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Prevalence of Ectoparasites of Fry Redbelly Tilapia (*Coptodon zillii*) Fishes from Euphrates River, Iraq

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ABSTRACT

Ectoparasitic infection represents one of the main challenges to freshwater and marine fish in all areas of the world. This study detected ectoparasite types in Fry Redbelly Tilapia Fishes. The specimens (250) were purchased from a market with a source of fish from the Euphrates River. Fishes were collected from December 2022 until the end of April 2023. The samples were used for macroscopic inspection, gross viscera, and microscopic exam - wet mount of fins, skin, gills and eyes. Ectoparasite species were obtained on fins, skin, and gills, and the eyes had no infection. The total prevalence was 140 (56%) of 250 fish. Significant fish types with weight and length are at ($P \leq 0.05$) and ($P \leq 0.01$), respectively. The mean and standard deviation according to weight were 71.4 and 14.7, while according to length, they were 15.6 and 1.9. Non-significant for genders in both types at ($P \geq 0.05$). The host *Coptodon zillii* is infested by four types of ectoparasites were included, with prevalence for each one being *Ichthyophthirius sp.*, 80/250 (32%); *Trichodina sp.*, 30/140 (12%); *Dactylogyrus sp.*, 20/250 (8%); *Gyrodactylus sp.*10/250 (4%). In conclusion, all parasites, namely single-host types. Management measures should be taken to save the procurement and import from reputable global markets.

Keywords: Ectoparasites, fish, Euphrates river, Iraq

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Introduction

Parasitic infestations represent one of the main challenges to freshwater and marine fish, including Tilapia Fishes (Coptodon zillii) in all areas of the world (Shinn et al. 2023). The protozoans Trichodina spp. and Ichthyophthirius multifiliis are common ectoparasites of cultured freshwater. Ichthyophthirius multifiliis is a protozoan ciliate that causes the disease Ichthyophthiriasis or white spot disease, with low host specificity and broad geographical distribution (Abu-Elala et al. 2021; Li et al. 2022). Trichodina species are commonly found on the skin and gills of fish and can cause dangerous problems, such as mortality, in heavily parasitized infections. Until now, about 400 Trichodina species have been found on various aquatic animals worldwide in a range of habitats, including freshwater, brackish water, and marine environments (Wang et al. 2020). Trichodina spp. Identification based on morphological characters is still difficult because of their high intraspecific variation, high interspecific similarity, and low host specificity (Wang et al. 2019). Gill Monogeneans (Platyhelminthes) Parasites are widely distributed in Iraq and worldwide. Dactylogyrus spp. are one of Platyhelminthes ectoparasites living on the gills. Dactylogyrus spp. are characterized by a high host specific (Al-Sa'adi et al. 2013; Abdullah 2009). Gyrodactylus spp. is a monogenean ectoparasite fluke, which lives on the body surface of freshwater fish (Dos Santos et al. 2019). Although there are many species of Gyrodactylus, there is little morphological and biological diversity. On a single species of fish host, numerous distinct Gyrodactylus

species were discovered. In contrast, some gyrodactylid species were host-specific (Abdullah 2021).

In recent years, many studies focusing on Ecto-endoparasites parasites species have been carried out worldwide (Shinn et al. 2023; Abu-Elala et al. 2021; Al-Sa'adi et al. 2013; Abdullah 2009; Dos Santos et al. 2019; Öztürk and Güven 2022; Blazhekovikj-Dimovska and Stojanovski 2021; Shigoley et al. 2023).

While the previous studies in the Euphrates River (AL-Zaidy 2013) were the first record of 21 Tilapia zilli collected from AI-Delmj marsh, middle Iraq, which is located between the cities of AI-Diwania (west) and Kut (east). While (Al-Faisal and Mutlak 2014) reported *Oreochromis niloticus* (Linnaeus, 1758) as first recorded from the Shatt Al-Arab River at Basrah (Abu-al-Khasib), Iraq. Another study of the same genus (Khalifa et al. 2018) reported Oreochromis aureus as first recorded in the Diyala river / Buhriz. But, (Abulheni and Abbas 2015) were recorded first of the Tilapia *Oreochromis niloticus* (Linnaeus 1758) in Euphrates River at Al-Hindia Barrage –Middle of Iraq. The main aim of the current investigation is to identify ectoparasites from the Fry Tilapia (*Coptodon zillii*) purchased from the market and the source of fish from the Euphrates River, Iraq.

Materials and Methods

Study Area

The greater part of the Euphrates Basin is in Turkey, Syria, and Iraq. The Euphrates source region is the Taurus Mountains. Firstly, three rivers add water to the Euphrates: the Khabur, the Balikh and the Sajur. The Khabur is the largest of these three, in terms of length from Turkey. These rivers rise in the foothills of the Taurus Mountains along the Syro–Turkish border and add comparatively little water to the river, from where the Khabur flows southeast past to Syria. Once the Euphrates enters Iraq, toward the Gulf at the south of Iraq, in its upper reaches, the Euphrates flows through the mountains of Southeast Turkey and their southern foothills. The length of the Euphrates from the source of the Murat River to the confluence with the Tigris is 3,000 km, of which 1,230 km is in Turkey, 710 km in Syria and 1,060 km in Iraq. The length is Approx. 2,800 km. Euphrates province is located at (31º-18ºnorth latitude, 47º- 26º east longitude). The river passes in cities in three countries: Birecik, Raqqa, Deir ez-Zor, Mayadin, Haditha, Ramadi, Habbaniyah, Fallujah, Kufa, Samawah, Nasiriyah. Then Al-Qurnah (Kurnah or Qurna, joint is a town in southern Iraq about 74 km northwest of Basra, where the Tigris and Euphrates rivers fused to form the Shatt al-Arab Sea.

Sampling

A total of 250 fish were purchased from different markets. The fish are selected randomly, and the source is the Euphrates River. The method was collected by gill nets. Fishes were collected from December 2022 until the end of April 2023. Fish was transferred and examined directly two to three times weekly. The pathological findings were obvious on the infected fish and affected the structure and function of the body. The observations were carried out by examining the alterations in the morphology, pigmentation, and tissue firmness of all external organs of the fish. Each specimen's weight and length were recorded before testing. The diagnostic methods included standard wet-mount specimens consisting of skin scraping (mucus smears), fin biopsy (fin clip) and gill biopsy (gill clip). The eyes were removed and put in a Petri dish for detecting infection.

The other operation made a longitudinal incision to search for other parasites like nematodes. The prevalence of infection was calculated as demonstrated by Margolis et al. (1982) 24. ectoparasites assessment under a light compound microscope at 4-10X magnification. Prevalence (P)= (number of infected fish/total number of examined fish) ×100; MI= (number of parasites/number of infected hosts).

Data Analysis

The total number of parasites was confirmed directly by numerical count. The number of fish sampled and the mean and Standard deviation of protozoa and helminth parasites were analyzed and interpreted according to (Shigoley et al. 2023). and was used to compare the data among weight and length with infection. Another comparison of genders with health status was made using SPSS version 24 for data analysis.

Results

Parasite diversity and prevalence

After examining 250 individual fish, four parasites were identified. We identified *Ichthyophthirius sp., Trichodina sp., Dactylogyrus sp., Gyrodactylus sp*. Fig 1.2.3and 4. The total prevalence was 56% (140/250), Table 1 Figure 1: The correlation of infection with weight and length differs significantly based on fish weight ($p<0.05$) and length ($p<0.01$), Table 2. The mean and standard deviation according to weight was (71.4, 14.7), while according to length was (15.6, 1.9), Table 3. Non-significant for genders in both types, Table 4. The prevalence for each one was *Ichthyophthirius sp.*, 80/140(57.1%); *Trichodina sp*., 30/140(21.4%); *Dactylogyrus sp.*, 20/140(14.3%); *Gyrodactylus sp.*10/140(7.1%) Table (5). The SPSS statistical software (version 24) is used to analyze data.

Table 1. Prevalence of infected and non-infected Fish.

	infected	Non- infected	total
No.	140	110	250
Percentage	56%	44%	100%

Clinical signs

Results of the analysis of the different infected fishes revealed the presence of several clinical signs Table 2. Indeed, we observed the appearance of necrotic areas, ulcerations and hemorrhage on the gills. We found Protozoa and Monogeneas parasites on other body parts that caused inflammations and mechanical injuries.

Table 2. Correlation of infection with weight and length.

Pearson correlation			
	weight	**Long(cm)**	**infected**
Weight (gm)	1	0.595**	0.396*
Length (cm)	0.595**	1	0.654**
infected	0.396*	0.654**	1

* Correlation is significant at 0.05 (2-tailed).
** Correlation is significant at the 0.01 level(2-tailed).

Table 3. Correlation of mean weight and length with standard deviation.

	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Weight (gm)	250	37.0	95.5	71.4	14.7
Length (cm)	250	13.0	19.6	15.6	1.9

Table 4. Correlation of genders with infection.

			health status		**Total**
			infected	**no infected**	
Gender	male	Count	110	60	170
		% within gender	65%	35%	100%
	female	Count	30	50	80
		% within gender	38%	63%	100%
Total		Count	140	110	250
		% within gender	56%	44%	100%
X^2	1.634	P value	**0.201**		

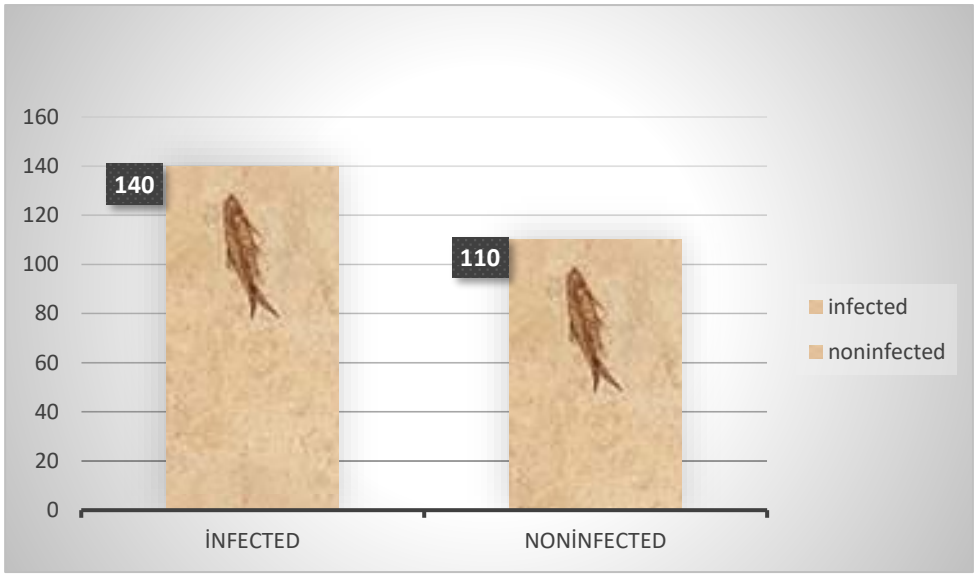
Non-significant at P ≥0.05

gender * health status Cross tabulation

Table 5. Prevalence of each parasite.

Species of nematodes	**No. of infected**	**%**
Ichthyophthirius multifiliis	80	32
Trichodina spp.	30	12
Dactylogyrus sp.	20	8
Gyrodactylus sp.	10	4
Total no. of infected	140	100

Figure 1. Distribution of infected and non-infected Fish.



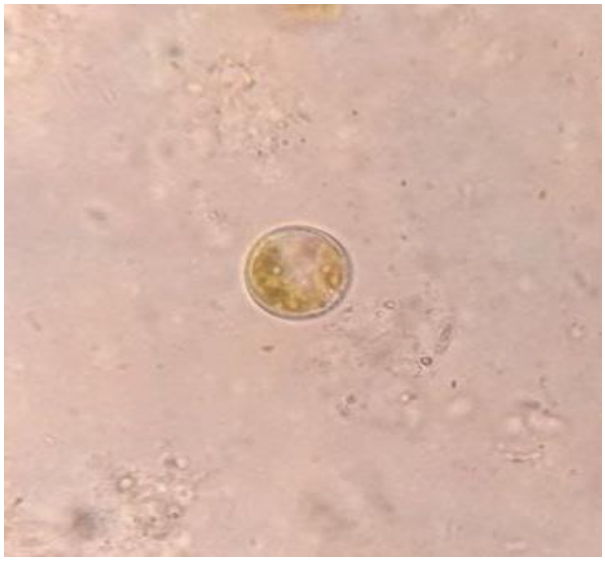


Figure 1. *Ichtyopthirius multifiliis* tomont stage X40.

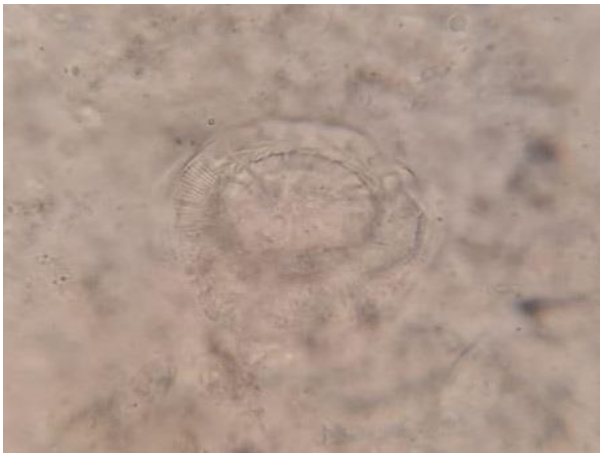


Figure 2. *Trichodina* sp. X100.

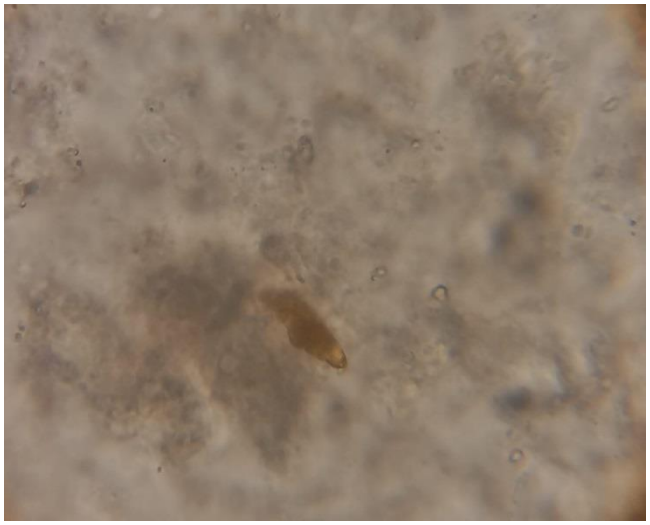


Figure 3. *Dactylogyrus* sp. X40.

Figure 4. *Gyrodactylus* sp. X40.

Discussion

Fish are regarded as a superior food source for humans and are preferred as the ideal diet due to their higher percentages of unsaturated fatty acids **(**Jain 2017). Head mucus, fin mucus, body mucus and gills of fish are the parts affected by ectoparasites (Iriansyah et al. 2020). For example, fish infect not only ectoparasites but endoparasites like the Anisakidae family's most crucial genus, Contracaecum, which contains various host species participating in their life cycles and considerably negatively influencing population health (Jawad et al. 2022). Therefore, this makes fish the source of basic income for millions of people and the economy globally (Blazhekovikj-Dimovska and Stojanovski 2021). Recently, several parasitic species have been recorded in various fish types. Although, several studies around the globe have revealed cases of infection in (*Tilapia*) with various ectoparasites (Iriansyah et al. 2020; Abu-Elala et al.2021; Shinn et al. 2023). These ectoparasitic infections can cause high morbidity among them, which may disrupt the environment and health of fish, leading to death and causing economic losses (Jain 2017).

In the current study, laboratory examination was performed on a set of 250 fish purchased from the market and selected randomly with the source of fish from the Euphrates River, Iraq. The total prevalence was (56%) with the detection one species *Ichthyophthirius multifilis*, and three genera *Trichodina sp.*, *Dactylogyrus sp.*, and *Gyrodactylus sp.; t*his result was similar to (Iriansyah et al. 2020) in freshwater fishes in Mulur Reservoir of Sukoharjo District, Indonesia who detected prevalence of more than 50% in tilapia (*Oreochromis niloticus*) and other fish types.

Accordingly, the environment was suitable, but there are some parasites that have infected tilapia. The infection by ectoparasites affected fish populations and caused several clinical signs. The current study reported the appearance of necrotic areas, ulcerations and hemorrhage on the gills.

We found Protozoa and Monogeneas parasites on other body parts that caused inflammations and mechanical injuries. These results agree with (Obaid

et al. 2021), who reported several injuries and ulcerations were observed within the gills, over fins and skin of infected fish, which were associated with high mortality rates in common carp (*Cyprinus carpio*) in aquacultures in the Erbil region. Another study by (Koyuncu et al.2022) in a private aquarium farm Koi fish (*Cyprinus carpio*) in Mersin, Turkey. The symptoms included melting of the fins, skin hyperemia, petechial haemorrhages and wounds drew attention, anorexia, swimming disorders, pale colour of the fins, melting, skin redness, haemorrhage and death.

The current study has no significant effect on infection between males and females. This agreement with another study on common carp Cyprinus carpio in AlForat River Al-Mussayab, which showed no recording of differences in chosen infection site and sex and host either in the skin, Fins and gills (Hussein 2018). In the current study, we detected a correlation of infection with weight and length (Shigoley et al.2023), and confirmed that new species in Morocco of gyrodactylids infecting hosts by specimens from the gills of 738 African cyprinid has a longer Hamulus total length, a longer hamulus root, a downward projecting toe, a trapezium shaped ventral bar membrane with slightly striated median portion and small rounded anterolateral processes.

Ichthyophthirius multifiliis is an important protozoan pathogen that infects the gills, skin and fins of freshwater fish, with broad geographical distribution and low host specificity. However, the epizootic occurrence of ichthyophthiriasis in high plateau has rarely been recorded (Li et al.2022). This ciliate cause threatens the global aquaculture, causing white spot disease in several countries (Yang et al.2023). Ichthyophthiriasis in Farmed Fishes in the Tigris River in Iraq was reported in four types, and the number of infections ranged from 10-35 (Khalifa et al.1983), while in the current study, ichthyophthiriasis infection was 32%. This result, in agreement with other studies (Hussein 2018), reported *Ichthyophthirius multifiliis* infection from 84 samples of common carp *Cyprinus carpio* in AlForat River Al-Mussayab, the presence of parasite on the skin, Fins and gills were examined. The highest percentage of infection recorded in April was 53.33% (Hussein 2018). Another study by (Blazhekovikj-Dimovska and Stojanovski 2021) who analyze protozoan distribution in farmed cyprinid fish from Macedonia. A total of 1134 fish samples were examined, from which parasite infestation with Protozoa was determined in 533 fish, with a total prevalence of 47%. Eight protozoan parasite representatives were identified: *Ichthyophthirius multifiliis* and *Trichodina* sp.

The second ectoparasites of numerous aquatic invertebrate and vertebrate hosts, Trichodinids, are well known in both wild and cultured fish (Öztürk and Güven, 2022). Trichodina spp. is an economically and ecologically important genus of ectoparasitic protozoan ciliates pathogen (Wang et al. 2020). In the present study, we reported a 12% infection rate. This result agreement with (Mansoor 2010) who detected *Trichodina domerguei* (21.6%) in cyprinid fish *Cyprinus carpio* from fish markets east of Baghdad city, while (Öztürk and Güven 2022) who confirmed prevalence range (7.5-100%) for *Trichodina rectuncinata, T. ovonucleata, T. jadranica,* and *T. domerguei* in four species of marine fishes from Sinop coasts of the Black Sea, Turkey. Additionally, some Cyprinid fish species living naturally in the Murat River in the Bingöl University Zoology Research Laboratory and parasite fauna and their distribution from 365 fish were examined, and 100 fish (27.4%) were infected with at least one Protozoan or Crustacean parasite (Korkut and Koyun 2022).

Many monogenean parasites have been co-introduced along with their fish hosts (García-Vásquez et al. 2021). In the current study, we reported Dactylogyrus sp. was (8%); this agreement with Al-Sa'adi et al. 2013, who diagnosed *Dactylogyrus dogieli* from gills of *Cyprinion kais, Alburnus sellal, Carassobarbus luteus, Mesopotamichthys sharpeyi* and *Ctenopharyngodon idella*. The prevalence was 1.7%, 4.3%, 5.2%, 50% and 50%, respectively, of 471 fishes from the Euphrates River at Al-Musaib City, Iraq. While (Abdullah 2009) recorded a prevalence range (of 15-33.33%) of 48 freshwater fishes belonging to four species of the family Cyprinidae were collected from Darbandikhan Lake, southwest Sulaimaniya City, Kurdistan region, in the north of Iraq. The inspection of gills revealed four species of monogenetic trematoda belonging to the genus Dactylogyrus, namely: *D. alatus*, *D. cyprinioni*, *D. macracanthus* and *D. microcirrus*.

Although several ectoparasites are found in domesticated fish than in wild fish (Iriansyah et al. 2020), this theory disagrees with the current source and the source of fishes from the wild environment. In the current study, *Gyrodactylus sp.* was (4%). This study agrees with the results by (Abdullah 2009), who reported an infection rate (5.2-28.5%) of *Gyrodactylus angorae* on the body of two nemachilid fishes (*Oxynoemacheilus zarzianus* and *Eidinemacheilus proudlovei*) from Iraq. Approximate *Gyrodactylus sp* abundance have reported (0.15-17.90) in Tilapian fish Mexico (García-Vásquez et al. 2021). Another study has similar results (20-100) on 10 fishes for each type. The results showed that 5 types of ectoparasites were *Epistylis* sp., *Ichthyophthirius multifilis*, *Trichodina* sp., *Dactylogyrus* sp., and *Gyrodactylus* sp.

(Iriansyah et al. 2020). In conclusion, the study referred to the nature of ectoparasites in freshwater environments. parasites infecting Fry Redbelly Tilapia (*Coptodon zillii*) in the Euphrates River from markets are *Ichtioptioptirius multifiliis*, *Trichodina* sp., *Dactylogyrus* sp.and *Gyrodactylus* sp. Before market sale, there should be a visual inspection of the fish industry. All parasites namely single-host types. Management measures should be taken to save the procurement and import from reputable global markets under the Food and Agriculture Organization (FAO) and World Health Organization (WHO) supervision.

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Ethical Statement

Ethics required are approved by the Ethical Committee of the College of Veterinary Medicine/University of Kerbala under acceptance number- UOK.VET. MI.2022.064.

Competing Interests

The authors declared that there is no conflict of interest.

Authors' Contributions

Firas Alali, Marwa Jawad and Sarah Mohammed ALSHEIKH: Writing- Original draft preparation, Conceptualization, Methodology, Funding acquisition, Investigation, Data analysis.

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Determination of Surface Water Quality Parameters of Creeks in Mogan Lake Basin (Ankara, Türkiye)

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ABSTRACT

The present research focuses on assessing the environmentally significant Mogan Lake and its feeder creeks water quality parameters, all of which hold recreational importance for Ankara. In light of the findings, a) The ranking of the creeks based on the average parameter values for each creek over three months is as follows: for TSS and TOC: Gölcük > Başpınar > Sukesen > Yavrucak; for TN: Başpınar > Gölcük > Sukesen > Yavrucak; for TP: Gölcük > Sukesen >Başpınar > Yavrucak, b) Based on dissolved oxygen values, Sukesen and Yavrucak Creeks were classified as Class I water quality, while in terms of pH values, all creek waters were in Class I. However, in terms of total phosphorus, they were classified as Class III water quality, c) TP, TOC were found to be controlled by wastewater reaching Başpınar and Sukesen Creek from both settlements and agricultural activities, while wastewater originating from agricultural activities was found to be directly responsible for DO, TN and TSS levels as in Gölcük and Yavrucak Creeks. In this context, monitoring the high TP, TN and TOC values of four creeks in the basin, which are exposed to multiple stressors, is important to protect the water quality of these creeks and to control their contribution to the eutrophication of Mogan Lake.

Keywords: Water quality parameters, nutrients, total suspended solids, creeks

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Mogan Gölü Havzası'ndaki (Ankara, Türkiye) Derelerin Yüzey Suyu Kalite Parametrelerinin Belirlenmesi

Öz: Bu araştırmada, Ankara açısından rekreaktif öneme sahip Mogan Gölü'nü besleyen Başpınar, Gölcük, Sukesen, Yavrucak Derelerinin yüzey suyu kalite parametrelerinin değerlendirilmesi amaçlanmıştır. Bulgular ışığında; a) Her bir derenin üç aya ilişkin ortalama parametre değerlerine göre sıralaması; AKM ve TOC için: Gölcük>Başpınar>Sukesen>Yavrucak; TN için: Başpınar>Gölcük>Sukesen>Yavrucak; TP için: Gölcük>Sukesen> Başpınar >Yavrucak olarak belirlenmiştir, b) Çözünmüş oksijen değerlerine göre Sukesen ve Yavrucak Dereleri, pH değerleri açısından tüm dere suları I. Sınıf, toplam fosfor açısından ise III. Sınıf su kalitesine sahiptir, c) TP, TOC'un Başpınar ve Sukesen Deresi'ne gerek yerleşim yerlerinden gerekse tarımsal aktivitelerden ulaşan atık sular tarafından kontrol edildiği, tarımsal faaliyetlerden köken alan atık suların ise, Gölcük ve Yavrucak Dere'lerinde olduğu gibi ÇO, TN ve AKM düzeylerinden doğrudan sorumlu olduğu saptanmıştır. Bu bağlamda, birden çok stres etkenine maruz kalan havzadaki dört dereye ilişkin yüksek TP, TN ve TOC değerlerinin izlenmesi, bu derelerin su kalitesini korumak ve Mogan Gölü'nün ötrofikasyonundaki katkılarını kontrol etmek açısından önem taşımaktadır.

Anahtar kelimeler: Su kalite parametreleri, besin elementleri, askıda katı maddeler, dereler

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Introduction

In terms of the sustainability of water resources, the determination and monitoring of water quality characteristics are important. Regular monitoring helps facilitate the provision of real-time data on water quality, thereby contributing to the preservation of public health and the assurance of water resource utilization safety.

Under the current 'Surface Water Quality Management Regulation' in effect in Türkiye, aquatic environments are categorized into water quality classes based on parameters such as pH, conductivity, oil and grease, dissolved oxygen,

chemical oxygen demand, biochemical oxygen demand, ammonium nitrogen, nitrate nitrogen, total kjeldahl nitrogen, total nitrogen, orthophosphate phosphorus, total phosphorus, fluoride, manganese, selenium, and sulfur. In this context, water quality monitoring results related to specific pollutants and priority substances are also evaluated according to the water body category (rivers/lakes, coastal and transitional waters) (TSWQR 2016). As stated in Anonymous (2022), in the Lake District Special Environmental Protection Plan for Gölbaşı, covering the period 2015-2019 and prepared by the General Directorate for Protection of Natural Assets of the Ministry of Environment and Urban Planning, the goal of achieving compliance with the standards set by the Surface Water Quality Management Regulation for Mogan-Eymir Lakes and the creeks feeding these lakes has been established.

The speed of eutrophication, which is a natural process in aquatic systems, gains momentum as a result of land use, sewage, domestic, agricultural and industrial wastewater reaching the water environment. As nutrient inputs continue to be introduced into aquatic systems in this manner, situations that are not suitable for the intended use of aquatic environments may arise. In this context, before starting to solve the eutrophication problem, a comprehensive analysis of nutrient sources should be carried out and separate management strategies should be developed for point, non-point and endogenous sources to control eutrophication (Pulatsü et al. 2014).

Mogan Lake (Ankara), which is one of the inland freshwater ecosystems exposed to eutrophication, is among the important wetlands in Türkiye nominated for Ramsar status. The increasing population, urbanization, changes in land use, sediment transport, the rise in the use of agricultural pesticides, mining activities, and factors like climate change in the lake basin have made year-to-year water-sediment quality changes in the lake inevitable. Since the early 1990s, the lake has possessed the characteristic of being a natural research area for some government institutions and universities, and due to its proximity to Ankara, it continues to be a focal point of interest (Pulatsü and Topçu 2023). Mogan Lake receives very low groundwater input, and the inflow of water into the lake mainly occurs during summers through irregularly flowing creeks, which often dry up. In this context, adverse interventions on the creeks and their beds can potentially impact both the flow rates and water quality conditions of the creeks that feed the lake. During the autumn season of 2022, rainfall across the country occurred below the normal levels and below the levels observed in the previous year. The autumn season rainfall across the country has shown a 27% decrease compared to the normal levels and a 9% decrease compared to the autumn season of the previous year (Anonymous 2023). The reflections of seasonal changes on all surface water resources are also valid for the Mogan Lake Basin.

In this study: a) the aim was to obtain current data related to some physicochemical parameters (water temperature, dissolved oxygen, pH, suspended solids, total phosphorus, total nitrogen, total organic carbon) for four significant creeks - Başpınar, Gölcük, Sukesen, and Yavrucak Creeks - which had been relatively underrepresented compared to other studies conducted in Mogan Lake, b) the water quality classes of creek waters based on the analyzed water quality parameters were determined, c) the estimation of nutrient and suspended solids loads of Sukesen and Yavrucak Creeks was conducted, d) to assess the impact of creek-specific pollutant sources on water quality parameters. It is believed that the findings are important for establishing the current situation in the context of basin-lake management.

Materials and Methods

Study Area

Mogan Lake is situated within the limits of the Gölbaşı Special Environmental Protection Area and is additionally recognized as one of the important wetlands in our nation proposed for Ramsar designation. The groundwater replenishment of Mogan Lake is quite limited, and during the summer months, water inflow primarily happens via sporadically flowing creeks that often run dry. The key screeks within this group include Sukesen, Başpınar, Gölova, Yavrucak, Çolakpınar, Tatlım, Kaldırım, and Gölcük, situated in the eastern-northwestern regions of the basin (Anonymous 2017). In this research, water samples were collected from four chosen creeks, which serve as pollution sources for Mogan Lake and contribute to its inflow (Figure 1).

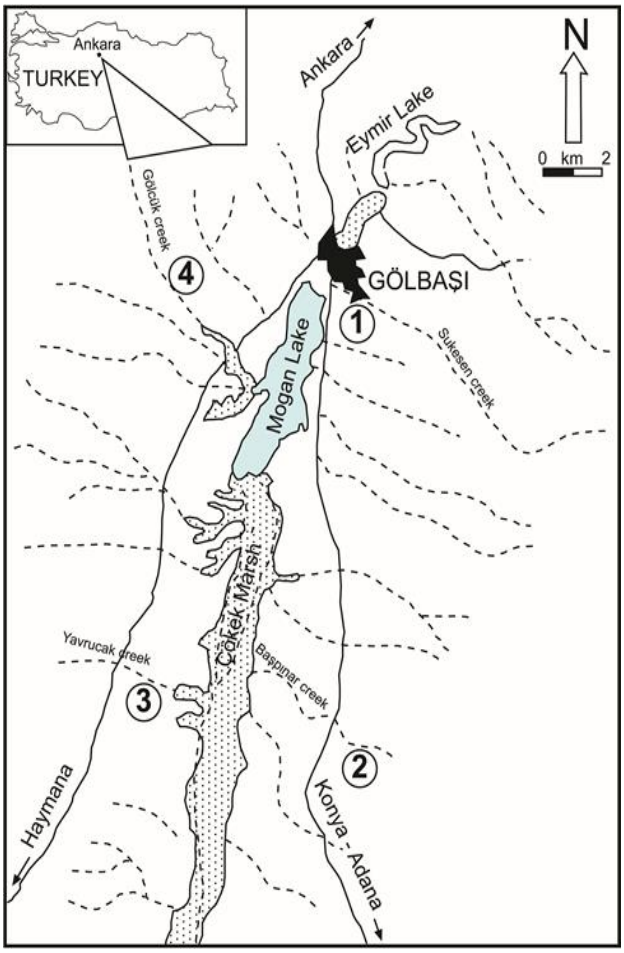
Methods

During this research, three sampling sessions were carried out on the creeks that supply Mogan Lake, precisely in December 2022, February 2023, and April 2023. The choice of sampling months was made by taking into account the potential for substantial rainfall, as indicated by historical meteorological data.

Fieldwork

The water samples were collected in plastic sample bottles after being thoroughly mixed with the surface waters of the mentioned creeks, ensuring there were no air bubbles, and then transported to the laboratory in a cold and dark environment. Water temperature (°C), dissolved oxygen (DO), and pH were measured in the field. Measurements of

DO and temperature were made using a portable oxygen meter (YSI Pro 20; temperature range: 5–45°C, sensitivity: ± 1°C; dissolved oxygen range: 0–15 ppm, sensitivity: ± 0.2 ppm). pH with a portable pH meter (YSI-Ecosense pH 100 A; ± 1°C sensitivity)



1: Sukesen Creek- 39°47,350"N, 32°48,266"E
2: Başpınar Creek- 39°46,616"N, 32°48, 149"E
3: Yavrucak Creek- 39°47,542"N, 32°48,205"E
4: Gölcük Creek-39°45,436"N, 32°45,568" E

Figure 1. Map of the study area and coordinates of the selected creeks

Laboratory work

The unfiltered samples were analysed for total phosphorus (TP), total nitrogen (TN), total organic carbon (TOC) and total suspended sediment (TSS). The analyses were conducted in a laboratory accredited by the Turkish Accreditation Agency (TÜRKAK) with four repetitions, and the methods used in the analyses are presented in Table 1.

Table 1. Methods used in creek water analyses

Parameters	**Methods**
Total phosphorus (TP)	TS EN ISO 17294-1.2
Total nitrogen (TN)	SM 4500 Norg B, SM 4110 B
Total organic carbon (TOC**)**	TS 8195 EN 1484
Total suspended solids (TSS**)**	SM 2540 D

Load estimation

To determine the loads of TP, TN, TOC, and TSS transported to Mogan Lake by the creeks, discharge values for these creeks are required. However, the latest records regarding creek discharge measurements in the basin are only available for Sukesen and Yavrucak Creeks, and they date back to the year 2008. In this context, the average flow values reported by Kapan (2011) were used for calculating the load values of the parameters. These values for December, February, and April are as follows (m^3): Sukesen Creek: 0.011, 0.013, 0.017 and Yavrucak Creek: 0.002, 0.012, 0.085 respectively. The load values for the same months were determined by multiplying the concentration values of the parameters by the daily discharge values.

Statistical analyses

The presence of a significant difference between the measurement-concentration values of parameters across months and creeks was tested using the Kruskal-Wallis test. The difference between which months and creeks were found was determined by Tukey test, one of the post hoc multiple comparisons tests. The presence of a significant relationship between the measurement-concentration values of parameters was obtained using the Spearman correlation coefficient. The correlation coefficient is interpreted as the direction, amount and strength of the relationship between two variables. The amount of the relationship varies between - 1.00 and + 1.00. As for the strength of the relationship correlations between 0.00 and 0.30 are considered low, correlations between 0.31 and 0.70 are moderate, and correlations between 0.70 and 1.00 indicate a high level of relationship between the two variables. In this study, Principal Component Analysis/Factor Analysis (PCA/FA) with varimax rotation was conducted to identify potential sources of water quality parameters. To determine the appropriate dataset for PCA, the Kaiser-Meyer-Olkin (KMO) and Bartlett's sphericity tests were performed. The KMO is a measurement of the acceptability of sampling since it indicates the common variance that might be induced by underlying factors. If the KMO value is close to 1, it suggests that the samples are acceptable, or PCA might be more effective (Kolassa 2020). All statistical analyses were performed by using SPSS 22.

Results

Within the scope of the study, the water temperature values of all the creeks in February and April were statistically significant ($P < 0.05$). The dissolved oxygen values also showed statistically significant differences across months in all creeks ($P < 0.05$). Minimum oxygen values in all creeks were measured in April. The pH values varied between 5.61 and 8.61 during the sampling months. The results of water temperature, dissolved oxygen and pH for the average values of the three months measured on the basis of creeks are shown in Figure 2.

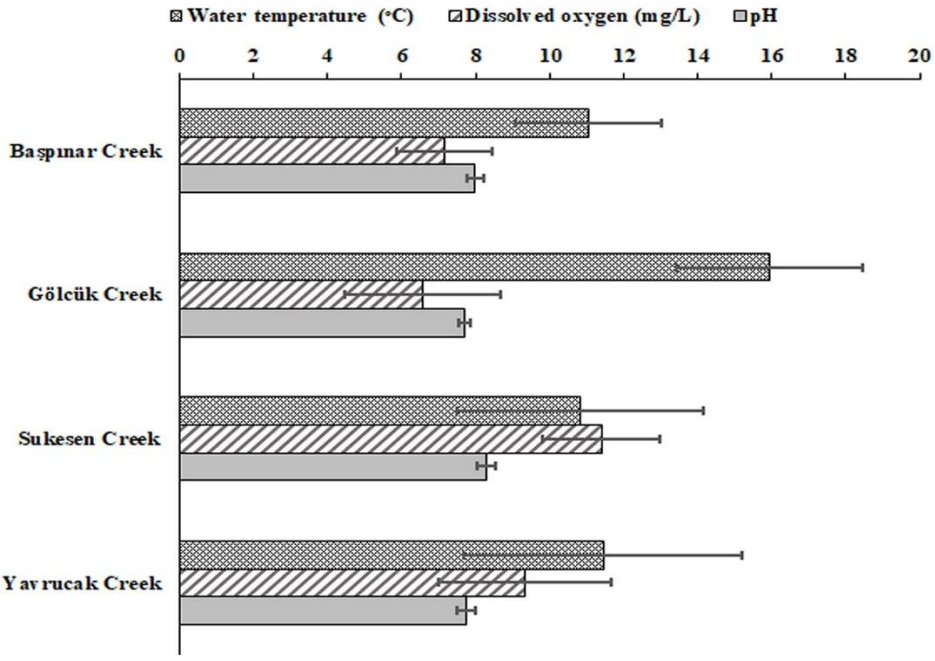


Figure 2. Seasonal variations in water temperature, dissolved oxygen, and pH values for creeks

The monthly variation in total suspended solids values was found to be statistically significant for each river ($P < 0.05$). Maximum total suspended solids values were determined as 23.45 and 3.38 mg/L in Başpınar and Yavrucak Creeks in December, respectively, and 40.30 and 19.63 mg/L in Gölcük and Sukesen Creeks in April, respectively. According to the values in Table 2, minimum TSS values were determined in February in Başpınar, Sukesen and Yavrucak creeks and in December in Gölcük creek. In terms of this parameter, the difference between the creeks in December and February was found to be statistically significant ($P < 0.05$).

As seen in Table 2, the monthly variation of total nitrogen in all creek waters was statistically significant ($P < 0.05$) The maximum total nitrogen value of 8.64 mg/L was determined in Gölcük Creek in April, while the minimum value (0.92 mg/L) belongs to Yavrucak Creek. In terms of this parameter, the difference between the creeks in February and April is statistically significant.

Table 2. Variation of water quality characteristics by months and creeks

		Months		
		December	**February**	**April**
Creeks	**Parameters**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Başpınar	TSS	$23.45 \pm 0.57^{aA*}$	16.13 ± 0.25^{bA}	19.18 ± 0.30^{cA}
	TN	6.10 ± 0.12^{aA}	6.75 ± 0.14^{bA}	7.29 ± 0.13^{cA}
	TP	0.07 ± 0.01^{aA}	51.40 ± 1.90^{bA}	18.90 ± 0.91^{cA}
	TOC	3.10 ± 0.11^{aA}	33.33 ± 0.40^{bA}	2.01 ± 1.18^{aA}
Gölcük	TSS	12.15 ± 0.41^{aB}	34.68 ± 0.38^{bB}	40.30 ± 0.42^{cB}
	TN	0.99 ± 0.05^{aB}	8.02 ± 0.08^{bB}	8.64 ± 0.06^{cB}
	TP	0.27 ± 0.02^{aB}	22.99 ± 0.98^{bB}	236.95 ± 3.21^{cB}
	TOC	$5.40 \pm 0.20B^{aB}$	36.60 ± 1.44^{bB}	2.75 ± 0.63^{cA}
Sukesen	TSS	7.50 ± 0.36^{aC}	2.63 ± 0.17^{bC}	19.63 ± 0.22^{cA}
	TN	2.19 ± 0.07^{aC}	4.49 ± 0.01^{bC}	1.00 ± 0.03^{cC}
	TP	0.07 ± 0.01^{aA}	77.45 ± 1.77^{bC}	4.20 ± 0.22^{cC}
	TOC	3.25 ± 0.61^{aA}	29.70 ± 0.86^{bC}	1.91 ± 1.00^{aA}
Yavrucak	TSS	3.38 ± 0.40^{aD}	1.25 ± 0.13^{bD}	1.28 ± 0.17^{bC}
	TN	3.08 ± 0.04^{aC}	2.95 ± 0.04^{bD}	0.92 ± 0.01^{cC}
	TP	0.01 ± 0.00^{aC}	3.99 ± 0.34^{bD}	2.49 ± 0.33^{cC}
	TOC	3.42 ± 0.22^{aA}	23.58 ± 2.17^{bD}	2.53 ± 0.89^{aA}

*The different lower-case letters in the same row show the differences between months in the same creek, while the different capital letters in the same column show the differences between the creeks at the same month ($P < 0.05$)

Monthly variation of total phosphorus values for each creek water was found to be statistically significant ($P < 0.05$). The minimum total phosphorus values in all creeks ranged between 0.01-0.27 mg/L and were measured in December. The highest total phosphorus value determined during the study period was 236.95 mg/L in April in Gölcük Creek (Table 2).

The total organic carbon values of all creeks showed a statistically significant difference in February ($P < 0.05$), while in April, there was not a statistically significant difference among the creeks in terms of this parameter ($P > 0.05$).

The limited number of study findings related to the considered creeks in this research are presented in Table 3. It is observed that the total phosphorus concentration values, total phosphorus concentration values have increased approximately 100 times in all creeks over the years and the ranking of creeks carrying excessive phosphorus changed depending on the type of point and non-point pollution sources affecting creek waters.

Table 3. Results of studies conducted in different periods at Mogan Lake (Total phosphorus-TP, total suspended solids-TSS, total nitrogen-TN)

Creeks	TP (mg/L)	Period	Reference
Başpınar	0.712	1992-1994	Pulatsü and Aydın (1997)
Gölcük	-		
Sukesen	0.325		
Yavrucak	0.478		
Başpınar	-	March 1997-April 1998	Burnak and Beklioğlu (2000)
Gölcük	0.198		
Sukesen	0.143		
Yavrucak	0.336		
Başpınar	0.712	January-December 2003	Fakıoğlu and Pulatsü (2005)
Gölcük	-		
Sukesen	0.292		
Yavrucak	0.478		

Creeks	TP (mg/L)	TSS (mg/L)	Period	Reference
Başpınar	0.22-0.66	108-227	July-1999 October-1999	Karakoç et al. (2003)
Gölcük	0.10-0.13	747-166		
Sukesen	0.35-0.07	< 10-18		
Yavrucak	0.27-0.7	190-85		

Creeks	TP (mg/L)	TSS (mg/L)	TN (mg/L)	Period	Reference
Başpınar	0.06	12.49	2.87	January-December 2008	Kapan (2011)
Gölcük	-	-	-		
Sukesen	0.55	330	11.08		
Yavrucak	0.13	48	2.69		
Başpınar	23.46	19.58	6.71	December 2022, February, April 2023	This study
Gölcük	86.73	29.04	5.88		
Sukesen	27.24	9.92	2.56		
Yavrucak	2.16	1.97	2.31		

In Table 4, water quality classifications for four rivers considered are presented. According to the Turkish Surface Water Quality Regulation (TSWQR 2016), Class I water quality waters are high quality waters and include surface waters with high potential to be drinking water, waters that can be used for recreational purposes including those requiring body contact such as swimming, waters that can be used for trout production and waters that can be used for animal production and farm needs. Within the scope of the study, among the parameters considered, Sukesen and Yavrucak creeks fall into Class I in terms of dissolved oxygen values, while all creek waters meet Class I water quality standards in terms of pH values. Under the same regulation, Class II waters, which are classified as slightly polluted waters, encompass waters that meet both Class I water quality standards and the irrigation water quality criteria determined by the relevant legislation for irrigation purposes. Başpınar and Gölcük Creeks are classified into this category based on their dissolved oxygen and total nitrogen values. Class III waters, classified as polluted waters, can be used for aquaculture and industrial purposes after suitable treatment, excluding facilities requiring high-quality water such as those in the food and textile industries. Within the scope of the study, the total phosphorus values of all creek waters considered indicate Class III water quality.

Table 4. Quality classes in creek waters according to the Turkish Surface Water Quality Regulation (TSWQR 2016)

	Water quality classes			This study			
Parameters	**I**	**II**	**III**	**B***	**G**	**S**	**Y**
Dissolved oxygen (DO) (mg/L)	> 8	6	< 6	II	II	I	I
pH	6-9	6-9	6-9	I	I	I	I
Total phosphorus (TP) (mg/L)	< 0.08	0.2	> 0.2	III	III	III	III
Total nitrogen (TN) (mg/L)	< 3.5	11.5	> 11.5	II	II	I	I

Load estimation results

The results regarding the TP, TN, TOC and TSS loads transported to Mogan Lake through the Sukesen and Yavrucak Creeks are presented in Figure 3. As can also be seen from the figure, in Sukesen Creek, the TSS load value is higher in April, while the TP load value is higher in February compared to Yavrucak Creek. In Yavrucak Creek, especially the TN and TOC load values are considerably higher than those in Sukesen Creek in April. The increase in flow values, in parallel with the increase in precipitation, seems to have led to an increase in the loads of the respective parameters, depending on the pollution sources of both creeks.

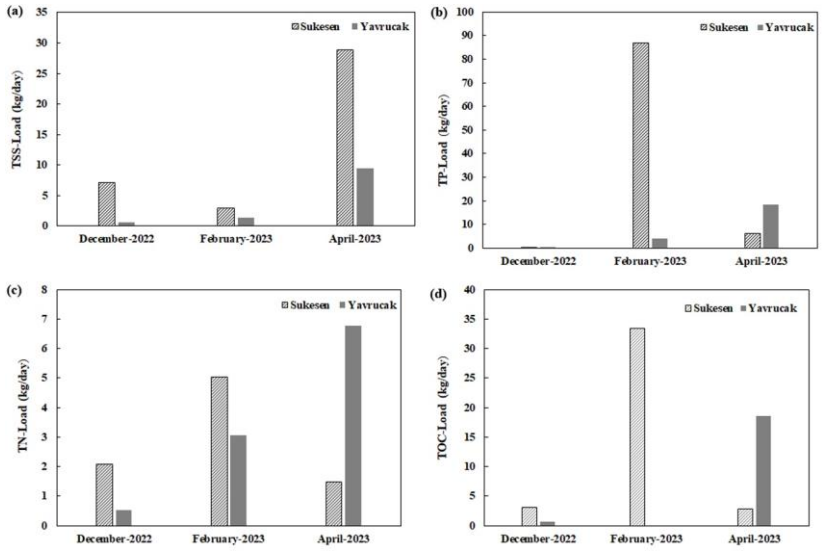


Figure 3. TSS, TP, TN and TOC loads for the Sukesen and Yavrucak Creeks

Correlation analysis

The Spearman correlation coefficient was used to determine whether there was a significant relationship between the measurement-concentration values of the 7 parameters considered in the study (Table 5). There is a moderately significant negative (opposite) relationship between water temperature and dissolved oxygen which is -0.564. In other words, as water temperature increases, the dissolved oxygen values decrease, which aligns with the phenomenon that dissolved oxygen in surface waters is primarily controlled by water temperature. Additionally, the positive correlation of 0.615 between pH values and dissolved oxygen indicates a moderately significant relationship. As can be seen in Table 5, it was found at the correlation between total nitrogen and total suspended solids was found to be at the highest level (0.647) compared to other parameters ($P < 0.05$). Furthermore, the correlation between total phosphorus and total suspended solids, total nitrogen, and total carbon was also found to be significant ($P < 0.05$). The statistically significant correlations among the mentioned four parameters indicate that these parameters likely originate from similar sources.

Table 5. Spearman correlation matrix analysis for all water quality parameters in the studied creeks

		Water temperature	DO	pH	TSS	TN	TP	TOC
Water temperature	r	1.000						
	p							
DO	r	-.564*	1.000					
	p	.000						
pH	r	-.328*	.615*	1.000				
	p	.023	.000					
SS	r	.196	-.152	.040	1.000			
	p	.183	.301	.788				
TN	r	-.079	-.169	-.068	.647*	1.000		
	p	.595	.249	.644	.000			
TP	r	.070	-.213	-.081	.324*	.581*	1.000	
	p	.634	.147	.585	.025	.000		
TOC	r	-.276	-.064	-.395*	-.114	.232	.311*	1.000
	p	.057	.664	.005	.440	.113	.032	

*P < 0.05

Possible sources of parameters in creeks

Factor analysis using the Principal Components method was conducted to determine the factorial structure of the seven measurements taken from Başpınar Creek. A KMO statistic value greater than 0.50 suggests the adequacy of sample size. Barlett's sphericity test assesses the suitability of the data for factor analysis, and a Sig. value lower than 0.05 is expected. According to Table 6, two factors with eigenvalues greater than 1 were found. The seven separate measurements were grouped into two subgroups. In the first factor, TP, TOC, TSS, and pH measurements were present, and these four measurements accounted for 62% of the measured structure. In the second factor, DO, water temperature, and TN measurements were included. Upon examining the factor loadings, it is evident that the TP measurement in the first factor is the most influential parameter for Başpınar Creek, and it has a negative direction. This result supports that the parameters TP and TOC are the most adversely affected by the pollution of this creek, particularly from wastewater originating from residential areas and agricultural activities. In the factor analysis conducted for Gölcük Creek, the first factor includes DO, TN, and TSS measurements, and these three measurements account for 61% of the measured structure. In the second factor, water temperature, TOC, TP, and pH measurements are included. In this context, the DO, TN, and TSS values for Gölcük Creek appear to be the parameters most affected by the pollution level of this creek particularly in the region where agricultural activities are intensive (Table 7).

Table 6. Total variance explaind and component matrixes for water quality parameters in Başpınar Creek

Component	Initial Eigenvalues			Variables	Component	
	Total	% of Variance	Cumulative %		1	2
1	4.308	61.541	61.541	TP	**-.996**	
2	2.583	36.907	98.448	TOC	**-.948**	.315
3	.061	.869	99.317	TSS	**.942**	.327
4	.040	.576	99.892	pH	**.884**	.448
5	.004	.059	99.951	DO		**.956**
6	.002	.025	99.976	Water temp.		**-.952**
7	.002	.024	100.000	TN	-.326	**-.931**

Extraction Method: Principal Component Analysis.

KMO=0.636 Bartlett's Test of Sphericity: Chi-Square=171.048 Df=21 Sig:0.000

Table 7. Total variance explained and component matrixes for water quality parameters in Gölcük Creek

Component	Initial Eigenvalues			Variables	Component	
	Total	% of Variance	Cumulative %		1	2
1	4.275	61.067	61.067	DO	**.993**	
2	2.551	36.438	97.505	TN	**.989**	
3	.168	2.404	99.909	TSS	**.965**	
4	.005	.073	99.982	Water temp.		**.990**
5	.001	.011	99.993	TOC	.502	**-.863**
6	000	.005	99.998	TP	.512	**.852**
7	.000	.002	100.000	pH	.549	**.754**

Extraction Method: Principal Component Analysis.

KMO=0615 Bartlett's Test of Sphericity: Chi-Square=224.997 Df=21 Sig:0.000

According to the results of the factor analysis conducted for Sukesen Creek, two factors with eigenvalues greater than 1 were found (Table 8). In the first factor, all parameters except dissolved oxygen were included, and it was determined that the contribution of wastewate for Yavrucak Creek waters, there is a significant contribution, especially on water temperature, TSS, DO, TP, and TN measurements (Table 9). Sukesen Creek was particularly high in terms of TOC and TP measurement values. In this context, it can be mentioned that the Yavrucak creek, where wastewater from agricultural activities reaches, has a negative impact on TSS and TP parameters.

Table 8. Total variance explained and component matrixes for water quality parameters in Sukesen Creek

Component	Initial Eigenvalues			Variables	Component	
	Total	% of Variance	Cumulative %		1	2
1	5.583	79.753	79.753	TOC	**.984**	
2	1.405	20.069	99.821	TP	**-.969**	
3	.008	.116	99.937	pH	**.957**	
4	.003	.049	99.987	TN	**.887**	.458
5	.001	.007	99.994	Water temp.	**.844**	.536
6	.000	.004	99.999	TSS	**-.729**	-.683
7	.000	.001	100.000	DO		**1.000**

Extraction Method: Principal Component Analysis.

KMO=0.665 Bartlett's Test of Sphericity: Chi-Square=260.613 Df=21 Sig:0.000

Table 9. Total variance explained and component matrixes for water quality parameters in Yavrucak Creek

Component	Initial Eigenvalues			Variables	Component	
	Total	% of Variance	Cumulative %		1	2
1	4.418	63.108	63.108	Water temp.	**-.992**	
2	2.487	35.523	98.631	TSS	**.947**	
3	.037	.535	99.165	DO	**.937**	-.342
4	.036	.507	99.673	TP	**-.788**	.599
5	.020	.279	99.952	TN	**.750**	.656
6	.003	.041	99.993	pH		**-.982**
7	.000	.007	100.000	TOC		**.972**

Extraction Method: Principal Component Analysis.

KMO=0.731 Bartlett's Test of Sphericity: Chi-Square=169.509 Df=21 Sig:0.000

Discussion

Anthropogenic factors in the Mogan Lake basin include the presence of a number of industrial facilities (such as aluminum coating factory, tile factory, machinery factories) due to the efficient highway transportation, unplanned housing development in parallel with the increasing population around the lake, the presence of hobby gardens increasing in number due to its proximity to Ankara as well as agricultural activities, andesite and quarries in operation. It is clear that the creeks that reach the lake by carrying the wastewater of all these elements also play a role in the pollution of the lake. This is in line with many recent studies in which pollution caused by surface waters flowing into inland water ecosystems in Türkiye originates from different anthropogenic sources (Anonymous 2013; Zeybek and Kalyoncu 2016; Tepe and Aydın 2017; Varol and Tokatlı 2023).

The temperature of surface waters is affected by latitude, altitude, season, time of day, air circulation, water flow and depth. Water temperature plays a significant role in determining the interaction and concentration levels of all water quality characteristics, especially the solubility of oxygen in water. As water temperature and salinity increase, the solubility of oxygen in water decreases (Pulatsü et al. 2014). It was determined that dissolved oxygen was generally low in the months when high temperatures were observed in the considered creek waters. The pH level, which is the measure of hydrogen ion concentration in waters, varies according to seasons and different times of the day, and it is observed that the pH measurement values in December are lower compared to the other two months (Figure 2).

Acceptable levels of total suspended solids (TSS) in water quality vary depending on the intended use of the water and represent the measure of the concentration of suspended particles in the water,

including organic and inorganic matter. Lai et al. (2013) categorized the waters based on the TSS value (mg/L) as follows: non-polluted <20; slightly polluted: 20-49; moderately polluted: 50-100; gross-polluted:>100. In this context, when considering average TSS values for all months, only Gölcük Creek (29.04 mg/L) falls into the 'slightly polluted' category. Our finding that Başpınar and Gölcük Creeks have higher TSS levels compared to the other two creeks is similar to the findings of Karakoç et al. (2003). However, the TSS values for Sukesen and Yavrucak Creeks were found to be lower than the values reported by Kapan (2011) (Table 3).

While the total organic carbon concentrations in surface waters are generally less than 10 mg/L, anthropogenic activities have a significant impact on the organic carbon budgets of aquatic ecosystems (Pulatsü et al. 2014). Especially the melting of snow, surface runoff from agricultural lands, domestic and industrial wastewater discharges, as well as rainwater overflows, can lead to significant increases in the levels of total and dissolved organic carbon in surface waters. In this study, it is thought that the maximum values of total organic carbon in creek waters, especially in February, are related to the increased surface runoff due to precipitation. In addition, Gölcük Creek has the highest TOC value (14.92 mg/L) based on the average of the three sampling months. This parameter gives an idea about the total pollution of dense organic compounds reaching this creek.

Çiçek et al. (2023) have indicated that the middle and lower sections of the Simav River (Susurluk Basin) are influenced by pollution originating from intensive agriculture, livestock farming, domestic, and industrial wastewater, and that pollution pressure has also started to increase in the upper sections. The increases observed in total phosphorus and total nitrogen concentrations in the creeks feeding Mogan Lake over the years (Table 3) are in parallel with the increase in settlement, industrial and agricultural activities in the basin. Over the years in the lake basin, it has been observed that anthropogenic activities have led to changes in hydrology. The dramatic increases in total phosphorus concentrations, especially among the parameters considered in the study, are noteworthy. It is clear that more effective reduction and management measures specific to this parameter are a necessity.

In the river mouth section of the Red River, a typical example of Southeast Asian rivers, a significant positive correlation (r^2 = 0.0757) was found between TSS and TP concentrations (Da et al. 2020). In this study, a moderate correlation (r= 0.581) was found between TP and TSS, while a positive high correlation (r = 0.647) was observed between TN and TSS. The result that TP and TN concentration levels in water samples from creeks in four geographical regions and seven provinces of Canada are positively correlated with the percentage of agricultural land in the watershed (Chambers et al. 2012) is in line with the result that the average three-month TP and TN values of Gölcük and Başpınar creeks, which pass through the region where agricultural activities are intense, are found to be high. The same researchers reported predefined trigger ranges according to the Canadian Council of Ministers of the Environment as follows: oligotrophic: ≤ 0.025 mg/L TP and ≤ 0.70 mg/L TN; mesotrophic: 0.025 to 0.075 mg/L TP and 0.70 to 1.5 mg/L TN; eutrophic: ≥ 0.075 mg/L TP and ≥ 1.5 mg/L TN. According to this, it is observed that all the creek waters considered in our study have eutrophic nutrient levels on a monthly average basis.

In surface water resources, several studies were conducted to assess spatial-temporal water quality changes and identify the main sources of pollution (Kagalou et al. 2001; Barakat et al. 2016; Chounlamany et al. 2017). The common point of these studies is related to estimating the sources of the parameters that characterize water quality (temperature, pH, EC, turbidity, total suspended solids, dissolved oxygen, ammonium, ammonia, total phosphorus, biological oxygen demand, chemical oxygen demand, etc.). In this study, the creeks feeding the lake originate from different pollutant sources. For example, Yavrucak and Gölcük Creeks transport pollutants primarily associated with agricultural activities to the lake, while Sukesen Creek carries pollutants from quarries and settlements, and Başpınar Creek conveys pollutants originating from residential areas and agricultural activities to the lake. In this context, changes in creek water quality are primarily believed to be associated with point source pollution (such as domestic and industrial wastewater), non-point source pollution (like agricultural activities), and natural processes (soil erosion). According to the results of the factor analysis conducted within the scope of the study, it can be said that TP and TOC are controlled by wastewater from both residential areas and agricultural activities in Başpınar and Sukesen Creeks. Both parameters are directly affected by urbanization and land use. Wastewater originating from agricultural activities was found to be directly responsible for DO, TN and TSS levels as in Gölcük and Yavrucak creeks. Nevertheless, the fact that Başpınar Creek enters Çökek Wetland before flowing into Mogan Lake provides an important advantage in terms of its contribution to the eutrophication of the lake. Furthermore, it is believed that the increases in various pollutant sources in the basin affect the

transport and diversity of nutrients in the creeks over the years.

Within the scope of this study, the estimated TSS and TP load values for Sukesen Creek also reflect the impact of quarries, in addition to domestic-oriented wastewater. The high TN and TOC load values for Yavrucak Creek indicate the significant amount of organic pollutants carried by agricultural-origin wastewater reaching this creek. Similar results were presented in various other studies. For example, it was reported that the wastewater from the Akçalar Town, located on the shores of Lake Uluabat, carried nutrient elements and suspended solids load to the Akçalar Creek (Musa Creek) and ultimately to Lake Uluabat (Katip et al. 2013). Similarly, it was reported that Solaklı and Sürmene Creeks in Trabzon and several other rivers flowing into the Black Sea carried nutrient elements and suspended solids loads into the sea (Boran and Sivri 2001). It was also reported that ten rivers flowing into Lake Hazar carried nutrient elements and suspended solids loads to the lake (Şen et al. 2002).

In Türkiye, numerous studies (Şen and Gölbaşı 2008, 2014; Baytaşoğlu and Şen 2015; Turan and Ülkü 2013; Gölbaşı and Şen 2019) were conducted to determine the water quality classes of surface waters that carried pollution loads to inland water sources such as lakes and reservoirs in different years. Our study findings, indicating that the dissolved oxygen and total nitrogen values for Sukesen and Yavrucak Creeks, as well as the pH values for all creeks, fall into Class I according to the Surface Water Quality Regulation, align with the results of studies on saline water sources within the Van Lake Basin (Atıcı et al. 2021) and Tersakan Creek in Muğla, which generally fall into the high-quality water class (Kasımoğlu and Yılmaz 2014). Our finding that all the creeks considered in the study fall into the category of polluted waters in terms of total phosphorus concentration supports the presence of potentially threatening pollutants in these creeks.

Within the scope of this study, the diversity of anthropogenic pollutant sources in the Mogan Lake Basin and the effects of uncontrolled increases were revealed through adverse changes in water quality parameters in the creeks. Significant increases, particularly in nutrient values compared to previous years, were detected, and this situation appeared to be related to factors such as land management practices (such as soil tillage methods, drainage applications, types of fertilizers) and climate change. Additionally, the control of water quality parameters in the creeks is challenging due to the diversity of factors contributing to pollution and the complex interactions among these factors. In this context, the development and use of seasonal and discharge-related regression models based on the monthly and yearly concentrations of the parameters considered in the study will be beneficial for making long-term predictions aimed at controlling the eutrophication level of Mogan Lake.

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The Presence and Population Status of Medicinal Leeches in the Some Wetlands in the Susurluk Basin

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ABSTRACT

In this study, the population status of the wetlands in the Susurluk Basin (except Uluabat and Manyas Lakes) in terms of medicinal leeches and the stock status of the existing areas were investigated. Between March and October 2022 and 2023, studies were carried out in a total of 108 areas, including 26 wetlands in Kütahya, 49 wetlands in Bursa and 33 wetlands in Balıkesir. Medicinal leech population and stock studies were carried out in the wetlands located in Keles Epçeler in Bursa, Balıkesir İvrindi Çelimler and Balıkesir Dursunbey Aşağımusalar regions. A total of 378 medicinal leeches (0.02-5.19 g weight and 10.48-153.92 mm) were sampled from the study areas. The catchable amount of medicinal leeches was determined as 4.956 g for Epçeler, 218 g for Çelimler and 44 g for Aşağımusalar. When the population status and stock amounts of medicinal leeches obtained from wetlands and their habitats were analysed, it was determined that there was a decrease in medicinal leeches as a result of hunting pressure and habitat change and loss. This situation has shown that breeding systems should be increased and the pressure on nature should be reduced against the increasing demand for medicinal leeches in recent years.

Keywords: Medicinal leeches, Susurluk Basin, Kütahya, Bursa, Balıkesir

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Susurluk Havzasında Yer Alan Bazı Sulak Alanların Tıbbi Sülük Varlığı ve Popülasyon Durumları

Öz: Bu çalışmada Susurluk Havzası'nda bulunan sulak alanların (Uluabat ve Manyas Gölleri hariç) tıbbi sülükler açısından popülasyon durumlarının tespiti ve mevcut alanların stok durumları araştırılmıştır. 2022 ve 2023 yıllarında Mart ve Ekim ayları arasında Kütahya'da 26, Bursa'da 49 ve Balıkesir'de 33 sulak alan olmak üzere toplam 108 alanda çalışmalar yürütülmüştür. Bursa'da Keles Epçeler; Balıkesir İvrindi Çelimler ve Balıkesir Dursunbey Aşağımusalar bölgelerinde yer alan sulak alanlarda tıbbi sülük popülasyon ve stok çalışmaları gerçekleştirilmiştir. Çalışma yapılan alanlardan toplam 378 adet tıbbi sülükte örneklenmiştir (0,02-5,19 g ağırlık ve 10,48-153,92 mm). Sulak alanlardan elde edilen tıbbi sülük popülasyon durumu ve stok miktarları ile habitatları incelendiğinde gerek av baskısı gerekse habitat değişimi ve kaybı sonucu tıbbi sülüklerde azalma olduğu tespit edilmiştir. Bu durum son yıllarda artan tıbbi sülük talebine karşı yetiştiricilik sistemlerinin artırılması ve doğa üzerindeki baskının azaltılması gerektiğini göstermiştir.

Anahtar kelimeler: Tıbbi sülükler, Susurluk Havzası, Kütahya, Bursa, Balıkesir

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Introduction

Leeches are a group of creatures in the Hirudinea class of the Annelida branch, which feed on blood and predators and have more than 800 species (Sağlam 2019; Ünal et al. 2023). Leeches are widely distributed all over the world in a variety of habitats (Zhang et al. 2008) and are also found in different ecosystems such as seas, deserts and oases, especially in fresh waters (Graf et al. 2006).

The leech species of the genus Hirudo are generally reported as Hirudo medicinalis, including in Türkiye, but as a result of detailed species diagnoses and molecular genetic studies, there are 6 species in this genus (*Hirudo medicinalis, Hirudo verbana, Hirudo orientalis, Hirudo troctina, Hirudo nipponia and Hirudo sulukii*) (Figure 1). The H. verbana species, which is also found in Türkiye, is widespread in the geography extending from Switzerland-Italy to Anatolia and Uzbekistan

(Siddall et al. 2007; Utevsky et al. 2010). *Hirudo verbana* and *Hirudo sulukii* species, which have been reported in species level studies in Turkey so far, are medicinally and economically important. However, recent genetic studies have indicated that the species *Hirudo medicinalis*, which documented in scientific literature in Türkiye, is distributed in Europe and is absent in Türkiye. The species found in Türkiye has been identified as *Hirudo verbana* (Utevsky et al. 2010; Trontelj and Utevsky 2012; Sağlam et al. 2016) (Figure 1).

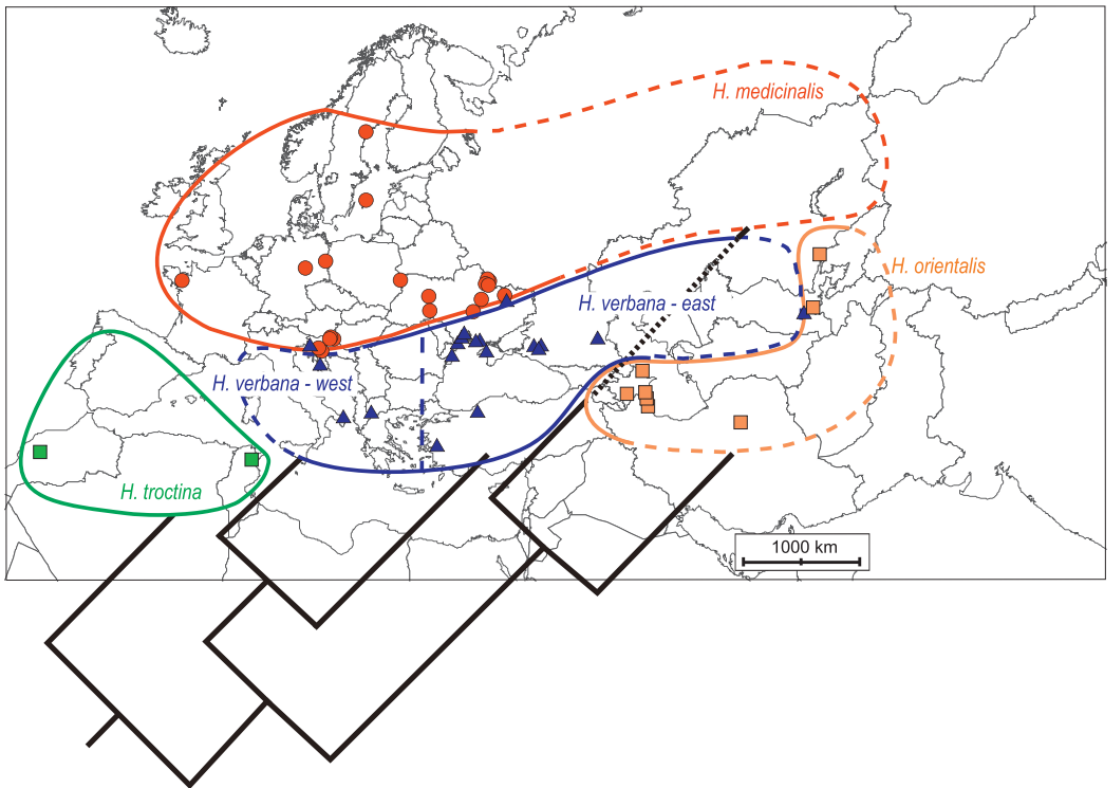

Figure 1. Distribution map of Hirudo species (Trontelj and Utevsky 2012)

They have been used for therapeutic purposes in human health since ancient times (Papavramidou et al. 2009; Gödekmerdan et al. 2011). In this context, the use of medicinal leeches has officially entered into force within the framework of the Traditional and Complementary Medicine Regulation issued by the Ministry of Health in 2014 (Anonymous 2014). Medicinal leeches, which have been used for such a long time and officially approved for use, are collected in Türkiye and exported abroad at an average price of 500 to 750 €/kg (Gödekmerdan et al. 2011). However, it is seen that higher amounts of sales are made especially abroad. For example, in Germany, medicinal leeches are sold for around 9.0-10.0 € each (Hirucult 2024), while in the USA, depending on their size, they are sold up to $ 18.15 per piece (Leeches USA 2024).

Leeches collected for medicinal purposes have experienced a dramatic decline in their populations in recent years as a result of hunting pressure, loss of general wetland habitats and pollution. In this direction, with the decline of medicinal leech species, it was added to the Red List of Threatened Species by the International Union for the Conservation of Nature and Natural Resources (IUCN) as a result of the efforts of international organizations (Trontelj et al. 2004)

The authority to manage the process related to the trade of medicinal leeches in Türkiye belongs to the Ministry of Agriculture and Forestry. As a result of this decrease in leech populations in recent years, the medicinal leech quota was applied as 8.000 kg in 2003 and 3.000 kg in 2013 (Anonymous 2002; Anonymous 2012a). Only 26.68% (1.601 kg) of the quota (6.000 kg) given by the Ministry of Agriculture and Forestry in 2010 could be exported. Accordingly, it is seen that even the quota cannot be filled and the amount exported decreases every year (Sağlam 2011). Currently, one of the most important leech exporting countries in the world is Türkiye. Türkiye is in a crucial position in the world in terms of leech exports. 86.54% of world trade is carried out from Türkiye. Under CITES, a quota is imposed by the Ministry of Agriculture and Forestry on the export of medicinal leeches from Türkiye. Since 2014, it has been applied as 2.000 kg. In 2023, it was determined

as 1.500 kg again (Anonymous 2012b and BSGM 2023).

Susurluk Basin is located in western Türkiye, between 39°-40° north latitude and 27°-30° east longitude (Anonymous 2018). Since the investigation of the medicinal leech species (*Hirudo verbana*) in the basin will contribute to both the international conservation status and the conservation and sustainable management of the relevant species, it is important to determine the population status, economic importance and to determine the level of fishable stock. In this study, the wetlands in the Susurluk Basin were screened in terms of medicinal leech Hirudo verbana populations, and the areas with medicinal leech and population status were analyzed economically and ecologically.

Material and Method

Study Area

The Susurluk Basin covers approximately 3.11% of Türkiye in terms of area and its total area is approximately 2.434.909 ha. In the basin, where the mountain system extends in the east-west direction is seen, there is Uludağ, the highest mountain belonging to the Marmara Region (Figure 2). The basin lies between the Mediterranean and the Black Sea climate. Throughout the West, summers are dry and hot, and winters are rainy and warm. As you go inland, the continental climate manifests itself. Especially in winters, these regions are cold and the coastal regions are mild in summers due to the effect of the Black Sea climate (Anonymous 2018). In the Susurluk Basin, which covers an area of more than 2 million hectares, there are many dam lakes, small lakes and ponds.

Figure 2. Susurluk Basin map (Anonymous 2018)

The study was conducted between March and October 2022 and 2023. In the study, wetland and aquatic areas (lake, dam lake, pond, etc.) located in the basin were examined. Wetlands were screened for medicinal leech population presence and economic level, and studies were conducted on population sizes, density conditions, and the amount of catchable stock (Figure 3)(Table 1).

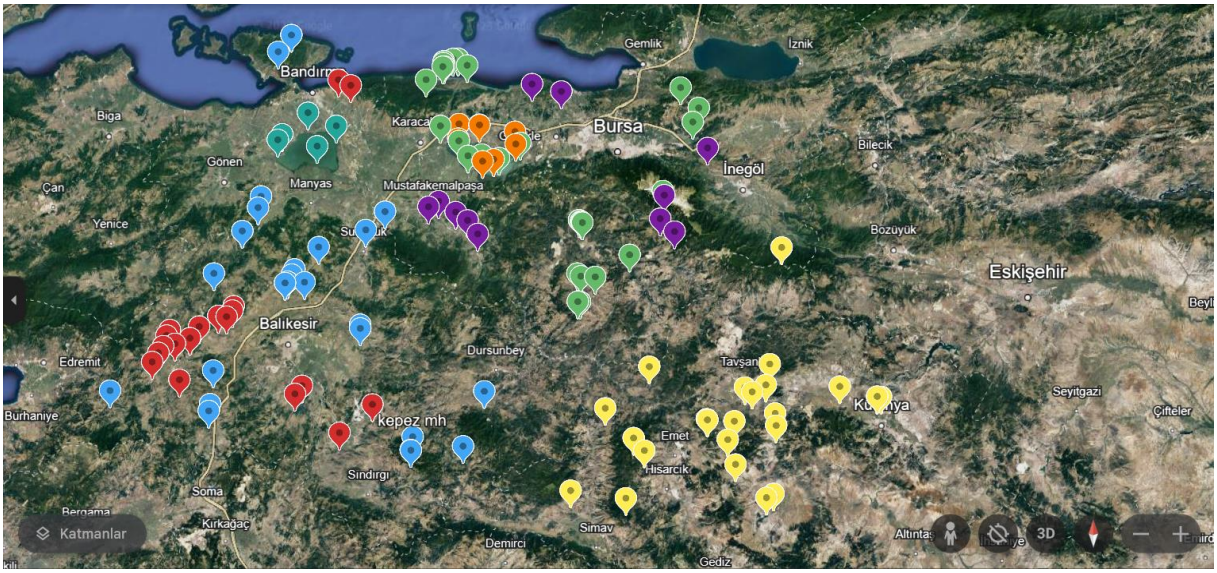

Figure 3. Medicinal leech sampling areas

Table 1. Study area sampling points and locations

No	Sampling point	Coordinate-1	Coordinate-2
	Bursa		
1	Bayramdere Pond	40°20'06.24"K	28°22'48.26"D
2	Longoz-1	40°22'40.80"K	28°25'15.74"D
3	Longoz-2	40°22'49.05"K	28°25'40.87"D
4	Longoz-3	40°22'29.64"K	28°26'32.99"D
5	Longoz-4	40°22'26.66"K	28°26'44.33"D
6	Longoz-5	40°22'17.64"K	28°26'19.01"D
7	Longoz-6	40°22'11.87"K	28°26'43.11"D
8	Dalyan Lake-1	40°23'30.27"K	28°28'18.52"D
9	Dalyan Lake-2	40°23'25.21"K	28°29'07.71"D
10	Dalyan Lake-3	40°23'33.11"K	28°29'35.48"D
11	Arapçiftliği Lake	40°22'24.69"K	28°31'21.28"D
12	Çapraz Creek	40°12'25.43"K	28°25'52.96"D
13	Karaoğlan	40°07'16.60"K	28°31'55.69"D
14	Fadıllı-1	40°08'00.51"K	28°40'24.41"D
15	Fadıllı-2	40°09'36.75"K	28°42'56.60"D
16	Akçalar	40°10'33.52"K	28°43'22.02"D
17	Kumkadı	40°10'00.49"K	28°29'51.56"D
18	Eskikaraağaç	40°12'40.72"K	28°33'58.44"D
19	Gürsu Ericek Pond	40°18'40.34"K	29°17'19.41"D
20	Kestel Nüzhetiye Gölcük Pond	40°15'26.83"K	29°21'11.45"D
21	Kestel Gölbaşı Lake	40°12'54.31"K	29°19'57.23"D
22	Keles Epçeler	40°00'30.92"K	29°13'16.49"D
23	Orhaneli Akçabük-1	39°56'38.73"K	28°55'25.61"D
24	Orhaneli Akçabük-2	39°56'29.74"K	28°55'52.63"D
25	Orhaneli Akçabük-3	39°56'21.94"K	28°56'00.75"D
26	Orhaneli Akçabük-4	39°56'15.98"K	28°56'08.10"D
27	Orhaneli Ağaçhisar	39°50'53.81"K	29°06'06.52"D
28	Büyükorhan Ciga Stream	39°47'14.81"K	28°58'45.90"D
29	Büyükorhan Cuma Stream	39°47'50.58"K	28°54'59.05"D
30	Büyükorhan Dam	39°47'21.30"K	28°55'42.64"D
31	Büyükorhan Kınık-1	39°43'08.36"K	28°55'30.01"D
32	Büyükorhan Kınık-2	39°43'09.67"K	28°55'02.66"D
33	Kestel Babasultan Dam	40°19'58.39"K	28°03'56.57"D
34	Ömerli	40°08'24.04"K	29°22'40.49"D
35	Kocaavşar-1	39°40'49.25"K	27°39'26.60"D
36	Kocaavşar-2	39°40'20.36"K	27°40'27.81"D
37	Narlı	39°41'32.17"K	27°41'4351"D

Table 1. Continue

38	Alidemirci Pond	39°42'29.65"K	27°41'57.21"D
39	Özgören Dam	39°26'00.31"K	28°11'44.00"D
40	Yörücekler Dam	39°21'33.12"K	28°04'26.97"D
41	Kemer	39°32'48.77"K	27°24'49.04"D
42	Susuzyayla	39°29'52.07"K	27°30'35.41"D
43	Yalıntaş Pond-M.K.Paşa	39°58'57.23"K	28°23'19.49"D
44	Behram	39°59'54.12"K	28°25'30.00"D
45	Hasköy	40°18'06.17"K	28°51'47.80"D
46	Çınarlı-Mudanya	40°19'21.81"K	28°45'30.39"D
47	Epçeler	40°00'30.92"K	29°13'16.49"D
48	Gököz-Keles	39°56'50.33"K	29°12'38.34"D
49	Keles Göl Kamp	39°54'41.26"K	29°15'39.30"D
	Balıkesir		
1	Erdek Yukarıyapıcı Pond	40°27'29.09"K	27°53'51.60"D
2	Erdek Pond	40°24'43.86"K	27°51'02.85"D
3	Erdek Strait	40°22'42.96"K	27°53'26.68"D
4	Manyas Necip	40° 0'33.94"K	27°47'35.68"D
5	Manyas Dam	39°58'42.61"K	27°46'58.69"D
6	Karacahisar Kocaçay	39°54'47.26"K	27°43'40.12"D
7	Balya Kocaçay	39°47'56.48"K	27°37'51.81"D
8	Susurluk	39°55'07.36"K	28°09'56.73"D
9	Susurluk Karapürçek Pond	39°58'10.65"K	28°14'05.93"D
10	Susurluk Reşadiye	39°52'14.55"K	27°59'59.23"D
11	Karesi Halkapınar	39°48'27.73"K	27°54'55.90"D
12	Karesi Karacaören	39°47'35.89"K	27°54'24.31"D
13	Karesi Karakolköy-1	39°46'23.72"K	27°53'30.88"D
14	Karesi Karakolköy-2	39°46'11.40"K	27°52'53.81"D
15	Karesi Davutlar	39°46'14.80"K	27°56'58.80"D
16	Kepsut Şerametler Kille Creek	39°38'48.66"K	28°08'54.47"D
17	Kepsut Şerametler-2	39°39'19.40"K	28°08'52.23"D
18	Savaştepe Çavlı	39°31'24.71"K	27°37'37.22"D
19	Savaştepe Sarıbeyler Dam-1	39°25'59.93"K	27°37'08.14"D
20	Savaştepe Sarıbeyler Dam-2	39°24'58.66"K	27°37'05.64"D
21	İvrindi Çelimler	39°27'57.23"K	27°15'54.08"D
22	Dursunbey Aşağımusalar	39°28'17.68"K	28°35'10.96"D
23	Sındırgı Karagöl	39°19'12.19"K	28°30'43.05"D
24	Sındırgı Kepez-1	39°18'33.90"K	28°19'36.77"D
25	Sındırgı Kepez-2	39°18'29.59"K	28°19'42.93"D
26	Sındırgı Kepez-3	39°18'21.33"K	28°19'53.59"D
27	Sındırgı Okçular-1	39°20'44.93"K	28°20'04.71"D
28	Bandırma Yeniziraatli Pond	40°19'08.11"K	28°06'40.21"D
29	İkizcetepeler Dam	39°29'05.38"K	27°56'34.53"D
30	Kocaçay	39°36'52.62"K	27°32'42.31"D
31	İvrindi Karaçepiş Pond	39°34'22.16"K	27°26'01.61"D
32	İvrindi Susuzyayla	39°29'53.59"K	27°30'34.85"D
33	İvrindi Saklıgöl	39°37'15.93"K	27°27'43.95"D
	Kütahya		
1	Domaniç Topuk Plateau	39°51'51.12"K	29°38'13.78"D
2	Kütahya Yedigöller Şehzadeler Park-1	39°26'42.59"K	29°59'10.74"D
3	Kütahya Yedigöller Şehzadeler Park-2	39°26'45.96"K	29°58'30.71"D
4	Kütahya Yedigöller Şehzadeler Park-3	39°26'41.97"K	29°58'28.22"D
5	Kütahya Yedigöller Şehzadeler Park-4	39°26'32.55"K	29°58'46.90"D
6	Kütahya İnköy Stream	39°26'42.52"K	29°59'38.07"D
7	Kütahya Enne Dam	39°28'33.26"K	29°50'31.59"D
8	Tavşanlı Devekayası	39°32'35.92"K	29°36'09.43"D
9	Tavşanlı Yağmurlu Orhaneli Creek	39°29'03.29"K	29°34'39.54"D
10	Tavşanlı Kayaboğazı Dam-1	39°24'14.81"K	29°36'42.84"D
11	Tavşanlı Kayaboğazı Dam-2	39°22'10.94"K	29°36'51.30"D
12	Tavşanlı Karacakaş	39°27'49.99"K	29°31'36.75"D
13	Tavşanlı Dağboğazı	39°28'38.46"K	29°30'20.49"D

Table 1. Continue

14	Tavşanlı Kayı Dam	39°23'04.17"K	29°27'59.42"D
15	Emet Konuş Pond	39°20'00.52"K	29°26'33.52"D
16	Emet İkibaşlı-1	39°23'20.81"K	29°22'17.92"D
17	Emet İkibaşlı-2	39°23'28.90"K	29°22'09.55"D
18	Tavşanlı Doğanlar Pond	39°32'14.29"K	29°10'10.02"D
19	Simav Toklar	39°25'25.08"K	29°00'44.91"D
20	Emet Yenice Pond	39°18'29.90"K	29°08'46.69"D
21	Emet Krater Lake	39°20'28.39"K	29°00'38.34"D
22	Simav Akdağ Örenli	39°11'40.54"K	28°53'24.86"D
23	Simav Gölcük Plateau Krater Lake	39°10'12.06"K	29°04'56.04"D
24	Emet Çerte Pond	39°15'43.54"K	29°28'02.10"D
25	Çavdarhisar Dam	39°10'12.24"K	29°34'42.59"D
26	Çavdarhisar	39°10'49.86"K	29°36'11.84"D

Medicinal Treatment and Evaluation Procedures

In the sampling of medicinal leeches, the study was carried out at water temperatures of 19 ºC and above, where they are actively present in the water (Figure 4) (Elliott and Tullett 1986). In this context; medicinal leeches were sampled with 1-hour operations in designated areas where leeches are likely to be found. In the study, firstly, the aquatic environment in which the medicinal leeches were found was walked and stirred manually and in the water environment. Thus, their activation was ensured. The floating leeches were collected with the help of a ladle or hand and placed in jars and biometric measurements were made with precision balances and digital calipers (Figure 5 and Figure 6) (Elliott 2008; Ceylan 2016).

Figure 4. Images from the sampling areas (a: Bursa Bayramdere Longoz; b: Bursa Keles Epçeler; c: Balıkesir Dursunbey Aşağımusalar; d: Kütahya Simav Toklar; e: Balıkesir İvrindi Çelimler; f: Bursa Orhaneli Akçabük)

Figure 5. Images from the sampling studies (a: Balıkesir Sındırgı Kepez; b: Balıkesir İvrindi Çelimler; c: Bursa Bayramdere)

Figure 6. Medicinal leeches measured with digital callipers

Determination of the Density of Medicinal Leeches:

In determining the densities of leeches, the method used by Sağlam (2011), Sağlam and Dörücü (2002) was applied and accordingly, the surface area (m^2) where the leeches were sampled was determined and the density of the leeches (pcs/m^2) was calculated by dividing the number of leeches sampled (pieces) by the surface area.

Estimating the Stock Status of Medicinal Leeches:

The "Area Scanning" method was used to estimate the stock amount of medicinal leeches (Sparre and Venema 1998; Avşar 2005). In this method, the approach based on determining the catchable stock amount by reflecting/proportioning the biomass value obtained in the sub-areas determined in the relevant wetland to the whole area was used. For this purpose, data on leech density per unit area, average weight of populations and the surface area where medicinal leeches can live were used. The number of leeches in each sample area was determined by multiplying the predicted surface area (m^2) where medicinal leeches can live by the density of medicinal leeches in the relevant sample area (pcs/m^2). Then, this value was multiplied by the average weight (g) of the relevant populations determined by individual weighing to estimate the catchable stock of medicinal leeches in the wetland (kg) (Ceylan 2016).

Determination of Water Quality Parameters

Each wetland was sampled and water samples were taken. For this purpose, water temperature (°C), dissolved oxygen (DO) (mg/L) concentration and oxygen saturation (OD) (%), pH, electrical conductivity (EC) (µS/cm (25°C)), total dissolved solids (TDS) (mg/L), salinity (ppt) parameters were determined in the field with WTW 3620i multiparameter meter. Ammonium nitrogen (NH_4-N) (mg/L), nitrite nitrogen (NO_2-N) (mg/L), nitrate nitrogen (NO_3-N) (mg/L), Ortho-phosphate (PO_4-P) (mg/L), sulfate (SO_4) (mg/L), hardness (mg/L

$CaCO_3$), turbidity (NTU), organic matter as $KMnO_4$ consumption (mg/L) were analyzed in the laboratory. For the analysis of the parameters, 1 L water samples were taken from each sampling locations and transported to the chemistry laboratory of Eğirdir Fisheries Research Institute Directorate. SO_4, organic matter and hardness were analyzed by titrimetric analysis, turbidity by turbidimeter, NH_4-N, NO_2-N, NO_3-N and PO_4-P by spectrophotometer in the laboratory (APHA 1971; APHA 1995; TSE 1996; Egemen and Ünlü 1996; WTW 2015).

Calculation of the Condition Factor

The condition factor is the formula that best controls the morphological structure in living organisms. It is one of the criteria for nutrition and development. In general, it is desired that the condition factor is close to 1. The condition factor is calculated by the following formula (Martinez and Vasquez 2001).

$$K = \frac{\text{(Medicinal leech weight (g))}}{\text{(Medicinal leech length (mm))}^3} \times 100$$

Regression Analysis

The length-weight relationship was calculated region by region for individuals and the whole population and the length-weight relationship curves were drawn. The equation is as follows.

$W=a*L^b$ (Bagenal and Tesch 1978)

was used to calculate length-weight relationships. In this equation; W= weight of medicinal leech (g), a and b are relationship constants, L= total length (mm). The parameters of the length-weight relationship were determined by linear regression transformation of the relation as below.

$LogW = Loga + b\ LogL$

Statistical Analysis

The data obtained as a result of the research were evaluated with the help of the SPSS 25.0 package program and Microsoft Excel 2021. The importance level was accepted as α=0.05 in all statistical tests (Özdamar 2011).

Results

During the study period, sampling was carried out in 49 areas excluding Uluabat Lake in Bursa province, 33 areas excluding Manyas Lake in Balıkesir province and 26 areas in Kütahya province (Figure 6). In the study, areas with the presence of medicinal leeches and areas where their presence was previously reported but could not be detected were determined. Medicinal leech sampling was carried out in Keles Epçeler in Bursa, İvrindi Çelimler and Dursunbey Aşağımusalar in Balıkesir (Figure 7). Although the presence of medicinal leeches was previously reported in Bayramdere Longoz and Orhaneli Akçabük in Bursa province; Manyas Necip and İvrindi Susuzyayla in Balıkesir province; and Simav Örenli and Toklar in Kütahya province, no samples were detected in the study. Only 2 medicinal leeches were obtained in Balıkesir Sındırgı Karagöl and were not evaluated statistically since sufficient samples could not be obtained (Figure 8). In other areas, no medicinal leech presence was found. The data of the medicinal leeches obtained in the study are given in Table 2, length-weight distribution regression graph is given in Figure 9, weight distribution histogram graph is given in Figure 10 and length distribution histogram graph is given in Figure 11.

Table 2. The lowest, highest and average data of the samples obtained from the areas with medicinal leeches in Bursa (Epçeler) and Balıkesir (Çelimler and Aşağımusalar) province

Bursa Keles Epçeler			
Number of samples		**226**	
	Minimum	**Maximum**	**Average**
Weight (g)	0.07	5.19	0.92±0.94
Length (mm)	10.48	153.92	81.24±0.94
Condition factor	0.05	0.45	0.14±0.05
Balıkesir Ivrindi Celimler			
Number of samples		**139**	
	Minimum	**Maximum**	**Average**
Weight (g)	0.02	1.92	0.15±0.26
Length (mm)	30.57	104.07	52.11±12.50
Condition factor	0.03	0.27	0.08±0.04
Balıkesir Dursunbey Aşağımusalar			
Number of samples		**13**	
	Minimum	**Maximum**	**Average**
Weight (g)	0.34	1.84	0.97±0.42
Length (mm)	74.20	119.95	93.95±14.29
Condition factor	0.08	0.17	0.11±0.02

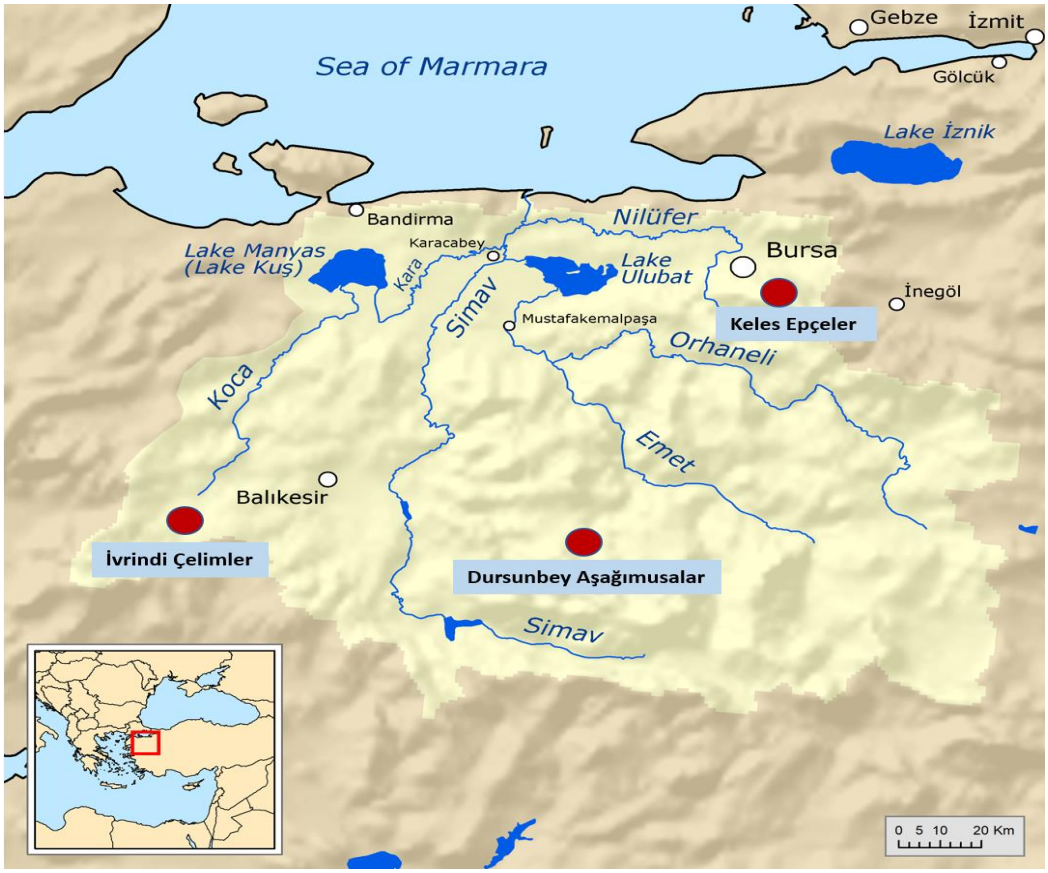

Figure 7. Map of medicinal leech sampling areas

Figure 8. Map of areas where the presence of medicinal leeches was previously reported but not found in the sampling study

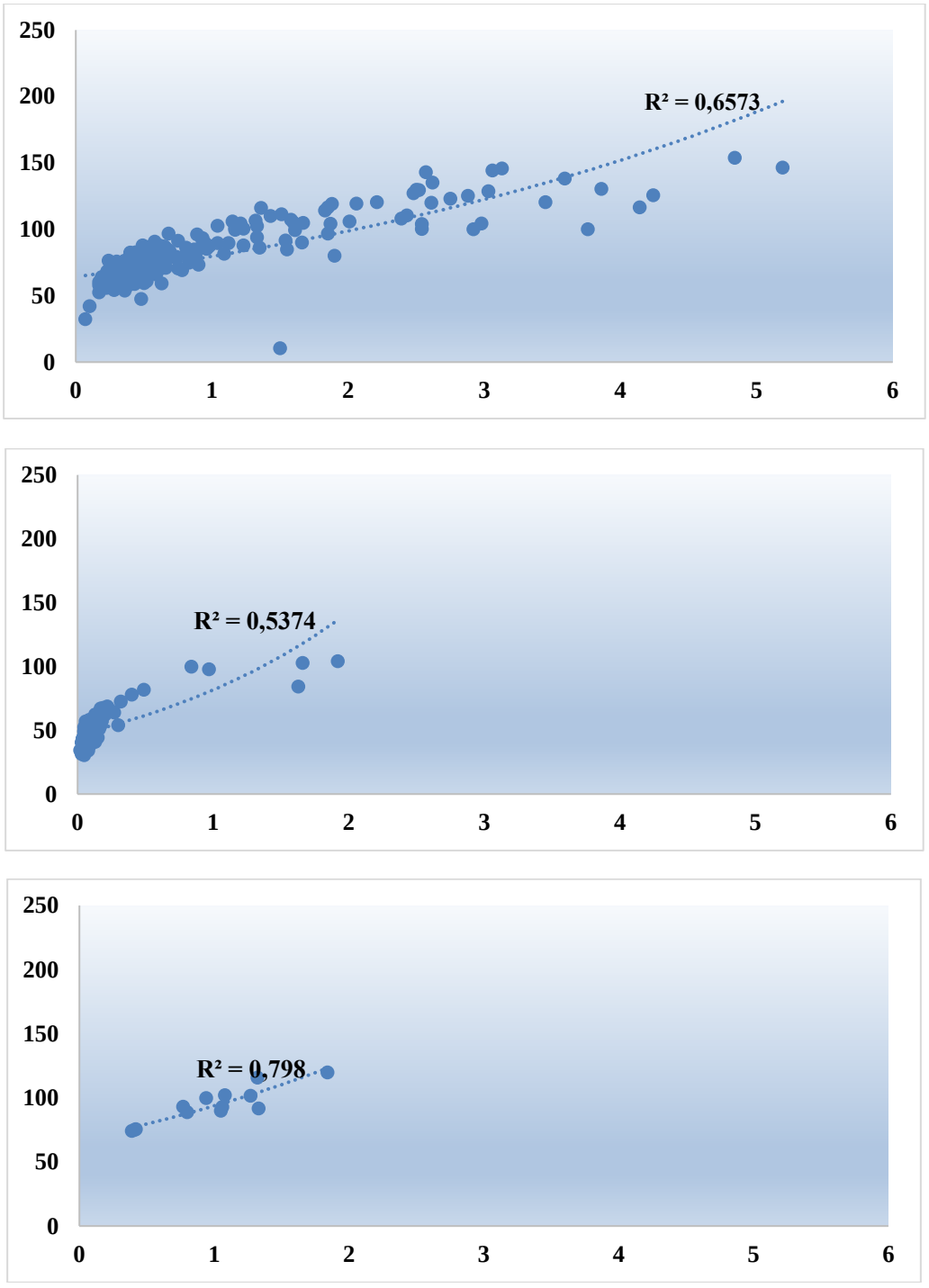

Figure 9. Epçeler (Bursa), Çelimler and Aşağımusalar (Balıkesir) medicinal leech length-weight distribution regression graph

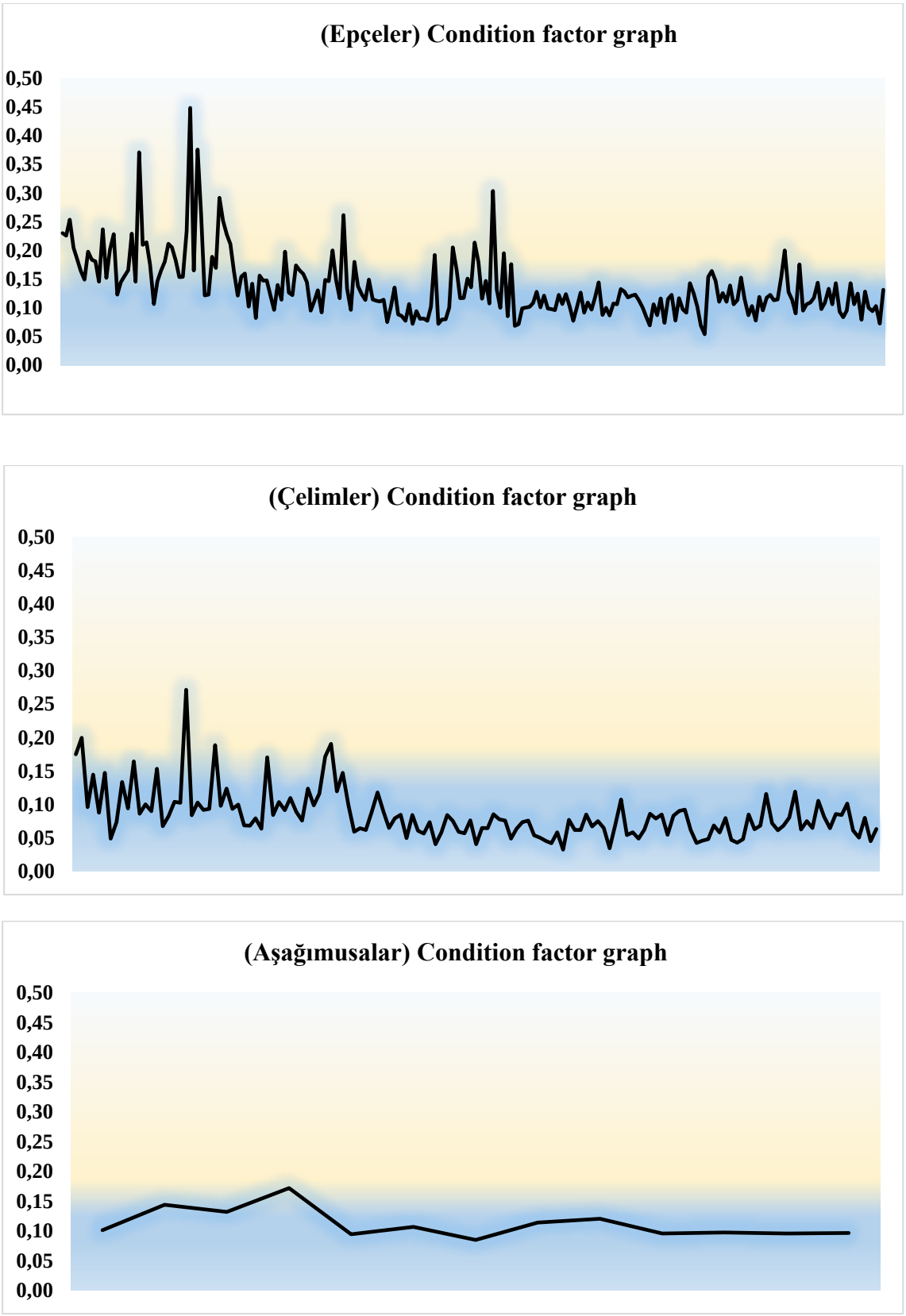

Figure 10. Epçeler (Bursa), Çelimler and Aşağımusalar (Balıkesir) medicinal leech condition factor graph

Densities and catchable stocks of medicinal leeches obtained from Bursa Keles Epçeler, Balıkesir İvrindi Çelimler and Dursunbey Aşağımusalar are given in Figure 11.

	**Bursa Keles Epçeler**
Medicinal Leech Density	3.77 pieces/m^2
Catchable Medicinal Leech Amount	4.956 g
	**Balıkesir İvrindi Çelimler**
Medicinal Leech Density	0.46 pieces/m^2
Catchable Medicinal Leech Amount	218 g
	**Balıkesir Dursunbey Aşağımusalar**
Medicinal Leech Density	0.04 pieces/m^2
Catchable Medicinal Leech Amount	44 g

Figure 11. Epçeler (Bursa) and Çelimler, Aşağımusalar (Balıkesir) densities of medicinal leeches and catchable leeches

The water quality results of the areas where medicinal leeches were obtained are given in Table 3. When the water quality data obtained from the wetlands where medicinal leeches are obtained are examined, it is seen that there is relatively nitrogen and phosphorus content, however, it contains conductivity values with an average range of 400-600 µS/cm and salinity values with a range of 0.0-0.2. It is also seen that the total alkalinity content is around 200 mg/L and the total hardness level is around 345 mg/L. This situation shows that medicinal leeches are organisms with high tolerance levels in terms of water quality, however, they are more effective especially in freshwater environment and prefer relatively clean waters.

Table 3. Water quality data of areas with medicinal leeches

	Bursa Keles Epçeler	Balıkesir İvrindi Çelimler	Balıkesir Dursunbey Aşağımusalar
Sampling time:	June 2022	July 2022	July 2022
Water temperature (^{o}C):	22.3	24.6	23.6
Dissolved oxygen (mg/L):	8.03	10.1	10.4
O_2 saturation (%):	93.2	112.1	113.1
pH:	9.01	8.56	8.65
Conductivity (µS/cm):	456	501	643
Total dissolved solids (mg/L):	0.342	0.377	0.498
Salinity (%):	0,2	0,2	0.2
Turbidity (NTU):	2,4	3.1	2.2
Total alkalinity(mg/L):	231.32	198.65	212.45
Total hardness (mg/L):	337	352	348
Ammonium (mg/L):	0.199	0.157	0.117
Nitrite (mg/L):	0.043	0.055	0.054
Nitrate (mg/L):	2.32	1.76	1.09
Ortho-phosphate (mg/L):	0.084	0.071	0.096

Discussion

In the study investigating the presence of medicinal leech populations and the size-weight distribution and stock amount of leeches in the areas where leeches were found, a total of 108 areas, including 26 wetlands in Kütahya, 49 wetlands in Bursa and 33 wetlands in Balıkesir, were surveyed in 2022 and 2023.

Kasparek et al. (2000), determined the presence of medicinal leeches in 42 of 65 wetlands surveyed in Türkiye using semi-quantitative method. The strongest populations were found in the Kızılırmak Delta, Yeşil Irmak Delta and Karagöl wetlands. Susurluk Basin is also one of the important wetlands of Türkiye in terms of the presence and population of medicinal leeches. Medicinal leeches have been identified in the wetlands of the basin, especially in Uluabat and Manyas Lakes, and have formed populations in certain areas.

Elliott (2008) used reduction-based "Maximum Likelihood" (Zippin 1956) and "Regression" (Leslie and Davis, 1939) methods to estimate *Hirudo medicinalis* population size at Jenny Dam. It was determined that the leech population ranged between 248-288 individuals over the years (1986-1992). It was found that the least represented group in the population was over 5 g with approximately 1% and the most represented group was the immature group (between 0.4-3.4 g). In this study, samples between 0.15 g and 0.97 g were obtained on average. Medicinal leeches collected in Çelimler region were found to be lower in weight compared to other regions.

Sağlam et al. (2008), reported that *Hirudo medicinalis* was found in 22 of 87 wetlands in the Eastern Anatolia Region. Ekman bucket and modified leech frame were used to capture leeches, and time-based collection method was preferred to determine leech density. It was reported that the average weight of leeches collected from the relevant wetlands was 1.90 g. With the genetic identification studies carried out in recent years, it has been determined that the species previously reported as *H. medicinalis* in Türkiye is actually *H. verbana*. When the data obtained in this study are evaluated with this study, it is concluded that medicinal leeches show that they grow well in areas with intense hunting pressure and low habitat destruction.

Ceylan (2023), in his study in Sındırgı Karagöl, reported that the number of medicinal leeches, which they found 12 in 2012, was 2 in 2022. In our study in the same wetland, 2 medicinal leeches were obtained.

Ceylan (2016) investigated the ecology, population size and hunting efficiency of medicinal leech *Hirudo verbana* populations in the wetlands around Lake Eğirdir and estimated the amount of medicinal leeches that can be hunted in the wetlands around Lake Eğirdir as 1,988,700 (593 kg). Ceylan et al. (2017) reported that they sampled leeches from 232 different habitats in wetlands within the borders of Afyonkarahisar, Burdur, Denizli, Isparta and Konya within the scope of the project "Investigation of the Leech Fauna and Economic Importance of the Lakes Region" carried out by TAGEM between

2011-2014, and as a result of the study, leeches were detected in 119 habitats. In their study carried out in the wetlands of the Lakes Region indicated a presence of a catchable stock of 142.46 kg (1.166.000 leeches) of medicinal leeches in the region. In the research Lake Eğirdir with 481.05 kg, Lake Gavur with 226.34 kg and Lake Karamık with 82.78 kg were determined as the habitats with the highest amount of catchable medicinal leeches. However, in recent years, wetlands in provinces such as Afyonkarahisar, Konya, Isparta, Denizli, especially in Lake Eğirdir, have seen serious population declines due to both the intense effects of local climate change, intense hunting pressure against medicinal leech populations and habitat change. In this context, it is significant to switch to sterile medicinal leech production as soon as possible due to excessive demand.

As a result, the use and popularity of medicinal leeches have increased in recent years and their stock amounts have been decreasing considerably due to hunting pressure and habitat loss in nature. Türkiye, which ranks first in exports in the world, has reduced the quota from 10 tonnes to 2 tonnes due to these problems. In this context, the cultivation of medicinal leeches should be started as soon as possible and hunting from nature.

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Conflict of Interest Declaration

The authors declare that there are no financial interests or personal relationships that may have influenced this work.

Ethical Approval Statement

Since experimental animals were not used in this study, Local Ethics Committee Approval was not obtained.

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Effect of low-dose irradiation on lipid quality and fatty acid composition in vacuum-packed hot smoked trout (*Oncorhynchus mykiss*) fillets during cold storage

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ABSTRACT

The effects of gamma irradiation at different doses (0, 3 and 5 kGy) on lipid quality and fatty acid composition in vacuum-packed hot smoked rainbow trout (*Oncorhynchus mykiss*) fillets during cold storage (2 °C) were investigated. The major fatty acids were identified as palmitic, oleic, linoleic and docosahexaenoic acids (DHA). The fatty acid compositions were not affected by the irradiation process initially. However, the increase on the total saturated fatty acids (SFA) of irradiated fillets was higher than the control group at the end of the storage. While a significant decrease was observed in the control group of total polyunsaturated fatty acids (PUFA), no change was observed in the groups irradiated with 3 and 5 kGy doses at the end of the storage. The TBA values of 0, 3 and 5 kGy irradiated groups were 1.27, 1.46 and 1.58 mg MA / kg, respectively, the PV values were 6.12, 9.18 and 9.97 meq / kg and the FFA values were 5.36%, 5.67% and 6.10%, respectively, at the end of the storage. Using a combination of techniques to various processed or fresh seafood products will likely play a significant role in enhancing the manufacture of safe meals with extended shelf lives.

Keywords: Irradiation, hot smoked, *Oncorhynchus mykiss*, fatty acids, lipid quality

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Düşük Doz Işınlamanın, Soğukta Muhafaza Sırasında Vakumla Paketlenmiş Sıcak Tütsülenmiş Alabalık (*Oncorhynchus mykiss*) Filetolarında Lipit Kalitesi ve Yağ Asidi Bileşimi Üzerine Etkisi

Öz: : Farklı dozlarda (0, 3 ve 5 kGy) gama ışınlamasının, soğukta (2 °C) depolanan vakum paketli sıcak tütsülenmiş gökkuşağı alabalığı (*Oncorhynchus mykiss*) filetolarında lipit kalitesi ve yağ asidi bileşimi üzerine etkileri araştırılmıştır. Başlıca yağ asitleri palmitik, oleik, linoleik ve dokosaheksaenoik asitler (DHA) olarak tanımlanmıştır. Yağ asidi bileşimleri başlangıçta ışınlama işleminden etkilenmemiştir. Ancak depolama sonunda ışınlanmış filetoların toplam doymuş yağ asitleri (SFA) artışı kontrol grubuna göre daha yüksek olduğu tespit edilmiştir. Depolama sonunda toplam çoklu doymamış yağ asitleri (PUFA) miktarında kontrol grubunda önemli bir azalma gözlenirken, 3 ve 5 kGy dozları ile ışınlanan gruplarda herhangi bir değişiklik gözlenmemiştir. Depolama sonunda 0, 3 ve 5 kGy ışınlanan grupların TBA değerleri sırasıyla 1,27, 1,46 ve 1,58 mg MA/kg, PV değerleri 6,12, 9,18 ve 9,97 meq/kg, FFA değerleri ise %5,36, %5,67 ve %6,10 olarak belirlendi. Çeşitli işlenmiş veya taze su ürünlerine yönelik tekniklerin bir kombinasyonunun kullanılması, uzun raf ömrüne sahip güvenli yiyeceklerin üretiminin arttırılmasında muhtemelen önemli bir rol oynayacaktır.

Anahtar kelimeler: Işınlama, sıcak tütsüleme, *Oncorhynchus mykiss*, yağ asitleri, lipit kalitesi

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Introduction

The great increase in foodborne poisoning and infections in recent years has revealed the need for investments in this area. Changes in consumer lifestyles and the demand for ready-made food, especially in developed and developing countries, have accelerated research on food processing and preservation methods. If appropriate additives are not added to ready-made foods, especially ready-to-use meat products, their shelf life is limited. Since meat and seafood products create an ideal environment for microorganisms, pathogenic organisms that may occur in these products pose a great risk to human health. Due to the recent trend towards foods that do

not contain food additives and the frequent problems in cold chain applications, the desired shelf life cannot be achieved in the final products and microbial risks are observed.

Consumers' preference for fresh products and their unwillingness to eat products with additives or frozen and thawed products has led producers to adopt methods that ensure food safety with minimal changes to the product. Since there is no significant temperature increase in the product during the "irradiation application" of foods, this method is called "cold sterilization". Irradiation causes minimal changes in the appearance of the food and can preserve the nutritional properties of the food better than other food processing methods. No chemical residue formation is observed in the environment with irradiation. For this reason, it enables the reduction of the use of additives used in foods today or the processing of foods of very different sizes and shapes (Yagiz 2008).

Food irradiation, in its simplest definition, is the treatment of any food substance with a certain type of energy. In this application, the food item in a package or box is exposed to ionizing radiation for a certain period. In this process, no matter how long the food is exposed to radiation or how much of the dose used is absorbed, the normal radioactivity in the food's own structure does not increase (ICGFI 1999). It has been proven that irradiated foods are safe for health and do not have any effects on humans (Tauxe 2001). The safety of food irradiation has been confirmed by the studies of many organizations such as USDA, FDA, FSIS, IAEA, FAO and WHO, and its effect on maintaining food quality safety has been demonstrated.

Smoked trout exports in Türkiye are just over 4000 tons according to TUİK (2021) data. The application of gamma irradiation to increase the export of healthy and reliable products with a longer shelf life without changing the taste of the smoked product brings to mind the idea that these products can create an attractive product class in the foreign market. Therefore, in this study, the combined effect of low doses (0, 3 and 5 kGy) irradiation with methods used in food preservation such as smoking and vacuum packaging on the shelf life of trout fillets was focused. For this aim, the effects of low-dose irradiation on lipid stability (TBA, FFA and PV) and fatty acid compositions in vacuum-packed hot smoked trout (*Oncorhynchus mykiss*) fillets during cold storage (2 °C) were investigated.

Materials and Methods

Fish Samples

Preparation, packaging and storage

The trout samples (*Oncorhynchus mykiss*) were obtained from a local processing company (Sasu A.Ş., Adana, Türkiye). The mean weight of the fish was 238.78±21.00 g. After gutting, beheading and skinning, the fish was filleted for the smoking process. The fillets were then kept waiting in a brine solution of 8-9 % salt for 12 hours and smoked at 60-70 °C in a smoking oven. After smoking, samples were divided into 150 g of fillets, wrapped in polyethylene bags and sealed under a vacuum (Özkütük 2002).

Irradiation Process

Packed samples were placed in styrofoam boxes (50x30x35 cm) surrounded with cooling cartridges and transported to the Sarayköy Nuclear Research and Training Center (SANAEM, Ankara) within 24 hours under cold conditions (0-2 °C). Control group samples were transported under the same conditions but were not subjected to irradiation. The fillets were irradiated with doses of 3 and 5 kGy using ^{60}Co source irradiator (PX-g-30 Issodovateji, dose rate 2.72 kGy/h and power 316,000 curries). Absorbance rate was measured by dosimeters (3042 Harwell Amber acrylic, polymethyl methacrylate, PMMA) stuck to the surfaces of the boxes. The absorbance rates measured for 3 kGy were 3.10, 3.08 and 3.52 and for 5 kGy, 5.17, 5.27 and 5.24 (Etyemez 2011). All groups were returned to seafood processing laboratory at University of Cukurova, Faculty of Fisheries, without breaking the cold chain.

Sampling

Samples stored in cold storage (2 °C) were analyzed on storage weeks 0, 2, 4, 6, 8, 11, 14, 17, 20 and 23. On each analysis day, three randomly chosen packages from all groups were evaluated chemically.

Analytical Methods

Thiobarbituric acid (TBA) value was determined according to Tarladgis et al. (1960) by using a spectrometer (Perkin Elmer Lambda 25 UV/VIS Spectrometer). The samples were analyzed in triplicates for each treatment group. 10 mg homogenized samples weighed out and 97.5 mL of distilled water and 2.5 mL of 1:2 HCl were added on to the samples. The mixture was distilled until 200 mL of distillates were gathered by using a distillation unit (Buchi Distillation Unit B-324). The distillates and TBA reactive were added in capped tubes in equal measures of 5 mL in duplicates and boiled in water for 35 minutes until the solution turned to a reddish color. After cooling at room temperature, the solutions were measured by using a spectrometer under 538 nm wavelength. The results were expressed as values of mg malonaldehyde in 1000 g of samples.

Peroxide value (PV) was determined according to AOCS 1994 method Cd8-53. The samples were

analyzed in triplicates for each treatment group. 50 mL of acetic acid: chloroform (60:40) solution was added and the samples were shaken until the lipid was dissolved. After adding 1 mL of satiated potassium iodide and shaking the solutions for 20 seconds, the solutions were kept waiting for 30 minutes in an enclosed dark closet. The samples were titrated with 0.002 M sodium thiosulphate until an opaque solution was observed, after adding 100 mL of distilled water and 4-5 drops of 1% starch solution. Calculations for PV content were carried out as follows:

$$Peroxide\ Value\ = \frac{2(C-B)}{W}\ \text{meq O2/kg}$$

C: Spent 0.002 M sodium thiosulphate (mL)

B: Spent 0.002 M sodium thiosulphate for null (mL)

W: Sample weight

Free fatty acid (FFA) analysis was determined according to AOCS 1994 method Ca 5a-40. The samples were analyzed in triplicates for each treatment group. Initially, 0.5 g of lipid samples were dissolved in 50 mL of diethylether:ethanol (50:50). After that 1 mL of 1 % phenolphthalein indicator was added to the samples. The aliquots were titrated under 0.1 M sodium hydroxide until a pinkish color was obtained (at least 15 seconds). % of free fatty acids in oleic acid were calculated as follows:

$$\%\ Free\ fatty\ acids = \frac{(C-B)\times 2.805}{W}$$

C: Spent 0.1 M sodium hydroxide (mL)

B: Spent 0.1 M sodium hydroxide for null (mL)

W: Sample weight

2.805: Conversion factor

Lipid samples (extracted by the method of Bligh and Dyer 1959) were converted to their fatty acid methyl esters as described by Ichihara et al. (1996). Transmethylation was carried out by 20 mg of extracted lipid sample (in duplicate) dissolved in 4 mL of *n*-heptane and mixed with 4 mL of 2 M KOH in methanol. The mixture was vortexed for 10 s and then centrifuged for 10 min at 4000 rpm. After the process *n*-heptane layer was taken for GC analyses. The fatty acid composition was analyzed by using a Clarus 500 gas chromatography with an autosampler (Perkin Elmer, Shelton, Conn., U.S.A.) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m × 0.32 mm ID × 0.25 m BP20 0.25 UM; SGE Analytical Science Pty. Ltd., Victoria, Australia). Initially, the oven temperature was held at 140 °C for 5 min. After that, the temperature was increased to 200 °C at a rate of 4 °C/min and then to 220 °C at a rate of 1 °C/min. The injector temperature was set at 220 °C and the detector temperature was at 280 °C. The carrier gas was controlled at 16 ps. The split used was 1 : 100. Fatty acids were identified by comparing the retention times of fatty acid methyl esters (FAMEs) with the Standard 37-component FAME mixture (Sigma-Aldrich Chemie Gmbh, Munich, Germany). Two replicate analyses were performed and the results were expressed as gas chromatographic area (%, mean ± standard deviation).

Statistical Analysis

Statistical analyses were performed using SPSS 14. The mean values and standard deviations were calculated from data obtained from the samples for each irradiation dose and storage time treatments. Data were subjected to analysis of variance (one-way ANOVA), Duncan's multiple range test and t-test at 5% confidence level.

Results

The fatty acid composition of smoked rainbow trout fillets irradiated at different doses (0, 3 and 5 kGy) is shown in Tables 1, 2 and 3. It was determined that the essential fatty acids of control and irradiated (3 and 5 kGy) smoked rainbow trout fillets were palmitic acid (C16:0), oleic acid (C18:1ω9), linoleic acid (C18:2ω6) and docosahexaenoic acid (DHA, C22: 6ω3). Similar results for rainbow trout were reported by Haliloğlu et al. (2004), Oraei et al. (2011) and Yıldız et al. (2006).

Initially, fatty acid values of control, irradiated at 3 and 5 kGy doses generally showed no statistically significant differences ($p>0.05$). Fluctuations in fatty acid values in all groups were observed during cold storage. The main saturated fatty acids (SFA) were palmitic acid (C16:0), myristic acid (C14:0) and stearic acid (C18:0) (Table 1). Palmitic acid was observed to be significantly ($p<0.05$) higher in the group irradiated with a dose of 5 kGy throughout the storage period than in the other groups. While the palmitic acid values of the control group were higher than the 3 kGy group at the beginning of storage, it was observed that the palmitic acid contents of the 3 kGy group were higher towards the end of storage. Similarly, it was observed that stearic acid rates were significantly ($p<0.05$) higher in the 5 kGy group throughout the storage than in the other groups. In addition, it was observed that the stearic acid values of the 3 kGy groups were generally higher than the control group during storage period. At the end of the

storage period, no significant change was observed in the myristic acid values of the control, 3 and 5 kGy dose irradiated groups ($p>0.05$). It was determined that the myristic acid values of the 5 kGy group were significantly higher than the group irradiated with a dose of 3 kGy in the 23rd week of storage ($p<0.05$). While no change was observed in palmitic acid values at the end of storage in the control group ($p>0.05$), a significant increase was observed in the irradiated groups (3 and 5 kGy) ($p<0.05$). Therefore, it can be concluded that there was an increase in total saturated fatty acid values in parallel with the increase in the irradiation dose applied during storage.

Table 1. Saturated fatty acids (SFA) changes during cold storage of non-irradiated (0 kGy), 3 kGy and 5 kGy irradiated smoked trout fillets

		Storage Period (Weeks)									
Fatty Acids		Day 0	2	4	6	8	11	14	17	20	23
C12	0	-	0.68 (0.01)^{dB}	-	0.34 (0.00)^{b} B		0.17 (0.07)^{aA}	0.43 (0.06)^{bc} A	0.49 (0.01)^{cA}	0.47 (0.01)^{cB}	-
	3	-	0.53 (0.01)^{eA} B	-	0.23 (0.01)^{ab} A	-	0.14 (0.08)^{aA}	0.28 (0.04)^{bc} A	0.44 (0.03)^{deA}	0.35 (0.01)^{bc} dA	-
	5	0.42 (0.01)^{d} A	0.38 (0.06)^{ab} cA	-	0.27 (0.03)^{a} A	0.39 (0.05)^{ab} cA	0.27 (0.12)^{aA}	0.42 (0.05)^{ab} cA	0.43 (0.01)^{bcA}	0.35 (0.06)^{ab} A	0.51 (0.06)^{c} A
C14	0	2.50 (0.23)^{ab} cA	2.20 (0.01)^{aA}	2.66 (0.10)^{bcd} B	2.41 (0.04)^{ab} A	2.21 (0.02)^{aA}	2.24 (0.10)^{aA} B	2.72 (0.04)^{cd} A	2.81 (0.06)^{dB}	2.72 (0.25)^{cd} A	-
	3	2.58 (0.04)^{ab} A	2.35 (0.05)^{aA}	2.27 (0.06)^{aA}	2.30 (0.09)^{a} A	2.35 (0.06)^{aA}	2.73 (0.30)^{bc} B	2.90 (0.07)^{cA}	2.38 (0.19)^{abA}	2.41 (0.06)^{ab} A	2.48 (0.04)^{ab} A
	5	2.99 (0.15)^{de} A	2.44 (0.09)^{bc} B	2.45 (0.01)^{bcA} B	2.40 (0.01)^{bc} A	2.35 (0.09)^{b} A	2.08 (0.11)^{aA}	3,14 (0.06)^{eB}	2.49 (0.01)^{bcA} B	2.59 (0.06)^{cA}	2.85 (0.13)^{d} B
C16	0	12.83 (1.00)^{bc} dA	12.50 (0.01)^{bc} B	12.26 (0.38)^{bcA}	12.46 (0.13)^{bc} B	11.46 (0.08)^{aA}	12.45 (0.23)^{bc} A	12.53 (0.09)^{bc} A	13.30 (0.40)^{cdA}	13.74 (0.05)^{d} A	-
	3	12.06 (0.27)^{ab} A	11.95 (0.13)^{ab} A	11.78 (0.18)^{aA}	11.64 (0.06)^{a} A	12.89 (0.43)^{bc} B	13.03 (1.29)^{bc} dA	13.16 (0.20)^{cd} AB	14.03 (0.25)^{dA}	13.88 (0.00)^{cd} A	13.17 (0.01)^{cd} A
	5	12.43 (0.57)^{aA}	12.64 (0.04)^{aB}	12.42 (0.06)^{aA}	12.69 (0.03)^{aB}	13.50 (0.12)^{bB}	12.67 (0.05)^{aA}	13.73 (0.31)^{bc} B	14.94 (0.07)^{eB}	14.43 (0.18)^{de} B	14.07 (0.30)^{cd} B
C17	0	0.10 (0.01)^{b} A	0.09 (0.00)^{b}	0.10 (0.01)^{bB}	0.09 (0.00)^{b}	0.06 (0.03)^{aA}	0.08 (0.00)^{ab} A	0.09 (0.01)^{bA}	0.09 (0.01)^{abA}	0.09 (0.01)^{ab} A	-
	3	0.10 (0.01)^{bc} A	0.09 (0.00)^{ab} cA	0.08 (0.00)^{aA}	0.09 (0.00)^{ab} cA	0.09 (0.01)^{ab} A	0.10 (0.01)^{bc} A	0.10 (0.00)^{cA}	0.09 (0.01)^{abA}	0.09 (0.06)^{ab} cA	0.09 (0.00)^{ab} cA
	5	0.09 (0.00)^{aA}	0.09 (0.00)^{aA}	0.09 (0.00)^{aA} B	0.09 (0.00)^{a} A	0.08 (0.00)^{aA}	0.10 (0.09)^{aA}	0.10 (0.01)^{aA}	0.08 (0.00)^{aA}	0.12 (0.00)^{aA}	0.12 (0.00)^{a} A
C18	0	3.27 (0.51)^{aA}	3.96 (0.01)^{cB}	3.34 (0.34)^{abA}	3.78 (0.01)^{ab} cB	3.55 (0.06)^{ab} cA	3.74 (0.14)^{ab} cA	3.77 (0.12)^{ab} cA	3.73 (0.05)^{abc} A	3.88 (0.11)^{bc} A	
	3	4.09 (0.06)^{cA}	3.89 (0.08)^{bc} AB	3.51 (0.15)^{abA}	3.41 (0.10)^{a} A	3.82 (0.37)^{ab} cA	3.46 (0.34)^{ab} A	3.91 (0.07)^{cA} B	4.10 (0.19)^{cA} B	4.08 (0.08)^{cA}	4.12 (0.20)^{c} A
	5	4.10 (0.11)^{ab} A	3.76 (0.01)^{aA}	3.74 (0.06)^{aA}	3.88 (0.09)^{aB}	4.09 (0.36)^{ab} A	3.81 (0.45)^{aA}	4.21 (0.06)^{ab} cB	4.38 (0.21)^{bcB}	4.63 (0.11)^{cB}	4.38 (0.28)^{bc} B

C20	0	0.12 (0.01)^{ab} A	0.11 (0.01)^{ab} A	0.11 (0.01)^{abA}	0.12 (0.00)^{ab} A	0.11 (0.00)^{ab} A	0.12 (0.00)^{ab} A	0.10 (0.01)^{aA}	0.13 (0.02)^{bA}	0.10 (0.00)^{aA}	-
	3	0.11 (0.00)^{ab} A	0.10 (0.03)^{aA}	0.10 (0.00)^{aA}	0.13 (0.00)^{ab} cA	0.12 (0.01)^{ab} A	0.14 (0.01)^{bc} A	0.15 (0.00)^{cB}	0.11 (0.03)^{abA}	0.11 (0.00)^{ab} A	0.15 (0.00)^{c} A
	5	0.12 (0.01)^{ab} A	0.13 (0.03)^{ab} A	0.12 (0.01)^{abA}	0.13 (0.01)^{ab} A	0.11 (0.01)^{aA}	0.15 (0.01)^{bc} A	0.11 (0.01)^{aA}	0.12 (0.01)^{abA}	0.10 (0.01)^{aA}	0.17 (0.01)^{cB}
C23	0	0.05 (0.01)^{ab} A	0.04 (0.00)^{aA}	0.07 (0.01)^{cB}	0.05 (0.00)^{ab} A	0.06 (0.00)^{bc} A	0.06 (0.00)^{bc} A	0.05 (0.00)^{ab} A	0.06 (0.01)^{bcA}	0.05 (0.00)^{ab} A	
	3	0.06 (0.00)^{ab} A	0.06 (0.00)^{ab} B	0.06 (0.00)^{abA} B	0.07 (0.01)^{b} B	0.06 (0.01)^{ab} A	0.06 (0.01)^{ab} A	0.05 (0.00)^{aA}	0.07 (0.01)^{bA}	0.07 (0.01)^{bB}	0.06 (0.00)^{ab} A
	5	0.06 (0.01)^{ab} A	0.08 (0.01)^{cC}	0.05 (0.00)^{aA}	0.06 (0.00)^{b} AB	0.05 (0.00)^{aA}	0.05 (0.00)^{aA}	0.08 (0.00)^{cB}	0.05 (0.00)^{aA}	0.05 (0.00)^{aA}	0.05 (0.00)^{a} A
C24	0	0.62 (0.09)^{bc} A	0.59 (0.00)^{ab} A	0.71 (0.01)^{dB}	0.55 (0.01)^{ab} A	0.59 (0.04)^{ab} cA	0.72 (0.02)^{d} A	0.56 (0.00)^{ab} A	0.65 (0.01)^{cdA}	0.52 (0.01)^{aA}	-
	3	0.62 (0.01)^{ab} A	0.69 (0.01)^{bc} C	0.60 (0.04)^{aA}	0.59 (0.01)^{aB}	0.62 (0.08)^{ab} A	0.73 (0.05)^{cA}	0.63 (0.03)^{ab} A	0.68 (0.06)^{abc} AB	0.60 (0.00)^{ab} B	0.96 (0.01)^{d} A
	5	0.75 (0.06)^{cd} eA	0.63 (0.01)^{ab} B	0.66 (0.02)^{abc} AB	0.58 (0.00)^{a} AB	0.70 (0.05)^{bc} dA	0.89 (0.05)^{fB}	0.81 (0.08)^{ef} B	0.77 (0.01)^{deB}	0.61 (0.01)^{ab} B	1.02 (0.01)^{g} B
ΣSFA	0	19.48 (0.82)^{bc} A	20.14 (0.06)^{cA}	19.24 (0.15)^{bB}	19.79 (0.18)^{bc} B	18.03 (0.06)^{aA}	19.58 (0.23)^{bc} A	20.25 (0.06)^{cA}	21.24 (0.36)^{dA}	21.54 (0.40)^{d} A	-
	3	19.99 (0.18)^{bc} A	19.64 (0.32)^{ab} A	18.39 (0.30)^{aA}	18.45 (0.03)^{a} A	19.93 (0.82)^{bc} B	20.37 (1.41)^{bc} dA	21.25 (0.05)^{cd} eB	21.88 (0.38)^{eA}	21.59 (0.09)^{de} A	21.02 (0.16)^{cd} eA
	5	21.25 (0.56)^{b} A	20.14 (0.01)^{aA}	19.52 (0.16)^{aB}	20.10 (0.11)^{aB}	21.25 (0.06)^{bB}	20.00 (0.54)^{aA}	22.57 (0.34)^{cC}	23.25 (0.31)^{cB}	22.85 (0.16)^{cB}	23.16 (0.67)^{cB}

(table continues)

[*] The values are expressed as mean ± standard deviation, n=3.

[a-e] Values in a same row followed by different letters indicate significant differences (P<0.05) during storage periods.

[A-C] Values in a same column followed by different letters indicate significant differences (P<0.05) of the parameter with respect to the irradiation treatment

It was found that the dominant fatty acids among monounsaturated fatty acids (MUFA) were oleic acid (18:1ω9), palmitoleic acid (C16:1) and vasenic acid (C18:1ω7) (Table 2). At the beginning of storage, palmitoleic acid, oleic acid and vasenic acid values, which were 3.35%, 21.27% and 2.76%, respectively, in the control group, were determined as 3.13%, 25.23% and 2.61%, respectively, in the irradiated group at a dose of 5 kGy. While palmitoleic and oleic acid values were detected as 3.43% and 24.51%, respectively, in the 3 kGy dose irradiated group, vasenic acid could not be detected. The differences observed in palmitoleic and oleic acid values because of irradiation treatment were not found to be statistically significant (p>0.05). The values of all fatty acids in MUFA fluctuated during storage (Table 2).

Table 2. Mono unsaturated fatty acids (MUFA) changes during cold storage of non-irradiated (0 kGy), 3 kGy and 5 kGy irradiated smoked trout fillets

		Storage Period (Weeks)									
Fatty Acids		Day 0	2	4	6	8	11	14	17	20	23
C14:1	0	0.04 (0.00)^{ab} A	0.01 (0.00)^{a} A	0.06 (0.03)^{b} A	0.02 (0.01)^{ab} A	0.05 (0.03)^{ab} A	0.05 (0.03)^{ab} A	0.01 (0.00)^{a} A	0.04 (0.00)^{ab} A	0.04 (0.00)^{ab} A	-
	3	0.04 (0.00)^{aA}	0.04 (0.00)^{a} B	0.04 (0.01)^{a} A	0.06 (0.02)^{aA}	0.06 (0.02)^{a} A	0.07 (0.04)^{a} B	0.05 (0.01)^{a} B	0.04 (0.01)^{aA}	0.04 (0.00)^{aA}	0.03 (0.00)^{a} A
	5	0.04 (0.00)^{abc} A	0.08 (0.01)^{d} C	0.03 (0.02)^{a} A	0.04 (0.01)^{ab} A	0.07 (0.01)^{cd} A	-	0.04 (0.00)^{ab} cB	0.06 (0.02)^{bc} dA	0.04 (0.00)^{abc} A	0.04 (0.01)^{ab} A
C15:1	0	0.03 (0.00)^{ab} A	0.03 (0.00)^{ab} A	0.17 (0.02)^{c} A	0.04 (0.01)^{ab} A	0.07 (0.06)^{b} A	0.02 (0.00)^{ab} A	0.03 (0.01)^{ab} A	0.01 (0.00)^{aA}	0.03 (0.01)^{ab} A	-
	3	-	0.05 (0.02)^{a} A	0.20 (0.08)^{b} A	0.03 (0.03)^{aA}	0.06 (0.01)^{a} A	0.02 (0.01)^{a} A	0.03 (0.01)^{a} A	-	-	-
	5	0.04 (0.00)^{bc} A	0.01 (0.00)^{a} A	0.11 (0.03)^{e} A	0.06 (0.00)^{cd} A	0.08 (0.02)^{d} A	0.04 (0.00)^{bc} B	0.02 (0.00)^{ab} A	-	0.02 (0.00)^{ab} A	0.01 (0.00)^{a} A
C16:1	0	3.35 (0.30)^{aA}	2.89 (0.01)^{a} A	3.29 (0.13)^{a} A	3.23 (0.03)^{aA}	3.16 (0.44)^{a} A	3.31 (0.38)^{a} A	3.24 (0.09)^{a} A	3.19 (0.19)^{aA}	2.92 (0.09)^{aA}	-
	3	3.43 (0.04)^{abc} dA	2.94 (0.06)^{a} A	3.04 (0.06)^{ab} A	3.66 (0.31)^{abc} dA	3.81 (0.50)^{bc} dA	3.91 (0.38)^{cd} A	4.26 (0.33)^{d} B	3.79 (0.77)^{abc} dA	3.58 (0.14)^{abc} dB	3.27 (0.01)^{ab} cA
	5	3.13 (0.01)^{ab} A	3.60 (0.10)^{ab} B	3.60 (0.49)^{ab} A	3.25 (0.02)^{ab} A	3.08 (0.16)^{a} A	3.22 (0.45)^{ab} A	3.76 (0.14)^{b} AB	3.45 (0.48)^{ab} A	3.40 (0.11)^{ab} B	3.37 (0.10)^{ab} B
C17:1	0	0.12 (0.01)^{abc} A	0.10 (0.01)^{a} A	0.13 (0.00)^{bc} B	0.11 (0.00)^{ab} A	0.11 (0.01)^{ab} A	0.11 (0.01)^{ab} B	0.14 (0.03)^{c} A	0.12 (0.01)^{abc} A	0.11 (0.01)^{ab} A	-
	3	0.13 (0.00)^{cA}	0.10 (0.00)^{ab} A	0.12 (0.01)^{ab} cA	0.11 (0.01)^{abc} A	0.10 (0.02)^{a} A	0.13 (0.02)^{bc} B	0.12 (0.00)^{ab} cA	0.12 (0.01)^{abc} A	0.11 (0.00)^{abc} AB	0.12 (0.00)^{ab} cA
	5	0.10 (0.03)^{bA}	0.13 (0.01)^{c} A	0.13 (0.00)^{c} B	0.11 (0.00)^{bc} A	0.11 (0.00)^{bc} A	0.06 (0.00)^{a} A	0.13 (0.01)^{c} A	0.11 (0.00)^{bc} A	0.13 (0.01)^{cB}	0.12 (0.00)^{bc} A
C18:1 ω9	0	21.27 (3.22)^{aA}	21.37 (0.01)^{a} A	20.31 (0.15)^{a} A	22.45 (0.21)^{aA}	21.37 (0.04)^{a} A	22.30 (0.28)^{a} A	22.37 (0.73)^{a} B	21.93 (0.93)^{aA}	21.81 (0.28)^{aA}	-
	3	24.51 (0.68)^{aA}	24.26 (0.69)^{a} B	21.81 (0.51)^{a} B	23.95 (0.26)^{aB}	23.66 (0.96)^{a} A	23.18 (4.75)^{a} A	20.44 (0.40)^{a} A	21.70 (4.45)^{aA}	23.53 (0.39)^{aB}	23.52 (0.01)^{a} A
	5	25.23 (1.43)^{aA}	25.11 (0.09)^{a} B	23.10 (0.52)^{a} B	23.58 (0.35)^{aB}	23.46 (1.37)^{a} A	24.67 (0.34)^{a} A	24.13 (0.26)^{a} C	23.61 (0.26)^{aA}	24.28 (0.06)^{aB}	25.11 (2.26)^{a} A
C18:1 ω7	0	2.76 (0.01)^{cd} B	2.71 (0.01)^{cd} A	2.38 (0.04)^{ab} A	2.81 (0.04)^{cd} A	2.93 (0.26)^{d} A	2.82 (0.09)^{cd} A	2.89 (0.04)^{d} B	2.59 (0.08)^{bc} A	2.14 (0.10)^{aA}	-
	3	-	2.58 (0.08)^{ab} A	2.48 (0.15)^{a} A	2.89 (0.11)^{cd} A	2.89 (0.28)^{cd} A	2.81 (0.01)^{bc} dA	2.39 (0.04)^{a} A	2.66 (0.01)^{abc} A	2.82 (0.02)^{bcd} B	3.07 (0.01)^{d} B

table continue

	5	2.61 (0.01)^{abc} A	2.61 (0.21)^{ab} cA	2.59 (0.01)^{ab} A	2.93 (0.06)^{ef} A	3.02 (0.05)^{f} A	2.82 (0.01)^{de} fA	2.46 (0.06)^{a} A	2.71 (0.06)^{bc} dA	2.88 (0.08)^{def} B	2.79 (0.06)^{cd} eA
C20:1	0	0.88 (0.06)^{dA}	0.78 (0.01)^{bc} A	0.63 (0.04)^{a} A	0.84 (0.01)^{cd} A	0.71 (0.00)^{b} A	0.83 (0.04)^{cd} A	0.82 (0.01)^{cd} A	0.80 (0.05)^{cA}	0.80 (0.01)^{cC}	-
	3	0.85 (0.01)^{bc} A	0.88 (0.03)^{c} B	0.75 (0.00)^{a} B	0.85 (0.01)^{bc} A	0.80 (0.06)^{ab} cA	0.98 (0.01)^{d} A	0.99 (0.01)^{d} B	0.86 (0.01)^{bc} A	0.77 (0.00)^{ab} B	0.80 (0.00)^{ab} cA
	5	0.81 (0.08)^{ab} A	0.77 (0.03)^{a} A	0.78 (0.02)^{a} B	0.85 (0.02)^{ab} A	0.82 (0.05)^{ab} A	0.96 (0.01)^{c} A	0.84 (0.01)^{ab} A	0.90 (0.01)^{bc} A	0.75 (0.00)^{aA}	0.83 (0.04)^{ab} A
C22:1 ω9	0	0.09 (0.01)^{abc} A	0.07 (0.00)^{a} A	0.09 (0.01)^{ab} cA	0.09 (0.00)^{bc} A	0.08 (0.00)^{ab} A	0.10 (0.01)^{c} A	0.07 (0.00)^{a} A	0.09 (0.01)^{abc} A	0.09 (0.00)^{bc} A	-
	3	0.10 (0.01)^{ab} A	0.10 (0.01)^{ab} B	0.09 (0.00)^{ab} A	0.08 (0.01)^{aA}	0.08 (0.01)^{a} A	0.11 (0.02)^{b} A	0.11 (0.00)^{b} C	0.10 (0.01)^{ab} A	0.08 (0.00)^{aA}	0.08 (0.01)^{a} A
	5	0.09 (0.00)^{ab} A	0.09 (0.00)^{ab} B	0.09 (0.00)^{ab} A	0.08 (0.01)^{aA}	0.08 (0.00)^{a} A	0.08 (0.00)^{a} A	0.10 (0.01)^{ab} B	0.11 (0.02)^{bA}	0.09 (0.01)^{aA}	0.09 (0.01)^{a} A
C24:1	0	0.04 (0.00)^{aA} B	0.08 (0.01)^{c} B	0.06 (0.01)^{b} A	-	0.04 (0.01)^{a} A	0.06 (0.01)^{b} A	-	0.04 (0.00)^{aA}	0.04 (0.00)^{aA}	-
	3	0.03 (0.00)^{aA}	0.05 (0.00)^{cd} A	0.04 (0.00)^{ab} cA	-	0.03 (0.00)^{a} A	0.05 (0.01)^{bc} d	0.05 (0.01)^{bc} dA	0.06 (0.01)^{dA}	0.04 (0.01)^{ab} A	0.04 (0.00)^{ab} cA
	5	0.05 (0.01)^{bc} B	0.04 (0.00)^{ab} A	0.05 (0.01)^{bc} A	-	0.04 (0.00)^{ab} A	-	0.05 (0.01)^{bc} A	0.06 (0.01)^{cA}	0.03 (0.00)^{aA}	0.04 (0.00)^{ab} A
ΣMUFA	0	28.56 (2.86)^{aA}	28.04 (0.07)^{a} A	27.08 (0.28)^{a} A	29.58 (0.28)^{aA}	28.65 (0.84)^{a} A	29.58 (0.11)^{a} A	29.56 (0.84)^{a} AB	28.78 (1.09)^{aA}	27.96 (0.28)^{aA}	-
	3	29.08 (0.72)^{aA}	30.99 (0.84)^{a} B	28.56 (0.52)^{a} AB	31.61 (0.21)^{aB}	31.69 (2.11)^{a} A	31.24 (4.22)^{a} A	28.42 (0.69)^{a} A	29.30 (3.59)^{aA}	30.96 (0.52)^{aB}	30.92 (0.01)^{a} A
	5	32.10 (1.46)^{aA}	32.43 (0.21)^{a} B	30.46 (1.10)^{a} B	30.88 (0.45)^{aB}	30.74 (1.65)^{a} A	31.85 (0.05)^{a} A	31.52 (0.04)^{a} B	30.99 (0.22)^{aA}	31.61 (0.01)^{aB}	32.38 (2.33)^{ab} A

* The values are expressed as mean ± standard deviation, n=3.

a-e Values in a same row followed by different letters indicate significant differences (P<0.05) during storage periods.

A-C Values in a same column followed by different letters indicate significant differences (P<0.05) of the parameter with respect to the irradiation treatment

The dominant fatty acids among polyunsaturated fatty acids (PUFA) were docosahexaenoic acid (DHA, C22:6ω3), linoleic acid (18:2ω6), linolenic acid (C18:3ω3), arachidonic acid (C20:4ω6) and eicosapentaenoic acid (EPA, C20:5ω3) (Table 3). As a result of irradiation, no statistically significant difference was observed in PUFAs except linoleic acid ($p>0.05$). Linoleic acid rate in the control group was found to be significantly higher than the irradiated groups at 3 and 5 kGy doses ($p<0.05$). The effect of irradiation on linolenic acid varied between groups during storage, but generally, no difference was observed between groups. Generally, no difference was observed in EPA values between the control and irradiated groups during the storage period (except for the 17th week). The effects of irradiation on DHA were remarkably observed and it was determined that the control group had generally higher values than the group irradiated at a dose of 5 kGy (Table 3). In this study, ω6 series fatty acids in total PUFA in rainbow trout fillets at the beginning of storage were determined as 19.12%, 17.01% and 16.20% in the control, 3 and 5 kGy dose irradiated groups,

respectively. Likewise, ω3 series fatty acids were determined as 20.77%, 18.49% and 17.97% in the control, 3 and 5 kGy dose irradiated groups, respectively. It is observed that the initial irradiation treatment was effective on the ω6 series fatty acid ratios, and the ω6 series fatty acid values of the control group were significantly higher than the group irradiated at a dose of 5 kGy ($p<0.05$). No significant effect of irradiation application was observed on the total ω6/ω3 ratios. While the lowest value of ω6/ω3 ratios was determined as 0.75 in the 2nd week in the control group, the highest value was determined as 1.03 in the 14th week in the 5 kGy dose irradiated group (Table 3).

Table 3. Polyunsaturated fatty acids (PUFA) changes during cold storage of non-irradiated (0 kGy), 3 kGy and 5 kGy irradiated smoked trout fillets

		Storage Period (Weeks)									
Fatty Acids		Day 0	2	4	6	8	11	14	17	20	23
C18:2ω6	0	14.76 (0.96)^{eB}	11.39 (0.01)^{aA}	13.81 (0.61)^{d} ^{eB}	12.64 (0.50)^{bc} ^{A}	11.38 (0.13)^{aA}	13.95 (0.26)^{de} ^{A}	11.81 (0.33)^{ab} ^{A}	13.40 (0.49)^{cd} ^{A}	13.40 (0.13)^{cd} ^{C}	-
	3	12.93 (0.16)^{ab} ^{cA}	12.55 (0.24)^{ab} ^{B}	12.39 (0.30)^{a} ^{bA}	12.91 (0.16)^{ab} ^{cA}	12.32 (0.17)^{ab} ^{AB}	13.66 (1.23)^{cA}	13.10 (0.01)^{bc} ^{B}	12.82 (1.20)^{ab} ^{cA}	13.02 (0.01)^{bc} ^{B}	11.90 (0.01)^{a} ^{B}
	5	12.44 (0.08)^{cd} ^{A}	12.02 (0.18)^{bc} ^{B}	11.19 (0.09)^{a} ^{A}	12.68 (0.50)^{cd} ^{A}	12.71 (0.49)^{dB}	12.69 (0.14)^{d} ^{A}	13.32 (0.26)^{eB}	12.45 (0.28)^{cd} ^{A}	12.46 (0.09)^{cd} ^{A}	11.54 (0.31)^{a} ^{bA}
C18:3ω6	0	0.24 (0.02)^{aA}	0.24 (0.01)^{ab} ^{A}	0.28 (0.01)^{b} ^{B}	0.24 (0.00)^{ab} ^{A}	0.25 (0.03)^{ab} ^{A}	0.26 (0.01)^{ab} ^{A}	0.26 (0.01)^{ab} ^{A}	0.26 (0.03)^{ab} ^{A}	0.27 (0.00)^{ab} ^{A}	-
	3	0.26 (0.01)^{aA}	0.25 (0.01)^{aA}	0.25 (0.00)^{a} ^{A}	0.28 (0.02)^{aA}	0.26 (0.05)^{aA}	0.29 (0.04)^{aA}	0.28 (0.03)^{aA}	0.28 0.00)^{aA}	0.28 (0.00)^{a} ^{A}	0.30 (0.00)^{a} ^{A}
	5	0.24 (0.01)^{ab} ^{A}	0.28 (0.03)^{ab} ^{A}	0.24 (0.00)^{a} ^{bA}	0.27 (0.03)^{ab} ^{A}	0.21 (0.10)^{aA}	0.31 (0.01)^{bB}	0.24 (0.00)^{ab} ^{A}	0.26 (0.03)^{ab} ^{A}	0.28 (0.04)^{ab} ^{A}	-
C18:3ω3	0	2.64 (0.18)^{cA}	2.23 (0.01)^{aA}	2.59 (0.08)^{b} ^{cB}	2.42 (0.01)^{ab} ^{A}	2.24 (0.04)^{aA}	2.45 (0.08)^{b} ^{A}	2.40 (0.06)^{ab} ^{A}	2.49 (0.08)^{bc} ^{A}	2.66 (0.03)^{c} ^{B}	-
	3	2.48 (0.04)^{aA}	2.37 (0.02)^{aB}	2.31 (0.06)^{a} ^{A}	2.45 (0.06)^{aA}	2.39 (0.09)^{aA} ^{B}	2.57 (0.28)^{aA}	2.32 (0.10)^{aA}	2.50 (0.28)^{a} ^{A}	2.48 (0.04)^{a} ^{A}	2.48 (0.00)^{a} ^{A}
	5	2.37 (0.09)^{bc} ^{A}	2.46 (0.02)^{cd} ^{C}	2.16 (0.01)^{a} ^{A}	2.41 (0.02)^{cd} ^{A}	2.46 (0.02)^{cd} ^{B}	2.18 (0.09)^{aA}	2.55 (0.09)^{d} ^{A}	2.25 (0.01)^{ab} ^{A}	2.47 (0.01)^{cd} ^{A}	2.51 (0.09)^{d} ^{A}
C20:2 cis	0	0.70 (0.08)^{b} ^{A}	0.83 (0.01)^{cC}	0.70 (0.00)^{b} ^{B}	0.69 (0.01)^{bB}	0.70 (0.03)^{bB}	0.68 (0.01)^{b} ^{A}	0.68 (0.01)^{bB}	0.63 (0.01)^{ab} ^{A}	0.60 (0.00)^{a} ^{A}	-
	3	0.59 (0.02)^{aA}	0.69 (0.01)^{cd} ^{B}	0.64 (0.01)^{a} ^{bcA}	0.60 (0.01)^{aA}	0.60 (0.01)^{aA}	0.57 (0.01)^{aA}	0.73 (0.01)^{dC}	0.67 (0.06)^{bc} ^{dA}	0.61 (0.00)^{ab} ^{A}	0.65 (0.01)^{a} ^{bcA}
	5	0.62 (0.00)^{ab} ^{cA}	0.57 (0.01)^{ab} ^{A}	0.65 (0.01)^{b} ^{cA}	0.68 (0.00)^{cB}	0.58 (0.03)^{ab} ^{A}	0.62 (0.01)^{ab} ^{cA}	0.53 (0.00)^{aA}	0.61 (0.01)^{ab} ^{cA}	0.65 (0.04)^{bc} ^{A}	0.60 (0.11)^{a} ^{bcA}
C20:3ω6	0	0.26 (0.03)^{ab} ^{A}	0.25 (0.00)^{aA}	0.29 (0.02)^{b} ^{B}	0.24 (0.00)^{aA}	0.23 (0.00)^{aA}	0.25 (0.00)^{aA}	0.26 (0.01)^{aA}	0.25 (0.01)^{a} ^{A}	0.23 (0.00)^{a} ^{AB}	-
	3	0.27 (0.01)^{bc} ^{dA}	0.27 (0.01)^{bc} ^{dA}	0.24 (0.01)^{a} ^{A}	0.25 (0.00)^{ab} ^{cA}	0.25 (0.01)^{ab} ^{cA}	0.29 (0.02)^{d} ^{A}	0.27 (0.00)^{cd} ^{A}	0.27 (0.02)^{bc} ^{dA}	0.23 (0.00)^{a} ^{A}	0.24 (0.00)^{a} ^{bA}

table continue

	5	0.26 (0.02)^{ab} A	0.25 (0.00)^{ab} A	0.23 (0.00)^{a} A	0.25 (0.01)^{ab} A	0.25 (0.01)^{ab} A	0.27 (0.01)^{b} A	0.26 (0.01)^{ab} A	0.26 (0.00)^{b} A	0.24 (0.00)^{ab} B	0.25 (0.01)^{a} bA
C20:4ω6	0	3.87 (0.33)^{b} A	3.97 (0.00)^{bB}	3.83 (0.01)^{b} A	3.67 (0.08)^{bA}	3.81 (0.12)^{b} A	3.97 (0.03)^{b} A	4.51 (0.10)^{cC}	3.63 (0.18)^{b} A	3.24 (0.04)^{a} A	-
	3	3.56 (0.03)^{b} A	3.42 (0.09)^{ab} A	3.46 (0.54)^{a} bA	3.60 (0.05)^{bA}	3.20 (0.27)^{ab} A	3.42 (0.49)^{ab} A	3.71 (0.04)^{bB}	3.57 (0.26)^{b} A	3.07 (0.08)^{ab} A	2.91 (0.08)^{a} A
	5	3.51 (0.39)^{bc} dA	3.36 (0.01)^{cd} eA	3.74 (0.11)^{e} A	3.64 (0.13)^{de} A	3.49 (0.24)^{cd} eA	3.29 (0.37)^{bc} deA	3.51 (0.04)^{bc} deA	3.13 (0.17)^{ab} cA	2.83 (0.23)^{a} A	2.86 (0.18)^{a} bA
C20:5ω3	0	2.91 (0.30)^{cd} A	2.41 (0.01)^{ab} A	2.81 (0.40)^{b} cdA	2.58 (0.03)^{ab} cA	2.48 (0.01)^{ab} cA	3.03 (0.03)^{d} A	2.73 (0.10)^{bc} dA	2.66 (0.15)^{ab} cdB	2.25 (0.01)^{a} A	-
	3	3.12 (0.11)^{cd} A	3.27 (0.16)^{dB}	2.64 (0.01)^{b} A	2.69 (0.09)^{bA}	2.77 (0.36)^{bc} A	3.18 (0.20)^{d} A	3.14 (0.01)^{cd} B	2.69 (0.13)^{b} B	2.19 (0.17)^{a} A	1.87 (0.05)^{a} A
	5	3.28 (0.51)^{d} A	3.04 (0.00)^{cd} B	2.73 (0.11)^{b} cA	2.65 (0.01)^{bc} A	3.06 (0.23)^{cd} A	3.34 (0.40)^{d} A	3.11 (0.04)^{cd} B	2.24 (0.01)^{ab} A	1.97 (0.06)^{a} A	1.96 (0.04)^{a} B
C22:2 cis	0	0.07 (0.00)^{b} A	0.06 (0.00)^{aA}	0.08 (0.01)^{b} cA	0.07 (0.00)^{bA}	0.06 (0.00)^{aA}	0.08 (0.00)^{cB}	0.06 (0.00)^{aA}	0.07 (0.00)^{b} A	0.06 (0.00)^{a} A	-
	3	0.06 (0.00)^{aA}	0.07 (0.01)^{ab} A	0.10 (0.04)^{b} A	0.06 (0.00)^{ab} A	0.06 (0.01)^{aA}	0.07 (0.01)^{ab} A	0.08 (0.01)^{ab} B	0.09 (0.01)^{ab} B	0.07 (0.00)^{ab} A	0.07 (0.00)^{a} bA
	5	0.06 (0.01)^{ab} cA	0.06 (0.01)^{ab} A	0.08 (0.00)^{c} A	0.07 (0.01)^{ab} cA	0.05 (0.00)^{aA}	0.07 (0.00)^{bc} AB	0.06 (0.00)^{ab} A	0.06 (0.00)^{ab} A	0.07 (0.01)^{bc} A	0.07 (0.01)^{a} bcA
C22:6ω3	0	14.82 (0.38)^{b} A	16.59 (0.01)^{dC}	16.48 (0.34)^{c} dB	15.27 (0.26)^{bc} B	15.95 (0.88)^{bc} dA	13.04 (0.33)^{aA}	15.10 (0.74)^{bB}	13.58 (0.81)^{a} A	12.87 (0.37)^{a} A	-
	3	12.89 (0.57)^{ab} cA	14.65 (0.81)^{cB}	14.55 (0.45)^{b} cA	14.49 (0.44)^{bc} AB	12.43 (1.20)^{ab} A	12.08 (2.04)^{aA}	13.85 (0.42)^{ab} cB	13.70 (0.22)^{ab} cA	12.35 (0.48)^{a} A	12.88 (0.01)^{a} bcB
	5	12.32 (3.25)^{aA}	12.97 (0.0.6)^{a} A	13.55 (0.64)^{a} A	13.76 (0.60)^{aA}	12.88 (1.50)^{aA}	11.30 (0.74)^{aA}	11.07 (0.02)^{aA}	12.90 (0.22)^{a} A	12.63 (0.27)^{a} A	12.20 (0.25)^{a} A
ΣPUFA	0	40.66 (2.86)^{b} A	37.97 (0.03)^{aB}	40.87 (0.01)^{b} C	37.80 (0.33)^{aA}	37.08 (0.82)^{aA}	37.70 (0.62)^{aA}	37.80 (0.35)^{aB}	36.95 (0.25)^{aB}	35.58 (0.57)^{a} B	-
	3	36.14 (0.24)^{ab} A	37.51 (0.48)^{bB}	36.57 (0.62)^{a} bB	37.31 (0.58)^{bA}	34.25 (1.07)^{ab} A	36.10 (4.37)^{ab} A	37.48 (0.59)^{bB}	36.58 (0.01)^{ab} B	34.29 (0.34)^{ab} A	33.28 (0.14)^{a} B
	5	34.85 (2.68)^{ab} cA	34.99 (0.11)^{ab} cA	34.55 (0.57)^{a} bcA	36.36 (0.71)^{cA}	35.74 (1.04)^{bc} A	33.96 (0.45)^{ab} cA	33.49 (0.35)^{ab} A	34.12 (0.24)^{ab} cA	33.58 (0.18)^{ab} cA	32.25 (0.31)^{a} A
EPA+DHA	0	18.13 (1.26)^{b} A	19.00 (0.03)^{bB}	19.29 (0.74)^{b} B	17.85 (0.28)^{bB}	18.43 (0.87)^{bB}	16.07 (0.25)^{aA}	17.83 (0.64)^{bB}	16.24 (0.66)^{a} A	15.12 (0.38)^{a} A	-

table continue

	3	16.01 (0.46)^{ab} cA	17.92 (0.64)^{cB}	17.19 (0.47)^{b} cA	17.18 (0.35)^{bc} AB	15.19 (0.83)^{ab} A	15.26 (2.24)^{ab} A	16.99 (0.42)^{bc} B	16.39 (0.09)^{ab} cA	14.54 (0.31)^{a} A	14.74 (0.06)^{a} B
	5	15.60 (2.74)^{aA}	16.01 (0.06)^{aA}	16.27 (0.54)^{a} A	16.40 (0.59)^{aA}	15.94 (1.27)^{aA} B	14.64 (1.13)^{aA}	14.17 (0.06)^{aA}	15.13 (0.21)^{a} A	14.60 (0.33)^{a} A	14.16 (0.21)^{a} A
PUFA/SFA	0	2.09 (0.06)^{dB}	1.89 (0.00)^{cA} B	2.13 (0.02)^{d} C	1.91 (0.03)^{cA}	2.06 (0.05)^{dB}	1.93 (0.05)^{cB}	1.87 (0.01)^{cC}	1.74 (0.01)^{b} C	1.65 (0.06)^{a} B	-
	3	1.81 (0.01)^{bc} AB	1.91 (0.06)^{cd} B	1.99 (0.00)^{d} B	2.02 (0.03)^{dB}	1.72 (0.13)^{b} A	1.77 (0.09)^{cA} B	1.77 (0.04)^{cB}	1.67 (0.03)^{ab} B	1.59 (0.01)^{a} B	1.59 (0.02)^{a} B
	5	1.64 (0.17)^{bc} A	1.77 (0.05)^{cd} A	1.77 (0.04)^{c} dA	1.81 (0.04)^{dA}	1.68 (0.06)^{cd} A	1.70 (0.03)^{cd} A	1.53 (0.04)^{ab} A	1.47 (0.01)^{a} A	1.47 (0.01)^{a} A	1.39 (0.06)^{a} A
Σω6	0	19.12 (1.34)^{dB}	15.85 (0.03)^{aA}	18.22 (0.66)^{c} dB	16.78 (0.03)^{ab} A	15.66 (0.04)^{aA}	18.43 (0.29)^{cd} A	16.83 (0.24)^{ab} A	17.53 (0.34)^{bc} B	17.14 (0.16)^{bc} C	-
	3	17.01 (0.20)^{bc} AB	16.48 (0.16)^{ab} cB	16.34 (0.23)^{a} bcA	17.03 (0.18)^{bc} A	16.02 (0.14)^{ab} A	17.65 (1.77)^{cA}	17.37 (0.06)^{bc} A	16.94 (0.11)^{bc} AB	16.59 (0.07)^{ab} cB	15.35 (0.09)^{a} B
	5	16.20 (0.16)^{cd} eA	15.91 (0.16)^{bc} A	15.39 (0.01)^{a} bA	16.80 (0.10)^{ef} A	16.72 (0.23)^{de} fB	16.46 (0.60)^{cd} eA	17.18 (0.31)^{fA}	16.08 (0.45)^{cd} A	15.79 (0.11)^{bc} A	14.93 (0.08)^{a} A
Σω3	0	19.12 (1.34)^{dB}	15.85 (0.03)^{aA}	18.22 (0.66)^{c} dB	16.78 (0.03)^{ab} A	15.66 (0.04)^{aA}	18.43 (0.29)^{cd} A	16.83 (0.24)^{ab} A	17.53 (0.34)^{bc} B	17.14 (0.16)^{bc} C	-
	3	18.49 (0.42)^{ab} cdA	20.28 (0.62)^{dB}	19.50 (0.40)^{c} dA	19.63 (0.40)^{cd} AB	17.58 (0.92)^{ab} cA	17.82 (2.52)^{ab} cA	19.31 (0.52)^{bc} dB	18.89 (0.18)^{ab} cdB	17.02 (0.27)^{a} A	15.35 (0.09)^{a} bA
	5	17.97 (2.84)^{aA}	18.46 (0.04)^{aA}	18.43 (0.54)^{a} A	18.81 (0.62)^{aA}	18.39 (1.24)^{aA}	16.82 (1.05)^{aA}	16.72 (0.04)^{aA}	17.38 (0.22)^{a} A	17.07 (0.34)^{a} A	16.67 (0.12)^{a} B
Σω6/Σω3	0	0.92 (0.00)^{d} A	0.75 (0.00)^{aA}	0.83 (0.06)^{b} cA	0.83 (0.01)^{bc} A	0.76 (0.03)^{ab} A	1.00 (0.00)^{eA}	0.84 (0.04)^{cA}	0.94 (0.05)^{de} A	0.96 (0.01)^{de} AB	-
	3	0.92 (0.03)^{cd} A	0.82 (0.04)^{aA} B	0.84 (0.01)^{a} bA	0.87 (0.01)^{ab} cAB	0.91 (0.14)^{cA}	0.99 (0.04)^{eA}	0.90 (0.01)^{cA}	0.90 (0.00)^{c} A	0.98 (0.01)^{de} B	0.89 (0.00)^{b} cA
	5	0.92 (0.13)^{ab} A	0.86 (0.01)^{aB}	0.84 (0.02)^{a} A	0.90 (0.02)^{ab} B	0.91 (0.07)^{ab} A	0.98 (0.10)^{ab} A	1.03 (0.02)^{bB}	0.93 (0.04)^{ab} A	0.93 (0.02)^{ab} A	0.90 (0.01)^{a} bA
DHA/EPA	0	5.25 (0.22)^{b} A	6.88 (0.03)^{dB}	5.93 (0.73)^{b} cA	5.92 (0.03)^{bc} A	6.45 (0.38)^{cd} A	4.31 (0.21)^{aB}	5.54 (0.47)^{bc} B	5.13 (0.59)^{ab} A	5.72 (0.13)^{bc} A	
	3	4.15 (0.32)^{ab} A	4.50 (0.47)^{ab} cdA	5.51 (0.14)^{c} dA	5.41 (0.35)^{cd} A	4.56 (1.03)^{ab} cdA	3.79 (0.40)^{aA} B	4.42 (0.14)^{ab} cA	5.10 (0.33)^{bc} dA	5.67 (0.66)^{d} A	6.91 (0.18)^{e} B
	5	3.88 (1.59)^{ab} cA	4.28 (0.01)^{ab} cA	4.98 (0.43)^{b} cdA	5.20 (0.24)^{cd} A	4.25 (0.82)^{ab} cA	3.40 (0.18)^{aA}	3.57 (0.04)^{ab} A	5.77 (0.11)^{d} A	6.42 (0.05)^{d} A	6.23 (0.26)^{d} A

table continue

N/A	0	11.31	13.85	12.82	12.84	16.25	13.15	12.40	13.04	14.93	-
	3	14.79	11.86	16.50	12.63	14.14	12.30	12.86	12.25	13.17	14.79
	5	11.81	12.45	15.49	12.68	12.28	14.20	11.43	11.65	11.98	12.22

* The values are expressed as mean ± standard deviation, n=3.
a-e Values in a same row followed by different letters indicate significant differences (P<0.05) during storage periods.
A-C Values in a same column followed by different letters indicate significant differences (P<0.05) of the parameter with respect to the irradiation treatment

PUFA/SFA ratios in this study were determined as 2.09, 1.81 and 1.64, respectively, in the control and irradiated groups at 3 and 5 kGy doses after irradiation. While the PUFA/SFA ratio of the control group was observed to be statistically higher than the irradiated group, especially at the 5 kGy dose ($p<0.05$), it was observed that there was no change in the PUFA/SFA ratios of the 3 kGy and 5 kGy dose irradiated groups ($p>0.05$). Statistical decreases in PUFA/SFA ratios were observed in all groups during storage ($p<0.05$) (Table 3). A similar picture was observed at the end of storage, and in general, the PUFA/SFA ratios of the 5 kGy dose irradiated group were observed to be low, while the control group was found to have higher ratios.

In this study, the initial TBA values of the control and the fillets that were irradiated with doses of 3 and 5 kGy were found as 0.96, 0.83 and 0.72 mg MA/kg (Table 4). Initially, while the group irradiated at 5 kGy had the lowest level of TBA, TBA values of 5 kGy dosed group increased quickly during storage and reached to higher values than the other groups. In addition, 3 kGy group had significantly higher values than the control group ($P<0.05$). Therefore, it can be said that gamma irradiation accelerated lipid oxidation in our study.

Table 4. The changes on thiobarbituric acid value (TBA, mg MA/kg) for smoked trout fillets irradiated with different doses during cold storage

Storage Period (Week)	Control (0 kGy)	3 kGy	5 kGy
0	0.96 ± 0.01^{cC}	0.83 ± 0.12^{aB}	0.72 ± 0.10^{aA}
2	0.99 ± 0.02^{dA}	1.04 ± 0.01^{cB}	1.22 ± 0.02^{bC}
4	0.90 ± 0.04^{bA}	0.94 ± 0.04^{bB}	1.50 ± 0.02^{eC}
6	1.21 ± 0.03^{fB}	1.00 ± 0.02^{bcA}	1.49 ± 0.09^{eC}
8	1.26 ± 0.01^{gA}	1.26 ± 0.05^{eA}	1.23 ± 0.11^{bA}
11	0.76 ± 0.02^{aA}	1.19 ± 0.04^{dB}	1.36 ± 0.01^{cC}
14	0.75 ± 0.01^{aA}	0.97 ± 0.04^{bB}	1.38 ± 0.01^{cC}
17	1.13 ± 0.03^{eA}	1.30 ± 0.05^{eB}	1.40 ± 0.01^{cdC}
20	1.27 ± 0.031^{gA}	1.40 ± 0.03^{fB}	1.45 ± 0.02^{deC}
23	---	1.46 ± 0.03^{gA}	1.58 ± 0.01^{fB}

* The values are expressed as mean ± standard deviation, n=6.
a-g Values in a same column followed by different letters indicate significant differences (P<0.05) during storage periods.
A-C Values in a same row followed by different letters indicate significant differences (P<0.05) of the parameter with respect to the irradiation treatment

The PVs of the control and irradiated groups during cold storage are shown in Table 5. Initially, the peroxide values of control, 3 kGy and 5 kGy groups were found to be 6.84, 7.03 and 5.63 meq/kg and fluctuations were detected in the following weeks. At the beginning of storage, it was seen that irradiation did not affect the peroxide value. However, peroxide values of the irradiated groups at 3 and 5 kGy doses at the 20th week of storage (11.35 and 12.08 meq/kg, respectively) were significantly higher than the control group (6.12 meq/kg) ($p < 0.05$). There was also a difference between the irradiated groups at 3 and 5 kGy doses at 23rd week (9.18 and 9.97 meq/kg, respectively) ($p<0.05$) and the peroxide value of irradiated group at 5 kGy dose was found to be higher.

Table 5. The changes on peroxide value (PV, meq/kg) for smoked trout fillets irradiated with different doses during cold storage

Storage Period (Week)	Control (0 kGy)	3 kGy	5 kGy
0	6.84±0.83^{aA}	7.03±1.76^{aA}	5.63±1.89^{aA}
2	7.99±0.39^{aB}	7.09±0.56^{aB}	4.99±0.55^{aA}
4	8.41±1.08^{aA}	9.18±1.01^{abAB}	10.74±1.01^{bcB}
6	10.39±1.47^{bA}	11.12±2.20^{bA}	12.06±1.90^{bcA}
8	7.48±1.03^{aA}	9.25±1.31^{abA}	13.13±1.08^{cB}
11	10.67±1.51^{cA}	11.94±1.43^{bA}	12.69±1.10^{bcA}
14	10.82±1.66^{cA}	11.74±1.59^{bA}	9.80±0.84^{bA}
17	8.22±0.95^{abA}	10.92±0.29^{bA}	10.40±1.99^{bcA}
20	6.12±0.73^{aA}	11.35±1.20^{bB}	12.08±1.07^{bcB}
23		9.18±1.56^{abA}	9.97±2.61^{bB}

* The values are expressed as mean ± standard deviation, n=3.
a-c Values in a same column followed by different letters indicate significant differences ($P<0.05$) during storage periods.
A-B Values in a same row followed by different letters indicate significant differences ($P<0.05$) of the parameter with respect to the irradiation treatment

As can be seen in Table 6, the FFA values of control and irradiated groups at 3 and 5 kGy doses were 1.89%, 2.40% and 2.54%, respectively, at the beginning of storage. Even if there were no significant differences between irradiated groups of 3 and 5 kGy doses, there was a significant difference between the control group and irradiated groups (3 and 5 kGy) ($p < 0.05$). The FFA values of the control group were significantly lower than the irradiated groups except for the 11th, 14th and 17th weeks of storage. The control group (5.36 %) had similar FFA values to the irradiated group at 3 kGy dose (5.62 %) at the 20th week ($p > 0.05$) but the 5 kGy irradiated group (5.95 %) had a significantly higher FFA value than the other groups ($p < 0.05$). Similarly, in the 23rd week of storage, the FFA values of irradiated group at 5 kGy dose (6.10 %) were found to be significantly higher than the irradiated group at 3 kGy dose (5.67 %) ($p < 0.05$).

Table 6. The changes on free fatty acids (FFA, % oleic acid) for smoked trout fillets irradiated with different doses during cold storage

Storage Periods (Week)	Control (0 kGy)	3 kGy	5 kGy
0	1.89±0.13^{aA}	2.40±0.06^{aB}	2.54±0.33^{aB}
2	1.98±0.13^{aA}	2.47±0.12^{aB}	2.63±0.28^{abB}
4	2.12±0.08^{abA}	2.76±0.29^{abB}	3.06±0.45^{abcB}
6	2.36±0.14^{abA}	3.15±0.30^{bcB}	3.16±0.22^{bcB}
8	2.53±0.13^{bA}	3.18±0.27^{bcB}	3.16±0.36^{bcB}
11	3.16±0.83^{cA}	3.55±0.15^{cdA}	3.51±0.15^{cA}
14	3.51±0.27^{cdA}	3.82±0.20^{deAB}	4.19±0.16^{dB}
17	3.86±0.27^{dA}	4.21±0.50^{eA}	4.57±0.15^{dA}
20	5.36±0.07^{eA}	5.62±0.14^{fA}	5.95±0.08^{eB}
23	-	5.67±0.46^{fA}	6.10±0.09^{eB}

* The values are expressed as mean ± standard deviation, n=3.
a-f Values in a same column followed by different letters indicate significant differences (P<0.05) during storage periods.
A-B Values in a same row followed by different letters indicate significant differences (P<0.05) of the parameter with respect to the irradiation treatment

Discussion

Surendra et al. (2018a) investigated the fatty acid changes in tilapia muscles irradiated at 1 kGy and 3 kGy levels. The researchers reported that while no change was observed in saturated fatty acids in the control and 3 kGy groups, there was a significant decrease in the samples irradiated at 1 kGy. In addition, a significant increase in PUFA was observed in tilapia samples irradiated with 1 kGy, while a decrease was reported in samples irradiated with 3 kGy. Therefore, researchers reported that the safest irradiation level for tilapia during ice storage was 1 kGy. El-Ghafour et al. (2018) studied the effects of commercially used gamma irradiation (0, 0.75, 1.5, 2.25 and 3 kGy) on fatty acids in mullet fish (*Mugil cephalus*). They observed that total SFA and MUFA values increased in direct proportion to the increase in irradiation dose. Significant decreases were observed in PUFA values between control and irradiated samples. Asamoah et al. (2022) observed on smoked Atlantic chub mackerel, that SFA and PUFA values increased significantly with smoking, but MUFA values decreased significantly. Researchers attributed the cause to dehydration. Al-Kuraieef (2021) irradiated fresh bolti fish and smoked herring and mackerel at different levels (1.5, 3.0 and 4.5 kGy). The researcher found that the total PUFA percentages decreased slightly with increasing radiation dose and reported that this may be due to lipid oxidation. It was also determined that there were no significant differences between control and irradiated samples in terms of saturated or unsaturated fatty acids.

Oraei et al. (2011), irradiated rainbow trout fillets at 0, 1, 3 and 5 kGy levels and found that irradiation did not initially cause any change in fatty acid levels. In our study, no statistically significant effect of irradiation was observed on other fatty acids except linoleic acid under initial 3 and 5 kGy doses of irradiation. In addition, researchers found that initial irradiation did not affect total SFA, MUFA and PUFA values, similar to the results obtained in this study. Oraei et al. (2011) found that low temperatures reduce the production of free radicals and thus slow down fatty acid changes. Mbarki et al. (2008) irradiated bonito fish (*Sarda sarda*) at 0, 1.5, 4.5, 6 and 7 kGy levels and stored them at 2 ºC. They detected an increase in SFA values and a significant decrease in PUFA values of the control and irradiated

groups during 21 days of storage. It was observed that irradiation was initially effective on EPA+DHA values, and as the amount of irradiation increased, there was a decrease in EPA+DHA values. Researchers stated that there was a decrease in EPA+DHA values during storage and that this decrease was high in the control group, but as the irradiation dose increased, the amount of decrease decreased. In our study, while a decrease was observed in the EPA+DHA values of the control group during storage, it was observed that storage did not cause any change in the groups irradiated at 3 and 5 kGy doses. Etyemez (2011) reported that irradiation did not cause any change in SFA and MUFA values in frog legs (*Rana esculenta*) irradiated at 4 and 5 kGy levels While some decrease was observed in PUFA values with irradiation, it was stated that there was no difference in PUFA values in the groups irradiated at a dose of 4 or 5 kGy.

It is stated in HSMO (1994) that the most appropriate ω6/ω3 ratio should be below 4.0. A low omega-6/omega-3 ratio has been linked to a lower risk of breast cancer in women and has been shown to benefit asthmatic patients. It was crucial to lower the ratio of w6 to w3 fatty acids in the human diet in order to lower the risk of cancer and coronary heart disease. (Durmuş 2019) The obtained ω6/ω3 ratios were included in these values. Yıldız et al. (2006) found the ω6/ω3 ratio to be 0.80 for rainbow trout. Likewise, Senadheera et al. (2012) found the ω6/ω3 ratio to be 0.98 in their study on rainbow trout. Similar results to our study of PUFA/SFA ratios were reported by Mbarki et al. (2008) and Gecgel (2011). It was also reported that the PUFA/SFA ratio recommended by HMSO (1994) should be at least 0.45. An index called PUFA/SFA is typically used to evaluate how food affects cardiovascular health (CVH). The theory suggests that while all SFAs contribute to elevated serum cholesterol, all PUFAs in the diet can suppress LDL-C and lower serum cholesterol levels. Therefore, the effect is more favorable the larger this ratio (Chen and Liu 2020). The lowest PUFA/SFA ratio detected in this research was 1.39.

Lipid oxidation is one of the main factors that affects fatty acids, especially PUFA and causes the spoilage of the food (Fernandez et al. 1997; Pearson et al. 1983). Lipid oxidation in fish can increase or decrease by several factors. Species, gender, size, process and storage conditions, prooxidants and antioxidants in fish, packaging methods and some other factors are quite effective on lipid oxidation (Polat and Tokur 2000; Ju-Woon et al. 2002; Mbarki et al. 2009). The first stage of the lipid oxidation starts with the connection of oxygen to the double bonds between carbon atoms on PUFA and the occurrence of peroxides. After that the second stage starts and peroxides degrade to aldehydes, ketones and carboxylic acids (Porter et al. 1992). Malonaldehyde, which was generated at this stage, reacts with thiobarbituric acid and a reddish pigment occurs (Fernandez et al. 1997). Rancidity can be determined by measuring this reddish pigment by calorimetric methods. Thiobarbituric acid value (TBA) is an important parameter that shows the rancidity levels of lipids (Piranavatharsan et al. 2023).

Many studies on gamma irradiation have obtained similar results (Lakshmanan et al. 1999; Cozzo-Siqueira et al. 2003; Chouliara et al. 2004; Özden et al. 2007; Mbarki et al. 2009). Moini et al. (2009) reported that ionizing radiation increases the formation of free radicals in lipids. Some researchers also reported that smoking has a significant effect on lipid oxidation and increases the TBA level by twofold or higher (Goulas and Kontominas 2005; Tokur 2007; Koral et al. 2009). Al-Kuraieef (2021) examined the effects of gamma irradiation (1.5 kGy, 3 kGy and 4.5 kGy) on raw tilapia and smoked herring and mackerel. Increases were observed in the TBA values of smoked fish compared to raw tilapia. In addition, TBA values increased in parallel with the increase in irradiation. The highest increase was observed in samples irradiated with 4.5 kGy (tilapia: 0.592 mg MDA/kg; herring: 0.635 mg MDA/kg and mackerel 0.722 mg MDA/kg) and thus it was concluded that peroxides and hydroperoxides decomposed more quickly into lower molecular weight compounds. Mbarki et al. (2009) stated that when low doses of radiation were used, an oxygen-impermeable form of packaging should be used to increase the storage life of fish in the refrigerator or on ice. Surendra et al. (2018a) examined the quality changes of tilapia stored in ice as a result of gamma irradiation (1 kGy and 3 kGy). The researchers reported that TBA values were between 0.014 and 0.003 mg MDA/kgduring ice storage and did not exceed the consumption limits.

One of the most important factors in the deterioration of the quality of the fish is the decrease of the polyunsaturated fatty acids and consequently an increase of the peroxide value (Oraei et al. 2012). In some studies, the peroxide value of 5 meq/kg or less was reported as fresh fish and between 5 meq/kg and 10 meq/kg as the start of the deterioration (Javanmard et al. 2006; Oraei et al. 2012). Additionally, Connell (1995) reported that over 10 meq/kg was marked as not suitable for human consumption. Egan et al. (1997) in their studies suggested that, deterioration in flavor perceived when the peroxide value reached between 20-40 meq/kg.

Many researchers have reported that gamma irradiation increases peroxide values (Hussain et al.

1985; Hampson et al. 1996; Javanmard et al. 2006; Al-Bachir and Zeinou 2009; Gecgel 2011). Reale et al. (2008) reported that one of the factors that increase lipid oxidation is microbial enzymes. The decrease in microbial load in the environment by gamma irradiation positively affected lipid oxidation. Al-Kuraieef (2021) observed the effects of gamma irradiation (1.5 kGy, 3 kGy and 4.5 kGy) on raw tilapia and smoked herring and mackerel. In the study, it was determined that the peroxide values of gamma-irradiated raw tilapia, smoked herring and mackerel fish (14.3, 15.9 and 13.9 meq O_2/kg at 4.5 kGy, respectively) were statistically higher than the control values (5.7, 7.5 and 8.3 meq O_2/kg). The researchers reported that peroxide values increased with increasing doses**.** However, Surendra et al. (2018a) found that there were fluctuations in the PV values of tilapia fish gamma irradiated (1 kGy and 3 kGy) during storage, and there was no statistically significant change in terms of the irradiation doses at the end of the study.

Hydrolytic degradation is caused by lipase enzymes and triggers the formation of free fatty acids (FFA). The FFA then undergoes oxidation to form low molecular weight compounds which produce unpleasant odors and taste in fish and seafood products (Pacheco-Aguilar et al., 2000). The development of lipid hydrolysis depends considerably on the hydrolytic enzyme contents under the influence of different internal and external factors. Separation of free fatty acids from the triglyceride matrix can increase the rate of lipid oxidation and unpleasant odor development (Jasour et al., 2011).

The observed values show that irradiation affects the FFA values of the smoked trout fillets and raises the FFA values. However, it was found that different irradiation doses (3 and 5 kGy) did not make a significant difference until the last 2 weeks. The study conducted by Surendra et al. (2018b) reported that the FFA values of control and yellowfin tuna (*Thunnus albacares*) irradiated at 10 kGy levels were quite high, but the samples irradiated at 5 and 7 kGy levels were at very low levels. Hussain et al. (1985) studied Indian mackerel (*Rastrelliger kanagurta*) irradiated at 0, 1, 1.5, 2 and 3 kGy levels and examined the free fatty acid change. While they found the FFA value to be 1.82% in the control group, they found the FFA values to be 1.63%, 1.95%, 2.65% and 2.26% in the groups irradiated at 1, 1.5, 2 and 3 kGy levels, respectively.

It was determined that irradiation did not cause a statistically significant difference in the fatty acid components of smoked trout fillets However, there was a statistical increase in the myristic,palmitic and stearic acid values of the irradiated groups during storage. The highest increase in total SFA level was observed in the 5 kGy irradiated group, while the lowest increase was in the control group. On the other hand, irradiation did not affect PUFAs except linoleic acid. As for linoleic acid, it was determined that linoleic fatty acid levels in the control group were higher than those irradiated at 3 and 5 kGy doses. It was determined that the total PUFA values of the groups irradiated at 3 and 5 kGy doses during storage were lower than the control group. In this study, it was observed that the lipid stability of the control and 3 kGy irradiated groups was better than the 5 kGy irradiated group. In addition to gamma irradiation, the use of other processing methods such as smoking and vacuum packaging was effective in reducing the effect on lipids. With this research, it is thought that the application of combined methods including low dose irradiation to different processed or fresh seafood products will bring important contributions to the production of safe foods with longer shelf life.

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Evaluation of the Aquatic Oligochatea Fauna and Seasonal Changes in Water Quality of the Büyükçekmece Dam Lake, which Provides Water to İstanbul

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ABSTRACT

This study was conducted at 4 stations in Büyükçekmece Dam lake in 2020. In this study, water and Oligochaeta samples were collected seasonally. Parameters such as secchi disk depth, water temperature, dissolved oxygen, pH, electrical conductivity, salinity, total hardness, nitrite nitrogen, phosphate and nitrate nitrogen were measured. Water quality parameters were qualified by SKKY (2004) ve TSWQR (2021). Also, the study, *Limnodrilus hoffmeisteri* (Claparède 1862) and *Limnodrilus udekemianus* (Claparède 1862) species belonging to Oligochaeta were found in the dam lake. The number of individuals per m^2 and % dominance ratios of species were also calculated. It is recommended that similar and more comprehensive studies be carried out periodically in the dam lake to monitor the water quality of the dam lake regularly and to determine the benthic macroinvertebrate fauna.

Keywords: Büyükçekmece, dam, lake, water quality

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İstanbul'a Su Sağlayan Büyükçekmece Baraj Gölü'nün Sucul Oligochatea Faunası ve Su Kalitesindeki Mevsimsel Değişimlerin Değerlendirilmesi

Öz: : Bu çalışma 2020 yılında Büyükçekmece baraj gölünde 4 istasyonda yapıldı. Çalışmada mevsimsel olarak su ve Oligochaeta örnekleri toplandı. Gölün secchi disk derinliği, su sıcaklığı, çözünmüş oksijen, pH, elektriksel iletkenlik, tuzluluk, toplam sertlik, nitrit azotu, fosfat ve nitrat azotu gibi parametreleri ölçüldü. Su kalite parametreleri SKKY (2004) ve TSWQR (2021)'na göre değerlendirildi. Çalışmada ayrıca, baraj gölünde Oligochaeta'ya ait *Limnodrilus hoffmeisteri* (Claparède 1862) ve *Limnodrilus udekemianus* (Claparède 1862) türleri bulundu. Türlerin m^2 deki birey sayıları ile % dominansi oranları da hesaplandı. Baraj gölünün su kalitesinin düzenli olarak izlenmesi ve bentik makroomurgasız faunasının belirlenmesi için benzer ve daha kapsamlı çalışmaların, gölde periyodik olarak yapılması önerilir.

Anahtar kelimeler: Büyükçekmece, baraj, göl, su kalitesi

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Introduction

The Büyükçekmece basin is located on the Çatalca Peninsula, in the western side of, the coast of the Marmara Sea, Istanbul. There is Küçükçekmece Lake to the east, Silivri settlement to the west, The Terkos drinking water basin to the north and the Marmara Sea to the south. Settlements are present in Büyükçekmece, Çatalca and Silivri districts within the basin. Büyükçekmece Lake is the second largest water source on the European side of Istanbul (Tekin 2010).

Aquatic Oligochaetas, which are important components of ecosystems, constitute an important part of the benthic fauna. Oligochaetas have a wide distribution in the world (Brinkhurst and Jamieson 1971). They are usually free-living on the ground, but some species are found in vegetation, in organic waste or between leaves. Most of the aquatic

Oligocheata species digest the sand and mud on the ground, as well as bacteria and other microorganisms, and return them to the environment. Thus, they provide the cleaning and aeration of the bottom mud (Arslan and Ahıska 2004). The Oligochaeta group is abundant in almost all seasons and in all kinds of waters increases their importance even more. It is also uses as a food source with high protein value for fish and is used as live feed in aquarium fisheries (Loden 1974).

Previous studies in the lake are mainly focused on fish fauna, fish parasites and fish biology. There have been very few studies on the benthic fauna of the lake. Koşal-Şahin (2006) revealed the benthic macroinvertebrate fauna of the lake in her phD thesis. Dorak (2019) conducted a preliminary study on the Rotifera group to determine the trophic structure of the lake and examined some physicochemical parameters seasonally.

Some studies of this kind in Turkey are by Arslan (2006), Yıldız and Balık (2006); Yıldız et al. (2008); Odabaşı et al. (2018); Fındık et al. (2019); Albayrak et al. (2023).

The aim of this study was to reveal the aquatic Oligochaeta fauna and seasonal changes in the water quality of Büyükçekmece Dam Lake, which provides water to Istanbul.

Materials and Methods

The Büyükçekmece Lake is located in northwestern Turkey, 50 km from the city center of Istanbul (Soyer 2003). Büyükçekmece Dam Lake, built on a lake located at the mouth of the Karasu Stream, flows into the Marmara Sea. As a result of the construction of a 11.4 m dam wall between the Marmara Sea and the lake by DSİ between 1983 and 1988, Büyükçekmece Dam Lake lost its lagoon characteristics and became a freshwater lake (Özuluğ 1999). It has been reported that the lake meets the drinking and potable water needs of Istanbul with 70 hm^3 of water per year (Aktan et al. 2006).

It has been reported that the total watershed area of Büyükçekmece Dam Lake is 622 km^2, with a surface area of 28.5 km, a length of 10 km and a width of 2.5 km (Soyer 2003). The maximum depth of the lake was reported to be 7.15 m by Meriç (1992). The main stream of the lake is The Karasu Stream in the north of the lake. It has many tributaries such as Delice, Karamurad, Tavşan, Ayva, Akalan, Kestanelik and Öncürlü. Keşliçiftiği on the west side of the lake and Çekmece Streams on the east side are other sources (Özuluğ 1999; Dorak 2019). The Büyükçekmece dam lake and sampling locations are shown in Figure 1.

Figure 1. Sampling locations

In 2020, research was planned to be conducted over four seasons. For this purpose, 4 stations were determined. The study was carried out in two phases: field and laboratory work. During the field study, the water the turbidity was measured in meters using a secchi disk. The water temperature of the stations was measured in ºC using a simple thermometer. A tape measure was used to measure the depth of the water at the stations which was determined in m. The pH, electrical conductivity (in µS/cm) and salinity (in ‰) of the water were measured during sampling using a YSI 556 model multiparameter meter.

The Total hardness (in ºFS), NO_2-N, NO_3-N and PO_4 values (in mg/l) were calculated (APHA, AWWA, WPCF 1989). Simultaneously to the water sampling, benthos were also sampled from the sediment part of the lake. The benthos samples taken using Ekman grab (15x15) were homogenized with lake water. Each station was sampled once with an Ekman grab collection. Three sieves with different mesh sizes (1.19 mm, 0.595 mm, 0.297 mm respectively) were passed through the sieves. The organisms remaining on the sieves were placed in plastic mud bottles containing 70% ethyl alcohol with the help of fine-tipped forceps and sealed with the station number. Mud bottles transported to the laboratory were removed from the deposit under a stereobinocular microscope and placed in glass tubes containing 70% alcohol. Sample collection, sieving, fixation The methods of Welch, 1948 were utilized in the identification and conservation. Oligochaeta specimens were identified under an Olympus CK2 inverted microscope by preparing permanent and temporary preparations (temporary preparations were made with glycerin and permanent preparations were made with polyvinyl lactophenol). Brinkhurst and Jamieson (1971), Timm (2009), Wetzel et al. (2000) were used for species identification. Identified organisms were also counted and the number of individuals per m^2 and dominance ratios were determined according to Bellan-Santini (1969).

Results

The seasonal measured values, minimum, maximum, average and quality classes of the stations according to the Turkish Surface Water Quality Control Regulation (TSWQR 2021) and Water Pollution Quality Control Regulation (SKKY 2004) are given in Table 1.

Table 1. Some Physicochemical Data of Büyükçekmece Lake Measured Seasonally

Stations	Variables	Spring	Summer	Autumn	Winter	Average	TSWQR, 2021	SKKY 2004
Station 1	Secchi (m)	0.5	1	1	1	0.8		
	W.T. (ºC)	5	21	15	4	13.2		I
	Depth (m)	1	1	1	1.5	1.2		
	D.O (mg/l)	8	6	7	12	8.6	I and II	
	pH	7.5	7	7.3	7.3	7.27	I	
	E.C (µS/cm)	453	550	498	500	500	I and II	
	Salinity (‰)	0.03	0	0	0.03	0.03		
	T.H. (^{0}FS)	13	13	12	17	13.4		
	NO_2-N (mg/l)	0	0.002	0	0	0.0005		I
	PO_4- (mg/l)	0	0.001	0	0.0103	0.0028		
	NO_3-N (mg/l)	0	0.001	0	0	0.0002	I	I
Station 2	Secchi (m)	0.5	1	0.5	1	0.7		
	W.T. (ºC)	5	24	15	5	14.4		I
	Depth (m)	2	2	2	2	1.9		
	D.O (mg/l)	10	5	8	9	8.8	I and II	
	pH	6.8	7	7	7	7.16		
	E.C (µS/cm)	452	550	514	526	510	I and II	
	Salinity (‰)	0.03	0	0	0.03	0.015		
	T.H (^{0}FS)	14	13	14	17.3	14.5		
	NO_2-N (mg/l)	0	0.001	0	0	0.0005		I
	PO_4 (mg/l)	0	0.001	0	0.008	0.0022		
	NO_3-N (mg/l)	0	0	0	0	0	I	

	(table continues)							
Station 3	Secchi (m)	1	1	0.5	2	1.2		
	W.T. (°C)	5	25	17	5	13		
	Depth (m)	2	2	2	2	2		
	D.O (mg/l)	10	7	7	11	8.75	I and II	
	pH	8	7	7	7.6	7.52	I	
	E.C (µS/cm)	470	545	500	510	506	I and II	
	Salinity (‰)	0.03	0	0	0.03	0.015		
	T.H.(^{0}FS)	13.4	13	12	18.9	14		
	NO_2- N mg/l)	0.026	0.001	0	0.068	0.023		III
	PO_4 (mg/l)	0	0.001	0	0.05	0.012		
	NO_3-N (mg/l)	0	0	0	0	0		
Station 4	Secchi (m)	0.5	1	1	1	1		
	W.T (°C)	5	25	17	5	13		I
	Depth (m)	7	7	7	5	6.5		
	D.O (mg/l)	10	7	7	11	8.75	I and II	
	pH	6	7	7	7.3	6.82		
	E.C (µS/cm)	480	540	514	498	508	I and II	
	Salinity (‰)	0.03	0	0	0.03	0.03		
	T.H. (^{0}FS)	12.9	12	13	18.9	14.2		
	NO_2-N (mg/l)	0.088	0.001	0	0.034	0.031	.	III
	PO_4 (mg/l)	0.011	0.001	0	0.0009	0.003		
	NO_3-N (mg/l)	0	0	0	0	0	I	

(W.T: water temperature; D.O:dissolved oxygen; E.C:electirical conductivity; T.H.:total hardness; NO_2-N:nitrite nitrogen, PO_4: phosphate; NO_3-N: nitrate nitrogen)

As a result of the identification of the Oligochaeta collected from the lake, it was found that 55.07% of the lake was composed of *L. udekemianus* and 44.93% of the lake was composed of *L. hoffmeisteri*. A total of 1976 individuals were detected in each m^2 of the lake. The highest number of individuals was 3333 individuals per m^2 at the 2nd station, followed by 2311 individuals per m^2 at the 1st station. A total of 1554 individuals were found at the 3rd station and 707 individuals were found at the 4th station. The distribution and dominance ratios of Oligochaeta species collected from the dam lake in terms of stations and seasons are presented in Table 2.

Table 2. Distribution and dominance ratios of species in Büyükçekmece dam lake according to seasons and stations

Seasons	Species	St. 1	St. 2	St. 3	St. 4	Total	Dominance %
Spring	*Limnodrilus hoffmeisteri*	-	-	400	-	400	5.06
	Limnodrilus udekemianus	179	311	-	311	800	10.12
Summer	*Limnodrilus hoffmeisteri*	844	533	400	44	1821	23,04
	Limnodrilus udekemianus	756	800	222	132	1910	24.17

(table continues)

Autumn	*Limnodrilus hoffmeisteri*	178	-	44	44	266	3.36
	Limnodrilus udekemianus	222	-	178	-	400	5.06
Winter	*Limnodrilus hoffmeisteri*	88	800	88	88	1064	13.47
	Limnodrilus udekemianus	44	889	222	88	1243	15.72
	Total	2311	3333	1554	707	7904	100

Limnodrilus hoffmeisteri	44.93 %
Limnodrilus udekemianus	55.07 %

When the dominance rates were analyzed, the highest dominance rate was found in summer with 47.21%, followed by winter with 29.19%. Oligochaeta were 15.18% in spring and 8.42% in autumn (Figure 2).

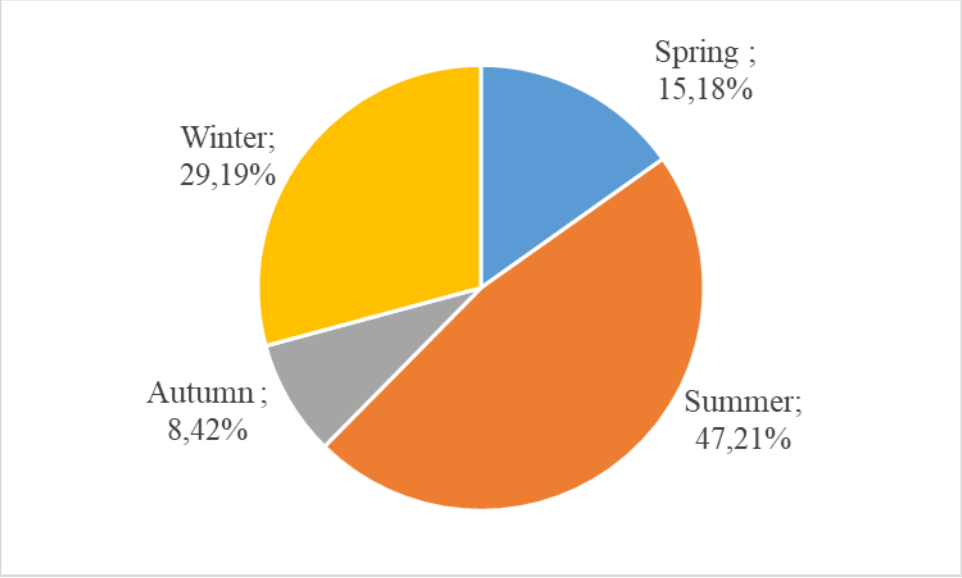

Figure 2. Seasonal Distribution of Oligochaeta in Büyükçekmece Dam Lake

Discussion

As a result of the study, 1976 individuals were detected in each m^2 of the lake. Two aquatic species of Oligochaetas, *L.hoffmeisteri* and *L.udekemianus* were detected in the lake. The detected species were observed in all seasons. The decrease in the predominance of Oligochaetas in spring and autumn can be attributed to the increase in water level due to the rainy seasons, and sampling could not be done completely from the lake.

When Büyükçekmece Lake is analyzed in terms of physicochemical data, it was observed that water temperature, pH, NO_2-N values are in 1st class water quality. DO and EC values were found to be between 1st and 2nd class water quality. NO_3-N values were not found at 2nd and 4th stations in all seasons. NO_2-N values were found to be in 1st and 2nd class water quality throughout the lake. NO_2-N value was found to be in the 4th quality in the winter season at station 3 and in the spring season at station 4 (TSWQR 2021; SKKY 2004).

Bayram and Kankal (2015) stated that one of the most important factors affecting DO concentration in surface waters is W.T. The increase in temperature decreases the solubility of oxygen in water. Based on this relationship, they reported that the amount of DO in cold water is higher than in warm water. In this case, DO concentration in surface waters increases in winter months when temperatures are low and decreases in summer months when temperatures are

high. In this study conducted in Büyükçekmece Dam Lake, it was determined that DO concentration was higher in winter and lower in summer. The graph of temperature and DO values determined during the seasons are plotted in Figure 3.

Koşal-Şahin (2006) reported that taxa such as *L.udekemianus, L.hoffmeisteri*, *Limnodrilus* sp., *Nais communis*, *Nais* sp., *Potamothrix hammoniensis*, *Psammoryctides albicola*, *Stylaria lacustris*, *Tubifex tubifex* were found. When our study is compared with this study, it is seen that there is a decrease in species diversity. Koşal-Şahin (2006) reported that 9 taxa were found in the lake. In this study, two species were found, only.

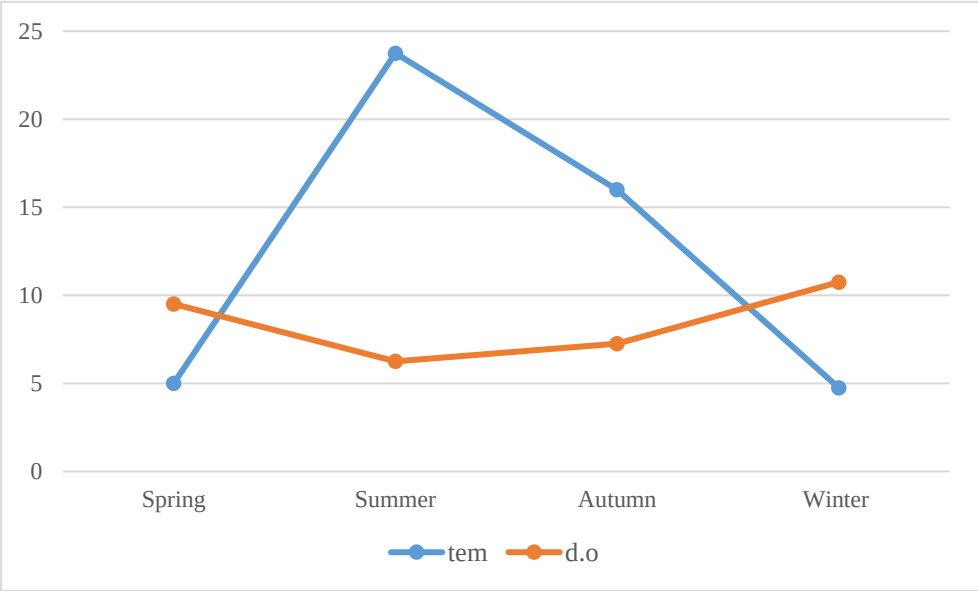

Figure 3. Temperature and dissolved oxygen graph of the lake

Koşal-Şahin (2006) reported that 939 per m^2 belonged to Oligochaeta in Büyükçekmece Lake. In this study, 1976 individuals were found in each m^2 of the lake. It was determined that the number of individuals per m^2 of Oligochaeta has doubled in the last 18 years. This may be because species that are indicators of pollution have adapted to the environment and proliferated, resulting in the disappearance of other species. The main impact of organic pollution in aquatic systems is seen on the community structure. Changes in the abundance and diversity of living organisms, especially with changing environmental conditions, occur in the form of an increase in the accumulation of organic matter on the substrates caused by the accumulation of wastes, which is preferred by some organisms. Accordingly, species that become dominant in an environment suppress other species, thus reducing species diversity (Kazancı et al.1997).

L. hoffmeisteri, one of the most common species in our study, is a highly pollution tolerant species and, together with other Tubificid species, is more frequently found in polluted habitats (Brinkhurst and Jamieson 1971; Timm 1999). It has been reported that *L. udekemianus* is a cosmopolitan species that can be found in many different environments, from oligotrophic habitats to habitats rich in organic matter (Timm 1970). In the study, the mean water temperature of the lake was found to be 13.4 ºC, the dissolved oxygen value was found to be 8.72 mg/L, the pH value was 7.19 and the salinity value was found 0.02 ppm. The electrical conductivity value of the lake was found 506 µS/cm and the secchi disk depth was 0.92 m.

Dorak (2019) reported the average water temperature of the lake as 16.3ºC and dissolved oxygen value as 9.5 mg/l. She found that the average pH value of the lake was 8.1 and the salinity value was 0.23 ppm. The electrical conductivity value of the lake was 473.1 µS/cm and the secchi disk depth was 0.8 m.

Arslan (2006) reported 1 potamodrilid, 4 aeolosomatids and 94 Oligochaeta species (1 lumbriculid, 1 haplotaxid, 46 naidids, 38 tubificids, 6 enchytraeids, 1 lumbricid and 1 criodrilid) from aquatic systems in different regions of Turkey. Information on the distribution of these species is described. Odabaşı et al. (2018) investigated the aquatic oligochaete fauna of the rivers in the Biga Peninsula and their seasonal variations. They reported that 33 taxa were identified as belonging to Oligochaeta. Their findings indicated that the pH and NO_3-N values of the streams fell within the 1st class

quality range, while the EC, DO and biological oxygen demand (BOD) values ranged between the 1st and 2nd class water quality levels. Fındık et al. (2019) conducted such a study in Damsa Dam Lake (Nevşehir). They reported 3 species of Oligochaeta; *Stylaria lacustris*, *Limnodrilus hoffmeisteri*, *Nais elinguis* species in the lake. They determined that the Oligochaetas were the most dominant group in the summer season. Yıldız and Balık (2006) determined the Oligochaeta fauna of Topçam Dam Lake. As a result of the research, they identified 11 Oligochaeta species. It was reported that *Limnodrilus hoffmesteri* was the dominant species in the lake and constituted 64.64% of the whole community. Yıldız et al. (2008) determined the macrobenthic fauna of Kemer reservoir. They found 10 taxa belonging to the Oligochaeta. They reported that 498 individuals were found in m^2 of the dam lake.

Albayrak et al. (2023) identified nine Oligochaeta taxa in their study of the Göksu stream. They reported that the DO values of the stream fell within the range of the 1st and 2nd classes, while the EC values are categorized in the 2nd class. Total phosphorus (TP) values are classified in the 3rd class, orthophosphate (o-PO_4) values in the 1st class, and ammonium nitrogen (NH_4-N) values were classified into the 2nd class.

As a result, two (*L.hoffmeiseri* and *L.udekemianus*) pollution indicator species of Oligochaeta were detected in the study conducted in Büyükçekmece dam lake for one year, seasonally. It was determined that Oligochaeta were represented by 1976 individuals in m^2. In the spring season at station 4 and in the winter season at station 3, NO_2-N variables decreased to 4th quality was determined.

It is recommended that the dam lake, which provides water to a large part of Istanbul, be examined in terms of various physicochemical, microbiological and toxicological parameters for all benthic macroinvertebrates and that these and similar studies be carried out at regular intervals.

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The Current Status of Viral Nervous Necrosis Disease in Türkiye

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ABSTRACT

The agent of Viral Nervous Necrosis (VNN) disease is betanodavirus and is a viral fish disease and VNN disease has been reported in many fish species in Türkiye and around the world. It is known to cause high mortality rates in aquatic animals living in both marine and fresh water. It has been reported that the RGNNV and RGNNV/SJNNV genotypes of the virus, which has four genotypes, were detected in Turkey by the end of 2023. RGNNV genotype was detected in sea bass (*Dicentrarchus labrax*) for the first time in our country in 2011 and after that has since been found in other fish species such as sea bream (*Sparus aurata*), red mullet (*Mullus barbatus*), and garfish (*Belone belone*) in the Mediterranean region. RGNNV genotypes have also been reported in sea bass (*D. labrax*) in the Black Sea, and in sea bass (*D. labrax*) and RGNNV/SJNNV genotypes have been reported sea bream (*S. aurata*) in the Aegean Sea. In this study, studies on VNN in Turkey were reviewed and it was aimed to discuss the current status of the disease as a whole.

Keywords: Betanodavirus, Türkiye, RGNNV, RGNNV/SJNNV

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Viral Nervöz Nekrozis Hastalığının Türkiye'deki Durumu

Öz: : Etkeni betanodavirus olan Viral nervöz nekrozis (VNN) hastalığı, Türkiye'de ve dünyada birçok balık türünde bildirimi yapılmış viral balık hastalığıdır. Hem deniz hemde tatlı sularda yaşayan sucul canlılarda yüksek mortaliteye neden olmaktadır. Dört genotipi bulunan virusun, Türkiye'de 2023 yılı sonuna kadar RGNNV ve RGNNV/SJNNV genotiplerinin tespit edildiği bildirilmiştir. Ülkemizde ilk defa 2011 yılında levrek (*Dicentrarchus labrax*) balığında RGNNV genotipi tespit edilmiştir. Sonrasında Akdeniz'de levrek (*D. labrax*), çipura *(Sparus aurata),* barbun *(Mullus barbatus)* ve zarganada (*Belone belone*) RGNNV virüs bulunmuştur. Karadeniz' de levrekte *(D. labrax)* RGNNV ve Ege denizinde levrekte (*D. labrax*) RGNNV ve çipurada *(S. aurata*) RGNNV/SJNNV genotipleri bildirilmiştir. Bu çalışmada, Türkiye'de VNN konusunda yapılmış çalışmalar taranmış ve Türkiye'de hastalığın güncel durumunun bir bütün olarak ele alınması amaçlanmıştır.

Anahtar kelimeler: Betanodavirus, Türkiye, RGNNV, RGNNV/SJNNV

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Introduction

Betanodaviruses, belonging to the Nodaviridae family, are known to cause viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) (Muroga 2001). VNN is a significant viral disease that affects marine fish farming worldwide. The disease causes neuropathological effects by attacking the central nervous system and retina through nerve degeneration and cellular vacuolation. While the virus can cause disease in adult fish, it is particularly devastating in fish larvae and juveniles (Totland et al. 1999; Johansen et al. 2004). Initially, VNN was thought to be specific to marine fish, but in recent years, cases of the illness have been reported in freshwater fish (Shetty et al. 2012; Costa and Thompson 2016). The VNN virus affects both farmed and wild fish living in marine and freshwater environments, and has been reported to infect more than 173 fish species (Bandin and Souto 2020).

The four genotypes of the viral nervous necrosis virus, Striped Jack Nervous Necrosis Virus (SJNNV), the Tiger Puffer Nervous Necrosis Virus (TPNNV), the Red-spotted Grouper Nervous Necrosis Virus (RGNNV), and the Barfin Flounder Nervous Necrosis Virus (BFNNV), were classified based on the T4 variable region of the RNA2 segment (Nishizawa et al. 1995; Panzarin et al. 2016). Turbot nervous necrosis virus (TNNV) has been proposed as a potential fifth genotype, but it remains unofficially classified (Johansen et al. 2004).

Betanodavirus's segmented genome allows for reassortment, a process where genetic segments from different genotypes combine to form new viral strains. Reassortant viruses combining SJNNV and RGNNV genotypes (RGNNV/SJNNV or SJNNV/RGNNV) have been frequently reported in sea bream and sea bass (Panzarin et al. 2014; Toffan et al. 2017; Volpe et al. 2020; Kaplan et al. 2022b). VNN virus was divided into three main serotypes by neutralization tests with polyclonal antibodies; SJNNV and RGNNV/SJNNV genotypes were classified in serotype A, TPNNV genotype in serotype B and BFNNV, RGNNV and SJNNV/RGNNV genotypes in serotype C (Nishizawa et al. 1997; Mori et al. 2003; Iwamoto et al. 2004; Panzarin et al. 2016).

Clinical signs of VNN in fish include abnormal swimming behavior, lethargy, skin discoloration (darkening), anorexia, and symptoms of nervous system malfunction caused by retinal and brain injuries. Both young and adult fish are susceptible to the disease, but it progresses more slowly in older fish, leading to individual mortality, while in larvae and juveniles, it progresses rapidly and leads to cumulative mortality. Another clinical sign is hyperinflammation in the air sac of the fish (Maltese and Bovo 2007; Nopadon et al. 2009; Vendramin et al. 2013). Typical symptoms of the disease include spiral swimming, abnormal response to stimulation, spinal deformation, exophthalmos, and opacification in the eyes (Gomez et al. 2009; Nopadon et al. 2009; Vendramin et al. 2013). Clinical signs of VNN include abnormal swimming behavior, lethargy, skin discoloration (darkening), anorexia, and symptoms of nervous system malfunction brought on by retinal and brain injuries. Young fish, as well as adult fish, are more susceptible to the disease. In older fish, the disease progresses more slowly and individual mortality occurs, while in larvae and juveniles, the onset of the disease is hyperacute and cumulative mortality occurs (Vendramin et al. 2014).

In this study, researchers in Türkiye collected data on the VNN virus, including the types of fish investigated, genotypes identified, and areas studied, and summarized it as a whole.

Geographical Distribution of the Virus

Water temperature significantly influences how viruses interact with their hosts. When the water temperature is suitable, it can demonstrate its capacity to cause illness. The infectious agent's capacity to colonize the host can be altered by temperature. VNN exhibits this temperature dependence, with different genotypes thriving at specific ranges. BFNNV genotype 15-20 ºC, TPNNV genotype 20 ºC, SJNNV 20-25 ºC, RGNNV 25-30 ºC water temperatures transpire. Therefore, different VNN genotypes are found in regions with varying water temperatures (Nylund et al. 2008; Maltese and Bovo 2007).

VNN virus has a wide geographical distribution due to its different genotypes. There have been reports of RGNNV in several fish species, and it is the most prevalent genotype in temperate and tropical fish species. RGNNV is extensively dispersed in farmed fish as well as wild fish throughout the Mediterranean basin and along the coastlines of Asia and Australia, according to recent research conducted in various geographic locations (Moody et al. 2009; Ciulli et al. 2007; Gomez et al. 2010; Gomez et al. 2004; Liu et al. 2015). Tuna sturgeon (*Acipenser gueldenstaedtii*), also freshwater species in Europe, Asia and Australia (Athanassopoulou et al. 2004), Goldfish (*Carassius auratus*) (Jithendran et al. 2011), Catfish (*Tandanus tandanus*) (Munday et al. 2002), Chinese catfish (*Parasilurus asotus*) (Chi et al. 2003), pikeperch (*Sander lucioperca*) (Bovo et al. 2011) reported as the only genotype associated with outbreaks. The BFNNV genotype is restricted to cold waters, mainly Japan, America and northern Europe. BFNNV was isolated from haddock (*Melanogrammus aeglefinus*), Atlantic and Pacific cod (*Gadus macrocephalus*), barfin halibut and Atlantic halibut in farms, and Atlantic cod and different wild fish species in Scandinavian coastal waters (Nylund et al. 2008; Korsnes et al. 2017). In contrast, the TPNNV genotype appears to be a minor variant. Because it was isolated from only one species in Japan (Nishizawa et al. 1997). The SJNNV genotype was thought to be restricted to Japan, as it was isolated only in kingfish (*Pseudocaranx dentex*) and red bream (*Pagrus major*) from farmed fish in Japan. However, it has also been reported in farmed sea bream (*Sparus aurata*), rock bass (*Argyrosomus regius*) and Solea senegalensis, a flatfish, in the Iberian Peninsula of Spain (Cutrín et al. 2007; Lopez-Jimena et al. 2010).

Research Conducted in Türkiye

The first study on VNN virus in Türkiye was conducted by Özkan Özyer et al. (2012). They sampled a total of 20 hatcheries and 33 fish farms were sampled from sea bass (*Dicentrarchus labrax*)

and sea bream (*S. aurata*) farms in the Aegean region, especially during periods when the water temperature reached 25 ºC and above. The collected samples were diagnosed by virus isolation method in SNN-1 cell line, Indirect Fluorescent Antibody Test (IFAT) method and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method. The researchers used conventional RT-PCR, which was performed using the primer set to amplify 427 bp of RNA2 (capsid protein gene), as reported by Nishizawa et al (1994). However, no positivity was obtained by all three methods (Özkan Özyer et al. 2012).

In 2011, 30 sea bass taken from a sea bass farm in Mersin province of the Mediterranean region and were tested for virüs isolation on SNN-1 cell line and CPE was observed in the 3rd passage, followed by immunofluorescent antibody test (IFAT) and RT-PCR technique. The results were positive and the first detection of VNN virus in Türkiye was reported. The primer set amplifying 427 bp of RNA2 (capsid protein gene) reported by Nishizawa et al. (1994) was used in the study. As a result of the sequencing, 99% RGNNV genotype according to polymerase protein (RNA-1) and 99% RGNNV genotype according to capsid protein (RNA-2) were determined (Özkan Özyer et al. 2014).

After the detection of the presence of the virus in 2011, the virus was detected again in fingerling sea bass and sea bream hatcheries that did not show symptoms. In 2014, the virus was detected in asymptomatic sea bass and sea bream fry, and the disease was clinically detected in fingerling-sized sea bass in a hatchery and marine facility with 5% and 10% mortality (Kalaycı et al. 2016). Until 2016, studies conducted in the Aegean and Mediterranean regions revealed the presence of VNN virus.

Since VNN virus is not a notifiable disease, there was not much data on its spread and prevalence in Türkiye (Kaplan 2019). The researchers investigated sea bass hatcheries (16) and (20) sea bass breeding facilities in Türkiye. Positivity was obtained in fingerling size fish from one hatchery. It was reported that the virus was not detected in the other fishes in the hatchery, due to the hatchery closed recirculating system, which provided the biosecurity; the positivity obtained was reported to be samples from the pools using direct seawater. Based on the RNA1 and RNA2 segments genome analysis, the positive isolate was shown to have the RGNNV genotype (Kaplan 2019). In the sea bass breeding farm, it was isolated from sea bass weighing between 3.2 and 10.4 g and 6-10 cm in length and the prevalence was reported to be 5%. The facility where it was found to be positive and the hatchery were found to be connected (Kaplan and Karaoğlu 2021a).

After the Mediterranean and Aegean regions, screening studies were conducted in the Black Sea region. In 2016, samples were collected from 6 fish farms and VNN virus positivity was obtained from one fish farming. It was determined that the virus obtained was in the RGNNV genotype and was close to the freshwater fish *Micropterus salmoides* (largemouth bass) and *S. lucioperca* in Italy. The analysis in terms of RNA2 also revealed close similarities with *D. labrax* (sea bass) in Cyprus and groupers in China, respectively. No positivity was obtained in the scans conducted in 2017-2019 (Kaplan et al. 2021b).

There are reports that molluscs may be a potential reservoir for aquatic viruses (Gomez et al. 2010; Panzarin et al. 2012). In Türkiye, between 2016 and 2020, samples were collected from carpet shell clam (*Ruditapes decussatus*) and black mussel (*Mytilus galloprovincialis*) stations in the North Aegean and Marmara Seas. Thirty carpet Shell clam and black mussels were taken from each station, and samples prepared from their hepatopancreas were tested by real Time RT-PCR, but no positivity was obtained (Kaplan et al. 2022a).

Until 2019, VNN virus was detected in all regions of the Aegean, Mediterranean and Black Sea regions and it was reported to be in the RGNNV genotype according to the sequence data but there were no reports on other genotypes. When the homogenates prepared from asymptomatic sea bream (*S. aurata*) weighing 90-100 g sampled from a fish farm in the Aegean region and samples were taken from the hatchery associated with this farm were analyzed, VNN virus was detected and it was reported to be RGNNV/SJNNV reassortant according to sequence data. In the study, RGNNV/SJNNV genotype was identified for the first time in Türkiye. While VNN virus strains detected between 2016 and 2019 were very similar among themselves, it was reported that isolates of the RGNNV/SJNNV genotype were very different genotipically (Kaplan et al. 2022b).

In addition, between 2019 and 2021, a total of 400 wild fish from 27 different species were sampled in the Mediterranean region. A high prevalence of VNN virus was detected in the garfish (*Belone belone*) sampled from Iskenderun Gulf in October 2019 and in the red mullet (*Mullus barbatus*) collected in Antalya Gulf in September 2021. According to the RNA1 segment, both isolates were identified to be RGNNV genotype, but according to the RNA2 segment, the isolate obtained from garfish was determined to be SJNNV and the isolate obtained from red mullet was determined to be RGNNV genotype. Therefore, while the isolate from garfish was reassortant RGNNV/SJNNV, the isolate from red mullet was determined as RGNNV genotype (Kaplan et al. 2023).

In a study on aquarium fish, a total of 180 fish samples were taken from koi (*Cyprinus carpio*) and

goldfish (*C. auratus*) species from 3 different aquarium farms operating in Antalya province. With this study, the presence of VNN virus in aquarium fish was investigated for the first time in Türkiye and positivity was not obtained (Oğuz et al. 2023). Information about the studies conducted in Türkiye is presented in Table 1.

Table1. Information on studies conducted in Türkiye

Fish species	Latin name	Status	Genotype	Methods	Country or Region	Year	References
Sea bass Sea bream	*Dicentrarchus labrax* *Sparus aurata*	Hatchery and Farm	Not determined	SNN-1 Cell IFAT, RT-PCR	Aegean	2010, 2011	Özkan Özyer et al. 2012
Sea bass	*Dicentrarchus labrax**	Farm	RGNNV	SNN-1 Cell , IFAT, RT-PCR	Mersin	2011	Özkan Özyer et al. 2014
Sea bass Sea bream	*Dicentrarchus labrax** *Sparus aurata**	Hatchery and Farm	RGNNV	SNN-1 Cell, IFAT, RT-PCR	Aegean	2012, 2014	Kalaycı et al. 2016
Sea bass	*Dicentrarchus labrax**	Hatchery and Farm	RGNNV	SNN-1 Cell, Real Time RT-PCR	Mediterranean and Aegean	2016,2017	Kaplan and Karaoğlu 2019
Sea bass	*Dicentrarchus labrax**	Farm	RGNNV	Real Time RT-PCR	Black Sea	2016	Kaplan et al.2021b
Carpet shell Mussel	*Ruditapes decussatus* *Mytilus galloprovincialis*	Farm	Not determined	Real Time RT-PCR	Aegean and Marmara Seas	2016, 2017, 2018, 2019,2020	Kaplan et al. 2022a
Sea bream	*Sparus aurata**	Hatchery	RGNNV/ SJNNV	Real Time RT-PCR	Aegean	2019	Kaplan et al. 2022b
Atlantic chub mackerel Lizzard fish Sea bream Bogue Goldbanded goatfish Round herring	*Scomber colias* *Saurida lessepsianus* *Sparus aurata* *Boops boops* *Upeneus moluccensis* *Etrumeus golanii*	Wild	Not determined	Real Time RT-PCR	Mediterranean	2019	Kaplan et al. 2023
Garfish Grey mullet Red mullet Annular seabream Bogue Sea bream	*Belone belone** *Mugil cephalus* *Mullus barbatus* *Diplodus annularis* *Boops boops* *Sparus aurata*	Wild	RGNNV Not determined	Real Time RT-PCR	Mediterranean	2020	Kaplan et al. 2023
Sea bass Sardine Common sole Lizzard fish	*Dicentrarchus labrax* *Sardinella aurita* *Solea solea* *Saurida lessepsianus*	Wild	Not determined	Real Time RT-PCR	Mediterranean	2020	Kaplan et al. 2023
Forkbeard Annular Seabream Meagre	*Phycis phycis* *Diplodus annularis* *Argyrosomus regius*	Wild	Not determined	Real Time RT-PCR	Mediterranean	2021	Kaplan et al. 2023

table-1 continue							
Zebrafish	*Diplodus cervinus*						
Saddled Seabream	*Oblada melanura*						
Salema	*Sarpa salpa*						
Common pandora	*Pagellus erythrinus*						
Yellow mouth barracuda	*Sphyraena viridensis*						
Mediterranean Horse mackerel	*Trachurus mediterraneus*	Wild	Not determined	Real Time RT-PCR	Mediterranean	2021	Kaplan et al. 2023
Red Sea goatfish	*Parupeneus forsskali*						
Crocodile toothfishes	*Champsodon nudivitis*						
Threadfin breams	*Nemipterus randalli*						
Slender rainbow sardine	*Dussumieria elopsoides*						
Morocco dentex	*Dentex moraccanus*						
Red cornetfish	*Fistularia petimba*						
Sardine	*Sardinella aurita*						
Lizzard fish	*Saurida lessepsianus*						
Red mullet	*Mullus barbatus**		RGNNV				
Common carp	*Cyprinus carpio*	Aquarium	Not determined	Real Time RT-PCR	Antalya	2023	Oğuz et al. 2023
Goldfish	*Carassius auratus*						

*VNN detected species.

Discussion and Conclusion

VNN virus has been detected in more than 173 species and has a very wide distribution. Approximately 90% of the detected VNN virus was genotyped and 86% of them were found to have the RGNNV genotype, 5.5% had the SJNNV genotype, while only 1.8% of VNN positive species had the BFNNV genotype (Bandin and Souto 2020). In Türkiye, RGNNV genotype was detected for the first time in 2011 and all detections made in the following years were of the same genotype (Özkan Özyer et al. 2014; Kalaycı et al. 2016; Kaplan 2019, 2021a; Kaplan et al. 2022b, 2023). Only in 2019, RGNNV/SJNNV was reported to be reassortant in a sample taken from a sea bream farm in the Aegean Sea and in garfish sampled from the Mediterranean Sea (Kaplan et al. 2022b, 2023).

The high prevalence of the RGNNV genotype in Turkish waters may be attributed to the warmer average sea temperatures compared to other regions. These temperatures might not be suitable for the survival or establishment of other VNN genotypes.

It is also commonly recognized that three distinct factors including the environment, host, and pathogen can affect an epidemic of a disease (Snieszko 1973). Recognizing that several environmental elements, such as stress, stocking density, and temperature, are predisposing factors for VNN epidemics is also important (Ma et al. 2015).

It has been shown that there is both horizontal and vertical transmission in various fish species. In studies conducted on Asian perch or barramundi (*Lates calcarifer*), European perch (*D. labrax*), bream (*S. aurata*), brown-marbled grouper (*Epinephelus fuscoguttatus*) and Senegal flounder, there was horizontal transmission from fish to fish or by water to fish. (Péducasse et al. 1999; Aranguren et al. 2002; Manin and Ransangan 2011; Hick et al. 2011; Souto et al. 2015). Vertical transmission has been reported in fish such as European sea bass (*D. labrax*), Asian sea bass (*L. calcarifer*) (Breuil et al. 2002; Azad et al. 2006). This occurs through viral shedding in the gonads, leading to infected eggs and seminal fluids (Valero et al. 2015; Nishizawa et al. 1994).

In studies conducted in Türkiye, it has been reported that horizontal transmission from water is the dominant mode. No detection of the virus in fish of different sizes from hatcheries with biosecurity measures supports this. However, the virus was found in areas that come into contact with seawater (Kaplan 2019; Kaplan and Karaoğlu 2021a; Kaplan et al. 2022b, 2023). On the other hand, there is no data or study on vertical transmission of the VNN. It has been reported that there have been significant increases in the number of Indo-Pacific fish through the Suez Canal, Atlantic fish through the Strait of Gibraltar, and species that find a chance to live in the Mediterranean through ship ballast water through both canals (Çınar and Bilecenoğlu 2015). In 2015, the Suez Canal was greatly expanded with the construction of a new canal, which was predicted to facilitate the introduction of species into the

Mediterranean. The Suez Canal is thought to be the entry point for 443 species of fish, macrophytes, and invertebrates into the Mediterranean, of which 89 have been documented in five or more nations (Galil et al. 2015). It has been reported that one of the first colonization areas of exotic species was the coasts of Türkiye due to its geographical location, and in the list reported in 2020, a total of 539 wild species were found in the seas of Türkiye, 404 of which were resident, while 105 species were invasive (Çınar et al. 2021).

Analysis of data from the General Directorate of Meteorology revealed significant differences in average seawater temperatures between 2010 and 2019 compared to 1970-1979. The Black Sea and Mediterranean Sea experienced the highest increase, with an average rise of 1.2 ºC. The Aegean and Marmara Seas saw increases of 0.9 ºC and 1.5 ºC, respectively (Kalıpcı et al. 2021). Warmer sea temperatures can create suitable habitats for certain non-native species, potentially facilitating their spread into the Mediterranean, Aegean, Marmara, and Black Seas. This trend aligns with reports of rapid population increases for species like pufferfish and lionfish, which were previously confined to the Mediterranean but have recently become more frequent in the Aegean (Kalıpcı et al. 2021).

It is believed that the rising temperatures of seawater in Türkiye may be caused by climate change. This, combined with the increased migration of fish through the Suez Canal and Strait of Gibraltar, could be contributing to the emergence of VNN in Turkish waters. The hypothesis is supported by the fact that VNN isolates found in Türkiye share a phylogenetic similarity with those isolated from Taiwan and Singapore, where VNN has previously occurred.

Encompassed by three seas and boasting abundant water resources (DSİ 2010), Türkiye heavily relies on a thriving aquaculture industry and fisheries sector. Fish health plays a critical role in ensuring the sustainability and economic viability of these sectors (Dönmez and Yılmaz 2018). Fish diseases pose a significant threat, particularly in aquaculture settings. They can hinder the sustainability of both aquaculture enterprises and natural fish populations. Viral diseases are one major category of concern (Dönmez and Yılmaz 2018).

As a result, all researchers emphasize that fish farms should take biosecurity measures, have their broodstock screened for diseases, disinfection of fertilized eggs with ozone or electrolyzed sea water, disinfection of the tools and equipment used in the farms, and the importance of vaccination practices. Although VNN is not classified as a notifiable disease in Türkiye, it poses a significant economic threat. Due to the lack of tracking, it is difficult to determine the current prevalence. Conducting extensive screening studies and research, particularly in freshwater ecosystems, is essential in mitigating the impact of this disease.

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