk, 2006; Emirbuyuran ve Çalık, 2016; Erdem vd., 2019), av kompozisyonu ve yakalanan miktarlar (Hoşsucu vd., 1995; Paşaoğlu, 2015) ile gırgır balıkçılığı ve sorunlarına (Kara, 1989; Hoşsucu vd., 1995; Erdem, 2016) yöneliktir. Bunların dışında Türkiye'nin en büyük bütçeli balıkçılık araştırma projesinde ise yine çoğunlukla gırgır ile yakalanan Karadeniz hamsisinin son yıllarda göç yolları ve balıkçılığının sömürülme şekilleri yeniden değerlendirilmiş ve hamsi için bazı önemli hasat kontrol stratejileri gözden geçirilmiştir (Gücü vd., 2017).

Karadeniz'de avlanan hamsi ve çaça (*Sprattus sprattus* Linnaeus, 1758)'dan dolayı Ege'nin toplam üretimde payı düşük olsa da son yıllarda birçok pelajik türün Ege Denizi'ndeki av miktarı diğer denizlerimize göre oldukça yükselmiştir. Gelinen bu durumla ilgili olarak 1967 yılından 2019 yılına kadar yaklaşık yarım asrı geçen süreçte önemli pelajik türlerin av kayıtları incelenmiş ve av miktarlarındaki dalgalanmalar balıkçılık yönetim uygulamaları (düzenlemeler) ile açıklanmaya çalışılmıştır. Çalışmada ayrıca Ege Denizi sürdürülebilir gırgır balıkçılığının önündeki temel sorunlar belirlenip, çözüm önerileri getirilmeye çalışılmıştır.

MATERYAL VE METOT

Bu çalışmada, 1967-2019 yılları arası 53 yıllık süreçte, Ege Denizi'nde gırgırda yakalanan önemli pelajik türlerin TUIK su ürünleri istatistikleri değerlendirmeye alınmıştır. Ege Denizi'nde avcılık yapan gırgırda önemli miktarlarda yakalanan pelajik türlerin başında sardalya, uskumru, kolyoz (*Scomber japonicus* Houttuyn, 1782), kupes (*Boops boops* Linnaeus, 1758) ve yuvarlak sardalya (*Sardinella aurita* Valenciennes, 1847) gelmektedir. Hamsi ve istavrit (*Trachurus spp.*) türlerinin Ege'de yakalanma miktarları az olduğundan burada bu türler değerlendirmeye alınmamıştır. Balıkçıların tirsi (*Alosa fallax* Lacepède, 1803) olarak isimlendirdiği, TUIK istatistiklerine giren tür, gerçekte yuvarlak sardalyadır. Bir karışıklığa neden olmaması için bu tür de detaylı bir değerlendirmeye alınmamıştır.

Av verilerinin tanımlamalı istatistiki değerlerinin hesaplanması ve yıllar itibarı ile av miktarlarını gösteren grafiklerin çizimi Ms Excel programında yapılmıştır.

1967-2019 yılları arasındaki av kayıtları, avcılık dönemlerinde uygulanan balıkçılık düzenlemeleri (sirküler ve tebliğler) ile ilişkilendirilmeye çalışılmıştır. Bu nedenle 1973-2020 yılları arasında yürürlüğe giren sirküler ve tebliğlerin, gırgır balıkçılığı ile ilgili genel ve Ege Denizi özelindeki ilgili bazı maddeleri kronolojik sırada düzenlenmiştir. Ancak çalışmada Ege Denizi'ne bazı dönemler diğer bölgelerden gelen tekneler ile avlanan gırgır tekne sayısı ve bunların motor güçleri ayrıca kullandıkları akustik aletler ve ağlarının uzunlukları ilgili kayıtlı ya da sağlıklı bir veri seti olmadığı için, bu tür balıkçılık eforu parametrelerinin av miktarını nasıl etkilediği tespit edilememiştir. Çalışmada ayrıca Ege gırgır balıkçılığının sorunları, alanda faaliyet gösteren Ege Bölgesi Gırgır Balıkçılığı Derneği yönetici ve üyeleri ile tartışılmış, bunlara çözüm önerileri getirilmeye çalışılmıştır.

BULGULAR

2019 yılı kayıtlarına göre sardalya, kupes, kolyoz, yuvarlak sardalya ve uskumru türleri en fazla miktarlarda Ege Denizi'nde yakalanmaktadır (Tablo 1). Eğer bu tablodaki hesaplamaya hamsi dahil edilmez ise yaklaşık %40'lık oran ile Ege, pelajik türlerin en fazla yakalandığı deniz olarak karşımıza çıkmaktadır. Son yıllarda Ege Denizi'nde de en fazla miktarda yakalanan tür hamsi olsa da %4,6'lık bu oran Türkiye toplamında oldukça düşüktür.

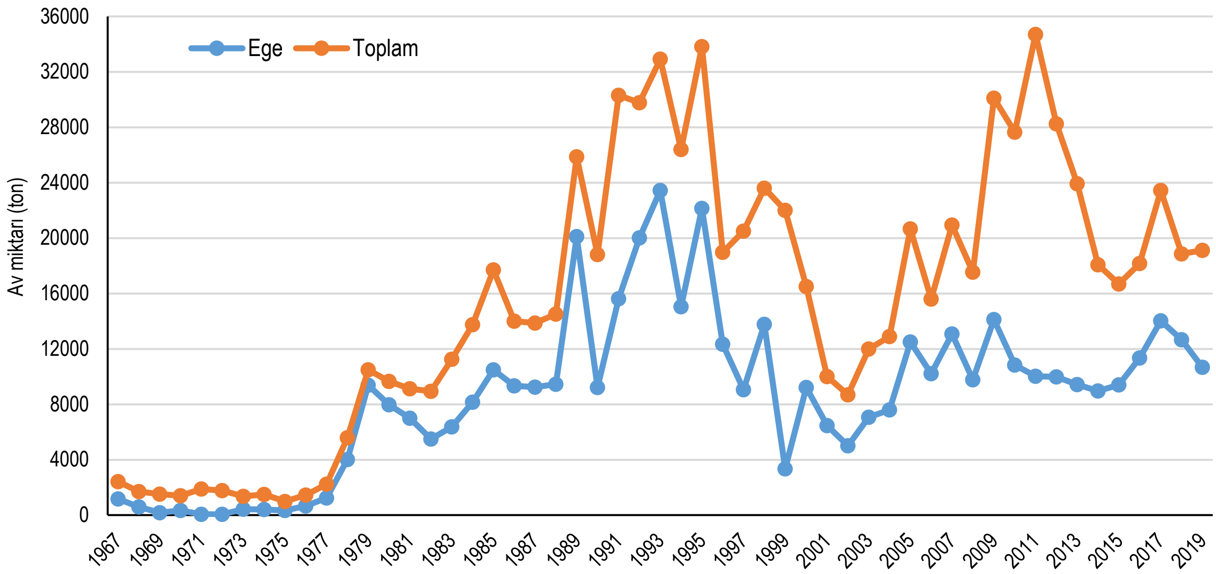
Tablo 1. TUIK 2019 kayıtlarına göre gırgır ile yakalanan bazı önemli pelajik türlerin av miktarları ve Ege'nin payı
Table 1. The catch amounts of some important pelagic species caught by purse seine and the ratio of the Aegean in TUIK 2019 records

2019	Toplam	Karadeniz	Marmara	Ege (%)	Akdeniz
Engraulis encrasicolus	262544,4	233083,6	17231,8	12141,4 (4,6)	87,6
Trachurus trachurus	13179,8	11190,0	1265,7	473,5 (3,6)	250,6
Trachurus mediterraneus	6325,4	2789,7	2443,9	899,3 (14,2)	192,5
Scomber japonicus	2334,2	36,1	36,4	1693,2 (72,5)	568,5
Boops boops	2865,0		76,1	2594,6 (90,6)	194,3
Pomatomus saltatrix	1213,7	584,4	400,9	152,6 (12,6)	75,8
Sarda sarda	1578,3	603,3	220,4	422,5 (26,8)	332,1
Sardina pilchardus	19119,2	244,4	4536,4	10682,8 (55,9)	3655,6
Sardinella aurita	1965,3	555,1	31,6	1371,6 (69,8)	7,0
Scomber scombrus	186,3	0,1	1,5	184,4 (99,0)	0,3
Belone belone	184,9	49,2	91,4	39,2 (21,2)	5,1
Toplam	311496,5	249135,9	26336,1	30655,1 (9,8)	5369,4

1967 ve 2019 yılları arasında ortalama 8655,3 ton ile sardalya, en çok avı Ege Denizinde vermiş olup, 1971, 57 ton ile en az, 1993, 23439 ton ile en yüksek av verdiği yıllardır (Tablo 2). 1967 ile 1977 yılları arasındaki 10 yıllık süreçte sardalya av miktarı, Ege Denizi ve Türkiye toplamında oldukça düşüktür (Şekil 1). 53 yılda avlanan toplam sardalya miktarının %55,7'si Ege Denizi'nde yakalanmış olup, Ege'nin ortalama yıllık sardalya yakalama oranı da %52 olarak hesaplanmıştır. 90'lı yıllar, sardalyanın Ege ve Türkiye denizlerinde en fazla miktarda avlandığı yıllardır. Gırgır tekne boy ve motor güçleri ile gırgır ağlarının boy ve derinliği deki artışlar sardalya av miktarına doğrudan yansımıştır. 2000'li yıllarda Ege'de 10000 tonlara düşen av miktarı, günümüze kadar bu seviyelerde devam etmiştir. Ege'den sonra sardalyanın en fazla miktarda yakalandığı Marmara Denizi'nde, 2020 yılında uygulamaya başlayan ışık yasağı, bu denizimizdeki sardalya üretimini doğrudan etkileyeceği düşünülmektedir.

Tablo 2. Sardalyanın 1967-2019 yılları arasında Türkiye denizlerindeki av miktarının tanımlamalı istatistiki değerleri
Table 2. The descriptive statistics of catch amounts of European pilchard in Turkish seas between 1967 and 2019

	Ege Denizi	Marmara Denizi	Akdeniz	Karadeniz	Toplam
Ortalama	8655,3	3727,9	2356,5	832,0	15541,3
Standart Hata	797,7	397,1	361,3	175,5	1353,6
Aralık	23382	15415	12120	6778	33720
En Küçük	57	282	2	0,1	989
En Büyük	23439	15697	12122	6778	34709
Sayı (yıl)	53	53	53	49	53
Güvenirlik Düzeyi (%95,0)	1600,74	796,85	724,93	352,96	2716,26



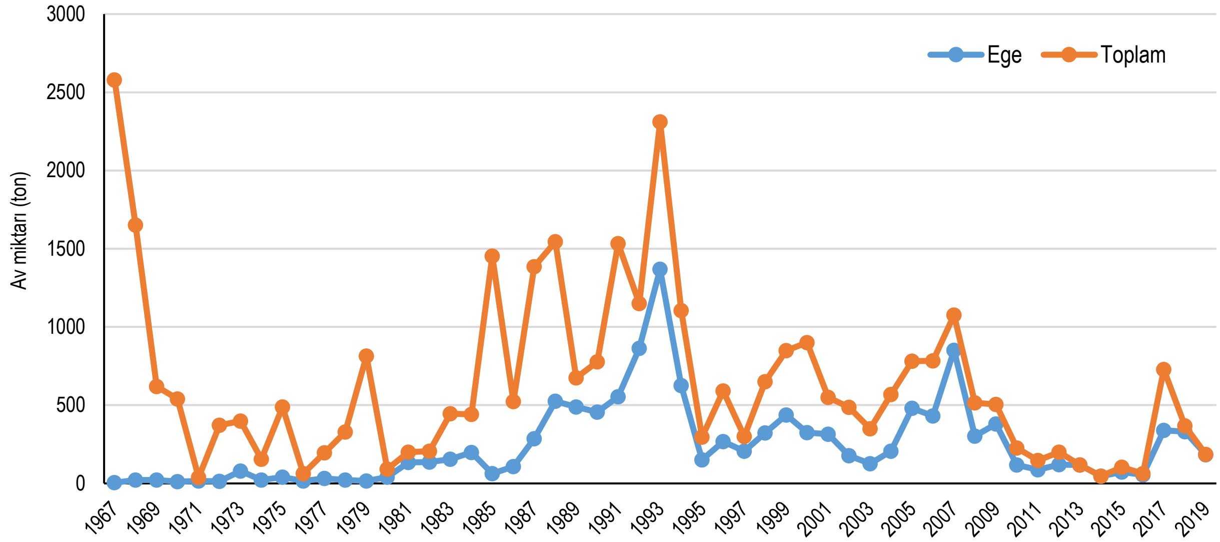
Şekil 1. 1967-2019 yılları arasında Ege Denizi ve Türkiye denizlerinde yakalanan sardalya balığı av miktarları
Figure 1. The catch amounts of European pilchard in the Aegean Sea and Turkish seas between 1967 and 2019

Uskumru, 1967 ve 2019 yılları arasında Ege Denizi'nde yıllık ortalama 240,9 ton av vermiştir (Tablo 3). En fazla uskumru, 1370 ton ile 1993 yılında yakalanmıştır ve bu rakam 53 yıllık süreçte 1000 tonun üzerinde gerçekleşen tek rakamdır.

Ege'de 90'lı yılların başlarındaki uskumru av miktarı artışı, 1995 yılından sonra sadece 2007 yılı hariç diğer yıllar 500 tonu geçmeyen rakamlar şeklinde devam etmiştir (Şekil 2). 53 yıllık süreçte uskumrunun %38,1'i, son otuz bir yılda (1989-2019) ise %57,1'i Ege Denizi'nden yakalanmış olup, 53 yılın Ege Denizi ortalama uskumru yakalama oranı %45 olarak hesaplanmıştır.

Tablo 3. 1967-2019 yılları arasında Ege Denizi ve Türkiye denizlerinde yakalanan toplam uskumru miktarının tanımlamalı istatistiki değerleri
Table 3. The descriptive statistics of catch amounts of mackerel in the Aegean Sea and Turkish seas between 1967 and 2019

	Ege Denizi	Toplam
Ortalama	240,9	631,7
Standart Hata	36,15	76,13
Aralık	1366	2542
En Küçük	4	38
En Büyük	1370	2580
Sayı (yıl)	53	53
Güvenirlik Düzeyi (%95,0)	72,53	152,77



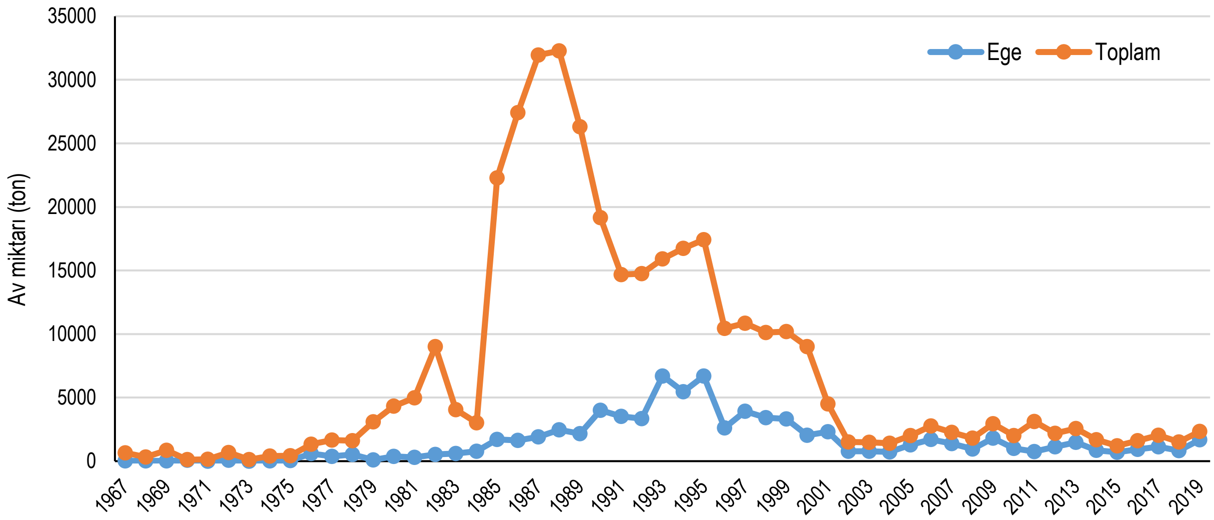
Şekil 2. 1967-2019 yılları arasında Ege Denizi ve Türkiye denizlerinde yakalanan uskumru balığı av miktarları
Figure 2. The catch amounts of mackerel in the Aegean Sea and Turkish seas between 1967 and 2019

Kolyoz, 1967 ve 2019 yılları arası 53 yıllık süreçte Ege Denizi ve Türkiye toplamında sırası ile yıllık ortalama 1539,3 ve 6928,9 ton av vermiştir (Tablo 4).

Ege'de 5000 tonun üzerinde kolyozun en fazla yakalandığı yıllar sadece 1993-1994-1995 ardışık yıllarıdır (Şekil 3). 1967'den günümüze kadar olan 53 yıllık süreçte kolyozun %22,2'si, 2002 yılından itibaren ise %54,6'sı Ege Denizi'nde yakalanmıştır. 53 yılın Ege Denizi ortalama kolyoz yakalama oranı ise %32'dir.

Tablo 4. 1967-2019 yılları arasında Ege Denizi ve Türkiye denizlerinde yakalanan toplam kolyoz miktarının tanımlamalı istatistiki değerleri
Table 4. The descriptive statistics of catch amounts of chub mackerel in the Aegean Sea and Turkish seas between 1967 and 2019

	Ege Denizi	Toplam
Ortalama	1539,3	6928,9
Standart Hata	221,1	1186,1
Aralık	6701	32168
En Küçük	7	112
En Büyük	6708	32280
Sayı (yıl)	53	53
Güvenirlik Düzeyi (%95,0)	443,75	2380,09

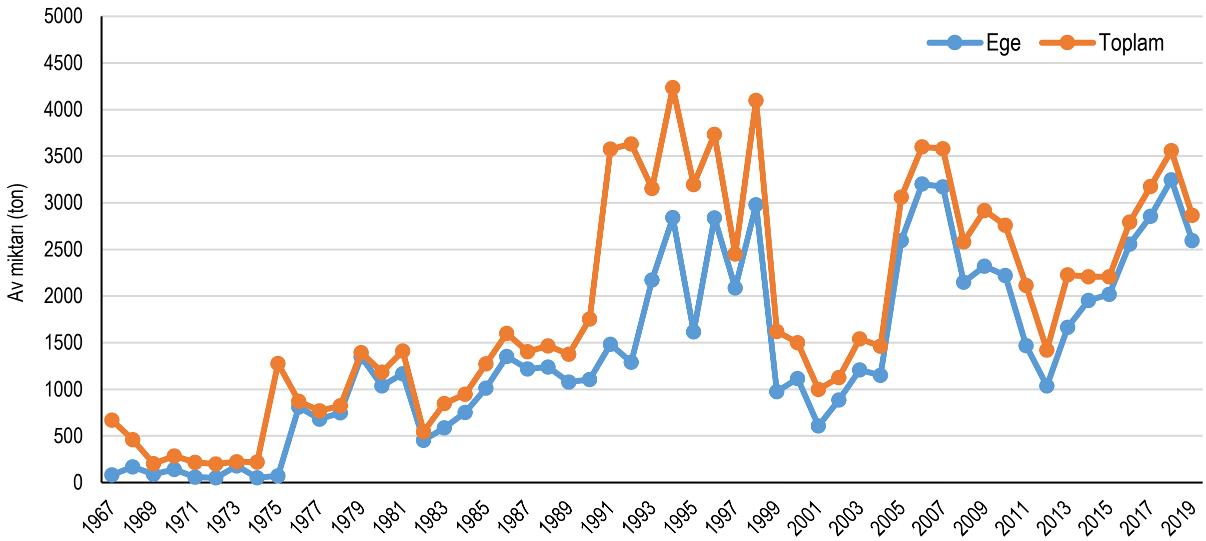


Şekil 3. 1967-2019 yılları arasında Ege Denizi ve Türkiye denizleri toplamında yakalanan kolyozun av miktarları
Figure 3. The catch amounts of chub mackerel in the Aegean Sea and Turkish seas between 1967 and 2019

Kupes'in, 1967-2019 yılları arası 53 yıllık süreçte Ege Denizi ortalama av miktarı 1392,1 ton iken Türkiye ortalaması 1864,7 tondur (Tablo 5). Bu süreçte Türkiye kupes avının ¾'ü, Ege Denizi'nde gerçekleşmiştir (Şekil 4). Yıllık 2500 ton civarı kupes, 2005 yılından itibaren Ege'de hemen hemen her yıl avlanmakta ve bunun toplamdaki oranı %85'in üzerindedir.

Tablo 5. 1967-2019 yılları arasında Ege Denizi ve Türkiye denizlerinde yakalanan toplam kupes miktarının tanımlamalı istatistiki değerleri
Table 5. The descriptive statistics of catch amounts of bogue in the Aegean Sea and Turkish seas between 1967 and 2019

	Ege Denizi	Toplam
Ortalama	1392,1	1864,7
Standart Hata	130,91	160,85
Aralık	3197	4037
En Küçük	51	199
En Büyük	3248	4236
Sayı (yıl)	53	53
Güvenirlik Düzeyi (%95,0)	262,69	322,76



Şekil 4. 1967-2019 yılları arasında Ege Denizi ve Türkiye denizleri toplamında yakalanan kupesin av miktarları
Figure 4. The catch amounts of bogue in the Aegean Sea and Turkish seas between 1967 and 2019

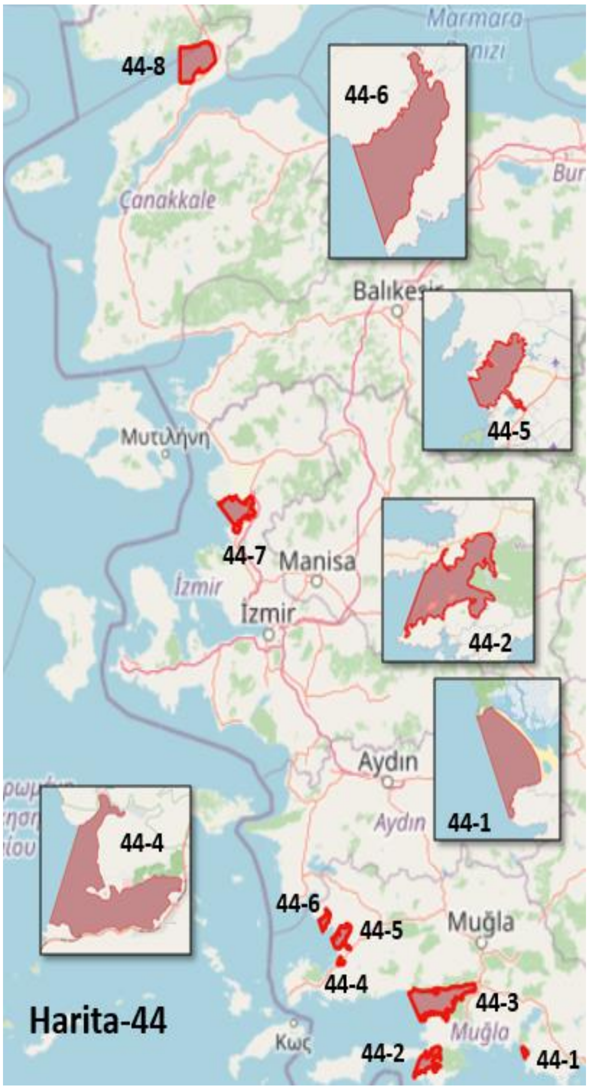
TARTIŞMA VE SONUÇ

1943 yılında uygulamaya alınan Meclis Kararname'sinde, memleketin bütün su avı yerlerinde 15 Nisandan Ağustos nihayetine kadar 4,5 aylık müddet içinde aletli balık avcılığı memnudur ile İktisat ve Ticaret Vekâleti'nin 1956-1973 yılları arasında çıkan sirkülerlerde balıkların yumurtlama zamanı olan 15 Nisan'dan Ağustos sonuna kadar dört buçuk aylık süre içinde bütün su avı yerlerinde balık avcılığı yapmak yasaktır düzenlemelerinden, 1943 öncesi ve sonrasında uygulanan Zabıtai Saydiye Nizamnamesi'ne göre 15-Nisan-31 Ağustos arası dönemde gırgır avcılığının Türkiye denizlerinde yasak olduğu anlaşılmaktadır. İktisat ve Ticaret Vekâleti (Ticaret Bakanlığı) ile Tarım Bakanlığının 1956-1990 yılları arasında çıkardığı sirkülerlerde, içsulara oranla deniz balıkları avcılığına ilişkin düzenlemenin çok az olması, bu yıllarda denizlerin sonsuz kaynak gibi görülmesi ve kullanılmasından kaynaklanmaktadır. Ancak 80'li yıllarda filo ve tekne boy ve motor güçlerindeki aşırı artış, bazı yasakların 90'lı yıllardan sonra daha yoğun uygulanmaya başlamasına neden olmuştur. Bunlara ilaveten balıkçı teknesi ruhsatlarına sınırlandırmalar getirilmiştir. Fakat, Ünal (2004) geçmişte lisans sistemi ile gemi sayısı sınırlamasının, hiçbir bilimsel çalışma veya araştırma raporu dikkate alınmadan kaldırıldığını rapor etmektedir. Her ne kadar 12 metreden büyük balıkçı teknelerinin ruhsatlandırılması 1991'de durdurulduysa da, Ünal ve Göncüoğlu (2012) her tür balıkçı teknesi ruhsatlandırılmasının 1997'de durdurulduğuna vurgu yapmaktadır. Ancak Ünal (2004) lisans sistemi ile gemi sayısı sınırlamasının, bilimsellikten uzak populist yaklaşımlarla, altı ay süreyle Haziran 2001'den 31 Aralık 2001'e kadar tekrar kaldırıldığını rapor etmektedir. Bu altı aylık süre boyunca (Haziran 2001- 1 Aralık 2001) çok fazla sayıda yeni balıkçı teknesinin balıkçılığa girmesine yasal olarak izin verilmiştir. Teknelere ruhsat verilmemesi 2002 yılından bu yana devam etmektedir. Şu anda, filo kapasitesinin daha fazla büyümesi, balıkçı filosuna ilave balıkçı gemilerinin girmesine izin verilmeyerek durdurulmuş görünüyor fakat Ünal ve Göncüoğlu (2012) bu uygulamada bir çelişki olduğunu ifade etmektedir. Ruhsat sistemi yürürlüktedir ancak mevcut sistem balıkçılık çabalarını sınırlamaktan oldukça uzaktır. Balıkçıların ve teknelerin ruhsatlandırılmasıyla balıkçılık çabalarının etkin kontrolü; sadece balık avlama izinleri, tekne sayıları ile değil, aynı zamanda avlanacak alanları ve süreleri ve hatta balıkların karaya çıkarılması gereken limanların da belirlenmesi ile mümkün olabilir. Günümüzde Karadeniz'den ve Marmara'dan bir çok balıkçı teknesi, dilediği zaman Ege ve Akdeniz'e gelip dilediği avlaklarda, dilediği türleri, dilediği miktarda avlamaya devam etmektedir (Ünal, 2004; Ünal ve Göncüoğlu, 2012).

1980'li yıllardaki gırgır çabasındaki artışların (tekne boy ve motor gücü ile gırgır ağının boy ve derinliğindeki artışlar, gemi inşa ve balık bulucu cihazlardaki gelişmeler vb.) olumsuz etkilerini azaltmak için, 90'lı yıllarda Ege gırgır balıkçılığına bazı düzenlemelerin geldiğini görmekteyiz. 2020 ile 2024 yılları arasındaki gırgır balıkçılığını düzenleyen, uygulanmakta olan son tebliğde ise bu kapsam çok daha genişletilmiş ve detaylandırılmıştır. Bu düzenlemeler gırgır dönem ve yer yasakları, ışık yer ve dönem yasakları, gırgır ışık yasakları, gırgır ağına ilişkin yasaklar ve gırgırda yakalanan pelajik türlere ilişkin düzenlemeler şeklinde detaylı olarak ele alınmıştır.

Yer yasakları (gırgır ve ışık)

1990 yılından itibaren Ege Denizi'nin körfez ve koylarına, gırgırla ile su ürünleri avcılığına yasaklar gelmeye başlamıştır. Bu alanlar Muğla Gülük Körfezi, Fethiye Körfezi, Bodrum Yarımadası, Gökova Körfezi, Güvercinlik Körfezi, Köyceğiz İztuzu Sahili, Aydın Akbük Körfezi ve Balıkesir Ayvalık Limanı'dır. 2020-2024 yılları arası dönemi kapsayan son tebliğde, gırgır yer yasakları aşağıdaki gibidir (Şekil 5). Bunun yanında ayrıca Ege Denizi'nde dönem boyu su ürünleri avcılığına yasak olan yerler de mevcuttur. Örneğin İzmir Körfezi'nde sağlık için sakıncalı olması ve dipteki kirletici maddeleri su yüzüne çıkararak ikinci bir kirlenmeye yol açması nedeniyle 1976 yılında Güzelyalı iskelesi, 1990 yılında Hava Eğitim Komutanlığı ve 1994 yılında Üçkuyular vapur iskelesi ile Bostanlı-Sazburnu arasında çekilen hattın doğusunda kalan körfez içinde gırgır ile avcılığa yasaklanmıştır. Tüm bu alanlar, gırgır balıkçılığının sürdürülebilirliği için birçok pelajik türe özel üreme ve büyüme rezerv alanı oluşturmuştur.

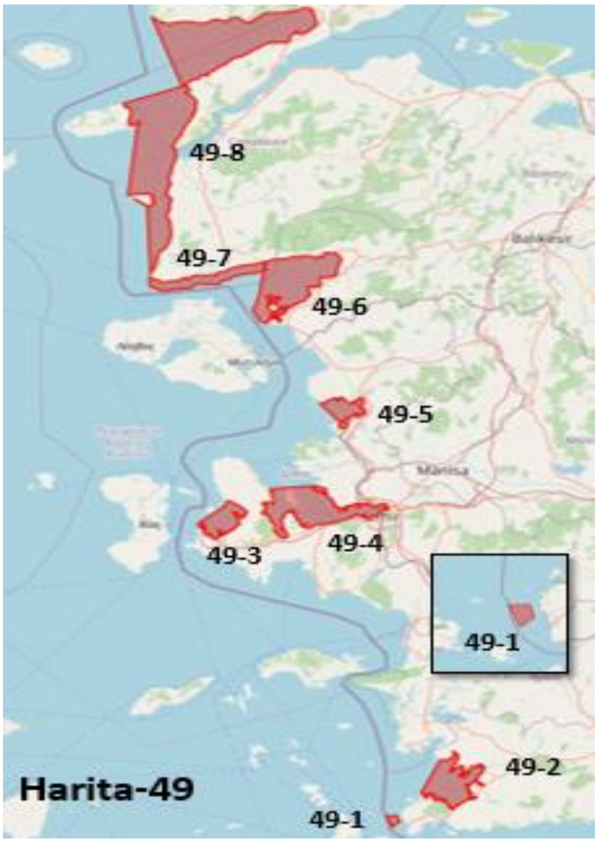


Şekil 5. Ege Denizi'nde gırgır avcılığına yasak olan yerler (Anonim, 2020)

Figure 5. The areas where the purse seine fishery is prohibited in the Aegean Sea (Anonim, 2020)

Ege Denizi'nde ışıkla dönem yasakları ise ilk kez 1 Nisan-31 Mayıs 1985 ile 15 Mart-31 Mart 1986 tarihleri arasında uygulanmaya başlamıştır. Sonrasında Meriç Nehrinin denize döküldüğü yer ile Edremit Körfezindeki Küçükkuyu limanı arasında kalan alanda, Edremit Körfezi Küçükkuyu Limanı ile Kuşadası Limanı arasında kalan alanda, Edremit Körfezi Küçükkuyu Limanı ile Çeşme Limanı arasında kalan alanda; İzmir Körfezinde, Çalıbaşı Burnu-Pelikan bankı Feneri-Abdullahağa Çiftliği hattının doğusunda kalan sahada, Çanakkale İli, Büyük Kemikli Burnu, Bozcaada, Batı Burnu ve Ayvacık Baba Burnu arasında çekilen hattın doğusunda kalan kısımda, Muğla ili, Güllük Körfezinde, Tekağaç Burnu ile İnce Burnu birleştiren hattın doğusunda kalan kısımda ışıkla ve gırgırla su ürünleri avcılığı tüm sezon boyunca yasaklanmıştır.

Son tebliğde ise aşağıdaki şekilde görüldüğü gibi Ege Denizi'nin birçok yeri ışıkla gırgır avcılığına kapatılmıştır (Şekil 6). Bu yerler, Kardak Kayalıklarının doğusu, Topan Adasının güneyi, Çatal Adanın kuzey burnu ve Çavuş Adası etrafı 1 Aralık-31 Ocak tarihleri arasında (Şekil 6; 49-1), Güllük Körfezinde; Kapalı Burnu ile Kızılyar Burnunu birleştiren hattın doğusunda kalan alan (Şekil 6; 49-2), Ildır Körfezinde; Çolak Burnu ile Çeşme Uçburnu arasında çekilen hattın doğusunda kalan alan (Şekil 6; 49-3), İzmir Körfezinde; Ardıç Burnu ile Kapan Burnu arasında çekilen hattın güneyinde kalan alan (Şekil 6; 49-4), Çandarlı Körfezinde; Kemikli Burnu ile Aliağa Taşlı Burun arasında çekilen hattın kuzeydoğusunda kalan alanda (Şekil 6; 49-5), Küçükkuyu Balıkçı Barınağı ile Küçük Maden Adası, Güneş Adası Feneri, Çıplak Ada Yumru Burnu ve Eğribucak Burnunu birleştiren hattın doğusunda kalan alan (Şekil 6; 49-6), Babakale ile Küçükkuyu Balıkçı Barınağı arasında kalan sahada 2 mil içerisinde (Şekil 6; 49-7) ve Babakaleyi, Bozcaada Eskifener Burnu, Bozcaada batı burnu, Gökçeada Aydıncık Burnu, Gökçeada Kaşkaval Burnu, Büyük Kemikli Burnu ve Boztepe Burnu ile birleştiren hattın doğusunda kalan alandır (Şekil 6; 49-8). Ayrıca Meriç Nehrinin denize döküldüğü yer ile Anamur Burnu arasında kalan karasularımızda 15 Nisan-31 Ağustos tarihleri arasında ışıkla avcılık yasaklanmıştır.



Şekil 6. Ege Denizi'nde ışıkla gırgır avcılığına yasak olan yerler (Anonim, 2020)

Figure 6. The areas where the purse seine fishery with light is prohibited in the Aegean Sea (Anonim, 2020)

Gırgır ışık yasakları

Gırgırda ilk ışık yasağı 1980 yılında toplam 8 bin mum'un üzerinde ışık veren elektrojen grupları ile avcılığın yasaklanması olmuştur. Daha sonra 1990 yılında takımların kullandığı ışık takatı, ana tekne, yardımcı ve taşıyıcı tekneler dahil toplam 8000 mum'u geçemez; 1991-1999 yıllarında takımda sadece bir teknede ışık kaynağı bulunabilir ve ışık takati, ana tekne, yardımcı ve taşıyıcı tekneler dahil toplam, akkor telli lambalarda 4000 watt'ı, florasan lambalarda 1000 watt'ı, buharlı lambalarda 500 watt'ı geçemez şeklinde uygulanmıştır. 2000-2024 yılları arasında ışık takatı, ana tekne, yardımcı ve taşıyıcı tekneler dahil toplam 8000 watt'ı geçemez ve ışık ile avcılıkta aydınlatma ancak su üstünde yapılır kuralları uygulamaya alınmıştır. Karadeniz ve Marmara Denizi'nde ışık kullanımının yasaklanması nedeniyle ışık ile avcılığın serbest olduğu alanlarda su ürünleri kaynaklarının üzerinde oluşan yoğun av baskının önüne geçmek, su ürünleri kaynaklarının etkin kullanımı ve sürdürülebilir işletiminin sağlanması amacıyla 2020 yılından itibaren ışık kaynağı olarak beyaz ışık kullanımı yasaklanmıştır. 1996-2006 arasında uygulanan ışıkla avcılıkta lamba teknelerinde ışık kaynağı olarak kullanılan jeneratörlerin gücü 12 kilowatt'dan fazla olamaz sınırlaması, sonraki tebliğlerden çıkarılmıştır. 1988 yılından itibaren iki ayrı takımın ışık tekneleri arasında en az 100 m; 1999-2024 yılları arasında 200 m mesafe ve lamba teknelerinde personel bulundurulması zorunludur ile 2004 yılından itibaren 30 m'den daha sığ sularda ışık yakılması düzenlemeleri uygulamaya alınmıştır.

Gırgır ağ yasakları

İlk kez 1986-1987 yılları arasında 5 m (3 kulaçtan) daha sığ sularda gırgır ağı kullanılması; 1988-1990 yılları arasında ise 8 m'den (5 kulaç) daha sığ sularda gırgır ağı kullanılması yasaklanmıştır. 1998 yılında ise İzmir İli özelinde Rauf Paşa Dalyanı ile Kırdeniz arasındaki sahil kesiminde 9 m (5 kulaç)'den daha sığ sularda gırgır ağı kullanımı yasaklanmıştır. 1991-2003 yılları arasında mekanik güç kullanılan gırgır ağlarının 16-18 m'den (10 kulaç), mekanik güç kullanılmayan gırgır ağlarının ise 10-11 m'den (6 kulaç) daha sığ sularda kullanılması Türkiye genelinde yasaklanmıştır. 2004-2011 yılları arasında bütün karasularımızda gırgır ağları ile 10 kulaç (18 m) derinlikten daha sığ sularda avcılık yapılması, 2012 yılından itibaren Ege Denizi'nde gırgır ağları ile kıyıdan itibaren 24 metre derinlikten sığ sularda avcılık yapılması yasaklanmıştır.

1988 yılında mavi yüzgeçli orkinos (*Thunnus thynnus* Linnaeus, 1758) avcılığında kullanılan ağların dışındaki gırgır ağlarının ağ uzunluğu 400 kulaç, ağ derinliği 80 kulaçtan; 1989-1996 yılında ise bu ağlarının sadece derinliği 80 kulaçtan fazla olamaz hükmü getirilmiştir. 2006 yılından itibaren ise mavi yüzgeçli orkinos avcılığında kullanılan gırgır ağları hariç, derinliği 164 m (90 kulaç)'den daha fazla gırgır ağlarının kullanımı yasaklanmıştır. Ancak gırgır ağların uzunluğu ile uygulamada olan bir sınırlama mevcut değildir.

Gırgır ile avlanan pelajik türlere ilişkin yasaklar

Tarım ve Orman Bakanlığının 1973 yılından 2020 yılına kadar yayınladığı tüm sirküler ve tebliğlerde, Ege Denizi'nde gırgır avcılığı ve hedef türlerine ilişkin bazı düzenlemeleri mevcuttur. 1 Nisan 1973-1 Mart 1974 av sezonuna ait sirkülerde 1 Haziran ile 15 Ağustos 1973 tarihleri arasında Ege'nin pek bilinmeyen pelajik balık türlerinden biri olan ve yerelde papalina olarak isimlendirilen çaça balığı için dönem yasağı getirilmiştir. Bu uygulama çok büyük olasılıkla yaz döneminde Kuzey Ege kıyılarında kıyı sürütme ağlarından ığrıp ve trata ile yakalanan, geleneksel olarak tüketilen papalina avcılığını önlemeye yönelik bir uygulama olabilir. Bu uygulama 4 yıl sürekli uygulandıktan sonra 1 Nisan 1977-31 Mart 1978 av sezonunda, sardalya dönem yasağı, 1 Mayıs-1 Kasım 1977 arası olarak uygulanmış ve sardalyaya ilk kez 10 cm çatal boy ilk yakalama boyu yasağı getirilmiştir. Bu boy yasağı daha sonra kaldırılmış olup, 1 Eylül 2012'den itibaren günümüze kadar 11 cm total boy olarak uygulanmaya başlamıştır. 1 Nisan 1977-31 Mart 1978 av sezonunda uskumruya da 1 Mart-1 Ağustos 1977 tarihleri arasında ilk kez dönem yasağı ile 31 Mart 1981 av sezonu sonuna kadar uygulanacak 22 cm'lik çatal boy ilk yakalama boy yasağı getirilmiştir. 1 Nisan 1981-31 Mart 1982 av sezonunda uskumru ve kolyoz türlerinin ilk yakalama boyları 20 cm çatal boy şeklinde değiştirilmiş, 1 Nisan 1987-31 Mart 1988 av sezonunda ise kolyozun ilk yakalama boyu 18 cm çatal boya düşürülmüştür. 1 Nisan 1990-31 Mart 1991 av sezonundan itibaren geçerli olmak üzere günümüze kadar uskumruya 20 cm, kolyoza da ise 18 cm total boy ilk yakalama boy yasağı getirilmiş ve uygulanmıştır.

1 Nisan 1991-31 Mart 1992 av sezonundan itibaren Ege'de ilk kez gırgır dönem yasağının 15 Nisan-1 Eylül 1991 tarihleri arasında uygulanmaya başladığı ve günümüze kadar devam ettiğini görmekteyiz. Ancak bazı dönemler bu yasağının 15 Nisan ile 15 Mayıs arasında başladığı, 25 Ağustos ile 1 Eylül arasında sonlandığı şeklinde değişik uygulamalar da vardır. Son olarak 1 Eylül 2006 av sezonundan itibaren günümüze kadar gırgır dönem yasağı 15 Nisan-31 Ağustos şeklinde Ege Denizi için uygulanmaktadır.

Türkiye, özellikle 2000'li yıllardan itibaren sorumlu balıkçılık ilkelerine uyum konusunda önemli ilerlemeler sağlamıştır (Anonim, 2018). Balıkçı filosunun sınırlandırılması, geri alım yoluyla küçültülmesi, balıkçılık yönetimi için sirküler ve tebliğlerle önemli düzenlemeler getirilmesi gibi pek çok önemli adımlar atılmıştır. Ancak kaynakların yıpranmasını önlemek için gelecekte daha fazla koruyucu tedbirlerin alınmasına ihtiyaç vardır. Üstelik bu tedbirler sadece ulusal ihtiyaçların bir gereği olmakla kalmayıp küreselleşen dünya içerisinde uluslararası zorunlulukları da beraberinde getirmektedir. Okyanuslar, denizler ve uluslararası paylaşılan stoklar, ülkelerin ortak varlıkları olarak görülmekte bu kaynaklardan faydalanmada uluslararası kurallara uyum konusu daha fazla gündeme gelmektedir. Bu nedenle sardalya başta olmak üzere tüm pelajik türlerin av kayıtları istatistiklerde eksiksiz olarak yer almalıdır.

Ege Denizi gırgır balıkçılığı özelinde güncel mevcut sürdürülebilirlik sorunlarının en başında, Ege Denizi'ne her gırgır av sezonunda özellikle Marmara başta olmak üzere birçok yerden çok sayıda gırgır teknesi (bazı dönemler 70'i geçmektedir) gelmekte ve Ege Denizi koy ve körfezleri ile özellikle açık sulardaki banklarda yoğun bir avcılık faaliyeti gerçekleştirmektedir. Hemen hemen her yıl aynı şekilde bu teknelerin tamamı Ege Denizi'nin kuzeyindeki körfez ve açığından başlayıp, o alanda balık kalmayana kadar avcılık yaptıktan sonra bir sonraki körfeze ilerlemektedir. Hoşsucu vd. (1995) Ege limanlarına kayıtlı 60 gırgır teknesinin olduğunu, ancak bu sayının diğer bölgelerden (Marmara ve Karadeniz) gelen tekneler ile 100'ü aştığı 1993-1994 av sezonu için belirtilmiştir. Gırgır teknelerdeki son teknoloji sonar ve echo-sounder gibi balık bulucu cihazlar ile ışık tekneleri sayesinde bu alanlardaki balık sürülerinin yakalanmama şansı yok gibidir. Bu sebeplerden dolayı Ege Denizi'nde gırgıra yasak olan koy ve körfezlerin, birer rezerv alanı olarak ne kadar büyük bir işlev gördüğü göz ardı edilmemelidir. Ancak bu düzenlemeler de Ege gırgır balıkçılığının sürdürülebilirliği için yeterli görülmemektedir. Bazı balıkçılara göre ya kıyılar (koy ve körfezler) ya da açıklar (banklar) gırgır balıkçılığına yasaklanmalıdır. Çünkü bu iki alandan biri pelajik türlerin devamlılığı için rezerv alanı olmak zorundadır (iki alandan biri rezerv alanı olarak korunmalıdır). 2000'li yıllardan sonra kolyoz başta olmak üzere bazı pelajik balık türlerin av miktarlarındaki düşüşün bir sebebi de açıkta çalışan gırgır teknelerinin olduğu ifade edilmektedir. Eskiden küçük boyutlu gırgır tekneleri ve ayrıca kıyı (voli) gırgırı olarak adlandırılan el gırgırları ile Ege'nin koy ve iç körfezlerinde yakalanan bu türler, artık bu koy ve körfezlere giriş yapmadan açıklarda büyük gırgır tekneleri ile yakalanmaktadır.

Ege Denizi'nde uskumru ve kolyoz av miktarları 2000'li yıllardan sonra oldukça azalmıştır. Bunun başlıca sebeplerinden biri de özellikle sezon sonu olan Mart 15-Nisan 15 döneminde stoğa yeni katılan aşırı miktarda jüvenil uskumru ve kolyozun (balıkçılar kolit demektedir) gırgır ağlarında yakalanmasıdır. Oysa Nisan ayında çok fazla miktarda yakalanan bu uskumru juvenilleri, 5,5 ay sonra başlayacak sezonun ergin bireyleri olacaktır. 2017 ve 2019 yıllarında TAGEM (Kasapoğlu vd., 2020) ve TÜBİTAK (Tosunoğlu vd., 2020) projelerinin İzmir Körfezi arazi çalışmalarında, Mart ayında stoğa yeni katılan sardalya, iri sardalya, istavrit ve uskumru genç bireylerinin gırgır ağ gözlerinden geçtiği ancak çok hızlı bir büyüme performansı gösteren uskumru genç bireylerinin, Nisan ayında gırgır ağ gözlerinden geçemediği gözlenmiştir. Özellikle sardalye ve hamsi ile birlikte hareket eden bu genç bireylerin gerek ışık teknesi altında gerekse sonarda yakalanmaları kaçınılmazdır. Bir kasa balığın (~14 kg) birkaç kg'ı bu yavru balıklardan oluşmaktadır. Yavru balık avcılığının önlenebilmesi teknik açıdan bir hayli zor görünmektedir. Bu nedenle özellikle Nisan ayında uskumru, kolyoz ve diğer türlerin yavru bireylerinin avcılığını engelleyecek balıkçılık düzenlemelerinin uygulamaya alınması pelajik türlerin sürdürülebilirliği açısından oldukça önemlidir. Burada dikkat edilmesi gereken en önemli husus, böyle bir düzenleme uygulandığında diğer bölgelerden gelen teknelerin oluşturduğu avcılık baskısı olacaktır. Gerek gırgır ve gerekse diğer tüm av araçları ile ilgili olarak bölgesel balıkçılığa geçilmesi ile yapılacak düzenlemeler, balık stokları üzerindeki baskıyı oldukça azaltabilir.

Gırgır balıkçılığında teknoloji ve eforun en üst düzeyde çıktığı günümüzde yakalanan balık miktarı, 90'lı yıllar ile benzerlik göstermektedir (Hoşsucu vd., 1995; Tosunoğlu vd., 2018, 2021). Nitekim Hoşsucu vd. (1995) Ege'de 1992 yılı için 55000 tonluk bir av miktarı tahmin edilmiştir. Bu miktarın 27000 tonu sardalya, 10000 tonu kolyoz, 5000 tonu kupez, 3500 tonu uskumru, 2500 tonu hamsi ve 7000 tonu da diğer türlerdir. Teknoloji ve çabanın en üst düzeyde olduğu bu dönemde, sürdürülebilirlik kurallarına uygun hareket edilmediği takdirde Ege Denizi pelajik balık stoklarında çöküş kaçınılmazdır. Nitekim 2020-2021 Ege Denizi gırgır av sezonu, bu yönde sinyaller vermiştir (Kişisel görüşme, Ege Bölgesi Gırgır Balıkçıları Derneği).

Başta aşırı avcılık baskısı ile Karadeniz ve Marmara'daki bazı ticari türlerin yok oluşu (Ulman vd., 2020) ya da stoklarındaki azalmalar (Demirel vd., 2020) benzer şekilde Ege Denizi'nde de gerçekleştiği değerlendirilmektedir. Çünkü Ege Denizi koy ve körfezlerinde son yıllarda gırgır av sezonunun sonlarına doğru hamsiden başka avlanılacak balık türüne neredeyse rastlanılmamaktadır (Tosunoğlu vd., 2020). Bu sebepten dolayı artık çoğu tekne sezon sonunu görmeden, yaklaşık bir ay öncesinden sezonu kapatmaktadır. Diğer tekneler ise sezon sonuna doğru önceki aylara göre balık çeşitliliği ve miktarındaki düşüş ve Mart ayında ışığa balık toplanmamasından dolayı, gündüz sonar ile balık takibine ve düşük miktardaki sürülere en az 2-3 kez ağ atmaktadır. Artık gırgır balıkçıları arasındaki "*çok balık yakalayarak çok para kazanma*" düşüncesi, yerini "*az balık yakalayıp, sürekli ve iyi fiyata değerlendirme*" ye dönüştürmelidir. Çünkü balık stoklarındaki azalma ve bu stoklardan avlanan tekne sayısındaki artış bu uygulamayı zorunlu kılmaktadır. Nitekim 2020-2021 sezonunda Ege gırgır pelajik balık türlerinin yakalanma miktarlarındaki düşüşler, bu durumu yaşatmıştır. Bunun yanında 2020-2021 av sezonundan itibaren Marmara'da ışıkla balık avcılığının tamamen yasaklanması, bu teknelerin Ege'ye gelişlerini daha da arttırmalarına neden olarak stoklardan yaralanma miktarını daha da azaltacaktır.

Ege Denizi koy ve körfezlerine dışardan büyük gruplar halinde gelen tekneler, balık stokları üzerinde aşırı avcılığa neden olmaktadır. Gırgır tekneleri için bölgesel balıkçılık uygulaması, kimi dönemlerde bazı yerlerde aşırı avcılığa neden olan yığılmaları engelleyebilir. Bölgesel balıkçılık ile getirilecek kota uygulamaları da yine bu stoklar üzerindeki baskıyı azaltabileceği düşünülmektedir. Bu husus birçok ortamda Ege gırgır balıkçıları tarafından talep edilse de kurallarının nasıl olacağına ilişkin ortaya konulmuş somut bir öneri yoktur.

Sonuç olarak yukarda bahsedilen konuların uygulamaya alınması ile Ege Denizi'nde hem pelajik balık stoklarının hem de Ege Denizinde avcılık yapan gırgır balıkçılarının sürdürülebilirliğine katkı sağlanacağı düşünülmektedir.

TEŞEKKÜR

Değerli görüş ve katkılarını aldığımız Tarım ve Orman Bakanlığı, Balıkçılık ve Su Ürünleri Genel Müdürlüğü eski çalışanlarından sayın Hamdi Arpa'ya ve ayrıca Ege Bölgesi Gırgır Balıkçıları Derneği yöneticileri ile gırgır reisi Süleyman Canbaz nezdinde tüm Ege'li gırgır balıkçılarına teşekkürü borç biliriz. Bu çalışma, hiçbir kurumun görüşlerini yansıtmayıp sadece yazarların düşüncelerini ifade eder.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 427-435 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.04

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The impacts of ultrasound-assisted protein hydrolysate coating on the quality parameters and shelf life of smoked bonito fillets stored at 4±1ºC

Ultrason destekli protein hidrolizatı kaplamanın tütsülenerek 4±1ºC'de depolanan palamut filetolarının kalite parametreleri ve raf ömrü üzerine etkileri

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Received date: 25.05.2021 Accepted date: 30.08.2021

How to cite this paper:
Balçık Mısır, G. & Koral, S. (2021). The impacts of ultrasound-assisted protein hydrolysate coating on the quality parameters and shelf life of smoked bonito fillets stored at 4±1ºC. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 427-435. DOI: 10.12714/egejfas.38.4.04

Abstract: Innovative bioactive protein hydrolysates (PH), produced from Rainbow trout (*Oncorhynchus mykiss* W., 1792) by-products, were used as a coating on smoked fillets of bonito (*Sarda sarda*) for extension of the quality and shelf life during chilled storage for 60 days. Three fillet groups were prepared as control group without coating (CG), normal hydrolysate coated group (NHCG), and ultrasound-assisted hydrolysate coated group (UHCG). Total volatile basic nitrogen (TVB-N) and TBA values exceeded the acceptable limits on the 39th and 33rd days for CG respectively; 60th day for NHCG and UHCG. However TBA of UHCG was significantly lower than NHCG. Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrophilic bacteria (TAPB) loads reached 6.32±0.06 and 7.30±0.12 log cfu/g for CG on the 39th day. TAMB and TAPB values of NHCG and UHCG have remained within the limits of consumable until the end of the storage. PH coatings prolonged the storage of fillets by delaying chemical, physical, and microbial degradation. Results showed that ultrasound-assisted PH could be used as a coating for smoked bonito at refrigerated conditions, delaying oxidation and microbial degradation.

Keywords: Bioactive coatings, protein hydrolysate, smoked fish fillet, ultrasound, food preservation

Öz: Gökkuşağı alabalığı (*Onchorhynchus mykiss* W., 1792) yan ürünlerinden üretilmiş yenilikçi biyoaktif protein hidrolizatı (PH), tütsülenerek 60 gün boyunca soğuk depolanan palamut filetolarının kalite ve raf ömrünü uzatmak amacıyla kaplama olarak kullanılmıştır. Kaplamasız kontrol grubu (CG), normal hidrolizat kaplı grup (NHCG) ve ultrason destekli hidrolizat kaplı grup (UHCG) olarak üç fileto grubu hazırlanmıştır. CG için toplam uçucu bazik nitrojen (TVB-N) ve TBA değerleri, kabul edilebilirlik sınırlarını sırasıyla 39. ve 33. günde; NHCG ve UHCG için ise 60. günde aşmıştır. Ancak UHCG'un TBA değeri NHCG'dan önemli ölçüde daha düşüktür. CG'na ait toplam aerobik mezofilik bakteri (TAMB) ve toplam aerobik psikrofilik bakteri (TAPB) yükleri 39. günde sırasıyla 6,32 ± 0,06 ve 7,30 ± 0,12 log kob/g'a ulaşmıştır. Bu değerler NHCG ve UHCG için depolamanın sonuna kadar tüketilebilirlik sınırları içinde kalmıştır. PH kaplamalar kimyasal, fiziksel ve mikrobiyal bozunmayı geciktirerek filetoların depolama süresini uzatmıştır. Sonuçlar ultrason destekli PH'nın, oksidasyon ve mikrobiyal bozunmayı geciktirerek, soğuk koşullarda depolanan tütsülenmiş palamut için kaplama olarak kullanılabileceğini göstermiştir.

Anahtar kelimeler: Biyoaktif kaplama, protein hidrolizatı, tütsülenmiş balık filetosu, ultrason, gıda muhafazası

INTRODUCTION

FAO authority stated, "Food loss and waste is a big challenge of our time". Food losses and waste occurs at all stages from farm to table including harvesting, transferring, processing, and storage. With the reduction of all these losses and wastes, productivity increases, more food can be supplied to people, and reduced economic and environmental problems such as energy and water are wasted (FAO, 2020).

Fish is an extremely perishable food, this spoilage occurs at every stage of the process from harvest to consumption, and rapid quality losses are experienced. This deterioration occurs not only how the fish is treated but by the biological reactions that occur in its structure and the metabolic activities of the bacteria (Lougovois and Kyrana, 2005). Also, high rate of unsaturated fatty acids is one of the leading causes of degradation by oxidation. As a result, loss of nutritional quality properties such as safety, color, flavor, and texture and shortening the shelf life of the fish (Sone *et al*., 2019). As a result, it needs particular care to be taken from harvesting and all necessary steps to preserve quality nutritional properties and decrease losses and waste.

A large part of the fish produced in the world is processed and presented for consumption. The smoking process not only prolongs the shelf life but also helps to improve the organoleptic characteristics of fish. It has been reported that the combined effects of the salt in the brine, the high temperature and the antibacterial and antioxidant compounds in the sawdust composition are responsible for the protection compared to non-smoked products. Drying the fish surface by

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the effect of the process also decreases the water activity (aw), resulting in increased inhibition of bacterial growth (Lerfall and Hoel, 2020). Although the above advantages, smoked fish are among semi-preserved products and always carry a risk for quality assurance (Kramarenko *et al*., 2016). To meet the consumer's demand for quality and prevent economic losses, the producers try to find innovative methods and new applications to produce safe-to-eat products with high quality and extended shelf life.

In recent years, the use of certain natural molecules such as tocopherols, ascorbic acid, plant extracts in the storage of such products has great potential. Edible coatings have been tried with an overall goal of improving safety, quality, and extend shelf life. The application of coatings achieves this with the corresponding functionality through the surface of the product. There is an increasing trend as artificial materials changing towards natural-based and eco-friendly alternatives on coating materials. PH's from marine sources has been reported to possess strong protection properties for the quality of fish (Hajfathalian *et al*., 2020). Especially protein hydrolysates produced from a by-product of aquatic organisms allow utilizing the lost and highly protein-rich materials as natural sources. The application of innovative technologies such as high hydrostatic pressure, irradiation, ultrasound, filtration, plasma technology, pulsed electric field, gamma irradiation, supercritical fluid extrusion, etc., can modify the protein structure and functional properties. Among these technologies, ultrasound technology modifies the enzymatic hydrolysis and could increase extraction yields and enhance the hydrolysate properties. Ultrasound is a novel technology is a safe, non-toxic, eco-friendly and easy to use. It can be divided into two categories; high frequency, low energy diagnostic ultrasound, low frequency, high energy power ultrasound, and have shown significant improvements in the food industry in the analysis and the modification of food products. Significant changes occur in physicochemical characteristics of food under the power ultrasonic frequency (between 16 kHz to 100 kHz). Ultrasonic waves cause gas dissolution and cavitation that result in physical, mechanical, or chemical, biochemical effects on modifying the product properties compared to conventional methods (Zink *et al*., 2016). One application is extracting on the recovery of protein and bioactive compounds (Wen *et al*., 2018). It could significantly improve the enzymatic hydrolysis and properties of proteins such as potential free radical-scavenging activities and capability of lipid oxidation-reduction (Balcik Misir and Koral, 2019a). But the ultrasound treatments still need new research to find out the ultrasound technology's effectiveness on food properties. Several studies have been achieved in the production of ultrasound-assisted PH from plant and animal origin raw materials and determining characteristics of the final products such as biochemical composition, molecular structure, amino acid composition, functional properties, antioxidant and antimicrobial activities. But very limited reported researches exist in investigating the effects of ultrasound-assisted hydrolysates coatings on shelf life and quality parameters of smoked fish.

Atlantic bonito (*Sarda sarda* Bloch, 1793), a fusiform fish species belonging to the Scombroid family, has a high market value globally and Turkey. It is a good source of food with high protein and fat contents (average 20.16g/100g and 8.4%, respectively) including reasonable amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in a range of 252.0–1169.1 mg/100 g and 712.1–3324.1 g/100 g, respectively, (Balçik Misir *et al*., 2014). But high rate of unsaturated fatty acids causes deterioration and degradation by oxidation of bonito flesh.

In this study, smoked bonito fillets were coated with PH produced from rainbow trout by-products (not include viscera) enzymatically normal and ultrasound-assisted. PH was produced and characterized biochemically, functionally, and structurally in a previous study (Balcik Misir and Koral, 2019a). Coatings were applied by the dipping method and coated fillets were stored at 4±1ºC. To determine the impact of coatings on fillet's quality and shelf life, physicochemical properties and bacterial counts of coated fillets were analyzed during 60 days.

MATERIAL AND METHODS

Fish protein hydrolysates; Normal hydrolysate (NH) was produced from rainbow trout by products and characterized previously. The production was conducted briefly by using Alcalase 2.4 L with enzyme/substrate ratio of 0.5%, pH 8, for 1 h at 60°C. The same conditions were used for ultrasound-assisted PH production. A probe-type ultrasound equipment (Sonics Vibra cell, USA, tapered micro tip, 142 x 6 mm) with 40% ultrasonic amplitude, pulse duration of 10 s on- time; 20 s off-time was applied during the production. NH contains 86.40% protein, 0.05% lipid, 1.36% moisture, and 6.25% ash, with the degree of hydrolysis 25.29 %. Ultrasound-assisted hydrolysate (UH) contains 86.75% protein 0.05% lipid, 2.10% moisture, and 5.95% ash with the degree of hydrolysis 23.67%. Copper (II) ion-reducing antioxidant capacities (CUPRAC) were 230.23 µM Trolox®/mg mixture and 244.89 µM Trolox®/mg mixture for NH and UH, in order. Iron (III) ion-reducing antioxidant power (FRAP) antioxidant activities were 12.161 µM FeSO4.7H2O/mg mixture and 13.75µM FeSO4.7H2O/mg mixturefor NH and UH, respectively (Balcik Misir and Koral, 2019a). Food-grade glycerol; (99.96% purity W252506, Sigma-Aldrich) at 20% (w/w, based on biopolymer content) was purchased from Sigma Aldrich (Steinheim, Germany) and used as a plasticizer. All other chemicals, solvents used in this work were of analytical grade.

Fish, thirty individuals of fresh bonito weighing 15.25 kg, were purchased from a local fish market in Trabzon, Turkey.

The bonitos were immediately stored in ice in polystyrene boxes and delivered to the laboratory. The average weight and length of the whole fish were 503.75±45.67g and 36.20±0.91 cm, respectively.

Smoking material; beechwood sawdust (purchased from a local supplier).

Smoking, coating, and storage of bonito fillets in the cold (+4±1°C) storage conditions; Bonitos were eviscerated, filleted, and washed, then drained for thirty minutes. Drained fillets were brined before smoking. Brining was carried out by immersing. The fillets were immersed in 10% sodium chloride (NaCl) solution for 1 h at 2°C for brining. After that, fillets were washed and drained again, then placed on the grills of the smoking kiln.

Smoking kiln specifications; A conventional smoking kiln (TERMAL Laboratuvar Aletleri, Istanbul, Turkey) made of stainless chrome with dimensions 120 x 80 x 80 cm (height x width x depth). On-board electronic thermostat (adjustable between 0-260°C) and humidity meter (0-100%) were available. For the heating of the kiln, A hidden resistance (220 V) was placed between two floors for heating. A separate oven was used to produce smoke and mouthed to the oven with a 13 cm diameter chrome pipe.

Smoking process conditions; The hot smoking process was performed according to Koral (2006). Firstly the fillets on the smoking kiln grills were dried in the smoking kiln for 30 min, at 30ºC and then the kiln temperature was increased to 60ºC and the process was continued at this temperature for 45 min. After that the temperature was allowed to reach 90ºC, and the fillets were kept at this temperature for another 30 minutes. Then, the smoking process was completed, and the smoked fillets were allowed to cool at room temperature (22±2°C).

Preparation and application of the coatings materials; NH and UH were dissolved in distilled water (10% w/w), and glycerol was added (2:1 protein/glycerol) as plasticizer (pH was adjusted to 7 with HCl or NaOH). Solutions were stirred slowly in a water bath (Daihan, Digital Precise Water Bath, WB, 06, Philippines) for 15 min at 60°C (Rodriguez-Turienzo *et al*., 2011). The coating materials were applied on one side of the fish with a silicone brush and left to stand for 5 minutes. After that, the same process was repeated for the other side. CG was prepared as uncoated. The fillets were placed in styrofoam plates individually, covered with stretch film for each day of analysis. Physical, chemical, and microbiological analyses were performed on the fresh fish and, 1st, 6th, 12th, 16th, 21st, 33th, 39th, 50th, and 60th days of storage. For the 0th day analysis, samples (n=6 different fillets; n=2 for each group) were kept at -80 ºC until analysis. The rest of the fillets (n=54 different fillets; n=2 fillets for each group) was stored at 4±1 ºC for analysis. Before each analysis, the film was taken off from the plates, and the fillets were homogenized with a knife by cutting into small particles.

Measurement of salt, aw, pH, and color

The salt content was determined by the Mohr method. According to the procedure, the aliquot was titrated with 0.1 N silver nitrate, and the amount of salt was calculated (Rohani *et al*., 2010). a_w was measured with Aqualab 3TE (0.100-1.000±0.003, Aqualab, Decagon Devices, Pullman, Washington, USA) brand device at 25 °C (±0.2°C). pH was measured with a probe-type pH meter (Mettler-Toledo AG, Seven Compact 8603 N, Sweden) with the procedure given by Koral (2012). Konica Minolta Colorimeter (CR 10, Tokyo, Japan) was used for color measurement of the coated fillets. The instrument was standardized with the standard white plate. CIE color table was used for evaluation. Three different points of the surface of two fish fillets were taken for the measurement.

Indicators of fish degradation and resistance to fat oxidation

Deterioration and fat oxidative stability of fillets during storage were assessed in TVB-N, TMA, and TBA analysis. The method described by Lücke and Geidel (1935) was used for TVB-N analysis. TBA analysis was carried out with the method used by Tarladgis *et al.* (1960). TMA analysis was done according to Boland and Paige (1971). TVB-N, TBA, and TMA analyses were performed as described previously by Balcik Misir and Koral (2019b). All experiments were performed in triplicate.

Microbiological analysis

The surface plate method was used for enumeration. All counts were expressed as log CFU/g and performed in triplicate.

Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrophilic bacteria (TAPB) counts

Standard plate count agar (PCA, Cat No. 105463, Merck, Darmstadt, Germany) was used for TAMB and TAPB. The methods determined by Bell *et al.* (2005) were applied for TAMB and TAPB analysis.

Yeast, mold, and coliform count

Potato dextrose agar (Cat. No: 1.10130.500, Merck, Darmstadt, Germany) was used for yeast and mold count, and violet bile agar (Cat. No: 1.01406.0500, Merck, Darmstadt, Germany) was used for the coliform count. The procedure was followed, explained by Bell *et al*., (2005).

All experiments were performed in triplicate.

Data analysis

All data analyses were done in triplicates, and means were compared statistically with Analysis of variance (one-way ANOVA), in case of significant differences, Tukey and

Mann Whitney U test (data not provided in the normality of assumptions) were applied with the program named "JMP 5.0.1" (SAS Institute. Inc. USA) and SPSS 18.0 (SPSS Inc., Chicago, IL) (Sokal and Rohlf, 1987). A significance level of 95% ($P<0.05$) was used throughout the analysis.

RESULTS AND DISCUSSION

Salt, a_w, pH and color

Salt contents of smoked, and coated fillets were analyzed and calculated as 6.45%, 7.60% and 7.88%, for CG, NHCG and UHCG, respectively. Codex Alimentarius (2013) stated that the salt concentration in the aqueous solution should be at least 5% to ensure completely effective protection at temperatures from 3 to 10 ºC. As it can be seen in Table 1, the initial a_w of the fresh bonito fillet was 0.993. A similar a_w value (0.994) was measured by Koral and Köse (2018), for the same species. In the current study, fluctuations have occurred in all groups' a_w values during storage. It was measured above the limiting level of this parameter to ensure microbial stability because it is generally accepted that no microbial growth will occur at $a_w< 0.66$ (*Castañeda-López et al.*, 2021). Decreasing of a_w value was assumed as a result of the application of salting and drying processes due to the nature of smoking technology. It has been determined that salt substitution and drying process decreases the a_w of foods (Pedro and Nunes, 2019). The acidity level of the fillets was determined by the pH measurement. The initial pH of the smoked fillet was 6.21±0.03. It was decreased in each group on the first day, and the differences between CG and NHCG and UHCG were not significant ($p<0.05$). It fluctuated for each group, but the general trends were reducing during the storage. No significant differences were observed between NHCG and UHCG except the 6th day of storage ($p>0.05$) (Table 1). This similarity can be attributed to the pH values of the two coatings that ultrasound treatment hadn't affected the pH of the PH (Balcik Misir and Koral 2019a). Several researchers have explained that the pH of smoked fish can be affected by some factors such as used fish species, smoking material, salting process, smoking procedure, and temperature of the process (Muñoz, et al., 2020; Jimenez *et al.*, 2020). The decreasing of the pH during storage was attributed to the salt content which has been said to have a highly significant linear decreasing effect on the pH, which was explained by the increase of the ionic strength of the solution inside of the cells (Leroi and Joffraud, 2000). This is confirmed by the present results. Color is one of the most important elements that influence consumer acceptance of food products. Coating materials affected the color of smoked fillets (Table 1); CG differed significantly ($p<0.05$) from coated fillets. Decreasing values have been experienced during the storage in this group. The color of UHCG was brighter and preserved the original color of fillets longer than CG and NHCG. L* value, which represents the lightness and varies from 0 (black) to 100 (white), was measured as 45.08 for fresh bonito fillets and increased in all groups after smoking. However, NHCG and UHCG have higher L* values than CG. L* values of CH have decreased during storage and measured as 41.03 on the 39th day of storage, where it sensibly fell below the consumable limit. L * values of NHCG and UHCG increased on the 1st day of storage and then decreased in the following days. Decreased values of L* demonstrated that fillets have become pale and darker. However, the barrier formed by the coating materials against environmental oxygen, other gases and moisture, made NHCG and UHCG more attractive than CG. Karnjanapratuma and Benjakul (2020) had determined the impact of the ultrasound-assisted process (UAP) on the yield and characteristics of Asian bullfrog skin gelatin. In their research, ultrasonication caused increased L* value, representing the lighter color of the gelatin. a* value of the CG had increased during the storage and reached the maximum value on the 39th day (16.68). Although a* values of NHCG and UHCG showed an increasing trend during the storage, they were lower than CG. Increasing a* value means increasing the redness in the color of fillets. The previous researchers also had similar results in their studies that mean generally smoking effect increase the redness of the fish fillets (Lerfall, and Hoel, 2021; Valø *et al.*, 2020). Having statistically different L*, a*, and b* values from other groups, UHCG has prevented the color of fillets more effectively. The structural changes produced by ultrasonication during protein hydrolysate production might have influenced the color parameters.

Table 1. Changes in a_w, pH and color (L*, a*, b*) parameters of smoked bonito fish preserved in cold storage (4 ± 1ºC)

Days	Sample	a_w	pH	L*	a*	b*
0	**Fresh bonito**	0.993±0.001	6.21±0.03	45.08±0.32	11.09±0.28	21.10±0.23
	CG	$0.980±0.001^{a}_{A}$	$5.43±0.03^{a}_{A}$	$45.10±0.22^{a}_{A}$	$13.40±0.08^{a}_{A}$	$24.75±0.13^{a}_{A}$
1	**NHCG**	$0.977±0.000^{a}_{A}$	$5.45±0.01^{a}_{A}$	$45.93±0.21^{b}_{A}$	$12.23±0.05^{b}_{A}$	$23.68±0.16^{b}_{A}$
	UHCG	$0.977±0.002^{a}_{A}$	$5.39±0.04^{a}_{A}$	$46.28±0.13^{b}_{A}$	$12.28±0.22^{b}_{A}$	$23.50±0.08^{b}_{A}$
	CG	$0.975±0.001^{a}_{B}$	$5.31±0.02^{a}_{B}$	$42.93±0.13^{a}_{B}$	$14.23±0.15^{a}_{B}$	$24.48±0.14^{a}_{A}$
6	**NHCG**	$0.977±0.001^{a}_{A}$	$5.64±0.01^{b}_{B}$	$46.15±0.24^{b}_{A}$	$13.90±0.14^{b}_{B}$	$23.18±0.18^{b}_{B}$
	UHCG	$0.972±0.001^{b}_{B}$	$5.69±0.01^{c}_{B}$	$47.15±0.10^{c}_{B}$	$12.73±0.10^{b}_{B}$	$22.20±0.12^{b}_{B}$
	CG	$0.977±0.001^{a}_{C}$	$5.42±0.01^{a}_{A}$	$42.97±0.25^{a}_{B}$	$14.87±0.15^{a}_{C}$	$24.07±0.06^{a}_{B}$
12	**NHCG**	$0.979±0.001^{a}_{A}$	$5.53±0.01^{b}_{C}$	$45.23±0.06^{b}_{B}$	$13.83±0.06^{b}_{B}$	$23.02±0.15^{b}_{B}$
	UHCG	$0.974±0.001^{b}_{A}$	$5.51±0.06^{b}_{C}$	$47.10±0.06^{a}_{B}$	$13.20±0.17^{c}_{C}$	$21.47±0.12^{c}_{C}$

Table 1. Continued

16	**CG**	$0.975\pm0.001^{a}_{B}$	$5.26\pm0.01^{a}_{C}$	$42.23\pm0.15^{a}_{C}$	$15.20\pm0.10^{a}_{D}$	$23.83\pm0.29^{a}_{C}$
	NHCG	$0.974\pm0.002^{b}_{A}$	$5.39\pm0.03^{b}_{D}$	$44.53\pm0.29^{b}_{C}$	$13.30\pm0.34^{b}_{C}$	$22.47\pm0.06^{b}_{C}$
	UHCG	$0.969\pm0.000^{c}_{C}$	$5.35\pm0.02^{b}_{A}$	$45.27\pm0.23^{c}_{C}$	$12.87\pm0.42^{b}_{B}$	$21.23\pm0.15^{c}_{C}$
21	**CG**	$0.976\pm0.001^{a}_{BC}$	$4.91\pm0.04^{a}_{D}$	$41.65\pm0.17^{a}_{D}$	$15.48\pm0.18^{c}_{E}$	$23.45\pm0.08^{a}_{D}$
	NHCG	$0.966\pm0.001^{b}_{B}$	$4.76\pm0.06^{b}_{E}$	$44.28\pm0.08^{b}_{D}$	$14.00\pm0.14^{b}_{B}$	$22.95\pm0.06^{b}_{B}$
	UHCG	$0.966\pm0.001^{b}_{D}$	$4.80\pm0.02^{b}_{D}$	$45.15\pm0.06^{c}_{C}$	$13.45\pm0.06^{a}_{C}$	$22.63\pm0.16^{c}_{D}$
33	**CG**	$0.976\pm0.001^{a}_{BC}$	$4.95\pm0.01^{a}_{D}$	$41.28\pm0.10^{a}_{E}$	$16.53\pm0.10^{a}_{F}$	$23.65\pm0.31^{a}_{E}$
	NHCG	$0.972\pm0.001^{b}_{A}$	$5.09\pm0.03^{b}_{F}$	$44.18\pm0.19^{b}_{E}$	$14.30\pm0.08^{b}_{D}$	$22.35\pm0.10^{b}_{C}$
	UHCG	$0.973\pm0.002^{b}_{AB}$	$5.06\pm0.02^{b}_{E}$	$44.88\pm0.13^{c}_{D}$	$14.25\pm0.06^{b}_{D}$	$22.02\pm0.19^{c}_{B}$
39	**CG**	$0.972\pm0.001^{a}_{D}$	$4.97\pm0.02^{a}_{D}$	$41.03\pm0.11^{a}_{F}$	$16.68\pm0.19^{a}_{F}$	$23.88\pm0.13^{a}_{C}$
	NHCG	$0.973\pm0.001^{a}_{A}$	$4.83\pm0.04^{b}_{E}$	$43.63\pm0.29^{b}_{F}$	$14.73\pm0.13^{b}_{E}$	$22.10\pm0.14^{b}_{D}$
	UHCG	$0.974\pm0.001^{a}_{A}$	$4.85\pm0.02^{b}_{D}$	$44.45\pm0.18^{c}_{E}$	$14.45\pm0.13^{b}_{E}$	$22.75\pm0.15^{c}_{D}$
50	**CG**	*	*	*	*	*
	NHCG	$0.973\pm0.001^{a}_{A}$	$4.88\pm0.03^{a}_{E}$	$43.17\pm0.13^{a}_{F}$	$15.23\pm0.06^{a}_{F}$	$21.63\pm0.06^{a}_{E}$
	UHCG	$0.971\pm0.001^{a}_{A}$	$4.87\pm0.02^{a}_{D}$	$44.00\pm0.10^{b}_{F}$	$15.07\pm0.15^{b}_{F}$	$21.13\pm0.12^{b}_{E}$
60	**CG**	*	*	*	*	*
	NHCG	$0.968\pm0.001^{a}_{B}$	$4.92\pm0.03^{a}_{E}$	$43.02\pm0.14^{a}_{F}$	$15.58\pm0.14^{a}_{G}$	$21.78\pm0.13^{a}_{E}$
	UHCG	$0.970\pm0.001^{a}_{A}$	$4.91\pm0.02^{a}_{D}$	$43.88\pm0.13^{b}_{G}$	$15.30\pm0.10^{b}_{G}$	$21.34\pm0.16^{b}_{E}$

Different lowercase letters (a, b, c) in the same column indicate the difference between the groups on the same day (P <0.05). Different capital letters in the same column (A, B, C, D) indicate the difference in the same group on different days (P <0.05). CG: Control. NHCG: Normal hydrolysate coated group. UHCG: Ultrasound-assisted hydrolysate coated group, *: Not analyzed

Indicators of fish degradation and resistance to fat oxidation

The degradation of protein and non-nitrogen components by factors such as microbiological activities of bacteria, enzymatic autolysis, lipid, and oxidation of proteins leads to the accumulation of organic amines, generally known as TVB-N. With the storage period, the TVB-N value increases depending on this accumulation. TVB-N contents are toxic and cause color and taste changes in the texture, thus affecting the acceptability in consumption (Cao *et al.*, 2019). Changes in TVB-N, TBA, and TMA values of smoked bonito fillets that were coated and stored at +4 °C, are shown in Table 2.

TVB-N of fresh bonito fillet was determined as 15.56 ± 0.50 mg /100 g. This value has increased statistically in all groups starting from the first day of the storage (P <0.05). Increases were significantly higher in CG than NHCG and UHCG (P <0.05). It exceeded the acceptability limit by reaching 41.88 ± 0.35 mg / 100 g for CG on the 39th day of storage, 43.12 ± 0.35 mg / 100g and 42.68 ± 0.30 mg / 100g for the NHCG and UHCG, on the 60th day of the storage, respectively. In addition to improved protective effects supplied by the combination of low moisture content by the effect of drying, salt content, and sawdust active ingredients, it can be said that coating materials could delay microbial deterioration and oxidation and made NHCG and UHCG's shelf lives longer than CG. The differences in TVB-N values of NHCG and UHCG were not statistically significant (P>0.05). Yu *et al.*, (2021) determined the effects of ultrasound-assisted chitooligosaccharide (COS-UA) and chitooligosaccharide (COS) coating on grass carp fillets during cold storage. Researchers stated that TVB-N values of the fillets increased slowly up to the 3rd day for each group, but this increase was not statistically important. TVB-N values of COS and COS-UA were lower than the control group (about 17-37 %). TVB-N value of COS-UA was found lower than COS on the 6th day. The control group's TVB-N value reached the minimum limit (11.39 mg/100 g) on the 6th day, but this limit was exceeded by COS and COS-UA on the 9th and 12th days, respectively. This was explained by the effect of ultrasound application that could help to inhibit the deterioration. El-Obeid *et al*., (2018) observed the shelf-life of smoked eel fillets treated with chitosan and thyme oil using vacuum packaging (VP) at 4 °C. They prepared smoked fillet groups as S (control, smoked, no antimicrobials added), ST with added thyme EO, SC with added chitosan, and SCT with added chitosan and thyme EO. They reported increasing trends in TVB-N content of all groups of smoked ell fillets had during the storage. They calculated the TVB-N values of S, ST, SC and, SCT as 31.5 mg N/100 g and 18.1, 14.9 and 13.1 mg N/100 g, respectively, on final days 35 and 42, 49 of storage. These previous studies' results had shown similar trends with the present study that ultrasound-assisted and/or normal treatments of natural coatings extended the shelf life of fish fillets during cold storage by delaying the deterioration. In the present research preventive effect of coating materials with their antioxidant activities (determined in our previous research paper (Balcik Misir and Koral 2019a) resulted in a lower increase in the TBA values of NHCG and UHCG than

CG. The TBA values of CG exceeded the consumable limit on the 33rd day of storage whereas, in the NHCG and UHCG, this limit was exceeded on the 60th day. As seen in Table 2, the TBA of UHCG was significantly lower than NHCG ($p<0.05$). Higher antioxidant activity of UHCG could be effective on this result (Balcik Misir and Koral, 2019a). Noman *et al*., (2020) reported, "US (ultrasound) technique can play a promising role in the production of protein hydrolysates and the improvement of their antioxidant properties." El-Obeid *et al*., (2018) explained the lower TBA values in the treated SC, SCT eel samples as the high antioxidant activity of chitosan and thyme EO which could reduce the level of oxidation in the eel samples.

Table 2. Changes in TVB-N, TBA and, TMA parameters of smoked bonito fillets coated with fish protein hydrolysate during storage at (4±1ºC)

Days	Sample	TVB-N (mg/100g)	TBA (mg MA/kg)	TMA (mg/100g)
0	Fresh Bonito fillets	15.56±0.50	0.46±0.06	0.48±0.06
1	CG	18.11±0.30$^{a}_{A}$	1.18±0.06$^{a}_{A}$	0.68±0.06$^{a}_{A}$
	NHCG	16.66±0.15$^{b}_{A}$	0.92±0.06$^{b}_{A}$	0.56±0.04$^{b}_{A}$
	UHCG	16.31±0.10$^{b}_{A}$	0.86±0.08$^{b}_{A}$	0.54±0.04$^{b}_{A}$
6	CG	22.28±0.15$^{a}_{B}$	2.72±0.12$^{a}_{B}$	0.89±0.05$^{a}_{B}$
	NHCG	18.14±0.17$^{b}_{B}$	1.54±0.08$^{b}_{B}$	0.66±0.06$^{b}_{A}$
	UHCG	18.68±0.15$^{b}_{B}$	1.39±0.10$^{c}_{B}$	0.60±0.04$^{b}_{A}$
12	CG	24.12±0.15$^{a}_{C}$	4.11±0.08$^{a}_{C}$	1.02±0.10$^{a}_{C}$
	NHCG	20.50±0.17$^{b}_{C}$	2.56±0.14$^{b}_{C}$	0.86±0.04$^{b}_{B}$
	UHCG	20.28±0.15$^{b}_{C}$	2.24±0.06$^{c}_{C}$	0.78±0.08$^{b}_{B}$
16	CG	27.88±0.17$^{a}_{D}$	5.76±0.14$^{a}_{D}$	1.68±0.06$^{a}_{D}$
	NHCG	23.48±0.17$^{b}_{D}$	3.48±0.10$^{b}_{D}$	1.18±0.06$^{b}_{C}$
	UHCG	23.08±0.15$^{b}_{D}$	3.25±0.08$^{c}_{D}$	1.09±0.04$^{b}_{C}$
21	CG	30.12±0.30$^{a}_{E}$	6.35±0.14$^{a}_{E}$	2.08±0.10$^{a}_{E}$
	NHCG	26.18±0.30$^{b}_{E}$	4.56±0.06$^{b}_{E}$	1.52±0.08$^{b}_{D}$
	UHCG	26.12±0.15$^{b}_{E}$	4.08±0.12$^{c}_{E}$	1.38±0.10$^{b}_{D}$
33	CG	33.88±0.17$^{a}_{F}$	7.28±0.10$^{a}_{F}$	2.72±0.06$^{a}_{F}$
	NHCG	29.26±0.10$^{b}_{F}$	5.02±0.08$^{b}_{F}$	1.82±0.10$^{b}_{E}$
	UHCG	29.18±0.15$^{b}_{F}$	4.70±0.06$^{c}_{F}$	1.66±0.08$^{b}_{E}$
39	CG	41.88±0.35$^{a}_{G}$	8.03±0.08$^{a}_{G}$	3.62±0.08$^{a}_{G}$
	NHCG	31.78±0.15$^{b}_{G}$	5.50±0.12$^{b}_{G}$	2.24±0.06$^{b}_{F}$
	UHCG	31.66±0.30$^{b}_{G}$	5.08±0.10$^{c}_{G}$	2.16±0.10$^{b}_{F}$
50	CG	*	*	*
	NHCG	34.28±0.35$^{a}_{H}$	5.98±0.10$^{a}_{H}$	2.64±0.06$^{a}_{G}$
	UHCG	33.96±0.17$^{a}_{H}$	5.38±0.12$^{b}_{H}$	2.56±0.10$^{a}_{G}$
60	CG	*	*	*
	NHCG	43.12±0.35$^{a}_{I}$	7.19±0.16$^{a}_{I}$	3.06±0.08$^{a}_{H}$
	UHCG	42.68±0.30$^{a}_{I}$	6.48±0.12$^{b}_{I}$	2.88±0.10$^{a}_{H}$

Different lowercase letters (a, b, c) in the same column indicate the difference between the groups on the same day ($P<0.05$). Different capital letters in the same column (A, B, C, D) indicate the difference in the same group on different days ($P<0.05$). CG: Control. NHCG: Normal hydrolysate coated group. UHCG: Ultrasound-assisted hydrolysate coated group, *: Not analyzed

Triethylamine oxide forms TMA by the enzymatic activity of bacteria, which is responsible for the unpleasant fishy odor (Olafsdóttir *et al.*,1997). The TMA values of all studied groups were well below the consumable limits throughout the entire store (Table 2). This situation could be due to both brining process used by the nature of the smoking process and the antioxidant and antimicrobial activities of the smoking material, and the temperature applied during the smoking. The microbial growth in the product was restricted under the influence of salt and temperature with decreasing moisture content. But lower TMA values of NHCG and UHCG than CG could be explained by coating materials used in these products. Coatings might have formed a protective layer on the surface of the fillets that prevented the passage of gasses, moisture, and microorganisms through the fillets and protected them. But similar to TVB-N, TMA values of NHCG and UHCG were not statistically different throughout the storage. There have been stated in some previous researches that edible coating materials of both vegetable and animal origins have shown antioxidant and antimicrobial effects in smoked fish species resulting in quite a long shelf life compared to control groups (El-Obeid *et al*., 2018; Martínez *et al.*, 2018).

Changes in the microbiological structure

The changes in the number of TAMB, TAPB, total coliform, and total mold and yeast in coated smoked bonito fillets are presented in Table 3. The initial TAMB, TAPB, coliform bacteria, and yeast/mold counts for fresh bonito fillets were 3.18±0.10, 2.94±0.08, <1.47, and <1.47, respectively.

The counts have decreased with the effect of the smoking process on the 1st day of storage, then started to increase. CG became inconsumable in terms of TAMB and TAPB loads on the 39th day, while in NHCG and UHCG, these loads have remained within the limits of consumable until the end of the storage period. TAPB loads of NHCG and UHCG have exceeded the consumable limits on the 60th day of storage. Although initially very low, there was an increase observed in the counts of coliform bacteria in CG on the 16th day of storage. Although the counts of yeast and mold increased in the CG on the 12th day, these counts increased on the 33rd day of storage in NHCG and UHCG. Yeast and molds are not found in normal flora in fish. The contamination can occur during catching, processing, from nets, tools, and other materials (Carrion-Granda *et al*., 2018). As seen from Table 3, similar to coliform bacteria, there was an increase occurred in the counts of yeasts and molds. Previous studies supported that protein-based edible films can extend the shelf life of the product by suppressing microbial growth (Xiong et al., 2021; Dinika *et al.*, 2020). Choulitoudi *et al.* (2017) applied plant extracts enriched with rosemary extracts as a coating material in the preservation of smoked ell. They expressed that the coating material shoved antimicrobial activity and prevented bacterial growth compared to the control group. Enriched the stability of smoked ell antimicrobial points.

Table 3. Changes in microbiological counts of smoked bonito fillets coated with fish protein hydrolysate during storage at (4±1ºC)

Days	Groups	Total Aerobic Bacteria (log CFU/g)			
		Mesophilic	Psychrophilic	Coliform Bacteria	Yeast and Mold
0	Fresh Bonito Fillets	3.18±0.10	2.94±0.08	<1.47	<1.47
1	CG	2.44±0.06$^{a}_{A}$	2.46±0.04$^{a}_{A}$	<1.47	<1.47
	NHCG	2.36±0.04$^{a}_{A}$	2.42±0.06$^{a}_{A}$	<1.47	<1.47
	UHCG	2.32±0.08$^{a}_{A}$	2.40±0.06$^{a}_{A}$	<1.47	<1.47
6	CG	2.68±0.06$^{a}_{B}$	2.78±0.04$^{a}_{B}$	<1.47	<1.47
	NHCG	2.40±0.08$^{b}_{A}$	2.48±0.08$^{b}_{A}$	<1.47	<1.47
	UHCG	2.38±0.04$^{b}_{A}$	2.40±0.10$^{b}_{A}$	<1.47	<1.47
12	CG	3.18±0.10$^{a}_{C}$	3.48±0.08$^{a}_{C}$	<1.47	1.58±0.06$_{A}$
	NHCG	2.68±0.10$^{b}_{B}$	2.90±0.12$^{b}_{B}$	<1.47	<1.47
	UHCG	2.70±0.14$^{b}_{B}$	2.86±0.10$^{b}_{B}$	<1.47	<1.47
16	CG	3.76±0.10$^{a}_{D}$	4.25±0.08$^{a}_{D}$	1.62±0.12$_{A}$	1.98±0.08$_{B}$
	NHCG	3.12±0.12$^{b}_{C}$	3.48±0.06$^{b}_{C}$	<1.47	<1.47
	UHCG	3.04±0.10$^{b}_{C}$	3.40±0.14$^{b}_{C}$	<1.47	<1.47
21	CG	4.63±0.06$^{a}_{E}$	5.18±0.06$^{a}_{E}$	1.98±0.04$_{B}$	2.38±0.10$_{D}$
	NHCG	3.72±0.10$^{b}_{D}$	4.12±0.10$^{b}_{D}$	<1.47	<1.47
	UHCG	3.60±0.08$^{b}_{D}$	4.02±0.08$^{b}_{D}$	<1.47	<1.47
33	CG	5.20±0.08$^{a}_{F}$	6.40±0.06$^{a}_{F}$	2.28±0.06$^{a}_{C}$	2.88±0.06$^{a}_{E}$
	NHCG	4.52±0.10$^{b}_{E}$	4.96±0.10$^{b}_{E}$	1.62±0.04$^{b}_{A}$	1.52±0.04$^{b}_{A}$
	UHCG	4.38±0.08$^{c}_{E}$	4.80±0.12$^{b}_{E}$	1.58±0.08$^{b}_{A}$	1.50±0.02$^{b}_{A}$
39	CG	6.3 2±0.06$^{a}_{G}$	7.30±0.12$^{a}_{D}$	2.82±0.10$^{a}_{D}$	3.26±0.12$^{a}_{F}$
	NHCG	4.90±0.08$^{b}_{F}$	5.36±0.06$^{b}_{F}$	1.88±0.08$^{b}_{B}$	1.72±0.06$^{b}_{B}$
	UHCG	4.96±0.12$^{b}_{F}$	5.24±0.10$^{b}_{F}$	1.80±0.06$^{b}_{B}$	1.70±0.08$^{b}_{B}$
50	CG	*	*	*	*
	NHCG	5.22±0.06$^{a}_{G}$	5.98±0.10$^{a}_{G}$	2.18±0.06$^{a}_{C}$	1.98±0.04$^{a}_{C}$
	UHCG	5.16±0.08$^{a}_{G}$	5.92±0.08$^{a}_{G}$	2.12±0.08$^{a}_{C}$	1.90±0.06$^{a}_{C}$
60	CG	*	*	*	*
	NHCG	5,72±0,08$^{a}_{H}$	6,82±0,10$^{a}_{H}$	2,42±0,06$^{a}_{C}$	2,22±0,06$^{a}_{D}$
	UHCG	5,60±0,10$^{a}_{H}$	6,68±0,06$^{a}_{H}$	2,36±0,04$^{a}_{C}$	2,16±0,08$^{a}_{D}$

Different lowercase letters (a, b, c) in the same column indicate the difference between the groups on the same day (P <0.05). Different capital letters in the same column (A, B, C, D) indicate the difference in the same group on different days (P <0.05). CG: Control. NHCG: Normal hydrolysate coated group. UHCG: Ultrasound-assisted hydrolysate coated group, *: Not analyzed

CONCLUSION

Normal and ultrasound-assisted protein hydrolysates were produced enzymatically. Resultant hydrolysates were used as a bioactive coating on smoked bonito fillets for preservation. Ultrasound technique could have a promising potential to get increased antioxidant/antimicrobial activities and improved physical, chemical properties for protein hydrolysates. The overall results clearly illustrated that both protein hydrolysates here-produced are a well-defined approach that could extend the shelf life of smoked fish fillets. Both coatings inhibited the microbiological growth and significant decreases obtained in the TVB-N, TBA, and TMA values. As a result, it is obvious that ultrasound-assisted protein hydrolysate produced from fish by-products may be used as a coating material with good antioxidant activity. However, ultrasonic applications in protein hydrolysate production are still in their early stages. It requires many future investigations to implement this technology on an industrial scale and explain the effect of ultrasound on the properties of protein hydrolysates.

ACKNOWLEDGEMENTS

This study is a part of the PhD thesis of Gülsüm Balçık Mısır, the author thanks to Directorate of Central Fisheries Research Institute for giving this opportunity, to perform this study during the work.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 437-447 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.05

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Size and structure of the Mediterranean medicinal leech, *Hirudo verbana* populations inhabiting wetlands around Lake Eğirdir, Turkey

Eğirdir Gölü (Türkiye) çevresindeki sulak alanlarda yaşayan Akdeniz tıbbi sülüğü, *Hirudo verbana* popülasyonlarının büyüklüğü ve yapısı

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Received date: 31.03.2021 *Accepted date: 08.09.2021*

How to cite this paper:
Ceylan, M. & Çetinkaya, O. (2021). Size and structure of the Mediterranean medicinal leech, *Hirudo verbana* populations inhabiting wetlands around Lake Eğirdir, Turkey. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 437-447. DOI: 10.12714/egejfas.38.4.05

Abstract: In the present study size and structure of the Mediterranean medicinal leech (*Hirudo verbana* Carena, 1820) populations inhabiting wetlands around Lake Eğirdir (Turkey) were investigated. Population size was estimated by removal methods, age classes were estimated for the first time in leeches (Hirudinea) using "Modal Progression Analysis" of body length frequencies using Bhattacharya's method. The population size and biomass of medicinal leeches around Lake Eğirdir were estimated to be 1,562,696 ± 805,613 leeches and 467.26 ± 172.91 kg, respectively. Three age classes were identified; the age group of 0+ was dominant with 78.6%. While 89.9% of the individuals weighed less than 1 g, whereas the broodstock was estimated to represent only 7%. Body weight, body length and condition factor were found to be 0.52 ± 1.19 g, 4.5 ± 2.4 cm and 0.620 ± 0.123, respectively. This study reveals that the population size of *H. verbana* in the wetlands around Lake Eğirdir is relatively well preserved and there is no significant threat to the sustainability of the exploitation of the medicinal leech populations. It is concluded that the stability of the lake's water budget, the variety and abundance of the host in the undamaged wetlands, the socioeconomic structure of the leech collectors, the sales policies, and the effective activities of conservation and control units might affect conservation status of the medicinal leech populations inhabiting wetlands around Lake Eğirdir.

Keywords: Removal methods, Bhattacharya's method, body length frequency, sustainable management, conservation

Öz: Bu çalışma ile Eğirdir Gölü çevresindeki sulak alanlarında yaşayan Akdeniz tıbbi sülüğü (*Hirudo verbana* Carena, 1820) popülasyonlarının büyüklüğü ve yapısının araştırılması amaçlanmıştır. Popülasyon büyüklüğü ürün azaltmaya dayalı metotlarla, yaş sınıfları ise sülüklerde (Hirudinea) ilk kez vücut boyu sınıfı frekansları kullanılarak Bhattacharya'nın "Modal Progression Analysis" metodu ile belirlenmiştir. Popülasyon büyüklüğü sırasıyla 1.562.696 ± 805.613 adet ve 467,26 ± 172,91 kg olarak belirlenmiştir. Üç farklı yaş sınıfı tespit edilmiş; 0+ yaş grubunun %78,6 ile baskın grup olduğu belirlenmiştir. 1 g'dan küçük bireylerin popülasyonun %89,9'unu temsil ettiği, buna karşın anaç sülüklerin oranının %7 olduğu tespit edilmiştir. Vücut ağırlığı, vücut boyu ve kondisyon faktörü sırasıyla 0,52 ± 1,19 g, 4,5 ± 2,4 cm ve 0,620 ± 0,123 olarak belirlenmiştir. Bu çalışma Eğirdir Gölü çevresindeki sulak alanlarda yaşayan *H. verbana* popülasyonlarının büyük oranda korunduğunu ve popülasyonların sürdürülebilir yönetimi bakımından tehdit bulunmadığını ortaya çıkarmıştır. Eğirdir Gölü çevresi sulak alanlarında yaşayan tıbbi sülük popülasyonlarının korunmasında gölün istikrarlı su bütçesinin, tahrip edilmemiş habitatlardaki konakçı çeşitliliği, sülük toplayıcılarının sosyoekonomik yapısı, sülük satış politikası ve koruma-kontrol birimlerinin faaliyetlerinin etkili olduğu düşünülmektedir.

Anahtar kelimeler: Ürün azaltma metodu, Bhattacharya metodu, vücut boyu frekansı, sürdürülebilir yönetim, koruma

INTRODUCTION

The trade of medicinal leech collected from nature is mostly based on *H. verbana* populations found in Turkish and Russian (Krasnodar Territory) wetlands (Trontelj and Utevsky, 2012; Sağlam *et al.*, 2016). Although Turkey is an important supplier of medicinal leeches, leech gathering has been done without information of their biological characteristics, reproductive behavior, and population structure (Kasparek, 1998). The intensive use of leeches in medicine, habitat destruction and over-collecting have led to a gradual decrease of medicinal leech populations in European wetlands and they have completely disappeared in some wetlands (Elliott and Tullett, 1984; Elliott and Kutschera, 2011; Sağlam, 2011; Elliott and Dobson, 2015; Ceylan and Çetinkaya, 2017). International medicinal leech trade, therefore, is conducted under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Collier *et al.*, 2016; CITES, 2021; Uğural and Serezli, 2020). Export quota was established to 10,000 kg/year in 1996 and has gradually declined for Turkey, which has about 80% of leech market of leeches collected from the wild, to 2,000 kg/year since 2014 (Sağlam 2011; Official Gazette, 2014; Official Gazette, 2020a). However, there is not enough information on the populations of *H. verbana* to form a basis for both export quota and international medicinal

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leech trade (Neubert and Nesemann, 1999; Kutschera, 2006; Kutschera and Roth, 2006; Elliott and Kutschera, 2011). Therefore, export quotas should be determined both realistically and reliably to manage the wild populations sustainably. For this purpose, sufficient and regularly updated information on the size and structure of the medicinal leech populations is required, especially in the wetlands where leech collection is conducted at the commercial level.

Because the population size is the most important information required for the conservation studies, scientific studies should be carried out especially on the exploited populations (Shaffer, 1981). The medicinal leech populations and their biological characteristics are largely neglected in scientific studies (Trontelj and Utevsky, 2005; Ceylan and Erbatur, 2012; Ceylan *et al*., 2019). Therefore, regular monitoring studies of medicinal leech populations and updated conservation policies are needed. In this study, it was aimed to investigate the structure and size of the medicinal leech, *H. verbana* populations inhabiting wetlands around Lake Eğirdir (Turkey), where leech collection has been carried out commercially. The removal methods to estimate population size were used for the first time for medicinal leech, *H. verbana* populations, and modal progression analysis of body length frequency to determine the age classes were used for the first time for all leech species (Hirudinea). The findings of the study can be used to conserve and manage sustainably the leech populations and habitats, and to provide more realistic export quotas for medicinal leech trade.

MATERIALS AND METHODS

Study area

This study was carried out in Akbük, Aşağı Tırtar, Boyalı, Gelendost and Kayaağzı wetlands around Lake Eğirdir, areas located between 38°16'41"-37°50'31"N and 030°44'49"-030°58'10"E in the province of Isparta, Turkey (Figure 1). The wetlands selected for the present study are the main areas, where the commercial leech collection is conducted, around Lake Eğirdir. The studied wetlands are distinctly isolated from the lake, being far from each other, rich in macrophytes, being shallow, and rich in food in the form of hosts for leeches (Ceylan, 2016; Ceylan and Çetinkaya, 2017; Ceylan *et al.*, 2021a).

The medicinal leeches were be collected in a total surface area of 110,300 m^2 in the studied wetlands. The suitable surface areas were estimated as 4,400 m^2, 9,000 m^2, 22,600 m^2, 24,000 m^2 and 50,300 m^2 in the wetlands of Boyalı, Akbük, Aşağı Tırtar, Kayaağzı and Gelendost, respectively.

Figure 1. The wetlands around Lake Eğirdir where the present study was conducted. 1: Boyalı, 2: Kayaağzı, 3: Aşağı Tırtar, 4: Akbük, 5: Gelendost.

Determination of population size

The removal methods, "Maximum likelihood" (MLM) (Zippin, 1956) and "Regression" (RM) (Leslie and Davis, 1939), were used to estimate the population size of *H. verbana*. Removal methods aim to estimate population size using the decreasing trend of leeches withdrawn by successive collection operations. These methods were used in medicinal leeches for the first time in *H. medicinalis* by Elliott (2008) and successful results were obtained.

The present study was conducted in May, June, July, and August of 2014 when the water temperature was suitable (>19°C) to activate the majority of leeches (Elliott and Tullett, 1986). The water temperature measured during the study per location and month was given in Table 1.

Table 1. The water temperature measured and number of collected leeches per location and month.

Locations	Months	Water temperature (°C)	Number of collected leeches (*n*)
Akbük	May	30.7	53
	June	31.7	276
	July	32.7	202
	August	28.4	39
Aşağı Tırtar	May	26.5	205
	June	28.1	431
	July	29.1	313
	August	28.8	39
Boyalı	May	21.6	56
	June	28	98
	July	29.8	10
	August	27.8	4
Gelendost	May	25.4	442
	June	26.7	751
	July	27.8	764
	August	27.9	355
Kayaağzı	May	21.7	28
	June	27.5	342
	July	30.4	293
	August	28.6	83
All habitats	May	25.2 ± 3.8	784
	June	28.4 ± 1.9	1,898
	July	30.0 ± 1.8	1,583
	August	28.3 ± 0.4	520

In total, four catch operations, each of which lasted for 45 minutes, were performed in each habitat by rotating clockwise in the certain surface areas. The leeches were stimulated to swim by disturbing the water by collectors. The leeches, which are actively swimming and attaching to the fishing boot, were collected, and put into 5L pet jars which were labelled accordingly. Between the catch operations, 15 minutes were left as the break. All leeches collected during the study (*n* = 4,784) were transported to the laboratory for counting and weighing, and afterwards all leeches were released in the wetlands, where they were collected. The collected leech number per location and month was given in Table 1.

The equations of the removal methods used in the present study are explained in detail below.

Maximum likelihood method: The population size in this method was estimated using following equations according to Zippin (1956).

$$N = \Sigma ni / (1 - q^s)$$

where "N" is the population size, "Σni" is the sum of all collected leeches, "s" is the number of catch operation runs, and "q" is the probability of a leech not being caught. "q" was determined following equation.

$$q = (\Sigma ni - C_1) / (\Sigma ni - C_s)$$

where "C_1" is the number of leeches caught in the first operation and "C_s" is the number of leeches caught in "s" operations.

Regression method: The population size in this method was estimated using following equation according to Elliott (2008).

$$N = m + (n / p)$$

where "N" is the population size, "m" is mean of the cumulative sum of the collected leeches except in the last catch operation, ""n" is the mean of the sum of the collected leeches for all catch operations and "p" is the slope of the regression line between the sum of the previous operations and the number of leeches caught on each successive catch operation.

These methods were applied in the sub-areas representing the studied wetlands. A proportion coefficient for each wetland was determined dividing the suitable surface area to sampling area. The estimated population size in the sub-areas was multiplied with proportion coefficient to estimate the total population size in each wetland.

Determination of biometric properties of leeches

All leeches were weighed, but since body length measurement has some technical difficulties, body length measurement was conducted in only some of them (n = 1,214). To measure the body length, leeches were anesthetized for 15 min in 2.5 ml/L phenoxyethanol solution (25 °C). Body length, the distance between the endpoints of the anterior and posterior suckers, was measured with a precision of 1 mm when they were anesthetized (Ceylan *et al*., 2021b). Condition factor (K) was calculated using the standard formula [K = (Body weight) / (Body length)^3 × 100] according to Ricker (1975). The body weight frequency of the leech populations was determined. Since the majority distribution was densified in small weights (right skewed), the axis showing the weight in the graph has been log scaled in especially small weights to show the weight distribution more clearly.

Determination of age classes

The age classes of the medicinal leech populations were estimated by Modal Progression Analysis (Bhattacharya's method) based on body length frequencies using FISAT II v.1.2.2 package software. Modal progression analysis uses the methodology which infers the growth from the apparent shift of modes or means in a time series length-frequency sample. The lowest separation index was considered as “2” for distinguishing age classes (Gayanilo *et al*., 2005).

Statistical analysis

Normality of data was tested by Kolmogorov-Smirnov test. One-way ANOVA test or Kruskal-Wallis rank order test was applied according to normality test results. While the Duncan post-hoc test was used for comparison of normally distributed data, Tukey HSD post-hoc test was used for non-normally distributed data. The results obtained from the removal methods used to determine the population size were evaluated by the Chi-Square Test of Independence. Relationship between average body weight and leech number per catch operation was determined using Regression analysis (Özdamar, 2011). The results are given as mean ± standard deviation ($\bar{X}$ ± SD). The significance level was accepted as α = 0.05. Data were analyzed using IBM SPSS Statistics version 25.0 for Windows package software (IBM Corp., Armonk, NY, USA).

Legal permissions and ethical statements

The medicinal leech, *Hirudo verbana* is an endangered species and whose international trade is regulated by the CITES. The present study, therefore, was carried out getting the legal permission (Date: 24.12.2012, Number: 05757) from the Ministry of Food, Agriculture and Livestock (currently Ministry of Agriculture and Forestry), General Directorate of Fisheries and Aquaculture, the management authority for the CITES in Turkey.

The present study was conducted under the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees of Turkey. Accordingly, only experiments conducted on vertebrate animals require review and approval by the Ethics Committees, and thus leeches did not require approval.

RESULTS

Estimated size of the medicinal leech populations

Since the response of leeches to collecting effort differs by season, and because the composition of the population size also differs according to the sampling date, the results of four months were averaged. The mean population size and biomass were estimated as 1,552,042 ± 778,378 leeches and 463.85 ± 166.43 kg, respectively by RM, and 1,562,696 ± 805,613 leeches and 467.26 ± 172.91 kg, respectively by MLM. The population size determined by methods of RM and MLM are very close to each other ($\chi 2$ = 2819.074, df = 19, P < 0.001). The difference in population size between the two methods is approximately 7‰.

Population size tended to increase from May to July. It was estimated to 829,806 leeches (357 kg) in May, increased to 2,565,122 leeches (715 kg) in July, and sharply decreased to 998,552 leeches (340 kg) in August.

The Boyalı wetland has the smallest population size with 2,106 ± 3,163 leeches (3.04 ± 4.15 kg) and the Gelendost wetland has the biggest population size with 1,205,999 ± 514,871 leeches (260.93 ± 139.18 kg). Gelendost wetland was followed by Aşağı Tırtar (117,045 ± 80,182 leeches – 111.55 ± 85.76 kg) and Kayaağzı (212,297 ± 208,751 leeches – 72.15 ± 49.48 kg), respectively. The difference in population size among habitats was significant (P < 0.05). The mean population size in studied wetlands was estimated as 1,562,696 ± 805,613 leeches and 467.26 ± 172.91 kg biomass. Monthly change of size of the medicinal leech populations per habitat is given in Table 2.

Table 2. Monthly change of size (abundance and biomass) of the medicinal leech, *H. verbana* populations per habitats.

Habitats	Parameters	Method (*)	Months (2014)				
			May	June	July	August	Average ($\bar{X}$ ± SD)
Boyalı	Abundance	RM	820 ± 3	6,833 ± 56	476 ± 25	251 ± 0	2,095 ± 3,167[a]
		MLM	851 ± 2	6,836 ± 35	486 ± 19	251 ± 0	2,106 ± 3,163[a]
	Biomass (kg)	RM	1.97 ± 0.00	9.16 ± 0.07	0.55 ± 0.03	0.38 ± 0.00	3.02 ± 4.16[A]
		MLM	2.04 ± 0.00	9.16 ± 0.04	0.56 ± 0.02	0.38 ± 0.00	3.04 ± 4.15[B]
Akbük	Abundance	RM	5,660 ± 129	32,970 ± 176	50,855 ± 309	11,551 ± 5	25,259 ± 20,709[a]
		MLM	5,480 ± 79	32,692 ± 106	51,124 ± 213	11,701 ± 3	25,249 ± 20,811[a]
	Biomass (kg)	RM	14.04 ± 0.20	12.86 ± 0.11	35.60 ± 0.26	16.06 ± 0.01	19.64 ± 10.72[A]
		MLM	13.59 ± 0.12	12.75 ± 0.07	35.79 ± 0.18	16.26 ± 0.00	19.60 ± 10.90[A]
Kayaağzı	Abundance	RM	19,290 ± 215	365,047 ± 1,891	443,416 ± 1,344	52,633 ± 207	220,097 ± 215,445[a]
		MLM	19,301 ± 148	335,901 ± 1,278	441,668 ± 953	52,316 ± 131	212,297 ± 208,751[a]
	Biomass (kg)	RM	19.68 ± 0.22	105.86 ± 1.02	128.59 ± 0.72	43.69 ± 0.19	74.46 ± 51.20[A]
		MLM	19.69 ± 0.15	97.41 ± 0.69	128.08 ± 0.51	43.42 ± 0.12	72.15 ± 49.48[A]
Aşağı Tırtar	Abundance	RM	77,652 ± 136	186,045 ± 383	178,649 ± 222	22,967 ± 81	116,328 ± 79,491[a]
		MLM	78,709 ± 94	186,718 ± 258	180,347 ± 145	22,406 ± 46	117,045 ± 80,182[a]
	Biomass (kg)	RM	224.41 ± 0.23	78.14 ± 0.25	114.34 ± 0.18	25.49 ± 0.03	110.60 ± 84.19[AB]
		MLM	227.47 ± 0.16	78.42 ± 0.17	115.42 ± 0.12	24.87 ± 0.05	111.55 ± 85.76[AB]
Gelendost	Abundance	RM	748,918 ± 2,013	1,282,646 ± 2,614	1,827,074 ± 4,033	894,416 ± 482	1,188,264 ± 481,788[b]
		MLM	725,465 ± 1,336	1,295,157 ± 1,964	1,891,497 ± 3,203	911,878 ± 339	1,205,999 ± 514,871[b]
	Biomass (kg)	RM	97.36 ± 0.73	256.53 ± 1.17	420.23 ± 1.93	250.44 ± 0.26	256.14 ± 131.87[B]
		MLM	94.31 ± 0.48	259.03 ± 0.88	435.04 ± 1.54	255.33 ± 0.18	260.93 ± 139.18[B]
All habitats	Abundance	RM	852,340	1,873,541	2,500,470	981,818	1,552,042 ± 778,378
		MLM	829,806	1,857,304	2,565,122	998,552	1,562,696 ± 805,613
	Biomass (kg)	RM	357.46	462.55	699.31	336.06	463.85 ± 166.43
		MLM	357.10	456.77	714.89	340.26	467.26 ± 172.91

(*) RM: Regression Method (Leslie and Davis, 1939), MLM: Maximum Likelihood Method (Zippin, 1956). The difference of the estimated number of leeches (abundance) and biomass of the populations among the habitats are shown by lower and upper cases respectively ($P < 0.05$).

In addition to the studied wetlands, leech collecting is carried out also in the wetlands of Yenice, Gençali and between Boyalı and Akkeçili located around Lake Eğirdir. However, the current study was conducted in only five wetlands due to technical possibilities. When the suitable surface area (approximately 30,000 m^2) of the nonstudied areas was taken into consideration, the total population size and biomass of medicinal leeches around Lake Eğirdir was estimated as 1,988,700 leeches and 593 kg, respectively.

Biometric features of the populations

The mean body weight, body length and condition factor of the medicinal leech populations in all habitats were estimated as 0.52 ± 1.19 g (min = 0.0182 g, max = 13.10 g), 4.5 ± 2.4 cm (min = 1.4 cm, max = 12.0 cm) and 0.620 ± 0.123 (min = 0.260, max = 1.252), respectively. 89.9% of leeches in the populations weighed less than 1 g. The smallest gravid leech in populations of *H. verbana* weighted 1.69 g (Ceylan, 2016). The percentage of the leeches bigger than 1.69 g, representing gravid specimens, was only 7.0% in this study. The frequency of the medicinal leech, *H. verbana* populations according to the weight classes is given in Figure 2.

The differences of the body weight, body length and condition factor among the habitats was significant considering the data of all months ($P < 0.05$). The biggest leeches (1.69 ± 1.70 g and 6.0 ± 2.2 cm) were collected from the Boyalı, which has the lowest catch amount and population size, while the smallest ones (0.21 ± 0.57 g and 3.5 ± 2.0 cm) were collected from the Gelendost, which has the highest catch amount and population size. The leeches having the lowest mean condition (0.590 ± 0.111) were found in Boyalı, which hosts the largest leeches. The highest mean condition (0.650 ± 0.135) of the populations was obtained from Kayaağzı (Table 3).

There was a negative significant correlation ($r = -0.620$, $P < 0.01$) and second order polynomial regression ($R^2 = 0.761$, $P < 0.001$) between the size and catch amount (Figure 3).

The differences of the sizes and condition factors of the leeches among the months were significant considering the data of all habitats ($P < 0.05$). The mean body weight and body length of the medicinal leech populations with 1.21 ± 2.09 g and 5.3 ± 3.2 cm in May decreased to 0.35 ± 0.94 g and 4.5 ± 2.4 cm in June and then gradually increased to 0.52 ± 0.67 g and 4.3 ± 1.7 cm in August. The mean condition factor was found to be the lowest in June with 0.602±0.107 and the highest in August with 0.664 ± 0.136 (Table 3).

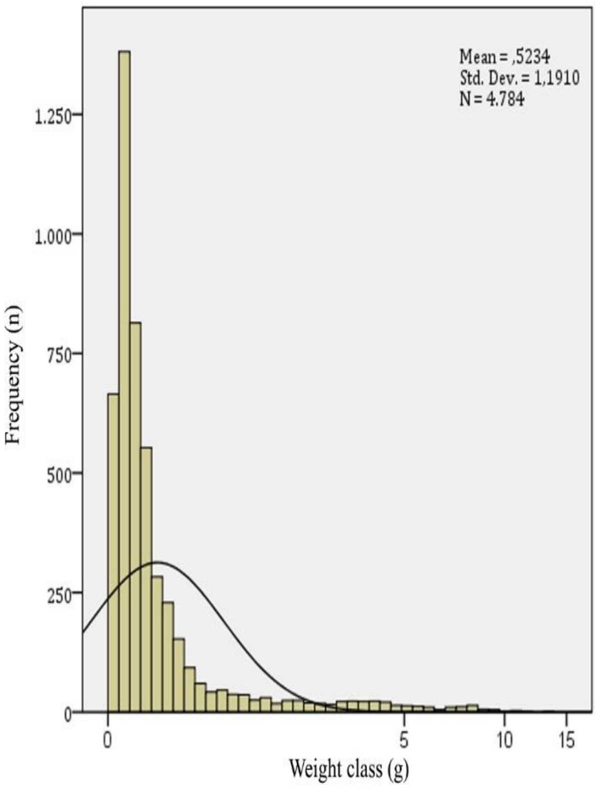

Figure 2. The body weight frequency of the medicinal leech, H. verbana populations.

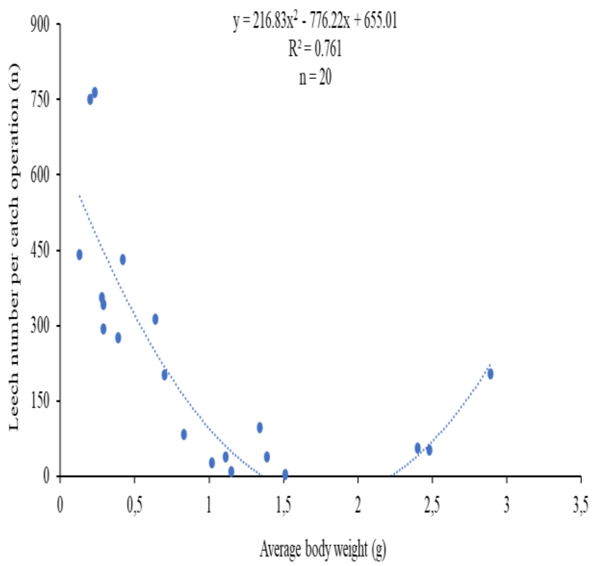

Figure 3. Relationship between average body weight and leech number per catch operation.

Table 3. The body weight, body length and condition factor the medicinal leeches by months and habitats ($\bar{X}$ ± SD).

Months	Habitats	Body weight (g)	Body length (cm)	Condition factor
May	Kayaağzı	1.02 ± 1.34^{Ab}	4.2 ± 2.8^{Abc}	0.593 ± 0.111^{A}
	Aşağı Tırtar	2.89 ± 2.55^{Aa}	6.4 ± 3.0^{Aa}	0.609 ± 0.114^{B}
	Gelendost	0.13 ± 0.48^{Bc}	3.2 ± 2.2^{Bc}	0.610 ± 0.133
	Akbük	2.48 ± 3.26^{Aa}	5.4 ± 3.6^{ABab}	0.644 ± 0.099^{AB}
	Boyalı	2.40 ± 1.56^{a}	6.6 ± 2.6^{a}	0.607 ± 0.159
	All habitats	1.21 ± 2.09	5.3 ± 3.2	0.614 ± 0.122
June	Kayaağzı	0.29 ± 0.82^{Bb}	3.9 ± 2.4^{ABb}	0.629 ± 0.121^{Aa}
	Aşağı Tırtar	0.42 ± 1.04^{Bb}	4.5 ± 2.4^{Bb}	0.620 ± 0.112^{ABa}
	Gelendost	0.20 ± 0.71^{ABb}	4.1 ± 2.7^{Ab}	0.599 ± 0.109^{ab}
	Akbük	0.39 ± 0.82^{Cb}	4.3 ± 2.0^{Bb}	0.562 ± 0.088^{Bb}
	Boyalı	1.34 ± 1.77^{a}	5.6 ± 2.2^{a}	0.592 ± 0.086^{ab}
	All habitats	0.35 ± 0.94	4.5 ± 2.4	0.602 ± 0.107
July	Kayaağzı	0.29 ± 0.48^{Bbc}	3.5 ± 1.5^{Bc}	0.633 ± 0.124^{Aa}
	Aşağı Tırtar	0.64 ± 0.96^{ABbc}	4.8 ± 2.1^{Bb}	0.672 ± 0.154^{Aa}
	Gelendost	0.23 ± 0.50^{Ac}	3.4 ± 1.7^{ABc}	0.595 ± 0.113^{ab}
	Akbük	0.70 ± 1.38^{Cb}	4.9 ± 2.0^{ABab}	0.599 ± 0.091^{BCab}
	Boyalı	1.15 ± 0.36^{a}	6.0 ± 0.8^{a}	0.529 ± 0.070^{b}
	All habitats	0.39 ± 0.79	4.0 ± 1.9	0.617 ± 0.123
August	Kayaağzı	0.83 ± 0.82^{Ab}	4.5 ± 1.6^{Abc}	0.728 ± 0.148^{Aa}
	Aşağı Tırtar	1.11 ± 0.65^{Bab}	5.4 ± 1.2^{ABab}	0.665 ± 0.075^{ABab}
	Gelendost	0.28 ± 0.49^{Ac}	3.3 ± 1.3^{Bc}	0.631 ± 0.139^{ab}
	Akbük	1.39 ± 0.31^{Ba}	6.0 ± 0.5^{Aa}	0.658 ± 0.122^{Aab}
	Boyalı	1.51 ± 0.35^{a}	6.4 ± 0.7^{a}	0.566 ± 0.060^{b}
	All habitats	0.52 ± 0.67	4.3 ± 1.7	0.664 ± 0.136
All months	Kayaağzı	0.38 ± 0.76^{a}	4.0 ± 2.0^{a}	0.650 ± 0.135^{c}
	Aşağı Tırtar	1.03 ± 1.75^{c}	5.3 ± 2.6^{b}	0.634 ± 0.123^{bc}
	Gelendost	0.21 ± 0.57^{a}	3.5 ± 2.0^{a}	0.608 ± 0.124^{ab}
	Akbük	0.76 ± 1.53^{b}	4.9 ± 2.4^{b}	0.603 ± 0.101^{ab}
	Boyalı	1.69 ± 1.70^{d}	6.0 ± 2.2^{c}	0.590 ± 0.111^{a}
	All habitats	0.52 ± 1.19	4.5 ± 2.4	0.620 ± 0.123

While the difference of body weight, body length and condition factor among the habitats was shown with lower case ($P < 0.05$), the difference among the months was shown with upper case ($P < 0.05$).

Age groups in the medicinal leech populations

Three distinct classes representing 0+, 1+ and 2+ ages were identified by analysis of body length frequencies using the Bhattacharya's method. The mean body length for each age class was estimated as 2.96 ± 1.35 cm (0+), 7.20 ± 1.01 cm (1+) and 9.79 ± 1.06 cm (2+), respectively (Table 4). According to the Table 4, body length of the leeches has decreased in general due to the continued participation of baby leeches in the population living in the water during the study. While the dominant age group was 0+ with 78.6%, the percentage of 1+ and 2+ age groups were 12.8% and 8.6%, respectively. The age classes of the medicinal leech, *Hirudo verbana* populations are given in Figure 4.

Table 4. Monthly changes of the body length, number of leeches, percentage, and the body length frequency separation index (S.I.) in age classes for all habitats.

Months	Parameters	Age classes		
		0+	1+	2+
May	Body length cm	2.50 ± 0.62	7.52 ± 1.69	10.56 ± 0.95
	Percentage (%)	146 (55.9%)	89 (34.1%)	26 (10%)
	S.I.	n.a.*	3.740	2.090
June	Body length cm	3.11 ± 0.81	8.20 ± 1.12	10.29 ± 0.73
	Percentage (%)	222 (76.3%)	41 (14.1%)	28 (9.6%)
	S.I.	n.a.	3.610	2.050
July	Body length cm	3.57 ± 0.86	5.50 ± 0.99	8.92 ± 1.98
	Percentage (%)	320 (83.6%)	28 (7.3%)	35 (9.1%)
	S.I.	n.a.	2.040	2.140
August	Body length cm	3.07 ± 1.35	6.07 ± 0.81	8.50 ± 1.24
	Percentage (%)	165 (70.8%)	65 (27.9%)	3 (1.3%)
	S.I.	n.a.	2.380	2.110
All months	Body length cm	2.96 ± 1.35	7.20 ± 1.01	9.79 ± 1.06
	Percentage (%)	1073 (78.6%)	175 (12.8%)	118 (8.6%)
	S.I.	n.a.	2.870	2.130

*n.a. methodologically not assessed.

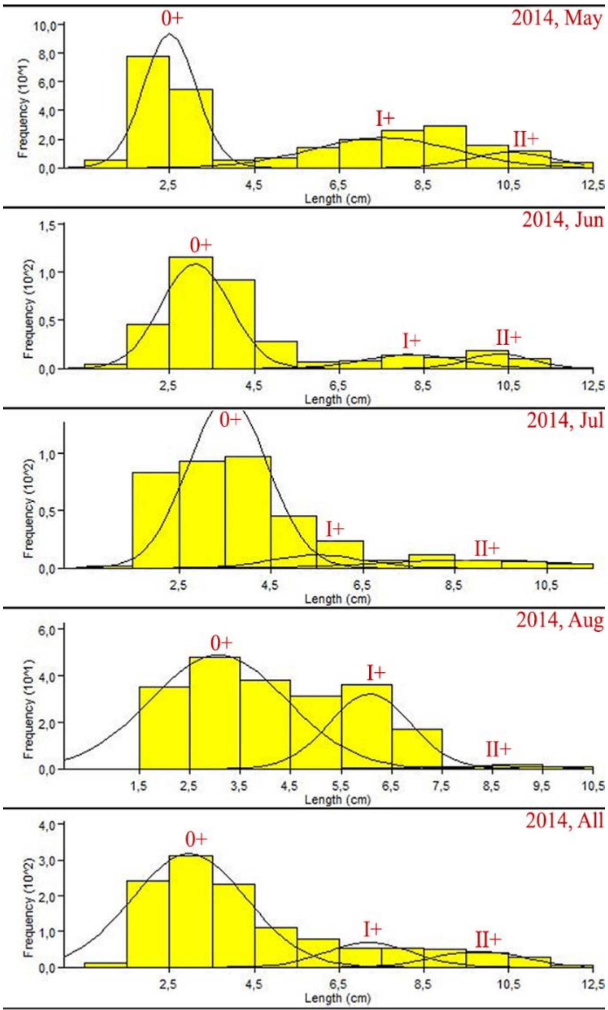

Figure 4. The age classes of the medicinal leech, *H. verbana* populations which were determined by the Bhattacharya's method.

DISCUSSION

In total, three age groups were identified in the studied medicinal leech populations. However, 4 size groups (age classes) were depicted in the *Hirudo medicinalis* populations inhabiting Jenny Dam (Lake District, England) (Elliott, 2008). In the study conducted by Elliott (2008) the oldest group, representing 4 years old leeches, represented only 1% of the population. This may indicate the difficulty of sampling large leeches that withdraw from the habitats subjected to leech harvesting before reaching older ages.

The body weight of the leeches varied between 0.0182 g and 13.10 g in this study. The body weight range of the leeches was 0.23 - 5.63 g in Akpınar Marsh, Turkey (Sağlam et al., 2018) and 0.1 – 15.8 g in Lake Efteni and Lake Poyrazlar, Turkey (Demirsoy et al., 2001). The studies conducted in some Turkish wetlands revealed that the mean body weight of the medicinal leech populations decline gradually from the spring months until June-July and then increase (Demirsoy *et al*., 2001; Ceylan, 2016). The mean body weight of the population was 1.21 ± 2.09 g in May, then decreased sharply to 0.35 ± 0.94 g in June and then gradually increased in the present study. This can be explained by the effects of the water level of the lake on the structure of medicinal leech populations. The gravid leeches of both *H. medicinalis* and *H. verbana* populations leave the water and deposit the cocoons in damp places in July, August and September (Wilkin, 1989; Elliott and Dobson, 2015; Ceylan, 2016). The water level gradually falls during this period. The cocoon incubation period lasts approximately 30 days in *H. verbana* populations (Kutschera and Roth, 2006; Petrauskienė *et al*., 2009; Ceylan *et al*., 2015). Decreasing the water level of lake during the cocoon incubation period causes leeches, which wait passively in the soil part of the habitat, to delay participation into populations living in the water. According to Ceylan (2016) the waiting period can last for 9-10 months, depending on both the water level rising and the distance of the overwintering cocoons to the water line of wetlands. Hatchlings live in the moist microhabitats during this period. When leeches started to deposit cocoons in July 2013, the water level of Lake Eğirdir was 917.77 m (Ceylan, 2016). However, the water level of the lake was able to rise to 917.68 m in following year (2014), then it gradually fell (Ceylan, 2016). As the highest water level in 2014 didn't reach the level at the beginning of the reproduction period in 2013, until June 2014 the entrance of offspring to the populations living in lake water continued. The mean body weight of the population decreased to the lowest value of 0.35 ± 0.94 g in this month. After this, the mean body weight of the populations increased gradually to 0.52 ± 0.67 g in August due to both the end of the entrance of offspring to the lake's water and that the leeches had the opportunity to feed regularly in lake's water.

In addition to the studied wetlands, also a couple of wetlands serve as habitat for medicinal leeches in Lake Eğirdir. Considering the surface areas of these habitats the size of medicinal leeches population in Lake Eğirdir is estimated at 1,988,700 leeches. According to this, the populations of *H. verbana* in the wetlands around Lake Eğirdir are much larger in comparison to the populations of *H. medicinalis* inhabiting Jenny Dam (248-289 leeches) (Elliott, 2008) and Dungeness (England) (more than 10,000 leeches) (Wilkin and Scofield, 1991). The size of medicinal leech populations in studied wetlands is higher than in the other 21 wetlands except for the population of the medicinal leech population in Doğubeyazıt Marsh, which was estimated to 17,500,000 leeches (Sağlam *et al*., 2008). The size of the studied medicinal leech populations is also higher than in the other 10 wetlands except for the population of the medicinal leech population in Beyşehir Lake, Turkey, which was estimated to 2,890,000 leeches (Anonymous, 1997).

It is thought that decline of the water level of the lake and the reproduction period are responsible for the sharp decrease in the estimated population size in August. In addition to this, it is considered that medicinal leeches (*Hirudo* sp.) becoming inactive after feeding for a long time can cause this result. Some of the leeches, which moved to the humid terrestrial areas of the habitats to lay cocoons, remain in these areas far from the water because of the decrease of lake water level. Sampling different sized leeches in the moist terrestrial areas of 200-300 m distance from the water and in the puddles revealed that some of the leeches passively wait in terrestrial areas without returning to the water until the water level increases. The ability of adaptation to arid conditions compared to other medicinal leech species should be considered as an assurance and the populations of *H. verbana* can tolerate the lake level cycle. The fact that the leeches remain in terrestrial environment due to the decrease in the water level of the lake provides an opportunity to naturally protect them from the pressure of leech-collecting, which occurs by catching individuals actively swimming in the water.

The medicinal leech population size was estimated as 1,796,000 leeches and biomass as 2,739 kg in the same wetlands 20 years ago in Lake Eğirdir (Anonymous, 1997). Although the population size in the present study is only slightly larger, the difference of the estimated biomass between both studies is quite high. The estimated biomass in the mentioned study was determined using an average individual body weight (1.525 g) for all wetlands. This difference occurred because of the low mean body weight (0.21 ± 0.57 g) of the population in Gelendost wetland, which has about 77% of the total estimated population size. It is thought that the number of leeches provides a more realistic assessment rather than biomass due to the fact that medicinal leeches can consume 8.9 times their own body weight in a feeding period (Dickinson and Lent, 1984).

Mediterranean medicinal leech, *H. verbana* populations living in wetlands around Lake Eğirdir deposit the cocoons from July to September when the water level of the lake decreases (Ceylan, 2016). Breeding season of *H. medicinalis* seems to be the same as of *H. verbana*, according to some studies conducted with *H. medicinalis* populations (Sawyer, 1986; Wilkin, 1989; Elliott and Kutschera, 2011; Kutschera and Elliott, 2014). However, the prohibition season is valid from 1 March to 30 June, according to the "Notification No. 4/1 Regulating Commercial Fisheries" in Turkey. It is thought that misapplying leech collection prohibition season may be due to insufficient knowledge on phenology and reproduction ecology of the *H. verbana* populations. Furthermore, also catch quantity restriction is not applied for medicinal leeches in Turkey (Official Gazette, 2020b). Despite commercial leech collection carrying out without the catch quantity restriction and misapplying the prohibition season, it is thought that the medicinal leech populations in wetlands around Lake Eğirdir are protected. Some biological, ecological, and operational factors are thought to be effective in this result. Long-term passive staying of medicinal leeches in bottom of habitats without any reaction after feeding period (Dickinson and Lent, 1984), the difficulties of leech collection due to habitat structure and decrease of the water level of the lake, and the collection on just only swimming leeches suggest that the collection pressure on leeches is not primary threat for medicinal leech populations.

The populations of *H. medicinalis* living in European wetlands have significantly declined (Elliott and Tullett, 1984; Elliott and Kutschera, 2011). The populations of *H. verbana* in the wetlands of the Black Sea Region (Turkey) where leech collection is the most intense in Turkey, also are declining day by day (Sağlam, 2011). However, the findings of the present study revealed that the populations of *H. verbana* living in the wetlands around Lake Eğirdir are protected. It is thought that stability in the withdrawal and rise of the water level of the lake, the variety and abundance of the host in the undamaged wetlands, the social structure of the leech collectors, the sales policies, and the effective activities of conservation and control units play important role on the protection of the ///medicinal leech populations living around Lake Eğirdir.

The marking methods (Wilkin and Scofield, 1991; Anonymous, 1997), Ekman dredge and modified unit square design methods (Sağlam *et al*., 2008) were used to estimate the medicinal leech population sizes. However, marking methods cause the injuries and deaths in leeches (Wilkin and Scofield, 1991). There are operational difficulties in using the Ekman dredge and modified unit square design methods due to habitat structure of the wetlands. It seems that the removal methods are more effective and leech-friendly compared to the other methods to estimate the population size of the medicinal leeches. Non-injury to the leeches, easy application, and taking quick results are the strengths of removal methods compared to other methods. However, removal methods are based on actively swimming leeches reacting to catch operations, the findings of the present study should be regarded as the catchable population size. The removal methods used in the present study, therefore, should be improved considering also inactive leeches.

This study revealed that both "Maximum Probability" and "Regression" methods used for the first time to estimate the population size of the medicinal leech, *H. verbana* can be applied reliably. In addition, the reliable results obtained from the Modal Progression Analysis (Bhattacharya's method), which was used for the first time in estimating the age classes for leeches (Hirudinea), may shape the future studies on medicinal leech populations. The methods regarding medicinal leech populations estimation used in the present study will serve to determine catch limits and export quotas in accordance with realistic and scientific norms needed to contribute to the monitoring and sustainable management of *H. verbana* populations, which have a significant share in the world's leech trade. Considering that the medicinal leech populations are declining day by day, and they are even completely extinct in many wetlands of Europe and Turkey, it is suggested that the wetlands around Lake Eğirdir should be regarded as model habitats in terms of conservation and sustainable management of medicinal leech populations. To contribute to the conservation of the medicinal leech *H. verbana* populations, the size of the medicinal leech populations should be determined at regular intervals and the annual leech collecting limits should be applied for each wetland, at least where commercial leech collection is conducted.

ACKNOWLEDGEMENTS

This study was supported by Süleyman Demirel University, Scientific Research Projects Coordination Unit (SDU-BAP 3341-D2-12). The authors are grateful to Eğirdir Fisheries Research Institute for laboratory support. We also thank Dr. Ramazan KÜÇÜKKARA and Ufuk AKÇİMEN for help in the laboratory.

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Ege Journal of Fisheries and Aquatic Sciences, 38(4), 449-459 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.06

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Monthly variation of micro- and macro-element composition in smooth scallop, *Flexopecten glaber* (Linnaeus, 1758), from the Çardak Lagoon (Çanakkale Strait, Turkey)

Çardak Lagünü'ndeki (Çanakkale Boğazı, Türkiye) deniz tarağının *Flexopecten glaber* (Linnaeus, 1758), mikro ve makro element kompozisyonundaki aylık değişimler

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Received date: 08.04.2021 *Accepted date: 16.09.2021*

How to cite this paper:
Vural, P. & Acarlı, S. (2021). Monthly variation of micro- and macro-element composition in smooth scallop, *Flexopecten glaber* (Linnaeus, 1758), from the Çardak Lagoon (Çanakkale Strait, Turkey). *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 449-459. DOI: 10.12714/egejfas.38.4.06

Abstract: The present study investigated the total elemental composition (Fe, Cu, Mn, Zn, Al, S, B, Na, Mg, K, Ca, P, S) in smooth scallop (*Flexopecten glaber*) meat collected monthly in the Çardak Lagoon (Turkey) in 2017- 2018. Considering the distribution of elements in terms of quantity during the year, the elements were sorted as S> Na> K> P> Mg> Ca> Fe> Zn> Mn> Al> B> Cu> Se and the heavy metals were below the detection limit. Although the elements were at their highest values during autumn and summer, they were at their lowest values during spring and winter. The differences between the months in terms of Fe, Mn, Mg, and Ca elements were found to be statistically significant (p <0.05). It was determined that the element composition of smooth scallop meat was suitable for consumption in terms of the area where it is collected and is beneficial for health when consumed in the amounts recommended by FAO/WHO, and CODEX. The Hazard Quotient (THQ) and the Hazard Index (HI), which was conducted to evaluate the health risks of consuming smooth scallop, were found below 1 all months. For this reason, smoth scallop consumption in this region does not have any negative effects on human health.

Keywords: *Flexopecten glaber*, smooth scallop, element, Çardak Lagoon, Çanakkale Strait

Öz: Bu çalışmada Çardak Lagünü'nden (Türkiye) 2017- 2018 tarihleri arasında bir yıl boyunca aylık olarak toplanan smooth scallop (*Flexopecten glaber*) etinin toplam element kompozisyonu (Fe, Cu, Mn, Zn, Al, Se, B, Na, Mg, K, Ca, P, S) belirlenmiştir. Elementlerin miktar açısından yıl içindeki dağılımına bakıldığında S> Na> K> P> Mg> Ca> Fe> Zn> Mn> Al> B> Cu> Se sıralamasında olduğu, ağır metallerin ise dedeksiyon limitinin altında olduğu gözlenmiştir. Elementler sonbahar ve yaz döneminde en yüksek değerde bulunmasına karşın, ilkbahar ve kış döneminde en düşük değerde gözlenmiştir. Fe, Mn, Mg ve Ca elementlerinde aylar arasındaki farklar istatistiksel olarak önemli bulunmuştur (p<0.05). Toplandığı bölge bakımından deniz tarağı etinin element kompozisyonunun tüketim için uygun olduğu ve FAO/WHO, CODEX tarafından tavsiye edilen miktarlarda tüketildiğinde sağlığa yararlı olduğu tespit edilmiştir. Deniz tarağı tüketmenin sağlık açısından risklerini değerlendirmek amacıyla yapılan Hedef Tehlike Katsayısı (THQ) ve Tehlike Indeksi (HI) tüm aylarda 1'in altında bulunmuştur. Bu nedenle bu bölgedeki deniz tarağı tüketiminin insan sağlığına olumsuz etkisi bulunmamaktadır.

Anahtar kelimeler: *Flexopecten glaber*, deniz tarağı, element, Çardak Lagünü, Çanakkale Boğazı

INTRODUCTION

Seawater, which forms oceans and seas, covers more than 70% of the earth's surface. Also, seawater is a mixture of 96.5% water, 2.5% salt and contains dissolved inorganic and organic substances and several atmospheric gases. Seawater is rich in elements (Sharp and Bryne *et al.*, 2020). Elements reach the sea from various sources. These are divided into natural resources and anthropogenic resources. Natural resources include mineral deposits, erosion of soil and rocks, dust caused by wind, volcanic activities, atmospheric and forest fires. Those anthropogenic resources include waste products, household wastes, pesticides, mines, petroleum refineries, protective paints, which are formed as a result of the use of elements and compounds in the industry (Ünsal, 2004). Elements that reach the seas from these sources are soluble in seawater or are present in the form of particles. Soluble ones are in the form of free metal ions or unstable organic and inorganic compounds (Çetingül and Aysel, 1998; Ünsal, 2004). Sea bottoms are areas of continuous sediment formation. Some of the sediments are materials transported by rivers, winds, and glaciers to the sea, and most of the sediments are the remains of marine creatures (Akkan, 1981). Aquatic organisms can take elements directly from the body surface and gills, as well as through digestion (Phillips and Rainbow, 1994; Bat and Raffaelli, 1998).

Lagoons are ecotones between freshwater, marine, and terrestrial biotopes (Ustaoğlu *et al.*, 2012; Maanan *et al.*,

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2015; Botello *et al*., 2018; Kükrer *et al*., 2020). Lagoons are connected to the sea via one or more canals. The water exchange with the sea and the transport of nutrients with it occur through tides, river flow, wind, and waves (Larson, 2012; Sevgi and Ulutarhan-Süzer, 2019). Due to the shallow transitional environments of lagoons such as rivers, streams, groundwaters and currents in lagoons, and also due to the influence of coastal geomorphological processes, hydrological processes, natural vegetation, and land use in the basin (Ustaoğlu *et al*., 2012), the physicochemical parameters frequently change. This affects the physiology and metal bioaccumulation in organisms distributed in lagoons (McLusky *et al*., 1986; Dame, 1996; Ulutarhan *et al*., 2019). Lagoons are among the most productive marine ecosystems in the world, serving as nutrition, habitat, migration routes, and nursery areas for many organisms such as phytoplankton, diatom, dinoflagellate, algae, fish, and bivalves (Din, 1992; Balls *et al*., 1997; Chapman and Wang, 2001). The Çardak lagoon chosen as a study area is abundant in terms of many economic fish species such as Mullet (*Mugil* sp.), Gilthead seabream (*Sparus aurata*), Scad (*Trachurus* sp.), Bluefish (*Pomatomus saltatrix*), eel fish (*Anguilla* sp.), picarel (*Spicara smaris*), common sole (*Solea* sp.), sparid fish (*Sarpa salpa*), striped sea bream (*Lithognathus mormyrus*), garfish (*Belone belone*), twaite shad (*Livoneca punctata*), flounder (*Platichthys flesus*) and bivalve species such as Mediterranean mussel (*Mytilus galloprovincialis*), manila clam (*Ruditapes philippinarum*), carpet shell clam, (*Ruditapes decussatus*), flat oyster (*Ostrea edulis*), razor clam (*Ensis sp.*), venerid clam (*Venus gallina*), lagoon cockle *(Cerastoderma glaucum)*(Vural and Acarlı, 2018).

Smooth scallop (*Flexopecten glaber*) is one of the bivalve organisms within the mollusks and is included in the *Pectinidae* order. *F. glaber* is distributed from the eastern Atlantic, Mediterranean, Black Sea, and Portugal to Morocco while in Turkey, it is seen as dense stocks from the south of Marmara, along the Aegean Sea, and throughout the Mediterranean (Aquamaps, 2019).

Bivalves are important marine organisms due to their nutritional value, taste, and reasonable selling prices (Orban *et al*., 2002; Orban *et al*., 2007). They have a rich nutritional composition, including high protein content, essential amino acids, low-fat content, high levels of ω3 fatty acids, elements, and antioxidants (Orban *et al*., 2002; Orban *et al*., 2007; Prato *et al*., 2019).

Microelements are accumulated in marine organisms (Plessi *et al*., 2001) and transferred to humans through the food chain (Gökoğlu *et al*., 2008). Microelements are especially of important in infants and children (WHO, 2006). Elements such as iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) are essential elements as they play an important role in biological systems (Hogstrand and Haux, 1991), however, their excessive consumption is toxic (Tarley *et al*., 2001). Generally, bivalves accumulate certain elements, especially zinc (Lök *et al*., 2010). Storelli *et al*. (2000) have reported that mollusks have a higher amount of Zn compared to fish species found in the Mediterranean. Manthey-Karl *et al*. (2015) have reported that smooth scallop meat is a good source of Zn. El Shenawy *et al*. (2016) observed that Al is dominant in sediments and bivalves. In humans, an average of 4% aluminum (Al) taken with nutrition is absorbed by the intestines and accumulates in bones, liver, lung, thyroid glands, and brain (Hellström-Westas and Rosen, 2006).

Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Phosphorus (P), and Sulfur (S) are macro elements. Na is an important element in the regulation of osmotic pressure (Tapiero *et al*., 2003; Yaşar and Melek, 2003), stimulation of nerves, and continuity of nerve and muscle functions. Also, Na plays a role in bone development together with Ca. Bivalves contain higher levels of Na than fish species (Spanish mackerel, Gray eel-catfish, Longtail shad; Cuttlefish, Prawn) (Nurnadia *et al*., 2013), while sea species contain higher levels of Na than freshwater species (Sidwell *et al*., 1977). K is one of the vital elements for humans. Of the K in the body, 98% is found inside the cell walls. K, together with Na, is responsible for the water balance in the body, in the passage of food into the cell, in the transmission of messages in the nervous system, in the formation of tissues such as bones, teeth, red blood cells, and muscles, and it maintains the healthy structure of the heart and other muscles. Also, K has beneficial effects in the development of bones together with Ca (Karadeniz, 2004, İnan and Gül, 2001; Cashman, 2006). It was determined that the macro element with the highest ratio in smooth scallop meat is usually K (*Bilandzic et al*., 2015, Manthey-Karl *et al*., 2015).

There are studies n the elemental composition of smooth scallop meat (Özden and Erkan, 2011; Berik *et al*., 2017; Prato *et al*., 2019a; Prato *et al*., 2019b). However, studies on the monthly element composition are limited (Prato *et al*., 2019a). Therefore, the present study aimed to monthly determine the element content of the meat of smooth scallop (*F. glaber*), one of the economic bivalves collected from the Çanakkale Strait Çardak lagoon in the Turkish Straits System, and also to determine its compliance with the maximum limits determined by health organizations. In addition, in this study, it was aimed to evaluate the potential health risk that may occur in case of consumption by adults by determining the Target Hazard Quotient (THQ) and Hazard Index (HI) values for the general population, taking into account the consumption frequency of the smooth scallop.

MATERIAL AND METHODS

Smooth scallop samples (53.95±3.70 mm in length) were collected by hand monthly between July 2017 and June 2018 in the Canakkale Province, Lapseki district, Çardak Lagoon in Turkey, (40° 22' 56"N, 21° 42' 58" E) (Figure 1). Çardak lagoon consists of 3 parts: Burunucu lagoon, Buruniçi lagoon and Ortagöl. The lagoon is a 180 ha saltwater lagoon located near the village of Çardak (Lapseki-Canakkale) on the southern coast of Marmara Sea. It is a lagoon with an average depth of 1.5 m (GTHB, 1997). The lagoon is under the influence of the sea, with its deep and open passage. There are many fish and bivalve species in the lagoon. Throughout the study, 30 smooth scallops per month were brought to the laboratory. Fouling organisms adhering on the smooth scallops were removed using a knife and the meat content of the smooth scallop was freeze dried after seperating the meat from the shell parts.

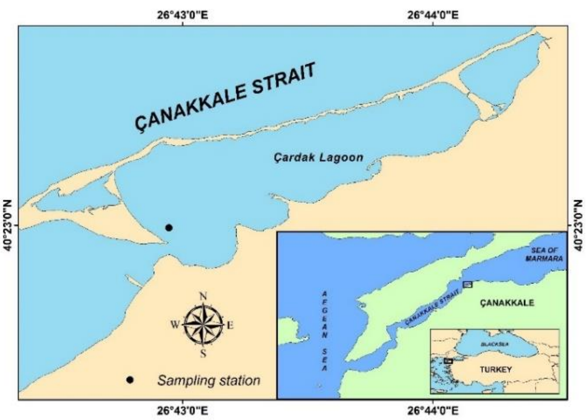

Figure 1. Map showing the sampling area Çardak Lagoon

A 0.5 g of dry smooth scallop meat and 10 ml of HNO_3 mixture were dissolved by burning in a microwave incinerator. It was then allowed to cool and filtered with distilled water. SpectroBlue inductively coupled plasma optical emission spectrometry (ICP-OES) was used to measure the element concentrations in smooth scallop meat after closed-vessel microwave digestion (EPA, 1994). Element content was expressed as percent of dry weight (dw).

Freeze-dried meat of the scallops was used to determine the element compositions. The dry weight of element concentration (mg/g) was converted to wet weight using to calculate the hazard quotient (THQ) and hazard index (HI). A coefficient was used in the conversion to wet meat. In the calculation of this coefficient, the method of El-Shenawy *et al.* (2016) was used (Table 1).

Table 1. Conversion coefficients of freeze dried *Flexopecten glaber* meat to wet *F. glaber* meat.

Months	Wet meat converison coefficients
July	0.25
August	0.26
September	0.24
October	0.26
November	0.24
December	0.22
January	0.22
February	0.25
March	0.25
April	0.23
May	0.23
June	0.23

The values of element in smooth scallop meat were used to calculate the estimated daily intake of elements (EDI), and target hazard quotients (THQ), hazard index (HI) seperately for adult individuals. In this evaluation, 70 kg of body weight refers to adult people (Yaman *et al.* 2014). The bivalve consumption rate in Turkey in 2010 is 1 g/ person·day (FAO, 2010).

Edible Daily Intake (EDI)

The EDI value was calculated with the following equation (Javed and Usmani, 2016; Alkan *et al.,* 2020; Tokatlı and Ustaoğlu, 2021):

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

$C_{element}$= Element concentration in smooth scallop wet weight (mg/kg),

$D_{food\ intake}$= Daily ingestion rate (kg/person.day) 1 g for bivalve)

BW= Average body weight (70 kg for adults).

Target Hazard Quotient (THQ)

The THQ is an estimation of the non-carcinogenic risk level and is used in its calculation with the following equation (USEPA,2019a; 2019b):

$$THQ = \frac{EDI}{RfDo \times AT}$$

RfD: Oral referans dose (mg/kg/day) (Table 2)

AT: Average exposure time for non-carcinogenic effects (365 days/year x ED)

ED: Exposure duration (70 years for a person is assumed in this study, equivalent to the average lifetimes),

HQ is categorized into six classes: HQ<1 (no health risk); 1<HQ<1.5 (low health risk); 1.5<HQ<2 (medium-low health risk); 2<HQ<2.5 (medium risk); 2.5<HQ<3 (next higher risk); 3<HQ (high risk) (USEPA, 1989; Çulha *et al*., 2016; *Alkan et al*., 2020).

Hazard Index (HI)

The HI can be calculated by the sum of the target hazard quotients of each metal (USEPA, 1989, 2011).

$$\mathrm{HI} = \Sigma \mathrm{THQ}$$

The significance of the monthly variations of element composition was determined using the one-way analysis of variance (ANOVA). Homogeneity of variance was determined using the Levene test. Investigation of the normality of data was carried out using the Kolmogorov-Smirnov test. Differences were analyzed using the post hoc Tukey test. Differences with values of p<0.05 were considered statistically significant.

The results were also examined using principal component analysis (PCA) using R Version 3.6.1., in order to identify the element that most contributes to the monthly variations of element content. PCA-based Biplots (Gabriel, 1971) were made for each element separately. PCA transformed the raw data into unit-less variables and also distribute variability into different factors or principal components. Biplot was drawn by using principal factors, which have most of the variability. Biplot was two dimensional scatter diagram that depicted the scattering pattern of elements and months. The method has been used to display objects and variables on the same graph in principal components analysis, row and column factors in correspondence analysis of two-way contingency tables, and detect interaction in two-way analysis of variance tables (Gower and Hand, 1996). Proximity between elements or months may be gleaned from these types of plots. Also, it is commonly used to interpret the axes in the biplot and treat the coordinates as scores on these axes. In the correlation analysis graphs, dark colors indicate a very high relationship and red colors indicate a negative relationship. As the darkness of the color increases, the strength of the relationship increases. As the color approaches white, the contribution value approaches 0.

RESULTS

The monthly changes in element concentrations are given in Table 2. The most abundant microelement was Fe, followed by Zn, Mn, and Al. The average Fe value was 0.31±0.06 mg/g dry weight ,dw (0.08±0.02 mg/g wet weight, ww), the highest Fe value was measured in March whereas the lowest Fe value was measured in June, and a statistically significant difference was found between the months (p<0.05). The average Zn value was 0.11±0.02 mg/g dw (0.03±0.00 mg/g ww), and Zinc did not present clear temporal trends. Mn showed a statistically significant difference especially in March and June (p<0.05). The average value of Al per month was found to be 0.04±0.02 mg/g dw (0.01±0.00 mg/g ww). The average B value was found to be 0.01 mg/g dw only in April and June, whereas 0.02 mg/g dw in the other months, and there was a statistically significant difference between the months (p<0.05). The average Cu value was 0.01±0.00 mg/g dw (1.01±0.36 µg/g ww), and the difference between the months was statistically significant (p<0.05). Selenium (Se) was detected in smooth scallop meat in trace levels (0.01 mg/g dw, 1.18 µg/g ww) only in December, and the differences between the months were significant (p<0.05) (Table 2.)

Of the macro elements, S was generally found to be low in winter and spring, whereas high in summer and autumn. It was observed that Na is usually high in summer and autumn, low in winter and spring, and statistically different between the months (p<0.05). The lowest K value was measured in April, whereas the highest value was measured in October. P values varied in the range of 4.86-7.10 mg/g dw (1.55±0.18 mg/g ww) during the study and the differences between the months were statistically significant (p<0.05). The average value of Mg was 2.34±0.36 mg/g dw (0.56±0.09 mg/g ww) and it varied between 1.64 mg/g dw (0.38 mg/g ww) in April and 2.92 mg/g dw (0.73 mg/g ww) in July and the difference was statistically significant (p<0.05). The average value of Ca was 1.12±0.34 mg/g dw (0.22±0.27 mg/g ww) and it varied between 0.61 mg/g dw (0.15 mg/g ww) in March and 1.90 mg/g dw (0.48 mg/g ww) in July (p<0.05). (Table 2.).

According to the correlation graph of the elements, negatively correlated were found between B and Fe, Se and Mn, Al and Fe, Mn, S and Ca. A positively correlated was observed between other elements (Figure 2).

There were three dimensions with ≥1 according to their eigen values. This showed that three dimensions will be sufficient to explain all elements (Table 3.)

According to the graph of the percentage of variances that can be explained, the elements were examined in four dimensions. Among these four dimensions, the first dimension explained 58.7% of the variances, while the second dimension explained 17.92% (Figure 3). Therefore, examining the first and the second dimensions (since it explained 76.62% of the variances) was sufficient for the evaluation of all elements.

Table 2. Monthly variation of element composition in smooth scallop (mg/g dw)[1]

Element	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.
Microelement												
Fe	0.39^{cd}	0.33^{abcd}	0.32^{abcd}	0.32^{abcd}	0.29^{abcd}	0.37^{bcd}	0.29^{abcd}	0.28^{abc}	0.20^{a}	0.23^{ab}	0.34^{abcd}	0.43^{d}
Cu	0.01	0.01	0.01	0.01	0.01	0.01	ND	0.01	0.01	ND	0.01	ND
Mn	0.11^{g}	0.07^{def}	0.06^{bcdef}	0.05^{bcde}	0.08^{efg}	0.05^{bcde}	0.03^{ab}	0.04^{bcd}	0.01^{a}	0.03^{abc}	0.07^{cdef}	0.08^{fg}
Zn	0.12	0.09	0.10	0.11	0.13	0.13	0.12	0.11	0.10	0.08	0.12	0.10
Al	0.02^{abc}	0.03^{bcde}	0.04^{de}	0.03^{cde}	$0.03b^{cd}$	0.05^{f}	0.08^{g}	0.07^{g}	0.04^{ef}	0.01^{a}	0.04^{cde}	0.02^{ab}
Se	ND	ND	ND	ND	ND	0.01	ND	ND	ND	ND	ND	ND
B	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01
Macroelement												
Na	16.98^{de}	15.69^{bcde}	16.11^{bcde}	16.31^{cde}	15.27^{bcde}	18.79^{e}	13.12^{abcde}	10.97^{abc}	10.37^{ab}	9.39^{a}	13.70^{abcde}	12.25^{abcd}
Mg	2.92^{c}	2.38^{abc}	2.47^{abc}	2.47^{abc}	2.35^{abc}	$2.80b^{c}$	2.56^{abc}	2.10^{abc}	1.91^{ab}	1.64^{a}	2.30^{abc}	2.14^{abc}
K	13.63	12.17	12.76	14.44	13.08	13.42	11.38	10.48	10.59	8.04	10.61	10.31
Ca	1.90^{b}	1.37^{ab}	1.10^{ab}	1.33^{ab}	1.09^{a}	1.37^{ab}	0.96^{a}	1.01^{a}	0.61^{a}	0.72^{a}	1.06^{a}	0.96^{a}
P	6.92	6.40	6.82	6.63	6.51	6.55	6.65	6.23	7.10	4.86	6.93	5.98
S	35.01	28.22	30.85	33.66	30.86	30.67	7.10	6.53	6.30	5.35	6.98	7.25

[1]Different superscript letters within rows represent significant differences ($p<0.05$) based on results of the post hoc Tukey test.
[2]N.D.: Not detected.

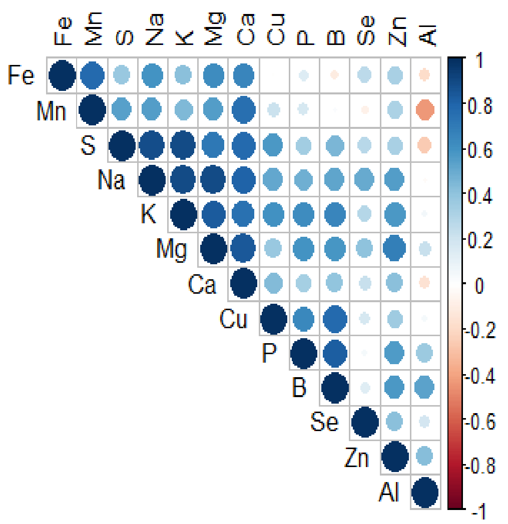

Figure 2. Correlation plot of elements (Fe, Mn, Cu, B, Se, Zn, Al, P, S, Na, K, Mg, and Ca)

Table 3. Eigen value of elements (Fe, Mn, Cu, B, Se, Zn, Al, P, S, Na, K, Mg, and Ca)

	Eigen Value	Variance (%)	Cumulative Variance (%)
Dimension 1	5.81	48.39	48.39
Dimension 2	2.52	21.02	69.41
Dimension 3	1.36	11.35	80.76
Dimension 4	0.96	7.97	88.72
Dimension 5	0.48	4.00	92.73
Dimension 6	0.35	2.89	95.61
Dimension 7	0.30	2.50	98.11
Dimension 8	0.15	1.24	99.35
Dimension 9	0.06	0.51	99.86
Dimension 10	0.01	0.10	99.96
Dimension 11	0.00	0.04	100.00

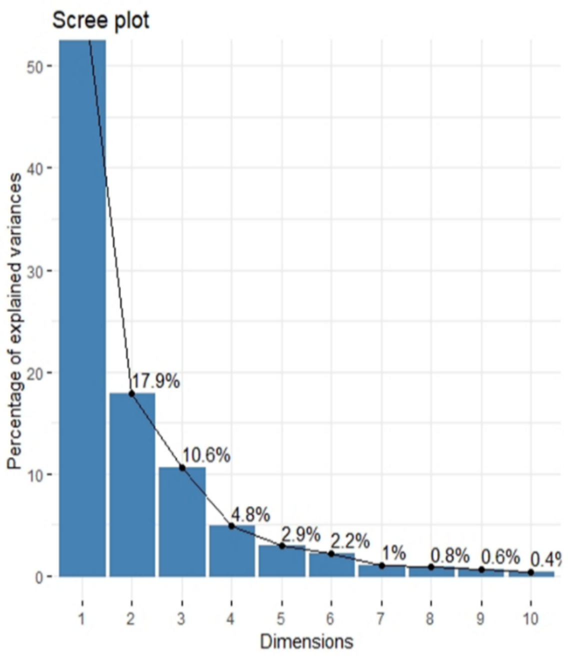

Figure 3. Percentage of explained variances of elements (Fe, Mn, Cu, B, Se, Zn, Al, P, S, Na, K, Mg and Ca)

According to the biplot graph, the contribution of Na, Mg, K, Ca, S, B elements were higher at Dimension 1. Furthermore, Al, Mn, B, and Fe elements among other elements were higher at Dimension 2. There were proximity were observed in May with Al, August with Fe, Mn, October with Se, Zn, Cu, P, December with K, October and November with Ca, S, Na, and Mg (Figure 4).

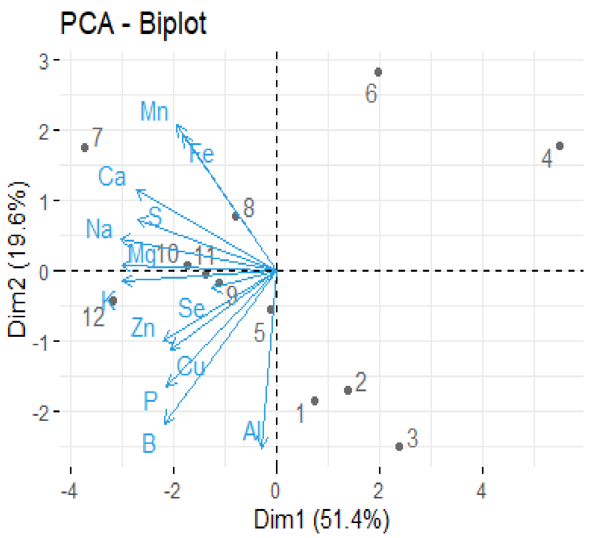

Figure 4. Principal component analysis (PCA)-Biplot of the composition of element values (Fe, Mn, Cu, B, Se, Zn, Al, P, S, Na, K, Mg and Ca,1:January,2: February,3:March,4: April, 5: May, 6: June,7:July,8:August, 9: September, 10: October, 11: November, 12: December).

THQ and HI values were used to determine the non-carcinogenic health risks posed by the elements in the consumption of smooth scallops. The THQ and HI values of the smooth scallop are given in Table 4. THQ and HI values below 1 in all months throughout the year, confirmed that continuous consumption of smooth scallop for 70 years had no adverse effects on human health.

Table 4. Rererence dose (Rfd), Estimated daily intake (EDI), Target hazard quotient (THQ) and Hazard index (HI) values of elements via consumption of smooth scallop

Elements	RfD (mg/kg bw-day)	EDI of smooth scallop (mg/70kg bw-day)	THQ of smooth scallop
Na	$2x10^{3}$	$4.83x10^{-5}$	$6.17x10^{-4}$
Mg	$3.5x10^{2}$	$8.01x10^{-6}$	$5.85x10^{-4}$
K	$2x10^{3}$	$4.04x10^{-5}$	$5.16\ x10^{-4}$
Ca	$8\ x10^{2}$	$3.87x10^{-6}$	$1.24\ x10^{-4}$
P	$3\ x10^{2}$	$2.22\ x10^{-5}$	$1.89\ x10^{-3}$
Fe	$7x10^{-1}$	$1.08\ x10^{-6}$	$3.93\ x10^{-2}$
Cu	$4x10^{-2}$	$2.16\ x10^{-8}$	$1.38\ x10^{-2}$
Mn	$1.4x10^{-1}$	$1.93\ x10^{-7}$	$3.53\ x10^{-2}$
Zn	$3x10^{-1}$	$3.77\ x10^{-7}$	$3.21\ x10^{-2}$
Al	1	$1.28\ x10^{-7}$	$3.27\ x10^{-3}$
Se	$5x10^{-3}$	$1.14\ x10^{-8}$	$5.83\ x10^{-2}$
B	$2x10^{-1}$	$5.9x10^{-8}$	$7.54\ x10^{-3}$
ΣHI			0.19

Rfd value data taken from WHO (2012), FAO/WHO (1993, 2002, 2010), Aksoy (2014), USEPA (2019a).

DISCUSSION

Bivalves taking elements not only from water and food but also from inorganic particles they digest (Warnau *et al*., 1996; El-Sikaily *et al.,* 2004). However, even though they are in the same region, the element contents in bivalves may differ. Differences in element levels in bivalves depend on the bioaccumulation capacity of each species, physiological conditions (reproductive activity, growth, filtration rate), biotic factors (age, height, and sex), genetic characteristics, abiotic factors (salinity, pH, temperature, and dissolved oxygen) depends on the chemical form of the elements, the structure of the sediment, and the area (O'Connor, 1996; Mendez *et al.*, 2001; Skinner *et al*., 2004; Fuentes *et al*., 2009; Ulutarhan *et al*., 2019).

In humans, Fe has several vital functions including transporting O_2 to tissues via the red blood cells hemoglobins, being a transport medium for electrons in cells, and being a part of important enzyme systems in various tissues (FAO/WHO, 2002). Fe is found in pigments (hemoglobin) that are found in the blood of many vertebrate and invertebrate organisms and carry oxygen and carbon dioxide for cell respiration (Clark, 1992). The increase in Fe concentrations are an indicator of the field's nutrient flow (drawdown), phytoplankton biomass, productivity (Fitzwater *et al*., 2000). Fe deficiency is seen in more than two million people around the world (Stoltzfus and Dreyfuss, 1998). Different consumption values are recommended for Fe based on the criteria including age, gender, etc. There is 300 mg Fe stored in the body, and 1 mg/day Fe in men, and 1.5 mg/day in women are excreted from the body (Aksoy, 2014). It is recommended to take 10 mg Fe for adult men and 15 mg/day for women. Fe intake is recommended as 15 mg/day (Aksoy, 2014) during pregnancy and breastfeeding, 11 mg/day for 0.5-1 year-olds (Schümann *et al*., 2007), 10 mg/day for babies and children from 6 months to 3 years (Aksoy, 2014). In the present study, the Fe values in smooth scallop were lower than those reported in the previous studies (Orban *et al*., 2007; Periyasamy *et al*., 2014).

Cu plays an important role in the functioning of various enzymes (Bajgas, 2000; Hambidge, 2000), carbohydrate metabolism, photosynthesis (Bajgas, 2000). It is also essential for the circulatory system (Walker *et al*., 2002). So it has to be consumed by humans, fish, bivalves, and other aquatic creatures (FAO/WHO, 2004). Excessive Cu has a toxic effect on the body and prevents the functions of some enzymes (Bajgas, 2000). FAO/WHO (2004) and USEPA (2019) temporarily determined the edible daily intake (EDI) as 0.4 mg/kg/day. The Cu value found in the present study was below the limits determined by FAO/WHO (2004) . It was thought that consuming 42.85 g smooth scallop daily will meet the amount recommended by FAO/WHO (2004) and USEPA (2019). Also, the Cu levels decrease when industrial and urban emissions, fertilizers, algaecides, fungicides, molluscicides, cyanobacteria in the region decrease (Moffett *et al*., 1997; Besada *et al*., 2002; Cheriyan *et al*., 2015). Cu is found in the greenish-blue pigment (hemocyanin) that carries oxygen of mollusks and crustaceans (Clark, 1992). The amount of influx and efflux of Cu element in bivalves vary among species (Cai and Wang, 2019).

The dissolved concentration of Zn in seawater ranges around <0.1 nmol/kg in surface waters. Zn plays a role in the synthesis of some enzymes that affect CO_2 and P uptake, photosynthesis, phytoplankton growth and microalgal growth, and the catabolism of carbohydrate, fat, protein, and nucleic acids (Morel *et al*., 1994; Bajgas, 2000; FAO/WHO, 2002; Shaked *et al*., 2006; Shariati and Yahyaabadi, 2006). In the present study, the Zn value was found to be lower than those in *P. maximus* (12.6-16.9 mg/kg wet meat) (Manthey-Karl *et al*., 2015), *D. incarnatus* (0.34 mg/g dry meat) (Periyasamy *et al*., 2014), and *C. gallina* (0.91-1.48 mg/100g wet meat) (Orban *et al*., 2007). Since Zn is an essential element observed in the cell wall of bacteria and diatoms, the death or decay of these microorganisms increases the amount of this element in the upwelling zone where coastal water and seawater are mixed (Lewis and Luther III, 2000; Monteiro and Roychoudhury, 2005; Baines *et al*., 2016; Grasse *et al*., 2016). In the present study, Zn values of smooth scallop were found to be safe for human consumption. Based on FAO/WHO (2004), it can be thought that consuming at max 350 g per week (7 g per day) would be sufficient.

The daily Al consumption should be 4-9 mg (Hellström-Westas and Rosen, 2006). The tolerable daily intake has been reported as 1 mg/kg body weight (WHO,1996, USEPA, 2019). Examining the values found in the present study, consuming 50 g per week(7 g per day) of the smooth scallop collected from the Çardak Lagoon is within the limits determined by EFSA (2013).

Se is an essential micronutrient with selenoproteins that perform various metabolic functions in animals and humans (Wen and Hu, 2010; Rayman, 2020). Many of the selenoproteins affect antioxidant metabolism (Steinbrenner *et al*., 2016). Se plays a role in protection from infections, promotes growth and development (FAO/WHO, 2002). Thyroid dysfunction may occur in Se deficiency. The Se concentration in the blood should be 60-100µ. Chronic high consumption of Se can cause changes in nail morphology, hair loss, diarrhea, central nervous system disorders, anorexia, kidney and liver damage (Selinus *et al*., 2005). The Se values in sediments and living things in the world vary due to different environmental conditions and agricultural practices (FAO/WHO, 2002). A daily intake of 55-75 µg Se is recommended (Aksoy, 2014). In the present study, Se was detected in trace levels in smooth scallop meat only in December and below the detection limit in other months. Therefore, it can be argued that consuming 5.5-7.5 g of smooth scallops per day from the Çardak Lagoon during December will meet the daily requirement.

Turkey ranks first in the world in the B source (Yiğitbaşıoğlu, 2004). B can be obtained from both foods and water. Evaluating the B element together with Ca, P, and Mg elements, contributes to the protection of vitamin D in the body (sufficient quantity of boron prevents vitamin D deficiency and, hence, osteoporosis and weakening and breaking of the bones) (Yakıncı and Kök, 2016). The B element is also effective in preventing the loss of Ca element in the body and in the transport of Ca element between cells (Aksoy, 2014). In the present study, a positively correlated was found between Ca and B ($p<0.05$). For adults, a daily intake of 0.4-0.5 mg/kg body weight B is recommended (CODEX, 2007). In line with the results obtained from the present study, it can be argued that the smooth scallop in the lagoon contains B elements throughout the year, and consuming 40 g smooth scallop per day will be sufficient to meet the B requirement.

Although the presence of 77 elements in seawater was detected, only 7-8 of them were abundant. In order of abundance, these elements are Cl, Na, Mg, S, Ca, K, Br, and C (Bat *et al*., 1998). In the present study, it was observed that the elements contained in the flesh of the smooth scallop were in a similar order except for sulfur. The anoxic nature of sedimentary affects the amount of sulfur in the water column (Büyükateş, 2017). In the present study, it can be argued that the high values of sulfur in the spring and summer months when the temperature increased was associated with the fact that the sediment was anoxic and, as a result, the water column was affected by this situation. In other words, the water properties of the environment may have affected the sulfur content of the smooth scallop.

In the present study, the Mg values in smooth scallop were much lower than those in *P. maximus* (330-356 mg/kg ww) (Manthey-Karl *et al*., 2015), *C. gallina* (74.3-89.4 mg/100g ww) (Orban *et al*., 2007), and *D. incarnatus* (60.54 mg/g dw) (Periyasamy *et al*., 2014). Mg is found in shell formation in small amounts as Mg-calcite (Broadaway, 2012). In the present study, it can be argued that the Mg in the smooth scallop will decrease due to its contribution to the shell growth in the spring. Evaluating in terms of nutrition, the daily recommended consumption is 350 mg for men and 280 mg for women (Aksoy, 2014). It can be argued that it would be sufficient to consume 500-628 g smooth scallop per day purchased from the Çardak Lagoon to meet this amount.

As a result of the death of organisms, a significant part of organic P dissolved or in particulate form in seawater turns into inorganic phosphate with the effect of phytoplankton species, the remaining part turns into inorganic phosphate by bacteria and this inorganic P turns into organic matter that phytoplankton and other marine creatures can benefit from (Karl *et al*., 1995; Geldiay and Kocataş, 1988; ÇŞB, 2020). Although P in seawater is maximum in summer, it was observed to decrease in autumn and reach a minimum level in the winter months. However, it was concluded that these changes depend on the regions (Geldiay and Kocataş, 1988). In the present study, although the P value in smooth scallop was stable, it was generally the lowest in spring whereas highest in autumn. Also, the results obtained in the present study were similar to those reported by Orban *et al.* (2007) for *C. gallina* (108-177 mg/100g wet meat). The recommended daily P consumption is 300-500 mg (Aksoy, 2014). Therefore, it can be argued that consuming 300-500 g smooth scallop per day in the present study will meet the P requirement.

Ca is of importance since it provides rigidity to the skeleton and plays an important role in many metabolic processes (FAO/WHO, 2002). Smooth scallop's shell consists of $CaCO_3$. In the present study, one of the reasons for the decrease in this element in spring may be the contribution of Ca to the shell formation. Ca value determined in smooth scallop in the present study was lower than those reported in previous studies (Orban *et al*., 2007; Manthey-Karl *et al*., 2015). In terms of human consumption, the daily requirement is recommended as 800 mg, and this value increases to 1200 mg for pregnant and breastfeeding women (Aksoy, 2014). In general, it can be recommended to consume daily 2 kg of smooth scallop in the Çardak Lagoon. In previous studies, it was stated that the macro element with the highest value in smooth scallop meat is usually the K element (Bilandzic *et al*., 2015, Manthey-Karl *et al*., 2015). The values found in this study were lower than the values found for *P. maximus* (3860-4118 mg/kg wet meat) (Manthey-Karl *et al*., 2015), *C. gallina* (221-257 mg/100g wet meat) (Orban *et al*., 2007), *D. incarnatus* (20.36 mg/g dry meat) (Periyasamy et al., 2014). The daily recommended K consumption for a healthy nervous system and a steady heart rhythm is 2000 mg (Aksoy, 2014). In this study, it can be recommended to consume 167 g of smooth scallop per day to meet the amount needed. The Na values obtained in the present study were higher than those reported in the previous studies (Orban *et al*., 2007; Manthey-Karl *et al.,* 2015), however, the values exhibited a similar pattern of change during the year (Orban *et al.,* 2007). The recommended daily minimum Na consumption is 500 mg (Aksoy, 2014) and the maximum recommended value is 2 g (WHO, 2012). According to the Na values in this study, it was thought that consuming 167 g of smooth scallop meat per day, especially in autumn and summer, will meet the daily Na amount required. Mn is one of the vital microelements. It is also a structural component of some enzymes (Wen and Hu, 2010). Considering the Mn values determined in the present study, the consumption of 13-33 g smooth scallop is within the limits determined by CODEX (2007).

CONCLUSION

The results obtained in the present study provide valuable information for both smooth scallop breeders and the consumers. For smooth scallop consumers, the data obtained from the present study can be a guide for the months, and consumption amounts for the smooth scallop in this region. Since the smooth scallop in Çardak Lagoon meets the criteria set by the Ministry of Agriculture and Forestry in terms of element composition, its cultivation is recommended in this region.

It was observed that *F. glaber* individuals collected from Çardak Lagoon were suitable for consumption in terms of their element content. Also, in the present study, the heavy metal content of smooth scallop meat was below the limit values specified by organizations such as the Turkish Food Codex, WHO, FAO, etc. In addition, the fact that the THQ and HI values of the smooth scallop were below 1 in all months throughout the year confirmed that their consumption did not have a negative effect on human health.

ACKNOWLEDGEMENTS

This study was supported by Çanakkale Onsekiz Mart University The Scientific Research Coordination Unit, Project number: FDK/1349. We would like to thank Asst. Prof. Dr. Burcu Mestav, who contributed to the statistical analysis of this article. We would also like to thank Assoc. Prof. Dr.Bayram Kızılkaya and Assoc. Prof. Dr. Semih KALE for their valuable comments. Lab experiments were carried out in the laboratory of Biochemistry, and the laboratory of Feed and Food Analysis, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 461-466 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.07

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

About the identity of *Granulina pusaterii* Smriglio & Mariottini, 2003 (Volutoidea: Granulinidae) from the Tunisian Plateau

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Received date: 15.05.2021 *Accepted date: 24.09.2021*

How to cite this paper:
Boyer, F., Renda, W. & Pagli, A. (2021). About the identity of *Granulina pusaterii* Smriglio & Mariottini, 2003 (Volutoidea: Granulinidae) from the Tunisian Plateau. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 461-466. DOI: 10.12714/egejfas.38.4.07

Abstract: On the ground of an extensive study of topotypes and of the shell morphology documented in comparable Mediterranean populations, *Granulina pusaterii* Smriglio & Mariottini, 2003 described from the Tunisian Plateau is proved to belong to the natural variability of *G. melitensis* Smriglio, Mariottini & Rufini, 1998 and it is proposed as junior synonym name of the latter.

Keywords: Granulinidae, *Granulina*, Mediterranean Sea, shell morphology, variability, synonymy

INTRODUCTION

Since the pioneering work of Gofas (1992), several new species of *Granulina* were described from the Mediterranean waters by various authors, most from circalittoral or upper bathyal levels, and a supraspecific revision of the granuliniform gastropods was proposed by Boyer (2017). More recently, few works were dedicated to the phenetic variability occurring in some *Granulina* species from Mediterranean, and partial specific revisions were proposed (Boyer *et al*., 2017 and Boyer *et al*., 2020). In most cases, an extensive documentation about the chromatism of the soft parts remains to be gathered for conclusive revisions. In some occurrences, it seems however that the shell morphology data allow by themselves to reach robust conclusions, if based on a representative material.

This consideration leads to dedicate the present article to a shell morphology-based revision of the taxon *G. pusaterii* Smriglio and Mariottini (2003) only reported from its type locality on the border of the Tunisian Plateau and never revised since its description. The identity of *G. pusaterii* has been originally defined by comparison with *G. melitensis* Smriglio *et al*.(1998), described from Malta, so the present work will proceed as a comparison between these two taxa, referring both to the morphologic variability displayed by a lot of topotypes of *G. pusaterii* recently discovered in the collection of Attilio Pagli, and to the morphologic variability of *G. melitensis* extensively pictured by Boyer *et al*. (2020) in their recent revision of the granulinid fauna from the coasts of Turkey.

MATERIAL AND METHODS

The documentation of this work is based on the variability of *G. melitensis* as documented by Boyer *et al*. (2020) in the frame of their study of the *Granulina* fauna from the waters of Turkey, and on an important lot of topotypes of *G. pusaterii* studied more recently by the authors. This lot of 181 topotypes constitutes the main part of a sampling of 201 similar shells sorted out of from shell grit dredged at 100 m on the Tunisian Plateau, 60 miles off Sfax, and saved by Dr Marco Taviani: 20 shells were selected firstly by Carlo Smriglio and Paolo Mariottini, as type material of their new species *G. pusaterii*, and the 181 remaining shells were sorted out later from grit of the same sample by the third author without conclusive identification.

The holotypes of *G. melitensis* and of *G. pusaterii* have been studied on the ground of new photos performed at higher resolution by Antonio Bonfitto (Museo di Zoologia di Bologna).

For the presentation of the morphologic variability in the nominal *G. melitensis*, the reader will refer to the extensive iconography given in Boyer *et al*. (2020) (Figures 6A-P, 10A, 10C-D, 10P, 11D, 11M-P), whereas the morphologic variability at work in the taxon *G. pusaterii* is illustrated herein through six contrasted topotypes (Figures 1G-I; 2A-I). The tentative of making morphometric graphics of this multifactorial variability range proved to be not operative neither probative in the present case, and the direct comparison of referred pictures

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looks to give more valuable information than any superposition of scatter graphs.

Abbreviations and Acronyms

BEL: Benthic Ecology Laboratory, University of Messina.

ISMAR : Istituto di Scienze Marine, Bologna.

MZB : Museo di Zoologia di Bologna.

APC : Attilio Pagli Collection.

FBC : Franck Boyer Collection.

WRC : Walter Renda Collection.

spm : specimen.

L : length.

Ib : ibidem, like the previous bibliographic reference.

e. g.: “exempli gratia”, for instance.

RESULTS

In the present study, *Granulina pusaterii* Smriglio & Mariottini, 2003 and *G. melitensis* Smriglio, Mariottini & Rufini, 1998 have been compared for their shell morphology on the ground of their respective type material, of topotypes of *G. pusateri*, and of extensive samples of *G. melitensis* previously studied by Boyer *et al*. (2020). This morphologic comparison is evidencing that *G. pusateri* belongs to the natural variability of *G. melitensis*.

Systematics

Phylum: Mollusca

Class: Gastropoda

Order: Neogastropoda

Superfamily: Volutoidea Rafinesque, 1815

Family: Granulinidae Coovert & Coovert, 1995

Genre: *Granulina* Jousseaume, 1888

Type-species: *Marginella pygmaea* Issel, 1869 (non-*Marginella pygmaea* Sowerby, 1846) = *Marginella isselli* Nevill and Nevill, 1875 (nom. nov.). By monotypy.

Granulina melitensis (Smriglio *et al.*, 1998) (Figure 1, A-C). Smriglio *et al*. (1998) (pp. 53-56, figures 1-7)

Type material

Holotype MZB (Figure 1, A-C), L= 2.2 mm, and 11 paratypes in the collections Smriglio, Mariottini, Ruffini and Engl.

Other material examined

Out of the material refered and illustrated in Boyer *et al*. (2020):

APC: 181 spm, 60 miles off Sfax, 100m.

FBC: typical form = 1 spm, Anzio, Coste Laziali, 250 m; large angular form = 11 spm, Capri Island, 200 m; 4 spm, Karpathos Island, no depth datum.

WRC: thick inflated form = 5 spm, Egadi Islands, 500 m.

BEL: 11 spm, Eolie Islands, 300 m.

The species was originally described by Smriglio *et al*. (1998: 53-54): « *Specie di piccola taglia, l’olotipo misura 2.2 x 1.4 mm, di forma ovoidale allungata anteriormente, con l’ultima spira avvolgente tutto l’insieme dei giri precedenti, che presenta una rottura del profilo anteriore accentuata. Rostrazione pronunciata e posta centralmente rispetto l’asse de avvolgimento del nicchio ; apertura boccale stretta ed allungata, labro ispessito che internamente presenta una serie di denticolazioni fitte e poco rilevate. La parete labiale esterna, osservata lateralmente si presenta notevolmente inspessita, quasi callosa, e nella parte terminale posteriore si arcua all’indietro verso il dorso del nicchio. La columella presenta quattro pliche che si raddopiano all’interno dell’apertura, e sono separate da una depressione, poco visibile, posta longitudinalmente. Superficie del nicchio liscia, lucida e brillante ; colorazione bianco lattea, lievemente trasparente nelle conchiglie fresche. Le dimenzioni medie di G. melitensis, calcolate dopo la misurazione di tutto il materiale tipico, sono pari a 2.1 x 1.4 mm, il rapporto H/D é pari a 1.49* ».

Type locality

Malta, 100-120 m.

Distribution

Documented from the northern Alboran Sea, the Tyrrhenian Sea, the Ionian Sea, Malta, the Gulf of Gabès, the Aegean Sea and the Turkish Levantine and Aegean coasts.

Remarks

Boyer *et al*. (2020) reported that the shell of *Granulina melitensis* seems to present a morphologic cline in Western Mediterranean, with transitional forms getting closer to the thick and rounded *G. minusculina* (Locard, 1897) in the Alboran Sea and parts of the Tyrrhenian (e.g.: Eolie Islands, 300 m and Egadi Islands, 500 m). Other distinctive variants are occurring in the Tyrrhenian, like a large angular form observed off Capri Island at 200 m, as well as various intergrades linking these variants to the typical form of *G. melitensis*.

In the Eastern Mediterranean Basin, the shell morphology of *G. melitensis* looks as less contrasted, even if a noticeable variability in observed in geographical populations, as demonstrated by Boyer *et al.* (2020) about the Turkish waters.

In their description article, Smriglio *et al.* (1998) are underlining the originality of the apical labrum morphology, looking as bending backwards when viewed laterally [Smriglio *et al*. (1998, 54), Description: " *The top of the posterior end of the outer lip is bending towards the dorsum of the shell*"], what is considered by them as a distinctive specific feature at the scale of the Mediterranean fauna (ib., Discussion : "character unmistakable for the identification of the species").

Granulina pusaterii Smriglio and Mariottini (2003) (Figure 1, D-I and Figure 2, A-I).Smriglio and Mariottini (2003) (pp. 286-288, figures 737a-740b).

Type material.

Holotype MZB-14683 (Figure 1, D-F), L = 1.8 mm, and 19 paratypes in the collections Smriglio and Mariottini (2003).

Other material examined

APC : 181 spm, 60 miles off Sfax, 100 m (topotypes).

Original description

Smriglio and Marriottini (2003: 286): « *Species small (holotype: 1.8 (H) x 1.3 (D) mm), ovoid in shape slightly pyriform, last whorl enclosing all the previous whorls, a break in the anterior profile just traced. Posterior rostration weak, anterior rostration slightly elongated, semi-central with respect to the columellar axis. Aperture narrow; outer labial varix not too thick, showing interiorly a series of small teeth, few, inequal and weak. Four strong columellar plicae. Surface of the shell smooth and bright. Colour milky white, slightly translucent even in fresh specimens. No microsculpture evident even at strong magnification* ».

Type locality

60 miles off Sfax, 100m.

Distribution

Only referred from the type locality.

Remarks

The name *Granulina pusaterii* is attributed by Smriglio and Mariottini (2003) to a phenon said to be « similar to *Granulina melitensis* Smriglio, Mariottini and Rufini, 1998, from which (it) differs by the presence of strong diagnostic characters, as follows:

1. smaller average size (1.8 x 1.3 mm) instead of 2.1 x 1.4 mm in *G. melitensis*,

2. general shape more inflated and less slender (H/D ratio=1.38), *G. melitensis*=1.49,

3. labial teeth smaller and more irregularly spaced,

4. stronger and more evident columellar plicae.

DISCUSSION

The type material of *G. pusaterii* is made of 20 specimens sorted out from a set of 201 similar shells matching closely the typical form of *G. melitensis*. The holotype of *G. pusaterii* (Figures 1D-F) looks as slightly diverging from the typical form of *G. melitensis* (Figures 1A-C) by its less pyriform and more oval outline, and by its less produced upper rostrum. Paratypes A and D selected by Smriglio and Mariottini (2003: figures 238a-b and 240a-b) are more oval-shaped, whereas paratype B (ib: figures 239a-b) is matching more closely the typical form of *G. melitensis*, despite a less sharp upper rostrum. The type material selected by Smriglio and Mariottini (2003) as representative of their new taxon *G. pusaterii* looks as corresponding to the 20 most « oval-shaped shells » among a series of 201 specimens belonging to the variability range of *G. melitensis*, even if this Tunisian population is showing to be less strongly rostrated in its whole than the holotype of *G. melitensis* (Figure 1, G-I and Figure 2, A-I). We note that various topotypes of *G. pusaterii* are intergrading between the oval and low-rostrated specimens selected in the type series of *G. pusaterii* and the typical form of *G. melitensis*: the holotype of *G. pusaterii* (Figure 1, D-F) is for instance matching closely the topotype illustrated in Figure 2, D-G and the three paratypes of *G. pusaterii* pictured in Smriglio and Mariottini (2003) (ib.) are closely matching the topotype illustrated in Figure 2, F-I.

On the ground of the variability observed in the population from the type locality of *G. pusaterii*, the comparison made by Smriglio and Mariottini (2003) between the « more oval shaped » *G. pusaterii* and the « more pyriform » *G. melitensis* must be corrected:

1. The specimens selected as type material of *G. pusaterii* are ranging from 1.7 mm to 2.0 mm, versus 1.8 mm to 2.1 mm for the 181 topotypes, whereas the type material of *G. melitensis* is sizing 1.8 mm to 2.2 mm. So the phenon *G. pusaterii* cannot be distinguished on the simple ground of its length size. We note that the diversified populations of *G. melitensis* observed from the coasts of Turkey are sizing 1.9 mm to 2.35 mm, that means a bit larger than the type material from Malta and the population studied from the Tunisian Plateau.

2. The more rounded and squatter look of *G. pusaterii* is just coming from the fact that the authors did select the specimens presenting the lowest rostrum. As a result, their length size is also shorter. Such « low rostrated » and « poorly pyriform » shells belong to the natural variability of *G. melitensis*, as demonstrated by Boyer *et al.* (2020) about the populations studied from the coasts of Turkey. In fact, all the intergrades are represented among the topotypes of *G. pusaterii*, from rather oval outline (Figure 2C) to strongly rostrated (Figure 1, G-I) and to heavily pyriform (Figure 2, E- H).

3. Tiny and irregularly spaced labial teeth are also found in more rostrated shells (Figure 2C), as well as quite smooth inner labrum (Figure 2, E-F). The size, number and distribution of the labial teeth prove to be deeply variable in *G. melitensis* (Boyer *et al*., 2020).

4. Strong and produced columellar plaits are also found in rostrated and pyriform shells (Figure 1, G-I). The position and the orientation of the columellar plaits show to be rather constant in *G. melitensis*, but their thickness and their elevation show to be noticeably variable (Boyer *et al*., 2020).

And last, we must notice that the holotype of *G. pusaterii* (Figure 1, D-F) as well as its topotypes (Figure 1, G-I) are all showing their apical labrum bending backwards, what was considered by the describers themselves as a specific feature characterizing *G. melitensis*, when they described this species (see above).

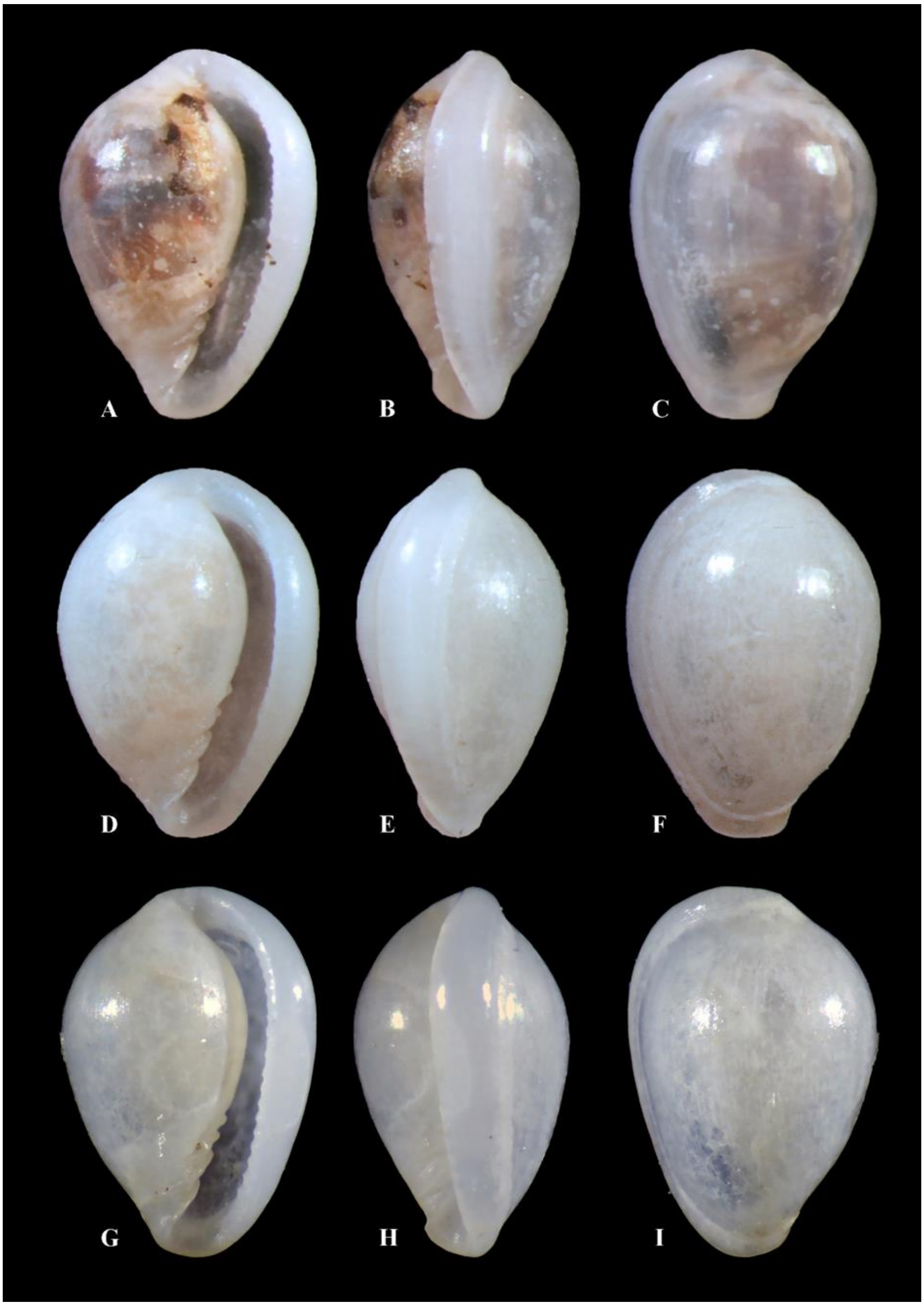

Figure 1. A-I. A-C. *Granulina melitensis*, holotype MZB, Malta, 100-120m, L = 2.2 mm. **D-F**. *G. pusateri*, holotype MZB, off Sfax, 100 m, L = 1.8 mm. **G-I**. *G. melitensis*, off Sfax, 100 m, L = 2.0 mm, APC.

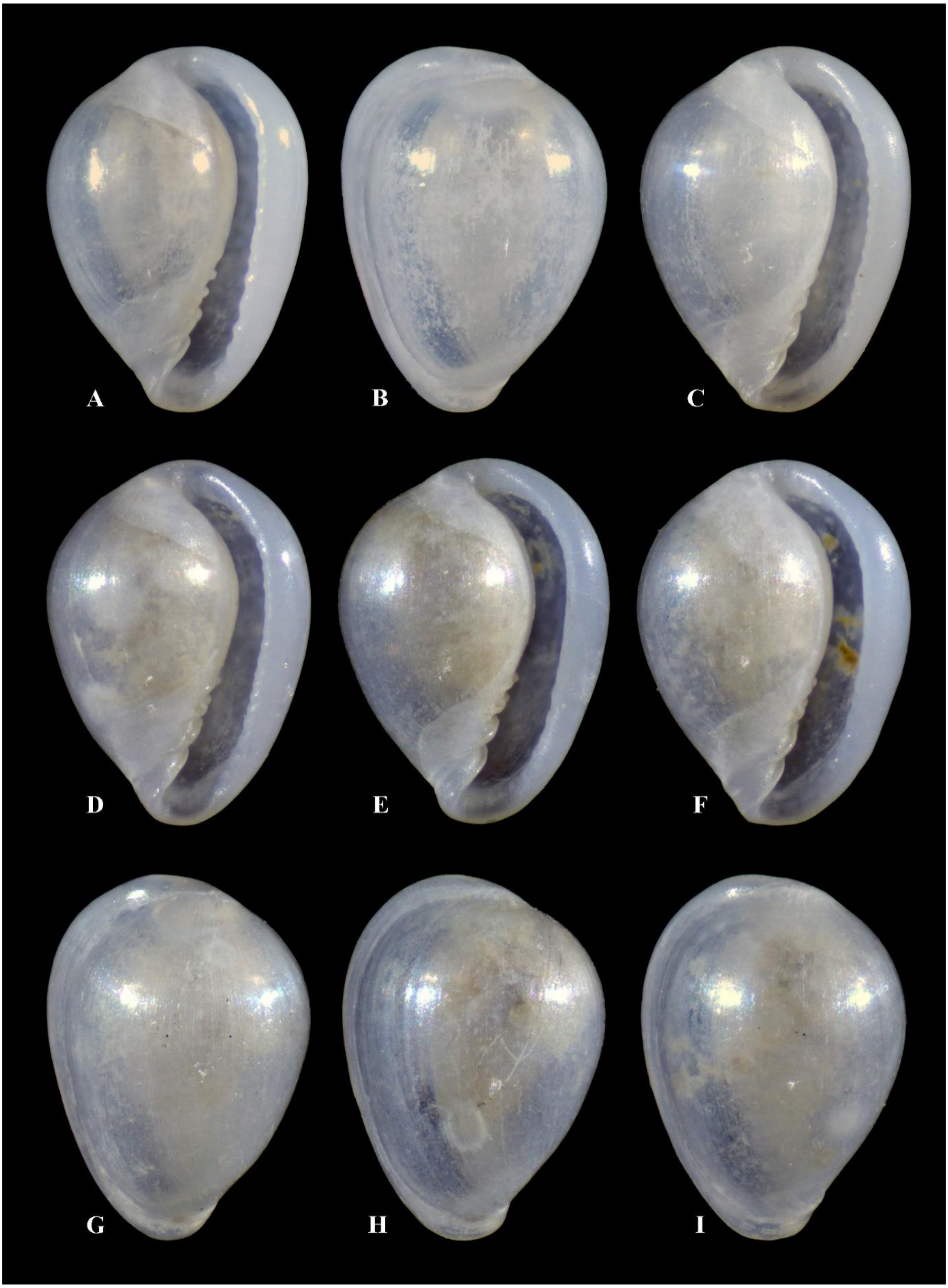

Figure 2. A-I. A-B. *Granulina melitensis*, off Sfax, 100 m, L = 2.1 mm. **C**. idem, L = 2.0 mm. **D**-**G**. idem, L = 2.0 mm. **E-H**. idem L = 2.0 mm. **F**-**I**. idem, L = 1.8 mm. All from APC.

CONCLUSION

On the ground of the shell morphology studied on a diversified material from its type locality (type material and topotypes), *G. pusaterii* shows to belong to the natural variability of the species *G. melitensis* and it must be considered as junior synonym name. In its whole, the population studied from the Tunisian Plateau looks to have slightly smaller and less rostrated shells than the typical population from Malta and the populations studied from the coasts of Turkey (Boyer *et al*., 2020). The higher disparities observed in the populations from Turkey is possibly coming from the wide dispersion of the numerous documented stations and from the diversified depths of the stations at infralittoral and circalittoral levels.

ACKNOWLEDGEMENTS

Thanks are due to Dr Marco Taviani (ISMAR) for the access to the shell grit sampled from the Tunisian Plateau. We are also greatly indebted to Pr Antonio Bonfitto (MZB) who performed sharp photos of the holotypes of *G. melitensis* and of *G. pusateri,* and to Prof. Salvatore Giacobbe (BEL) for the loan of specimens from the Aeolian Islands. We are also indebted to Prof. Dr. Bilal Öztürk (EGE University, Izmir) for adjusting the form of the manuscript and for his constant help.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 467-477 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.08

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Diversity of benthic macroinvertebrates and water quality of Karasu Stream (Black Sea)

Karasu Deresi'nin (Karadeniz) bentik makroomurgasız çeşitliliği ve su kalitesi

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Received date: 31.03.2021 *Accepted date: 11.10.2021*

How to cite this paper:
Aydemir Çil, E., Özbek, M., Yardım, Ö., Yıldız, S., Taşdemir, A., Rasouli, H. & Gürbüzer, P. (2021). Diversity of benthic macroinvertebrates and water quality of Karasu Stream (Black Sea). *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 467-477. DOI: 10.12714/egejfas.38.4.08

Abstract: The study was conducted in Karasu Stream (Sinop Province, Black Sea Region of Turkey). The purpose of this study is, to determine the benthic macroinvertebrate composition of the stream, together with some of its environmental characteristics (water temperature, pH and dissolved oxygen) to evaluate the trophic level of the stream. Samplings of benthic macroinvertebrates and environmental variables were performed monthly at ten stations between February 2013 and January 2014. As a result, 18260 specimens were investigated and 175 taxa were determined. Chironomidae and Oligochaeta were the higher groups in terms of species richness with 48 and 38 taxa, respectively. The BMWP and ASPT indices indicate that all the stations belong to "slightly polluted (Class II)" or "unpolluted (Class I)" water quality levels.

Keywords: Stream, water quality, benthos, macroinvertebrate, Turkey

Öz: Çalışma; Karasu Çayı'nda (Türkiye'nin Karadeniz Bölgesi, Sinop İli) yürütülmüştür. Bu çalışmanın amacı, akarsuyun bentik makroomurgasız tür kompozisyonunu belirlemek, bazı çevresel özellikleri (su sıcaklığı, pH ve çözünmüş oksijen) ile birlikte akarsuyun trofik seviyesini değerlendirmektir. Makrobentik omurgasızların ve çevresel değişkenlerin örneklemesi, Şubat 2013 - Ocak 2014 tarihleri arasında 10 istasyonda aylık olarak gerçekleştirilmiştir. Sonuç olarak, 18260 örnek incelenmiş ve 175 takson belirlenmiştir. Tür zenginliği açısından Chironomidae ve Oligochaeta sırasıyla 48 ve 38 takson ile en yüksek gruplardır. BMWP ve ASPT endeksleri, tüm istasyonların "hafif kirli (Sınıf II)" veya "kirlenmemiş (Sınıf I)" su kalitesi seviyelerine ait olduğunu göstermektedir.

Anahtar kelimeler: Akarsu, su kalitesi, bentos, makroomurgasız, Türkiye

INTRODUCTION

The physico-chemical parameters can reflect temporary water quality levels of the region and cannot give reliable data about the amount of pollution in streams or rivers. Which can be helpful but insufficient when considering a long-term water quality assessment (Demir, 2005) but organisms such as macroinvertebrates, fish, etc. can be more adapted to a specific environment. Benthic macroinvertebrates constitute a major component of the aquatic biota in freshwater environments. Most of them have constricted ecological demands and are very beneficial as bioindicators in determining the characteristics of aquatic environments (Benetti and Garrido, 2010). They are the group of organisms most frequently used in biomonitoring studies of running waters because their responses to all kind of pollution have been extensively proven (Thorne and Williams, 1997).

Various studies have been conducted on the assessment of benthic macroinvertebrates in Sinop province (Akbulut,1996; Bat *et al*. 2000; Akbulut, 2001; Akbulut *et al.* 2001;2002; Ertorun and Tanatmış, 2004; Öktener, 2004; Tanatmış, 2004; Şendoğan, 2006; Tanatmış and Ertorun, 2008; Yardım *et al*. 2008; Aydemir-Çil, 2014; Yardım *et al*. 2017). There is no study carried out on the diversity of benthic macroinvertebrate of the Karasu Stream.

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The objective of this study is to determine both the benthic macro-invertebrate composition of the stream and some environmental parameters (water temperature, pH, and dissolved oxygen) and to assess the ecological quality of the stream

MATERIALS AND METHODS

Karasu Stream, which has approximately 80 km in length, originates from Boyabat district, passes along Erfelek town in Sinop province, and flows into the Black Sea (Figure1). The stream and Erfelek Dam supply the drinking water of the surrounding settlements.

Environmental variables and benthic materials were sampled at 10 sites between February 2013 and January 2014 in monthly intervals (Figure 1, Table 1). In total, seven sites were located on the mainstream (two of them – upstream the dam) and three sites were on tributaries. Karasu River flows into the Black Sea through an estuarine system, thus the sites A1 and A2 are under the influence of water from the sea in spring and winter due to waves and currents. The A3 and A6 sites are located on Karasu Creek, while the A4, A5 and A7 sites were selected from the tributaries of the stream. A9 and A10 were selected before the Erfelek Dam. The substrate types, vegetation, and geographic data of the sites are given in Table 1.

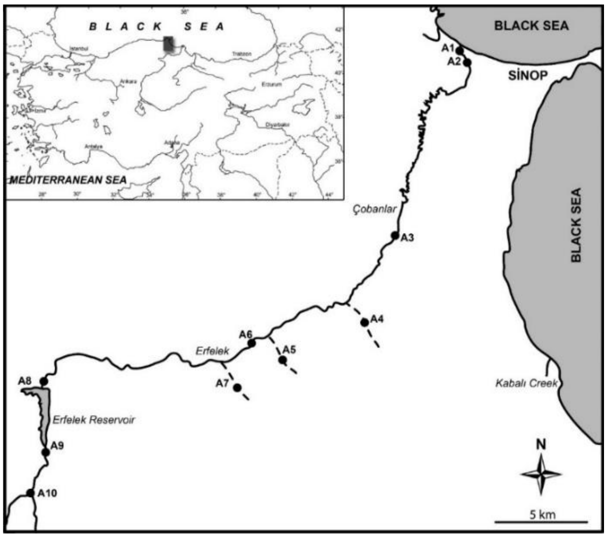


Figure 1. The geographical location of the study area (Karasu Stream) and the sampling sites.

The environmental variables were measured using Hanna 9829 HI model multiparameter device. Samples were collected by the Kick-sampling method (Letovsky *et al.* 2012) from a 1 m^2 area with a 5-minute collection standard (kick-net mesh size 180 µm).

Table 1. Geographical and ecological data about the sampling sites

Station	Latitude	Longitude	Substrate Type	Depth(m)	Riparian Vegetations
A1	42°01'56"N	35°03'33"E	sand and mud	0-4 m	grassland and reedbed
A2	42°01'52"N	35°03'34"E	sand and mud	0-4 m	grassland and reedbed
A3	41°55'05"N	35°06'14"E	stone and mud	0-1 m	grassland and moss
A4	41°54'16"N	34°59'49"E	mud	0-0.5 m	grassland
A5	41°53'32"N	34°56'04"E	mud	0-0.5 m	grassland
A6	41°52'46"N	34°51'23"E	stone and mud	0-0.5 m	grassland and *Chara* sp.
A7	41°52'59"N	34°47'45"E	rocks and mud	0-1 m	grassland and green algae
A8	41°50'53"N	34°46'31"E	calcareous rocks	0-0.5 m	-
A9	41°50'26"N	34°46'47"E	mud	0-0.5 m	grassland and *Astiboles* sp.
A10	41°49'24"N	34°46'17"E	rocks and mud	0-1 m	grassland and moss

The samples were transferred into 500-1000 ml plastic jars and fixed in 4% formalin solution in the field. Then, in the laboratory, the collected materials were washed under tap water to remove formaldehyde and filtered through 0.5- and 1-mm sieves to sort the macroinvertebrates based on size. The, organisms were transferred into small jars with 75% ethanol solution.

Fauna Europaea (2021) database was used for the current names of the taxa. All the macroinvertebrate samples were identified to the genera-species level whenever possible.

The Index of Diversity (H') (Shannon and Weaver, 1949), Pielou's Evennes Index (J') (Pielou, 1975), Similarity Index (Brayand Curtis, 1957), frequency (Soyer, 1970), and dominance values (Bellan-Santini, 1969) of the determined taxa were calculated and used to describe the characteristics of the sites and Karasu Stream. For assessing the water quality of the studied locations, Biological Monitoring Working Party (BMWP) scores (Paisley *et al.* 2013) and Average Score Per Taxon (ASPT) (Armitage *et al.* 1983) were calculated. These scores were obtained from ASTERICS 3.3.1 (AQEM/STAR Ecological River Classification System; AQEM Consortium 2002) software.

The similarity of the studied localities followed by cluster analysis (UPGMA, Unweighted Pair Group Average) was calculated starting from the quantitative data of the macroinvertebrate taxa; the Multivariate Statistical Package (MVSP) program version 3.1 (Kovach, 1998) was used to perform the cluster analysis.

The results of the physico-chemical measurements and biological analysis were evaluated according to the National Surface Water Quality Regulations of Republic of Turkey Ministry of Agriculture and Forestry (Anonymous, 2016) to classify the water quality levels of the sites.

RESULTS

Environmental variables

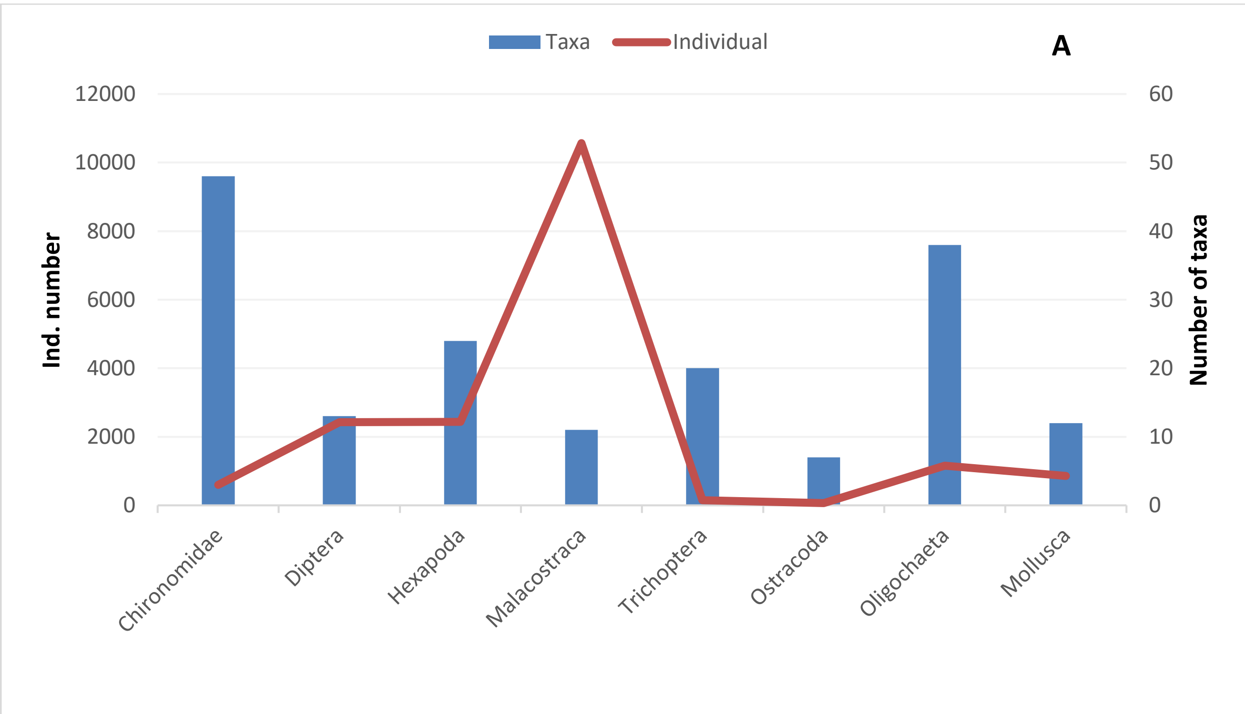
During the study, the lowest water temperature (4.06 °C, in February) was observed at the A5 station while the highest (27.6 °C, in August) was measured at A1 and A2 sites. The dissolved oxygen value (DO) was the highest at the A1 station (17.7 mg/l, in April) and the lowest at the A4 station (1.07 mg/l, in July). Similarly, the highest pH value was observed at the A2 station (11.62) in January and the lowest at the A10 station (6.18) in May. The general pattern of the pH values suggests that Karasu Stream has a slightly alkaline character (Table 2).

Table 2. Maximum and minimum values of the measured environmental variables (T: water temperature, DO: dissolved oxygen)

Months	T (°C)	pH	DO (mg/l)
February	4.06 - 6.73	8.09 - 8.48	5.7 - 8.3
March	4.08 - 8.03	8.27 - 10.44	9.18 - 11.32
April	6.66 - 10.9	7.48 - 8.39	10.7 - 11.7
May	8.34 - 14.7	6.18 - 8.57	8.09 - 11.1
June	7.81 - 14.8	8.3 - 9.17	4.8 - 8.76
July	7.5 - 17.2	7.92 - 8.41	1.07 - 8.5
August	12.7 - 27.6	7.79 - 8.85	1.24 - 5.47
September	12.7 - 24.6	7.64 - 8.27	3.7 - 9.7
October	13.6 - 27.5	8.18 - 9.04	1.9 - 10.4
November	11.2 - 16	7.55 - 8.55	5.5 - 10.3
December	9.42 - 14.93	7.75 - 8.46	10.1 - 11.8
January	7.83 - 8.53	7.19 - 11.98	5.3 - 8.4

Benthic macroinvertebrates

In total 18,260 individuals belonging to 175 taxa were determined. Almost all of them (170 taxa) except for that of Ephemeroptera (5 taxa were reported by Ertorun and Tanatmış, 2004) are new records for the Karasu Stream. The benthic macroinvertebrates diversity of the stream consisted of Mollusca (12 taxa, 860 ind.), Oligochaeta (38 taxa, 1157 ind.), Malacostraca (11 taxa, 10568 ind.), Ostracoda (7 taxa, 65 ind.), Hexapoda (26 taxa, 2437 ind.), Trichoptera (20 taxa, 151 ind), Chironomidae (48 taxa, 595 ind.) and other Dipterans (13 taxa, 2427 ind.) (Figure 2). The family of Chironomidae has the highest number of taxa among the groups. The list of the identified taxa and their occurrence, dominancy and frequency values per station are given in Table 3.



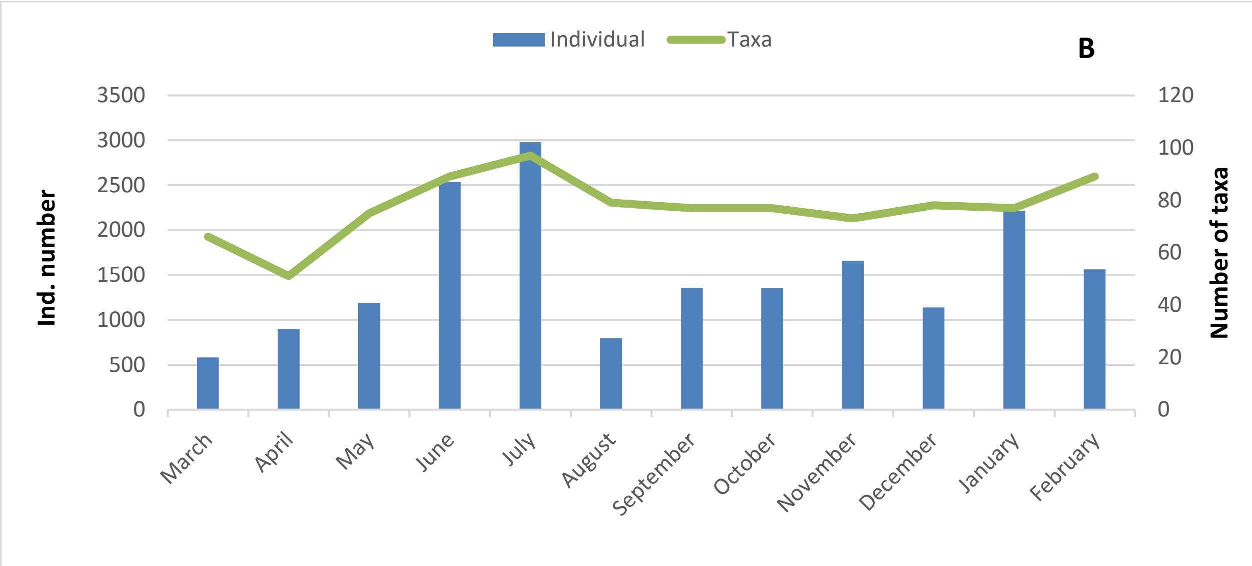


Figure 2. The number of individuals and taxa of the identified systematic groups for the whole study (A), and in time scale (B).

Table 3. List of the identified taxa and their annual abundance (ind/m²), dominance (%D), and frequency (%F) values at the sites (Ent.: Entomobryomorpha; Dec.: Decapoda).

Phylum	Classis	Ordo	Family	Taxa	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	% D	%F
Annelida	Clitellata	Tubificida	Naididae	*Dero digitata* (Müller, 1774)	0	4	0	0	0	0	0	0	0	0	0.02	10
				Nais barbata Müller, 1774	0	2	0	0	0	1	0	0	0	0	0.02	20
				N. bretscheri Michaelsen, 1899	0	1	0	0	0	0	0	0	23	3	0.15	30
				N. christinae Kasprzak, 1973	0	0	0	0	0	2	0	0	0	0	0.01	10
				N. communis Piguet, 1906	1	0	0	0	0	0	0	48	0	0	0.27	20
				N. elinguis Müller, 1774	3	2	0	0	1	9	0	16	0	0	0.17	50
				N. pardalis Piguet, 1906	0	1	0	0	0	0	0	0	0	0	0.01	10
				N. stolci Hrabě, 1981	0	0	7	0	0	0	0	0	0	7	0.08	20
				Ophidonais serpentina (Müller, 1774)	0	0	5	166	0	1	0	0	0	0	0.94	30
			Pristinidae	*Pristina menoni* (Aiyer, 1930)	0	1	0	0	3	1	0	0	3	0	0.04	40
				P.sima (Marcus, 1944)	0	2	0	0	0	0	0	1	0	2	0.03	30
			Tubificidae	*Aulodrilus limnobius* Bretscher, 1899	0	0	0	1	0	0	0	0	0	0	0.01	10
				A. pigueti Kowalewski, 1914	0	1	1	1	0	0	0	0	0	0	0.02	30
				pluriseta (Piguet, 1906)	0	1	1	2	1	1	0	0	3	0	0.05	60
				Limnodrilus claparedeanus Ratzel, 1868	0	0	18	2	0	0	0	0	0	0	0.11	20
				L.hoffmesteri Claparede, 1862	21	76	48	34	1	27	0	2	0	0	1.14	70
				L.hoffmeisteri f. parvus Southern, 1909	14	8	14	1	2	29	1	4	3	0	0.42	90
				L.udekemianus Claparede, 1862	10	3	2	3	1	4	0	0	1	0	0.13	70
				Potamothrix hammoniensis (Michaelsen, 1901)	0	0	0	0	0	0	0	0	1	0	0.01	10
				Psammoryctides albicola (Michaelsen, 1901)	0	1	0	0	1	0	0	0	0	0	0.01	20
				P.deserticola (Grimm, 1876)	14	27	0	0	2	0	0	0	10	0	0.29	40

Phylum	Classis	Ordo	Family	Taxa	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	% D	%F
				Tubifex blanchardi Vejdovsky, 1891	9	15	13	56	1	6	0	0	0	0	0.55	60
				T. newaensis (Michaelsen, 1903)	0	0	2	0	0	0	0	0	26	5	0.18	30
				T. tubifex (Müller, 1774)	2	3	6	4	3	0	3	0	0	0	0.12	50
				Tubificoides sp.	1	0	0	1	0	0	0	0	0	0	0.01	20
				Haber speciosus (Hrabě, 1931)	0	2	0	0	0	0	0	0	0	0	0.01	10
				Spirosperma ferox Eisen, 1879	0	0	0	0	0	0	0	0	2	0	0.01	10
				S.nikolskyi (Lastockin & Sokolskaya, 1935)	0	0	0	0	0	0	0	0	3	0	0.02	10
				Spirosperma sp.	0	0	0	0	0	0	0	0	3	0	0.02	10
			Enchytraeidae	*Cognettia glandulosa* (Michaelsen, 1889)	36	12	1	4	0	4	19	0	4	0	0.44	70
				Henlea ventriculosa (Udekem, 1854)	4	7	0	2	3	5	14	3	40	1	0.43	80
				Henlea sp.	1	0	0	2	0	0	0	0	0	0	0.02	20
				Marionina riparia Bretscher, 1899	0	0	0	0	0	0	6	0	1	0	0.04	20
				Fridericia spp.	0	1	0	0	0	0	2	1	0	0	0.02	30
				Mesenchytraeus sp.	0	3	0	0	14	12	2	1	1	1	0.19	70
			Haplotaxidae	*Haplotaxis gordioides* (Hartmann, 1821)	0	2	0	0	0	8	1	0	1	0	0.07	40
			Lumbriculidae	*Lumbriculus variegatus* (Müller, 1774)	1	10	3	0	0	18	3	3	14	2	0.30	80
			Lumbricidae	*Eiseniella tetraedra* (Savigny, 1826)	0	0	0	0	0	4	0	0	0	0	0.02	10
Mollusca	Gastropoda		Zonitidae	*Zonites algirus* (Linnaeus, 1758)	0	0	0	0	0	0	0	0	1	0	0.01	10
			Planorbidae	*Gyraulus albus* (O. F. Müller, 1774)	25	78	2	4	1	0	0	0	0	0	0.60	50
			Bithyniidae	*Bithynia tentaculata* (Linnaeus, 1758)	13	3	0	6	1	14	0	2	1	10	0.27	80
			Lymnaeidae	*Potamopyrgus jenkinsi* Smith, 1889	2	2	0	2	0	11	0	1	0	0	0.10	50
				*Radix peregra*Müller, 1774	2	13	0	0	0	0	0	0	0	0	0.08	20
			Succineidae	*Succinea putris* (Linnaeus, 1758)	38	38	1	1	2	9	0	0	0	1	0.49	70
	Bivalvia		Unionoida	*Anodonta cygnea* (Linnaeus, 1758)	2	0	0	0	0	0	0	0	0	0	0.01	10
				Unio pictorum (Linnaeus, 1758)	0	8	120	1	0	0	0	0	0	0	0.71	30
			Mytilidae	*Mytilus galloprovincialis* Lamarck, 1819	5	0	0	0	0	0	0	0	0	0	0.03	10
			Mesodesmatidae	*Donacilla cornea* (Poli, 1791)	15	2	0	0	0	0	0	0	0	0	0.09	20
			Veneridae	*Chamelea gallina* (Linnaeus, 1758)	1	0	0	0	0	0	0	0	0	0	0.01	10
			Sphaeriidae	*Pisidium casertanum* (Poli, 1791)	5	10	17	279	25	54	2	3	27	0	2.31	90
Arthropoda	Ostracoda	Podocopida	Candonidae	*Candona candida* (O. F. Müller, 1776)	0	0	0	1	0	3	0	0	1	0	0.03	30
				C. neglecta G.O. Sars, 1887	0	1	2	14	6	8	2	1	3	0	0.20	80
			Cyprididae	*Heterocypris* sp.	0	0	0	0	0	1	0	0	0	0	0.01	10
			Ilyocyprididae	*Ilyocypris* sp.	0	0	0	1	0	0	0	0	0	0	0.01	10
				Prionocypris zenkeri (Chyzer and Toth, 1858)	1	1	0	0	0	0	0	0	0	0	0.01	20
				Psychrodromus olivaceus (Brady and Norman, 1889)	0	0	3	4	3	2	2	0	4	0	0.10	40
				Tonnacypris lutaria (Koch, 1838)	0	0	0	0	0	1	0	0	0	0	0.01	10
	Malacostraca	Isopoda	Detonidae	*Armadilloniscus littoralis* Budde-Lund, 1885	4	4	1	7	0	1	0	2	1	0	0.11	70
			Asellidae	*Asellus aquaticus* Odenwall, 1927	35	93	5	6	0	3	0	0	2	0	0.79	60
			Trichoniscoidea	*Haplophthalmus* sp.	2	0	0	0	0	0	0	0	0	0	0.01	10
				Trichoniscus sp.	5	0	3	2	0	1	0	1	0	0	0.07	50
			Ligiidae	*Ligia italica*	0	0	3	1	0	0	0	1	0	0	0.03	30
		Amphipoda	Gammaridae	*Gammarus balcanicus* Schäferna, 1922	1	0	1	49	3	13	15	16	69	0	0.91	80
				G.komareki Schäferna, 1922	3	0	34	31	62	77	1431	3170	944	25	31.64	90
				G.pulex pulex (Linnaeus, 1758)	0	1	0	45	13	6	29	12	64	2	0.94	80
				G.uludagi Karaman, 1975	0	0	22	287	265	77	2456	455	542	1	22.48	80
			Niphargidae	*Niphargus sp.*	13	8	7	0	0	0	0	0	0	1	0.16	40
		Dec	Potamidae	*Potamon* sp.	2	4	32	0	30	21	0	9	5	32	0.74	80
	Entognatha	Ent.	Isotomidae	*Isotoma* sp.	0	0	1	0	0	2	3	0	0	0	0.03	30
		Ephemeroptera	Beatidae	*Baetis* sp.	10	3	56	59	131	185	180	49	38	82	4.34	100
			Caenidae	*Caenis* sp.	0	0	0	0	0	0	1	0	0	0	0.01	10
			Ephemeridae	*Ephemera* sp.	1	5	23	0	2	15	0	0	8	36	0.49	70
			Heptageniidae	*Heptagenia* sp.	0	6	16	334	14	55	8	3	3	43	2.64	90
			Leptophlebiidae	*Leptophlebia* sp.	0	0	0	163	68	9	0	0	4	42	1.54	50
		Odonata	Calopterygidae	*Calopteryx* sp.	10	14	36	6	2	0	1	0	0	10	0.43	70
			Euphaeidae	*Euphaea* sp.	0	0	0	0	0	0	0	0	0	1	0.01	10
			Coenagrionidae	*Coenagrion* sp.	9	18	5	5	0	0	0	0	0	0	0.20	30
			Cordulegastridae	*Cordulegaster* sp.	0	0	6	3	3	0	6	1	4	1	0.13	70
			Gomphidae	*Gomphus* sp.	0	0	16	2	3	5	5	0	2	0	0.18	60

Phylum	Classis	Ordo	Family	Taxa	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	% D	%F
			Lestidae	*Lestes* sp.	18	12	9	11	2	0	1	0	1	5	0.32	80
			Libellulidae	*Libellula* sp.	2	2	2	41	4	0	0	0	0	0	0.28	50
		Plecoptera	Capniidae	*Capnia* sp.	0	4	0	0	21	36	3	15	2	21	0.56	70
			Perlodidae	*Perlodes* sp.	0	0	0	0	0	0	3	1	0	0	0.02	20
			Perlidae	*Agnetina* sp.	0	0	0	0	2	0	1	0	0	15	0.10	30
			Leuctridae	*Leuctra* sp.	0	0	0	0	0	19	5	6	3	24	0.31	50
			Nemourida	*Nemoura* sp.	0	0	0	20	49	35	4	17	6	34	0.90	70
			Taeniopterygidae	*Taeniopteryx* sp.	0	0	1	0	0	0	1	0	0	0	0.01	20
		Coleoptera	Carabidae	*Carabus* sp.	0	0	0	0	0	0	3	0	0	1	0.02	20
			Dytiscidae	*Dytiscus* sp.	0	1	0	11	25	7	2	16	3	2	0.37	80
			Hydrophilidae	*Hydrophilini* sp.	0	0	0	0	1	0	2	13	1	0	0.09	40
			Tenebrionidae	*Tenebrio* sp.	0	0	0	0	0	0	18	0	0	0	0.10	10
		Hemiptera	Notonectidae	*Notonecta* sp.	0	1	0	5	22	9	0	0	0	0	0.20	40
			Pleidae	*Plea* sp.	0	0	0	0	0	0	0	3	0	0	0.02	10
			Pentatomidae	*Pentatoma* sp.	0	0	0	0	0	0	0	0	0	1	0.01	10
		Trichoptera	Phryganeidae	*Agrypnia obsoleta* Martynov, 1928	0	0	0	1	0	0	0	0	0	0	0.01	10
			Hydropsychidae	*Diplectrona felix* McLachlan, 1878	0	0	0	0	0	0	0	2	1	1	0.02	30
			Ecnomidae	*Ecnomus tenellus* (Rambur, 1842)	0	0	0	0	0	0	0	0	3	1	0.02	20
			Limnephilidae	*Glyphotaelius pellucidus* (Retzius, 1783)	0	0	0	0	1	0	0	0	0	0	0.01	10
			Hydropsychoidae	*Hydropsyche angustipennis* (Curtis, 1834)	0	0	4	0	0	15	2	3	0	21	0.25	50
				H.instabilis (Curtis, 1834)	0	0	0	0	0	0	3	0	0	6	0.05	20
				H.pellucida (Curtis 1834)	0	0	0	0	0	1	0	0	0	0	0.01	10
				Hydropsyche sp.	0	0	0	0	0	0	0	0	0	3	0.02	10
			Psychomyiidae	*Lype reducta* (Hagen, 1868)	0	0	0	0	0	9	0	0	0	0	0.05	10
			Limnephilidae	*Micropterna lateralis* Stephens 1837	0	0	0	0	0	5	0	0	0	0	0.03	10
			Molannidae	*Molanna angustata* Kolenati, 1858	0	0	0	9	0	4	0	1	3	0	0.09	40
			Sericostomatidae	*Notidobia ciliaris* (Linnaeus, 1761)	0	0	0	0	0	0	0	0	8	0	0.04	10
			Polycentropodidae	*Polycentropus flavomaculatus* (Pictet, 1834)	0	0	0	0	0	1	0	0	0	0	0.01	10
				P.irroratus (Curtis, 1835)	0	0	0	0	10	1	0	0	0	1	0.07	30
				P.kingi McLachlan, 1881	0	0	0	0	0	0	0	0	2	0	0.01	10
			Rhyacophilidae	*Rhyacophila dorsalis persimilis* McL	0	0	0	0	0	0	4	3	0	0	0.04	20
				R. munda Navas, 1936	0	0	0	0	0	0	1	0	0	0	0.01	10
				R.septentrionis McLachlan, 1865	1	0	0	1	0	1	3	1	0	0	0.04	40
				Rhyacaphila sp.	0	0	0	0	0	0	0	6	4	0	0.05	20
			Sericostomatidae	*Sericostoma* sp.	0	0	0	0	0	4	0	0	0	0	0.02	10
		Diptera	Athericidae	*Atherix* sp.	1	5	25	0	7	9	0	0	1	37	0.47	80
			Tabanidae	*Tabanus* sp.	7	5	19	37	28	26	15	20	9	17	1.00	100
			Dolichopodidae	*Dolichopus* sp.	0	0	0	0	0	1	1	6	1	1	0.05	50
			Ephydridae	*Ephydra* sp.	2	0	1	0	0	5	34	5	8	0	0.30	60
			Syrphidae	*Syrphus* sp.	0	0	0	0	0	0	2	0	1	0	0.02	20
			Stratiomyidae	*Stratiomys* sp.	0	0	5	1	4	10	0	12	2	1	0.19	70
			Fannidae	*Fannia* sp.	0	0	0	0	0	1	0	0	0	0	0.01	10
			Tipulidae	*Tipula* sp.	24	4	88	38	18	28	42	18	27	23	1.70	100
			Limoniidae	*Limonia* sp.	0	3	0	1	1	5	7	2	8	12	0.21	80
			Ceratopogoninae	*Bezzia* sp.	19	40	83	104	6	202	19	26	50	32	3.18	100
			Simuliidae	*Simulium* sp.	3	1	180	48	131	252	374	21	50	32	5.98	100
			Culicidae	*Aedes* sp.	0	1	0	6	16	0	0	0	0	0	0.13	30
			Psychodidae	*Psychoda* sp.	0	0	0	0	0	1	0	8	1	0	0.05	20
			Chironomidae	*Ablabesmyialongistyla* Fittkau, 1962	5	11	14	11	20	4	0	1	0	4	0.38	80
				Apsectrotanypus sp.	0	0	0	2	0	0	0	0	0	0	0.01	10
				Conchapelopia sp.	0	0	6	8	10	14	1	1	3	2	0.25	80
				Procladius (*Holotanypus*) sp.	3	2	3	18	3	0	0	0	0	1	0.16	60
				Telopelopia sp.	1	0	0	6	2	0	1	0	12	4	0.14	60
				Potthastia gaedii (Meigen, 1838)	0	0	4	0	0	5	1	0	0	0	0.05	30
				Prodiamesa olivacea (Meigen, 1818)	0	0	2	4	1	0	0	0	0	0	0.04	30

Phylum	Classis	Ordo	Family	Taxa	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	% D	%F
				Brillia flavifrons (Johannsen, 1905)	1	0	6	0	0	0	0	0	2	3	0.07	40
				B.modesta (Meigen, 1830)	0	0	0	3	7	5	10	6	10	2	0.24	70
				Cardiocladius fuscus Kieffer, 1924	0	0	0	0	0	0	0	1	0	0	0.01	10
				Cricotopus sp.	4	4	5	5	0	4	2	1	5	3	0.18	90
				Cricotopus sylvestris (Fabricius, 1794)	2	1	0	0	0	0	0	0	0	0	0.02	20
				C.triannulatus Macquart, 1826	0	0	0	0	0	2	0	0	0	0	0.01	10
				Eukiefferiella sp.	1	0	2	0	1	1	0	0	3	3	0.06	40
				Eukiefferiella claripennis (Lunnbeck, 1898)	1	1	1	0	0	0	0	0	0	0	0.02	30
				E.gracei (Edwards, 1929)	0	0	0	0	0	1	0	0	1	0	0.01	20
				Orthocladius sp.	2	0	1	0	1	1	0	0	0	2	0.04	40
				Parametriocnemus sp.	1	0	1	0	1	1	0	1	1	0	0.03	60
				Paratrissocladius sp.	0	0	1	0	0	0	0	0	0	0	0.01	10
				Psectrocladius sp.	0	0	0	0	0	0	0	0	1	0	0.01	10
				Rheocricotopus fuscipes Kieffer, 1909	0	0	1	0	0	0	0	1	0	0	0.01	20
				Rheocricotopus sp.	0	0	4	1	6	1	9	1	4	0	0.14	70
				Smittia sp.	1	1	2	0	0	0	1	0	0	0	0.03	40
				Thienemannimyia sp.	0	0	4	2	3	7	1	2	7	2	0.15	80
				Tvetenia sp.	0	0	0	0	1	0	1	0	0	0	0.01	20
				Chironomus sp.	8	8	6	8	10	3	0	0	4	2	0.27	80
				Cryptochironomus denticulatus (Goetghebuer, 1921)	0	0	1	0	0	0	0	0	0	0	0.01	10
				Dicrotendipes lobiger (Kieffer, 1921)	0	1	0	5	3	0	0	0	0	0	0.05	30
				D.nervosus (Stæger, 1839)	2	1	0	5	0	0	0	1	0	0	0.05	40
				D.notatus (Meigen, 1818)	1	1	0	0	1	0	0	0	0	0	0.02	30
				Endochironomus dispar (Meigen, 1830)	4	5	0	5	2	0	2	0	1	0	0.10	60
				E.lepidus (Meigen, 1830)	1	0	0	2	2	0	0	0	0	0	0.03	30
				Endochironomus sp.	2	1	0	1	0	0	0	0	1	0	0.03	40
				Kiefferulus sp.	4	7	0	12	3	0	0	0	0	0	0.14	40
				Microtendipes pedullus (De Geer, 1776)	0	0	1	1	1	0	0	2	1	0	0.03	50
				Phaenopsectra sp.	1	1	0	1	2	0	0	0	0	0	0.03	40
				Polypedilum laetum (Meigen, 1818)	0	2	2	0	1	0	0	0	1	2	0.04	50
				P.albicorne (Meigen, 1838)	0	0	0	0	0	1	0	2	0	0	0.02	20
				P.convictum (Walker, 1856)	0	0	0	0	0	0	1	1	0	1	0.02	30
				P.nubeculosum Meigen, 1804	2	2	0	0	0	0	0	0	0	0	0.02	20
				P.pedestre (Meigen, 1830)	0	1	2	0	1	1	0	0	1	0	0.03	50
				P.scalaenum (Schrank, 1803)	0	0	1	0	0	0	0	0	0	0	0.01	10
				P.tritum (Walker, 1856)	1	1	0	0	0	0	0	0	0	0	0.01	20
				P.uncinatum (Goetghebuer, 1921)	1	1	0	0	0	0	0	0	0	1	0.02	30
				Polypedilum sp.	6	10	1	0	1	0	0	0	1	5	0.13	60
				Micropsectra sp.	0	0	0	0	1	2	1	0	3	1	0.04	50
				Paratanytarsus sp.	0	2	1	0	2	0	0	0	0	1	0.03	40
				Tanytarsus sp.	0	2	2	4	1	0	1	2	0	0	0.07	60

According to Soyer's frequency index, 175 taxa were observed continuously all year long (Table 3). The highest number of taxa was determined in July (98 taxa), June (90 taxa), and February (89 taxa), while the least in April (52 taxa). The highest number of individuals was sampled in July (2986 ind./m²) and June (2539 ind./m²), while the lowest in March (589 ind./m²) (Figure 2).

A1 and A2 sites were located near the mouth of the stream, thus the sampled benthic organisms consist of mainly salinity-tolerant taxa such as *N. pardalis*, *P. jenkinsi*, *D. cornea*, *M. galloprovincialis*, *C. gallina*, *T. blanchardi*, and *T. tubifex*. Similarly, A3, A4, A5, and A6 sites were in the middle part of the stream where pure freshwater forms were observed (such as *G. albus*, *L. hoffmeisteri*, *H. ventriculosa*, *S. putris*, *G. uludagi*, and *G. pulex pulex*). A7, A8, A9, and A10 sites were in the upper part of the stream and are the cleanest ones because of the lack of pollutants around them. *G. balcanicus* and *G. uludagi* were the two typical taxa at these sites.

G. komareki has the highest dominance among the determined species with 31.64% and was followed by *G. uludagi* with 22.48%. Individuals belonging to the orders of Hemiptera and Trichoptera with dominance of 0.01%, also make up the least common groups.

The dominant taxon of A1 and A2 was *A. aquaticus*. All the dominant taxa observed at these sites are tolerant to organic pollution. The bottom of the A3 station consists of stone, gravel, and sand and the flow rate of water varies significantly throughout the year. The dominant taxa at the station were *Simulium* sp. and *U. pictorum*. A4 and A5 are creeks that join the Karasu Stream, and they have a rich riparian zone. The villages near the creeks were the main pollutants because of discharging wastes. The dominant taxa of these sites were *G. uludagi* and *P. casertanum*. The latter is cosmopolitan and euryoecious, which is mostly found in oligo- or beta-mesosaprobic freshwater environment (Subba Rao, 1989). Karasu River at site A6 passes through a sparse forest with a low flow rate, it has shallow with a stony-gravelly bottom and a dense *Chara* sp. population. *Simulium* sp., *Bezzia* sp., *G. komareki*, and *G. uludagi* were the dominant taxa at the station. A7 has 1–1.5 m depth, a rocky and stony bottom with rich terrestrial and aquatic plants. *G. uludagi* and *G. komareki* were the dominant taxa at the station. A8 was a small, clean waterfall outflowing to a reservoir near Erfelek town. The bottom of the station has rocks, stones, and pebbles. *G. komareki*, *G. uludagi*, and *Baetis* sp. were the dominant taxa at the station. A9 has a sandy bottom and is located near the Tatlıca Waterfall. The dominant taxa of the station were *G. komareki, G. uludagi, Bezzia* sp., and *Simulium* sp. A10, which has a rocky and stony bottom, is located above the Tatlıca Waterfall. The dominant taxa of these sites were *G. komareki and G. uludagi*.

Biological indices

According to BMWP scores, the water quality of the sites A4, A5, A6, A9, and A10 was of the first class. Only around the sites of A1, A2, and A3, settlements and agricultural activities occur; the other localities are not under the pressure of such negative effects. Results of BMWP analysis showed that A1, A2, A3, A7, and A8 sites were classified in slightly polluted (Class II) groups. Similarly, ASPT analysis indicated that A1, A2, A6, and A8 were in the third class while the remaining ones in the second class (Table 4).

Table 4. The BMWP and ASPT scores and diversity indices of the sites (S: Total number of taxa, N: Total number of individuals, D: Margalef Species Richness, J': Pielou's Evenness Index, H': Shannon-Weiner Diversity Index, 1-λ': Simpson Index of Diversity)

	BMWP			ASPT			Diversity Indices					
	Score value	Quality class		Score value	Quality class		S	N	D	J'	H'	1-λ'
A1	111	II.	Good	4.6	III.	Poor	68	453	10.96	**0.85**	**3.59**	**0.96**
A2	144	II.	Good	4.6	III.	Poor	77	642	**11.76**	0.77	3.36	0.94
A3	144	II.	Good	5.1	II.	Fair	72	1024	10.24	0.75	3.21	0.93
A4	159	I.	Excellent	5.1	II.	Fair	74	2030	9.59	0.69	2.96	0.91
A5	170	I.	Excellent	5.3	II.	Fair	74	1102	10.42	0.69	2.98	0.90
A6	180	I.	Excellent	4.8	III.	Poor	80	1445	10.86	0.73	3.19	0.92
A7	143	II.	Good	5.1	II.	Fair	60	4774	**6.96**	0.35	1.45	0.64
A8	141	II.	Good	4.7	III.	Poor	60	4033	7.11	**0.25**	**1.01**	**0.37**
A9	189	I.	Excellent	5.2	II.	Fair	78	2119	10.05	0.48	2.10	0.73
A10	175	I.	Excellent	5.5	II.	Fair	60	638	9.14	0.81	3.31	0.95

Both Shannon-Weiner and Simpson indices resulted in high scores. Station A1 has the highest diversity value (H'=3.59), while station A8 has the lowest (H'=1.01). Nearly all the sites have high values in terms of richness but A2 has the highest taxa while A7 has the lowest. Similarly, the evenness index suggested that A2 has the highest score (J'=0.85) while station A8 has the lowest (J'=0.25) (Table 4).

The UPGMA analysis grouped the sites with a similarity of more than 50% according to the occurred taxa. In general, all the localities have high level of similarities (more than 50%) to each other. A10 (with a stony and gravel bottom) was out grouped from the others. A7 and A8, which have a rocky bottom, were grouped; both were separated from all the other sites except A10 and constitute another group. Within this group, A1 and A2 constituted a separate group with almost 70

% similarity. Both sites are in the Abramis zone (lower part of the stream) and have a slightly brackish character. The other group was constituted by the remaining sites, which are purely freshwater. A3 with a stony and muddy bottom was separated within this group. Although the bottom structure of the other sites A4, A5, A6, and A9 were different, all have a muddy bottom and similarities in terms of faunal components; they form another cluster (Figure 3).

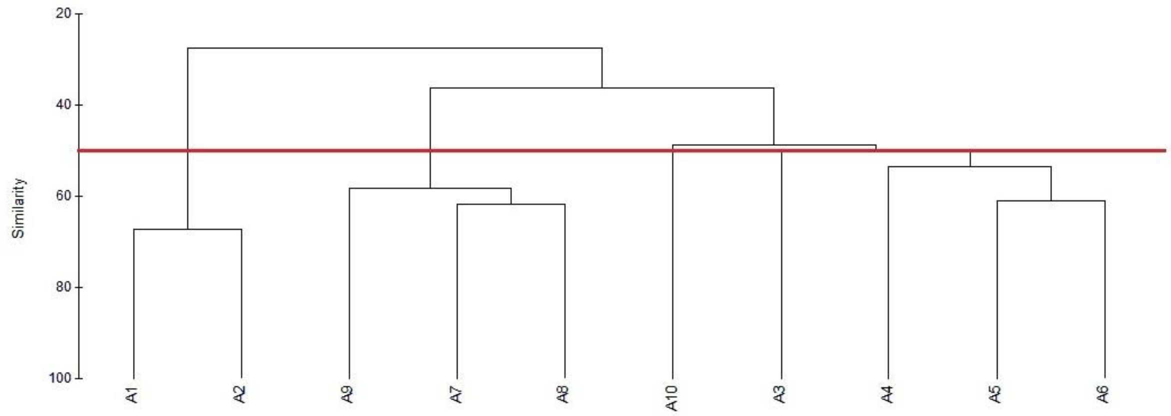


Figure 3. The UPGMA dendrogram showing the similarity of the sites

DISCUSSION

Long-term changes in water quality can be estimated more accurately if biological indicators are used as they are adapted to specific environmental conditions for a long time. For this reason, if any changes occur in running waters, they can be detected using the compositions and structures of aquatic organisms (Zamora-Muñoz and Alba-Tercedor, 1996).

In general, diverse and productive fish and macroinvertebrate communities prefer slightly alkaline aquatic environments, where pH values were between 6.5 and 8.5 (NAS, 1972). High pH and low oxygen concentrations have a lethal effect on living organisms (Tanyolaç, 2004). By having slight alkaline character, Karasu Stream offers a comfortable settlement for various benthic macroinvertebrate species.

Water temperature and dissolved oxygen values were the determinant factors in species richness at the sites. Pollution-sensitive taxa or clean water indicator species were observed frequently at the A4, A5, A6, A9, and A10 sites, where diversity values were high andwater quality levels were in first class.

Climate and regions are the main predictors for the temperature periodicity patterns (Ward, 1985) and seasonality may become weak or harsh according to locations of the stream (e.g. Hopkins, 1971). The water temperature of the sites varies between 4.06-27.6°C throughout the year and is within the seasonal norms.

Macroinvertebrates inhabit different parts of a waterbody. They can live in the sediment or water's surface, water itself, etc. Environmental conditions such as submerged rocks, leaf litter, or water velocity can be determinant which macroinvertebrates can live (Tanyolaç, 2004).

The BMWP and ASPT scores are frequently used to determine the stream quality. Biological indices are usually specific for certain types of pollution since they are based on the presence or absence of indicators organisms, which are unlikely to be equally sensitive to all types of pollution. They are considered the sensitivities of macroinvertebrates to pollution and the BMWP scores give the how much clean or polluted the sites (Chapman, 1996). In the present study, the BMWP scores, suggested that all of the localities have high (more than 100) scores and it means all of them are in good (even excellent) conditions. On the other hand, the results of ASPT indicated that A1, A2 and A8sites were in poor conditions while the other ones in fair conditions.

The content of the species is expected to have high similarities at successive sampling sites, such as A1-A2 and A7-A8 or A5-A6. Because habitat transitions are close to one another, species compositions may be similar (Figure 3). Although A3 and A10 stations are far from each other in terms of location, they have high similarity to each other. Having similar environmental characteristics such as water depth, bottom structure, presence of riparian vegetation can be main reason for the high similarity.

The species compositions expected to have high similarities at successive sampling sites, such as A1-A2 and A7-A8 or A5-A6. Because habitat transitions are close to one another, species compositions may be similar (Figure 3). Although A3 and A10 stations are far from each other in terms of location, they have high similarity to each other. Having similar environmental characteristics such as water depth, bottom structure, presence of riparian vegetation can be main reason for the high similarity. In addition, both sites have natural environmental conditions, isolated from roads and settlements.

A6 has the highest species richness (82 taxa). Rich riparian zone, diverse bottom structure (stone, gravel, sand, and mud), and relatively low water flow rates can be the main reasons for the higher macroinvertebrate diversity at the station. Reversely, A7 (with 62 taxa) has the lowest species richness where weak aquatic vegetation, rocky bottom, and rapid water flow occur. The main restrictive factor for species diversity at the station can be the high velocity of water flow. The members of the genus *Gammarus* constituted the dominant group at the station.

CONCLUSIONS

Karasu Stream is a suitable habitat for benthic macroinvertebrates in terms of its location, bottom structure, water quality, and other ecological characteristics. The biological monitoring studies should be carried out for the sustainable use of the Karasu Stream. In this way, biodiversity and water quality will remain at the desired level and its unique habitats can be protected from the destructive effects of human pressure and pollution.

ACKNOWLEDGMENTS

This study was financially supported by S.U. Scientific Research Council (BAP) (Project no: SÜF- 1901-12-12). The authors wish to thank Yakup ERDEM, Uğur ÇARLI, Ethem ERTAŞ and Erkut ÖZCAN for their help during the field studies and to BAP for their support.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 479-486 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.09

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

The effect of mercury, copper, and zinc on paraoxonase (PON) enzyme activity in Bonito (*Sarda sarda*) fish

Palamut *(Sarda sarda)* balığında paraoksonaz (PON) enzim aktivitesi üzerine cıva, bakır ve çinkonun etkisi

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Received date: 21.04.2021 *Accepted date: 14.10.2021*

How to cite this paper:
Şahin, B. & Çenesiz, S. (2021). The effect of mercury, copper, and zinc on paraoxonase (PON) enzyme activity in Bonito (*Sarda sarda*) fish. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 479-486. DOI: 10.12714/egejfas.38.4.09

Abstract: Since heavy metal dirtiness, which we frequently encounter in environmental pollution causes harmful effects on the organism through biochemical enzyme reactions, in this study, the effects of mercury (Hg^{+2}), copper (Cu^{+2}), and zinc (Zn^{+2}) heavy metal ions, which are common in environmental pollution, on PON (paraoxonase) enzyme activity in muscle tissue of bonito (*Sarda sarda*) were investigated. In the study, 25 bonito (*S. sarda*) fish muscle tissues freshly obtained from the Samsun region sea were used. The changes in PON enzyme activity were determined by adding different volumes of heavy metal solutions. PON enzyme activities of Hg^{+2} heavy metal ion used in different volumes were calculated as 30.9383 U/mLdak, 29.0598 U/mLdak, 26.3799 U/mLdak, 23.9443 U/mLdak, 20.6725 U/mLdak, PON enzyme activities of Cu^{+2} heavy metal ion used in different volumes were calculated as 19.7949 U/mLdak, 19.4807 U/mLdak 19.1864 U/mLdak, 19.1200 U/mLdak, 18.9037 U/mLdak and PON enzyme activities of Zn^{+2} heavy metal ion used in different volumes were calculated as 23.8305 U/mLdak, 23.0781 U/mLdak, 22.9073 U/mLdak, 22.4324 U/mLdak, 21.8159 U/mLdak. As a result of these obtained data, activity (%) values were calculated and activity (%) graphs were drawn. As a result of the study, it was determined that increasing concentrations of Cu^{+2} and Zn^{+2} heavy metal ions caused a decrease in PON enzyme activity, but there was no statistically significant difference between the different concentrations used. It was determined that increasing concentrations of Hg^{+2} heavy metal ion inhibited the PON enzyme activity, caused a statistically significant decrease between the activities depending on the different concentrations used ($p < 0.05$).

Keywords: Bonito (*Sarda sarda*), heavy metals, copper (Cu), mercury (Hg), zinc (Zn), paraoxonase (PON)

Öz: Çevre kirliliğinde sık rastlanan ağır metal kirlilikleri, biyokimyasal enzim reaksiyonları üzerinden organizmada zararlı etkiler meydana getirdiği için bu çalışmada çevre kirliliğinde sık rastlanan cıva (Hg^{+2}), bakır (Cu^{+2}) ve çinko (Zn^{+2}) ağır metal iyonlarının palamut (*Sarda sarda*) balığı kas dokusunda bulunan PON (paraoksonaz) aktivitesi üzerine etkileri araştırılmıştır. Çalışmada Samsun bölgesi denizinden taze olarak temin edilen 25 adet palamut balığının kas dokuları kullanıldı. Ağır metal çözeltilerinden farklı hacimlerde eklenerek PON enzim aktivitesindeki değişiklikler tayin edildi. Farklı hacimlerde kullanılan Hg^{+2} ağır metal iyonunun PON enzim aktiviteleri 30,9383 U/mLdak, 29,0598 U/mLdak, 26,3799 U/mLdak, 23,9443 U/mLdak, 20,6725 U/mLdak olarak hesaplandı, farklı hacimlerde kullanılan Cu^{+2} ağır metal iyonunun PON enzim aktiviteleri 19,7949 U/mLdak, 19,4807 U/mLdak 19,1864 U/mLdak, 19,1200 U/mLdak, 18,9037 U/mLdak olarak hesaplandı ve farklı hacimlerde kullanılan Zn^{+2} ağır metal iyonunun PON enzim aktiviteleri 23,8305 U/mLdak, 23,0781 U/mLdak, 22,9073 U/mLdak, 22,4324 U/mLdak, 21,8159 U/mLdak olarak hesaplandı. Elde edilen bu veriler sonucunda % aktivite değerleri hesaplanarak % aktivite grafikleri çizildi. Çalışma sonucunda Cu^{+2} ve Zn^{+2} ağır metal iyonlarının artan derişimlerinin enzim aktivitesinde azalmaya neden olduğu fakat kullanılan farklı derişimler arasında istatistiksel olarak anlamlı bir fark olmadığı belirlendi. Hg^{+2} ağır metal iyonun artan derişimlerinin PON enzim aktivitesini inhibe ettiği, kullanılan farklı derişimlere bağlı olarak aktiviteler arasında istatistiksel olarak anlamlı bir azalmaya neden olduğu belirlendi ($p < 0.05$).

Anahtar kelimeler: Palamut (*Sarda sarda*), ağır metal, bakır (Cu), cıva (Hg), çinko (Zn), paraoksonaz (PON)

INTRODUCTION

Paraoxonase (PON) is a serum esterase with arylesterase and paraoxonase activities (aryldialkylphosphatase; E.C.3.1.8.1), which is expressed from the liver and able to hydrolyze paraoxone, which the active metabolite of parathion (Primo-Parma *et al.*, 1996; Memişoğulları and Orhan, 2010). The paraoxonase enzyme hydrolyzes toxic oxon metabolites of insecticides such as chlorpyrifos, parathion, and diazinon (Mackness *et al.*, 1996), OP (organophosphate) nerve agents such as sarin, tabun, and soman (Broomfield and Ford, 1991; Baillie *et al.*, 1993), and ester substrates such as phenylacetate (Eckerson *et al.*, 1983; Sorenson *et al.*, 1995). The gene which is responsible for PON activity has three members and is called PON1, PON2, PON3 (Primo-Parma *et al.*, 1996). After PON1 and PON3 are synthesized in the liver, they are delivered to the blood, the plasma is transported together with HDL (high density lipoprotein). PON2, on the other hand, is not found in serum and synthesized in many different tissues (*Teiber et al.*, 2007). It has been hypothesized that the receptor that provides the relationship between PON and HDL is scavenger

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receptor B1 (SR-B1). It has been reported that this receptor HDL binding to the membrane of the cell and providing material exchange between the cell and lipoproteins and that PON is synthesized abundantly from the liver (Deakin and James, 2004).

There are many defense mechanisms to prevent the formation of reactive oxygen species (ROT) in living and the damage which is caused (Prior and Cao, 1999). One of them is the PON enzyme (Costa *et al*., 1999; La Du *et al*., 1999). The paraoxonase enzyme exists depending on HDL and has a protective effect against the formation of lipid peroxides by oxidation of LDL (low density lipoprotein) (Mackness *et al*., 2001; Carey *et al*., 2005). It shows an antioxidant property by limiting lipid oxidation in LDL thanks to HDL, which is dependent on the structure of this enzyme (Elana *et al*., 2006).

Heavy metals are defined as metals which have toxic or poisoner effects even at low concentration and they have a density of more than 5 g/cm^3 in terms of physical properties (Kahvecioğlu *et al*., 2003; Seven *et al*., 2018). These metals, even if found in small quantities, accumulate in the body of aquatic organisms at increasing rates and reach levels that will do a toxic effect (Ikuta, 1985). Although some metals, like copper and iron, are necessary for life at certain levels, metals in the state of mercury and lead can be toxic even at trace levels (Hu, 2002). Heavy metals are taken from the external environment by fish and are transported to tissues and organs through the blood tract by binding with carrier proteins, reaching high concentrations holding by metal-retaining proteins in tissues (Kaptan, 2014). Metals of physiological importance are stored from metals that can be excreted out of the body by participating in different metabolic processes in the living structure, but if these are toxic metals, these can disrupt enzyme structures (Yazkan *et al*., 2004). Mercury (Hg) levels in the tissues of fish found in these regions have also increased due to the increase of industrial enterprises on the sea coast (Vural, 1993). Hg is highly toxic to fish, even in very low amounts. Organic Hg compounds usually enter the living body through food, causing chronic toxic disorders after accumulating (Dökmeci, 2001; Kaya and Akar, 2002). Copper (Cu) is taken from the environment by fish usually through the gill and food, its excretion outside the body is through feces and urine (Sağmanlıgil, 1994; Cicik, 2003). Excess copper in the water accumulates in the gill tissue within a short period, as well as reaching higher concentrations in the liver tissue depending on the duration of action (Kalay and Erdem,1995). Besides, the Cu metal increases the outflow of sodium ions by fracking the energy-bound sodium/potassium pump in the gill tissue (Cicik, 2003; Coppock and Nation, 2007). In addition to the fact that the mechanism of action of zinc (Zn) metal in fish is not fully known, it has been found to cause damage to gill tissue (Cicik, 2003; Kaya and Akar, 2002; Ağcasulu, 2007).

Heavy metals, which reach from the first step to the last step of the food chain and showing accumulation, in bonito (*S. sarda* Bloch, 1793) fish, which is of great importance in protein-rich human nutrition, the accumulation in increasing concentrations shows a toxic effect and negatively affects human health. To find a solution to such a problem, further research of some metals is needed. How the activity of the PON enzyme with physiological function in metabolism, is affected by some heavy metals is extremely important for animal and environmental health. This study has investigated the effects of mercury (Hg^{+2}), copper (Cu^{+2}), and zinc (Zn^{+2}) heavy metal ions on PON enzyme activity in muscle tissue of bonito (*S. sarda*) fish.

MATERIAL AND METHODS

A total of 25 bonito (*S. sarda*) fish, which an average weight of 600-800 g and a length of 40 to 45 cm consisted of the material of the study. In the study, muscle tissue of bonito (*S. sarda*) fish freshly taken from the sea of Samsun region in October, which is the seasonal season of this fish species, and brought to the laboratory environment by the cold chain in a short time was used. Tissue samples taken from bonito (*S. sarda*) were weighed 0.3 g and taken into dry centrifuge tubes, then 1.5 mL Tris-HCl buffer was added to them and homogenized. The homogenized tissues were centrifuged in a cooled centrifuge at +4 °C and 3000 rmp speed for 30 minutes and supernatants were separated. Then, the separated supernatants were used on the same day.

PON enzyme activity determination method recommended by Gülcü and Gürsu (2003), was used for PON enzyme activity determination. In activity determination, 50 µL Tris-HCl buffer, 50 µL substrate (calcium chloride+paraoxone) solution were added to cuvettes and the value at 405 nm absorbance was read in ELISA. 50 µL supernatant solution was added to the measured cuvettes and the change occurring in the absorbance at 405 nm, at 37 °C in ELISA it was read in 30 seconds. In this way, the enzymatic conversion speed of paraoxone to p-nitrophenol was defined as PON activity (1 U/L).

The activity measurement in the paraoxonase enzyme of the mercury II chloride ($HgCl_2$) solution: In paraoxonase enzyme activity determination, 50 µL tris-HCL buffer, 50 µL substrate (calcium chloride+paraoxone) solution, and 50 µL supernatant solution were added to the cuvettes, and the value at 37 °C and 405 nm absorbance in ELISA it was read in 30 seconds. Then, the change in enzyme activity was determined, after adding different volumes 10 µL, 20 µL, 30 µL, 40 µL, 50 µL 0.001 M $HgCl_2$ solution to the measuring cuvette. The enzyme activity determined in the environment without inhibitor was used as 100% activity.

The activity measurement in the paraoxonase enzyme of the copper II chloride ($CuCl_2$) solution: In paraoxonase enzyme activity determination, 50 µL tris-HCL buffer, 50 µL substrate (calcium chloride+paraoxone) solution, and 50 µL supernatant solution was added to the cuvettes and the value at 37 °C and 405 nm absorbance in ELISA it was read in 30 seconds. Then, the change in enzyme activity was determined, after adding different volumes 10 μL, 20 μL, 30 μL, 40 μL, 50 μL 0.001 M $CuCl_2$ solution to the measuring cuvette. The enzyme activity determined in the environment without inhibitor was used as 100% activity.

The activity measurement in the paraoxonase enzyme of the zinc ıı chloride ($ZnCl_2$) solution: In paraoxonase enzyme activity determination, 50 μL tris-HCL buffer, 50 μL substrate (calcium chloride+paraoxone) solution, and 50 μL supernatant solution were added to the cuvettes, and the value at 37 °C and 405 nm absorbance in ELISA it was read in 30 seconds. Then, the change in enzyme activity was determined, after adding different volumes 10 μL, 20 μL, 30 μL, 40 μL, 50 μL 0.001 M $ZnCl_2$ solution to the measuring cuvette. The enzyme activity determined in the environment without inhibitor was used as 100% activity.

Measurement of the total oxidant capacity (TOC): The TOC was measured by a colorimetric method based on the cumulatively oxidize of ferrous ion to the ferric ion of oxidant molecules present in the supernatant (Erel, 2004, 2005). Calculations were made according to the formula in the procedure.

Measurement of the total antioxidant capacity (TAC): The TAC was measured by the method of decolorization of the colored radical in proportion to the total concentration of the antioxidant molecules by reducing dark blue-green colored ABTS cationic radical of all antioxidant molecules present in the supernatant (Erel, 2004, 2005). Calculations were made according to the formula in the procedure.

Calculation of the oxidative stress ındex (OSI): The OSI was obtained by calculating from the Total oxidant capacity (μmol H_2O_2Equiv/L)/Total antioxidant capacity (mmol TroloxEquiv/L) × 10 formulation.

SPSS statistical (version 22) package program was used for statistical analysis. The analysis of the samples was done with a One-Way Analysis of Variance (One-Way Anova). While the difference between the samples was not significant for $p>0.05$ value, the difference was regarded to be significant for $p<0.05$ value. For the samples with a value of $p<0.05$, the homogeneity of the samples was examined using the homogeneity of variance test, and the samples with a significant difference between them were compared with the Tukey test.

RESULTS

The enzyme activity table and graph were drawn by repeating the measurements made by taking different volumes from $CuCl_2$, $HgCl_2$, and $ZnCl_2$ solutions and studying in duplicate and calculating the average of the results.

Paraoxonase enzyme activity (%) values obtained by using $HgCl_2$ solutions of different concentrations (0.6 × 10^{-4} M, 1.1 × 10^{-4} M, 1.6 × 10^{-4} M, 2.1 × 10^{-4} M, 2.5 × 10^{-4} M) were given in Table 1.

Table 1. Activity (%) values of paraoxonase enzyme determined in $HgCl_2$ environment

Heavy metal	Tris-HCl Buffer (μL)	Supernatant Solution Volume (μL)	Substrate Solution Volume (μL)	Metal Solution Volume (μL)	Metal Solution concentration (1x10-4 M)	ΔOD (405nm)	Activity (U/mL dak)	Activity (%)
Hg	50	50	50	---	---	1.1822	19.3910	100.00
				10	0.6	1.8862	30.9383	159.55
				20	1.1	1.7717	29.0598	149.86
				30	1.6	1.6082	26.3799	136.04
				40	2.1	1.4598	23.9443	123.48
				50	2.5	1.2603	20.6725	106.61

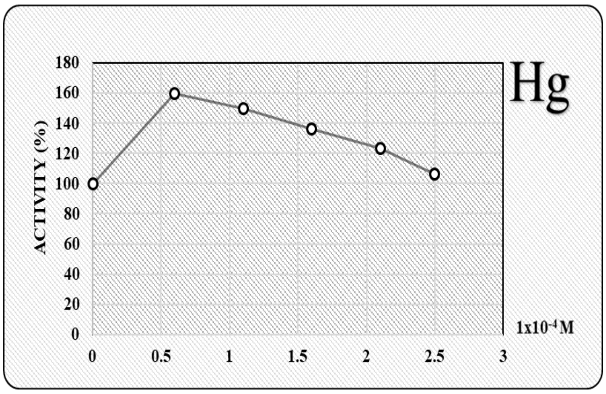

Figure 1. Activity determination for paraoxonase enzyme in $HgCl_2$ environment

The solution concentrations and activity (%) graph specified in Table 1 for paraoxonase enzyme in $HgCl_2$ environment were given in Figure 1.

After determining enzyme activity, PON enzyme activity was calculated as 159.55% when added a 10 μL-0.6 × 10^{-4} M $HgCl_2$ solution to cuvettes, while enzyme activity decreased as a result of increased concentrations and was calculated as 106.61% when added a 50 μL-2.5 × 10^{-4} M $HgCl_2$ solution. When the activity (%) graph was examined as a result of the measurements made, it was determined that Hg^{+2} heavy metal ion caused a decrease in paraoxonase enzyme activity ($p<0.05$). Paraoxonase enzyme activity (%) values obtained by using $CuCl_2$ solutions of different concentrations (0.6 × 10^{-4} M, 1.1 × 10^{-4} M, 1.6 × 10^{-4} M, 2.1 × 10^{-4} M, 2.5 × 10^{-4} M) were given in Table 2.

Table 2. Activity (%) values of paraoxonase enzyme determined in $CuCl_2$ environment

Heavy Metal	Tris- HCl Buffer (µL)	Supernatant Solution Volume (µL)	Substrate Solution Volume (µL)	Metal Solution Volume (µL)	Metal Solution Concentration ($1x10^{-4}$ M)	ΔOD (405nm)	Activity (U/mL dak)	Activity (%)
Cu	50	50	50	---	---	1.0730	17.5997	100.00
				10	0.6	1.2068	19.7949	112.49
				20	1.1	1.1877	19.4807	110.69
				30	1.6	1.1697	19.1864	109.02
				40	2.1	1.1657	19.1200	108.64
				50	2.5	1.1525	18.9037	107.41

The solution concentrations and activity (%) graph specified in Table 2 for paraoxonase enzyme in $CuCl_2$ environment were given in Figure 2.

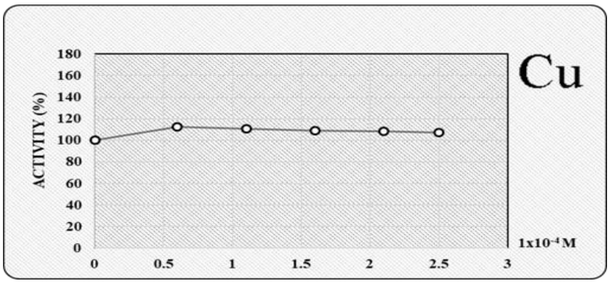

Figure 2. Activity determination for paraoxonase enzyme in $CuCl_2$ environment

After determining enzyme activity, PON enzyme activity was calculated as 112.49% when added a 10 µL-0.6 × 10^{-4} M $CuCl_2$ solution to cuvettes, while enzyme activity decreased as a result of increased concentrations and was calculated as 107.41% when added a 50 µL-2.5 × 10^{-4} M $CuCl_2$ solution. When the activity (%) graph was examined as a result of the measurements made, it was determined that Cu^{+2} heavy metal ion caused a decrease in paraoxonase enzyme activity but there was no statistically significant difference ($p>0.05$).

Paraoxonase enzyme activity (%) values obtained by using $ZnCl_2$ solutions of different concentrations (0.6 × 10^{-4} M, 1.1 × 10^{-4} M, 1.6 × 10^{-4} M, 2.1 × 10^{-4} M, 2.5 × 10^{-4} M) were given in Table 3.

Table 3. Activity (%) values of paraoxonase enzyme determined in $ZnCl_2$ environment

Heavy Metal	Tris- HCl Buffer (µL)	Supernatant Solution Volume (µL)	Substrate Solution Volume (µL)	Metal Solution Volume (µL)	Metal Solution Concentration ($1x10^{-4}$ M)	ΔOD (405nm)	Activity (U/mL dak)	Activity (%)
Zn	50	50	50	---	---	1.3021	21.3574	100.00
				10	0.6	1.4529	23.8305	111.58
				20	1.1	1.4070	23.0781	108.06
				30	1.6	1.3958	22.9073	107.26
				40	2.1	1.3676	22.4324	105.03
				50	2.5	1.3300	21.8159	102.15

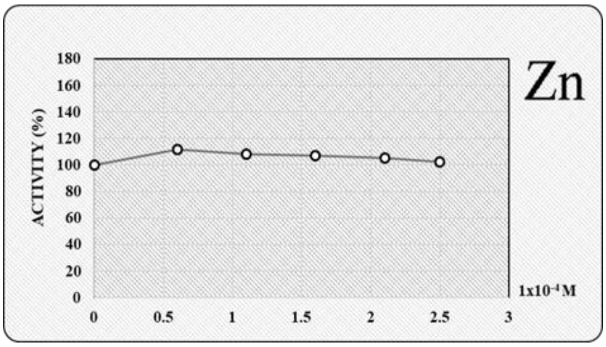

Figure 3. Activity determination for paraoxonase enzyme in $ZnCl_2$ environment

The solution concentrations and activity (%) graph specified in Table 3 for paraoxonase enzyme in $ZnCl_2$ environment were given in Figure 3.

After determining enzyme activity, PON enzyme activity was calculated as 111.58% when added a 10 µL-0.6 × 10^{-4} M $ZnCl_2$ solution to cuvettes, while enzyme activity decreased as a result of increased concentrations and was calculated as 102.15% when added a 50 µL-2.5 × 10^{-4} M $ZnCl_2$ solution. When the activity (%) graph was examined as a result of the measurements made, it was determined that Zn^{+2} heavy metal ion caused a decrease in paraoxonase enzyme activity but there was no statistically significant difference ($p>0.05$). The TOC, TAC, and OSI index values obtained as a result of the analyses were given in Table 4.

Table 4. Total antioxidant capacity (TAC), total oxidant capacity (TOC), and oxidative stress index (OSI) values (mean values ± standard error)

		Mean ± SE
TAC	(mmol TroloxEquiv/L)	0.55 ± 0.29
TOC	(μmol H_2O_2Equiv/L)	10.72 ± 3.52
OSI	(AU)	2.45 ± 1.41

DISCUSSION AND CONCLUSION

Recently, the pollution of surface water resources by heavy metals, which is one of the common environmental pollutants, has started to increase all over the world (Uncumusaoglu *et al*., 2016; Mutlu and Kurnaz, 2017; Huang et al., 2020) and the level of metals in the environment has become very dangerous for the health of water and land life (Pamukoglu and Kargi, 2007). Especially, It has been shown in many studies that fish, which are among aquatic organisms, are the living group most affected from heavy metal pollution (Yilmaz et al., 2016; Aytekin and Kargın, 2019; Çoğun and Kargın, 2020). Among marine creatures, fish accumulate pollutants from the aquatic environment and therefore widely used in pollution monitoring systems of the aquatic environment (Henry *et al*., 2004). Due to pollution from chemicals and waters, fish are constantly exposed to heavy metals (Ashraf, 2005). An increasing heavy metal accumulation in the bodies of fish can disrupt the structure of enzymes by showing toxic effect (Mackness *et al*., 2000; Mackness *et al*., 2001). Heavy metals exhibit toxicity by complexing with organic compounds and if they bind to these groups, they can switch to the inactive enzyme form (*Ekinci et al.,* 2007). Since PON activity is important in environmental pollution, in this study the effects of Hg^{+2}, Cu^{+2}, and Zn^{+2} heavy metal ions on the activity of paraoxonase enzyme, which has detoxification, antioxidant and antibacterial activity in muscle tissue of bonito (*S. sarda*) fish was examined and the oxidative stress index of the muscle tissue at normal levels was determined.

When the effect of Cu^{+2} heavy metal ion on PON enzyme activity, it was determined that increasing concentrations caused a decrease in PON enzyme activity, but there was no statistically significant difference between increasing concentrations of Cu^{+2} heavy metal ion. Sayın *et al.* (2012), in a study, examined in vitro the inhibition effect of Cu^{2+}, Ni^{2+}, Cd^{2+}, and Hg^{2+} metal ions on PON enzyme activity in *Scyliorhinus canicula* fish. Cu^{2+}, Ni^{2+}, Cd^{2+}, and Hg^{2+} metal ions showed an inhibitory effect on this enzyme activity and were determined that the strongest effect was by Cu^{2+} (Sayın *et al*., 2012). But when the activity (%) graphs were examined in this study, it was found that Cu^{+2} heavy metal ion did not cause a significant difference between enzyme activities, but Hg^{+2} heavy metal ion significantly caused a decrease in enzyme activity, causing a greater effect on activity. Hg is a metal that acts toxic by binding to thiol groups and inactivating proteins and enzymes when it enters the cell (Misra, 1992). Erdös *et al.* (1960), in a study, stated that Hg, Cu, and Ni salts inhibit PON1 activity at low concentration due to interacting with a thiol group at the catalytic center (Erdös *et al*., 1960). Therefore, Hg heavy metal ion is thought to inactivate the enzyme by binding to the free sulfhydryl (thiol) group in the cysteine at the 284 th position of the PON enzyme. During the oxidation of Cu^{+1}/Cu^{+2} ions, it has also been reported that it may be responsible for the partial inactivation of PON, replacing the Ca ion required for PON's paraoxonase activity (Aviram, 1999). In this study, when the activity (%) graph was examined, It was determined that the Cu^{+2} heavy metal ion acted in this way, causing a partial decrease in paraoxonase enzyme activity but there was no significant difference between statistically measured activities.

When the effect of Zn^{+2} heavy metal ion on PON enzyme activity was examined, it was determined that increasing concentrations caused a decrease in PON enzyme activity, but there was no statistically significant difference between increasing concentrations of Zn^{+2} heavy metal ion. In a study investigating the effect of zinc on PON enzyme activity in fish, plasma PON activity of *Capoeta capoeta* fish kept in tanks containing 5 and 10 mg/L $ZnSO_4$ for 10 days decreased compared to the control group. They reported that this decrease in PON enzyme activity may be due to the absorption relationship between Ca^{+2} and Zn^{+2} cations (Deveci *et al*., 2015). In this study, when the activity graph of the Zn^{+2} heavy metal ion was examined, it was observed that it caused a decrease in PON enzyme activity, and this decrease in activity was thought to be due to the absorption relationship between Ca^{+2}/Zn^{+2} ions. Because the Ca^{+2} ions required for the stability and activity of the PON enzyme due to HDL in plasma are directly related to the absorption of Zn^{+2} ions (Babacan *et al*., 2011; Cicik, 2003). Also, Zn is taken by an apical Ca^{+2} channel found in mitochondrial-rich ion carrier cells in the gills of fish (Bury *et al*., 2003; Zhang and Wang, 2006).

It was observed that Hg^{+2} heavy metal ion showed a statistically significant decrease in PON enzyme activity compared to Cu^{+2} and Zn^{+2} ions due to increasing concentrations and inhibited the enzyme. Especially when enzyme activities were compared, it was determined that there were significant differences between the 0.6×10^{-4} M and $2.1\text{-}2.5 \times 10^{-4}$ M samples used and between 1.1×10^{-4} M and 2.5×10^{-4} M samples. According to the results obtained, it was seen that the Hg^{+2} heavy metal ion inhibited by showing more effect compared to Cu^{+2} and Zn^{+2} ions on enzyme activity. In a study examined the effect of different metals on paraoxonase enzyme activity in carp (*Cyprinus carpio)* fish, it was determined that Co, Hg, Cu, and Cd heavy metals inhibited different levels of PON activity (Beyaztaş *et al*., 2007).

In another study in scaly carp (*C. carpio*) fish, the effect of malathion, which is known to be toxic such as heavy metals on paraoxonase and arylesterase enzyme activities were researched and it was observed that PON activity decreased due to increasing concentrations of malathion (Kılıç and Yonar, 2017). In a study using the chromium oxide (CrO_3)

form of chromium, a heavy metal such as copper, mercury and zinc, it was determined that chromium applied at concentrations of 15, 30 and 60 ppb for 28 days reduced the activity of carp (*C. carpio*) serum PON (Yonar *et al*., 2012).

In a study conducted in bonito (*S. sarda*) fish, it was examined the effect of some heavy metals on glutathione transferase enzyme, which has detoxification and antioxidant properties such as the PON enzyme. Güller *et al.* (2014), in this study, the effects of Pb^{2+}, Cr^{2+}, Fe^{3+} Ag^{+}, Cu^{2+}, Cd^{2+}, and Zn^{2+} metal ions on the enzyme activity were examined and it was reported that Cu ion showed the strongest inhibitor effect and, Zn ion showed weakest inhibitor effect (Güler *et al.*, 2014). When the activity (%) graphs were examined also in our study, it was found that Zn heavy metal ion caused a more significant decrease in PON enzyme activity compared to Cu heavy metal ion.

Besides studies carried out among fish, in the studies also carried out in rats, humans, bull, and sheep, the effect of heavy metals on PON enzyme activity was investigated. Pla *et al*. (2007), in a study, examined the effects of some metal ions on PON1 enzyme activity purified from rat liver. In the present study they have done, inhibition effects of Mn^{+}, Cu^{+2}, Hg^{+2} ve Co^{+2} heavy metal ions were determined and it was determined that Hg^{+2} heavy metal ion was the strongest inhibitor and Cu^{+2} ion was the weakest inhibitor for PON1 (Pla *et al*., 2007). In this study, when the activity (%) graphs were examined, it was determined that the inhibitor effect of Hg^{+2} ion on the enzyme activity more than the effect of Cu^{+2} ion. When looked the effect of Hg^{+2} and Cu^{+2} heavy metal ions on PON enzyme activity studied in bonito (*S. sarda*) fish, it is seen that it causes a decrease in enzyme activity similar to the PON1 enzyme activity in rats. Samra *et al*. (2010), examined the in vitro inhibitor effects of some metal ions, at 1.0 mM concentration on human PON1 enzyme activity. It was determined that Mg^{+2} and Mn^{+2} ions did not show any effect on human PON1 enzyme activity, Pb^{+2}, Co^{+2}, and Zn^{+2} ions decreased the activity, while Ni^{+2}, $Cd^{+2,}$ and Cu^{+2} ions inhibited the PON1 enzyme activity (Samra *et al*., 2010). Dedeoğlu *et al.* (2014), in a study, determined changes occurring in PON1 enzyme activity purified from bull semen in the presence of Cu^{+2}, Mn^{+2}, Cd^{+2}, Zn^{+2}, Ni^{+2}, and Pb^{+2} heavy metal ions in different cuvette concentrations. While Cd^{+2} ions increased PON1 activity, other heavy metal ions were found to inhibit PON1 at micromolar levels (Dedeoğlu *et al*., 2014). It was determined that Cu^{+2} and Zn^{+2} heavy metal ions used in this study also caused at low levels decrease of PON enzyme activity in bonito (*S. sarda*) fish but there was no statistically significant difference. Therefore, it is seen that Cu and Zn heavy metal ions affect PON enzyme activity studied in bonito (*S. sarda*) fish, similar to that of human PON1 enzyme and PON1 enzyme activity purified from bull semen. Erol *et al.* (2013), in another study, examined the effect of some metal ions on PON1 enzyme activity purified from blood samples taken from Merino and Kivircik sheep breeds. It was determined that Mn^{+2}, Hg^{+2}, Co^{+2}, Cd^{+2}, $Ni^{+2,}$ and Cu^{+2} metal ions showed different levels of inhibition effect on PON enzyme activity and Cu^{+2} heavy metal ion caused strongest inhibitor effect for PON (Erol *et al*., 2013). When examined the effects of heavy metal ions used in this study on PON enzyme activity in bonito (*S. sarda*) fish, it is seen that Cu^{+2} metal ion caused a partial decrease in the enzyme activity compared to other heavy metal ions (Hg^{+2}, Zn^{+2}) used in the study. Therefore, it is seen that the Cu^{+2} heavy metal ion caused a decrease at different levels of PON enzyme activity in Merino and Kivircik sheep breeds by the PON enzyme of bonito (*S. sarda*) fish.

In the study, the effect of Hg^{+2}, $Cu^{+2,}$ and Zn^{+2} heavy metal ions was researched on paraoxonase enzyme activity, which an antioxidant enzyme in muscle tissue of bonito (*S. sarda*) fish, and it was found Cu^{+2}, Zn^{+2} ions caused a statistically insignificant decrease in enzyme activity. It is thought that the inhibitory effect of Cu and Zn ions, which are present in excess amount in the environment, on the PON enzyme may be due to the substitution of Ca ions, which are the cofactor of the enzyme. Therefore, it is thought of these ions cause a decrease in activity by causing inhibition of enzymatic activity. It is thought that Zn^{+2} and Cu^{+2} ions to be used in higher concentrations in studies where the effects of heavy metals on PON enzyme activity will be investigated, may cause a significant decrease in enzyme activity. Hg^{+2} heavy metal ion is thought to inhibit the enzyme activity and show its inhibitory effect on the enzyme by binding to the free sulfhydryl (thiol) group in the cysteine at the 284 th position of the PON enzyme. It is thought that controlled experimental studies may be conducted to investigate the effects of heavy metals taken in higher concentrations on TOC, TAC and PON enzyme activity and our study may also create an example.

ACKNOWLEDGEMENTS

The authors thank the support provided by the Ondokuz Mayıs University Scientific Research Projects Commission Presidency with the Project number PYO.VET.1904.19.011.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 487-497 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.10

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Histological changes in the gills, livers and muscles of *Oreochromis niloticus* fed with steriod, natural hormone and pawpaw seed based diets

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Received date: 06.04.2021 *Accepted date: 18.10.2021*

How to cite this paper:
Orose, E., Wokeh, O. K. & Odioko, E. (2021). Histological changes in the gills, livers and muscles of *Oreochromis niloticus* fed with steriod, natural hormone and pawpaw seed based diets. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 487-497. DOI: 10.12714/egejfas.38.4.10

Abstract: This study was conducted to evaluate the histological changes of 17αmethyl-testosterone (MT), wet testes of bull (*Bos indicus*), boar (*Sus domesticus*), bull/mud catfish testes (*B. indicus/C. gariepinus*) and pawpaw (*Carica papaya*) seed powder-based diet on the Nile tilapia fry in an indoor experimental pond. Histological changes and efficacies in producing monosex males were determined after four months. Fifty-five fry were assigned randomly to three experimental ponds in triplicates. Five experimental feeds composed of soya-bean, fish meal, wheat bran, cassava flour were formulated by adding 0.06g of 17α- methyl-testosterone, 17.47g of bull wet testes, 25.59g of boar wet testes, 20.48g of bull/catfish and 20g of pawpaw seed powder per kg of feed to group 1, 2, 3, 4, 5 respectively. Obtained results from the morphological examination revealed a significant difference ($p < 0.05$) in phenotypic males of the Nile tilapia fry fed with the experimental diets. The highest indistinct percentage was recorded in the 17αmethyl-testosterone-treated diet while the lowest was recorded in diets 3 and 4 with no significant differences. All the groups showed histopathological differences in the livers, gills, and muscles. However, the liver histology of groups 1 and 5 showed central vessels that are mildly congested with abundant glycogen vacuolation and somewhat fat infiltration, while the histology of the muscles of group 5 showed pathological lesions including moderate to severe necrosis. These alterations can be considered an indication of the performance and health of fish in monosex culture medium indicating effects of plant extract and MT.

Keywords: Histological changes, *Oreochromis niloticus,* monosex, 17α- methyl-testosterone, pawpaw seed

INTRODUCTION

Overstocking of tilapia in an enclosed pond causes stunted growth due to the unavailability of natural food, especially in the semi-intensive system (Meske and Vogt, 2014). To achieve more productivity in growing tilapia *Oreochromis niloticus*, it is important to produce a monosex culture that constitutes males (El-Greisy and El-Gamal, 2012), because male grows faster than female because of unnecessary spawning. The most efficient method of producing only males is using steroid hormones fed to newly hatched fry with unknown sex (Ajiboye *et al*., 2015). This is done by exposing the fish to hormones (testosterone or estrogen) in their diets orally for several weeks when the fish start feeding, which leads to sex reversal. The application of synthetic hormones is under serious criticism due to their health and environmental hazards. More so, there is little literature on the tangible effect of the residuals on the environment and human health (Desprez *et al*., 2003, Mengumphan *et al*., 2006).

Thus far, the caused by the synthetic hormone on environmental damage or harm to humans used for sex inversion has no substantial proof; however, there is a huge amount of MT used for sex reversal compared to the actual dose (Xu *et al*., 2015; Mlalila *et al*., 2015), which may lead to building up of MT chemical in tilapia farming. Hormone treated fish is restricted in some countries except it is proven that it will not pose risks to human health (Megbowon and Mojekwu, 2014). However, the potential disadvantage of synthetic hormone treatment increased the risk of long-term exposure of workers handling methyltestosterone during food preparation and feeding may cause adverse effects on their health (Green *et al*., 1997). There have been reports that hormones excreted by treated fish from undigested feeds can build up in a closed water system; accumulate in water residue and aquatic biota (Dauda *et al*., 2014). Kefi *et al.* (2013) in their trial with tilapia fish, observed an induced sex inversion in waters not directly applied with methyl-testosterone, although is not well understood; the effect on water pollution.

Owing to the detrimental toxicity of hormone on human health which results in mostly carcinogenic and endocrine disorder and on the environment, researchers has deliberated on its use in aquaculture production (Dergal *et al*., 2016). Furthermore, the use of anabolic steroids has been banned because of the harmfulness to humans, fish and the environment (Suseno *et al*., 2020); based on the decision from the Republic of Indonesia with number KEP.52/MEN/2014 of the Ministry of Marine Affairs and Fisheries.

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In recent times, the use of MT in the seed production of Oreochromis species has increased despite inadequate information about the impact of the androgen on fish physiology; though, its long-term impacts on the pathology of livers, gills and muscle remain unclear (Kefi *et al*., 2013). Curtis et al. (1991) established the fact that metabolizes of plasma testosterone are quickly excreted from the body. There are few published articles on the histological changes of *O. niloticus* subjected to sex steroids in particular MT, animal, and plant extract; hence, the need to evaluate the histological changes associated with the gills, livers, and muscles of *O. niloticus* subjected to MT, animal hormone and plant extract. Therefore, this study will help to understand the effects of *O. niloticus* exposed to the different sex reversals-based diets and provide fishery resources managers and decision-makers information on the most fish-friendly and effective sex reversal-based diet.

MATERIAL AND METHODS

A total of 825 *Oreochromis niloticus* (Nile tilapia) fry with an average weight of 0.013g; were obtained from the Tilapia family testing unit of the Africa Regional Aquacultural Center (ARAC) Aluu, Port Harcourt, Rivers State. The experiment was carried out in 15 different experimental ponds at ARAC and was distributed randomly into five groups; fifty-five (55) fry were stocked in each in triplicate. The methyl-testosterone was acquired from Lagos State (University of Lagos) Nigeria while the testes of boar bull fish were procured from Port Harcourt market, while pawpaw fruits were collected from the University of Port Harcourt Farm which was sun-dried, ground and prepared as described by (Orose *et al*., 2016).

Hormone preparation

The hormone was prepared by adding 0.06 g of 17 α-Methyltestosterone, 4 g of catfish testes, 10 g of boar and bull testes to a beaker containing 500 ml of ethanol (95%) respectively, while 5.7 g of pawpaw seed powder was dissolved in water for hormonal analysis (testosterone/estrogen) at the Chemical Pathology Laboratory, University of Port Harcourt Teaching Hospital (UPTH) for hormonal analysis. Total testosterones from the serum of each experimental animal were analyzed using an Enzyme Immunoassay Testosterone test kit (Table 1). The 1000 g compounded feed was formulated with the addition of the steroid hormone and plant extract. Group 1 was added with 0.06g of 17α- methyl-testosterone, group 2 was prepared with 17.47 g of bull testes, group 3 was prepared with 25.59g of boar testes, group 4 was prepared by adding 20.48 g of bull/ catfish testes while group 5 was prepared by adding 20 g of pawpaw seed powder-based diet (Orose *et al*., 2018).

Exposure of test organisms

One thousand grams of feed was formulated based on the working composition obtained from the feed mill of ARAC, using 40% crude protein. The formulated feed materials were composed of wheat bran, fish meal soya-bean, groundnut cake, vitamins etc. in various percentages as described by Orose and Vincent-Akpu, 2016. The feeding frequency was three times daily at 20% of fish body weight for 42 days hormonal trial with final weight for group 0.42g, 0.52g, 0.78g, 0.60g and 0.69g for group 1 to 5 respectively. Thereafter, the fry were reared for 4 months for histological studies with final weights of 7.30g, 10.43g, 11.73g, 10.67g and 10.40g for groups 1 to 5 respectively.

Table 1. Total testosterone of steroid, animal and pawpaw seed powder

Treatments	Sample (gram)	Concentration
Treatment 1 (MT)	0.06 g	16.25
Treatment 2 (bull testes)	10 g	9.3
Treatment 3 (boar testes)	10 g	6.3
Treatment 4 (catfish testes)	4 g	1.8
Treatment 5 (PSP)	5.7 g	0.3

Sex determination

After four months of growth stage, sexing of fingerlings was determined, each group was collected in all the replicates and confirmed by examining the external genitals (Figure 1).

Figure 1. Male (left) and female (right) genital papilla (Fuentes – Silva *et al*., 2013)

Histological Changes

Fish samples were prepared with standard methods for histological analysis as detailed in the guideline as describe by Avwioro, (2010). Livers, gills, and muscles of 5 fish per treatment were surgically removed and placed in 10% Davison's solution and taken to the Veterinary Department of the Federal University of Agriculture, Abeokuta. The livers gills and muscles were fixed and prepared for sectioning involved dehydration, clearing and infiltration, embedding and sectioning using a microtome. The process for dehydration involved fixation of tissue in ascending grades alcohol of 30%, 50% 70% and 90% alcohol each for 1 hour; 95% alcohol for 12 hours; and absolute alcohol for 1 hour. The clearing process involved immersion of dehydrated tissue in xylene for 1 hour. The infiltration process involved immersion of the cleared samples in molten paraffin wax (between 56°C – 60°C) for two hours. The embedded tissues were sectioned using a microtome before placing them on slides then staining with Haematoxylin for 3 minutes and 1% solution of Eosin stain for 5-10minutes (H and E method). The prepared slides were viewed under a light microscope, Olympus CX31RTSF.

Data Analysis

All data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 21, analysis of variance (ANOVA) was used to determine significant differences among treatments. Differences were regarded as significant at $P < 0.05$; mean significant was compared using Duncan Multiple Range Test (DMRT).

RESULTS

Effect of experimental diet on masculinization of Nile Tilapia (*O. niloticus*)

The results for the morphological examination on sex ratio are presented in Table 2. There was no significant difference between group 3 and group 4, although there were significant differences ($p < 0.05$) among group 1, group 2 and group 5. The lowest male value was observed in group 5 (73.98±2.04%). The results on indistinct (sex differentiation that was not clear) showed that group 1 had a higher indistinct (5.00±0.58), while there was no significant difference ($p \geq 0.05$) between group 3 and group 4.

Effect of experimental diets on mortality rate of Nile Tilapia (*O. niloticus*)

The result on the mortality rate of Nile tilapia fries after the four months experimental period is shown in Figure 2. The result showed that group 1 recorded the highest mortality (51%) during the experiment, followed by group 2 (18%). However, there were no significant differences in mortality rates among group 3, group 4 and group 5 with mortality rates of 10%, 11%, and 10% respectively.

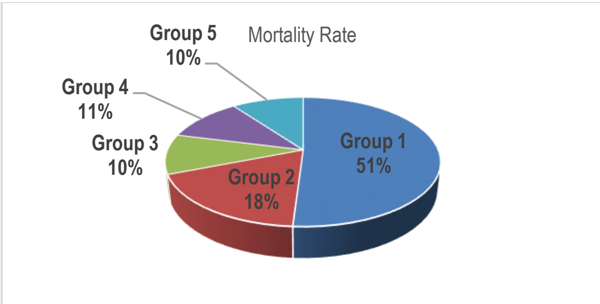

Figure 2. Mortality rate of Nile tilapia fed five experimental diets (5 groups)

Table 2. Morphological effect of treatments on sex ratio of *O niloticus*

	Numbers of Fish Observed per Group (percentages are in parentheses)				
Sex	**1**	**2**	**3**	**4**	**5**
Male	19.00 ± 1.53^{c} (79.17 ± 0.74^{bc})	30.33 ± 2.00^{b} (79.82 ± 1.03^{b})	37.67 ± 1.20^{a} (91.88 ± 2.11^{a})	36.67 ± 0.88^{a} (91.68 ± 0.59^{a})	30.33 ± 0.88^{b} (73.98 ± 2.04^{b})
Female	0.00 ± 0.00^{d} 0.00 ± 0.00^{d})	3.67 ± 0.33^{b} 9.66 ± 0.27^{b})	2.67 ± 0.33^{c} 6.51 ± 0.68^{c})	3.00 ± 0.00^{bc} 7.50 ± 0.60^{c})	7.33 ± 0.33^{a} 17.88 ± 0.60^{a})
Indistinct	5.00 ± 0.58^{a} 20.83 ± 0.74^{a})	4.00 ± 1.00^{a} 10.52 ± 1.98^{b}	0.67 ± 0.67^{b} 1.61 ± 1.63^{c})	0.33 ± 0.21^{b} 0.82 ± 0.80^{c})	3.34 ± 0.88^{a} 8.14 ± 2.05^{d})
Total	24 (100)	38 (100)	41 (100)	41 (100)	41 (100)

a, b, c and d are superscripts to indicate significant differences among different groups/ Mean values (mean ± standard error) in the same row with different superscript are significantly different ($p < 0.05$).

Histological examination

Histological changes in the livers of *Oreochromis niloticus* fed experimental diets

The histological analysis of the livers revealed that there were pathological differences among the groups. Group 1 photomicrograph of fish liver showed Central vessels that are mildly congested with mildly thickened vascular wall (white arrow), the sinusoids (black arrow) appear normal and not infiltrated by inflammatory cells while the hepatocytes showed abundant glycogen vacuolation and somewhat fat infiltration (blue arrow) (Figure 3), Group 2 showed section with normal central vessels that are not congested (white arrow), however, there was mild perivascular infiltration seen (gray arrow), the sinusoids (black arrow) appeared normal and not infiltrated by inflammatory cells, the hepatocytes showed abundant glycogen vacuolation (blue arrow) (Figure 4), group 3 showed normal central vessels that are not congested (white arrow), the sinusoids (black arrow) appeared normal and not infiltrated by inflammatory cells, the hepatocytes showed moderate glycogen vacuolation (blue arrow) (Figure 5), additionally, group 4 showed normal central vessels that are not congested (white arrow), the sinusoids (black arrow) appeared normal and not infiltrated by inflammatory cells, the hepatocytes showed abundant glycogen vacuolation (blue arrow) Figure 6. On the other hand, group 5 showed mildly congested central vessels (white arrow), the sinusoids (black arrow) appeared normal and not infiltrated by inflammatory cells, the hepatocytes showed abundant glycogen vacuolation and fat degeneration (blue arrow) (Figure 7). However, there appeared to be differences in their hepatocytes: group 1 showed abundant glycogen vacuolation and somewhat fat infiltration, group 2 and 4 showed abundant glycogen vacuolation, group 3 showed moderate glycogen vacuolation while group 5 showed abundant glycogen vacuolation with fat degeneration.

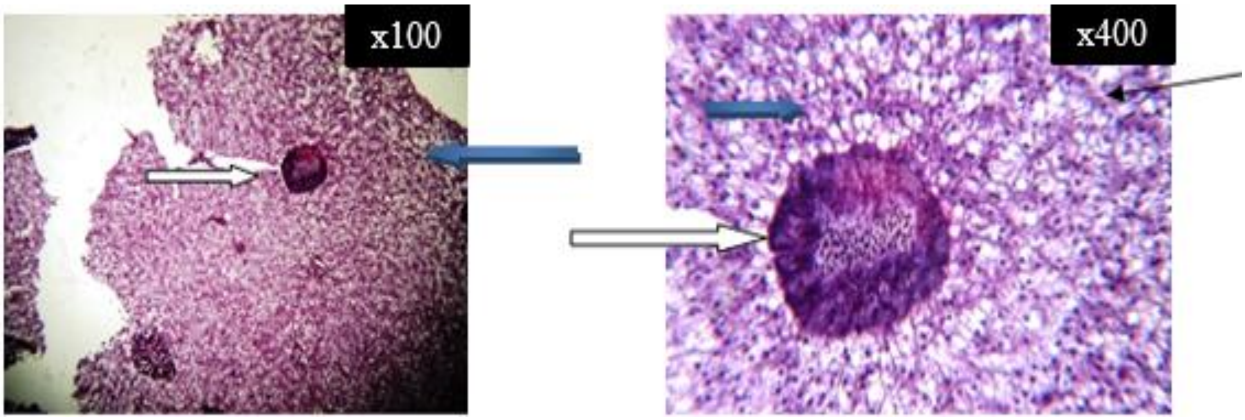

Figure 3. Photomicrograph of group 1 fish liver section

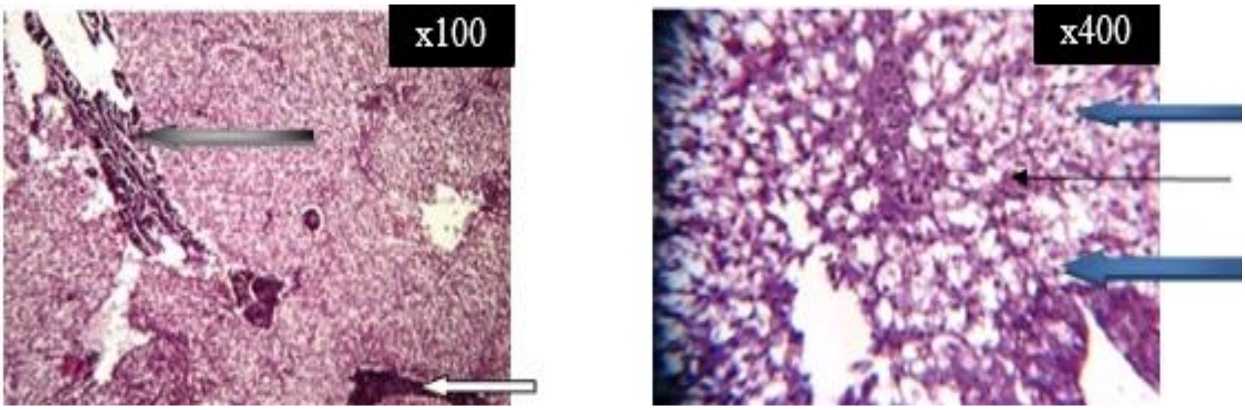

Figure 4. Photomicrograph of group 2 fish liver section

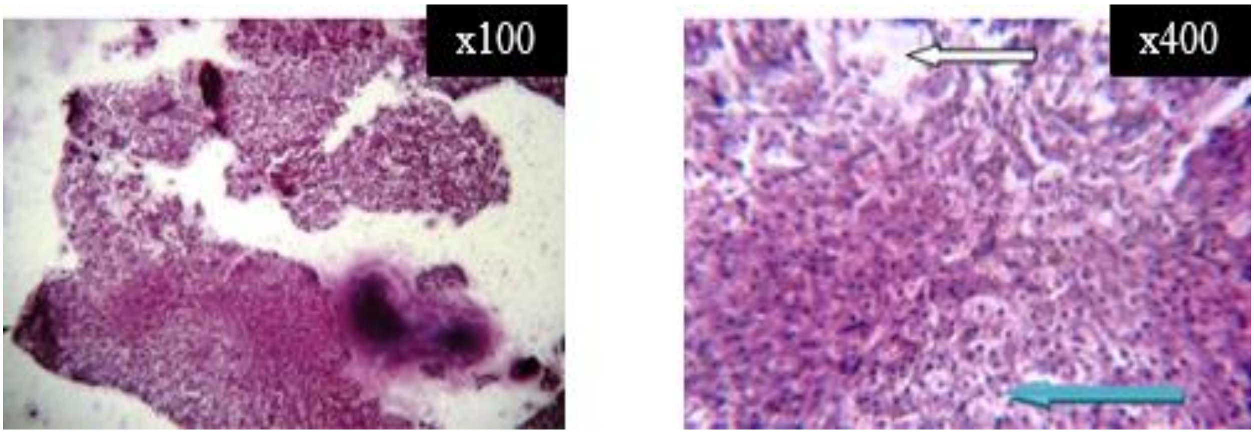

Figure 5. Photomicrograph of group 3 fish liver section

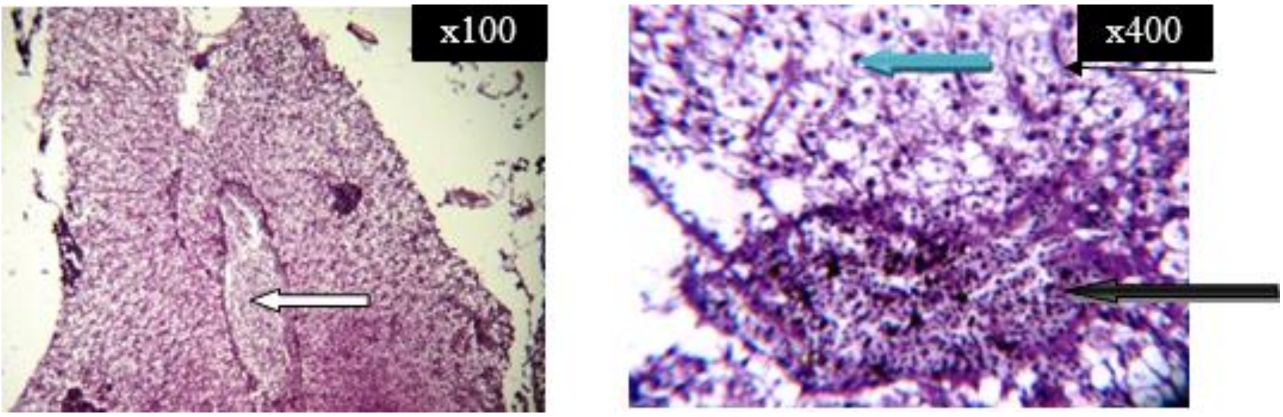

Figure 6. Photomicrograph of group 4 fish liver section

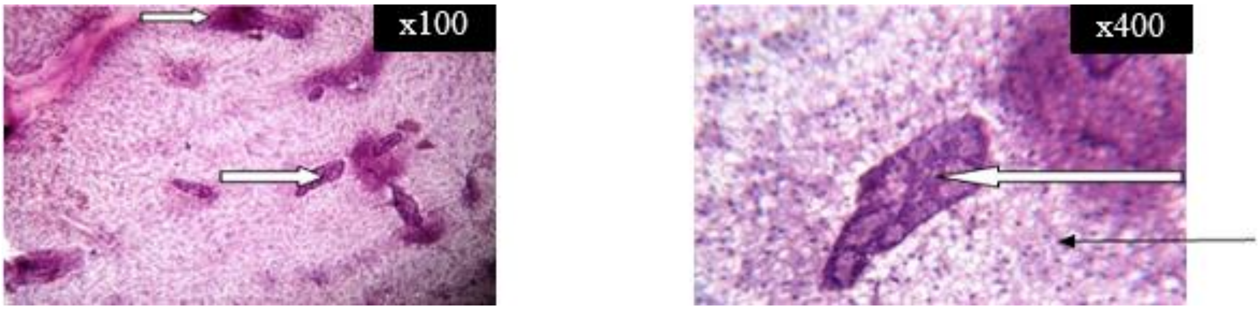

Figure 7. Photomicrograph of group 5 fish liver section

Histological changes in the gills of *Oreochromis niloticus* fed experimental diets

The histopathological study of the gills section revealed that the gills section for group 1 showed normal gill lamellae (white arrow) and arch (blue arrow) lamellae consist of normal erythrocytes, chloride cells and pillar cells (black arrow), while the gill arch showed normal osteocyte-like cells (Figure 8). Group 2 had a normal gill lamella (white arrow) and arch (blue arrow) the lamellae consist of normal erythrocytes, chloride cells and pillar cells (black arrow) while the gill arch showed normal osteocyte-like cells (Figure 9). Group 3 showed poorly arranged gill lamellae (white arrow) and arch (blue arrow). The lamella consists of normal chloride cells and pillar cells (black arrow), while the gill arch showed normal osteocyte-like cells (Figure 10). Group 4 showed shortened gill lamellae (white arrow) and arch the lamellae consisting of normal erythrocytes and few chloride cells and pillar cells (Figure 11), while group 5 showed normal gill lamellae (white arrow) and arch (blue arrow) (Figure 12). The lamellae consist of normal erythrocytes, chloride cells and pillar cells (black arrow), while the gill arch showed normal osteocyte-like cells.

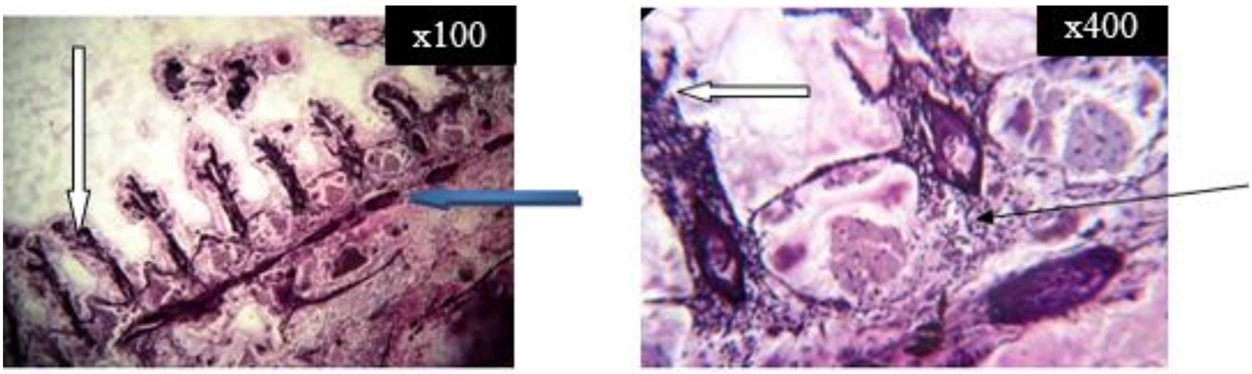

Figure 8. Photomicrograph of group 1 fish gill section

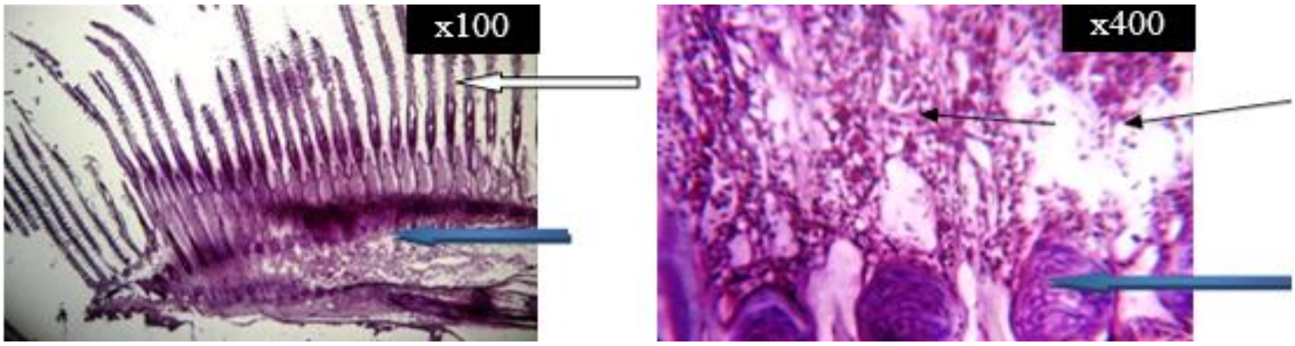

Figure 9. Photomicrograph of group 2 fish gill section

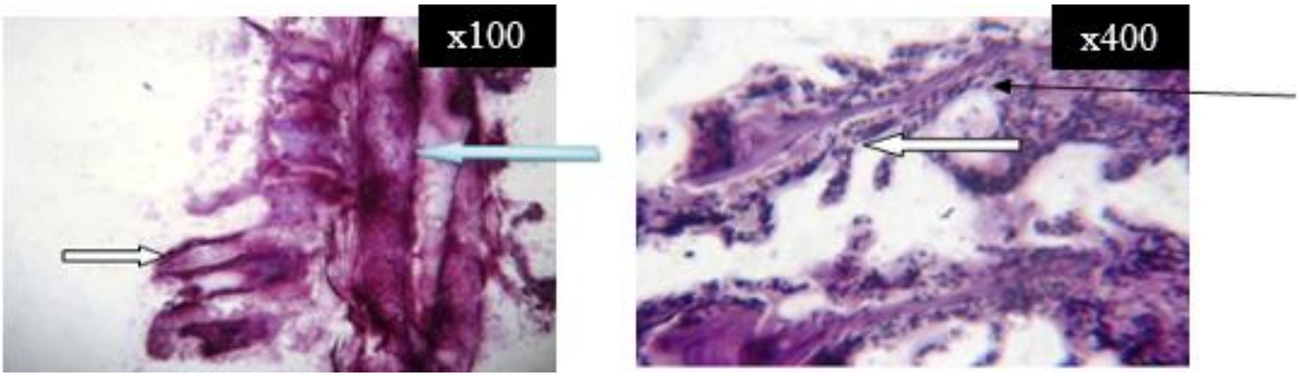

Figure 10. Photomicrograph of group 3 fish gill section

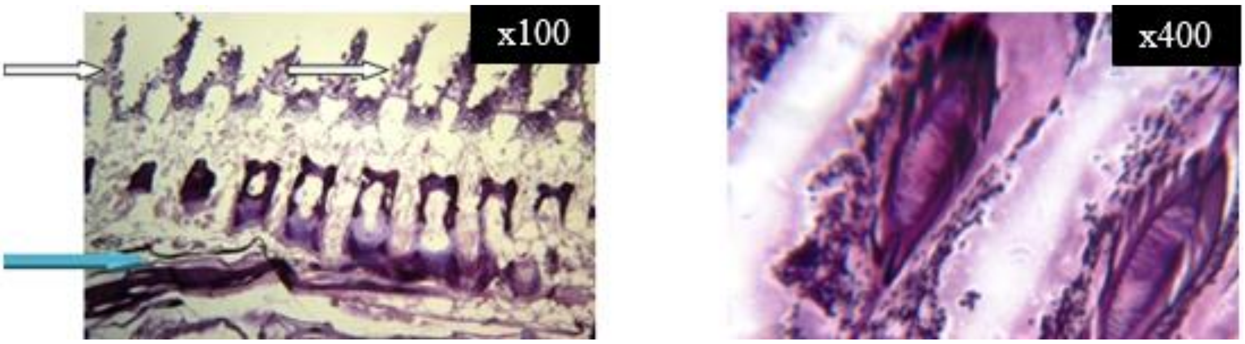

Figure 11.Photomicrograph of group 4 fish gill section

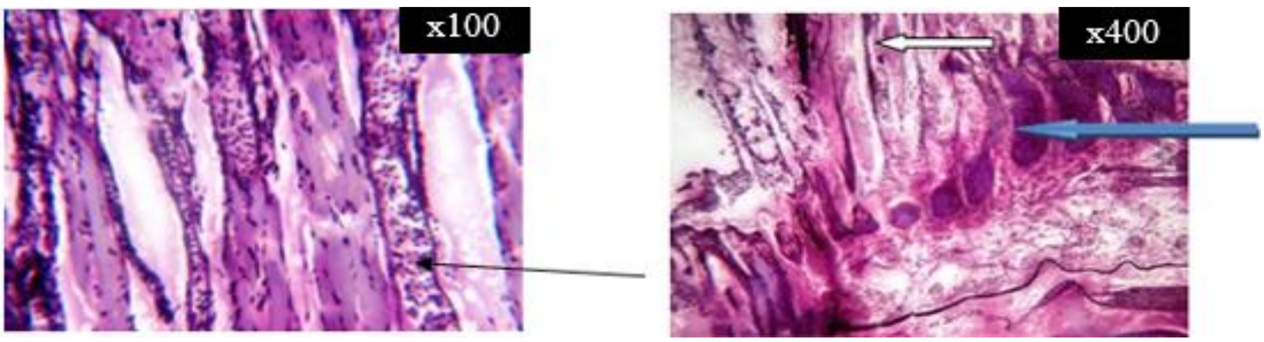

Figure 12. Photomicrograph of group 5 fish gill section

Histological changes in the muscles of *Oreochromis niloticus* fed experimental diets

The histopathological examination of the muscles revealed that muscles sections of group 1 showed normal architecture (white arrow), the pathological lesion was not seen (Figure 13), group 2 showed normal architecture (white arrow), with no pathological lesion (Figure 14).

Group 3 showed normal architecture (white arrow), without pathological lesion (Figure 15), while group 4 showed moderate architecture (white arrow), with no pathological lesion found (Figure 16) whereas group 5) (Figure 17) showed a poor architecture, but with the presence of pathological lesion including moderate to severe necrosis (black arrow).

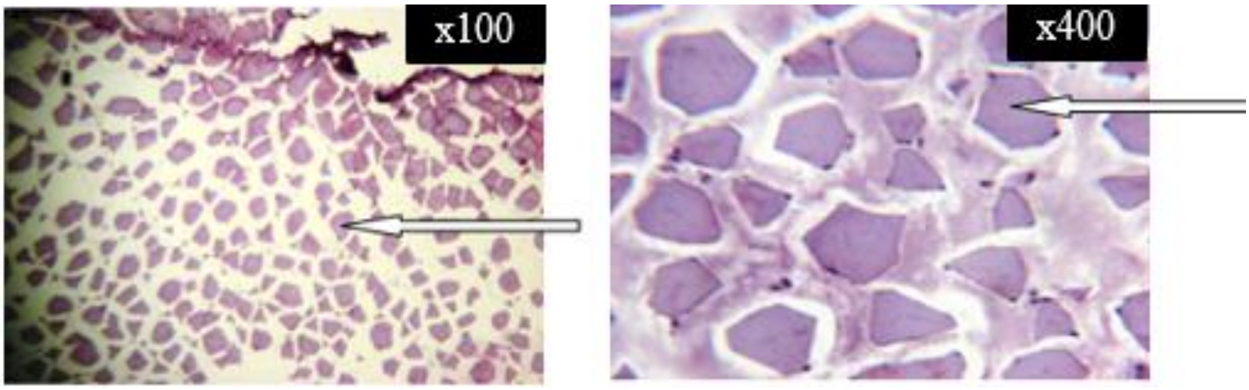

Figure 13. Photomicrograph of group 1 fish muscle section

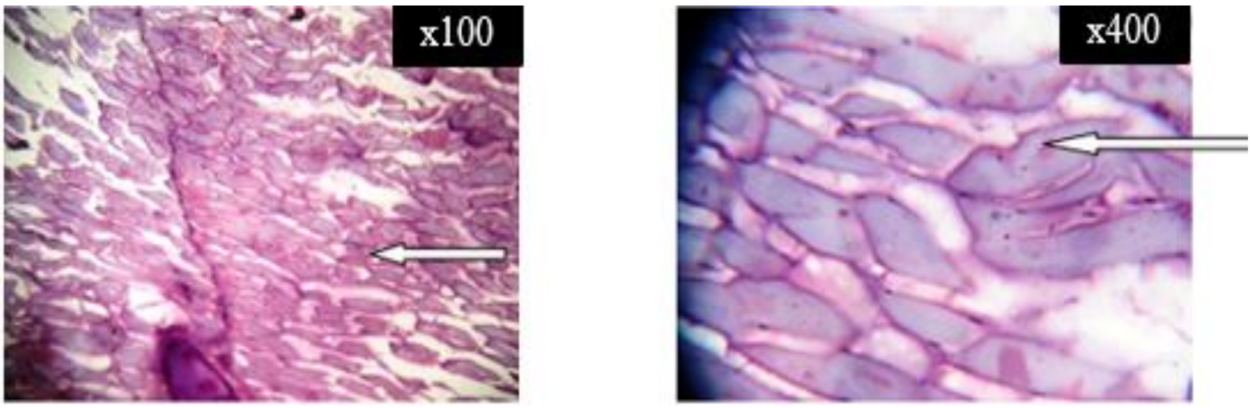

Figure 14.Photomicrograph of group 2 fish muscle section

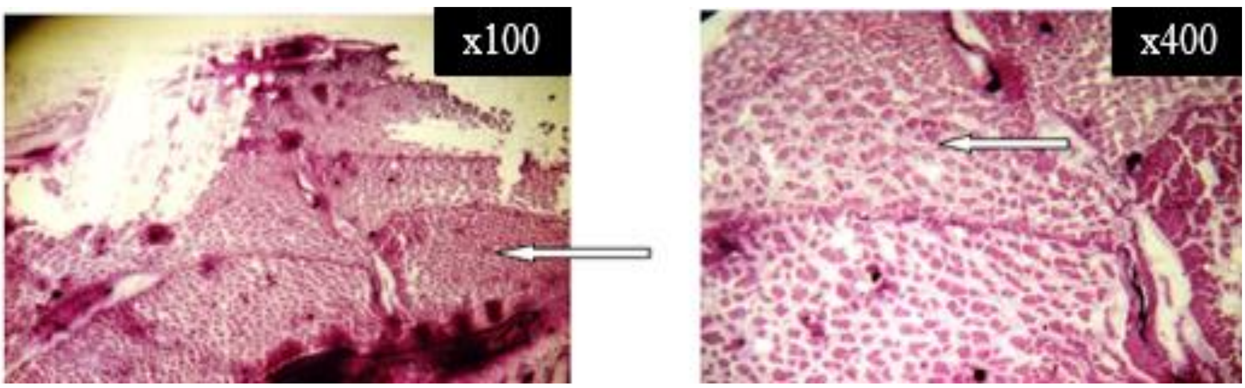

Figure 15.Photomicrograph of group 3 fish muscle section

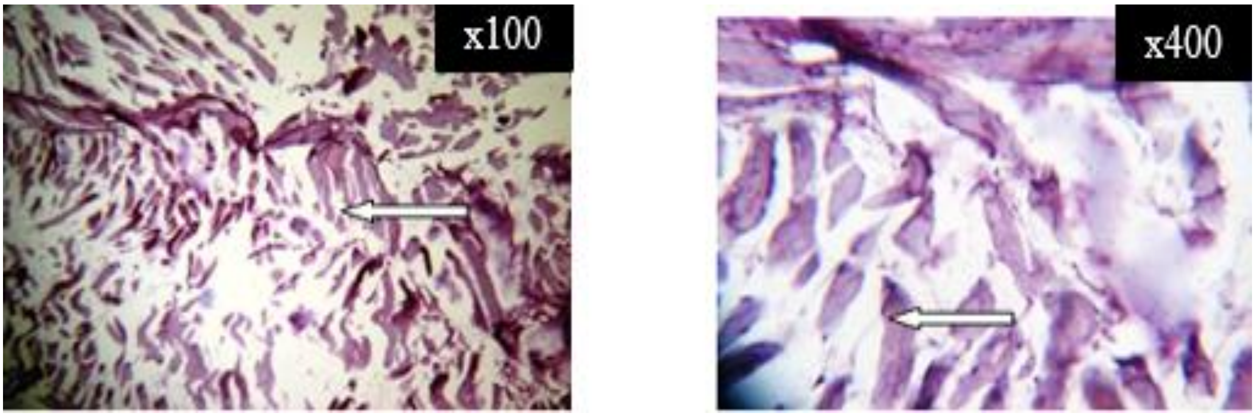

Figure 16.Photomicrograph of group 4 fish muscle section

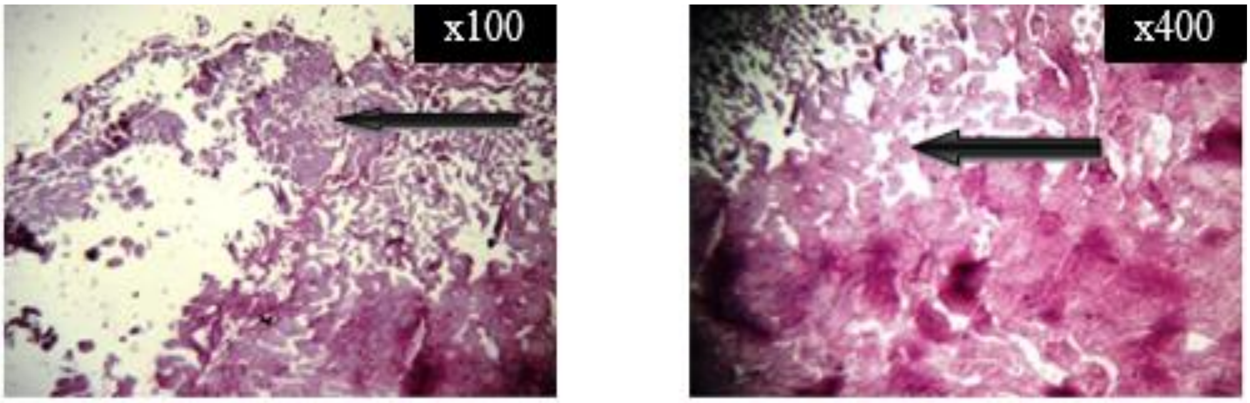

Figure 17.Photomicrograph of group 17 fish muscle section

DISCUSSION

The examination of external genital papillae of *O. niloticus* showed that the natural hormone and plant extract had the highest male percentage. However, MT treated diet had the highest indistinct percentage rate and mortality rate. These results are in line with the findings of Mohamed (2019) that reported the variation between morphological and gonad results. Mohamed (2019) examined 50 fish from a group; morphologically, 40 of the fishes were presumed to be males, but after gonad and histological examination of the gonad, it was observed that there were 42 males, seven females and one hermaphrodite. The result from this study showed that MT had the lowest percentage of phenotypic males (79.26%). Group 3 had the highest percentage male ratio. The differences among the groups were scientifically significant. The lowest male ratio was observed in the MT diet, this may be due to the high percentage indistinct found in group 1 compared to all other groups upon observation, while groups 3 and 4 had the lowest rate.

Hussain (2004) and Rahma *et al*. (2015) reported that the sexual dimorphism of tilapia is expressed in genital papillae. Furthermore, at the time of sex determination, few fish were less than 4 g in weight; at this stage, sex determination of the gonad is difficult. Fish with reduce weight and abnormalities were regarded as indistinct. In this study, fish genital papilla that was not clear at the time of sex determination were regarded as indistinct. In addition, Ekwu and Sikoki (2001), sexed tilapia at 2 g and observed indistinct fish by gross examination. They reported that those indistinct fish turned out to be female after histological sectioning, which was not in line with Berger and Rothbard (1987) that reported that indistinct fish were regarded as male.

The liver section of the present study (Figure 3-8) showed pathological differences among the groups, mildly congested central vessels that were with mildly thickened vascular wall, although group 2 were normal in the central vessels, however, there was mild perivascular infiltration seen in groups 3 and 4. Groups 3 and 4 showed normal central vessels that were not congested, on the other hand, group 5 showed mildly congested central vessels, the sinusoids appeared normal and not infiltrated by inflammatory cells. This finding agrees with a similar study by Khater (1998) in studying the effect of different doses (15, 30, 60, 90 mg) of 17α-methyltestosterone on the liver for 28 days and indicated that the hepatic parenchyma had diffused vacuolar degeneration while the central veins and hepatic sinusoids were congested, on the contrary, Deborah, (1990) and Kefi *et al*. (2013) found that there was no deviation from the normal morphology in livers. However, in this study, there appeared to be differences in the hepatocytes, abundant glycogen vacuolation in groups 2 and 4. But group 1 showed abundant glycogen vacuolation and somewhat fat infiltration, group 3 showed moderate glycogen vacuolation, while group 5 showed abundant glycogen vacuolation with fat degeneration. Khater (1998), also reported that liver tissue treated with 60 mg MT for 14 days showed diffuse hydropic degeneration, congestion of central vein and haemorrhage was seen in the hepatic parenchyma. Furthermore, Dias-Neto *et al*. 2016 observed that only the larvae that received hormone had hydropic degeneration on day 28, while the fish fed with and without hormone had more lesions and severe lesions (lipid degeneration).

The liver histological changes in this study were pronounced more in the fish exposed to methyl-testosterone and pawpaw seed basal diet. Although, Myers *et al*. (1987) reported that alterations are often associated with a degenerative-necrotic condition. Similarly, Gayão *et al*. (2013) found a high level of protein in fish that received steroid hormone with higher hepatic alterations. Additionally, Sayed *et al*. (2018) observed hydropic degeneration in the hepatocytes and blood congestion in the central vein of tilapia fish produced using 17alpha-methyltestosterone. In the same vein, diffuse vacuolar degeneration followed by mild and severe vacuolation of the hepatocytes was described by Hasheesh *et al*. (2011). Furthermore, Suseno *et al*., 2020 stated that vacuole degeneration was reversible when exposed to toxic substances and at the end of administrating MT; cells could be returned to normal, and congestion was preceded by degeneration of liver cells in which an enlarged vacuole was filled with erythrocytes that cause sinusoid to widen which was accumulated in blood and haemorrhage. Whereas Sipahutar *et al*. (2013) highlighted that there is no cure for necrosis; if it is exposed to tissue activity continuously, cell activity will decrease, and cells will lose some parts leading to death.

The gill section of the present study (Figure 8–12) showed normal gill lamellae and arch with the lamellae consisting of normal erythrocytes, chloride cells and pillar cells while the gill arch showed normal osteocyte-like cells. This result agrees with the study of Vallada˜o *et al*. (2013) that reported severe alterations in gill structure, compromising the function of its filaments. On the other hand, poorly arranged gill lamellae and arch with the lamellae consisting of normal chloride cells and pillar cells, with the gill arch showed normal osteocyte-like cells was also observed in this study. Lamellar oedema is the most common lesion in fish and may progress to necrosis of the lamellar epithelium. This can lead to osmoregulatory and respiratory distress, which may result in health compromise, development, and survival of fish (Yang and Albright 1992).

According to the study of Suseno *et al*. 2020, larger than normal gill lamella looked unclear between the primary and secondary lamellas which is a result of hypertrophy (cell enlargement). Furthermore, Dias-Neto *et al*. 2016 also reported that Edema of branchial lamellae was less pronounced in the fish fed with natural feed at the end of the experiment.

The histopathological examination of the muscles revealed that muscles sections showed normal, moderate and poor structure throughout the experimental period. In contracts, Khalil *et al*., 2011 and Curtis *et al*. (1991) stated that there was no change in muscle composition and traces of hormone accumulation in the muscle tissue of fish. Additionally, the residual value of 17 α- methyltestosterone in *Oreochromis niloticus* fry muscle dropped significantly after the withdrawal period of 6 weeks but was still higher than their control group (El- Nemr *et al*., 1999). In this study, we also observed pathological lesions including moderate to severe necrosis.

Pandian and Kirankumar (2008) observed that a high concentration of MT was found in the muscle and flesh; because the MT metabolite has been absorbed into the muscle and flesh of fish, which might cause accumulation every month of this hormone in the flesh. In addition, excretion of methyltestosterone and rapid metabolism by fish treated early with the extended period needed to produce a marketable size fish results in a safe consumer product (Phelps, 2001).

CONCLUSION

The obtained results in this study showed the superior effects of plant extract, steroid and natural hormone in the sex reversal rates of *Oreochromis niloticus*. Although, the highest indistinct percentage and mortality rate was recorded in the MT-treated diet while the least was recorded in diets 3 and 4 with no significant difference. Consequently, all groups showed histopathological differences in the livers, gills, and muscles. However, the liver histology of groups 1 and 5 showed central vessels that are mildly congested with abundant glycogen vacuolation and somewhat fat infiltration while muscle histology of group 5 showed pathological lesions including moderate to severe necrosis. These changes can be measured as a sign of fish performance indicating the effects of plant extract and MT. However, further studies are essential for a better understanding of its effects.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 499-505 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.11

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Global growth trend in fisheries and current situation in Turkey

Dünya su ürünleri gelişim trendi ve Türkiye'nin durumu

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Received date: 24.03.2021 *Accepted date: 25.10.2021*

How to cite this paper:
Arısoy H., Bayramoğlu Z., Ağızan K. & Ağızan S. (2021). Global growth trend in fisheries and current situation in Turkey. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 499-505. DOI: 10.12714/egejfas.38.4.11

Abstract: In recent years the most salient change in the global food sector has been witnessed in the range of fisheries. Due to the gravity of fishery, particularly in meeting the needs of an adequate and balanced diet, its total share in production, consumption and trading has been on the rise on a global scale. The aim of this study is to detect the factors impinging upon growth trend and foreign trade of global fishery sector. To that end, data from 1990-2017 period of 10 countries meeting 72% of global fisheries and data from Turkey have been employed. In this study panel data analysis method has been applied by using 11 cross-section data and 28-time series. Importation and exportation models have thus been set. An increase by 100% in production of fisheries climbs exportation share by 15% and degrades importation share by 1%. 1-unit increase in income heightens importation by 8 units. Slowness in the growth rate of production of fisheries can be associated with supplying the vast majority of total production by only a few select countries thus threatening global exportation of fisheries. Turkey and relevant countries could gain exportation advantage by conducting a better analysis of their existing potential.

Keywords: Fisheries, foreign trade, exportation, panel data analysis, Turkey

Öz: Son yıllarda dünya gıda sektörü içerisindeki en önemli gelişme su ürünleri alanında görülmektedir. Özellikle yeterli ve dengeli beslenme bakımından olan önemi nedeniyle dünya toplam üretimi, tüketimi ve ticareti artmaktadır. Bu çalışma ile dünya su ürünleri sektörünün gelişme trendi ve dış ticaretini etkileyen faktörlerin belirlenmesi amaçlanmıştır. Bu amaçla dünya su ürünleri üretiminin %72'sini kapsayan 10 ülke ve Türkiye'nin 1990-2017 dönemi verileri kullanılmıştır. Çalışmada 11 yatay kesit verisi ve 28 zaman serisi kullanılarak panel veri analiz yöntemi kullanılmıştır. İthalat ve ihracat modelleri kurulmuştur. Su ürünleri üretimindeki %100'lük artış ihracatı %15 artırmakta, ithalatı %1 azaltmaktadır. Gelirde görülen 1 birimlik artış ithalatı 8 birim artırmaktadır. Su ürünleri üretiminin artış hızının yavaşlaması, toplam üretimin büyük kısmını birkaç ülkenin karşılıyor olması dünya su ürünleri ihracatı açısından tehdit olarak değerlendirilebilir. Türkiye gibi ülkeler mevcut potansiyelini daha iyi değerlendirerek ihracat avantajı yakalayabilir.

Anahtar kelimeler: Su ürünleri, dış ticaret, ihracat, panel veri analizi, Türkiye

INTRODUCTION

In recent times policies on food security and safety have grabbed much wider attention due to a number of reasons such as; global rise in population, scarcity of agricultural lands due to misuse, rising demands in consumption, failure to stop food waste and unequal distribution of food, hunger and environmental risks. Studies suggest that world population being 7.6 billion presently will be over 9 billion in 2050 (FAO, 2017) and protein demand of humans will multiply by 70% (Özdemir, 2019).

An adequate and balanced diet plays a critical role in the development of societies. Currently animal protein consumption per person is a salient criterion in measuring development level of states. According to data released by World Health Organization (WHO), a healthy individual must consume 1 gr. of protein for every kilogram of total body weight and 42% of this intake should be animal-based (Özuğur *et al*., 2019). One of the best resources to meet protein deficiency in a cheap and effective way is fisheries of which value has now grabbed an increasing focus. Having acknowledged this trend, countries seek ways to maximally use water products to further enrich their animal protein sources. Hence, many countries have an economic and sustainable fisheries management (Düzgüneş and Erdoğan, 2008).

Fishery sector holds an economic value too by virtue of its direct or indirect links with the food and manufacturing industry, health, environment, tourism and transportation sectors. Since it has no equivalent match in food pyramid and provides a positive contribution on employment and foreign trade, significance of water-products sector climbs even higher (Anonymous, 2012).

Production of fisheries has gained rapid impetus as of post Second World War and been soaring since then. Food and Agriculture Organization (FAO) highlights water products as the fastest-growing food sector globe wide (Tatlıdil *et al*., 2009). Production of fisheries is practiced under two categories namely; sea and territorial sea hunting & aquaculture (fish farming) in territorial seas, seas and territorial waters. A great ratio of production is through hunting but in recent years' fishery through hunting has been losing its share in total production whereas fishery through aquaculture has been following a rapid growth. One of the most apparent causes is failure to increase the quantity of products obtainable from the seas through hunting. To ensure sustainable fishery we have now reached the utmost level in the quantity of huntable fish (Anonymous, 2012).

There has been a rise in the global production of fisheries and trading. Turkey is a peninsula surrounded by seas on three sides. Turkey lies within an 8,333 km. of seashore and 177,714 km-long rivers. Besides it holds dam lakes of 342,377 hectare expanding each new year. Total surface area of seas and inland water resources of Turkey is measured as 25 million hectares; this figure is close to the totality of agricultural lands of Turkey (Anonymous, 2012). Irrespective of this potential Turkey's global share in the production of fisheries is 0.3%, its global share in the exportation of fisheries is 0.5%. Foreign-trade surplus of fisheries merely 405 million dollars (FAO, 2019).

The fishery sector that, by virtue of added-value and employment it creates, holds a strategic interest has been developing in Turkey as also witnessed globe wide. Studies that analyze the contribution of sector on national economy and foreign trade are of vital importance (Akyol and Ceyhan, 2010; Candemir and Dağtekin, 2020; Demir, 2011; Emiroğlu, 2018; Hoşsucu *et al.,* 2001; Koşar, 2009; Sarıözkan, 2016; Tekelioğlu *et al.*, 2007; Yıldırım and Okumuş, 2004). In addition, many studies have been carried out on production (Alçiçek, 2009; Başçınar, 2007; Çeliker, 2006; Demir, 2011; Doğan, 1997; Emiroğlu, 2018; Köse *et al.*, 2010; Yüngül *et al*., 2012), trade (Başçınar, 2007; Dağtekin and Orhan, 2007; Kuşat and Kuşat, 2019), consumption (Akbay *et al*., 2013; Aydın and Karadurmuş, 2013; Bayraktar *et al*., 2019; Çolakoğlu *et al.*, 2006; Dağtekin and Orhan, 2007; Dereli *et al*., 2016; Özuğur et al., 2019; Sağlam and Samsun, 2018; Sayğı *et al*., 2015; Şenol and Saygı, 2001), competition (Kuşat and Kuşat, 2019), and organization (Ünal and Yercan, 2006; Yılmaz *et al.*, 2009), and it is important to determine the foreign trade potential and the factors affecting this potential in order to increase competitiveness in today's conditions where the market structure is changing very rapidly. Thus, it was aimed to increase the competitiveness of Turkey in this field by determining measures, strategies and policies to improve foreign trade in fisheries products.

MATERIALS AND METHODS

Research material consists of secondary-data collected from 10 countries (China, Indonesia, India, Vietnam, USA, Russia, Japan, Peru, Bangladesh, the Philippines) that meet 72% of global-production of fisheries as well as data from Turkey.

In scientific research data categories are generically recognized and utilized as cross-section and time series, but in case there is inadequacy of monitoring the quantity in constructing applicable models, panel data model is opted for. In relation to relevant variable in data sets constructed via panel data, there is data in both time and also cross dimension. Within the context of this study too, panel data set was used in analyzing the factors affecting importation and exportation of fisheries among select countries. Panel data analysis is defined as estimation of economic relations by utilizing cross series of time dimension (Pazarlıoğlu, 2001). It is feasible to repeat the observations in panel data sets. From this point of view, it is detected that on the basis of panel data analyses repetitive variance analysis and variance analysis models are formed (Greene, 2003). In the panel-data set used in this study there are 11 cross-section data and 28-time series. This study focuses on explaining importation and exportation practices of 10 countries with the highest rate of global fish production and data from Turkey. Importation, exportation, production, consumption, income and currency parities of the said countries for the period between 1990-2017 were compiled and a panel data set constructed with a total of 308 observation values was formed.

The model based on panel data analysis is as follows (Greene, 2003).

$$Y_{it} = \alpha_i + \beta' X_{it} + u_{it}$$

Xit contains a K regressor. But the constant term is not included, α_{it}, is constant over time t, describing the special effect for cross-section units i. This is an ordinary regression model. If α_{it} all units of 's are the same, this dataset can be analyzed by the ordinary least squares method and the parameters are consistent and unbiased. Panel data models are examined in two groups as fixed effects and random effects methods. The difference between these two methods is to determine a constant coefficient for each group. In the fixed effects method, each group is considered heterogeneous and the fixed coefficient for each group is estimated. However, the same constant coefficient is estimated for each group in the random effects method. Some tests are needed to decide which method to use in panel data models. These tests are the F test, the Breusch-Pagan test and the Hausmann test.

F test: It aims to test if panel data has fixed effect or not. Based on this test, pooled or fixed-effect panel data model is estimated. As the model is estimated via Classical Least Squares Method (LSM), F test is applied and hypothesis is tested. Given that F Statistics is significant *(< 0.05)* H_0 hypothesis is rejected. Hypotheses of F test are as listed below.

H_0 = Pooled Regression Model is Fit.

H_1 = Fixed Effects Model is Fit.

In the second stage, by harnessing **Breusch-Pagan** Test Pooled Model-Random effects, the model is tested. In a different saying it tests whether or not panel data have random effects. Model is estimated through classical LSM and by administering "Breusch-Pagan LM" test, hypothesis is tested. Given that Breusch-Pagan LM Statistics is significant *(< 0.05)* H_0 hypothesis is rejected. Hypotheses are such;

H_0 = Pooled Regression Model is Fit.

H_1 = Random Effects Regression Model is Fit.

In the third stage by using Hausmann test**,** a choice is drawn between fixed effects model and random effects model. Based on this test fixed or random effect panel data model is estimated. To conduct Hausman test, firstly, the model should be estimated through "Random Effect Panel Data Model" method. Hypotheses of Hausmann test are as depicted below.

H_0 = Random effects Model is Fit.

H_1 = Fixed effects Model is Fit.

As a result of the tests, it was determined whether the model has a random or constant effect and the estimation of the model was made with the help of the STATA package program.

In this study two models were built to explain importation and exportation. Variables mentioned in the model are given below. All of the variables out-of-parity were measured per person and included in the model.

Importation (IM): explains per person fisheries importation quantity of countries (kg/year)

Exportation (EX): explains per person fisheries exportation quantity of countries (kg/year)

Production (PR): explains per person production quantity of fisheries in countries (kg/year)

Consumption (CM): explains per person consumption quantity of fisheries in countries (kg/year)

Income (IN): explains gross-domestic-product income per person in countries (dollar/year)

Parity (PR): explains dollar parity of national currency in countries (dollar)

RESULTS

Current status in fishery sector

Data of top-ranking 10 countries in the global production of fisheries and data from Turkey are as listed in Table 1. There is a steady growth in production. Currently total production is twice above the production quantity (103 million tons to 206 million tons) computed in 1990 (FAO, 2019). Main cause of the global rise in the production of fisheries is adding new resources to present resources rather than effective management of existing resources. Indeed, there is an abundance of evidence on the diminished fish efficiency in current resources. Environmental damages, pollution of coast waters, insensible hunting are some of the negative factors on global fish population (Longer, 2000). Presently half ratio of global production of fisheries nearly 206 million tons, is met by China and Indonesia. In these countries growth in the production of fisheries outpaces the rise in entire world. Compared to year 1990, in China, production multiplied around 5 times and in Indonesia production increased approximately 7 times above.

Likewise, there is a global increase in the consumption of fisheries. While in 1990 annual consumption quantity per person was computed by 13.2 kg/year the same percentage climbed to 19.2 kg/year in 2017. Countries with maximum consumption per person are listed as maritime countries such as Japan, China, Vietnam and Indonesia in which the production ratio is also at its peak. Turkey is far below the world average in terms of both production of fisheries and also in consumption per person.

There is also a corresponding climb in the global trading of fisheries. In 1990 sum of exportation value in fisheries measured as 35 billion dollars reached to 158 billion dollars in 2017. Among the most significant exporter countries are China, Norway and Vietnam respectively and these three countries constitute around one fourth of total exportation ratio. The most significant importer countries are USA, Japan and China respectively and these three countries constitute around one third of total importation ratio (FAO, 2019). All over the world, the most popular fisheries globally exchanged are prawns, tuna and salmon fish (Anonymous, 2012).

Table 1. Production, consumption and foreign trade status in select countries (2017) Source: FAO (2019)

Countries	Production (tons)	Per Person Consumption (kg/year)	Exportation (000 $)	Importation (000 $)	Per Person Production (kg/year)	Per Person EX (kg/year)	Per Person IM (kg/year)
China	79,935,168	39.13	20,701,805	11,027,653	55.03	2.94	3.36
Indonesia	22,632,380	30.95	4,383,228	398,007	85.52	4.01	1.12
India	11,632,313	6.74	7,183,336	113,732	8.69	1.05	0.03
Vietnam	7,108,815	36.24	8,586,492	1,765,991	75.15	19.28	6.62
USA	5,480,131	22.23	6,246,034	21,842,536	16.86	5.25	8.64
Russia	5,065,176	20.72	4,524,995	2,025,068	34.81	15.27	4.28
Japan	4,295,728	46.21	2,112,314	15,352,351	33.69	4.66	19.35
Peru	4,285,648	24.21	2,875,635	311,286	136.29	49.98	4.31
Bangladesh	4,134,436	24.65	496,166	104,930	25.89	0.38	0.85
Philippines	4,127,777	28.68	883,537	585,047	39.25	3.06	4.69
Turkey	627,797	5.03	862,127	456,745	7.74	2.01	3.27
WORLD	205,580,364	19.20	158,102,263	148,605,591	27.24	10.44	5.12

The global ratio of total food exchange in fishery trading is also on the rise. In recent years' liberalization policies, technological innovations, improvements in processing, packaging, and transportation, as well as changes in distribution and marketing, have further accelerated this trend while facilitating the emergence of complex supply chains in which goods often cross national borders several times before final consumption (Bellmann *et al*., 2016).

Developing countries play a key role in global trading of fisheries. With respect to exchange value of fisheries in total, the share of developing countries group in 1990 was computed as 40% while in 2017 the ratio jumped to 52% (FAO, 2019).

Analysis results

Panel fitness of data tests were conducted for the data utilized in this research. The first test is F test aimed at checking whether or not panel data renders a fixed-effect. Accordingly, as can be seen in Table 2 since F Statistics is < *0.005* H_0 hypothesis is rejected thus for both models it is essential to apply fixed-effects regression model (Yerdelen Tatoğlu, 2013).

Another test aims to check whether or not panel data leads to a random effect. Based on this model pooled or "Random Effect Panel Data Model" is estimated. As displayed in Table 2 in both models probability values (prob.) of Breusch-Pagan LM were measured as 0.000. This value is < *%5* hence based on this equation H_0 hypothesis is rejected. This finding validates that in the model fixed-term is randomly determined in the average of population and differences cause an effect equal to error term for every unit.

Based on this finding it can reasonably be argued that there is a greater advantage in using "Random Effect Panel Data Model". It was also concluded that based on F test and Breusch-Pagan LM, test estimation should be processed through panel data models. That being the case, however, it is essential to make a choice between Fixed-effect and Random Effect models as both being panel data models. To that end Hausman Test was conducted to check if panel data has fixed or random effect. Based on Hausman test statistics, since both models are > *0.05*, data are fit for fixed effects model.

Table 2. F Statistics, Breusch-Pagan LM Test and Hausmann Statistics

Model	Statistics	Value Statistics	Prob.
Exportation	F Statistics	2670.719	0.000
Importation	F Statistics	16101.99	0.000
Exportation	Breusch-Pagan LM	639.1554	0.000
Importation	Breusch-Pagan LM	442.1815	0.000
Exportation	Hausmann	79.09318	0.074
Importation	Hausmann	3.993122	0.406

Source: Author's calculations

Table 3 exhibits parameters of exportation and importation model. Firstly, R^2 that validates significance of the model was analyzed. In exportation model R^2 was computed as 95%, in importation model as 97%. R^2 indicates explanatory power of the model and presents to what extend independent variables can affect dependent variable (Kalaycı, 2010). Significance of the model is visualized by F test. Accordingly, in two models alike F Statistics was measured to have a statistical-significance level by 1%. Autocorrelation problem in the model was analyzed via Durbin Watson Statistics. As a result of the DW statistics, autocorrelation could not be determined since the 1.275590 statistics included in the export model are between the minimum-maximum value in the DW 5% significance level table (1.028-1.850). However, since the DW test result obtained in the import model is lower than the minimum value in the test table, the autocorrelation problem has been detected. Therefore, in order to solve the autocorrelation problem, the one degree lagged values of the import model were included in the model and re-estimated.

Regarding the 11 countries selected within the scope of this research importation and exportation models have been explained via fixed-effect panel model. There is always a linear relationship between importation and exportation and this deduction has been validated in a wide array of studies too (Tatlıdil *et al*., 2009).

Similarly, in the context of this research too, exportation and importation variable signs of both models were detected as positive as expected. It can be argued that in exportation model; 100% climb in importation quantity would lead to rise by 78% in exportation quantity. However, in importation model it is suggested that 100% climb in exportation quantity would lead to a rise of merely 6% in importation quantity (Table 3). Based on the results of these models the fact that importation has a greater effect on the rise of exportation could be explained by two reasons. The first reason is re-exportation. In another saying countries can re-export the kind of products imported in a lower cost margin of their own production cost. Second reason is that countries can process the products they import and then export the same products. Thus in both scenarios exportation is made dependent on importation and in such cases importation acts like the source of constant exportation.

Another parameter in these models is consumption. In exportation model consumption is statistically significant and as expected, its sign was detected to be negative. In that case it can be argued that when consumption quantity climbs by 100% exportation quantity will fall by 24%. In the importation model, however, there is a significant and linear relationship between importation and consumption. In that sense, as consumption quantity rises by 100% importation quantity falls by 23%.

Table 3.Parameters of exportation and importation model

EXPORTATION				IMPORTATION			
Variables	**Coefficient**	**t-Statistics**	**Prob.**	**Variables**	**Coefficient**	**t-Statistics**	**Prob.**
Consumption	-0.241049	-3.379675	0.0008	DConsumption	0.233546	1.269585	0.0012
Production	0.158651	1.813343	0.0000	DProduction	-0.043256	-7.854236	0.0000
Income	6.14E-05	0.994756	0.3207	DIncome	6.23E-05	3.126358	0.0000
Importation	0.788944	3.631783	0.0003	DExportation	0.063561	2.452369	0.0004
Parity	0.000323	1.920341	0.0558	DParity	-5.83E-05	-3.221529	0.2215
C	2.145.094	1.661833	0.0976	C	-0.456923	-2.752169	0.0463
China	8.927895			China	2.707073		
Indonesia	1.496104			Indonesia	-0.344443		
India	2.835507			India	-4.372223		
Vietnam	1.321334			Vietnam	-1.347603		
USA	-4.935283			USA	8.643756		
Russia	1.630721			Russia	4.322457		
Japan	-2.684346			Japan	-2.042774		
Peru	2.675309			Peru	0.344436		
Bangladesh	-3.231482			Bangladesh	0.034670		
Philippines	-0.908791			Philippines	-2.345374		
Turkey	1.700477			Turkey	1.373377		
R^2	0.955842			R^2	0.935693		
Adjusted R^2	0.953573			Adjusted R^2	0.935458		
S.E.of regression	4.273755			S.E.of regression	1.156985		
F-statistics	421.3729			F-statistics	758.6582		
Prob (F-statistic)	0.000000			Prob(F-statistic)	0.000000		
D.Watson stat.	1.275590			D.Watson stat.	1.652892		

Source: Author's calculations

In production variable, on the other hand, there is an inverse relationship as for consumption. In exportation model there is a significant and linear relationship between production and exportation and if production climbs by 100% exportation escalates by 15%. In importation model, there is an inverse but significant relationship between production and importation. As production jumps up by 100% importation goes down by 4%. Based on these parameter results, trading of water products is remarkably effective to raise national income level since they are categorized as products with high value of exportation potential and render a rise in added value.

As we delve into the relationship between income variable and importation and exportation not any statistically significant relationship could be measured between income and exportation whilst in importation model a significant relationship was identified between income and importation. It can thus be projected that 1-unit rise in income would climb importation 8 times above. Indeed, previous studies evidenced that production of water products leads to a high elasticity in income demands (Tatlıdil *et al.*, 2009). That being the case a change of one unit in income would lead to a greater increase in consumption. Then rising demand unviable to meet through domestic consumption would thus be met via importation.

One of the most effective factors in foreign trade is national currency value. Given that a state's national currency gains value against the currency of other nations, exportation becomes tougher for the said country while importation becomes easier. On that account parity-variable sign is expected to be positive in importation model but negative in exportation model. Within the context of this study, a significant relationship in a range of 10% could not be determined between importation and parity. Between exportation and parity, a significant and yet not positive relationship could be detected. In exportation model sign of parity variable is expected to be negative. The main reason for obtaining a positive sign is that lately there has been a value-gain against dollar in the national currency of China and Japan as holding 41% share in fish production and 14% share in exportation.

Finally, the country results are evaluated in the Table, and the differences of the countries in imports and exports of each country are shown. Accordingly, countries with a high constant coefficient have a higher impact on imports or exports. For example, in the export model, China and the USA were determined as the countries with the highest impact, and in the import model, the USA and Russia had the highest impact. In addition, the changes in the consumption, production, income, import and parity variables in the USA, Japan, Bangladesh and Philippines in the export model affect exports negatively. In the import model, it was determined that the changes in consumption, production, income, import and parity variables in Indonesia, India, Vietnam, Japan and the Philippines affected imports negatively. As a matter of fact, the USA and Japan are among the important importing countries in fisheries products, and the change in the demand for fishery products will have a significant impact on world exports. Similarly, Indonesia, India and Vietnam countries, especially China, are known as important exporters, and the differences in the production potential of these countries or depending on market conditions affect world imports significantly. For this reason, the effects of these countries on both exports and imports are high. In Turkey, the per capita consumption of fishery products (5.03 kg/year) is below the world average (19.20 kg/year), and it has not yet reached the desired level of competition in aquaculture. In this context, it was aimed to establish policies to increase competitiveness and it was emphasized that the factors affecting import and export should be taken into consideration.

DISCUSSION

Fisheries is acknowledged as an indispensable sector to satisfy escalating animal-protein need of global population on the rise. Consequently, all over the world and in Turkey alike there has been a continuous upward trend in production, consumption and trading of fisheries. Nevertheless, despite its geographical advantage Turkey has failed to grab the lion's share from this expanding market. In the last decades, although a major rise in production has been achieved through aquaculture, total sum of production is significantly far below Turkey's real potential. Backwardness of Turkey in the production of water products has also been echoed in its consumption and trading.

In relation to this research, it was aimed to detect certain factors affecting foreign trade of fisheries all over the world. Doubtless to say that there are many factors impinging upon foreign trade. A few of these factors are resolutions taken by international organizations such as World Trade Organization, bilateral trade agreements between countries, foreign trade policies effectuated in countries and political developments. In line with the objective of this research, by using 28 years of data obtained from 10 countries constituting 72% of total production of fisheries in the world and data from Turkey, importation and exportation models have been structured. Production, consumption, income, importation, exportation and parity independent variables were integrated into the models. Explanatory power of independent variables for these models were computed to be significantly high, thus results to obtain from the model are qualified to steer foreign-trade policies of fisheries.

In exportation model a positive relationship was unveiled between exportation and production. Thus any lag in the growth rate of global production of fisheries and supply of majority of total production by a few select countries can be evaluated as a threat for the global exportation of fisheries. Turkey and similar countries could thus grab an advantage in exportation provided that its current potential is better evaluated.

As a result, to provide competitive advantage in the aquaculture sector in Turkey, first, it is necessary to determine the production and marketing potentials of fishery products. In this context, first of all, food supply chain, supply chain and value chain analyze in aquatic products should be made and the production and marketing channels of the sector, value added elements and all factors that increase competition should be determined. For this reason, the measures to be taken to improve the competition in national and international markets in seafood are given below. These measures are;

1. To prepare the production planning of seafood products

2. Giving the supports by rearranging them according to the feed utilization rate and input/cost parity through cooperatives.

3. To provide competitive power-enhancing supports

4. To ensure their control and traceability in hunting and aquaculture practices

5. Accelerate breeding of species that require less or no feed

6. Considering the principles of environmental sustainability in the creation of hunting policies

7. To increase the publication and promotion activities for the consumption of fishery products

8. Reducing waste and losses by increasing cold chain applications in seafood

9. Reducing bureaucratic procedures for aquaculture facilities

10. Establishing a fisheries market

11. It can be listed as increasing exports by processing all kinds of aquatic products in a way that creates added value.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 507-514 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.12

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Bioaccumulation of heavy metals in the water, sediment and the tissues of *Carassius gibelio* (Bloch, 1782) from Eber Lake

Eber Gölü'nde (Türkiye) su, sediment ve *Carassius gibelio* (Bloch, 1782) dokularında ağır metal birikimi

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Received date: 05.04.2021 *Accepted date: 26.10.2021*

How to cite this paper:
Gümüş, N. E. & Akköz, C. (2021). Bioaccumulation of heavy metals in the water, sediment and the tissues of *Carassius gibelio* (Bloch, 1782) from Eber Lake. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 507-514. DOI: 10.12714/egejfas.38.4.12

Abstract: In this research, in order to observe the seasonal change between April 2014 and February 2015, heavy metal contents in the water, sediment and the *Carassius gibelio* (Bloch, 1782) tissues (muscle, gill and liver) growing in Eber Lake were determined. The obtained results showed that trace elements were founded in water, sediment and fish tissues. Among the average annual concentrations of water samples, the highest concentration was found for Mn (0.1584 mg/L) element and the lowest concentration was found for Pb element in the spring season. the maximum Average annual concentration was calculated for Mn (0.1584 mg/L, Spring) and the minimum was determined for Pb in water. In the sediment, Fe (16245.84 mg/kg, Spring) was found to be maximum and Co was the minimum. In fish, the highest concentrations were found in liver (Mo, Ni, Pb, Co, Cu, Fe), while the lowest concentrations were found in gill (Mn, Zn) and muscle (Cd, Cr, Se) tissues While the highest heavy metal concentrations were observed in the sediment, the minimum was found in the fish tissues (the ranking was the following; liver, gill and muscle). It was concluded that the heavy metal levels measured in the Lake Eber have no risk to the environment and to the public health.

Keywords: Eber Lake, heavy metal, water, sediment, fish tissues

Öz: Bu çalışmada Nisan 2014 ile Şubat 2015 arasında Eber Gölü'nden alınan su, sediment ve *Carassius gibelio* (Bloch, 1782) dokularındaki (kas, solungaç ve karaciğer) ağır metal içeriklerinin mevsimsel değişimlerini belirlemek amaçlanmıştır. Su, sediment ve balık dokularında iz elementler tespit edilmiştir. Su örneklerinde ortalama yıllık konsantrasyonlar içerisinde en yüksek Mn (0,1584 mg/L) İlkbahar mevsimde en düşük konsantrasyon ise Pb elementinde bulunmuştur. Sediment örneklerinde ortalama yıllık konsantrasyonlar içerisinde en yüksek Fe (16245,84 mg/kg) İlkbahar mevsimde en düşük konsantrasyon ise Co elementinde bulunmuştur. Balıklarda, en yüksek konsantrasyonlar karaciğer dokusunda (Mo, Ni, Pb, Co, Cu, Fe) bulunurken, en düşük konsantrasyonlar solungaç (Mn, Zn) ve kas dokularında (Cd, Cr, Se) tespit edilmiştir. En yüksek ağır metal konsantrasyonları sedimentte gözlenirken, en düşük ağır metal konsantrasyonları balık dokularında sırasıyla karaciğer, solungaç ve kasda bulunmuştur. Eber Gölü'nde ölçülen ağır metal seviyelerinin çevre ve halk sağlığı açısından herhangi bir risk taşımadığı tespit edilmiştir.

Anahtar kelimeler: Eber Gölü, ağır metal, su, sediment, balık dokusu

INTRODUCTION

The different types of water pollutions observed in many countries will lead to life-threat problems all around the world. Heavy metal pollution is considered to be one of the most significant environmental hazards. Streams form the wetland areas which are considered as receivers by collecting the pollutants within the soil including the heavy metals as well (Brzozowska *et al*., 2011). Heavy metals are shown among those pollutants which have the abilities to cause problems. Heavy metals are important because they accumulate in biotic and abiotic elements, can stay in the environment for a long time, show toxic effects on aquatic organisms and pass on to other living things through the food chain (Shrivastava et al., 2003; Withanachchi *et al*., 2008). Therefore many studies were carried out recently both in Turkey and also worldwide related to the accumulation of the heavy metals (Icaga, 2007; Emiroğlu and Arslan, 2015; Fidan *et. al*., 2008; Zhu *et al*., 2015; Rajeshkumar *et al*., 2018; Tokatlı, 2019; Varol & Sunbul 2019).

The heavy metals accumulated within the sediment of the aquatic environments damage particularly the benthic organisms and also the other living organisms through the food chain (Mohiuddin *et al*., 2011; Arslan *et al*., 2009). In recent years, researches on trace element accumulation in lentic ecosystems have accelerated (Siddiqui *et al.* 2019; Milačič *et al*., 2019; Özparlak *et al*., 2016).

Lake Eber is one of the most important wetland areas concerning this issue. The lake is an important biodiversity area which has the characteristics of a wetland area however if the necessary measures are not taken in a very short time it will lose this characteristic (Gümüş and Akköz, 2020). Due to this critical situation Lake Eber was chosen as the research

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area. The most important surface water which feeds the Eber Lake is Akarçay. Akarçay carries the waste of sewage system, the sugar, beer and milk factories of Afyonkarahisar, a meat and fish combine, alkaloids and enamel factories located in Bolvadin and the waste of many small industrial places around Afyonkarahisar and Bolvadin (Kıvrak *et al*. 2012; Gümüş, 2021). This has led to intense eutrophication especially in Lake Eber (Fidan *et al.* 2008). Therefore, it was aimed to determine the heavy metal values seasonally in the lake's water, sediment and in the muscle, gill and liver tissues of the fishes.

MATERIAL AND METHODS

Study area

Ecologically it is one of Turkey's most shallow and eutrophic lakes. Almost the lake's entire surface is covered with reeds and canes, there are some open water surfaces (lake mirrors) which do not exceed 5-10 hectares. The average depth of the lake does not exceed 3-4 meters. Lake Eber is a tectonic lake situated in the Lake District in the north of the Sultan Mountains and the south of the Emir Mountain between the geographic coordinates of 38° 40'N and 31° 12' S. It is 65 km far from the city of Afyonkarahisar and the altitude of the water surface is 966 m. The water, sediment and fish tissues (muscle, gill, liver) were gather seasonally among April 2014 and March 2015. When determining the stations during the field studies five points were selected which reflected the structure of the lake homogenously including the inflow of Akarçay which is the most important river feeding the lake with high pollution load. The station 1. is in the shallowest part of the lake. Station 2 was located on the where the eber village settlement is dominated. Station 3 and station 4 was located on the where it could reflect all domestic, agricultural, industrial discharges and natural–geologic effects. Station 5. is located in the area at the entrance of Akarçay Stream and exposed to agricultural and domestic discharges. Water and sediment samples taken from the stations were collected using appropriate containers. The fish (*Carassius gibelio*) samples were also caught from the same localities. In each sampling season, 40 fish (160 fish in total) were caught by making a net with the help of professional fishermen from the same field. In the heavy metal analysis all samples were brought with cold chains from the land to the laboratory. The geographical locations of the stations and the locations on Lake Eber were given in Figure 1.

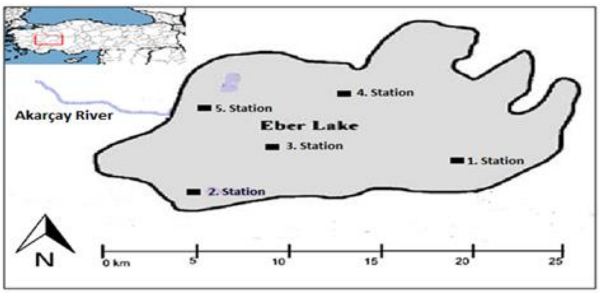

Figure 1. Eber Lake sampling stations

Element analysis

The water samples brought to the laboratory were passed over a 45 µm membrane filter (Whatman) (GF/C) and were put into 500 mL coloured flasks, 5mL of nitric acid was added and the flask were stored in the refrigerator at 4 ºC until the analysis was made (Skujins, 1998). The sediment samples were taken from the same areas as the water samples. An Ekman Grab was used to collect the sediment samples. Some of the sediment samples (1 kg) were put into glass petri plates after they had been sifted, and they were dried by being kept in the drying oven at 105ºC for 24 hours. The solubilisation process was performed in the microwave oven (CEM Mars Xpress 5) after 0.2 g. samples adding 5 mL of HCl, 3 mL of HNO_3 and 2 mL of pure water. After the tubes taken out of the oven were kept at room temperature, the solution was transferred to the falcon tubes with a 45 µm membrane filter and completed with 25 mL of distilled water (Mohiuddin *et al*., 2011). The samples taken from the tissues were awaited for 24 hours at 105 ºC. The sample tissues (each of them weighting 0.2 g) taken from the fish tissues were taken to separate polypropylene containers. The solubilisation process of the taken tissues were carried out in a microwave oven (CEM Mars Xpress 5) after adding 6 mL of HNO_3 (% 65) and 4 mL distilled water. The tubes which were removed from the oven later were cooled down to room temperature and the solution in the tubes was transferred to falcon tubes through 45 µm membrane filter. The amount of the solution within the tubes was completed to 25 mL with pure water. The prepared samples were stored at +4°C before analysis (UNEP, 1984). Samples ready for analysis were analyzed by ICP-OES (Varian Vista). The wavelengths of the elements measured in the device were respectively Co 238.892 nm, Mo 202.032 nm, Cd 214.439 nm, Cr 267.716 nm, Cu 327.395 nm, Fe 238.204 nm, Mn 257.610 nm, Ni 231.604 nm, Pb 220.353 nm, Se 203.985 nm and Zn 213.857 nm.

The averages were taken after reading the samples 3 times. In addition the accuracy of the results was tested with the prepared fish reference materials. The measuring device was tested by fish reference material TORT-2 (Lobster hepatopancreas) in order to verify the validity of its analytical method. According to these results the accuracy of the recorded values was between 87% and 95% according to the reference value (Table 1).

Table 1. Values of metals found in certified and observed reference material TORT-2

Elemental value (mg/kg)	Certified value	Observed value	Recovery (%)	RSD (%)
Cd	26.7±0.6	22.6319	87	12.2
Cr	0.77±0.15	0.7376	95	3.6
Co	0.51±0.09	0.4727	92	5.2
Cu	106±10	94.6928	88	11.2
Fe	105±13	99.7398	94	7.4
Pb	0.35±0.13	31.6438	90	6.7
Ni	2.50±0.19	2.2319	89	4.1
Se	5.63±0.67	5.1448	91	2.7

Statistical analysis

The statistical differences of heavy metal accumulations between the fish tissues and stations were determined by a Duncan Multiple Comparison Test ($p<0.05$) one-way anova analysis of variance using the SPSS 21packet program. The results of heavy metals are given as mean values (Steel *et al.* 1996; Barlas, 2005).

RESULTS

The seasonal heavy metal values of Lake Eber's water and sediments were determined and the annual average heavy metal levels are given in Table 2 and Table 3.

The minimum Cd level in water samples was detected at the first station (0.0003 mg/L) in autumn. The maximum Cd level was detected at the fifth station (0.0008 mg/L) in winter. The maximum Co level in the water was observed at the fifth station in summer while the minimum level was recorded at the second station in winter. The Co concentration was measured to be higher in summer than in the other seasons. The Co level at the fifth station was significantly higher statistically compared to the other stations ($p<0.05$). The maximum Cr value was detected in the first station (0.0017 mg/L) at the first station. In spring, the highest Cr concentration was found at the first station (0.0017 mg/L). Cr could not be detected at the second station in autumn and at the first and third station in the winter. The highest Fe concentration in the water was detected at the first station (0.1259 mg/L). The annual average Mn level in the water varied between 0.0181and 0.0488 mg/L. The minimum level in was detected at the fifth station (0.0003 mg/L) in winter. The maximum level was detected at the fifth station (0.0008 mg/L) in spring. The Mo concentration in the water was higher in the winter months than in the other months. The maximum Mo concentration was observed at the third station in winter (0.0049 mg/L) while the lowest Mo concentration was observed at the first station (0.0013 mg/L) in the summer. The fifth station (0.0049 mg/L) had the highest Ni concentration found in summer. Significant difference was determined among the stations in relation to the Ni values of the water. The Ni concentration was statistically higher at the fifth station. The fifth station (0.0008 mg/L) had the highest Pb concentration in spring. Pb was not detected in water at any station in the summer. Statistically significant difference was determined in the Pb values among the station ($p<0.05$). The Se was not observed in the water during all seasons except 3 stations. The fifth station (0.0507 mg/L) had the highest Zn concentration found in spring. It was not detected except the fourth station during the winter. Significant differences were not determined in the Cr, Se, Zn, Mo, Fe, Mn and Cd values among the different stations ($p>0.05$).

In the sediment of Lake Eber, the highest Cd level was found at the fifth station in the autumn (5.6435 mg/kg). This value was above the toxic effects threshold (TET) value (4.98 mg/kg) determined by MacDonald *et al* (2000). The values observed at all the other stations during all seasons were lower than the threshold effect level (TEL) (0.596 mg/kg), lowest effect level (LEL) (0.6 mg/kg) and minimal effect threshold (MET) (0.9 mg/kg) values (MacDonald et al., 2000). The Cd level was significantly higher statistically at the fifth station than at the other stations ($p<0.05$) (Table 3). The highest Co concentration was founded at the first station (1.796 mg/kg) in the summer while the lowest was seen at the second station (0.2767 mg/kg) in the spring. The highest Cr concentration in the sediment was observed generally in the summer season except the fourth and fifth stations. The highest Cr level of sediment samples was observed at the first station (41.1762 mg/kg) in the summer. The Cr concentration values obtained from the sediment were below the MET (55 mg/kg) and TET (111 mg/kg) values and above the TEL (3.3 mg/kg) and LEL (26 mg/kg) values at some of the stations. The highest Cu level in the sediment was observed at the fifth station (32.1933 mg/kg) during the autumn while the lowest level was seen at the third station (5.5892 mg/kg) during the spring. The Cu values obtained from the sediment were all below the TET value (149 mg/kg). The Cu and Cr concentration was statistically significantly higher at the first and fifth stations compared to the other stations ($p<0.05$).

The Fe level was significantly higher statistically at the first and fifth stations compared to the others ($p>0.05$). The Fe levels of Lake Eber's sediment were observed between 3285.455 and 12424.48 mg/kg during the spring, between 2633.244 and 14524.79 mg/kg during the summer and 3551.377 and 16245.84 mg/kg during the autumn and between 3971.337 and 16199.74 mg/kg during the winter. The maxium Mn level of the sediment was found at the third station (775.2915 mg/kg) in the spring while the minimum concentration was observed at the first station (180.9605 mg/kg) during the summer. The Mn concentration was statistically significantly higher at the third and fourth stations compared to the other stations ($p<0.05$) (Table 3). The Mn concentrations of the sediment taken from Lake Eber was recorded between 192.0395 and 533.6344 mg/kg during the spring, between 180.9605 and 417.6687 mg/kg during the summer, between 267.4343 and 775.2915 mg/kg during the autumn, between 613.7977 and 16199.74 mg/kg during the winter. The highest Mo concentration in the sediment was observed at the first station (775.2915 mg/kg) in the winter, while the lowest concentration was found at the fifth station (180.9605 mg/kg) during the winter. The Mo levels in the sediment was recorded between 1.3607 and 2.0979 mg/kg in the spring, between 0.907 and 2.27 mg/kg during the summer, between 1.3804 and 3.2159 mg/kg during the autumn and between 1.2465 and 3.2773 mg/kg during the winter.

The highest Ni concentration in the sediment was recorded at the first station (46.30458 mg/kg) in spring. The Ni values obtained from the sediment were below the TET value (48.6 mg/kg). The maximum Pb concentration in the sediment was founded at the fifth station in winter (17.194 mg/kg) while the minimum concentration was measured at the fourth station (3.4189 mg/kg) in summer. The Pb concentration was significantly higher statistically at the first and fifth stations compared to the other stations (p<0.05). The Pb values obtained from the sediments were below the values of MacDonald *et al* (2000) quality criteria. The highest Se level in the sediment was observed at the first station (3.0274 mg/kg) in the spring. It was not detected at the fourth and fifth stations during the summer, at the fourth station during the autumn and at the third station during the winter. The highest Zn level was measured at the fifth station (91.1524 mg/kg) in spring, while the lowest level was observed at the fourth station (15.6252 mg/kg) in summer. The Zn concentration was considerably higher at the fourth station compared to the other stations (p<0.05). Significant differences were not determined in the Co, Mo, and Ni values among the different stations (p>0.05).

The heavy metal levels in the tissues (muscles, gill and liver) of *Carassius gibelo* (Bloch, 1782) taken from Lake Eber seasonally are shown in Table 4. The heavy metal concentrations within the muscle tissue of the fish were compared to the quality criteria of the Turkish Food Codex (2011). The highest Cd concentration in the fish tissue was found in autumn in the muscle tissue (0.0598 mg/kg) (Table 4). Significant difference was not determined in the Cd values within the fish tissues (p>0.05). The Co concentration in the fish tissues was the highest in the liver (0.2206 mg/kg) and the lowest in the gill (0.0987 mg/kg) in spring. In the summer the lowest Co concentration was observed in the gill (0.0112 mg/kg) and the highest in the liver 0.1214 mg/kg, in autumn the highest Co concentration was observed in the muscle 0.1415 mg/kg while the lowest was in the liver 0.0716 mg/kg, in winter the highest Co concentration was found in the muscle 0.1685 mg/kg while the lowest was found in the gill (0.0766 mg/kg). Statistically significant difference was not determined in the Co values of the fish tissues among the stations. In spring the Co concentration in the liver was considerably higher statistically compared to the other seasons and tissues (p<0.05). The Cr concentration in the fish tissues was the highest in the muscle 0.7989 mg/kg and the lowest in the liver 0.4803 mg/kg in spring, during the summer the lowest Cr concentration was observed in the liver 0.0111 mg/kg and the highest in the muscle 0.782 mg/kg, in autumn the highest Cr concentration was observed in the gill (0.3645 mg/kg) while the lowest was in the liver 0.1156 mg/kg, in the winter the highest Cr concentration was found in the gill 0.2112 mg/kg while the lowest was found in the liver 0.0877 mg/kg. The Cr concentration of some freshwater fish was reported as 1.32-4.20 mg/kg by Alaş *et al*., (2014). The Cr level in the fish tissue was significantly higher in the muscles during the spring compared to the other seasons and tissues. The Cu concentration in the fish tissue was found to be the highest in the liver in all seasons except summer. The values obtained from the muscle tissue in all seasons were lower than the acceptable limit values given by the Turkish Food Codex (20 mg/kg). Statistically significant difference was determined in the Cu values of the fish tissues between the stations (p<0.05). The highest Cu concentration was found in the liver tissue during winter (2.1995 mg/kg) (Table 4). The Fe accumulation in the fish tissue of Lake Eber were the following in all seasons liver>gill>muscle. In this study the amount of Fe was determined between 11.3897 and 208.0796 mg/kg. The Fe level in the fish tissue was statistically significantly higher in the liver (208.0796 mg/kg) (Table 4) during autumn compared to the other tissues and seasons (p<0.05). In this study, the amount of Mn was determined between 0.0379 and 14.7615 mg/kg. The Mo concentration in the fish tissues was the highest in the liver 0.1511 mg/kg and the lowest 0.0832 mg/kg ins spring, during the summer highest Mo concentration was observed in the muscle 0.1044 mg/kg and the lowest in the gill 0.1214 mg/kg, in autumn the highest Mo concentration was observed in the gill 0.0997 mg /kg while the minimum was in the muscle 0.0633 mg/kg, in the winter the highest Mo concentration was found in the gill 0.0943 mg/kg while the lowest was found in the gill 0.0742 mg/kg. The Mo level was significantly higher statistically in the liver in summer compared to the other seasons and tissues (p<0.05). In our study the highest Ni value was observed in the liver (0.7328 mg/kg) in spring (Table 4). The values measured in the gill and liver during the winter were below the measurable limit values. The Ni level in the liver in spring was statistically significantly higher than in other tissues in other seasons (p<0.05). Lead was detected below the measurable limit values in the gills and liver during the summer and winter season and in the muscle during the autumn. The highest Pb values was measured during the spring in the liver (0.0876 mg/kg). Significant difference was determined in the Pb values within the fish tissue (p<0.05). Selenium was found below the limit value in the muscle during spring, in the gill during the summer, and in the liver during the autumn and winter. In this research, the highest Se level was measured in the muscle tissue in winter (0.336008 mg/kg) (Table 4). Significant difference was determined in the Se values within the fish tissue (p<0.05).

Table 2: Seasonal and annual average heavy metal values of water taken from Eber Lake stations (mg/L)

Season	Station	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Se	Zn
Spring	1	0.0008	0.0022	0.0017	0.0021	0.1259	0.022	0.0015	0.0021	ND	ND	0.0386
	2	0.0006	0.0029	0.0004	0.0013	ND	0.0113	0.0033	0.0023	ND	ND	0.0156
	3	0.0004	0.0018	0.0005	0.00005	ND	0.0094	0.0037	0.0019	ND	0.0004	0.0179
	4	0.0005	0.0016	0.0002	ND	ND	0.0092	0.0033	ND	ND	ND	0.0106
	5	0.0004	0.0036	0.0013	ND	0.0969	0.1584	0.003	0.0053	0.0008	ND	0.0131
Summer	1	0.0005	0.0015	0.0001	0.0016	ND	0.0106	0.0013	0.0012	ND	ND	0.0099
	2	0.0005	0.0032	0.0004	ND	0.0049	0.0618	0.0043	0.0004	ND	ND	0.0136
	3	0.0006	0.0021	0.0003	ND	ND	0.0616	0.0026	0.0012	ND	0.0063	0.0079
	4	0.0005	0.0023	0.0003	ND	ND	0.0094	0.0015	0.0016	ND	ND	0.011
	5	0.0005	0.0049	0.0009	ND	0.0117	0.0109	0.0019	0.0095	ND	ND	0.0036
Autumn	1	0.0003	0.0019	0.0003	0.0013	ND	0.031	0.0025	0.0014	ND	ND	0.0265
	2	0.0005	0.002	ND	ND	ND	0.0092	0.0038	0.0027	ND	ND	0.0191
	3	0.0008	0.0016	0.0006	ND	ND	0.0284	0.0021	0.00002	ND	ND	0.0267
	4	0.0005	0.0025	0.0001	ND	ND	0.0554	0.0016	0.0004	ND	ND	0.0194
	5	0.0006	0.0022	0.0002	ND	ND	0.0175	0.0049	ND	0.001	ND	0.0507
Winter	1	0.0005	0.0023	ND	ND	ND	0.0087	0.0036	0.0015	ND	ND	0.0074
	2	0.0004	0.0012	0.0004	ND	0.0911	0.0124	0.0042	0.0008	0.0002	ND	0.0034
	3	0.0005	0.0024	ND	ND	0.0167	0.0116	0.0049	0.0017	ND	ND	0.0009
	4	0.0005	0.002	0.00006	ND	ND	0.0101	0.0041	0.0012	ND	ND	ND
	5	0.0008	0.0025	0.0002	ND	ND	0.0085	0.0044	0.002	ND	0.0063	0.0015
Annual Mean	1	0.0005^{a*}	0.002^{b}	0.0005^{a}	0.0013^{a}	0.0314^{a}	0.0181^{a}	0.0022^{a}	0.0016^{ab}	ND	ND	0.0206^{a}
	2	0.0005^{a}	0.0023^{ab}	0.0003^{a}	0.0003^{b}	0.024^{a}	0.0237^{a}	0.0039^{a}	0.0015^{ab}	0.00005^{b}	ND	0.0129^{a}
	3	0.0006^{a}	0.002^{b}	0.0003^{a}	0.00001^{b}	0.0041^{a}	0.0278^{a}	0.0033^{a}	0.0012^{ab}	ND	0.0016^{a}	0.0133^{a}
	4	0.0005^{a}	0.0021^{b}	0.0001^{a}	ND**	ND	0.021^{a}	0.0026^{a}	0.0008^{b}	ND	ND	0.0102^{a}
	5	0.0006^{a}	0.0033^{a}	0.0006^{a}	ND	0.0271^{a}	0.0488^{a}	0.0035^{a}	0.0042^{a}	0.0004^{a}	0.0015^{a}	0.0172^{a}

*Averages indicated by different letters in the same column are statistically different ($p<0.05$) ** ND: Not Detected <0,0001 mg/kg

Table 3. Seasonal and annual average heavy metal values of sediment taken from Eber Lake stations (mg/kg)

Season	Station	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Se	Zn
Spring	1	0.3624	1.5429	29.012	25.6042	12424.48	192.0395	1.9669	46.3045	12.7267	3.0274	66.4788
	2	0.1667	0.2767	11.862	10.169	5368.347	353.9277	1.3607	19.6738	5.0009	2.374	31.3378
	3	0.133	0.3842	5.0594	5.5892	3285.455	533.6344	1.5259	15.8469	3.6497	0.2635	25.0848
	4	0.1665	0.5154	10.0565	11.0674	4382.358	355.7712	2.0979	19.8341	5.3219	1.5143	32.2830
	5	3.1074	0.6269	27.3750	29.9756	9778.956	200.8294	2.0909	25.9991	10.2345	2.5967	91.1524
Summer	1	0.275	1.796	41.1762	25.5576	13331.67	180.9605	1.4054	22.3749	12.272	2.5117	51.4702
	2	0.1666	0.3846	14.4272	12.6427	6621.718	224.6447	2.27	23.625	5.8811	0.5383	27.5589
	3	0.115	0.3537	12.0906	10.6679	5034.166	319.7483	1.4291	14.5262	5.7035	1.9036	22.1982
	4	0.1006	0.3186	5.5649	6.8079	2633.244	417.6687	1.2943	13.9233	3.4189	ND	15.6252
	5	3.8949	0.6504	30.7425	24.7902	14524.79	246.2176	0.907	31.1456	14.63	ND	64.3621
Autumn	1	0.2843	0.3909	25.7425	26.7874	15864.94	303.2335	1.3804	25.9143	12.2412	2.0762	51.2592
	2	0.1966	0.5485	13.7591	17.1517	8835.225	416.925	3.2159	21.7762	8.0622	0.9626	31.8664
	3	0.1257	0.7858	6.0432	9.159	4620.155	775.2915	2.5177	13.29	5.3555	2.3851	20.6419
	4	0.1129	0.9328	4.6858	7.1566	3551.377	710.626	2.4275	10.7329	4.69	ND	18.5806
	5	5.6435	0.8497	34.7876	32.1933	16245.84	267.4343	1.3892	36.064	17.1591	2.2285	78.9697
Winter	1	0.3072	0.671	14.8066	18.9685	13707.47	457.6013	3.3277	18.2166	11.7165	2.1832	45.3732
	2	0.198	0.8413	14.5196	15.0903	7548.085	429.8555	2.0681	17.6466	10.1386	2.1119	33.243
	3	0.1238	0.619	5.3709	8.0475	3971.337	613.7977	2.2252	10.525	5.7073	ND	20.3113
	4	0.1585	0.557	10.2756	16.6193	5474.896	600.4953	2.1735	19.0524	7.5022	0.6107	24.5946
	5	0.9725	0.6896	33.485	26.4259	16199.74	371.3087	1.2465	30.1273	17.194	1.6401	65.0904
Annual Mean	1	0.3072^{b*}	1.1002^{a}	27.6844^{a}	24.2294^{a}	13832.14^{a}	283.4587^{b}	2.0201^{a}	28.2026^{a}	12.2393^{a}	2.4496^{a}	53.6453^{b}
	2	0.182^{b}	0.5128^{a}	13.642^{b}	13.7634^{b}	7093.34^{c}	356.3382^{ab}	2.2287^{a}	20.6804^{a}	7.2707^{b}	1.4967^{ab}	31.0015^{c}
	3	0.1244^{b}	0.5357^{a}	7.1410^{b}	8.3659^{b}	4227.77^{b}	560.618^{a}	1.9245^{a}	13.547^{a}	5.104^{b}	1.138^{ab}	22.0591^{c}
	4	0.1347^{b}	0.581^{a}	7.6457^{b}	10.4128^{b}	4010.46^{b}	521.1403^{a}	1.9983^{a}	15.8857^{a}	5.2332	0.5312^{ab}	22.7709^{c}
	5	3.404^{a}	0.7041^{a}	31.5976^{a}	28.3462^{a}	14187.33^{a}	271.4475^{b}	1.4084^{a}	30.834^{a}	14.8044^{a}	1.6163^{b}	74.8936^{a}

*Averages indicated by different letters in the same column are statistically different ($p<0.05$) ** ND: : Not Detected <0,0001 mg/kg

Table 4. Heavy metals concentrations in different tissues of *Carassius gibelio* (Bloch, 1782) from Eber Lake (mg/kg, dry weight)

Season	Tissue	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Se	Zn
Spring	M-	0.0358^{*a}	0.1147^{bc}	0.7989^{a}	0.9949^{bc}	50.9^{d}	1.9283^{d}	0.085^{abc}	0.583^{ab}	0.017^{b}	ND	26.3751^{c}
	S.D.	0.0091	0.0391	0.6953	1.075	16.8372	0.4069	0.0624	0.5326	0.0188	-	6.0022
	G-	0.0479^{a}	0.0987^{bc}	0.6727^{ab}	1.2879^{bc}	121.38^{bc}	8.2728^{c}	0.0832^{abc}	0.4237^{bc}	0.0095^{b}	0.0354^{b}	127.2273^{a}
	S.D.	0.0167	0.0485	0.3507	0.6106	30.9984	3.6508	0.053	0.1373	0.0005	0.0054	46.9508
	L-	0.0443^{a}	0.2206^{a}	0.4803^{abc}	1.6975^{b}	182.0846^{a}	1.5688^{d}	0.1511^{ab}	0.7328^{a}	0.0876^{a}	0.1553^{ab}	84.3947^{ab}
	S.D.	0.0167	0.2107	0.1621	0.9208	33.7451	0.2511	0.1634	0.3429	0.0912	0.069	21.1185
Summer	M-	0.0352^{a}	0.1203^{bc}	0.775^{a}	1.1367^{bc}	21.0209^{d}	1.6744^{d}	0.0848^{abc}	0.1735^{cd}	0.0328^{b}	0.0552^{b}	15.147^{c}
	S.D.	0.0116	0.0274	0.782	0.418	13.9103	1.179	0.0179	0.0118	0.008	0.0552	1.7352
	G-	0.0542^{a}	0.1378^{abc}	0.5258^{abc}	0.4144^{c}	39.0372^{d}	6.067^{c}	0.0507^{c}	0.1819^{cd}	ND	ND	50.3372^{bc}
	S.D.	0.0008	0.0112	0.0705	0.1155	12.7286	1.3584	0.0307	0.1265	-	-	3.0521
	L-	0.0346^{a}	0.11098^{bc}	0.3534^{abc}	1.0031^{bc}	167.192^{ab}	1.6248^{d}	0.1773^{a}	0.1677^{cd}	ND	0.1221^{ab}	112.369^{a}
	S.D.	0.0031	0.0991	0.009	0.2674	10.9506	0.0309	0.0635	0.0475	-	0.0849	23.0366
Autumn	M-	0.0598^{a}	0.1415^{abc}	0.1188^{c}	0.6691^{c}	11.3897^{d}	1.1452^{d}	0.0633^{bc}	0.0039^{d}	ND	0.0981^{b}	15.0727^{c}
	S.D.	0.0725	0.0425	0.0467	0.1709	4.1343	0.1696	0.0066	0.0117	-	0.0849	2.1739
	G-	0.0448^{a}	0.1227^{bc}	0.358^{abc}	0.6605^{c}	107.194^{c}	14.7615^{a}	0.0997^{abc}	0.1224^{d}	0.0124^{b}	0.1038^{b}	52.5166^{bc}
	S.D.	0.0164	0.0384	0.0771	0.1177	54.757	4.3054	0.0546	0.1402	0.0264	0.0809	8.2681
	L-	0.0408^{a}	0.0716^{c}	0.1156^{c}	1.4625^{bc}	208.079^{a}	1.1771^{d}	0.0895^{abc}	0.0702^{d}	0.011^{b}	ND	29.6267^{c}
	S.D.	0.0153	0.0173	0.0613	0.2964	68.36	0.2337	0.0683	0.1524	0.0031	-	3.2853
Winter	M-	0.0388^{a}	0.1685^{ab}	0.1468^{c}	1.0723^{bc}	13.9041^{d}	1.0921^{d}	0.0752^{bc}	0.0552^{d}	0.05^{ab}	0.336^{a}	17.6810^{c}
	S.D.	0.005	0.0089	0.0475	0.2871	0.1977	0.0809	0.0229	0.0054	0.005	0.1939	2.8817
	G-	0.0475^{a}	0.0766^{c}	0.2112^{bc}	0.74234^{c}	60.4995^{d}	11.7071^{b}	0.0943^{abc}	ND**	ND	0.0072^{b}	57.6319^{bc}
	S.D.	0.0099	0.0173	0.0561	0.0265	3.4687	0.694	0.0042	-	-	0.0041	2.2548
	L-	0.0199^{a}	0.0894^{bc}	0.0877^{c}	2.1995^{a}	126.127^{bc}	1.1999^{d}	0.0742^{bc}	ND	ND	ND	24.9595^{c}
	S.D.	0.0017	0.0415	0.0072	0.6344	39.585	0.054	0.0448	-	-	-	4.1375

*Averages indicated by different letters in the same column are statistically different ($p<0.05$) ** ND: Not Detected <0,0001 mg/kg

DISCUSSION

At all stations and in all season the heavy metal values for all metals except Mn were first class according to the Turkish water quality control regulations (WPCR) and were found to be under the limit values according to EPA (Environmental Protection Agency) (EPA, 2018) and European Communities (EC, 2006). During the spring season Pb and Se were detected only at one station, while Cu was detected at two and Fe at three stations. Ni was not detected only at the fourth station. In the summer, Cu and Se could be detected only at one station while Fe could be detected at two stations. Pb was not detected at any of the stations (Table 2). In this research, the Mn was the most recorded metal in the water of the Lake Eber, and it was followed respectively by Fe, Zn, Mo, Co, Ni, Se, Cd, Cr, Cu and Pb. In this study, metals generally reach their highest values in warm seasons, while these values decrease in winter months. Comparing this study to the study conducted on the Lake Kovada (Kayrak and Tekin-Özan, 2018) and the Pondicherry Lake (Satheeshkumar and Senthilkumar 2011) in this study Mn and Fe were the most detected elements in the water. The high Mn accumulation is of anthropogenic origin due to the discharge of the Akarçay river.

In this study, Fe was the mostly recorded metal in the sediment, and followed respectively by Mn, Zn, Ni, Cr, Cu, Pb, Se, Mo, Cd, Co. Comparing to the studies which were carried out on Aktan and Tekin-Özan (2012), Habbaniya (Al-Saadi *et. al*., 2002) and Hazar Lake (Karadede-Akın, 2009) also in this research Fe then Mn were the most observed metals in the sediment. Barlas *et al*., (2005) reported the highest mean levels metals Cu (1.19 mg/kg), Cr (4.88 mg/kg), Pb (2.39 mg/kg), Cd (0.12 mg/kg) and Ni (8.93 mg/kg) in May 2002 in sediment samples. The highest concentration of Zn (8.36 mg/kg) was recorded in September 2002. The excess amount of Fe in the sediment of the river and lake can be explained with the fact that the Fe is the most often observed metal within the Earth's crust.

In present study, the amount of metals in the sediment as a state bonding to the metal has varied but in general the highest accumulation was observed at the fifth station. This can be caused by two reasons: one of them is the fact that Akarçay, which is one of the main source feeding the lake, enters the lake by being subjected to organic and industrial waste and the other one is the collapse of the suspended solids onto the sediment which bind the metals in the water (Kıvrak.and Uygun, 2012; Gümüş, 2021)

The amounts of Zn was determined between 4.2504 and 127.2273 mg/kg. In all seasons, the amount of Zn which was obtained from the muscle tissue was below the acceptable limit values of the Turkish Food Codex 50 mg/kg and FAO 40 mg/kg (FAO, 1989; TFC 2011). Significant difference was determined in the Zn values within the fish tissue ($p<0.05$). The Zn concentration of some fish was reported as 1.25–1.32 mg/l 100 g Zn by Özyurt *et al*., (2009). Nawaz *et al*., (2010) determined the levels of Cd, Pb, Hg, Zn, and Cu as 0.35–0.45, 2.1–3.0, 37.85–40.74, and 1.39–2.93 mg/kg in freshwater fish species of the River Ravi in Pakistan. *Malik et al.*, (2010) stated that in Lake Bhopal (India) the majority of the metals accumulated in the liver of the *Labeo rohita* and *Ctenopharyngodon idella* species while the least of the metals accumulated in the muscles. Zn accumulated at the highest percentage among the metals while Hg was deposited at the lowest rate. Mohammadi *et al.*, (2011) determined that within the body of *Barbus grypus* which is living in River Karoon and Dez most of the metals accumulated within the liver while in *Barbus xanthopterus* the maximum concentration rate of the metals was determined in the gill. In this study the highest concentration of the heavy metals was detected in the gill and liver of the fish tissue. In the study conducted by Fidan *et al.*, (2008) on Lake Eber, some heavy metals were determined in the muscle, gills and liver tissues of the *Carassius carassius L.,* 1758 fish. The concentration of the metals in the tissue was the following muscle<gill <liver, the most accumulated metal was Fe and Zn. Altundağ *et al.,* (2019) found the following levels of Cu<Fe<Zn in muscle tissue of carp caught from the Sapanca Lake. Uysal, (2010) the Zn level in which the highest Zn accumulation in the tissues of the *C. carpio*, *Carassius carassius* and *Rutilus rutilusspecies* were found in the gill tissue. These data showed similarities with the data which was obtained in our study. According to this study in *Carassius gibelio* (Bloch, 1782) fish the highest concentration of Co, Mo, Ni, Cu, Fe, Pb, metals were determined in the liver, while the maximum concentration of Mn and Zn were detected in the gill tissue and the highest level of Cd, Cr and Se were determined within the muscle tissue. According to the findings obtained from Lake Eber the accumulation of the metals varied according to the tissue and to the type of the metal. Canpolat and Catla (2003) reached the highest values for heavy metals in the tissue of the *Capoeta capoeta umbla* fish during the spring. These results are in line with the data obtained in our study.

CONCLUSIONS

The water and sediment quality of Lake Eber and the River Akarçay which feeds the lake with agricultural pollutants are under the influence of Afyonkarahisar's industrial and municipal discharges. The water of Lake Eber was found in normal quality except the Mn element according to criteria of WPCR, EPA and EC. In the sediment all the metals except of Cd remained below the toxic effect value given by MacDonald. The highest value of all the metals except Zn in spring was found in the sediment. The water and sediment quality of Eber Lake is affected by agricultural, industrial, and domestic discharges from Afyonkarahisar. According to the heavy metal bioaccumulations detected in tissues of the *Carassius gibelio* species, Eber Lake's biotic components are also adversely affected by anthropogenic activities. The results of the heavy metals within the muscle tissue of the *Carassius gibelio* fish were compared to the quality criteria of the Turkish Food Codex and FAO and it was observed that they remained below the limit values. In the fish tissue the highest values for heavy metals were observed in the liver and gill tissue. According to these results although there was not a conclusion which could cause risk in the biotic and abiotic factors considering the fact that some of the values exceeded the limit values and the anthropogenic activities in Lake Eber increased gradually recently, reveals the need to subject the biotic and abiotic factors to continuous monitoring.

ACKNOWLEDGEMENTS

PhD thesis of Numan Emre GÜMÜŞ was financially supported by the Selçuk University Scientific Research Project Coordinator with the project coded (BAP-14201044) and Tübitak BİDEB. Also, I would like to thank to Dr. Buğrahan EMSEN for his contribution to the statistical analysis for the interpretation of the data.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 515-521 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.13

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Preliminary study on otolith chemistry and otolith morphology of two demersal fish species, European hake (*Merluccius merluccius* Linnaeus, 1758) and striped red mullet (*Mullus surmuletus* Linnaeus, 1758) in the Sea of Marmara

Marmara Denizi'nde iki demersal balık türünün bakalyaro (*Merluccius merluccius* Linnaeus, 1758) ve tekir (*Mullus surmuletus* Linnaeus, 1758)'in otolit kimyası ve otolit morfolojisi üzerine ön çalışma

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Received date: 25.05.2021 *Accepted date: 09.11.2021*

How to cite this paper:
Bal, H. & Esen, S. (2021). Preliminary study on otolith chemistry and otolith morphology of two demersal fish species, European hake *Merluccius merluccius* (Linnaeus, 1758) and striped red mullet (*Mullus surmuletus*, Linnaeus, 1758) in the Sea of Marmara. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 515-521. DOI: 10.12714/egejfas.38.4.13

Abstract: Otoliths are calcium carbonate ($CaCO_3$) accumulations. Under the influence of different ecosystems morphological and chemical composition change. In this study, economically important two demersal fish species; European hake *Merluccius merluccius* (Linnaeus, 1758) and Stripet red mullet (*Mullus surmuletus* Linnaeus, 1758) was examined. Otoliths (Sagitta) belonging to these two species have been studied both chemically and morphologicall. Morphometric measurements of otoliths (length, mm; width, mm; area, mm^2; perimeter, mm) in the sagittal of each species was made by the Leica M125 tri-ocular microscope. In the chemical analysis of otoliths, strontium (Sr), magnesium (Mg) and calcium (Ca) trace element amounts, the ratios of Sr and Mg trace elements to Ca element (Sr/Ca and Mg/Ca) were determined. For the micro-chemical analysis of otoliths ICP-MS was used. The highest magnesium (24.92±9.57 mmol/mol) and strontium (26.17±1.81 mmol/mol) element values were found in the otolith of red mullet. The difference between strontium (Sr) and magnesium (Mg) amounts for two fish species was found to be significant ($P<0.05$). In addition to it was found that the difference between them in the amount of calcium is significant ($P<0.001$). The shape indexes of otoliths are significantly different between the two fish species. Especially in terms of roundness (R_D) and aspect ratio (A_R) ($P<0.001$). The results of this study provide information about the habitats of two economic importance demersal fish species. Since such studies can give information about the habitat areas of fish species, they are important for tracking stocks, migration routes and sustainable fisheries.

Keywords: Demersal fish, otolith chemistry, otolith morphology, Sea of Marmara

Öz: Otolitler kalsiyum karbonat ($CaCO_3$) birikimleridir. Farklı ekosistemlerin etkisi altında morfolojik ve kimyasal olarak değişmektedir. Bu çalışmada, ekonomik olarak önemli iki demersal balık türü; Bakalyaro *Merluccius merluccius* (Linnaeus, 1758) ve tekir (*Mullus surmuletus* Linnaeus, 1758) balığına ait otolitler (Sagitta) hem kimyasal hem de morfolojik olarak incelenmiştir. Otolitlerin (uzunluk, mm; genişlik, mm; alan, mm^2; çevre, mm) morfometrik ölçümleri Leica M125 tri-oküler mikroskop ile yapılmıştır. Otolit yapısındaki stronsiyum (Sr), magnezyum (Mg) ve kalsiyum (Ca) iz element miktarları ile Sr ve Mg eser elementlerinin Ca elementine (Sr/Ca ve Mg/Ca) oranları belirlenmiştir. Otolitlerin mikro kimyasal analizi için ICP-MS kullanılmıştır. En yüksek magnezyum (24,92±9,57 mmol/mol) ve stronsiyum (26,17±1,81 mmol/mol) element değerleri tekir otolitinde tespit edilmiştir. İki balık türü için stronsiyum (Sr) ve magnezyum (Mg) miktarları arasındaki fark önemli bulunmuştur ($P<0,05$). Ayrıca kalsiyum miktarı açısından aralarındaki farkın oldukça önemli olduğu tespit edilmiştir ($P<0,001$). Otolitlerin şekil indeksleri iki balık türü arasında önemli ölçüde, özellikle yuvarlaklık (R_D) ve en boy oranı (A_R) açısından önemli olduğu tespit edilmiştir ($P<0,001$). Bu çalışmanın sonuçları, ekonomik önemi olan iki demersal balık türünün habitatları hakkında bilgi verdiğinden; stokları, göç yolları ve sürdürülebilir balıkçılığın takibi açısından önem arz etmektedir.

Anahtar kelimeler: Demersal balık, otolit kimyası, otolit morfolojisi, Marmara Denizi

INTRODUCTION

Otolith are stable structures in terms of metabolic activity and calcium carbonate ($CaCO_3$) deposits (Degens *et al*., 1969; Pannella, 1971; Campana, 1999). There are three pairs of otoliths in sagitta, lapillus and asteriscus in bony fishes. They undertake the task of providing balance and hearing (Popper and Coombs, 1980; Campana, 1999; Campana and Thorrold, 2001).

Otolith has been the subject of studies in many areas such as fish biology, fish ecology, fish stock detection, diet assessment, as well as age and growth (Campana, 1999;

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Friedland and Reddin, 1994; Tracey *et al*. 2006; Gonzalez-Salas and Lenfant, 2007; Barrett, 1990; Martucci et al., 1993; Velando and Freire, 1999; Turan, 2006; Morat *et al*., 2014; Başusta and Khan, 2021).

Otolith constitute a source of information and data for the identifying ichthyological taxa with systematic and fleet genetic studies. Scientific studies and applications on otolith are not limited to ichthyology. It is also possible to access in-depth information about historical processes by using them in fields such as paleontology, stratigraphy, archeology and zoo-geography (Schwarzhans, 1999).

The best evidence of fish movement is observing and following their movements from one place to another. However, these data are difficult to obtain for multiple life stages (Gillanders, 2005). Although the potential importance of movement, relatively little is known about how many fish species vary within and between populations (Quinn, 1993; Gowan *et al*., 1994). Therefore, a robust and cost-effective method is needed to determine the habitat origins and movements of fish and to describe the stock structure of fish species. Otolith element composition has recently proven to be a robust natural tag for tracking population structure, species life history, habitat areas and migration routes (Campana and Neilson, 1985; Rieman *et al*., 1994; Campana *et al*., 1995; Thorrold et al., 1997; *Wells et al*., 2000; Thorrold *et al*., 2001). The structures of otolith undergo differentiation ontogenetically under the influence of different ecosystems. Their structure is three-dimensional, but not all dimensions grow equally and at the same rate. It also varies significantly among species in terms of size, shape and chemical content (Campana and Thorrold, 2001). One of the fastest growing areas of fisheries science is the use of these calcined structures (otolith) to answer ecological questions about fish movement and habitat areas. Otolith's two properties make it particularly suitable for keeping records of the environment in which the fish live (Gillanders, 2005). European hake and stripet red mullet are the most economically important demersal fish species in Turkey Seas (Yıldız and Karakulak, 2018). It mainly inhabits rocky bottoms and soft substrates and undergoes vertical movements between 5 and 100 m in depth (Froese and Pauly, 2008).

However, stocks are gradually decreasing due to overfishing, which have been decreasing recently. According to the Turkey Statistical Institute (2018-2019) 1.019 tons of *M. merluccius*, 2.914 tons *M. surmuletus* and 1269 tons *M. merluccius*, 2341 tons *M. surmuletus* were caught, respectively (TUİK, 2021).

In this study, the amount of trace elements and morphological structures of otoliths was examined and evaluated comparatively to monitor the stocks of two important demersal fish species.

MATERIAL AND METHODS

A total of 10 *M. merluccius* and 10 *M. surmuletus* were obtained from fishing vessels in the Sea of Marmara (west of "Kapıdağ Peninsula"). Otoliths were examined both morphologically and in microchemical analysis.

Otolith chemistry

Whole otoliths (right and left) were used for otolith chemistry. For each otolith to reach a constant weight, it has been subjected to drying in glass tubes for 2 h in an oven set at 120 °C. The otolith samples whose drying was completed were weighed with a sensitive scale sensitive to approximately 0.001 g and transferred to the vessels in the microwave incinerator unit. Then, 5 ml of nitric acid (HNO_3) and 2 ml of hydrogen peroxide (H_2O_2) were added to each vessel and subjected to heat-controlled microwave combustion using the SK-12 rotor in the Easy model Ethos (Rooker *et al*., 2001; Correia *et al*., 2011). After the extract reached room temperature, the final volume was diluted with 25 ml of distilled water and analyzed in solution mode on a Perkin Elmer brand Nexion 350X model Inductively Coupled Plasma Mass Spectrometer ICP-MS (Jarvis and Jarvis, 1992).

At the end of the analysis, strontium (Sr), calcium (Ca) and magnesium (Mg) trace element concentrations and elemental calcium ratios (Sr/Ca and Mg/Ca) were determined.

Otolith morphology

Firstly, the length (with a height measuring scale with 0.01 mm precision) and weight measurements (in 0.01 g precision balance) of the fish samples were examined. The sagitta otoliths were removed and soaked in 3% hydrogen peroxide (H_2O_2) and 1% nitric acid (HNO_3) for 5 min, respectively, to purify the blood, tissue and other surface contaminants. It was then kept in distilled water for 5 min to remove the acid (Rooker *et al*., 2001). There were no differences between right and left otolith of both species (*t*-test, P>0.05). Therefore, the right otolith was used for the measuring the otolith morphology of two fish species. Otolith length (OL) and otolith width (OW_i) (±0.001 mm) were determined by Leica M125. Otolith length was defined as the greatest distance between the anterior and posterior edge and otolith width was described as the greatest distance from dorsal to the ventral edge. To illustrate how measurements of otolith morphology are made *M. merluccius* otolith measurements are given as an example (Figure 1).

The raw data obtained at the end of the measurement [otolith length (OL, mm), otolith width (OW, mm), otolith area (OA, mm^2) and otolith perimeter (OP, mm)] was evaluated and the shape indexes of was calculated. The otolith shape identification process helps construct the otolith atlas and identify the species (Tuset *et al*., 2008). The formulas given in Table 1 were used to determine the shape indexes of otoliths (Tuset *et al*., 2003; Ponton, 2006).

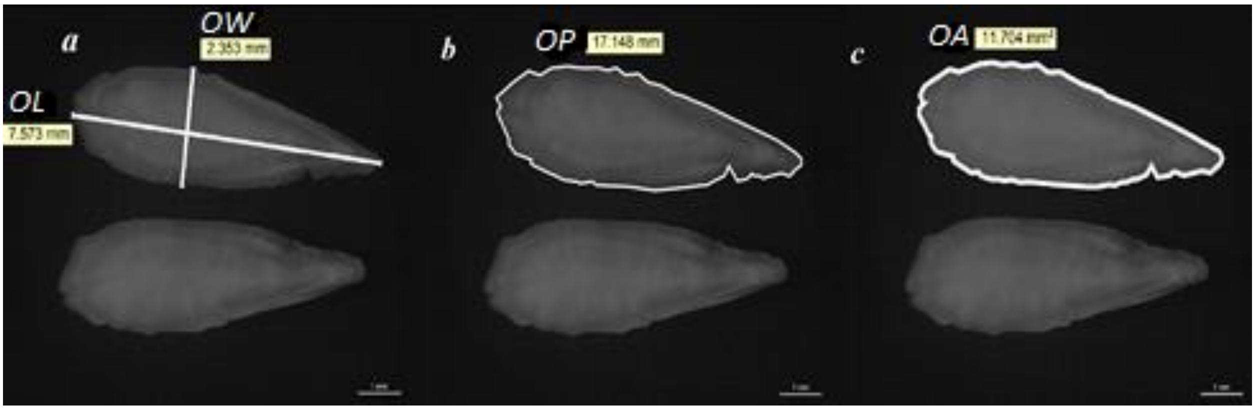

Figure 1. a: OL otolith length (mm); OW: otolith width (mm); b: OP otolith perimeter (mm); c: OA otolith area (mm^2); measurement: 1mm. Photos by H. BAL)

Table 1. Formulas to be used for the shape index otoliths of the species

Parameters	Shape indexes	Formula
OP (otolith perimeter, mm)	Form Factor (F_F)	$4.\pi.OA/(OP)^2$
OA (otolith area, mm^2)	Circularity (C_I)	$(OP)^2/OA$
OL (otolith length, mm)	Roundness (R_D)	$4.OA/\pi.(OL)^2$
OW_I (otolith witdh, mm)	Width/Length (W/L)	$OL.OW_I^{-1}$

The results of this study provide information about the habitats of two demersal fish species that have economic importance and share the same habitat. Since such studies can give information about the habitat areas of fish species, they are important for tracking stocks, migration routes and sustainable fisheries.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using the SAS package program (Version: 6.0.0) to determine the differences between two demersal fish species on otolith chemistry and morphology.

RESULTS

Both chemical and morphological analysis was performed on otolith belonging to two demersal fish species.

Otolith chemistry

Chemical trace elements (Sr, Mg and Ca) in otoliths belonging to two demersal fish species were investigated. It was determined that the highest value among the three trace elements was in otoliths belonging to *M. surmuletus* species and the average amount of strontium as 26.17±1.81 mmol/mol was determined. Similarly, it was determined that the highest amount of Mg was in *M. surmuletus* (24.92±9.57 mmol/mol). However, calcium trace element (Ca) content of *M. merluccius* was found to be 10.2±0.07 mmol/mol higher than *M. surmuletus*. The difference between strontium (Sr) and magnesium (Mg) amounts for the two fish species was found to be significant ($P<0.05$). It was found that the difference between them in the amount of calcium is significant ($P<0.001$). Data of fish size and otolith chemistry for two demersal species are given in Table 2.

Table 2. Data on otolith chemistry of two demersal fish species

Species	TL (cm) Min-max Mean±SE	W (g) Min-max Mean±SE	Sr (µmol/mg) Min-max Mean±SE	Mg (µmol/mg) Min-max Mean±SE	Ca (µmol/mg) Min-max Mean±SE	Sr/Ca (mmol/mol) Min-max Mean±SE	Mg/Ca (mmol/mol) Min-max Mean±SE
Merluccius merluccius	27.10-36.50 31.54±0.98	141.87-413.14 264.97±28.53	3.97-30.28 15.51±2.99	0.60-1.98 1.26±0.18	9.97-10.67 10.25±0.07	0.38-2.96 1.52±2.11	0.05-0.19 0.12±0.11
Mullus surmuletus	13.50-18.0 15.35±0.38	25.75-75.83 43.01±4.27	20.46-38.30 26.17±1.81	5.63-105.96 24.92±9.57	7.49-10.50 9.30±0.29	2.42-3.64 2.79±0.45	0.57-14.17 2.94±1.20
Sig.	***	***	**	***	***	**	***

TL: Total fish length (cm), W: Total fish weight (g); Sr: Strontium (mmol/mol); Mg: Magnesium (mmol/mol); Ca: Calcium (mmol/mol); SE: Standard error; Sig.: Significance, **: $P<0.01$, ***: $P<0.001$.

Otolith morphology

It was determined that the shape indexes obtained from the morphological analysis of the otoliths belonging to both fish species differ from each other. The otolith morphology of the two species was determined to be significantly different from each other. The minimum, maximum, mean and standard error of fish size and otolith measurement are given in Table 3. The raw data obtained at the end of the measurement [otolith length (OL, mm), otolith width (OW_i, mm), otolith area (OA, mm^2) and otolith perimeter (OP, mm)] was evaluated and the shape indexes of was calculated. The shape indexes differ significantly from each other. Especially in terms of roundness (R_D) and aspect ratio (A_R) (P<0.001). Data of morphological and shape indexes are given in Table 4.

Table 3. Descriptive statistics on fish size and otolith measurements

Fish size and otolith measurements	Species				
	Merluccius merluccius		*Mullus surmuletus*		
	Min-max	Mean±SE	Min-max	Mean±SE	Sig.
TL (cm)	27.10-36.50	31.54±0.98	13.50-18.0	15.35 ± 0.38	***
W (g)	141.87-413.1	248.51±2.05	25.75-75.83	43.01 ± 4.27	***
OW (mg)	75.0-159.0	107.70±7.81	4.20-10.80	6.08±0.64	***
OL (mm)	10.09-13.33	11.45 ± 0.37	2.11-3.01	2.31 ± 0.09	***
OW_i (mm)	3.96-5.82	4.65 ± 0.17	1.41-1.99	1.60±0.06	***
OA (mm^2)	28.57-52.20	37.13 ± 2.24	2.05-4.08	2.48±0.19	***
OP (mm)	22.73-30.82	25.98 ± 0.83	5.28-8.34	6.32±0.31	***

TL: Total fish length (cm), W: Total fish weight (g), OW: Otolith weight (mg), OL: Otolith length (mm), OW_i: Otolith width (mm), OA: Otolith area (mm^2), OP: Otolith perimeter (mm), SE: Standard error; Sig.: Significance, ***: P<0.001.

Table 4. Fish size and otolith shape analysis

Species	TL (cm) Min-max Mean±SE	W (g) Min-max Mean±SE	Circularity (C_i) Min-max Mean±SE	Form factor (F_F) Min-max Mean±SE	Roundness (R_D) Min-max Mean±SE	Aspect ratio (A_R) Min-max Mean±SE
Merluccius merluccius	27.10-36.50 31.54±0.98	141.87413.14 248.51±2.05	17.33-1977 18.30±0.22	0.635-0.725 0.680±0.008	0.325-0.378 0.358±0.006	2.292-2.600 2.464±0.029
Mullus surmuletus	13.50-18.0 15.35±0.38	25.75-75.83 43.01±4.27	12.87-20.05 16.33±0.83	0.626-0.976 0.789±0.042	0.547-0.642 0.592±0.009	1.322-1.620 1.445±0.030
Sig.	***	***	*	*	***	***

TL: Total fish length (cm); W: Total fish weight (g); OW: Otolith weight (mg); SE: Standard error; Sig.: Significance; *: P<0.05; ***: P<0.001.

DISCUSSION

Although the potential importance of the movement, relatively little is known about the populations of many fish species (Quinn, 1993; *Gowan et al*., 1994). Therefore, there is a need for a robust and cost-effective method to determine the habitat origins and movements of fish and to describe the stock structure of fish species. Otolith element composition has recently proven to be a powerful natural label for tracking population structure, species life histories, habitat ranges, and migration routes (Campana and Neilson, 1985; Rieman *et al*., 1994; Campana *et al*., 1995; Thorrold *et al*., 1997; *Wells et al*., 2000; Thorrold *et al*., 2001).

Although *M. merluccius* and *M. surmuletus* are demersal fish species, it has been determined that the chemical element amounts and ratios of otoliths belonging to *M. surmuletus* were higher than *M. merluccius*. Especially, when compared to the otolith size of *M. surmuletus*, Sr/Ca and Mg/Ca ratios are observed to be higher although it is much smaller than *M. merluccius*. Similarly, although *M. surmuletus* otolith is lighter (Mean±SE; 6.08±0.64 mg) than *M. merluccius* otolith (Mean±SE; 107.70±7.81 mg), it contains higher amounts of Sr and Mg trace elements (Table 2 and Table 3). But, calcium (Ca) trace element content of *M. merluccius* was found to be 10.2±0.07 mmol/mol higher than *M. surmuletus.* According to the results of scientific studies, it has been determined that salinity, temperature and conditional environmental effect the otolith chemistry (Secor *et al*., 1995; Hoff and Fuiman, 1995; Elsdon and Gillanders, 2004). It is thought that the differences between species in the inclusion of elements in our study may be partially related to environmental conditions, differences in metabolic and otolith deposition rate (Hamer and Jenkins, 2007).

This study is the first to examine the otolith chemistry and morphology of these two demersal species for Turkish waters and there is no study using this method for *M. merluccius* and *M. surmuletus* fish species in Turkey Seas. Therefore, no comparison could be made.

Only one study has been detected as research that examines both the morphological and analysis of the chemical structure of otoliths in Turkey Seas. Within the scope of the research, the horse mackerel (*Trachurus mediterraneus* Steindachner, 1868) was examined and a significant contribution was made to the separation of the stocks related to the species (Turan, 2006). Apart from this study, otolith Sr/Ca ratios were examined to determine the migration characteristics of the European eel (*Anguilla anguilla* Linnaeus, 1758) species in the Asi River (Lin *et al*., 2011). The average ratio of Sr/Ca in otoliths of *A. anguilla* taken from the Asi River was determined as 2.79×10^{-3} µm, which is approximately twice time (1.4×10^{-3} µm) the ratio of Sr/Ca in otliths of samples taken from the Garonne and Dordogne rivers (Lin *et al*., 2011). Thus, it was determined that the same fish species exist in different habitats by using otolith chemistry.

However, there are many studies examining otolith chemistry and morphology of two demersal fish species in different seas of the world (Torres *et al.,* 2000; *Lombarte et al*., 2003; Morales-Nin *et al.,* 2005; Swan *et al*., 2006; Leakey *et al.,* 2009; Mahe *et al*., 2014; Morales-Nin *et al.,* 2014; Bakkari *et al*., 2020).

In different study, it has been determined that Mg and Sr amounts in otoliths of fish samples (*M. merluccius)* taken from five different geographical locations (in north-east Atlantic and the Western Mediterranean Sea) is significantly different between the sample locations (Morales-Nin *et al*., 2005).

In another study on otolith chemistry was used to determine the relationship among populations of European hake; it was found that the ratio of Sr:Ca in the otolith core was higher than the otolith edge of the European hake. Also, the otolith edge had lower ratios for all elements except Mg (Morales-Nin *et al*., 2014). This situation shows that the habitat areas in which the juvenile stages, which are the first life stages of the fish, may differ from the adult stages. Another idiom on the otolith morphology of European hake was investigated for two different areas (North-eastern Atlantic-Cantabrian Sea and the Mediterranean Sea-Gulf of Lion). In the study, fish lengths are between 14 and 77 cm and 21-70 cm, and the average otolith area, otolith perimeter and otolith length of the samples in the North Atlantic were calculated as 80.15 mm^2, 102.01 mm, and 17.71 mm, respectively. In the Mediterranean, the average otolith area, otolith perimeter and otolith length were calculated as 102.78 mm^2, 118.8 mm, and 19.6 mm, respectively (Torres *et al.*, 2000). Another idiom conducted in the Mediterranean, trace elements of the European otoliths were examined.

All otoliths from *M. merluccius* separate areas differed not only in elemental concentrations but also in the distribution of elements among different individuals. For example, significant differences were found in the otolith concentrations between Mg, Mn and Sr among individuals in the Mediterranean region (Swan *et al*., 2006).

Although many factors such as the amount of accumulation of chemical trace elements, fish physiology, stress and genetics are effective, it may vary in proportion to environmental conditions, especially temperature and salinity (Reis Santos *et al*., 2013; Avigliano *et al*., 2014; Sarimin and Mohamed, 2014). The amount of strontium element increases positively depending on the salinity of the water (Kraus and Secor, 2004; Sturrock *et al*., 2012; Avigliano and Volpedo, 2013). However, barium is negatively affected (Miller, 2011; Avigliano *et al*., 2014). Calcium (Ca) is physiologically included in otoliths (Popper *et al*., 2005). However, magnesium increases depending on the metabolic activity and growth in the ecosystem where the fish is located (Martin and Thorrold, 2005; Sturrock *et al.,* 2015; Grammer *et al*., 2017). Also, strontium (Sr) and magnesium (Mg) are included in the otolith chemistry according to the natural abundance of trace elements carried by the fish habitat and chemical property of water (Farrell and Campana, 1996).

Additionally, environmental conditions affect the morphology of otoliths may differ between populations of the same species (Congiu et al., 2002). Determination of shape indices such as circularity and roundness by using the structural shapes and sizes of otoliths that change in a morphological sense and identification of species are secondary areas of use that help to determine the differences between stocks of the same species depending on geographical distance (Ponton, 2006; Campana and Casselman, 1993; Torres et al., 2000; Monteiro *et al*., 2005). In this study, the otolith morphology of two demersal fish species was investigated. Although samples from different regions cannot be taken and analyzed, in the present study findings are expected to form complementary data that will be a source for future scientific research on similar species. There are many studies examining the morphology of otoliths for descripting fish stocks in different parts of the world and in Seas of Turkey (Campana and Casselman, 1993; Campana *et al*., 1995; Monteiro *et al*., 2005; Özpiçak *et al*., 2017; Bal *et al.*, 2018). They made a significant contribution to the stock descriptions of the investigated species in these studies.

Since the same trace elements and the same morphological features were not examined in other studies, a comprehensive comparison could not be made with the present study.

CONCLUSION

The differences between the stocks of demersal species and their habitat areas are not yet well known. It is of great importance to define the habitats of these important fish species, which can be considered the basic stone of the marine ecosystem, the characteristics of their habitat areas and the stocks. Because; if the stocks and habitat areas of the species are not analyzed well, it is obvious that the extinction of the species will be endangered as these areas cannot be protected since stocks cannot be distinguished and defined.

However, samples could not be taken from different regions due to some limiting factors such as the costs required for laboratory analysis. To determine the differences between stocks in more detail, complementary studies are needed. It is thought that the findings obtained from this study will be a source for future research.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 523-525 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.14

SHORT COMMUNICATION

KISA MAKALE

Length-weight relationship and condition factor of three endemic fish species, *Ponticola bathybius*, *Neogobius caspius* and *Neogobius pallasi* (Perciformes: Gobiidae) from the Southern Caspian Sea basin, Iran

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Received date: 07.03.2021 *Accepted date: 24.08.2021*

How to cite this paper:
Nikmehr, N., Eagderi, S., Poorbagher, H. & Abbasi, K. (2021). Length-weight relationship and condition factor of three endemic fish species, *Ponticola bathybius*, *Neogobius caspius* and *N. pallasi* (Perciformes: Gobiidae) from the Southern Caspian Sea basin, Iran. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 523-525. DOI: 10.12714/egejfas.38.4.14

Abstract: In this study, the length-weight relationships (LWRs) and condition factors were estimated for 445 specimens belonging to three gobiid species *viz. Ponticola bathybius* (Kessler, 1877), *Neogobius caspius* (Eichwald, 1831) and *Neogobius pallasi* (Berg, 1916) from the Southern Caspian Sea basin. Total length and total weight of the examined specimens ranged 4.9-28.3 cm and 0.64-263.4 g, respectively. The length-weight parameter *b* of the studied species found between 2.47 (*N. caspius*) and 3.45 (*N. pallasi*) with r^2 ranging 0.831 to 0.988. LWR and K parameters are reported first time for *N. pallasi* from the Caspian Sea basin. The condition factor estimated was calculated from 0.94 (*N. pallasi*) to 0.98 (*N. caspius*) and 1.00 (*P. bathybius*). The basic biological information on the LWRs of these three gobies will be useful as baseline information for further biological research in the region.

Keywords: Gobiidae, goby, condition factor, Southern Caspian Sea basin

INTRODUCTION

Study of the length-weight relationship (LWR) is important in fisheries science as it provides valuable information on fish condition (Bagenal, 1978). It is applied to compare the interregional morphological variation of a single species' populations (Froese, 2006; Ali *et al.*, 2013), to estimate the weight of a specimen from its length and vice versa, to estimate the biomass, understanding the life cycle, evaluation of the fish stocks, ontogenetic changes and growth studies (Froese, 2006; Cherif *et al.,* 2008; Mouludi-Saleh and Eagderi, 2019; Eagderi et al., 2020a; Eagderi *et al.,* 2020b; Mouludi-Saleh *et al.*, 2021).

Condition factor (K) states condition of fish species *viz*. well-being of a certain species and its degree of fatness, the state of sexual maturity, the degree of food sources availability, and age and sex of some species and is significance for management and conservation of natural populations (Bagenal, 1978; Sarkar *et al.*, 2009; *Muchlisin et al.*, 2010).

In the Iranian coast of the Caspian Sea, 43 species of the family Gobiidae in 15 genera have been reported (*Esmaeili et al.*, 2018; Eagderi *et al.*, 2020c). The species members of the genus *Neogobius* is three (Froese and Pauly, 2008; Esmaeili *et al.,* 2018; Eagderi *et al.*, 2020c) and those of the genus *Ponticola* is six in the southern Caspian Sea (Eagderi *et al.*, 2020c). These genera are great importance due to their relatively large size (10 to 35 cm) (Berg, 1949; Rahimov, 1991). Therefore, in this study, LWRs and K are provided for *Ponticola bathybius*, *Neogobius caspius* and *N. pallasi* from the Southern Caspian Sea basin.

MATERIAL AND METHODS

A total of 165 specimens of *P. bathybius*, 102 *N. caspius* and 178 *N. pallasi* were collected between September 2012 and April 2019 using seine nets (mesh size: 4, 6 and 8 mm) from The Anzali Wetland, Turkman, Langrud, Chaboksar, Sefidroud and Lisar shores of the Southern Caspian Sea. All sampled fishes were anesthetized with a 1% clove oil solution and fixed with 10% buffered formalin then transferred to the laboratory for further studies. For each specimen, total length (*TL*) was measured with a digital caliper to the nearest 0.01 mm and body weight (*BW*) was recorded using a digital scale to the nearest 0.1 g.

The length-weight relationship was determined by the method of least squares using the equation of $W = aL^b$ and logarithmically transformed into Log (W) = Log (a) + b log (L) (Froese, 2006), where W is the total body weight (g), L is the total length (cm) and "a" is the intercept and "b" is the slope. Prior to regression analyses, log–log plots of the length–weight pairs were performed to identify outliers (Froese *et al.*, 2011). Outliers perceive in the log–log plots of all species were removed from the regression. The student's t-test (ts)

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was used to determine whether the parameter *b* is significantly different from the expected or theoretical value of 3. Fulton's condition factor (*K*) was estimated by the equation: $K = 100\times(W/TL^3)$ (Fulton, 1904), where *W* is the total body weight (g), L is the total length (cm) and scaling factor of 100 was used to fetch the K close to the unit factor. All statistical analyses were performed in Excel 2016 and PAST v 2.17b (Hammer *et al.*, 2001) software.

RESULTS AND DISCUSSION

The length-weight relationship (LWR**)** data of fish species is an important parameter in their dynamic population studies, playing a key role in fisheries assessments. In the current study, LWR parameters of 445 gobiid fishes belonging to three species were estimated. Total lengths and weight of *P. bathybius*, *N. caspius* and *N. pallasi* ranged 6.95-28.36 cm, 2.3-263.4 g and 8.20-13.20 cm, 7.0-21.6 g, 4.92-12.55 cm and 0.6-24.6 g, respectively. The results of LWR data of the three studied species, including values of the slope parameter (*b*), intercept (*a*), coefficient of determination (r^2) and 95% CL of *b*, *a* and condition factor (*K*) of the studied species are presented in Table 1.

The results showed *b*-values of the studied species ranging 2.47 (*N. caspius*) to 3.45 (*N. pallasi*) and coefficient of determination (r^2) 0.83 to 0.98, indicating the length increased with increasing weight of the fish (Konan *et al.*, 2007; *Tah et al.*, 2012; Koffi *et al.*, 2014; Cicek *et al.*, 2019). The *b*-value based on Froese, 2006 is between 2.5 and 3.5 or 2–4 (Tesch 1971), which all calculated *b*-values were within this expected ranges in this study.

Table1. Descriptive statistics and length-weight relationship parameters for three gobiid species from the Southern Caspian Sea basin 2012–2019

Species	n	Total length (cm)	Total weight (g)	LWR parameters					Condition factor (*K*)	Growth pattern	*P*	t
		min-max	min-max	*a*	*b*	r^2	95% CL of *b*	95% CL of *a*				
P. bathybius	165	6.95-28.36	2.3-263.4	0.003	3.32	0.988	3.28-3.35	0.002-0.0047	1.00±0.18	A^+	<0.05	38.78
N. caspius	102	8.20-13.20	7.0-21.6	0.032	2.47	0.831	2.25-2.70	0.013-0.026	0.98±0.12	A^-	<0.05	-75.81
N. pallasi	178	4.92-12.55	0.6-24.6	0.003	3.45	0.902	3.25-3.67	0.002-0.005	0.94±0.18	A^+	<0.05	59.48

N= number of individuals; Min= minimum; Max= maximum; a= intercept; b= slope; CL= confidence limits; r^2= coefficient of determination.

In LWRs, *b*-values which are higher and lower than 3 indicated positive and negative allometric growth patterns, respectively. Based on the results, allometric growth pattern was positive for *P. bathybius* and *N. pallasi,* and negative for *N. caspius*. Abdoli *et al.* (2009) estimated the *b*-value of *P. bathybius* as 2.44 lower than our result (3.32). *Aghajanpour et al.* (2016) and Mahdipour *et al.* (2017) in their study reported *b*-value for *N. caspius* 2.85-3.0 and 3.17 which is different with our study. LWR and *K* parameters have been reported for the first time for *N. pallasi* from the Caspian Sea basin. LWRs may change during the events of life cycle, growth, and onset of maturity, gut fullness, sampling techniques, and availability of food (Le Cren, 1951).

Fulton's condition factor (*K*) of the studied species ranging 0.94 to 1.00. Higher value of the condition factor indicates suitability of a specific water body for growth of fish (Mouludi-Saleh and Eagderi, 2019; Abbasi *et al.*, 2019). The condition factor fluctuating is based on the seasonal variations of the gonads and feeding intensity (Biswas, 1993). Finding of the present study will be helpful in biological studies and fisheries management.

ACKNOWLEDGEMENTS

This study is a product of project No. 97014470, financially supported by the Iran National Science Foundation and University of Tehran.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 38(4), 527-531 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.15

SHORT COMMUNICATION **KISA MAKALE**

Characterization of *Planococcus dechangensis* isolated from a water sample of Çamaltı Saltern

Çamaltı Tuzlasının su örneğinden izole edilen *Planococcus dechangensis*'in karakterizasyonu

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Received date: 30.04.2021 *Accepted date: 06.08.2021*

How to cite this paper:
Çağlayan, P. (2021). Characterization of Planococcus dechangensis isolated from a water sample of Çamaltı Saltern. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 527-531. DOI: 10.12714/egejfas.38.4.15

Abstract: In the present study, strain MHDS3 was isolated from a water sample of Çamaltı Saltern and identified using conventional and molecular methods. 16S rRNA gene sequence analyses showed that the strain MHDS3 belonged to *Planococcus dechangensis* species. It gave a positive result in the Gram staining test. The cells were coccus, non-motile, aerobic, catalase positive, oxidase negative and the colony pigmentation was yellow-orange. It showed negative results for citrate utilization, indole production from tryptophane, Voges-Proskauer and methyl red. This isolate was able to grow at 10-45°C (optimally 35°C), pH 6-8 (optimally pH 7) and 3-20% NaCl (optimally 10% NaCl). It was not able to grow at 4°C, 10°C, 50°C, salt-free, 0.5%, 25%, %30 total salt, pH 4-5, and pH 9-12. Glucose, ribose, fructose, sucrose, maltose were used by the test isolate as carbon sources. Different amino acids found in the structure of animal hide such as L-lysine, L-arginine, L-cysteine, L-alanine, L-tyrosine, L-histidine were also utilized by the bacterium. During the salt production process, this bacterium may contaminate the salt which is used in the food and leather industries. The activities of harmful moderately halophilic bacteria should be prevented by effective antimicrobial applications.

Keywords: Moderately halophilic bacterium, Çamaltı Saltern, salt production, *Planococcus dechangensis*

Öz: Bu çalışmada, MHDS3 suşu Çamaltı Tuzlasının su örneğinden izole edilmiştir ve geleneksel ve moleküler yöntemlerle tanımlanmıştır. 16S rRNA gen dizi analizleri bu suşun *Planococcus dechangensis* türü olduğunu göstermiştir. Gram pozitif, kok formunda, hareketsiz, aerobik, katalaz pozitif, oksidaz negatiftir ve sarı-turuncu koloni pigmentasyonu göstermiştir. Bu suş sitrat kullanımı, triptofanda indol üretimi, Voges-Proskauer ve metil kırmızısı testlerinde negatif sonuç vermiştir. Bu izolat 10-45°C'de (optimum 35°C), pH 6-8'de (optimum pH 7) ve %3-20 NaCl konsantrasyonunda (optimum %10 NaCl) gelişebilmektedir. 4°C, 10°C, 50°C'de, tuzsuz ortamda, %0.5, %25, %30 toplam tuz oranında, pH 4-5 ve pH 9-12'de gelişme göstermemiştir. Glukoz, riboz, fruktoz, sukroz, maltoz bu izolat tarafından karbon kaynağı olarak kullanılmıştır. Hayvan derisinin yapısında bulunan L-lizin, L-arjinin, L-sistein, L-alanin, L-tirozin, L-histidin gibi farklı amino asitler bu izolat tarafından da kullanılmıştır. Tuz üretimi aşamasında, bu bakteri gıda ve deri endüstrisinde kullanılmak üzere üretilen tuzu kontamine edebilir. Ilımlı halofil bakterilerin zararlı etkileri uygun antimikrobiyal uygulamalar ile engellenmelidir.

Anahtar kelimeler: Ilımlı halofil bakteri, Çamaltı Tuzlası, tuz üretimi, *Planococcus dechangensis*

INTRODUCTION

Moderately halophilic aerobic microorganisms can grow at 3-15% NaCl, 0-45ºC and pH 5-10. Salt tolerance and salt requirement vary among the different moderately halophilic species (Ventosa *et al.*, 1998). Moderately halophilic bacteria produce important metabolites such as compatible solutes, enzymes, exopolysaccharides, pigments, which have industrial and commercial values (Galinski and Louis, 1998; Ventosa *et al.*, 1998; Shivanand and Mugeraya, 2011). Compatible solvents such as ectoine, hydroxyectoine, glycine, proline, and betaine found in moderately halophilic bacterial cells, are also called organic intracellular solutes. They provide osmotic balance and prevent denaturation of proteins and enzymes caused by drying, heating and freezing in the cells (Imhoff, 1986; Galinski and Louis, 1998; Ventosa *et al.*, 1998). Compatible solvents have been used as stress-protective agents in medicine (Shivanand and Mugeraya, 2011). In molecular biology, new halophilic restriction endonuclease enzymes and also other halophilic enzymes have been discovered in moderately halophilic microorganisms that inhabit saline environments. The pigment carotenoids produced by orange or pink moderate halophile colonies have been used in the food industry as a food-colouring and as additives in health food products. These microorganisms may also have the potential for novel and diverse extracellular salt-adapted enzymes (*Ventosa et al.,* 1998, Rohban *et al.*, 2009; Ventosa *et al.*, 2011). In previous studies, protease, amylase, pullulanase, xylanase, DNase, inulinase, cellulase and lipase enzymes were produced by moderately halophilic bacteria isolated from saline environments (Sánchez-Porro *et al.*, 2003; *Rohban et al.,* 2009; Ventosa *et al.*, 2011; Akpolat *et al.*, 2015). These microbial enzymes have been applied for starch liquefaction

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in the paper, leather, pharmaceutical, food, meat, baking, dry cleaning, textile, and brewing industries (Ventosa *et al*., 1998; Gupta *et al*., 2016; Kami *et al.,* 2020). Due to moderately halophilic microorganisms can tolerate various pH, temperature and salt concentration, they have significant potential in harsh industrial applications. The researchers were isolated the *Planococcus* genus from different places such as sea, salted food, fermented food, marine beach (Hao and Komagata, 1985; Junge *et al.,* 1998; Engelhardt *et al.,* 2001; Romano *et al.*, 2003). *Planococcus* spp. produce proline, glycine-betaine at high salinities. The researchers applied rhamnolipid biosurfactant produced by two *Planococcus* species against pathogenic bacteria and they reported that it exhibited bactericidal properties (Gaur *et al*., 2020). Moreover, those *Planococcus* species which may be used in food industry were able to emulsify various vegetable oils (Gaur *et al.,* 2020). Thus, the investigation of industrially interesting compounds in moderately halophilic bacteria is important. The investigations with different moderately halophilic species as well as *Planococcus dechangensis* in several industrial applications are still promising. Hence, scientific researchers about their industrial applications should be carried out. Therefore, this study was aimed to characterize a moderately halophilic bacterial isolate (strain MHDS3) from a water sample of Çamaltı Saltern and to investigate its biochemical properties.

MATERIAL AND METHODS

The water sample collected from Çamaltı Saltern (38°29'25.6''N, 26°56'39.2''E) in Izmir (Turkey) was placed in a sterile plastic bottle, then the plastic bottle was placed in ice and immediately brought to the laboratory. 10 mL of water sample were added to a flask containing 90 mL 10% NaCl solution (10^{-1} dilution of water sample) was placed in a shaking incubator at 90 rpm and 24ºC for 2 hours. Aliquots of 100 µL direct and 10^{-1} dilution of water sample were separately spread onto the surface of the petri plate containing Complex Agar Medium (CAM) containing 5 g yeast extract. The final salt concentration of CAM was adjusted to 10% with the following composition: 0.0026% (w/v) NaBr, 0.96% (w/v) $MgSO_4$, 0.7% (w/v) $MgCl_2$, 8.1% (w/v) NaCl, 0.2% (w/v) KCl, 0.036% (w/v) $CaCl_2$ and 0.006% (w/v) $NaHCO_3$ (Ventosa *et al.,* 1989). The plates were incubated at 35°C for 24 h (Ventosa *et al.,* 1989). After the incubation period, yellow-orange bacterial colonies were restreaked several times to obtain pure cultures, then subjected to phenotypic and genotypic analysis.

QIAamp DNA Mini Kit (Qiagen) and QIAquick PCR Purification Kit (Qiagen) were respectively used for isolation of genomic DNA and PCR purification. The isolation and purification were conducted according to the manufacturer's instructions. 16S rRNA gene was amplified by Polymerase Chain Reaction using two primers: Reverse Primer 16R1488 (5'-CGGTTACCTTGTTAGGACTTCACC-3') and Forward Primer 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3') (Mellado *et al.*, 1995). The 16S rRNA gene sequence analysis (1194 bp) were determined by IONTEK Laboratory in Istanbul (Turkey). 16S rRNA gene sequence similarity (100%) between the test isolate and closely related species was detected by using ChromasPro Software (ChromasPro 2.1, Technelysium Pty Ltd, Australia) and the web-based EzTaxon-e program (Kim *et al.*, 2012). The gene sequence data of the strain MHDS3 were deposited in GenBank under accession number (MH748798). Gram reaction, morphology and motility of the exponentially growing cells were examined on wet mounts under the light microscope (Caglayan *et al.,* 2017). The growth of test bacterium at different salt percentages (0, 0.5%, 3%, 5%, 7.5%, 10%, 12.5%, 15%, 20%, 25%, %30), different pH (4, 5, 6, 7, 8, 9, 10, 11, 12), and different temperatures (4°C, 10°C, 20°C , 28°C, 32°C, 35°C, 37°C, 40°C, 45°C, 50°C) were investigated on CAM (Caglayan *et al.,* 2018). Catalase, oxidase, citrate utilization, indole production, Voges-Proskauer and methyl red tests were tested according to earlier described procedures (Johnson and Case, 2010; De la Haba *et al.*, 2011). Utilization of different amino acid sources (L-valine, L-ornithine, L-lysine, L-arginine, L-cysteine, L-alanine, L-tyrosine, L-histidine, L-threonine) by the isolate was investigated using 1% (w/v) amino acid, 0.05% (w/v) dextrose, 0.0005% (w/v) cresol red, 0.5% (w/v) beef extract, 0.5% (w/v) peptone, 0.001% (w/v) bromocresol purple, 0.0005% (w/v) pyridoxal in 10% saline water. A positive test result was indicated by purple colour in the test tube after 24-hour incubation. The test isolate was also investigated for its sugar requirement as the sole carbon source using seven different sugars (glucose, galactose, ribose, fructose, lactose, sucrose, maltose). 0.001% (w/v) phenol red, 0.5% (w/v) yeast extract and 1% (w/v) of each sugar source was used for the medium. The colour change from red to yellow was accepted as a positive result, indicating pH change to acidic (Johnson and Case, 2010, Caglayan et al., 2018).

RESULTS AND DISCUSSION

The 16S rRNA gene sequence (1194 bp) of strain MHDS3 showed 100% similarity with the corresponding gene sequence of the GenBank accession numbers for the 16S rRNA of the isolate is MH748798.

The results of Gram staining, cell morphology, motility, catalase, oxidase, citrate utilization, indole production, Voges-Proskauer, methyl red, minimum, optimum and maximum growth ranges for temperature, pH and total salt of the test bacterium were shown in Figure 1 and Table 1.

The cells were Gram-positive, coccoid, non-motile, catalase-positive, oxidase-negative. The pigmentation of the bacterial colony was yellow-orange. This isolate showed negative results for citrate utilization, indole production, Voges-Proskauer and methyl red tests. This isolate showed growth at 10-45°C (optimally 35°C), at pH 6-8 (optimally pH 7) and at 3-20% NaCl (optimally 10% NaCl). It did not show growth at 4°C, 10°C, 50°C, without salt, 0.5%, 25%, %30 total salt, pH 4-5, pH 9-12 (Table 1).

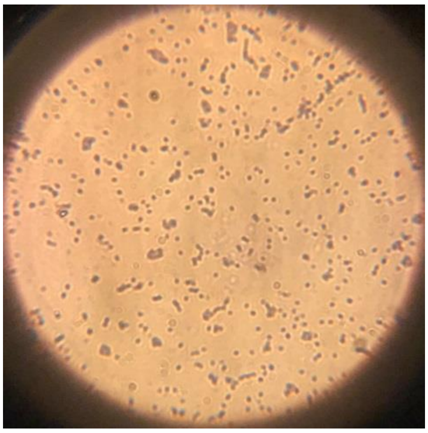

Figure 1. Gram stained cells of strain *Planococcus dechangensis* MHDS3 taken through light microscope

Table 1. Biochemical characteristics of moderately halophilic *Planococcus dechangensis*

Carbon sources				
			minimum	10
Glucose	+	Temperatures range (°C)	optimum	35
Galactose	-		maximum	45
Ribose	+		minimum	6
Fructose	+	pH range	optimum	7
Lactose	-		maximum	8
Sucrose	+		minimum	3
Maltose	+	Total salt range (%)	optimum	10
			maximum	20
Amino acids				
L-valine	-	Gram staining		positive
L-ornithine	-	Cell morphology		cocci
L-lysine	+	Pigmentation		yellow-orange
L-arginine	+	Motility		-
L-cysteine	+	Catalase		+
L-alanine	+	Oxidase		-
L-tyrosine	+	Citrate Utilization		-
L-histidine	+	Indole production from tryptophane		-
L-threonine	-	Voges-Proskauer test		-
		Methyl red test		-

Acid is produced from glucose, ribose, fructose, sucrose and maltose, but not from galactose and lactose (Table 1). The test isolates utilized 71% of the tested sugar sources. While the isolate utilized L-lysine, L-arginine, L-cysteine, L-alanine, L-tyrosine, L-histidine, it did not use L-valine, L-ornithine, L-threonine (Table 1). The skin structure contains L-lysine (%2.9), L-arginine (4.9%), L-cysteine (0.1%), L-alanine (10.9%), L-tyrosine (0.3%), and L-histidine (%0.5) (Szpak, 2011). *Planococcus dechangensis* strain NEAU-ST10-9T was first isolated from saline and alkaline soils in Dechang Township in China and identified by Wang *et al.* (2015). In that study, the growth of the test bacterium was investigated at different salt percentages (0, 1%, 2%, 3%, 4%, 6%, 7%, 8%, 10%, 15%, 17%, 20%), different pH (3-11), and different temperatures (0°C, 4°C, 28°C, 30°C, 32°C, 35°C, 37°C, 40°C, 42°C, 50°C, 60°C). The strain was reported as a moderately halophilic bacterium and was able to grow at 2-10% NaCl concentrations (optimal 4% NaCl), at 4-50°C (optimal 30°C) and pH 6-10 (optimal pH 7) (Wang *et al.*, 2015). In the present study, while *Planococcus dechangensis* strain MHDS3 was optimally able to grow at 10% NaCl concentration, pH 7, and 35°C, it showed growth between 3% and 20% NaCl, pH 6-8, 10°C-45°C ranges (Table 1). The strain *Planococcus dechangensis* strain MHDS3 was able to grow narrow ranges of pH and temperature than the strain *Planococcus dechangensis* strain NEAU-ST10-9T. Both, strain NEAU-ST10-9T (Wang *et al.*, 2015) and strain MHDS3 in the present study were able to use glucose and fructose. Although strain MHDS3 produced acid from ribose, strain NEAU-ST10-9T did not. Bacterial and archaeal populations of Çamaltı Saltern were investigated several times (Yaşa *et al.*, 2008; Poli *et al.*, 2012; Mutlu and Guven, 2009; Guven *et al.*, 2010; Mutlu and Guven, 2011; Erdogmus *et al.*, 2013; Mutlu and Guven, 2015). Extremely halophilic archaeal species *Haloferax alexandrinus* and *Halobacterium salinarum* were isolated from soil samples obtained from Çamaltı Saltern (Yaşa *et al.*, 2008). Enzyme producing bacterial and archaeal species were isolated from water samples in Çamaltı Saltern by Guven *et al.* (2010). Mutlu and Guven (2011) investigated microbial community in Çamaltı Saltern using FISH and Real-Time PCR. They reported that the samples contained approximately 10^7 cells in one mL belonging to *Bacteria* and *Archaea* domains. Poli *et al.* (2012) have isolated a new moderately halophilic bacterium phylogenetically related to *Halomonas smyrnensis*, which was optimally grown at 10% NaCl (w/v). *Haloferax* sp., *Halorubrum* sp., *Halobacterium* sp. and *Haloarcula* sp. found in Çamaltı Saltern were stated as aromatic hydrocarbon-degrading *Archaea* by Erdogmus *et al.* (2013). In the study of Mutlu and Guven (2015), 17 different bacterial isolates were found to be phylogenetically related to *Halobacillus, Virgibacillus, Salinibacter* and *Halomonas* genera. Halotolerant *Bacillus licheniformis* was isolated from crude salt samples of Çamaltı Saltern by the researchers (Kırtel *et al.*, 2021).

In Çamaltı Saltern, solar salt is produced by solar evaporation. In this method, the seawater of Aegean Sea is pumped into crystallizer multiponds (the salinity is about 0.35%) in spring. After the evaporation, the final salinity is measured approximately 26.5% in shallow ponds. Under supersaturation levels, salt begins to crystallize (Guven *et al.*, 2010; Mutlu and Guven, 2015). It is known that moderately halophilic bacteria are able to grow at a wide range of salt concentrations (Ventosa *et al.*, 1998). Therefore, moderately halophilic bacteria found in crystallizer multiponds, can also grow at the final solar salt produced in Çamaltı Saltern.

CONCLUSION

This is the first study that *Planococcus dechangensis*, a moderately halophilic bacterium, was isolated from Çamaltı Saltern's water sample. This isolate may produce industrially important enzymes, compatible solutes, other compounds which may be active and stable under saline conditions. These biomolecules should be detected and their biotechnological properties should be determined.

The bioactive compounds produced by moderately halophilic bacteria will be suitable for application in various industrial processes where conditions are saline.

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Ege Journal of Fisheries and Aquatic Sciences, 38(4), 533-544 (2021)

Su Ürünleri Dergisi (2021)
DOI: 10.12714/egejfas.38.4.16

OLGU SUNUMU **CASE REPORT**

Türkiye balıkçılık ve su ürünleri yetiştiriciliği sektöründeki iş kazalarının istatistikleri üzerine bir analiz; 2013-2019 dönemi

An analysis on the statistics of occupational accidents in Turkish fisheries and aquaculture sector; 2013-2019 period

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Received date: 28.02.2021 *Accepted date: 22.08.2021*

How to cite this paper:
Soykan, O. (2021). An analysis on the statistics of occupational accidents in Turkish fisheries and aquaculture sector; 2013-2019 period. *Ege Journal of Fisheries and Aquatic Sciences,* 38(4), 533-544. DOI: 10.12714/egejfas.38.4.16

Öz: Bu çalışma Türkiye'deki balıkçılık ve su ürünleri yetiştiriciliği sektörünü iş sağlığı ve güvenliği istatistikleri açısından bütünsel ve paydaşları özelinde değerlendirmek ve Türkiye'deki iş sağlığı ve güvenliği istatistikleri ile kıyaslayarak sektörünün durumunu ortaya koymak amacıyla gerçekleştirilmiştir. Balıkçılık ve su ürünleri yetiştiriciliği sektöründe yıllık ortalama 312 (±147,6) kaza olduğu, bunların 2,14 (±1,57)'ünün sürekli iş göremezlik ve 1,57 (±1,51)'sinin ölümle sonuçlandığı tespit edilmiştir. Türkiye'deki genel kaza sıklığı, ölümlü kaza sıklığı, sürekli iş göremezlik kaza sıklığı ve iş kazası sıklık hızı sırasıyla 2211,8 (±574) 103,6 (±13,4), 5,88 (±2,41) ve 8,36 (±1,93) olarak hesaplanırken balıkçılık ve su ürünleri yetiştiriciliğinde bu değerler sırasıyla 3579,1 (±1549,3) 175,65 (±152,34), 240,67 (154,62) ve 13,65 (±5,84) olarak bulunmuştur. 2019 yılında Türkiye'deki ölümlü kaza oranı genel olarak %0,27 bulunmuş, maden sektöründe %0,34, inşaatta %0,77, nakliyede %0,80 olarak tespit edilmiştir. Balıkçılık ve su ürünleri yetiştiriciliğinde ise bu oran %1 olarak hesaplanmıştır. Denizlerde yapılan avcılık ve yetiştiricilik faaliyetlerinde tatlı sulardakilere göre daha fazla iş kazası ve ölümlü kaza olduğu tespit edilmiştir. Denizlerde yapılan avcılık ve yetiştiricilik faaliyetleri ile tatlı sularda yapılan avcılık faaliyetlerinde yaşanan iş kazası sayısı ve iş kazası sonucu geçici iş göremezlik süreleri arasındaki farkın da önemli olduğu saptanmıştır (p<0,05). Yetiştiricilik sektöründe meydana gelen ölümlü iş kazası sayısının ise avcılığın 4 katından fazla olduğu bulunmuştur. Balıkçılık ve su ürünleri yetiştiriciliğinin tahmin edilenden çok daha riskli bir sektör olduğu ortaya konmuştur. Mevcut durumun iyileştirilmesi için birtakım değerlendirme ve öneriler ortaya konsada, sistemsel ve gücünü mevzuattan alan bir yaklaşımın daha efektif olacağı düşünülmektedir. Bu yüzden, balıkçılık ve su ürünleri yetiştiriciliği ekonomik faaliyet koluna ait tüm alt çalışma alanlarına ait tehlike sınıflarının "çok tehlikeli" sınıfa yükseltilmesi uygun bir yaklaşım olacaktır. Bu sayede sektör çalışanlarının mesleki riskler konusunda aldıkları eğitim süresi artacak, sektörde çalışan İSG profesyonellerinin unvan ve sayıları artarak daha nitelikli bir uzmanlaşma gerçekleşecek, kontrol ve denetim faaliyetlerinin daha sıklaşması sağlanmış olacaktır.

Anahtar kelimeler: İş kazası, iş güvenliği, balıkçılık, yetiştiricilik

Abstract: This study was carried out to make a holistic evaluation on the occupational health and safety (OHS) of fisheries and aquaculture sector with its subsectors and to reveal the actual situation of fisheries and aquaculture sector by comparing relevant OHS statistics reported in Turkey. Annual average of accidents in the fisheries and aquaculture sector was found to be 312 (± 147.6), of which 2.14 (± 1.57) resulted in permanent incapacity and 1.57 (± 1.51) of them with fatality. While general incidence rate, fatal incidence rate, permanent incapacity incidence rate and work accident frequency rate were calculated as 2211.8 (±574) 103.6 (±13.4), 5.88 (±2.41) ve 8.36 (±1.93), these values for fisheries and aquaculture sector were determined to be 3579.1 (±1549.3) 175.65 (±152.34), 240.67 (154.62) ve 13.65 (±5.84), respectively. In 2019, the fatal accident rate in Turkey was found to be 0.27% in general, 0.34% in the mining sector, 0.77% in construction and 0.80% in transportation. This ratio was calculated as 1% in the fisheries and aquaculture sector. It was determined that there were more work accidents and fatal incidents in fisheries and aquaculture activities in the seas than in fresh water. Difference between number of work accidents and the duration of temporary incapacity as a result of work accidents in fisheries and aquaculture activities in marine and fresh waters were also significant (p<0.05). Number of fatal accidents in aquaculture sector was found to be more than 4 times than in fisheries. It was determined that the all types of incident rates, work accident frequency rates and fatality rates per work accident in fisheries and aquaculture sector were higher than that of Turkey in general. Evaluation of occupational accidents in the fisheries and aquaculture activity was found to be riskier than expected. Although some evaluations and suggestions are put forward to reduce the number of accidents and the severity of these accidents, a systematic and legislative approach will be more effective. Therefore, it would be a suitable approach to raise the hazard classes of fisheries and aquaculture economic activity to the "very dangerous" class. In this way, the training period of the sector employees on occupational risks and the titles and numbers of OHS professionals working in the sector will be increased, a more qualified specialization will be realized, and control and audit activities will be more frequent.

Keywords: Work accident, occupational safety, fisheries, aquaculture

GİRİŞ

Günümüzde iş sağlığı ve güvenliği insan odaklı yaklaşımın bir sonucu olarak sürekli gelişen, ilerleyen, dinamik ve çok disiplinli bir bilim dalı olarak ifade edilmektedir. İş sağlığı ve güvenliği uygulamalarının temel amaçları tüm sektörlerdeki çalışanları proaktif yaklaşım ile iş kazalarından ve meslek hastalıklarından korumak, işletmenin güvenliğini sağlamak, üretim ve kaliteyi arttırmaktır. Tüm dünyada olduğu Türkiye'de de iş kazaları ve buna bağlı olarak ölüm sayılarının en yüksek olduğu ekonomik faaliyetler istihdamın da fazla olmasıyla maden, inşaat, metal ve nakliye sektörlerine aittir

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(Goetsch, 2010; Bayraktar vd., 2018). Her mesleğin veya sektörün kendine özgü çalışma koşulları olsa da iş sağlığı ve güvenliği kapsamındaki çalışmalar farklı üretim proseslerine sahip sektörleri de kapsamakta ve bu sektörlerin sorunlarına çözüm üretebilmektedir. Kendine özgü çalışma koşullarına sahip olan sektörlerden biri de balıkçılık ve su ürünleri yetiştiriciliğidir. Balıkçılık ve su ürünleri yetiştiriciliği dünyadaki en eski sektörlerden biri olmakla beraber köklü geleneklere de sahiptir (Soykan, 2018). 2018 yılında dünya su ürünleri üretimi 179 milyon tona ve buna karşılık gelen 401 milyar dolarlık ekonomik büyüklüğe ulaşmıştır (FAO, 2020). Dünya genelinde 58 milyon kişi balıkçılık ve akuakültür sektöründe çalışmakta, bunlardan 38 milyonu ise avcılık sektöründe görev yapmaktadır (ILO, 2018). Türkiye'de 2019 yılında 836 bin 524 ton su ürünleri üretimi gerçekleştirilmiştir. Üretimin %44,8'ini avcılık yoluyla elde edilen deniz balıkları, %6,8'ini avcılık yoluyla elde edilen diğer deniz ürünleri, %3,8'ini avcılık yoluyla elde edilen iç su ürünleri ve %44,6'sını yetiştiricilik ürünleri oluşturmuştur. Türkiye'nin su ürünleri ihracat miktarı 1 milyar doları geçmiştir (TÜİK, 2020).

Türkiye'de balıkçılık sektörünü üç ana grupta ele almak mümkündür. Bunlar; avcılık, yetiştiricilik ve işleme sektörleridir. Bu alt sektörlere ait resmi istihdam sayısı yalnızca avcılık sektörü için mevcut olup, 2019 yılı için bildirilen rakam 28717'dir (TÜİK, 2020). Buna ek olarak, Türkiye'de yetiştiricilik sektöründeki çalışan sayısı konusunda resmi veri olmamakla birlikte üretim, işleme, pazarlama, alet ve ekipman temini, balık yemi üretimi ve bu sektörle ilişkili işyerlerinde yaklaşık 25.000 kişinin çalışmakta olduğu bildirilmiştir (Aydın, 2016; Gülşahin vd., 2020). Balıkçılık dünyanın birçok ülkesinde en tehlikeli ve riskli mesleklerin başında gelmektedir (ILO, 2010). Buna karşın balıkçılık ve su ürünleri yetiştiriciliği, Türkiye'de, 26.12.2012 tarihinde yayınlanarak yürürlüğe giren "İş Sağlığı ve Güvenliğine İlişkin Tehlike Sınıfları Tebliği"ne göre tehlikeli işler kategorisinde bulunmaktadır.

Geçmişten günümüze Türkiye'de iş sağlığı ve güvenliği konusunda yaşanan en önemli gelişme şüphesiz ki kavramın bir kanun haline geldiği 2012 yılında olmuştur. Bu yılda yürürlüğe giren 6331 sayılı "İş Sağlığı Ve Güvenliği Kanunu" balıkçılık sektörü de dâhil olma üzere, kamu ve özel sektöre ait bütün işlere ve işyerlerine, bu işyerlerinin işverenleri ile işveren vekillerine, çırak ve stajyerler de dâhil olmak üzere tüm çalışanlarına faaliyet konularına bakılmaksızın uygulanmaktadır. Buna ilaveten kamu kurumlarında çalışan memurlar ile 50'den az çalışanı olan az tehlikeli işletmeler için kanunun yürürlüğe girme tarihi 31 Aralık 2023'e ertelenmiştir. Ayrıca bazı çalışan grupları (MİT personeli, TSK personeli, Sivil savunma personeli vd.) bu kanunun istisnaları arasında yer almaktadır. Bu kanun kapsamına dâhil edilen her türlü olay ve/veya süreç (iş kazası, meslek hastalığı, vb.) T.C Sosyal Güvenlik Kurumu tarafından kayıt altına alınmaktadır. İş sağlığı ve Güvenliği Kanunu'nun yürürlüğe girmesinin ardından bu kanunun farklı sektörler üzerindeki etkisini ortaya koyan ve bu kanun sonucu oluşan farkındalığın birçok sektör üzerindeki yansımalarını konu alan pek çok ulusal ve uluslararası bilimsel çalışma üretilmiştir. Madencilik sektöründeki iş kazalarının analizi (Bayraktar vd., 2018), tekstil sektöründeki istihdam-iş kazası ilişkisi (Güllüoğlu ve Taçgın, 2018), Orman (Akyüz vd., 2016) ve inşaat (Ceylan, 2014) sektörlerindeki iş kazası istatistikleri Türkiye'de yapılan önemli bilimsel çalışmalardandır. Balıkçılık ve yetiştiricilik alanında uluslararası birçok çalışma varken (Kaplan ve Kite-Powell, 2000; Myers ve Durborov, 2012; Pfeiffer ve Gratz, 2016; Watterson, 2018), balıkçılık ve su ürünleri sektöründeki iş sağlığı ve güvenliğini konu alan ulusal ölçekteki bilimsel makale ve proje sayısı azdır (Mert ve Ercan, 2014; Akyol vd., 2016; Ulukan, 2016; Doğanyılmaz Özbilgin ve Tok, 2017; Soykan, 2018; Köken vd., 2018; Gülşahin vd., 2020; Aydoğan, 2020).

Bu çalışmanın amacı balıkçılık sektörünü iş sağlığı ve güvenliği istatistikleri açısından bütünsel ve paydaşları özelinde değerlendirmek ve Türkiye'deki iş sağlığı ve güvenliği istatistikleri ile kıyaslayarak balıkçılık sektörünün gerçek durumunu ortaya koymaktır. Ayrıca balıkçılıktaki iş sağlığı ve güvenliği kavramının geliştirilmesi ve konunun öneminin anlaşılabilmesi için bilimsel temelli katkı ve eleştirilerde bulunulmuştur. Bu çalışma ile Türkiye balıkçılık sektöründeki iş sağlığı ve güvenliği konusuna bilimsel katkı yapılarak, ulusal literatürü zenginleştirmek ve gelecekte yapılacak kapsamlı çalışmalara önemli bir altyapı oluşturmak amaçlanmıştır.

MATERYAL VE YÖNTEM

Bu çalışmanın veri setini T.C. Sosyal Güvenlik Kurumu (SGK) istatistik yıllıklarına ait "iş kazası ve meslek hastalığı" ve "sigortalı ve işyeri istatistikleri" oluşturmaktadır. SGK istatistik yıllıklarında iş sağlığı ve güvenliği alanındaki veriler 2007 yılından itibaren verilmektedir. Bu çalışmada, iş sağlığı ve güvenliği kanununun yürürlüğe girdiği 2012 yılından sonraki veriler (2013-2019) incelenmiştir. Türkiye'de balıkçılık ve su ürünleri yetiştiriciliği "İş Sağlığı ve Güvenliğine İlişkin İşyeri Tehlike Sınıfları Tebliği"ne göre deniz balıkçılığı, tatlı su balıkçılığı, deniz ürünleri yetiştiriciliği ve tatlı su ürünleri yetiştiriciliği olmak üzere 4 başlık altında toplanmıştır. Bu nedenle çalışmada iş kazası ve meslek hastalıklarına ait istatistikler bu başlıklar altında incelenmiştir. Balıkçılık ve su ürünleri yetiştiriciliğindeki iş kazaları, meslek hastalıkları, ölüm sayıları, geçici iş göremezlik süreleri ve sürekli iş göremezlik sayısı değerlendirmeye alınmıştır. Balıkçılık ve su ürünleri yetiştiriciliğindeki çalışan sayıları SGK'nın yayınladığı sigortalı ve işyeri istatistiklerinden alınmıştır. Bu veri seti içerisinde balıkçılık ve su ürünleri yetiştiriciliğinde çalışan sayıları alt sektörlere göre sınıflandırılmadığından istihdam sayısı tüm sektörü kapsayacak şekilde verilmiştir. Türkiye Cumhuriyeti Aile, Çalışma ve Sosyal Hizmetler Bakanlığı'nın 2017 ve sonrasında çalışanlarla ilgili verileri 4a ve 4b olarak sınıflaması nedeniyle 2017, 2018 ve 2019 yılına ait veriler bu iki grubun toplamını içermektedir.

İş kazaları ile ilgili karşılaştırmalarda, uluslararası geçerliliği olan çeşitli kıyaslama ölçütleri kullanılmakta, bunlardan ulusal ve uluslararası mevzuatta en yaygın olanları genel kaza sıklığı, sürekli iş görmezlik kaza sıklığı, ölümlü kaza sıklığı, standardize iş kazası oranı ve iş kazası sıklık hızıdır (Eurostat, 2013; SGK, 2016). Bu çalışmada, balıkçılık ve su ürünleri sektöründe meydana gelen kazaların büyüklüğünü değerlendirebilmek için Türkiye çapında faaliyet gösteren işkolu ve sektörlerin toplamı ile balıkçılık ve su ürünleri yetiştiriciliği sektöründe meydana gelen iş kazalarına ait istatistiksel veriler bu parametreler aracılığıyla hesaplanmış ve karşılaştırmalı olarak sunulmuştur. Bunlara ek olarak iş kazası başına düşen ölüm oranı da verilmiştir.

Genel iş kazası sıklığı (GKS): Bir yılda 100.000 çalışan başına düşen iş kazası sayısıdır ve aşağıdaki formülle hesaplanmaktadır;

GKS= $\frac{KSx100000}{ÇİS}$

KS: Kaza sayısı, ÇİS: Çalışan işçi sayısı kavramlarını ifade etmektedir.

Sürekli iş görmezlik kaza sıklığı (SİGKS): Bir yılda 1.000.000 çalışan başına düşen sürekli iş göremezlik sayısı olup formülü aşağıda verilmiştir.

SİGKS= $\frac{SİGSx1000000}{ÇİS}$

SİGS: Sürekli iş göremezlik sayısı, ÇİS: Çalışan işçi sayısı kavramlarını ifade etmektedir.

Ölümlü iş kazası sıklığı (ÖKS): Bir yılda 1.000.000 çalışan başına düşen ölüm sayısıdır. Formülü aşağıda verilmiştir:

ÖKS= $\frac{ÖSx1000000}{ÇİS}$

Formülde verilen ÖS: Ölüm sayısı, ÇİS: Çalışan işçi sayısıdır.

Standardize iş kazası oranı (SİKO): Bir yıl içerisinde, incelenen faaliyet kolunda meydana gelen iş kazası sayısının o faaliyet kolundaki beklenen kaza sayısına oranının yüzdesel ifadesidir. Diğer bir ifade ile ilgili sektörde bir yılda çalışan sigortalı sayısı ve meydana gelen iş kazası sayısına göre hesaplanan sıklık hızının, ülke için o yıl beklenen iş kazası sıklık hızına göre artış oranını ifade etmektedir. SİKO herhangi bir sektörün bir yıl içindeki performansını genel kaza sıklığını baz alarak Türkiye geneli ile karşılaştırmak için kullanılan bir parametredir. Bu çalışmada Türkiye için her yıl referans alınan SİKO değeri diğer birçok çalışmada (Güllüoğlu ve Güllüoğlu, 2019; Güllüoğlu ve Taçgın, 2018) verildiği gibi %100 olarak alınmıştır. SİKO oranı hesabı için kullanılan bu formüller aşağıda verilmiştir (Karadağ, 2010);

SIKO (%)= (Yılda faaliyet kolundaki iş kazası sayısı x 100) / Beklenen iş kazası sayısı

Beklenen iş kazası sayısı = Genel iş kazası hızı x İş kolundaki zorunlu sigortalı sayısı

Genel iş kazası hızı = Kaydedilen toplam iş kazası sayısı / Toplam sigortalı sayısı

İş kazası sıklık hızı (İKSH): Bir takvim yılı içerisinde ölümlü ya da ölümlü olmayan iş kazası sayısının incelenecek sektör içinde yer alan işçilerin çalışma saatine bölünmesi ile elde edilen orandır. Farklı hesaplama yöntemleri olmasına karşın, ülkeler ve sektörler arasında karşılaştırma yapmaya olanak sağladığı için en fazla tercih edilen parametredir (Akyüz vd., 2016). İKSH genel olarak iki farklı yöntemle hesaplanır. Birinci yöntem ($İKSH_1$); bir yıl içerisinde çalışılan her 1.000.000 iş saatine karşılık gelen kaza sayısı olarak tanımlanmaktadır.

$İKSH_1$= İKS / (PTEGS x 8) x 1.000.000

İKS: İş kazası geçiren sigortalı sayısı,

PTEGS: Toplam prim tahakkuk eden gün sayısı (PTEGS, her gün için 8 saatlik tam çalışma ile çarpılarak tüm sigortalıların bir yıl içinde toplam çalışma saati bulunur), olarak ifade edilmektedir.

PTEGS, SGK istatistik yıllıklarında her bir faaliyet kolu için ayrı ayrı verilmemektedir. Balıkçılık ve su ürünleri yetiştiriciliği için toplam prim tahakkuk eden gün sayısını hesaplanırken Türkiye genelinde ilgili yılda çalışan başına düşen ortalama prim tahakkuk eden gün sayısı bulunmuş ve Balıkçılık ve su ürünleri yetiştiriciliğinde çalışan sigortalı sayısıyla çarpılmıştır (Akyüz vd., 2016).

İkinci yöntem ($İKSH_2$); tam gün çalışan her 100 kişi arasında kaç kaza olduğunu ifade etmekte ve aşağıdaki formülle hesaplanmaktadır:

$İKSH_2$= İKS / (PTEGS x 8) x 225.000

Formülde 100 sigortalının haftada 45 saat, yılda 50 hafta çalıştığı kabul edilmektedir (SGK, 2016).

İş kazası başına düşen ölüm oranı (İKBDÖO): Bir yıl içerisinde, incelenen faaliyet kolunda meydana gelen ölümlü iş kazası sayısının o faaliyet kolundaki toplam iş kazası sayısına oranının yüzdesel ifadesidir.

İKBDÖO= $\frac{\text{Sektördeki ölümlü iş kazası} * 100}{\text{Sektördeki toplam iş kazası}}$

Balıkçılık ve su ürünleri yetiştiriciliğindeki iş kazaları, ölümlü iş kazası sayıları, iş kazası istatistikleri, iş kazası sıklık değerleri ve sıklık hızları MS Office Excel programında değerlendirilmiş, bu verilere ilişkin ortalama (ort), varyans (var) ve standart sapma (std.sap) değerleri hesaplanmıştır. Deniz balıkçılığı-deniz yetiştiriciliği, deniz balıkçılığı-tatlı su balıkçılığı, deniz yetiştiriciliği-tatlı su yetiştiriciliği ve deniz-tatlı su çalışmalarındaki (avcılık ve yetiştiricilik toplam olmak üzere) kaza sayıları ve iş kazası sonrası geçici iş göremezlik süreleri arasındaki farklar p=0,05 önemlilik derecesinde non parametrik Mann Whitney U testi ile Statistica 12.0 paket programında test edilmiştir.

BULGULAR

Türkiye'de balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolu, İş Sağlığı ve Güvenliğine İlişkin İşyeri Tehlike Sınıfları Tebliği'nde deniz balıkçılığı, tatlı su balıkçılığı, deniz ürünleri yetiştiriciliği ve tatlı su ürünleri yetiştiriciliği olarak 4 ana grupta incelenmektedir. Bu alt faaliyetler "az tehlikeli", "tehlikeli" ve "çok tehlikeli" olarak ilgili tebliğde sınıflandırılmıştır (Tablo 1). Buna karşın Sosyal güvenlik kurumu (SGK)'nun kuruluşundan önce var olan Sosyal Sigortalar Kurumu (SSK), balıkçılık ve balıkçılığın içerdiği tüm alt sektörleri "Balıkçılık" başlığı altında toplamıştır.

Tablo 1. İş Sağlığı ve Güvenliğine İlişkin İşyeri Tehlike Sınıfları Tebliği'ne göre Türkiye'de balıkçılık ve su ürünleri yetiştiriciliğine ait faaliyet kolları ve tehlike sınıfları (T.C. Resmi Gazete, 28509, 26.12.2012)

Table 1. Economic avtivity codes and danger classes of subsectors belonging to fisheries and aquaculture sector in Turkey according to danger class communiqué of occupational health and safety (Republic of Turkey, Official Newspaper 28509, 26.12.2012)

NACE Rev.2_Altılı Kod	NACE Rev.2_Altılı Tanım	Tehlike Sınıfı
03	**Balıkçılık ve su ürünleri yetiştiriciliği**	
03.1	**Balıkçılık**	
03.11	**Deniz balıkçılığı**	
03.11.01	Deniz ve kıyı sularında yapılan balıkçılık (gırgır balıkçılığı, dalyancılık dahil)	Tehlikeli
03.11.02	Deniz kabuklularının (midye, ıstakoz vb.), yumuşakçaların, diğer deniz canlıları ve ürünlerinin toplanması (sedef, doğal inci, sünger, mercan, deniz yosunu, vb.)	Çok Tehlikeli
03.12	**Tatlı su balıkçılığı**	
03.12.01	Tatlı sularda (ırmak, göl) yapılan balıkçılık (alabalık, sazan, yayın vb.)	Tehlikeli
03.2	**Su ürünleri yetiştiriciliği**	
03.21	**Deniz ürünleri yetiştiriciliği**	
03.21.01	Denizde yapılan balık yetiştiriciliği (çipura, karagöz, kefal vb. yetiştiriciliği ile kültür balığı, balık yumurtası ve yavrusu dahil)	Tehlikeli
03.21.02	Denizde yapılan diğer su ürünleri yetiştiriciliği (midye, istiridye, ıstakoz, karides, eklembacaklılar, kabuklular, deniz yosunları vb.) (balık hariç)	Tehlikeli
03.22	**Tatlı su ürünleri yetiştiriciliği**	
(Değişik: RG-31/1/2018-30318) 03.22.01	Tatlı sularda yapılan balık yetiştiriciliği (süs balığı, kültür balığı, balık yumurtası ve yavrusu dahil)	Az Tehlikeli
03.22.02	Tatlısu ürünleri yetiştiriciliği (yumuşakçalar, kabuklular, kurbağalar vb.) (balık hariç)	Tehlikeli

Türkiye'de 4a (hizmet akdine bağlı çalışanlar) ve 4b (kendi adına ve hesabına bağımsız çalışanlar) statüsünde çalışan sigortalılardan 2019 yılında 422837 iş kazası ve 1091 meslek hastalığı bildirilmiş olup balıkçılık ve su ürünleri yetiştiriciliğinde 375 iş kazası meydana gelmiş buna karşın hiç meslek hastalığı rapor edilmemiştir. Türkiye'de 2019 yılında yaşanan toplam iş kazalarının 1147 tanesi ölümle sonuçlanırken, ölümlü kaza oranı %0,27 olarak tespit edilmiştir. Bu oran maden (kömür ve linyit çıkarılması (05.10.01), metal cevheri madenciliği (07.10.01, 07.21.03, 07.21.04, 07.29.01-06), diğer madencilik ve taş ocakçılığı (08.11.01-07, 08.12.01-03, 08.91.01-05, 08.92.01, 08.93.01-02, 08.99.01-90), madenciliği destekleyici hizmet faaliyetleri (09.10.01-03)) inşaat (bina inşaatı (41.10.01-03, 41.20.01-05), bina dışı yapıların inşaatı (42.11.01-03, 42.12.01, 42.13.01-02, 42.21.01-05, 42.22.01-07, 42.91.01-04, 42.99.01-04), özel inşaat faaliyetleri (43.11.01, 43.12.01-02, 43.13.01, 43.21.01-03, 43.22.03-07, 43.29.01-05, 43.32.01-03, 43.33.01-02, 43.34.01-03, 43.39.01-02, 43.91.01, 43.99.01-15)) ve nakliye (kara taşımacılığı ve boru hattı taşımacılığı (49.10.01, 49.20.01, 49.31.01-90, 49.32.01-02, 49.39.01-90, 49.41.01-90, 49.42.01, 49.50.01-90), su yolu taşımacılığı (50.10.12-90, 50.20.17-91, 50.30.08-09, 50.40.05-08), hava yolu taşımacılığı (51.10.01-03, 51.21.17, 51.22.02), taşımacılık için depolama ve destekleyici faaliyetler (52.10.02-90, 52.21.04-90, 52.22.06-90, 52.23.03-90, 52.24.08-11, 52.29.01-90)) sektörleri (parantez içinde NACE 2 kodları ile verilen tüm ilgili faaliyet kolları dâhil olmak üzere) için sırasıyla % 0,34, % 0,77 ve % 0,8 olarak hesaplanmıştır. Aynı yıl içinde balıkçılık ve su ürünleri yetiştiriciliği sektöründe ölümle sonuçlanan iş kazası sayısı 4 olup, ölümlü kaza oranı %1 olarak bulunmuştur. Bu oranlar baz alındığında 2019 yılında Türkiye'de kaza başına düşen ölüm oranının en fazla olduğu faaliyet balıkçılık ve su ürünleri yetiştiriciliğidir. Bununla beraber 2013-2019 yılları arasında balıkçılık ve su ürünleri yetiştiriciliğindeki iş kazası başına düşen ölüm oranı %0,71 iken, Türkiye'deki tüm sektörlerin ortalaması %0,51 olarak tespit edilmiştir (Tablo 2).

Tablo 2. 2013-2019 yılları arasında balıkçılık ve su ürünleri yetiştiriciliğindeki iş kazası ve ölümlü iş kazası sayılarının Türkiye'deki genel durum ile kıyaslanması
Table 2. Comparing the number of work accidents and fatal incidents in fisheries and aquaculture sector and Turkey in general

	Türkiye'deki tüm sektörler toplam			Balıkçılık ve su ürünleri yetiştiriciliği		
	İş kazası sayısı	Ölüm sayısı	İKBDÖO*	İş kazası sayısı	Ölüm sayısı	İKBDÖO*
2013	191389	1360	0,71	118	3	2,54
2014	221366	1626	0,73	169	0	0,00
2015	241547	1252	0,52	300	1	0,33
2016	286068	1405	0,49	582	0	0,00
2017	359653	1633	0,45	273	2	0,73
2018	430985	1541	0,36	344	1	0,29
2019	422463	1147	0,27	375	4	1,07
Var.	8127828060	29848,82	0,02	19652,49	1,96	0,69
Ort.	307638,71	1423,43	0,51	308,71	1,57	0,71
Std.sap.	90154,47	172,77	0,16	140,19	1,40	0,83

*İKBDÖO: iş kazası başına düşen ölüm oranı

6331 sayılı İş Sağlığı ve Güvenliği Kanunu yürürlüğe girmeden önce Türkiye'de 2007-2012 yılları arasında balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolunda toplam 230 iş kazası gerçekleşmiş, bu kazalardan 4 tanesi sürekli iş göremezlikle ve 8 tanesi de ölümle sonuçlanmıştır. Bu dönemde resmi kayıtlara geçen hiç meslek hastalığı olmamıştır.

Balıkçılık ve su ürünleri sektöründe ve bu sektörün alt dallarına ilişkin iş kazası sayıları, sürekli ve geçici iş göremezlik sayıları, iş kazası nedeniyle ölüm sayıları 2013-2019 dönemi için Tablo 3'te verilmiştir.

Tablo 3. 2013-2019 yılları arasında balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolundaki bazı iş kazası istatistikleri (Den. bal: deniz balıkçılığı; Tat. Bal: Tatlı su balıkçılığı; Den Yet: Deniz yetiştiriciliği; Tat. Yet: Tatlı su yetiştiriciliği)
Table 3. Some work accident statistics of fisheries and aquaculture sector between 2013 and 2019

Yıl	Faaliyet	Sigortalı çalışan sayısı	İş kazası Sayısı	İş kazası sonucu geçici iş göremezlik (gün)	İş kazası sonucu sürekli iş göremezlik (kişi)	İş kazası nedeniyle ölüm (kişi)
2013	Deniz. Bal.	8148	38	591	2	1
	Tat. Bal.		17	65		0
	Den. Yet.		48	401		1
	Tat. Yet.		15	158		1
Ara Toplam			118	1215	2	3
2014	Den. Bal.	7582	96	790	0	0
	Tat. Bal.		18	194		0
	Den. Yet.		71	638		0
	Tat. Yet.		11	136		0
Ara Toplam			196	1758	0	0
2015	Den. Bal.	8041	169	478	2	0
	Tat. Bal.		13	65		0
	Den. Yet.		101	1289		0
	Tat. Yet.		17	475		1
Ara Toplam			300	2307	2	1
2016	Den. Bal.	8467	374	959	2	0
	Tat. Bal.		10	69		0
	Den. Yet.		190	1989		0
	Tat. Yet.		8	92		0
Ara Toplam			582	3109	2	0
2017	Den. Bal.	9062	17	432	1	0
	Tat. Bal.		11	378		0
	Den. Yet.		224	2105		1
	Tat. Yet.		21	529		1
Ara Toplam			273	3444	1	2
2018	Den. Bal.	9306	32	57	5	0
	Tat. Bal.		13	30		0
	Den. Yet.		277	1060		1
	Tat. Yet.		22	93		0
Ara Toplam			344	1240	5	1
2019	Den. Bal.	9784	37	789	3	1
	Tat. Bal.		5	10		0
	Den. Yet.		309	4104		3
	Tat. Yet.		24	364		0
Ara Toplam			375	5267	3	4
Genel Toplam			2188	18340	15	11
Varyans			18664	1806306,29	2,12	1,96
Ortalama			312,6	2620	2,14	1,57
Standart sapma			147,6	1451,67	1,57	1,51

Çalışmanın kapsadığı dönemde tatlı su balıkçılığında gerçekleşen 87 iş kazasında hiç ölüm rapor edilmemesine karşın, tatlı su yetiştiriciliğinde 118 kazanın 3'ünün, deniz yetiştiriciliğinde 1220 kazanın 6'sının ve deniz balıkçılığında

763 kazanın 2'sinin ölümle sonuçlandığı bildirilmiştir. İş kazası başına düşen ölüm oranın en yüksek olduğu faaliyetin %2,54 ile tatlı su yetiştiriciliği olduğu, bunu sırasıyla %0,49 ve %0,26 ile deniz yetiştiriciliği ve deniz balıkçılığının izlediği tespit edilmiştir. İncelenen 6 yıllık dönemde hiç meslek hastalığı buna bağlı iş göremezlik ve ölüm rapor edilmemiştir. Yapılan istatistiksel değerlendirmelerde deniz balıkçılığı ve deniz yetiştiriciliği faaliyet alanlarında yaşanan iş kazası sayıları ve iş kazası sonucu geçici iş göremezlik süreleri arasındaki farkların önemsiz tespit edilmiştir ($p>0,05$). Buna karşın deniz balıkçılığı-tatlı su balıkçılığı ve deniz yetiştiriciliği-tatlı su yetiştiriciliği karşılaştırıldığında bu farkların her iki durum içinde önemli olduğu bulunmuştur ($p<0,05$). Daha genel bir değerlendirme ile denizlerde yapılan avcılık ve yetiştiricilik faaliyetleri ile tatlı sularda yapılan avcılık faaliyetlerinde yaşanan iş kazası sayısı ve iş kazası sonucu geçici iş göremezlik süreleri arasındaki farkın da önemli olduğu saptanmıştır ($p<0,05$) (Tablo 4)

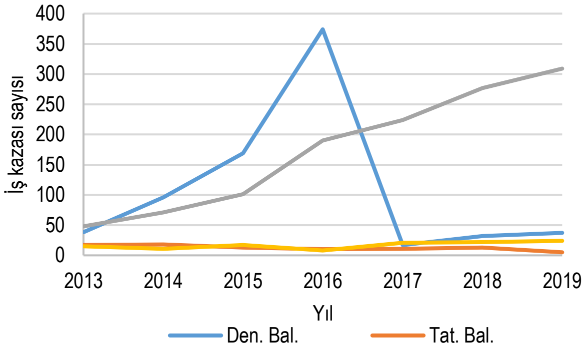
Tablo 4. Denizlerde ve tatlı sularda gerçekleştirilen faaliyetlerin iş kazası sayısı ve iş kazası sonucu geçici iş göremezlik süresi açısından Mann Whitney-U testi ile karşılaştırılması

Table 4. Comparing number of work accidents and days of temporary incapacity in marine and freshwater activities by Mann Whitney-U test

Kıyaslanan faaliyetler	İş kazası sayısı	İş kazası sonucu geçici iş göremezlik süresi
Deniz balıkçılığı - Deniz yetiştiriciliği	p=0,12	p=0,054
Deniz balıkçılığı – Tatlı su balıkçılığı	p=0,004	p=0.015
Deniz yetiştiriciliği – Tatlı su yetiştiriciliği	p=0.002	p=0.004
Deniz (avcılık ve yetiştiricilik toplam) – Tatlı su (avcılık ve yetiştiricilik toplam)	p=0.002	p=0.002

İncelenen tarih aralığında en fazla ölümlü iş kazasının 2019 yılında (n=4) olduğu, 2014 ve 2016 yılları arasında ise yaşanan kazaların ölümle sonuçlanmadığı tespit edilmiştir. İş kazaları ve ölümle sonuçlanan iş kazalarının faaliyet bölgesine göre dağılımında denizlerde yapılan avcılık ve yetiştiricilik faaliyetlerinin 1983 iş kazası ve 8 ölümlü kaza ile rapor edildiği, tatlı sularda ise 205 kazaya karşın, 3 ölümlü kaza meydana geldiği bildirilmiştir (Tablo 3). Çalışmanın kapsadığı dönemde yetiştiricilik sektöründe (deniz ve tatlı su toplam) 1338 kazaya karşılık 9 ölümlü iş kazası meydana gelmiş, avcılık sektöründe (deniz ve tatlı su toplam) ise 850 kazaya karşın 2 ölümlü iş kazası rapor edilmiştir (Tablo 3). Bu periyotta ölümlü iş kazası yaşanmayan tek alt sektör tatlı su balıkçılığı olmuştur. 2013-2019 yılları arasında balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolunda meslek hastalığından kaynaklı geçici ve kalıcı iş göremezlik ve meslek hastalığı nedenli ölüm bildirilmemiştir. İş kazası sonucu sürekli iş göremez olarak nitelendirilen çalışan sayısı en yüksek seviyeye (n=5) 2018 yılında ulaşılmış, 2019 yılında 3 olarak tespit edilmiş, 2013, 2015, 2016 yıllarında 2, 2017 'de 1 ve 2014'te 0 olarak rapor edilmiştir. İş kazası sonucu geçici iş göremezlik verilerinde en yüksek sayı 5267 ile 2019 yılında ve en düşük sayı 1215 ile 2013 yılında verilmiştir. Çalışmanın kapsadığı dönemde geçici iş göremezlik sürelerinin en fazla olduğu alt sektörün deniz yetiştiriciliği olduğu (11586 gün), bunu sırasıyla deniz balıkçılığı (4096 gün), tatlı su yetiştiriciliği (1847 gün) ve tatlı su balıkçılığının (811 gün) izlediği tespit edilmiştir. Yetiştiricilik (deniz ve tatlı su toplam) sektöründeki 13433 günlük geçici iş göremez süresine karşın balıkçılıkta bu sayı 4907 olmuştur.

Balıkçılık ve su ürünleri yetiştiriciliğine ait alt sektörlerde 2013-2019 yılları arasında yaşanan iş kazalarının dağılımında deniz balıkçılığındaki kaza sayılarının 2013'ten 2016 yılına kadar arttığı ve 2016'da pik noktaya ulaştığı görülmektedir. 2017 yılında deniz balıkçılığında yaşanan kaza sayısı dramatik bir düşüş göstermiş ve 2018 ve 2019 yıllarında az miktarda artmıştır. Buna karşın çalışmanın kapsadığı dönemde deniz yetiştiriciliği alanında meydana gelen kaza sayıları sürekli artış göstermiştir (Şekil 1).



Şekil 1. Balıkçılık ve su ürünleri sektörünün alt dallarına ilişkin iş kazası sayılarının 2013-2019 yılları arasındaki dağılımı

Figure 1. Distribution of work accident numbers between 2013 and 2019 within the subsectors of fisheries and aquaculture

Çalışmada incelenen 3 adet kaza sıklık değeri Tablo 5'de verilmiştir. Balıkçılık ve su ürünleri yetiştiriciliğindeki genel kaza sıklık değerleri 2013 yılı hariç Türkiye genelinde bildirilen genel kaza sıklık değerlerinin üzerinde bulunmuştur. Ayrıca 2016 yılında hesaplanan kaza sıklık değeri Türkiye genelinin yaklaşık 5 katıdır. Sürekli iş göremezlik kaza sıklık değerinde ise 2014 yılında balıkçılık ve su ürünleri yetiştiriciliği sektöründe sürekli iş göremezlikle sonuçlanan kaza gerçekleşmediğinden bu değer 0 olarak tespit edilmiştir. Bunun dışındaki yıllarda ise sektörde hesaplanan oran Türkiye genelindeki oranlardan çok yüksek bulunmuştur. 2014 ve 2016 yıllarında balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolunda ölümlü iş kazası bildirilmemiştir ve bu nedenle ölümlü kaza sıklığı değeri bu yıllarda 0'dır. Diğer yıllarda ise sektördeki ölümlü kaza sıklık değerleri Türkiye genelinde tespit edilen değerlerden 1,9 ile 5 kat daha fazla olarak tespit edilmiştir. Standardize iş kazası oranları incelendiğinde minimum değerin 2013, maksimum değerin ise 2016 yılında elde edildiği görülmektedir (Tablo 6). Balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolundaki iş kazası sıklık hızları da 2013 yılı hariç Türkiye geneli için verilen değerlerin üzerinde hesaplanmıştır. Son 7 yıllık dönemde balıkçılık ve su ürünleri yetiştiriciliğinde her 1000000 iş saatinde ortalama olarak 13,65 iş kazası meydana gelirken Türkiye geneli için bu değer 8,36'dır. Bununla beraber incelenen faaliyet kolunda her 100 çalışana 3'ün üzerinde kaza düşerken, Türkiye geneli için 2'nin altındadır (Tablo 7).

Tablo 5. 2013-2019 yılları arasında balıkçılık ve su ürünleri yetiştiriciliğindeki iş kazası sıklık değerleri ve Türkiye'deki tüm sektörlerle karşılaştırılması (GKS: Genel kaza sıklığı, SİGKS: Sürekli iş göremezlik kaza sıklığı, ÖKS: Ölümlü kaza sıklığı)
Table 5. Incident rates in fisheries and aquaculture sector and Turkey in general between 2013-2019 (GKS: General incidence rate, SİGKS: Permanent incapacity incidence rate, ÖKS: Fatal incidence rate)

	Türkiye'deki tüm sektörler toplam						
	Çalışan sayısı	İş kazası sayısı	*Sürekli iş göremez sayısı	Ölümlü iş kazası sayısı	GKS	SİGKS	ÖKS
2013	12484113	191389	74	1360	1533,06	5,93	108,94
2014	13240122	221366	115	1626	1671,93	8,69	122,81
2015	13999398	241547	5	1252	1725,41	0,36	89,43
2016	13775118	286068	94	1405	2076,7	6,82	102,00
2017	14447817	359866	85	1636	2490,8	5,88	113,24
2018	14229170	431276	94	1542	3030,93	6,61	108,37
2019	14314313	422837	98	1149	2953,95	6,85	80,27
Var.	421720756485	8153541487	1089,06	29905,3	329508,9	5,8	180,1
Ort.	13784293	307764	80,7	1424,2	2211,8	5,88	103,6
Std.sap	649400	90296	33,0	172,9	574,0	2,41	13,4
	Balıkçılık ve su ürünleri yetiştiriciliği						
2013	8148	118	2	3	1448,21	245,459	368,189
2014	7582	196	0	0	2585,07	0	0
2015	8041	300	2	1	3730,88	248,725	124,363
2016	8467	582	2	0	6873,75	236,211	0
2017	9062	273	1	2	3012,58	110,351	220,702
2018	9306	344	5	1	3696,54	537,288	107,458
2019	9784	375	3	4	3832,79	306,623	408,831
Var.	525625,26	18664	2,12	1,95	2400477	23906,3	23208,6
Ort.	8627,14	312,57	2,14	1,57	3597,12	240,67	175,65
Std.sap	725,0	136,62	1,46	1,40	1549,35	154,62	152,34

*İlgili yılda iş kazası geçirip sürekli iş göremez durumuna düşen çalışan sayısı

Tablo 6. Balıkçılık ve su ürünleri yetiştiriciliği sektöründe standardize iş kazası oranlarına ilişkin parametreler ve bu oranların yıllık dağılımları (GİKH: Genel iş kazası hızı, SİKO: Standardize iş kazası oranı)

Table 6. Annual distribution of standardized work accident rates in fisheries and aquaculture sector (GİKH: General work accident frequency, SİKO: Standardized work accident rate)

	Çalışan sayısı		İş kazası sayısı			SİKO	
	Türkiye geneli	Balıkçılık ve su ürünleri yetiştiriciliği	Türkiye geneli	Balıkçılık ve su ürünleri yetiştiriciliği	GİKH	Türkiye geneli	Balıkçılık ve su ürünleri yetiştiriciliği
2013	12484113	8148	191389	118	0,015	100	94,47
2014	13240122	7582	221366	196	0,017	100	154,62
2015	13999398	8041	241547	300	0,017	100	216,23
2016	13775118	8467	286068	582	0,021	100	330,99
2017	14447817	9062	359866	273	0,025	100	120,95
2018	14229170	9306	431276	344	0,030	100	121,96
2019	14314313	9784	422837	375	0,030	100	129,75

Tablo 7. Balıkçılık ve su ürünleri yetiştiriciliğindeki iş kazası sıklık hızına ilişkin veriler ve bu verilerin Türkiye'deki tüm sektörlerin geneli ile karşılaştırılması (PTEGS: Prime tahakkuk eden gün sayısı, $İKSH_1$: İş kazası sıklık hızı (1. yöntem), $İKSH_2$: İş kazası sıklık hızı (2. yöntem))

Table 7. Data and results for incidence rates of occupational injuries in fisheries and aquaculture sector and comparison with the values reported for Turkey in general (PTEGS: number of days of premium accrued represents total working days of all insured persons during calendar year, $İKSH_1$: This method represents the number insured persons who had an occupational accident per 1.000.000 working hours (1. method), $İKSH_2$: This method represents the number insured persons who had an occupational accident per 100 person (2. method))

	Türkiye'deki tüm sektörler toplam					Balıkçılık ve su ürünleri yetiştiriciliği				
	Çalışan sayısı	İş kazası sayısı	PTEGS	$İKSH_1$	$İKSH_2$	Çalışan sayısı	İş kazası sayısı	PTEGS	$İKSH_1$	$İKSH_2$
2013	12484113	191389	4069831784	5,88	1,32	8148	118	2656255	5,55	1,25
2014	13240122	221366	4248428182	6,51	1,47	7582	196	2432877	10,07	2,27
2015	13999398	241547	4462091444	6,77	1,52	8041	300	2562944	14,63	3,29
2016	13775118	286068	4524501578	7,90	1,78	8467	582	2781026	26,16	5,89
2017	14447817	359866	4524383875	9,94	2,24	9062	273	2837797	12,03	2,71
2018	14229170	431276	5006245563	10,76	2,42	9306	344	3274128	13,13	2,95
2019	14314313	422837	4907005930	10,76	2,42	9784	375	3353996	13,98	3,14
Var.				3,76	0,19				34,12	1,72
Ort.				8,36	1,88				13,65	3,07
Std.sap				1,93	0,43				5,84	1,31

TARTIŞMA VE SONUÇ

Bu çalışmada, Türkiye ekonomisine yüksek katma değer sağlayan Türkiye balıkçılık ve su ürünleri yetiştiriciliği sektörünün iş sağlığı ve güvenliği özelinde içinde bulunduğu durumu ortaya koyabilmek için iş kazası ve meslek hastalığı istatistikleri değerlendirilmiştir. Türkiye'deki balıkçılık ve su ürünleri yetiştiriciliği iş kolunun son 7 yılına ait iş kazası ve meslek hastalığı istatistiklerini değerlendiren bu makale ile sektörün iş sağlığı ve güvenliğine ilişkin bazı tespitler ilk kez yapılmıştır. Bu tespitlerin en önemlilerinden birisi son 7 yıllık istatistiklerin ortalamasında balıkçılık ve su ürünleri yetiştiriciliğindeki ölümlü iş kazası oranının Türkiye ortalamasından yaklaşık %50 fazla olmasıdır. 2019 yılında Türkiye'deki ölümlü kaza oranı genel olarak %0,27 bulunmuş, maden sektöründe %0,34, inşaatta %0,77 ve nakliyede %0,80 olarak tespit edilmiştir. Balıkçılık ve su ürünleri yetiştiriciliğinde ise bu oran %1 olarak hesaplanmış ve en riskli faaliyet kollarından olan madencilik, inşaat ve nakliye sektörleri, kaza başına düşen ölüm sayısında balıkçılık ve su ürünleri yetiştiriciliğinin gerisinde kalmıştır. Daha genel bir ifade ile değerlendirmek gerekirse balıkçılık ve su ürünleri yetiştiriciliği sektöründeki iş kazası başına düşen ölüm oranı Türkiye ortalamasının 4 katına yakın olmuştur. Bu bağlamda balıkçılık ve su ürünleri sektörü 2019 yılı için Türkiye'de kaza başına düşen ölüm oranının en yüksek olduğu çalışma alanı olarak karşımıza çıkmaktadır. Kaplan ve Kite-Powell (2000), bu bulguya paralel olarak, balıkçılık mesleğindeki ölüm oranlarının polislik ve itfaiyecilik dahil koruma hizmeti sektöründeki ölüm oranlarından önemli ölçüde daha yüksek olduğunu bildirmiştir. Balıkçılık ve su ürünleri sektöründe kaza başına düşen ölüm oranlarının yüksek olmasının en önemli nedenlerinden birisinin çalışma ortamlarının genel olarak profesyonel sağlık hizmeti sunan servislerden uzak olması ve iş kazası sonucu oluşan yaralanmalara kısa sürede müdahale edilememesi olduğu düşünülmektedir. Kaplan ve Kite-Powell (2000) balık ve diğer denizel kaynakların üretim süreçlerinin genelde zorlu hava ve deniz şartlarında gerçekleştirildiğini böyle zamanlarda kaza ve yaralanmaların sayısının oldukça yüksek olduğunu bildirmiştir.

Türkiye'de meslek hastalığı kavramı tüm sektörlerde üzerinde önemle durulması gereken bir konudur. Mevcut çalışmanın bulgularında en dikkat çeken noktalardan biri balıkçılık ve su ürünleri yetiştiriciliği sektöründe hiç meslek hastalığı rapor edilmemesidir. Meslek hastalığının rapor edilmemesi, meslek hastalığının bu sektörde hiç yaşanmadığı anlamına gelmemektedir. Bir ülkede beklenen meslek hastalıkları sayısı konusunda Harrington vd., (1998) tarafından yayınlanan "Occupational Health" isimli çalışma temel literatürlerden bir tanesidir. Harrington vd., (1998) bir ülkenin iş sağlığı alanındaki gelişmişlik düzeyiyle bağlantılı olarak saptanması gereken meslek hastalığı sayısı binde 4-12 arasında olması gerektiğini ifade etmiştir. Bu kriter baz alındığında 2013-2019 yılları arasında balıkçılık ve su ürünleri faaliyet kolunda beklenen meslek hastalığı sayısının 32 ile 120 arasında olması gerektiği değerlendirilmiştir. Meslek hastalıklarının tanısı ve raporlanması aşamasında birtakım eksiklikler ve yetersizlikler yaşanmaktadır ki bunlar gerçek sonuçların ortaya konmasındaki en önemli sorunlardır (Aydoğan, 2020). Özveri (2018), meslek hastalıklarının tespitine ilişkin hukuki sürecin, teknik ve hukuki araçlardan yoksun, uzun, yetersiz, keyfi şekilde seyrettiğini, meslek hastalığının tespitinde SGK sağlık sunucularının sorumluluk almaktan kaçındığını ve ilgili vakaların meslek hastalıkları hastanelerine yollandığını bildirmiştir. Meslek hastalıkları hastanelerinin ise kadro ve bilgi birikimi açısından ciddi şekilde erozyona uğradığı araştırıcı tarafından eklenmiştir. Diğer taraftan, meslek hastalıklarının tespit edilmesindeki güçlük, bazı meslek hastalıklarının çok uzun dönemlerde ortaya çıkması ve çalışanların bu süreçte sektör değiştirmeleri meslek hastalığı bildiriminin önündeki engeller olarak ifade edilmektedir (Aydoğan, 2020). Avcılık, yetiştiricilik, işleme gibi zorlu üretim ve süreçlerde çalışan balıkçılık ve su ürünleri yetiştiriciliği çalışanları fiziksel, kimyasal ve biyolojik risk etmenlerine maruz kalarak çalışmakta bu yüzden de çeşitli meslek hastalıklarına yakalanma ihtimalleri artmaktadır. Bu nedenle çalışanların ve işverenlerin meslek hastalığı konusunda farkındalığı arttırılmalı ve meslek hastalığı konusundaki mevzuat ve süreç titizlikle takip edilmelidir.

Çalışmada, balıkçılık ve su ürünleri yetiştiriciliği sektöründeki iş kazası sıklık değerleri (GKS, SİGKS ve ÖKS) Türkiye geneliyle kıyaslanmış ve sektörde veri bildirimi yapılan hemen her yıl hesaplanan değer (GKS için 2013, SİGKS için 2014, ÖKS için 2014 ve 2016) Türkiye genelinde elde edilen değerlerin ve yüksek istihdama sahip birçok sektörün üzerinde bulunmuştur. Örneğin 2016 yılında tekstil sektöründe bildirilen genel kaza sıklığı 1935 iken (Güllüoğlu ve Taçgın, 2018) balıkçılık ve su ürünleri yetiştiriciliğinde (6873,5) bunun yaklaşık 4 katıdır. Ceylan (2014) Türkiye'de 2004-2010 yılları arasında inşaat sektöründe ölümlü kaza sıklık değerlerinin 127-350 arasında ve sürekli iş göremezlik kaza sıklığını 196-460 olarak bildirilmiştir. 2013-2019 yılları arasını kapsayan bu çalışmada balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolunda veri bildirilen yıllarda ölümlü kaza sıklığının 107-480 ve sürekli iş göremezlik kaza sıklığının 110-537 arasında değiştiği tespit edilmiştir. Her iki sektöre ait verilen değerler göz önünde bulundurulduğunda balıkçılık ve su ürünleri yetiştiriciliğine ait maksimum değerlerin daha fazla olduğu görülmektedir. Sektöre ait iş kazası sıklık hızı da 2013 yılı hariç Türkiye genelinin hep üzerinde seyretmiştir. 2013-2016 yılları arasında balıkçılık ve su ürünleri yetiştiriciliği sektöründe elde edilen kaza sıklık hızları tekstil sektörünün yaklaşık 4 katına ulaşmaktadır. Bayraktar vd. (2018), 2015 yılında Türkiye'de en riskli sektörlerden olan madencilik, metal ve inşaat sektörlerindeki iş kazası sıklık hızlarını sırasıyla 8,05, 4,8 ve 1,68 olarak rapor etmişlerdir. Aynı yıl balıkçılık ve su ürünleri yetiştiriciliğinde hesaplanan değer 3,29 olup inşaat sektöründeki değerin yaklaşık 2 katıdır. Standardize iş kazası oranı (SİKO) bir sektörün belirli bir yıldaki performansını genel kaza sıklığı açısından Türkiye geneli ile kıyaslamak için kullandığı bir karşılaştırma ölçütüdür. Her ne kadar SGK istatistik yıllıklarında 2012 yılından sonra kullanılmasa da halen güncel akademik çalışmalarda (Güllüoğlu ve Güllüoğlu, 2019; Güllüoğlu ve Taçgın, 2018; Akyüz vd., 2016) yer verilen bir parametredir. Çalışmada hesaplanan standardize iş kazası oranları incelendiğinde, Türkiye genelinde gerçekleşen iş kazalarını standardize oranı 100 olarak kabul edildiğinde, balıkçılık ve su ürünleri yetiştiriciliğinde iş kazalarının en yoğun 2016 (SİKO=330,99) ve en az 2013 (SİKO=94,47) yıllarında gerçekleştiği görülmektedir. Tüm bu istatistiki değerlendirmeler balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolunun Türkiye'deki en riskli sektörlerden biri olduğunu göstermektedir. Buna ek olarak sadece balıkçılık (avcılık faaliyeti) mesleği ölümlü kaza oranları ve çalışan başına düşen ölüm oranları nedeniyle dünyanın en tehlikeli ve riskli mesleklerinden biridir (ILO, 2010). Dünyada balıkçılık konusunda söz sahibi olan bazı ülkelerde bildirilen çalışan başına düşen ölüm oranları genellikle 1 ölüm/1000 çalışan civarındadır (Jensen vd., 2014) (Tablo 8). Türkiye bu kriterlere göre değerlendirme yapmak veri eksikliği nedeniyle oldukça güçtür. Çalışmanın kapsadığı dönemde Türkiye'de sadece 2013 ve 2019 yıllarında deniz balıkçılığında 1'er ölüm bildirilmiş ve bu yıllarda balıkçılıkta çalışan sayısı sırasıyla 33455 ve 28717 olarak verilmiştir. Bu nedenle Türkiye balıkçılığında çalışan başına düşen ölüm oranı diğer ülkelere nazaran oldukça düşük bulunmuştur (Tablo 8). Bu durumun temel nedeninin balıkçılıktaki iş kazası ve çalışan sayısı istatistiklerindeki veri toplama süreci ve konuya gerekli önemin verilmemesi olduğu düşünülmektedir. Buna ek olarak dünya çapında yetiştiricilik sektöründeki iş kazaları ve meslek hastalıklarını konu alan çalışma sayısı oldukça sınırlı sayıdadır. Bu çalışmaların en önemlilerinden birinde Norveç yetiştiricilik sektöründe 1982-2015 yılları arasında toplam 35 ölümlü iş kazası rapor edilmiştir (Holen vd., 2018). Başka bir ifade ile Norveç yetiştiricilik sektöründe yıllık ortalama ölüm sayısı 1,06 iken Türkiye yetiştiriciliğinde bu oran 1,28 olup, Norveç'tekinden %20 daha fazla olduğu tespit edilmiştir.

Tablo 8. Bazı ülkelerde avcılık sektörü için bildirilen ölüm oranları (Jensen vd., 2014)
Table 8. Fatality rates (per 1,000 fishermen) of fishery sector in some countries (Jensen vd., 2014)

Ülke	Veri dönem	Oran (ölüm/çalışan sayısı)
Norveç	1998-2006	0,2/1000
İzlanda	1980-2005	0,5/1000
Danimarka	2000-2009	1/1000
İngiltere	1992-2006	1,26/1000
Polonya	1960-1999	0,9/1000
Kanada	1999-2010	0,24/1000
ABD	2000-2010	1,24/1000
Türkiye	2013-2019	0,03/1000

Balıkçılık ve su ürünleri yetiştiriciliğine ait alt sektörlerde 2013-2019 yılları arasında yaşanan iş kazalarının dağılımı incelendiğinde; tatlı su balıkçılığı ve yetiştiriciliğindeki iş kazası sayılarının yıllık dağılımlarında önemli değişiklikler olmamıştır. Buna karşın deniz balıkçılığında kayda değer dalgalanmaların olduğu ve deniz yetiştiriciliğinde kaza sayısının düzenli artış eğilimi gösterdiği görülmektedir. Tatlı sularda yapılan balıkçılık ve yetiştiricilik faaliyetlerinde 2013-2019 yılları arasındaki kaza sayıları ise diğer iki sektöre nazaran farklılıklar göstererek nispeten küçük değişimlerle sabit bir eğilim ifade etmektedirler. Genel olarak, denizlerde yapılan üretim faaliyetlerinde kaza ve ölümlerin daha çok olduğu bunun da sektörel bazdaki istihdam ile ilgili olduğu değerlendirilmektedir. Ülkemizde kayıt dışı istihdam ve bildirilmeyen iş kazası sayısının çok fazla olduğu düşünülürse, SGK tarafından açıklanan verilerin aslında gerçeği tam yansıtmadığı (Ergin, 2016), her sektörde olduğu gibi balıkçılık ve su ürünleri yetiştiriciliği alanında da yaşanan kazaların sayısının kayıtlarda belirtilenden daha fazla olduğu ortaya çıkmaktadır.

Bu çalışmanın balıkçılık ve su ürünleri sektörü için ortaya çıkardığı en önemli tartışmalardan biri de Türkiye'de balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolundaki çalışan sayısı üzerinedir. SGK verilerine göre 2013-2019 yılları arasında balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolunda, çalışan sayısı 8148-9784 arasında değişmesine rağmen, TÜİK su ürünleri istatistiklerinde yalnızca balıkçılıktaki istihdamın bu 7 yıllık süreçte 33455 ile 28717 kişi arasında olduğu görülmektedir. Buna ek olarak 150.000 çalışanın yetiştiricilik ve 100.000 kişinin balıkçılık olmak üzere su ürünleri sektörünün 250.000 çalışana istihdam sağladığı bildirilmiştir (Kalkınma Bakanlığı, 2014). Ayrıca, Aydın (2016), Türkiye'de kültür balıkçılığı sektöründeki çalışan sayısı konusunda sağlıklı bilgi olmadığını, bununla birlikte üretim, işleme, pazarlama, alet ve ekipman temini, balık yemi üretimi ve bu sektörle ilişkili işyerlerinde 25.000 kişinin çalışmakta olduğunu rapor etmiştir. Bildirilen bu rakamlar arasındaki büyük farklılıklar ciddi bir tutarsızlık oluşturmaktadır. Bu tutarsızlığın en önemli yansıması şüphesiz ki iş kazası sıklık oranının hesaplanmasında ortaya çıkacaktır zira bu oranın hesabında kullanılan parametrelerden biri çalışan sayısıdır. Bu çalışmada SGK sigortalı ve işyeri istatistiklerinde bildirilen çalışan sayıları baz alınmış olmasına rağmen yukarıda tartışılan diğer literatürlerdeki istihdam sayılarıyla yapılacak hesaplamalar çok farklı sonuçlara neden olacaktır. Bir diğer konu ise balıkçılık ve su ürünleri yetiştiriciliğine ait alt faaliyet kollarının sınıflandırılmasıdır. SGK iş kazası ve meslek hastalığı istatistiklerinde balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolu deniz balıkçılığı, tatlı su balıkçılığı, sünger avcılığı, deniz ürünleri yetiştiriciliği ve tatlı su ürünleri yetiştiriciliği olmak üzere 5 başlık altında toplanmasına karşın İş Sağlığı ve Güvenliğine İlişkin İşyeri Tehlike Sınıfları Tebliği'nde deniz balıkçılığı, tatlı su balıkçılığı, deniz ürünleri yetiştiriciliği ve tatlı su ürünleri yetiştiriciliği olarak 4 grupta verilmiştir. Günümüzde sünger avcılığı artık bir endüstri kolu veya ana geçim kaynağı olan bir iş olmadığından SGK istatistiklerinin tehlike sınıfları tebliğine göre düzenlenmesi ilgili mevzuatların tutarlı ve güvenilir olmasını sağlayacaktır.

İş sağlığı ve güvenliği bütün mesleklerde; çalışanların sağlıklarını sosyal, ruhsal ve bedensel olarak en üst düzeyde sürdürmek, çalışma koşullarını sağlığa uygun hale getirmek, çalışanları zararlı etkilerden ve tehlikelerden koruyup daha güvenli bir çalışma ortamı yaratarak, işin ve çalışanın; birbirine uyumunu sağlamak üzere kurulmuş bir bilim dalıdır. Güvenlik kültürünün halen tam olarak oluşmadığı Türkiye'de iş kazaları ve meslek hastalıklarına ilişkin istatistiklerin her sektör için detaylı şekilde ele alınması ve bu çalışmalara ilişkin sonuçların topluma çeşitli yollarla (resmi-akademik yayınlar, kamu spotları ve sosyal medya) aktarılması gerekmektedir. Her ne kadar tüm meslekler gerek tanımsal gerekse de mevzuat bakımından iş sağlığı ve güvenliğinin kapsamı dâhiline alınmışda olsa balıkçılık ve su ürünleri yetiştiriciliği gibi bazı ekonomik faaliyetler iş sağlığı ve güvenliği çalışmaları açısından yeterli akademik ve bürokratik ilgiyi görememektedir. Balıkçılık ve su ürünleri yetiştiriciliği faaliyet kolunda iş kazaları ve meslek hastalıklarının değerlendirildiği bu çalışma sonucunda ilgili çalışma alanının tahmin edilenden çok daha riskli bir sektör olduğu ortaya konmuştur.

Sektörde yaşanan kaza sayılarının düşürülmesi ve bu kazaların sonuçlarının hafifletilebilmesi bir takım önlem, eğitim ve kontroller ile bir dereceye kadar mümkün olabilir. Bu önlem, eğitim ve kontrol faaliyetlerinin en önemlileri, önemli hastalıklara ve yaralanmalara karşı acil eylem planlarının hazırlanması, ilgili çalışmaya ve çalışana uygun, iş güvenliği standartlarına sahip kişisel koruyucuların kullanılması, sektör çalışanlarının ilk yardım ve acil durum eğitimi almalarıdır. Bu noktada ilkyardım eğitimi çok önemlidir çünkü çoğu kaza ve yaralanma sektörde yapılan çalışmaların fiziki ortamı gereği profesyonel sağlık hizmeti sunucularına oldukça uzak mesafelerde gerçekleşmektedir. Diğer yandan, balıkçılık ve su ürünleri yetiştiriciliğinde yapılan çalışmaların çoğunlukla zorlu hava ve deniz şartlarında gerçekleştiği de yadsınamaz bir gerçektir. Ayrıca sektör çalışanları yapılan işi doğası gereği fiziksel ve ergonomik risk etmenlerine maruz kalarak çalışmaktadır. Dolayısıyla kaza sayılarının ve bu kazalara ilişkin ciddiyet derecelerinin azaltılması için yukarıda bahsedilen önerilerden ziyade sistemsel ve gücünü resmi mevzuattan alan bir yaklaşıma ihtiyaç duyulmaktadır. Bu yüzden, balıkçılık ve su ürünleri yetiştiriciliği ekonomik faaliyet koluna ait tüm alt çalışma alanlarının tehlike sınıflarının “çok tehlikeli” sınıfa yükseltilmesi uygun bir yaklaşım olacaktır. Bu sayede sektör çalışanlarının mesleki riskler konusunda aldıkları eğitim süresi artacak, sektörde çalışan İSG profesyonellerinin unvan ve sayıları artarak daha nitelikli bir uzmanlaşma gerçekleşecek, kontrol ve denetim faaliyetlerinin daha sıklaşması sağlanmış olacaktır. Bu öneriler doğrultusunda Türkiye ekonomisine önemli katkı yapan balıkçılık ve su ürünleri yetiştiriciliği sektörünün çok daha güvenli bir şekilde hizmet vereceği değerlendirilmektedir.

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