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İÇİNDEKİLER CONTENTS

ARAŞTIRMA MAKALELERİ RESEARCH ARTICLES

OLGU SUNUMU CASE REPORT

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Definition of textural deterioration in squid samples: Three different tools supported by microbial, visual and physico-chemical analysis

Kalamar örneklerinde tekstürel bozulmanın tespiti: Üç farklı aracın mikrobiyolojik, görsel ve fiziko-kimyasal analizlerle desteklenmesi

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Abstract: The aim of the present study was to reveal the textural profile changes like hardness, cohesiveness, springiness, and adhesion supported by total mesophilic aerobic bacteria count (TMABc), pH, and some visual sensory characteristics in squid samples stored at 4 ºC. Three different Brookfield Texture Analyzer tools, named TA7, TA9, and TA18 were used to observe the textural changes more clearly. The difference of TMABc between the storage days reached from 4.32 log CFU/g to 6.32 log CFU/g. The hardness value of the squid samples, detected by TA18 and TA9 tools, increased while the hardness value obtained from TA7 was higher. The highest change in cohesiveness value in the squid samples was defined by the TA9 tool as ~63%. Once springiness values detected by TA9 were sharply decreased from 4.9 mm to 2.1 mm, those of TA7 and TA18 were slowly decreased. The most increase in adhesion value (0.08 mJ to 0.21 mJ) was obtained in the TA7 tool. Depending on the textural quality changes, the pH value was increased, and as visual and sensory, dark, or yellow spots were observed. The present study results revealed that especially the TA18 tool could be effectively used to determine the quality changes of the squid samples.

***Keywords*:** Texture, hardness, cohesiveness, springiness, adhesion, squid

Öz: Bu çalışmanın amacı, 4 ºC'de depolanan kalamar örneklerinde toplam mezofilik aerobik bakteri sayısı (TMABc), pH ve bazı görsel duyusal özelliklerin desteklediği sertlik, kohezyon, yaylanma ve yapışma gibi dokusal profil değişikliklerini ortaya çıkarmaktır. Tekstürel değişiklikleri daha net gözlemlemek için Brookfield Tekstür Analiz cihazının TA7, TA9 ve TA18 kodlu üç farklı aracı kullanılmıştır. Depolama günleri arasındaki TMABc 4,32 log KOB/g'dan 6,32 log KOB/g'a ulaşmıştır. TA18 ve TA9 araçlarıyla tespit edilen kalamar numunelerinin sertlik değeri artarken TA7'den elde edilen sertlik değeri daha yüksek olmuştur. Kalamar örneklerinde kohezif yapışkanlık değerindeki en yüksek değişiklik TA9 aracı ile ~%63 olarak tanımlanmıştır. TA9 tarafından tespit edilen elastikiyet değerleri, 4,9 mm'den 2,1 mm'ye keskin bir şekilde düşerken, TA7 ve TA18'in değerleri daha yumuşak bir azalış göstermiştir. Adezif yapışkanlık değerinde en fazla artış (0.08 mJ'den 0.21 mJ'ye) TA7 aracıyla elde edilmiştir. Tekstürel değişimlerle birlikte, pH değeri artmış, üründe bölgesel kararma veya sarı lekeler gözlemlenmiştir. Bu çalışmanın sonuçları, kalamar örneklerinin kalite değişikliklerini belirlemede, özellikle TA18 aracının mikrobiyolojik ve fiziko-kimyasal analizlerle, daha ileri kalamar doku çalışmalarına rehberlik edebileceğini ortaya koymuştur.

***Anahtar kelimeler*:** Doku, sertlik, kohezif yapışkanlık, elastikiyet, adezif yapışkanlık, kalamar

INTRODUCTION

Seafood is a food group with high protein value, rich in omega-3 fatty acids such as EPA, DHA and unsaturated fatty acids (Watters et al., 2012). Numerous studies have shown that people who consume seafood have a lower risk of developing diabetes and cardiovascular disease (Ceylan, 2014). Also, fish meat is known as the most consumed food material among seafood. However, there has been an increase in the interest in shellfish in recent years as well. Especially the consumption of shrimp and squid plays an important role among them. Since seafood is highly perishable, the rapid increase in some quality changes such as microbiological, sensory, chemical, and textural deterioration can be more easily observed (Cheng et al., 2014; Rodrigues et al., 2017; Yu et al., 2017). Like in many food materials, the growth in total mesophilic bacteria is used as an indicator to determine the shelf life of seafood (Armani et al., 2016; Fazlara et al., 2014).

Especially microbial growth in seafood and sensory properties can give an idea to evaluate the products for the consumers. Moreover, the changes in sensory characteristics like odor could be linked with microbiological spoilage in seafood (Shalini et al., 2015; Sutikno et al., 2019). In this respect, when the squid samples are thought to be among the highly perishable seafood, particularly the rapid changes in the color of the squid samples reveal the quality changes of the products during the cold storage period. For example, occurring pink spots on the surface of the squid samples could be easily detected (Sungsri-in et al., 2011). Besides the parameters which can be rapidly evaluated by the consumers, the changes in pH can have an important role in the squid samples (Márquez-Ríos et al., 2007). The squid samples are treated with different processing applications and the pH or textural properties could be affected before consumption. Also, textural applications in food science are named as one

of the practical methods that provide lots of advantages such as cost-effectiveness and giving speedy results with spending less time. Textural properties can define the preferences of the consumers as well. Analytical tools applied to evaluate fish freshness, such as chemical and microbiological analyzes, are time-consuming and require various chemicals (Rodrigues et al., 2017). Texture analyzes are quick, expertise-free, and non-destructive analyzes. With these analyzes, accurate assessment of fish quality, shortening the analysis time, and preventing sample loss can be provided. In addition, more sensitive results are obtained from subjective measurements based on human senses. Despite these advantages, there are very few studies using texture analysis as an indicator of fish freshness quality (Ceylan and Meral, 2018; Ceylan, et al., 2020a). This case is acceptable for the squid samples as well.

Besides the above-mentioned properties of squids, squid samples are widely exported globally. Therefore, squid has economical importance for the public as well. In this sense, initial quality, or the observation of the quality changes of squid samples stored at usually -18 ºC and then thawing at 4 ºC are very significant for the economic model of the countries and for the food safety. Thusly, the main aim of the present study was to reveal the microbial quality changes of squid samples stored at -18 ºC and then thawed at 4 ºC for getting ready consumption. Also, it is widely known that the textural properties of squid are also important, so the second target of the study was to investigate the different textural properties of the same samples by using three different texture tools. The final goal of the study was to support the textural changes with the pH, sensory, photographic images, and microbiological changes for the consumers and food industry.

MATERIAL AND METHODS

Materials

Frozen imported squid (*Uroteuthis duvauceli*) samples were obtained from the wholesale market at -18 ºC, transported to food processing department in an icebox, thawed, and stored at 4 ºC (Figure 1). Stored samples were analyzed for four days at room temperature.

Figure 1. Fresh squid sample

Methods

Total mesophilic aerobic bacteria count: TMABc in squid samples were determined. For each sample, 10 g squid was homogenized for 150 s in a stomacher (HG 400 V, Mayo International srl, Novate Milanese, Italy) with 90 mL peptone water (0.1%). Serial dilutions were prepared from 10^{-1} to 10^{-6} for each sample.

1 ml from the diluted sample was placed plate count agar (Merck-VM888763 930) by cast plate method and incubated (WiseCube® Wisd Digital Incubator WIG-50, DAIHAN Scientific Co., Ltd, South Korea) at 35 °C for 48 h to estimate TMABc in squid samples stored at 4 ºC for 4 days (Maturin and Peeler, 2001).

pH: Before the pH measurements, all samples were homogenized using WiseTis HG-15D Digital Homogenizer (DAIHAN Scientific Co., Ltd, South Korea) separately. The homogenized squid samples were stirred by using distilled water (1:10). pH values were measured with a pH meter (HANNA HI9124, Hanna Instruments, Romania). The pH analysis was repeated for the samples three times.

Photographic and quality evaluation: A well-ventilated and lighted sensory analysis room were presented to the judges for better observation of the sensory changes. In this study, comments related to the quality evaluation are presented. Photographic images were obtained by using a cell phone having 3024x4032 pixels and 72 dpi.

Texture Analysis: For texture analysis, Brookfield CT3-1000 (Brookfield Engineering Laboratories, Middleboro, USA), Accessories Rotary Base Table (TA-RT-KIT), Ball: (TA18 12.7 mm diameter, stainless steel, 30 g), Needle: (TA9, 1.0 mm diameter, 43 mm length, 10^0 Maximum taper) and Knife-edge: (TA7, 60 mm wide, clear acrylic, 8 g) were used in the present study (Figure 2). Test parameters for Ball, Needle and Knife-edge measurements were determined as Trigger Value (TV) 0.03 N, Deformation (D) 2 mm, TV 0.03 N, D 5 mm, and TV 0.06 N, D 4 mm, respectively. In this respect, the changes in hardness (N), cohesiveness, springiness (mm), and adhesion (mJ) properties of the samples were revealed during the experimental period. In order to reveal the textural changes in the squid samples, a method by Ceylan et al. (2020a) was modified and used in the present study. The analysis was repeated for the samples in 10 mm depth three times at room temperature.

Figure 2. Ball (TA18), needle (TA9), and knife-edge (TA7) probes left to right

Statistical evaluation

For statistical analysis, all measurements were repeated with three replications. Obtained data were subjected to analysis of variance (ANOVA) to evaluate the textural changes, pH, and microbial growth every analysis day. GraphPad Prism Software Version 5.00 (California Corporation, CA) was performed to reveal significant differences between the analysis days, and comparisons of all differences among them were evaluated by Tukey's Multiple Range Test ($p < 0.05$).

RESULTS AND DISCUSSION

Textural changes

Hardness: Textural changes revealed by hardness, cohesiveness, adhesion, and springiness properties of imported squid samples are given in Table 1, 2, and 3. Hardness value is defined as the strength of food to resist the applied force according to Ertaş and Doğruer (2010). The hardness results obtained TA18 probe (Table 1) revealed that depending on the increase in storage period, hardness values of the samples were increased from 0.5 N to 3.39 N ($p< 0.05$).

Table 1. Texture measurements with ball (TA18) probe depending on the storage time

Analysis Days	0. Day	3. Day	4. Day
Hardness (N)	0.5 ± 0.17^{c}	1.64 ± 0.09^{b}	3.39 ± 0.26^{a}
Cohesiveness	0.57 ± 0.03^{b}	0.63 ± 0^{a}	0.795 ± 0.2^{a}
Springiness (mm)	1.6 ± 0.14^{a}	1.35 ± 0.07^{b}	1.35 ± 0.21^{b}
Adhesion (mJ)	0.06 ± 0.05^{c}	0.1 ± 0.01^{b}	0.17 ± 0.006^{a}

$^{a, b, c}$ letters define the statistical significant differences between storage days ($p < 0.05$)

Also, an increase in hardness value obtained from the TA9 probe (Table 2) was observed ($p < 0.05$). But, the ratio in an increase of TA18 and TA9 was different from each other. In another word, a rapid and sharp increase was observed using the TA18 probe.

Table 2. Texture measurements with needle (TA9) probe depending on the storage time

Analysis Days	0. Day	3. Day	4. Day
Hardness (N)	0.84 ± 0.04^{c}	0.9 ± 0.07^{b}	1.06 ± 0.21^{a}
Cohesiveness	0.89 ± 0.11^{a}	0.33 ± 0.18^{b}	0.51 ± 0.32^{b}
Springiness (mm)	4.9 ± 0.00^{a}	2.1 ± 0.14^{b}	2.6 ± 0.7^{b}
Adhesion (mJ)	0.15 ± 0.025^{a}	0.20 ± 0.025^{a}	0.18 ± 0.035^{a}

$^{a, b, c}$ letters define the statistical significant differences between storage days ($p < 0.05$)

On the other hand, according to the results of TA7 (Table 3), the increase of the storage period was associated with the decline in hardness value (6.13 N to 1.28 N).

Table 3. Texture measurements with knife-edge (TA7) probe depending on the storage time

Analysis Days	0. Day	3. Day	4. Day
Hardness (N)	6.13 ± 0.93^{a}	2.34 ± 0.99^{b}	1.28 ± 0.16^{c}
Cohesiveness	0.57 ± 0.09^{a}	0.6 ± 0.02^{a}	0.59 ± 0.04^{a}
Springiness (mm)	1.80 ± 0.14^{a}	1.50 ± 0.00^{c}	1.65 ± 0.07^{b}
Adhesion (mJ)	0.08 ± 0.03^{c}	0.10 ± 0.02^{b}	0.21 ± 0.015^{a}

$^{a, b, c}$ letters define the statistical significant differences between storage days ($p < 0.05$)

In this sense, Cheng et al. (2014) noted that softness would be evaluated as a loss for texture in seafood. Also, some studies reported that depending on the storage, hardness was decreased (Alasalvar et al., 2001; Jain et al., 2007). These declines in hardness are widely associated with chemical and enzymatic reactions as stated by Chéret et al. (2005). In this respect, the probe type in the present study played a key role in order to detect the changes or deterioration in imported squid samples. Furthermore, the hardness values of the samples were detected to be unstable during the experimental period.

Cohesiveness: The cohesiveness value of the samples was also investigated using three different probes. In this respect, Mousavi et al. (2019) stated that cohesiveness is determined to be the forces of inner bond links that maintain the product as perfect, and it could be expressed as the force content, which could cause deform a material. Also, this value could be associated with consumers' preferences. According to the results of the present study, the initial value of the samples, which was detected by the TA18 probe was determined to be 0.57, but at the end of the storage period, that was found as 0.795 ($p < 0.05$). Also, similarly, TA9 probe results showed that depending on the increase in the storage period, cohesiveness was decreased from 0.89 to 0.33 ($p < 0.05$). On the other hand, TA7 was not found to be effective to determine the quality changes based on cohesiveness.

Springiness: In addition to cohesiveness and hardness, springiness value in the imported squid samples was observed. All probe types revealed that springiness value was declined. Furthermore, this decline was carried out for ball probe (TA18) as 15.6% (1.6 to 1.35 mm). For needle probe (TA9), this decline was observed in the range of 57.14% (4.90 to 2.1 mm) and for knife-edge probe (TA7), it was 8.33% (1.80 to 1.65 mm). Sutikno et al. (2019) reported that the springiness value of raw squid samples was 1.14 mm. According to Narasimha Murthy et al. (2018), icing methods such as chilling onboard or slurry ice could play a key role to determine the springiness value. Also, different types of

probes could be effectively used to define the quality changes in the squid samples.

Adhesion: It was observed that adhesion values of the imported squid samples were increased. These increases revealed by TA18 and TA17 probes were defined to be rapid as compared to the results of the TA9 probe. Also, there were statistical differences between the storage periods ($p < 0.05$). Moreover, in TA7, the initial value was increased from 0.08 mJ to 0.21 mJ at the end of the experimental period. Dunnewind et al. (2004) noted that there might be a correlation between adhesion value and sensory changes in some foods. So, investigation of AD values could be much more important for the squid consumers.

Total mesophilic aerobic bacteria growth

TMABc of the imported squid samples during the experimental period are given in Table 4. As known, TMAB growth in food samples plays a key role to define the quality (Ceylan, et al., 2020b). In this respect, besides the definition of textural quality by using three different texture probes of the imported squid, the relationship between textural deterioration and microbial spoilage is gaining today much more important in food science (Ceylan, et al., 2020a). In this study, the initial TMAB load of the imported squid samples was found as 4.39 log CFU/g. In this period, higher textural results were obtained as compared with the other days of the storage period. By the increase of the time, TMABc began to increase and on the 3rd day of the cold storage it reached 5.28 log CFU/g, and then in the last period, TMAB load of the imported squid samples had 6.30 log CFU/g. Bacterial spoilage in squid can cause other chemical amines such as biogenic, which can be hazardous for the consumers (Kim et al., 2009). Already, while the TMABc of the imported squid samples was increasing, the adhesion value was also increasing. However, the springiness value obtained from three different probes was significantly decreased. Furthermore, cohesiveness and some hardness values were depended on the probe type. For example, in hardness, according to the TA18 probe results, the value was rapidly increased with the increase of TMABc. But, when TA7 values were declined also, TMABc was increasing. Therefore, the study especially revealed that the used probe-type should be illustrated with the microbiological growth in the squid samples.

pH

The changes in pH values of the imported squid are presented in Table 4. The pH value could be evaluated as one of the most effective indicators of meat quality as described by Gokoglu et al. (2017). Besides revealing the potential relationship between the TMAB growth and textural changes in the squid samples, observation in the pH changes could be evaluated as an important parameter. In this regard, at the beginning of the storage period, the pH value was detected to be 7.88. When the TMABc reached the highest value, the pH value was also reached 8.16. It could be observed that the pH value might decrease after death because of converting from glycogen to lactic acid in meat and then an increase could be widely detected with the increased time (Ceylan et al., 2018). Also, by this study, the increase in the pH value of the squid could be clearly associated with the increase in adhesion and the decrease in springiness.

Table 4. Quality parameters and changes for squid samples in regards to storage time

Analysis Days	0. Day	3. Day	4. Day
pH	7.88 ± 0.01^{b}	7.76 ± 0.03^{c}	8.16 ± 0.03^{a}
TMAB (log CFU/g)	4.39 ± 0.09^{c}	5.28 ± 0.08^{b}	6.30 ± 0.01^{a}
Observations	*Hard, bright structure*	*Mild smell, water release, color fading, formation of spots,*	*Rubberization, stickness, drying, growth of yellow spots,*

$^{a, b, c}$ letters define the statistical significant differences between storage days ($p < 0.05$)

Photographic and quality evaluation

Figure 3 reflects the photographic changes in the squid samples depending on storage time. As can be seen from the figure, there was a smooth and clear meat surface at the beginning of the study. In another word, there were no yellowish, pink, or reddish structures on the surface of the squid meat. Particularly, depending on the loss in the textural properties, different colors and spots were defined on the surface of the squid samples. These images had a good relationship with quality evaluation as well. Because off-odor, dried and the changes in hardness, which may occur in loss of water were clearly seen. As know that different colorimetric measurements such as image analysis are used to define the quality of the meat products (Ceylan et al., 2018; Ünal Şengör et al., 2019). But, instead of costly methods, simple applications like textural measurement are recently preferred to determine the quality of food products. Moreover, this kind of study may play a guiding role to be built data-base systems for textural properties such as hardness, cohesiveness, springiness.

Figure 3. Visual changes of squid samples on day 0, 3, and 4

CONCLUSION

Different texture tools (TA7, TA9, and TA18) were effectively used to reveal the textural changes. TMABc of the squid samples were increased with time, at the same analysis periods, the textural changes were also observed by the three tools in the same period. Particularly, the clear changes in the hardness value were more successfully revealed by TA18 and TA9 probes.

During the analysis period, the changes of the cohesiveness value in the squid samples were observed as ~63% by the TA9 tool. Sharply decrease in springiness value was also obtained with the TA9 probe. On the other hand, the TA7 tool showed that adhesion value could increase by the increase of the storage period. In terms of the observation of the textural changes, the use of three different textural tools provided clear, understanding, and comprehensive opportunities. These changes were also observed by the other quality changes such as sensory, visual, and pH. The present study results suggested that the use of the TA18 probe could be more efficient as compared to the others.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The role of nitrosative and oxidative stress in rainbow trout (*Oncorhynchus mykiss*) liver tissue applied mercury chloride ($HgCl_2$)

Civa klorür ($HgCl_2$) uygulanan gökkuşağı alabalığı (*Oncorhynchus mykiss*) karaciğer dokusunda nitrozatif ve oksidatif stresin rolü

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Abstract: The aim of this study was to determine oxidative stress caused by mercury chloride ($HgCl_2$) in rainbow trout (*Oncorhynchus mykiss*) liver tissue. For this purpose, the LD_{50} value of $HgCl_2$ on rainbow trout was determined as 551 µg/L. In the study, 40 fish in four groups were exposed to 25% and 50% (138 and 276 µg/L) of the two subletal doses of $HgCl_2$ for 2 and 7 days, with 10 fish (n=10) in each group. To determine oxidative stress; peroxynitrite ($ONOO^-$), total oxidant level (TOS), total antioxidant level (TAS), oxidative stress index (OSI) and malondialdehyde (MDA) were analyzed. In the study, it was observed that the differences between the groups in terms of $ONOO^-$, TOS, TAS and OSI levels in the liver tissues was significant ($P<0.05$), however, this difference was not significant ($P>0.05$) in terms of MDA values. As a result, it can be concluded that $HgCl_2$ increases $ONOO^-$, TOS, TAS, OSI and MDA levels in liver tissue and even small doses of mercury are toxic to fish.

***Keywords*:** *Oncorhynchus mykiss,* mercury toxicity, oxidant/antioxidant, peroxynitrite, liver

Öz: Çalışmanın amacı, gökkuşağı alabalığı (*Oncorhynchus mykiss*) karaciğer dokusunda civa klorürün ($HgCl_2$) neden olduğu oksidatif stresi belirlemektir. Bu amaçla gökkuşağı alabalığı üzerine $HgCl_2$'nin LD_{50} değeri 551 µg/L olarak tespit edilmiştir. Çalışmada, her grupta 10 balık (n=10) olacak şekilde dört grupta toplam 40 adet balık 2 ve 7 gün süreyle $HgCl_2$'ün iki subletal dozun %25' ine ve %50' sine (138 ve 276 µg/L) maruz bırakılmıştır. Oksidatif stresi belirlemek için peroksinitrit (ONOO-), toplam oksidan seviyesi (TOS), toplam antioksidan seviyesi (TAS), oksidatif stres indeksi (OSİ) ve malondialdehit (MDA) tayinleri yapılmıştır. Çalışma sonucunda, genel olarak karaciğer dokularındaki $ONOO^-$,TOS, TAS ve OSİ düzeyleri açısından gruplar arasındaki fark istatistiksel olarak önemli ($P<0.05$) çıkarken, MDA değerleri açısından bu fark istatistiksel olarak önemsiz ($P>0.05$) çıkmıştır. Sonuç olarak $HgCl_2$'nin karaciğer dokusunda ONOO-, TOS, TAS, OSİ ve MDA düzeylerini arttırdığı ve civanın küçük dozlarının bile balıklar için toksik olduğu belirlenmiştir.

***Anahtar kelimeler*:** Gökkuşağı alabalığı, civa toksisitesi, oksidan/antioksidan, peroksinitrit, karaciğer

INTRODUCTION

In parallel with the increase in industry, settlement and agricultural areas, the pollution of water resources also increases. It is causing pollution of water resources and disruption of natural balance; organic substances, metals, petroleum derivatives, artificial agricultural fertilizers, detergents, radioactive, pesticides, inorganic salts, artificial organic chemicals. Environmental conditions and water resources have to be considered and the metals causing environmental pollution are the most dangerous. The reason for this is due to the metals cannot be physically decomposed and persists for a long time. In particular, heavy metals, such as mercury, accumulate because they cannot be disposed of by natural physiological mechanisms and have a toxic effect if regulations are exceeded inside. In this accumulation, fish living in the water and people who feed on these fish may risk their lives (Dural et al., 2007; Gunes et al., 2019; Kasassi et al., 2008; Kırıcı et al., 2013).

Mercury is expressed as one of the 20 most toxic substances in the World. Today, mercury is widely used as a fungicide in agricultural applications and in the chlor-alkali industry, the manufacture of electrical equipment, the pharmaceutical industry, the cellulose and paper industry, and the production of plastics. Mercury is a metal that is highly toxic, even at trace levels, for all living things, both inorganic and organic form (Plessi et al., 2001). Mercury is mostly found as inorganic mercury compounds or in the aquatic

environment as methylmercury (CH_3Hg^+) (Driscoll et al., 1994). There are two different ways for the bioaccumulation of mercury in aquatic organisms. These are the result of direct (by the metal in the water) or trophic exposures (by the metal of the food) (Boudou and Ribeyre, 1983). Mercury can also suppress important defense mechanisms of cells and cause lipid peroxidation by causing free oxygen species formation and oxidative stress (Berntssen et al., 2003). Mercury has a high affinity for the -SH groups of cellular biomolecules. For this reason, it can be attached to low molecular weight thiols and thyol containing proteins such as mercury, cysteine and glutathione after being taken into the body, and it can remain in tissues and organs for a long time, causing free radicals that cause lipid, protein and DNA oxidation (Perottoni et al., 2004). Considering that mercury compounds can easily spread in water, fish can be used as a good bioindicator of pollution in water ecosystems (Has-Schon et al., 2015).

Reactive oxygen species (ROS) such as superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical, and reactive nitrogen types (RNS) such as nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^-$), are produced in fish as a result of intracellular metabolic processes and after exposure to genotoxic agents such as heavy metals. ROS or RNS-mediated oxidative or nitrosative injury occurs as a result of fish exposure to heavy metals (Berntssen et al., 2003; Mieiro et al., 2010; Wang et al., 2015). NO reacts with O_2 to produce $ONOO^-$ anion, a powerful oxidant that can cause lipid peroxidation. $ONOO^-$ inhibits mitochondrial electron transport, oxidizing thiol compounds and DNA (Powell et al., 2005). Malondialdehyde (MDA) is a product of lipid peroxidation in fish as well as in all highly vertebrates, and it is considered as one of the most important indicators of oxidative stress occurring in cell components (Morales et al.,2004).

Mercury chloride ($HgCl_2$), in industrial world in Turkey, is widely used both in scientific and agricultural purposes. For example, it is used as a fungicide in agriculture and as a topical antiseptic and disinfectant in medicine (Baser et al., 2003). So far, many researches have been done to determine the toxic effects of heavy metals, especially in relation to mercury compounds (Gül et al., 2008; Pandey et al., 2005; Terzi & Verep, 2012; Thongra-ar et al., 2003; Verep et al., 2007). The aim of this study is to investigate the changes of nitrosative and oxidative stress ($ONOO^-$, TOS, TAS, OSI and MDA) parameters that occur in rainbow trout liver tissue, where $HgCl_2$ is applied.

MATERIAL AND METHODS

Fish material and experimental application

The application of the study was carried out at the Aquaculture Laboratory of Faculty of Agriculture and Molecular Biology Laboratory of the Faculty of Arts and Sciences in Bingöl University. Rainbow trout (*Oncorhynchus mykiss*) (59.43±3.73 g and 17.24±1.64 cm) was purchased from the trout facility of Keban district of Elazığ province and brought to the laboratory as live. The fish brought to the laboratory were placed in 600 lt tanks and for their adaptation to the environment, it was fed with a commercial feed of 2% of its live weight twice a day for 15 days. During the study, water temperature, dissolved oxygen level and alkalinity were observed as 14±3 °C, 8.24±0.5 mg/L and 128±11 mg/L, respectively, and total hardness was measured as 132±29 mg/L and pH 7.3±0.2 as $CaCO_3$.

In order to determine the LD_{50} value, $HgCl_2$ was applied to Rainbow trout in the groups (n=10 fish) with the dose of 100, 200, 500, 750, 1000 and 1500 µg/L. The fish in the groups were checked 3 times a day for 96 hours, and those who died were removed from the aquarium and noted. After 96 hours, the LD_{50} value was calculated as 551 µg/L. Then, 2 subletal doses (25% LD_{50} = 138 µg/L and 50% LD_{50} = 276 µg/L) were determined to apply. The fish were treated with subletal doses for 2 and 7 days. In the study, 10 fish were used in each group. No deaths occurred in any of the groups during the study. The use of fish and the experimental protocol were approved by Bingöl University Animal Experimentation Ethics Committee (Bingöl, Turkey).

Preparation of homogen

After euthanasia of the fish, liver tissues were removed by performing the necessary autopsy. Tissues were kept at -80°C in the freezer until use. Frozen liver tissue samples were homogenized individually in a 1:10 (w/v) ratio (10 mM Tris-buffer (pH= 7.4), 0.1 mM NaCl, 1% TritonX-100, 0.2% SDS, 2.5 mM ethylenediaminetetraacetic acid.

Determination of $ONOO^-$ value

Evaluation of nitrosative stress status in liver tissue is obtained by determining $ONOO^-$value. $ONOO^-$ value was measured by phenol nitration (Ahlatci et al., 2014; Al-Nimer et al., 2012; Vanuffelen et al., 1998). To obtain a final volume of 2 ml, 10 µl of sample was added to 5 mM phenol in 50 mM sodium phosphate buffer (pH 7.4). After 2 hours of incubation in a dark place at 37°C, 15 µl of 0.1 M NaOH was added and the absorbance of the samples at 412 nm wavelength was recorded. Nitrophenol yield was calculated from ε= 4400/M/cm. Results were expressed as µmol/g wet tissue. Biochemical measurements were made using a spectrophotometer (Shimadzu U 1601, Japan).

Determination of TAS, TOS and OSI values

TAS and TOS values of liver tissues were measured by Rel Assay brand commercial kits (Rel Assay Kit Diagnostics, Turkey). Trolox, a water-soluble analog of vitamin E, was used as calibrator for TAS tests. Results are expressed as mmol Trolox equiv/L (Erel, 2004). Hydrogen peroxide was used as calibrator for TOS tests. Results are expressed as µmol H_2O_2 equiv./L. While calculating OSI, which is expressed as the percentage of the ratio of TOS levels to TAS levels, the mmol value in the unit of the TAS test was

converted to µmol as in the TOS test (Erel, 2005). The results were calculated according to the formula below.

$$OSI = \frac{\text{TOS, µmol } H_2O_2\text{equiv./L}}{\text{TAS, mmol Trolox equiv./Lx10}}$$

MDA measurements

MDA determination of tissue samples was made by method of Ohkawa et al. (1979) according to the method, 200 µl of each group was taken and 200 µl of 8.1% SDS was added. Then it was kept in a boiling water bath at 95 °C for one hour and then cooled and vortexed by adding a mixture of 1 ml distilled water and 5 ml of n-butanolpyridine in a ratio of 15: 1 (v/v). After centrifuging at 4000 rpm for 15 minutes, the top organic layer was taken and measured spectrophotometrically at 532 nm wavelength, and the results were recorded in nmol/ml.

Statistical analysis

SPSS 20.0 package program was used to calculate the statistical analysis of the data obtained. One-way analysis of variance (oneway ANOVA) was used to determine the differences between the groups and Duncan Test was used to compare the groups.

RESULTS

As a result of the study, the 96-hour LD_{50} value of $HgCl_2$ in rainbow trout was 551 µg/L. Groups were created based on the LD_{50} value. The groups were formed from five groups (1 control and 4 treatments); 2 and 7 days with 25% (138 µg/L) and 50% (276 µg/L) of the control group and LD_{50}.

The mean values of TAS, TOS, OSI, $ONOO^-$and MDA of the control and experimental groups were statistically interpreted. While the difference between TAS, TOS, OSI and $ONOO^-$ values was statistically significant ($P<0.05$), the difference in MDA levels was insignificant ($P>0.05$).

When TAS values were analyzed, the highest group was found as Group 1 (0.72 mmol/L), while the lowest group was found as group 2 and group 3 (0.62 mmol/L). As a result of comparison of control and experimental groups in TAS values; There was no statistically difference between the control group (group 5) and other groups. However, when the groups were compared among themselves, a statistically significant difference was found between groups 1, 4 and groups 2, 3 ($P<0.05$).

When TOS values were examined, the highest group was 3 (8.35 µmol/L) and the lowest was 5 (5.82 µmol/L). Also, when the groups were compared, the difference between the control group and the other groups was found statistically significant ($P<0.05$).

In terms of OSI values, the difference of group 3 from all other groups was statistically significant ($P<0.05$). In MDA values, the difference of group 3 from all other groups was statistically significant ($P<0.05$).

TAS (mmol/L), TOS (µmol/L), OSI, $ONOO^-$ (mmol/L) and MDA (nmol/mg protein) values of liver samples are given in Table 1.

Table 1. TAS, TOS, OSI, $ONOO^-$ and MDA values of liver samples

	Trial Groups ($\bar{x}$±SD)*				
	1	2	3	4	5
TAS	0.72±0.06^{b}	0.62±0.06^{a}	0.62±0.05^{a}	0.70±0.05^{b}	0.67±0.08^{ab}
TOS	6.54±0.95^{ab}	7.07±0.83^{bc}	8.35±0.49^{d}	7.54±0.45^{c}	5.82±0.53^{a}
OSI	90.42±17.85^{a}	113.08±10.58^{b}	134.42±10.25^{c}	107.92±8.40^{b}	87.39±9.60^{a}
ONOO$^-$	34.64±10.04^{a}	41.79±4.94^{ab}	54.64±14.17^{bc}	70.39±26.98^{c}	33.61±2.38^{a}
MDA	12.40±3.15^{a}	17.10±6.84^{a}	31.06±12.38^{b}	14.00±3.81^{a}	9.69±2.80^{a}

*The difference between average values carrying different letters in the same line is statistically significant ($p<0.05$).

TAS=Total Antioxidant Level, TOS=Total Oxidant Level, OSI=Oxidative Stress Index, $ONOO^-$=Peroxynitrite, MDA=Malondialdehyde, 1= 25% LC50 2 Days, 2=50% LC50 2 Days, 3=25% LC50 7 Days, 4=50% LC50 7 Days, 5=Control group

DISCUSSION

Mercury is a heavy metal that is not necessary for biological functions and can be very toxic even at very low levels. Given that a total of 40,000-50,000 tons of mercury reaches the atmosphere and 4,000 tons of water every year, mercury poses a great risk for humans and other living things. Mercury is listed by the International Chemical Safety Program (IPCS) as one of the most dangerous chemicals in the environment (Gilbert and Grantwebster, 1995). This heavy metal is also included in the most dangerous xenobiotic class with its toxicological effects such as neurotoxic, embryotoxic and cytotoxic, and wide spread and permanence in the environment (Gundacker et al., 2006). Mercury mixed with water is converted to methylciva by bacteria and organisms. Planktons get into the food chain with small fish and mussels that eat them, and large fish and marine mammals that feed on small fish (Güven et al., 2004). The increase in water temperature increases the solubility of the mercury in the water in the summer and affects the increase of the mercury concentration in the fish (Gül et al., 2004). Mercury accumulates in many fish species, causing kidney and liver lesions, endocrine disorders and changes in the membranes of cells in the central nervous system (Bano and Hasan, 1990; Iliopoulou-Georgudaki and Kotsanis, 2001; Veena et al., 1997).

Since hepatic blood flow is proportionally slower than cardiac blood flow in fish liver, it is more sensitive to damage caused by toxic substances. Also, because the liver is a detoxification organ, heavy metals accumulate most in this organ. Indeed, in a study, it was observed that the highest

elimination percentage (up to 64% in the liver, 20% in the brain and 3% in the muscle) was recorded in the liver in European seabasses exposed to MeHg for 28 days (Maulvault et al., 2016). Hg can cause liver damage, as shown in bream (Guardiola et al., 2016), *Salvelinus alpinus* (de Oliveira Ribeiro et al., 2002) and tiger fish (Elia et al., 2003). In a study (Guardiola et al., 2016), it was stated that MeHg exposure to sea bream (*Sparus aurata*) fish increased SOD, CAT activities and ROS levels in blood serum while decreasing antioxidant potential. In our study, it was determined that rainbow trout (*Oncorhynchus mykiss*) increased the oxidative stress parameters of $HgCl_2$ in liver tissue. Besides, it was found that rainbow trout of $HgCl_2$ caused toxic effects on liver tissue.

The most important feature of the antioxidant defense system is that all components of the system act in a way that creates a synergy against reactive oxygen types (Chaudiere and Ferrari-Iliou, 1999). Therefore, all antioxidants are vital in ensuring homeostasis in living things (Doyotte et al., 1997). As a result of the oxidant and antioxidants in the blood acting together, more oxidant and antioxidant effects occur than each one creates alone. For this reason, it is reported that TOS and TAS measurement may be more useful to determine the total oxidant/antioxidant balance instead of measuring the oxidant and antioxidants individually (Erel, 2004, 2005). Indeed, there are many studies in this direction. For example; Doğan et al. (2011) investigated the biochemical effects of subletal concentrations of fenpyroximate acaricide in the liver tissue of adult guppies by looking at TAS and TOS values, they noted that the doses of subletal acaricide administered did not cause any changes in antioxidant activity, and 25 and 50 µg/l acaricide caused oxidative stress. Kaya et al (2014), in their study investigating the effect of tebuconazole used as fungicide in *Cyprinus carpio* (L., 1758) on serum TAS and TOS levels, found that serum TAS levels decreased in groups treated with tebuconazole compared to the control group and increased TOS levels. In this study, while the TOS values were increasing, no statistically significant difference was found between the control group and the other groups in TAS values. However, a statistically significant difference was found between TAS and time-dependent groups in TAS values ($P<0.05$).

MDA is considered as indicators of oxidative stress caused by the damage caused by free radicals to the membrane complements of cells (Yonar et al., 2016). Many researchers have reported a relationship between MDA and $HgCl_2$-induced stress in fish (Fathi et al., 2018; Ibrahim, 2015; Thirumavalavan, 2010). The findings of this study showed that liver MDA levels were higher than the control group (Table 1). This is most likely explained by the production of hyperreactive oxygen species, which may be associated with a lowering of the antioxidant enzyme level and thus lead to lipid peroxidation.

As a result, it has been determined that $HgCl_2$ increases $ONOO^-$, TOS, TAS, OSI and MDA levels in liver tissue and even small doses of mercury are toxic to fish. These results show the effect of $HgCl_2$ on the exhaustion of antioxidant mechanisms. More studies are needed to clarify the basic mechanisms in $HgCl_2$'s long-term toxicity profile in rainbow trout and to make sense of the toxicity mechanism. This study plays a role in understanding our $HgCl_2$ exposure, its potential impact and improving our knowledge of $HgCl_2$ ecotoxicology and risk assessment. However, it is a known fact that some fish species are more susceptible to mercury toxicity than others. Therefore, toxicological pathology caused by mercury in fish is affected by factors such as species, age, environmental conditions, exposure time and exposure concentration. Considering these factors, studies should be carried out in different doses and durations in different fish to make sense of the toxicity mechanism of $HgCl_2$ on fish.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Contribution of fishery and aquaculture products to Turkish foreign trade: An evaluation by a hybrid multi-criteria decision-making method

Balıkçılık ve su ürünlerinin Türk dış ticaretine katkısı: Hibrit çok kriterli karar verme yöntemi ile bir değerlendirme

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Abstract: Fisheries and aquaculture industry, which provides a continuous and high-return market advantage to potential countries, is also one of the priority industries for Turkey. Although Turkey has important advantages with its geographic position and bio-diversity, the industry's contribution to foreign trade is not at an acceptable level. Accordingly, it is important to evaluate the capture fishery and aquaculture products, which provide the best contribution to the foreign trade of Turkey. Thus, the present paper aims to provide foreign trade executives with an intuition about the most appropriate products to invest in and contributing products to Turkish foreign trade within the scope of market strategies. Entropy-WASPAS, which is a hybrid multi-criteria decision-making method, is used for evaluating the contribution of fishery and aquaculture products to Turkish foreign trade. This model includes both criteria (production, import quantity, import value, export quantity, export value) and alternatives (trout, sea bream, sea bass, sprat, atlantic bonito, anchovy, horse mackerel, pilchard, sea snail, prawn, cuttle fish, mussel). Entropy is used to determine the criteria weights, and WASPAS is applied for ranking the fishery and aquaculture products. According to the results, export value is the most important criteria while Sea Bream is found as the most significant product for Turkey's foreign trade.

***Keywords*:** Fishery products, Turkey, decision making, Entropy, WASPAS

Öz*:* Ülkelere sürekli ve yüksek getiri sağlayarak pazar avantajı sağlayan balıkçılık sektörü, Türkiye için de öncelikli sektörlerden biridir. Türkiye coğrafi konumu ve biyolojik çeşitliliği ile önemli avantajlara sahip olmakla birlikte, sektörün dış ticarete katkısı olması gereken düzeyde değildir. Buna göre, Türkiye'nin dış ticaretine en çok katkıyı sağlayan balıkçılık ve su ürünlerinin değerlendirilmesi önemlidir. Bu nedenle, bu makale dış ticaret yöneticilerine, Türk dış ticaretine katkı sağlayan ve yatırım yapılabilecek en uygun ürünlerin neler olduğuna dair bir öngörü sağlamayı amaçlamaktadır. Balıkçılık ve su ürünlerinin Türk dış ticaretine katkısının değerlendirilmesinde hibrit bir Çok Kriterli Karar Verme yöntemi olan Entropi-WASPAS yöntemi kullanılmıştır. Bu model kriterleri (üretim, ithalat miktarı, ithalat değeri, ihracat miktarı, ihracat değeri) ve alternatifleri (alabalık, çipura, levrek, çaça, palamut, hamsi, uskumru, sardalya, deniz salyangozu, karides, mürekkep balığı, midye) içermektedir. Kriter ağırlıklarını belirlemek için Entropi kullanılmış olup, balıkçılık ve su ürünleri yetiştiriciliği ürünlerinin sıralanması için WASPAS yöntemi uygulanmıştır. Sonuçlara göre, en önemli kriter olarak ihracat değeri, Türkiye'nin dış ticaretine katkı sağlayan en önemli ürün ise çipura olarak bulunmuştur.

***Anahtar kelimeler*:** Balıkçılık ürünleri, Türkiye, karar verme, Entropi, WASPAS

INTRODUCTION

In the last 50 years, global changes and threats have led to a high global awareness of aquatic ecosystems and the need of managing the ecosystems in a sustainable manner, thus the importance of responsible consumption for fisheries and aquaculture resources has been realized and prioritized globally. The fisheries and aquaculture industry is one of the four sub-industries of agriculture. It has a significant importance in contributing to animal/human nutrition and providing raw materials to important industries. Due to the lack of any other nutritional equivalent, the awareness and demand for seafood consumption are increasing in order to ensure adequate and balanced nutrition of societies (Hixson, 2014).

In the period of 1961–2017, the average annual growth rate of total food fish consumption increased by 3.1%, outpacing annual population growth rate (1.6%). Also, in per capita terms, food fish consumption rose from 9.0 kg (live weight equivalent) in 1961 to 20.3 kg in 2017, at an average rate of about 1.5% per year (FAO, 2020). According to the expectations of being 9.6 billion in 2050, the researchers estimate that future generations will experience a significant nutritional problem. Therefore, it is expected that the industry's foreign trade value will increase and the countries, which are successful in trade politics and marketing strategies, will have great opportunities for gaining a high market share.

The consumption of seafood has also led to a rapid increase in fishery and aquaculture production. According to STATISTA (2020) statistics, global fish production amounted to 177.8 million metric tons in 2019, of which 86.5 million metric tons came from aquaculture production, while 91.3 million metric tons of fish were captured. It is expected that the contribution of aquaculture to global fish production will continue to grow and surpass total capture fisheries by 2024. As seen in Figure 1, by 2029, aquaculture production is projected to reach 105 million tonnes.

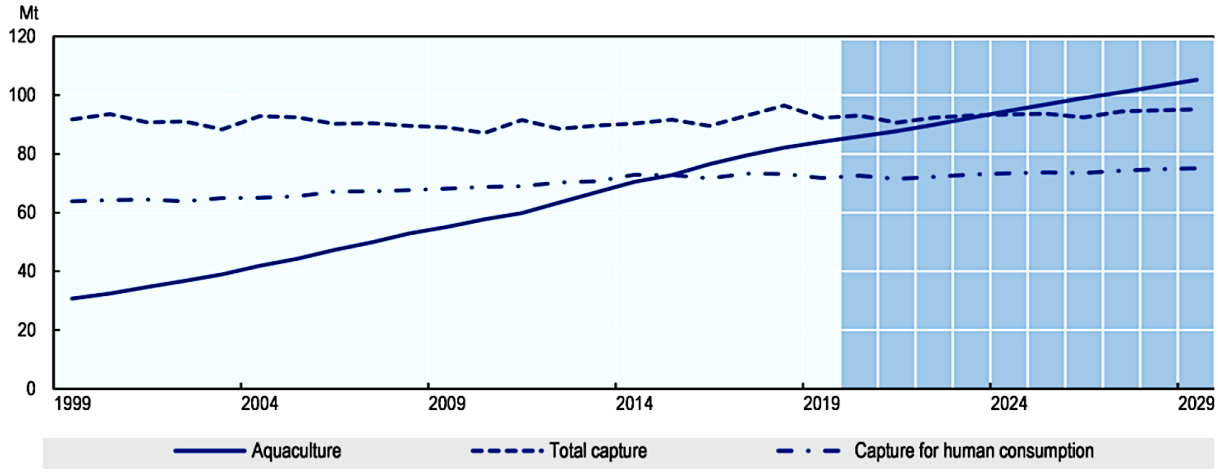

Figure 1. World aquaculture and capture fisheries (OECD library, 2020)

Increases in consumption and growth of aquaculture production have led seafood to be one of the most traded food categories in the world today. From 1976 to 2018, the value of global exports of fish products increased at an annual rate of 8% in nominal and 4% in real terms. In 2018, 67 million tonnes of fish (live weight equivalent) were traded internationally (FAO, 2020). The global seafood market was valued at 130 billion U.S. dollars in 2018 and it is expected to reach approximately 155.32 billion U.S. dollars by the year 2023 (STATISTA, 2020). The top exporters and the importers of fishery and aquaculture products in 2018 worldwide is given in Figure 2.

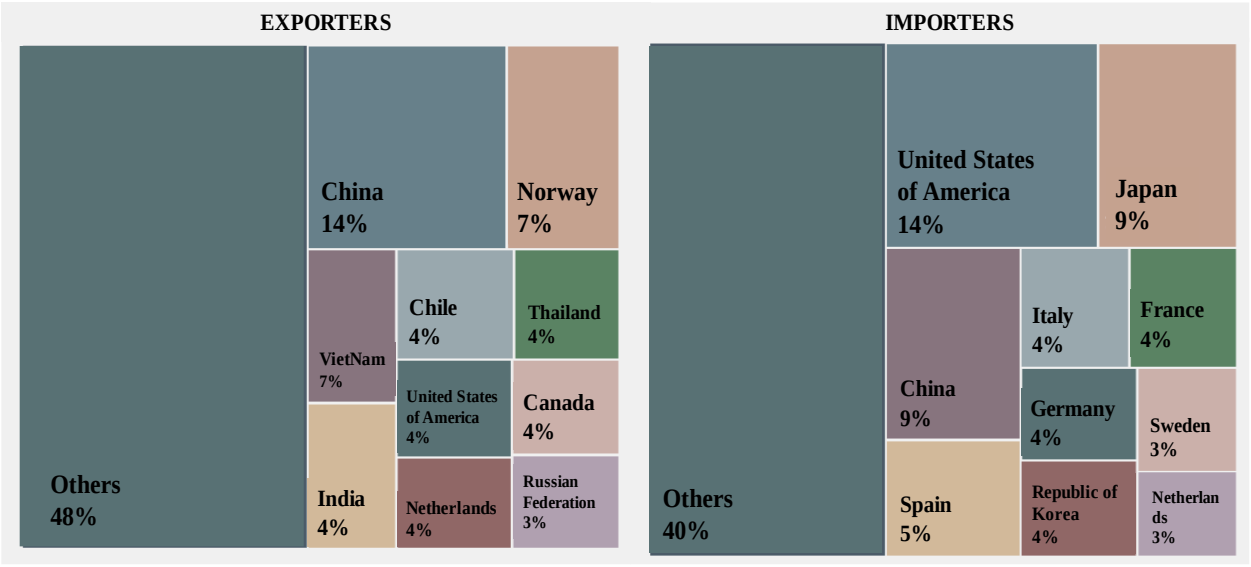

Figure 2. Leading exporting-importing countries in 2018 (FAO, 2020)

Turkey is an emerging country in the industry with its geographic features, biodiversity, and capturing and aquaculture production facilities. There are approximately 500 species in the seas and 370 species in the inland waters, of which about 100 are hunted commercially. The production in Turkey is carried by sea and inland capturing and aquaculture production. However, similar to the world, considering the limitation of capturing resources, aquaculture production has

showed significant improvements in the last thirty years. Especially in recent years, aquaculture production has gained its place among the fastest growing industry. In 2019, the aquaculture production reached 373.356 tonnes, while the capturing production amounted to 463.168 tonnes (Turkish Statistical Institute - TUIK, 2020). Aquaculture production of Turkey is expected to surpass the capturing in the forthcoming years (General Directorate of Agricultural Research and Policy - TAGEM, 2019). The industry also became one of the leading industries for Turkey's foreign trade thanks to increasing numbers in production. Turkey has an increasing export capacity and enlarging its target markets.

In 2019, Turkey exported to 81 countries with a quantity of about 200.226 tonnes with a total sale value estimated at 1.025 billion U.S. dollars. 62% of the exports were made to the EU countries, and the Netherlands, Italy, and Russia were the most exported countries (TUIK, 2020). However, in the current situation, Turkey has not yet shown its real potential for foreign trade sufficiently. In order to manage effective foreign trade policy, it is crucial to focus on product selection and evaluate which of them contribute to foreign trade effectively. Therefore, this research aims to evaluate the current contribution of leading fishery and aquaculture products (given in Table 1) to the foreign trade of Turkey and also specify the products which are more valuable and able to create currency inflow. The results provide intuition to the foreign trade executives about the most contributing fishery products to Turkish foreign trade. Also, it offers the occasion of showing the most appropriate products to invest in within the scope of a market development strategy. Hence, this is a multi-criteria decision problem, so Entropy and WASPAS methods, which are among the MCDM methods, were used for the efficiency analysis of the determined products.

In this study, Entropy and WASPAS methods were used in an integrated way. When the literature is examined separately for Entropy and WASPAS methods, many studies stand out. However, only five studies in which two methods were used together were found.

Ali et al. (2021) focused on developing an effective way to cope with multi-criteria group decision making problems and they evaluated a supplier selection problem with Entropy-WASPAS method. Akçakanat et al. (2017), evaluated the performance of small, medium and large-scale banks using Entropy, and WASPAS methods. Criterion weights were determined by the Entropy method and the WASPAS method was used to rank the banks. Ural et al. (2018), evaluated the performance of three state-owned banks in an integrated manner with the data obtained from the financial statements for the period of 2012-2016 and applied the Entropy and WASPAS methods. Similarly, the performances of participation banks operating in Turkey are examined with the help of Entropy and WASPAS methods by Gezen (2019). Çelik (2020), utilized the logistic performance of OECD countries with WASPAS based Entropy method. It was observed that a similar study was applied to the forestry industry by Bayram (2020). Bayram (2020) evaluated the forest industry products of Turkey regarding their economic contribution by Entropy – TOPSIS.

Although it is revealed that Entropy and WASPAS methods are used in the banking and logistics industry in an integrated manner, no previous study has been found in the fisheries and aquaculture industry. Moreover, since there is also a research gap for fisheries and aquaculture industry that focuses on the impact on Turkish foreign trade and economy, this study is expected to contribute to the literature.

In order to meet the purpose, the research framework has been determined as follows.

Step 1: The products were determined according to their average production amounts for the last 5 years and divided into three different groups. The most captured fish, other fish products, and fishes from aquaculture production were evaluated for the Turkish foreign trade.

Step 2: The decision criteria, used for evaluating the products, were determined for the analysis.

Step 3: The selected criteria in the decision matrix were weighted by Entropy method.

Step 4: The products are evaluated and rated according to their contribution to foreign trade by WASPAS method.

Following the introduction section, the importance and purpose of the research are clarified with industrial information and the statistics of Turkey and World. Then, the methods applied in the study are explained in detail. Afterwards, the empirical application has been implemented within the scope of the study's methodology. In the conclusion part of the study, the results are evaluated and interpreted, and suggestions were made for future studies.

MATERIAL AND METHODS

In this paper, a hybrid MCDM model, Entropy-WASPAS, was utilized for an objective rating. The weights of criteria were calculated by Entropy and the alternatives were evaluated by WASPAS method. In this section, the methods are explained briefly.

Entropy method

The concept of entropy was developed by using probability theory to measure uncertainty in knowledge by Shannon and Weaver in 1947 (Shemshadi et al., 2011), and was adapted from thermodynamics to information systems by Shannon in 1948 (Santos et al., 2019). Entropy method, which is used in multi-criteria decision-making (MCDM) problems, is an objective weighting method and provides the weighting of the evaluation criteria in the decision matrix (Wu and Lin, 2012). The implementation steps of the method are summarized as follows (Li et al., 2011; Ghorbani et al., 2012):

Step 1: Formation of decision matrix: x_{ij} values are created for *m* alternatives and *n* criteria.

$$X = \begin{bmatrix} x_{11} & x_{12} & \dots & x_{1n} \\ x_{21} & x_{22} & \dots & x_{2n} \\ \vdots & \vdots & \dots & \vdots \\ x_{m1} & x_{m2} & \dots & x_{mn} \end{bmatrix} \quad (1)$$

i=1,2,…,*m*; *j*=1,2,…,*n*

Step 2: Normalization: This process is carried out with the help of the following formula.

$$p_{ij} = x_{ij} / \sum_{i=1}^{m} x_{ij} \quad (2)$$

Step 3: Calculation of entropy values(e_j):

$$e_j = -k \sum_{i=1}^{m} p_{ij} \,.\, ln(p_{ij}) \quad (3)$$

Where k is a constant number provides the expression 0 ≤ ej ≤ 1 and is calculated as$k$=1/ln(m).

Step 4: Determination of degree of difference: The higher the d_j values, the higher the significance of the relevant criterion.

$$d_j = 1 - e_j \quad (4)$$

Step 5: Calculation of weights:

$$w_j = d_j / \sum_{j=1}^{n} d_j \quad (5)$$

Weighted aggregated sum product assessment (WASPAS)

WASPAS was developed by Zavadskas et al (2010), as a combined version of Weighted Sum Model (WSM) and Weighted Product Model (WPM). Steps of the methodology are explained briefly (Chakraborty et al., 2015):

Step 1. Developing the decision matrix.

Step 2. Normalizing the decision matrix.

$$\bar{x}_{ij} = \frac{x_{ij}}{max\, x_{ij}} \quad \text{for beneficial criteria} \quad (6)$$

$$\bar{x}_{ij} = \frac{min\, x_{ij}}{x_{ij}}$$

for cost criteria

Step 3. The total relative importance of the alternatives is calculated by WSM.

$$\mathbf{Q_i^{(1)}} = \sum_{\mathbf{j=1}}^{\mathbf{n}} \mathbf{w_j r_{ij}} \quad (8)$$

Step 4. The total relative importance of the alternatives is calculated by WPM.

$$\mathbf{Q_i^{(2)}} = \prod_{\mathbf{j=1}}^{\mathbf{n}} \mathbf{r_{ij}^{w_j}} \quad (9)$$

Step 5. The following formula is used to determine the relative and total significance levels of alternatives (Sengupta et al., 2017).

$$\begin{aligned} Q_i &= \lambda Q_i^{(1)} + (1-\lambda) Q_i^{(2)} \\ &= \lambda \sum_{j=1}^{n} \bar{x}_{ij} w_j + (1 \\ &- \lambda) \prod_{j=1}^{n} (\bar{x}_{ij})^{w_j}, \lambda \\ &= 0,0.1,\dots,1 \end{aligned} \quad (10)$$

RESULTS

The products, which were included in the study, were determined according to their average production amounts for the last 5 years which are divided into three different groups as the most captured fishes, other sea products, and fishes from aquaculture production. The production amounts of products were taken from TUIK given in Table 1. The global data as export value, export quantity, import value, and import quantity data were taken from the international trade database TradeMap. Each product's data was selected according to 8 digits of Harmonized Systems (HS) Codes. Therefore, fresh, frozen, dried product types classified under "*chapter 03 - Fish and crustaceans, molluscs and other aquatic invertebrates"* and prepared-preserved product types classified under the *chapter 16 - Preparations of meat, of fish or crustaceans, molluscs or other aquatic invertebrates"* were added to the product selection. Ultimately, a detail product selection was made and all products, which were subjected to foreign trade activities of Turkey, were included in all calculations

Table 1. The most captured and produced species in turkey (tonnes)

Type of fish	2015	2016	2017	2018	2019
					Quantity of aquaculture production (the most produced)
Trout (all types)	108038.45	107013.02	109657.33	114497.09	125745.87
Sea bream	51844.33	58254.08	61090.04	76680.56	99730.67
Sea bass	75164.44	80847.23	99971.79	116915.07	137419.11
					Quantity of caught sea fish (the most captured)
Sprat	76995.60	50224.90	33949.50	20056.60	38077.60
Atlantic bonito	4573.00	39459.60	7577.60	30920.40	1578.30
Anchovy	193492.30	102595.20	158093.80	96451.70	262544.40
Horse mackerel	14290.40	8859.80	8065.60	14221.80	13179.80
Pilchard	16693.40	18162.10	23425.70	18854.00	19119.20
			Quantity of caught other sea products (crustaceas, molluscs) (the moct captured)		
Sea snail	8795.30	10353.70	9194.10	9672.30	11646.30
Prawn (all types)	3995.20	4500.90	4730.30	4536.10	5136.60
Cuttle fish	744.70	925.10	986.00	1041.90	940.10
Mussel (all types)	37649.40	21014.00	35476.70	45137.40	37796.90

Source : TUIK, 2020

The decision matrix was created before starting the analysis. The alternatives were chosen as trout, sea bream, sea bass, sprat, atlantic bonito, anchovy, horse mackerel, pilchard, sea snail, prawn, cuttle fish and mussel. The alternatives were decided according to the statistics of the Republic of Turkey Ministry of Agriculture and Forestry (TAGEM, 2019). The criteria were settled as production quantity, import quantity, import value, export quantity, and export value.

Evaluating the weights of criteria by Entropy method

The decision matrix is constituted with the last 5 years data of each alternative and criterion. The weighted mean was used for the value-related data while arithmetic mean was used for quantity related data. Table 2 shows the decision matrix.

The weights of the data are calculated and shown in Table 3.

Table 2. Decision matrix

	Production (tonnes)	Import quantity (tonnes)	Import value ($1000)	Export quantity (tonnes)	Export value ($1000)
Trout	112990.00	735.00	7083.11	23477.40	117494.84
Sea bream	69519.60	1251.60	2967.88	68232.20	392972.63
Sea bass	102063.20	667.80	2120.82	17615.00	185824.45
Sprat	43860.84	11913.20	188.62	109.00	188.62
Atlantic bonito	16821.78	548.40	1233.10	12680.80	53183.73
Anchovy	162635.48	4695.00	3117.40	13084.00	54751.30
Horse mackerel	16195.98	265.20	178.99	156.60	489.22
Pilchard	19250.88	11974.20	8967.69	5764.60	27066.66
Sea snail	9932.34	223.20	847.24	1375.80	10681.42
Prawn	4579.82	1522.20	6046.45	674.00	5949.44
Cuttle fish	927.56	1192.20	9156.10	336.20	1953.99
Mussel	35414.88	477.20	1718.72	16.80	232.42

Table 3. The weights of the criteria

	Production (tonnes)	Import quantity (tonnes)	Import value ($1000)	Export quantity (tonnes)	Export value ($1000)
w_j	0.135	0.220	0.112	0.262	0.271

Evaluating the alternatives by WASPAS method

After calculating the weights, WASPAS method was used for evaluating the alternatives. According to the Equations (6-7), normalized decision matrix is found as in Table 4. After normalizing the decision matrix, applying Equations (8-10), the relative and total significance levels of alternatives are calculated as in Table 5.

Scenario analysis

In the last part of the application, to search the differences in the rankings and the robustness of proposed methodology, a scenario analysis consists of 5 scenarios is conducted and the results are compared. The result of scenario analysis is shown in Table 6. Also, Figure 3 shows the graphical display.

Table 4. Normalized decision matrix

	Production (tonnes)	Import quantity (tonnes)	Import value ($1000)	Export quantity (tonnes)	Export value ($1000)
Trout	0.695	0.304	0.025	0.344	0.299
Sea bream	0.427	0.178	0.060	1.000	1.000
Sea bass	0.628	0.334	0.084	0.258	0.473
Sprat	0.270	0.019	0.949	0.002	0.000
Atlantic bonito	0.103	0.407	0.145	0.186	0.135
Anchovy	1.000	0.048	0.057	0.192	0.139
Horse mackerel	0.100	0.842	1.000	0.002	0.001
Pilchard	0.118	0.019	0.020	0.084	0.069
Sea snail	0.061	1.000	0.211	0.020	0.027
Prawn	0.028	0.147	0.030	0.010	0.015
Cuttle fish	0.006	0.187	0.020	0.005	0.005
Mussel	0.218	0.468	0.104	0.000	0.001

Table 5. The relative and total significance levels of alternatives

	$Q_i^{(1)}$	$Q_i^{(2)}$	Q_i (λ = 0.5)	*Rating*
Trout	0.335	0.264	0.299	*3*
Sea bream	0.637	0.445	0.541	*1*
Sea bass	0.364	0.320	0.342	*2*
Sprat	0.148	0.008	0.078	*8*
Atlantic bonito	0.205	0.182	0.194	*4*
Anchovy	0.240	0.141	0.191	*5*
Horse mackerel	0.312	0.023	0.168	*7*
Pilchard	0.063	0.051	0.057	*10*
Sea snail	0.264	0.078	0.171	*6*
Prawn	0.046	0.026	0.036	*11*
Cuttle fish	0.047	0.013	0.030	*12*
Mussel	0.144	0.008	0.076	*9*

Table 6. Results of scenario analysis

Scenarios	1		2		3		4		5	
Weights	w_1	0.135	w_1	0.271	w_1	0.135	w_1	0.135	w_1	0.135
	w_2	0.220	w_2	0.220	w_2	0.271	w_2	0.220	w_2	0.220
	w_3	0.112	w_3	0.112	w_3	0.112	w_3	0.271	w_3	0.112
	w_4	0.262	w_4	0.262	w_4	0.262	w_4	0.262	w_4	0.271
	w_5	0.271	w_5	0.135	w_5	0.220	w_5	0.112	w_5	0.262
	c_j	ranking	c_j	ranking	c_j	ranking	c_j	ranking	c_j	ranking
Trout	0.299	*3*	0.342	*3*	0.300	*3*	0.235	*4*	0.300	*3*
Sea bream	0.541	*1*	0.478	*1*	0.501	*1*	0.386	*1*	0.541	*1*
Sea bass	0.342	*2*	0.359	*2*	0.335	*2*	0.273	*2*	0.340	*2*
Sprat	0.078	*8*	0.102	*8*	0.079	*9*	0.163	*8*	0.078	*8*
Atlantic bonito	0.194	*4*	0.188	*5*	0.206	*4*	0.195	*6*	0.194	*4*
Anchovy	0.191	*5*	0.271	*4*	0.185	*7*	0.175	*7*	0.191	*5*
Saurel	0.168	*7*	0.184	*6*	0.194	*6*	0.269	*3*	0.168	*7*
Pilchard	0.057	*10*	0.062	*10*	0.054	*10*	0.049	*10*	0.057	*10*
Sea snail	0.171	*6*	0.178	*7*	0.204	*5*	0.201	*5*	0.171	*6*
Shrimp	0.036	*11*	0.038	*11*	0.041	*11*	0.039	*11*	0.036	*11*
Cuttle fish	0.030	*12*	0.030	*12*	0.036	*12*	0.033	*12*	0.030	*12*
Mussel	0.076	*9*	0.096	*9*	0.090	*8*	0.089	*9*	0.076	*9*

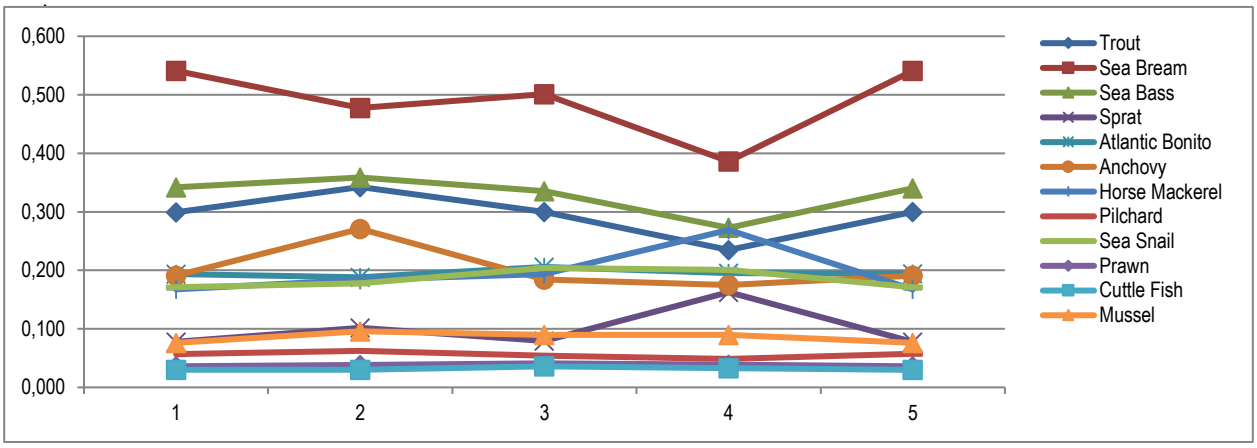

Figure 3. Scenario analysis

As can be seen from the scenarios, when we change the weights of the criteria, the order of fishery products does not change. For all the scenarios, Sea Bream is found as the most valuable fishery product. And also, cuttle fish is found as the least valuable fishery product for Turkey.

CONCLUSION

The fisheries and aquaculture industry is one of the leading industries in Turkey which has a significant importance in contributing to nutrition and food security, as well as offering a wide range of employment opportunities and having a high trade potential. Scientific researches of the last 50 years have much improved the understanding of fisheries and aquaculture industry, and global awareness for the need of managing the industry in a sustainable approach. However, less attention has been given to its important role in foreign trade which plays as one of the key drivers of economic activities, in generating regional employment and as a source

of foreign exchange. In this context, this paper focused on showing this importance by the help of determining the products providing the greatest contribution to the foreign trade of Turkey. Furthermore, Entropy-WASPAS, which is a hybrid multi-criteria decision-making method was applied to evaluate the products.

Following the steps of Entropy method, the results in Table 3 shows that the most important criterion is the export value (0.271), whereas the least important is import value (0.112). Then WASPAS method was used for ranking the alternatives. The results show that sea bream is the most valuable and cuttle fish is found as the least valuable fishery product for Turkey. Since fishery products are evaluated in terms of trade data, products with high import data like cuttle fish, prawn and pilchard are found to contribute less to the economy. Besides, since its export value and quantity is high, although its production is less, sea bream is considered to be more valuable for the country's economy than its closest competitors, Trout and Sea Bass.

Evaluating the stability of the results, scenario analysis was conducted. According to 5 scenarios, supporting the paper, sea bream is found as the most valuable and cuttle Fish is found as the least valuable fishery product for Turkey. Thusly, the robustness of the methodology is shown.

There are some limitations about the field of the study and methods. In this study we investigated the most produced and found fishery products in Turkey. For further research, all fishery products in Turkey can be investigated. On the other hand, Entropy and WASPAS methods are applied in this study. Besides, using different Multi-Criteria Decision Making models such as SWARA, ELECTRE, VIKOR etc. in future research can contribute to the literature.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Nasturtium officinale R.Br. ve *Mentha aquatica* L. taksonlarının farklı kurşun elementi konsantrasyonlarındaki tepkilerinin araştırılması

Investigation of *Nasturtium officinale* R.Br. and *Mentha aquatica* L. taxa reaction in different lead element concentrations

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Öz: Bu çalışmada tarımsal faaliyetlerin ve kurşun kirliliğinin Umurbey Çayı'nda (Çanakkale) yaşayan *Nasturtium officinale* ve *Mentha aquatica* makrofitleri üzerindeki morfolojik ve fizyolojik etkinin belirlenmesi ile bitkiler arası rekabet araştırılmıştır. Araştırma materyali olan makrofitler Çanakkale ili, Umurbey ilçesi, Umurbey Çayı'nın her iki tarafında meyve bahçeleri ile kaplı olan Gökköy Geçemeği'nin alt kısmından Mayıs-Haziran 2016 tarihlerinde toplanmıştır. Makrofitler 3 gün süreyle besin çözeltisi içerisinde adaptasyon sürecine bırakılmıştır. İlk aşamada kurşun kirliliğinin makrofit türlerinin morfolojisi ve fizyolojisi üzerine etkisi 1, 5 ve 10 ppm Pb konsantrasyonlarında tekli kültürde incelenmiştir. Sonra karışık kültürde ara doz olan 5 ppm Pb konsantrasyonunda iki makrofit türünün rekabet kabiliyeti incelenmiştir. Ayrıca klorofil a ve b, karotenoit, serbest prolin, protein, toplam fenolik bileşik miktarı, adsorblanan kurşun miktarı ile toplam potasyum, fosfor, demir ve magnezyum miktarlarına bakılmıştır. Su teresinin morfolojik olarak en fazla etkilendiği 5 ppm Pb dozunda, P, Fe, Mg, K, Pb ve protein içeriği de en fazla seviyeye ulaşmıştır. Aynı zamanda bu dozda su teresi fotosentetik pigment ve serbest prolin seviyesini en aza indirmiştir. Su nanesinin morfolojik olarak en çok etkilendiği doz 10 ppm Pb dozudur. Bahsi geçen dozda su nanesinin P, Fe, Mg ve K miktarı en yüksek değerine ulaşmıştır. Bu çalışmada kurşun stresinin olmadığı, sucul bitkilerin bir arada yetiştirildiği ikili kontrol gruplarında, su nanesinin morfolojik ve fizyolojik olarak daha dirençli olduğu kanıtlamıştır. Kurşunun stres olarak uygulandığı durumda ise, su teresinin daha fazla mücadeleci olduğu morfolojik ve fizyolojik olarak gösterilmiştir. Bu çalışma ile su teresinin, su nanesine göre absorpladığı kurşun miktarının iki katından daha fazla kurşun adsorplayarak iyi bir kurşun akümülatörü olduğu belirlenmiştir.

***Anahtar kelimeler*:** Umurbey Çayı, kurşun, *Nasturtium officinale*, *Mentha aquatica*, rekabet

Abstract: In this study, it was aimed to determine the morphological and physiological effects of agricultural activities and lead pollution on *Nasturtium officinale* and *Mentha aquatica* macrophytes living in Umurbey Stream (Çanakkale), and also the competition between plants in this region was investigated. Macrophytes were collected between May-June 2016 from the lower part of Gökköy Passage, which is covered with orchards on both sides of Çanakkale province, Umurbey district, Umurbey Stream. The macrophytes were left in the nutrient solution for 3 days for the adaptation process. In the first stage, the effect of lead contamination on the morphology and physiology of macrophyte species was investigated in a single culture at 1, 5 and 10 ppm Pb concentrations. Then, the competitiveness of two macrophyte species at an intermediate dose of 5 ppm Pb in a mixed culture was examined. In addition to the amount of, chlorophyll a and b, carotenoid, free proline, protein, total phenolic compound amount, adsorbed lead amount and total potassium, phosphorus, iron and magnesium amounts were examined. P, Fe, Mg, K, Pb and protein content reached the highest level at the dose of 5 ppm Pb where *N. officinale* was affected the most morphologically. At the same time, *N officinale* minimized the photosynthetic pigment and free proline levels at this dose. The dose at which *M. aquatica* was most affected morphologically was 10 ppm Pb dose. P, Fe, Mg and K contents of *M. aquatica* at that dose reached the highest value. This study proved that *M. aquatica* was morphologically and physiologically more resistant in two control groups where there was no lead stress and aquatic plants were grown together. Morphologically and physiologically, it has been shown that *N. officinale* was more combative when lead was applied as stress. With this study, it was determined that watercress is a good lead accumulator by adsorbing more than twice the lead amount absorbed by water mint.

***Keywords*:** Umurbey Stream, lead, *Nasturtium officinale*, *Mentha aquatica*, competition

GİRİŞ

Tatlı sular gerek ekolojik gerekse ekonomik yönleriyle önemli ekosistemlerdir. Hızlı kentsel dönüşüm, sanayi atıklarının kimyasallarından arıtılmadan su sistemlerine verilmesi, tarım alanlarında yaygın şekilde pestisit kullanımı, evsel atıklar gibi birçok etken su kaynaklarının kirlenmesine neden olmaktadır. Sanayileşme, maden işletmeleri ve tarımsal alanlardaki insan faaliyetlerine bağlı ortaya çıkan çevre kirliliği günümüzde küresel bir problem haline gelmiştir. Kirleticiler arasında ağır metaller, tarımsal ilaçlar, organik maddeler ve radyoaktif atıklar önemli bir yer tutmaktadır (El-Sikaily vd., 2004; Okcu vd., 2009; Martin ve Coetzee, 2014).

Makrofitlere çeşitli fiziksel ve kimyasal faktörlerin uygulanması ve verdikleri cevabın izlenmesiyle ilgili birçok çalışma yapılmıştır (Lee vd., 1991; Banerjee ve Sarker, 1997; Cardwell vd., 2002; Demirezen ve Aksoy, 2004; Saygıdeğer ve Doğan, 2005; Doğan vd., 2009; Singh vd., 2010; Favas vd.,2012).

Çalışma konusunu oluşturan *Nasturtium officinale* R.Br. ve *Mentha aquatica* L. ile ilgili Kara, (2005); Özen, (2009); Bahramikia ve Yazdanparast, (2010); Duman ve Öztürk, (2010); Öztürk vd., (2010); Namdjoyan ve Kermanian, (2013);

Zeb, (2015); Giallourou vd., (2016) ile Zurayk vd., (2001); Aslan vd., (2003); Száková vd., (2011); Benabdallah vd., (2016); Nazari vd., (2017); Nazari vd., (2018) tarafından yapılan çalışmalarda da iki sucul bitkinin ağır metal alım ve biriktirme kapasitelerinin oldukça fazla olduğu tespit edilmiştir.

Bazı çalışmalarda ise biyotik faktörlerin yanında abiyotik faktörlerin de araştırıldığı görülmektedir. Türler arası rekabet konusunda Abernethy vd., (1996), James vd., (1999), Agami ve Waisel, (2002), Spencer ve Rejmánek, (2010), Stiers vd., (2011), Martin ve Coetzee, (2014), Srivastava vd., (2014), Shields ve Moore, (2016), Türker vd., (2016), Zheng vd., (2016) çalışmaları mevcuttur.

Çalışılan taksonlardan *N. officinale,* rizomlu, çok yıllık ve emers bir tatlı su makrofiti olup, ekolojik ve ekonomik bakımdan tatlı su makrofitlerinin en önemlilerindendir. Omega-3 yağ asitlerince zengin gıdalardan olan yeşil yapraklı bitkilerden su teresi, yıllardır insanlar tarafından gıda olarak kullanılmakla birlikte tıbbi bir bitki olarak da değerlendirilmektedir (Lee ve Newman, 1997). *M. aquatica*, keskin kokulu, genellikle mor ve çok değişken yapıda olan çok yıllık otsu emers bir bitkidir. Uçucu yağlar, fenolikler ve flavanoidler açısından zengin, antibakteriyel ve antioksidan etkisi yüksektir (Száková vd., 2011). Esansiyel yağ açısından zengin olan bu takson, yapısındaki yağlar sayesinde güçlü bir antioksidan özelliğe sahiptir (Riahi vd., 2013).

Bu çalışmada Umurbey Çayı'nda aynı ortamı paylaşan *N. officinale* ve *M. aquatica* taksonlarının, Umurbey Çayı'na ulaşan kurşun madeninden etkilenip etkilenmediklerini belirlemek için abiyotik faktörlerden Pb ağır metali, biyotik faktörlerden ise rekabet ele alınmıştır. Bu taksonların tekli ve ikili kültür ortamlarında Pb stresi altında ve Pb stresi olmaksızın rekabete girdiklerinde nasıl tepkiler vereceğini belirlemek amacıyla yapılmıştır**.**

MATERYAL VE METOT

The Araştırma materyali olan *N. officinale* ve *M. aquatica* Çanakkale ili, Umurbey ilçesi, Umurbey Çayı'nın her iki tarafı şeftali ve kiraz bahçeleri ile kaplı olan Gökköy Geçemeğinin alt kısmından Mayıs-Haziran 2016 tarihlerinde toplanmıştır.

Bitkilerin muamele ortamı ve düzeneği

Çalışma materyali *N. officinale* ve *M. aquatica* makrofitlerinin Pb alımından nasıl etkilediklerini belirlemek amacıyla Kurşun (II) Asetat Trihidrat bileşiği kullanılmıştır. Kurşun (II) Asetat Trihidrat bileşiğinden, litrede 1 gram Pb olacak şekilde Pb stok çözeltisi hazırlanmıştır ve bu stok çözeltiden belirlenen miktarlarda eklenerek seyreltik derişimli çözeltiler oluşturulmuştur. Ön çalışmalar sonucunda 1, 5 ve 10 mg/L Pb konsantrasyonlarında çalışmaya karar verilmiştir. Ön çalışma sonucu yüksek konsantrasyonlarda bitkilerin fazla etkilenmesinden dolayı, ikili kültürler için 5 mg/L Pb konsantrasyonu tercih edilmiştir. Ön çalışmada, 15 gün boyunca adaptasyon sürecine bırakılan sucul bitkiler için bu sürenin uzun olduğu gözlenmiş ve bu süre 3 gün olarak belirlenmiştir.

Kontrol çalışması için kurşunsuz ortam hazırlanılmıştır. Makrofitlerin gelişmesi için gerekli olan besin çözeltisi Hoagland ve Arnon (1950)'a göre hazırlanmıştır. Makrofitleri yetiştirme ortamına % 10 oranında bu çözeltiden konulmuştur. Çözeltilerin pH'ını ayarlamak için 0.1 N NaOH (Merck) ve 0.1 N Asetik asit (Merck) kullanılmıştır. Makrofitler nötre yakın pH'larda iyi geliştiği için (Saygıdeğer vd., 2004) test çözeltilerinin başlangıç pH'ları 6.5-7.0 düzeylerine ayarlanmıştır. Deney süresince de akvaryum pH'ları düzenli ölçülerek bahsi geçen pH'lar ayarlanmıştır.

Deney düzeneği ve yapılışı

Umurbey Çayı'ndan toplanarak ortam suyu ile birlikte laboratuara getirilen bitkiler tek tek yıkanarak kültür ortamlarına yerleştirilmiştir. Tür teşhisleri için Seçmen ve Leblebici (2008)'nin kitabından yararlanılarak yapılmıştır. Köklerin güneş ışığından etkilenmemesi için pet şişeler siyah poşet ile sarılmıştır. Laboratuar ortamında sadece güneş ışığı alarak 3 gün süresince bitkiler kültür ortamında adaptasyona bırakılmışlardır. Bu süreçte uygun Pb konsantrasyonunu belirleyebilmek için iki bitki türü ve Umurbey çayından alınan su örneği Çanakkale Gıda Kontrol Laboratuvarı'na Pb analizi için gönderilmiştir. Alınan sonuca göre ne suda ne de bitkilerde kurşuna rastlanmamıştır. Bu sonuç iyi bir kontrol grubu sağlamaktadır.

Asıl deney için akvaryumların konulduğu ve güneş ışığı almayan siyah cam kapaklı, her rafta iki florasan (5058 lx) bulunan bir dolap yaptırılmış, her bir akvaryum suyu eşit güçte oksijen pompalayan hava pompası ile havalandırılmıştır. Böylece fiziksel koşullar tamamen sabit tutulabilmiştir. Akvaryumların çapı 19 cm, yüksekliği 20 cm olup, her bir akvaryuma 3 bitki yerleştirilmiştir. 16 saat aydınlık, 8 saat karanlık olacak şekilde aydınlanma, gün uzunluğu düşünülerek belirlenmiştir. Akvaryum sularının sıcaklığı florasanların açık ve kapalı olmasına bağlı olarak 24-26 ^{0}C değerleri arasında değişiklik göstermiştir. 3 günlük adaptasyon sürecinden sonra bitkiler ayrı ayrı kültüre alınarak her bir akvaryuma %10 'luk Hoagland besin çözeltisi eklenmiş ve 1 mg/L Pb, 5 mg/L Pb ve 10 mg/L Pb konsantrasyonlarında statik 3 tekrarlı olarak çalışılmıştır. Kontrol grupları için sadece %10 'luk Hoagland besin çözeltisi konulmuştur. İkili kültürler için orta doz olan 5 mg/L Pb konsantrasyonu tercih edilmiş olup, ikili kültür kontrol gruplarına da sadece %10'luk Hoagland besin çözeltisi

eklenmiştir. Deney süresince günlük morfolojik değişimler kaydedilmiştir. Tekli ve ikili kontrol gruplarında hafif morfolojik değişimler görülmesine rağmen yüksek konsantrasyonlarda bitkiler canlılıklarını kaybetmeye başladığı için 5. günün sonunda (120 saat) deneyin sonlandırılmasına karar verilmiştir.

Bitkilerin örneklerin analizlere hazırlanması

120 saat sonunda deney sonlandırıldığında, bitkiler önce bol çeşme suyu ile ardından saf su ile yıkanarak yapılacak analizler için ayrı ayrı etiketlenip poşetlenerek -18 °C 'de saklanmıştır.

Bitki örneklerine uygulanan analizler

Deney periyodu bitiminde *N. officinale* ve *M. aquatica* makrofitlerinin fotosentetik pigment (klorofil-a, klorofil-b ve karotenoid), serbest prolin, protein, toplam fenolik bileşikleri taze örneklerde çalışılmıştır. Bitkilerin absorpladığı toplam Pb (kurşun) ve Mg (magnezyum), Fe (demir), P (fosfor) ve K (potasyum) miktarları bitki kısımlarının tamamında çalışılarak Çanakkale Onsekiz Mart Üniversitesi Bilim ve Teknoloji Uygulama ve Araştırma Merkezi'nde hizmet alımı ile gerçekleştirilmiştir. Mineral içerikleri ve kurşun miktarları ICP-OES cihazında Epa 200.7 metoduna göre belirlenmiştir. 1000 ppm' lik karışık standartlar kullanılmıştır. P, K ve Mg için 0-25 ppm, Pb ve Fe için ise 0-1000 ppb kalibre aralığında çalışılmıştır.

Makrofitlerdeki klorofil a, klorofil b ve karotenoid hesaplamaları Lichtenthaler ve Wellburn (1985)'e göre aşağıdaki formüller kullanılarak yapılmıştır. Fotosentetik pigment analizleri bitkinin tepe noktasındaki 4.-5. sıradaki yıkanmış taze makrofit yaprakları ile yapılmıştır.

Klorofil a= 11.75A662 – 2.35A645

Klorofil b = 18.61A645 – 3.96A662

Karotenoid = 1000A470 – 2.27 Klorofil a – 81.4 Klorofil b /227

Makrofitlerdeki serbest prolin miktarları Bates vd. (1973)'larının yöntemine göre belirlenmiştir. Bitkilerin en tepe ve onun altındaki yaprakları analiz için kullanılmıştır. Serbest prolin değerlerini belirlemek için L-prolin standardı kullanılmış, standartlar 40-50 µg/ml. aralığında hazırlanmış ve hesaplamalarda standart eğri grafiğinden elde edilen aşağıda belirtilen formül kullanılmıştır.

$y = 0,0359x1,0711 \quad R^2 = 0,9994$

Makrofitlerdeki protein analizi Lowry vd. (1951)'larının belirledikleri yönteme göre belirlenmiştir. Su nanesinde tepe noktasındaki yapraklarda, su teresinde ise üst ve orta yapraklar ve yaprakların yan dallarında çalışılmıştır. Protein değerlerini belirlemek için, standart eğri çizimi 20-70 mg/ml aralığında hazırlanan bovine serum albüminle çalışılmış ve hesaplamalarda standart eğri grafiğinden elde edilen aşağıdaki formül kullanılmıştır.

$y=0,0104x+0,0738 \; R^2=1$

Makrofitlerdeki fenolik bileşik miktarı Ratkevicius vd. (2003)'na göre yapılmıştır. Su teresi ve su nanesinde orta yapraklar ve yaprakların bulunduğu yan dallarda çalışılmıştır. Toplam fenolik bileşik değerleri belirlemek için, standart eğri çizimi 20-35 mg/ml aralığında hazırlanan gallik asitle çalışılmış ve hesaplamalarda standart eğri grafiğinden elde edilen aşağıdaki formül kullanılmıştır.

$y= 0,0347x-0,3177 \quad R^2= 0,9998$

İstatistiksel analizler

Bu araştırmada tanımlanmış gruplar içinde ölçülen parametrelerin ortalama değerleri arasında fark var mıdır hipotezi test edilmiştir. Bu amaçla α= 0.05 alınmıştır. Kurulan hipotezde; H0: Gruplar arasında fark yoktur. H1: En az bir grup diğerlerinden farklıdır. Bu hipotezler SPSS (SPSS 15.0 for Windows) paket programı kullanılarak yapılmıştır. Hangi grubun ya da grupların farklı olduğunu belirlemek amacıyla tekli kültürler için 'One-Way ANOVA LSD testi' ve ikili kültürler için 't testi' uygulanmıştır. Aynı zamanda sucul bitkilere yapılan analiz sonuçlarının aralarında ilişki olup olmadığını belirleyebilmek için korelasyon analizi (Özdamar, 2004) yapılmıştır.

BULGULAR VE TARTIŞMA

Su teresi ve su nanesi bitkilerine uygulanan farklı konsantrasyonlardaki Pb uygulamaları sonucunda bitkilerde meydana gelen değişimlerden elde edilen ortalama değerler Tablo 1'de verilmiştir. Bitkiler metabolik faaliyetlerini devam ettirebilmek ve hayatlarını sürdürebilmek için minerallere ihtiyaç duymaktadırlar. Birçok stres faktörünün bitkilerin bu elementleri alımını ve kullanımını sınırlandırdığı ile ilgili çalışmalar mevcuttur.

Tablo 1. *Nasturtium officinale* ve *Mentha aquatica* taksonlarının farklı Pb konsantrasyonu uygulamalarındaki analiz sonuçlarının ortalama değerleri

Table 1. The average values of the analysis results of *Nasturtium officinale and Mentha aquatica* taxa in different Pb concentration applications

Nasturtium officinale	P (ppm)	Fe (ppm)	Mg (ppm)	K(ppm)	Pb(ppm)	Kl-a	Kl-b	Karotenoid	Serbest Prolin (ppm)	Fenolik Bileşik (ppm)	Protein (mg/ml)
kontrol	548	71926,67	1274	16430	3818,67	11,74	3,44	2,87	10,49	29,04	177,54
1 ppm Pb	578,33	83506,67	1152,33	14006,67	35403,33	11,68	3,69	2,59	43,87	31,82	201,49
5 ppm Pb	718,43	108273,3	1342,33	16773,33	152100	7,9	2,69	2,17	26,02	33,01	222,26
10 ppm Pb	267,26	79843,33	954	9074,67	90013,33	10,31	3,34	2,52	39,35	38,56	210,72
ikili kontrol	693,63	78926,67	1383,67	24686,67	5832,33	9,73	2,36	2,72	16,23	36,24	228,15
ikili 5 ppm Pb	546,63	91930	1503,67	22230	52396,67	11,5	3,17	2,67	13,41	35,33	134,95
Mentha aquatica	**P (ppm)**	**Fe (ppm)**	**Mg (ppm)**	**K(ppm)**	**Pb(ppm)**	**Kl-a**	**Kl-b**	**Karotenoid**	**Serbest Prolin (ppm)**	**Fenolik Bileşik (ppm)**	**Protein (mg/ml)**
kontrol	349,4	58870	1121,5	10286,33	3423	12,3	3,48	2,69	6,43	29,79	147,02
1 ppm Pb	362,83	77120	1127	10344,33	32913,33	17,3	4,3	3,36	8,31	33,03	167,04
5 ppm Pb	374,9	65810	1298,67	8902	70426,67	13,7	4,23	3,14	7,55	48,03	261,36
10 ppm Pb	701,4	87023,33	1511,67	13906,67	54460	12,9	3,66	2,7	6,7	44,25	179,18
ikili kontrol	267,27	63856,67	776,1	5179,33	1706,33	15,8	4,69	3,44	7,28	43,09	238,54
ikili 5 ppm Pb	432	64013,33	1339	12226,67	55710	14,7	4,08	3,2	5,05	27,94	129,31

N. officinale ve *M. aquatica* taksonlarının toplam fosfor miktarı

Su teresi tekli kültürlerindeki fosfor (P) değerlerinin ortalamaları arasındaki fark istatistiksel olarak çok önemli bulunmuştur (F: 90,990; df:3; P<0,001). Su teresi tekli kültürlerinde en fazla P değerine 5 ppm Pb dozunda ulaşılmıştır. Bunun sebebi bu dozda Pb stresi ile metabolik faaliyetlerin artmasına bağlı olarak ATP'yi bu faaliyetlerde kullanıp P açığa çıkması sonucunda P miktarı artmakta ve bitki Pb stresi ile en fazla mücadeleyi bu konsantrasyonda vermekte diyebiliriz. En düşük P değerine ise 10 ppm Pb dozunda ulaşılmıştır. Bunun nedeni ise; bitki artık ölüm evresine girdiği için ortamdaki aşırı Pb'yi alamamakta, var olan fosforu da metabolik olaylarda kullanmakta ya da artık yeni fosfor alamamakta olabilir. Hatta bitkiler için mutlak gerekli olan P elementinin 10 ppm Pb dozunda aşırı düşmesi, bu konsantrasyonda bitkinin ölüm evresine girmesine neden olmuş olabilir.

Su nanesi tekli kültürlerindeki P miktarları ortalamaları arasındaki fark istatistiksel olarak çok önemli bulunmuştur (F:34,894; df:3; P<0,001). 10 ppm Pb uygulanan tekli kültürdeki su nanesi P miktarı istatistiksel olarak tüm uygulamalardan farklı bulunmuş, 10 ppm Pb dozunda P değeri kontrol grubu P değerine oranla %100,7 artmıştır. Bulgular neticesinde sucul bitkilerin tekli kültürlerinde su teresi en fazla fosfor değerine 5 ppm Pb dozunda, su nanesi ise 10 ppm Pb dozunda ulaşmıştır. Su nanesi Pb stresine daha çok dayanmış gibi görünse de su teresi 10 ppm Pb dozunda 90013,33 ppm Pb biriktirirken su nanesi 54460 ppm Pb biriktirmiştir.

Su nanesi tekli kültürdeki kontrol grubuna göre ikili kültürdeki kontrol grubunda P miktarını azaltırken, su teresi ise P miktarını arttırmıştır. Su nanesinin P miktarının azalması, bu dozda su nanesinin daha fazla mücadele vererek, P elementini metabolik olaylarda kullanmasına bağlayabiliriz. İkili kültürdeki 5 ppm Pb doz uygulamasında ise su teresi P miktarını tekli kültüre göre azaltırken, su nanesi P miktarını arttırmıştır. Bu doz uygulamasında da su teresi P elementini metabolik olaylarda kullanarak, daha baskın olduğunu göstermektedir.

N. officinale ve *M. aquatica* taksonlarının toplam demir miktarı

Su teresi tekli kültürlerindeki demir (Fe) miktarı ortalamaları arasındaki fark istatistiksel olarak çok önemli bulunmuştur (F:25,488; df:3; P<0,001). Bitkinin biriktirdiği Pb konsantrasyonu ile Fe miktarı arasında pozitif güçlü bir ilişki bulunmuştur. Biriktirilen Pb miktarı arttıkça Fe miktarı artmış ve 5 ppm Pb dozunda en yüksek değerine ulaşmıştır. 10 ppm Pb dozunda biriktirilen Pb miktarının düşmesiyle Fe miktarı da düşmüştür. Dolayısıyla su teresi Fe miktarındaki bu

değişikliğin Pb stresinden kaynaklı olduğu sonucuna varılabilir. 10 ppm Pb dozunda su teresi ölüm evresine girdiği için demiri metabolik aktivitelerde kullanmış olabilir.

Su teresi tekli kültürdeki 5 ppm Pb uygulamasının Fe miktarı ile ikili kültürdeki 5 ppm Pb uygulamasının Fe miktarı arasındaki fark istatistiksel olarak önemli bulunmuş (t: 6,610; df:4; P<0,01) ve Fe miktarı azalmıştır. Bu azalma Fe'in metabolik olaylarda kullanıldığının ve su teresinin ikili kültürde Pb stresi altında mücadele verdiğinin kanıtıdır.

Su nanesi tekli kültürdeki grupların Fe miktarı ortalamaları arasındaki fark istatistiksel olarak çok önemli bulunmuştur (F: 19,865; df:3; P<0,001). Su nanesi tekli kültürlerinde Fe miktarı (87023,33 ppm) en fazla 10 ppm Pb dozunda artmış olup, kontrol grubuna göre %47,8 oranında bir artış görülmüştür. Pb uygulamalarında Fe değeri, kontrol grubunun Fe değerinin altına hiç düşmemiştir.

Sucul bitkilerin tekli kültürlerinde su teresi en fazla Fe değerine 5 ppm Pb dozunda, su nanesi ise 10 ppm Pb dozunda ulaşmıştır. Su teresinin en fazla tepkiyi 5 ppm Pb dozunda, su nanesinin ise 10 ppm Pb dozunda verdiği sonucunu Fe miktarı da desteklemektedir. Su nanesi tekli ve ikili kültürler arasında Fe miktarı açısından istatistiksel olarak fark bulunmamıştır (P>0,05).

N. officinale ve *M. aquatica* taksonlarının toplam magnezyum miktarı

Su teresi tekli kültürlerindeki farklı Pb uygulamalarında magnezyum (Mg) miktarı ortalamaları arasındaki fark önemli bulunmuştur (F: 11,020; df:3; P<0,01). Su teresi tekli kültürlerinde kontrol grubuna göre 1 ppm Pb dozunda Mg değerinde azalma görülmüştür. Bu durum, bitkide bu derişimde su içeriğinin fazla olmasından dolayı Mg'in çözünebilir hale geldiğini ve çeşitli metabolik faaliyetlerde kullanıldığını düşündürmektedir. Mg içeriği en fazla değerine 5 ppm Pb dozunda, en düşük değerine ise 10 ppm Pb dozunda ulaşmıştır. 10 ppm Pb dozunda Mg miktarının düşmesinin nedeni, ölüm evresine girmiş olan bitkinin ATP üretebilmek için Mg kullanması veya artık Mg alamaması olabilir. Yüksek Pb dozunda Mg miktarının düşmesinin bir sebebi de; Pb ile Mg atomunun yer değiştirmesi olabilir. Ağır metal etkisine bırakılan submers makrofitlerle yapılan çalışmalarda, ağır metalin elementlerle yer değiştirdiği ve bitkide zarar meydana getirdiği belirtilmiştir. Aşırı dozda Pb, klorofilin yapısında bulunan Mg ile yer değiştirerek klorofil ile birleşmekte ve yer değiştirmeden etkilenen klorofil molekülü fotosentez için gerekli olan ışığı toplayamamaktadır (Kacar ve Katkat, 2007; Karabulut ve Bellitürk, 2013). Su teresi tekli kültürdeki 5 ppm Pb uygulaması ile ikili kültürdeki 5 ppm Pb uygulaması Mg miktarı arasındaki fark önemli bulunmuştur (t: 2,937; df:4; P<0,05).

Su nanesi tekli kültürlerindeki Mg miktarı ortalamaları arasındaki fark önemli bulunmuştur (F: 6,210; df:3; P<0,05). Su nanesi tekli kültürlerinde ise Mg değeri Pb dozu arttıkça artmıştır. 10 ppm Pb dozunda en yüksek değerine (1511,67 ppm) ulaşmıştır. Mg miktarının artışı, Pb stresine giren bitkinin tepkisi olarak protein miktarını da arttırdığını düşündürmektedir.

Tekli kültürdeki kontrol grubu ile ikili kültürdeki kontrol grubu su nanesinin Mg değerleri farklı bulunmuştur (t: 4,572; df:4; P<0,05). Tekli kültürdeki kontrol gruplarına göre, ikili kültürdeki kontrol grubu su teresi Mg miktarını arttırırken, su nanesi Mg miktarını azaltmıştır. Bu dozda Mg miktarını azaltan su nanesi, Mg elementini metabolik faaliyetlerde kullanıyor ve su teresine göre daha fazla mücadele ediyor tespitimizi güçlendirmektedir.

N. officinale ve *M. aquatica* taksonlarının toplam potasyum miktarı

Su teresi tekli kültürdeki potasyum (K^+) miktarı ortalamaları arasındaki fark istatistiksel olarak anlamlı bulunmuştur (F:6,470; df:3; P<0,05). Su teresi tekli kültürlerinde kontrol grubuna göre 1 ppm Pb dozunda K^+ miktarında önemsiz bir azalma görülmüştür. Bunun nedeni; suyun fazla olduğu bu konsantrasyonda K^+ iyonunun ATP sentezinde, hücre büyümesinde ve enzimatik aktivitelerde kolayca kullanılabilir olması olabilir. K^+ miktarında en fazla artış 5 ppm Pb dozunda görülmüş olup, bu artışta kontrol grubuna göre önemli bulunmamıştır. 5 ppm Pb dozunda ise hücreler fazla su almış, osmotik basıncını arttırmış ve K^+ miktarlarını yükseltmiş olabilirler. Su teresi tekli kültürlerinde 10 ppm Pb dozunda, kontrol grubuna göre K^+ içeriği %44,8 oranında azalmıştır. 10 ppm Pb dozunda ölüm evresine giren su teresi hücrelerinde solunumun olumsuz etkilenmesi sonucu negatif elektriği azalmış ve hücre dışına önemli miktarda K^+ çıkışı olmuş olabilir. K^+ bulunmamasına bağlı olarak hücre su kaybeder, stomalar kapanır, büyüme geriler, kloroz ve nekroz görülür. Aynı zamanda K^+ eksikliğinde bitki gövdesinde yatma eğilimi görülür (Kacar ve Katkat, 2007; Yıldız ve Terzi, 2007; Wang vd., 2013; Horuz vd., 2016). Bu çalışmada 10 ppm Pb dozu uygulanan su teresinde K^+ eksikliğine bağlı olarak tüm bu morfolojik bulgulara rastlanmıştır.

Su nanesi tekli kültürlerindeki K^+ miktarı ortalamaları arasındaki fark istatistiksel olarak çok önemli bulunmuştur (F: 19,626; df:3; P<0,001). 10 ppm Pb dozu uygulanan su nanesinin K^+ miktarı diğer tüm uygulamaların K^+ miktarından farklı bulunmuştur. Kontrol grubuna göre 10 ppm Pb dozunda K^+ değeri %35,2 oranında artmış ve en yüksek değerine ulaşmıştır. Tekli kültürde K^+ açısından Pb stresine verilen tepkiler karşılaştırıldığında su nanesi daha dirençli gözükmektedir. Su teresi 10 ppm Pb dozunda K^+ içeriğini önemli ölçüde azaltırken, su nanesi ise bu dozda önemli ölçüde K^+ içeriğini arttırmıştır.

Tekli kültürdeki kontrol grubu su teresi K^+ değeri ile ikili kültürdeki kontrol grubu su teresi K^+ değeri birbirinden farklı bulunmuştur (t: 4,146; df:4; P<0,05). Tekli kültürdeki kontrol

grubu su nanesi ile ikili kültürdeki kontrol grubu K^+ değeri birbirinden farklı bulunmuştur (t: 6,795; df:4; P<0,01). Su nanesi K^+ elementini tekli kültürdeki kontrol grubuna göre ikili kültürde kontrol grubunda %49,6 oranında azaltırken, su teresi %50,25 oranında arttırmıştır. Bu sonuçta su nanesinin rekabet ortamında K^+'yı metabolik aktivitelerde kullandığının, mücadele ettiğinin kanıtıdır.

Tekli kültürdeki 5 ppm Pb uygulanan su teresinin K^+ miktarı ile ikili kültürdeki 5 ppm Pb uygulanan su teresinin K^+ miktarı farklı bulunmuştur (t: 3,197; df:4; P<0,05). Tekli kültürde 5 ppm Pb uygulanan su nanesi K^+ miktarı ile ikili kültürdeki 5 ppm Pb uygulanan su nanesi K^+ miktarı birbirinden farklı bulunmuştur (t: 3,861; df:4; P<0,05). İkili kültür 5 ppm Pb uygulamasında ise su nanesinin K^+ miktarını su teresine göre daha fazla arttırdığı belirlenmiştir. Su teresi K^+ elementini metabolik aktivitelerinde kullandığı için bu dozda daha fazla mücadelecidir.

N. officinale ve *M. aquatica* taksonlarının adsorpladığı toplam kurşun miktarı

Su teresi tekli kültürlerindeki farklı konsantrasyonlardaki Pb uygulamalarında, su teresinin adsorbe ettiği Pb miktarı ortalamaları arasındaki fark çok önemli bulunmuştur (F: 177,034; df:3; P<0,001). 5 ppm Pb uygulamasında su teresi en yüksek Pb birikimini göstermiştir. 10 ppm Pb uygulamasında ise 5 ppm Pb uygulamasına göre Pb miktarı %40,82 oranında azalmıştır.

Tekli kültürdeki 5 ppm Pb uygulanan su teresinin adsorpladığı Pb miktarı ile ikili kültürde 5 ppm Pb uygulanan su teresinin biriktirdiği Pb miktarı birbirinden farklı bulunmuştur (t: 51,030; df:4; P<0,001). Tekli kültürde 5 ppm Pb uygulanan su teresinin adsorpladığı Pb miktarına göre ikili kültürde 5 ppm Pb uygulanan su teresi Pb miktarı %65,5 oranında azalmıştır.

Su nanesi tekli kültürdeki adsorplanan Pb miktarı ortalamaları arasındaki fark istatistiksel olarak çok önemli bulunmuştur (F:212,81; df:3; P<0,001). En yüksek kurşun birikimine 5 ppm Pb değerinde ulaşılmıştır. 5 ppm Pb uygulamasına göre 10 ppm Pb uygulanmış su nanesi Pb miktarını %22,67 oranında azaltmıştır.

Tekli kültürde 5 ppm Pb uygulanan su nanesi ile ikili kültürde 5 ppm Pb uygulanan su nanesi Pb değerleri arasındaki fark istatistiksel olarak anlamlı bulunmuştur (t: 3,729; df:4; P<0,05). Tekli kültürdeki 5 ppm Pb uygulamasına göre ikili kültürde 5 ppm Pb uygulanan su nanesinin Pb değeri %20,9 oranında azalmıştır.

Su nanesi ve su teresi en fazla kurşunu 5 ppm Pb uygulamasında biriktirmişlerdir. Su nanesi 5 ppm Pb uygulamasında 70426,67 ppm Pb adsorplarken, su teresi aynı doz uygulamasında 152100 ppm Pb adsorplamıştır. Su teresi tüm Pb uygulamalarında su nanesine göre daha fazla kurşun adsorplamıştır. Su teresi iyi bir Pb alıcı olarak değerlendirilebilir.

N. officinale ve *M. aquatica* yapraklarında klorafil-a miktarı

Su teresi tekli kültürlerindeki klorofil a değerlerinin ortalamaları arasındaki fark istatistiksel olarak önemli bulunmuştur (F: 17,59; df:3; P<0,01). Klorofil a değeri açısından 5 ppm Pb uygulanan su teresi diğer tüm uygulamalardan istatistiksel olarak farklı bulunmuştur. Kontrol grubuna göre 5 ppm Pb uygulanan su teresinin klorofil a değeri %32,7 oranında azalma göstermiştir. Kontrol grubunun klorofil a değerine göre tüm uygulamalarda klorofil a değerinde azalma söz konusudur.

Tekli kültürde 5 ppm Pb uygulanan su teresi ile ikili kültürde 5 ppm Pb uygulanan su teresinin klorofil a değerleri istatistiksel olarak farklı bulunmuştur (t: 4,85; df:4; P<0,01). Tekli kültürde 5 ppm Pb uygulanan su teresine göre ikili kültürde 5 ppm Pb uygulanan su teresinin klorofil a değerinde %45,57 oranında artış olmuştur.

Su nanesi tekli kültürlerindeki klorofil a değerlerinin ortalamaları arasındaki fark istatistiksel olarak önemli bulunmuştur (F:14,72; df:3; P<0,01). Klorofil a açısından 1 ppm Pb uygulanan su nanesi diğer tüm uygulamalardan farklı bulunmuştur. Kontrol grubuna göre 1 ppm Pb uygulanan su nanesinin klorofil a değeri %40,29 oranında artış göstermiştir ve en yüksek klorofil a değerine bu konsantrasyonda ulaşılmıştır.

Su nanesinin tekli kültürdeki kontrol grubu ile ikili kültürdeki kontrol grubu arasında klorofil a açısından istatistiksel olarak önemli bir fark bulunmuştur (t: 4,21; df:4; P<0,05). Tekli kültürdeki kontrol grubuna göre ikili kültürdeki kontrol grubu klorofil a değerini %28,43 oranında arttırmıştır.

N. officinale ve *M. aquatica* yapraklarında klorofil-b miktarı

Su teresi tekli kültürlerindeki klorofil b ortalama değerleri arasındaki fark önemli bulunmuştur (F:5,63; df:3; P<0,05). 5 ppm Pb uygulamasında su teresinin klorofil b değeri en düşük değerini almıştır. 5 ppm Pb uygulaması, kontrol grubuna göre klorofil b değerini %21,8 oranında, 1 ppm Pb uygulamasına göre %27,1 oranında azaltmıştır. 10 ppm Pb uygulamasında klorofil b değeri tekrar yükselmiş, ancak kontrol grubundaki değerine ulaşamamıştır. En yüksek klorofil b değerine 1 ppm Pb dozunda ulaşılmıştır. Ancak kontrol grubu ile 1 ppm Pb dozundaki klorofil b değeri arasında istatistiksel olarak önemli bir fark yoktur (P>0,05).

Su teresi tekli kültürdeki kontrol grubu ile ikili kültürdeki kontrol grubu arasında klorofil b değeri açısından fark önemli bulunmuştur (t:7,29; df:4; P<0,01). Tekli kültürdeki kontrol grubuna göre ikili kültürdeki kontrol grubu klorofil b değerini %31,7 oranında azaltmıştır.

Su nanesi tekli kültürlerindeki klorofil b değerleri ortalamaları arasındaki fark istatistiksel olarak önemli bulunmuştur (F: 4,57; df:3; P<0,05). Kontrol grubuna göre 1 ppm Pb uygulaması klorofil b değerini %25,5 ppm Pb

uygulaması klorofil b değerini %21,5 ve 10 ppm Pb uygulaması klorofil b değerini %5,2 oranında artış göstermiştir. En yüksek klorofil b değerine 1 ppm Pb dozunda ulaşılmıştır.

Tekli kültürdeki su nanesinin kontrol grubu ile ikili kültürdeki kontrol grubu klorofil b değeri arasındaki fark istatistiksel olarak anlamlı bulunmuştur (t: 5,72; df:4; P<0,01). Tekli kültürdeki kontrol grubuna göre ikili kültürdeki kontrol grubu klorofil b değerini %34,7 oranında arttırmıştır.

***N. officinale* ve *M. aquatica* yapraklarında karotenoit miktarı**

Su teresi tekli kültürlerinin karotenoit ortalama değerleri arasındaki fark önemsiz bulunmuştur (F: 3,47; df:3; P>0,05). Sadece kontrol grubu ile 5 ppm Pb uygulanan su teresi karotenoit değerleri arasında fark bulunmuştur (P<0,05). Kontrol grubuna göre diğer tüm uygulamalarda karotenoit değeri düşüş göstermiştir.

Karotenoit değeri açısından tekli ve ikili kültürler karşılaştırıldığında; sadece tekli kültürdeki 5 ppm Pb uygulaması ile ikili kültürde 5 ppm Pb uygulanan su teresi karotenoit değerleri arasındaki fark anlamlı bulunmuş olup (t: 3,142; df:4; P<0,05), ikili kültürdeki 5 ppm Pb uygulanmış su teresi, tekli kültürdeki 5 ppm Pb uygulanmış su teresine göre karotenoit değerini %23 oranında arttırmıştır.

Su nanesi tekli kültürdeki karotenoit değeri ortalamaları arasındaki fark önemli bulunmuştur (F: 9,012; df:3; P<0,01). Kontrol grubuna göre 1 ve 5 ppm Pb uygulamalarındaki karotenoit değerlerinde sırasıyla %25 ve %16,7 oranında bir artış belirlenmiştir. 10 ppm Pb dozu uygulanan su nanesi karotenoit değeri kontrol grubuna yakın bir değer almıştır.

Su nanesi ikili kültür değerleri incelendiğinde, sadece tekli kontrol grubu ile ikili kültürdeki kontrol grubu karotenoit değeri arasında bir fark bulunmuştur (t: 2,842; df:4; P<0,05). İkili kültürdeki kontrol grubu tekli kültürdeki kontrol grubuna göre karotenoit değerini %27,8 oranında arttırmıştır.

Tekli kültürlerde su teresi Pb uygulamalarıyla fotosentetik pigment içeriklerini kontrol grubu pigment içeriğine göre daha da azaltırken, su nanesi Pb uygulamalarıyla fotosentetik pigment içeriklerini kontrol grubu pigment içeriğine göre daha da arttırmıştır. Su teresi fotosentetik pigment miktarı ile Pb birikimi arasında negatif güçlü bir ilişki bulunmuştur. Bu durum stres koşullarına karşı bitkinin geliştirdiği bir adaptasyon yanıtı olarak yorumlanabilir. Yüksek Pb birikiminde fotosentetik pigment içeriğinin azalmasının nedenleri; besin eksikliğinden, klorofil sentezinin engellenmesinden, artan klorofilaz aktivitesine bağlı olarak var olan klorofilin bozulmasından ve reaktif oksijen türleri tarafından kloroplast membran lipitlerinin ve pigmentlerinin peroksidasyonla bozunmasından dolayı olabilir (Gupta ve Chandra, 1996).

Tekli kültürdeki kontrol grubu su teresine göre ikili kültürdeki kontrol grubu su teresi fotosentetik pigment içeriğini azaltırken, su nanesi fotosentetik pigment içeriğini arttırmıştır. İkili kültür kontrol gruplarında fotosentetik pigment içerikleri bakımından su nanesi daha mücadelecidir.

Ancak ikili kültür ortamına 5 ppm Pb dozu uygulandığında, bu defa su teresi daha mücadelecidir. İkili kültürde, kontrol grubuna göre 5 ppm Pb uygulanan su teresi fotosentetik pigment içeriğini arttırırken, su nanesi fotosentetik pigment içeriğini azaltmıştır. Ortamda Pb stresi olmadığında iki sucul bitkinin sadece aynı ortamı paylaşmasından dolayı maruz kaldıkları stresler karşısında su nanesi rekabette üstünlük kazanmıştır. Ancak bu ortama Pb stresi de eklendiğinde, su teresinin daha fazla mücadele verdiğini fotosentetik pigment içerikleri sonuçlarımızda desteklemiştir.

***N. officinale* ve *M. aquatica* taksonlarının serbest prolin miktarları**

Su teresi tekli kültürlerinin serbest prolin ortalama değerleri arasındaki fark istatistiksel olarak çok önemli bulunmuştur (F: 102,98; df:3; P<0,001). Kontrol grubuna göre; 1, 5 ve 10 ppm Pb uygulanan su tereleri serbest prolin değerlerini sırasıyla %318,6, %148,3 ve %277 oranında arttırmışlardır. Su teresinin yüksek oranda prolin biriktirmesi bitkinin osmoregülasyon mekanizması ve antioksidatif özelliğine dayandırılabilir. Pb stresi altında su teresinin prolin miktarını yükseltmesi; bitkinin stresi tolere etmeye ve daha az zarar görmeye çalıştığının kanıtıdır (Hare ve Cress, 1997; Aziz vd., 1998; Sharmila ve Pardha Saradhi, 2002; Özden vd., 2009).

Su teresi tekli kültürdeki kontrol grubu ile ikili kültürdeki kontrol grubu arasında serbest prolin miktarı ortalamaları açısından fark anlamlı bulunmuştur (t: 4,436; df:4; P<0,05). Tekli kültürdeki kontrol grubuna göre ikili kültürdeki kontrol grubu su teresi serbest prolin değerini %54,7 oranında arttırmıştır.

Su teresi tekli kültürdeki 5 ppm Pb uygulaması ile ikili kültürdeki 5 ppm Pb uygulaması serbest prolin ortalama değerleri arasındaki fark anlamlı bulunmuştur (t: 6,723; df:4; P<0,01). Tekli kültürde 5 ppm Pb uygulanmış olan su teresine göre ikili kültürde 5 ppm Pb uygulanmış su teresi serbest prolin değerini %48,5 oranında azaltmıştır.

Su nanesi farklı konsantrasyonlardaki Pb stresi altında serbest prolin içeriğinde istatistiksel olarak önemli bir değişiklik görülmemiştir. Su nanesi kontrol grubuna göre diğer Pb uygulamalarında prolin miktarında artış göstermiş, ancak bu artışlar önemli bulunmamıştır.

***N. officinale* ve *M. aquatica* taksonlarının protein miktarları**

Su teresi tekli kültürlerinin protein değerlerinin ortalamaları arasındaki fark önemli bulunmuştur (F: 11,74; df:3; P<0,01). Kontrol grubunun toplam protein miktarına göre 1, 5 ve 10 ppm Pb uygulanan su terelerinin protein miktarı sırasıyla %13,3, %25,2 ve %18,7 oranında artış göstermiştir.

Su nanesi tekli kültürlerinin protein miktarı ortalamalarının arasındaki fark çok önemli bulunmuştur (F:39,73; df:3;

P<0,001). 5 ppm Pb uygulanmış su nanesinin protein değeri diğer tüm uygulamalardan istatistiksel olarak önemli derecede farklı bulunmuştur (P<0,001). 5 ppm Pb dozunda su nanesi en yüksek protein değerine ulaşmış olup, kontrol grubuna göre protein miktarını %77,8 oranında arttırmıştır.

Her iki sucul bitkide de Pb konsantrasyonu arttıkça protein değeri artış göstermiş ve 5 ppm Pb konsantrasyonunda en yüksek protein değerine ulaşılmıştır. Protein içeriği, bitkilerde oksidatif metal stresinin güvenilir bir göstergesidir. Bu çalışmada, 5 ppm Pb dozuna kadar protein içeriğinin artması; ağır metal dayanıklılığını sağlayan stres enzimlerini de içine alan farklı proteinlerin işlev gösterdiğinin bir kanıtıdır. Ağır metal stresine maruz kalan bitkilerin, uygulamanın ilk evrelerinde değişen çevre koşullarına tepki olarak stres proteinleri üretmek suretiyle hayatta kalmaya çalıştıkları da bilinmektedir (Öztürk vd., 2010).

Tekli kültürlerde su teresi kontrol grubuna göre 5 ppm Pb dozunda protein değerini %25,20 arttırırken, su nanesi kontrol grubuna göre 5 ppm Pb dozunda protein değerini %77,77 oranında arttırmıştır. Su nanesi düşük Pb uygulamasında Pb stresine karşı daha fazla stres proteini üreterek, bu stres dozuna su teresine göre daha fazla tepki vermiştir diyebiliriz. 10 ppm Pb konsantrasyonunda ise her iki türde protein içeriğini azaltmıştır. Su teresi 5 ppm Pb uygulamasına göre 10 ppm Pb dozunda protein içeriğini %5,2 oranında, su nanesi ise 5 ppm Pb uygulamasına göre 10 ppm Pb dozunda protein içeriğini %31,32 oranında azaltmıştır. Su teresindeki bu düşüş istatistiksel olarak önemsiz, su nanesinde ise önemli bulunmuştur. Su nanesinde 10 ppm Pb dozunda protein değerinin bu denli düşmesi; artık bitki canlılığı yitirmeye başladığı için protein yıkımının daha fazla olduğu yönünde yorumlanabilir. Yüksek Pb dozunda (10 ppm Pb) protein içeriğinin azalmasının nedenleri; protein sentezinin inhibisyonu, serbest radikallerin artması, oksidatif streste üretilen ROT'ların proteolisizi tetiklemesi ve protein yapısının bozulması olabilir. Ancak her iki türde de hiçbir Pb uygulamasında protein değeri kontrol grubu protein değerine kadar düşmemiştir.

İkili kültürlerde kontrol grubu su teresi protein miktarını arttırmıştır ancak bu artış su nanesinin gösterdiği artış kadar önemli değildir. Su teresi tekli kültürdeki kontrol grubuna göre ikili kültürdeki kontrol grubunda protein içeriğini %28,6 oranında arttırırken, su nanesi %62,25 oranında arttırmıştır. Bu deney sonucu da ikili kültürdeki kontrol gruplarında su nanesinin üstünlüğünü desteklemektedir. İkili kültürdeki türlere 5 ppm Pb uygulandığında ise su teresi protein içeriğini tekli kültüründeki 5 ppm Pb uygulamasına göre %39,3 azaltırken, su nanesi %50,5 oranında azaltmıştır. İkili kültürde ortama Pb eklendiğinde su teresinin daha rekabetçi olduğu yorumu yapılabilir.

N. officinale ve *M. aquatica* taksonlarının toplam fenolik bileşiklerin miktarları

Su teresi tekli kültürlerindeki kontrol grubu, 1, 5 ve 10 ppm Pb uygulanan türlerinin toplam fenolik bileşik değerlerinin ortalamaları arasındaki fark önemli bulunmuştur (F: 4,96; df:3; P<0,05) Pb konsantrasyonu arttıkça, toplam fenolik bileşik değeri artış göstermiştir. Kontrol grubuna göre 10 ppm Pb dozu toplam fenolik bileşik değeri %32,8 oranında artış göstermiştir ve su teresi en yüksek toplam fenolik bileşik miktarına 10 ppm Pb uygulamasında ulaşmıştır.

Su nanesi tekli kültürlerindeki toplam fenolik bileşik değeri ortalamaları arasındaki fark çok önemli bulunmuştur (F: 21,134; df:3; P<0,001). Kontrol grubuna göre 1,5 ve 10 ppm Pb uygulanmış su nanelerinin toplam fenolik bileşik değerleri sırasıyla %10,8, %61,2 ve %48,5 oranında artış göstermiştir. Su nanesi 5 ppm Pb uygulamasında en yüksek toplam fenolik içeriğe ulaşarak antioksidant yeteneği ile dikkatleri çekmektedir.

İkili kültürdeki kontrol grubu su teresi tekli kültürdeki kontrol grubu su teresine göre toplam fenolik içeriğini %24,8 oranında arttırırken, su nanesi %44,6 oranında arttırmıştır. Bu deney sonucu da ikili kültürdeki kontrol gruplarında su nanesinin daha rekabetçi olduğunu desteklemektedir.

İkili kültürdeki türlere 5 ppm Pb uygulamasında, su teresi tekli kültürdeki 5 ppm Pb uygulamasına göre toplam fenolik bileşik içeriğini %7,03 oranında arttırırken, su nanesi %41,8 oranında azaltmıştır. Bu deney sonucu da ikili kültürde 5 ppm Pb dozunda su teresinin üstünlüğünü ortaya koymaktadır.

SONUÇ

Aynı habitatta aynı çayda yayılış gösteren canlılar birbirlerinin gelişimini etkileyebilirler. Su teresi ve su nanesi de Umurbey Çayı'nda yanyana gelişen sucul bitkiler oldukları için tercih edilmiştir. Böylece aralarındaki rekabet durumu ortaya konulabilmiştir. Tekli kültürlerde su teresinin iyi bir Pb alıcısı olduğu ve en fazla tepkiyi 5 ppm Pb dozunda verdiği morfolojik gözlemlerimiz ve fizyolojik deneylerimizle tespit edilmiştir. Tekli kültürdeki su nanesi ise su teresine göre daha az Pb (hatta yarısından bile az) biriktirmiştir. Bunun sonucu olarak ta en fazla doz olan 10 mg/L Pb dozuna kadar dayanabilmiştir.

Umurbey Çayı'nda doğal ortamlarında da beraber gelişen bu sucul bitkiler aynı kültür ortamında yetiştirilmiştir. Pb stresinin olmadığı, sucul bitkilerin sadece bir arada yetiştirildiği ikili kontrol gruplarında, su nanesi morfolojik ve fizyolojik olarak üstünlüğünü kanıtlamıştır. Mayıs-2016 döneminde yapılan arazi gözlemlerimizde, Pb elementine rastlanmayan dönemde su nanesi ve su teresi çayda yan yana gelişmekte, ancak su nanesi çay boyunca yayılış gösterirken, su teresi sadece bir bölgede öbek halinde bulunmaktaydı. Bu gözlemde deney sonuçlarımız ile örtüşmekte ve Pb yokluğunda doğal ortamında ve deney ortamında su nanesi üstünlüğünü kanıtlamaktadır. Mayıs-2018 döneminde yapılan arazi çalışmasındaki gözlemlerimizde ise su teresine rastlanmamış, su nanesinin ise çay boyunca çok fazla yayılış gösterdiği gözlemlenmiştir. Umurbey Çayı'nda ise aşırı kirlilik ve su yüzeyinin alglerle kaplandığı görülmüştür. Bu aşırı kirlilik karşısında su

nanesinin bu denli fazla gelişim göstermesi onun Pb elementi yokluğunda ne kadar dayanıklı bir tür olduğunu destekler niteliktedir.

İkili kültürdeki sucul bitkilerimize 5 mg/L dozunda Pb stresi uyguladığımızda, su teresinin daha fazla mücadeleci olduğu morfolojik ve fizyolojik olarak ispatlanmıştır. Aynı ortamı paylaşmaktan doğabilecek olan streslerde su nanesi üstünken, ortama Pb stresi de eklendiğinde su nanesinin üstünlüğünü su teresine kaptırdığı görülmüştür. Doğal ortamda Pb yokluğunda geniş yayılış gösteren su nanesi, ileri de Umurbey Çayı'na olası bir Pb sızmasında su teresinden daha fazla etkileneceği, su teresinin yanında varlığını uzun süre sürdüremeyeceği yorumu yapılabilir. Su teresi ise iyi bir Pb akümülatörü olarak bu stresle daha iyi başedip, hayatta kalmayı başarabilir ve bünyesinde fazlaca biriktirdiği Pb ile çevreci bir sucul bitkisi olarak adlandırılabilir. Ayrıca önemli bir aminoasit olan prolin miktarının da bitki ağır metale maruz kaldığında dört katına kadar arttığı görülmüştür. Bu da bu bitkiden yararlanma adına oldukça önemli bir göstergedir.

Su teresi iyi bir Pb akümülatörü olarak Pb elementine maruz kalabilecek olan akarsu kenarlarında kültüre edilebilir. Böylece herhangi bir Pb sızıntısında Pb'yi fazla miktarda adsorplayarak çevreye yayılmasına engel olabilir. Su nanesi ise kökleriyle çok hızlı yayılmakta ve Umurbey Çayı'nda geniş yayılış göstermektedir. Çok geniş ve hızlı yayılış göstermesi, kirliliğe karşı dayanıklı olması, ileride yayılmacı bir tür olmasına neden olabilir. İki yıl içerisinde su nanesinin çayda aşırı yayılış göstermesi ve su nanelerinin çok büyük boyutlara ulaşmaları bunun kanıtıdır. Bu yüzden bu sucul bitkinin geniş yayılış gösterdiği çaylarda ekologlar çalışmalarını genişletebilir. Bu çalışmada abiyotik faktörlerden Pb ağır metalini, biyotik faktörlerden ise rekabeti ele almıştık. Umurbey Çayı etrafında kullanılan pestisitlerin içerisinde yer alan Pb elementi ve bölgede yer alan Pb madeni açısından Pb stresi altında kalabilme ihtimali yüksek bir çaydır. Aynı çayda yayılış gösteren iki sucul makrofitin Pb ağır metali karşısında birbirleriyle ışık, yer ve besin için rekabet ettiklerinde hangisinin mücadeleyi kazandığını belirlemek, ilerideki çalışmalara önemli katkı sağlayacaktır.

TEŞEKKÜR

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Growth and reproduction properties of endemic *Capoeta kosswigi* and *Barbus ercisianus* in the Deliçay Stream (Van, Turkey)

Deliçay (Van, Türkiye)'da yaşayan endemik *Capoeta kosswigi* ve *Barbus ercisianus*'un büyüme ve üreme özellikleri

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Abstract: In this study, the growth and reproductive characteristics of *Capoeta kosswigi* Karaman, 1969 and *Barbus ercisianus* Karaman, 1971, which are endemic to the Lake Van Basin, in Deliçay (Van) were investigated. A total of 309 *C. kosswigi* and 288 *B. ercisianus* specimens were caught by electrofishing between April and August 2018. The fork length of *C. kosswigi and B. ercisianus* ranged from 3.7-26.1 cm and from 4.3-22.7 cm, and the total weights ranged from 0.6-227.4 g and from 1.2-140.0 g, respectively. The most intensive catching was in *C. kosswigi* population of 5.0-6.9 (27.5%) cm and 0-19.9 (67.0%) g in the groups, and 12.0-13.9 (24.7%) cm and 0-9.9 (23.3%) g groups in *B. ercisianus.* Condition factor was determined as 1.305±0.008 (0.798-1.886) in *C. kosswigi* and 1.158±0.006 (0.864-1.559) in *B. ercisianus.* The length-weight relationships were calculated as $W = 0.01435 \times L^{2.952}$ ($r^2 = 0.996$) for *C. kosswigi* and $W = 0.01276 \times L^{2.959}$ ($r^2 = 0.994$) for *B. ercisianus.* The M:F ratio was calculated as 1:0.15 in *C. kosswigi* and 1:0.64 in *B. ercisianus.* It was established that *C. kosswigi* attained sexual maturity when they reached to 11 cm fork length for males, 18 cm fork length for females. Maturation of *B.ercisianus* individuals occurred at 9 cm fork length in males and 12 cm fork length in females. The spawning in *C. kosswigi* was observed from 1st week of May to 2nd week of July, and in *B. ercisianus* from 1st week of May to 1st week of August. It may be suggested that minimum catching size must be 20 cm fork length for *C. kosswigi* and 15 cm fork length for *B. ercisianus.*

***Keywords*:** *Barbus ercisianus, Capoeta kosswigi*, growth, length–weight relationships, reproduction

Öz: Bu çalışmada Van Gölü Havzası'na endemik olan *Capoeta kosswigi* Karaman, 1969 ve *Barbus ercisianus* Karaman, 1971 türlerinin Deliçay (Van)'da büyüme ve üreme özellikleri araştırılmıştır. Nisan 2018 - Ağustos 2018 tarihleri arasında toplam 309 adet *C. kosswigi* ve 288 adet *B. ercisianus* elektrikle avcılık yolu ile yakalanmıştır. Çatal boylar *C. kosswigi*'de 3,7-26,1 cm, *B. ercisianus*'ta 4,3-22,7 cm; ağırlık ise *C. kosswigi*'de 0,6-227,4 ve *B. ercisianus*'ta 1,2-140,0 g arasında belirlenmiştir. En yoğun avcılık *C. kosswigi* populasyonunda 5.0-6,9 (%27,5) cm ve 0-19,9 (%67,0) g'lık gruplarda, *B. ercisianus*'ta ise 12,0-13,9 (%24,7) cm ve 0-9,9 (%23,3) g'lık gruplarda gerçekleşmiştir. Kondisyon faktörü *C. kosswigi*'de 1,305±0,008 (0,798-1,886), *B. ercisianus*'ta 1,158±0,006 (0,864-1,559) olarak belirlenmiştir. Boy-ağırlık ilişkisi *C. kosswigi*'de $W = 0,01435 \times L^{2.952}$ ($r^2 = 0,996$), *B. ercisianus*'ta ise $W = 0,01276 \times L^{2.959}$ ($r^2 = 0,994$) olarak hesaplanmıştır. Erkek:dişi oranı *C. kosswigi*'de 1:0,15 ile *B. ercisianus*'ta 1:0,64 olarak hesaplanmıştır. *C. kosswigi'nin* cinsel olgunluğa erkeklerde 11 cm, dişilerde ise 18 cm çatal boyda ulaştığı tespit edilmiştir. *B. ercisianus*'ta cinsel olgunluk boyu erkeklerde 9 cm, dişilerde ise 12 cm çatal boyda gerçekleşmiştir. *C. kosswigi*'de üremenin Mayıs ayının 1. haftası ile Temmuz ayının 2. haftası arasında, *B. ercisianus*'ta ise Mayıs ayının 1. haftası ile Ağustos ayının ilk haftası arasında gerçekleştiği belirlenmiştir. Türlerin devamlılığı için avlanma boyunun *C. kosswigi* için 20 cm çatal boy, *B.* ercisianus için ise 15 cm çatal boy altında olmaması gerekmektedir.

***Anahtar kelimeler*:** *Barbus ercisianus, Capoeta kosswigi*, büyüme, boy-ağırlık ilişkisi, üreme

INTRODUCTION

Determination of the population dynamics parameters such as reproduction, growth, mortality, and length weight relationships (LWRs) in monitoring natural fish populations and using them efficiently and sustainably is the main subject of fisheries biology (Haimovici and Velasco, 2000).

LWRs provide a quick effective method for assessing the weight of a particular fish species by estimating the weight from length observations obtained on the field and the equations can be used to estimate fish stock biomass from limited data (Kimmerer et al., 2005; Froese et al., 2011). LWRs can be used to measure changes in the health of a fish population, determine the relative condition of small fish compared to large fish and compare the condition of a fish population (Froose, 2006). Fish can show either isometric or allometric growth (Sakar et al., 2013). Isometric growth (b=3) indicates that both length and weight of the fish are increasing at the same rate. Allometric growth can be either positive or negative. Positive allometric (b > 3) implies that the fish becomes stouter, or heavier or deeper-bodied as its length increases. Negative allometric (b<3) implies the fish becomes slender or lighter as its length increases (Wootton, 1998).

Fulton's Condition factor (K) is an estimation of general well being of fish and a useful index for estimating growth rate and age and for assessing environmental quality (Ricker,

1975). The Fulton condition factor of 1.0 or greater shows the good condition of fish while less than 1.0 indicates poor condition (Abobi, 2015). The condition factor may differ due to one or more factors such as season, sex, type of food organism consumed by fish, age of fish, amount of fat reserved and environmental conditions (Bagenal and Tesch, 1978; Sakar et al., 2013).

Reproduction is an important physiological system that is crucial in the life cycle of fish and has many characters unique to aquatic life (Bagenal and Tesch, 1978). The reproductive success of a species is determined by its genetic capacities depending on ecological conditions. Reproduction is of vital importance in fish, as in other living things, to ensure the continuity of species. Although a fish can grow and develop in a water source or its environment, it is not considered to have adapted to that environment if it does not have reproductive characteristics. Therefore, it is necessary to determine the growth and reproductive biology of the species to develop successful fisheries management. The gonadosomatic index (GSI), is described as gonad mass as a percentage of total body weight. This index is widely used as a simple measure of the extent of reproductive investment, gonadal development and maturity of fish in relation to spawning. GSI of fish increases with maturity and abruptly declines after spawning. Thus, GSI is particularly helpful in identifying season of spawning (Wootton, 1992; Çetinkaya et al., 2005; Karataş et al., 2005).

Capoeta and *Barbus* genera (Familia: Cyprinidae) shows a widespread distribution in Asia and the Middle East, while the *Barbus* genus shows a distribution in Europe. The different species live in many water sources (Geldiay and Balık, 2009; Türkmen et al., 2002).

Located at the east of Turkey, which features a closed basin, the Lake Van Basin is very rich in terms of diversity of fish species. Five of 9 fish species in the basin are endemic (Şen et al., 2018). *C. kosswigi* and *B. ercisianus* are among the endemic fish of the basin (Elp et al., 2016; Elp, 2017).

C. kosswigi and *B. ercisianus* are firstly recorded by Karaman et al, (1969) and (1971) in Erciş, Karasu and Hoşap streams which are flow into the Lake Van. While *C. kosswigi* has a couple of short barbels, *B. ercisianus* has two pairs of barbels. The body of both species are long and covered with a large number of small scales, and reproductive tubercles are observed on the body, especially in the head, during the reproductive period. They prefer sandy and pebbly bottoms and with flowing, clean, cold and high oxygen content water (Geldiay and Balık, 2009).

This study was carried out for the purpose of determination of growth and reproduction properties and evaluate the current situation of *C. kosswigi* and *B. ercisianus* populations in Deliçay Stream.

MATERIAL AND METHODS

Study area

The present study was carried out in Deliçay Stream, which flows into the Lake Van. Deliçay Stream is formed by the rain and small waters coming from the Morgedik Dam and the hills around it and the melted snow waters. It has approximately 45-50 km length and an average annual flow rate of 2.8 m^3 (Çetinkaya, 1993). There is also a regulator on the river (Figure 1).

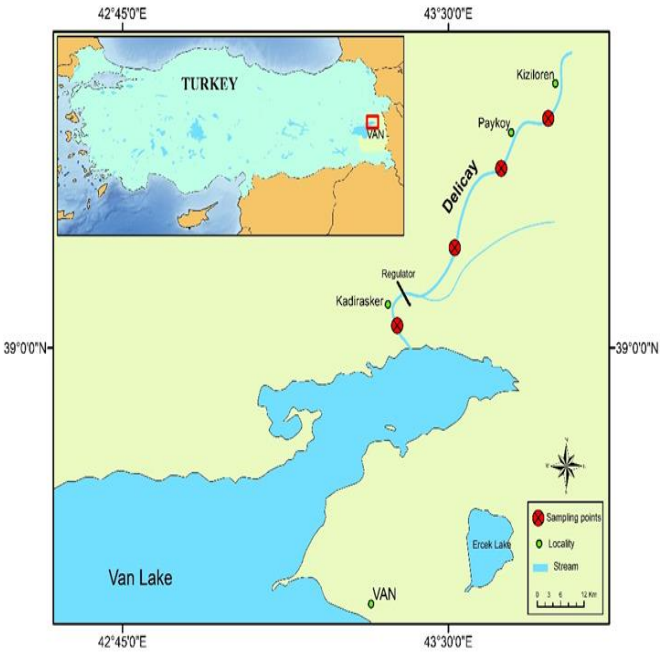

Figure 1. Sampling sites at Deliçay Stream

Fish collection

An electrofishing method (SAMUS 725 MS) was used to catch fish samples with the permission of the Van Yüzüncü Yıl University (Turkey) Animal Researches Local Ethic Committee. During the study, a total of 309 *C. kosswigi* and 288 *B. ercisianus* were monthly collected from April 2018 to August 2018 at four stations: Kadirasker, Deliçay, Payköy and Kızılören (Figure 1).

Laboratory procedures

Fish samples were measured to the nearest 0.1 cm for fork length (FL) and to the nearest 0.1 g for total weight (W). Total length-weight relationships were determined using the equation: $W = a \times L^b$, where W is weight (W), L is length (L), a is the intercept, and b is the slope (Le Cren, 1951). Fulton's Condition Factor was calculated using $K= (W / L^3) \times 100$, where W= weight of fish (g), L= total length of fish (cm) (Ricker, 1975).

The gonads were removed and observed morphologically. Sex was recorded for each fish. The sex of the fish was categorized as male, female, or juvenile. The first

reproduction length and weight of the fish were determined according to the maturity status of the gonads (Crim and Glebe, 1990). Gonadosomatic Index value was calculated using the formula GSI= (Gw / W) × 100, where Gw= gonad weight of fish (g), W= total weight of fish (g) (Karataş et al., 2005).

Statistical analysis

The growth types for the specimens were determined using the Student t-test. The t-test statistics values were calculated and compared with critical values from the T-Table to check if the growth type is isometric (b =3) or allometric (b ≠ 3) (Pajuelo and Lorenzo, 1998). The sex ratio was tested by the chi-square test (χ^2) to indicate whether there was a deviation from a 1:1 ratio (Zar, 1999). The means, variance, standard error, regression, correlation values, and the comparisons of population parameters obtained from the study were performed with SPSS 21.0 and Microsoft Excel 2016.

RESULTS

Growth in length

Fork length values of 309 *C. kosswigi* specimens in the population were ranged from 3.7 to 26.1 cm (n = 309) for all population, 4.8 to 21.8 cm (n = 113) for males, 18.0 to 26.1 cm (n = 17) for females, and 3.7 to 11.4 cm (n =179) for juveniles. The lengths were grouped into 2 cm and the length frequency distributions were examined. It was determined that the most dominant groups were found as 5.0-6.9 cm (27.5%) for all samples, 13.0-14.9 cm (9.7%) for males, 19.0-20.9 cm (1.9%) for females, and 5.0-6.9 cm (26.9%) for juveniles (Table 1).

Table 1. Length groups of *Capoeta kosswigi* in the Deliçay Stream

Fork length groups (cm)	Male		Famale		Juvenil		Total	
	n	%n	n	%n	n	%n	n	%n
3.0-4.9	2	0.6	0	0.0	64	20.7	67	21.7
5.0-6.9	2	0.6	0	0.0	83	26.9	85	27.5
7.0-8.9	7	2.3	0	0.0	27	8.7	34	11.0
9.0-10.9	9	2.9	0	0.0	4	1.3	13	4.2
11.0-12.9	17	5.5	0	0.0	1	0.3	18	5.8
13.0-14.9	30	9.7	0	0.0	0	0.0	30	9.7
15.0-16.9	23	7.4	0	0.0	0	0.0	23	7.4
17.0-18.9	15	4.9	2	0.6	0	0.0	17	5.5
19.0-20.9	6	1.9	6	1.9	0	0.0	12	3.9
21.0-22.9	2	0.6	5	1.6	0	0.0	7	2.3
23.0≤	0	0.0	4	1.3	0	0.0	4	1.3
Total	**113**	**36.6**	**17**	**5.5**	**179**	**57.9**	**309**	**100.0**

The fork lengths for *B. ercisianus* were varied from 4.3-22.7 cm (n = 288) for all samples, 8.0-15.9 cm (n =145) for males, 12.4-22.7 cm (n = 92) for females and 4.3-7.8 cm (n = 51) for juveniles. According to 2 cm length groups, it was observed that the largest length groups were found as 12.0-13.9 cm-group (24.7%) for all samples, 12.0-13.9 cm-group (23.0%) for males, 16.0-17.9 cm-group (12.5%) for females, and 6.0-7.9 cm-group (9.4%) for juveniles (Table 2).

Table 2. Length groups of *Barbus ercisianus* in the Deliçay Stream

Fork length groups (cm)	Male		Famale		Juvenil		Total	
	n	%n	n	%n	n	%n	n	%n
4.0-5.9	0	0.0	0	0.0	24	8.3	24	8.3
6.0-7.9	0	0.0	0	0.0	27	9.4	27	9.4
8.0-9.9	23	8.0	0	0.0	0	0.0	23	8.0
10.0-11.9	40	13.9	0	0.0	0	0.0	40	13.9
12.0-13.9	66	23.0	5	1.7	0	0.0	71	24.7
14.0-15.9	16	5.5	8	2.8	0	0.0	24	8.3
16.0-17.9	0	0.0	36	12.5	0	0.0	36	12.5
18.0-19.9	0	0.0	34	11.8	0	0.0	34	11.8
20.0≤	0	0.0	9	3.1	0	0.0	9	3.1
Total	**145**	**50.3**	**92**	**31.9**	**51**	**17.7**	**288**	**100.0**

Growth in weight

The ranges of total weight in *C. kosswigi* population were found between 0.6-227.4 g (n = 309) for all the samples, 1.2-134.2 g (n = 113) for males, 78.5-227.4 g (n = 17) for females, and 0.6-17.6 g (n = 179) for juveniles.

The weights were grouped into 20 g sample groups and the weight-frequencies were investigated. It was determined that the dominant weight groups were found as 0.0-19.9 g (67.0%) for all samples, 20.0-39.9 g (10.4%) for males, 100.0-119.9 g (1.9%) for females, and 0.0-19.9 g (57.9%) for juveniles (Table 3).

The total weight values of *B. ercisianus* were ranged from 1.2 to 140.0 g (n = 288) for all samples, 5.6 to 44.6 g (n = 145) for males, 23.9 to 140.0 g (n = 92) for females, and 1.2 to 5.6 g (n = 51) for juveniles. According to 10 g weight groups, it was determined that the largest weight groups were found as 0-9.9 g (23.3%) for all samples, 20.0-29.9 g (19.8%) for males, 70.0-79.9 g (6.6%) for females, and 0-9.9 g (17.7%) for juveniles (Table 4).

Table 3. Weight groups of *Capoeta kosswigi* in the Deliçay Stream

Weight groups (g)	Male		Famale		Juvenile		Total	
	n	%n	n	%n	n	%n	n	%n
0-19.9	28	9.1	0	0.0	179	57.9	207	67.0
20.0-39.9	32	10.4	0	0.0	0	0.00	32	10.4
40.0-59.9	28	9.1	0	0.0	0	0.00	28	9.1
60.0-79.9	17	5.5	2	0.6	0	0.00	19	6.1
80.0-99.9	4	1.3	1	0.3	0	0.00	5	1.6
100.0-119.9	3	1.0	6	1.9	0	0.00	9	2.9
120.0-139.9	1	0.3	3	1.0	0	0.00	4	1.3
140.0≤	0	0.0	5	1.6	0	0.00	5	1.6
Total	113	36.6	17	5.5	179	57.9	309	100.0

Table 4. Weight groups of *Barbus ercisianus* in the Deliçay Stream

Weight groups (g)	Male		Famale		Juvenile		Total	
	n	%n	n	%n	n	%n	n	%n
0-9.9	16	5.6	0	0.0	51	17.7	67	23.3
10.0-19.9	52	18.1	0	0.0	0	0.0	52	18.1
20.0-29.9	57	19.8	5	1.7	0	0.0	62	21.5
30.0-39.9	15	5.2	4	1.4	0	0.0	19	6.6
40.0-49.9	5	1.7	9	3.1	0	0.0	14	4.9
50.0-59.9	0	0.0	18	6.3	0	0.0	18	6.3
60.0-69.9	0	0.0	18	6.3	0	0.0	18	6.3
70.0-79.9	0	0.0	19	6.6	0	0.0	19	6.6
80.0-89.9	0	0.0	9	3.1	0	0.0	9	3.1
90.0-99.9	0	0.0	5	1.7	0	0.0	5	1.7
100≤	0	0.0	5	1.7	0	0.0	5	1.7
Total	**145**	**50.3**	**92**	**31.9**	**51**	**17.7**	**288**	**100.0**

Length-weight relationships

Length-weight relationships for both species are shown in Table 5. Strong relationships were found between length and weight for these species in the study (r^2 = 0.948 to 0.996).

The length-weight relationships of *C. kosswigi* were calculated as $W = 0.01435 \times L^{2.952}$ (r^2 = 0.996) for all samples, $W = 0.01854 \times L^{2.858}$ (r^2 = 0.987) for males, $W = 0.02079 \times L^{2.843}$ (r^2 = 0.952) for females, and $W = 0.01574 \times L^{2.895}$ (r^2 = 0.980) for juvenile. The isometric growth pattern was observed for all the samples, females and juveniles ($p>0.05$), whereas the growth pattern for males was negative allometric ($p<0.05$) (Table 5).

The length-weight relationship equations for *B. ercisianus* were calculated as $W = 0.01276 \times L^{2.959}$ (r^2 = 0.994) for all samples, $W = 0.01476 \times L^{2.901}$ (r^2 = 0.962) for males, $W = 0.01949 \times L^{2.813}$ (r^2 = 0.948) for females, and $W = 0.01343 \times L^{2.927}$ (r^2 = 0.960) for juveniles. It was determined that the growth pattern for *B. ercisianus* for all groups was found as isometric ($p>0.05$) (Table 5).

Table 5. The descriptive statistics and estimated parameters of length-weight relationships of *C. kosswigi* and *B. ercisianus* populations in the Deliçay Stream

Species	Sex	n	Regression parameters				Student's t-test	p	Growth type
			a	b	SE_b	r^2			
C. kosswigi	Male	113	0.01854	2.858	0.031	0.987	-2.190	0.044*	A (-)
	Female	17	0.02079	2.843	0.166	0.952	-0.422	0.746	I
	Juvenil	179	0.01574	2.895	0.031	0.980	-0.493	0.656	I
	Total	309	0.01435	2.952	0.011	0.996	-0.380	0.723	I
B. ercisianus	Male	145	0.01476	2.901	0.048	0.962	-0.762	0.489	I
	Female	92	0.01949	2.813	0.069	0.948	-1.905	0.129	I
	Juvenil	51	0.01343	2.927	0.085	0.960	-0.771	0.521	I
	Total	288	0.01276	2.959	0.013	0.994	-1.958	0.122	I

n = number of individuals, a = proportionality constant, b = slope of the relationship; SE_b, standard error of b; r^2 = coefficient of determination; I = isometric growth; A = allometric growth.
* Statistically different ($p<0.05$).

Fulton's condition factor

Fulton's condition factor, K, was used to assess the degree of well-being of *C. kosswigi* and *B. ercisianus* in the Deliçay Stream which provides information on the environmental quality and suitability.

Fulton's condition factor of *C. kosswigi* was calculated as 1.305±0.008 (0.798-1.886) for all samples, 1.287±0.013 (0.798-1.870) for males, 1.288±0.022 (1.154-1.470) for females, and 1.319±0.010 (0.911-1.886) for juveniles. The mean lowest condition value was determined in August (1.272±0.012), whereas the highest in May (1.364±0.074) (Figure 2).

Fulton's condition factor for *B. ercisianus* was determined as 1.158±0.006 (0.864-1.559) for overall, 1.159±0.008 (0.884-1.491) for males, 1.143±0.010 (0.890-1.559) for females, and 1.180±0.016 (0.864-1.509) for juveniles. The mean condition factor ranged from 1.125±0.010 in July to 1.236±0.042 in May (Figure 2).

Reproduction

In the population of *C. kosswigi*, 113 (86.9%) of the specimens collected were male and 17 (13.1%) were female. Male:female ratio was calculated as 1:0.15. The chi-square analysis showed that the sex ratio was significantly different from the expected ratio (χ^2 test, $p<0.05$) (Table 6).

Sex was determined on 237 specimens for *B. ercisianus*. 145 (61.2%) samples were male and 92 (38.8%) samples were female. The M:F ratio was determined as 1:0.64. The sex ratio was found to be significantly different from equality 1:1 (χ^2 test, $p<0.05$) (Table 6).

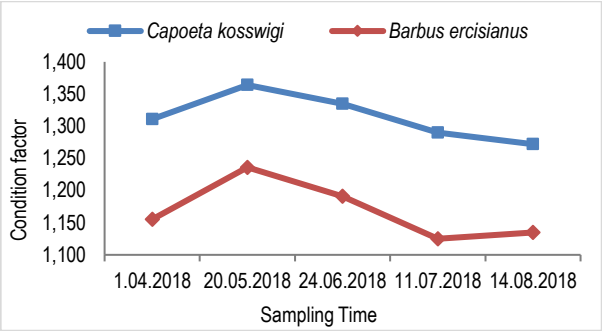

Figure 2. Fulton's condition factor changes for *Capoeta kosswigi* and *Barbus ercisianus* in the Deliçay Stream

Table 6. M:F ratio of the *Capoeta kosswigi* and *Barbus ercisianus* populations in the Deliçay Stream

Species	Male		Female		M+F	M:F	χ^2	Result
	n	%n	n	%n				
C. kosswigi	113	86.9	17	13.1	130	1:0.15	70.89	p<0.05
B. ercisianus	145	61.2	92	38.8	237	1:0.64	12.30	p<0.05

The sexual maturity for *C. kosswigi* was determined on 130 samples. Total 116 of the overall samples was mature and 14 was immature. Of the 113 males, 99 were identified as mature, while all the females were found as mature. When the sexual maturation was examined according to 1 cm length groups, it was determined that maturation occurred at 11 cm (100%) FL for males and 18 cm (100.0%) FL for females (Table 7).

The sexual maturity for *B. ercisianus* was determined on 237 samples. Total 216 of the overall samples was mature and 21 was immature. Of the 145 males, 124 were identified as mature, while all the females were found as mature.

According to 1 cm length groups, males matured at 9 cm (85.7%) fork length and females at 12 cm (100.0%) fork length (Table 8).

Table 7. Sexual maturity groups of *Capoeta kosswigi* in the Deliçay Stream

Fork length groups (cm)	Male		Female	
	Mature (+) (%n)	Mature (-) n (%n)	Mature (+) n (%n)	Mature (-) n (%n)
≤10	6 (30.0)	14 (70.0)	0	0
11	11 (100.0)	0	0	0
12	6 (100.0)	0	0	0
13	9 (100.0)	0	0	0
14	21 (100.0)	0	0	0
15	15 (100.0)	0	0	0
16	8 (100.0)	0	0	0
17	7 (100.0)	0	0	0
18	8 (100.0)	0	2 (100.0)	0
19	4 (100.0)	0	2 (100.0)	0
20	2 (100.0)	0	4 (100.0)	0
21	2 (100.0)	0	2 (100.0)	0
22≤	0	0	7 (100.0)	0
Total	99	14	17	0

Table 8. Sexual maturity groups of *Barbus ercisianus* in the Deliçay Stream

Fork length groups (cm)	Male		Female	
	Mature (+) (%n)	Mature (-) n (%n)	Mature (+) n (%n)	Mature (-) n (%n)
≤8.9	3 (33.3)	6 (66.7)	0	0
9	12 (85.7)	2 (14.3)	0	0
10	13 (86.7)	2 (13.3)	0	0
11	21 (84.0)	4 (16.0)	0	0
12	37 (84.1)	7 (15.9)	2 (100.0)	0
13	22 (100.0)	0	3 (100.0)	0
14	10 (100.0)	0	4 (100.0)	0
15	6 (100.0)	0	4 (100.0)	0
16	0	0	14 (100.0)	0
17	0	0	22 (100.0)	0
18	0	0	19 (100.0)	0
19	0	0	15 (100.0)	0
20	0	0	5 (100.0)	0
21	0	0	3 (100.0)	0
22≤	0	0	1 (100.0)	0
Total	**124**	**21**	**92**	**0**

The GSI values were calculated to determine the reproduction for both populations. The total GSI for *C. kosswigi* was calculated between 0.389 and 16.000 with an average of 2.392±0.243. This value was determined as 2.059±0.252 (0.389-16.000) for males and 3.880±0.605 (1.259-9.776) for females. The mean GSI for all samples peaked in May (5.621±2.930), whereas the lowest in July (1.227±0.162) (Figure 3). The total GSI value for *B. ercisianus* ranged from 0.282 to 16.924 with an average of 4.184±0.212. This value was calculated as 4.032±0.265 (0.282-15.441) for males and 4.388±0.346 (0.540-16.924) for females. The highest GSI for all samples was in May (6.877±1.084), while the lowest in August (1.429±0.091) (Figure 3).

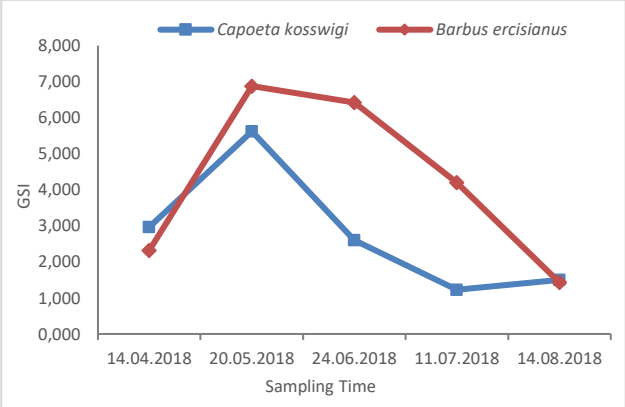

Figure 3. GSI changes for *Capoeta kosswigi* and *Barbus ercisianus* in the Deliçay Stream

DISCUSSION

In this study, the sample size ranged from 309 specimens for *C. kosswigi* to 288 specimens for *B. ercisianus*. The lenght and weight ranges for males was lower than for females in both species.

The maximum length and weight values for *C. kosswigi* were smaller than the previous studies in Lake Van Basin (Table 9). The b value is considered as an indicator showing the nutritional status and growth of the fish. It is affected by the water temperature, the abundance of nutrients in the environment, and other properties. In practice, values of b smaller, equal and larger than 3 indicate isometry, negative allometry and positive allometry respectively (Wootton, 1998). The growth types of *C. kosswigi* for all the samples, females, and juveniles were isometric growth ($p>0.05$), while the growth type for males was negative allometric growth ($p<0.05$). On the other hand, the b values for *C. kosswigi* in our study were within the values (2.5-3.5) reported by Froese (2006). Population characteristics of *C. kosswigi* presented different studies are given in Table 9.

Fulton's condition factor value is related to the body shape of the fish. It is used as an indicator of long-term changing nutritional balance in comparison of fish populations of the same species living under similar or different conditions such as condition factor, nutrient density, climatic conditions, determination of gonad maturity time and duration and nutritional activity (Bogler and Connolly, 1989; Yılmaz et al., 2003; Çetinkaya et al., 2005). Wootton (1992) reported that fish with higher K values (> 1) are in a better condition than fish with lower K values (< 1). In this study, K values ranged from 0.798 to 1.886 with a mean value of 1.305 (Table 9) which indicates that fish were in good condition during the study period in the aquatic ecosystem. Fulton's condition factor values of the *Capoeta* species are shown in Table 9. In the previous studies conducted in our study, the condition factor ranged between 1.234 (Nazik Lake) and 1.452 (Karasu River).

The sex ratio was close to 1:1 in many species, and it sometimes can vary from species to species, even from different sources of the same species in different years or different populations (Nikolsky, 1963). In the present study, the overall M:F ratio of the population was 1:0.15, for all of the investigated samples in *C. kosswigi.* The chi-square analysis showed that the sex ratio was significantly different from the expected 1:1 ratio (χ^2= 70.89, $p<0.05$). In this study, the males were found to be dominant. Due to the large size of females, prey pressure is thought to be more intense on females. It was reported in Table 9 that the males were dominant in Nazik Lake (1:0.77), Karasu River (1:0.85) and Çığlı Stream (1:0.77), whereas females were dominant in Koçköprü (1:1.43) and Zernek Dam Lakes (1:1.72).

Sexual maturation for *C. kosswigi* was occured at 11 cm FL for males and 18 cm FL for females in this study. In Lake Van Basin, females reach sexual maturity for *C. kosswigi* at a larger size than males. These sexual maturity sizes were observed at 15 cm for males and 22 cm for females from Nazik Lake (Şen et al., 1999), 23 cm for males and 33 cm for females from Koçköprü Dam Lake (Elp and Karabatak, 2007), 14 cm for males and 22 cm for females from Zernek Dam Lake (Şen et al., 2008), 11 cm for males and 23 cm for females from Karasu River (Elp and Şen, 2009), 10 cm for males and 18 cm for females from Çığlı Stream (Şen et al., 2014). In addition, it was determined that the *C. kosswigi* population in Deliçay Stream had reached sexual maturity size before the population of *C. kosswigi* from Nazik Lake, Zernek Dam Lake and Koçköprü Dam Lake. The water temperature and nutrition may be effective in this result.

Reproduction in fish is a very complex vital activity, and reproduction has vital importance to ensure species survival in fish as in other living things. (Karataş et al., 2005). The mean GSI value for *C. kosswigi* piked in May (5.621±2.930) and then declined. The lowest GSI value was determined in July (1.227±0.162). Spawning season of *C. kosswigi* took place intensively from 1st week of May to 2nd week of July in Deliçay Stream. Spawning season of *C. kosswigi* was reported in Lake Van Basin between May and July from Nazik Lake (Şen et al., 1999), May and June from Koçköprü Dam Lake (Elp and Karabatak, 2007), June and July from Zernek Dam Lake (Şen et al., 2008) and May and July from Çığlı Stream (Şen et al., 2014). It has been observed that *C. kosswigi* population reproduced at similar periods from various regions in Lake Van Basin

Table 9. Population characteristics of *Capoeta kosswigi* genus presented different studies in Lake Van Basin

Locality	Species*	N	M	F	J	M:F	FL (min-max)	W (min-max)	a	b	r^2	Mean K (min-max)	GSI (min-max)	Reference
Deliçay Stream	*C. kosswigi*	309	113	17	179	1:0.15	3.7-26.1	0.6-227.4	0.01435	2.952	0.996	1.305 (0.798-1.886)	0.39-16.00	The present study
Nazik Lake	*C. capoeta*	603	331	254	18	1:0.77	1.9-48.3	0.1-1379.5	0.01349	2.960	0.993	1.234 (0.494-1.514)	1.16-8.65	Şen et al. (1999)
Koçköprü Dam Lake	*C. capoeta*	1234	507	727	-	1:1.43	3.2-39.8	0.4-755.6	0.01262	2.999	0.998	1.275 (1.078-1.434)	1.05-10.10	Elp and Karabatak (2007)
Zernek Dam Lake	*C. capoeta*	586	158	272	156	1:1.72	4-41	0.7-1060.4	0.0137	2.992	0.990	1.314 (0.997-1.793)	0.23-7.85	Şen et al. (2008)
Karasu Stream	*C. capoeta*	427	216	183	28	1:0.85	4-37.5	0.7-676.2	0.0129	3.039	0.989	1.452 (1.069-2.135)	-	Elp and Şen (2009)
Çığlı Stream	*C. capoeta*	535	254	196	85	1:0.77	3.8-33.2	0.8-432.3	0.0170	2.887	0.985	1.280 (0.665-1.866)	1.33-11.33	Şen et al. (2014)

*In binomial nomenclature, *C. capoeta* was used as the synonym of *C. kosswigi*.

The maximum size for *B. ercisianus* was found as 22.7 cm and 140.0 g. These values are very higher than Çığlı Stream (Table 10). Bilici et al. (2017) explained the size differences in the populations with the selectivity of the sampling nets used, fishing pressure and moreover, ecological differences between lakes and streams. In Deliçay Stream, weight increased isometric with size since the values of b had not a significant difference from the value 3.0 ($p>0.05$). The regression equation for length-weight relationship of *B. ercisianus* shows that the species exhibited an isometric growth pattern. This indicates that there was dimensional proportionality (in body weight and total length) at the same rate. Froese (2006) reported that if b = 3, then small number of specimens in the fish sample have the same form and condition as large specimens. The b value in our study is similar to the value reported for Koçköprü Dam Lake and Çığlı Stream (Table 10). According to their results, the growth type of population was isometric in Çığlı Stream.

The mean Fulton's condition factor values for *B. ercisianus* in present study were lower than Koçköprü Dam Lake and Çığlı Stream (Table 10). In present study, the highest value was reached in May, being higher in the feding months and just prior to spawning. These results were similar with Koçköprü Dam Lake and Çığlı Stream.

The sex ratio of male to female for *B. ercisianus* was 1:0.64 and the difference was statistically significant (χ^2= 12.30, $p<0.05$). In other studies, the male:female ratio has been presented in Table 10. The similar studies showed that the males were dominant. Generally, it is reported that the ability of males to hatch is higher than females in freshwater, but the proportion of males gradually decreases in the upper age classes and the proportion of females becomes quiet dominant in a population (Yıldırım et al., 2001).

The first sexual maturity size for *B. ercisianus* individuals in Deliçay was observed at 9 cm in males, and 12 cm in females. It was reported as 7.0-7.9 cm for males and 15.0-15.9 cm for females from Koçköprü Dam Lake (Elp et al., 2006) and 6.0-6.9 cm for males and 10.0-10.9 cm for females from Çığlı Stream (Şen and Kara, 2016). Our results were close to the values reported for Koçköprü Dam Lake and Çığlı Stream. Moreover, males may grow slower than the females or males may mature earlier than the females in Lake Van Basin.

The GSI peak for *B. ercisianus* was observed on May (6.877±1.084), whereas the lowest GSI was in August (1.429±0.091). Spawning took place intensively from 1st week of May to 1st week of August. Şen and Kara (2016) determined that spawning period for *B. ercisianus* was happened between May and August in Çığlı Stream. The results of the present study are similar to the results given for *B. ercisianus* population in Çığlı Stream.

In conclusion, from these measurements and calculations, it can be argued that growth and condition in the population had suitable values. It was established that *C. kosswigi* attained sexual maturity when they reached to 11 cm (100%) for males and 18 cm (100.0%) for females. The individuals maturation for *B. ercisianus* occurred at 9 cm (85.7%) for males and 12 cm (100.0%) for females. The spawning period for *C. kosswigi* was observed from 1st week of May to 2nd week of July, and for *B. ercisianus* from 1st week of May to 1st week of August. It is expected that the results of the presently reported study will contribute to the sustainable fishery for *C. kosswigi* and *B. ercisianus* in the Deliçay Stream. It may be suggested that fishing should be forbidden between April and August and minimum catching size must be 20 cm fork length for *C. kosswigi* and 15 cm fork length for *B. ercisianus*.

Table 10. Population characteristics of *Barbus ercisianus* presented different studies

Locality	Species*	N	M	F	J	M:F	FL (min-max)	W (min-max)	a	b	r^2	Mean K (min-max)	GSI	Reference
Deliçay Stream	*B. ercisianus*	288	145	92	51	1:0.64	4.3-22.7	1.2-140.0	0.01276	2.959	0.994	1.158 (0.864-1.559)	4.184	The present study
Koçköprü Dam Lake	*B. ercisianus*	204	85	68	51	1:0.80	3-33.8	0.1-428	0.014	2.934	0.992	1.242 (0.790-2.226)	-	Elp et al. (2006)
Çığlı Stream	*B. plebejus*	196	119	76	1	1:0.64	4.3-16.6	1.2-65.8	0.0146	2.934	0.976	1.260 (0.954-1.632)	1.71-10.03	Şen and Kara (2016)

*In binomial nomenclature, *B. plebejus* was used as the synonym of *B. ercisianus*.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Türkiye'deki barbungiller balıkçılığının TÜİK mikro-veri setine dayalı olarak değerlendirilmesi

Assessment of goatfish fisheries in Turkey based on the microdata set of official landing statistics

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Öz: Barbungiller (Mullidae Rafinesque, 1815), Türkiye balıkçılığının en önemli türleri arasında yer almaktadır. Buna karşın, barbungil balıkçılığının genel karakteristik özellikleri ve iller bazında değişimi üzerine geniş ölçekli bir değerlendirme ile karşılaşılmamıştır. Bu çalışma kapsamında Türkiye İstatistik Kurumu (TÜİK) tarafından 2014-2017 yılları için derlenen mikro-veri seti kullanılarak ticari balıkçılık filomuz tarafından gerçekleştirilen barbun (*Mullus barbatus* Linnaeus, 1758) ve tekir (*Mullus surmuletus* Linnaeus, 1758) avının şehirlere göre değişimi incelenmiş, küçük (tekne <10m) ve büyük ölçekli (tekne >10m) balıkçılar ile farklı balıkçılık yöntemlerinin toplam ava katkısı karşılaştırılmış ve rapor edilen ıskarta oranları değerlendirilmiştir. Çalışma sonucunda Doğu Akdeniz'de barbun, Batı Karadeniz'de ise tekir balığı avının daha fazla olduğu görülmüştür. Diğer taraftan, literatürde balıkçılıktan bağımsız olarak yapılan araştırmaların sonuçları incelendiğinde Türk balıkçılık filosunun esas olarak barbun avladığı görülmüştür. Dolayısıyla, TÜİK istatistiklerindeki barbun ve tekir kayıtlarının farklı türlerden ziyade yerel isimlendirme farklılıklarını yansıttığına dair güçlü bir şüphe oluşmuştur. Akdeniz, Ege ve Karadeniz'de barbun avının çoğu büyük ölçekli balıkçılar tarafından karşılanırken, Marmara'da küçük ölçekli balıkçıların toplam ava katkısı daha yüksektir. Toplam avın Karadeniz'de %88'i, Ege'de %92'si ve Akdeniz'de %87'si dip trolü avcılığından karşılanmaktadır. Dip trolü avcılığının yasak olduğu Marmara Denizi'nde ise, toplam barbun avının %40'ı gırgır balıkçıları tarafından rapor edilmiştir. Mikro-veri setinde rapor edilen ıskarta oranının türler, denizler ve av araçları arasındaki değişimi istatistik açıdan önemli bulunmamış olup, barbun ve tekirin toplamı için %0,47 olarak hesaplanmıştır.

Anahtar kelimeler: Av istatistikleri, balıkçılığın karakterizasyonu, Mullidae*, Mullus barbatus, Mullus surmuletus,* barbun, tekir balığı

Abstract: Although goatfishes (Mullidae Rafinesque, 1815) are among the most important commercial fishes in Turkey, no research has been found investigating the characteristics and spatial patterns of goatfish fishery. Here, we assessed the goatfish fishery of Turkey based on the microdata set of Turkish National Fishery Statistics gathered by Turkish Statistical Institute (TÜİK) between 2014 and 2017. In this context, we investigated the variation of total goatfish catch by cities. In addition, we compared the contribution of small (boat <10m) and large scale (boat >10m) fishers as well as different fishing techniques to the total goatfish catch in Turkey. Finally, an evaluation was made on the discard rates recorded in the official landing statistics. The results showed that higher red mullet (*Mullus barbatus* Linnaeus, 1758) catch was reported in the eastern Mediterranean, whereas surmullet (*Mullus surmuletus* Linnaeus, 1758) catch was significantly higher in the western Black Sea. On the other hand, fishery-independent investigations revealed that the Turkish fishery fleet mostly catches red mullet throughout the coasts of Turkey. Therefore, the separate records of red mullet and surmullet in the landing statistics likely represent the local names of red mullet rather than two different species. Large scale fishers were the main source of fishery pressure in the Mediterranean Sea, the Aegean Sea and the Black Sea. However, the majority of the catch was landed by small scale fishers in the Marmara Sea. The bottom trawl fishery landed 88, 92 and 87% of total goatfish catch in the Black Sea, the Aegean Sea and the Mediterranean Sea, respectively. Purse seiners provided the largest part of total goatfish catch (40%) in the Marmara Sea, where the bottom trawl fishery is prohibited. The overall average for the discard rate was found to be 0.47%. There were no statistically significant differences among the discard rates of two species, marine regions or fishing methods.

Keywords: Fishery characterization, landing statistics, Mullidae*, Mullus barbatus, Mullus surmuletus,* red mullet, surmullet

GİRİŞ

Ülkemiz denizlerinde şimdiye kadar beş barbungil (Mullidae Rafinesque, 1815) türü kayıt edilmiş olup, bunlardan barbun (*Mullus barbatus* Linnaeus, 1758) ve tekir balığı (*Mullus surmuletus Linnaeus, 1758*) tüm denizlerimizde, Nil barbunu (*Upeneus moluccensis* (Bleeker, 1855)) ve benekli barbun (*Upeneus pori* Ben-Tuvia & Golani, 1989) Akdeniz ile Ege'de, Kızıldeniz barbunu ise (*Parupeneus forsskali* (Fourmanoir & Guézé, 1976)) yalnızca Akdeniz'de yayılım göstermektedir (Bilecenoğlu vd., 2014). Barbun ve tekir balığı Atlantik-Akdeniz kökenli olup; bölgemizin yerli türleri arasında yer almaktadır. Diğer üç tür ise Lesepsiyen olup; Hint-Pasifik kökenlidir ve Akdeniz'e Süveyş Kanalı yoluyla girmişlerdir (Golani vd., 2006; Bariche vd., 2013).

Türkiye kıyılarında dağılan tüm barbungil türleri ekonomik açıdan önemli olmakla birlikte (Froese ve Pauly, 2020), yerli türlerin ticari değerinin daha yüksek olduğu bilinmektedir (Mavruk ve Avşar, 2007). Resmi balıkçılık istatistiklerinde barbun, tekir ve Nil barbunu olmak üzere üç barbungil türünün av miktarları rapor edilmekte olup; toplam avın neredeyse tamamı barbun ve tekir balığına aittir. Bu istatistiklere göre,

Türkiye'de 2017 yılı itibariyle barbungil avcılığından elde edilen mali değer 80 milyon Türk Lirası'nın üzerindedir. Ülkemizde gerçekleştirilen barbungil avcılığının önemli bir kısmı Akdeniz, Ege ve Batı Karadeniz'den gelmekte olup (TÜİK, 2019); bu bölgelerde hangi illerin bu avcılıkta önemli rol üstlendiği henüz araştırılmamıştır. Ayrıca, Türkiye'de barbungil balıklarının avcılığının dip trolü başta olmak üzere sade ve fanyalı uzatma ağlarıyla yapıldığı bilinse de (Hoşsucu, 2000); hangi av araçlarının toplam ava hangi ölçüde katkı sağladığına dair bir değerlendirme ile henüz karşılaşılmamıştır.

Türkiye'de barbungil balıkçılığı önemli bir mali değer oluştursa da bu balıkçılığın özellikleri hakkında bilinenler sınırlıdır. Bu doğrultuda, tüm Türkiye kıyılarını içeren detaylı araştırmalara gereksinim duyulmasına karşın; böylesi araştırmalar lojistik ve ekonomik açıdan ciddi maliyetler getirmektedir. Diğer taraftan ulusal balıkçılık istatistikleri bu eksikliğin giderilmesinde önemli bir potansiyele sahiptir. Bilinen birçok problemine rağmen (Ulman vd., 2013; Pauly vd., 2014), ulusal balıkçılık istatistiklerinin avdaki alan ve zamansal değişimleri başarıyla yansıttığına dair önemli bulgular mevcuttur (Mavruk 2020). Bu çalışmada TÜİK tarafından derlenen mikro-veriyi kullanmak suretiyle Türkiye genelindeki barbungil avının illere ve av araçlarına göre dağılımı değerlendirilerek, barbungil balıkçılığının temel karakteristik özellikleri ortaya konmuştur.

MATERYAL VE YÖNTEM

Türkiye'de deniz balıkları avcılığı istatistikleri 1967 yılından bu yana TÜİK tarafından derlenmektedir. Bu kapsamda veri, 5m'den büyük tekneye sahip profesyonel balıkçılarla yapılan anketler yoluyla, TÜİK ile T.C. Tarım ve Orman Bakanlığı'nda görevli personel tarafından toplanmaktadır. Anketler 2014 yılından bu yana 10m'den küçük balıkçılardan Tabakalı Sistematik Örnekleme Metoduna göre seçilen örneğe, yılda iki kez olmak üzere sezonluk olarak uygulanmakta iken; 10m'den büyük balıkçıların tamamına aylık olarak uygulanmaktadır. Anketlerde türler bazında bir önceki ay ya da sezonun toplam av değerleri sorulmakta, ayrıca gemi özelliklerine ilişkin, motor gücü, kullanılan av aracının türü gibi bilgiler de toplanmaktadır (TÜİK, 2019). Anketler kapsamında toplanan av verisi türler bazında gruplandırılarak 5 bölge halinde (Akdeniz, Ege, Marmara, Batı Karadeniz ve Doğu Karadeniz) Biruni Veri Tabanı (https://biruni.tuik.gov.tr) üzerinden erişime açılmaktadır. Ham veriye "Türkiye İstatistik Kurumu Mikro Veriye Erişim ve Kullanımı Hakkında Yönerge" kapsamında imzalanan protokol ile TÜİK bölge müdürlüklerinde yer alan Veri Araştırma Merkezleri aracılığıyla erişilebilmektedir.

Barbun ve tekir balığı avının iller ve av araçlarına göre değişimini incelemek amacıyla, 2014-2017 yıllarının Türkiye deniz balıkları avcılığı istatistiklerine ait mikro-veriye TÜİK ile imzalanan protokol ile erişilmiştir. Veri talebi onaylandıktan sonra TÜİK Adana Bölge Müdürlüğü'nde yer alan Veri Araştırma Merkezi'ne gidilerek gerekli veri indirilmiş ve incelenmek üzere TÜİK personeline teslim edilmiştir. Gizlilik ilkeleri gereği TÜİK personeli tarafından kişisel bilgilerden arındırılan veri teslim alınarak çalışmalara başlanmıştır.

Barbungiller, TÜİK tarafından 707 kodlu Barbunya (*M. barbatus*), 708 kodlu barbunya-paşa barbunu (*U. moluccensis*) ve 766 kodlu tekir (*M. surmuletus*) adlarıyla üç farklı tür halinde kayda alınmaktadır. Bu çalışma kapsamında Barbunya balığı "barbun" adıyla değerlendirilmiş, toplam Mullid avı ise "barbungiller" adıyla ele alınmıştır. Mikro-veride teknenin kayıtlı olduğu liman ile avcılığın gerçekleştirildiği liman ayrı ayrı kayda alınmaktadır. Bu çalışma kapsamındaki değerlendirmeler avcılığın gerçekleştirildiği liman üzerinden yapılmıştır.

Veri analizi ve görselleştirme süreçlerine geçmeden önce olası hatalı girişler incelenerek Mersin ili için kayıt edilen ortasu trolü tekneleri dip trolüne dönüştürülmüş, Marmara Denizi'nde dip trolü avcılığı yasak olduğundan (Resmi Gazete, 2020), Marmara Denizi'nde kayıtlı olan dip trolcüleri tarafından yine Marmara Denizi'nden rapor edilen barbungil avı diğer denizlere dağıtılmıştır. Bu kapsamda; İstanbul, Kocaeli ve Tekirdağ illerinden rapor edilen av Karadeniz'e, Balıkesir'den rapor edilen av ise Ege Denizi'ne eklenmiştir.

Av değerleri büyük ölçekli balıkçılardan tamsayım yöntemiyle toplandığından, toplam avın illere göre değişimi büyük ölçekli balıkçıların rapor ettiği değerler üzerinden incelenmiştir. Bu amaçla iller bazında toplam av değerleri hesaplanmış ve illerin av miktarları arasındaki farklılıklar Kruskall-Wallis Testi ile analiz edilmiştir (Sokal ve Rohlf, 2012). Büyük ve küçük ölçekli balıkçıların toplam ava katkılarının incelenmesi amacıyla, bölgeler bazında büyük ölçekli balıkçıların toplam av değerleri mikro-veriden hesaplanmıştır. Ardından küçük ölçekli balıkçıların av miktarını hesaplamak amacıyla, büyük ölçekli balıkçıların av değerleri bölgesel toplam av değerlerinden çıkarılmıştır. Büyük ve küçük ölçekli balıkçıların av miktarları arasındaki farklar Wilcoxon Sıra Sayıları Toplamı Testi ile analiz edilmiştir.

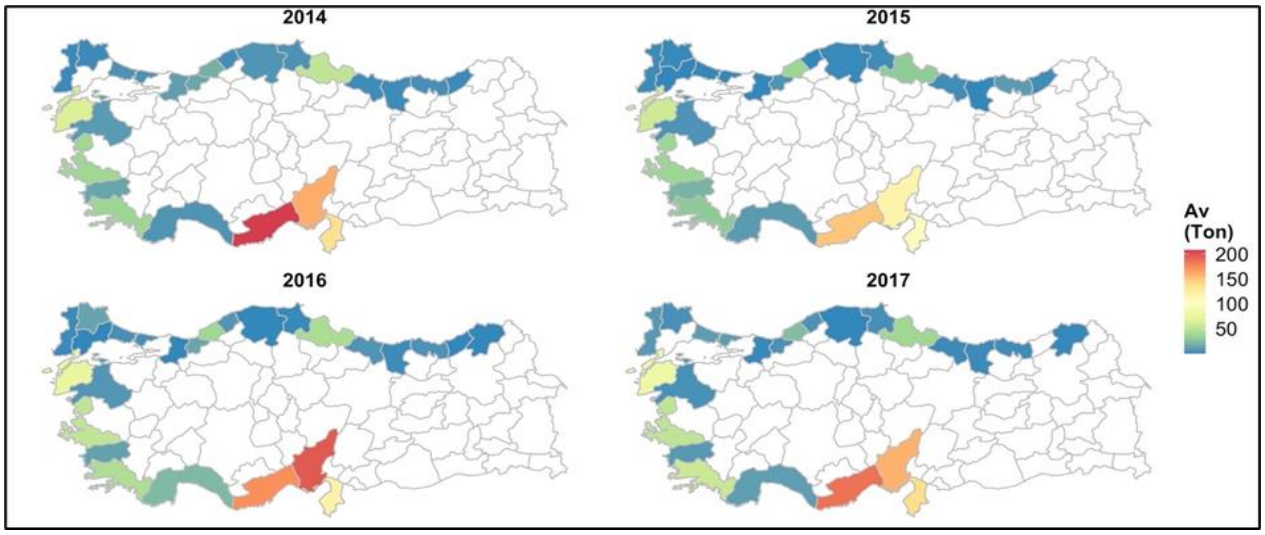
Barbungillerin av araçlarına göre değişimlerini incelemek amacıyla, mikro-veride 20 farklı sınıf altında kayda alınan av araçları; algarna, çevirme ve voli ağları, dip trolü, gırgır, ortasu trolü, paraketa ve olta, uzatma ağları ve diğerleri olmak üzere 8 kategori altında toplanmıştır. Toplam avın av araçlarına göre değişimini incelemek amacıyla yalnızca büyük ölçekli balıkçıların raporlarından faydalanılmış, her bir avcılık kategorisi için toplam av değerleri hesaplanmıştır. Mikro-veri setinde balıkçıların toplam av miktarları ve karaya çıkarılan net miktarlar ayrı ayrı rapor edilmiştir. Iskarta miktarı, toplam avdan net miktarın çıkarılması suretiyle hesaplanmıştır.

Çalışma genelinde ortalama ± standart sapma değerleri hesaplanarak raporlanmıştır. Tüm istatistiki analizler ve haritalama işlemlerinde R İstatistiksel Hesaplama Ortamı ve Programlama Dili (R Core Team, 2019) ile "ggplot2" paketinden yararlanılmıştır (Wickham, 2009).

BULGULAR

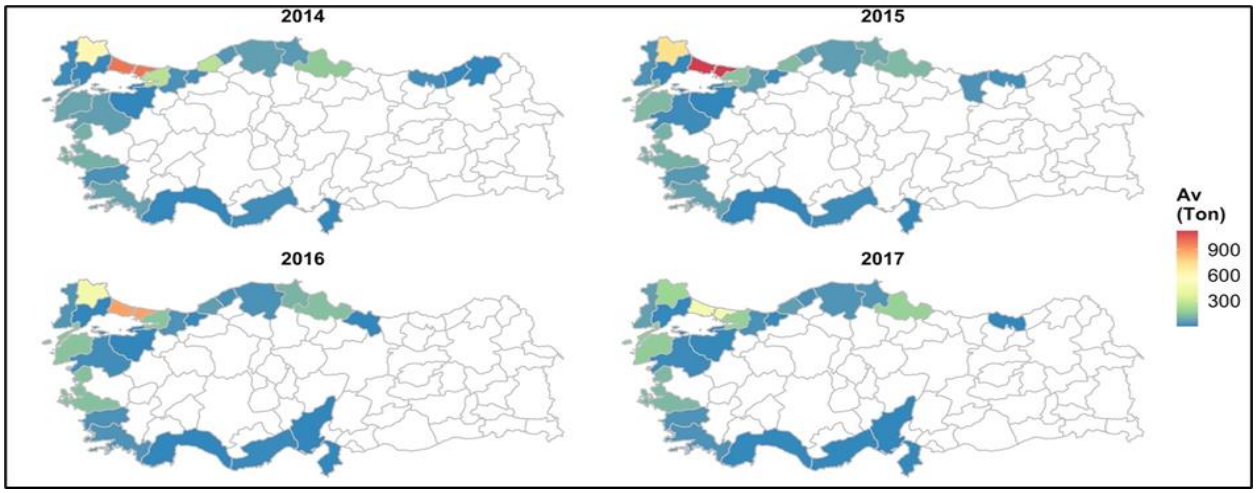
Barbungil avcılığının şehirlere göre değişimi

Türkiye genelinde barbun avının şehirlere göre değişimi istatistiki açıdan önemli bulunmuştur (Kruskal-Wallis χ^2=81,802, serbestlik derecesi = 24, p<0,01). En yüksek av değerlerinin kaydedildiği ilk üç şehir, İskenderun ve Mersin Körfez'lerinin kıyısında yer alan Mersin, Adana ve Hatay illeridir. Mikro-verinin değerlendirildiği 2014-2017 yıllarında 10m'den büyük tekneler tarafından Mersin iline bağlı balıkçı barınaklarından karaya çıkarılan yıllık ortalama barbun miktarı 179 ± 24,3 ton iken; bu rakam Adana için 158 ± 32,8 ton, Hatay için ise 125 ± 16,3 ton olarak gerçekleşmiştir. Bu şehirlerin kendi aralarındaki sıralamaları yıllar itibariyle değişim gösterebilse de Türkiye genelinde istikrarlı bir de ilk üçü oluşturdukları görülmektedir (Şekil 1). Dört yıllık süreçte Kocaeli ilinden hiç barbun rapor edilmemiş olması dikkat çekicidir.



Şekil 1. Büyük ölçekli balıkçıların (tekne > 10m) yıllık toplam barbun avının şehirlere göre değişimi
Figure 1. Total red mullet catch of large scale fishers (boat > 10m) by cities

Barbuna benzer şekilde yıllık toplam tekir balığı avının da şehirlere göre değişimi istatistiki açıdan önemli bulunmuştur (Kruskal-Wallis χ^2= 90,091, serbestlik derecesi = 27, p<0,01). Ancak bu kez en yüksek değerlerin kaydedildiği ilk üç şehir Batı Karadeniz kıyılarımızda yer almaktadır. Bu bağlamda, 2014-2017 yılları arasında, 10 m'den büyük ticari balıkçı tekneleri tarafından kaydedilen yıllık ortalama tekir avı bakımından İstanbul ili 867 ± 280 ton ile birinci sırayı alırken; bunu 500 ± 230 ton ile Kırklareli ve 184 ± 55,8 ton ile Kocaeli takip etmektedir (Şekil 2). Adana da dahil olmak üzere bazı kıyı illerimizden hiç tekir balığı rapor edilmediği dikkat çekmektedir.



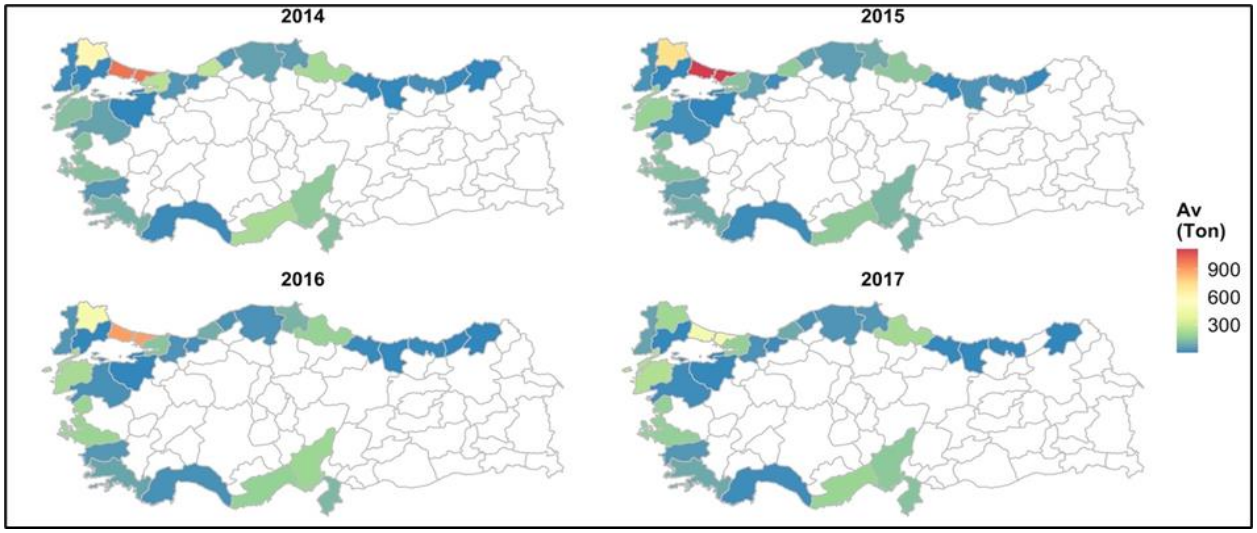
Şekil 2. Büyük ölçekli balıkçıların (tekne >10m) yıllık toplam tekir balığı avının şehirlere göre değişimi
Figure 2. Total surmullet catch of large scale fishers (boat > 10m) by cities

Barbun ve tekir balığı avının toplamının da mekansal değişimleri istatistiki açıdan önemli bulunmuştur (Kruskal-Wallis χ^2= 102,76, serbestlik derecesi= 27, p<0,01). Tekir balıklarında olduğu gibi, ilk iki şehrin Batı Karadeniz'de yer alan İstanbul (872 ± 276 ton) ve Kırklareli (505 ± 229 ton) olduğu görülmektedir (Şekil 3).

Barbungil avcılığının av araçlarına göre değişimi

Türkiye geneli değerlendirildiğinde, küçük (Tekne <10m) ve büyük ölçekli (Tekne >10m) balıkçıların toplam barbun üretimleri arasındaki fark istatistiki açıdan önemli bulunmamıştır (Wilcoxon Sıra Sayıları Toplamı Testi (W)= 107, p=0,84).

Diğer taraftan denizler itibariyle ele alındığında, Akdeniz'de barbun avının çoğu büyük ölçekli balıkçılar tarafından sağlanırken (W = 16, p <0,05); Ege Denizi (W = 4, p=0,34) ve Marmara'da (W=1, p=0,2) büyük ve küçük ölçekli balıkçıların payının aşağı yukarı eşit olduğu, Karadeniz'de ise avın çoğunun küçük ölçekli balıkçılar tarafından sağlandığı (W = 0, p <0,05) görülmektedir (Tablo 1).



Şekil 3. Büyük ölçekli balıkçıların (tekne >10m) yıllık toplam barbungil avının şehirlere göre değişimi
Figure 3. Total mullet catch of large scale fishers (boat > 10m) by cities

Tablo 1. Denizler itibariyle büyük ve küçük ölçekli balıkçıların barbun ve tekir balığı avı ile bunların toplamına katkıları (ton/yıl; ± değerler standart sapmadır)
Table 1. Red mullet, surmullet and total mullet catch of small (boat <10m) and large scale (boat >10m) fishers by marine areas (tonnes/year; ± standard deviation)

Tür	Ölçek	Akdeniz	Ege	Marmara	Karadeniz
Barbun	<10m	145 ± 26	199 ± 30	5 ± 3	268 ± 56
	>10m	474 ± 63	179 ± 27	1 ± 2	114 ± 21
Tekir Balığı	<10m	6 ± 3	80 ± 20	139 ± 63	398 ± 113
	>10m	14 ± 6	377 ± 36	17 ± 3	2024 ± 628
Toplam	<10m	150 ± 24	279 ± 27	144 ± 64	666 ± 124
	>10m	488 ± 63	556 ± 61	18 ± 5	2138 ± 632

Türkiye geneli değerlendirildiğinde, küçük (Tekne <10m) ve büyük ölçekli (Tekne >10m) balıkçıların toplam tekir balığı avı miktarları arasındaki fark istatistiki açıdan önemli bulunmamıştır (W = 152, p = 0,3809). Diğer taraftan denizler itibariyle ele alındığında, Akdeniz, Ege ve Karadeniz'de tekir balığı avının çoğu büyük ölçekli balıkçılar tarafından sağlanırken (W=16, p <0,05); Marmara Denizi'nde küçük ölçekli balıkçıların toplam ava katkısı en üst düzeydedir (W = 0, p <0,05) (Tablo 1).

Barbun ve tekir balığı için ayrı ayrı gözlenen eğilim, her iki türün toplamı için de geçerliliğini korumaktadır. Türkiye genelinde küçük ve büyük ölçekli balıkçıların toplam barbungil avına katkısının benzer düzeylerde olduğu bulunmuştur (W=165, p = 0,1713). Ancak denizler bazında değerlendirildiğinde, yalnızca Marmara'da küçük ölçekli balıkçılığın baskın olduğu (W = 0, p <0,05), diğer denizlerimizde ise avın çoğunluğunun büyük ölçekli balıkçılar tarafından karşılandığı (W = 16, p <0,05) görülmektedir (Tablo 1).

Türkiye İstatistik Kurumu kayıtlarına göre, Türkiye kıyılarında barbungil balıkları toplam 18 farklı av aracıyla yakalanmaktadır. Bunlar dışında 1219 kayıtta av aracı rapor edilmemiş, dokuz kayıt ise "Diğer" av aracı şeklinde rapor edilmiştir. Dört yıl boyunca alınan kayıt sayısı bakımından değerlendirildiğinde, 13948 kayıt ile en çok rapor veren filo segmenti dip trolü kullanan balıkçılardır. Bunu 2158 kayıt ile fanyalı ve 2142 kayıt ile sade uzatma ağları takip etmektedir. 598 kayıt ile gırgır ise dördüncü sırada yer almaktadır. Diğer taraftan tekne başına rapor edilen ortalama av miktarları ele alındığında, yalnızca bir kez barbungil kaydı vermiş olan ığrıp (Kocaeli/Darıca; 10000 kg/tekne) ve 169 kez barbungil kaydı vermiş olan ortasu trolü'nün (1639 kg/tekne) ilk iki sırada yer aldığı görülmektedir (Tablo 2).

Büyük ölçekli balıkçılar tarafından karaya çıkarılan toplam barbun ve tekir balığı avı değerlendirildiğinde, Marmara hariç tüm denizlerimizde barbun avının çoğunun dip trolü balıkçıları tarafından sağlandığı görülmektedir. Karaya çıkan toplam barbunun Akdeniz'de %87'si (± %4,34), Ege'de %88'i (± %2,80) ve Karadeniz'de %81,1'i (± %3,99); tekir balığının ise Akdeniz'de %84'ü (± %16,7), Ege'de % 93,9'u (± %2,64) ve Karadeniz'de %89,2'si (± %4,20) dip trolü avcılığıyla yakalanmaktadır. Denizler itibariyle yıllık toplam barbungil avının avcılık metotlarına dağılımı Tablo 3'de verilmiştir.

Tablo 2. 2014-2017 yılları arasında farklı av araçlarının kayıt sayıları ve tekne başına düşen av değerleri
Table 2. Number of records and landing per boat values of different fishing methods between 2014 and 2017

Av Aracı	Tekne Başına Av (kg)	Adet
Dip trolü	809	13948
Fanyalı uzatma ağları	143	2158
Sade uzatma ağları	151	2142
Bilinmiyor	135	1219
Gırgır	1431	598
Karma uzatma ağları (fanyalı-sade)	174	291
Çevirme-voli ağları (fanyalı)	191	222
Paraketa	68	203
Algarna	241	181
Ortasu trolü	1689	169
Olta	40	113
Çevirme-voli ağları (sade)	129	72
Çevirme-voli ağları (karma)	346	26
Diğer	118	9
Tekneye çekilen sürütme ağları, manyat	441	7
Tuzaklar (sepet, çömlek)	288	2
Çökertme ağları (dalyanlar)	200	1
Hidrolik dreçler	120	1
Kıyıya çekilen sürütme ağları, ığrıp	10000	1
Kıyıya çekilen sürütme ağları, trata	1000	1

Iskarta oranının değişimi

Mikro-veri incelendiğinde, toplam 21364 kayıttan yalnızca 1707 tanesinde (%8) ıskarta oranının sıfırın üzerinde rapor edildiği görülmektedir. Tüm raporlar ele alındığında ıskarta oranı barbun için %0,29 ± 2,20, balığı için %0,62 ± 2,93, her iki türün toplamı için ise %0,47 ± 2,59 olarak hesaplanmıştır. Rapor edilen ıskarta oranının türler (Wilcoxon W= 16, p=0,318), denizler (Kruskal-Wallis χ^2= 3,33, serbestlik derecesi = 3, p=0,343), av araçları (Kruskal-Wallis χ^2= 11,83, serbestlik derecesi = 7, p=0,106) ve tekne boy grupları (Kruskal-Wallis χ^2= 9,14, serbestlik derecesi = 6, p=0,166) arasındaki değişimi istatistiki açıdan önemli bulunmamıştır.

Tablo 3. Büyük ölçekli balıkçıların yıllık toplam barbungil avının (ton) denizler ve avcılık metotlarına göre değişimi (± standart sapma)
Table 3. Annual total mullet catch (tonnes) of large scale fishers by marine areas and fishing methods (± standard deviation)

Avcılık	Deniz			
	Akdeniz	Ege	Marmara	Karadeniz
Dip trolü	423 ± 55	512 ± 69	-	1884 ± 516
Uzatma ağları	9 ± 3	31 ± 9	2 ± 1	28 ± 17
Ortasu trolü	-	-	-	71 ± 20
Gırgır	55 ± 19	10 ± 10	8 ± 3	140 ± 145
Diğer	1 ± 1	3 ± 1	8 ± 6	15 ± 10

TARTIŞMA VE SONUÇ

Çalışma sonuçlarına göre, Türkiye genelinde barbungillerin avcılığında en baskın yöntemin dip trolü balıkçılığı olduğu belirlenmiştir. Bu avcılık Türkiye'de büyük ölçekli balıkçılar tarafından gerçekleştirilmekte olup; Marmara Denizi'nde tümüyle yasaktır (Resmi Gazete, 2020). Bu doğrultuda Akdeniz, Ege ve Karadeniz'de barbungil avcılığının önemli bir kısmı büyük ölçekli balıkçılar tarafından gerçekleştirilirken; Marmara'da küçük ölçekli balıkçıların toplam ava katkısı daha yüksek düzeyde bulunmuştur. Bu durum Marmara Denizi'ndeki dip trolü yasağının küçük ölçekli balıkçılar için önemli bir alan açtığının da göstergesi olarak kabul edilmiş ve yalnızca barbungil popülasyonlarının sürdürülebilirliği açısından değil, geleneksel balıkçılığın desteklenmesi açısından da olumlu bir uygulama olduğu değerlendirilmiştir.

Su ürünleri avcılığı istatistiklerinde zaman zaman dağılım alanı sınırları dışında kayıt altına alınan türler ya da ilgili bölgede kesinlikle kullanılmayan av araçları gibi bariz hataların varlığı dikkat çekmiştir. Dolayısıyla, veri kaynağı olarak av istatistiklerinin kullanıldığı çalışmalarda veri kontrolünün dikkatlice yapılması ve olası hataların tespit edilerek değerlendirmeye alınmaması çalışmanın güvenirliği açısından büyük önem arz etmektedir.

Çalışma kapsamında barbunun en yoğun Hatay, Adana ve Mersin illerine bağlı barınaklardan karaya çıkarıldığı, buna karşın bu bölgelerde gerçekleşen tekir balığı avının son derece düşük düzeylerde olduğu görülmüştür. Balıkçılıktan bağımsız olarak gerçekleştirilen araştırma faaliyetleri de bu bulguları doğrular niteliktedir. Doğu Akdeniz'de dip trolü ile gerçekleştirilen çalışmalarda barbun bolluk bakımından ilk sıralarda yer alırken; tekir balığı ile ya nadiren karşılaşılmakta ya da hiç rapor edilmemektedir (Başusta vd., 2002; Yemişken vd., 2014; Gökçe vd., 2016; Mavruk vd., 2017; Özyurt vd., 2018). Diğer taraftan tekir balığı avı incelendiğinde, barbunun tersine bir mekansal eğilimin söz konusu olduğu ve tekir balığı avı bakımından ilk üç ilin Batı Karadeniz kıyılarımızda yer alan İstanbul, Kırklareli ve Kocaeli olduğu görülmektedir. Dahası bu illerde barbun kayıtlarının son derece düşük düzeylerde olduğu ve hatta Kocaeli'de hiç barbun avı rapor

edilmediği dikkat çekmektedir. Oysaki bölgede dip trolleriyle yapılan araştırma faaliyetlerinde baskın türün tekir balığı değil barbun olduğu açıkça görülmektedir (Yıldız ve Karakulak, 2018; Yıldız vd., 2019). Dolayısıyla bölgede barbun baskın iken; ağırlıklı olarak tekir balığının rapor edilmesi isimlendirmede yüksek ihtimalle bir karışıklık olduğuna işaret etmektedir. Diğer taraftan, tekirin ilk avlanma boyunun barbundan küçük olması nedeniyle (Resmi Gazete, 2020) balıkçıların küçük bireyleri özellikle tekir olarak kaydedebilecekleri de göz ardı edilmemelidir.

Balıkçılık istatistiklerinin dağılımı incelendiğinde de balıkçıların rapor ederken barbun ve tekir balığı arasında tür düzeyinde bir ayrım yapmadığına dair önemli kanıtlar göze çarpmaktadır. Örneğin çalışmanın konusunu oluşturan 2014-2017 yılları arasındaki dört yıllık süreçte, Kocaeli kıyılarında avlanan balıkçılardan hiç barbun rapor edilmezken, önemli miktarlarda tekir balığı rapor edilmiştir. Oysaki komşu iller olan İstanbul ve Sakarya'da her iki türün de bol miktarda avlandığı kayıtlara geçmiştir. Ayrıca Kocaeli'nde rapor edilen tekir balığı miktarı, Sakarya'ya nazaran önemli ölçüde yüksek düzeylerdeyken; barbun ve tekir balığının toplam avı değerlendirildiğinde bu farkın kapandığı görülmektedir.

Balıkçılık istatistiklerinde 18 farklı av aracı tarafından barbungil balıklarının avlandığına dair kayıtlar bulunmaktadır. Her ne kadar ülkemiz balıkçılığıyla ilgili kaynaklarda barbungil avcılığının genel olarak dip trolü ve barbun uzatma ağları olmak üzere iki av aracıyla gerçekleştirildiği vurgulanmış olsa da (Hoşsucu, 2000); normalde pelajik balıkların avcılığı için kullanılan gırgır ve ortasu trolü gibi av araçlarıyla da barbungil avlanabildiği görülmüştür. Dahası, tekne başına rapor edilen av değerleri ve toplam karaya çıkarılan ürün değerleri incelendiğinde, bu avcılık türlerinin katkısının azımsanamayacak düzeylerde olduğu anlaşılmaktadır.

Ülkemiz balıkçılık mevzuatında ortasu trolü avcılığı yalnızca Karadeniz'de serbesttir. 2016-2020 yılları arasında yürürlükte olan 4/1 nolu Ticari Amaçlı Su Ürünleri Avcılığını Düzenleyen Tebliğ'e göre, ortasu trolü avcılığı yapan teknelerde barbun, tekir balığı gibi dip balıklarının bulundurulması yasaktır (Resmi Gazete, 2016). Diğer taraftan bu çalışmada 2016 yılından sonra da ortasu trolü tekneleri tarafından barbun rapor edildiği görülmüştür. Benzer bir durum algarna avcılığı için de söz konusudur. 2016 yılına kadar yürürlükte olan 3/1 nolu tebliğde yalnızca Marmara Denizi'nde algarna ile karides avcılığına müsaade edilmiş ve karidesin %15'i kadar ıskartaya izin verilmiştir. Karadeniz'de gerçekleştirilen algarna avcılığı için ise özel bir düzenleme yapılmamıştır (Resmi Gazete, 2012). Diğer taraftan, 2016 yılında yürürlüğe giren 4/1 nolu tebliğde algarnanın Karadeniz'de deniz salyangozu, Marmara'da ise karides avcılığı dışında kullanımı yasaklanmış, yine karides avcılığında hedef türün %15'i kadar ıskartaya izin verilmiştir. Bu çalışma kapsamında Karadeniz'de bazı algarna teknelerinin 2016 yılından sonra da barbun rapor ettikleri dikkat çekmiştir. Yürürlükte olan mevzuata göre bu avcılığın yasa dışı olması kaçınılmazdır. Bu bağlamda, mevcut mevzuatın uygulanmasında daha sıkı denetimlere ihtiyaç duyulduğu açıktır.

Su ürünleri anket formunda toplam avlanan balığın yanı sıra, atılan balıklarla ilgili de soru bulunmaktadır (TÜİK, 2019). Bu çalışma kapsamında kayıtların yaklaşık %8'inde balıkçıların atılan balıkları da rapor ettiği görülmüştür. Diğer taraftan bu çalışmanın sonuçlarından da açıkça görüldüğü üzere, toplanan verideki ıskarta oranı ortalama %0,47 olarak hesaplanmış olup; ıskarta oranında türler, bölgeler ve av araçları bazında herhangi bir değişim görülmemektedir. Barbun balıkçılığında baskın yöntem olan dip trolü avcılığında ıskarta oranını ele alan araştırmalar incelendiğinde, ıskarta oranının TÜİK istatistiklerinde rapor edilene kıyasla son derece yüksek olduğu dikkat çekmektedir. Örneğin barbunda ıskarta oranının İskenderun Körfezi'nde %9,47 (Yemişken vd., 2014), Batı Karadeniz'de ise %11,45 olduğunu rapor edilmiştir (Yıldız ve Karakulak, 2017). Diğer taraftan Doğu Karadeniz'de, barbun avcılığında önemli bir yere sahip olan galsama ağları için rapor edilen ıskarta oranı %0,3 olup (Balık, 2020), bu değer TÜİK tarafından kaydedilen değerden dahi düşüktür.

Dünya genelinde toplanan balıkçılık istatistiklerinde balıkçıların ıskartayı ve yasa dışı avı rapor etmedikleri kabullenmeleri mevcuttur (Garibaldi, 2012; Pauly ve Zeller, 2015). Hatta, bu kabullenmeler esas alınarak geçmişe dönük istatistiklerin yeniden inşası ve hataların düzeltilmesine yönelik çok sayıda çalışma bulunmaktadır (Pauly ve Zeller, 2016; 2017; Zeller ve Pauly, 2016). Ülkemizde toplanan balıkçılık istatistikleri de benzer varsayımlarla ele alınmış ve yeniden inşa edilmiştir (Ulman vd., 2013). Oysaki, ülkemizde 2014–2017 yılları arasında kaydedilen balıkçılık istatistiklerinin avın yanı sıra ıskartayı da kapsadığı görülmektedir. Dahası, ortasu trolü gibi dip balıklarının avcılığında kullanılması yasak olan (Resmi Gazete, 2016) av araçlarıyla yakalanan barbun balıklarının da kayda alındığı dikkat çekmektedir.

Özetle, ulusal balıkçılık istatistiklerinde barbun ve tekir balıklarına atfen kaydedilen avın büyük ölçüde barbun avını temsil ettiği görülmüştür. İstatistiklerin toplanması sürecinde türlerin doğru teşhis edilmesine özen gösterilmeli, yerel isimlendirme farklılıklarına dikkat edilmelidir. Farklı avcılık yöntemleri ve bölgelerde avın barbungil türlerine göre oransal dağılımı tespit edilerek av istatistiklerinin geçmişe dönük olarak düzeltilmesi de faydalı bir uygulama olabilir. TÜİK istatistiklerinde yasa dışı olarak avlanan balıklar ile ıskartaya ait kayıtların da tutulduğu görülmektedir. Diğer taraftan bu kayıtların gerçek durumu ne ölçüde yansıttığı ileri çalışmalarda ele alınmalıdır. Dolayısıyla, TÜİK tarafından derlenen su ürünleri istatistiklerinin balıkçılık yönetiminde son derece faydalı olabilecek bir veri kaynağı oluşturduğu, ancak veri toplama ve raporlama süreçlerinin geliştirilmesi gerektiği sonucuna varılmıştır.

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Destekleri dolayısıyla TÜİK'e ve veri erişimindeki yardımları dolayısıyla TÜİK Adana Veri Araştırma Merkezi çalışanlarına teşekkürü borç bilirim.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Karyotype, C-band and NOR phenotype of Anatolian endemic fish *Squalius anatolicus* (Bogutskaya, 1997) (Teleostei, Leuciscidae)

Anadolu'ya endemik *Squalius anatolicus* (Bogutskaya, 1997) (Teleostei, Leuciscidae) türünün karyotip, C-bant ve NOR fenotipi

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Ünal Karakuş, S. & Gaffaroğlu, M. (2021). Karyotype, C-band and NOR phenotype of Anatolian endemic fish *Squalius anatolicus* (Bogutskaya, 1997) (Teleostei, Leuciscidae). *Ege Journal of Fisheries and Aquatic Sciences,* 38(3), 311-315. DOI: 10.12714/egejfas.38.3.07

Abstract: The karyotype and distribution of constitutive heterochromatin and nucleolus organizer regions (NORs) of Anatolian leuciscine endemic to Lake Beysehir, *Squalius anatolicus* (Bogutskaya, 1997) were analyzed respectively using conventional Giemsa-staining, C-banding and Ag-impregnation. Diploid chromosome number was *2n* = 50 and karyotype consisted of 7 pairs of metacentric, 13 pairs of submetacentric, 5 pairs of subtelo- to acrocentric chromosomes, NF value equaled 90. Heteromorphic elements indicating sex chromosomes were not detected. C-banding revealed clear pericentromeric constitutive heterochromatin blocks in several chromosomes. Ag-impregnation revealed the size heteromorphism of NORs that covered almost the entire short arms of the middle-sized submetacentric chromosome pair. The karyotype pattern and simple NOR phenotype of *S. anatolicus* are nearly identical with that found not only in *Squalius* species analyzed to date but also in many other representatives of the Eurasian leuciscine cyprinids, which indicates remarkable chromosome stasis in this leuciscid lineage.

Keywords: Leuciscid cytotaxonomy, fish cytogenetics, chromosome banding, major rDNA sites, *Squalius anatolicus*

Öz: Beyşehir Gölü'ne endemik Anadolu leuciscini *Squalius anatolicus* (Bogutskaya, 1997)'un karyotipi, konstitütif heterokromatin dağılımı ve çekirdekçik organize edici bölgeleri (NOR'lar) sırası ile geleneksel Giemsa-boyama, C-bantlama ve Gümüş emdirme teknikleri kullanılarak analiz edilmiştir. Diploid kromozom sayısı *2n* = 50 olmak üzere; karyotipinin 7 çift metasentrik, 13 çift submetasentrik, 5 çift subtelo – akrosentrik kromozomdan oluştuğu ve toplam kol sayısının 90 olduğu tespit edilmiştir. Cinsiyet kromozomlarını temsil eden heteromorfik yapılar gözlenmemiştir. C-bantlama birçok kromozomda belirgin perisentromerik heterokromatin blokları ortaya çıkarmıştır. Gümüş emdirme ile orta boy submetasentrik kromozom çiftinin neredeyse kısa kolunun tamamını kaplayan NOR'ların büyüklük heteromorfizmi tespit edilmiştir. *S. anatolicus*'un karyotip şekli ve temel NOR fenotipi sadece bugüne kadar analiz edilen *Squalius* türlerinde değil, aynı zamanda bu leuciscid soyunda büyük ölçüde kromozom durumunu gösteren Avrasya leuciscinlerinin diğer birçok temsilcisinde bulunanla hemen hemen aynıdır.

Anahtar kelimeler: Leuciscid sitotaksonomisi, balık sitogenetiği, kromozom bantlama, major rDNA bölgeleri, *Squalius anatolicus*

INTRODUCTION

The genus *Squalius* was recognized within the genus *Leuciscus* for a long time until morphological and molecular data demonstrated that *Leuciscus* represents another leuciscine lineage (Zardoya and Doadrio, 1999). The *Squalius* genus belongs to the Leuciscinae subfamily and comprises at least 45 species that are commonly named chub (Özulug and Freyhof, 2011). The genus represented by 22 species includes *Squalius adanensis, S. anatolicus, S. aristotelis, S. berak, S. cappadocicus, S. carinus, S. cephaloides, S. cephalus, S. cii, S. fellowesii, S. irideus, S. kosswigi, S. kottelati, S. lepidus, S. orientalis, S. orpheus, S. pursakensis, S. recurvirostris, S. semae, S. seyhanensis, S. spurius,* and *S. turcicus* in Anatolia (Çiçek et al., 2020).

Squalius species are present in almost every water body in Anatolia (Stoumboudi et al., 2006) while only a few populations have been defined in sufficient detail in Anatolia and adjacent basins. Some species of *Squalius* were reported from the Tigris, the Euphrates, the Orontes and the Beysehir drainages, respectively. Bogutskaya (1997) identified populations of Lake Beysehir basin as *S. anatolicus* (known as Beysehir dace) (Turan et al., 2009). This species has restricted distribution in central Anatolia, but it exists also in the Manavgat River, draining to the Mediterranean east of Antalya. The current population trend is reported as decreasing and it was known as endangered until 2006 (Özulug and Freyhof, 2011) but recently it is listed as the least concern in the IUCN Red List (Freyhof, 2014).

The subfamily Leuciscinae includes virtually 70 freshwater genera nonetheless approximately 25 leuciscine genera have been cytogenetically investigated (Rab and Collares-Pereira, 1995; Bianco et al., 2004; Rossi et al., 2012). The primary challenge in leuciscines' cytogenetic analysis relevant in

effectively descrying species at the karyotype level, fundamental for advanced chromosomal studies (Pereira et al., 2013). In earlier studies, *S. anatolicus* was described as morphologically and analysed as parasitologically (Turan et al., 2013; Aydogdu et al., 2015) while there are no cytogenetic data reported. The aim of the study is to investigate the karyotype, distribution of constitutive heterochromatin and NORs' phenotype that it means number and position of major rDNA/NOR sites of Anatolian leuciscine endemic to Beysehir Lake, *S. anatolicus* (Bogutskaya, 1997) using conventional Giemsa-staining, C-banding, and Ag-impregnation.

MATERIAL AND METHODS

Eight adult individuals of *S. anatolicus* (four females and four males) were collected from Lake Beyşehir basin, Konya, Turkey (37°52' N, 31°35' E) using electrofishing. The alive individuals were transported to the laboratory and kept in a well-aerated aquarium until analysis. The specimens were deposited in a fish collection of the Genetic Research Laboratory of Kırşehir Ahi Evran University, Kırşehir, Turkey.

The chromosome preparations were obtained according to standard protocol of Collares-Pereira (1992), using direct air drying technique. The slides were stained with 4% Giemsa staining solution (with Sorenson's phosphate buffer, pH 6.8). The metaphase plates were examined and photographed using Leica DMLB. The total sum of metaphases was 411 and metaphase number was not evenly ratio for per each of eight analysed specimens. The C-bands were obtained according to Sumner (1972). The Ag-impregnation method of Howell and Black (1980) was applied to determine NORs. The chromosomes were classified using a modified version of Levan et al. (1964), and the fundamental arm number (NF) was identified by scoring the metacentric (m) and submetacentric (sm) as biarmed and subtelo-acrocentric (st/a) chromosomes as uniarmed chromosomes.

Ethical Statement

Permissions of sampling and laboratory study on fish have issued by respectively the Republic of Turkey Ministry of Agriculture and Forestry (the number and date of fieldwork permission: B.12.0.KKG.0.17/106.01-11-01/3007840-02.02.2010), and the Kırşehir Ahi Evran University Animal Experiments Local Ethics Committee (the number and date of fieldwork permission: 68429034/02-14.02.2019).

RESULTS

All analyzed *S. anatolicus* specimens showed *2n* = 50 chromosomes. Karyotype consisted of 7 pairs of m, 13 pairs of sm, 5 pairs of st/a chromosomes (Table 1). The fundamental number of chromosome arms equaled NF = 90 (Figure 1). The morphologically differentiated sex chromosomes were not detected.

Table 1. Cytogenetic data of *Squalius anatolicus*

Species	Specimen number	Metaphase number	*2n*	NF	Chromosome morphology		
					m	sm	st/a
Squalius anatolicus	8	411	50	90	14	26	10

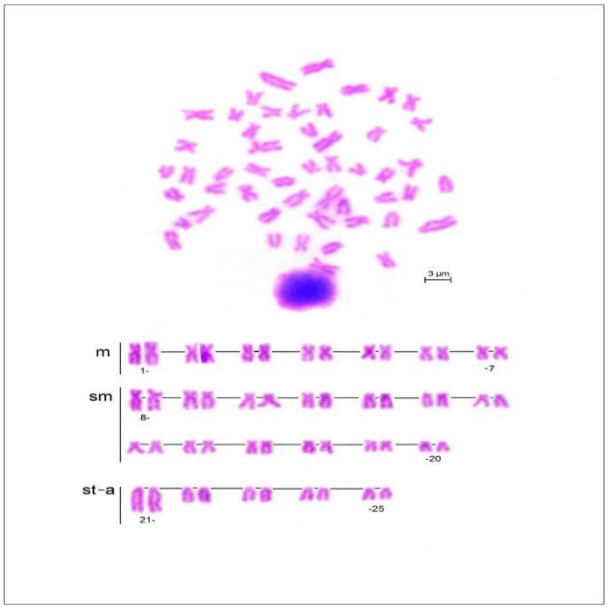

Figure 1. Giemsa stained metaphase and corresponding karyotype of *Squalius anatolicus*. Scale bar = 3 µm

C-banding procedure showed blocks of constitutive heterochromatin (CH) mostly in (peri) centromeric regions of several chromosomes. However, intercalar or terminal C-bands were also visible on some chromosomes (Figure 2).

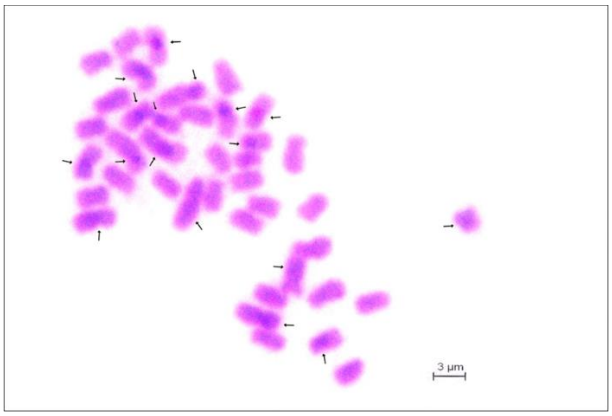

Figure 2. Metaphase chromosomes of *S. anatolicus* after C-banding. Arrows indicate C-bands. Scale bar = 3 µm

The NORs were located terminally on short arms of one sm chromosome pair (Figure 3). Besides, the size heteromorphism of NORs in the homologous chromosomes were identified by Ag-impregnation. NORs covered almost the entire short arms of one pair of the homologous

chromosomes, while the others were identified to be more restricted to the end of the short arm.

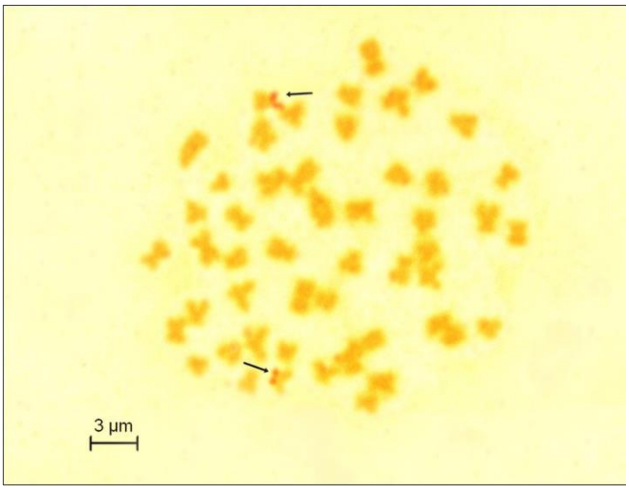

Figure 3. Metaphase chromosomes of *S. anatolicus* after Ag-impregnation. Arrows indicate NORs. Scale bar = 3 µm

DISCUSSION

Bogutskaya (1997) previously recognized that the *Squalius* population in Lake Beyşehir is a different species from *S. cephalus*, and it was identified as endemic species *S. anatolicus*.

The karyotype and chromosomal characteristics of *S. anatolicus* were analyzed in this study for the first time. The diploid chromosome number was *2n* = 50 and NF was 90. Within the individuals examined no karyotype variation was identified among the individuals and their sex. Acrocentric chromosomes were represented by 5 pairs of chromosomes, while the sm chromosomes were represented by 13 pairs, being thus the prevalent morphological chromosome type within the karyotype.

Rab and Collares-Pereira (1995) reported that the diploid chromosome numbers within leuciscins are usually *2n* = (48) 50 (52) and that their karyotype macrostructure is composed of 6 to 8 pairs of m, 12 to 14 pairs of sm, and 2 to 4 pairs of st/t chromosomes. Amemiya and Gold (1990), on the other hand, observed that while 90% of North American cyprinids had a karyotype morphology of *2n* = 50, this *2n* could vary from 48 to 52, and that the NF value could vary from 80 to 100. In terms of *2n* and karyotype macrostructure, results from the present study are in line with above mentioned reports.

Cytogenetic studies of this leuciscine lineage demonstrated that *Squalius carolitertii* (Syn: *Leuciscus carolitertii*) has *2n* = 50 and karyotype with 6 pairs of m, 15 pairs of st, and 4 pairs of a chromosomes, *S. pyrenaicus* (Syn: *Leuciscus pyrenaicus*) *2n* = 50 and karyotype with 6 pairs of m, 16 pairs of sm, and 3 pairs of a chromosomes (while the morphology of the chromosomes of *S. carolitertii* is stable, karyotype of *S. pyrenacius* is variable) (Collares-Pereira et al., 1998) (Table 2). Recent cytogenetic analysis of *S. carinus*, *S. fellowesii* (Karasu Ayata, 2020) and *S. seyhanensis* (Unal and Gaffaroğlu, 2016) have shown the same *2n* as in other *Squalius* and *Leuciscus* species and karyotypes consisted of 12 pairs of m, 10 pairs of sm and 3 pairs of st/a chromosomes (NF = 94); 10 pairs of m, 10 pairs of sm and 5 pairs of st/ chromosomes (NF = 90); 8 pairs of m, 14 pairs of sm and 3 pairs of st/a chromosomes (NF = 94) respectively. Boron et al. (2009) shown that *L. idus* has *2n* = 50 and NF = 86, and karyotype with 5 pairs of m, 13 pairs of sm, 3 pairs of st and 4 pairs of a chromosomes and *L. leuciscus* has *2n* = 50 and NF = 86, karyotype with 6 pairs of m, 12 pairs sm, 4 pairs of st and 3 pairs of a chromosomes. The same study (Boron et al., 2009) have also indicated that *L. leuciscus* (Syn: *Leuciscus leuciscus baicalensis kirgisorum*), *L. schmidti* and *L. bergi* have *2n* = 50 and NF = 90, and karyotype with 9 pairs of m, 11 pairs of sm and 5 pairs a chromosomes (Mazik et al., 1986). In all the previously analyzed species of *Leuciscus*, the number of m chromosomes, in *L. leuciscus* and *L. idus* the number of sm chromosomes higher and, the NF similar to the present study. In terms of NF, results presented in this study are in agreement with above mentioned studies. On the other hand, cytogenetic data of *S. cephalus* (Syn: *Leuciscus cephalus*) vary from one study to another. Al-Sabti (1986) revealed *2n* = 50 and NF = 84, karyotype with a karyotype composed of 17 pairs of m-sm and 8 pairs of st/a chromosomes, while Boron et al. (2009) identified *2n* = 50 and NF = 82, with a karyotype composed of 5 pairs of m, 11 pairs of sm, 5 pairs of st and 4 pairs of a chromosomes. Karyotype characteristics of *S. cephalus* in those studies are different from *S. anatolicus*. In addition, in the karyotypes of *S. alburnoides*, *S. lucumonis*, *S. aradensis* and *S. torgalensis* were determined chromosome structure as distinct from *S. anatolicus* (Table 2) (Gromicho and Collares-Pereira, 2004; Rossi et al., 2012; Nabais et al., 2013).

In *S. cephalus, L. idus*, and *L. leuciscus*, the heterochromatic blocks were identified in the pericentromeric regions of most chromosomes (Boron et al., 2009). Karasu Ayata (2020) and Unal and Gaffaroğlu (2016) found CH blocks in pericentromeric and distal part in chromosomes of *S. carinus*, *S. fellowesii* and *S. seyhanensis*. These studies are fairly similar to results obtained in *S. anatolicus*. While NORs are generally observed at the end of the short arms of st and sm chromosomes, they can sometimes be observed at the end of the long arms of st and sm chromosomes, on the arms of m and a chromosomes; between telomeres and centromeres, and adjacent to centromeres (Galetti et al., 1984). The present study supports the results of Galetti et al. (1984) in that NORs were localized on the short arms of sm chromosomes.

Table 2. Cytogenetic data of some *Squalius* species

Species	*2n*	Chromosome morphology	NF	References
S. carolitertii	50	10-12m+30-32sm+8st/a	92	Collares-Pereira *et al.* (1998)
S. pyrenaicus	50	12m+32sm+6st/a	94	Collares-Pereira *et al.* (1998)
S. pyrenaicus	50	16m+28sm+6a	-	Gromicho and Collares-Pereira (2004)
S. alburnoides	50	16m+30sm+4st/a	96	Gromicho and Collares-Pereira (2004)
S. lucumonis	50	16m+26sm+8st/a	-	Rossi *et al.* (2012)
S. aradensis	50	10m+36sm+4st/a	96	Nabais *et al.* (2013)
S. torgalensis	50	10m+36sm+4st/a	96	Nabais *et al.* (2013)
S. seyhanensis	50	16m+28sm+6st/a	94	Unal and Gaffaroğlu (2016)
S. carinus	50	24m+20sm+6st/a	94	Karasu Ayata (2020)
S. fellowesii	50	20m+20sm+10st/a	90	Karasu Ayata (2020)
S. anatolicus	50	14m+26sm+10st/a	90	Present study

Karasu Ayata (2020) and Unal and Gaffaroğlu (2016) reported that two NORs were on the short arms of sm chromosomes in *S. carinus*, *S. fellowesii* and *S. seyhanensis* but NORs were reported that on the long arms of second largest sm chromosomes in *L. idus* (Boron et al., 2009). Rossi et al. (2012) observed two heteromorphic in size NORs on the short arms of a medium-sized sm chromosome pair in *S. lucumonis* and this occurrence was explained that by FISH using the 45S rDNA probe. According to this study, it was evident that the 5S signals were proximal and co-localized with the distal NORs and this character should be ancestral within the entire Leuciscinae lineage. The presence of NORs on the sm chromosome pair of *S. anatolicus* is in line with NOR phenotypes and size heteromorphism of NORs presented in these studies.

In conclusion, the karyotype having typically the largest st/a chromosome pair of *S. anatolicus* is characteristic of leuciscines in terms of the chromosome number and morphology, the distribution of CH and NOR phenotype. These findings support that consistency of karyotypes and chromosomal banding patterns in especially leuciscines. In addition, the presented cytotaxonomic characters should be scrutiny by molecular methods for further cytogenetic studies to understand evolutionary processes within the related leuciscid lineages.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Terzialan Göleti'nin (Çan, Çanakkale) sulama suyu kalitesi açısından mevsimsel değişimlerinin değerlendirilmesi

Evaluation of seasonal changes in terms of irrigation water quality of Terzialan Pond (Çan, Çanakkale)

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Öz: Tarımsal sulama amaçlı yapılan göletlerin su kalitesinin belirlenmesi gerek zirai üretim gerekse sucul canlıların yaşamı açısından büyük önem taşımaktadır. Bu çalışmada, Terzialan Göleti'nde suyun fiziko-kimyasal parametrelerinin ve metal konsantrasyonlarının mevsimlere bağlı değişimleri incelenmiştir. 2019 yılında, dört örnekleme noktasından, mevsimsel olarak alınan su örneklerinin fiziko-kimyasal analizleri APHA (2012) standart metoduna göre, ağır metal konsantrasyonlarının saptanması ise ICP-OES ile yapılmıştır. Göletin su kalitesi Yüzeysel Su Kalitesi Yönetimi Yönetmeliği (YSKYY), İnsani Tüketim Amaçlı Sular Hakkında Yönetmelik (İTASHY) ve Türk Standardı İnsani Tüketim Amaçlı Sular (TS266) sınıflarına göre değerlendirilmiştir. Sonuç olarak Terzialan Göleti'nin, Yüzeysel Su Kalitesi Yönetimi Yönetmeliğindeki sınıflandırmaya göre fosfat konsantrasyonu açısından II. sınıf su kalitesi, diğer parametreler açısından I. sınıf su kalitesine sahip olduğu saptanmıştır. Gölet, ABD tuzluluk laboratuvarına göre C2-S1 sınıfında, Wilcox diyagramına göre çok iyi sınıfında olup tarımsal sulama suyu olarak kullanılabilir niteliktedir. Doğal etkilerin yanında mevsimlere bağlı tarımsal ve rekreasyonel faaliyetlerle ilişkili antropojenik etkiler yüzünden su kalitesinde değişimler görülmüştür. Göletin periyodik olarak izlenmesi, çevresel yönetim için yararlı olacaktır.

Anahtar kelimeler: Su kalitesi, ağır metal kirliliği, Terzialan Göleti, Çan, Çanakkale

Abstract: Determining the water quality of agricultural irrigation ponds has great importance both for agricultural production and for aquatic organisms. In this study, seasonal changes of the physico-chemical parameters and metal concentrations of water in Terzialan Pond were investigated. In 2019, physico-chemical analysis of the water samples taken from four sampling points are made according to APHA (2012) standard method, determining metal concentrations with ICP-OES. The water quality of the pond is evaluated according to YSKYY, ITASHY and TS266 classes. Terzialan Pond, according to Surface Water Quality Management Regulations is found to be second quality in terms of phosphate concentration and first quality in other parameters. The pond can be used as agricultural irrigation water being in C2-S1 class according to US Salinity Laboratory diagram and very good- good class according to Wilcox diagram. Changes in water quality are observed due to anthropogenic effects depending on seasonal agricultural and recreational activities, besides natural effects. Periodic monitoring of the pond will be beneficial for environmental management.

Keywords: Water quality, heavy metal pollution, Terzialan Pond, Çan, Çanakkale

GİRİŞ

Son yıllarda ülkemizde mevcut su kaynaklarının teknik ve ekonomik yönden daha pratik ve daha az maliyetli kullanılmasına olanak sağlayan göletler, su temini, sel taşkınlarını önleme, elektrik enerjisi elde etme, tarım arazileri için sulama suyu sağlama gibi amaçlarla inşa edilen barajlara alternatif olmuştur. Küçük derelerin, yağmurların ve eriyen kar sularının, genellikle bir set ardında biriktirilmesiyle oluşturulan ve böylelikle daha kısa sürede zirai kullanıma sunulabilen göletler, aynı zamanda sportif olta balıkçılığı, mesire yeri olarak kullanım gibi rekreasyonel faaliyetler açısından da bölge halkının ilgisini çekmektedir (Fayrap, 2011; Aşık, 2016).

Sulama amaçlı kullanılan su kaynakları, bilinçsiz yapılan gübreleme ve ilaçlama gibi tarımsal faaliyetler ile diğer antropojenik etkilere bağlı ve sürekli olarak kirliliğe maruz kalmaktadır. Yetiştirilen ürün için büyük önem arz eden sulama suyunun kalitesindeki olumsuz değişimler gerek toprak verimliliğini gerekse tarımsal üretimi sınırlamaktadır (Parlak ve Parlak, 2006). Ayrıca çeşitli yollarla sucul ortama karışan kirleticiler, bu sularda yaşayan organizmaların dokusunda ve organlarında birikerek biyoçeşitliliğin önemli derecede azalmasına neden olmakta ve besin zincirinin en üst basamağında bulunan insan için de risk oluşturmaktadır (Dodson vd., 2000). Dolayısıyla su kalitesinin ekolojik açıdan değerlendirilmesi, düşük kaliteye sahip su kaynaklarının iyileştirilerek sürdürülebilirliğinin sağlanması, sucul ekosistemlerinin korunması ve yüzey sularının yönetimi için büyük önem taşımaktadır.

Ülkemizdeki sucul ekosistemlerin kirlilik problemini ortaya koymak amacıyla yapılan birçok çalışma mevcuttur (Selvi vd., 2015; Türkmen ve Akbulut, 2015; Kalyoncu vd., 2016; Ünlü ve Alpar, 2016; Selvi vd., 2017a, 2017b; Mutlu ve Kutlu, 2017; Hamidi vd., 2018; Baki, 2019; Çavuş ve Şen, 2020). Ancak

Terzialan (Çan, Çanakkale) çevresinde zirai ve endüstriyel faaliyetler yoğun olarak yapılmasına rağmen; bölgenin önemli sulama kaynaklarından biri olan Terzialan Göleti'nde su kalitesinin belirlenmesi kapsamında bir çalışma bulunmamaktadır.

Bu çalışmada, Terzialan Göleti'nin fiziko-kimyasal özelliklerine ait parametrelerin ve sudaki ağır metal konsantrasyonlarının mevsimsel değişimlerinin belirlenmesi, sonuçların Yüzeysel Su Kalitesi Yönetimi Yönetmeliği (YSKYY, 2016), İnsani Tüketim Amaçlı Sular Hakkında Yönetmelik (İTASHY, 2013) ve Türk Standardı İnsani Tüketim Amaçlı Sular (TS 266/T2, 2005) ulusal standartlar ile karşılaştırılarak değerlendirilmesi ve elde edilen verilerin Terzialan Göleti'nde önümüzdeki yıllarda su kalitesiyle ilgili yürütülecek çalışmalar için bir veri tabanı oluşturması amaçlanmıştır. Gölette su kalitesi ile ilgili yapılan ilk çalışma olması sebebiyle önemlidir.

MATERYAL ve YÖNTEM

Çalışma alanı ve örnekleme

Çalışma, Çanakkale'nin Çan ilçesinin yaklaşık 10 km güneybatısında yer alan Terzialan Göleti'nde (39°57'31" K – 27°00'19" D) gerçekleştirilmiştir. Çevresindeki derelerin, yağmurların ve eriyen kar sularının beslediği, 0,242 km^2 yüz ölçümüne sahip gölet, tarımsal sulama ve hayvan içme suyunu temin etmek, bölgedeki su taşkınlarını önlemek amacıyla kurulmuştur (Şekil 1). Göletin mevsime bağlı yüzeysel su kalitesinin durumunu tespit etmek için gölet üzerinden 4 farklı örnek noktası belirlenerek 2019 yılının Ocak, Nisan, Temmuz ve Ekim aylarında mevsimsel örnekleme yapılmıştır. Örnekler 3 tekrar olacak şekilde 500 ml alınarak, +4°C koşullarda soğuk zincir ile laboratuvara taşınmıştır.

Çalışma bölgesi, bulunduğu konum itibariye geçiş iklimi özellikleri göstermektedir ve genel karakteriyle Akdeniz iklimi özelliklerini yansıtmaktadır. Yaz aylarında yağış miktarı oldukça düşük olmaktadır. Yağışların en fazla görüldüğü mevsim kış ve ilkbahar aylarıdır.

Terzialan Göleti'nde, göletin su özelliklerini homojen olarak sağlayabilecek dört adet istasyon belirlenmiştir. Tarım alanlarıyla çevrili olan göletin 1. ve 4. istasyon olarak seçilen bölgelerin arasında kalan alanlar bölge halkının piknik alanı olup, olta balıkçılarının da sıklıkla konuşlandığı yerlerdir. İnsan kaynaklı kirliliğin etkilerini belirlemek için bu bölgelerden numune alınmıştır. Göletin güneyinde çevre köyleri birbirine bağlayan işlek bir yol ve yolun üst kısmında birkaç hane ile hayvan damları bulunmaktadır. Ayrıca tarımsal amaçlı kullanılan suyu çekmek için kullanılan pompalar sıklıkla bu bölgededir. Dolayısıyla antropojenik etkinin tespit edilebilmesi açısından 2. istasyon, bu yolun gölete en yakın olduğu noktadan seçilmiştir. Göleti besleyen küçük derelerin su kalitesi açısından etkisini belirlemek için de göletin geleğenlerine yakın noktalar da 3. ve 4. istasyon olarak seçilmiştir.

Şekil 1. Terzialan Göleti ve örnekleme noktalarının konumu
Figure 1. Terzialan Pond and location of sampling points

Suyun fiziko-kimyasal parametre analizleri

Suyun fiziko-kimyasal özelliklerinin tespiti için pH, sıcaklık, çözünmüş oksijen, elektriksel iletkenlik ve tuzluluk gibi parametreler, çoklu parametre ölçüm cihazı (YSI Pro Plus, ABD) kullanılarak arazide ölçülmüştür. Diğer su kalite parametrelerinden, kimyasal oksijen ihtiyacı (KOİ), biyokimyasal oksijen ihtiyacı ($BOİ_5$), toplam fosfat (PO_4^{3-}), nitrat azotu (NO_3^--N), nitrit azotu (NO_2^--N), amonyum azotu (NH_4^+-N), sodyum (Na^+), potasyum (K^+), klorür (Cl^-) toplam sertlik ve (Ca^{+2} ve Mg^{+2}) analizleri standart metoda göre yapılmıştır (APHA, 2012). Ayrıca aşağıda verilen formüllere göre su örneklerinin magnezyum oranı (MR), Kelly indeksi (KI), sodyum yüzdesi (%Na) ve sodyum adsorbsiyon oranı (SAR) hesaplanmıştır (Özer ve Köklü, 2019; Demer ve Hepdeniz, 2018; Bouderbala, 2017).

$$MR = \frac{Mg^{+2}}{Ca^{+2}+Mg^{+2}} x100 \quad (1)$$

$$KI = \frac{Na^{+2}}{Ca^{+2}+ Mg^{+2}} \quad (2)$$

$$\%Na = \left(\frac{Na^{+2} + K^{+2}}{Ca^{+2}+ Mg^{+2}+ Na^{+2}+K^{+2}}\right) x\ 100 \quad (3)$$

$$SAR = \frac{Na^{+2}}{\sqrt{\frac{Ca^{+2}+ Mg^{+2}}{2}}} \quad (4)$$

Suda ağır metal analizleri

Sudaki ağır metal konsantrasyonunu belirlemek için alınan su numunelerine (demir, bakır, çinko, kurşun, kadmiyum) ön ekstraksiyon işlemi yapıldıktan sonra sonuçlar İndüktif Eşleşmiş Plazma Atomik Emisyon Spektrometresi (ICP-OES) (Varian Liberty Sequential) ile belirlenmiştir. Ön ekstraksiyon işlemi, örneklerin 0,45 µm şırınga filtreden geçirilerek üzerine 5 ml HNO_3 damlatılması ile yapılmıştır (Smith vd., 2007). Analizi gerçekleştirilen her metal için stok standart kalibrasyon çözeltileri kullanılmış olup ölçüm aralığına uygun kalibrasyon standart çözeltileri hazırlanmıştır. Okutulan her bir parametre türü için önce kalibrasyon eğrisi oluşturularak örnekler cihaza verilmiş ve sonuçlar alınmıştır.

İstatiksel analizler ve değerlendirme

Sonuçların istatistiksel değerlendirilmesi SPSS 20 (SPSS Inc., Chicago, IL, USA) programı kullanılarak gerçekleştirilmiştir. Sudaki ağır metal konsantrasyon bulgularına iki yönlü varyans analizleri uygulanarak, konsantrasyon-istasyon ve konsantrasyon-zaman ikili interaksiyonu belirlenmiştir. Gruplar arasındaki varyans, P <0,05 olarak değerlendirilmiştir (Logan, 2010).

Elde edilen verilerin yıllık ortalama değerleri, Yüzeysel Su Kalitesi Yönetimi Yönetmeliği (YSKYY, 2016), İnsani Tüketim Amaçlı Sular Hakkında Yönetmelik (İTASHY, 2013) ve Türk Standardı İnsani Tüketim Amaçlı Sular (TS 266/T2, 2005) ulusal standartlar ile karşılaştırılarak, sulama suyu kriterlerine göre değerlendirilmiştir. Ayrıca tarımsal sulama amaçlı kullanılan suyun sınıflandırmasını belirlemede ABD tuzluluk ve Wilcox diyagramlarından yararlanılmıştır.

BULGULAR

Terzialan Göleti'nin mevsimsel su kalitesinin tespiti için Terzialan Göleti'nden belirlenen istasyonlardan elde edilen suyun fiziko-kimyasal parametreleri YSKYY ve İTASHY standartlarına göre karşılaştırılarak sonuçlar Tablo 1'de gösterilmiştir.

Analiz bulgularına göre en yüksek pH değeri, göletin su kütlesinin nispeten azaldığı yaz mevsiminde belirlenmiştir. Ayrıca pH değerinin kış mevsiminde de artış gösterdiği tespit edilmiştir. Çalışmada ölçülen çözünmüş oksijen konsantrasyonunun, yüzey suyu sıcaklıklarına bağlı olarak yaz aylarında azaldığı saptanmıştır (Tablo 1). Çalışmada 0,70 – 6,21 mg/L arasında ölçülen biyolojik oksijen ihtiyacı değeri, yaz mevsiminde en düşük ve sonbahar mevsiminde en yüksek konsantrasyona ulaşmış olup, YSKKY (2016)'ya göre I. ve II. sınıf su olarak değerlendirilmiştir. Çalışmada ölçülen en yüksek kimyasal oksijen ihtiyacı miktarı yaz mevsiminde ölçülmesine rağmen Terzialan Göleti'nin su kalitesi, kimyasal oksijen ihtiyacı bakımından YSKKY (2016) 'ya göre I. sınıf su olarak belirlenmiştir. Terzialan Göleti'nde 0,38 – 2,02 mg/L arasında ölçülen NO_3^--N konsantrasyonu, sonbahar mevsiminde artış göstermiş olsa da YSKYY (2016) 'ya göre I. sınıf su kalitesindedir. Terzialan Göleti'nde ölçülen sudaki toplam fosfat konsantrasyonu, 0,02 – 0,63 mg/L arasında değişmektedir. En yüksek fosfat konsantrasyonu yaz mevsimde tespit edilmiştir ve YSKYY (2016) 'ya göre II. sınıf su kalitesindedir. Çalışmada 19 – 36 mg/L arasında ölçülen klorür konsantrasyonunun, İTASHY (2013) tarafından belirtilen limitlerinin oldukça altında olduğu tespit edilmiştir. Terzialan Göleti'nden alınan su örneklerinde ölçülen elektriksel iletkenlik değerinin en yüksek olduğu mevsim sonbahar mevsimidir. Gölet sularının elektriksel iletkenliği 235–359 µS/cm arasında değişmekte olup, YSKYY (2016) 'ya göre I. sınıf su olarak belirlenmiştir. Çalışmada ölçülen suların sertlik değeri 376 – 539 mg/L arasında değişmekte olup, Terzialan Göleti sularının sert sular sınıfına girdiği tespit edilmiştir.

Tablo 1. Belirlenen istasyonlardan alınan su örneklerinin mevsimlere göre ortama fiziko-kimyasal parametre değerleri ile YSKYY ve İTASHY standartları ile kalite kriterlerinin karşılaştırılması

Table 1. Comparison of the physico-chemical parameter values of the water samples taken from the determined stations according to the seasons and the YSKYY and İTASHY standards and quality criteria

P	B	M	1. İstasyon	2. İstasyon	3. İstasyon	4. İstasyon	İTASHY[1]	YSKYY[2] I	II	III	IV
T	°C	K	7,79±0,04 Bd	7,97±0,04 Ad	7,83±0,04 Bd	8,01±0,01 Ad	25	-	-	-	-
		İ	17,23±0,02 Bb	18,06±0,04 Ab	16,79±0,02 Db	16,89±0,07 Cb					
		Y	21,64±0,25 Ba	23,10±0,07 Aa	23,36±0,18 Aa	21,60±0,11 Ba					
		S	10,28±0,03 Bc	10,59±0,12 Ac	10,58±0,09 Ac	10,10±0,09 Cc					
pH	-	K	7,94±0,05 Ab	8,02±0,02 Ab	7,90±0,02 Aab	7,92±0,01 Aa	6,5-9,5	6-9	6-9	6-9	6-9
		İ	7,75±0,04 Ac	7,69±0,02 Bc	7,51±0,02 Cc	7,79±0,01 Ac					
		Y	8,15±0,03 Ba	8,08±0,02 Ca	7,98±0,02 Da	8,25±0,03 Ab					
		S	7,62±0,02 Ad	7,54±0,02 Bd	7,57±0,05 ABc	7,63±0,02 Ad					
Çözünmüş Oksijen	mg/L	K	8,80±0,08 Ba	8,90±0,04 ABa	8,90±0,06 ABa	8,95±0,03 Aa	-	>8	6	3	<3
		İ	7,38±0,02 Bc	7,17±0,06 Cc	7,70±0,12 Ab	7,38±0,06 Bc					
		Y	5,79±0,19 Bd	5,38±0,06 Cd	6,09±0,05 Ac	5,84±0,05 Bd					
		S	7,99±0,04 Ab	7,87±0,04 Bb	7,91±0,02 Bb	7,73±0,02 Cb					
Tuzluluk	ppt	K	0,13±0,00 Aa	0,13±0,00 Aa	0,13±0,00 Aa	0,13±0,00 Aa	-	-	-	-	-
		İ	0,13±0,00 Aa	0,14±0,00 Aa	0,14±0,00 Aa	0,13±0,00 Aa					
		Y	0,13±0,00 Aa	0,14±0,00 Aa	0,13±0,00 Aa	0,13±0,00 Aa					
		S	0,14±0,00 Aa	0,13±0,00 Aa	0,14±0,00 Aa	0,13±0,00 Aa					
İletkenlik	µS/cm	K	255,66±10,06 Bb	266,00±10,44 ABbc	271,00±13,45 ABb	284,00±8,88 Ab	2500	<400	1000	3000	>3000
		İ	251,66±24,78 ABb	248,00±26,66 Bc	261,33±16,56 Ab	235,33±14,01 Cc					
		Y	294,33±9,07 Aa	297,00±10,53 Ab	286,00±14,17 Bb	293,33±15,30 Ab					
		S	317,33±21,22 Ba	337,00±16,52 ABa	318,66±10,01 Ba	359,00±18,73 Aa					
KOİ	mg/L	K	5,83±1,63 Ab	6,06±0,4 Ab	6,02±0,32 Ab	5,98±0,32 Ab	-	<25	50	70	>70
		İ	5,30±0,91 Ab	6,04±0,3 Ab	5,47±0,24 Ab	5,38±0,10 Ab					
		Y	17,70±1,33 Ba	18,58±0,61 ABa	19,47±0,53 Aa	17,60±0,50 Ba					
		S	5,63±1,12 Ab	5,55±1,18 Ab	6,06±1,00 Ab	5,36±1,10 Ab					
$BOİ_5$	mg/L	K	2,38±0,18 Ab	2,26±0,14 Ab	2,32±0,16 Ab	2,37±0,26 Ab	-	<4	8	20	>20
		İ	1,40±0,17 Bc	1,55±0,16 Bc	1,92±0,08 Ac	1,83±0,12 Ac					
		Y	0,70±0,08 Bd	0,77±0,04 ABd	0,83±0,05 Ad	0,82±0,06 ABd					
		S	5,45±0,22 Ba	6,21±0,17 Aa	6,10±0,17 Aa	5,36±0,06 Ba					
NH_4^+-N	mg/L	K	öd	öd	öd	öd	-	-	-	-	-
		İ	öd	öd	öd	öd					
		Y	öd	öd	öd	öd					
		S	öd	öd	öd	öd					
NO_2^- - N	mg/L	K	öd	öd	öd	öd	-	<3	10	20	>20
		İ	öd	öd	öd	öd					
		Y	öd	öd	öd	öd					
		S	öd	öd	öd	öd					

Tablo 1. Devamı
Table 1. Continued

NO_3^- - N	**mg/L**	**K**	0,38±0,17 ABb	0,60±0,17 Ab	0,53±0,33 Ab	0,53±0,22 Ab	-	<3	10	20	>20
		İ	öd	öd	öd	öd					
		Y	öd	öd	öd	öd					
		S	1,82±0,34 ABa	1,75±0,36 ABa	2,01±0,35 Aa	2,02±0,41 Aa					
PO_4^{3-}	**mg/L**	**K**	0,04±0,02 Ac	0,03±0,03 Aa	0,05±0,01 Ac	0,02±0,01 Ac	-	<0,05	0,16	0,65	>0,65
		İ	0,13±0,03 Ab	0,06±0,02 Bb	0,14±0,04 Ab	0,18±0,03 Ab					
		Y	0,63±0,08 Aa	0,61±0,18 Ba	0,53±0,10 Aa	0,48±0,18 Aa					
		S	0,04±0,02 Ac	0,03±0,03 Ab	0,07±0,01 Ac	0,04±0,03 Ac					
Cl^-	**mg/L**	**K**	20,00±3,65 Cc	19,00±2,36 Cc	27,00±3,95 Bb	32,00±4,51 Ab	250	-	-	-	-
		İ	25,00±4,11 Bb	21,00±2,45 Cb	24,00±2,57 Bc	28,00±3,57 Ac					
		Y	18,00±2,68 ABd	14,00±1,98 Cd	19,00±2,09 Ad	17,00±1,45 Bd					
		S	29,00±4,23 Ba	25,00±3,22 Ca	30,00±4,26 Ba	36,00±4,15 Aa					
Na^+	**mg/L**	**K**	13,95±0,23 Aa	12,79±0,12 Ab	13,58±0,12 Ab	14,53±0,17 Aa	-	-	-	-	-
		İ	11,25±0,19 Ac	11,63±0,05 Ac	11,96±0,15 Ac	11,49±0,18 Ac					
		Y	12,35±0,15 Ab	12,81±0,16 Ab	13,24±0,22 Ab	12,91±0,06 Ab					
		S	14,21±0,17 Aa	14,09±0,07 Aa	14,52±0,15 Aa	14,01±0,15 Aa					
Ca^{+2}	**mg/L**	**K**	35,21±0,63 Aa	34,85±0,75 Aa	34,65±0,61 Aa	33,21±0,61 Aa	-	-	-	-	-
		İ	27,32±0,13 Ac	28,58±0,36 Ac	28,05±0,18 Abc	28,41±0,53 Ab					
		Y	29,15±0,04 Ab	29,54±0,50 Ab	28,64±0,33 Ab	27,88±0,13 Ac					
		S	27,64±0,48 Ac	28,11±0,10 Ac	27,43±0,12 Ac	28,17±027 Abc					
Mg^{+2}	**mg/L**	**K**	25,11±0,28 Ad	24,32±0,12 Ad	25,12±0,75 Ac	25,87±0,12 Ac	-	-	-	-	-
		İ	29,15±0,31 Aa	29,35±0,33 Aa	28,96±0,50 Aa	29,15±0,32 Aa					
		Y	27,36±0,06 Ab	28,25±0,50 Ab	26,49±0,34 Ab	26,66±0,48 Ab					
		S	26,32±0,10 Ac	27,19±0,68 Ac	26,09±0,41 Ab	26,75±0,55 Ab					
K^+	**mg/L**	**K**	8,65±0,60 Aab	8,55±0,44 Ab	8,82±0,16 Aa	8,74±0,47 Aa	-	-	-	-	-
		İ	7,79±0,77 Ab	7,62±0,12 Ab	7,93±0,38 Aa	7,35±0,51 Ab					
		Y	8,78±0,82 Ab	8,92±0,74 Aa	8,65±0,82 Ab	8,83±0,28 Aa					
		S	8,01±0,47 Aa	8,17±0,05 Ac	8,15±0,12 Ac	8,09±0,25 Ac					
Sertlik	**mg/L**	**K**	485,00±26,32 Ab	425,00±22,54 Ab	437,00±24,38 Ab	471,00±26,85 Ab	-	-	-	-	
		İ	512,00±30,28 Aa	528,00±36,25 Aa	506,00±31,97 Aa	539,00±35,41 AAa					
		Y	392,00±24,51 Ac	381,00±21,94 Ac	365,00±23,47 Ac	376,00±21,82 Ac					
		S	428,00±28,16 Ab	430,00±23,77 Ab	394,00±25,89 Ac	411,00±29,12 Ab					

*Büyük harfler istasyonlar arası, küçük harfler ise mevsimler arasındaki farklılıkları göstermektedir (P <0,05). *P: Parametre, B: Birim, M: Mevsim, K: Kış, İ: İlkbahar, Y: Yaz, S: Sonbahar *öd: Ölçülemeyecek düzeydedir.

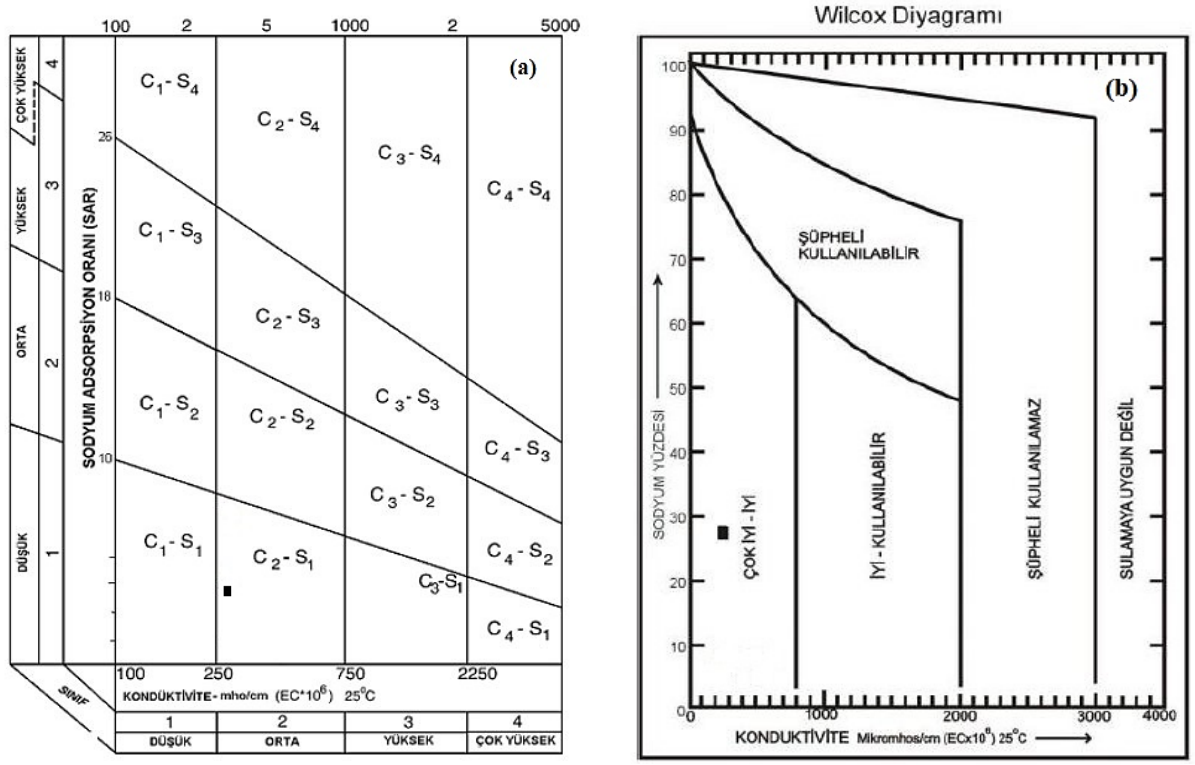
[1]İTASHY: 07/03/2013 tarihli ve 28580 sayılı Resmî Gazete'de yayımlanan İnsani Tüketim Amaçlı Sular Hakkında Yönetmelikte Değişiklik Yapılmasına Dair Yönetmelik [2]YSKYY: 10/08/2016 tarihli ve 29797 sayılı Resmî Gazete'de yayınlanan Yüzeysel Su Kalitesi Yönetimi Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik

Tablo 2 incelendiğinde; Terzialan Göleti'nin SAR değerinin 2,11-2,81 arasında değiştiği ve yıllık ortalamanın 2,46 olduğu görülmektedir. %Na değeri ise 24,65-29,75 arasındadır ve yıllık ortalama 27,36 olarak saptanmıştır. MR değeri 41,10-51,62 arasında değişmekte olup, yıllık ortalama 47,62 olarak belirlenmiştir.

Buna ek olarak KI değeri 0,19-0,27 arasında olup yıllık ortalama 0,23'tür.Terzialan Göleti'nin, mevsimsel ortalamalara göre ABD tuzluluk diyagramında C2-S1 sınıfında olduğu belirlenmiştir. Wilcox diyagramında ise çok iyi sınıfında yer aldığı saptanmıştır (Şekil 2).

Tablo 2. Terzialan Göleti'nin MAR, KI, %Na ve SAR değerleri (Özer ve Köklü, 2019)
Table 2. Terzialan Pond's MR, KI, %Na and SAR values (Özer ve Köklü, 2019)

P	M	1. İstasyon	2. İstasyon	3. İstasyon	4. İstasyon	
MR	K	41,63	41,10	42,03	43,79	
	İ	51,62	50,66	50,80	50,64	<50 Uygun
	Y	48,42	48,88	48,05	48,88	>50 Uygun değil
	S	48,78	49,17	48,75	48,71	
KI	K	0,23	0,22	0,23	0,25	
	İ	0,20	0,20	0,21	0,20	<1 Uygun
	Y	0,22	0,22	0,24	0,24	>1 Uygun değil
	S	0,26	0,25	0,27	0,26	
%Na	K	27,26	26,51	27,26	28,26	<20 Mükemmel
	İ	25,20	24,94	25,86	24,66	20-40 İyi
	Y	27,22	27,33	28,42	28,50	40-60 İzin verilebilir
	S	29,17	28,70	29,75	28,69	60-80 Şüpheli
						>80 Uygun değil
SAR	K	2,54	2,35	2,48	2,67	<20 Mükemmel
	İ	2,11	2,16	2,24	2,14	20-40 iyi
	Y	2,32	2,38	2,52	2,47	40-60 İzin verilebilir
	S	2,74	2,68	2,81	2,67	60-80 Şüpheli
						>80 Uygun değil



Şekil 2. Terzialan Göleti'nin (a) ABD tuzluluk ve (b) Wilcox diyagramları
Figure 2. (a) US salinity and (b) Wilcox diagrams of Terzialan Pond

Terzialan Göleti'nin ağır metal konsantrasyonları TS266 ve YSKYY göre karşılaştırılarak sonuçlar Tablo 3'te verilmiştir. Çalışmada en düşük demir konsantrasyonu 2. istasyonda yaz mevsiminde belirlenmiş olup, mevsimler arası istatistiksel fark önemlidir (P <0,05). Bununla birlikte en yüksek demir konsantrasyonu, 3. istasyonda sonbahar mevsiminde belirlenmiş ve istasyonlar arası fark istatistiksel olarak önemli bulunmuştur (P <0,05). Yıllık ortalama demir (179 µg/L) konsantrasyonunun, TS266 'ya göre Tip II Sınıf II ve YSKYY 'e göre I. Sınıf grubunda yer aldığı belirlenmiştir. Gölette belirlenen en düşük bakır konsantrasyonu 3. istasyonda ve kış mevsiminde olup, mevsimler arası farkların anlamlı olduğu görülmektedir. İlkbahar mevsiminde 3. istasyonda en yüksek ölçülen bakır derişiminin istasyonlar arası ve mevsimler arası farkları istatistiksel olarak anlamlıdır (P <0,05). Sudaki konsantrasyonu yıllık ortalama 18,9 µg/L olarak belirlenen bakır, TS266 'ya göre Tip I Sınıf I, YSKYY 'e göre I. Sınıf grubunda yer aldığı belirlenmiştir. Sudaki çinko konsantrasyonları değerlendirildiğinde ise en yüksek konsantrasyon 78,13 µg/L olarak kış mevsiminde ve 3. istasyonda ölçüldüğü anlaşılmaktadır. Ayrıca istasyonlar arası fark istatistiksel olarak önemlidir (P <0,05). En düşük çinko konsantrasyonu (24,19 µg/L) yaz mevsiminde ve 1. istasyonda ölçülmüş olup gerek istasyonlar gerekse mevsimler arasındaki farkların önemli olduğu belirlenmiştir (P <0,05). Yıllık ortalama çinko konsantrasyonun (47,2 µg/L), TS266 ve YSKYY 'ya göre Sınıf I grubunda yer aldığı belirlenmiştir. Çalışmada ölçülen kurşun konsantrasyonları en yüksek 1,41 µg/L olarak sonbahar mevsiminde ve 2. istasyonda tespit edilmiştir. Bununla beraber istasyonlar arası fark istatistiksel olarak önemlidir (P <0,05). En düşük kurşun konsantrasyonu ise 0,11 µg/L olarak 4. istasyonda ve kış mevsiminde ölçülmüş olup, mevsimler arası farklar önemlidir (P <0,05). Kurşun konsantrasyonları yıllık ortalama olarak değerlendirildiğinde (0,64 µg/L); TS266 ve YSKYY 'ya göre Sınıf I grubunda yer aldığı belirlenmiştir. En düşük kadmiyum konsantrasyonu (0,14 µg/L) kış mevsiminde ve 4. istasyonda ölçülmüştür. Bununla birlikte istasyonlar arası farklar önemlidir. En yüksek kadmiyum konsantrasyonu (0,82 µg/L) yaz mevsiminde 2. istasyonda belirlenmiş olup hem mevsimler hem de istasyonlar arası farklar istatistiksel olarak anlamlıdır (P <0,05). Ayrıca yıllık ortalama 0,51 µg/L olarak ölçülen kadmiyum konsantrasyonunun, TS266 ve YSKYY 'ya göre Sınıf I grubunda yer aldığı belirlenmiştir.

Tablo 3. Terzialan Göleti'nde belirlenen istasyonlardan alınan su örneklerinin mevsimlere göre metal konsantrasyonu ile YSKYY ve TS266 standartlarına göre kalite kriterleri (µg/L)

Table 3. The metal concentration of the water samples taken from the stations determined from Terzialan Pond according to the seasons and the quality criteria according to YSKYY and TS266 standards (µg/L)

Metal	M	1. İstasyon	2. İstasyon	3. İstasyon	4. İstasyon	TS266[1] Kılavuz Değerler		YSKYY[2]			
						Tip 1 Sınıf 1	Tip 2 Sınıf 2	I. Sınıf	II. Sınıf	III. Sınıf	IV. Sınıf
Fe	K	131,52±5,93Bb	113,58±13,7Cb	173,45±8,76Ac	184,24±11,18Aa	50	200	<300	1000	5000	>5000
	İ	239,70±18,58Aa	231,72±18,0Aa	261,72±22,88Ab	190,43±8,66Ba						
	Y	90,90±4,40Ac	73,21±6,60Bc	88,93±5,45Ad	74,53±3,91Bb						
	S	263,02±12,15Ba	210,40±7,18Ca	351,41±28,70Aa	187,96±8,49Ca						
Cu	K	15,4±0,06Ab	12,8±0,05Bd	10,9±0,04Bd	12,3±0,04Cbc	100	2000	<20	50	200	>200
	İ	23,8±0,36Ba	26,2±0,11Ba	35,6±0,08Aa	25,8±0,17Ba						
	Y	24,4±0,10Aa	16,0±0,11Cc	19,4±0,04Bc	13,5±0,04Db						
	S	15,2±0,04Cb	18,5±0,05Bb	21,6±0,05Ab	11,3±0,03Dc						
Zn	K	51,01±0,64Cb	35,30±1,07Da	78,13±1,07Aa	61,74±0,54Ba	100	5000	<200	500	2000	>2000
	İ	48,79±0,97Cb	25,27±3,52Db	73,53±2,27Ab	31,06±1,17Bd						
	Y	24,19±1,76Dc	32,52±1,49Ba	28,00±1,16Cc	43,85±2,13Ac						
	S	66,13±3,24Ba	33,62±1,08Da	70,60±2,77Ab	50,80±1,37Cb						
Pb	K	0,18±0,05Cd	0,38±0,03Ad	0,25±0,03Bb	0,11±0,02Cd	10	10	<10	20	50	>50
	İ	0,59±0,01Db	0,93±0,02Ab	0,82±0,01Ba	0,71±0,01Ca						
	Y	0,34±0,03Dc	0,78±0,02Bc	0,86±0,03Aa	0,51±0,03Cb						
	S	1,25±0,09Ba	1,41±0,11Aa	0,81±0,03Ca	0,26±0,03Dc						
Cd	K	0,42±0,03Bab	0,58±0,15Ac	0,37±0,06Bc	0,14±0,02Cc	5	5	<2	5	7	>7
	İ	0,39±0,02Cb	0,44±0,03Cb	0,62±0,04Bb	0,79±0,07Aa						
	Y	0,46±0,03Ca	0,82±0,04Aa	0,71±0,01Ba	0,66±0,03Bb						
	S	0,32±0,03Cc	0,49±0,02Bb	0,66±0,04Aab	0,22±0,02Dc						

*Büyük harfler istasyonlar arası, küçük harfler ise mevsimler arasındaki farklılıkları göstermektedir (P <0,05). *M: Mevsim, K: Kış, İ: İlkbahar, Y: Yaz, S: Sonbahar
*Fe: Demir, Cu: Bakır, Zn: Çinko, Pb: Kurşun, Cd: Kadmiyum
[1]TS266: Türk Standardı, İnsani Tüketim Amaçlı Sular (2005) [2]YSKYY: 15 Nisan 2015 Tarihli ve 29327 sayılı Resmî Gazete'de yayınlanan Yüzeysel Su Kalitesi Yönetimi Yönetmeliğinde Değişiklik Yapılmasına Dair Yönetmelik

TARTIŞMA VE SONUÇ

Su kalitesi parametreleri, sudaki bileşiklerin toksisitesi ile suda yaşayan türlerin verimliliğini ve fizyolojik durumlarını önemli ölçüde etkilemektedir. Çalışmada, Terzialan Göleti su kalitesinin mevsimlere bağlı olarak değişimi değerlendirilerek durum tespiti yapılmıştır.

Bölgedeki kayaçların karbonat içeriği, mevsimsel yağışlarla göl sularına karışarak suyun bazik özellik kazanmasına neden olabilmektedir. Suyun asitliğini veya alkalinitesini gösteren pH, tatlı sularda doğal faktörlerin yanı sıra antropojenik girdiler nedeniyle büyük ölçüde değişebilmekte ve sucul yaşamı önemli ölçüde etkilemektedir (Abdelaty, 2018). Atıcı (2020), Van'da bulunan Dönerdere, Yumruklu, Değirmigöl ve Dolutaş Göletlerinin pH değerini 8,88-9,00 saptamıştır. Çiçek vd. (2017) tarafından yapılan çalışmada ise Eskişehir'de yer alan Keskin, Çukurhisar, Borabey Kanlıpınar, Sarısungur ve Yukarıkartal Göletlerinin pH değerinin 8,4-8,9 arasında değiştiği bildirilmiştir. Yaptığımız çalışmada, Terzialan Göleti'nin pH değerinin 7,51-8,25 arasında değiştiği ve literatürdeki verilere göre düşük olduğu belirlenmiştir. Bu durum arazilerin jeolojik karakterlerinin farklı olmasından kaynaklanmaktadır (Rahman vd., 2017).

Sudaki çözünmüş oksijen miktarı, sucul canlıların metabolik faaliyetlerinde görevli olup, su kalitesinin belirlenmesinde indikatör olarak kullanılmaktadır (Ustaoğlu vd., 2020). Yüzey suyu sıcaklığı ise biyolojik aktivite hızını artıran ve oksijen doygunluğunu azaltan önemli bir parametredir. Cüce vd. (2020), Çambaşı Göleti'nin (Ordu), çözünmüş oksijen miktarını en düşük yaz mevsiminde (5,60 mg/L), en yüksek ilkbahar mevsiminde (7,70 mg/L) olarak tespit etmişlerdir. Çalışmamızda da benzer şekilde en düşük çözünmüş oksijen değeri yaz aylarında 5,79 mg/L olarak ölçülmüştür. Yaz mevsiminde sudaki oksijen seviyesinin düşmesine, sıcaklık artışına bağlı olarak artan organik madde ayrışması ve fotosentetik aktivite neden olmaktadır (Lone vd., 2020). Bunun nedeni ılık suyun oksijen tutma kapasitesinin, soğuk sudan daha düşük olması ve çözünmüş oksijenin, su kaynağının sıcaklığından etkilenmesidir (WHO, 2017).

Kimyasal oksijen ihtiyacı, sudaki kirlenme derecesini oksijen miktarı cinsinden ifade etmek için kullanılmaktadır (Haldar vd., 2020). Mutlu ve Paruğ (2018), Dereköy Göleti'nin kimyasal oksijen ihtiyacının en düşük değerini aralık ayında (2,04 mg/L), en yüksek değerini haziran ayında (7,36 mg/L) saptamışlardır. Benzer şekilde çalışmamızda en yüksek KOİ değerleri yaz mevsiminde tespit edilmiştir. Bunun nedeni yaz aylarında özelikle göletin çevresinde sıklıkla görülen tarımsal ve rekreasyonel faaliyetlerin sudaki kirliliği hızlandırmasıdır. Hacısalihoğlu ve Karaer (2020), kimyasal oksijen ihtiyacındaki artışın, su kaynaklarına karışan evsel ve endüstriyel atık sularla birlikte tarım ve hayvancılık faaliyetlerinden kaynaklandığını belirtmişlerdir.

Azotlu ve fosforlu bileşiklerin konsantrasyonu, su kalitesini gösteren önemli parametrelerdendir. Bu bileşiklerin konsantrasyonundaki artış, su kalitesinin düşmesine neden olmaktadır (Ustaoğlu vd., 2020). Bitkiler için temel besin maddesi olan nitrat bileşiklerinin sudaki konsantrasyonu genellikle düşüktür. Bu su kaynaklarındaki artan nitrat konsantrasyonu, alglerin büyümesini hızlandırarak su kalitesinde bozulmalara neden olabilmektedir (Ayandiran vd., 2018). Dolayısıyla yüzey sularındaki azot türevlerinin konsantrasyonundaki artış, genellikle noktasal olmayan tarımsal kirliliğin göstergesi olarak kabul edilmektedir (Ji vd., 2020). Çalışmamızda, Terzialan Göleti'nin nitrit azotu içeriği tüm mevsimlerde ölçüm limitleri altında belirlenmiştir. Benzer şekilde nitrat azotu da yaz ve ilkbahar mevsiminde ölçüm limitleri altında saptanmıştır. Ayrıca nitrat azotu konsantrasyonu en yüksek sonbahar mevsiminde (2,02 mg/L) tespit edilmiştir. Literatürde, nitrat azotunun yaz aylarında nispeten düşük seviyelerde olduğunu belirten benzer çalışmalar yer almaktadır (İleri vd.,2014; Mutlu ve Tepe, 2014). Sucul organizmalar için önemli ölçüde toksik olmayan amonyum iyonu, alg ve makrofitler tarafından doğrudan alınabilmekte olup, Terzialan Göleti'ndeki tüm istasyonlarda amonyum azotu ölçüm limitlerinin altındadır. Temiz ve bol oksijenli sularda amonyum bileşikleri çok düşük düzeylerde bulunmaktadır. Artan konsantrasyonlarda suda oksijen tüketiminin artmasına ve ötrofikasyonun hızlanmasına neden olabilmektedir (Haralambous vd.,1992; Taş, 2011).

Çalışmamızda, fosfat konsantrasyonunun en yüksek 0,63 mg/L yaz mevsiminde, en düşük 0,02 mg/L kış mevsiminde ölçülmüştür. Kar ve Leblebici (2020) araştırmalarında, yüzey sularının fosfat konsantrasyonundaki artışın, ötrofikasyona neden olduğunu ve suyun kalitesini önemli oranda düşürdüğünü ifade etmişlerdir. Sağın ve Şen (2018), Kabalar Göleti'nin (Kastamonu) fosfat konsantrasyonunu en düşük 0,10 mg/L mart ayında, en yüksek 0,82 mg/L Eylül ayında saptamışlardır. Tüm istasyonlarda en yüksek fosfat konsantrasyonunun yaz mevsiminde olmasının nedeninin, mevsime bağlı tarımsal faaliyetlerde kullanılan yüksek azot ve fosfat içerikli gübrelerden kaynaklandığı söylenebilir. Literatürdeki veriler ile karşılaştırıldığında; Terzialan Göleti'nin, tarımsal kirlilik açısından daha iyi durumda olduğu söylenebilir.

Yüzey sularındaki yüksek klorür içeriği, tuzluluğu ve dolayısıyla yüksek elektriksel iletkenliği gösterirken, su kalitesinin belirlenmesinde indikatör olarak kullanılmaktadır (Zeybek ve Kalyoncu, 2016). Çağlar ve Saler (2014), canlı metabolizması için oldukça önemli olan klorür iyonunun, tuzlu su girişinin olmadığı su kaynaklarında genellikle düşük olduğunu bildirmektedir. Taş (2011), Gaga Gölü'nün (Ordu) klorür içeriğini en yüksek yaz mevsiminde 1,0 mg/L olarak saptamıştır. Terzialan Göleti'nin yaz mevsimindeki klorür içeriği ise 14,00-18,00 mg/L arasında değişiklik göstermiştir. Buna göre Terzialan Göleti'nin klorür içeriğinin Gaga Gölü'nden daha yüksek olduğu belirlenmiştir. Çalışmalar arasındaki farklılıklar iklimsel faktörlerden kaynaklanabilir. Sudaki klorür konsantrasyonunda, mevsimsel yağış miktarındaki artışların ve karların eriyerek su kaynaklarına

karışmasının etkili olduğunu ifade edilmektedir (Gündoğdu ve Çarlı, 2020).

Suyun elektrik kapasitesinin bir ölçüsü olan iletkenlik, sularda çözünmüş katıların konsantrasyonlarındaki değişimi ifade etmektedir. Ayrıca suda çözünmüş tuzlar fotosentezde kullanıldığından, suyun biyolojik verimliliğinde oldukça önemlidir (Gevera vd., 2020). WHO (2017) standartlarına göre, 1000 µS/cm 'yi aşan su kaynaklarının elektrik iletkenliğinin suda çözünen tuzlara, suyun sıcaklığına ve yoğunluğuna bağlı olarak değiştiği bildirilmiştir. Kurnaz vd. (2016), Çiğdem Göleti'nin (Kastamonu) en düşük elektrik iletkenliği değerini ilkbahar mevsiminde 171,10 µs/cm ve en yüksek değeri sonbahar mevsiminde 280,13 µs/cm tespit etmişlerdir. Terzialan Göleti'nde de en düşük elektrik iletkenlik değeri ilkbahar mevsiminde 235,33 µs/cm ve en düşük değeri sonbahar mevsiminde 359,00 µs/cm saptanmıştır.

Suların sertliği, suda çözünmüş olarak bulunan toplam Ca^{+2} (kalsiyum) ve Mg^{+2} (magnezyum) iyonlarının miktarının $CaCO_3$ (kalsiyum bikarbonat) eşdeğeri olarak tanımlanır (Abbasnia vd., 2019; Ustaoğlu vd., 2020). Bununla birlikte sodyumun sudaki Ca^{+2} ve Mg^{+2} miktarlarına oranı olarak ifade edilen SAR değeri de alkali (tuzluluk) riskinin bir ölçüsü olarak sulama suyunun kalitesinde önemli bir göstergedir (Rahman vd., 2017; Adimalla ve Wu, 2019; Gevera vd., 2020). SAR değeri arttıkça toprağın geçirgenliği azalarak, toprak sistemindeki hava ve su dolaşımı kısıtlanmaktadır (Tomaz vd., 2020). Bu nedenle SAR değerinin yüksek olması, toprak verimsizliğine yol açarak tarladaki mahsulün veriminin düşmesine neden olabilmektedir (Haldar vd., 2020). Sulama amaçlı kullanılan suyun sınıflandırmasında, suyun 25°C 'deki iletkenliğinin (µS/cm) ve SAR değerinin baz alınarak hesaplandığı ABD tuzluluk diyagramından yararlanılmaktadır (Öztürk, 2004). Terzialan Göleti, mevsimsel ortalamalara göre ABD tuzluluk diyagramında C2-S1 sınıfında olup, tarımsal sulama suyu olarak kullanılabilir (Öztürk, 2004). Ayrıca Wilcox diyagramında yüzeysel ve yeraltı sularının, sodyum ve iletkenlik yüzdesi değerleri dikkate alınarak sulamaya uygunluğu hesaplanmaktadır. Hesaplamalar, EC ve sodyum yüzdesi değerleri kullanılarak gerçekleştirilmekte ve sulama sınıfları çok iyi, iyi kullanılabilir, şüpheli kullanılabilir, şüpheli kullanılamaz ve sulamaya uygun değil şeklinde 5 sınıf altında değerlendirilmektedir (Şener ve Güneş, 2015). Buna göre Terzialan Göleti'nin, sulama suyu olarak çok iyi sınıfında olduğu belirlenmiştir. Benzer şekilde Aydın (2017), Van Çaldıran Ovası yüzey sularından alınan örneklerin, Şener ve Güneş (2015) ise Aksu (Isparta) Ovası yüzey sularından alınan tüm örneklerin çok iyi sınıfta yer aldığını bildirmiştir.

Magnezyum oranının (MR) %50'den fazla olması toprağı alkali yaparak, mahsul verimini etkilemektedir. Bu nedenle %50'den fazla MR değerine sahip suların sulama amacı için uygun olmadığı bildirilmektedir (Shukla ve Saxena, 2020). Terzialan Göleti için bu değer yıllık ortalama 41,61-48,00 arasında değişiklik göstermektedir.

Kelley İndeksi (KI), Ca^{+2} ve Mg^{+2} ile ölçülen Na^{+} seviyesine göre su kalitesi hakkında bilgi veren parametredir. Bu oranın sulama suyu için kullanılacak sularda <1 olması gerektiği bildirilmektedir (Özer ve Köklü, 2019). Terzialan Göleti'nin KI değeri, istasyonlar arasında yıllık ortalama 0,23-0,24 arasında değişiklik göstermektedir. Dolayısıyla Terzialan Göleti'nin sulama için uygun olduğu söylenebilmektedir.

Demir (Fe), bakır (Cu), çinko (Zn) gibi metaller, düşük konsantrasyonlarda metabolik faaliyetlerde gerekliyken yüksek konsantrasyonlarda toksik etki gösterebilmektedir. Kurşun (Pb), kadmiyum (Cd) gibi ağır metaller ise düşük konsantrasyonlarda bile sucul ekosistem ve halk sağlığı açısından ciddi bir tehlikedir. Çeşitli çevresel faktörlerin etkisiyle yüzey sularına ulaşan metaller, adsorpsiyon ve çökeltme süreçleri ile tortularda birikme eğilimindedir (Ayandiran vd., 2018). Bu nedenle farklı su kaynaklarında ağır metal konsantrasyonlarının karşılaştırılması, kirliliğin değerlendirilmesi açısından önemlidir.

Demir, doğal ortamdaki kayaçlarda bol miktarda bulunan bir metaldir. Bitkilerde klorofilin sentezinde ve fotosentez olayının gerçekleşmesinde rol oynayan demir, bölgenin jeolojik yapısından, demir içeren gübrelerden, maden işletmelerinden ve evsel atıklardan doğal su ortamlarına taşınmaktadır (WHO, 2017). Suyun debisinin düştüğü, rüzgarların ve yağışların azaldığı yaz mevsiminde metaller, suların durgunlaşmasıyla birlikte sedimente çökebilmektedir. Çalışmamıza benzer olarak birçok çalışmada yaz aylarında sudaki demir konsantrasyonlarının azaldığı, ayrıca sedimentteki metal birikiminin sudakinden daha yüksek olduğu rapor edilmiştir (Öner ve Çelik, 2011; Kır ve Tumantozlu, 2012). Metabolik faaliyetlerin gerçekleşmesinde esansiyel bir metal olan bakır, yüksek konsantrasyonlarda canlı fizyolojini bozarak besin alınımını ve solunum mekanizmasını olumsuz etkilemektedir (Yerli vd., 2020). Terzialan Göleti'nde, ilkbahar mevsiminde tüm istasyonlardaki ortalama bakır konsantrasyonundaki artış, bölgenin jeolojik yapısından, tarımsal faaliyetlerde kullanılan fosfatlı gübrelerden ve pestisit yoğunluğundan kaynaklanabilir (ATSDR, 2004). Sulama suyundaki çinkonun yüksek konsantrasyonu, bitkilerde kök ve gelişiminin gecikmesine ve bitkinin erken yaşlanmasına neden olmaktadır (Asati vd., 2016). Çalışmamızda ilkbahar ve kış mevsimlerinde suda arttığı belirlenen çinko konsantrasyonlarının, bölgenin topoğrafik yapısından kaynaklandığı ve yağışlar sebebiyle suya karıştığı birçok çalışmada benzer şekilde bildirilmiştir (Okonkwo ve Mothiba, 2005; Kaptan ve Özan, 2014; Sökmen vd., 2018; Oruçoğlu ve Beyhan, 2019).

Yaşamsal faaliyetler için esansiyel olmayan kadmiyum ve kurşun, düşük konsantrasyonlarda dahi sucul canlıların solungaç, böbrek, karaciğer gibi hayati organlarında artan oranlarda birikerek toksik etki yapmaktadır. Kurşun, yerkabuğunda çok düşük miktarlarda bulunsa da tarım ilaçları ve egzoz gazları önemli oranda kirliliğe sebep olmaktadır (ATSDR, 2007). Çalışmamızda mevsimlere ve istasyonlara göre farklılıklar gösteren kurşun konsantrasyonunun, bilinçsiz

tarım uygulamalarından, araba egzozlarından ve piknik yapanların atıklarını gölet civarında bırakması gibi antropojenik etkilerden kaynaklandığı söylenebilir. Kadmiyum canlılar için toksik bir metaldir. Evsel ve endüstriyel atık suların deşarjı, bilinçsizce pestitisit ve fosfatlı gübre kullanımı ile atmosferik birikintiler nedeniyle toprak ve su kaynaklarında kadmiyum derişimi artmaktadır. Bitkilerde enzim aktivitesini engelleyerek, solunum ve fotosentez gibi yaşamsal faaliyetleri olumsuz etkilemektedir (Asri ve Sönmez, 2006). Mutlu ve Güzel (2019), Gümüşsuyu Göleti'nin (Sinop) kadmiyum konsantrasyonunu en yüksek Eylül ayında 0,50 µg/L olarak belirlemiştir. Terzialan Göleti'nin kadmiyum değeri ise en yüksek ilkbahar (0,79 µg/L) ve yaz (0,82 µg/L) mevsimlerinde tespit edilmiştir. Yaz mevsimindeki yüksek kadmiyum birikiminin, yapay gübre kullanımından ve pestisit içeren drenaj sularından kaynaklandığı bildirilmektedir (Abdeldayem ve Zaghlou, 2018).

Sonuç olarak; Terzialan Göleti'nin tarımsal sulama için risk oluşturmadığı tespit edilmiştir. Su kalitesi parametrelerindeki bölgesel ve mevsimsel farklılıkların, mevsimsel yağışlara, tarımsal uygulamalara ve diğer antropojenik faktörlere göre değiştiği anlaşılmaktadır. Bu nedenle Terzialan Göleti'nin sulama amaçlı sürdürülebilirliği için periyodik olarak izlenmeli ve sonraki çalışmalarda bor, kalıntı sodyum karbonat (RSC), çözünebilir sodyum yüzdesi (TDS), çözünebilir sodyum yüzdesi (SSP) ve potansiyel tuzluluk (PI) parametreleri de kullanılarak göletin sulamaya uygunluğunu daha geniş yelpazede değerlendirilmelidir. Ayrıca su kalitesindeki bozulmaların, besin zincirinde önemli bir yer tutan sucul canlılar üzerindeki etkisi de göz ardı edilmemelidir. Su kaynaklarının sürdürülebilirliği ve sucul canlıların yaşam kalitesi, bu suların kaliteli ve temiz olması ile mümkündür. Dolayısıyla bölgede yapılacak çalışmalarda su kalitesinin belirlenmesinin yanı sıra sediment, balık, sucul omurgasız, alg kullanarak biyoindikatör çalışması niteliğinde bir veri grubu elde edilmeli ve sonuçlar karşılaştırılarak bölgenin su ve canlı etkileşimine bağlı verimi değerlendirilmelidir.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Production of dry fermented fish sausages by using different fish species and determination of the microbiological qualities

Farklı balık türleri kullanarak fermente balık sucuklarının üretimi ve mikrobiyal kalitelerinin belirlenmesi

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Abstract: In this study frozen-thawed rainbow trout (*Onchorhynchus mykiss*) and seabass (*Dicentrarchus labrax*) fillets were used for preparing of dry fermented fish sausages. The total mesophilic aerob bacteria (TMAB), total psychrophilic aerob bacteria (TPAB), yeast–mould (YM), lactic acid bacteria (LB), *Enterobactericeae* (EB), *Staphylococcus aureus* (SA) changes of these dry fermented sausages were examined in the refrigerated storage at 6-8˚C. At the end of the storage period of 90 days, the results of the TMAB, TPAB, YM, LB, EB and SA counts of dry fermented seabass sausages were determined as 6.25, 7.01, 3.61, 5.31, <1.0 ve <1.0 log cfu/g, while, TMAB, TPAB, YM, LB, EB and SA counts of dry fermented trout sausages were found as 6.57, 7.20, 4.44, 5.14, <1.0 ve <1.0 log cfu/g, respectively. In this study both fermented fish sausages were determined as too much dried and exceeded the microbiological limit of TMAB at the end of the storage period of 90 days in the refrigerator. However, fermented seabass sausage reached the maximum level of YM count on the 10th day of storage, whereas fermented trout sausage reached this level on the 30th day of storage. Therefore, it is suggested that they should be packaged in vacuum packaging because of preventing too much drying and the growth of undesirable moulds. Additionally, the identification of microorganisms in fermented fish sausages would also be advised to determine desirable and undesirable microorganisms. Dry fermented fish sausage would be an alternative product to traditional dry fermented meat sausage in Turkey because of the health benefits of fish.

Keywords: Fish species, dry fermented fish sausage, microbiological quality

Öz: Bu çalışmada dondurulmuş çözündürülmüş alabalık (*Onchorhynchus mykiss)* ve levrek (*Dicentrarchus labrax)* filetoları kullanılarak balık sucukları üretilmiştir. 6-8˚C'de buzdolabında depolanan alabalık ve levrek sucuklarının toplam mezofil aerob bakteri (TMAB), toplam psikrofil aerob bakteri (TPAB), maya-küf (MK), laktik asit bakteri (LB), *Enterobactericeae* (EB) ve *Staphylococcus aureus* (SA) değişimleri incelenmiştir. Depolamanın 90. gününde fermente levrek sucuklarının TMAB, TPAB, MK, LB, EB ve SA bakteri sayıları sırasıyla 6,25; 7,01; 3,61; 5,31; <1,0 ve <1,0 log kob/g olarak tespit edilirken, alabalıktan üretilen fermente balık sucuklarının TMAB, TPAB, MK, LB, EB ve SA bakteri sayıları sırasıyla 6,57; 7,20; 4,44; 5,14; <1,0 ve <1,0 log kob/g olarak saptanmıştır. Çalışmada her iki fermente balık sucuğunun da buzdolabında 90 günlük depolama periyodu sonunda çok fazla kurudukları ve TMAB açısından mikrobiyolojik limit değerini geçtikleri saptanmıştır. Buna karşın, en yüksek MK değerlerine levrek sucukları depolamanın 10. gününde ulaşırken, alabalık sucukları depolamanın 30. gününde ulaşmıştır. Bu nedenle depolama esnasında çok fazla kurumanın ve istenmeyen küf gelişiminin önlenmesi için fermente balık sucuklarının vakum paketleme kullanılarak paketlenmesi önerilir. Ayrıca fermente balık sucuklarında mikroorganizmaların tanımlanması da arzu edilen ve istenmeyen mikroorganizmaların belirlenmesi için tavsiye edilir. Türkiye'de balık sucukları sağlığa yararlı olmaları nedeniyle geleneksel etten üretilen sucukların yerine alternatif olabilir.

Anahtar kelimeler: Balık türleri, fermente balık sucuk, mikrobiyal kalite

INTRODUCTION

The improvement of safety and standardization of fermented meat products with the typical characteristics is very essential and can be achieved naturally or sometimes by using native starter cultures that influence appearance, colour, odour, flavor and texture of these products (Cruxen et al., 2019). The most important part of these fermented meat products are fermented fish products (Kılınç et al., 2006) that they have been also produced and consumed in some parts of the world (Kılınç, 2003; Kılınç, 2004). In many parts of the world fermented sausages are traditional products that they have been consumed by most of the consumers for centuries (Bou et al., 2017). Additionally, the process of the manufacture of these fermented meats is a very important part of the meat industry in many countries (Fernandez et al., 2000). The raw materials of the manufacture of fermented sausage are derived from variety of meats and fat tissue. Nonmeat ingredients such as water, salt, spices, sugars, ascorbates and phosphates have been also used for the manufacturing of sausages to improve the flavor and taste characteristics of sausages (Lonergan et al., 2019). The qualities of dry fermented products depend on many factors such as the microbial microflora, the chemico-physical variables and the hygienic procedure of these products during processing stage etc. (Suzzi and Gardini, 2003). Many dry fermented products have been produced in different formulation in the many parts of the world (Incze, 1998; Kılınç, 2004; Papamanoli et al., 2003; Nordvi et al., 2007; Ordonez et al., 2010; Vignolo et al., 2010; Papavergou, 2011; Khem et

al., 2013; Holck et al., 2017; Sidira et al., 2019; Gonzalez-Mohino et al., 2020). Sucuk is one of the most important dry fermented meat product of Turkey. In Turkey, this product has been frequently produced from beef or lamb by adding spices, salt, and tail fat into these meats (Kaban and Kaya, 2006). Then prepared sucuk dough has been filled into casings before fermentation process, which occured under uncontrolled conditions (Bozkurt and Bayram, 2006). Fermentation could be formed by the natural microbial flora of sucuk (Kaya and Gökalp, 2004). The microflora of fermented products commonly have been consisted of lactic acid bacteria, coagulase negative coccus, enterococcus, and yeasts (Rantsiou and Cocolin, 2006). The lactic acid bacteria counts of fermented sucuks generally have been changed from 10^2 to10^4 cfu/g. However, these values were reported by Apaydın et al. (2009) could be risen during the process of fermentation. The microorganisms, which were responsible for the process of fermentation, notified by Kaya and Gökalp (2004) that were *Kocuria*, *Staphylococcus*, *Lactobacillus* and *Pediococcus*. According to Turkish Standards Institution; *Lactobacillus (L. plantarum, L. pentosus, L. curvatus, L. sake), Pediocoocus (P. pentosaceus, P. acidilactici, Micrococcus (Korucia varians)* and *Debaryomyces (D. hansenii)* have been used as the starter cultures for the production of sucuks (TSE, 2002). Con and Gökalp (2000) informed that the group of lactic acid bacteria were not only responsible for the process of fermentation, but also they could be inhibited spoilage and pathogenic bacteria during fermentation process. In recent years heat treatments have been applied on sucuks in terms of inhibiting the harmful microbial flora of sucuks. But, these heat treatments had bad effects on the formation of flavours described by Ercoşkun, (2006). Spices and ingredients, which have been added into sucuks, improved the flavour and colour characteristics of sucuks (Bozkurt and Erkmen, 2007). Sucuk has been produced through microbial fermentation and drying process (Aksu and Kaya, 2004). When looking at the literatures; many studies have been done about dry fermented meat products which have been produced from beef, sheep, goat, buffalo, camel etc. (Bozkurt and Erkmen 2002; Suzzi and Gardini, 2003; Kara et al., 2012; Atik, 2013; Yoo et al. 2016; Cunha et al., 2018; Adab et al., 2020). Many studies also have been done about dry fermented fish sausauges (Khem et al., 2013; Stollewerk et al. 2014; Wang et al., 2017) in the world. In addition to this, some studies have been done about fish sausages (Dinçer et al., 2007a, Dinçer et al. 2007b; Özpolat, 2012; Dinçer and Çaklı, 2015; Dinçer et al., 2017; Çoban, 2020), fish jambon (Eren, 2011) and shark meat sausage (Yılmaz and Berik, 2013), whereas limited studies have been done about dry fermented fish sausages (Arslan et al., 2001; Berik and Kahraman, 2010) in Turkey.

Fish compounds were beneficial to health. However, many people prefer to eat little or no fish. Therefore, the development of convenient and new fish products, which were easy to eat to increase the fish intake, was necessary reported by Nordvi et al. (2007). In addition to this; there have been no production and consumption of dry fermented fish sausages which are made from fish species in Turkey. For this reason; the aim of this study was to produce dry fermented fish products from sea bass and rainbow trout. And also microbiological changes of these dry fermented fish sausages were determined during storage period.

MATERIAL AND METHODS

Material and the preparation of fermented fish sausages

In this study frozen at -18˚C for 3 months and then thawed rainbow trout (*Onchorhynchus mykiss*) and seabass (*Dicentrarchus labrax*) fillets were used. The preparation of dry fermented fish sausage is shown in Figure 1. Dry fermented fish sausages were prepared by using fish, beef tail fat, salt, garlic, red pepper, hot red pepper, black pepper, ginger, cinnamon, cumin, sucrose, allspice and potassium sorbate. All spices, which were sold as open, were bought from the spice shop in the Bornova province of Turkey. All formulations contain 1200 g fish meat, 75 g beef tail fat, 20 g salt, 41.0 g garlic, 30.3 g red pepper, 20.3 g hot red pepper, 9.4 g black pepper, 7.25 g ginger, 7.0 g cinnamon, 9.3 g cumin, 7.3 g sucrose, 3.72 g allspice, % 0.03 potassium sorbate in their recipe. After the fish meat was homogenized by using the blender, all spices were added and then they were mixed for 10 minutes. After leaving the mixture in the refrigerator (at 6-8 ˚C) overnight, the sausage filling machine (Sfinx, Czech Republic) were used to be stuffed this mixture into the natural casings to be about approximatelly 10 cm and the end parts were tied with rope and cut. Prepared rainbow trout and seabass sausages were put into refrigerator at 6-8 ˚C and the microbiological changes were examined during storage period.

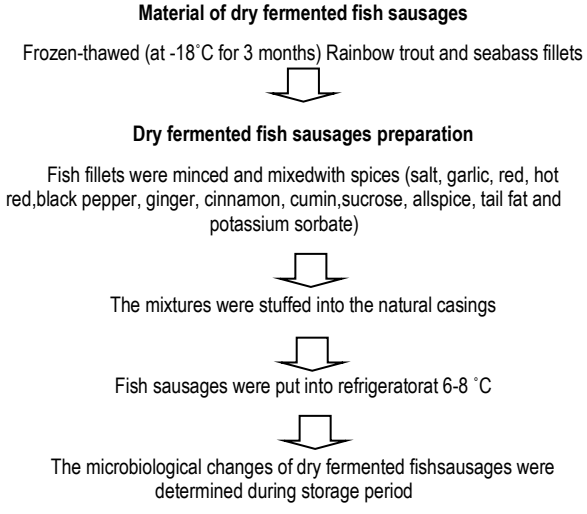

Figure 1. The preparation of dry fermented fish sausages

Microbiological analyses

Sampling for microbiological analyses

Three dry fermented fish sausages of each type were removed in the 1st, 5th, 10th and 15th, 30th, 60th and 90th day of storage period. 10 g of samples was removed from each dry fermented fish sausage and homogenized in a stomacher (IUL, Barcelona, Spain) containing 90 ml of 0.1% peptone water (Difco, 0118-17-0). Other serial dilutions were prepared from this solution. Double trial for each dilution were tested.

Microbiological methods

The TMAB counts of dry fermented sausages were done according to method of (ICMSF, 1983). The Pour Plate Method was used for determining TMAB and TPAB counts of dry fermented fish sausages. One mililiter of inoculum were put into the petridishes and then Plate Count Agar (PCA, Oxoid, CM0325) was poured onto the inoculum. Inoculated petri dishes were incubated for 48 hrs at 30°C for the TMAB analysis. After incubation, colonies on petridishes were enumerated and converted into log cfu/g. The TPAB counts were determined according to method of (Merck, 1998). Plate Count Agar (PCA, Oxoid, CM0325) was also used for the TPAB count as the medium. One mililiter of inoculum was taken from each dilution and then put onto the petri dishes. Then approximately 15-20 ml of Plate Count Agar (PCA, Oxoid, CM0325) was poured onto the each inoculum. Inoculated petri dishes were incubated for 14 days at 4°C for the TPAB analysis. Colonies were enumerated as the TPAB counts and converted into log cfu/g. The LB counts of dry fermented fish sausages were done according to method of (DeMan et al., 1960). Double layer plate method was used for the determination of the LB counts of samples. One mililiter of inoculum was put into the petridishes and then Man Rogosa Sharpe Agar (MRSA) (LAB^{M} 93) was poured onto the inoculum as double layer. Inoculated petri dishes were incubated for 5 days at 30°C for determining the LB counts of samples. Colonies were enumerated after the incubation as the LB. For the YM counts; Qxytetracycline Yeast Extract Agar (LAB^{M} X89) was used as medium and the incubation period was for 3-5 days at 30°C. The YM counts were done according to method of (Harrigan and McCance, 1976) by using the Pour Plate method. The *Enterobacteriaceae* counts of dry fermented sausages were done according to method of (Vanderzant and Splittstoesser, 1992). Violet Red Bile Dextrose Agar (VRBD, Merck, 1.10275.0500) was used as medium for the EB counts. The inoculation was done by using the double layer plate method. After inoculation, incubation was done for 24 hrs at 37°C. The *Staphylococcus* bacteria counts were done according to method of (Mossel and Moreno-Garcia, 1985). For the SA counts of dry fermented sausages, Baird Parker Agar (BPA, Merck, 1.05406.0500) was used as medium and the egg yolk tellurite emulsion (Merck, 103785) was used as supplement. After inoculation, inoculated petri dishes were incubated for 30 hrs at 37°C (Mossel and Moreno-Garcia, 1985). All microbiological analyses were done triplicate.

Statistical analysis

Statistical analysis was performed by using the Statistical Program for Social Sciences (SPSS ver. 25.0). The effect of storage periods on to the groups were analyzed. Kruskal-Wallis and Mann-Whitney tests were used for determining the differences of bacteria counts between the groups (Gamgam and Altunkaynak, 2017). The level of significance was indicated as $p<0.05$ and the level of not significance was as $p>0.05$.

RESULTS AND DISCUSSION

The microbiological quality of fermented seabass sausage are shown in Table 1. At the beginning of fermentation process; TMAB, TPAB, YM, LB, EB and SA counts of fermented seabass sausages were determined as 2.49, 2.41, 1.15, 2.02, 2.09,<1.0 log cfu/g, while at the end of the storage period of 90 days, the results of the mesophilic, TMAB, TPAB, YM, LB, EB and SA counts of fermented seabass sausage were determined as 6.25, 7.01, 3.61, 5.31, <1.0 ve <1.0 log cfu/g, respectively.

Table 1. The results of the microbiological counts of dry fermented seabass sausage during storage period

Day	Microbiological analyses of dry fermented seabass sausage					
	TMAB (log cfu/g)	TPAB (log cfu/g)	YM (log cfu/g)	LB (log cfu/g)	EB (log cfu/g)	SA (log cfu/g)
1	2.49±0.14 ^{a}	2.41±0.18 ^{a}	1.15±0.21 ^{a}	2.02±0.03 ^{a}	2.09±0.13 ^{a}	<1.0 ^{a}
5	3.91±0.07 ^{b}	3.83±0.03 ^{b}	1.81±0.04 ^{b}	2.26±0.01 ^{b}	1.85±0.05 ^{b}	<1.0 ^{a}
10	4.31±0.18 ^{c}	4.60±0.19 ^{c}	2.02±0.09 ^{c}	3.35±0.05 ^{c}	<1.0 ^{c}	<1.0 ^{a}
15	4.85±0.09 ^{d}	4.93±0.05 ^{d}	2.21±0.05 ^{cd}	4.02±0.03 ^{d}	<1.0 ^{c}	<1.0 ^{a}
30	5.37±0.29 ^{e}	5.52±0.15 ^{e}	2.34±0.06 ^{de}	4.49±0.13 ^{e}	<1.0 ^{c}	<1.0 ^{a}
60	5.43±0.10 ^{e}	5.67±0.07 ^{e}	2.44±0.11 ^{e}	4.75±0.07 ^{f}	<1.0 ^{c}	<1.0 ^{a}
90	6.25±0.37 ^{f}	7.01±0.19 ^{f}	3.61±0.11 ^{f}	5.31±0.06 ^{g}	<1.0 ^{c}	<1.0 ^{a}

n=3 (Mean value ± Standard deviation), the mean value within each column with different small letters are statistically different ($p<0.05$) according to storage period. TMAB: Total mesophilic aerob bacteria, TPAB: Total psychrophilic aerob bacteria, MY: Yeast-Mould, LB: Lactic acid bacteria, EB: Enterobactericeae , SA: *Staphylococcus aureus*

The microbiological quality of dry fermented trout sausage are given in Table 2. At the beginning of fermentation process; TMAB, TPAB, YM, LB, EB and SA counts of fermented trout sausage were found as 2.47, 2.35, 1.00, 1.95, 1.24 and <1.0 log cfu/g, while at the end of the storage period of 90 days; TMAB, TPAB, YM, LB, EB and SA counts of fermented trout sausage were found as 6.57, 7.20, 4.44, 5.14, <1.0 ve <1.0 log cfu/g, respectively.

Table 2. The results of the microbiological counts of dry fermented trout sausage during storage period

Day	Microbiological analyses of dry fermented trout sausage					
	TMAB (log cfu/g)	TPAB (log cfu/g)	YM (log cfu/g)	LB (log cfu/g)	EB (log cfu/g)	SA (log cfu/g)
1	2.47±0.07^{a}	2.35±0.06^{a}	1.00±0.00^{a}	1.95±0.07^{a}	1.24±0.34^{a}	<1.0^{a}
5	3.82±0.18^{b}	3.83±0.02^{b}	1.59±0.16^{b}	2.32±0.19^{b}	1.15±0.21^{b}	<1.0^{a}
10	4.34±0.19^{c}	4.37±0.17^{c}	1.88±0.04^{c}	3.90±0.07^{c}	<1.0^{c}	<1.0^{a}
15	4.91±0.06^{d}	4.96±0.01^{d}	1.93±0.04^{cd}	4.21±0.06^{d}	<1.0^{c}	<1.0^{a}
30	5.01±0.05^{e}	5.04±0.06^{e}	2.11±0.09^{de}	4.42±0.17^{e}	<1.0^{c}	<1.0^{a}
60	5.02±0.09^{e}	5.29±0.81^{e}	2.25±0.07^{e}	4.55±0.07^{f}	<1.0^{c}	<1.0^{a}
90	6.57±0.08^{f}	7.20±0.14^{f}	4.44±0.33^{f}	5.14±0.10^{g}	<1.0^{c}	<1.0^{a}

n=3 (Mean value ± Standard deviation), the mean value within each column with different small letters are statistically different (p<0.05) according to storage period. TMAB: Total mesophilic aerob bacteria, TPAC: Total psychrophilic aerob bacteria, YM: Yeast-mould, LB: Lactic acid bacteria, EB: Enterobactericeae, SA: *Staphylococcus aureus*

TMAB, TPAB, YM, LB counts of fermented seabass and trout sausages were increased during storage period. Significant differences (p<0.05) were determined in microrganism counts during storage, but not significant differences (p>0.05) were determined in microorganism counts between the groups according to time of storage. TMAB counts of dry fermented seabass and trout sausages were determined as 2.49 and 2.47 log cfu/g on the first day of storage. After 90 days of storage period, TMAB counts of fermented seabass and trout sausages increased to 6.25 and 6.57 log cfu/g, respectively.

TPAB counts of fermented seabass sausage increased from 2.41 to 7.01 log cfu/g, while TPAB counts of fermented trout sausages increased from 2.35 to 7.20 log cfu/g at the end of the storage period. The initial YM counts of fermented seabass and trout sausages were found as 1.15 and 1.00 log cfu/ g., respectively. After 90 days of the storage, these values increased to 3.61 log cfu/g for the fermented seabass sausage and 4.44 log cfu/g for the fermented trout sausage. The LB counts of both fermented sausages increased during storage period. The LB counts of fermented seabass sausages were determined as 2.02, 2.26, 3.35, 4.02, 4.49, 4.75, 5.31 log cfu/g on the 1th, 5th, 10th, 15th, 30th, 60th and 90th day of storage period, respectively. The LB counts of fermented trout sausage increased from 1.95 log cfu/g to 4.21 log cfu/g on the 15th day of storage. During storage period the LB counts of fermented trout sausage found as 4.42, 4.55, 5.14 log cfu/g, on the 30th, 60th, 90th day of storage, respectively. The EB counts of fermented seabass and trout sausages were determined as 2.09 and 1.24 log cfu/g at the begining of the storage, respectively. However, this bacteria counts inhibited on both fermented sausages on the 10th day of storage. The SA was not determined in any of fermented sausages during storage period.

According to the Kruskal Wallis test, there was no significant difference (p>0.05) between the mean values of fermented seabass and trout sausages in terms of the TMAB counts (p-value=0.772). However, the difference between days was statistically significant (p<0.05) for both fish species in terms of the TMAB (p-value=0.000). The Mann Whitney test was done for determining the differences between the days. The difference between the days was not significant (p-value=0.818) on the 30th and 60th days of storage while the difference between all other days was statistically significant (p<0.05) in both groups. There was no significant difference (p>0.05) between the average values in terms of the TPAB counts of fermented seabass and trout sausages (p-value=0.792). However, the difference between two fish species in terms of the days for the TPAB counts found to be statistically significant (p-value=0.000). The Mann Whitney test was also done for determining the differences between the days. The difference between the days was not significant (p-value=0.132) on the 30th and 60th days of storage, while the difference of the TPAB counts between all other days was statistically significant (p<0.05) in both groups. According to the Kruskal Wallis test, there was no significant difference (p>0.05) between the mean values of YM counts for fermented seabass and trout sausages (p-value=0.792), but the difference between days for the two fish species was statistically significant (p-value=0.000). The difference between the days was checked by using the Mann Whitney test. The difference between the days was not significant on the 10th and 15th days of storage was (p-value=0.180), 15th and 30th days of storage was (p-value=0.180), 30th and 60th days of storage was (p-value=0.180). However, the difference between all other days was found as statistically significant (p<0.05). There was no significant difference between the mean value of of LB counts of fermented seabass and trout sausages (p-value=0.85), but the difference between days was statistically significant (p<0.05) for the both fish species (p-value=0.000).

The difference between all days was checked by using the Mann Whitney test. Statistically significant difference ($p<0.05$) between all days were determined between the both groups.

The TMAB count was not only a good indicator for deciding the acceptability of fermented food products but also, it should be considered together with the number of LB of these products whether or not they were suitable for consumption (Ünlütürk and Turantaş, 2003). Ekici et al. (2015) reported that the TMAB counts of dry fermented sausages were determined as ~8.5 log cfu/g. In another report, the TMAB in fermented sausages was reported to be between 6.00 and 7.00 log cfu/g (Ekici and Omer, 2018). In our study, the TMAB counts of fermented seabass and trout sausages were determined as 6.25 and 6.57 log cfu/g at the end of the storage period of 90 days. During storage period at 6-8 ˚C, the TPAB counts of the both fermented fish sausages were determined higher than the TMAB counts of the fermented fish sausages after 5th day of storage. The total number of TMAB in fermented sausages obtained under hygienic conditions should be below 6.0 log cfu/g (Öksüztepe et al., 2011). Both fermented seabass and trout sausages exceeded this limit (6.0 log cfu/g) on the 90th day of storage in our study.

Arslan and Soyer (2018) reported that sausages were exposed to the high humidity during the fermentation process, which could be caused the growth of both desirable and undesirable fungi on the surface of sausages. Furthermore, controlled molds growth was reported that they gave sausages a typical flavor as a result of degredation of lipids and proteins. However, uncontrolled mould growth was indicated that they could be responsible for the discoloration of the surface of sausages as well as spoilage. In our study, the YM counts of fermented sea bass sausage changed from 1.15 to 3.61 log cfu/g, while the YM counts of fermented trout sausages changed from 1.00 to 4.44 log cfu/g after 90 days of storage. Arslan et al. (2001) found that the number of YM counts were determined increasing in all groups of fermented *Cyprinus carpio* sausages from 1 to 30th day of storage. At the end of the 30th day of storage; the YM counts of four different *Cyprinus carpio* sausages were found to be as 4.59, 4.88, 4.79 and 4.60 log cfu/g.

In this study the authors reported that the excessive number of the YM counts of fermented products could be due to the growing the ability of the YM in these type of products easily. Besides, the authors denoted that the fermented products could be contaminated with the food additives, particulary from the spices (Arslan et al., 2001). In another study; the YM counts of the traditional fermented sausages were described as varied from 3 log cfu/g to 5 log cfu/g (Erkmen and Bozkurt, 2004). Ekici et al. (2015) defined that exhibited significant variations among the YM counts of samples ranging from 3.54 log cfu/g to 5.21 log cfu/g. The yeast and mould counts of Milano type of traditional fermented sausages were found as 4.2 ±0.08 and 4.8 ±0.03 log cfu/g, respectively (Haouet et al., 2017). In our study, the YM count of the dry fermented seabass sausage increased to 3.61 log cfu/g, while the YM count of fermented trout sausage increased to 4.44 log cfu/g at the end of the storage period. Our results were well accordance with the above studies (Arslan et al., 2001; Erkmen and Bozkurt, 2004; Ekici et al., 2015; Haouet et al., 2017) about the number of YM of fermented products.

According to Institute of Turkish Standards (2002), the maximum level of the YM counts of dry fermented meat product was defined as microbiologically to be 2.00 log cfu/g.

In the study, fermented seabass sausage reached this limit on the 10th day of storage, whereas fermented trout sausage reached this limit on the 30th day of storage. The high number of YM on dry fermented sausages could be from the spices that used in the study. For this reason, the authors thougt that the spices should be prefered at the best hygienic quality for producing dry fermented fish sausages. The LB counts of both fermented fish sausages increased during storage period. At the end of the storage period; the LB counts of fermented seabass sausage increased from 2.02 to 5.31 log cfu/g while the LB counts of fermented trout sausages changed from 1.95 to 5.14 log cfu/g.

In one report; high number of LB in the fermented sausages was reported by the authors that this group of bacteria was to be the predominant flora of the fermented sausages (Arslan and Soyer, 2018). In another report; the LB counts of fermented sausages were reported to be responsible for the quality development of these products during processing as well as the qualities of fermented sausages could be affected by the LB during marketing (Yaman et al., 1998). Adab et al. (2018) reported that the acidifying activity of the LB that an important role in the stability of the dry fermented meat products, following the inhibition of the growth of the pathogenic and spoilage microorganisms. In our study, the results of the increasing of the LB counts in the dry fermented fish sausages were in agreement with those of (Yaman et al., 1998; Arslan and Soyer, 2018; Adab et al. 2018).

The authors revealed in one study that the addition of the spice, especially cinnamon into the dry fermented sausages was an advisable to improve the sensory quality and also the inhibition of the growth of *Enterobacteriaceae* (Sun et al., 2018). The result of our study was well correlated with this above study (Sun et al., 2018) about the inhibition of the growth of the EB in dry fermented fish sausages. The EB count of fermented sausages was a good indicator for the sanitary of the production conditions (Arslan and Soyer, 2018). In one report, the number of EB species in the processing period of fermented meat products decreased due to the acidification. Moreover, fast acidification was resulted in a significant reduction in the number of this type of bacteria was reported by (Lücke, 1985). In another study, the authors also indicated that the EB count decreased during the production process of the fermented meat product (Kaban

and Kaya, 2009). In our study, the EB count decreased in the both groups of the fermented fish sausages on the 5th day of storage and then it was determined to be lower than the detection limit on the 10th day of storage. Our results were determined well correlation with these above investigations (Lücke, 1985; Kaban and Kaya, 2009) about decreasing and inhibition of this type of microorganisms in dry fermented sausages.

Kaban and Kaya (2006) reported that *S. aureus* was an important foodborne pathogenic bacteria in fermented meat sausage. In one report the authors showed that pathogenic and spoilage bacteria could not grow in the finished fermented fish sausages because of good hygienic procedure of processing. In their study the authors determined the decreasing of bacteria during processing. Additionally, they reported that the pathogenic bacteria was not detected during storage (Stollewerk et al., 2014). Scetar et al. (2013) also reported that the good hygienic quality during the entire processing and storage as no pathogens were detected in their study. Similar results were observed in our study with these above studies that the SA was not detected in the both groups of fermented fish sausages during storage period in the refrigerator.

CONCLUSION

In conclusion, dry fermented fish sausage can be a healty alternative product to fermented meat products in Turkey because of health benefits of fish. In our study; we produced fermented fish sausages by using seabass and trout fillets in laboratory conditions. However, cheap, discarded and economical fish species can be evaluated for producing fermented fish sausages as well. In the study, both fermented fish sausages were determined as too much dried and exceeded the microbiological limit (6.0 log cfu/g) of TMAB on the 90 th day of storage in the refrigerator at 6-8˚C. Moreover, the maximum level of the YM counts of dry fermented meat product was defined as microbiologically to be 2.00 log cfu/g according to the Turkish Standards (2002). Therefore, fermented seabass sausage reached this limit on the 10th day of storage, whereas fermented trout sausage reached this limit on the 30th day of storage. As a result, it is suggested that fish sausages should be packaged in vacuum packaging because of preventing too much drying and the growth of undesirable moulds. Additionally, good hygienic qualified spices or natural compounds should also be used for preventing the growth of undesirable moulds on dry fermented fish sausages. Limited studies have been done about the production and determination of the qualities of dry fermented fish sausages. For this reason, much more studies should be advised to be done about dry fermented fish sausages. The studies should also be done about the identification of microorganisms of fermented fish sausages during storage period to determine desirable and undesirable microorganisms. The production, standardization and consumption of dry fermented fish sausage just like traditional dry fermented meat products in Turkey would be possible in the near future.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Pullu sazan (*Cyprinus carpio* Linneaus, 1758)'da ellajik asidin büyüme ve bazı antioksidan parametrelere etkisi

Effect of ellagic acid on growth and some antioxidant parameters in scaly carp (*Cyprinus carpio*)

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Öz: Bu çalışmada, farklı oranlarda yeme katılan ellajik asidin pullu sazanda (*Cyprinus carpio*) büyüme performansı ve antioksidan durum üzerine etkisi araştırıldı. Balıklar, bir kontrol ve üç farklı düzeyde ellajik asit içeren (50, 100 ve 200 mg/kg yem) yemlerle 60 gün süresince beslendi. Çalışmanın 30. ve 60. günlerinde büyüme performansı [canlı ağırlık artışı, oransal büyüme ve spesifik büyüme oranı] ve oksidan/antioksidan parametreler [malondialdehit düzeyi, katalaz ve glutatyon S-transferaz aktivitesi ile redükte glutatyon düzeyi] analiz edildi. Kontrol ve ellajik asit uygulanan grupların canlı ağırlık artışları, oransal büyüme ve spesifik büyüme oranlarında istatistiksel olarak herhangi bir farklılık belirlenmedi ($p > 0,05$). Ellajik asit uygulanan grupların karaciğer ve böbrek malondialdehit düzeyleri kontrol grubuna göre istatistiksel olarak önemli düzeyde azaldı ($p < 0,05$). Ellajik asit uygulanan grupların karaciğer ve böbrek katalaz ve glutatyon S-transferaz aktiviteleri ile redükte glutatyon düzeyleri kontrol grubuna göre istatistiksel olarak önemli düzeyde arttı ($p < 0,05$). Ellajik asidin balıklarda antioksidan olarak kullanılabileceği sonucuna varıldı.

Anahtar kelimeler: Antioksidan, büyüme parametreleri, ellajik asit, oksidatif stres, sazan

Abstract: In the present study, it was investigated the effects of various levels of dietary ellagic acid on growth performance and antioxidant status in scaly carp (*Cyprinus carpio*). Fish were fed with the control diet and three different experimental diets containing three graded levels of ellagic acid (50, 100 and 200 mg kg^{-1} diet) for 60 days. On 30th and 60th days of experiment, the growth performance [live weight gain, relative growth and specific growth rate] and oxidant/antioxidant parameters [malondialdehyde level, catalase and glutathione-S-transferase activities and reduced glutathione level] were analysed. There was no statistically significant difference in the live weight gain, relative growth and specific growth rates of the control and ellagic acid treated groups ($p > 0.05$). When compared to the control group, the liver and kidney malondialdehyde levels of ellagic acid treated groups were significantly decreased ($p < 0.05$). The liver and kidney catalase and glutathione-S-transferase activities and reduced glutathione levels of ellagic acid treated groups were significantly increased when compared to the control group ($p < 0.05$). It was concluded that ellagic acid can be used as an antioxidant in fish.

Keywords: Antioxidant, growth parameters, ellagic acid, oxidative stress, carp

GİRİŞ

Tüm dünyada olduğu gibi ülkemizde de su ürünleri üretimi her yıl ciddi bir büyüme göstererek gelişimini devam ettirmektedir. Sürdürülebilir yetiştiricilik açısından balıkların uygun koşullarda yetiştirilmesi ve hastalıklardan korunması büyük önem taşımaktadır. Su ürünleri yetiştiriciliğinde meydana gelen ekonomik kayıpların hemen hemen yarısı hastalıklardan kaynaklanmakta ve ekonomik kayıplara neden olan faktörlerin başında hastalıklara bağlı sorunlar gelmektedir (Arda vd., 2005). Buna karşın günümüzde balık hastalıklarının birçoğunun tedavisi için halen etkin bir çözümün geliştirilememesi ve var olan tedavi yöntemlerinin de balıklar için ekstra bir stres oluşturması bilim insanlarını balık sağlığını arttırmaya yönelik çalışmalara yöneltmiştir (Talpur ve Ikhwanuddin, 2013; Mişe Yonar, 2019; Yonar vd., 2019; Yousefi vd., 2019). Diğer taraftan balıklarda görülen hastalıkların tedavisinde kemoterapötik maddeler kullanılmaktadır. Ancak balıkların karaciğer, böbrek, bağırsak, deri gibi organlarına zarar vermesi, kas dokusunda birikerek insanlara geçmesi, mikroorganizmaların kemoterapötiklere direnç kazanması, sedimentasyon oluşturması, bağışıklık sistemini olumsuz yönde etkilemesi, etkisinin kısa süreli olması, oksidatif strese neden olması ve antioksidan mekanizmayı baskılaması, tüm enfeksiyonlara karşı etkili olmaması kemoterapötik ilaçların kullanımını sınırlandırmaktadır (Sağlam ve Yonar, 2009; Yonar vd., 2011; Yonar, 2012). Bu sebeple son zamanlarda hastalığın çıkmasını engelleyecek korunma önlemlerinin alınması oldukça önem kazanmıştır. Koruma önlemleri içerisinde aşılama, doğal ya da sentetik immunostimulanlar ile balıkların direncini azaltarak hastalıkların oluşumuna sebep olan stres faktörlerine karşı antioksidanların kullanılabilirliği konusu da son yıllarda bir hayli dikkat çekmektedir. İmmunostimulan ve antioksidan özelliğe sahip maddelerin kullanılması bu bağlamda önemli bir çözüm olarak görülmektedir (Yonar vd., 2019; Mişe Yonar, 2019).

Ellajik asit, doğada böğürtlen, çilek, nar, ahududu, kırmızı üzüm, badem ve ceviz gibi birçok bitkide serbest ve

ellajitanen glikozitleri halinde bulunan fenolik karakterde antioksidan bileşiklerdir. Ellajik asit, ellagitaninlerin bazı kimyasal işlemler geçirmesi sonucunda oluşur. Ellajik asit [2,3,7,8-tetrahidroksi(1)benzopirano (5,4,3-cde)(1)benzopiran-5,10 dion]'in kimyasal formülü $C_{14}H_6O_8$ ve molekül ağırlığı 302,197 g/mol'dür. Genellikle açık sarı renkte ve toz halinde bulunur. Doğada zayıf bir asit olan ellajik asit, 360 ºC' nin üzerinde yüksek erime noktasında çok kararlı bir bileşik olur. Yapısında bulunan dört fenolik grup, ellajik asidin kalsiyum ve magnezyum gibi metal iyonları ile kompleks form oluşturmasını sağlar (Erenoğlu, 2012; Diop, 2013; Seyhan, 2013; Tıkırdık, 2019). Ellajik asit serbest radikallerin olumsuz etkilerini bloke edici özelliği olan bir antioksidandır. Etki mekanizması tam olarak bilinmemesine rağmen süperoksit ve hidroksil anyonları gibi reaktif oksijen türevlerini güçlü bir şekilde temizlediği bildirilmektedir (Losso vd., 2004; Landete, 2011; Çeribaşı vd., 2012).

Lipid peroksidasyon, hücre membranında bulunan doymamış yağ asitlerinin, serbest oksijen radikalleri tarafından peroksitler, alkoller, aldehitler, hidroksi yağ asitleri, etan ve pentan gibi çeşitli ürünlere yıkılması reaksiyonudur. Lipid peroksidasyonunun en önemli ürünü olan malondialdehit (MDA), iyon alışverişini değiştirerek hücre membranındaki bileşenlerin çapraz bağlanmasına, enzim aktivitesi ve iyon geçirgenliğinin değişmesine neden olur. MDA, lipid peroksid düzeyinin belirlenmesinde sıklıkla kullanılmaktadır (Morales vd., 2004). Serbest oksijen radikallerinin oluşmasını engellemek ve bu radikallerin yol açtığı oksidatif hasardan korunmak için vücutta katalaz (CAT), glutatyon peroksidaz (GSH-PX), süperoksit dismutaz (SOD), glutatyon redüktaz (GR) ve glutatyon-S-transferaz (GST) gibi antioksidan enzimler bulunmaktadır (Droge, 2002; Storey, 1996).

Bu araştırmada, yeme farklı oranlarda katılan ellajik asidin pullu sazanda büyüme ve oksidan/antioksidan parametrelere etkisinin araştırılması amaçlanmıştır. Bunun için balıklara ellajik asidin farklı dozları uygulanmış, büyüme, oksidatif stres ve antioksidan parametrelerdeki değişimler incelenmiştir. İncelenen parametreler ışığında ellajik asidin pullu sazandaki antioksidan potansiyeli ortaya konulmuştur.

MATERYAL VE METOT

Başlangıç ağırlığı yaklaşık 50 ± 5 g olan 240 adet canlı pullu sazan (*Cyprinus carpio*) 33 x 100 x 60 cm boyutlarındaki 12 farklı cam akvaryum (3 tekrar ve her bir tekrar için 4 akvaryum, her akvaryum için 20 ve toplamda 240 balık) içerisine yerleştirildi. Deneysel çalışma başlamadan önce balıklar bu ortama 15 gün süreyle adapte edildi. Adaptasyon süresince balıklara günde iki kere alabildikleri kadar ticari balık yemi verildi.

Çalışmada kullanılan ellajik asit Sigma-Aldrich' den (katalog no: E2250), laboratuvar analizleri sırasında kullanılan kimyasallar ise Sigma-Aldrich, Merck, Serva, Isolab, VWR Chemicals, Fluka, AppliChem, ABCR firmalarından elde edildi.

Deneme yemlerinin hazırlanması için ellajik asit 50, 100 ve 200 mg/kg yem oranında tartıldı. Daha sonra özel bir firmadan alınan ve toz haline getirilen pelet yemlerle karıştırıldı. Hamur haline getirilen karışım kıyma makinesinden geçirilerek tekrar pelet haline dönüştürüldü. Hazırlanan peletler tepsilere yerleştirilip yem fırınında oda ısısında kurutuldu. Yemler kullanılıncaya kadar koyu renkli cam muhafaza kapları içerisinde ve 4 °C' de muhafaza edildi.

Adaptasyon süresi sonunda balıklar aşağıdaki gibi dört grubu ayrıldı.

K: Kontrol grubu,

EA-50: 60 gün için 50 mg/kg yem ellajik asit uygulanan grup,

EA-100: 60 gün için 100 mg/kg yem ellajik asit uygulanan grup,

EA-200: 60 gün için 200 mg/kg yem ellajik asit uygulanan grup.

Yemler balıklara sabah ve akşam olmak üzere iki defa ve doyuncaya kadar verildi. Ellajik asidin uygulanan dozları için Mişe Yonar vd. (2014) ve Mişe Yonar (2019)'ın çalışmaları referans alındı. Çalışma Fırat Üniversitesi Hayvan Deneyleri Etik Kurulu Başkanlığı tarafından onaylandı (Protokol No: 2019/62).

Araştırmanın 30. ve 60. günlerinde her bir tekrardan 10 balık alınarak 25 mg/L konsantrasyonundaki benzokain (25 mg/l) yardımıyla anestezi edildi (San ve Yonar, 2017). Anestezi altındaki balıklar büyüme parametrelerinin belirlenmesi amacıyla tartıldı. Daha sonra usulüne uygun bir şekilde otopsisi yapılan (Arda vd., 2005) balıkların karaciğer ve böbreği çıkarılarak folyolara sarıldı ve -20ºC' de derin dondurucuda saklandı. Karaciğer ve böbrek örnekleri 30 gün içerisinde işlendi.

Büyüme oranının belirlenmesi için canlı ağırlık artışı (CAA) (Çelikkale, 1988), oransal büyüme (OB) (Çelikkale, 1988) ve spesifik büyüme oranı (SBO) (Halver, 1989) aşağıdaki formüller kullanılarak hesaplandı.

CAA = Çalışma Sonu Ortalama Ağırlığı (g) - Çalışma Başı Ortalama Ağırlığı (g)

OB = (Çalışma Sonu Ortalama Ağırlık - Çalışma Başı Ortalama Ağırlık) / (Çalışma Başı Ortalama Ağırlık)] x 100

SBO = [[(Log_e Çalışma Sonu Ortalama Ağırlık) - (Log_e Çalışma Başı Ortalama Ağırlık)] / Çalışma Süresi]] x 100

Karaciğer ve böbrek örneklerinden oksidan/antioksidan parametrelerin belirlenmesi için homojenatlar hazırlandı. Homojenatların hazırlanması için doku örnekleri, serum fizyolojik (%0,09 NaCl) ile yıkandı. İki süzgeç kâğıdı arasında suyu alındıktan sonra %1.15'lik KCl ile 1:10 oranında sulandırılarak cam-cam homojenizatör içerisinde homojenize edildi. Elde edilen homojenatlar 50 ml'lik propilen tüplerde soğutmalı santrifüjde 3200 rpm'de 4°C'de 10 dakika santrifüj edildikten sonra süpernatantlar alındı (Sakin vd., 2011; Mişe

Yonar vd., 2017). Süpernatantlarda lipit peroksidasyonun bir göstergesi olarak malondialdehit (MDA) düzeyi (Placer vd., 1966), antioksidan parametrelerden ise katalaz (CAT) aktivitesi (Aebi, 1983), glutatyon S-transferaz (GST) aktivitesi (Habig vd., 1974) ve redükte glutatyon (GSH) düzeyi (Ellman vd., 1959) belirlendi.

Dokulardaki CAT ve GST spesifik enzim aktivitesi ile MDA ve GSH düzeylerini hesaplamak için ölçülen doku protein düzeyleri Lowry vd. (1951) tarafından bildirilen yönteme göre tespit edildi.

Sonuçların istatistiksel analizleri için SPSS 21.0 istatistik programı kullanıldı. Kontrol ve deneme gruplarının incelenen parametrelerinde oluşan değişimler p<0,05 düzeyinde tek yönlü varyans analizi ile (ONEWAY – ANOVA) test edildi. Gruplar arasındaki farklılıklar ise Least Significant Difference (LSD) ile test edildi. Bağımlı gruplarda istatistiksel farklılığı ortaya çıkarabilmek amacıyla tekrarlı ölçümlerde varyans analizi kullanıldı. Sonuçlar ortalama ± standart hata olarak verildi.

BULGULAR

Çalışmaya başlamadan önce 15 gün süreyle adaptasyonu sağlanan balıklarda herhangi bir ölüm olayı yaşanmadı. Yine 60 günlük deneme süresince kontrol ve deneme grubu balıklarında da herhangi bir ölüm gözlenmedi. Adaptasyon ve deneme süresince kontrol ve deneme grubu balıklarının yem alımlarında herhangi bir aksaklık yaşanmadı. Deneme gruplarının ellajik asit içeren deneme yemlerini aldıkları görüldü. Kontrol grubuna kıyasla ellajik asit verilen grupların büyüme parametrelerinde herhangi bir değişiklik gözlemlenmezken oksidan/antioksidan parametrelerde önemli farklılıklar tespit edildi.

Kontrol grubu ve ellajik asit uygulanan gruplardaki balıkların, CAA, OB ve SBO değerleri Tablo 1' de verilmiştir.

Tablo 1. Kontrol ve deneme gruplarının büyüme parametreleri
Table 1. Growth parameters of the control and experimental groups

		Gruplar			
		K	EA-50	EA-100	EA-200
BA(g)		50,47±2,74	51,12±3,10	49,88±2,17	50,06±2,33
FA(g)	30.gün	71,33±3,47	72,11±3,62	72,45±4,69	72,83±3,70
	60.gün	90,17±3,82	91,30±4,23	91,89±3,76	92,33±4,12
CAA(g)	30.gün	20,86±2,11	20,99±2,45	22,57±1,86	22,77±2,38
	60.gün	39,70±3,41	40,18±2,89	42,01±3,28	42,27±3,56
OB(%)	30.gün	41,33±2,74	41,06±3,20	45,24±3,95	45,48±3,48
	60.gün	78,66±3,88	78,59±3,76	84,22±4,45	84,43±4,29
SBO(%)	30.gün	0,500±0,03	0,497±0,04	0,543±0,04	0,543±0,05
	60.gün	0,420±0,04	0,420±0,04	0,443±0,03	0,443±0,04

BA: Başlangıç ağırlığı, FA: Final ağırlığı, CAA: Canlı ağırlık artışı, OB: Oransal büyüme, SBO: Spesifik büyüme oranı, K: Kontrol grubu, EA-50: 50 mg ellajik asit uygulanan grup, EA-100: 100 mg ellajik asit uygulanan grup, EA-200: 200 mg ellajik asit uygulanan grup

Kontrol grubu ve sırasıyla 50, 100 ve 200 mg/kg yem oranında ellajik asit verilen EA-50, EA-100 ve EA-200 gruplarının CAA, OB ve SBO değerlerinde çalışmanın hem 30. hem de 60. günlerinde istatistiksel herhangi bir farklılık görülmedi (p> 0,05). Ayrıca yalnız ellajik asit verilen EA-50, EA-100 ve EA-200 grupları kendi içerisinde kıyaslandığında hem 30. hem de 60. gündeki büyüme parametreleri değerlerinde istatistiksel olarak herhangi bir farklılık belirlenmedi (p> 0,05).

Kontrol grubu ve ellajik asit uygulanan gruplardaki balıkların karaciğer ve böbrek MDA düzeyleri Tablo 2' de verilmiştir.

Tablo 2. Kontrol ve deneme gruplarının karaciğer ve böbrek MDA düzeyleri (nmol/mg protein)
Table 2. Liver and kidney MDA levels (nmol/mg protein) of the control and experimental groups

		Gruplar			
		K	EA-50	EA-100	EA-200
Karaciğer	30.gün	2,88±0,13^{c}	2,47±0,10^{b}	2,19±0,12^{a}	2,18±0,10^{a}
	60.gün	2,85±0,11^{c}	2,23±0,13^{b,*}	2,01±0,11^{a,*}	2,02±0,12^{a,*}
Böbrek	30.gün	3,95±0,10^{c}	3,61±0,11^{b}	3,33±0,13^{a}	3,31±0,12^{a}
	60.gün	3,96±0,12^{c}	3,38±0,12^{b,*}	3,10±0,14^{a,*}	3,08±0,10^{a,*}

K: Kontrol grubu, EA-50: 50 mg ellajik asit uygulanan grup, EA-100: 100 mg ellajik asit uygulanan grup, EA-200: 200 mg ellajik asit uygulanan grup; ^{a,b,c} Aynı satırda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır (p < 0,05); * Aynı grup içinde 30. günden farkı göstermektedir (p < 0,05).

Çalışmanın 30. gününde kontrol grubuna göre ellajik asit uygulanan EA-50, EA-100 ve EA-200 gruplarının karaciğer ve böbrek MDA düzeylerinin istatistiksel olarak önemli düzeyde azaldığı belirlendi (p<0,05). EA-50, EA-100 ve EA-200 grupları kendi içerisinde kıyaslandığında ise EA-100 ve EA-200 gruplarının karaciğer ve böbrek MDA düzeylerinin EA-50 grubundan istatistiksel olarak daha düşük olduğu görüldü (p<0,05).

Denemenin 60. gününde ellajik asit uygulanan EA-50, EA-100 ve EA-200 gruplarının karaciğer ve böbrek MDA düzeylerinin kontrol grubuyla kıyaslandığında istatistiksel olarak önemli düzeyde azaldığı belirlendi (p<0,05). EA-50, EA-100 ve EA-200 grupları kendi arasında karşılaştırıldığında ise EA-100 ve EA-200 gruplarının karaciğer ve böbrek MDA düzeylerinin EA-50 grubundan daha düşük olduğu tespit edildi (p <0,05).

EA-50, EA-100 ve EA-200 gruplarının 60. gündeki karaciğer ve böbrek MDA düzeylerinin 30. güne kıyasla istatistiksel olarak daha düşük olduğu saptandı (p <0,05).

Kontrol grubu ve ellajik asit uygulanan gruplardaki balıkların karaciğer ve böbrek CAT aktiviteleri Tablo 3' de verilmiştir.

Tablo 3. Kontrol ve deneme gruplarının karaciğer ve böbrek CAT aktiviteleri (k/mg protein)
Table 3. Liver and kidney CAT activities (k/mg protein) of the control and experimental groups

		Gruplar			
		K	EA-50	EA-100	EA-200
Karaciğer	30.gün	3,66±0,18^{a}	3,95±0,21^{b}	4,22±0,25^{c}	4,20±0,23^{c}
	60.gün	3,65±0,22^{a}	4,20±0,32^{b,*}	4,64±0,29^{c,*}	4,68±0,26^{c,*}
Böbrek	30.gün	3,14±0,15^{a}	3,42±0,21^{b}	3,89±0,18^{c}	3,91±0,22^{c}
	60.gün	3,16±0,18^{a}	3,80±0,24^{b,*}	4,03±0,17^{c,*}	4,02±0,24^{c,*}

K: Kontrol grubu, EA-50: 50 mg ellajik asit uygulanan grup, EA-100: 100 mg ellajik asit uygulanan grup, EA-200: 200 mg ellajik asit uygulanan grup; ^{a,b,c} Aynı satırda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır ($p < 0,05$); * Aynı grup içinde 30. günden farkı göstermektedir ($p < 0,05$).

Denemenin 30. gününde ellajik asit uygulanan EA-50, EA-100 ve EA-200 gruplarının karaciğer ve böbrek CAT aktivitelerinin kontrol grubuyla kıyaslandığında istatistiksel olarak önemli düzeyde arttığı belirlendi ($p <0,05$). EA-50, EA-100 ve EA-200 grupları kendi arasında karşılaştırıldığında ise EA-100 ve EA-200 gruplarının karaciğer ve böbrek CAT aktivitelerinin EA-50 grubundan daha yüksek olduğu tespit edildi ($p <0,05$).

Uygulamanın 60. gününde kontrol grubuna göre ellajik asit verilen EA-50, EA-100 ve EA-200 gruplarının karaciğer ve böbrek CAT aktivitelerinin istatistiksel olarak önemli düzeyde arttığı belirlendi ($p <0,05$). EA-50, EA-100 ve EA-200 grupları kendi içerisinde kıyaslandığında ise EA-100 ve EA-200 gruplarının karaciğer ve böbrek CAT aktivitelerinin EA-50 grubundan istatistiksel olarak daha yüksek olduğu görüldü ($p <0,05$).

EA-50, EA-100 ve EA-200 gruplarının 60. gündeki karaciğer ve böbrek CAT aktivitelerinin 30. güne kıyasla istatistiksel olarak daha yüksek olduğu belirlendi ($p <0,05$).

Kontrol grubu ve ellajik asit uygulanan gruplardaki balıkların karaciğer ve böbrek GST aktiviteleri Tablo 4' de verilmiştir.

Çalışmanın 30. gününde kontrol grubuna göre ellajik asit uygulanan EA-50, EA-100 ve EA-200 gruplarının karaciğer ve böbrek GST aktivitelerinin istatistiksel olarak önemli düzeyde arttığı belirlendi ($p <0,05$). EA-50, EA-100 ve EA-200 grupları kendi içerisinde kıyaslandığında ise EA-100 ve EA-200 gruplarının karaciğer ve böbrek GST aktivitelerinin EA-50 grubundan istatistiksel olarak daha yüksek olduğu görüldü ($p <0,05$).

Denemenin 60. gününde ellajik asit uygulanan EA-50, EA-100 ve EA-200 gruplarının karaciğer ve böbrek GST aktivitelerinin kontrol grubuyla kıyaslandığında istatistiksel olarak önemli düzeyde arttığı belirlendi ($p <0,05$). EA-50, EA-100 ve EA-200 grupları kendi arasında karşılaştırıldığında ise EA-100 ve EA-200 gruplarının karaciğer ve böbrek GST aktivitelerinin EA-50 grubundan daha yüksek olduğu tespit edildi ($p<0,05$).

Tablo 4. Kontrol ve deneme gruplarının karaciğer ve böbrek GST aktiviteleri (µmol/dakika/mg protein)
Table 4. Liver and kidney GST activities (µmol/dakika/mg protein) of the control and experimental groups

		Gruplar			
		K	EA-50	EA-100	EA-200
Karaciğer	30.gün	113,47±8,22^{a}	135,86±11,30^{b}	159,87±12,49^{c}	161,04±10,63^{c}
	60.gün	115,01±7,05^{a}	138,08±9,27^{b}	156,35±11,02^{c}	160,18±12,41^{c}
Böbrek	30.gün	98,63±4,35^{a}	119,20±6,47^{b}	136,41±5,33^{c}	134,19±5,87^{c}
	60.gün	97,14±3,91^{a}	117,73±4,86^{b}	140,02±6,18^{c}	139,00±7,14^{c}

K: Kontrol grubu, EA-50: 50 mg ellajik asit uygulanan grup, EA-100: 100 mg ellajik asit uygulanan grup, EA-200: 200 mg ellajik asit uygulanan grup; ^{a,b,c} Aynı satırda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır ($p < 0,05$); * Aynı grup içinde 30. günden farkı göstermektedir ($p< 0,05$)

EA-50, EA-100 ve EA-200 gruplarının 60. gündeki karaciğer ve böbrek GST aktivitelerinin 30. güne kıyasla istatistiksel olarak herhangi bir farklılık göstermediği saptandı ($p> 0,05$).

Kontrol grubu ve ellajik asit uygulanan gruplardaki balıkların karaciğer ve böbrek GSH aktiviteleri Tablo 5' de verilmiştir.

Tablo 5. Kontrol ve deneme gruplarının karaciğer ve böbrek GSH düzeyleri (µmol/mg protein)
Table 5. Liver and kidney GSH levels (µmol/mg protein) of the control and experimental groups

		Gruplar			
		K	EA-50	EA-100	EA-200
Karaciğer	30.gün	74,49±4,02^{a}	89,80±5,11^{b}	102,03±5,89^{c}	101,01±4,21^{c}
	60.gün	75,06±3,69^{a}	90,73±4,28^{b}	103,56±4,62^{c}	103,31±5,04^{c}
Böbrek	30.gün	46,58±3,15^{a}	61,02±4,51^{b}	73,73±3,19^{c}	75,10±4,09^{c}
	60.gün	45,94±4,30^{a}	61,28±3,47^{b}	75,52±4,01^{c}	74,70±3,66^{c}

K: Kontrol grubu, EA-50: 50 mg ellajik asit uygulanan grup, EA-100: 100 mg ellajik asit uygulanan grup, EA-200: 200 mg ellajik asit uygulanan grup; ^{a,b,c} Aynı satırda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır ($p < 0,05$); * Aynı grup içinde 30. günden farkı göstermektedir ($p < 0,05$)

Denemenin 30. gününde kontrol grubuna göre ellajik asit uygulanan EA-50, EA-100 ve EA-200 gruplarının karaciğer ve böbrek GSH düzeylerinin istatistiksel olarak önemli düzeyde arttığı tespit edildi ($p <0,05$). EA-50, EA-100 ve EA-200 grupları kendi içerisinde kıyaslandığında ise EA-100 ve EA-200 gruplarının karaciğer ve böbrek GSH düzeylerinin EA-50 grubundan istatistiksel olarak daha yüksek olduğu belirlendi ($p <0,05$).

Uygulamanın 60. gününde ellajik asit verilen EA-50, EA-100 ve EA-200 gruplarının karaciğer ve böbrek GSH düzeylerinin kontrol grubuyla kıyaslandığında istatistiksel olarak önemli düzeyde arttığı saptandı ($p <0,05$). EA-50, EA-100 ve EA-200 grupları kendi arasında karşılaştırıldığında ise EA-100 ve EA-200 gruplarının karaciğer ve böbrek GSH düzeylerinin EA-50 grubundan daha yüksek olduğu görüldü ($p<0,05$).

EA-50, EA-100 ve EA-200 gruplarının 60. gündeki karaciğer ve böbrek GSH düzeylerinin 30. güne kıyasla istatistiksel olarak herhangi bir farklılık göstermediği belirlendi ($p > 0,05$).

TARTIŞMA VE SONUÇ

Üretim ve karlılıkla direkt bağlantılı olarak kısa süre içerisinde büyümede sağlanabilecek maksimum artış su ürünleri yetiştiriciliğinde oldukça önemlidir ve özel ilgi duyulan bir konudur (Wang vd., 2015). Büyüme parametreleri üzerine immunostimulan ve antioksidan karakterdeki doğal maddelerin etkileri farklı balık türlerinde farklı araştırmacılar tarafından rapor edilmiştir (Nya ve Austin, 2009a; Nya ve Austin, 2009b; Zahran vd., 2014; Musthafa vd., 2018; Mehrabi vd., 2019; Li vd., 2019; Yousefi vd., 2019; Yonar vd., 2019; Farsani vd., 2019). Mişe Yonar (2019) tarafından yapılan bir çalışmada, 8 hafta süreyle 50, 100 ve 200 mg ellajik asit/kg yem verilen alabalıkların ağırlık kazancı, spesifik büyüme oranı ve yem dönüşüm oranında kontrol grubuna kıyasla istatistiksel herhangi bir farklılık olmadığı ifade edilmiştir. Benzer sonuçlar bu çalışmada da elde edilmiş, ellajik asit verilen deneme gruplarının CAA, OB ve SBO değerlerinde kontrol grubuna göre istatistiksel olarak herhangi bir farklılık tespit edilmemiştir. Bu sonuç ellajik asidin pullu sazanın büyüme performansı üzerine olumlu herhangi bir etkisinin olmadığını göstermiştir. Bunun nedeni ellajik asitte bulunan ve antibesinsel bir faktör olan tanen ile açıklanabilir. Diğer taraftan elde edilen sonuçlar ellajik asidin büyümeyi olumsuz etkilemediğini de göstermiştir. Çünkü kontrol grubuna göre ellajik asit verilen gruplara göre deneme gruplarının büyüme parametrelerinde olumsuz herhangi bir sonuç belirlenmemiştir.

Serbest radikallerin en önemli etkileri karbohidrat, protein, lipit ve nükleik asitlerin yıkımına sebep olmalarıdır. Doymamış yağ asitlerinin oksidatif yıkımı sonucu oluşan, bir başka ifadeyle lipid peroksidasyon sonucu açığa çıkan aldehitlerden biri olan malondialdehit MDA düzeyinin ölçülmesi hücrelerde oluşan oksidatif hasarın belirlenmesinde kullanılan en önemli göstergelerden biridir (Morales vd., 2004; Fontagné vd., 2006). Bu çalışmada ellajik asit uygulanan EA-50, EA-100 ve EA-200 deneme gruplarında, karaciğer ve böbrek MDA düzeylerinin kontrol grubuna göre istatistiksel olarak önemli düzeyde azaldığı, yine EA-100 ve EA-200 deneme gruplarında karaciğer ve böbrek MDA düzeylerinin EA-50 grubundan daha düşük olduğu belirlenmiştir. Benzer sonuçlar 21 gün süreyle 50, 100 ve 150 mg (Mişe Yonar vd., 2014) ve 8 hafta süreyle 50, 100 ve 200 mg (Mişe Yonar 2019) dozunda ellajik asit uygulanan gökkuşağı alabalıklarında da elde edilmiştir. Her iki araştırmada da ellajik asit uygulanan balıkların karaciğer, böbrek ve dalağında MDA düzeyinin azaldığı belirlenmiştir. Ural vd. (2015) tarafından yapılan ve pullu sazanda malathion pestisitine karşı ellajik asit koruyuculuğunun araştırıldığı başka bir çalışmada ise 14 gün süreyle 100 mg/kg balık dozunda yalnız ellajik asit verilen grupların karaciğer, böbrek ve solungaç MDA düzeyleri araştırılmıştır. Sonuç olarak bu çalışmadan elde edilen verilerin aksine karaciğer ve böbrek MDA düzeyinde istatistiksel olarak önemli olmayan bir düşüş, solungaç MDA düzeyinde ise yine istatistiksel açıdan önemsiz bir artış tespit edilmiştir.

Balıklarda antioksidanlar diğer yüksek omurgalılarda olduğu gibi enzimatik (süperoksid dismutaz, katalaz, glutatyon peroksidaz ve glutatyona bağlı diğer enzimler) ve non-enzimatik (redükte glutatyon, vitamin E ve C, β karoten vb.) olarak sınıflandırılırlar (Belló vd., 2000; Mourente vd., 2002; Puangkaew vd., 2005). Süperoksit radikalinin dismutasyonuyla oluşan hidrojen peroksit radikalini temizleyen ve çok önemli bir antioksidan enzim olan CAT, oksidatif strese karşı savunmada antioksidan sistemin öncelikli bir komponentidir. Ural vd. (2015) pullu sazanda malathion pestisitine karşı ellajik asit koruyuculuğunu araştırmış ve 100 mg/kg balık dozunda 14 gün süreyle yalnız ellajik asidin verildiği gruplarda karaciğer, böbrek ve solungaç CAT aktivitelerinin arttığını gözlemlemişlerdir. Diğer taraftan 50, 100 ve 150 mg/kg dozlarında 21 gün süreyle ellajik asidin uygulandığı gökkuşağı alabalığında her üç deneme grubunun CAT aktivitesi kontrol grubuna göre istatistiksel olarak artış göstermiştir. Fakat deneme grupları kendi içerisinde karşılaştırıldığında belirlenen artışlar önemsiz bulunmuştur (Mişe Yonar vd., 2014). 50, 100 ve 200 mg/kg dozlarında ve 8 hafta süreyle ellajik asit verilen gökkuşağı alabalıklarında kontrol grubuna göre deneme gruplarının CAT aktivitesinin arttığı belirlenmiştir. Yine aynı çalışmada 100 ve 200 mg ellajik asit uygulanan grupların CAT aktivitesi 50 mg ellajik asit uygulanan gruptan yüksek bulunmuştur (Mişe Yonar 2019). Bu araştırmada ise ellajik asidin her üç dozunun uygulandığı deneme gruplarında karaciğer ve böbrek CAT aktivitesinin çalışmanın hem 30. gününde hem de 60. gününde kontrol grubuna göre arttığı belirlenmiştir. Ayrıca 100 ve 200 mg dozunda ellajik asit verilen grupların karaciğer ve böbrek CAT aktivitesi 50 mg dozunda ellajik asit verilen gruptan da yüksek bulunmuştur.

Serbest radikaller ve peroksitlerle reaksiyona girerek hücreleri oksidatif stresten koruyan tripeptit karakterdeki GSH, çok önemli bir antioksidan olup non-enzimatik ve endojen özelliktedir. Protein yapısındaki sülfhidril gruplarını indirgenmiş halde tutan GSH böylece çoğu protein ve enzimin inaktive olmasını önler (Hayes ve McLellan 1999). Diğer taraftan GSH ile birlikte elektrofilik gruplar arasındaki konjugasyonu katalizleyen GST, ksenobiyotik ve endojen bileşiklerin detoksifikasyonu ve biyotransformasyonunda görevli faz II enzim ailesinin bir üyesidir (Hamed vd., 2003). Ural vd. (2015) 14 gün süreyle 100 mg/kg balık dozunda ellajik asit verilen grupların karaciğer, böbrek ve solungaç GST aktivitelerini araştırmıştır. Sonuç olarak karaciğer ve solungaç GST aktivitesinde istatistiksel olarak önemli olmayan bir artış, böbrek GST aktivitesinde ise yine istatistiksel açıdan önemsiz bir düşüş tespit edilmiştir. Bu çalışmada ise ellajik asit verilen deneme gruplarının karaciğer ve böbrek dokusundaki GSH düzeyi ve GST aktivitesi çalışmanın hem 30. hem de 60. gününde kontrol grubuna

göre istatistiksel olarak önemli bir artış göstermiştir. Her iki çalışmadan elde edilen sonuçlar arasındaki farklılık ellajik asidin uygulanan dozu ve uygulama süresiyle açıklanabilir.

Özetle ellajik asidin pullu sazanın büyüme performansı üzerine herhangi bir etkisinin olmadığı görülmüştür. Kontrol ve ellajik asit uygulanan grupların CAA, OB ve SBO değerlerinde istatistiksel olarak herhangi bir farklılık belirlenmemiştir. Bununla birlikte ellajik asidin büyümeyi olumsuz etkilemediği de tespit edilmiştir. Diğer taraftan farklı dozlarda ellajik asit verilen gruplarda karaciğer ve böbrek MDA düzeylerinin düştüğü, bir başka ifadeyle oksidatif stresin azaldığı belirlenmiştir. Ayrıca farklı dozlarda ellajik asit uygulanan grupların karaciğer ve böbrek CAT ve GST aktiviteleri ile GSH düzeylerinin yükseldiği dolayısıyla da antioksidan kapasitenin arttığı görülmüştür. Sonuç olarak, ellajik asit uygulaması oksidatif stresi azalttığı ve antioksidan kapasiteyi arttırdığı için bu madde balıklarda antioksidan olarak kullanılabilir. Fakat farklı balık türlerinde, farklı doz ve süreler için farklı yöntemler kullanılarak ellajik asit uygulamasının sonuçlarına ihtiyaç olduğu görülmektedir.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Feeding of *Barbus cyclolepis* Heckel, 1837 (Teleostei: Cyprinidae) and its relationship with benthic macroinvertebrate fauna in the Istranca Stream (İstanbul, Turkey)

Barbus cyclolepis Heckel, 1837 (Teleostei: Cyprinidae)'in beslenmesi ve Istranca Deresi (İstanbul, Türkiye)'ndeki makroomurgasız faunası ile ilişkisi

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Abstract: The aims of the study are to determine what the organisms *Barbus cyclolepis* consume as food in its feeding environments and to compare prey consumption with prey abundance in the environment. The study was conducted in the Istranca Stream located in Istanbul (Turkey) during the spring and summer of 2012. A total of 142 *B. cyclolepis* specimens were captured and it is determined that 94 of them had full digestive tracts. Diet analyses of *B. cyclolepis* showed that its food spectrum consisted of 11 different food types, and the species was found to feed on insects, mainly Diptera (IRI%= 92.26%). The most abundant macroinvertebrate organisms in the environment were Diptera and Gastropoda. The electivity index of *B. cyclolepis* was positive for Diptera in the spring (E= 0.49), but the value was below the expected value of 0.6 for high selectivity. The electivity values for other macroinvertebrate groups, consumed in low proportions, were negative. In summer, the fish fed on Diptera, Plecoptera, and Trichoptera, but a high electivity index value was found only for Trichoptera (E= 0.87). Results showed that *B. cyclolepis* mainly consumed Diptera as food and did not consume Gastropoda, although it is the second most abundant macroinvertebrate group in the environment. A relationship was determined between the proportion of food groups consumed in the digestive tracts of fish and the ratios of macroinvertebrates in the environment, and as a result, it was specified that the fish was selective on Diptera.

Keywords: *Barbus cyclolepis*, diet, selectivity, benthic insects, stream, freshwater fish

Öz: Çalışmanın amacı, *Barbus cyclolepis*'in beslenme ortamında hangi besinleri tükettiğini belirlemek ve av tüketimini çevredeki av bolluğu ile karşılaştırmaktır. Çalışma 2012 yılı ilkbahar ve yaz aylarında İstanbul (Türkiye)'da bulunan Istranca Deresi'nde yürütülmüştür. Toplam 142 adet *B. cyclolepis* yakalanmıştır ve bunlardan 94 adedinin sindirim kanalının dolu olduğu tespit edilmiştir. *Barbus cyclolepis*'in diyet analizleri, türün besin spektrumunun 11 farklı besin türünden oluştuğunu ve başlıca Diptera (%IRI= %92,26) olmak üzere böceklerle beslendiği göstermiştir. Çalışma ortamında sayısal olarak en bol bulunan makroomurgasız organizmalar Diptera ve Gastropoda olarak belirlenmiştir. *Barbus cyclolepis*'in seçicilik indeksi ilkbaharda Diptera için pozitif olarak belirlenmiş (E= 0,49) ancak değer, yüksek seçicilik açısından beklenen 0,6 değerinin altında bulunmuştur. Düşük oranlarda tüketilen diğer makroomurgasız grupları için ise seçicilik değerleri negatif olarak saptanmıştır. Yaz aylarında, Diptera, Plecoptera ve Trichoptera ile beslenen türde sadece Trichoptera (E= 0,87) için yüksek bir seçicilik indeksi değeri tespit edilmiştir. Sonuçlar, *B. cyclolepis*'in ağırlıklı olarak Diptera'yı besin olarak tükettiğini ve buna karşılık çevrede sayısal olarak en bol bulunan ikinci makroomurgasız grubu olmasına rağmen Gastropoda ile beslenmediğini saptamıştır. Balıkların sindirim sistemlerinde tüketilen besin gruplarının oranı ile çevredeki makroomurgasızların oranları arasında bir ilişki tespit edilmiş ve sonuçta türün Diptera üzerinde seçici olduğu belirlenmiştir.

Anahtar kelimeler: *Barbus cyclolepis,* besin, seçicilik, bentik böcekler, dere, tatlısu balığı

INTRODUCTION

Fish diet studies provide information about the food preferences of fish. The food items that the fish are fed are revealed by examining the contents of the stomach and information about their consumption rates are obtained. It is an important question whether the fish are fed randomly or by choosing the organisms found in the environment. The answer of this question can be found by comparing the number of each organism found in the stomach contents of fish and their proportions with the organisms living in the environment (Tupinambas et al., 2015)

Benthic macroinvertebrate species have an important role in the diet of many benthic fish species. Macroinvertebrates have an important place in the biodiversity of rivers and lakes, and they also act as nutrient recyclers, primary and secondary consumers, and food for wildlife (Keiper et al.,

2002). These organisms can also serve as ecological indicators to determine the productivity and water quality of aquatic environments (López-López and Sedeño-Díaz, 2015).

Species of the genus *Barbus* are bottom-feeders and use their barbels to locate food (mostly bottom-dwelling and drifting benthic organisms) in the sediment. However, they are mostly small-sized rheophilic cyprinids, except a few species, (Antal et al., 2016) and usually found in riffle areas of streams because strong flow enhances the abundance of drift organisms such as macroinvertebrates in these gravel and rocky bottomed areas. *Barbus cyclolepis* Heckel, 1837, one of the members of this genus, spreads in a limited area in south-eastern Europe (Kottelat and Freyhof, 2007). In the study conducted on the diet and feeding habits of this species, it was reported that the food of the population living in the Meriç River Basin is dominated by Chironomid larvae, followed by plant detritus and Gammarids (Rozdina et al., 2008). In the present study, the aim was to find answers to the following questions by temporally and spatially comparing the diet of *B. cyclolepis* in the Istranca Stream, where it inhabits together with various macroinvertebrate species:

1) what are the organisms that *B. cyclolepis* consume as food?
2) what is the selectivity of the fish in its feeding environment?

The presence/absence of available food sources or their abundance are the factors that can influence the feeding preference of a fish and in many of the feeding ecology studies. However, it is seen that the stomach contents of the fish are not compared with the possible food groups living in the environment. It is thought that the findings to be obtained as a result of the answers to these questions will contribute to other studies that will examine the interaction of fish species with other organisms in their habitats as well as their trophic levels.

MATERIAL AND METHODS

Study area

The study was conducted in the endorheic Istranca Stream, which is located in the northwest of Istanbul and flows into Lake Durusu (Figure 1, see Saç and Özuluğ, 2020a,b). A total of 20 fish species belonging to seven families (Acheilognathidae, Cyprinidae, Cobitidae, Esocidae, Gobiidae, Gobionidae and Leuciscidae) inhabit the stream (Saç and Özuluğ, 2017). Fish and macroinvertebrate samplings were performed at six stations:

Taşlıgeçit Creek (St. 1 – 41.33098°N, 28.24897°E) is a small and shallow stream with a mostly stony substrate. The stream bed mostly shows riffle characteristics,

Danamandıra Creek (St. 2 – 41.31415ºN, 28.24893ºE) is a shallow stream and its substrate is mostly sandy and rarely stony, especially in riffle areas. It contains dense filamentous algae and submerged macrophytes in warm seasons as it is affected by domestic and agricultural pollution and animal waste,

Şeytan Creek (St. 3 – 41.41750ºN, 28.13845ºE) is a small and shallow stream with stony (cobbles) substrate. It is wooded along the bank and the roots of the trees extend into the stream,

Binkılıç Creek (St. 4 – 41.39901ºN, 28.19366ºE) shows different water depths depending on seasons and its substrate is mostly stony (gravel and cobbles). It is influenced by sewage discharge and domestic pollution,

Karamandere Creek (St. 5 – 41.37920ºN, 28.29610ºE) is the deepest one with a mostly stony substrate (boulders and cobbles),

Karacaköy Creek (St. 6 – 41.39946ºN, 28.38352ºE) shows different water depths depending on seasons and is influenced by sewage discharge and domestic pollution. The substrate is mostly sandy (Saç and Özuluğ, 2017).

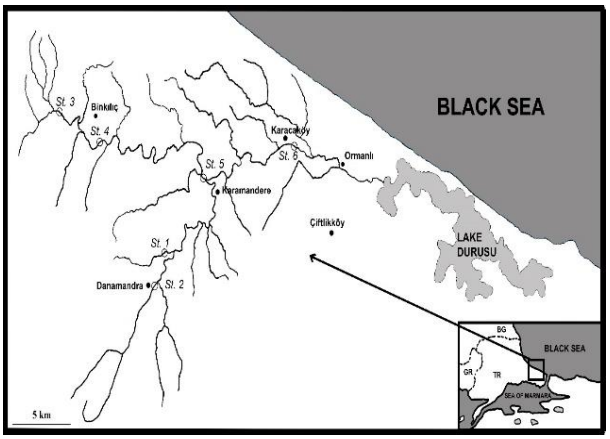

Figure 1. Study area (Istranca Stream, Lake Durusu Basin, Turkey) and sampling stations (St.1: Taşlıgeçit Creek; St.2: Danamandıra Creek; St.3: Şeytan Creek; St.4: Binkılıç Creek; St.5: Karamandere Creek; St.6: Karacaköy Creek)

Fish and macroinvertebrate sampling

Fish and aquatic macroinvertebrate samples were collected from the stations once a month (in spring 2012: March and April; in summer 2012: June and August). Since the aim of the study is to reveal the feeding relationship between fish and their possible preys, field surveys were conducted in the spring and summer months, when the food supply in the environment and the feeding activity of the fish were the highest. Fish specimens were captured by electrofishing (SAMUS 725G portable electro-shockers; frequency 50-55 Hz; the grab net has a mesh size of 3 mm) from the same points of the stations (approximately 50 m long sampling sections) and transferred to the laboratory in cold conditions (portable freezer, -18°C). The aquatic macroinvertebrate samples were collected by a special hand-net with a 0.5 mm mesh-size against the direction of the water flow within a 1 m^2 quadrat area. Organisms were collected

into the net by moving this hand-net slowly over the fixed distance of the quadrat. Then, the collected samples were stored in plastic bottles and fixed with 4% formaldehyde solution.

Laboratory studies

Fish samples were measured for standard length (SL) to the nearest 0.1 cm and their total body weight (W) was weighed using a digital balance with a 0.01 g accuracy. To determine the diet composition, the digestive tracts were removed from the fish specimens and fixed in 4% formaldehyde solution. The prey items were identified to the lowest possible taxonomic level using a binocular microscope. Each taxonomic group was counted individually and then oven dried at 80°C and dried items weighed to the nearest 0.0001 g.

The bottom sediment samples which contain macroinvertebrates were washed under high-pressure tap water using sieves (mesh size 1; 0.25; 0.16 mm), and each organism was preserved in 70% ethyl alcohol. The macroinvertebrates both in the environment and in the digestive tract of fish samples were identified according to Askew (1988), Nilsson (1996, 1997), Zwick (2004), Oscoz et al. (2011) and Bouchard (2012).

Data analyses

The feeding habit of the fish was determined by indices of relative importance. The modified index of relative importance (MI%, see Hayse, 1990) for major prey items (such as Crustacea, Algae, Detritus, etc.) was estimated as:

$$MI\% = [(F\% \times W\%) / \Sigma(F\% \times W\%)] \times 100.$$

The index of relative importance (IRI%) only for each countable insect groups was estimated as:

$$IRI\% = [((N\% + W\%) \times F\%) / \Sigma((N\% + W\%) \times F\%)] \times 100,$$

where F% is the percentage of frequency of occurrence [(number of digestive tracts containing a food item/total number of digestive tracts with food)×100], N% is the numerical percentage of digestive tracts with a certain food item against the total number of digestive tracts, and W% is the gravimetric percentage of a certain food item against the weight of all consumed taxa (Hyslop, 1980).

To determine the niche breadth of *B. cyclolepis*, Levins' standardized niche breadth (Hurlbert, 1978) index was calculated: $B_A = (B-1)/(n-1)$, where *B* is Levins' measure of niche breadth (Levins, 1968), P_j is the proportion of individuals found using resource *j*, and *n* is the number of possible resource states. Levins' standardized B_A determines the measure of niche breadth on a scale from 0 to 1. A B_A value close to 0 indicates a narrower diet (minimum niche breadth) and more specialization, and a B_A value close to 1 indicates a broader diet (species consumes available food items in equal proportion) and more generalization (Krebs, 1998). Values of B_A are considered high when higher than 0.6, intermediate when between 0.4 and 0.6, and low when below 0.4 (Novakowski et al., 2008).

The macroinvertebrates in the environment were identified and the specimens of each taxon were counted for the determination of N_e% (relative abundance of each food item in the environment) with the following equation;

N_e% = specimen numbers of a taxon (N_e) / total specimen number of all taxa (total N_e)×100 (Kocataş, 2008).

The Ivlev's electivity index (E) was calculated to evaluate the prey preferences of the fish. The index formula is as follows: $E = (r_i - P_i)/(r_i + P_i)$, where E= electivity index, r_i= percentage of food item *i* in the diet (N_f%), P_i=percentage of food item *i* in the environment (N_e%). The value of E ranges from -1 to +1 and it indicates positive selectivity when it is above 0 and negative when less than 0. The selectivity is accepted as high for values equal or higher than 0.6 (Pinto and Uieda, 2007).

RESULTS

Fish diet and feeding ecology

A total of 142 *B. cyclolepis* specimens were captured (spring: 58 specimens, 2.6-10.6 cm SL, 0.42-21.28 g W; summer: 84 specimens, 2.2-14.2 cm SL, 0.21-57.01 g W) and 66% of them (72% in spring, 2.6-10.6 cm SL, 0.42-21.28 g W; 62% in summer, 3.1-11.6 cm SL, 0.61-35.52 g W) had full digestive tract. The individual distribution of the fish according to the stations and the digestive tract occupancy rates are as follows: in St.1, 9 specimens with 89%; in St. 2, 44 specimens with 80%; in St. 3, 24 specimens with 63%; in St. 4, 12 specimens with 25%; in St. 5, 20 specimens with 35%; St. 6, 33 individuals with 79%. The diet of *B. cyclolepis* consisted of five major food components: Insecta, Arachnida (Acaridae), Crustacea (Gammaridae), Algae and detritus. It was determined that the fish mainly fed on insects (MI% was 96.15% in spring and 98.04% in summer, see Table 1). The insects consumed were represented by seven different groups (Diptera, Ephemeroptera, Plecoptera, Odonata, Trichoptera, Coleoptera, Hymenoptera) and Diptera was preferred as the main food with the highest values of relative importance among these groups (Table 2). MI% and IRI% values of food items were also estimated for each sampling station and shown in Table 3. In each habitat, fish has mainly consumed insects and among them, Diptera was the main food item in every station except St. 4.

Levins' standardized niche breadth (B_A) for all specimens captured in the study period was estimated at 0.32. In spring, B_A value was 0.40, and 0.31 in summer. The results showed that the food spectrum of *B. cyclolepis* was low in both seasons. Niche breadth values were also calculated separately according to the stations and were recorded as 0.70, 0.41, 0.49, 0.79, 0.60 and 0.60, respectively.

Table 1. The major food composition in the diet *B. cyclolepis* in the Istranca Stream (n: number of fish, F (%): The percentage of frequency of occurrence, W (%): The percentage of dry gravimetric composition, MI (%): modified index of relative importance for major prey items)

Food components	Spring (n = 42)			Summer (n = 52)		
	F%	W%	MI%	F%	W%	MI%
Insecta	66.67	85.10	96.15	82.69	83.41	98.04
Algae	9.52	4.10	0.66	5.77	7.74	0.63
Crustacea (Gammaridae)	4.76	0.88	0.07	5.77	1.46	0.12
Arachnida (Acaridae)	4.76	0.34	0.03	5.77	0.12	0.01
Detritus	19.05	9.58	3.09	11.54	7.26	1.19

Table 2. The values of the index of the relative importance for Insecta groups in the diet of *B. cyclolepis* in the Istranca Stream (n: number of fish, F (%): The percentage of frequency of occurrence, W (%): The percentage of dry gravimetric composition, N (%): Numerical percentage, IRI (%): index of relative importance for countable Insecta groups)

Food items	Spring (n = 42)				Summer (n = 52)			
	F%	W%	N%	IRI%	F%	W%	N%	IRI%
Diptera	52.38	26.01	96.27	93.25	75.00	48.14	95.74	90.58
Ephemeroptera	7.14	0.35	0.25	0.06	21.15	14.66	1.71	2.91
Plecoptera	2.38	0.08	0.17	0.01	7.69	0.92	0.20	0.07
Trichoptera	30.95	5.88	3.23	4.14	40.38	16.61	2.21	6.38
Hymenoptera	2.38	52.78	0.08	1.85	1.92	0.85	0.05	0.01
Odonata	-	-	-	-	1.92	1.89	0.02	0.03
Coleoptera	-	-	-	-	3.85	0.34	0.07	0.01

Table 3. The values of the importance indices of food items in the diet of *B. cyclolepis* for each station in the Istranca Stream (n: number of fish, MI (%): modified index of relative importance for major prey items; IRI (%): index of relative importance for countable Insecta groups)

	Station-1 (n=8)		Station-2 (n=35)		Station-3 (n=3)		Station-4 (n=15)		Station-5 (n=7)		Station-6 (n=26)	
Food items	MI%	IRI%	MI%	IRI%	MI%	IRI%	MI%	IRI%	MI%	IRI%	MI%	IRI%
Algae	-		0.20		-		-		3.14		5.73	
Gammaridae	13.70		-		0.07		-		-		-	
Acaridae	-		0.14		0.02		-		0.14		-	
Detritus	-		3.15		5.23		-		-		6.46	
Insecta	86.30		96.52		94.68		100.00		96.73		87.81	
Diptera		71.89		90.96		85.21		37.57		54.83		99.56
Ephemeroptera		5.95		0.87		2.18		61.36		-		-
Plecoptera		1.12		-		0.95		-		-		-
Trichoptera		20.84		5.67		11.26		1.07		45.17		0.44
Hymenoptera		-		2.44		-		-		-		-
Odonata		-		0.06		-		-		-		-
Coleoptera		0.20		-		0.10		-		-		-

Macroinvertebrate composition on the environment

A total of 1272 specimens of macroinvertebrates were sampled during the study period (601 specimens in spring; 671 specimens in summer). Specimens were grouped within the same level as food items in the digestive tract of sampled fish.

Detailed information about the taxa and the numbers of specimens is given in Table 4. In spring, the most abundant macroinvertebrates were dipteran larvae (N_e: 198; N_e%:32.94) followed by Gastropoda (N_e: 196; N_e%: 32.61). Similarly, in summer, dipterans were also prevailing (N_e: 446; N_e%: 66.47). However, the number of gastropods did not increase as much as dipterans. The distribution of macroinvertebrates and their proportions in each sampling station are shown in Table 5. Macroinvertebrates diversity and the number of individuals were highest at St. 2, followed by St. 5 and St. 6.

Table 4. Macroinvertebrate taxa and their seasonally specimen numbers (N_e) and percentage (N_e%) in the environment (Istranca Stream)

Taxa	Spring		Summer	
	N_e	N_e%	N_e	N_e%
Diptera	198	32.95	446	66.47
Ephemeroptera	36	5.99	20	2.98
Plecoptera	4	0.67	1	0.15
Trichoptera	23	3.83	1	0.15
Odonata	10	1.66	19	2.83
Coleoptera	1	0.17	6	0.89
Gammaridae	123	20.47	6	0.89
Gastropoda	196	32.61	151	22.50
Bivalvia	1	0.17	7	1.04
Hemiptera	9	1.50	14	2.09
Total N	**601**		**671**	

Table 5. Macroinvertebrate taxa and their specimen numbers (N_e) and percentage (N_e%) in each sampling station in the Istranca Stream

Taxa	Station-1		Station-2		Station-3		Station-4		Station-5		Station-6	
	N_e	N_e%	N_e	N_e%	N_e	N_e%	N_e	N_e%	N_e	N_e%	N_e	N_e%
Diptera	-	-	272	59.52	29	49.15	139	97.89	38	9.79	166	83.00
Ephemeroptera	6	24.00	14	3.06	17	28.81	3	2.11	5	1.29	11	5.50
Plecoptera	2	8.00	-	-	2	3.39	-	-	1	0.26	-	-
Trichoptera	7	28.00	14	3.06	1	1.69	-	-	1	0.26	1	0.50
Odonata	4	16.00	8	1.75	6	10.17	-	-	4	1.03	7	3.50
Coleoptera	-	-	-	-	-	-	-	-	-	-	6	3.00
Gammaridae	-	-	127	27.79	1	1.69	-	-	-	-	1	0.50
Gastropoda	-	-	2	0.44	1	1.69	-	-	338	87.11	6	3.00
Bivalvia	-	-	7	1.53	-	-	-	-	-	-	1	0.50
Hemiptera	6	24.00	13	2.84	2	3.39	-	-	1	0.26	1	0.50
Total N	25		457		59		142		388		200	

Food selectivity

According to the values of the electivity index, *B. cyclolepis* had a high selectivity (+1) for Hymenoptera and Arachnida (Table 6). Diptera was the main food item of *B. cyclolepis* sampled in the area during spring (Table 6), but the electivity index was not high (E<0.6). Other macroinvertebrate groups were consumed in low proportions by the fish and the electivity was calculated as negative. In summer, the fish consumed Diptera, Plecoptera and Trichoptera, but a high electivity index was estimated only for Trichoptera (E > 0.6). When analyzed according to the stations, there was selectivity in all stations except the St. 4 on Diptera, but the index value was significant in terms of high selectivity in only two stations (St. 1 and St. 5). In St. 3 and St. 5, *B. cyclolepis* showed high selectivity on Trichoptera, as well (Table 7).

Table 6. Electivity index (E) values calculated for *B. cyclolepis* sampled in the Istranca Stream during spring and summer seasons (r_i=percentage of food item *i* in the diet (N_f%), P_i= percentage of food item *i* in the environment (N_e%)). Values in boldface indicate positive selectivity by the fish

Food items	Spring			Summer		
	r_i (%)	P_i (%)	E	r_i (%)	P_i (%)	E
Diptera	95.95	32.95	**0.49**	95.54	66.47	**0.18**
Ephemeroptera	0.25	5.99	-0.92	1.71	2.98	-0.27
Plecoptera	0.17	0.67	-0.60	0.25	0.15	**0.25**
Trichoptera	3.22	3.83	-0.09	2.18	0.15	**0.87**
Odonata	-	1.66	-1	0.02	2.83	-0.98
Coleoptera	-	0.17	-1	0.07	0.89	-0.85
Hemiptera	-	1.50	-1	-	2.09	-1
Gammaridae	0.17	20.47	-0.98	0.07	0.89	-0.85
Hymenoptera	0.08	-	**1**	0.05	-	**1**
Bivalvia	-	0.17	-1	-	1.04	-1
Gastropoda	-	32.61	-1	-	22.50	-1
Arachnida	0.17	-	**1**	0.10	-	**1**

Table 7. Electivity index (E) values calculated for *B. cyclolepis* captured in each station in the Istranca Stream. Values in boldface indicate positive selectivity by the fish

Food items	Station-1	Station-2	Station-3	Station-4	Station-5	Station-6
Diptera	**1**	**0.24**	**0.28**	-0.31	**0.80**	**0.09**
Ephemeroptera	-0.57	-0.37	-0.83	**0.91**	-1	-1
Plecoptera	-0.60	-	-0.27	-	-1	-
Trichoptera	-0.17	-0.36	**0.56**	**1**	**0.96**	**0.35**
Odonata	-1	-0.96	-1	-	-1	-1
Coleoptera	**1**	-	**1**	-	-	-1
Hemiptera	-1	-1	-1	-	-1	-1
Gammaridae	**1**	-1	-0.72	-	-	-1
Hymenoptera	-	**1**	**1**	-	-	-
Bivalvia	-	-1	-	-	-	-1
Gastropoda	-	-1	-1	-	-	-1
Arachnida	-	**1**	**1**	-	**1**	-

DISCUSSION

The present study proved that *B. cyclolepis* is a typical benthophagous and insectivorous fish that was also feeding on a small number of plants and other animals (Table 1). In both seasons, Insecta ranked first in this species' food preference. Diptera and Trichoptera played the most important role in the diet of *B. cyclolepis* and the domination of dipteran larvae in the diet of other species of barbels was also reported from many studies (Collares-Pereira et al., 1996; Piria et al., 2005; Rozdina et al., 2008; Sapounidis et al., 2015). Dipterans are one of the most abundant macroinvertebrate groups in freshwater environments and these prolific organisms produce large populations (Keiper et al., 2002; Gülbunar et al., 2018). Dipteran larvae sometimes attach themselves to structures on the bottom but are often free-swimming and suspended off the bottom. High nutritional value, stabile availability, abundance and visibility make dipteran larvae easy prey for many fish species. Considering that Diptera was the most abundant insect group in the Istranca Stream, it is not surprising that they were the most important food for the benthic *B. cyclolepis.* Although Diptera is consumed as the main food by fish, the electivity index did not show a significant value on it as it is still the most abundant group in the environment. The total amount of algae, Crustacea and Arachnida, which are other food groups in the stomach, is very low compared to Insecta.

When the seasonal diet of the species was compared, it was observed that the diversity and abundance of food consumed increased during the summer months (Table 1, Table 2). While Ephemeroptera and Trichoptera were consumed in both seasons, their importance values increased in summer. Besides, it has been observed that Odanata and Coleoptera, which are not found in the stomach in spring, are consumed in summer feeding. However, since these two food groups are represented with low values in the environment, it is an expected result that the values found in the stomach in summer are also low.

No spatial difference was determined in the diet of the species and it preferred Insecta and especially Diptera as the main food at each station. Station 1 and St. 3 are streams

located in the headwater of the Istranca Stream and display similar habitat characteristics and their food supplies have reflected in the food preference of fish. EPT (Ephemeroptera, Plecoptera, and Trichoptera) species mostly prefer the clean headwaters of the river systems (Hamid and Rawi, 2017), and their presence in the diet of fish may be related to their preference for those stations. However, the water quality in St. 4 was influenced by sewage discharge and domestic pollution (Saç and Özuluğ, 2017), and it is thought that this situation has influenced both the presence of fish as well as macroinvertebrates and the feeding of the fish. The environment was represented by only two macroinvertebrate species (Diptera and Ephemeroptera) and in addition to these, only one individual of Trichoptera was found in the digestive tract of the fish (Table 3 and Table 5). Despite the density of plants, the fish mainly fed on insects instead of them at St. 2. However, it was determined that the food preference of the fish was especially focused on Diptera and Trichoptera at St. 5 and St. 6 where insects decreased numerically, and the value of plants increased relatively in the diet of the fish due to the poor food supply of the environment.

According to Rozdina et al. (2008), the diet of *B. cyclolepis* inhabited in the Meriç River Basin consisted of 14 food components and was dominated by Chironomid larvae (Diptera), followed by plant and Gammaridae. The results of our study largely overlap with the results of this previous study in terms of both the food groups consumed by the fish and the dominance of Diptera in its diet. The other similarity of the results of these two studies is the seasonal activity in feeding and that both populations had a peak in summer.

When the macroinvertebrates in the environment are examined, the most abundant group is Insecta, followed by Gastropoda and Gammaridae. As in the stomach content, the most abundant group in Insecta is Diptera. Diptera has numerically increased 2.25 times in summer (Table 3). This result may be related to the active reproduction activity of different dipteran groups at different times of the year (Thorp and Covich, 2001). Nevertheless, the consumption proportions of the Diptera by the fish are very close in spring (93.25%) and summer (90.58%) (Table 2).

During the two sampling seasons, Gastropoda was the second most abundant macroinvertebrate group in the environment. However, no gastropods were found in the digestive tract of *B. cyclolepis*. It is thought that the fact that the fish had not fed on the most abundant Gastropoda after Diptera in the stream may be related to the assumption that these relatively immobile gastropods seem like stones to the fish due to their hard shells. *Barbus cyclolepis* is a rheophilic species that prefers riffle and run habitats at all stations along the Istranca Stream (Saç and Özuluğ, 2017). In addition, riffle zones are rich feeding areas for this fish, as strong currents increase invertebrate density (Freyhof, 1996). In these habitats, the fish are thought to prefer more mobile insects that drift with the current rather than immobile organisms that fix themselves on the ground, such as the gastropods. Rozdina et al. (2008) also could not find gastropods in the digestive tract of *B. cyclolepis* and thought that molluscs, which were detected at a very low rate, probably accidentally got into the diet of the fish. In addition, while some species belonging to the genus do not prefer gastropods or other molluscs as food (Dadebo et al., 2013; Sapounidis et al., 2015), in some other species these prey items were represented with low proportions (Collares-Pereira et al., 1996; Pires et al., 2001; Piria et al., 2005).

Crustacea found in the diet of *B. cyclolepis* consists of Gammaridae. While the abundance of Gammaridae in the environment was high in spring, this value has sharply decreased in the summer. Gammarids reproduce throughout most of the year but there is a pronounced peak of activity in spring and early summer. Additionally, stream warming has considerable negative effects on the reproductive processes of some Gammaridae species that would potentially reduce the reproductive capacity of these organisms (Pöckl et al., 2003). Therefore, it is thought that the decrease in the abundance of Gammaridae in summer might be related to the water temperature (Saç and Özuluğ, 2017).

The values of the electivity index, which ranged from -1 to +1, are related to the presence/absence of food items in the digestive tract of fish in relation to their abundance in the environment. Hemiptera, Gastropoda and Bivalvia were captured in the study area during these two seasons but none of them was found in the digestive tract of the fish; so, the electivity index for these groups was -1. The values of the electivity index also showed that *B. cyclolepis* had a high selectivity (+1) for Hymenoptera and Arachnida. The hymenopteran parts or extremities found in the digestive tract of *B. cyclolepis*, belong to Formicidae (ants). Formicidae is not aquatic, but it is possible that specimens drifted by flow were accidentally consumed by the fish. Arachnida found in the diet of *B. cyclolepis* consists of members of the Acaridae. According to Gerecke and Di Sabatino (2007), a typical macroinvertebrate sample that could provide a more representative idea about the investigated mite fauna is often not adequate for two reasons: (1) collecting is generally done with a too large mesh size (300 µm or more); and (2) sorting generally concentrates on animals of a larger size at low magnification; consequently, many mites are overlooked. Water mites were probably not represented in the benthos sample because the mesh size of the special hand net grab was too large (250 µm) to retain these small-bodied macroinvertebrates or they may have been overlooked during the sorting of samples.

The proportions of some macroinvertebrates (Ephemeroptera, Plecoptera, Odonata, Coleoptera and Hemiptera) in the environment were relatively low. The low values of these organisms overlapped by the low percentage of their occurrence in the diet of fish (Table 4). However, it is noteworthy that, especially in summer, the value of Trichoptera in the environment is quite low while its value in the stomach is high (Table 2, Table 3). The fact that

macroinvertebrates were collected from a limited area at each sampling station and that conversely, *B. cyclolepis* is relatively more mobile along the stream is considered as evidence that the fish exhibits selective feeding on Trichoptera (Table 4).

CONCLUSION

In conclusion, it is thought that the answers to the targeted questions were reached with the results of this study. Firstly, the diet of *B. cyclolepis* consisted of 11 different food items collected in five major groups, mainly Insecta. The main food item in its diet is Diptera larvae, which have high importance values, so this feeding habit has resulted in a narrow niche width. Secondly, there was a relationship between the proportion of consumed food items by the fish and their ratios in the environment. The fish was fed with every organism detected in the environment except Gastropoda, Bivalvia and Hemiptera. However, despite the relatively high proportions of some organisms such as Crustacea and Gastropoda in the environment, *B. cyclolepis* was selective mostly on Diptera, which is the easiest prey for itself. Seasonal activity, both in the feeding of fish and occurrences of the macroinvertebrates in the environment resulted in high values in summer.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Artificial seagrass experiments in the Northeast Mediterranean

Kuzeydoğu Akdeniz'de yapay deniz çayırı denemeleri

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Abstract: Seagrasses provide important nursery grounds, shelter and natural habitats for juvenile fish. In this study, we evaluated if artificially created seagrass areas can play the same role as the natural seagrass (NS) habitats. The study was carried out in three different stations on the coast of Yumurtalık, Adana, selected according to the seagrass areas. Artificial seagrass (AS) was made of polypropylene ribbon and fixed on the ground in the designated areas with a depth of 0.5 m on average. Sampling was carried out with a beach seine net once a week at stations between 28 April 2016 and 11 August 2016. Sampled fish were identified to the lowest possible taxonomic level. Based on our results, the fish abundance and species richness of NS and AS habitats were not statistically different, whereas the both parameters were significantly lower in sandy (S) habitats ($p<0.001$). Moreover, the species composition of NS and AS habitats was found to be similar each other, whereas the composition was significantly different in S habitats. This study, conducted in the Northeast Mediterranean, shows that AS habitats effect the distribution of juvenile fish.

Keywords: Meadows, juvenile fish, recruitment, nursery, Levant Basin

Öz: Deniz çayırları yavru balıklar için barınak, stoğa katılma ve doğal yaşam alanları sağlar. Bu çalışmada, yapay olarak oluşturulmuş deniz çayırı alanlarının doğal deniz çayırı (NS) habitatlarıyla aynı rolü oynayıp oynayamayacağı değerlendirilmiştir. Çalışma Adana, Yumurtalık kıyısında deniz çayırı alanlarına göre seçilen üç farklı istasyonda gerçekleştirilmiştir. Yapay deniz çayırı (AS) polipropilen şeritten yapılmıştır ve belirlenen alanlarda ortalama 0,5 m derinliğe sahip zemine sabitlenmiştir. Örnekleme 28 Nisan 2016 ile 11 Ağustos 2016 tarihleri arasında istasyonlarda haftada bir ığrıp ile gerçekleştirilmiştir. Örneklenen balıklar mümkün olan en düşük taksonomik seviyede tespit edilmiştir. Sonuçlarımıza göre, NS ve AS habitatlarının balık bolluğu ve tür zenginliği istatistiksel olarak farklı değildir. Fakat her iki parametre de kumlu (S) habitatlarda daha düşük bulunmuştur ($p <0,001$). NS ve AS habitatlarının tür kompozisyonu birbirine benzerken, S habitatlarında önemli ölçüde farklı bulunmuştur. Kuzeydoğu Akdeniz'de yapılan bu çalışma, AS habitatlarının yavru balıkların dağılımını etkilediğini göstermektedir.

Anahtar kelimeler: Çayır, yavru balık, stoğa katılma, yuva, Levant Baseni

INTRODUCTION

Seagrasses are quite important for the coastal ecosystems as a source of natural habitat, shelter, oxygen and food (Gullstrom et al., 2008; Hemminga and Duarte, 2000; Tuya et al., 2014). Seagrass habitats can support high invertebrate abundance and richness and this provide important source of food for many fish species (Jenkins et al., 1997; Orth, 1992; Orth et al., 1984; Pihl et al., 2006). Moreover, they also play important role in decreasing the water movement and therefore stabilizing the sand, along with securing the quality of water and producing oxygen (Becker and Choresh, 2006). One of the most important functions of seagrasses is their role as a shelter for juvenile fish by protecting them from the other predators as they can hide in the leaves of seagrasses (Beck et al., 2001; Deegan et al., 2002; Mattila et al., 1999; Pollard, 1984; Spalding et al., 2003).

There are 66 known species of seagrasses throughout the world (Kuo and Den Hartog, 2007), five of which, *Zostera marina, Zostera noltii, Cymodocea nodosa, Posidonia oceanica* and *Halophila stipulacea* distribute in Turkish coasts (Demirci and Karakan, 2006), including the northeastern coasts of Levant Basin (Aysel et al., 2006).

The total destruction or the decline of seagrasses cause failing of its ecosystem services and resulted with adverse impacts both economically and environmentally (Orth et al., 2006; Waycott et al., 2006). Coastal structures, industrialization, marine pollution, illegal trawling along coasts are the major threats damaging the seagrass habitats (Boudouresque et al., 1994; Meinesz et al., 1991). Hence, in order to protect seagrass habitats, conservation measures have been taken over the coasts of most of Mediterranean Countries (Protocol, 1995). Additionally, habitat rehabilitation studies are in progress in disrupted areas (Irving et al., 2010). In this context, creating AS can also simulate the positive impacts of seagrasses on aquatic life. For instance Lee and Low (1991) reported that in the areas of AS, fish family increased from 12 to 14, fish species increased from 16 to 30 and created a habitat for fish. Similarly, Saad et al. (2011) identified 497 fish belonging to 17 fish families in the field AS

in their study and reported that AS created an important nutrient-rich habitat for marine fish. Upston and Booth (2003) showed that there were no significant differences in species richness and diversity between AS and NS area in Botany Bay, New South Wales. Although many studies have been performed about the function of AS on fish settlement, there is no empirical evidence in the Northeast Mediterranean.

In this study, we investigate the impact of AS areas on the settlement of fish in a relatively undisturbed coastal area in Northeast Mediterranean. For this purpose, we compared the species richness, composition, and total fish abundance between the S habitats, NS and AS habitats over the course of a four-month period when NS were available.

MATERIALS AND METHODS

The study was conducted in İskenderun Bay, along the coast of Yumurtalık (35°40'50"E ile 36°50'40"N) (Figure 1). The average depth of a large part of Yumurtalık Bay is 2.8 meters, which is quite shallow (Avşar et al., 1999). The study area was built 20 meters away from the shore, on a seabed at an average depth of 0.5 meters. The site was safe from the impacts of currents, waves and wind effect and away from sites and human activities. The stations were arranged taking seagrasses into consideration as can be seen below (Table 1, Figure 1).

Table 1. Habitats at stations in the study

	Station 1 (S1)	Station 2 (S2)	Station 3 (S3)
NS	100m²	-	100m²
AS	-	25m²	100m²
S	100m²	25m²	100m²

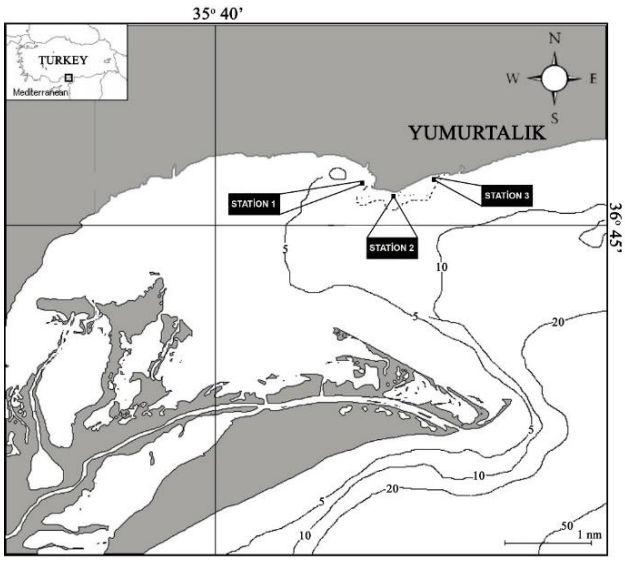

Figure 1. The locations of the stations on the map

Green thine polypropylene ribbon (d = 0.946 gr/cm³) was used in the making of AS leaf. The ribbons were attached a wire mesh fence. The fence was 1m width and 20m long and has 5 x 5 cm square. Based on the previous studies, the abundance and number of species are higher in the seagrasses the leaves of which are between 20 and 40 cm (Mattila et al., 1999). Therefore, we kept the leave length as 30 cm. Then AS model was placed into the 2nd Station (Figure 2) on 8 April 2016 and into the 3rd station on 27 May 2016. The models were fixed on the ground by using T-shaped rods.

Sampling at the stations was done weekly at the same time. Sampling in S1 and S2 started on April 28, and S3 on June 17, 2016 and ended on August 11, 2016.

Figure 2. The AS area created for Station 2

The specimens were identified at the lowest possible taxonomic level using the following references Whitehead et al. (1986), Golani et al. (2006), Turan (2007) and Froese and Pauly (2016). The number of individuals were recorded for each species. After the procedures, alive specimens were released back into the sea and the ones those were already dead were fixed in a 10% formaldehyde solution for further investigations.

The change of total abundance and total number of species based on day of sampling, habitat type and station were analyzed by using generalized additive models. The models were fitted following the protocols suggested by Zuur et al. (2009) using MASS library (Venables and Ripley, 2002) in R environment (Team, 2017). The changes of species composition were analyzed with Constrained Analyze of Principle Coordinates (CAP) using vegan and Biodiversity R libraries of R environment (Kindt and Coe, 2005; Oksanen et al., 2013)

RESULTS

A total of 29 different species of juvenile fish were detected in the samplings in NS areas. The most dominant species was found to be *Atherina boyeri* (68.92%) followed by *Siganus rivulatus* (16.46%), *Gobius niger* (6.36%) and *Diplodus sargus* (4.18%) (Figure 3.).

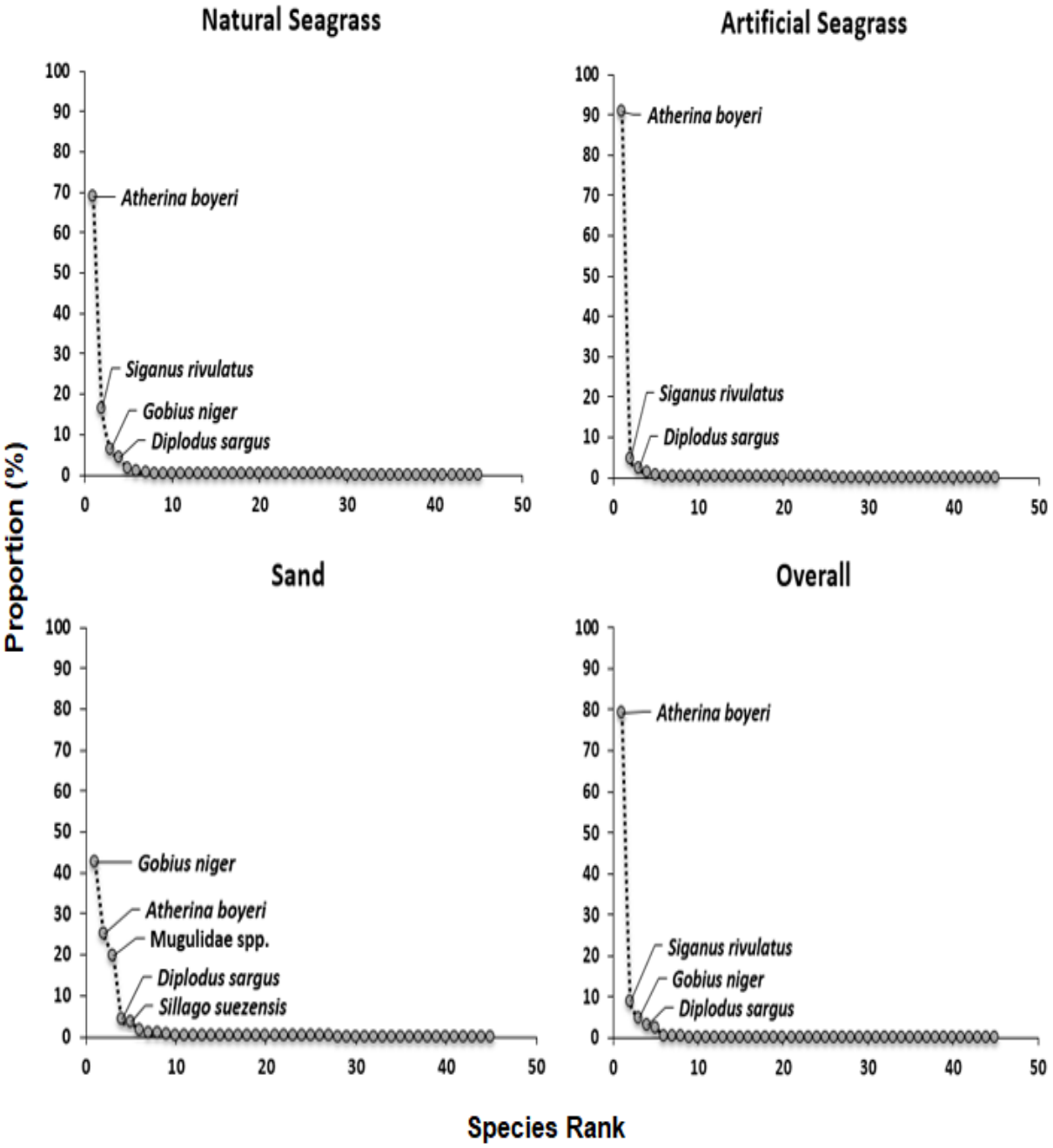

Figure 3. Rank abundance curves of NS, AS and Sand habitats, and overall study area

Results of GAMs revealed that overall average fish abundance and number of species were similar in AS and NS habitats and the both were significantly lower in S habitats (Table 2, Table 3, Figure 4, Figure 5).

Fish abundance did not reveal any significant trends along time in NS, and S habitats of S1 and S2. On the other hand, two contrasting patterns were appeared in AS. In S2, fish abundance revealed a clear increment after 80th days of experiments, whereas it monotonously decreased in S3. In stations, the number of species remained similar or decreased along the experiments in S and NS habitats, respectively. Change in AS habitat of S2 was not significant whereas GAM revealed a significantly fluctuating pattern in AS habitat of S3.

Table 2. Estimated regression parameters and approximate significance of smooth terms of negative binomial (NB) generalized additive model for total fish abundance

$Abundance_{ijk} \sim NB(\mu_{ijk})$; $\ln(\mu_{ijk}+1) = Intercept + H_i + s(DoY_k : H_i : S_k) + \varepsilon_{ijk}$				
Parametric coefficients:				
	Estimate	**Std. Error**	**z value**	**p value**
Intercept	**6.030**	**0.183**	**32.918**	**<0.001**
Habitat NS	0.101	0.272	0.371	0.711
Habitat S	**-1.851**	**0.234**	**-7.901**	**<0.001**
Approximate significance of smooth terms:				
	edf	Ref.df	Chi.sq	p-value
s(DoY) : S_1, H_{NS}	1.000	1.000	0.701	0.403
s(DoY) : S_1, H_S	2.642	3.282	4.775	0.211
s(DoY) : S_2, H_{AS}	**6.893**	**7.978**	**71.813**	**<0.001**
s(DoY) : S_2, H_S	3.687	4.550	9.464	0.088
s(DoY) : S_3, H_{AS}	**1.000**	**1.000**	**29.742**	**<0.001**
s(DoY) : S_3, H_{NS}	2.463	3.010	7.641	0.054
s(DoY) : S_3, H_S	**2.237**	**2.739**	**11.270**	**0.008**
R^2 (adj) = 0.798, Deviance explained = 81.6%, n = 91				

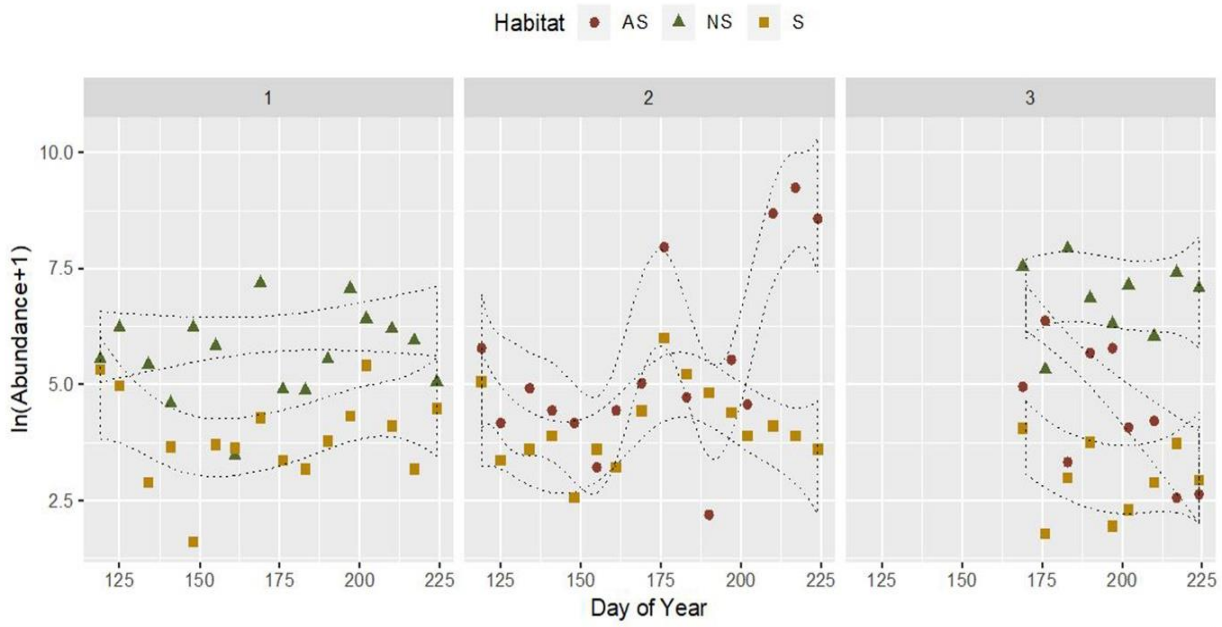

Figure 4. Fit of the negative binomial GAM in Table 2 for the total abundance

Table 3. Estimated regression parameters and approximate significance of smooth terms of quasi-Poisson (QP) generalized additive model for the richness of fish species

$Richness_{ijk} \sim QP(\mu_{ijk})$; $\ln(\mu_{ijk}+1) = Intercept + H_i + s(DoY_k : H_i : S_k) + \varepsilon_{ijk}$				
Parametric coefficients:				
	Estimate	Std. Error	z value	p value
Intercept	**1.443**	**0.099**	**14.599**	**<0.001**
Habitat NS	0.198	0.134	1.477	0.144
Habitat S	**-0.435**	**0.132**	**-3.308**	**0.001**
Approximate significance of smooth terms:				
	edf	Ref.df	Chi.sq	p-value
s(DoY) : S_1, H_{NS}	**1.000**	**1.000**	**5.743**	**0.019**
s(DoY) : S_1, H_S	1.242	1.444	3.058	0.105
s(DoY) : S_2, H_{AS}	1.000	1.001	3.412	0.069
s(DoY) : S_2, H_S	1.000	1.000	0.163	0.687
s(DoY) : S_3, H_{AS}	**5.382**	**5.897**	**2.653**	**0.017**
s(DoY) : S_3, H_{NS}	**2.966**	**3.581**	**6.993**	**<0.001**
s(DoY) : S_3, H_S	**2.512**	**3.055**	**4.165**	**0.008**
R^2 (adj) = 0.605, Deviance explained = 61.8%, n = 91				

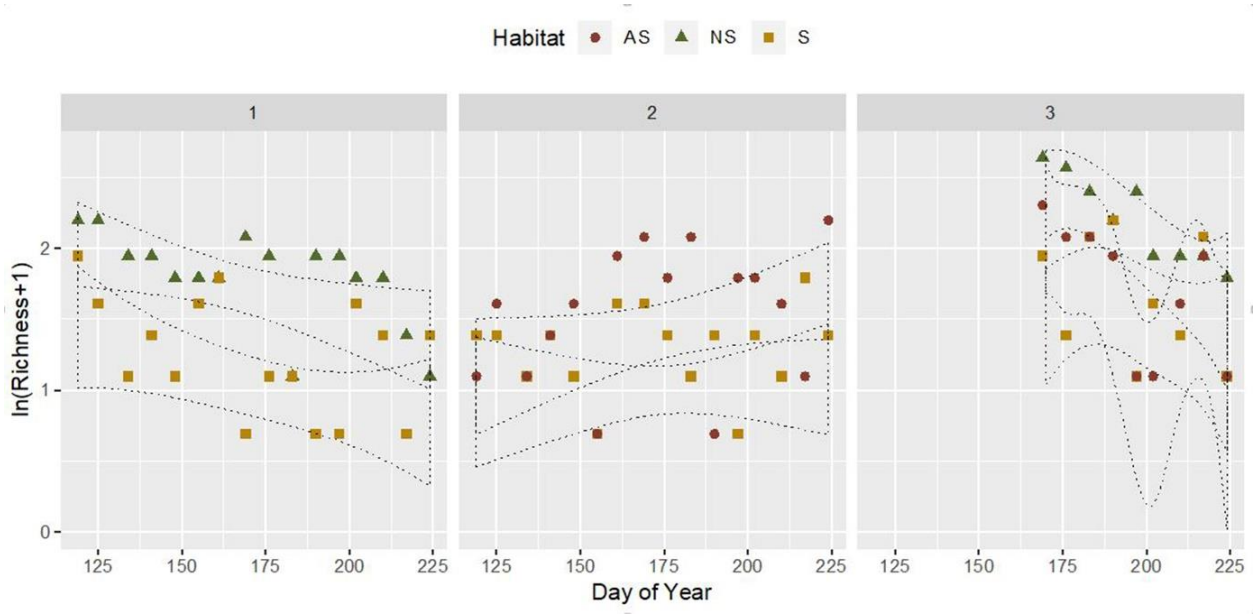

Figure 5. Fit of the quasi-Poisson GAM in Table 3 for the total richness

CAP and SimProf results revealed that the species composition was significantly changed among habitats and stations (Table 4).

Whereas artificial and NS possessed a similar composition, S habitats of all three stations were different than each other (Figure 6).

Table 4. Eigenvalues and its proportion in total and constrained inertia of first two constrained analysis of principle coordinates (CAP) axes, and the results of marginal permutation test for CAP

	CAP1	CAP2	Constrained Total	Total
Eigenvalue	0.328	0.216	0.686	0.759
Proportion in Total Inertia	0.432	0.284	0.903	1.000
Proportion in Constrained Inertia	0.478	0.314	1.000	-
Source of Variation	df	SS	F	p value
Habitat	**2**	**0.263**	**2.664**	**0.041**
Station	**2**	**0.394**	**3.988**	**0.002**
Residual	2	0.099		

n of permutations= 999

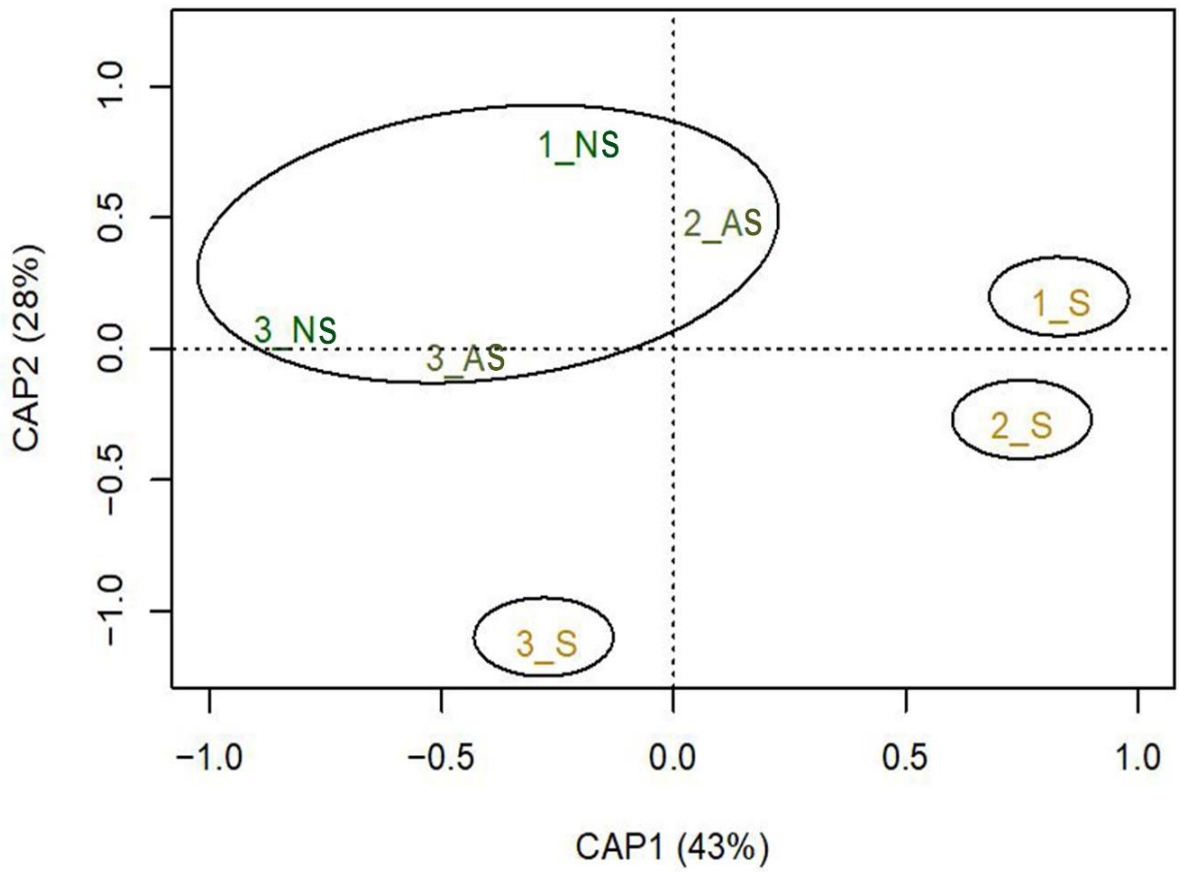

Figure 6. Results of constrained analysis of principle coordinates (CAP) ordination. Ellipses show statistically significant clusters at 95% confidence level based on a similarity profiling (SimProf) analyze

DISCUSSION AND CONCLUSION

In context of our study, a total of 45 species were detected between April and August in the Northeast Mediterranean (Table 5). In the same area also Başusta et al. (2002) reported 33 species. These numbers are not close to each other. Because of our study is in shallow water while the other study is at depths of up to 30 meters.

Table 5. Abundance of fish species (individual per 100m²) detected in context of the study (NS: Natural Seagrass, AS: Artificial Seagrass, S: Sand)

FAMILY / SPECIES	Station I		Station II		Station III		
	NS	S	AS	S	NS	AS	S
Atherinidae							
Atherina boyeri	2609	103	24028	460	9122	653	57
Atherinomorus lacunosus	2	1	4	4	-	-	-
Bothidae							
Arnoglossus laterna	-	-	-	-	-	-	2
Arnoglossus spp.	-	-	-	-	1	-	-
Blenniidae							
Blenniidae spp.	-	-	-	-	1	-	-
Parablennius gattorugine	-	-	-	-	1	-	-
Parablennius sanguinolentus	4	-	28	-	4	-	-
Callionymidae							
Callionymus filamentosus	-	-	-	-	-	1	2
Carangidae							
Seriola dumerili	-	-	4	4	-	-	-
Trachinotus ovatus	-	-	12	4	-	-	-
Dussumieriidae							
Dussumieria elopsoides	-	-	4	-	-	-	-
Engraulidae							
Engraulis encrasicolus	-	-	-	4	-	-	-
Fistulariidae							
Fistularia commersonii	-	-	-	-	-	-	1
Gobidae							
Gobiidae spp.	1	-	4	-	-	-	-
Gobius niger	1064	456	132	528	19	7	71
Haemulidae							
Pomadasys stridens	-	-	-	-	8	-	-
Leiognathidae							
Equulites klunzingeri	-	-	-	-	28	26	3
Monacanthidae							
Stephanolepis diaspros	-	-	-	-	3	-	-
Moronidae							
Dicentrarchus labrax	25	-	-	-	-	-	-
Mugilidae							
Mugilidae spp.	257	326	316	160	22	21	-
Mullidae							
Mullus barbatus	8	-	-	-	1	16	2
Mullus surmuletus	1	-	-	-	3	-	-
Upeneus pori	-	-	-	-	-	2	9
Serranidae							
Mycteroperca rubra	-	-	-	-	31	1	3
Serranidae spp.	-	-	-	-	-	-	1
Siganidae							
Siganus luridus	6	-	24	-	1	4	-
Siganus rivulatus	1284	-	564	12	1518	692	28
Sillaginidae							
Sillago suezensis	-	22	36	64	-	1	1
Soleidae							
Buglossidium luteum	1	2	20	-	1	3	-
Microchirus ocellatus	-	-	4	-	-	-	-
Pegusa lascaris	-	-	-	4	1	-	2
Solea solea	-	3	-	-	-	-	-
Sparidae							
Diplodus sargus	679	10	492	84	33	68	7
Lithognathus mormyrus	-	-	4	-	-	-	-
Pagellus erythrinus	3	1	-	-	-	-	-
Sparidae spp.	2	-	-	-	-	-	1
Sparus aurata	88	13	4	4	5	2	2
Sphyraenidae							
Sphyraena chrysotaenia	-	-	-	-	4	-	-
Sphyraena sphyraena	-	-	-	-	-	1	2
Syngnathidae							
Nerophis ophidion	-	-	-	-	5	-	-
Syngnathus abaster	-	-	-	-	-	1	-
Syngnathus phlegon	-	-	-	-	1	-	-
Terapontidae							
Pelates quadrilineatus	145	-	-	-	9	-	-
Trachinidae							
Echiichthys vipera	8	-	4	12	12	2	6
Trachinus araneus	-	-	-	-	-	-	3

Results of generalized additive models and similarity profiling analysis revealed that there was not statistically significant difference of the species composition, number of species and total abundance between AS and NS, whereas all three parameters were significantly different in S habitats. This situation demonstrated that the AS could simulate the NS. Similarly, Guidetti (2000) reported that seagrass areas are more preferred than sand areas. Factors such as nutrition, predators, the abundance of food come into play as reasons for fish to prefer seagrass as their habitat (Heck et al., 1997).

As larval stage, juvenile fish assemblages are also temporary associations (Miller, 2002), depends on the seasonality of spawning and settlement processes (Ak, 2004; Banbul, 2014). Accordingly, we detected the highest richness in April when the highest rate of ichthyoplankton richness and abundance were reported in the study area (Mavruk et al., 2018). In accordance with ichthyoplankton samplings performed by Mavruk et al. (2018) our richness were contentiously decreased in all stations and habitats over the periods of experiments depending on the decreasing spawning activity in the study area.

The habitats for adult and juvenile fish was ranked by Beck et al. (2001) from most to least efficient as seagrass, swamp, muddy areas, sand habitats in this order. The areas with seagrass were preferred by fish to areas without plants such as sand grounds and loams (Ferrell and Bell, 1991; Mattila et al., 1999; Pihl et al., 2006). Similarly, in our study, NS and AS have higher fish abundance values than sand habitats. The fish abundance and richness were relatively stable in NS and S habitats, as seen in the sampling. Whereas, AS revealed a fluctuating pattern in these two parameters. On the other hand, species compositions of S habitats were more variable. This may be due to the insufficient amount of sampling.

Different species preferred different kinds of habitats (Mattila et al., 1999). *Atherina boyeri*, which are carnivorous, and which represent the 79.31% of the total abundance of fish, were the predominant species in this study. Considering the fact that 31.67% of this species was observed in areas with seagrass, 66.64% in areas with AS, 1.67% in S habitats, it can be said to be a resident species of NS and AS.

High-density and low-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyester (PS), polyvinyl terephthalate (PET) make up 90% of the world production as the most commonly used plastics (Andrady and Neal, 2009). Therefore, it is known that most of the substances polluting the coastal and marine environments are composed of these materials (Andrady, 2011; Engler, 2012). Increasing sea pollution along with microplastics and marine debris which are discussed in many researches pose a threat to the ecosystem (Gündoğdu and Çevik, 2017; Gündoğdu et al., 2017). Nowadays, it is widely accepted that plastic pollution affects numerous marine species ranging from zooplanktons to whales (Andrady, 2011; Cole et al., 2013; Cole et al., 2011). Plastic wastes harm marine species as their gills can be clogged by plastics and they can get tangled in them and/or swallow them (Gregory, 2009; Li et al., 2016). Therefore, the use of plastic (polypropylene) as a material in AS is controversial. The general consensus is that preferring natural fibers when choosing materials is more of an eco-friendly approach and is predicted to prevent possible controversies.

Seagrass habitats have important roles in the coastal ecosystems. Therefore, they are extremely vulnerable to various stressors and declining all over the world. In this study, AS was tested as an alternative to the declining seagrass. Our study revealed that the abundance of fish is higher in the NS and AS in comparison with S habitats. This study, conducted in the Northeast Mediterranean, shows that AS habitats effect the distribution of juvenile fish.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The visual characteristics and quality of cultured gilthead seabream (*Sparus aurata*) in earthen ponds and net cages in Turkey

Türkiye'de toprak havuzlarda ve ağ kafeslerde yetiştirilen çipura balığı'nın (*Sparus aurata*) görsel karakteristikleri ve kalitesi

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Abstract: The objective of this study is to investigate the quality differences and visual characteristics between cultured gilthead sea bream obtained from earthen ponds and net cage habitats. No significant differences from obtained in two different habitats are determined cultured gilthead sea bream in terms of chemical and microbiological quality. It has been determined that it is a safe food for consumption since it does not contain pathogenic microorganisms such as *Escherichia coli* and *Salmonella* spp. that negatively affect food safety. Visual quality differences are distinguished like the skin color, reddish color on the operculum cover, the head shape and the tail transparency from each other. Having unique sensorial characteristics fishes from two different habitats do offer valuable nutrient sources for consumers. Thanks to the controlled aquaculture conditions, safe production of cultured gilthead sea bream is carried out according to food safety in Turkey. Therefore owing to their delicious food source of cultured gilthead sea bream it is preferred for consumption in Turkey and all ower the world.

***Keywords*:** Gilthead seabream, breeding, quality, visual characteristics, food safety

Öz: Bu çalışmanın amacı, toprak havuzlarda ve ağ kafeslerde yetiştirilen çipura balıkları arasındaki kalite farklılıklarını ve görsel özelliklerini araştırmaktır. Her iki habitatda yetiştiriciliği yapılan çipura balıklarında kimyasal ve mikrobiyolojik kalite açısından önemli bir farklılık tespit edilmemiştir. *Escherichia coli* ve *Salmonella* spp. gibi gıda güvenliğini olumsuz yönde etkileyen patojen mikroorganizma içermemesi bakımından tüketim için güvenli gıda olduğu belirlenmiştir. Görsel kalite farklılıkları olan deri rengi, solungaç kapağı üzerindeki kırmızımsı renk, kafa şekli ve kuyruk şeffaflığı ile birbirinden ayırt edilmektedir. Toprak havuzlarda ve ağ kafeslerde yetiştirilen çipura balıkları, farklı duyusal özellikleri ile tüketiciler için değerli besin kaynaklarıdır. Türkiye'de yetiştiriciliği yapılan çipura balıklarının, kontrollü akvakültür koşulları sayesinde gıda güvenliğine uygun üretimi yapılmaktadır. Bu nedenle kültüre alınan çipura balıkları, lezzetli besin kaynağı olması nedeniyle hem ülkemizde hem de dünyada tercih edilen ve güvenle tüketilen önemli bir ticari türdür.

***Anahtar kelimeler*:** Çipura, yetiştiricilik, kalite, görsel karakteristikler, gıda güvenliği

INTRODUCTION

Aquaculture is the only alternative to wild capture fishing able to satisfy the increasing consumer demand for sustainable production of safe seafood of the highest quality and nutritional value (Claret et al., 2016). The decrease in the sustainability of natural aquatic food sources due to adverse environmental conditions in the aquatic environment, overfishing, increase in the invasive fish population, etc. creates difficulties every day in the consumption of safe fisheries. Thanks to the successful production in fish farming all over the world, it is possible to meet the important animal protein needs for human nutrition. The gilthead seabream (*Sparus aurata*) is an economically cultured fish species and a rich protein food source. Turkey is one of the leading aquaculture producing countries for farmed gilthead sea bream and sea bass together with Greece, Italy, and Spain. López Ales (2018) explained that there are gilthead seabream productions in 20 different countries, where through the years, the total production of gilthead sea bream has been increasing, Turkey is the main producer with 72.000 t (34.8 % total production), Greece with 51.000 t (24.6%), Egypt with 26.000 t (12.6%) and Spain with 13.642 t (6.6%). Marine fish farming was started at the beginning of the 1990s in Turkey. Generally marine fish was mostly cultured in the Mediterranean region in net cages. Aquaculture production gradually increases day by day owing to the increasing consumption of farmed marine fish all over the world. Total aquaculture fishery production of Turkey is approximately 276.502 tons, of which 99.971 and 61.090 tons of sea bass

and sea bream respectively (TurkStat, Fishery Statistics, 2017). Total aquaculture fishery production of Turkey is 314.537 tons of which 209.370 tons is cultured marine fish and 105.167 tons is freshwater fish farming. 76.680 tons of cultured marine fish is gilthead sea bream and 116.915 tons is sea bass. It's projected that the amount of cultured gilthead sea bream and sea bass production will reach a total of 402.951 tons. (Tagem, 2019). It is clear from the statistics and the projections provided by the Republic of Turkey Ministry of Agriculture and Forestry that aquaculture production increases steadily at a high pace. Today, some of the producers around Muğla province contribute to the national economy by breeding gilthead sea bream in earthen ponds that are not on land unsuitable for agriculture and they produce fish similar to wild fish in terms of sensory characteristics. Others of the fish farmers are farming gilthead seabream in net cages offshore. Over the past decade demand for freshly cultured gilthead seabream in Turkey and Europe has increased significantly due to flavor, texture, total quality, availability throughout the year, and cost efficiency to consumers. Gilthead seabream is one the most and increasingly preferred cultured fish species worldwide. Factors affecting the sensorial and microbiological quality of cultured fish may vary depending on features of fish species, cultivation conditions, fish feeding regime, practices during harvesting and post-harvesting (Caggiano, 2000). Excessive feeding of fish increases the microbial load by enzyme activities in their digestive systems. Therefore the shelf life of these types of fish is short. The sources of contamination are soil, water, weather, human, and tool devices. Papadopoulos et al. (2003) reported that based on sensory and microbiological analyses gutted sea bass have a shorter shelf life than ungutted specimens. If the soil which is the natural environment for many microorganisms, is contaminated especially by the sewage systems or feces there would be an increase in both the species and number of microorganisms. Similarly, water may become another source of microbial contamination unless a sanitary condition for farmed fish is provided. The amount of bacteria in the aquatic waters has a significant effect on fish quality. Pollution and the presence of sediment deposition indicate the existence of the pathogenetic bacteria in waters used for aquaculture fish production. Microorganisms may cause a pathogenetic impact responsible for the deteriorating quality of fish flesh. The most common pathogens in fish are *Salmonella typhi*, *E. coli*, *Pseudomonas florescens*, *Aeromonas hydrophilla*, *Proteus vulgaris*, *Staphylococus aureus*, *Shigella spp*. (Obasohan et al.,2010). Bacteria present on the fish are normally associated with those found in their natural environment and influenced by the season and the harvesting condition. The proportion of the initial population can easily be changed after the harvesting process depending on the ability of the bacteria to adapt to the new conditions (ICMSF, 1998). Live fish is normally considered to be sterile but microorganisms exist on the skin and gills and in the alimentary tract of live and newly caught fish in varying numbers. A normal range of 10^2-10^7 cfu/cm^2 on the skin and between 10^3-10^9 cfu/g in the gills and intestines have been observed (Liston, 1980). Spoilage bacteria are predominant on newly caught fish but some pathogenic bacteria could also be present in the skin, gills, or guts (Jha et al., 2010). To ensure sensorial and microbiological quality in cultured fish it is necessary to control environmental factors, take measures to prevent the contamination of seawater, and hygiene practices. If the fish are stressed and alive, the lactic acid level will be increased, therefore the pH value will decrease dramatically; in case of prolonged stress, all glycogen will be consumed, leading to a high level of pH. Meantime psychrophile and psychotropic aerobic, gram-negative bacteria, usually living on the fish begin to multiply and cause the characteristic odors affecting sensorial quality (Caggiano, 2000).

There are some studies on quality evaluation of cultured gilthead sea bream (Kyrana et al., 1997; Alasalvar et al., 2002 a, b; Grigrakis et al., 2002; 2007; Lougovoisa et al., 2003; Beklevik et al., 2005; Kılınc et al., 2007; Alvarez et al., 2008; Yıldız et al., 2008a, Yıldız, 2008b; Attouchi and Sodak, 2010; Cardinal et al., 2011). However, the aim of this study was to determine quality parameters and visual characteristics of gilthead sea bream cultured in different habitats.

MATERIAL AND METHODS

Gilthead seabream is cultivated in net cages and/or in earthen ponds by some of the producers around Muğla province in Turkey. In this research fresh gilthead seabream with an average weight of 300-350 g and an average length of 26-28 cm cultured in net cages (by Kılıc Deniz Co. in Bodrum, Turkey) and earthen ponds (by Association of Inland Aquaculture Farmer and Producers in Milas, Turkey) harvested in every two months were used. The fish were slaughtered by immersing in ice-cold water (hypothermia) and delivered to the laboratory (whole) within 12 h after harvesting, packed in separate thermo-insulated polystyrene boxes with ice. Figure 1 shows cultured gilthead sea bream. The photo on the left side represents in net cage cultured fish (a) and the right side represents farmed fish in earthen ponds (b).

Physico-chemical analyses

During analyzing period pH, TVB-N, and NH_3-N analyzes were measured for both of the groups. pH values of fish flesh (1:10 v/w) were measured with Thermo Scientific Model Orion Star A 214 (USA) pH meter. NH_3 measurement of fish flesh was measured according to the ISE procedure by Pivarnik et al. (1998). To determine NH_3 value of fish flesh, 5 g of flesh fish, 95 ml pure water, and 2 ml alkali ISA were homogenized for 2 minutes. After calibrating the Thermo Scientific Model Orion Star A 214 (USA) pH/ISE meter, NH_3 measurement had been carried out. The results of NH_3 were provided as mg/100 g. The method reported by Schormüller (1968) was used to determine TVB-N values of fish samples. MgO was added to the homogenized fish samples and then it was distilled. After distillation, distilled ammonia materials were titrated with 0.1 N NaOH. The results were explained as mg TVB-N/100 g muscle.

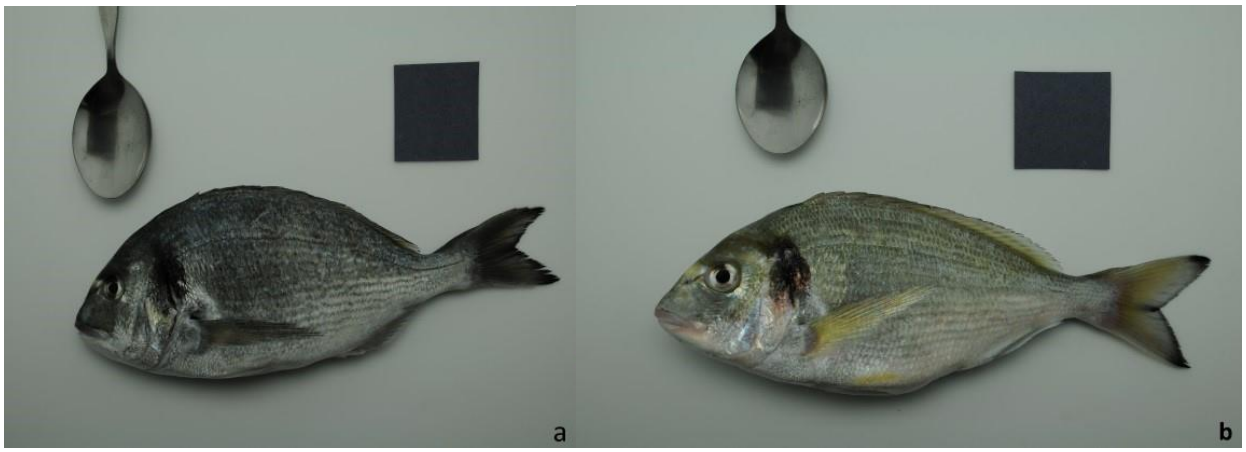

Figure 1. Cultured gilthead seabream (*Sparus aurata*) in net cage[a] and in earthen ponds[b] (Original photograph)

Color measurement

Color analyses were determined from each of five fish using Minolta CR-400 chromometer (Minolta Camera Co., Japan). The Minolta colorimeter was calibrated with a standard white plate (D65, Y=93.9, x=0.3155, y=0.3319) before each use L*, a*, b* values were measured under D65 illumination. By using five fish for each value L*, a*, b* values were measured three times from different spots on dorsal parts of skin, flesh, and operculum of each fish separately. Finally, the average value was calculated and recorded as result. L* value represents lightness from black to white between 0 and 100 a* value represents color from red (+) to green (-) and b* value represents color from yellow (+) to blue (-) in the color measurement of fish.

Sensorial analyses

Raw and cooked fish samples

The sensorial attributes of cooked fish were evaluated by a panel of ten experienced judges during each sampling period. Fish samples (100 g of fillets) were cooked individually in a steam cooker with a Pyrex lid (Raks Buharlım, Manisa, Turkey) which had been preheated to 100 °C for about 30 min until the internal temperature of each fillet reached 70 °C at the thickest part measured with a thermocouple probe. After reaching the desired internal temperature samples were immediately presented to the panelists (each panelist evaluating approximately 20 g of fish sample).

Panelists were asked to score the odor, taste, and texture of fish using a 0–10 descriptive hedonic scale (Chang et al., 1998). The same panelists also scored QIM on the significant sensory parameters of raw fish using characteristic parameters and a scoring system from 0 to 3 demerit points (Huidobro et al., 2000).

Microbiological analyses

Total mesophilic aerobic bacteria count (TMABc) and yeast and mold count (TYMc) were determined as stated by Maturin and Peeler (1998) and Anonymous (2005) respectively. Each fish sample of 10 g was homogenized for 150 sec in a stomacher (IU Instruments, Spain) with 90 ml peptone water (0.1%).Serial dilutions (from 10^1 to 10^4) were prepared for each sample obtained from obtaining different aquaculture habitats. Diluted samples were placed on plate count agar (PCA, Merck) and incubated at 35 °C for 48 h to estimate TMABc count. Also dicloran rose bengal agar (DRBC, Merck) was used to estimate TYMc by incubating at 23°C for 5 days. Microbiological measurements were conducted on five whole fish samples and each measurement was repeated eight times resulted in sixteen (n=16) measurements for each enumeration.

Detection of salmonella was determined as stated by Rall et al. (2005). During pre-enrichment 25 g of fish samples were homogenized with 225 ml of buffered peptone broth in a stomacher for one minute. The fish samples were pre-enriched in buffered peptone broth which was incubated between 18 and 20 h at 35°C. After pre-enrichment of the samples, selective enrichment was carried out, in this sense, 0.1 ml (approximately) of the pre-enriched sample was transferred to 10 ml of Rappaport-Vassiliadis broth (RV). 1 ml of pre-enriched sample was transferred to 10 ml of tetrathionate broth (TT) and then both media were incubated at 42ºC for 24 hours. Besides during plating on solid selective media, they were shaken and streaked onto plates of CHROM agar Salmonella Plus medium and Xylose lysine desoxycholate (XLD) agar. Incubation of the samples was carried out at 35°C for 24 hours, after the incubation period of the plates, the samples were examined for typical *Salmonella* colonies.

The isolation of *E. coli* was determined as stated by (FDA-BAM) (Feng et al., 2011). In this respect, a 25 g fish sample was transferred in 225 ml of tryptone phosphate (TP) broth and incubated at 44°C for 24 h for the pre-enrichment period. A volume of enriched broth was plated onto eosin-methylene blue (EMB; colonies produce a green metallic sheen) agar and MacConkey agar plates (colonies are brick red).

Statistical analysis

Kolmogorov-Smirnov and Shapiro-Wilk normal tests were used to determine the statistical significance of the results of the study. The groups which were normal distribution was used Student-t, and the groups which weren't normal distribution was used Mann-Whitney U. Once the results of normal distribution groups were indicated as average ± standard deviation, abnormal group results were showed as a min-max median. The result of the study was evaluated as $p<0.05$ and the results were considered statistically significant.

RESULTS AND DISCUSSION

The identification of the color characteristics in the body parts is a determining factor in distinguishing the gilthead sea bream fish grown in different aquaculture habitats from each other.

The skin, operculum, and flesh of color values of gilthead seabream are provided in Figures 2 to 8. The lightness values (L*) of the skin of cultured gilthead seabream in net cage ranged from 59.31 to 86.74 and the values of red and yellow color, a* and b*, ranged respectively from -0.46 to -1.03 and -4.06 to 0.17 depending on harvesting times. On the other hand the lightness values (L*) of the skin of cultured gilthead seabream in earthen ponds ranged from 62.63 to 85.91 and the values of red and yellow color, a* and b*, ranged respectively from -0.30 to -1.69 and -5.02 to 2.02 in the same harvesting periods. The skin color of the net cage cultured gilthead seabream was darker than those of gilthead sea bream from earthen ponds (Figure 1, 2 and 3). Especially, except in April, skin L* values of cultured gilthead seabream from earthen ponds were statistically higher in June, August, October, and December than those of the net cage cultured gilthead seabream. The transparency and the number of rays of the tail fin of gilthead seabream cultured in earthen ponds were more distinctive than those of the gilthead seabream cultured in net cages.

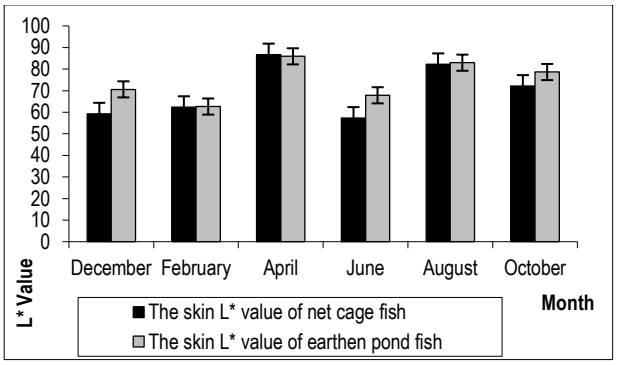

Figure 2. The skin L* values of cultured gilthead seabream.

The L* and a* values of the flesh of cultured gilthead seabream are provided in Figures 4 and 5. It is determined that the average L* and a* values of the flesh of cultured gilthead seabream in earthen ponds ranged from 45.83 to 81.82 and from -0.52 to 3.63 respectively. On the other hand, the average L * value and a* values of the flesh of cultured gilthead seabream in the net cage ranged from 49.86 to 77.03 and from -0.33 to 4.22 respectively.

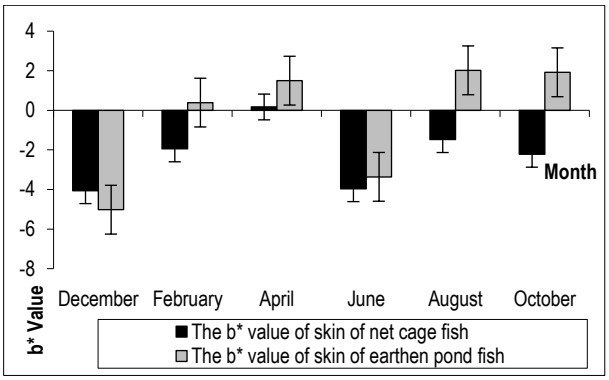

Figure 3. The skin b* values of cultured gilthead seabream

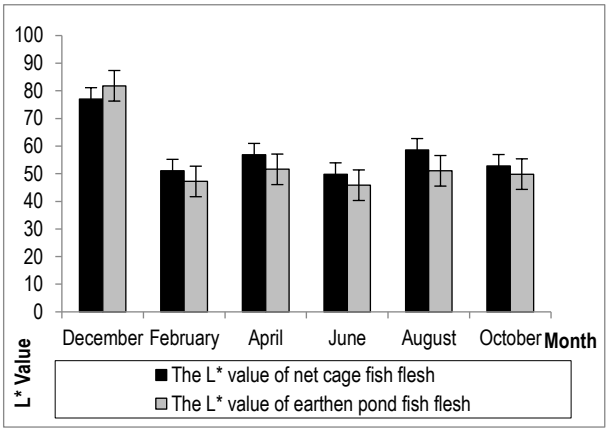

Figure 4. The flesh L* values of cultured gilthead seabream

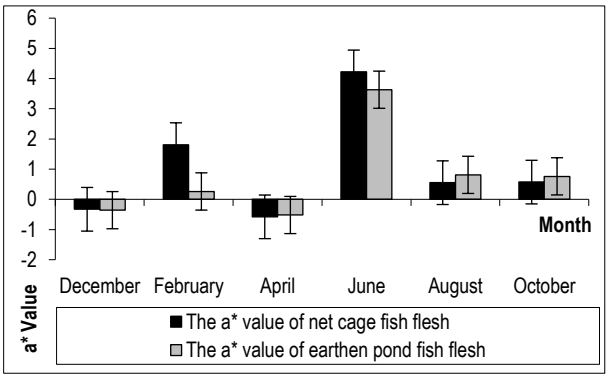

Figure 5. The flesh a* values of cultured gilthead seabream

It was determined that the L* values of the flesh of cultured gilthead seabream in the net cages were brighter than that of the gilthead seabream from earthen ponds only in December whereas during the other months it was exactly the opposite as statistically. However, the a* value of flesh of cultured gilthead seabream in net cages was brighter in February and June whereas, on the contrary, the a* values of flesh of cultured gilthead seabream in earthen ponds were

brighter in August and October. There was no difference in the a* values of the flesh of cultured gilthead seabream both from the net cage and earthen ponds in December and April ($p>0.05$).

Cardinal et al. (2011) reported that lightness values of the raw fillet seabream ranged from 41.2 to 43.1 and the values of red and yellow color, a* and b*, ranged respectively from-1.5 to-0.5 and from -0.4 to -0.04, according to the period. A significant difference was only observed on the a* parameter of the samples caught in March presenting a slightly less red color. In general, the a* color values of both groups of fish fillets were slightly reddish in December and April whereas it was markedly reddish flesh color in June. These a* color value results are comparable to reported color values by Cardinal et al. (2011). It was determined that the ideal flesh color was similar to that of wild fish in December referring to L* and a* values for both groups of fish fillets. The operculum L*, a*, and b* values of cultured gilthead seabream can be seen in Figures 6, 7 and 8. As provided in Figure 7, the a* values of operculum cultured gilthead seabream in earthen ponds ranged from 0.19 to 2.48. Whereas, it was determined that the redness, a* values of the operculum of cultured gilthead seabream in the net cage were mostly under 1. Statistically, in all harvesting periods operculum, a* value of cultured gilthead seabream in earthen ponds was found more reddish than those of the cultured fish in the net cage ($p<0.05$). Also in all periods except in December operculum b* values of gilthead sea bream cultured in earthen ponds were yellowish than those of the cultured fish in the net cage ($p<0.05$).

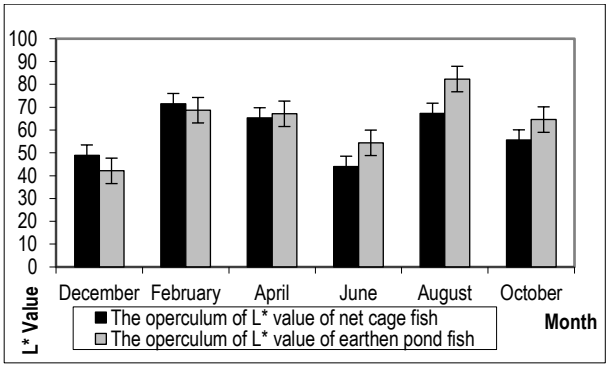

Figure 6. The operculum L* values of cultured gilthead seabream

The wild and cultured seabream could be separated from each other by visual differences such as body shape, color, skin, stamps, teeth, and odor (Grikorakis et al., 2002). Body-color differences of cultured gilthead seabream were found significant between cultured in the net cage and earthen ponds ($p<0.05$). They could be distinguished from each other considering visual characteristics such as skin color, head shape, redness of the operculum, and transparency and color of the tail fin. In this study, the most significant color difference between the two groups of fish was the skin color. The skin L*value of cultured gilthead seabream from earthen ponds was determined brighter than that of the cultured gilthead seabream in the net cage. Moreover, cultured gilthead seabream in earthen ponds had distinct red color on the operculum.

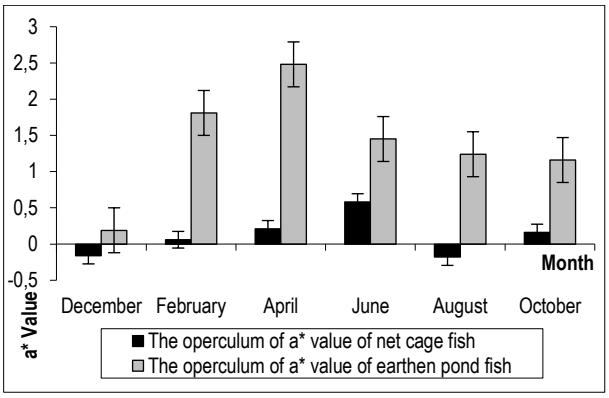

Figure 7. The operculum a* values of cultured gilthead seabream

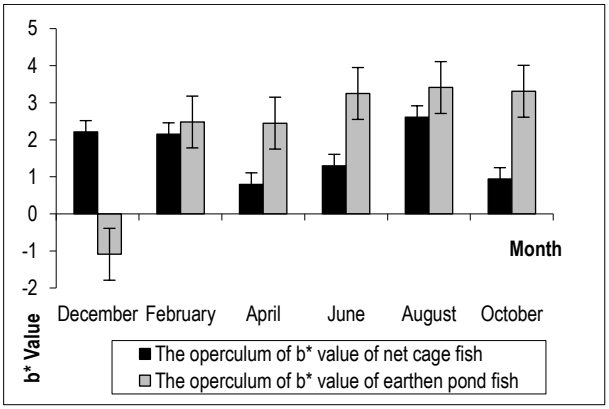

Figure 8. The operculum b* values of cultured gilthead seabream

Besides, gilthead seabream cultured in earthen ponds was separated easily than the other fish samples thanks to the brightness and transparency of the tail fin. While cultured gilthead sea bream in earthen ponds had thinner head just as wild fish, cultured gilthead seabream from net cage had almost a big round-shaped head. Because of the brightness of skin L* value and the redness of a* value on the operculum cultured gilthead seabream in earthen ponds were significantly different than that of the cultured fish in the net cage ($p<0.05$). Visual characteristics of cultured gilthead seabream in earthen ponds were quite similar to those of the wild gilthead seabream which was also in line with the findings of Grigorakis et. al (2002).

Sensory evaluations of raw and cooked cultured gilthead seabream both from two different habitats are provided in Figures 9 and 10. The quality is generally defined with nutritional, microbiological, biochemical, and physicochemical, but none of these terms are solely enough to define the quality. Sensorial perception and consumer acceptance scores should be considered along with these properties to define the overall quality. The fish quality should be

maintained seamlessly from fishing to the consumer. The quality index method (QIM) is the commonly used method to determine fish quality lately. The total score represents all quality properties of fish and this score is named as the quality index. For fresh fish, a zero score is given and the score increases when the fish sample gets worse (Nielsen et al., 2002). Papadopoulos et al. (2003) reported that rigor mortis, metallic sheen, and iridescence of the skin, as well as glossy, bright red gills processing seaweedy and shellfish odor, should be considered as attributes of extreme freshness, whereas loss of brilliance and iridescence, fading of skin colors and bleaching of the gills in patches would indicate stale fish. In Figure 9, the sensorial average score of cultured gilthead sea bream in the net cage was 3.3 where cultured gilthead seabream in earthen ponds had a 2.8 average sensorial score. Although the differences weren't statistically meaningful for the sensorial quality of raw fish ($p>0.05$) however probably these differences could depend on harvesting conditions, feeding regime, sea or freshwater temperature, the effectiveness of cold chain, and transport conditions.

Cardinal et al. (2011) concluded that sensory criteria chosen for seabream cooked fillet related to the appearance, odor, flavor, and texture were identified for odor; global intensity, for appearance, color homogeneity, for texture, firmness, for flavor, global intensity. The taste, odor, and flavor properties of cooked cultured gilthead seabream are shown in Figure 10.

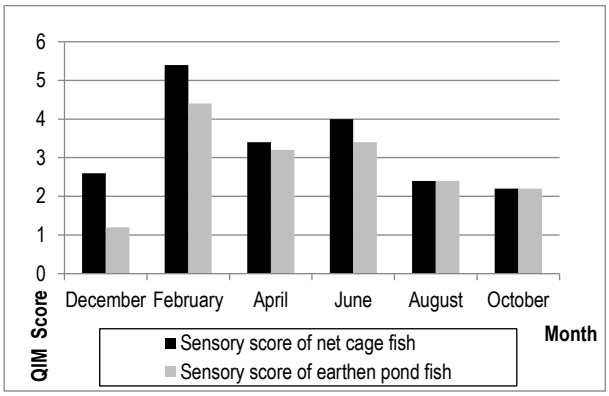

Figure 9. QIM score of raw cultured gilthead seabream. Points represent mean values of ten determinations ± standard error

The textural properties of cooked cultured gilthead seabream in the net cage were scored the best quality except in February and April by panelists' preferences. On the other hand, in terms of odor characteristics except for December the best quality was determined for cultured gilthead seabream in the net cage. The texture, odor, and flavor of cultured gilthead seabream in the net cage had a great influence on the preferences of the panelists with a higher score in October than cultured fish in earthen ponds.

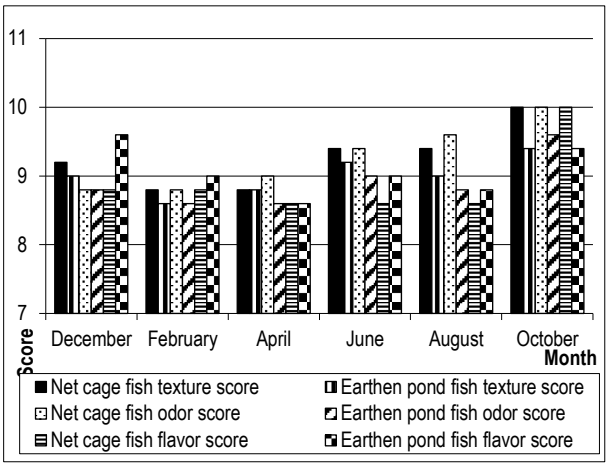

Figure 10. The sensory score of cooked cultured gilthead seabream. Points represent mean values of ten determinations ± standard error

It was clear that cooked gilthead seabream cultured in earthen ponds had softer flesh tissue than the other fish group. Furthermore, the most preferred group with salty flesh taste cultured gilthead seabream in earthen ponds was noticed. For these reasons, cultured gilthead seabream in earthen ponds were mostly preferred due to the juiciness of fillets by panelists. On the other hand, cultured gilthead seabream in the net cage had harder flesh tissue and less taste than the other fish group. But it was noticed that the cultured gilthead seabream in the net cage showed higher quality through textural and odor parameters. A statistically significant difference couldn't be found between the sensorial properties (texture, odor, and taste) of the two fish groups. The feeding regime is one of the important parameters affecting the cultured fish quality. During feeding periods the digestive tract of the fish contains many bacteria that produce digestive enzymes capable of causing intense postmortem autolysis resulting in strong odors and flavors especially in the abdominal area (Huss, 1995).

Table 1 shows that the total number of mesophilic bacteria in the flesh of gilthead seabream fish was 1.40-3.31 log cfu/g in net cage fish; 1.61-2.99 log cfu/g in the earthen ponds fish. Yeast and mold were determined 2.07-2.90 log cfu/g in the net cage fish and 0.74-3.68 log cfu/g in the earthen pond fish. Yeast and mold were determined 2.07-2.90 log cfu/g in the net cage fish and 0.74-3.68 log cfu/g in the earthen pond fish.

There was a difference between the groups in terms of yeast and mold in December, April, and August ($p<0.05$). The total mesophilic bacteria of gilthead seabream vary in months and groups ($p <0.05$). In December and February, the number of mesophilic bacteria of cultured fish in the net cage was higher than those of the earthen pond fish. Whereas in April the number of mesophilic bacteria in the earthen pond fish was found to be higher than those of the cultured seabream in the net cage. Attouchi and Sadok (2012) reported that mesophilic bacterial count in fresh farmed seabream was 2.83

log cfu/g. In our study low initial mesophilic bacterial counts in both cultured fish were found to be similar to those reported in cultured seabream by Attouchi and Sadok (2012). According to ICMSF (1986) that the limit value of mesophilic bacteria for human consumption of fresh fish is 7 log cfu/g. The total mesophilic bacteria counts of fish groups did not exceed the maximum level for gilthead seabream regardless of the month harvested. Both groups were found to be suitable for human consumption in terms of total mesophilic bacterial load, yeast, and mold in fish meat.

Table 1. Changes in microbiological bacteria count of cultured gilthead seabream

Mesophilic	Net cage fish	Earthen pond fish	*p*
December	3.31±0.05[a]	2.11±0.06[b]	0.001
February	2.72±0.06[a]	2.59±0.05[b]	0.006
April	1.40±0.08[a]	1.61±0.08[b]	0.005
June	2.27±0.16[a]	2.09±0.07[a]	0.065
August	2.02±0.07[a]	2.20±0.20[a]	0.096
October	3.14±0.10[a]	2.99±0.23[a]	0.217

Yeast and	Net cage fish	Earthen pond fish	*p*
December	3.80±0.08[a]	2.42±0.02[b]	0.001
February	3.12±0.12[a]	2.97±0.10[a]	0.055
April	1.40±0.08[a]	1.61±0.08[b]	0.005
June	2.59±0.14[a]	2.40±0.19[a]	0.096
August	2.31±0.12[a]	2.52±0.08[b]	0.015
October	3.60±0.14[a]	3.42±0.20[a]	0.140

(*) a and b determine statistical differences between the two groups during analysis period (n=5)

Besides, *Salmonella spp*. and *E. coli* are important for food safety. The presence *E. coli* in food indicates fecal contamination in general directly or indirectly depending on the fecal origin. High levels of *E. coli* in food give a general idea that food is produced and stored under inappropriate or inadequate hygiene and sanitation conditions (Temiz, 1998). *Salmonella spp.* is a disease pathogen and should never be present in food. If sanitary procedures are implemented by good manufacturing practices the production of good eating quality is assured. The pathogenic bacteria release toxins into the substrate, which can be a food product, or the human gastrointestinal system can cause food poisoning (Kanduri and Eckhardt, 2002). According to the microbiological analysis results, *Salmonella spp*. and *E. coli* were not detected in any month for two cultured fish samples. The absence of *Salmonella spp*. and *E. coli* in any fish groups was an indicator that the consumption of gilthead seabream was suitable for food safety.

The difference in chemical qualities of cultured gilthead seabream in both aquacultural conditions was found to be significant, namely pH, TVB-N, and NH_3 values ($p<0.05$). (Table 2). Cultured gilthead seabream were found to have pH values of 6.18 to 6.34, TVB-N values of 11.40 to 25.29 mg/100 g, and NH_3 values of 0.91 to 2.70 mg/100 g in net cage fish. Whereas the cultured gilthead seabream in earthen ponds had pH values of 6.16 to 6.32, TVB-N values of 12.80 to 27.85 mg/100 g, and NH_3 values of 1.01 to 4.32 mg/100 g. The small changes in TVB-N values between the flesh of cultured gilthead seabream in two fish groups were statistically significant in December, February, and April ($p<0.05$). Also, pH values in the flesh of cultured gilthead seabream between two fish groups were found to be statistically significant only in April ($p<0.05$). While the TVB-N values of cultured gilthead seabream in the earthen ponds were higher in December and February than that of the cultured gilthead seabream in the net cage. On the other hand, the TVB-N values of cultured gilthead seabream in the net cage were higher amount TVB-N value in April than that of the fishes of the earthen pond.

Table 2. Changes in chemical quality indices of cultured gilthead seabream

TVB-N	Net cage fish	Earthen pond fish	*p*
December	11.40±0.40[a]	12.80±0.46[b]	0.016
February	25.29±0.69[a]	27.85±0.40[b]	0.005
April	24.73±1.11[a]	20.84±1.39[b]	0.019
June	23.03±1.64[a]	23.03±0.82[a]	0.999
August	20.90±1.53[a]	18.50±0.79[a]	0.073
October	15.12±1.00[a]	13.61±0.48[a]	0.079

NH_3 analysis	Net cage fish	Earthen pond fish	*p*
December	1.53±0.03[a]	1.44±0.04[b]	0.037
February	1.08±0.01[a]	1.80±0.01[b]	0.001
April	2.61±0.24[a]	4.32±0.25[b]	0.001
June	0.91±0.08[a]	1.01±0.07[a]	0.189
August	2.37±0.06[a]	2.16±0.04[b]	0.006
October	2.70±0.03[a]	2.64±0.04[b]	0.136

pH	Net cage fish	Earthen pond fish	*p*
December	6.25±0.01a	6.25±0.01a	1.000
February	6.34±0.04a	6.28±0.02[a]	0.084
April	6.29±0.07[a]	6.16±0.04[b]	0.038
June	6.18±0.03[a]	6.19±0.01[a]	0.492
August	6.30±0.03[a]	6.32±0.04[a]	0.566
October	6.24±0.02[a]	6.23±0.01[a]	0.252

*a and b determine statistical differences between two groups during analysis period (n=3)

The difference between the fish groups was significant due to the ammonia values measured in February, April, August, and December in cultured gilthead seabream ($p<0.05$).

Together with the increase in TVB-N level in fish meat, the unpleasant taste and odor development in fish characteristically emerge. This is an indication that the chemical quality of the fish flesh has deteriorated beyond the consumable limit value. High ammonia values in fish flesh texture can only be detected when there is significant deterioration.

The total volatile base nitrogen value, which is a sign of fish freshness is a widely used indicator for quality assessment. The European Commission (Council Regulation No. 95/149 EEC of March 1995) reported the acceptable limit

value for TVB-N as 30-35 mg/100 g in the sensory evaluation of different fish species. In the case of sensory rejection of degraded aquatic products, TVB-N value is supported by the idea that levels of undesirable tastes, which alone are not responsible for seafood spoilage (Dalgaard, 2000). Kyrana et al. (1997) reported that it may be a consequence of the high level of non-proteinaceous nitrogen content in the initial TVB-N level of gilthead seabream flesh. It was determined that the TVB-N value and NH_3 value of cultured gilthead seabream from two different habitats in our study were different from each other. The difference is thought to be due to changes in feeding conditions and feed content, or in fish post-mortem conditions. This can lead to loss of quality and limiting the shelf life of fish during cold storage.

CONCLUSION

As a result, it is thought that the small differences between the total quality scores of the cultured gilthead seabream depend on the harvesting conditions, water temperature, and the effective cold chain conditions. Both groups of fish have features that can appeal to the consumers because of their unique quality characteristics. According to the panelists; soft flesh texture and salty taste can be described as tastier in the earthen ponds culture fish while the hard texture and characteristic aromatic properties of net cage fish can be the reason for consumers' acceptance.

The visual differences between the two cultured fish are distinguished by the color differences in the skin, operculum, head shape, and transparency of rays in the fin tail. It is almost impossible to differentiate the wild gilthead seabream from the cultured gilthead seabream from earthen ponds due to similarities in reddish color on the operculum and light skin color.

There is no microbiological evidence that could negatively affect the consumption of edible fish flesh in terms of total bacterial load, yeast, and mold. Furthermore, the absence of *Salmonella spp.* and *E. coli* is the indication that fish are suitable for consumption in terms of food safety.

Water quality and aquaculture practices play important roles in determining fish quality. Cultured fish production is not permitted near the Turkish coastline by the Ministry of Food, Agriculture, and Livestock authorities. In Turkey, the cultured fish pass through the HACCP control system before sales, be individually marked, and are sold at retail fish markets. The fish production companies are certified by the authorities and granted a working and aquaculture production permit. The quality of fresh fish from the farm to the fork is regularly checked and reported by Ministerial Inspector. Thus cultured fish are trustable marketed to national and international markets.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Determination of important enzymes and antimicrobial resistances of gram-positive haloalkaliphilic bacteria isolated from Salda Lake

Salda Gölü'nden izole edilen gram-pozitif haloalkalifik bakterilerin önemli enzimlerinin ve antibiyotik dirençlerinin belirlenmesi

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Abstract: As an extreme environment, soda lakes harbor various haloalkaliphilic microorganisms. Salda Lake is one of the natural soda lake (pH>9) in Turkey. Haloalkaliphiles are unique microorganisms in their ability to live in high alkaline and high saline conditions, and play an important role in biodegradation and bioremediation of hydrocarbons. Hence, the aims of this study were to isolate haloalkaliphilic bacteria from water sample of Salda Lake, to identify these isolates by both conventional and molecular methods, to screen their industrially important enzymes, and to investigate their antimicrobial resistance profiles. Six isolates were identified as *Bacillus horneckiae*, *Bacillus subtilis*, *Bacillus paramycoides*, *Bacillus pumilus*, *Staphylococcus epidermidis*, *Bacillus haynesii* according to 16S rRNA gene sequencing analysis. The industrially important enzymes (amylase, cellulase, pullulanase, lipase, urease, protease, caseinase, oxidase, catalase) were produced by haloalkaliphilic isolates. These enzymes maybe used in alkaline and saline industrial processes. Although *Bacillus subtilis* was susceptible to all antibiotics, other isolates showed resistance to at least one antibiotic. The resistance against antibiotics were found as ampicillin/sulbactam 83%, amoxycillin/clavulanic acid 83%, ampicillin 67%, mupirocin 67%, chloramphenicol 50%, tetracycline 50%, imipenem 50%, meropenem 50%, cefadroxil 17%. These bacteria may have develope resistance to antibiotics that entering their natural environment in different ways.

***Keywords*:** Microbial enzymes, antibiotic resistance, soda lake, haloalkaliphiles, biodiversity, gram positive bacteria

Öz: Aşırı ortamlar olarak soda gölleri, çeşitli haloalkalifilik mikroorganizmaları barındırır. Salda Gölü, Türkiye'deki doğal soda göllerinden (pH>9) biridir. Haloalkalifiller yüksek alkali ve yüksek tuzlu koşullarda yaşama kabiliyetleri bakımından benzersiz mikroorganizmalardır ve hidrokarbonların biyolojik olarak parçalanması ve biyoremediasyonunda önemli bir rol oynarlar. Bu nedenle, bu çalışmanın amacı haloalkalifilik bakterileri Salda Gölü'nden izole etmek, geleneksel ve moleküler metotlarla tanımlamak, ürettikleri önemli endüstriyel enzimleri ve antibiyotik direnç profillerini araştırmaktır. 16S rRNA gen dizi analizine göre altı adet haloalkalifilik izolat (*Bacillus horneckiae*, *Bacillus subtilis*, *Bacillus paramycoides*, *Bacillus pumilus*, *Staphylococcus epidermidis*, *Bacillus haynesii*) tanımlanmıştır. Endüstriyel öneme sahip amilaz, selülaz, pullulanaz, lipaz, üreaz, proteaz, kazeinaz, oksidaz, katalaz enzimleri haloalkalifilik izolatlar tarafından üretilmiştir. Bu enzimler alkali ve tuzlu endüstriyel işlemlerde kullanılabilir. *Bacillus subtilis* tüm antibiyotiklere duyarlı olmasına rağmen, diğer izolatlar en az bir antibiyotiğe direnç göstermiştir. Antibiyotiklere direnç; ampisilin/sulbaktam %83, amoksisilin/klavulanik asit %83ampisilin %67, mupirosin %67, kloramfenikol %50, tetrasiklin %50, imipenem %50, meropenem %50, sefadroksil %17 olarak bulunmuştur. Bu bakteriler doğal ortamlarına farklı yollardan giren antibiyotiklere karşı direnç geliştirebilirler.

***Anahtar kelimeler*:** Mikrobiyal enzimler, antibiyotik direnci, soda gölü, haloalkalifiller, biyoçeşitlilik, gram pozitif bakteri

INTRODUCTION

Extreme environments harbor extremophile microorganisms such as alkaliphiles, halophiles, thermophiles, psychrophiles and acidophiles. These environments have enormous potential for novel enzymes which are salt tolerant, thermostable, active at high pH. Microorganisms that can survive under extreme pH values are referred as alkaliphiles (Raval et al., 2015). The enzymes of alkaliphiles are used in different industries for various applications (Table 1).

The most widely studied enzymatic group is alkaline proteases (Chand and Mishra, 2003). The researchers reported that *Bacillus licheniformis*, *B. subtilis*, *B. amyloliquefaciens* and *B. majovensis* produce alkaline protease enzymes (Gupta et al., 2002). Alkaliphilic bacterial species were reported by other researchers. Alkaliphilic *Bacillus cohnii*, *B. pseudofirmus*, and *B. clarkii* were isolated from bauxite residue in the southern region of Minas Gerais (Brazil) (Nogueira et al., 2017). Haloalkaliphilic bacterial strains belonging to the genera *Bacillus*, *Staphylococcus*, *Halobacillus*, *Virgibacillus*, *Oceanobacillus* were isolated from saline desert of Little Rann of Kutch in India (Bhatt et al., 2018). These isolates were able to produce protease, cellulase, carboxymethyl cellulase (CMCase) and amylase enzymes at high salt concentration and high pH (Bhatt et al., 2018). The researchers also reported that these isolates showed resistance against ampicillin, amikacin, augmentin, cefaclor, colistin, cefoperazone, cefuroxime, cefotaxime, cefixime, erythromycin, azithromycin, co-norfloxacin, trimoxazole, amoxycillin, cefadroxil, cefpodoxime, penicillin and gentamycin (Bhatt et al., 2018).

Table 1. Microbial enzymes and their industrial applications

Microbial enzyme	Industries using enzymes	The use of enzymes	References
Cellulase	Textile	Softening and shining of clothes	Aygan and Arikan, 2008
	Detergent	Polishing fabrics	Wang et al., 2009
	Agriculture	Production of biofuel from cellulosic material	
		Conversion of agricultural biomass into useful products	
Protease	Detergent	Laundry additives	Chand and Mishra, 2003
	Food	Formulations of detergents	Mitra and Chakrabartty, 2005
	Animal feed	Peptide sythesis	
	Baking	Fish sauce preparation	
	Biomedical	Dehairing goak skin	
	Brewing		
	Cheese		
	Chemistry		
	Tanning		
	Leather		
	Pharmaceutical		
Lipase	Detergent	Detergent additives	Jaeger and Holliger, 2010
	Food	Enantioselective biocatalyst for the Production of fine chemicals	Babu et al., 2008
	Paper	Esterification	
		Trans-esterification	
		Amynolysis	
Xylanase	Food	Production of coffee, feed and flour	Ratnakar, 2013
	Paper	Removal of lignin from pulp	Khandeparker and Numan, 2008
	Pulp	Increasing loaf volume	
	Baking	Production of biofuel	
		Starch production from lignocellulose	
Pullulanase	Food	Biocatalysis in organic solvents and super critic fluids	Delgado-García et al., 2015
Amylase	Textile	Starch saccharification	Ratnakar, 2013
	Food	Strach hydrolysis	Ammar et al., 2002
	Brewing	Saccharification of marine microalgae	Kikani et al., 2010
	Distilling	Removal of starch from clothes	
	Detergent	Desizing process	
		Production of syrups	
		Reduction of turbidity of fruit juice	
DNase	Food	Acid 5'-guanilic and acid 5'-inosinic as flavor agents	Delgado-García et al., 2015
Urease	Beverage	Removal of urea from wine	Liu et al., 2012
Caseinase	Food	Degrading casein in milk	Johnson and Case, 2010

Salda Lake, which is an extremely alkaline, slightly saline and closed system, is located in the lakes district of Turkey (Balci et al., 2018). Salda Lake is close to Yesilova, located in 44 km^2 area and nearly 200 m deep. In Salda Lake region, agriculture, animal husbandry, livestock, tourism and fishing activities are carried out. The pollutants caused by these activities are mixed into the water of the Salda Lake. The contamination of lake water with antibiotic resistant bacteria may be resulted from the livestock-related or agricultural activities. The researchers reported that the water of the Salda Lake may be used as drinking water in the future (Varol et al., 2021). It should be known which microbial populations live in Salda Lake so that the lake water can be decontaminated with the correct chemicals or methods. Therefore, it is important to isolate and identify haloalkaliphilic bacteria found in Salda Lake water resistant to antibiotics used in human and veterinary medicine.

There are a few reports on the microbial populations found in Salke Lake. *Lyngbya majuscula*, *Gloeocapsa aeruginosa*, *Synechococcus* sp., *Microcystis aeruginosa*, *Chroococcus turgidus* and *Oscillatoria limnosa* were found in Salda Lake by the researchers (Braithwaite and Zedef, 1996; Kazanci et al., 2004; Shirokova et al., 2013). In another study, new generation sequence analysis showed that 97.3% of the lake belongs to *Bacteria* domain (*Gammaprotobacteria* 39.6%, *Alphaprotobacteria* 25.6%, *Bacilli* 23.7%, *Cyanobacteria* 5.3%, *Betaproteobacteria* 2%, *Actinobacteria* 1.7%) (Balci et al., 2018).

Antibiotics are used in food animals to treat, control and prevent diseases (Mirzaagha et al., 2011). Although antibiotics are commonly used in animal husbandry to promote growth, in human and veterinary medicine to treat infections caused by bacteria, antibiotic-resistant bacteria increased worldwide because of the misuse and overuse of antibiotics. While the researchers focused on the antibiotic resistance in human, agriculture and animal, much less information is available about antibiotic-resistant bacteria in natural environments. The natural environments contain various reservoir of antimicrobial resistance genes and genetic elements (Berkner et al., 2014). These genes constitute the resistome of environment which are an important part of resistome of human pathogens (Gillings, 2013).

Carbapenems (imipenem, meropenem); cephalosporins 1st generation (cefadroxil); penicillins-β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanic acid, ampicillin, ampicillin/sulbactam); amphenicols (chloramphenicol); tetracyclines (tetracycline) are among the antibiotics which are used in both human and veterinary medicine (WHO, 2017). Hence, chloramphenicol, cefadroxil, ampicillin, tetracycline, mupirocin, imipenem, meropenem, ampicillin/sulbactam and amoxycillin/clavulanic acid were selected as test antibiotics.

According to the literature review, there are no studies in which haloalkaliphilic bacteria were isolated from Salda Lake and identified, and their enzymatic functions and antibiotic susceptibilities were determined. In this respect, this study provided original results. Hence, this study focused on the haloalkaliphilic bacterial diversity of Salda Lake, their enzymatic fuctions in alkaline ecosystem and their antibiotic resistance profiles. Therefore, haloalkaliphilic bacterial species were isolated from water sample collected from Salda Lake; these bacterial isolates were identified at the species level using conventional and molecular methods; their industrially important enzymes were determined; and antibiotic resistance profiles of haloalkaliphilic wild-type bacteria were investigated in the present study.

MATERIALS AND METHODS

Isolation of haloalkaliphilic bacteria

Haloalkaliphilic medium containing 5 g glucose, 4 g $Na_2B_4O_7.10{\times}H_2O$, 3 g $NaNO_3$, 1 g NH_4Cl, 0.5 g KH_2PO_4, 1 g yeast extract, 0.5 g casamino acids, 100 g NaCl, 1000 ml distilled water was used for isolation from water sample of Salda Lake (Burdur/Turkey). The final pH was adjusted to 9. After overnight incubation at 35°C, different bacterial colonies were selected and restreaked several times to obtain pure cultures, then subjected to phenotypic and genotypic analysis (Gonzales et al., 1978; Rodríguez-Montalvo et al., 1998; Harley and Prescott, 2002).

Amplification and sequencing of 16S rRNA genes

Chromosomal DNA were extracted and PCR products were purified as described by the manufacturer's instructions (QIAamp DNA Mini Kit, QIAquick PCR Purification Kit, Qiagen). The 16S rRNA genes were amplified using specific universal 1492R (5'TACGGYTACCTTGTTACGACTT3') and 27F (5'AGAGTTTGATCMTGGCTCAG 3') primers (Sambrook and Russell, 2001). The 16S ribosomal RNA gene sequences were determined by IONTEK Laboratory (Istanbul/Turkey). 16S rRNA gene similarities were further determined between isolates and closely related species by using ChromasPro and EzTaxon-e tool (Kim et al., 2012).

Characterization of the haloalkaliphilic isolates

Exponentially growing broth cultures of six isolates were examined for cell morphology on prepared wet mounts using light microscopy. Colony pigmentation was observed by growing colonies on haloalkaliphilic agar medium. Gram staining, catalase and oxidase activities were carried out according to the standard procedures (Arahal et al., 1996; Johnson and Case, 2010; Sánchez-Porro et al., 2011). Salt requirement and salt tolerance of the isolates were investigated on plates containing haloalkaliphilic agar medium in which the NaCl concentration was varied (0%, 1%, 1.5%, 2%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20% NaCl). The pH tolerance of the isolates was tested on haloalkaliphilic agar

medium adjusted to pH values of 5-13. To determine optimum growth temperature of the isolates, the plates inoculated with each isolate were incubated at different temperatures (4°C-60°C).

Enzymatic activities of the haloalkaliphilic isolates

Amylase activity was detected using haloalkaliphilic agar medium supplemented with 0.5% (w/v) soluble starch. After incubation, the plate was flooded with 0.3% I_2-0.6% KI solution. Clear halos around the colonies indicated starch hydrolysis. The DNase test agar was used to determine DNase activity. After incubation, the plate was flooded with 1N HCl. Clear zones around the colonies showed hydrolysis of DNA (Sánchez-Porro et al., 2003). The cellulose medium agar plate containing 0.2% (w/v) carboxymethyl cellulose was used to detect production of cellulase. After incubation, 0.1% congo red test reagent was flooded on the colonies and left for 30 min. Then, the colonies were washed with 1 M NaCl solution. Clear zones around the colonies showed cellulase activity (Birbir et al., 2007). Hydrolysis of casein was tested with the Plate Count Agar medium containing 2% skim milk. After incubation, clear zones around the colonies were interpreted as caseinase production (Sánchez-Porro et al., 2011). Lipase activity was screened on Tween 80 agar medium containing 1% (w/v) Tween 80. After incubation, opaque zones around the colonies were accepted as evidence of lipase activity (Caglayan et al., 2018). Protease activity was screened on gelatin agar medium containing 2% gelatin (w/v). After incubation, the plate was flooded with Frazier solution. Clear zones around the colonies were interpreted as positive protease activities (Sánchez-Porro et al., 2003). Urease activity was detected on Christensen Urea Agar. After growth was obtained, the tube was examined for pink or red color changes (Johnson and Case, 2010). To detect pullulolytic and xylanolytic activities of the isolates, plates containing the chromogenic substrates such as azurine-cross-linked (AZCL)-pullulan and AZCL-xylan were respectively used. Clear zones around the colonies were accepted as positive pullulolytic and xylanolytic activities (Sánchez-Porro et al., 2003; Caglayan et al., 2017). The pH of all media was adjusted to 9.

Nucleotide accesion number

16S rRNA sequence data of the haloalkaliphilic water isolates S10, S4A, S6, S2, S1, S4, reported in this article have been deposited in NCBI and GenBank nucleotide sequence database under the respective accession numbers: MH752438, MH748635, MH748638, MH748643, MH748647, MH748673.

Antimicrobial susceptibility tests

The haloalkaliphilic isolates were grown in Mueller Hinton Broth containing 1% (w/v) NaCl. After the overnight incubation at 35°C, the optical densities of the bacterial suspensions were adjusted to McFarland Standard No 0.5 (1×10^8 CFU/mL) with sterile saline solution (1% NaCl). The Kirby-Bauer disc diffusion method was used to determine the resistance of isolates to antibiotics such as chloramphenicol (30 µg), cefadroxil (30 µg), ampicillin (10 µg), tetracycline (30 µg), mupirocin (20 µg), imipenem (10 µg), meropenem (10 µg), ampicillin/sulbactam (10/10 µg; 20 µg) and amoxycillin/clavulanic acid (20/10 µg; 30 µg). The surface of the Mueller Hinton Agar plates were inoculated with bacterial suspension of each test isolate and antibiotic discs (Oxoid, UK) were placed on the surface. After 24 hours incubation of plates at 35°C, the inhibition zone diameters were mesured and evaluated according to zone diameter distributions of wildtype microorganisms and ECOFF information explained by European Committee on Antimicrobial Susceptibility Testing as resistant and susceptible (EUCAST, 2014).

RESULTS

Phylogenetic analysis of haloalkaliphilic bacteria

The 16S ribosomal RNA gene sequences and the 16S rRNA the pairwise sequence similarities of the haloalkaliphilic isolates were respectively found as 1155-1299 bp and 98.1-100%. Two different genera, *Staphylococcus* (1 isolate) and *Bacillus* (5 isolates), were determined in *Firmicutes* (Table 2). The colonies of haloalkaliphilic bacterial isolates on haloalkaliphilic medium agar plates were yellow, cream and white. All of the isolates were Gram-positive. Cell morphologies of the isolates were coccus and rod-shaped. All isolates grew at 0-7.5% salt concentrations and pH range 7-12. Four and two isolates respectively showed optimum growth at 1.5% and 1% salt concentrations. All isolates showed optimum growth at pH 9. While five isolates grew at 10-55°C, only one isolate grew at 10-60°C. Four isolates, one isolate and one isolate were respectively exhibited optimum growth at 35°C, 37°C and 35-37°C (Table 2).

Catalase (100%), oxidase (67%), protease (50%), cellulase (50%), lipase (33%), caseinase (33%), urease (17%), amylase (17%), and pullulanase (17%) were produced by the isolates (Table 2). Xylanase and deoxyribonuclease were not produced. The isolates showed combined enzymatic activities. *Bacillus haynesii* (catalase, protease) and *Bacillus horneckiae* (catalase, cellulase) produced two enzymes. *Bacillus subtilis* produced oxidase, catalase and cellulase. *Bacillus paramycoides*, *Bacillus pumilus*, and *Staphylococcus epidermidis* respectively produced seven (oxidase, catalase, amylase, cellulase, urease, protease, caseinase), five (oxidase, catalase, lipase, protease, caseinase) and four (oxidase, catalase, pullulanase, lipase) enzymes. Among the isolates *Bacillus paramycoides* produced all enzymes except pullulanase, xylanase, lipase and deoxyribonuclease. It is known that the haloalkaliphilic bacteria and their enzymes are active and stable under harsh conditions. Therefore, the test isolates may have potential for novel enzymes due to their ability to be stable under saline conditions and high pH. The enzymes of haloalkaliphiles such as oxidase, catalase amylase, cellulase, pullulanase, lipase, urease, protease,

caseinase may be used in several industrial applications. In order to characterize the properties of these enzymes and determine their biochemical reactions, more detailed studies are needed to be performed.

Table 2. Characteristics of haloalkaliphilic bacteria isolated from water sample in Salda Lake

Characteristics	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Bacillus pumilus*	*Bacillus paramycoides*	*Bacillus haynesii*	*Bacillus horneckiae*
Isolate code	S10	S4A	S6	S2	S1	S4
Gram staining	+	+	+	+	+	+
Colony pigmentation	yellow	cream	cream	white	cream	yellow
Cell morphology	coccus	rod-shaped	rod shaped	rod-shaped	rod-shaped	rod-shaped
NaCl range (%)	0-10	0-10	0-7.5	0-10	0-10	0-12.5
Optimum NaCl (%)	1	1.5	1.5	1	1.5	1.5
Temperature range (°C)	10-55	10-55	10-55	10-55	10-60	10-55
Optimum temperature (°C)	37	35	35	35-37	35	35
pH range	7-12	7-12	7-12	7-12	7-12	7-12
Optimum pH	9	9	9	9	9	9
Oxidase	+	+	+	+	-	-
Catalase	+	+	+	+	+	+
Amylase	-	-	-	+	-	-
Cellulase	-	+	-	+	-	+
Pullulanase	+	-	-	-	-	-
Xylanase	-	-	-	-	-	-
Lipase	+	-	+	-	-	-
Urease	-	-	-	+	-	-
Protease	-	-	+	+	+	-
Caseinase	-	-	+	+	-	-
Deoxyribonuclease	-	-	-	-	-	-

Antibiotic resistance profiles of haloalkaliphilic bacteria

The zone diameter breakpoints were determined for chloramphenicol (30 µg ≥18mm susceptible), cefadroxil (30 µg ≥12mm susceptible), ampicillin (10 µg ≥12mm susceptible), tetracycline (30 µg ≥22mm susceptible), mupirocin (20 µg ≥30mm susceptible), imipenem (10 µg ≥24mm susceptible), meropenem (10 µg ≥25mm susceptible), ampicillin/sulbactam (20 µg ≥17mm susceptible) and amoxycillin/clavulanic acid (30 µg ≥19mm susceptible) according to zone diameter distributions of wildtype microorganisms and ECOFF information (EUCAST, 2014).

The diameter of inhibition zones around chloramphenicol with the highest inhibition zone observed against *Bacillus horneckiae* (23 mm), followed by *Staphylococcus epidermidis* (22 mm), *Bacillus subtilis* (21 mm); around cefadroxil with the highest inhibition zone observed against *Bacillus paramycoides* and *Bacillus haynesii* (20 mm), followed by *Bacillus horneckiae* (19 mm), *Bacillus pumilus* (18 mm), *Bacillus subtilis* (17 mm); around ampicillin with the highest inhibition zone observed against *Bacillus haynesii* (22 mm), followed by *Bacillus subtilis* (21 mm); around tetracycline with the highest inhibition zone observed against *Staphylococcus epidermidis* and *Bacillus horneckiae* (25 mm), followed by *Bacillus subtilis* (23 mm); around mupirocin with the highest inhibition zone observed against *Bacillus haynesii* (34 mm), followed by *Bacillus subtilis* (32 mm); around imipenem with the highest inhibition zone observed against *Bacillus paramycoides* (26 mm), followed by *Bacillus subtilis* and *Bacillus horneckiae* (25 mm); around meropenem with the highest inhibition zone observed against *Bacillus pumilus* (30 mm), followed by *Bacillus subtilis* (28 mm) and *Bacillus haynesii* (27 mm). The diameter of inhibition zones around ampicillin/sulbactam and amoxycillin/clavulanic acid against *Bacillus subtilis* were respectively measured as 18 mm and 22 mm (Table 3).

Moreover, *Staphylococcus epidermidis*, *Bacillus pumilus*, *Bacillus paramycoides*, *Bacillus haynesii*, *Bacillus horneckiae* showed no zone of inhibition around cefadroxil, ampicillin, mupirocin, imipenem, meropenem, ampicillin/sulbactam, amoxycillin/clavulanic acid; chloramphenicol, ampicillin, tetracycline, mupirocin, imipenem, ampicillin/sulbactam, amoxycillin/clavulanic acid; chloramphenicol, ampicillin, tetracycline, mupirocin, meropenem, ampicillin/sulbactam, amoxycillin/clavulanic acid; chloramphenicol, tetracycline, imipenem, ampicillin/sulbactam, amoxycillin/clavulanic acid; ampicillin, mupirocin, meropenem, ampicillin/sulbactam, amoxycillin/clavulanic acid (Table 3).

Table 3. Antibiotic resistance profiles of haloalkaliphilic bacteria isolated from water sample in Salda Lake

Antibiotics	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Bacillus pumilus*	*Bacillus paramycoides*	*Bacillus haynesii*	*Bacillus horneckiae*	R%	S%
Chloramphenicol	S (22 mm)	S (21 mm)	R (0)	R (0)	R (0)	S (23 mm)	50	50
Cefadroxil	R (0)	S (17 mm)	S (18 mm)	S (20 mm)	S (20 mm)	S (19 mm)	17	83
Ampicillin	R (0)	S (21 mm)	R (0)	R (0)	S (22 mm)	R (0)	67	33
Tetracycline	S (25 mm)	S (23 mm)	R (0)	R (0)	R (0)	S (25 mm)	50	50
Mupirocin	R (0)	S (32 mm)	R (0)	R (0)	S (34 mm)	R (0)	67	33
Imipenem	R (0)	S (25 mm)	R (0)	S (26 mm)	R (0)	S (25 mm)	50	50
Meropenem	R (0)	S (28 mm)	S (30 mm)	R (0)	S (27 mm)	R (0)	50	50
Ampicillin/sulbactam	R (0)	S (18 mm)	R (0)	R (0)	R (0)	R (0)	83	17
Amoxycillin/clavulanic acid	R (0)	S (22 mm)	R (0)	R (0)	R (0)	R (0)	83	17
Multidrug resistant bacteria	7	0	7	7	5	5		

R%, Percentage of isolates resistant to antibiotics; S%, Percentage of isolates susceptible to antibiotics

All isolates showed resistance to at least one antibiotic tested except *Bacillus subtilis*. This isolate was susceptible to all antibiotics. While more than half of the isolates were resistant to ampicillin/sulbactam (83%), amoxycillin/clavulanic acid (83%), ampicillin (67%), mupirocin (67%), less than half of the isolates were resistant to cefadroxil 17%. Fifty percent of the isolates exhibited resitance to chloramphenicol, tetracycline, imipenem, and meropenem (Table 3). Multidrug resistant bacteria, which are able to survive and grow in the presence of two or more antibiotics, were detected as *Bacillus horneckiae*, *Bacillus paramycoides*, *Bacillus pumilus*, *Staphylococcus epidermidis* and *Bacillus haynesii* (Table 3).

DISCUSSION

Bacillus species are widely distributed in water, seawater, soil, marine sediments, and salt. In previous studies, the species isolated and tested in the present study, were isolated from different locations. *Bacillus horneckiae* was isolated from a spacecraft-assembly clean room in the Kennedy Space Center (Vaishampayan et al., 2010). In that study, *Bacillus horneckiae* showed weak protease and lipase activities (Vaishampayan et al., 2010). However, this isolate was protease and lipase negative in the present study. *Bacillus subtilis* was isolated from human gastrointestinal tract (ileum biopsies, faecal samples) (Hong et al., 2009) and dairy effluent (Vijayalakshmi and Murali, 2015). Similar to the results obtained from the study performed by Hong et al. (2009), we observed that *Bacillus subtilis* was amylase negative, and was able to grow at 10-50°C. *Bacillus paramycoides* was isolated from sediment of the South China Sea (Liu et al., 2017) and soil samples collected from rhizospheres of crop plants (Osman and Yin, 2018). *Bacillus paramycoides* was found as amylase and protease positive in the study of Liu et al. (2017) and in the present study. It was reported that this species showed growth at 15-39°C (optimum 30°C), 0-5% NaCl concentration (optimum 0.5% NaCl), 5-9 pH range (optimum pH 7) (Liu et al., 2017). This species was reported as plant growth-promoting rhizobacteria (Osman and Yin, 2018). *Bacillus pumilus* was isolated from coastal environment of Cochin in India (Parvathi et al., 2009) and marine ecosystems (Liu et al., 2013). Similar to the results of this study, *Bacillus pumilus* showed negative deoxyribonuclease activity, and positive lipase and protease activities (Parvathi et al., 2009). Daptomycin-resistant *Staphylococcus epidermidis* was isolated from bovine udders (Brown et al., 1969) and patients (Eladli et al., 2018). *Bacillus haynesii* was isolated from desert soil (Dunlap et al., 2017). According to the results of that study, *Bacillus haynesii* was able to grow at pH 5.5-10 (optimum pH 7), temperatures of 15-60°C (optimum 37°C), and at 0-12% NaCl concentrations (Dunlap et al., 2017).

This is the first study on the isolation and characterization of haloalkaliphilic bacteria from water sample of Salda Lake, screening their industrial enzymes, and testing antibiotic resistance profiles. The haloalkaliphilic *Bacillus horneckiae* *Bacillus subtilis*, *Bacillus paramycoides*, *Bacillus pumilus*, *Staphylococcus epidermidis*, *Bacillus haynesii* species, isolated from Salda Lake, were first reported in the present study. These isolates were found resistant to different antibiotics in the present study. The agricultural, livestock, tourism and fishing activities are considered, these antibiotic resistant bacteria may have harmful impacts on water

sources. Furthermore, amylase, cellulase, pullulanase, lipase, urease, protease, caseinase, oxidase, catalase produced by these isolates would be important candidates for various industrial applications due to enzymatic activity and stability in a broader range of NaCl and pH.

CONCLUSION

Haloalkaliphilic bacteria display different morphological, physiological, molecular and cultural characteristics, Gram staining reaction, biochemical features, antibiotic susceptibility profiles, enzyme and metabolite production. Alkaline environments harbor diverse haloalkaliphilic bacteria producing stable enzymes. Due to the stability and activity of alkaline enzymes in harsh conditions, they have potential to be used in biotechnological applications. Future performed investigations with their metabolites are very important considering their ability to survive under high pH. The presence of multidrug resistant bacteria in Salda Lake was detected. Antibiotic resistant *Bacillus* and *Staphylococcus* species present in the Salda Lake may be transmitted by human, animal, air, soil, or wastewater. These genes are present in natural environments worldwide.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Comparison of the visual texture calculation methods by image analysis, applied to mirror and scaled carp skin

Aynalı ve pullu sazan derisine uygulanan görüntü analizi ile görsel tekstür hesaplama yöntemlerinin karşılaştırılması

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Abstract: Regions of interest (ROI) representative of the visual texture of images of mirror carp *Cyprinus carpio carpio* and scaled carp *Cyprinus carpio* were taken. Red, green, blue and grayscale (R, G, B, GS) histograms of these ROI were calculated. The following methods of visual texture calculations were performed on the ROIs: 1) image energy based on histograms, 2) image entropy based on histograms, 3) image energy based on co-occurrence matrices, 4) image entropy based on co-occurrence matrices, 5) texture based on fractal dimensions, 6) texture based on texture primitives method. Calculations were performed for color and grayscale images. The identification of the smoothest and roughest ROIs depended on the method used. The largest range between the minimum and maximum values was found in the co-occurrence matrix-based entropy calculation. A close second was the texture change index (TCI) method.

***Keywords*:** *Cyprinus carpio,* visual texture, histograms, co-occurrence matrices, fractals

Öz: Aynalı sazan (*Cyprinus carpio carpio)* ve pullu sazan (*Cyprinus carpio)* görüntülerinin görsel tekstürü temsil eden ilgili bölgeleri (ROI) değerlendirilmiştir. Bu ROI'lerin kırmızı, yeşil, mavi ve gri (R, G, B, GS) histogramları hesaplanmıştır. ROI'lere aşağıdaki görsel doku hesaplama yöntemleri uygulanmıştır: 1) Histogramlara dayalı görüntü enerjisi, 2) Histogramlara dayalı görüntü entropisi, 3) Eşdizimlilik matrislerine dayalı görüntü enerjisi, 4) Eşdizimlilik matrislerine dayalı görüntü entropisi, 5) Fraktal boyutlara dayalı tekstür, 6) Tekstür pirimitif yöntemine dayalı tekstür'dür. Hesaplamalar renkli ve gri tonlamalı resimler için yapılmıştır. En düz ve en kaba ROI'lerin tanımlanmasının kullanılan yönteme bağlı olduğu bulunmuştur. Minimum ve maksimum değerler arasındaki en büyük aralık, entropi hesaplamasına bağlı eşdizimlilik matrisinde tespit edilirken ikinci büyük aralık tekstür değişim indeksi (TCI) yöntemiyle bulunmuştur.

***Anahtar kelimeler*:** *Cyprinus carpio*, görsel doku, histogramlar, eşdizimlilik matrisleri, fraktallar

INTRODUCTION

In most agricultural materials, colors are distributed non-uniformly over the surface of the material (Balaban, 2008). Regarding the surface of most fruits (apple, mango, watermelon, ripe banana, etc.), fish, meat, grains, the colors are not uniformly distributed. In the case of e.g., meat, this stems from the difference in color between the muscle fiber and fat and their distribution. In the case of fish, it is the differences in the scales and the colorings that are genetic in nature. In the case of most fruits, it is the distribution of the individual components (carotenoids, anthocyanins, etc). Therefore, the amount / level of colors as well as their distribution is important.

Color, size, shape and visual texture are among the properties that can be obtained by image analysis of foods (Sharma et al., 2001; Zheng et al., 2006). Visual texture is different from the touch / squeeze-based texture; it refers to visual non-uniformity, and uneven patterns of the surface.

An image is composed of pixels containing color or intensity information. A pixel in a color image, generally has red (R), green (G), and blue (B) components. The R, G, and B values change from 0 to 255. Bits per pixels of a grayscale image are different than those of a color image. For a grayscale image, the pixels have values changing from 0 to 255; 0 being black and 255 being white.

Tamura et al. (1978) started the research on the lexicon of visual texture. The images that they describe are grayscale, and not related with food. There are a very limited number of studies on the visual texture of foods. Watermarking in salmon is a good example of visual texture (Oliveira et al, 2006). Balaban et al. (2014) quantified the change in the redness value in the skin of gurnard, as well as its distribution. However, they did not quantify the visual texture. Bharati et al. (2004) used visual texture methods to classify rolled steel sheets into various quality grades. Gray level (Partio et al., 2002) or color analysis (Hendrawan et al., 2019) can be used to develop texture properties of surfaces. Parameters can be developed based on histograms or based on co-occurrence matrices. Histograms are very easy to develop for an image, since they represent the counts of individual R, or G, or B, or grayscale values of every pixel in

the image. Co-occurrence matrices are slightly more complicated, since they require a direction and a step size for the definition of matrix elements. However, they are also easy to develop.

The objective of this study was to apply image analysis based visual texture calculation methods to regions of interest of carp pictures. The visual texture parameters were measured by 6 different methods, and the sensitivity of the methods were compared.

MATERIALS AND METHODS

Fish samples and image acquisition

Images of common carp (mirror and scaled) raised in concrete tanks of Republic of Turkey Ministry of Agriculture and Forestry, Mediterranean Fisheries Research Production and Training Institute, Kepez, Antalya, Turkey were used.

Pictures were taken using a modified light box (120-cm high, 60-cm wide, and 60-cm deep) designed by Luzuriaga et al. (1997) under a Nikon D610 DSLR camera (Nikon Corp., Tokyo, Japan) with a 24-300 mm zoom Nikon lens with a circular polarizing filter that camera settings are given in Table 1. The details of light box and color reference were given in the study carried out by Gümüş et al. (2021).

Table 1. The Nikon D610 camera control settings for front-lighting and back-lighting images.

Camera settings	Front-lighting	Back-lighting
Exposure mode	manual	manual
Shutter speed	1/2.5 sec	1/4 sec
Aperture	f/9	f/9
Exposure compensation	0 EV	0 EV
ISO sensitivity	200	200
White balance	Preset 1	Preset 1
Image small size (pixels)	3008*2008	3008*2008

Pictures were taken with two-image method described by Alçiçek and Balaban (2012). Then, the images using the silhouette (back-lighted) picture were segmented with using the LensEye-NET software (Engineering and Cyber Solutions, Gainesville, FL, USA).

Image analysis

Corel PhotoPaint (Corel Corp., Ottawa, Ontario, Canada) was used to clear the images taken by camera to isolate the color reference. All visual texture analyses (energies and entropies based on histograms and co-occurrence matrices, fractal dimensions), and texture primitive analyses were performed by using the software LensEye-NET. Two mirror carp images, and two scaled carp images were chosen at random.

In Figure 1, Rectangular Regions of Interest (ROIs) are shown. The size and location of the ROIs were selected to show representative regions of the fish. ROI 1 was selected to show a lighter area of the mirror carp 1, closer to the head without the interference of the pectoral fin or gills. ROI 2 was selected to show the mid-section area close to the tail, again without interference from the anal fin. ROI 3 shows a similar area in mirror carp 2, similar to mirror carp 1. Since mirror carp 2 has more width, ROI 3 area is larger than ROI 1 area. ROI 4 was selected in mirror carp 2, in an area similar to ROI 2 in mirror carp 1. Again, this area is larger than that of ROI 2. Once the 4 ROIs were selected in mirror carp, they were replicated as areas in scaled carp. The only difference is that they were placed close to the midline of the fish to represent the scales without other interference.

For the mirror carp, the surface looks smooth, with little visual texture roughness. For the scaled carp, the scales constitute a fairly ordered visual texture. The ROIs close to the head have more distance between the scales compared to the ROI close to the tail.

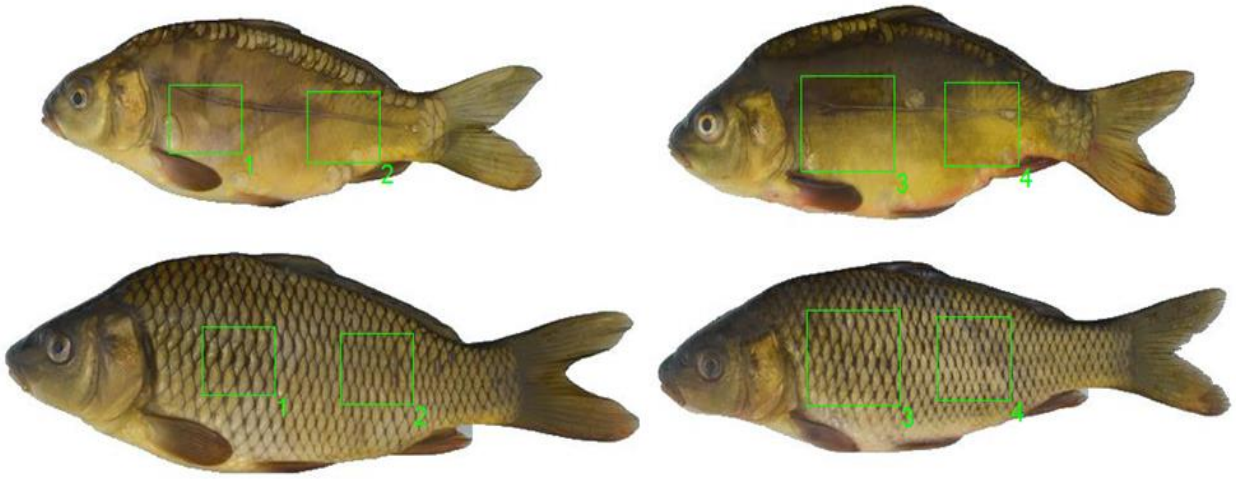

Figure 1. Regions of interest for mirror carp (above), and scaled carp (below)

Calculation of the energy, entropy, fractals, and texture change index (tci)

Histogram-based energies and entropies: A histogram is the distribution of colors and counts the number of occurrences of R, G and B values for each pixel of the object. A grayscale (GS) (Figure 2) histogram can also be constructed, and its counts gives the number of pixels of the image. The conversion of a pixel with (R, G, B) colors to grayscale is (Padmavathi and Thangadurai, 2016):

Grayscale Value = 0.3 * R + 0.59 * G + 0.11 * B (*Eqn. 1*)

Figure 2. Grayscale images and regions of interest in mirror carp (left), and scaled carp (right) images

Once a histogram is obtained (R, G, B or GS), the energy of an image can be calculated as:

$$E = \sum_{k=0}^{M-1} (p_k)^2 \qquad (Eqn. 2)$$

where M = 256, and E is the energy of the image, based on R, G, B, or GS histograms.

The entropy of an image is given by the equation (Larkin, 2016):

$$H = -\sum_{k=0}^{M-1} p_k \, log_2(p_k) \qquad (Eqn. 3)$$

Where M = 256, and H is the entropy of the image, based on the R, G, B, and GS histograms.

Co-occurrence matrix-based energies and entropies: The co-occurrence matrix is a symmetric square matrix, with 256 rows (from 0 to 255), and 256 columns (0 to 255). This matrix can be considered as an accumulator, counting the number of times neighboring pixels have certain defined characteristics. In the generation of the matrix requires a direction as 0, 45, 90 and 135 degrees and a step size d measured in pixels (Zucker and Terzopoulos, 1980). The co-occurrence matrix is used as a "statistical" method in the traditional analysis of visual texture (Pathak and Barooah, 2013). The energy equation according to Tuceryan (1998) becomes:

$$E = \sum_i \sum_j P^2{}_d \, (i,j) \qquad (Eqn. 4)$$

The entropy equation becomes:

$$H = -\sum_i \sum_j P_d \, (i,j) \log P_d(\mathrm{i,j}) \qquad (Eqn. 5)$$

Fractal Analysis: Calculation the fractal dimension of an image is used the box counting method. At a given point p (X, Y), the sum of all R, G, B, and grayscale intensities (0-255) are added, at increasingly larger neighborhoods (Varma and Garg, 2007). Then the log of these sums vs log(d) is plotted. This plot is expected to be a straight line, with a slope and an intercept given in Eqn.5.

The sum of the intensities at a given point p, for a given d is used to calculate the local fractal dimension D(p):

$$log\left[\sum I(p,d)\right] = D(p)\log(d) + L(p) \qquad (Eqn. 6)$$

where the slope D(p) is the local fractal dimension (also called the Holder exponent), L(p) is the local intercept local log fractal length, p is a given point, and d is the neighborhood size.

Texture Primitives and Texture Change Index: Another method to quantify the distribution of colors is the "primitives" method (Zheng et al., 2007; Balaban, 2008). The intensity difference in case of a texture primitive is:

$$\Delta I_t = \sqrt{(R_i - R_j)^2 + (G_i - G_j)^2 + (B_i - B_j)^2} \quad (Eqn. 7)$$

For a given threshold, the image may display many texture primitives. Intuition tells us that the more primitives, the more textured the image. Texture Change Index (TCI) described by Balaban (2008) was used to calculate texture primitives:

$$\text{TCI} = \frac{(\text{Sum of primitive intensity differences})(\text{Number of primitives})}{(\text{Sum of distances between primitives})(\text{Object area})} \quad (Eqn. 8)$$

The intensity threshold was arbitrarily set to 5. The TCI values of color images and grayscale images (Figure 2) were calculated.

RESULTS AND DISCUSSION

Figures 3, 4, 5 and 6 represent the R, G, B, and GS histograms of the ROIs for mirror carp and scaled carp images. It is evident that except for the 3rd ROI of mirror carp which is darker, the R histograms are relatively comparable. The same can be said for the G histogram (Figure 4): the 3rd ROI is darker, therefore it has relatively lower G values. For the B histogram, the 2nd, 3rd and 4th ROIs of mirror carp stand separate from the rest. In the grayscale histogram (Figure 6) the 3rd ROI for mirror carp again stands out, since this ROI is darker, it has relatively lower grayscale values.

Table 2 presents the results of histogram-based energy calculations for R, G, B, and GS histograms. Because the histograms are different, there are differences in R, G, B, and GS energies. The individual energy values are very small, in the 10^{-2} range. This may be disadvantageous if fine separation of visual texture using histogram-based energy is required.

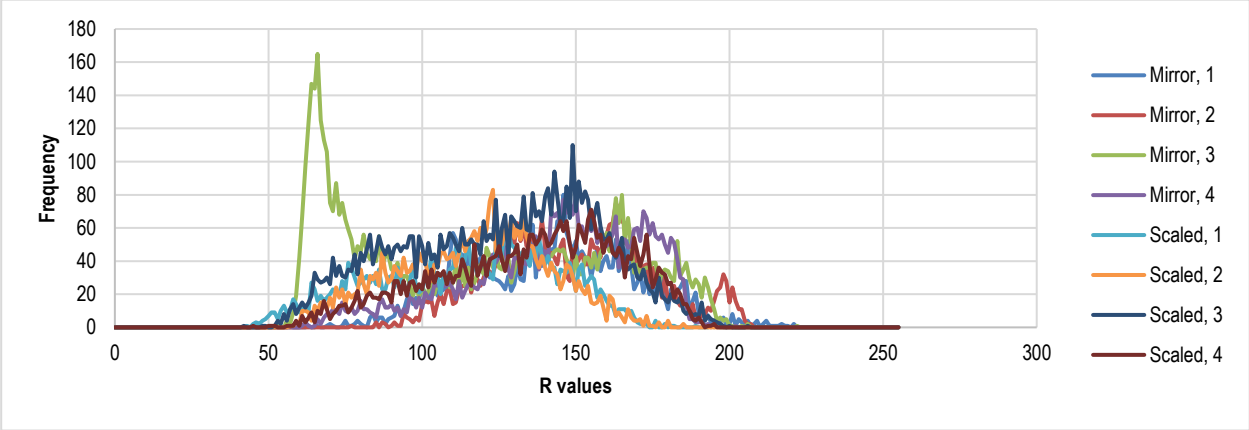

Figure 3. R histograms of mirror and scaled carp image objects

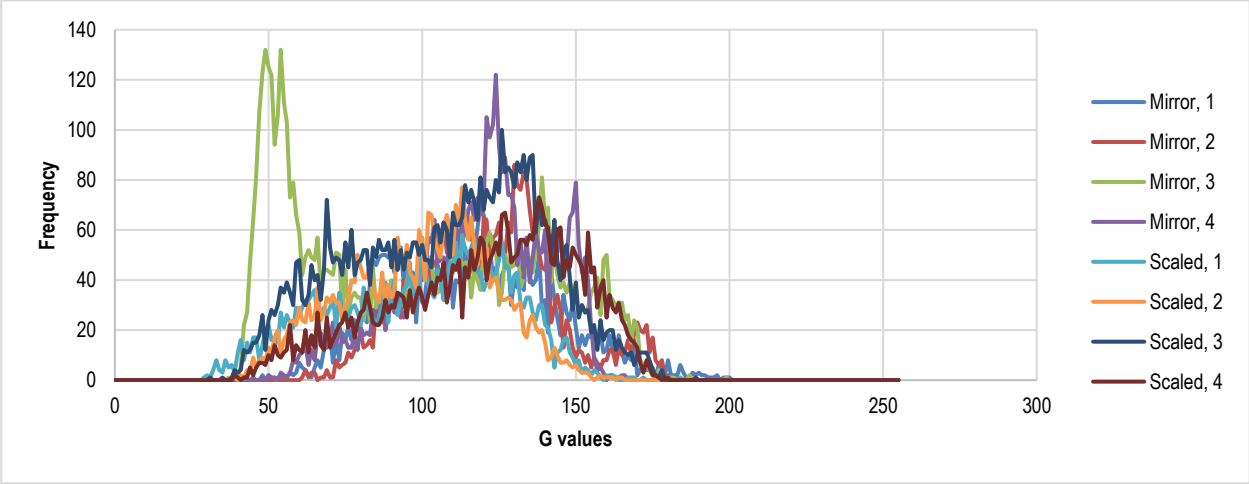

Figure 4. G histograms of mirror and scaled carp image objects

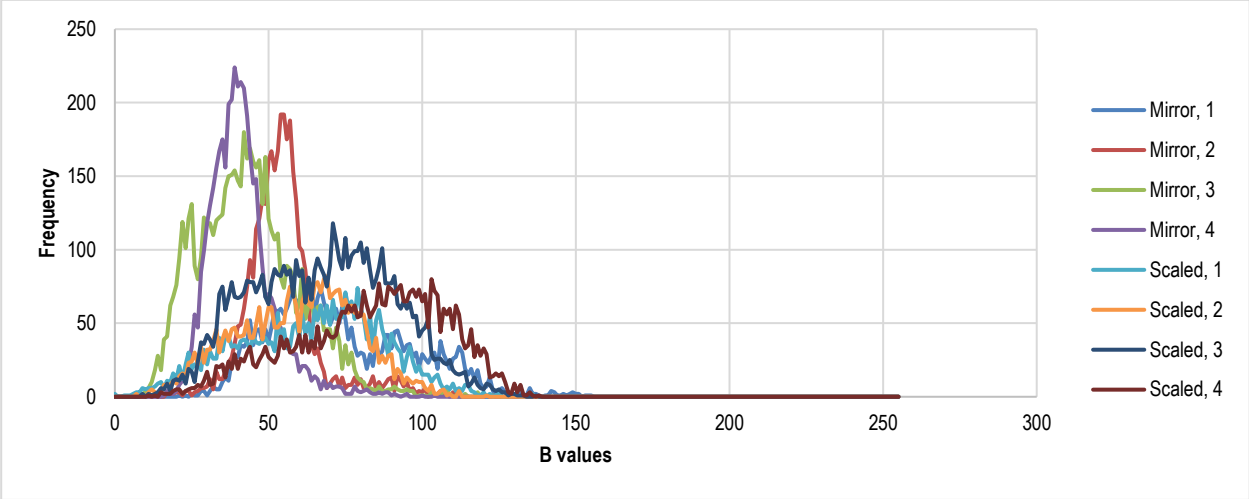

Figure 5. B histograms of mirror and scaled carp image objects

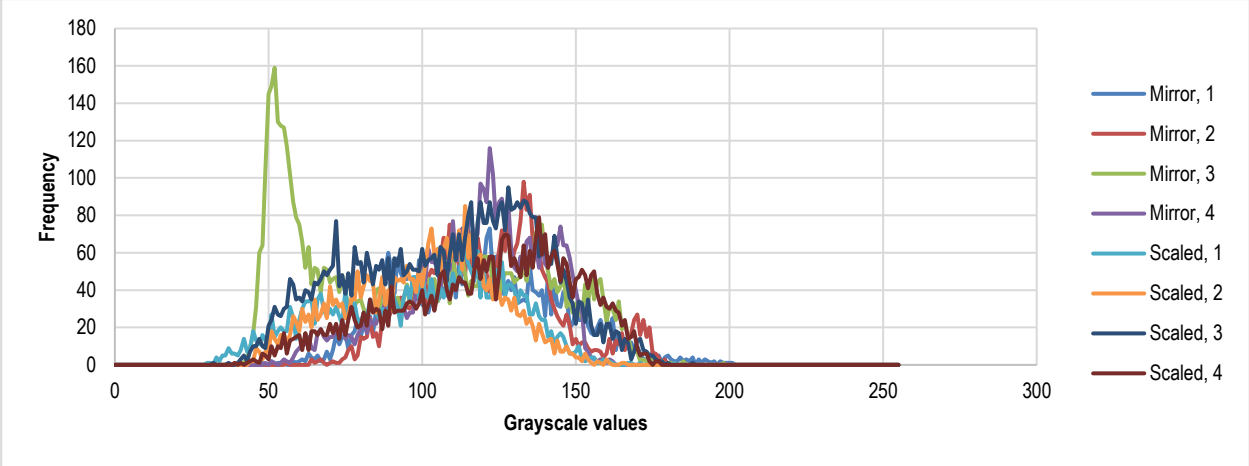

Figure 6. Grayscale histograms of mirror and scaled carp image objects

Table 2. Histogram based energies of carp image objects

Object	Mirror carp				Scaled Carp			
	1	2	3	4	1	2	3	4
R energy	0.011	0.010	0.010	0.011	0.010	0.012	0.009	0.010
G energy	0.011	0.010	0.010	0.013	0.010	0.012	0.009	0.010
B energy	0.013	0.012	0.018	0.033	0.012	0.014	0.012	0.012
Avg. RGB	0.012	0.011	0.012	0.019	0.011	0.013	0.010	0.010
Std RGB	0.001	0.001	0.005	0.012	0.001	0.002	0.002	0.001
GS energy	0.011	0.010	0.010	0.014	0.010	0.012	0.010	0.010

It can be observed that the 3rd ROI for mirror carp stands different than the rest. There is also not much difference between the color-based values and the grayscale-based values of the energies: the average energy values of R, G, and B are within one standard deviation of the GS energy value. The minimum energy value in the table is 0.009 for scaled carp, ROI 3, R-based histogram.

The maximum value is 0.033 for mirror carp, ROI 4, B histogram. Most publications first convert a color image to grayscale (and cause loss of information), but deal with one value (grayscale) instead of 3 (R, G, B) (Basset et al.2000; Perez-Nieto et al., 2010). Based on this, the smallest grayscale value for energy is 0.009, for scaled carp, ROI 3. This means that the 3rd ROI has the most textured surface.

The largest energy value for grayscale in Table 2 is 0.013 for mirror carp, ROI 4. This ROI is the "smoothest" based on this calculation.

Table 3 shows the histogram-based entropies of R, G, B and GS histograms. Again, the values are different for the R, G, B and GS. The entropy values are in the order of 10, they are bigger than the energy values, and can possibly perform better in the fine separation of visual texture. The largest GS entropy value is 6.854 for scaled carp, ROI 3. This is the same ROI that has the lowest energy. Also, the smallest GS entropy value is 6.376 for mirror carp, ROI 4. This is the same ROI that has the largest energy. Therefore, it is confirmed that energy and entropy work in reverse of each other.

Table 3. Histogram based entropies of carp image objects

	Mirror carp				Scaled Carp			
Object	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
R entropy	6.721	6.587	6.951	6.692	6.810	6.601	6.948	6.839
G entropy	6.695	6.451	6.905	6.426	6.783	6.568	6.892	6.825
B entropy	6.453	5.367	6.002	5.254	6.543	6.280	6.514	6.571
GS entropy	6.678	6.382	6.830	6.376	6.753	6.532	6.854	6.789

Table 4 displays the results of co-occurrence matrix-based calculation of energy values (Equation 4) for 0 degrees and step size = 1. Notice that the energy values of scaled carp (more visual texture) are almost an order of magnitude smaller than those of the mirror carp (less visual texture). Therefore, in this method of calculation, the separation of visual texture is better. The maximum GS energy value in Table 4 is 2.782 for mirror carp, ROI 3, as in the case of histogram-based energy calculations (Table 2). Also, the minimum energy value for GS is 0.226 for scaled carp, ROI 1. This is different than the case of Table 2 (ROI 3). Looking at Figure 1, it can be argued that scaled carp ROI 1 has the most visual texture, but this assertion is not based on sensory evaluation.

Table 4. Co-occurrence matrix-based energies of carp image objects

	Mirror carp				Scaled Carp			
Object	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
R energy	0.704	0.881	2.611	1.001	0.218	0.257	0.499	0.275
G energy	0.726	1.008	2.538	1.246	0.227	0.261	0.516	0.277
B energy	0.759	2.270	3.877	2.949	0.269	0.323	0.664	0.328
GS energy	0.734	1.067	2.782	1.272	0.226	0.264	0.533	0.283

Table 5 presents the results of co-occurrence matrix-based calculation of entropy values (Equation 5) for 0 degrees, step size = 1. The magnitudes of the entropy values compared to Table 3 are much larger, in the range of 10^2, which may be advantageous if fine separation of the visual texture is required. The largest grayscale entropy is 221.897 for scaled carp, ROI 3, as before. The smallest entropy is 99.887 for mirror carp, ROI 2. This is different than Table 3 where the minimum entropy was for mirror carp, ROI 4. This emphasizes the possibility of different results of evaluating visual texture based on different methods of entropy calculation.

Table 5. Co-occurrence matrix-based entropies of carp image objects

	Mirror carp				Scaled Carp			
Object	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
R entropy	105.201	103.687	162.404	125.166	131.720	133.344	224.804	163.436
G entropy	104.508	101.095	161.833	119.467	130.994	133.220	223.407	163.178
B entropy	102.489	82.928	136.63	93.886	127.342	127.907	211.441	158.238
GS entropy	104.078	99.887	158.651	118.272	130.981	132.614	221.897	162.346

Table 6 shows the average slopes for R, G, B, and GS based fractal calculations. The average R^2 values are high (above 0.99). Again, concentrating on the GS values, for mirror carp the GS slope averages are all close to 1.6 for the four ROIs. The standard deviations are in the range of 0.1. This shows that all the GS slope averages are within 1 standard deviation of each other.

Therefore, there is no statistical difference between the grayscale slopes for any ROI. The same is true for the scaled carp GS slope averages: they are all close to 1.65. The standard deviations are all in the range 0.11. Again, the average GS slopes are within one standard deviation from each other. Furthermore, the GS average slopes of mirror carp and scaled carp are within one standard deviation of each other. Therefore, although there is significant visual texture difference between the mirror carp and the scaled carp, the fractals method used here cannot differentiate this difference in visual texture.

Table 6. Fractal dimensions of carp image objects

Object	Mirror carp				Scaled carp			
	1	2	3	4	1	2	3	4
R slope, avg	1.651	1.654	1.690	1.672	1.654	1.654	1.689	1.669
St dev	0.124	0.119	0.118	0.101	0.137	0.124	0.124	0.115
R^2 avg	0.998	0.998	0.998	0.998	0.997	0.997	0.997	0.997
G slope, avg	1.651	1.655	1.691	1.673	1.656	1.654	1.690	1.670
St dev	0.127	0.120	0.123	0.098	0.144	0.127	0.128	0.117
R^2 avg	0.998	0.998	0.998	0.998	0.997	0.997	0.997	0.997
B slope, avg	1.655	1.654	1.691	1.669	1.660	1.657	1.691	1.673
St dev	0.143	0.132	0.141	0.135	0.161	0.131	0.136	0.116
R^2 avg	0.997	0.997	0.997	0.997	0.996	0.997	0.997	0.997
GS slope, avg	1.651	1.654	1.691	1.672	1.655	1.654	1.690	1.670
St dev	0.126	0.119	0.121	0.099	0.142	0.126	0.127	0.116
R^2 avg	0.998	0.998	0.998	0.998	0.997	0.997	0.997	0.997

Table 7 presents both the color and grayscale-based TCI values of mirror carp and scaled carp ROIs calculated based on the texture primitives with an intensity threshold of 5. The smallest TCI values (the smoothest visual texture) are that of mirror carp, ROI 3. The largest TCI values are that of scaled carp, ROI 4. Also, there is enough range between the minimum (11) and maximum (101) values for fine separation between visual textures.

Another tool of the texture primitives method is the texture circles, shown in Figures 7 and 8. In the case of mirror carp (Figure 7), the visual texture is relatively smooth. This results in large circles, but few in number. However, in the case of scaled carp (Figure 8), with much visual texture, the circles are small and there are many of them. This gives another indication of the relative visual texture between samples.

Table 7. Texture Change Index values of carp image objects

Object	Mirror carp				Scaled carp			
	1	2	3	4	1	2	3	4
Color TCI	24.371	19.834	12.478	20.173	105.452	80.939	95.657	113.169
GS TCI	23.107	15.318	11.077	12.511	96.477	73.261	78.649	101.128

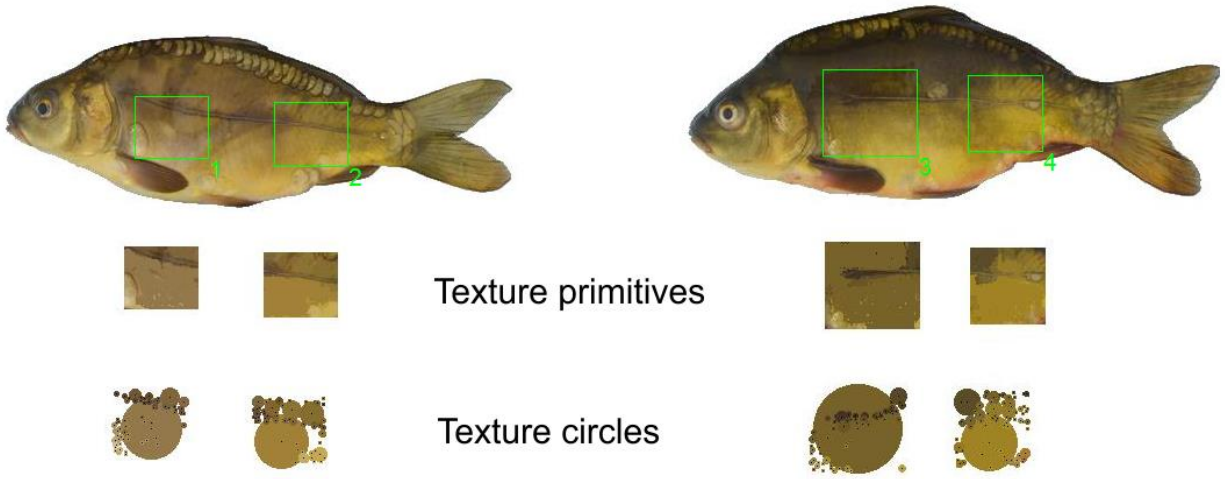

Figure 7. Texture primitives and texture circles for mirror carp

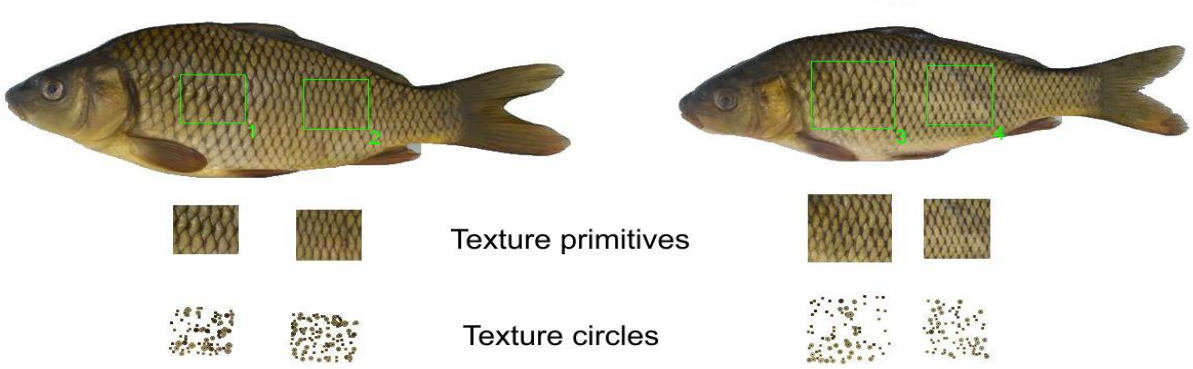

Figure 8. Texture primitives and texture circles for scaled carp

CONCLUSION

Table 8 summarizes the findings of this study. It is apparent that different image analysis-based methods to calculate visual texture may result in different conclusions. In Table 8 the minimum and maximum energy and entropy values differ depending on which method is used. Also, the identification of the smoothest and most textured images is different for different methods. An obvious solution to the selection of method is to correlate the results with that of a sensory study. However, sensory studies that correlate machine calculations to sensory panel results are difficult to find in the literature. This is mainly due to the difficulties in finding existing and established methods to quantify sensory panel evaluations of visual texture.

Another important criterion in the selection of method is the range between the minimum and maximum values. If the range is small, e.g., histogram-based energy, then the fine resolution of images with similar visual texture will be difficult.

Table 8. Summary of minimum and maximum values of parameters for different methods

Object	Min value	Max value	Min ROI	Max ROI	Range
Histogram energy	0.009	0.013	Scaled 3	Mirror 4	0.004
Histogram entropy	6.375	6.854	Mirror 4	Scaled 3	0.478
Co-occurrence energy	0.226	2.781	Scaled 1	Mirror 3	2.555
Co-occurrence entropy	99.887	221.897	Mirror 2	Scaled 3	122.011
Texture Change Index	11.077	101.128	Mirror 3	Scaled 4	90.051

This study suggests that the co-occurrence matrix-based analysis of entropy resulted in the largest range for our samples. The TCI method was a close second. However, the identification of the smoothest and roughest samples in these two methods is different. Therefore, it is clear that the correlation and merging of quantitative visual information, especially for foods, with sensory analysis must be done to bring meaning to the computer-based parameters.

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CASE REPORT **OLGU SUNUMU**

Observation of *Vibrio mediterranei* (Pujalte and Garay 1986) / *Vibrio shiloi* (Kushmaro et al. 2001) bacteria from skin ulcers of the cultured sea cucumber *Holothuria poli* (Delle Chiaje, 1823)

Kültürü yapılan *Holothuria poli* (Delle Chiaje, 1823) türü deniz hıyarının deri ülserlerinden *Vibrio mediterranei* (Pujalte ve Garay 1986) / *Vibrio shiloi* (Kushmaro vd. 2001) bakterisinin gözlenmesi

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Abstract: Skin ulcer syndrome is frequently reported as a serious disease affecting the health, growth and mortality of stocks in sea cucumber aquaculture. In this study, bacteria isolated predominantly from skin ulcers of sea cucumber *Holothuria poli* (Delle Chiaje, 1823), a new candidate for aquaculture in the Mediterranean, were investigated. Morphological and biochemical tests, and molecular analysis methods were used to examine the dominant bacteria in the lesions of *H. poli* showing skin ulceration, peristome tumour and visceral ejection symptoms in rearing tanks. Present study is the first report for isolation and identification of *Vibrio mediterranei* (Pujalte and Garay 1986) (called also *Vibrio shiloi* Kushmaro et al. 2001) as a predominant gram-negative bacterium in the skin ulcers of *H. poli*. Reference data provided from the present study would lead to understand possible major pathogens causing skin ulceration syndrome and is crucial for the prophylaxis and treatment of such disease in holothuriculture.

Keywords: Bacteria, *Holothuria poli,* holothuriculture, sea cucumber, skin ulceration syndrome, *Vibrio mediterranei, Vibrio shiloi*

Öz: Deri ülseri sendromu deniz hıyarı yetiştiriciliğinde stokların sağlığını, büyümesini ve ölüm oranını etkileyen ciddi bir hastalık olarak sıklıkla rapor edilmektedir. Bu çalışmada, Akdeniz'de yetiştiricilik için yeni bir aday olan *Holothuria poli* (Delle Chiaje, 1823) türü deniz hıyarının deri ülserlerinden baskın halde izole edilen bakteriler incelenmiştir. Yetiştirme tanklarında deri ülseri, peristomal tümör ve bağırsak çıkarma semptomları gösteren deniz hıyarlarının deri lezyonlarındaki dominant bakterileri incelemek amacıyla morfolojik ve biyokimyasal testler, ve moleküler analiz yöntemleri kullanılmıştır. Bu çalışma, *H. poli'nin* cilt ülserlerinde dominant gram-negatif bir bakteri olarak *Vibrio mediterranei* (Pujalte ve Garay 1986) 'nin (*Vibrio shiloi* Kushmaro vd. 2001 olarak da adlandırılır) izolasyonu ve tanımlanması konusunda ilk rapordur. Bu çalışmadan sağlanan referans veriler, deri ülseri sendromuna neden olan olası baskın patojenlerin anlaşılmasına yardımcı olurken deniz hıyarı kültüründe bu tür hastalıkların önlenmesi ve tedavisi için yol gösterici olması yönünden önemlidir.

Anahtar kelimeler: Bakteri, *Holothuria poli,* deniz hıyarı yetiştiriciliği, deniz hıyarı, deri ülseri sendromu, *Vibrio mediterranei, Vibrio shiloi*

INTRODUCTION

Sea cucumbers in all seas of the world, are in high demand and commercially exploited, particularly for food, cosmetics, and medical usage mainly by Far-East countries (Sicuro and Levine, 2011). Sea cucumber farming is reported as one of the fastest-growing aquaculture industries (Conand et al., 2014). Nearly fifteen species and more than 176 500 tons (FAO, 2021) of sea cucumbers are produced in holothuriculture facilities particularly in China, Russian Federation Indonesia, Sri Lanka, Malaysia, Korea, Madagascar, Vietnam, New Caledonia, Zanzibar and Japan (Sicuro and Levine, 2011). Moreover, the temperate sea cucumber species like *Holothuria tubulosa* (Gmelin 1788), *Holothuria poli* (Delle Chiaje 1823), *Holothuria forskali* (Delle Chiaje, 1823), *Holothuria arguinensis* (Koehler and Vaney, 1906) and *Holothuria mamata* (Grube 1840) in the Atlantic and Mediterranean Sea are new candidates close to the commercial production level (Domínguez-Godino et al., 2015, Tonn et al., 2016, Tolon, 2017, Domínguez-Godino and González-Wangüemert, 2018, Rakaj et al., 2018, Rakaj et al., 2019).

The diseases of sea cucumbers that lead to mass mortality and significant economic loss in production farms are getting more important as a major factor limiting productivity (Li et al., 2012). Skin ulceration syndrome is the most critical disease among all, which is responsible for 95% of deaths in sea cucumber rearing tanks (Deng et al., 2009). The high infective disease has symptoms like decolorization

of the skin, increased viscous mucus and lesions primarily formed on the mouth and cloacal openings of sea cucumbers. Becker et al., (2004), who first named this infection as "Skin Ulceration Syndrome", isolated and identified *Vibrio sp*., *Bacteroides sp*. and α-Proteobacterium type bacteria from the skin lesions of *H. scabra* by microscopic and biomolecular techniques. Vast majority of studies about skin ulceration syndrome are reporting various species of Vibrio genus as pathogenic bacteria in high-commercial sea cucumbers like *H. scabra, Apostichopus japonicus* (Selenka, 1867), and *Isostichopus fuscus* (Ludwig, 1875) (Wang et al., 2005, Ma et al., 2006, Wang, 2006, Zhang et al., 2006, Deng et al., 2008, Deng et al., 2009, Liu et al., 2010). However, it has been reported that *Vibrio* type bacteria isolated from skin lesions make the diagnosis and treatment of diseases difficult by hosting more than one strain according to sea cucumber species (Lane and Birkbeck, 1999; Choquet et al., 2003).

Vibrio mediterranei (former synonym for *V. shiloi*) are bacteria of the genus *Vibrio* isolated from bivalve populations, including oysters and mussels (Tarazona et al., 2014) and reported to cause significant mortality in other marine species (Kushmaro et al., 2001). *Vibrio shiloi*, first seen as the causative agent of bleaching disease of coral *Oculina patagonica* along the Israeli coastline, was later identified as a synonym for *Vibrio mediteranei* (Thompson et al., 2001).

There are limited or no studies on skin ulceration syndrome with sea cucumber species, particularly candidate for holothuriculture. However, case observations and mortality in breeding studies suggest that this disease affects these species as well as commercial ones. *H. poli,* a recent candidate for Mediterranean aquaculture, is one of these species which suffer from the skin ulceration syndrome in rearing conditions.

In this paper, predominant strains associated with the skin ulceration syndrome of the Mediterranean Sea cucumber, *H. poli* were addressed for the first time. Therefore, bacteria isolated from the skin lesions of *H. poli* reared in aquaculture tanks were identified and characterised by using morphological and biochemical tests, and 16S rDNA gene sequence analysis in order to analyse the physiological and biochemical characteristics of the predominant strains. Reference data provided from the present study would help to understand possible pathogen bacteria associated with the skin ulceration syndrome and crucial for the prophylaxis and treatment of such disease in holothuriculture.

MATERIAL AND METHODS

Sampling and physiologic – biochemical characterization of the bacteria

Adult sea cucumber *H. poli* individuals (92 ± 2.4 g) showing symptoms of skin ulceration, peristome tumescence, visceral ejection or in moribund condition were collected from the rearing tanks of the aquaculture facility in Ege University, Faculty of Fisheries, Urla Research Unit (Izmir, Turkey). The water parameters in recirculating aquaculture system were 22-26 C°, 38-40 ppm salinity, 6.8-7.0 ppm dissolved oxygen and 8,00 pH.

Tissue samples were taken from the skin lesions, mucous and coelomic fluid of 15 dissected sea cucumbers under sterile conditions. In order to prevent the external contamination of the samples by microorganisms and aquatic microflora, the skin of the sea cucumbers and the tools were treated by 98% ethanol, prior to sampling. Samples were streaked onto the Tryptone Soy Agar (TSA, Oxoid-CM0131) containing 1.5 % NaCl (w/v) and Thiosulphate-Citrate-Bile Salt-Sucrose agar (TCBS, Difco). All cultures incubated at 25 ± 2 °C for 24 - 48 hours. Following the incubation period, the suspected colonies (yellow and green) selected and streaked onto TCBS agar to ensure the purity of the culture. Morphologic and biochemical tests like cytochrome oxidase, resistance to O/129 (10 µg), gram staining, catalase, Triple Sugar Iron Agar (TSI), Voges–Proskauer (VP), citrate utilization, indole production, gelatinase, ONPG and decarboxylase of amino acids were performed to identify the selected culture (Bergey et al., 1939, Alsina and Blanch, 1994). Phenotypic characterization of the isolate was performed by API 20 E Identification Strip (Bio Merieux).

DNA extraction, PCR amplification and sequencing

DNA was performed from pure cultures of the bacteria using Roche High Pure PCR Template Preparation kit according to the manufacturer's instructions. Amplification of the 16S rDNA gene fragments were achieved using universal 16S rDNA bacterial primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTACGACTT-3') and Taq DNA polymerase kit (HelixAmpTM). PCR was conducted in 50 µl reaction volumes containing 1µl of each primer, 1µl of each dNTP, 5 µl 10× Taq polymerase buffer, 10 µl 5× Tune-Up Buffer, 1.25 units of Taq polymerase, 5 µl DNA, and molecular grade water. PCRs were run on SimpliAmp Thermal Cycler (Applied Biosystem), with initial denaturation at 95 °C for 2 minutes, followed by 35 cycles of 95 °C for 20 seconds, 53 °C for 40 seconds, and 72 °C for 90 seconds. The final elongation step performed at 72°C for 5 min. PCR products were electrophoresed in a 1 % agarose gel in 1×Tris-borate-EDTA (TBE) buffer stained with GelRed. The PCR amplification products were purified and sequenced by AltigenBio Laboratories (Izmir, Turkey). The sequencing reactions were performed using an ABI 3730 DNA analyser (Applied Biosystem).

Sequences were assembled using the BioEdit 7.0.5.2 (Hall, 1999) and consensus sequence was compared with other bacterial 16S rDNA sequences for DNA similarity in the GenBank database of the National Centre for Biotechnology Information (NCBI) by the nucleotide Basic Local Alignment Search Tool (BLAST) searches.

RESULTS

Symptomatic observation

Symptomatic observations in sea cucumber rearing tanks showed similar signs of disease in all sea cucumbers with skin ulceration disease. The symptoms characterized as anorexia, constipation, loss of adhesion ability to the substrates and inertia in very early stages of the disease. The body colour returns to matte palish brown and lost its vibrancy. Dense increase in viscous mucous secretion covering the whole-body surface was the most characteristic sign related with the skin ulceration syndrome. Ulcer spots were appeared primarily on the mouth and cloacal openings and spread to the various parts of the body. In mid stages, infected sea cucumbers became motionless, stop feeding completely and eviscerated their viscera. The body wall became flat and extremely soft without any muscular response to physical contact (Figure 1).

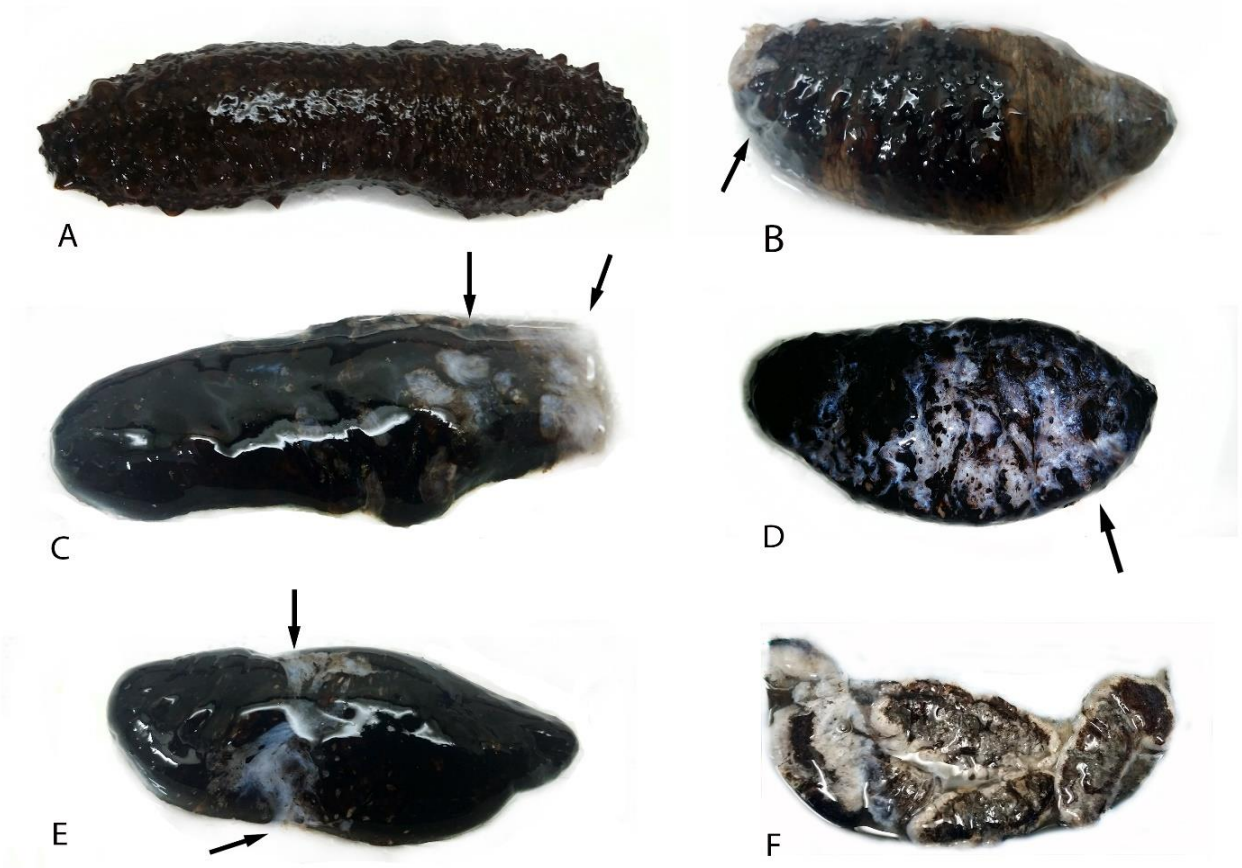

Figure 1. Arrows indicated lesions on the skin; A: healthy sea cucumber; B: early stage of ulceration close to cloacal opening; C: viscous mucus covering the whole-body wall and lesion patches close to mouth; D and E: ulceration on body wall; F: ulceration on whole body

The last stage of the disease characterised by extreme mucus secretion, dense white lesions covered the whole body, mass erosion of body wall and death within 3 to 5 days. Symptoms are observed on several sea cucumber individuals in various stages however, after the first appearance of the disease, there was a rapid increase in the number of sea cucumbers showing symptoms of the skin ulceration syndrome.

Bacterial morphology and biochemical characters

Biochemical characterization was established by detecting the dominant bacterial isolate in the streaking results from the body tissues and coelomic fluid of the infected sea cucumbers reared at 22-26 °C. It was determined that the cultured bacteria were highly homogenous and the isolated colonies in the TCBS agar were yellow, round, and smooth, and the bacteria cells were gram-negative. The reactions to indole, glucose, mannitol, sorbitol, melibiose, sucrose, melibiose, amylose, oxidase and catalase were positive; however, arginine dihydrolase, lysine decarboxylase ornithine decarboxylase, citrate, H_2S, urease, Voges-Proskauer, gelatinase, inositol, rhamnose and arabinose reactions were negative. Based on morphological and biochemical analyses, the dominant bacterium isolated from the skin ulcers of infected *Holothuria poli* was identified as *Vibrio mediterranei / Vibrio shiloi.*

Molecular analysis

The universal primers successfully amplified 1427 bp fragment of the 16S rDNA gene fragment from one isolate. In sequence similarity analysis using BLASTn tool, the sequence of the present *Vibrio* species isolate showed 100% the highest similarity with other *V. mediterranei* strains (MN874182, MN843962, MN843755, HF541963, JQ409387, MT860483, KX904712, MK452737.1, KM041189) and with uncultured *Vibrio* sp. (MG554521) and 99.51% the minimum similarity with *Vibrio shiloi* strain S0901 (HQ658895).

DISCUSSION

Skin ulceration syndrome is an important epidemic infection that is the most common in sea cucumber aquaculture. In previous studies, *Pseudoalteromonas nigrifacien*, *Vibrio tapetis*, *Vibrio splendidus*, *Marinomonas dokdonensi* (Deng et al., 2008), *Shewanella sp*., *Pseudoalteromonas tetraodonis* (Liu et al., 2010), *Vibrio alginolyticus* (Zhang et al., 2015), *Aeromonas salmonda* and *Aeromonas media* (Wang et al., 2007) have been reported as the bacteria isolated from the skin ulcers of infected sea cucumbers in aquaculture tanks. Liu et al., (2010) reported *Pseudoalteromonas* sp. and *Pseudoalteromonas tetraodonis* as the pathogenic bacteria responsible for the skin ulceration syndrome in experimental or naturally infected *Apostichopus japonicus* individuals, however, the healthy *Holothuria scabra* adults used in the same study failed to be experimentally infected with those bacteria. All findings derived from those studies are indicating *Vibrio* species, as the major pathogenic agent of skin ulceration syndrome (Becker et al., 2004, Wang et al., 2005).

In the current study, Mediterranean-specific *V. mediterranei / V. shiloi* were isolated for the first time from the skin ulcers of *H. poli*, which suggests that the different bacteria species would be effective in skin ulceration syndrome among the geographical regions. *Vibrio mediterranei* was first reported in 1992 (Pujalte et al., 1992) to cause coral albinism but harmless to humans and animals. However, this study revealed that the mentioned bacterium is predominantly present in the skin ulcers of *Holothuria poli* and would be susceptible from the disease.

V. mediterranei/V. shiloi has a wide range of identification from marine sediments, planktons, bivalves (mussels, oysters, and crustaceans), marine invertebrates (corals, sponges, shrimps, and sea urchins), and fish (shield, amberjack, and spotted rose snapper), however, a limited number of studies has identified this bacteria strain as a pathogenic agent in marine organisms. León-Palmero et al., (2018) have identified *V. mediterranei* in the feces of healthy sea cucumbers, *H. tubulosa*, and *H. forskali,* sampled from the experimental aquaculture tanks but could not successfully associate with any disease. It is considered that *V. mediterranei / V. shiloi*, which is frequently present in the marine environment, may be effective in skin ulceration syndrome with the effect of high-water temperatures (23-25°C) in the aquaculture environment. Similarly, Toren et al., (1998) reported that *V. mediterranei*, the bleaching agent in *Oculina patagonica* corals in the Mediterranean had no effect at 16°C but became more virulent with the increasing water temperature (23–29°C). Also in this study, *V. mediterranei / V. shiloi* was isolated from *H. poli* reared in high water temperatures like 22-26°C. However, the effect of different water temperatures and other abiotic variables on the virulence of this bacteria should be examined in further studies.

In this study, *V. mediterranei / V. shiloi* was reported for the first time in literature as the dominant bacterium, isolated from the skin ulcers of Mediterranean Sea cucumber, *H. poli*. The analysis of 16S rDNA gene sequence similarity of BLAST search revealed that the present *Vibrio* species isolate was showed the highest similarity ranging from 99.51% to 100% to all other *V. mediterranei* strains. Disease symptoms, morphology and biochemical characters of bacteria also support that the identified *Vibrio* species is the *V. mediterranei / V. shiloi.*

Information derived from this study suggests that different bacterial isolates would be effective on skin ulceration syndrome of sea cucumbers among the species and geographical regions. Therefore, future studies about skin ulceration syndrome should be conducted as specific to the species and geographical locations.

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