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Corresponding Address
Su Ürünleri Dergisi
Ege University Faculty of Fisheries
35100 Bornova-İzmir, Turkey
Phone: +90 232 311 3838
Fax: +90 232 388 3685
E-mail: editor@egejfas.org

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Tel: +90 232 311 3838 Fax: +90 232 388 3685 http://www.egejfas.org info@egejfas.org

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Population parameters of the pontic shad, *Alosa immaculata* Bennett, 1835 in the Fatsa coast of the south-eastern Black Sea

Güney-doğu Karadeniz'in Fatsa kıyılarında tirsi balığının, *Alosa immaculata* Bennett, 1835 populasyon parametreleri

İsmet Balık

Akdeniz University, Kemer Maritime Faculty, Dumlupınar Bulvarı 07058 Kampüs, Antalya, Turkey https://orcid.org/0000-0003-2168-8572

ibalik@akdeniz.edu.tr

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Abstract: The aim of this study is to estimate population parameters of pontic shad, *Alosa immaculata* Bennett, 1835 in the Fatsa coast of the south-eastern Black Sea. A total of 314 pontic shad specimens were collected from study area using artisanal fishing gears from March 2013 to February 2014. In the study, parameters of the von Bertalanffy growth equation were found as L_∞=43.05 cm; k=0.430 per year and t_0=-0.451 year. The growth performance index (Φ') was estimated as 2.90. The total mortality (Z), natural mortality (M), fishing mortality rates (F) were calculated as 1.33 $year^{-1}$, 0.75 $year^{-1}$ and 0.58 $year^{-1}$, respectively. The annual instantaneous fishing mortality rate was greater than both the target (F_{opt}=0.375 $year^{-1}$) and limit (F_{limit}=0.50 $year^{-1}$) biological reference points. Similarly, the present level of exploitation rate (E=0.43) was higher than the exploitation ratio for maximum yield per recruit (E_{max}=0.375) suggesting that overexploitation occurred. These results showed that this species has been over-exploited in the Fatsa coast of the south-eastern Black Sea. Measures should be taken to reduce the current exploitation rate for sustainable fishing of pontic shad in the Fatsa coast of the south-eastern Black Sea.

Keywords: Black Sea, pontic shad, *Alosa immaculata*, growth, mortality, exploitation

Öz: Bu çalışmanın amacı, Güney-doğu Karadeniz'in Fatsa kıyılarında tirsi balığı *Alosa immaculata* Bennett, 1835'nın popülasyon parametrelerini belirlemektir. Geleneksel uzatma ağları kullanılarak, Mart 2013 ile Şubat 2013 tarihleri arasında yapılan örnekleme avcılığı çalışmalarında toplam 314 tirsi balığı yakalanmıştır. Çalışmada, von Bertalanffy büyüme denkleminin parametreleri L_∞=43,05 cm, k=0,430 $yıl^{-1}$ ve t_0=-0,451 yıl olarak bulunmuştur. Büyüme performans indeksi ise (Φ') 2,90 olarak hesaplanmıştır. Toplam ölüm (Z), doğal ölüm (M) ve balıkçılık ölümü (F) oranları sırasıyla 1,33 $yıl^{-1}$, 0,75 $yıl^{-1}$ ve 0,58 $yıl^{-1}$ olarak tahmin edilmiştir. Yıllık anlık balıkçılık ölümü oranı, hem optimum balıkçılık ölümü oranı (F_{opt}=0,375 $yıl^{-1}$) hem de limit (F_{limit}=0,50 $yıl^{-1}$) biyolojik referans noktalarından daha yüksek bulunmuştur. Mevcut sömürülme oranı (E=0,43), stoka katılım başına düşen maksimum verim için hesaplanan maksimum sömürülme oranından (E_{max}=0,375) daha yüksektir. Bu sonuçlar, tirsi balığının Güney-doğu Karadeniz'in Fatsa kıyılarında aşırı av baskısı altında olduğunu göstermektedir. Tirsi balığının sürdürülebilir avcılığı için mevcut sömürü oranını azaltacak önlemler alınmalıdır.

Anahtar kelimeler: Karadeniz, tirsi, *Alosa immaculata*, büyüme, ölüm oranı, sömürülme

INTRODUCTION

In Black Sea including the Sea of Azov, three shad species of the genus *Alosa* are known: Pontic shad, *Alosa immaculata* Bennett, 1835; Caspian shad, *Alosa tanaica* Grimm, 1901 and Black Sea shad *Alosa maeotica* Grimm, 1901 (Tiganov et al., 2013). The Pontic shad, *Alosa immaculata* Bennet, 1835 is the largest species of the family Clupeidae in the Black Sea. It is an anadromous fish species which inhabits Black Sea and Azov Sea, and for spawning enters the big rivers (Navodaru, 2001; Višnjić-Jeftić et al., 2013; Raikova-Petrova, 2013; Rozdina et al., 2015; Lenhardt et al., 2016). Pontic shad is an endemic fish species for the Black Sea (Turan et al., 2015) and it is found off the coasts of the Black Sea and the Marmara Sea in Turkey (Turan et al., 2007). According to TUIK data, the total catches of *Alosa* species caught from Turkish waters between 2009 and 2018 are seen in Figure 1.

Pontic shad is a fish species that lives scattered in mid-August until December, Turkey's Black Sea waters. They are

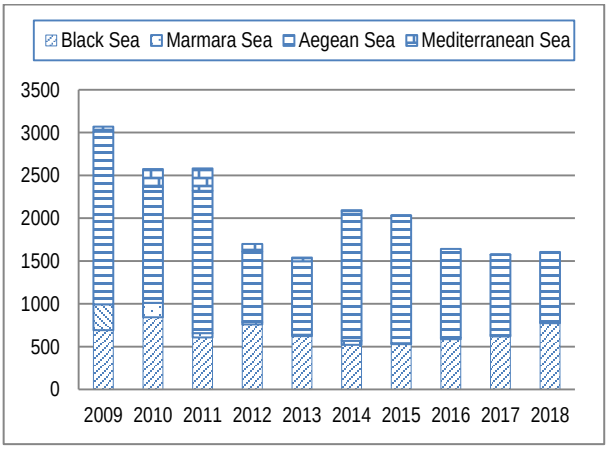

Figure 1. Total catches of *Alosa* species caught from Turkish seas between 2009 and 2018 (Ton)

usually fished with midwater trawl, purse seine and gillnets (Erguden, 2007; Özdemir et al., 2010; Tiganov et al., 2016). The meat has a high fat content and is a good source of nutrients. Therefore, consumption is particularly recommended. Although the meat of this species is excessively bony, it is a very tasty (Polat and Ergun, 2008).

This species migrates upriver to spawn at 3 years, rarely earlier and only a few individuals spawn two seasons. It appears along coast in March-April, enters rivers when temperatures reach about 6-9°C, between late March and late April. Migration usually peaks in May. Spawning starts when temperature rises above 15°C, in April-August. Spent individuals migrate back to sea to feed. Juveniles inhabit floodplain and shallow riverine habitats, migrate to the sea or estuarine habitats during first summer; in autumn, they move to the sea, remaining there until they mature. At sea, feeds on a wide variety of zooplankton (especially crustaceans) and small fish. Impoundment of main rivers (all happened more than 10 years ago) has significantly reduced available spawning sites and migration routes. The current threat to the species is overfishing, at sea and in the rivers during the migration runs, which is causing a population decline of unknown levels (Freyhof and Kottelat, 2008).

In IUCN (2018) reported that pontic shad is a vulnerable fish species. Overfishing is the major current threat to the species. It is caught both at sea and in the lower courses of rivers during the migration. Dam construction in the Black Sea basin (over 10 years ago) has led to the loss of large areas of spawning grounds.

The sustainable exploitation of this commercial fish species requires detailed study of the population and mortality parameters of populations. The species has been regularly studied on the Turkish coast of the Black Sea (Samsun, 1995; Özdamar, 1995; Kalaycı et al., 2007; Ergüden et al., 2007; Yılmaz and Polat, 2011; Samsun et al., 2017). In this study, population parameters such as the growth, mortality and exploitation rate for pontic shad caught from the Fatsa coasts of the south-eastern Black Sea were investigated.

MATERIALS AND METHODS

Study area

The study was carried out in Fatsa (Ordu) coast of Black Sea (Figure 2). Pontic shad specimens were monthly collected by using multifilament gillnets with mesh sizes of 32, 34, 36 and 38 mm, from March 2013 to February 2014. The fishing experiments were conducted three times for each month except for July and August. In July and August, fishing experiments could not be conducted due to in maintenance of fishing boat. During the sampling, the total length (L) of each specimen collected was measured.

Data analysis

For data analysis was used software package FiSAT II (FAO - ICLARM Stock Assessment Tool) based on length frequency distribution (Gayanilo et al., 2003).

The estimation of the growth parameters

The growth parameters (L_∞, k) were estimated by the length frequency analysis using the ELEFAN model implemented by the FiSAT II program (Gayanilo et al., 2005). The theoretical age at birth (t_0) was calculated using the empirical formula (Pauly, 1979):

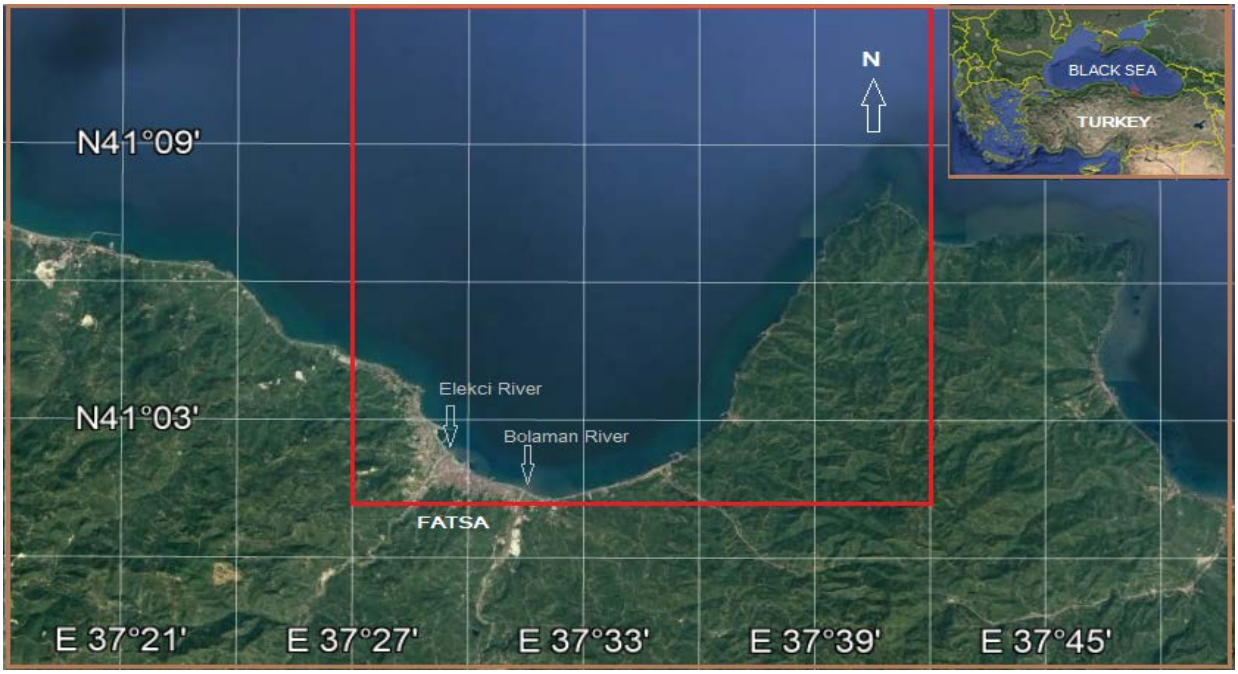

Figure 2. Study area

$Log(-t_0)=-0.3922 - 0.275*Log_{10}(L_\infty) - 1.038*Log_{10}(k)$. The general equation of the increase in length as a function of age (Von Bertalanffy) is: $L_t=L_\infty*[1-e^{-k*(t-t_0)}]$.

Where; L_t = length at age t, L_∞ = the asymptotic length of fish; k = curvature parameter that determines how fast the fish approaching to L_∞; t_0= the theoretical age at which the fish length is 0.

The growth performance index was calculated from the below expressed equation:

$(\Phi') = 2*Log(L_\infty) + Log(k)$ (Munro and Pauly, 1983).

The mortality

The estimation of mortality rates represent an important component of fisheries management. Total mortality (Z) was computed using the length converted catch curve analysis method in FiSAT II computer software package. The natural mortality (M) was estimated by the Pauly's empirical formula, using a mean surface temperature (T). Water temperature was monthly measured using thermometer and determine as 17.7 °C. $Log\ M = -0.0066 - 0.279*Log(L_\infty) + 0.6543*Log(k) + 0.4634*Log(T)$ (Pauly, 1980). Where; *M* is the natural mortality, L_∞ is the asymptotic length, *T* is the mean surface temperature and k refers to the growth rate coefficient of the VBGF. Fishing mortality (F) was calculated using the relationship: F=Z-M (Gulland, 1971), where; *Z* is the total mortality, *F* is the fishing mortality and *M* is the natural mortality. The exploitation level (E) was obtained using the relationship: E=F/Z (Gulland, 1971). Fishing mortality rate with target (F_{opt}) and limit (F_{limit}) biological reference points were estimated by formula of Patterson (1992): $F_{opt}=0.5M$ and $F_{limit}=2/3M$.

Probabilty of capture

The ascending left arm of the length converted catch curve incorporated in FİSAT II tool was used to estimate the probability of length at first capture (L_c) in addition to the length at both 25 and 75 captures which corresponded to the cumulative probability at 25% and 75% respectively. The probability of capture gives clear idea about the estimate of the real size of the fish in the fishing area that is being caught by specific gear. It is an important tool for fisheries managers in sustainably managing a target fishery, because it helps would be managers determining the minimum mesh size of a fishing fleet.

Length at recruitment (Lr) and Length at first capture (Lc)

Length at recruitment (Lr) was estimated also in the same manner by applying the growth equation of Von Bertalanffy: $Lr = = \bar{L}-k*(L_\infty-L')/Z$. Where; *L'* is the length for which all fish of that length and longer are under full exploitation, $\bar{L}$ is the mean length and *Z* is the instantaneous total mortality coefficient. Length at first capture (Lc) was investigated from the equation of Beverton and Holt (1956) which applies the growth constants of Von Bertalanffy: $Lc = \bar{L} - k(L_\infty - \bar{L}) / Z$. Where; $\bar{L}$ is the mean length of the catch, *k* and L_∞ are the constants of Von Bertalanffy equation and Z is the instantaneous total mortality coefficient.

Relative yield per recruit (Y/R) and relative biomass per recruit (B'/R)

Relative yield per recruit (Y/R) was based on the Beverton and Holt model (1966). The relative biomass per recruit (B'/R) was estimated as B'/ R=(Y'/R)/F. E_{max} depicts exploitation rate producing maximum yield. $E_{0.1}$ highlighting exploitation rate at which the marginal increase of *Y'/R* is 10% of its virgin stock with $E_{0.5}$ implying exploitation rate under which the stock is reduced to half its virgin biomass were computed using the procedure incorporated using the Knife-edge option fitted in the FiSAT II Tool. The length frequency data were pooled into groups with 1cm length intervals. Then the data was analyzed using the FiSAT II (FAO-ICLARM Stock Assessment Tools) software (Gayanilo et al., 1993).

RESULTS

Growth parameters

A total of 314 pontic shad were collected during the sampling studies. The total lengths of the fish studied were between 13 and 41 cm, with an average value of 30.5 cm.

Table 1. Mean, minimum and maximum total lengths (L) of pontic shads collected from study area (N: Fish number, SD: Standard Deviation)

Month	N	L	SD	min.	max.
March 2013	68	29.5	5.7	13.0	37.0
April	30	34.0	2.7	28.0	38.0
May	8	30.1	2.9	27.0	35.0
January	8	32.1	3.0	29.0	38.0
September	20	29.5	3.8	26.0	39.0
October	82	31.2	4.1	19.0	41.0
November	30	30.7	2.8	26.0	36.0
December	17	29.8	3.0	26.0	34.0
January 2014	27	28.6	3.0	23.5	33.5
February	24	29.6	5.5	21.0	41.0
Total	314	30.5	4.4	13.0	41.0

The L_∞ and k were estimated as 43.05 cm and 0.430 $year^{-1}$ from length frequency data using ELEFAN I by FiSAT II software (Figure 3). Since very few pontic shads were caught in some sampling periods, the data were seasonally evaluated. Then, the t_0 was calculated as -0.451 year using the empirical formula.

The von Bertalanffy growth function fitted to size-at age relationship for pontic shad in the Fatsa coast of the south-eastern Black Sea was shown in Figure 4. The growth performance was calculated as Φ'=2.90 from L_∞, k and mean annual water temperature (17.7 ºC).

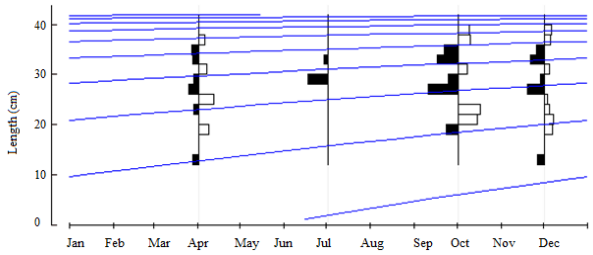

Figure 3. Restructured Length frequency distribution output from FiSAT II with superimposed growth curves (Dark bars= actual frequency bars & White bars=reconstructed bars)

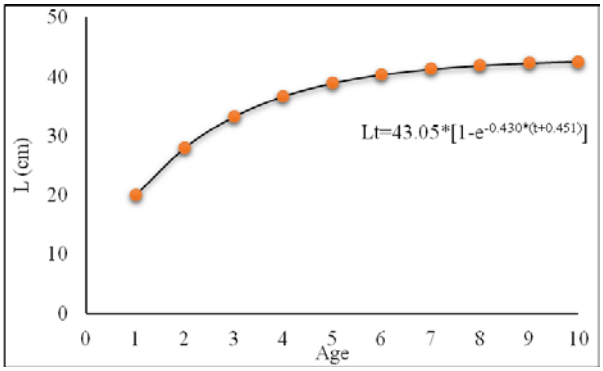

Figure 4. The von Bertalanffy growth function fitted to size-at age relationship for pontic shad in the Fatsa coasts of the south-eastern Black Sea

Mortality coefficients and current exploitation rate

The instantaneous rate of natural mortality (M) was estimated to be 0.75 year^{-1} by using Pauly (1980)'s empirical formula, with an average temperature of 17.7 °C. A length-converted catch curve (Figure 5) was utilized for the calculation of the instantaneous total mortality at Z=1.33 (0.88-1.79) year^{-1}. The fishing mortality (F) was estimated to be 0.58 year^{-1} which was much greater than both the target (F_{opt}=0.375 year^{-1}) and limit (F_{limit}=0.50 year^{-1}) biological reference points. The exploitation rate (E) was estimated to be 0.43 year^{-1}.

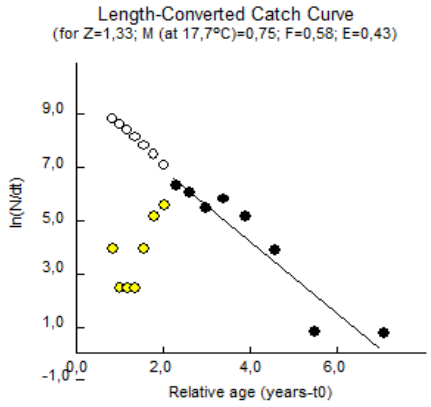

Figure 5. FiSAT II output of linearized length-converted catch curve for pontic shad in Fatsa coast of the south-eastern Black Sea (Yellow and white dots were not used in calculations)

Length at first capture (Lc)

The typical selectivity for pontic shad caught in the Fatsa coast of the south-eastern Black Sea showed that at least 25% of fish of 26.93 cm, 50% of the fish of 29.53 cm and 75% of all fish of 32.13 cm total length were caught by gillnets (Figure 6). The midpoint of lower length classes in the sampled data was used as a length at recruitment which is 20.8 cm. The length at first capture (Lc) was adopted as 26.4 cm.

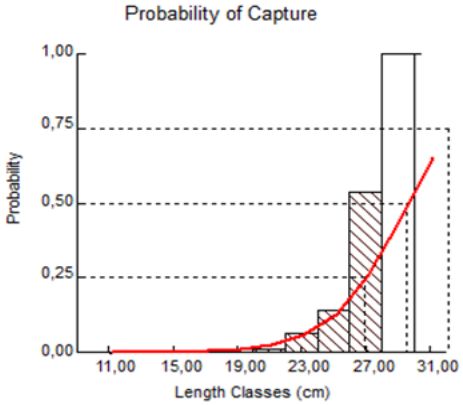

Figure 6. Estimated probability of capture by length of pontic shad caught from the Fatsa coast of the south-eastern Black Sea

FiSAT II output of the probability of capture for pontic shad in the fisheries waters of Fatsa coast of the south-eastern Black Sea (0.25, 0.50 and 0.75 relates to 25%, 50% and 75% respectively)

Relative yield per recruit (Y'/R)

The Beverton and Holt relative yield per recruit model in Figure 7 showed that the indices for sustainable yield were 0.244 for optimum sustainable yield ($E_{0.5}$), 0.375 for the maximum sustainable yield (E_{max}) and 0.307 for economic yield target ($E_{0.1}$). The current exploitation rate was estimated 0.43 from the analysis of mortality rates, which was already above the maximum, optimum and economic yield indices.

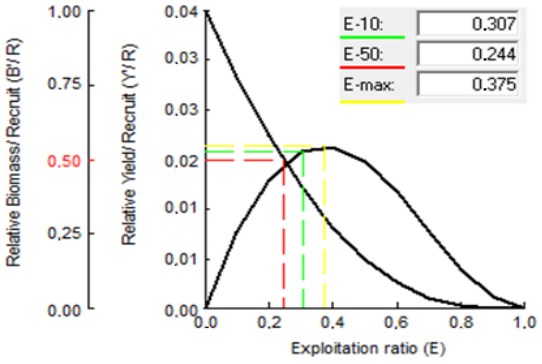

Figure 7. Beverton and Holt's relative yield per recruit and average biomass per recruit models, showing levels of yield indices for pontic shad in the Fatsa coast of the south-eastern Black Sea (Red dashes=$E_{0.1}$, Green dashes=$E_{0.5}$ and Yellow dashes=E_{max})

DISCUSSION

Maximum length of pontic shad was determined as 41 cm in the south-eastern coast of the Black Sea which was slightly larger than the values reported by Yılmaz and Polat (2011), Samsun (1995), Özdamar (1995), Kalaycı et al. (2007), Özdemir et al. (2010), Yankova et al. (2011), Özdemir and Duyar (2013) in the Black Sea and by Ibănescu et al. (2017) in the Romanian section of the Danube river and Rozdina et al. (2013) in the Bulgarian sector of the Danube.

The growth parameters L_∞, k and t_0 are constants in an equation we can predict the fish body size when it reaches a certain age. The asymptotic length (L_∞=43.05 cm) and the growth coefficient k (0.430 year^{-1}) of the pontic shad were close to the values reported in the some studies. For example, the L_∞ and k were found to be L_∞=40.06 cm, k=0.32 year^{-1} in the Turkish coast of the Black sea (Özdemir et al., 2018), L_∞=40.43 cm, k=0.380 year^{-1} in the Romanian section of the Danube river (Ibănescu et al., 2017), L_∞=35.7 cm, k=0.493 year^{-1} in the Bulgarian sector of the Danube river (Rozdina et al., 2013), L_∞=57.38 cm, k=0.1067 year^{-1} in the Danube river (Kolarov, 1980), L_∞=40.43 cm, k=0.27 year^{-1} in the Black Sea (Kolarov, 1983). In the present study, the phi-prime index (Φ') of Munro was calculated as 2.90. This value is higher than values reported in the Romanian section of the Danube river (Ibănescu et al., 2017), Bulgarian sector of the Danube river (Rozdina et al., 2013), the Danube river (Kolarov, 1980) and Black Sea (Kolarov, 1983). These comparisons show that the pontic shad grow faster in the Fatsa coast than in some other regions of the Black sea and the Danube river.

The growth difference among different populations of the same species may be due to environmental conditions, gonad development and reproduction period (Bagenal and Tesch, 1978), as well as sampling site, sampling time, characteristics of sampling nets and sampling methods (Tiraşin, 1993). For example, our research area and Samsun coasts are very close each other. However, the results show significant differences. Probably, this difference may be due to the sampling method. While the samples of our study were collected with gillnets, Özdemir et al. (2018) investigated samples collected by midwater trawl from the Samsun coastal areas in 2011-2012.

Morphometric and meristic structure of Black Sea Shad populations sampled from Marmara Sea (Adalar) and Black Sea (Şile, Sinop, Samsun, Trabzon) were investigated by Turan et al. (2010). According to the results of that study, Trabzon and Sinop populations were different morphologically from other populations and from each other. It is thought that these differences may be due to the geographical location of the rivers preferred by the populations. In this study, it is stated that populations move away from each other with geographical distance increase. These results show that, apart from reproductive migration, pontic shads in the Black Sea do not migrate long-distance. In the Fatsa coast, this species enter into the Bolaman and Elekçi rivers for breeding. Many rivers flow into the Black Sea coastal area of Turkey. Each of these rivers has different characteristics. This may also affect the growth of fish. Because, the migration from the sea to the river the fish cease the feeding, spend a lot of energy to reach the spawning sites and lose weight. This inevitably reduces the fish condition (Rozdina et al., 2015).

Contrary to the values (M=0.51 year^{-1} and F=0.69 year^{-1}) reported by Özdemir et al. (2018) in Samsun coast of the Black Sea, the natural mortality (M=0.75 year^{-1}) was greater than the fishing mortality (F=0.58 year^{-1}). In the Black Sea coast of Turkey, the pontic shad is usually fished with gillnets and midwater trawl. In the Black Sea coast of Turkey, the pontic shad is usually caught with gillnets and midwater trawl. However, while fishing with midwater trawl is free on the shores of Samsun, it is prohibited on the shores of Fatsa. Therefore, the fishing mortality rate of pontic shad was higher in Samsun region than Fatsa region.

Patterson (1992) reported that fishing mortality rates above 2/3 M are often associated with stock declines, whereas fishing mortality rates below this level have resulted in stock recovery. Therefore, we consider that fishing mortality rates above 2/3 M to represent an undesirable state for the resource, and a situation which management action should avoid, in essence a limit reference point (F_{limit}) for fishery managers. Exploitation rates above F_{limit} have been associated with stock declines whilst below this level the tendency has been towards stock recovery. Exploitation below F_{opt} allows stock to increase in size. In our study the fishing mortality (F=0.58 year^{-1}) was considerably greater than both the target (F_{opt}=0.375 year^{-1}) and limit (F_{limit}=0.50 year^{-1}) biological reference points, suggesting that this species is being over-exploited in the Fatsa coast of the south-eastern Black Sea. Pontic shad, like all other alosines species, are highly sensitive to multiple stresses, both natural and anthropogenic (Smederevac-Lalić et. al., 2018). Therefore, changes in the population structure and stock size of the pontic shad in the Black Sea must be monitored continuously. Estimating mortality rates are important for maintaining fish stocks at the desired level, so to avoid the over-exploitation of fishery resources. In present study, the analysis showed an E_{max} to be 0.375 against the present exploitation rate (E) of 0.43 thus indicating that the stock of this species is under fishing pressure in the Fatsa coast of the south-eastern Black Sea. It could be concluded that the pontic shad stock in the Fatsa coast of the south-eastern Black Sea is in a situation of overexploitation and for the management purpose the current exploitation rate should be reduced to the level of 0.375.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Some oxidative stress parameters in heart tissue of Zebrafish (*Danio rerio*) caused by mancozeb

Zebrabalığı (*Danio rerio*) kalp dokusunda mancozeb'in neden olduğu bazı oksidatif stres parametreleri

Figen Esin Kayhan[1*] ● Güllü Kaymak[2] ● Harika Eylül Esmer Duruel[3] ● **Şeyma Kızılkaya**[4]

[1]Biology Department, Arts and Science Faculty, Marmara University, 34722, Istanbul, Turkey https://orcid.org/0000-0001-7754-1356
[2]Biology Department, Arts and Science Faculty, Marmara University, 34722, Istanbul, Turkey https://orcid.org/0000-0001-6309-0208
[3]Biology Department, Arts and Science Faculty, Marmara University, 34722, Istanbul, Turkey https://orcid.org/0000-0002-0792-2062
[4]Biology Department, Arts and Science Faculty, Marmara University, 34722, Istanbul, Turkey https://orcid.org/0000-0001-8065-217X

Corresponding author: fekayhan@marmara.edu.tr
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Abstract: The potential toxic effects of mancozeb exposure on some antioxidant enzyme were investigated on heart tissue of zebrafish in this study. Zebrafish groups were exposed to different doses of mancozeb (Group A: 5 mgL^{-1} and Group B: 7.5 mgL^{-1}) for 120 hours. In this study, catalase (CAT) activity, malondialdehyde (MDA) level and total protein (TP) level were determined with spectrophotometer. Our results showed that CAT activity was found 2.541±0.771 mg L^{-1} in A group and 2.011±0.201 mg L^{-1} in B group in this study. CAT and MDA activity levels decreased in the experiment group according to control group. MDA levels were found 0.025±0.003 mg L^{-1} in A group and 0.025±0.003 mg L^{-1} in B group. TP levels were found 9.75±1.51 mg L^{-1} in A group and 10.18±0.32 mg L^{-1} in B group. TP levels increased in the all experiment groups according to control group. We observed that the changes in the CAT activity and MDA levels were time and as well as mancozeb dose dependent. As a result, mancozeb is a very toxic substance for zebrafish and other aquatic organisms.

Keywords: Mancozeb, oxidative stress, catalase, malondialdehyde, zebrafish

Öz: Bu çalışmada, zebra balığı kalp dokusunda mancozeb'in bazı antioksidan enzimler üzerindeki potansiyel toksik etkileri araştırılmıştır. Zebra balığı grupları, 120 saat boyunca farklı dozlarda mancozeb (Grup A: 5 mg L^{-1} ve Grup B: 7,5 mg L^{-1}) maruz bırakıldı. Bu çalışmada, katalaz (CAT) aktivitesi, malondialdehit (MDA) ve total protein (TP) seviyeleri spektrofotometre ile belirlenmiştir. Elde ettiğimiz sonuçlar CAT aktivitesinin ve MDA seviyesinin tüm deney gruplarında azaldığını gösterdi. Bu çalışmada CAT aktivitesi A grubunda 2,541±0,771 mg L^{-1}, B grubunda ise 2,011±0,201 mg L^{-1} olarak bulunmuştur. CAT ve MDA aktiviteleri tüm deney gruplarında kontrol grubuna göre azalmıştır. MDA seviyeleri A grubunda 0,025±0,003 mg L^{-1} ve B grubunda 0,025±0,003 mg L^{-1} olarak bulunmuştur. TP seviyeleri A grubunda 9,75±1,51 mg L^{-1}, B grubunda ise 10,18±0,32 mg L^{-1} olarak bulunmuştur. Deney grubunda TP düzeyi kontrol grubuna göre artmıştır. CAT aktivitesi ve MDA seviyelerindeki değişikliklerin deney süresine ve mancozeb dozuna bağlı olduğu belirlenmiştir. Sonuç olarak; mancozeb zebra balığı ve diğer sucul organizmalar için oldukça toksik bir maddedir.

Anahtar kelimeler: Mancozeb, oksidatif stres, katalaz, malondialdehit, zebra balığı

INTRODUCTION

Aquatic biota of existing agricultural areas are often exposed to broad spectrum of pesticides that reach water ecosystems through unintended direct application, sprey drift and runoff, thus posing a possible risk for nontarget aquatic organisms (Wagner et al., 2014; Faggio et al., 2016). Pesticides are chemicals used to control pests, their primary use is to increase agricultural yields and prevent the spread of vector-borne disease that have implications to health and the economy (Kumar et al., 2019). Mancozeb (Manganese ethylenebis/dithiocarbamate) is one of the most widely used fungicide in agriculture particularly in many fungal diseases in a various agriculture areas (EPA, 2005). Fish expose to various stressors in their environment such as heavy metals, pesticides, industrial wastes etc. In recent years many of their natural habitats are being altered by anthropogenic effects, including industrial waste and urban pollutants. Zebrafish (*Danio rerio*) is a significant vertebrate model organism. Recently the zebrafish has frequently been used for toxicological studies in various organs and tissues such as liver, gills, intestine, pancreas, brain and heart. Although the stuctural organization of zebrafish organ and tissues different from humans, the two species are similar in terms of function of metabolic pathways of antioxidant enzymes (Qiu et al., 2019). The use of zebrafish as a toxicity model for mammals and humans is well established (McCollum et. al., 2011; Ribas and Pifeffer, 2013). CAT, SOD and GPx enzymes constitute the first step of the antioxidant defence systems (Percin and Sogut, 2010). In this study different mancozeb levels present in the water were investigated in zebrafish under laboratory conditions to describe maximum concentrations allowed in the water and to determine concentrations of fish intake appropriate for human consumption (Andreu-Sanchez et al., 2012). Thus to better

understand the harmful effects of mancozeb and oxidative stress process induced by mancozeb, this study examined dose dependent oxidative stress in zebrafish heart tissue by determining the MDA and protein level and CAT activity.

MATERIAL AND METHODS

Experimental design

Mancozeb is the member of ethylene bisdithiocarbamates (EBDC) fungicides a broad spectrum protectant from fungi for the control of a wide range of diseases in agricultural areas (EPA 2005). First, a stock solution of mancozeb was prepared. The mean body lengths of zebrafish were body length =31.6 ± 1.17 mm (n=25). Zebrafish acclimatized for two week under standard laboratory conditions. Fish were fed twice a day on well-aerated tap water in glass aquariums and with a photoperiod consisting of 14h light/10h dark period during experiments. Healty zebrafish groups were divided into two experimental groups (A and B) and a control (C) group. Different doses of mancozeb were applied in the experimental groups 5 and 7.5 mgL^{-1} respectively. 120 hours exposure of mancozeb was accepted as an indicator of chronic effect during the tests. After 120 hours fish were dissected immediately with sterile surgical instruments. The heart tissues of fish were removed. The heart tissue samples added cold 0.1M phosphate buffer (pH 7.4) and homogenized by using glass beads with ice bath cooling. Then homogenates centrifugated at 10.000g for 30 min at 4^0C to obtain the supernatant for analysing the biochemical parameters the supernatants were stored at 4^0C and all applications were performed under ice-bath cooling to keep the enzyme activities stable. CAT, MDA and TP levels were detected using spectrophotometric methods.

Catalase (CAT) activity assay

CAT activity was determined according to the method by Aebi. The principle of assay is based on the determination of rate constant of hydrogen peroxide decomposition by the CAT. Briefly the activity was determined by measuring the decrease in absorbance at 240 nm of a reaction mixture consisting of H_2O_2, in phosphate buffer, pH7.0 and requisite volume of tissue sample (Aebi, 1984).

Malondialdehyde (MDA) estimation assay

MDA levels was determined according to the method by Ledwozyw. The MDA content was measured after incubation at 95^0C with thiobarbituric acid in aerobic conditions. The pink color produced by the reactions was measured with spectrophotometer at 532 nm. Specific activity was defined as the unit of activity per milligram protein (Ledwozyw, 1986).

Total protein (TP)

Soluble protein concentration was measured with the Coomassie Brilliant blue G-250 using bovine serum albümin as a standard at 595 nm (Bradford, 1976).

Statistical analysis

The SPSS 23.0 package program was used for analysis. Study data were given as arithmetic means and standard deviations. The one way analysis of variance and student t-test were used for the determination of the significance of the differences between the groups. A value of $p<0,05$ was considered statistically significant.

RESULTS

Our results show that mancozeb cause changes at a biochemical level in heart tissue of zebrafish. In this study, we determined low CAT activity in the heart tissue of samples after both of mancozeb treatment. Our results demostrated reduced levels of CAT and MDA in zebrafish heart tissue when compared to the control group. The decrease in CAT activity could be due to the excess production of superoxide radicals. MDA which is itself responsible for some of the damaging effect of free radicals on cell membranes whereas severe oxidative stress in the cells can cause cell injury and death of cell. The decreased MDA levels may be consequence of cellular oxidative damage due to pesticide exposure. Total results of this study including MDA and protein levels, CAT activity in heart tissue of zebrafish with or without exposed to mancozeb are in Table 1.

Table 1. Catalase (CAT) activity, malondialdehyde (MDA) and total protein (TP) level changes related to after different doses of mancozeb exposure (Values were expressed as mean± SD)

	Exposure concentration mg L^{-1}		
	A group (Mancozeb 5 mg L^{-1})	B group (Mancozeb 7,5 mg L^{-1})	C group (Control group)
CAT	2.541± 0.771	2.011 ± 0.201	3.230 ± 0.698
MDA	0.025± 0.003	0.025 ± 0.003	0.118 ± 0.012
TP	9.75 ± 1.51	10.18 ± 0.32	8.46 ± 1.49

DISCUSSION

All pesticides cause nondegredable residues on soil, water and living organisms. CAT plays a significant protective role in animal tissues against reactive oxygen species (ROS) attack. CAT is one of the primary enzyme which involves in peroxide detoxification and has a special importance for the clearance of H_2O_2. In this study we found low CAT activity in the heart tissue of zebrafish. Similar results have also been reported in various fish species. (Plhalova et al. 2017) investigated the subchronic effects of neem-azal T/S (it is a biopesticide and containing 1% of the active ingredient azadirachtin A) on the mortality, growth and histopathology of juvenile zebrafish. Also the researchers reported that influenced indices of oxidative stress on liver of zebrafish. They found that the results of their study indicate that these tested concentrations of neem-azal T/S affect fish growth and have a negative effects on the indices of oxidative stress in the juvenile stage of zebrafish, as well as cause mild

histopathological changes in liver tissue. Blahova et al.(2013) reported a considerable decline in CAT activity in all test groups of zebrafish exposed to atrazine (Blahova et al., 2013). Environmental toxicants such as pesticides cause oxidative damage by directly increasingly cellular concentration of ROS and also by reducing the cellular antioxidant capacity. In our study, catalase activity decreased in heart tissue of zebrafish after exposed to mancozeb for 120 hours. This situation may be as the consequence of cellular oxidative damage due to pesticide exposure at different concentrations during the experiment. So, we can infer that oxidative balance might play an important role in prevention of antioxidative enzyme activity due to mancozeb exposure. If the decrease of CAT levels because of high superoxide radicals, it can be cause to inhibit CAT activity. Zhu et al., (2019) reported that fenobucarb (2-sec-butylphenyl methylcarbamate) is a possible risk factor for cardiovascular and cerebrovascular systems in fish. They found that fenobucarb induced severe heart failure, reduced heart contractions and myocardial apoptosis in their study (Zhu et.al., 2019). Our results demonstrated that pesticides could induce organ failure in a dose dependent manner. Our results mean that pesticides would have potential to cause harmful effects in aquatic animals.

Lipid peroxidation (LPO) is one of the most important early events in cell degeneration leading to necrosis and occurs primarily in the cell membrane (Tabassum 2016). Environmental toxicants such as heavy metals, pesticides, can generally cause oxidative stress and LPO has been widely used as a marker of oxidative stress in living cells (San and Yonar 2017; Tsaboula et al., 2016). MDA is one of the most preferred indicator of LPO in all living organisms. MDA is the product formed as a result of LPO and is a parameter extensively used to show the oxidative damage on cells and tissues (Zengin, 2018). Alterates on the levels of main metabolites such as MDA and on the activity of some antioxidant enzymes such as CAT have been described as biomarkers of oxidative stress (Pascual et al., 2003; Morales et al., 2004. According to Clasen et. al., (2018) pesticides have severe adverse consequences in fish and their potential risk to human health due to their bioaccumulation in farmed fish too (Clasen et. al., 2018).

Although proteins are now used generally as a marker of ROS, they infrequently have been investigated in aquatic pollution. TP levels are one of the main targets for the explanation of effects of environmental toxicants such as pesticides or heavy metals in aquatic organisms. Miron et al. (2008) reported that exposure to clomazoneon *Leporinus obtusidens* for 8 days, resulted as increased protein levels in fish liver (Miron et al., 2008). Moraes et al. (2011) also reported similar results related with the total protein levels when carps were exposed to imizethapy+imizapic pesticides (Moraes et al., 2011). In addition, according to Karaca et. al. (2014) the antioxidant enzyme activities on fish may be considered sensitive markers of organochlorine pesticide exposure on their nature habitats (Karaca et.al., 2014). In this study, exposed to mancozeb, demonstrated with the increase in protein levels in heart tissue and caused oxidative stress. At the same time levels of TP had also significantly increased in the heart tissue of samples after mancozeb exposure. According to a study, high activity of antioxidant enzymes LPO have been found in the blood of the captive Northern Bluefin Tuna samples. Konyalıoğlu and Perçin (2017) have been found that high activity of antioxidant enzymes (LPO) in the blood of the captive Northern Bluefin Tuna (NBT) samples in their study. When compared GSH levels of blood have turned out to be low in comparison to the wild NBT samples. Their findings show that captive NBTs suffer from stress more than wild NBTs. This is important to know the differences in enzyme levels in wild fish species (Konyalıoğlu and Perçin, 2017). Vieira et. al., (2018) indicated that high concentrations of imidaclobrid (IMI) induced a marked increase in LPO in liver and kidney tissues of fish. According to researchers, pesticide exposure caused oxidative stress on various biological molecules, including lipid, proteins and antioxidant enzymes (Vieira et. al., 2018). Our results support these findings in this study. Maharajan et. al., (2018) reported that pyriproxyfen could cause harmful effect on early developmental stages such as egg and larvae of zebrafish at higher concentration. They showed that these damages that will occur during the development stage of organs and tissues such as liver, pancreas or heart will affect the whole life of the fish (Maharajan et. al., 2018).

In conclusion this study investigated the existance of oxidative stress biomarkers in zebrafish heart tissue under laboratory conditions and found that decreased level of MDA and CAT activity with an increased level of TP in relation to oxidative stress. The results are significant for reporting acute mancozeb toxicity in terms of biochemical changes: mancozeb is substantially toxic to fish. Special studies such as detecting biomarkers in polluted aquatic environments, recommended to ensure continue the sustainability of healthy environment.

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RESEARCH ARTICLE
ARAŞTIRMA MAKALESİ

New records of halacarid mites (Acari: Halacaridae) from the Levantine coast of Turkey

Türkiye'nin Levant Denizi kıyılarından yeni halacarid kayıtları

Furkan Durucan

Işıklar Caddesi No. 16, Antalya, Turkey
https://orcid.org/0000-0002-6168-2135

f_durucan@hotmail.com

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Abstract: This paper reports on six marine halacarid species collected from the coasts of Antalya (3 stations) and Mersin (1 station), Turkey. The species are respectively: *Actacarus bacescui*, *Camactognathus tesselatus*, *Coloboceras drachi*, *Halacarus rismondoi*, *Rhombognathus paratonops*, and *Rhombognathus parvulus*. Among the species, 2 genera (*Camactognathus* and *Coloboceras*) and 4 species (*Camactognathus tesselatus*, *Coloboceras drachi*, *Halacarus rismondoi* and *Rhombognathus parvulus*) are being newly reported from the coasts of Turkey for the first time. Each species found in the area was briefly described with comments on their habitat preferences and geographical distributions.

Keywords: Systematics, meiofauna, Antalya, Mersin

Öz: Bu çalışmada, Antalya (3 istasyon) ve Mersin (1 istasyon) kıyılarından elde edilen 6 deniz halacarid kaydı verilmiştir. Bu türler sırasıyla, *Actacarus bacescui*, *Camactognathus tesselatus*, *Coloboceras drachi*, *Halacarus rismondoi*, *Rhombognathus paratonops* ve *Rhombognathus parvulus*'tur. Bunlardan 2 cins (*Camactognathus* ve *Coloboceras*) ve 4 tür (*C. tesselatus*, *C. drachi*, *H. rismondoi* ve *R. parvulus*) Türkiye kıyılarından daha önce kaydedilmemiştir. Çalışmada, her bir türün tanımları, habitat bilgileri ve dünyadaki dağılımları verilmiştir.

Anahtar kelimeler: Sistematik, meiofauna, Antalya, Mersin

INTRODUCTION

The family Halacaridae is meiobentic and generally live in submerged habitats in a variety of substrata (e.g. on many animal groups and algae) (Bartsch, 2004; 2006; Giere, 2009). Many species are predators, but some genera evolved the ability to feed on macroalgae (Pepato et al., 2018). The family includes marine, brackish and freshwater species occupying areas from littoral to the deep sea (Bartsch, 2006). To date about 1200 species of Halacaridae have been reported worldwide (Bartsch, 2009; WoRMS Editorial Board, 2019). The first study about halacarids in Turkey was started by Dr. Ilse Bartsch (2000), who described a halacarid species "*Isobactrus ponticus*" new to science from the Sinop coast. She (2001, 2004, 2013 and 2015) also reported some species from the Black Sea region. Afterwards, three new species records were given from the Sea of Marmara (Bostanci coast, Istanbul) by Bilecenoğlu et.al. (2013), Kapiris et al. (2014), Durucan and Boyaci (2016). The first study on the Levantine Sea (Antalya, Kaş) halacarids was started by Mytilineou et al. (2016), who gave a new record of the species "*Agauopsis microhyncha*". Recently, several papers (Durucan and Boyaci, 2017; 2018a; 2018b; Durucan, 2018; 2019; Stamouli et al. 2017; Chartosia et al. 2018) have been published about the distribution of halacarid mites on the coast of Antalya.

In the present study, six species belonging to five genera were recorded and discussed from the Levantine coast of Turkey.

MATERIAL AND METHODS

Samples of *Cystoseira barbata* and various size of sand habitats (interstitial, medium coarse and fine sand from 0,5 m to 12 m depths) were collected by hand using snorkelling and SCUBA diving at 4 stations along the coasts of Antalya and Mersin in 2017 (Figure 1 & Table 1). Immediately after collection, mites were extracted by washing the substrates. The meiofauna were retained in the set of sieves (63 μm, 500 μm, 1000 μm). The halacarids were sorted under binocular stereo microscope (Nikon SMZ10). In the laboratory, mite specimens were cleared in lactic acid and mounted in Hoyer's medium. Figures were drawn with the aid of a camera lucida (Nikon Eclipse E400). All measurements are given as micrometers (μm). The specimens were kept in the author's personal collection in Antalya, Turkey.

Table 1. List of recorded halacarid species in this study with their locations and habitats

Stations	Coordinates	Species	Habitat
Sta.1 Anamur	36.069263°N 32.866953°E	*Actacarus bacescui*	interstitial water (50 cm)
		Rhombognathus paranotops	*Cystoseira barbata* (6 m)
Sta.2 Yakamoz	36.845556°N 30.799167°E	*Camactognathus tesselatus*	well sorted fine sand (2 m)
Sta.3 Bilem	36.854722°N 30.743889°E	*Coloboceras drachi*	medium-size sand (6 m)
		Rhombognathus parvulus	*Cystoseira barbata* (6 m)
Sta.4 Kaş	36.156944°N 29.628333°E	*Halacarus rismondoi*	fine sand (12 m)

Figure 1. Map of sampling locations

RESULTS

SYSTEMATICS

Family Halacaridae Murray, 1877

Genus *Actacarus* Schulz, 1937

Actacarus bacescui Konnerth-Ionescu, 1970 (Figure 2)

Material examined. Sta. 1; interstitial water at 50 cm depth below on the shore near the water, 5 ♀♀, 7 ♂♂, 5 deutonymphs.

Morphology. Idiosoma of female 210-225 µm long, 90-100 µm wide, that of male 170-200 µm long, 75-85 µm wide, that of deutonymphs between 175-187 µm long, 63-85 µm wide. Anterior dorsal plate 65 µm long, 80 µm wide in female. Ocular plate 12 µm long. Posterior dorsal plate 150 µm long, 90 µm wide in female. Anterior epimeral plate 85 µm long, 100 µm wide. Posterior epimeral plate 85 µm long, 17 µm wide (Figure 2A, B & E-G). Male genital opening surrounded by 20 perigenital setae and 2 pairs of subgenital setae (Figure 2D). Female genitoanal plate with 3-4 pairs of perigenital setae. Gnathosoma 55 µm long 50 µm wide. Palps are slender and 4 segmented. Total palp length 61 µm long (Figure 2C).

Remarks. The morphological characteristics and habitat preferences of the specimens reported here accord with the previously given records by Durucan and Boyaci (2018a) and original descriptions by Morselli and Mari (1979). This species is well characterized by possesses a median spine on the tectum which easily separates it from related species (*A. ponticus* and *A. pygmaeus*) (Bartsch, 1999).

Habitat. The species is found in sandy bottom in tidal areas (Bartsch, 2009).

Distribution. Atlantic coast, Mediterranean and Black Sea (Bartsch, 2009; Durucan and Boyaci, 2018a).

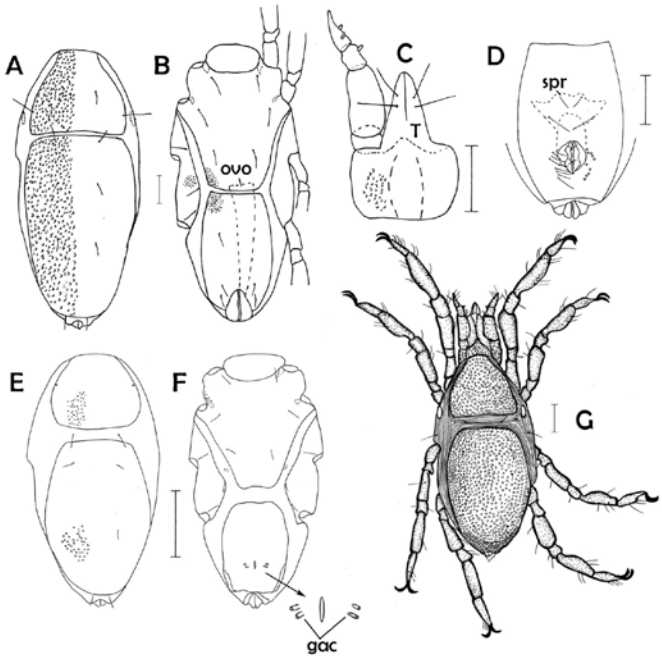

Figure 2. *Actacarus bacescui* Konnerth-Ionescu, 1970: A. idiosoma, dorsal view, female; B. idiosoma, ventral view, female; C. gnathosoma, ventral view, female; D. Genitoanal plate of male; E. idiosoma, dorsal view, deutonymph; F. idiosoma, ventral view, deutonymph; G. dorsal view, female (gac; genital acetabula, ovo; ovopositor, spr; spermatopositor, T; tectum) Scale bars: A-C= 50 µm; D-G= 25 µm

Genus *Camactognathus* Newell, 1984

Camactognathus tesselatus (Morselli & Mari, 1982) (Figure3)

Material examined. Sta. 2; well sorted fine sand (2 m), 1 ♀.

Morphology. Idiosoma 260 µm long, 127 µm wide. Dorsal plates uniformly foveated. Anterior dorsal plate 88 µm long, 75 µm wide. Ocular plate 85 µm long, 23 µm wide. Posterior dorsal plate 174 µm long, 98 µm wide. Dorsal setae-1 on anterior dorsal plate, dorsal setae-2 on integument. Anterior epimeral plate 105 µm long, 114 µm wide, epimeral pores lacking. Posterior epimeral plate 100 µm long, 50 µm wide. Anterior epimeral plate and genitoanal plate almost equal in length. Genitoanal plate with four pairs of perigenital setae (Figure 3A,B & D). Gnathosoma missing. Leg I 180 µm long. The chaetotaxy of leg I as follows from trochanter to tarsus 1, 2, 4, 4, 8, 5 (Figure 3C).

Remarks. This is the second record of this species from Turkey. The morphological characteristics, habitat preferences and body sizes of the specimens reported here accord with the previously given records by Bartsch (2013).

Habitat. The species was previosly recorded from mixture of shell remains and muddy sand (3 m) in Sinop, Turkey by Bartsch (2013).

Distribution. Mediterranean and Black Sea (Bartsch, 2009; 2016).

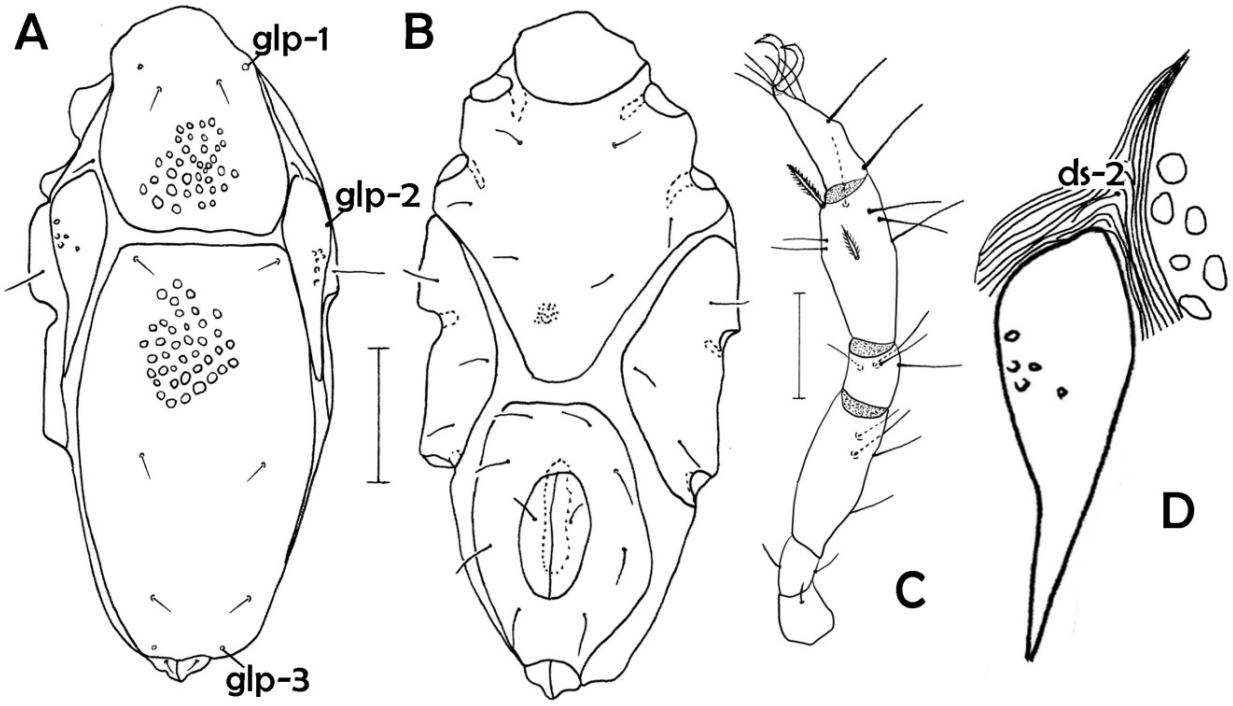

Figure 3. *Camactognathus tesselatus* (Morselli & Mari, 1982) (female): A. idiosoma, dorsal view; B. idiosoma, ventral view; C. leg I, lateral view; D. detail of ocular plate and second dorsal setae (ds-2; second dorsal setae, glp; gland pore) Scale bars: 50 µm

Genus *Coloboceras* Trouessart, 1889

Coloboceras drachi Monniot, 1962 (Figure 4)

Material examined. Sta. 3; medium-size sand (6 m), 1 ♀, 1 deutonymph.

Morphology. Idiosoma of female 450 µm long, 185 µm wide. Anterior dorsal plate 150 µm long, 100 µm wide in female. Ocular plate 85 µm long, 17 µm wide in female. Posterior dorsal plate 260 µm long, 125 µm wide in female. Female anterior epimeral plate 235 µm long, 210 µm wide without epimeral vesicles or epimeral pores. Genitoanal plate 161 µm long, 105 µm wide with three pairs of perigenital setae and two pairs of subgenital setae in female (Figure 4A & B). Idiosoma colour is slightly brown.

Gnathosoma missing in both female and deutonymph. Idiosoma of deutonymph 310 µm long, 185 µm wide (Figure 4C).

Remarks. This is the first record of this species from the Eastern Mediterranean, Levantine Sea of Turkey. With regard to the external morphological characters, the specimen corresponds with the record given by Monniot (1962).

Habitat. Coloboceras drachi was found in subtidal sediment (Bartsch, 2009).

Distribution. Northeastern Atlantic, Mediterranean Sea (Bartsch, 2009).

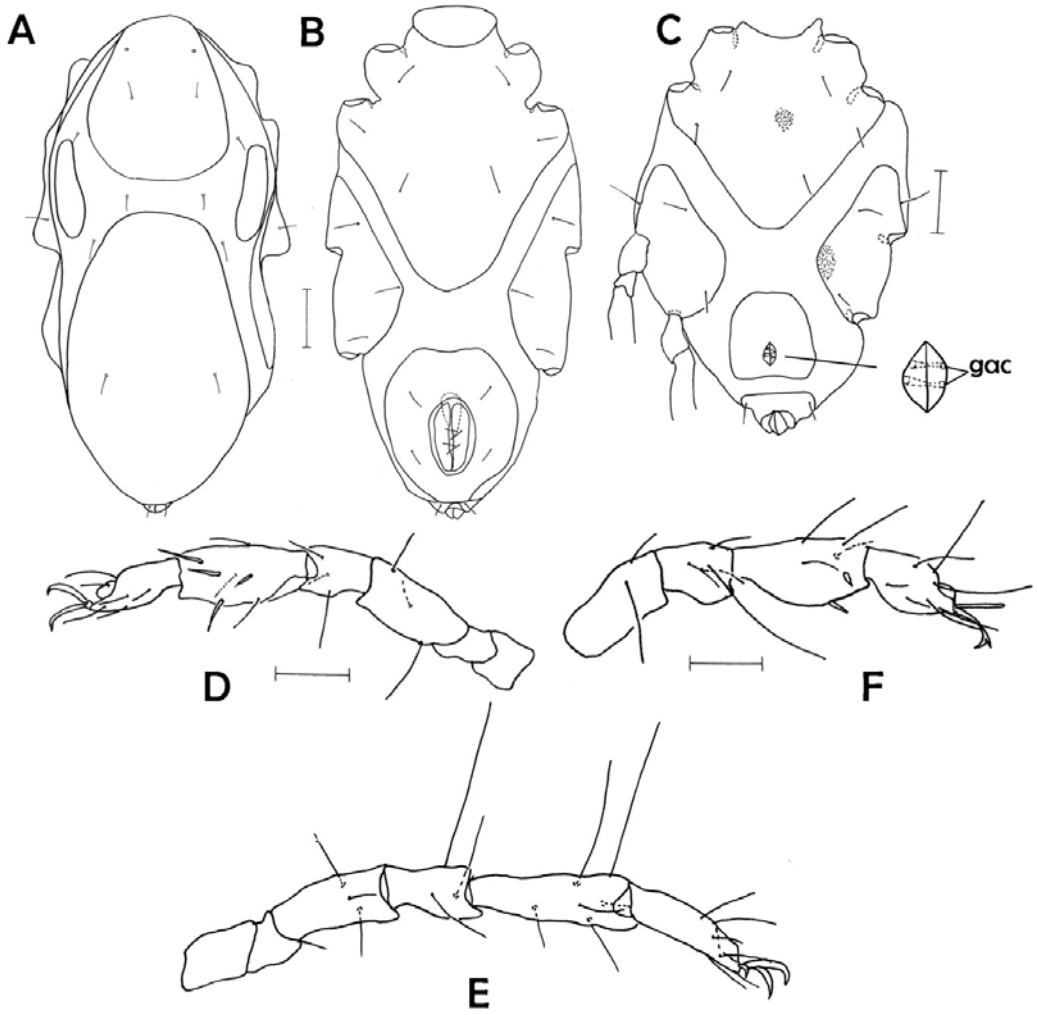

Figure 4. *Coloboceras drachi* Monniot, 1962: A. idiosoma, dorsal view, female; B. idiosoma, ventral view, female; C. idiosoma, ventral view, deutonymph; D. leg II, lateral view, female; E. leg IV, medial view, female; F. leg I (from telofemur to tarsus), lateral view, deutonymph. (gac; genital acetabula) Scale bars: 50 µm

Genus *Halacarus* Gosse, 1885

Halacarus rismondoi Viets, 1940 (Figure 5)

Material examined. Sta. 4; fine sand (12 m), 2 ♀♀, 1 ♂.

Morphology. Idiosoma of female 375 µm long, 183 µm wide, that of male 365 µm long, 190 µm wide, anterior dorsal plate in male 88 µm long, 65 µm wide with frontal process. Ocular plate with corneae and porus canalicus. Posterior dorsal plate 89 µm long, 65 µm wide. Anterior epimeral plate 88 µm long, 163 µm wide. Posterior dorsal plate 137 µm long, 65 µm wide with 1 dorsal and 3 ventral setae. Dorsum with 6 pairs of idiosomatic setae. First dorsal setae on anterior dorsal plate, second to fifth on integument, sixth dorsal setae on posterior dorsal plate. Epicuticula on plates, gnathosoma and legs with striae in parallel and fingerprint-like arrangement. Pair of first dorsal setae posterior to first gland pore. Pairs of gland pore 3 and gland pore 4 striated integument, gland pore 5 on posterior dorsal plate. Male genital opening surrounded by 34 perigenital setae, 4 pairs of outlying setae. Female genitoanal plate with pair of crescent cerotegumental areas; anterior pair of perigenital setae on genito-anal plate (Figure 5A-C). Gnathosoma 87 µm long 62 µm wide. Palps slender and 4 segmented. Total palp length 78 µm long (Figure 5D). Leg I 355 µm long. The chaetotaxy of leg I as follows from trochanter to tarsus 1, 2, 8, 10, 10, 8 (Figure 5E).

Remarks. *Halacarus rismondoi* was originally described by Viets (1940) from the Adriatic Sea (Croatia-Rovinj). The morphology of the specimen is similar to given by Viets (1940) and Bartsch (2017) .

Habitat. This halacarid mite was found by Viets (1940) among variety of macroalgae (*Geodia* sp., *Udothea* sp. and *Cystoseira* sp.)

Distribution. Mediterranean Sea (Rovinj, Croatia) (Bartsch, 2009).

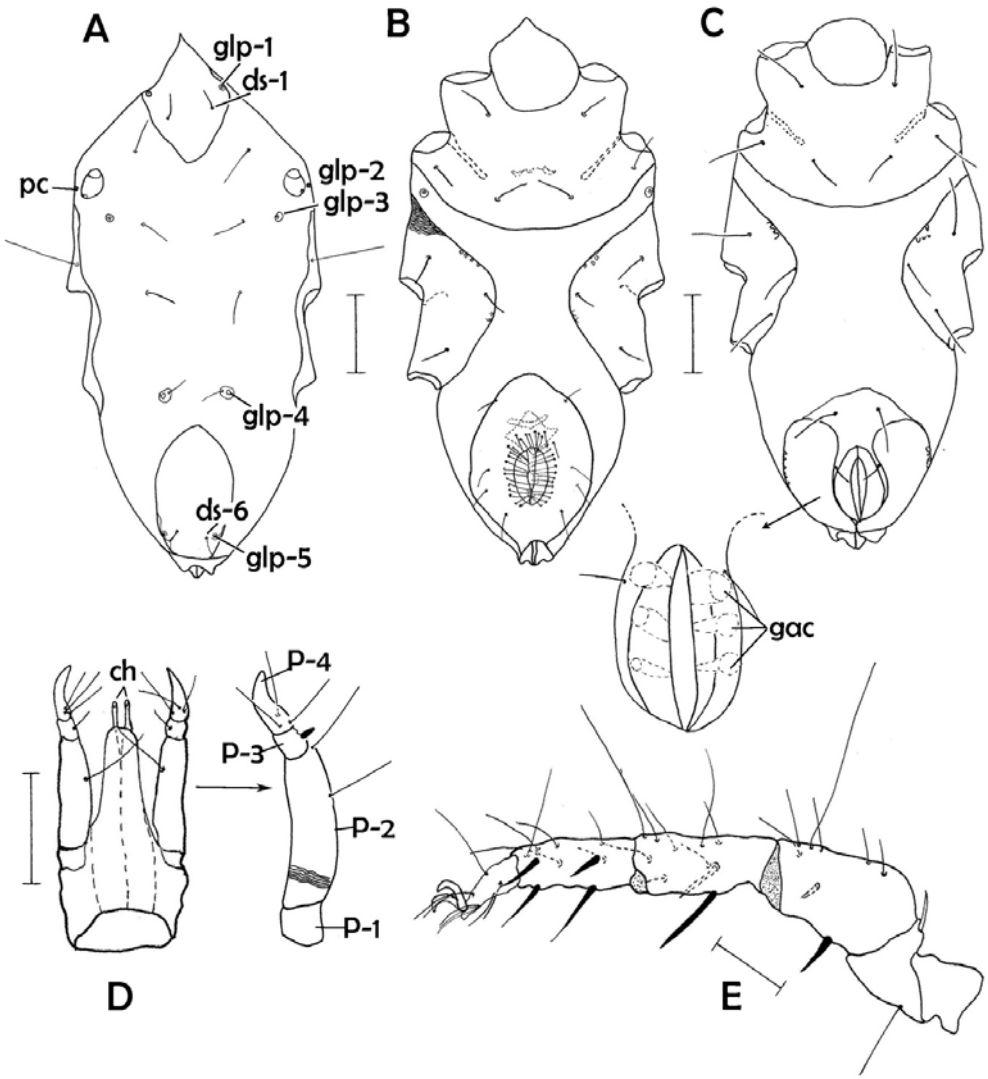

Figure 5. *Halacarus rismondoi* Viets, 1940: A. idiosoma, dorsal view, male; B. idiosoma, ventral view, male; C. gnathosoma, ventral view, female; D. gnathosoma, ventral view, male; E. leg I, lateral view, male (ch. chelicerae; ds-1 to ds-6. dorsal setae, from anterior to posterior; gac. genital acetabula; glp-1 to glp-5. gland pore/s, from anterior to posterior; P-1 to P-4. first to fourth segments of palp; pc. porus canalicus) Scale bars: 50 µm

Genus *Rhombognathus* Trouessart, 1888

Rhombognathus paranotops Bartsch, 1986 (Figure 6)

Material examined. Sta 1; *Cystoseria barbata* (6 m), 5 ♂♂.

Morphology. Idiosoma of male 288 µm long, 186 µm wide. Anterior dorsal plate 93 µm long, 118 µm wide. Ocular plate 83 µm long, 65 µm wide with two corneae. Uniformly reticulated posterior dorsal plate 155 µm long, 96 µm wide. First dorsal setae (37 µm) on anterior dorsal plate, ds-2 to ds-4 (striated in integument). All ventral plates fused. Male GO surrounded by 22-24 perigenital setae and 2 pairs of subgenital setae (Figure 6A & B). Palps are slender and 4 segmented. Total palp length 90 µm long (Figure 6C). Leg I 275 µm long. The chaetotaxy of leg I as follows from trochanter to tarsus 1, 2, 7, 4, 5, 6 (Figure 6D & E).

Remarks. *Rhombognathus paratonops* is common in the Mediterranean Sea, and is distinguished from the other congener, *R. tonops* by having an accessory process and reticulation on the plates. In *R. paratonops*, the posterior dorsal plate is uniformly reticulated whereas in *R. tonops* the median and the pair of lateral foveate portions of the plate separated by longitudinal areas which are delicately porose but lack of a foveate or reticulate ornamentation; the accessory process on the claws of *R. paratonops* bear 13-14 tines, those of *R. tonops* 8 tines (Bartsch, 1986;1996).

Habitat. The species previously was found from shallow water among seagrass and various red algae (*Corallina officinalis*, *Cystoseria barbata* and C. *crinita*) (Bartsch, 2009).

Distribution. Mediterranean and Black Sea (Bartsch, 2009).

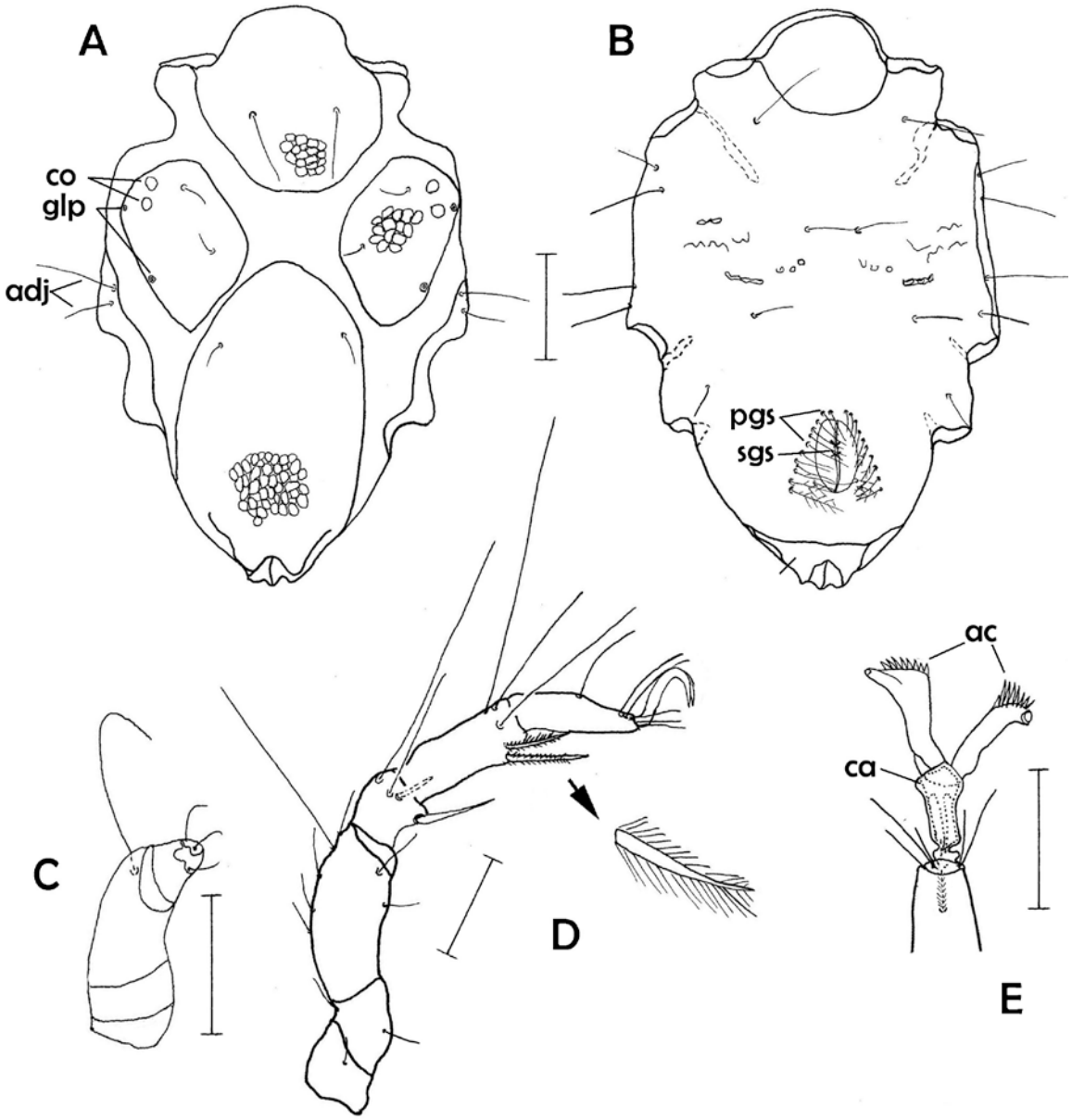

Figure 6. *Rhombognathus paranotops* Bartsch, 1986 (male): A. idiosoma, dorsal view; B. idiosoma, ventral view; C. palp, lateral view; D. Leg I, lateral view; E. tarsus I, lateral view (ac. accessory process with tines; adj. adjunct seta; ca. carpite; co. corneae; glp. gland pore; pgs. perigenital seta; sgs. subgenital seta) Scale bars: 50 µm

Rhombognathus parvulus Viets, 1939 (Figure 7)

Material examined. Sta.3; *Cystoseria barbata* (6 m), 1 ♀.

Morphology. Idiosoma of female 287 µm long, 165 µm wide. All dorsal and ventral plates fused. Ocular plate with 2 corneae. Five pair of perigenital setae are found on the sides of the genital opening. Ventral shield separated from anal plate by striated integument (Figure 7A & B). Gnathosoma 75 µm long and 50 µm wide (Figure 7C). Tibia I with two bipectinate setae (Figure 7D).

Remarks. The general morphological characteristics, habitat preferences and body sizes of the specimens reported here agree with the previously given records by Viets (1939) and Bartsch (1986; 2009). *Rhombognathus parvulus* is similar to *R. peltatus*. But, two species are distinguished eachother by long of carpite and comb structure on claws. In *R. parvulus*, carpite not conspicuously long while in *R. peltatus* carpite long. In *R. parvulus*, accessory process with teeth present, but no long claw comb while in *R. peltatus*, teeth of claw comb running along the concave side to base of the claw accoding to the Bartsch (1986).

Habitat. The species has been found in association with a variety of macroalgae (Bartsch, 2009).

Distribution. Mediterranean Sea (Bartsch, 2009).

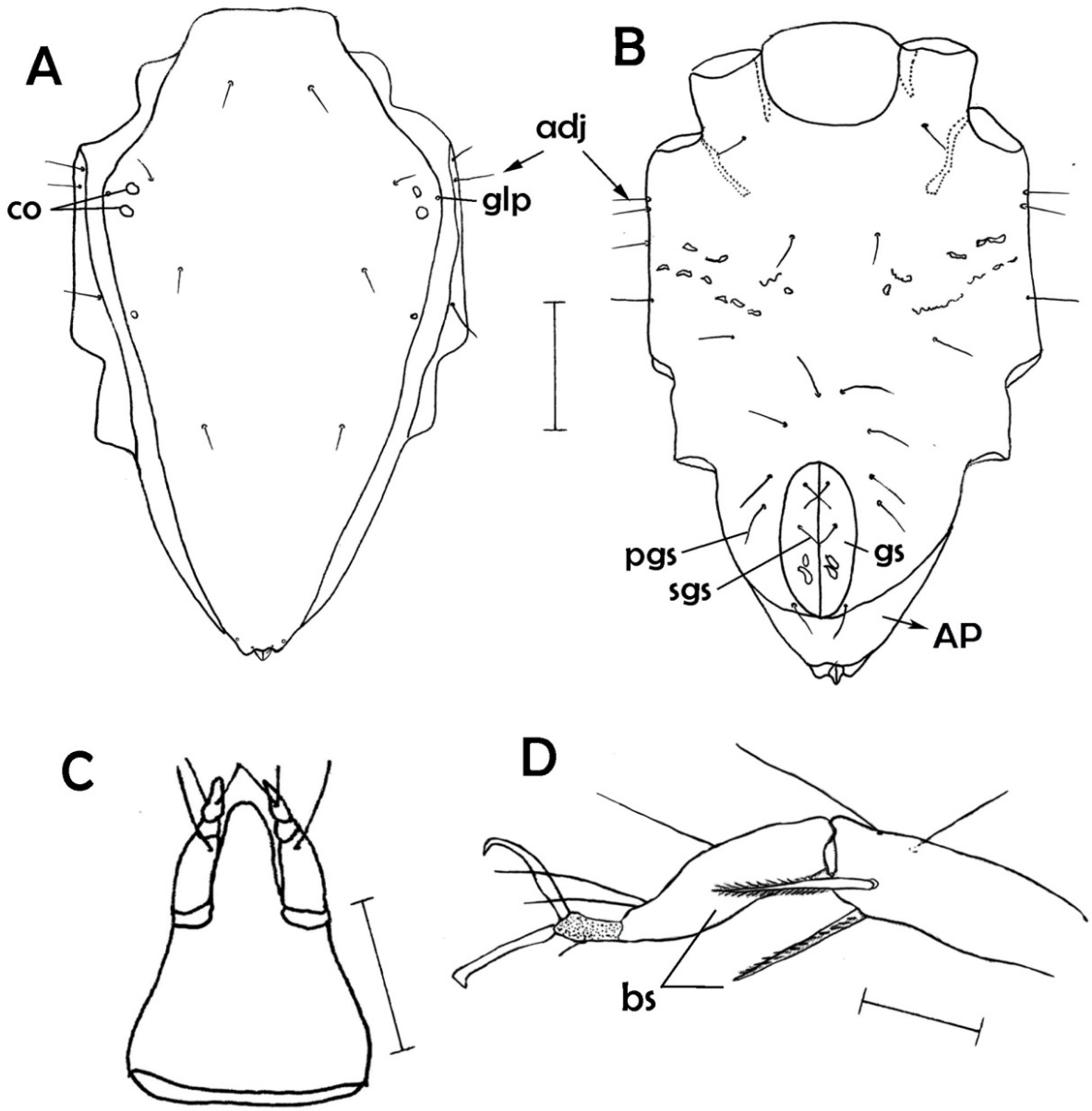

Figure 7. *Rhombognathus parvulus* Viets, 1939 (female): A. idiosoma, dorsal view; B. idiosoma, ventral view; C. gnathosoma, dorsal view; D. tarsus and tibia I, lateral view adj. adjunct seta; AP. anal plate; bs. bristle shaped setae; co. corneae; glp. gland pore; gs. genital sclerite; pgs. perigenital setae; sgs. subgenital setae) Scale bars: 50 µm

CONCLUSION

Six halacarid species belonging to five genera were found from various habitats of the Eastern Mediterranean, Levantine Sea of Turkey (Antalya and Mersin) in the present study. The genera *Camactognathus* and *Coloboceras* and the species of *Coloboceras drachi*, *Halacarus rismondoi* and *Rhombognathus parvulus* have not previously been recorded from Levantine Sea of Turkey. The summary of my findings are contributed to an increase in the number and better knowledge of halacarid diversity. Knowledge about marine halacarid mites has increased considerably within the last few years. Further reports of new halacarid taxa from Turkey aimed to improve our knowledge in terms of their biodiversity and ecological remarks.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Atatürk Baraj Gölü'ne dökülen Kahta Çayı'nın (Adıyaman) su kalitesi

Water quality of Kahta Stream (Adıyaman) discharging into Atatürk Dam Lake

Selami Gölbaşı[1*] ● **Bülent Şen**[2]

[1]Devlet Su İşleri Genel Müdürlüğü 13. Bölge Müdürlüğü, Antalya
[2]Fırat Üniversitesi, Su Ürünleri Fakültesi, Elazığ

https://orcid.org/0000-0003-2310-2993
https://orcid.org/0000-0001-8875-8702

Corresponding author: selamigolbasi@gmail.com

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Öz: Bu çalışmada, Nisan 2012-Mart 2013 tarihleri arasında bazı hidrolojik ve fiziko-kimyasal özellikleri izlenerek, Atatürk Baraj Gölü'ne dökülen Kahta Çayı'nın su kalitesinin belirlenmesi amaçlanmıştır. Bu amaçla, kaynaktan mansaba doğru belirlenen beş istasyonda aylık ölçümler yapılmış ve su örnekleri alınmıştır. İzleme periyodu boyunca örnekleme noktalarında su sıcaklığı 5,0-25,2 °C; elektriksel iletkenlik 216-359 µS/cm; toplam çözünmüş katı madde 147-244 mg/L; çözünmüş oksijen 8,67-13,36 mg/L ve pH 7,08-8,76 aralığında ölçülmüştür. Askıda katı madde 2-138 mg/L; bulanıklık 0,92-96,70 NTU; toplam alkalinite 102-200 mg $CaCO_3$/L; klorür 0,71-5,02 mg/L; amonyum azotu 0,007-0,400 mg/L; nitrit azotu 0,003-0,060 mg/L; nitrat azotu 0,12-1,19 mg/L; çözünmüş reaktif fosfor 0,007-0,033 mg/L; silika 5-23 mg/L; sülfat 8-33 mg/L ve kimyasal oksijen ihtiyacı 0,63-9,18 mg/L arasında tayin edilmiştir.

Hafif alkali karaktere sahip Kahta Çayı, izlenen parametreler bakımından; Yerüstü Su Kalitesi Yönetmeliği'nin Çevresel Kalite Standartlarına göre I. Sınıf (yüksek kaliteli su) özelliğine sahip olduğu belirlenmiştir. Bu karakteristiğiyle Kahta Çayı'nın içme suyu temini için kullanılma potansiyelinin yüksek olduğu rekreasyonel amaçlı kullanım, alabalık yetiştiriciliği, hayvan üretimi ve diğer çiftlik ihtiyaçları için kullanılabilir olduğu tespit edilmiştir. Ayrıca değişkenlerin zamansal ilişkisi istatistiksel açıdan incelendiğinde, SiO_2 hariç diğer tüm değişkenler arasında anlamlı bir fark bulunmuştur ($p<0,05$). Bu sonuç, değişkenlerin mevsimsel değişikliklerinden etkilendiğini ortaya koymaktadır.

Anahtar kelimeler: Kahta Çayı, Atatürk Baraj Gölü, su kalitesi, limnoloji

Abstract: In this study, it is aimed to determine the water quality of Kahta Stream which is poured into Atatürk Dam Lake by following some hydrological and physico-chemical characteristics between April 2012 and March 2013. For this purpose, monthly measurements were made at five stations from the source to the downstream and water samples were taken. During the monitoring period, water temperature 5.0-25.2 °C; electrical conductivity 216-359 µS/cm; total dissolved solids 147-244 mg/L; dissolved oxygen 8.67-13.36 mg/L and pH were measured in range of 7.08-8.76. Suspended solid 2-138 mg/L; turbidity 0.92-96.70 NTU; total alkalinity 102-200 mg/L; chloride 0.71-5.02 mg/L; ammonium nitrogen 0.007-0.400 mg/L; nitrite nitrogen 0.003-0.060 mg/L; nitrate nitrogen 0.12-1.19 mg/L; dissolved reactive phosphorus 0.007-0.033 mg/L; silica 5-23 mg/L; sulphate 8-33 mg/L and chemical oxygen demand were determined betwen 0.63-9.18 mg/L.

Kahta Stream with slightly alkaline character, in terms of the parameters monitored, it is determined that the Surface Water Quality Regulation has the characteristics of Class I (high quality water) according to the Environmental Quality Standards. With this characteristic, it has been determined that Kahta Stream has a high potential to be used for drinking water supply, it can be used for re-use, trout farming, animal production and other farm needs. In addition, when the temporal relationship of variables was analyzed statistically, a significant difference was found among all variables except SiO_2 ($p<0.05$). This result shows that the variables are affected by seasonal changes.

Keywords: Kahta Stream, Atatürk Dam Lake, water quality, limnology

GİRİŞ

Hızla artan dünya nüfusu ve insanoğlunun daha iyi yaşam standartlarını yakalama arzusu, doğal kaynaklar üzerinde baskı oluşturmaktadır. Günümüzde yerüstü su kaynaklarının sahip oldukları su kalitesinin belirlenmesi ve buna bağlı oluşturulan su kalite yönetimi tüm dünyada önemli araştırmalar arasında ciddi bir yer tutmaktadır. Su kalitesi ile ilgili yapılan çalışmalar, suyun kullanım amacının belirlenmesinde çok önemli olmasının yanı sıra, yüzey su kaynaklarının sürdürülebilir kullanımı açısından da önem arz etmektedir. Bu nedenle yüzey su kaynaklarının su kalitesinin sürekli ve düzenli izlenmesi gerekmektedir.

Ülkemizde son zamanlarda lotik sistemler ile ilgili olarak çeşitli çalışmalar yapılmıştır (Şen vd., 1999, 2002; Boran ve Sivri, 2001; Taşdemir ve Göksu, 2001; Tepe vd., 2006; Şen ve Gölbaşı, 2008, 2014; Varol ve Şen, 2009; Mutlu vd., 2016; Zeybek ve Kalyoncu, 2016; Sönmez ve Battal, 2017). Bu çalışmaların çoğunluğunda akarsuların fiziksel ve kimyasal özeliklerinin izlenmesi ön plana çıkmaktadır.

Kahta Çayı, Adıyaman İli sınırları içerisinde kalan ve Çakal Çayı, Kalburcu Çayı, Eğri Çayı ve Ziyaret Çayı ile birlikte Atatürk Baraj Gölü'nü besleyen başlıca akarsulardan biridir (Anonim, 2003). Kahta çayı üzerine sadece arazi kullanımı, jeomorfolojisi, sedimantasyon ve morfometrik özellikleri ile ilgili birkaç çalışma (Elmastaş, 2008; Sunkar ve Karataş, 2012, 2013, 2014) yürütülmüştür. Buna karşılık Atatürk Baraj Gölü'ne dökülen önemli bir akarsu olmasına rağmen, araştırıldığı kadarıyla çayın su kalitesi üzerine

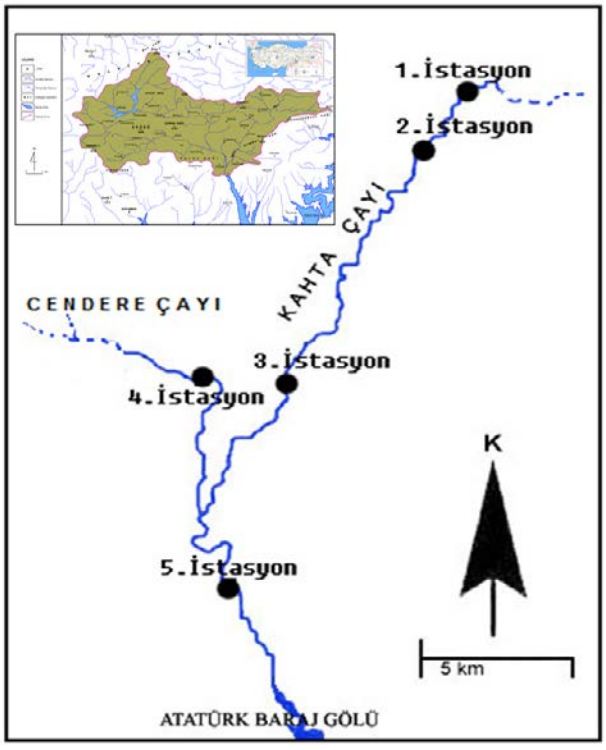
yayınlanmış bir araştırma verisine ulaşılamamıştır. Bu araştırma Kahta Çayı'nın su kalite özelliklerinin belirlenerek, bu hususta eksikliğini gidermek ve gelecek yıllarda yapılacak çalışmalara referans olabilmesi amacıyla gerçekleştirilmiştir. Bu amaç doğrultusunda kaynaktan mansaba doğru belirlenen beş istasyondan bir yıl süresince su kalitesi verileri toplanmış ve Kahta Çayı su kalitesinin ulusal Yerüstü Su Kalitesi Yönetmeliği'ne göre sınıflandırılması yapılmıştır.

MATERYAL VE METOT

Çalışma alanı ve örnekleme noktaları

Adıyaman il sınırları içerisinde kalan ve Atatürk Baraj Gölü'nü besleyen, kaynağını Malatya ile Adıyaman havzaları arasında kalan Güneydoğu Toros Dağları'ndan alan Kahta Çayı, Fırat Nehri'nin önemli kollarından biridir. Kaynağını dağlık alanlardaki karstik kaynaklardan alan Kahta Çayı, 46 km uzunluğa sahip olup, havzanın kapladığı alan ise yaklaşık 1575 km^2'dir (Elmastaş, 2008).

Kahta Çayı'nın su kalitesini belirlemek amacıyla Nisan 2012-Mart 2013 tarihleri arasında periyodik olarak her ayın üçüncü haftası içinde akarsuyu kaynaktan mansaba doğru en iyi temsil edecek şekilde belirlenen 5 örnekleme noktasında (Şekil 1) izleme çalışması yürütülmüştür.



Şekil 1. Araştırmanın yapıldığı Kahta Çayı Havzası ve örnekleme noktaları (Elmastaş, 2008'den düzenlenmiştir)

Figure 1. Kahta Stream Basin and sampling points where the research was conducted (Elmastaş was composed in 2008) the research was conducted (Elmastaş was composed in 2008)

I. İstasyon (38°01'39.18"N ve 38°43'29.74"E) deniz seviyesinden 877 m yükseklikte bulunmakta olup, taban yapısı kayalardan ve taşlardan oluşmaktadır. Yatağı çok geniş olmayıp iki vadi arasından akmaktadır. Yer yer akarsu yamaçlarında tarım arazileri bulunmaktadır. II. İstasyon (38° 00'40.67"N ve 38°41'51.17"E) deniz seviyesinden 819 m yükseklikte yer almaktadır. Taban yapısı taş ve çakıllardan oluşmaktadır. Akarsu çevresi ağaçlarla kaplıdır. III. İstasyon (37°57'03.29"N ve 38°39'46.03"E) deniz seviyesinden 680 m yükseklikte bulunmaktadır. Taban yapısı kaya ve taşlardan oluşmaktadır. Yamaçları dik olup yatağı dardır. Cendere Çayı üzerinde Cendere Köprüsü mevkii IV. istasyon (37°55'58.04"N ve 38°36'31.34"E) olarak seçilmiştir. Örnekleme noktası deniz seviyesinden 615 m yükseklikte yer almaktadır. Taban yapısı taş, çakıl ve kumdan oluşmaktadır. Cendere Köprüsü'nden sonra akarsu yatağı genişlemekte ve eğimi oldukça azaldığından akış hızı yavaşlamakta olup sediment birikimi ortaya çıkmaktadır. Yaklaşık 4-4,5 km sonra Kahta Çayı ile birleşmektedir. V. İstasyon (37°52'03.03"N ve 38°37'07.73"E) deniz seviyesinden 555 m yükseklikte bulunmaktadır. Taban yapısı çakıl ve kumdan oluşmaktadır. Yatağı oldukça genişlemiş olup sediment birikimi fazladır. Cendere Çayı ve Kahta Çayı'nın sularının birleşmesiyle akan akarsu, örgülü bir ağ şeklinde geniş bir yatağa yayılarak Atatürk Baraj Gölü'ne boşalmaktadır.

Analiz metotları

Kahta Çayı'nda su sıcaklığı (T), çözünmüş oksijen (DO), pH, elektriksel iletkenlik (EC) ve toplam çözünmüş katı madde (TDS) Hach-HQ40d model multi-parametre ölçüm cihazıyla arazide ölçülmüştür. Su örnekleri 1,5 litrelik polietilen şişelere hava boşluğu kalmayacak şekilde doldurularak alınmıştır. Alınan örnekler gün içerisinde laboratuvara ulaştırılarak analizleri gerçekleştirilmiştir.

Bulanıklık HACH 2100P model türbidimetre ile laboratuvarda ölçülmüştür. Askıda katı madde (SS) tayini içerisine 0,45 µm filtre kağıdı yerleştirilmiş olan Gooch krozesinden belirli hacimdeki numune süzüldükten sonra Gooch krozesinin 100–105 ºC'de kurutularak tartılmasıyla gravimetrik olarak (TSE, 2007), toplam alkalinite (TA), titrasyon metodu ile ölçülmüştür (TSE, 1998).

Klorür (Cl^-), nitrat azotu (NO_3^--N) ve sülfat (SO_4^-) (TSE, 2012) Dionex ICS Model iyon kromatografi ile tayin edilmiştir.

Nitrit azotu (NO_2^--N), Hach-Lange LCK 341 nolu test kiti; amonyum azotu (NH_4^+-N), Hach-Lange LCK 304 nolu test kiti; çözünmüş reaktif fosfor (PO_4^{-3}-P) Hach-Lange LCK 349 nolu test kiti, silika (SiO_2), Hach-Lange PP 2429600 nolu test kiti ve kimyasal oksijen ihtiyacı (KOİ), Hach-Lange LCK 414 nolu test kiti ile Hach-Lange DR 5000 marka spektrofotometre kullanılarak tayin edilmiştir.

İstatistik analizler

Verilerin değerlendirilmesi ve kutu grafiklerin çiziminde GraphPad Prism 5.0 paket programı, verilerin istatistiksel

olarak analizinde ise IBM SPSS Statistic 22.0 paket programı kullanılmıştır. İncelenen parametreler arasındaki ilişki Pearson korelasyonuna göre yapılmıştır. Değişkenler ve mevsimler arasında bir fark olup olmadığını tespit etmek amacıyla parametrik değişkenler için tek yönlü varyans analizi, parametrik olmayan değişkenler için ise Kruskal-Wallis testi yapılmıştır. Ortalama değerler ± standart hata ile birlikte verilmiştir.

BULGULAR

Kahta Çayı'nı karakterize ettiği düşünülen beş istasyonda ölçülen bazı su kalite parametrelerinin aylık değişimi Şekil 2-5'de gösterilmiştir. Değişkenler arasındaki ilişki Pearson korelasyon analizi ile yapılmış olup sonuçlar Tablo 1'de verilmiştir.

Su sıcaklığı en düşük Aralık 2012'de 5,0 °C, en yüksek ise Temmuz 2012'de 25,2 °C ölçülmüş olup, yıllık ortalama değer 13,23±0,65 °C olarak bulunmuştur (Şekil 2). Mevsimler arasındaki fark önemli bulunmuştur ($F_{(3-16)}$=19,934; p=0,000; r=0,89; p<0,05).

Çözünmüş oksijen konsantrasyonu en düşük Eylül 2012'de 8,67 mg/L, en yüksek ise Nisan 2012'de 13,36 mg/L olarak kaydedilmiş olup, yıllık ortalama değer 10,62±0,14 mg/L olarak hesaplanmıştır (Şekil 2). Mevsimler arasındaki fark önemli bulunmuştur ($F_{(3-16)}$=8,460; p=0,001; r=0,87; p<0,05).

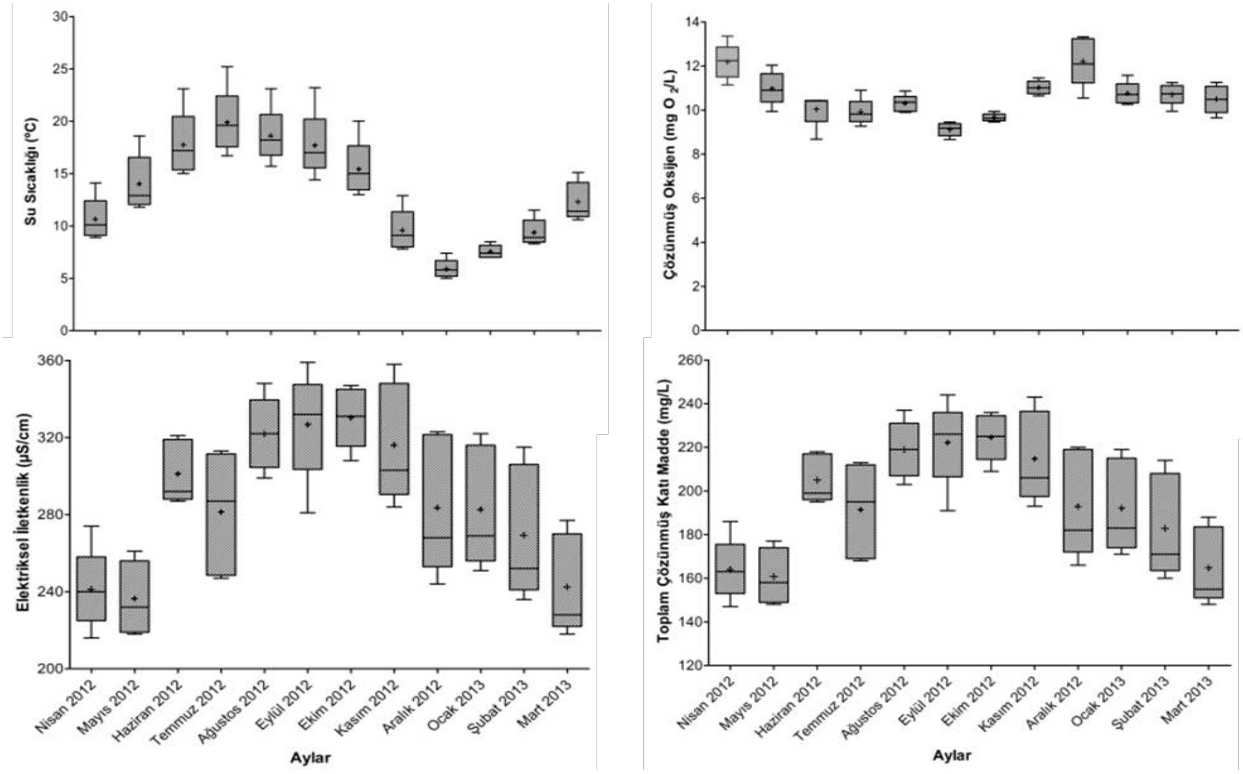
Elektriksel iletkenlik değerleri en düşük Mayıs 2012'de 216 µS/cm, en yüksek ise Eylül 2012'de 359 µS/cm kaydedilmiştir. Yıllık ortalama değer 287±5,42 µS/cm olarak hesaplanmıştır (Şekil 2). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($F_{(3-16)}$=16,672; p=0,000; r=0,95; p<0,05).

Toplam çözünmüş katı madde konsantrasyonu en düşük Nisan 2012'de 147 mg/L, en yüksek ise Eylül 2012'de 244 mg/L ölçülmüş olup, yıllık ortalama değer ise 194,53±3,58 mg/L olarak kaydedilmiştir (Şekil 2). Mevsimler arasındaki fark önemli bulunmuştur ($F_{(3-16)}$=16,672; p=0,000; r=0,87; p<0,05).

Askıda katı madde değerleri en düşük Ekim 2012'de 2 mg/L, en yüksek ise Nisan 2012'de 138 mg/L olarak tayin edilmiştir. Yıllık ortalama değer 47,96±5,06 mg/L olarak hesaplanmıştır (Şekil 3). Mevsimler arasındaki fark önemli bulunmuştur (χ^2(3)=16,760; p=0,001; p<0,05).

Bulanıklık değerleri en düşük Ekim 2012'de 0,92 NTU, en yüksek ise Ocak 2013'te 96,70 NTU olarak tespit edilmiştir (Şekil 3). Yıllık ortalama değer 12,34±2,24 olarak bulunmuştur. Mevsimler arasındaki fark önemli bulunmuştur (χ^2(3)=15,823; p=0,001; p<0,05).

pH değerleri en düşük Mayıs 2012'de 7,08 en yüksek ise Mart 2013'te 8,76 olarak ölçülmüştür. Yıllık ortalama değer 8,20±0,05 olarak hesaplanmıştır (Şekil 3). Mevsimler arasındaki fark önemli bulunmuştur (χ^2(3)=12,897; p=0,005; p<0,05).



Şekil 2. Kahta Çayı'ndaki su sıcaklığı, çözünmüş oksijen, elektriksel iletkenlik ve toplam çözünmüş katı madde değerlerinin aylık değişimi

Figure 2. Monthly change of water temperature, dissolved oxygen, electrical conductivity and total dissolved solids values in Kahta Stream

Toplam alkalinite konsantrasyonları en düşük Mart 2013'te 99 mg $CaCO_3$/L, en yüksek ise Kasım 2012'de 200 mg $CaCO_3$/L olarak tespit edilmiştir. Yıllık ortalama değer 127,17±3,08 mg $CaCO_3$/L olarak hesaplanmıştır (Şekil 3). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($\chi^2(3)=14,701$; $p=0,002$; $p<0,05$).

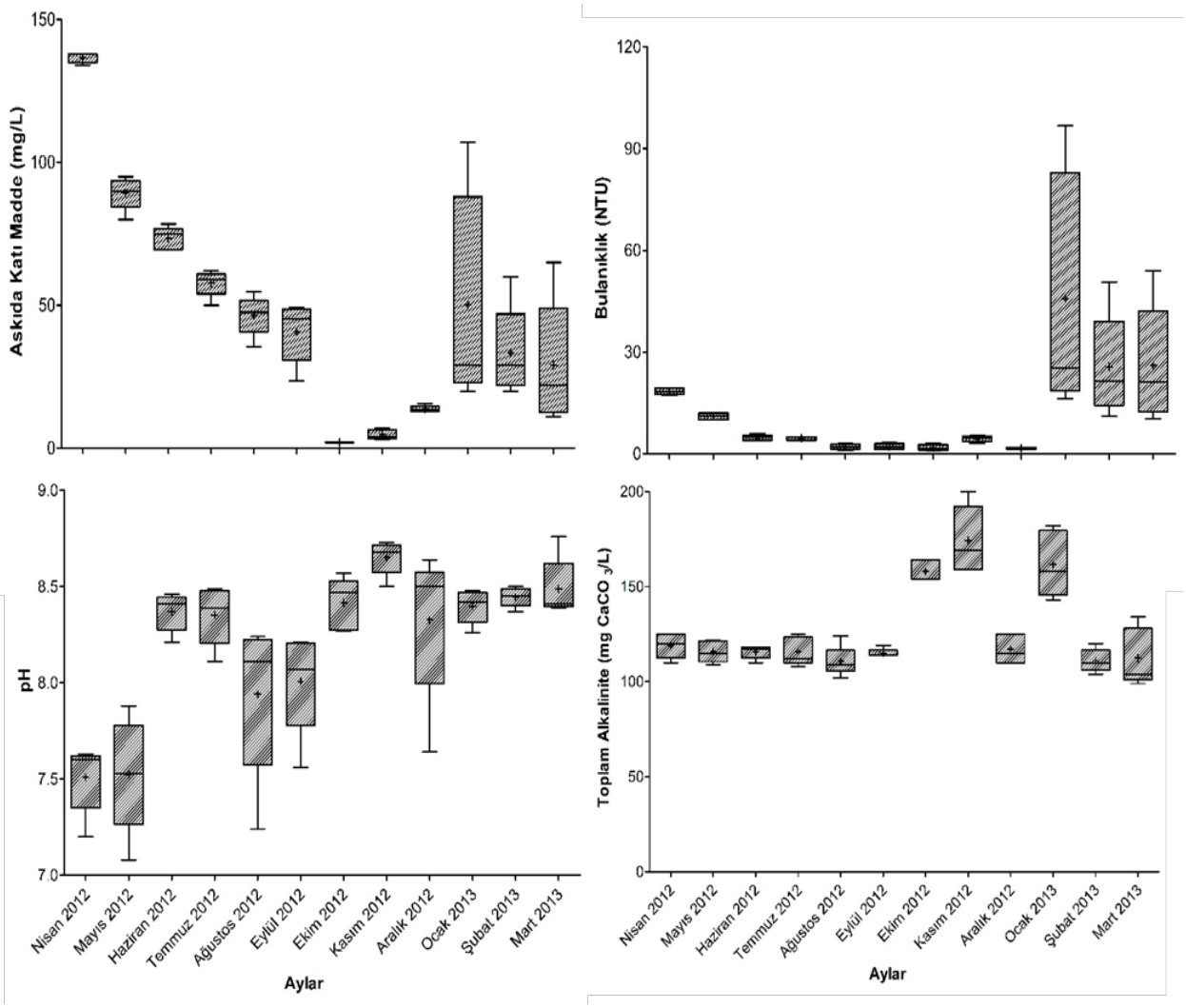
Klorür değerleri en düşük Mart 2013'te 0,71 mg Cl^-/L, en yüksek ise Eylül 2012'de 5,02 mg Cl^-/L olarak kaydedilmiş olup, yıllık ortalama değer 2,89±0,15 mg Cl^-/L olarak hesaplanmıştır (Şekil 4). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($F_{(3-16)}=100,902$; $p=0,000$; $r=0,97$; $p<0,05$).

Amonyum azotu değerleri en düşük Haziran 2012'de 0,007 mg NH_4^+-N/L, en yüksek ise Mayıs 2012'de 0,400 mg NH_4^+-N/L olarak bulunmuştur. Yıllık ortalama değer 0,07±0,01 mg NH_4^+-N/L olarak hesaplanmıştır (Şekil 4). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($\chi^2(4)=17,596$; $p=0,001$; $p<0,05$).

Nitrit azotu değerleri en düşük Haziran ve Temmuz 2012'de 0,003 mg NO_2^--N/L, en yüksek ise Ocak 2013'de 0,060 mg NO_2^--N/L olarak kaydedilmiş olup yıllık ortalama değer 0,02±0,002 mg NO_2^--N/L olarak hesaplanmıştır (Şekil 4). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($\chi^2(3)=16,476$; $p=0,001$; $p<0,05$).

Nitrat azotu konsantrasyonları en düşük Mayıs 2012'de 0,12 NO_3^--N/L, en yüksek ise Eylül 2012'de 1,19 NO_3^--N/L olarak tayin edilmiş olup, yıllık ortalama değer 0,58±0,03 mg NO_3^--N/L olarak hesaplanmıştır (Şekil 4). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($F_{(3-16)}=7,272$; $p=0,003$; $r=0,76$; $p<0,05$).

Çözünmüş reaktif fosfor değerleri en düşük Nisan 2012'de 0,007 mg PO_4^{-3}-P/L, en yüksek ise Aralık 2012'de 0,033 mg PO_4^{-3}-P/L olarak kaydedilmiştir. Yıllık ortalama değer 0,015±0,001 mg PO_4^{-3}-P/L olarak hesaplanmıştır (Şekil 5). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($\chi^2(3)=14,008$; $p=0,003$; $p<0,05$).

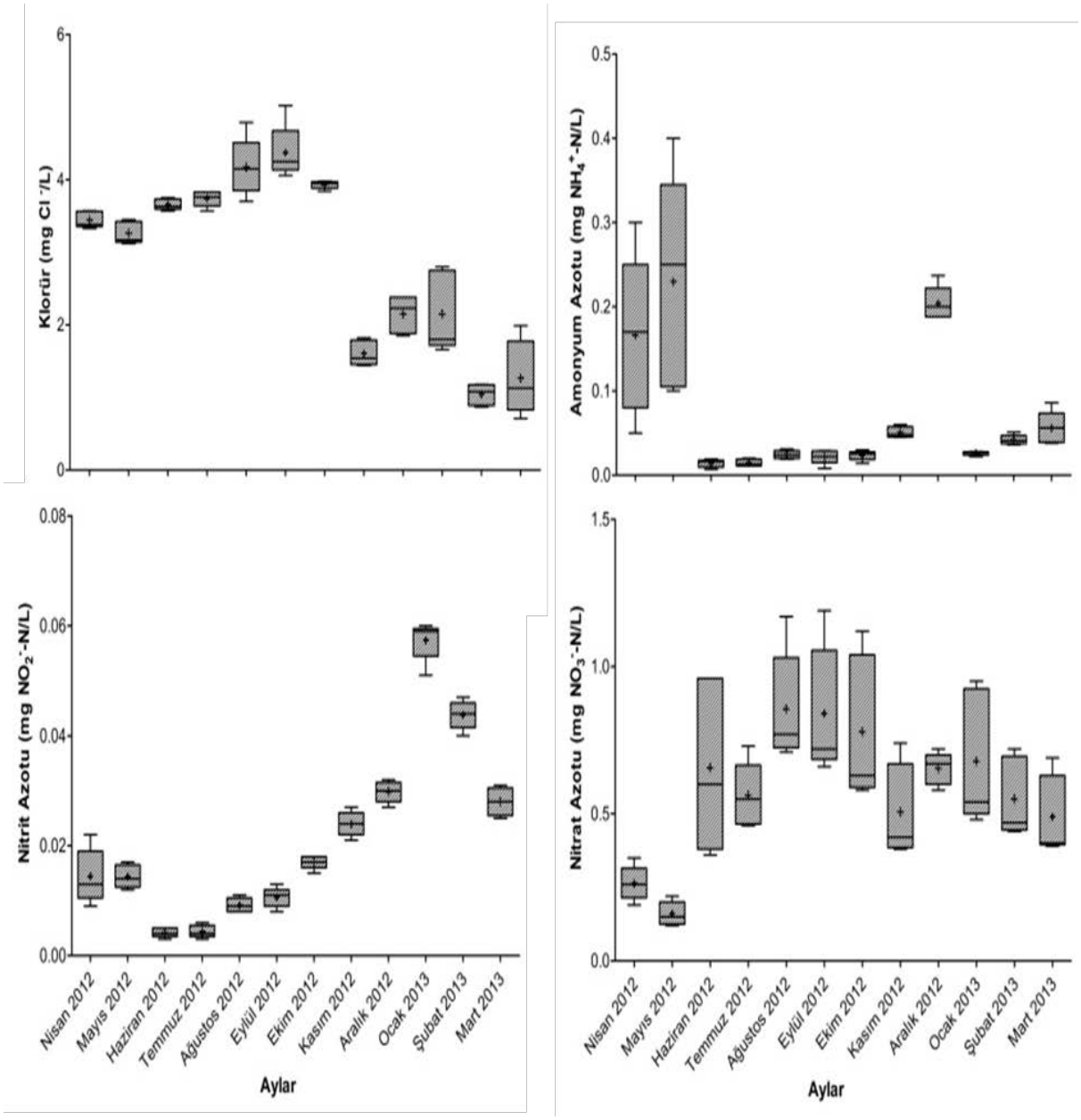


Şekil 3. Kahta Çayı'ndaki askıda katı madde, bulanıklık, pH ve toplam alkalinite değerlerinin aylık değişimi
Figure 3. Monthly change of suspended solids, turbidity, pH and total alkalinity values in Kahta Stream

Sülfat konsantrasyonu en düşük Mart 2013'te 8 mg SO_4^{-2}/L, en yüksek ise Aralık 2012'de 35 mg SO_4^{-2}/L olarak kaydedilmiş olup, yıllık ortalama değer 18,28±0,79 mg SO_4^{-2}/L olarak hesaplanmıştır (Şekil 5). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($\chi^2(3)=11,274$; p=0,010; p<0,05).

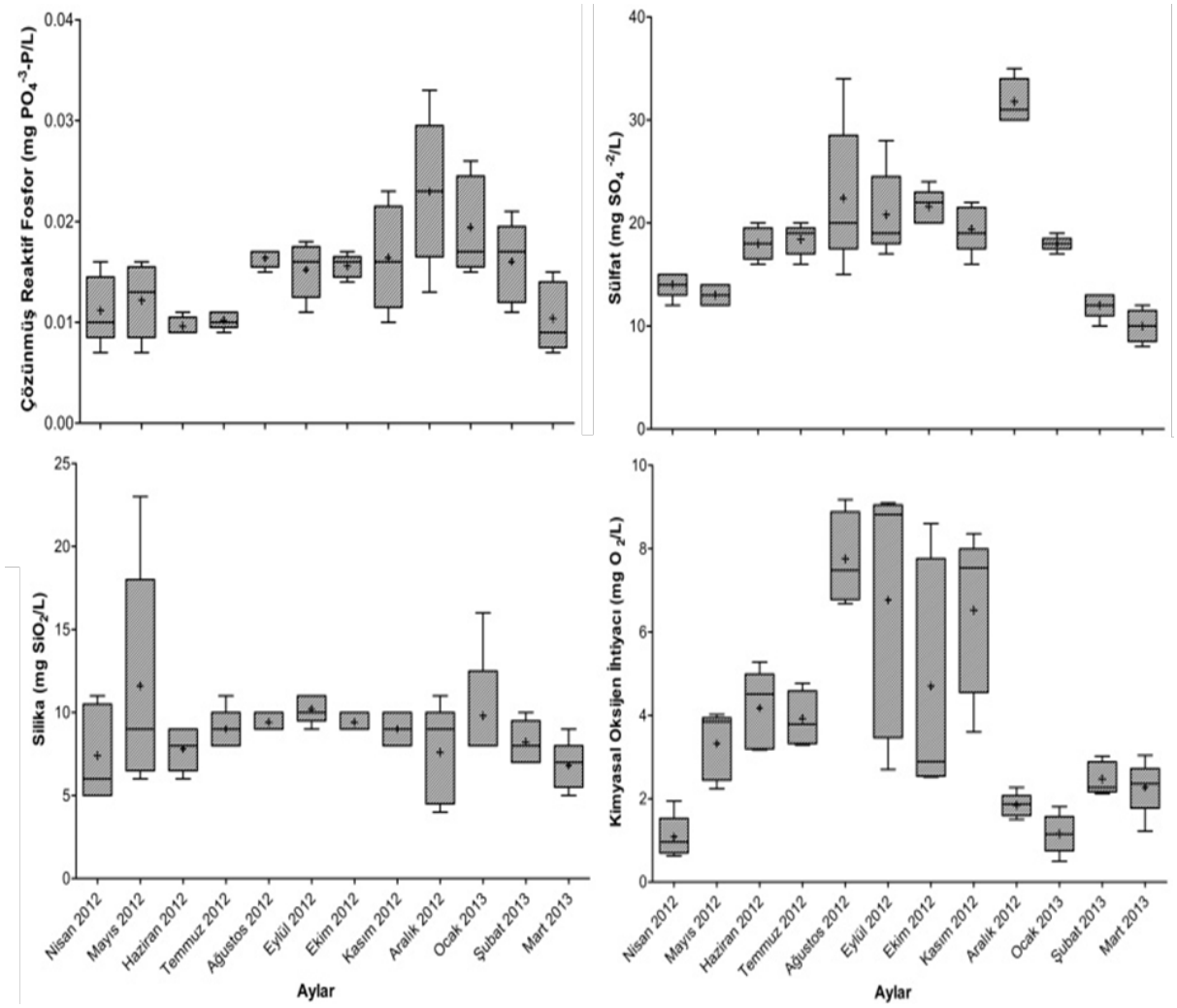
Silika konsantrasyonu en düşük Mart 2013 ve Nisan 2012'de 5 mg SiO_2/L, en yüksek ise Mayıs 2012'de 23 mg SiO_2/L olarak kaydedilmiştir. Yıllık ortalama değer 8,85±0,35 mg SiO_2/L olarak hesaplanmıştır (Şekil 5). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmamıştır ($F_{(3-16)}=0,536$; p=0,664; r=0,30; p>0,05).

Kimyasal oksijen ihtiyacı değerleri en düşük Nisan 2012'de 0,63 mg/L, en yüksek ise Ağustos 2012'de 9,18 mg/L olarak tayin edilmiştir. Yıllık ortalama değer 3,83±0,32 mg/L olarak bulunmuştur (Şekil 5). Mevsimler arasındaki fark istatistiksel açıdan önemli bulunmuştur ($\chi^2(3)=14,840$; p=0,002; p<0,05).



Şekil 4. Kahta Çayı'ndaki klorür, amonyum azotu, nitrit azotu ve nitrat azotu konsantrasyonlarının aylık değişimi

Figure 4. Monthly changes in chloride, ammonium nitrogen, nitrite nitrogen and nitrate nitrogen concentrations in Kahta Stream



Şekil 5. Kahta Çayı'ndaki çözünmüş reaktif fosfor, sülfat, silika ve kimyasal oksijen ihtiyacı konsantrasyonlarının aylık değişimi
Figure 5. Monthly change of dissolved reactive phosphorus, sulfate, silica and chemical oxygen demand concentrations in Kahta Stream

Tablo 1. Kahta Çayı'nda izlenen su kalitesi değişkenleri arasındaki Pearson korelasyon analizi
Table 1. Pearson correlation analysis among water quality variables in Kahta Stream

	T	EC	TDS	SS	**Bulanıklık**	pH	DO	TA	Cl^-	NO_3^--N	NO_2^--N	$NH4^+$-N	PO_4^{-3}-P	SiO_2	SO_4^{-2}	**KOİ**
T	1															
EC	0,295*	1														
TDS	0,322*	0,986**	1													
SS	0,149	-0,376**	-0,374**	1												
NTU	-0,306*	-0,150	-0,167	0,334**	1											
pH	-0,182	0,148	0,141	-0,657**	0,025	1										
DO	-0,689**	-0,409**	-0,424**	0,212	0,063	-0,225	1									
TA	-0,252	0,334**	0,359**	-0,336**	0,192	0,312*	-0,033	1								
Cl^-	0,693**	0,335**	0,381**	-0,149	-0,338**	-0,456**	-0,404**	-0,143	1							
NO_3^-N	0,317*	0,716**	0,726**	0,349**	0,007	0,158	-0,439**	0,186	0,274*	1						
NO_2^--N	-0,721**	-0,150	-0,184	-0,377**	0,616**	0,315*	0,269*	0,357**	-0,721**	0,006	1					
NH_4^+-N	-0,368**	-0,410**	-0,414**	-0,275*	-0,024	-0,407**	0,596**	-0,175	-0,103	-0,485**	-0,008	1				
PO_4^{-3}-P	-0,394**	0,263*	0,244	0,329*	0,166	0,061	0,173	0,268*	-0,170	0,284*	0,439**	0,073	1			
SiO_2	0,181	0,242	0,237	-0,326*	0,044	-0,311*	-0,124	0,157	0,242	0,114	-0,050	-0,044	0,168	1		
SO_4^{-2}	-0,067	0,484**	0,519**	0,016	-0,351**	0,073	0,051	0,154	0,276*	0,429**	-0,099	0,064	0,482**	0,038	1	
KOİ	0,453**	0,505**	0,534**	-0,357**	-0,450**	0,070	-0,470**	0,079	0,416**	0,308*	-0,441**	-0,345**	-0,028	0,196	0,249	1

* : p<0,05; **: p<0,01

TARTIŞMA

Su sıcaklığının azalıp artmasına etki eden faktörler arasında enlem, yükseklik, mevsimler, atmosfer şartları, akıntı hızı ve akarsu yatağının önemli yer tuttuğu (Chapman ve Kimstach, 1996; USEPA, 1997; Taşdemir ve Göksu, 2001; Hauraki District Council, 2003) Kahta Çayı'nda sonbahar sonu ve kış başlangıcında oldukça azalan su sıcaklıkları, ilkbahardan itibaren havaların ısınmasına paralel olarak artmış ve yaz ortasında en yüksek seviyesine ulaşmıştır. Ayrıca genel olarak aynı aylar içerisinde üst akarsu bölgesinden alt akarsu bölgesine gidildikçe rakıma, suyun akış hızına ve akarsu yatağının genişlemesine bağlı olarak (Şen ve Gölbaşı, 2008; Zeybek ve Kalyoncu, 2016) su sıcaklıklarında azalıp ve artmalar meydana gelmiştir.

Doğal suların oksijen içeriği sıcaklık, tuzluluk, su karışımları, atmosferik basınç, fotosentez aktiviteleri ve bazı biyolojik aktiviteler nedeniyle değişmektedir (Chapman ve Kimstach, 1996; Webb ve Walling, 1992). Soğuk sular daha fazla oksijen tutma kapasitesine sahip olduğundan, akarsularda çözünmüş oksijen konsantrasyonlarının kışın daha yüksek, yazın ise daha düşük olduğu ifade edilmiştir (Hem, 1985).

Kahta Çayı'nda çözünmüş oksijen ile sıcaklık ve çözünmüş katı madde arasında negatif yönde ($p<0,01$) bir ilişki bulunmuş olup, düşük çözünmüş oksijen konsantrasyonlarına çözünmüş katı maddenin ve kimyasal oksijen ihtiyacının yüksek olduğu sonbahar başlangıcında rastlanırken, yüksek konsantrasyonlara ise sıcaklığın nispeten düşük, akış hızı ve yüzey suyu havalanmasının yüksek olduğu ilkbahar aylarında rastlanılmıştır.

Elektriksel iletkenliğin, su akışları vasıtasıyla bölgenin jeolojisi tarafından birinci derecede etkilendiği ve sıcaklığa bağlı olarak azalıp artığı (USEPA, 1997); bir akarsuyun kondüktivitesinin ani artışının bölgede bulunan çözünmüş iyonların artışıyla ilgili olduğu (Vaishali ve Punita, 2013) ve elektriksel iletkenliğin akım ile ters, sıcaklık ve çözünmüş katı madde miktarıyla ise doğru orantılı olarak değiştiği (Şen ve Gölbaşı, 2008) rapor edilmiştir.

Kahta Çayı'nda ilkbahar aylarında yağışların artmasıyla birlikte çözünmüş maddelerin akarsu içinde seyrelmesi ile elektriksel iletkenlik değeri azalırken, yağışların durduğu ve yüksek sıcaklığın devam ettiği sonbahar aylarında ise artmıştır. Ayrıca, sıcaklık ($p<0,05$) ve çözünmüş katı maddeyle ($p<0,01$) arasında pozitif yönde bir ilişki bulunmuştur. Çalışmamızın bulguları USEPA (1997), Vaishali ve Punita (2013), Şen ve Gölbaşı (2008)'nın yaptıkları çalışmaların sonuçlarıyla benzerlik göstermiştir.

Toplam çözünmüş katı maddelerle ilgili olarak göl ve akarsu karakteristikleri arasındaki başlıca farkların, maddelerin akarsu boyunca dağılımı, bileşimi ve nispi konsantrasyonuyla ilgili olduğu ve drenaj havzasının jeokimyasal yapısının yanı sıra yüzey akışlardaki ve düşen yağış miktarındaki mevsimsel değişimlerin de akarsuların bileşimini etkilediği bildirilmiştir (Reid, 1961; Jain, 2002). Kahta Çayı'nda da benzer sonuçlar bulunmuş olup, yağışların ve yüzey akışların artmasıyla birlikte düşük değerler ilkbahar aylarında, yüksek değerler ise akımın azaldığı ve sıcaklığın arttığı yaz sonu ve sonbahar aylarında kaydedilmiştir.

Allan (1995) ve Lewis vd. (2002), askıda katı madde miktarlarının akarsu akımının düşmesi ve yükselmesi ile azalıp arttığını ve askıda katı madde miktarında görülen farklılıkların havzanın eğiminden, jeolojisinden, toprak yapısından, bitkilerden ve kara kullanımından kaynaklandığını bildirmişlerdir. Çalışmamızda düşük değerlere yağışların tamamen durduğu sonbahar aylarında, yüksek değerlere ise yağışların ve yüzey akışların devam ettiği ilkbahar aylarında rastlanılmıştır. Elde edilen bulgular, bu araştırmacıların bulgularıyla benzerlik göstermiştir.

Webb ve Walling (1992), bulanıklık ile askıda katı madde arasında pozitif bir ilişkinin olduğunu; Dodds (2002), akarsularda yağışlardan sonra bulanıklığın arttığını ve bunun karadan sediment girişinin artışına bağlanabileceğini belirtmişlerdir. Kahta Çayı'nda genellikle düşük değerleri yağışların tamamen durduğu yaz sonu ve sonbahar aylarında, yüksek değerler ise yağışların etkisiyle ilkbahar ve kış aylarında kaydedilmiştir. Ayrıca, askıda katı madde ile arasında pozitif ($p<0,01$) bir ilişki bulunmuş olup ve yapılan çalışmaların bulgularıyla benzerlik göstermiştir.

Hauraki District Council (2003), suyun pH seviyesinin önemli ölçüde akarsu havzasının toprak yapısı ve jeolojisinden etkilendiğini ve genellikle pH aralığının 6,5-8,5 arasında değiştiğini kaydetmiştir. Meybeck ve Helmer (1989), volkanik kaya tipi havzaya sahip akarsular için pH değerlerinin genel olarak 7,2 ve kireç taşı tipi havzaya sahip akarsular için ise 7,9 olduğunu ifade etmişlerdir.

Kahta Çayı ortalama pH değeri 8,20 olarak hesaplanmış olup, alkali su sınıfına girmektedir. Akarsu havzasının karstik bir yapıya sahip olması bunda oldukça etkili olmuştur. Ölçülen pH değerleri Meybeck ve Helmer (1989)'in bulgularıyla benzerlik göstermektedir.

Bir suyun alkalinitesi, o suyun asitleri nötralize edebilme kapasitesi olarak tanımlanır. Tatlı sularda başlıca bikarbonat, karbonat ve hidroksil iyonlarından kaynaklandığı ifade edilmiştir (Wetzel, 1975).

Kahta Çayı'nda toplam alkalinite değerleri sonbahar ve kış aylarında diğer aylara nazaran daha yüksek bulunmuştur. Elektriksel iletkenlik ve toplam çözünmüş katı madde ile arasında pozitif yönde ($p<0,01$), askıda katı madde arasında ise negatif yönde ($p<0,01$) bir ilişki bulunmuş ve bu ilişki yapılan diğer araştırmaların (Şen vd., 2002; Tepe vd., 2006; Şen ve Gölbaşı, 2008) sonuçlarıyla paralellik göstermiştir. Gedik vd. (2010), doğal suların alkalinite değerlerinin su

havzasının jeolojisiyle yakından ilişkili olduğunu ve 5-500 mg $CaCO_3$/L arasında değiştiğini ifade etmişlerdir. Bu bulgu, yaptığımız çalışmamızdaki toplam alkalinite konsantrasyonları ile ilgili bulguları desteklemektedir.

Samsunlu (1999), sularda klorür içeriğinin normal olarak mineral içeriğinin artması ile arttığını ve dağlık alanlardaki su kaynaklarının çok düşük klorür konsantrasyonları içerdiğini ifade etmiştir. Allan (1995), dünya nehirlerinin klorür içeriğinin ortalamasını kirlenmemiş doğal sularda 5,8 mg/L olarak, insan aktivitelerinden etkilenen nehirlerin ortalamasını ise 8,3 mg/L olarak bildirmiştir.

Kahta Çayı'nda düşük klorür değerlerine yüzey akışların devam ettiği kış sonu ve ilkbahar başlangıcında, yüksek değerlere ise yüzey akışların tamamen durduğu, buharlaşmanın ise yüksek olduğu yaz sonu ve sonbahar başlangıcında rastlanılmıştır. Samsunlu (1999) ve Allan (1995)'ın bulguları, çalışmamızın bulguları ile benzerlik göstermiş olup, Kahta Çayı'nın kirlenmemiş doğal akarsu sınıfına girdiğini göstermektedir.

Wetzel ve Likens (1991), çoğu alkalin (pH> 9) şartlar hariç, tatlı sularda amonyağın (NH_3) çoğunun iyonik formda (NH_4^+) bulunduğunu ve amonyumun akarsularda ve göllerde yüksek yapılı bitkiler, algler ve bakteriler için azotun önemli bir kaynağını oluşturduğunu bildirmişlerdir. Vaishali ve Punita (2013), doğal olarak kirlenmemiş nehirlerde amonyum konsantrasyonun genellikle kışın yüksek olduğunu ve nehirlerdeki nitrifikasyon işlemlerinin yüksek yaz sıcaklıklarından daha çok etkilendiğini ifade etmişlerdir. OWW (2005), güçlü yüzey akışların olduğu dönemlerde toprak partikülleri tarafından adsorbe edilmiş amonyumun akarsular ve göller içine taşındığını rapor etmiştir.

Kahta Çayı'nda nitrifikasyonun bir sonucu olarak, amonyum azotu ile sıcaklık ve nitrat azotu arasında negatif yönde ($p<0,01$) bir ilişki bulunmuş olup, en yüksek değerlere kış ve ilkbahar aylarında, en düşük konsantrasyonlara ise yaz ve sonbahar aylarında rastlanılmıştır. Bu bulgular, Vaishali ve Punita (2013) ve OWW (2005)'nin sonuçları ile paralellik göstermiştir.

Nitrit, nitrat ve amonyum arasında bir ara ürün olup diğerlerine nazaran kararlı değildir. İyi oksijenlenmiş sularda nitrit oldukça düşük seviyelerde bulunur. Amonyumca zengin atık suların alıcı ortamlara boşalması sonucu artış gösterir ve bu özelliğinden dolayı sularda kirlilik indikatörü olarak kullanılır (Hauraki District Council, 2003).

Kahta Çayı'nda nitrit azotu ile sıcaklık arasında negatif yönde ($p<0,01$) bir ilişki bulunmuş ve genellikle düşük değerler yaz ve sonbahar aylarında, yüksek değerler ise kış ve ilkbahar aylarında kaydedilmiştir. Varol (2004)'un, Hazar Gölü'ne dökülen Behrimaz Çayı'nda en düşük nitrit azotu konsantrasyonlarını akışın az olduğu aylarda, en yüksek konsantrasyonları ise akışın yüksek olduğu aylarda; Sönmez ve Battal (2017), Han Çayı'nda yaptıkları çalışmada en yüksek nitrit değerini kış ayı olan aralık ayında tespit etmiş olmaları çalışmamızın bulgularını desteklemektedir.

Eberhardt ve Larson (2000), nitratın nehir ve akarsularda topraktan, hayvan ve bitki atıklarından, atık sulardan ve gübrelerden kaynaklandığına; Chapman ve Kimstach (1996), nitrat konsantrasyonun mevsimsel dalgalanmasının ekosistemdeki bitkilerin gelişimi ve bozulmasıyla doğrudan etkilendiğine işaret etmişlerdir.

Yapılan araştırmalarda (Taşdemir ve Göksu, 2001; Boran ve Sivri, 2001; Tepe vd., 2006; Şen ve Gölbaşı, 2014) nitrat azotu değerlerinin düşük sıcaklıklarda ve yağışların - karların erime dönemi olan ilkbahar aylarında tarımsal arazilerden gelen gübrelerin karışmasıyla artış gösterdiği, buna karşın sıcaklığın artmasıyla birlikte yüzey akışların azalmasının bir sonucu olarak organik madde girdisinin azalması ve alg gelişimine bağlı olarak ise nitrat kullanımının fazla olmasından dolayı düzenli bir şekilde azalma gösterdiği bildirilmiştir. Kahta Çayı'nda nitrat konsantrasyonunun ilkbahar aylarında düşük, yaz sonu ve sonbaharda yüksek değerlerde kaydedilmiş olması yukarıdaki çalışmaların bulgularıyla uyumlu olmamıştır. Bununla birlikte bu bulgu, Mutlu vd. (2016)'nin, Çınarlı Çayı'nda nitratın mevsimsel değişimiyle ilgili sonuçlarına benzerlik göstermektedir.

Jain (2002), çözünmüş reaktif fosfor miktarının gübrelerin yanı sıra toprak yapısının bozulması ve aşınmasıyla arttığını; Bordalo vd. (2001), fosfor değerlerinin yağışlı mevsimlerde yüksek, kurak mevsimde ise düşük olduğunu ifade etmişlerdir. Bu çalışmaların sonuçları Kahta Çayı'nın bulguları ile paralellik göstermiş olup, akarsu boyunca yüksek reaktif fosfor değerlerine sonbahar ve kış ayalarında, düşük değerlere ise ilkbahar ve yaz aylarında rastlanılmıştır. Çözünmüş reaktif fosfordaki mevsimsel dalgalanmaların fosfatlı gübreler ve biyolojik olaylar ile ilgili olduğu düşünülmektedir. Meybeck ve Helmer (1989), kirlenmemiş nehirlerde ortalama reaktif fosfor (PO_4^{-3}-P) konsantrasyonunu 0,01 mg/L olarak bildirmişlerdir. Araştırmamızda ortalama reaktif fosfor konsantrasyonlarının 0,015 mg/L olarak hesaplanmış olup Meybeck ve Helmer (1989)'in bildirdiği değerlere oldukça yakın bulunmuştur.

Allan (1995), silikanın genellikle silikat taşlarının aşınmasıyla oluştuğunu ve bu yüzden konsantrasyonların bölgenin jeomorfolojisi ile değiştiğini bildirmiştir. Hem (1985), doğal sularda silika konsantrasyonlarının değişim aralığını 1-30 mg/L olduğunu ve yüzey suları için ortalama silika değerini ise 14 mg/L olarak bildirmiştir. Kahta Çayı'ndaki silika konsantrasyonları tüm istasyonlarda Hem (1985)'in bildirdiği sınırlar içerisinde bulunmuştur. Havza jeolojisine bağlı olarak düşük silika konsantrasyonlarının kireç taşı yapıdaki havzaya sahip akarsularda, yüksek silika konsantrasyonlarının ise granit ve bazalt yapıda havzaya sahip akarsularda ortaya çıktığı (Meybeck, 1986) düşünüldüğünde, kireç taşı tipi bir

havzaya sahip olan Kahta Çayı'nda görülen düşük silika konsantrasyonunun havza jeolojisiyle ilgili olduğu anlaşılmaktadır.

Allan (1995), sulardaki sülfatın kirlilik ve sedimentte bulunan taşların aşınmasıyla kaynaklanabileceğini ifade etmiştir. Wetzel (1975), sularda sülfat miktarının 5-30 mg SO_4^{-2}/L arasında değiştiğini ve ortalama değerin ise 11 mg SO_4^{-2}/L olduğunu bildirmiştir.

Kahta Çayı'ndaki sülfat miktarı en yüksek (35 mg SO_4^{-2}/L) seviyesine kış başlangıcında ulaşmıştır. Bu sonuç tarımsal amaçlı kullanılan sülfatlı gübrelerin yüzey akışlar vasıtasıyla suya karışmasıyla açıklanabilir. Kahta Çayı'nın ortalama sülfat konsantrasyonu (18,28 mg SO_4^{-2}/L), bildirilen ortalama değerlerden yüksek olmasına rağmen, Aralık 2013 ayı hariç tespit edilen sülfat miktarları Wetzel (1975)'in bildirdiği sınırlar içerisinde kalmıştır.

Chapman ve Kimstach (1996), kirletilmemiş yüzey sularında KOİ konsantrasyonun 20 mg/L civarında iken, atık su deşarjı yapılan sularda ise 200 mg/L ve üzerinde olduğunu bildirmiştir.

Kahta Çayı'nda, organik maddenin parçalanması sırasında oksijen tüketilmesinin (Uslu ve Türkman, 1987) bir sonucu olarak, KOİ konsantrasyonları ile çözünmüş oksijen arasında negatif yönde ($p<0,01$), sıcaklık arasında ise pozitif yönde ($p<0,01$) bir ilişki bulunmuş olup, düşük KOİ değerlerine kış ve ilkbahar aylarında rastlanırken, yüksek değerlere ise yaz ve sonbahar aylarında rastlanılmıştır. Tespit edilen değerler, Chapman ve Kimstach (1996)'ın bildirdiği kirlenmemiş sulardaki KOİ konsantrasyonunun altında bulunmuştur.

Yaptığımız çalışmada, Yerüstü Su Kalitesi Yönetmeliği (T.C. Resmi Gazete, 2012), Kıtaiçi Yerüstü Su Kaynaklarının Genel Kimyasal ve Fizikokimyasal Parametreler Açısından Sınıflarına Göre Kalite Kriterleri dikkate alındığında; pH, iletkenlik, çözünmüş oksijen, amonyum azotu, nitrat azotu, reaktif fosfor ve kimyasal oksijen ihtiyacı parametrelerinin ortalama değerleri bakımından, hafif alkali karaktere sahip Kahta Çayı'nda izlenen örnekleme noktalarının I. Sınıf Su Kalitesine sahip olduğu belirlenmiştir. Bu karakteristiğiyle Kahta Çayının içme suyu temini için kullanılma potansiyelinin yüksek olduğu, rekreasyonel amaçlı kullanım, alabalık yetiştiriciliği, hayvan üretimi ve diğer çiftlik ihtiyaçları için kullanılabilir olduğu sonucuna ulaşılmıştır. Ayrıca değişkenlerin, zamansal ilişkisi istatistiksel açıdan incelendiğinde, SiO_2 hariç diğer tüm değişkenler arasında anlamlı bir fark bulunmuştur ($p<0,05$). Bu sonuç, değişkenlerin mevsim değişikliklerinden etkilendiğini ortaya koymaktadır.

TEŞEKKÜR

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RESEARCH ARTICLE
ARAŞTIRMA MAKALESİ

Assessment of environmental applicability of TiO_2 coated self-cleaning glass for photocatalytic degradation of estrone, 17β-estradiol and their byproducts

Golnar Matin[1*] ● Ali Reza Amani-Ghadim[2] ● Amir Abbas Matin[3] ● Navid Kargar[4] Hasan Baha Buyukisik[5]

[1] Department of Marine-Inland Water Sciences and Technology, Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey https://orcid.org/0000-0002-1286-8611
[2] Department of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University, 53714-161 Tabriz, Iran https://orcid.org/0000-0003-3279-5170
[3] Department of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University, 53714-161 Tabriz, Iran https://orcid.org/0000-0001-8264-8414
[4] Department of Marine-Inland Water Sciences and Technology, Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey https://orcid.org/0000-0002-6939-0284
[5] Department of Marine-Inland Water Sciences and Technology, Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey https://orcid.org/0000-0002-5855-4300

Corresponding author: golnarmatin@gmail.com
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Abstract: Optimization of photocatalytic degradation of two natural estrogenic compounds, estrone (E1) and 17β-estradiol (17β-E2) in aqueous medium was performed on TiO_2 coated Pilkington Activ™ self-cleaning glass as a novel approach to eliminate free nano-TiO_2 releasing to the intended environment after treatment. The active glass was characterized by Atomic Force Microscopy (AFM), X-ray diffraction (XRD), and Raman spectroscopy to characterize the TiO_2 nanoparticles. The main purposes were mineralization of target compounds in the treated water during the photocatalytic reaction and also to investigate the oxidation by products. Response Surface Methodology (RSM) has been applied to optimize the photocatalytic degradation by changing time, pH, and light intensity as effective factors. According to the results, time was the more effective parameter. The maximum efficiency degradation was achieved in alkaline media. Due to interactive effects between variable factors, 1 mg/L aqueous solution of E1 and 17β-E2 in water was totally decomposed by TiO_2 photocatalyzed reactions under UV-C irradiation of 10.08 W/m² for 52.49 min at pH 9.42. Results of GC-MS analysis were introduced 17-deoxy Estrone and 2-Hydroxyestradiol as intermediate products for E1 and 17β-E2, respectively. All of the peaks finally disappeared after 170 min. Optimized conditions were applied for real sample of wastewater, presenting 30.40% and 56.84% in the efficiency degradation of E1 and 17β-E2, respectively.

Keywords: Photocatalytic degradation, TiO_2, Endocrine Disrupting Chemicals (EDCs), Estrone, 17β-estradiol, Response surface methodology

INTRODUCTION

Endocrine Disrupting Chemicals (EDCs) comprise a group of active organic compounds which interfere with regular function of the hormonal system in the organisms (Deguchi et al., 2008; Noppe et al., 2008). EDCs are exogenous biologically active compounds by both natural and anthropogenic origins. Human's body, animals, also plants and fungi synthesize biologically derived estrogens. Synthetic EDCs can be found as a constituent component of various daily used products like plasticizers, pesticides, herbicides, detergents, pharmaceuticals, personal care products, and others (Iwanowicz & Blazer, 2011; Song et al., 2018; X. Zhang et al., 2009). Estrogenic Endocrine Disrupting Chemicals (EEDCs) have emerged as concern chemicals over the last two decades (Hamid & Eskicioglu, 2013; Mockler et al., 2017; Wert et al., 2009). Lipophilic character and long biological half-life of these group of chemicals make them more stable and easily accumulated in organisms body (Adeel et al., 2017; Cai et al., 2015). Exposure of aquatic organisms to EEDCs leads to inappropriate genetic induction for estrogen sensitive genes, abnormal development of reproductive organs and gametes, fertility reduction especially in males, reduction in the quality and quantity of male sperm, the vitellogenin elevation in male fishes, existence of intersex forms, disturbance in sex change of protandrous hermaphrodites and high female-to-male (F/M) ratios, also EEDCs modulate immune responses of fishes (Adeel et al., 2017; Blanchfield et al., 2015; Iwanowicz & Blazer, 2011; Long et al., 2014).

Factors such as distributed chemical substance type, duration and frequency of exposure, season, sex, and developmental stage could change the severity of EEDCs effects on organism (Iwanowicz & Blazer, 2011; D.-G. Wang et al., 2011). The introduction of EEDCs into the natural environment is a consequence of both natural estrogenic compounds such as estrone (E1), 17α-estradiol (α-E2),17β-estradiol (17β-E2) and estriol (E3) and synthetic ones such as ethinyl estradiol (EE2) which are the main components in oral contraceptive pills and hormone therapy, that was originated from exorcise the human urinary and fecal. Meanwhile, liquid and solid waste effluences from livestock, poultry, and fish crop farming system that contains great amounts of estrogenic compounds (Adeel et al., 2017; Song et al., 2018; Vulliet & Cren-Olivé, 2011). Degradation of E1 and 17β-E2 has concerned in this study due to their abundant excreting and toxic effects on natural environment.

Numerous studies have indicated aquatic environment sufferance from EEDCs pollution due to adverse wastewater treatment systems. Notable residual concentrations of the estrogens in treated wastewater effluents and conventional wastewater treatment plants (WWTPs), are considered as the major source for continuous entry of estrogens into the environment (Marfil-Vega et al., 2010; Wert et al., 2009; A. Zhang & Li, 2014). There is no official limitation for EEDCs in treated wastewaters, nonetheless, there has been increased concern regarding accumulation characteristic and adverse ecological and health effects of EEDCs, thereby advanced technologies and innovation needs to reinforce conventional wastewater treatment systems to decrease EEDCs effluent flowing into the aquatic environment.

Advanced Oxidation Processes (AOPs) are widely utilized as treatment techniques for persistent organic pollutants by high chemical stability and low biodegradability property. Photocatalytic processes are a successful way to mineralize the contaminants into carbon dioxide, water, and inorganics during AOPs (Blanco-Galvez et al., 2007). Photocatalytic treatment has proven as a convenient method for organic pollutants degradation, whereas the biological treatment processes are insufficient for treating trace levels of this group of chemicals (Mayer et al., 2019; Paredes et al., 2019). Photocatalysis are chemical processes that are considered as environmentally friendly approach in purification system (FUJISHIMA et al., 2008; Han et al., 2012a). Electronic structure, light absorption properties, charge transport features, and excited life time attribute metal oxides to their usage as photocatalyst. Among these metal oxides, TiO_2, ZnO, SnO_2, and CeO_2 due to their biocompatibility and exceptional stability in various conditions, have been extensively used as photocatalyst (Belver et al., 2019; Oturan & Aaron, 2014). Two oxidation and reduction at least occur in photocatalytic system which equilibrium between two steps is of system efficiency determination (Daghrir et al., 2013). Oxidation process in photocatalytic reactions focused on the toxic chemicals mineralization into CO_2 and H_2O. TiO_2 which has selected as catalyst in this study, is useful for the water and air purification, cleaning up and sterilization of surfaces, photolytically split water to hydrogen and oxygen, and also to perform selective reactions in organic chemistry (Kurtoglu, Longenbach, & Gogotsi, 2011). Rutile, anatase and brookite are three main types of TiO_2 structures with different stability depending on particle size by similar photocatalytic effect which has been proved by previous studies, hence it is essential to choose the more suitable structure and apply method regarding the research's purpose (FUJISHIMA et al., 2008). TiO_2 Degussa P25-slurry treatment has been attracting great interest in estrogenic pollutants elimination (Gmurek et al., 2017; Han et al., 2012a; Ohko et al., 2002; Sornalingam et al., 2016; Y. Zhang et al., 2007) although it has disadvantages such as: adversely effects of particles on UV transmission and inhibiting the photocatalysis reaction, release of free ions to the intended environment and cause to secondary pollution (Huy et al., 2019), negative effects of nano-TiO_2 as a variety of inorganic pollutant on aquatic natural life (Battin et al., 2009; Christian et al., 2008; Lee et al., 2011; Marfil-Vega et al., 2010; Mueller & Nowack, 2008; Tong et al., 2013). Also, it has been demonstrated that phosphate ions which abundantly found in waste water, adsorb on TiO_2 and inhibits the reaction rate (Blanco-Galvez et al., 2007).

To the best of our knowledge, there is no study about the photocatalytic E1 and 17β-E2 degradation with the anatase TiO_2 nanocrystals in the immobilized form on glass. To maximize the efficiency degradation, Response Surface Methodology (RSM) was applied as it has been utilized successfully to evaluate the significance of several factors affecting on the prcesses response even in the presence of complicated interactions in the processes (H.Myers et al., 2016; Khayet et al., 2011; Zodi et al., 2010). Also, some recent publications have reported the RSM prosperity for optimize the EDCs photodegradation (Chong et al., 2010; Daghrir et al., 2013).

MATERIAL AND METHOD

Experimental

Chemicals and reagents

E1 and 17β-E2 analytical standards were obtained from Sigma–Aldrich (USA). HPLC gradient grade acetonitrile and water were purchased from Chem-Lab NV (Belgium).LC-MS grade water, methanol, acetone, 1-Octanol, hydrochloric acid and sodium hydroxide were purchased from Merck (Germany). Ultrapure 18.2 MΩ.cm water was collected from a sartorius arium® 611DI purification system (Sartorius AG, Gottingen, Germany). Pilkington Activ™ TiO_2 coated glass (self-cleaning glass) was purchased from Pilkington (Pilkington UK Ltd, U.K.). Accurel® PP Q3/2 polypropylene hollow fiber (inner diameter: 600 µm, wall thickness: 200 µm, pore size: 0.2 µm) was purchased from Membrana GmbH (Wuppertal, Germany).

Apparatus and instruments

Photocatalysis reactor equipped with UV-C TUV 15W lamps (100-280 nm), model SLV/25 (Phillips, Netherlands) was made in our laboratory. Magnetic mini-stirrer from IKA (Germany) was used for solutions agitation. TiO_2 coated on self- cleaning glass was characterized by X-ray diffraction patterns (XRD) that recorded by Bruker AXS model D8 advanced diffractometer (Cu Kα radiation (λ=1.54187 Å) at 40 kV and 35 mA with Bragg angle ranging from 3 to 70). Surface morphology of the self-cleaning glass was studied by atomic force microscopy (FlexAFM, Nanosurf, Switzerland). Raman spectra were recorded by Renishaw InVia

spectrometer (Renishaw RM1000, Gloucestershire, UK). A high-performance liquid chromatograph model SY-8100 from BRFL Co. (China) equipped with an isocratic pump, a reversed phase C_{18}column (250×4.6 mm I.D, 3 µm particle size) from Macherey Nagel (Germany) and a UV detector was used for following the estrogens degradation via photocatalytic reaction. A 7890 A model gas chromatograph hyphenated with a 5970-model mass spectrometer (Quadrupole mass analyzer and EI source) equipped with DB-5MS capillary column (30m×0.25 mm I.D., 0.25 µm film thicknesses) from Agilent (USA) was used for separation and identification of photocatalytic reaction byproducts. A WTW pH meter, model 3110 (Germany) was used for pH adjustments.

Photocatalytic procedure

The reactor box (50 cm × 50 cm × 120 cm) was made of medium-density fiber board and equipped by ventilation fan. Two 15W UV-C lamps were installed in the center of ceiling of the reactor to irradiate the solutions from the top with a total power of 30W. The photocatalytic glass was placed in the crystallization dishes with nickel-chrome stands in direction of the UV source.

Different radiation intensity of the experimental setup was attained by adjusting distance of active surface of glass and UV lamps. The 1 $mg.L^{-1}$ mix solution of E1 and 17β-E2 was prepared by dissolving appropriate amounts of them in methanol and diluted to the mark with ultra-pure water, stored in refrigerator at the temperature of 4 ºC, and protected from light.

Aqueous solutions of estrogenic compounds were filed in the dishes in the way that rose about 1 cm from the active level of glass. All of the solutions were stirred during tests. The experimental setup used in the photocatalytic tests consisted of irradiating solutions in selected values of independent variables proposed by RSM. The concentrations of target compounds at different conditions followed by high performance liquid chromatography that were obtained through sampling 1 mL of treated solutions. Separation of the analyses were performed on C_{18} column (250×4.6 mm I.D, 3 µm particle size), eluted by acetonitrile: water (80:20) at 1 mL min^{-1} flow rate. The E1 and 17-β E2 concentrations were measured by UV detector at 220 nm. In order to identification byproducts degradation, treated samples at optimized conditions that were obtained from RSM were analyzed by GC/MS. Separations were performed on DB-5MS capillary column (30m×0.25 mm I.D., 0.25 µm film thickness) under temperature programming. Initial temperature was set at 50 ºC for 2 min, raised to 300 ºC at 10 ºC min^{-1} rate and hold for 15 min. Ionization source (EI: 70 eV) and transfer line temperatures were set at 250 ºC and 260 ºC, respectively. Scan range was 25-500 (m/z). The efficiency degradation was calculated from the following formula:

$$X = \frac{C - C_0}{C_0} \quad (1)$$

Where C_0 and C are the initial concentration and concentration of E1 and 17β-E2 at sampling times ($mg.L^{-1}$).

Experimental design

The photocatalytic wastewater treatment methods are influenced by various factors. Controllable variables can optimize to maximize the response (dependent variable).So in this study, solution pH, irradiation time (min) and light intensity ($W.m^{-2}$) were investigated as independent variables. Coded and actual values of RSM input variables are given in Table 1. The coded values of variables were applied for RSM optimization procedure, according to the following equation:

$$X_i = \frac{Z_i - Z_0}{\Delta Z} \quad (2)$$

Where X_i is the dimensionless coded level, Z_i, Z_0 and ΔZare the actual value, center point of the variable and interval variation, respectively.

Table 1. Coded and actual values of operational parameters (independent variables) utilized in experimental design

Input variables	Symbol	actual values of coded levels				
		-α[a]	-1	0	+1	+α
Light Intensity, (W/m^2)	X_1	8.29	10	12.5	15	16.70
pH	X_2	4.47	5.5	7	8.5	9.52
Time (min)	X_3	4.47	15	30	45	55.22

α=1.682

To assay the individual and interactive effects of three independent variables on the response (photocatalytic efficiency degradation), Central Composite Design (CCD) was utilized as the most popular design of RSM. 20 experiments with 8 orthogonal two levels full factorial design points (coded as ±1), six axial points (coded as ±α= 1.682) and six replications of the central points were performed based on CCD procedure. The design of experiments and analysis of experimental data were performed by Design Expert 10. The matrix of experiments is given in Table 2.

RESULTS AND DISCUSSIONS

Characterization of TiO_2 coated self- cleaning glass as photocatalyst

Surface of the proposed photocatalytic Pilkington Activ™ self-cleaning glass was studied by Atomic Force Microscopy (AFM). From the top-down view (TiO_2-coated surface of glass), the average TiO_2 nanoparticle diameter is about 90±30 nm (Figure. 1a). The hemispherical peaks are observable in the topographical view (Figure 1b). From the sectional AFM view (Figure 1c) the thickness of TiO_2 layer on the glass is about 8±3 nm. The results indicate the

appropriate roughness with high surface for the photocatalyticaly active surface of glass. The top-down view (bare surface of glass) AFM images exhibit very smooth surface in comparison with active surface (Figure 1d-f).

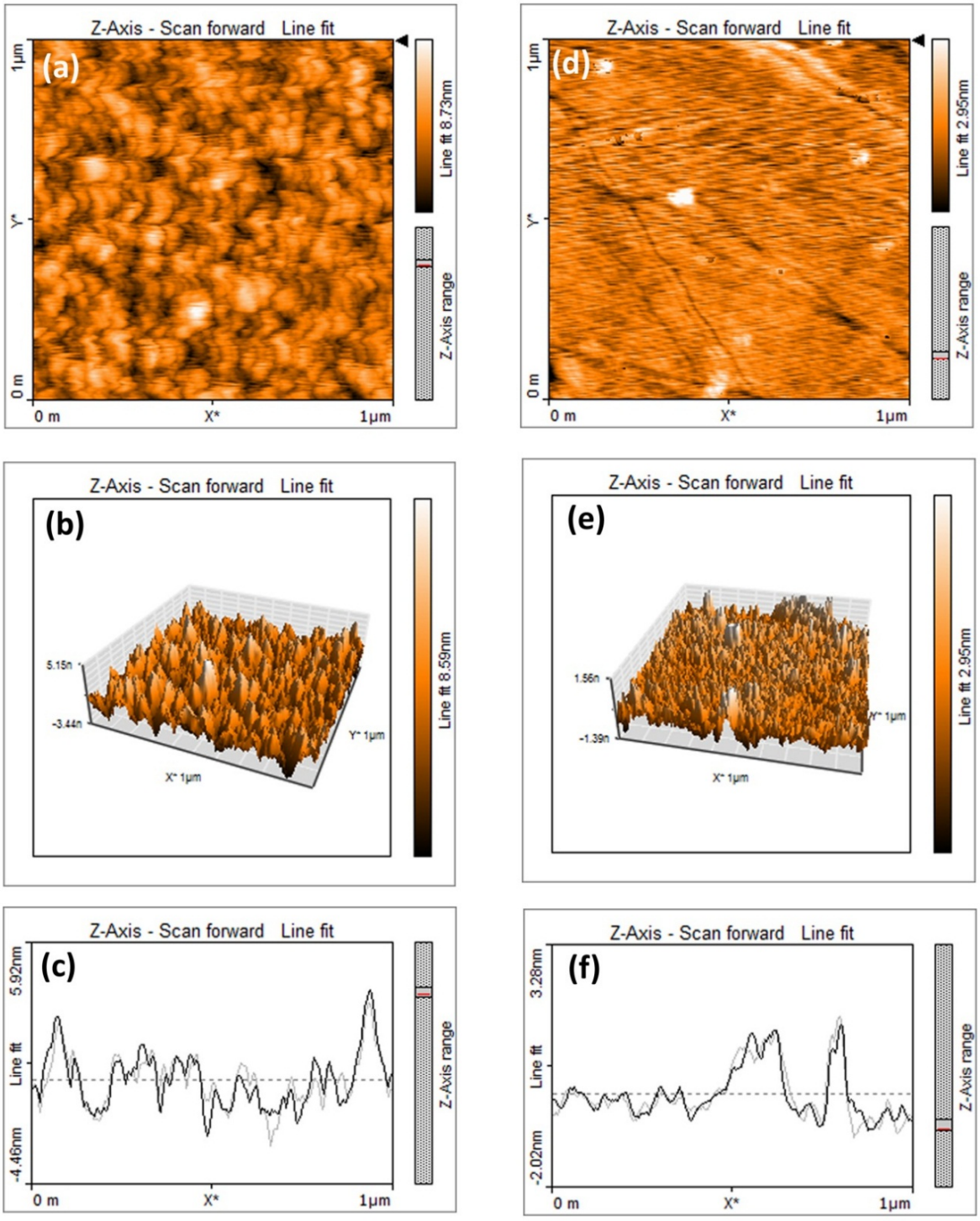


Figure 1. Demonstrates AFM images of Pilkington Activ™ glass in (a, b) a top-down view and (c, d) a topographical view, (e, f) sectional view for active and bar surfaces, respectively

The existence of TiO_2 on the active surface of glass was investigated by XRD and Raman spectra. Figure 2a demonstrated Recorded XRD pattern. A sharp peak at $2\theta = 25.28^{o}$ confirms the existence of TiO_2 as anatase phase. No

peaks related to the rutile phase of TiO_2 are observed. The characterization of TiO_2 phase coated on the active surface of glass was more investigated by Raman spectroscopy which would be considered as an accurate way for the analysis of surface morphology of thin TiO_2 films (Kurtoglu, Longenbach, & Gogotsi, 2011; Kurtoglu, Longenbach, Reddington, et al., 2011). The Raman spectra of surface film showed an obvious characteristic peak of TiO_2 anatase phase at 144, 396, 514 and 636 cm^{-1} frequencies (Figure 2b).

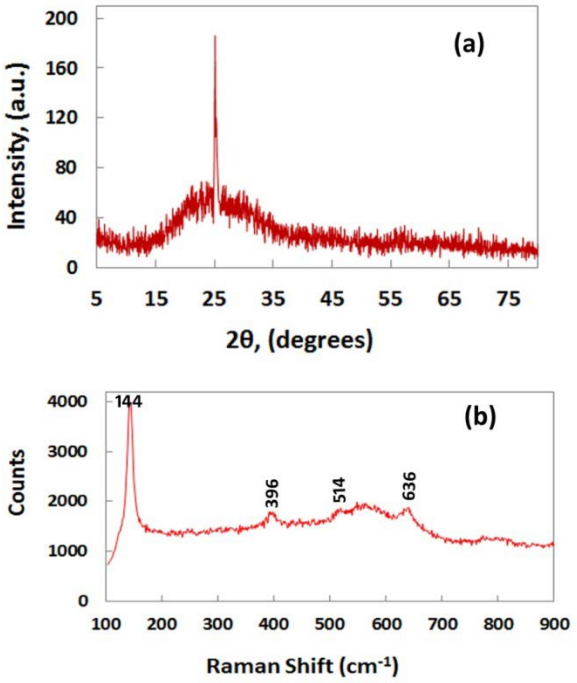


Figure 2. (a) XRD pattern and (b) Raman spectra of Pilkington Activ™ glass active surface

Photocatalytic performance of Pilkington ActivTM glass in degradation of E1 and 17β-E2

Figure 3 demonstrated the efficiencies degradation of E1 and 17β-E2 vs. time in adsorption (in dark), direct photolysis (without active glass), and photocatalytic (with active glass under UV irradiation) processes. Under UV light irradiation, the efficiency degradation of E1 and 17β-E2after 120 min was 61.26 and 41.52%, respectively. The removal efficiency of estrogenic compounds in dark (adsorption process) was negligible. As shown in Figure 3 significantly greater degradation of each estrogenic compound is obtained from the coated TiO_2 on the active surface of Pilkington Activ™ glass in compared to direct photolysis. At the same UV irradiation (10.08 W/m^2) and time (120 min) more than 90% of degradation occurs in the existence of coated TiO_2 on the active surface of Pilkington Activ™ glass for both E1 and 17β-E2. The observed difference in efficiencies degradation is due to the fact that the dominant mechanism of degradation becomes hydroxyl or superoxide anion radical mediated advanced oxidation when active glass is placed in the exposing of UV light.

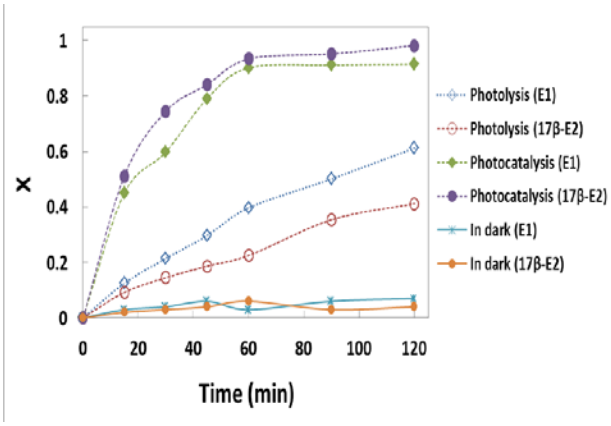


Figure 3. Efficiency Degradation E1 and 17β-E2 indirect photolysis (in absence of active glass and UV irradiation) and photocatalysis (by existence of active glass and UV light intensity of 10.08W/m^2)

Modeling, effect of operational parameters and optimization of the photocatalytic degradation process by RSM

Model development and analysis

A second-order polynomial or quadratic regression equation (Eq. (3)) was utilized to fit the experimental results that were achieved from CCD. This response surface modelprovides polynomial experimental results estimation.

$$y = b_0 + \sum_{i=1}^{n}(b_i X_i) + \sum_{i=1}^{n}(b_{ii} X_{ii}^2) + \sum_{i,j=1}^{n}(b_{ij} X_i X_j) \quad (3)$$

Wherey and bare the predicted responses (efficiency degradation, %) and regression of coefficients. For each experiment, the efficiency degradation was experimentally obtained from the experimental design results (Table 2), the quadratic equations for specifying the photocatalytic degradation of E1 and 17β-E2 by TiO_2 coated self-cleaning glass were obtained from the least square approximation.

The equations are presented as equations 4 and 5 for E1 and 17β-E2, respectively:

$$y_{E1} = 0.66 - 0.021X_1 - 0.015X_2 + 0.19X_3 + 0.022X_1^2 + 0.022X_2^2 - 0.047X_3^2 - 1.125\times10^{-3}X_1X_2 + 1.125\times10^{-3}X_1X_3 + 1.125\times10^{-3}X_2X_3 \quad (4)$$

$$y_{17\beta-E2} = 0.73 - 0.035X_1 - 0.019X_2 + 0.18X_3 + 0.046X_1^2 + 0.067X_2^2 - 0.053X_3^2 - 8.625\times10^{-3}X_1X_2 - 3.875\times10^{-3}X_1X_3 - 2.625\times10^{-3}X_2X_3 \quad (5)$$

Table 2. Applied central composite design matrix and efficiency degradation values for photocatalytic degradation of the estrogens

Run order	L.I (W/m²)	pH	Time (min)	Degradation efficiency E1		Degradation efficiency 17β-E2	
				Experimental	Predicted	Experimental	Predicted
1	15	8.5	15	0.45	0.431	0.578	0.583
2	15	8.5	45	0.791	0.811	0.902	0.907
3	12.5	9.52	30	0.708	0.699	0.894	0.886
4	15	5.5	15	0.471	0.466	0.621	0.626
5	12.5	7	30	0.62	0.6611	0.752	0.731
6	12.5	7	30	0.675	0.661	0.711	0.731
7	12.5	4.47	30	0.784	0.749	0.964	0.956
8	10	8.5	15	0.477	0.478	0.622	0.627
9	12.5	7	30	0.671	0.661	0.721	0.731
10	8.29	7	30	0.802	0.759	0.964	0.956
11	12.5	7	30	0.658	0.661	0.749	0.731
12	12.5	7	30	0.64	0.661	0.751	0.731
13	10	8.5	45	0.818	0.853	0.971	0.976
14	12.5	7	55.22	0.909	0.844	0.931	0.923
15	10	5.5	15	0.498	0.508	0.64	0.645
16	12.5	7	30	0.697	0.661	0.701	0.731
17	12.5	7	4.77	0.193	0.214	0.247	0.239
18	16.70	7	30	0.688	0.687	0.772	0.764
19	10	5.5	45	0.83	0.878	0.99	0.995
20	15	5.5	45	0.812	0.840	0.965	0.970

Table 3. Analysis of variance (ANOVA) for the developed models

Source	DF*	ANOVA for E1 degradation				**ANOVA for 17β-E2 degradation**			
		SS**	Adj-MS***	F-Value	P-Value	SS	Adj-MS	F-Value	P-Value
Regression	9	0.54	0.06	35.55 (Critical F-value=3.02)	0.0001<	0.63	0.07	62.02 (Critical F-value =3.02)	0.0001<
Residual error	10	0.017	1.685×10^{-3}	-	-	0.011	1.133×10^{-3}	-	-
Lack-of-fit	5	13×10^{-3}	2.627×10^{-3}	3.53 (Critical F-value =5.05)	0.0962	8.77×10^{-3}	1.754×10^{-3}	3.42 Critical F-value =5.05)	0.1017
Pure error	5	3.72×10^{-3}	7.438×10^{-3}	-	-	2.56×10^{-3}	5.13×10^{-4}	-	-
Total	19	0.56	-	-	-	0.64	-	-	-

* DF = Degrees of freedom. ** SS = Sum-of-squares. ***Adj-MS = Adjusted mean squares.

The experimental and predicted efficiencies degradation of E1 and 17β-E2 (from Eqs. (4) and (5)) has shown in Table 2.

Table 3 demonstrates the analysis of variance (ANOVA) results. The ANOVA is carried out to evaluate the significance and adequacy of the obtained response surface models. Based on the ANOVA results, the Fisher F-values of both regressions were remarkably higher than the tabulated F-value (35.55 and 62.02 for E1 and 17β-E2, respectively at 95% significance) implying that the obtained models predict the experimental results, suitably. Furthermore, P-values that are related to the Fisher test are lower than 0.05 signifying that the response surface models are significant by a statistical point of view. The next criterion for evaluating the achieved models was Lack of Fit (LOF) test. Indeed, it compares the residual with pure error. The pure error calculated by the replicated experiments at the central level of variables from the ANOVA results, the F-values that are related to the LOF of response surface models were less than tabulated ones. Also, the P-values of LOF test were equal to0.0962 and 0.1017, respectively. The results of LOF have shown the insignificant lack of fit for the developed models for predicting the degradation efficiency of E1 and 17β-E2.

The coefficient of determination (R^2) and Adj-R^2 values, which compared experimental and predicted degradation efficiencies, were equal to 96.97% and 94.24% for developed model of E1 degradation and they were equal to 98.24% and 96.60%, for 17β-E2, respectively (Figure 4).

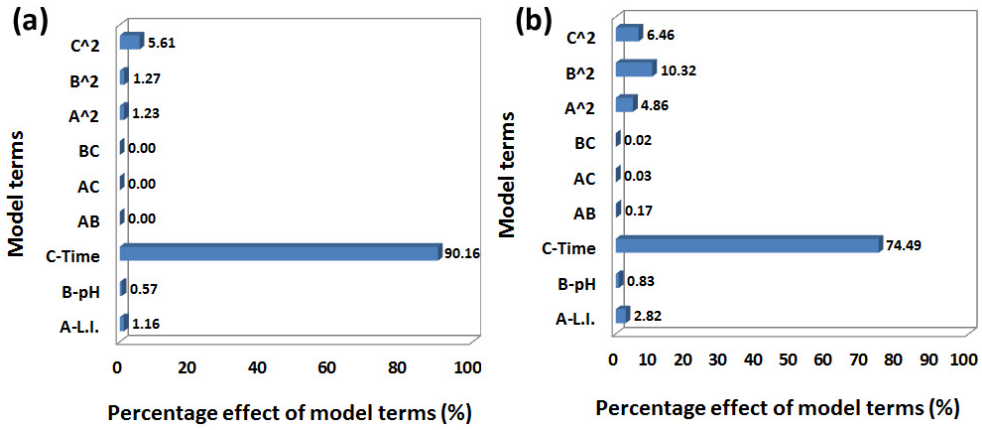


Figure 4. Experimental degradation efficiency vs. calculated ones by developed models for a) E1 and b) 17β-E2

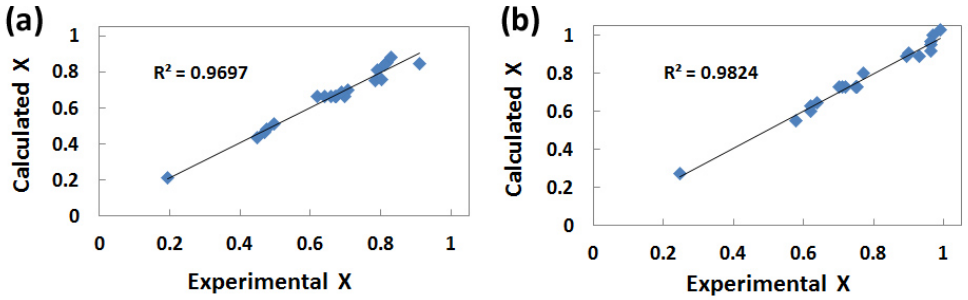


Figure 5. Experimental efficiency degradation vs. calculated ones by developed models for a)E1 and b) 17β-E2

Determination of importance of model terms

The well-known Pareto analysis was used in order to determine the effective terms in the response surface models. In Pareto analysis the percentage of independent variables (P_i) was calculated to evaluate the effectiveness of model parameters using the following formula:

$$P_i = \left(\frac{b_i^2}{\sum_{i=1}^{n} b_i^2} \right) \times 100 \qquad i \neq 0 \qquad (6)$$

According to the Pareto results (Figure 5), during the photocatalytic degradation process the irradiation time showed the most effect (90.16% and 74.49% for E1 and 17β-E2, respectively) on the efficiency degradation of the studied hormones. The coefficient of the quadratic effects of time, pH and light intensity were also significant. In this mechanism, the maximum efficiency degradation is achieved by alkaline media. Therefore, pH effect as 10.32% quadratic terms predicted by Pareto is expectable.

Optimized conditions for photocatalytic degradation of E1 and 17β-E2

The main purpose in designing experiments by RSM is achieving the optimized conditions of operational factors to attain the desired response. The optimized values of the variables for obtaining the maximum efficiency degradation of E1 and 17β-E2 are given in Table 4. Other experiments were performed to optimized conditions proposed by RSM and experimental efficiency degradations for both estrogenic compounds that were obtained to verify the optimized values of the factors. The experimental results demonstrated that the maximum degradation efficiency was equal to 0.921 and 0.986 for E1 and 17β-E2 which were in a good agreement with the predicted ones.

Table 4. Optimum values of factors to obtain the maximum photocatalytic degradation of the E1 and E2

Degradation process	Predicted optimized values of factors			Predicted degradation efficiency	Experimental degradation efficiency
	Light intensity (W/m^2)	pH	Time (min)		
E1	10.084	9.421	52.498	0.915	0.921
17β-E2	10.084	9.421	52.498	1.040	0.986

Effects of variables on estrogens' efficiency degradation

Figure 6 demonstrates the three-dimensional response surface plots for investigating the effect of pH and light intensity and irradiation time on the degradation of studied hormones. It is clear that the efficiency degradation of hormones increased by irradiation time increasing. Also, the light intensity had a negligible effect on photocatalytic degradation in comparison with irradiation time. The photocatalytic degradation mechanism is summarized as follow:

$$TiO_2 + h\nu \rightarrow h^+ + e^- \quad (7)$$

$$h^+ + e^- \rightarrow heat \quad (8)$$

$$H_2O + TiO_2 \rightarrow H_2O_{ads} - TiO_2 \quad (9)$$

$$OH^- + H^+ + TiO_2 \rightarrow OH^-_{ads} - TiO_2 + H^+ \quad (10)$$

$$Estrogen + TiO_2 \rightarrow [Estrogen]_{ads} - TiO_2 \quad (11)$$

$$H_2O_{ads} - TiO_2 + h^+ \rightarrow {}^{\cdot}OH_{ads} - TiO_2 + H^+ \quad (12)$$

$$OH^-_{ads} - TiO_2 + h^+ \rightarrow {}^{\cdot}OH_{ads} - TiO_2 \quad (13)$$

$${}^{\cdot}OH_{ads} + [Estrogen]_{ads} \rightarrow [Intermediate] \quad (14)$$

$$OH^-_{ads} + TiO_2 h^+ \rightarrow {}^{\cdot}OH_{ads} \quad (15)$$

$$HO_2^{\cdot} + H^+ + e^- \rightarrow H_2O_2 \quad (16)$$

$$H_2O_2 + e^- \rightarrow {}^{\cdot}OH_{ads} + OH^- \quad (17)$$

$$O_2^{\cdot -} + H^+ \rightarrow HO_2^{\cdot} \quad (18)$$

$$HO_2^{\cdot} + H^+ + TiO_2 e^- \rightarrow H_2O_2 \quad (19)$$

$$H_2O_2 + TiO_2 e^- \rightarrow {}^{\cdot}OH_{ads} + OH^- \quad (20)$$

$${}^{\cdot}OH_{ads} + [Estrogen]_{ads} \rightarrow [intermediates] \quad (21)$$

$$O^{\cdot -}_{2(ads)} + [Estrogen]_{ads} \rightarrow [intermediates] \quad (22)$$

The reaction between hydroxide ions and positive holes on TiO_2 surface can lead to formation of hydroxyl radicals (Eq. 13). The hydroxyl radicals can be easily formed in alkaline media by oxidizing more hydroxide ions. The photocatalytic efficiency degradation of both estrogens is enhanced in alkaline solution more significantly, even though the efficiency increases for both acid and alkaline pH. From Eqs. 14 - 17, the formation of the superoxide anion radicals and their conversion of the hydroxyl radicals is favorable at low pH. Even though at the acidic solution, oxygen reduction by electrons in conduction band may play an important role in the degradation, highest efficiency degradation of target compounds were observed under alkaline conditions. For achieve better sight in photocatalytic degradation of E1 and 17β-E2, in comparison to other previous studies was reported in Table 5.

Table 5. A compilation of comparative studies on AOPs in degradation of EEDCs

Degradation Process	Target	Experimental conditions	Intermediates	Efficiency %	Ref.
Photocatalytic	E1	P25 TiO_2* suspension 50 mg L^{-1} /UVA 60 min /Irradiating Batch system	-	95	(Han et al., 2012b)
Photocatalytic	17β-E2	P25 TiO_2* suspension 100 mg L^{-1} /UV-C 60 min /Irradiating Batch system		>85	(Orozco-Hernández et al., 2019)
Photocatalytic	17β-E2 (C_0=0.003 mg/L)	P25 TiO_2 suspension 1.00 g L^{-1} /UVA 180 min / irradiation intensity: 6mW.cm^{-2} / Irradiating Batch system	10ε-17β-Dihydroxy-1,4-estradien-3-one and testosterone like species	100	(Ohko et al., 2002)
Photocatalytic	17β-E2	1.8 $\times 10^{-5}$ M $FeCl_3$/$NaNO_2$ under natural light irradiation / 1440 min / Irradiating Batch system	-	>86.6	(L. Wang et al., 2007)
Photocatalytic	E1 and 17β-E2	PTT*** / UV-C -LED / ~120 / irradiation intensity: 0.390 mW.cm^{-2}		100 for E1 and inefficient degradation of 17β-E2	(Arlos et al., 2016)
Photocatalytic	E1 and 17β-E2	Anatase TiO_2 as glass coated immobilized form / UV-C 52.5 min / irradiation intensity: 10.084 W.cm^{-2} / Irradiating Batch system	17-deoxy Estrone & 2-Hydroxyestradiol	>92 for E1 and >98 for 17β-E2	This study

*P25 TiO_2 : Titanium dioxide, anatase nanopowder, Particle Size ≤ 25 nm, CAS Number: 1317-70-0
**EDDS: Ethylenediamine-N,N´-disuccinic acid
***PTT: Porous Titania-TiO_2

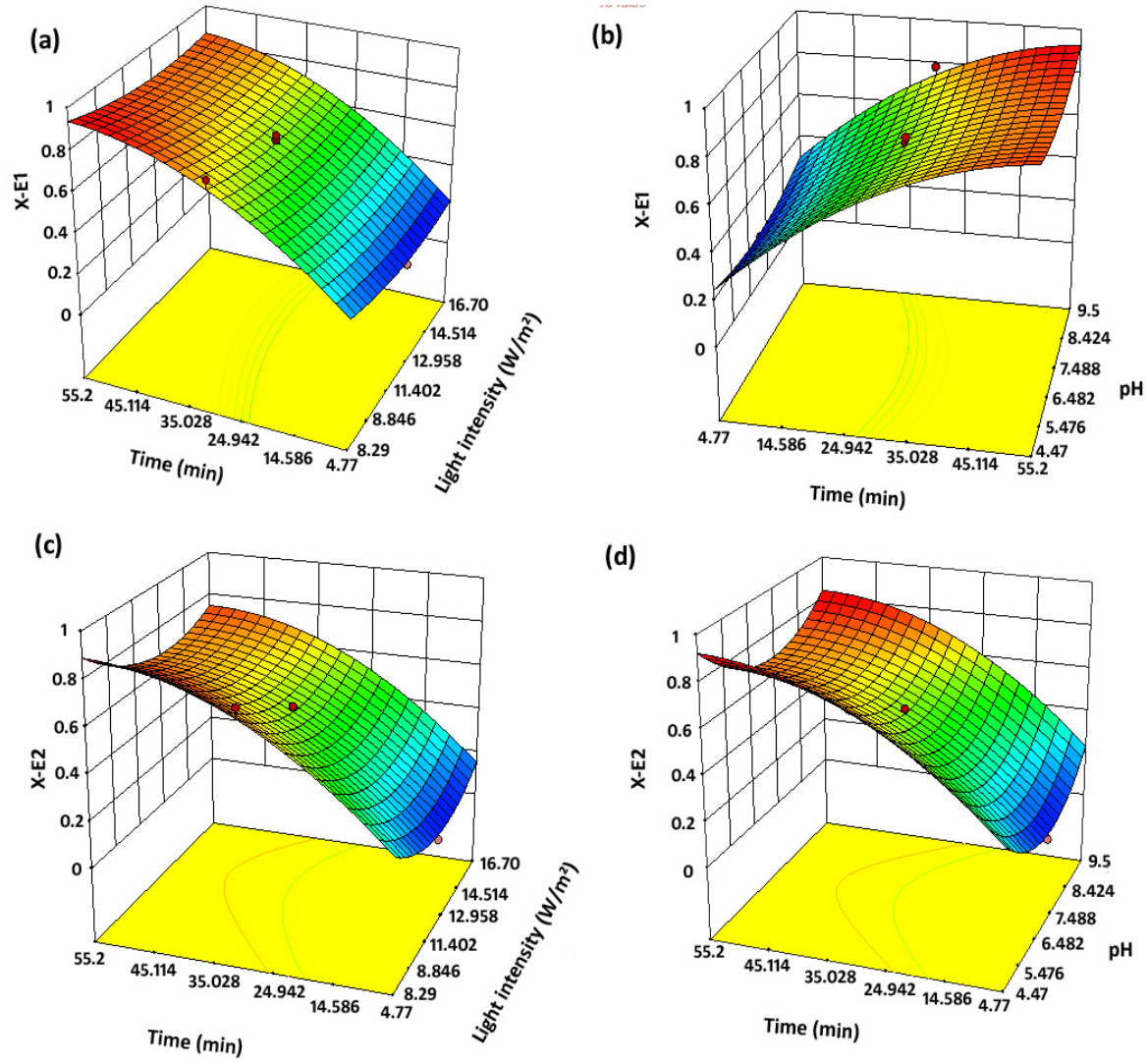


Figure 6. Response surface plots for investigating the time and light intensity effects on photocatalytic degradation of (a) E1 and (c) 17 β-E2 and effect of pH on (b) E1 and (d) 17 β-E2 efficiency degradation

For identification of the active species in the photocatalytic degradation process, a series of experiments were accomplished. Hydroxyl radicals($OH^{\cdot}$), trapped photogenerated holes on the TiO_2 and superoxide anion radicals(O_2^{-}) are the main reactive oxidative species in photocatalytic degradation of estrogenic hormones. In the new series of experiments, three test were separately conducted in the existence of tert-butyl alcohol (t-BuOH), benzoquinone (BQ), and ammonium oxalate (AO) as a hydroxyl radical, superoxide anion radicals and photogenerated holes scavengers, respectively. The photocatalytic tests were performed at the optimized conditions obtained from RSM (Table 4). Figure 7 shows the ranking of different radical scavengers effect of efficiency degradation was in order of BQ > t-BuOH >AO. Therefore, the superoxide anion radicals and hydroxyl radicals are the main oxidative specie for degradation of both estrogenic compounds.

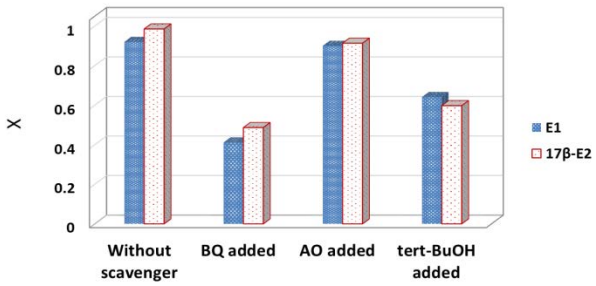
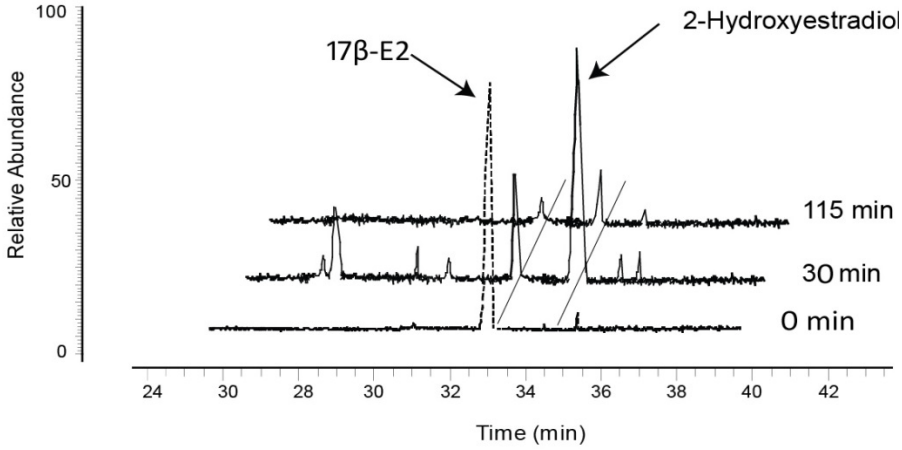


Figure 7. Effect of different radical scavengers on photocatalytic degradation of of E1 and 17β-E2 (1 mg/L of mix solution, light intensity=10.08 W/m2, pH=9.421 and irradiation time=52.5 min)

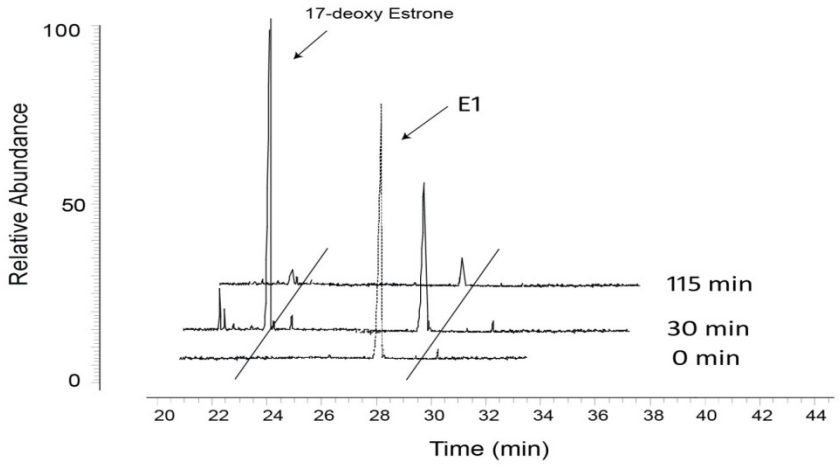
HPLC and GC/MS study of the photocatalytic degradation of E1 and 17β-E2 in aqueous media

Photocatalytic tests were also performed at the optimized conditions that were proposed by RSM and the degradation of E1 and 17β-E2 was studied by high performance liquid chromatography with determining the changes in the hormones concentrations during tests. The chromatograms of mixed estrogen solutions under optimized pH, light intensity, and varying times were investigated. It has clearly shown that E1 and 17β-E2 peaks were disappeared during 0-115min of UV-C irradiation with 10.08W/m^2 light intensity and 9.42 solution pH in the existence of self-cleaning glass as proposed photocatalyst. From comparison of the HPLC chromatograms, it could be seen growth of the new peaks at retention times 4.45 and 5.15 min, which was related to degradation byproducts of the studied hormones. The first peak growth was continued till 115 min and then with further light irradiation that was disappeared (HPLC chromatogram for170 min). The second peak reached to maximum at 22.5 min and at longer irradiation times the peak disappears from 22.5 up to 60 minutes.

The test solutions of individual hormones were examined by GC/MS in order to identify reaction byproducts. Figure 8 shows the total ion chromatograms of GC/MS analysis. As can be seen the peaks that are related to the E1 and 17β-E2 were disappeared photocatalytic degradation. However, the byproduct peaks grew at the first minutes of processes and then omitted. The proposed byproduct compounds are 2-hydroxyestradiol as intermediate compound before complete oxidation and mineralization of 17β-E2 and 17-deoxy estrone as intermediate compound in photocatalytic degradation of E1 which were identified by mass spectra of GC/MS analysis.



(a)



(b)

Figure 8. Total ion chromatogram of individual (a) 17β-E2 and (b) E1 during photocatalytic degradation process that performed at optimized conditions proposed by RSM

.

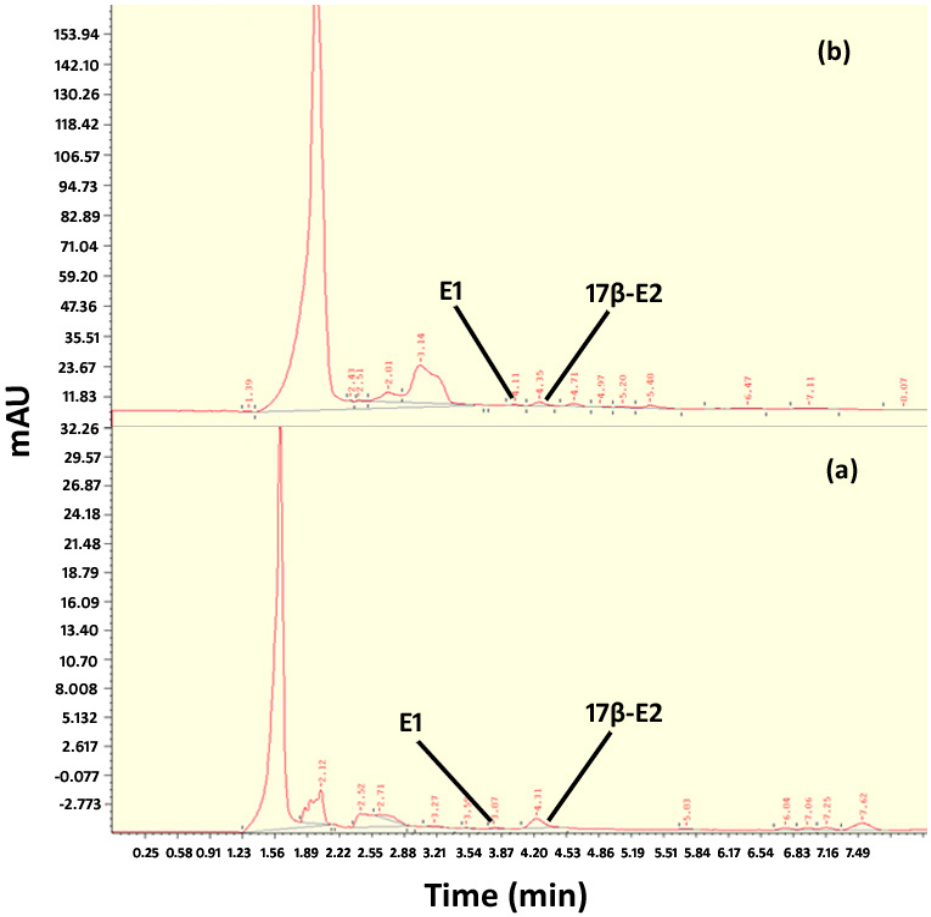


Figure 9. HPLC chromatograms of real sample of wastewater effluent of MBR system before (a) and after (b) photocatalytic degradation at RSM optimized conditions

Evaluation of applicability the method for real wastewater matrix

To evaluate the optimized method applicability, a real sample from effluent of a WWTP which membrane bioreactor (MBR) technology employed as treatment process, were collected. Profile of mentioned WWTP's influent was in the ration of 40% domestic (originated from ~ 7000 personnel) and 60% industrial approximately.120 mL of real sample was treated under optimized conditions that were established by RSM. 50 mL of treated samples were extracted following the hollow-fiber liquid-phase micro extraction procedure.

HPLC analyses were performed and Figure 9 shows the HPLC chromatograms. Efficiency degradation of E1 and 17β-E2 was equal to 30.40% and 56.84%, respectively; which is lower than performance of the system for synthetic wastewater; it can be in the result of containing complex matrices of organic matter and other competing chemicals in real samples and higher level of organic content of them naturally(Bodhipaksha et al., 2017); in addition, phosphate ions which abundantly found in wastewater, can inhibit the reaction rate by adsorption on TiO_2 crystals (Blanco-Galvez et al., 2007). This result highlights the importance of moderation of AOPs in pilot tests before real scale application. Regard to main role of superoxide and the hydroxyl radicals in the case, existence of effective electron scavenger (e.g. phosphate and nitrate anions) over illuminated TiO_2 being able to compete successfully with molecular oxygen for the photogenerated electrons (Gu et al., 2002).

CONCLUSION

Deficiency of conventional WWT systems to decrease EEDCs effluent flowing on the aquatic environment alert needs an advanced treatment systems development. E1 and 17β-E2 as a representing estrogenic compound undergo photocatalytic degradation process under UV irradiation in the existence of TiO_2coated on Pilkington Activ™ glass. UV irradiation is conducted at room temperature which is near to realistic conditions for feature real scale tests. The optimal efficiency degradation was obtained in alkaline pH under 10.08 W/m^2 UV light irradiation and after52.49min of process which were obtained from RSM. According to the Pareto analysis, the irradiation time is the most important parameter

in treatment process which should be suit to remove of primary compounds. Anatase crystal phase of TiO_2 as glass coated immobilized form is used by obtaining self-cleaning commercial glass Pilkington Activ™ which is available to purchase for real scale photo reactors is advantageous due to eliminate secondary pollution of aquatic ecosystems. The results of this study provide new insight for the benefits and efficiency of using the TiO_2 coated self-cleaning glass to purify the wastewater. In comparison with other previous studies, achieved results in this study provide further justification for AOP development applications in real scale WWT systems. Presented evidences advices to ideal management practices, use this material in reinforce treatment systems after further ecotoxicological researches, approach for the degradation of organic micro pollutants especially EEDCs in WWTPs.

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RESEARCH ARTICLE
ARAŞTIRMA MAKALESİ

Individual rearing of common octopus (*Octopus vulgaris* Cuvier, 1797) in tanks: Preliminary results

Ahtapot (*Octopus vulgaris* Cuvier, 1797)'un tanklarda bireysel **yetiştiriciliği: İlk** sonuçlar

Halil Şen

Department of Aquaculture, Faculty of Fisheries, Ege University, İzmir, Turkey
https://orcid.org/0000-0003-0878-3583

halil.sen@ege.edu.tr

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Abstract: The effects of individual rearing technique on the growth and survival of *Octopus vulgaris* (Cuvier, 1797) were investigated. Therefore, wild octopuses were reared in the transparent, perforated and caped polyethylene terephthalate (PET) pots (10 l). The assay was established as one free group rearing as a control (12.7 kg/m^3 of stoking density) and two individual rearing groups as I1 and I2 (13.8 kg/m^3 and 18.3 kg/m^3 of stoking density, respectively). The individuals were fed mainly fresh or thawed fish at 5% body weight per day. The experiments lasted for 120 days. The acclimatization period of the octopuses lasted within 3-5 days in the individual rearing and 7-10 days in the free group rearing. The final survival rates were 100% for the control and I1 or 85.7% for I2 (P>0.05). Statistically differences were not found among the growth or specific growth rates (between 0.5 and 0.6%/day for the trials) (P>0.05). The maximum density (30.7 kg/m^3) was obtained from the I2 trial.

Keywords: Octopus vulgaris, individual rearing, growth, survival

Öz: Bireysel yetiştiriciliğin *Octopus vulgaris* (Cuvier, 1797)'in gelişimi ve yaşama oranı üzerine etkileri araştırıldı. Bunun için doğadan yakalanan ahtapotlar, 10 litre hacmindeki şeffaf, delikli ve kapaklı polietilen tereflat (PET) kaplarda yetiştirildi. Denemeler, bir serbest grup yetiştiricilik (12,7 kg/m^3 stok yoğunluğunda, kontrol grubu) ve iki bireysel yetiştiricilik (I1, ve I2 olarak sırasıyla, 13,8 kg/m^3 ve I2, 18,3 kg/m^3 stok yoğunluğunda) grubu olarak kuruldu. Ahtapotlar, ağırlıklı olarak taze ve/veya taze donmuş çözülmüş balık ile günlük olarak vücut ağırlıklarının %5'i oranında beslendi. Denemeler 120 gün sürdü. Adaptasyon periyodu bireysel yetiştiricilik gruplarında 3-5 günde, serbest grup yetiştiricilik grubunda 7-10 günde tamamlandı. Hayatta kalma oranları kontrol ve I1 için %100, I2 için %85,7 olarak hesaplandı (P>0,05). Ahtapotların gelişimleri veya spesifik gelişim oranları (denemeler için 0,5 ve 0,6%/gün) arasında istatistiksel olarak önemli farklılıklar saptanmadı (P>0,05). Maksimum stok yoğunluğu (30,7 kg/m^3) stoklamanın en fazla olduğu I2 denemsinden elde edildi.

Anahtar kelimeler: Octopus vulgaris, bireysel yetiştiricilik, gelişim, yaşama oranı

INTRODUCTION

Octopus vulgaris (Cuvier, 1797), has become a potential species for the aquaculture industry, due to the high growth rate, high nutritional value, an easy adaptation to controlled conditions and high market demand (Vaz-Pires et al., 2004; Iglesias et al., 2004; Iglesias et al., 2007; García García et al., 2009). However, *O. vulgaris* culture has cannot be performed as commercially yet, due to the low survival rate of the paralarvae and lack of specific live and/or compound diets for its paralarvae and subadults (Vaz-Pires et al., 2004; Iglesias et al., 2007; Valverde et al., 2015). Despite these constraints, fattening of *O. vulgaris*, using floating cages in Spain (Galicia, NW) was achieved in 1998 and 1999 (FAO, 2002).

In recent years, most studies on the improvement of growth in *O. vulgaris* is concentrating on the development of specific enrichments and compound or formulated diets (Valverde et al., 2013; Valverde et al., 2015; Morillo Velarde et al., 2015; Querol et al., 2015a; Querol et al., 2015b). On the other hand, a few studies have been performed in order to test new rearing systems and/or techniques for *O. vulgaris* ongrowing (Chapela et al., 2006; Rodríguez et al., 2006; Estefanell et al., 2012a; Estefanell et al., 2012b).

Further, in the previous studies, it's clearly pointed out that better growth results in the common octopus have been obtained by free group rearing technique, but higher survival rates have been achieved by individual rearing (Chapela et al., 2006; García García and Valverde, 2006; Petza et al., 2006; Rodriguez et al., 2006; Valverde et al., 2008; Biandolino et al., 2010; Prato et al., 2010; García Garrido et al., 2011; Estefanell et al., 2012a). Therefore, the individual rearing seems to be a highly considerable method for *O. vulgaris* on growing when consideration of the handicaps of the free group rearing technique for *O. vulgaris* that mentioned above. The purpose of the current study was to test the growth and survival of *O. vulgaris* by individual rearing technique, and also whether it can solve the main problems in the free group rearing method.

MATERIALS AND METHODS

Wild live octopuses were obtained from local fishermen using by a jigging line, of Urla Port (Izmir Bay, Aegean Sea).

The octopuses were transferred with no mortality to the laboratory immediately after collection. They kept together till coming into the laboratory in a 40 l tank with the water being renewed every 20 minutes; the temperature was 16-18°C and oxygen above 6.5 mg/l. The specimens were weighed one by one after the sex determination. Then, the octopuses were randomly distributed according to the rearing technique (individual or free group). The adaptation period of the octopuses was performed in circular polyester 430 l tanks and open flow-through filtered seawater system (860 l/h). In the free group rearing trial, polyvinyl chloride (PVC; 110 mm in diameter and 350 mm in length) tubes as shelters, and a net cover to prevent octopuses from escaping were provided. In the individual rearing groups, transparent, perforated and with caps polyethylene terephthalate (PET) pots (10 l) were used (Figure 1). During the period, the octopuses were fed as ad libitum once a day at 09:00 hour, with a mixed diet including fresh or thawed bogue (*Boops boops*), annular seabream (*Diplodus annularis*), picarel (*Spicara smaris*) and mantis shrimp (*Squilla mantis*), provided on consecutive days.

Totally 17 octopuses (1132.9 ± 218.2 g) were weighed following controlled of their sexes and were stocked at different initial densities (Table 1). The experiment was established as one free group rearing as a control (12.7 kg/m^3 of stoking density) and two individual rearing groups as I1 and I2 (13.8 kg/m^3 and 18.3 kg/m^3 of stoking density, respectively). No significant differences were found in the weights of the individuals (P>0.05). The rearing experiments were carried out in the same tank conditions as mentioned above. In the control group, a net cover and the PVC tubes as the same number of the octopuses were used. In the individual rearing groups, the octopuses were placed one by one into the PET pots (Figure 1).

Table 1. Beginning data of the experiment (mean ± SD).

	C	I1	I2
N_i	5	5	7
Female:Male	3:2	1:4	5:2
Average W_i (g)	1093 ± 243^a	1187 ± 313^a	1122 ± 135^a
Min W_i (g)	784	809	937
Max W_i (g)	1447	1450	1324
D_i (kg/m^3)	12.7	13.8	18.3

N_i, initial number; D_i, initial density; Different superscript letters show the significant

During the trials, the specimens were fed a mixed diet as fresh and/or thawed fish (93%; 48% of *D. annularis*, 33% of *S. smaris*, 12% of *B. boops*) and mantis shrimp (*S. mantis* as 7%) once a day as 5% body weight per day (Domingues et al., 2010). The next morning, remaining foods were collected from the tanks by hand or net, and were weighed before feeding. This was used to determine food ingestion of the octopuses. The food ratio was arranged after each weighting period. The specimens were weighed individually every 15 days during the assay.

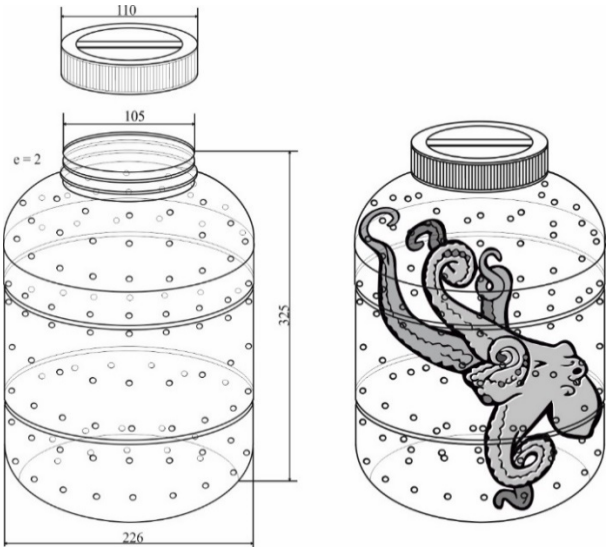

Figure 1. The polyethylene terephthalate (PET) pot (10 L) (dimensions in mm)

The experiments were finished in the 120th days of the trial since at this time mating behavior began among the octopuses in the control group.

Through the experimental period, each experimental tank illuminated by a 40W daylight fluorescent lamp. Photoperiodicity was natural (38˚21'K, 26°46'D; December to April). The water parameters were measured by YSI EcoSense DO 200A for oxygen and temperature, YSI EcoSense pH 100A for pH, and YSI EcoSense EC300A for salinity.

For every sampling period, the weight data were used to calculate: absolute growth rate (AGR)= (Wf - Wi) / t; specific growth rate (SGR) = (LnWf - LnWi) × 100 / t, where Wf and Wi are the final and initial weights of the octopuses, respectively, Ln the natural logarithm, and t the number of days of the experimental period; feeding rate (FR)= [IF / Average W(t)] × 100, where IF is the ingested food and Average W(t) is the average weight of the octopus during that period; food conversion (FC) = (Wf - Wi) / IF, where Wf - Wi is the weight gained by the octopus during that period; octopus density (D) per tank = total biomass of the octopuses (kg) × (1000 / 430); where 430 was available water volume (l) of the tank. Survival (%) was calculated by counting for each tank for each sampling period.

Statistical analyses of the data (means ± SD) were performed with SPSS 25.0 statistical software in 95% confidence interval (P < 0.05); following the ANOVA to test differences in weight, growth rate, feeding rates and feed

conversion rates, the significance of differences was tested using a Tukey multiple comparison test. The normal distribution analysis after the square root transformation of the data was carried out with the Kolmogorov-Smirnov test. Homogeneity of variances was tested with Levene's test (Zar, 1999). Differences in survival rates between were tested with the chi-square (χ^2) test.

RESULTS

The acclimatization periods of the octopuses in the individual and free group rearing completed within 3-5 days and 7-10 days, respectively. In addition, mortality related to cannibalism was not detected. During the period, the water temperature, salinity, oxygen, and pH were 15.2-17.3°C, 37.7-38.6‰, 8.5-9.6 mg/l and 8.1-8.2, respectively. During the assays, the experimental water parameters were given in Table 2.

Table 2. Experimental water parameters (Mean ± SD)

	C	I1	I2
Temperature (°C)	13.4 ± 1.3 a.	13.3 ± 1.2 a.	13.9 ± 0.9 a.
Salinity (‰)	39.4 ± 0.8 a.	39.4 ± 0.9 a.	39.4 ± 0.8 a.
Oxygen (mg/l)	9.4 ± 0.7 a.	9.4 ± 0.6 a.	9.2 ± 0.7 a.
pH	8.0 ± 0.07 a.	8.0 ± 0.07 a.	8.0 ± 0.07 a.

Different superscript letters show the significant differences (P < 0.05)

The final survival of 85.7 for I2 and 100% for I1 and control groups were recorded (P > 0.05). The mortality occurred in I2 with one octopus on the 90th day of the experiment.

The average weight gains of the octopuses were shown in Figures 2 and 3, respectively. The absolute growth rates, the specific growth rates, the feeding rates, and the food conversion rates were not significantly affected by the method of rearing (P> 0.05). The experimental results were summarized in Table 3.

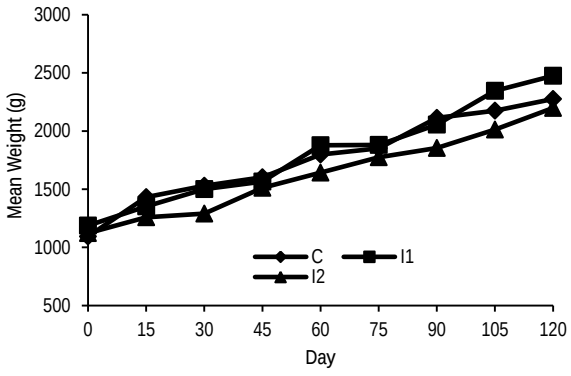

Figure 2. The mean weights (g) of *O. vulgaris*

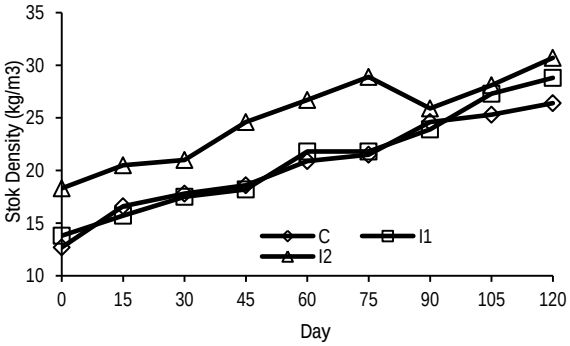

Figure 3. The stock densities of *O. vulgaris*

Table 3. The experimental results (mean ± SD).

	C	I1	I2
Average W_f (g)	2275 ± 235[a]	2475 ± 705[a]	2197 ± 370[a]
Min W_f (g)	1980	1749	1894
Max W_f (g)	2564	3491	2853
D_f (kg/m^3)	26.5[a]	28.8[a]	30.7[a]
S (%)	100[a]	100[a]	85.7[a]
AGR (g/day)	63.6 ± 21.2[a]	51.3 ± 5.3[a]	50.8 ± 11.7[a]
SGR (%/day)	0.6 ± 0.5[a]	0.6 ± 0.4[a]	0.5 ± 0.3[a]
FR (%/day)	21.9 ± 8.3[a]	20.8 ± 9.8[a]	20.1 ± 4.7[a]
FC (%)	41 ± 26[a]	39 ± 22[a]	50 ± 42[a]

Different superscript letters show the significant differences (P < 0.05); D_f, final density

In the control group, except cannibalism, food competition, aggressiveness, injuries, escape and mating behaviors were recorded. In the individual groups, these behaviors were not detected.

DISCUSSION

In this study, the individual rearing method for *Octopus vulgaris* ongrowing was applied for the first time. The present results showed that the individual rearing technique can be used for the octopus on growing due to the higher growth and survival rates.

It's pointed out that in the prior studies, the acclimatization period completes within 7-15 days for *O. vulgaris* in free group rearing method (Cagnetta, 2000; Iglesias et al., 2000a; García García and Valverde, 2006; Domingues et al., 2010; Delgado et al., 2011; Estefanell et al., 2012a; Estefanell, et al., 2012b). However, Şen (2012) notified that this period lasted 3-5 days for *O. vulgaris* by individual rearing technique. These results were found parallel to the present findings for both techniques. According to the current results, the individual rearing technique reduced the adaptation period of the octopus by almost one of the third when comparing to that of the free group rearing method.

The water parameters during the experimental period were optimal for the development of the octopuses, but especially the salinity value (41‰) was relatively higher than the previous data (García García et al., 2009; Delgado et al., 2011; Boletzky and Hanlon, 1983; Aguado Gimenez and García García, 2002). However, it's thought that the salinity data did not affect to the results.

For the free group rearing method, it's suggested that initial stocking density should be up 10 kg/m^3 for *O. vulgaris* ongrowing (Iglesias et al., 2000a; García García et al., 2004; Rodriguez et al., 2006). However, the present survival rates showed that culture density up to 18 kg/m^3 could be used for on growing of the octopus by the individual technique. On the other hand, reason of the mortality in I2 could not have been understood, clearly.

Common octopus reaches 2.5-3 kg in 3-5 months in the optimal conditions (García García et al., 2009). However, the rearing period should not exceed 3.5 months in order to commercial farming of the octopus, otherwise, the rearing practice would not be economically successful (García García et al., 2004; García García et al., 2009). In the present study, according to the current growth results, the octopuses reached almost to the commercial market size within 120 days (4 months). The major factor related to the delay in the growth of the specimens was the water temperature, which was under the suggested values (18-21°C) (García García et al., 2009). Likewise, the feeding with mainly fish might have been caused by the delay of the growth. Because, Aguado and García García (2002) reported that a diet mainly of fish, leads to comparatively poor growth.

The current growth rates (below to 1%/day) were nearly close to the ones reported for *O. vulgaris* at lower temperatures (Otero et al., 1999; Iglesias et al., 2000a; Chapela et al., 2006). Also, these data were lower than those reported for the ones rearing at a higher temperature (García García and Giménez, 2002). Temperature is the major factor that effects to cephalopod growth, food conversions and ingestion (Domingues et al., 2010).

Reported feeding rates for *O. vulgaris* range 2-8% in the previous studies (Sanchez et al., 1998; Iglesias et al., 2000a,b; García García and Valverde, 2006; Biandolino et al., 2010; Domingues et al., 2010; Estefanell et al., 2012a). However, given the average feeding rates, the current values were below the reported rates. Further, it's thought that the low FRs in this study might stem from the low water temperature and/or mainly fish diet (Aguado Giménez and García García, 2002).

Food conversions between 30 and 60% are reported for *O. vulgaris* (Aguado Giménez and García García, 2002; Vaz-Pires et al., 2004). In the present study, the food conversions got from the trials stayed within this range. Besides, food conversions in the trials indicated that the rearing methods did not affect normal growth, and the conditions were convenient for the on-growing of octopuses. More, since feeding rates in the assays were similar for the three groups, the octopuses in I2 did not consume more energy in order to metabolic purposes and biomass increment. This might indicate that the initial density of the octopuses could be raised up to 18 kg/m^3 by the individual rearing technique.

In the free group rearing trial, it was observed that the female and male octopuses kept with together demonstrates mating behavior ending with spawning. These events were also emphasized in previously (Otero et al., 1999; Iglesias et al., 2000a,b; Vaz-Pires et al., 2004; García García and Valverde, 2006; Domingues et al.,2010). Although there were no contact possibilities between the octopuses in the individual rearing method, only the one female octopus laid eggs in I2 group, interestingly. It is thought to be that the female octopus was mating in its natural environment before being caught, probably. Because, in *O. vulgaris*, 580 g females and 250 g males can reach sexual maturity and reproduce (Silva et al., 2002).

In conclusion, using the individual rearing method is a common application for high priced and highly cannibalistic farmed species (Nicholson et al., 2008; Perez et al., 2010). So, it's thought that the present results supported the authors' findings. Because it's obvious that the individual rearing technique for *O. vulgaris* on growing can be used due to the positive effects on the survival and growth of the octopuses. Furthermore, according to the present results, the individual rearing method presented important advantages, when compared to the free group rearing; no mortality related to cannibalism, no shelter, no aggressiveness, no food competition, a high survival rate, high growth rate, high initial stocking density, co-rearing of female and male octopuses, co-rearing of large and small octopuses, a short adaptation period, and any marketing size harvesting possibility. As a result, the preliminary findings showed that the individual rearing technique could solve the main problems in the free group. Finally, the preliminary results showed that for *O. vulgaris* on growing, the individual rearing technique could solve the main problems in the free group. However, there are needed more detailed studies should be carried out to the improvement of this technique.

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RESEARCH ARTICLE
ARAŞTIRMA MAKALESİ

Effects of various antioxidants on oxidative stability of anchovy (*Engraulis encrasicolus*) oil

Çeşitli antioksidanların hamsi (*Engraulis encrasicolus*) yağının oksidatif stabilitesi üzerine etkileri

Mehmet Gökhan Soydan[1] ● **Fatime Erdoğan**[2*]

[1]Graduate School of Natural and Applied Sciences, Muğla Sıtkı Koçman University, 48600, Muğla, Turkey https://orcid.org/0000-0002-9756-0797
[2]Ortaca Vocational School, Department of Aquaculture, Muğla Sıtkı Koçman University, 48600, Muğla, Turkey https://orcid.org/0000-0002-4376-4372

**Corresponding author: ferdogan95@hotmail.com*
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Abstract: The aim of study was to investigate four commercial available antioxidants (groups A (300 mg propyl gallate (PG)+10 mg rosemary extract (RE)/1000 mg), B (240 mg butylated hydroxy anisole (BHA)+80 mg PG+80 mg citric acid (CA)/1000 mg), C (120 mg BHA+120 mg PG+50 mg CA)/1000 mg), D (150 mg butylated hydroxytoluene (BHT)+100 mg BHA+10 mg PG)/1000 mg) used to evaluate oxidation during the storage in fish oil. Antioxidants were added to the fish oil to determine which ones were most effective in preventing oxidation, and fish oil was stored in the amber bottles at room temperature (20 °C) for 90 days. The control group samples were stored under the same conditions and antioxidant was not added. To determine the effect of antioxidants, the recommended by the manufacturer dose of commercial antioxidant (1000 mg kg^{-1} fish oil) was used in the experimental groups. The formation of the primary and secondary oxidation products in fish oil storage trial was examined by conducting the peroxide value (PV) and p-anisidine value (AV) analyses. The total oxidation value (TOTOX) was calculated based on the PV and AV measurements. Minor changes were observed in the PV of the fish oil during the first 30 days. In the study, antioxidant added samples (groups B, C, D > 5 meq kg^{-1}) were oxidized on the 45th day; on the other hand, both control and group A oxidized on the 75th day. A possible prooxidative effect was seen for some of the antioxidants. There was a very little change secondary oxidation of fish oil and no significant effects of all four antioxidant groups on the changes of AV (<20) during the storage period (P>0.05). In addition TOTOX was calculated under GOED (<26) limit during the storage for 90 days. At the end of the study, control samples were not significantly different from the other samples with antioxidant-added. Due to the results obtained at the end of the 90-day study, it was found that none of the antioxidants were used efficiently in this study.

Keywords: Fish oil, antioxidants, lipid oxidation, stabilization

Öz: Bu çalışmanın amacı, balık yağında depolanma sırasındaki oksidasyonu değerlendirmek için kullanılan dört ticari antioksidanı (A (300 mg propil gallat (PG)+10 mg biberiye ekstraktı (BE)/1000 mg, B (240 mg bütil hidroksi anisol (BHA)+80 mg PG+80 mg sitrik asit (SA)/1000 mg, C (120 mg BHA+120 mg PG+50 mg SA)/ 1000 mg, D (150 mg bütil hidroksi toluene (BHT)+100 mg BHA+10 mg PG)/1000 mg) incelemektir. Antioksidanlar, hangilerinin oksidasyonun önlenmesinde en etkili olduğunu belirlemek için balık yağına eklenmiş ve balık yağları, 90 gün boyunca oda sıcaklığında (20 °C) amber şişelerde saklanmıştır. Kontrol numuneleri aynı koşullar altında muhafaza edilmiş ve antioksidan eklenmemiştir. Antioksidanların etkisini belirlemek için, deney gruplarında üretici firma tarafından önerilen ticari antioksidan dozu (1000 mg kg^{-1} balık yağına) kullanılmıştır. Balık yağı depolama denemesinde birincil ve ikincil oksidasyon ürünlerinin oluşumu, peroksit değeri (PV) ve p-anisidin değeri (AV) analizleri yapılarak incelenmiştir. PV ve AV ölçümlerine dayanarak toplam oksidasyon değeri (TOTOX) hesaplanmıştır. İlk 30 gün boyunca balık yağının peroksit değerlerinde küçük değişiklikler gözlenmiştir. Çalışmada, antioksidan takviyeli örnekler B, C, D > 5 meq kg^{-1}), 45. günde okside olmuş, ancak kontrol ve A grubu 75. günde okside olmuştur. Bazı antioksidanlar için olası bir prooxidatif etki görülmüştür. Balık yağının sekonder oksidasyonunda küçük değişiklikler gerçekleşmiş ve depolama süresi boyunca dört antioksidan grubunun da AV değişiklikleri üzerinde önemli bir etkisi olmamıştır (p>0.05). İlaveten TOTOX 90 günlük depolama boyunca GOED (< 26) limiti altında hesaplanmıştır. Çalışmanın sonunda kontrol örnekleri ile antioksidan ilaveli örnekler arasında önemli fark bulunmamıştır (P>0,05). 90 günlük çalışmanın sonunda elde edilen sonuçlara göre antioksidanların hiçbirinin verimli bir şekilde kullanılmadığı tespit edilmiştir.

Anahtar kelimeler: Balık yağı, antioksidanlar, yağ oksidasyonu, stabilizasyon

INTRODUCTION

Fish meal and fish oil are used as feed ingredient in aquaculture. Small pelagic and demersal species having no economic value are preferred for the production of fish meal and oil. Fish used for obtaining fish oils for commercial production today are herring, cod, sardine, anchovy, menhaden, horse mackerel, sharks and dolphins (Kasbo, 2011; Boran, 2004). Fish oil is the major source of unsaturated omega-3 fatty acids ie. eicosapentaenoic acid (20:5 n−3, EPA) and docosahexaenoic acid (22:6 n−3, DHA) (Fakir and Waghmare, 2015). However, due to their high level of unsaturation these omega-3 PUFA are extremely susceptible to oxidative spoilage (Morales et al., 2015).

The content of EPA and DHA in fish oil is dependent on the type of fish, the fish diet, sea water temperatures and geographic location of the catch (Kasbo, 2011). Due to the fish oil structure and environmental factors such as enzyme, light, metal ions, temperature it oxidizes rapidly during the processing and storage (Baek, 2012; Palupi et al., 2016). Oxidation of fish oil produces undesirable flavors. It may also

reduce the nutritional quality and safety of the oils (Eritsland, 2000; Korkut et al., 2007). Thus, studies that are intended to prevent oxidation in fish oil and prolong its shelf life are required.

There are several methods to protect the oil from oxidation. The most common methods are use of metal inactivators, minimizing exposure to air, heat and light, minimizing the loss of naturally occurring antioxidants and adding additional antioxidants (Fennema, 2008).

Antioxidants are chemical compounds which have been shown to prevent and delay oxidative deterioration in oil. These chemical compounds have an effect at the beginning of oxidative and auto oxidative processes and thus inhibiting oxidation which leads to the formation of the reaction products (Fennema, 2008).

Many studies have been carried out so far to protect fish oil against oxidation. Natural and synthetic antioxidants were used in these studies. Some researchers focused on antioxidative effects of rosemary extract (Hraš et al., 2000). Morales et al., (2015) compared the effects of natural and synthetic antioxidants on oxidation in sardine oil and the following researchers tested the effects of synthetic antioxidants on oxidative stability, in herring oil (Carvajal et al., 2014; Baek, 2012), mackerel oil (Fakir and Waghmare, 2015), sardine oil (Chandrasekar et al., 2016) and cod liver oil (Kasbo, 2011), respectively. However, there have not been any studies carried out to investigate the effects of the addition of newly released commercial antioxidants in the market prepared with the addition of different antioxidant combinations in anchovy oil obtained from the Black Sea region on oxidative stability. In this study, four different (A (300g PG+10 g RE)/1000 g, B (240 g BHA+80 g PG+80 g CA)/1000 g, C (120 g BHA+120 g PG+50 g CA)/ 1000 g, D (150 g BHT+100 g BHA+10 g PG)/1000 g) commercial antioxidants were added to fish oil obtained from anchovy fish and their resistance to oxidation in the storage conditions was tested.

MATERIALS AND METHODS

Materials

Anchovy oil was obtained from Sürsan Incorporated Company (Samsun). Fatty acid composition of anchovy fish oil is analyzed in EcoSmyrna Laboratories (by gas chromatography). Fatty acid composition of anchovy oil is shown in Table 1.

Table 1. Fatty acid composition of anchovy oil used in the study

Fatty Acids		%
14:00	myristic acid	8.46
16:00	palmitic acid	26.20
18:00	stearic acid	4.51
18:01	oleic acid	22.90
18:3 -3	α-linolenic acid	1.31
20:3 ω-3	eicosatrienoic acid	0.30
20:5 ω -3	eicosapentaenoic acid (EPA)	11.30
22:5 ω-3	decosapentaenoic acid	0.59
22:6 ω -3	docosahexaenoic acid (DHA)	15.30
ω-3 Total	**ω**-3 Total	28.80
18:2 ω -6	linoleic acid	1.31
18:3 ω-6	gamma linolenic acid	0.28
20:2 ω-6	eicosadienoic acid	0.23
20:3 ω-6	eicosatrienoic acid	0.27
20:4 ω-6	arachidonic acid	0.25
ω -6 Total	**ω**-6 Total	2.82
18:1 Trans	trans oleic acid	0.04
18:2 Trans	trans-linoleic acid	0.48
18:3 Trans	trans linolenic acid	0.05
23:0 Trans	trans tricosanoic acid	0.00
	Total	93.78

Anchovy oil, tocopherol content was analyzed in Süleyman Demirel University Applied Basic Sciences and Technologies Research Laboratory (with HPLC RF–10AXL Fluorescence detector). Tocopherol content of anchovy oil is shown in Table 2.

Table 2. Tocopherol content of anchovy oil (ppm) used in the study

	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol
Anchovy Oil	71.59	<0.10	0.37	<0.005

Antioxidants; Miaradox L PV 301, Miaradox L AP 248, Miaradox L AP 1212 were supplied Miavid Company (Germany) and Oxy-Nil Eqzero Plus Liquid was supplied Nutriad Company (United Kingdom).

The antioxidants presented are propyl gallate (PG), rosemary extract (RE), butylated hydroxyanisole (BHA), citric acid (CA), butylated hydroxytoluene (BHT).

The contents of the antioxidants used in the experiment are as follows:

Group Control no antioxidant added

Group A (Miaradox L PV 301) (300.000 mg PG +10.000 mg RE) kg^{-1}
Group B Miaradox L AP 248 (240.000 mg BHA + 80.000 mg PG + 80.000 mg CA) kg^{-1}
Group C Miaradox L AP 1212 (120.000 mg BHA + 120.000 mg PG + 50.000 mg CA) kg^{-1}
Group D Oxy-Nil Eqzero Plus Liquid (150.000 mg BHT + 100.000 mg BHA + 10.000 mg PG) kg^{-1}

Sample preparation

This study was carried out in the laboratories of Skretting Incorporated Company. Fish oil was weighed and placed in separate plastic containers.

Commercial antioxidants were added to fish oil at the recommended rate (1000 mg kg^{-1}) by the manufacturer. The antioxidants weighed in petri dishes were added to the fish oil and mixed with a plastic-tipped beater for 5 minutes to be homogenized. The sample without antioxidants was used for the control group. The fish oil was then filled into the amber bottles, each of 100 ml with a screw cap. In this study a total of 70 amber bottles was used. The bottles were stored in a dark room at 20±1 ^{o}C.

The determination of oxidative stability

Fish oil samples were measured by the use of peroxide value (PV), p-anisidine value (AV) and free fatty acid value (FFA). Samples were analyzed every two weeks during the trial. All samples were tested in duplicate.

The peroxide value (PV)

The primary oxidation of fish oil was determined by measuring the peroxide value. GOED has defined a limit of PV 5 meq kg^{-1} for accepted quality of oil (GOED, 2019).

The PV of the fish oil were measured according to a modification of the method of the American Oil Chemist's Society Official method Cd 8b-90 (AOCS 2017b). Approximately 5 g of fish oil was weighed into a 250 ml Erlenmeyer flask. To this flask was added 18 ml of acetic acid/chloroform mixture (3/2) (v/v) and the contents were continuously stirred in order to fully dissolve the fish oil. 0.5 ml potassium iodide (KI) solution was added and the flask was allowed to stand for 1 min with occasional shaking. Distilled H_2O (30 ml) was added to the flask and was titrated against 0.01 N sodium thiosulphate ($Na_2S_2O_3$) using 2 ml of 1% starch indicator. Blank samples were determined by titration of samples which did not contain fish oil. The PV was calculated as follows:

PV (meq O_2 kg^{-1} oil) = (S - B) * N *1000 / sample weight (1)

where, S is sample titre (ml); B is blank titre (ml) and N is Normality of $Na_2S_2O_3$.

The p-Anisidine value (AV)

The measurement of the AV is a common method for determining the level of secondary oxidation products in oils. GOED has been defined a limit of AV 20 for accepted quality of fish oil (GOED, 2019).

p-Anisidine value was determined according to AOCS Cd 18-90 (AOCS 2017a). This method determines the amount of aldehydes (principally 2–alkenals and 2.4–dienals) in animal and vegetable fats and oils, by reaction in an acetic acid solution of the aldehydic compounds in the oil and the p-anisidine, and then measuring the absorbance at 350 nm.

The anisidine values were calculated as in Eq. (2)

pAV= (25*1.2 As- Ab)/ m (2)

As is the absorbance of oil after reaction with p-anisidine, Ab is the absorbance of oil in isoocatane, m is the weight of anchovy oil used for analysis (g).

The total oxidation value (TOTOX)

The total oxidation values (TOTOX) of the oil samples were used in this study as an indication of overall oxidative stability. TOTOX was defined as the addition of both the peroxide and anisidine value;

TOTOX value = (2 PV + p-AV) (3)

PV: Peroxide value
AV: p-anisidine value

The GOED monograph states TOTOX of 26 as the limit for oxidative status (GOED, 2019).

The free fatty acid (FFA)

The formation of FFA is an important measure of food rancidity. Determination of FFA, expressed as percentage of oleic acid, were done by acidimetric titration after adding ethanol and using phenolphthalein as an indicator, following AOAC (1990) method. Hertrampf and Piedad-Pascual (2000) has defined a limit of FFA 5 % for accepted quality of oil.

FFA= (M_1*0.25*28.2) / M_2 (4)

Free fatty acid = (FFA) (as oleic acid %)
M_1: amount of sodium thiosulfate consumption (ml)
M_2: sample amount (gr)

Statistical analysis

Statistical analysis comprised a one-way ANOVA using the probability level of 0.05. The significant differences between the means of parameters were determined using Duncan's test. All statistical analyses were performed using SPSS 14.0 for Windows (SPSS INC. Chicago, IL, USA). Each replicate is expressed as mean ± SE. All experiments were replicated two times.

RESULTS AND DISCUSSION

The effect on peroxide value (PV)

Peroxides are the first compounds formed when polyunsaturated fatty acids oxidized, and this is the first step of lipid oxidation. The level of primary oxidation products, hydroperoxides, can be measured by using PV as a method

(Kasbo, 2011). Changes in peroxide values of anchovy oil during 90 days of storage are shown in Table 3.

Table 3. Changes in peroxide value of anchovy oil during 90 days of storage

Days	Groups				
	Control	A	B	C	D
Initial	3.93 ± 0.06^{aBC}	3.93 ± 0.06^{aBC}	3.93 ± 0.06^{aE}	3.93 ± 0.06^{aC}	3.93 ± 0.06^{aE}
15th Day	4.23 ± 0.13^{aBC}	4.33 ± 0.09^{aB}	4.53 ± 0.03^{aC}	4.85 ± 0.04^{aB}	4.70 ± 0.38^{aCDE}
30th Day	3.66 ± 0.04^{dC}	3.92 ± 0.17^{cdBC}	4.22 ± 0.12^{bcCDE}	4.65 ± 0.01^{aB}	4.43 ± 0.01^{abDE}
45th Day	3.97 ± 0.14^{cBC}	4.42 ± 0.35^{bcB}	5.12 ± 0.17^{abB}	5.61 ± 0.17^{aA}	5.69 ± 0.43^{aAB}
60th Day	2.64 ± 0.02^{dD}	3.48 ± 0.05^{bC}	3.00 ± 0.02^{cF}	3.69 ± 0.06^{aC}	3.13 ± 0.00^{cF}
75th Day	4.44 ± 0.27^{bABC}	4.28 ± 0.07^{bB}	4.11 ± 0.04^{bDE}	5.11 ± 0.05^{aB}	5.33 ± 0.29^{aBC}
90th Day	5.32 ± 0.67^{aA}	5.74 ± 0.25^{aA}	5.59 ± 0.16^{aA}	5.92 ± 0.24^{aA}	6.14 ± 0.00^{aA}

* Values are presented as mean ±SE, n=2

[a-b] Values that contain different superscript lowercase letters in the same row are significantly different (P<0.05)

[A-B] Values that contain different superscript uppercase letters in the same column are significantly different (P<0.05)

The PV of the fish oil, stored at 20 °C, changed insignificantly till the 30th day during the storage in all the experimental groups (P>0.05). Control samples were not distinguished from samples with added antioxidants until the 45th day. For the first time the B (5.12±0.17 meq kg^{-1}), C (5.61±0.17 meq kg^{-1}) and D (5.69±0.43 meq kg^{-1}) groups exceeded over the limits of GOED (PV 5 meq kg^{-1}) on the 45th day. However, there was a limited increase in control (3.97±0.14 meq kg^{-1}) and group A (4.42±0.35 meq kg^{-1}). The effects of antioxidants are dependent on the concentration. Too high concentration of antioxidants may change the action of antioxidants to work as prooxidants (Kasbo, 2011). This result could be due to the possible effects of prooxidative effects of antioxidants as stated by Kasbo (2011). PV values of all fish oil samples decreased at 60th day. The decrease in the PV of oil samples, as stated by Hras et al., (2000) may be attributed to a decline in the rate of hydroperoxide formation in associated with a rise in the production of the secondary oxidation products.

The control and the group A remained below the PV 5 meq kg^{-1} throughout 75 days. It has been determined that all groups have exceeded the limit of PV 5 meq kg^{-1} on the 90th day. As of 90th day, the control samples did not distinguish from samples with antioxidant added.

At the beginning of the study, the tocopherol level of anchovy oil was measured as 71.59 ppm. It can be considered that the α-tocopherol naturally found in anchovy oil might have protected fish oil from oxidation. This situation could explain why the PV of control group was lower than the groups supplemented with antioxidant throughout the trial. Similar to our results, Morales et al., (2015) stated that the low concentrations of α-tocopherol (50 ppm) in sardine oil together with ascorbyl palmitate was effective in eliminating the hydroperoxides. This is due to the destruction ability of α-tocopherol with non-radical processes like the elimination and reduction of hydroperoxides or hydrogen donation. It was reported in the same study that the high levels of α-tocopherol demonstrate prooxidative behaviors and thus accelerating the oxidation of fish oil (Kulas and Ackman, 2001; Drusch et al., 2008; Frankel, 2005).

Throughout the trial, it was revealed that there was lower PV in the group (A) in which PG and rosemary extract were added when compared to the other groups supplemented with antioxidants. However, the group A exhibited similar PV with the control group but it could not demonstrate a better performance than the control group. It was reported by Tsimidou et al., (1995) and O'Sullivan et al., (2005) that when rosemary was added to the mackerel oil and cod liver oil, there was a similar decrease in oxidation.

The effect on p-Anisidine values (AV)

AV is an indicator of the formation of secondary oxidation products such as aldehydes and ketones formed by the decomposition of peroxide and hydroperoxides. AV can be used as a rough estimator of future storage stability of freshly processed oil (Frankel, 2005). Changes in p-anisidine values of anchovy oil during 90 days of storage are shown in Table 4.

Table 4. Changes in p-anisidine value of anchovy oil during 90 days of storage

Days	Groups				
	Control	A	B	C	D
Initial	6.61 ± 0.25^{aD}	6.61 ± 0.25^{aBC}	6.61 ± 0.25^{aC}	6.61 ± 025^{aB}	6.61 ± 0.25^{aC}
15th Day	6.68 ± 0.38^{aD}	6.44 ± 0.46^{aC}	6.75 ± 0.54^{aC}	6.65 ± 0.50^{aB}	7.30 ± 0.04^{aBC}
30th Day	6.69 ± 0.02^{aD}	6.85 ± 0.21^{aBC}	6.97 ± 0.22^{aC}	6.75 ± 0.10^{aB}	7.02 ± 0.28^{aBC}
45th Day	6.77 ± 0.34^{aCD}	7.44 ± 0.13^{aAB}	7.21 ± 1.04^{aBC}	7.12 ± 0.58^{aB}	6.64 ± 0.31^{aC}
60th Day	7.57 ± 0.09^{cBC}	7.57 ± 0.03^{cAB}	7.95 ± 0.07^{abABC}	8.18 ± 0.07^{aAB}	7.81 ± 0.06^{bcBC}
75th Day	8.01 ± 0.01^{aAB}	8.02 ± 0.09^{aA}	8.59 ± 0.07^{aAB}	9.58 ± 1.03^{aA}	8.05 ± 0.02^{aAB}
90th Day	8.48 ± 0.02^{aA}	8.06 ± 0.51^{aA}	8.84 ± 0.13^{aA}	7.84 ± 0.04^{aB}	9.09 ± 0.86^{aA}

* Values are presented as mean ±SE, n=2

[a-b] Values that contain different superscript lowercase letters in the same row are significantly different (P<0.05)

[A-B] Values that contain different superscript uppercase letters in the same column are significantly different (P<0.05)

Small changes were recorded for AV which was found to be 6.61 ± 0.25 (P> 0.05) at the beginning of the experiment but there was a difference between the groups on the 60th day (P <0.05). At the end of the experiment, the lowest values were observed in the group C (7.84±0.04) and the AV of all groups were found to be similar (P>0.05). At the end of the experiment, the AV remained below the GOED (<20) limits in all groups. Fakir and Waghmare (2015) in their study added synthetic antioxidants in mackerel oil and AV showed small differences throughout the storage and the addition of antioxidant did not have an effect on this parameter (P>0.05). This result revealed that the secondary oxidation was very little throughout the storage. These results are compatible

with our results regarding the AV. Wang et al., (2011) determined that in addition to the concentration and type of the antioxidant added to the fish oil and the temperature of the environment was effective on the fish oil oxidation.

The total oxidation value (TOTOX)

PV and AV change over time as hydroperoxides are produced and decomposed in fish oil. TOTOX gives complete information about the oxidative state of the oil, combines the history of the oil (AV) with the present status (PV). TOTOX considers both primary and secondary oxidation products (Kasbo, 2011). Changes in total oxidation value of anchovy oil during 90 days of storage are shown in Table 5.

Table 5. Changes in total oxidation value of anchovy oil during 90 days of storage

	Groups				
Days	Control	A	B	C	D
Initial	14.48±0.00^{aDE}	14.48±0.00^{aE}	14.48±0.00^{aEF}	14.48±0.00^{aD}	14.48±0.00^{aD}
15th Day	15.13±0.12^{aCD}	15.10±0.63^{aDE}	15.80±0.48^{aCDE}	16.36±0.59^{aBCD}	16.69±0.80^{aBC}
30th Day	14.01±0.11^{bDE}	14.69±0.56^{abE}	15.41±0.47^{abDE}	16.05±0.08^{aCD}	15.88±0.26^{aCD}
45th Day	14.71±0.61^{bDE}	16.27±0.58^{abBC}	17.46±0.71^{abB}	18.34±0.92^{aAB}	18.01±1.17^{aBC}
60th Day	12.86±0.04^{dE}	14.53±0.14^{bE}	13.95±0.03^{cF}	15.56±0.05^{aCD}	14.07±0.05^{bD}
75th Day	16.88±0.55^{aBC}	16.58±0.04^{aB}	16.81±0.01^{aBC}	19.80±1.13^{aA}	18.72±0.59^{aB}
90th Day	19.12±1.04^{bA}	19.54±0.01^{abA}	20.02±0.45^{abA}	19.69±0.51^{abA}	21.38±0.84^{aA}

* Values are presented as mean ±SE, n=2

a-b Values that contain different superscript lowercase letters in the same row are significantly different (P<0.05)

A-B Values that contain different superscript uppercase letters in the same column are significantly different (P<0.05)

The TOTOX of the fish oil was calculated as 14.48 at the beginning of the trial. Insignificant increases were observed from the first day to the 15th in all groups but there were not significant differences between the groups (P>0.05). On the 30th and 45th days, TOTOX continued to increase and except for the group D, all groups exhibited similarities (P>0.05). On the 60th day, based on the decreases in peroxide values, very clear changes in TOTOX caused significant differences among the groups (P<0.05). It was also verified by O'Sullivan et al., (2005) that the decrease of PV occurred due to the reduction of hydroperoxides. On the 75th day, the TOTOX increased in all the groups (P>0.05). At the end of the storage, on the 90th day the TOTOX in group D was considerably higher than the control group (P<0.05); however, it was found similarly in the other groups (P>0.05). For 90th day storage, the TOTOX was calculated below the limits of GOED (26) just like the PV and AV.

Similar to our findings, the results shown by Kasbo 2011 the added different commercial antioxidants stated that due to the unclear results, the most efficient antioxidants and concentrations were not obtained. However, O'Sullivan et al., (2005) carried out a study in which they added natural antioxidants in cod (*Gadus morhua*) and white pollack (*Pollachius pollachius*) oils and they reported that the TOTOX in the groups supplemented with antioxidants was quite lower than the control group (P<0.05). According to their findings, they stated that the natural antioxidants added in the fish oil were quite successful in stabilizing. The results obtained from the different studies reveal that the effectiveness of the antioxidants depend on such factors as the types of fish oil, storage temperature, and synergic effect of the antioxidants (Baek, 2012; Kasbo, 2011; Wang et al., 2011; O'Sullivan et al., 2005).

The effect on free fatty acids (FFA)

FFA formation is due to the hydrolysis of triglycerides; this process may be promoted by the reaction of oil with moisture (Iqbal and Bhanger, 2007). Lipid rancidity gives in increase in the number of effects such as hydrolysed and oxidation rancidity. In particular, polyunsaturated acids easily oxidized by air, producing peroxide which breaks down into aldehydes and ketones. The production of aldehydes and ketones causes unpleasant taste (Wang et al., 2011). Changes in free fatty acid value of anchovy oil during 90 days of storage are shown in Table 6.

Table 6. Changes in free fatty acid value of anchovy oil during 90 days of storage

	Groups				
Days	Control	A	B	C	D
Initial	1.53±0.02^{aCD}	1.53±0.02^{aBC}	1.53±0.02^{aAB}	1.53±0.02^{aE}	1.53±0.02^{aD}
15th Day	1.48±0.02^{aD}	1.38±0.02^{aBC}	0.95±0.18^{bC}	1.24±0.08^{abF}	1.23±0.03^{abE}
30th Day	1.53±0.09^{bcCD}	1.40±0.18^{cC}	1.35±0.16^{cB}	2.03±0.02^{aA}	1.86±0.00^{abA}
45th Day	2.48±0.17^{aA}	1.95±0.05^{bBC}	1.84±0.14^{bA}	18.2±0.02^{bBC}	1.67±0.03^{bC}
60th Day	1.75±0.06^{aBC}	1.70±0.03^{aD}	1.76±0.03^{aA}	1.72±000^{aCD}	1.72±0.02^{aBC}
75th Day	1.67±0.01^{aBCD}	1.62±0.00^{aABC}	1.71±0.05^{aA}	1.67±0.05^{aD}	1.68±0.04^{aC}
90th Day	1.80±0.02^{bB}	1.95±0.02^{aA}	1.76±0.03^{bA}	1.93±0.00^{aAB}	1.79±0.03^{bAB}

* Values are presented as mean ±SE, n=2

a-b Values that contain different superscript lowercase letters in the same row are significantly different (P<0.05)

A-B Values that contain different superscript uppercase letters in the same column are significantly different (P<0.05)

FFA values decreased on the 15th day of fish oil storage study (ranging from 0.95 to 1.48 %) but on the 30th day FFA increased in all the groups (ranging from 1.35 to 2.03 %). The highest FFA contents (2.48 %) were observed in the control group (P<0.05) on the 45th day while C and D showed the lowest FFA contents (1.82% and 1.67% respectively) on the same day of the storage. Except for the control sample, the FFA levels of the other groups showed no difference in the 45th days. High FFA increases were not detected during the storage between the 60th – the 90th days. There were no statistically significant differences between the control and with antioxidant added groups. This is explained by the lack of

hydrolysis due to the low moisture content in stabilized oil (Aidos et al., 2002). Throughout the trial, the moisture level was between 0.15 and 0.37% in all the groups and did not reach the upper limit of 1% during the trial. Similarly, the FFA level was between 0.95 and 2.48%, but did not reach 5% higher in all groups. (Hertrampf and Piedad-Pascual, 2000). Similar to our results, Morales et al., (2015) stated that during the storage period, FFA content of oils stabilized with different concentrations of α-tocopherol did not increase, but a slight decrease occurred. On the other hand, Wang et al., (2011) reported that different concentrations of carnosic acid (CA) and TBHQ provided much better FFA than the control group at two different temperatures (4-30 °C) in the 66-day storage study and the highest FFA was detected in the control group. Researchers reported that although oils have been supplemented with antioxidants, the oils should be stored at low temperatures and that low moisture content should be maintained during the storage.

CONCLUSION

The control group and the group A oxidized on the 75th day and PV exceeded 5 meq kg^{-1} (<5 meq kg^{-1} GOED limits). The groups B, C and D got oxidated in a shorter time on the 45th day. AV showed an increase at a small level throughout 90 days but it was below the GOED limits (<20) in all the groups. TOTOX was calculated under GOED (<26) limit during the storage throughout 90 days. FFA values increased slightly during the trial. There are many aspects connected to the oxidation of fish oil and one has to consider each of them when choosing an antioxidant. Factors such as EPA and DHA content of the fish oil, temperature of the setting, light, access to oxygen, and metal ion concentration are effective on the oxidation of oil. Considering the results, the antioxidants used in the experiment were not effective in anchovy oil. It is thought that the new studies should be carried out with these commercial antioxidants in different fish oil and temperature environments.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Kapalı devre sistemde tatlı su ve ‰5 tuzlulukta yetiştirilen Avrupa yayın balığının (*Silurus glanis* L.) büyüme performansının karşılaştırılması

Comparison of growth performance of European catfish (*Silurus glanis* L) rearing in freshwater and 5 ‰ salinity in recirculating system

Sevim Hamzaçebi[1*] ● Ramazan Serezli[2]

[1]İzmir Kâtip Çelebi Üniversitesi, Su Ürünleri Fakültesi, 35620, Çiğli, İzmir, Türkiye http://orcid.org/0000-0002-2179-1900
[2]İzmir Kâtip Çelebi Üniversitesi, Su Ürünleri Fakültesi, 35620, Çiğli, İzmir, Türkiye http://orcid.org/0000-0003-4819-6607

*Corresponding author: sevim.hamzacebi@ikc.edu.tr
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Öz: Avrupa yayın balığı (*Silurus glanis* L.) hızlı büyümesi ve etinin lezzetli olmasından dolayı ekonomik değeri yüksek olan bir balıktır. Bu çalışmada, kapalı devre sistemde, hem tatlı suda hem de ‰5 tuzluluktaki suda yayın balıklarının büyüme performansları araştırılmıştır. Çalışma, ortalama ağırlığı 507±13,02 g ve ortalama total boyu 42,85±0,45 cm olan balıklar kullanılarak, üç ay boyunca tatlı su (Y1, Y2, Y3) ve ‰5 tuzluluktaki artezyen suyunda (T1, T2, T3) olmak üzere üçer tekerrür şeklinde dizayn edilmiştir. Balıklara günde iki öğün ağırlıklarının %3'ü kadar besleme yapılmıştır. Deneme sonucunda ortalama canlı ağırlık artışı, total boy artışı, yem tüketimi miktarı ve oranı, SGR ve FCR oranları Y grubunda daha yüksek bir değer göstermiştir (P<0,05). Grupların yaşama oranları arasında herhangi bir istatistiksel fark görülmemiştir (P>0,05).

Sonuç olarak, bu türün hem tatlı su hem de ‰5 tuzluluğa sahip sularda yetiştiriciliğinin yapılabileceği kanaatine varılmıştır.

Anahtar kelimeler: Avrupa yayın balığı, Silurus glanis, kapalı devre sistem, yetiştiricilik, balık besleme

Abstract: European catfish (*Silurus glanis* L.) is a fish that has a high economic value due to its rapid growth and delicious flesh. In this study, the growth performance of European catfish was investigated in freshwater and 5‰ salinity in recirculating system. The study was carried out using fish with an average weight of 507±13.02 g and an average total length of 42.85±0.45 cm in freshwater (Y1, Y2, Y3) and 5‰ salinity of ground water (T1, T2, T3) designed as three replications for three months. Fish were fed with 3% of the total weight two meals a day. The mean body weight gain, total lenght increase, amount and percentage of feed consumption, specific growth rate (SGR) and feed conversion rate (FCR) were higher in group Y (P<0.05). No statistical difference was observed between the survival rates of the groups (P>0.05). As a result, it is concluded that this species can be reared in both fresh water and 5‰ saline water.

Keywords: European catfish, Silurus glanis, recirculating system, aquaculture, fish feding

GİRİŞ

Avrupa yayın balığı (*Silurus glanis*) 100 yılı aşkın süredir Avrupa'da yetiştiriciliği yapılan tatlı su balığı türüdür (Linhart vd., 2002). Yayın balığı Güney ve Güneydoğu Anadolu hariç Türkiye'deki hemen hemen tüm iç sularda doğal olarak bulunan bir tür olup, hızlı büyümesi ve etinin lezzetli olmasından dolayı ekonomik değeri yüksek olan bir türdür (Çelikkale, 1994). Maksimum 5 m boy, 306 kg ağırlığa ulaşabilen bu balık, tatlı su balıkları arasında en büyük balık olarak görülmektedir (Copp vd., 2009). Orta ve Doğu Avrupa ile Batı Asya akarsu ve göllerinde yaşamaktadır. Birçok Avrupa ülkesinde kültür koşullarında yetiştiriciliği yapılmaktadır. Türkiye'de ise yetiştiriciliği konusunda önemli bir uygulama bulunmamaktadır (Alpbaz, 2005). Geçmişte bu türün yoğun yetiştiricilik şartlarına uygun olmadığı düşünülse de, günümüzde yetiştiriciliği önem kazanmaya başlamış olup, özellikle monokültür olarak yoğun yetiştiriciliği mümkün görülmektedir (Talpeş vd., 2009). Etinin lezzetli olması, yüksek stok yoğunluğunda yetiştirilebilmesi, pelet yem ile beslenebilmesi, ılık sularda yaşayabilmesi üretiminde artışa yol açmıştır (Mocanu vd., 2012). Stok yoğunluğu ile ilgili çalışmalar oldukça az olup 60 kg/m^3'e kadar stoklanabileceği belirtilmekle birlikte (Placinta vd., 2014) Mocanu vd. (2012) yaptıkları çalışmada başlangıç stok yoğunluklarını 1,46 ve 2,78 kg/m^3 olarak belirlemişlerdir. Bu tür ile ilgili stok yoğunluğu çalışması sınırlıdır. Yayın balığının 1993 yılındaki toplam üretimi 602 ton, 2002 yılında 2000 ton (Linhart vd., 2002), 2010 yılında 3515 ton, 2016 yılında 3699 ton, 2017 yılında ise 3871 ton olarak gerçekleşmiştir (FAO, 2019). Nispeten düşük sıcaklıklarda (<10°C) bile büyüyebildikleri bildirilmektedir (David, 2006). Yayın balığının kapalı devre sistemlerde pelet yem ile hem monokültür hem de polikültür olarak (sazan (*Cyprinus carpio*), kadife balığı (*Tinca tinca*) ve mersin balığı (*Acipenser* sp.)) yoğun yetiştiriciliği yapılabilmektedir (Linhart vd., 2002; Ulikowski, 2003). Kapalı devre sistem yetiştiricilikte diğer sistemlere göre ortam şartları daha iyi kontrol edildiğinden daha yoğun bir üretim yapılabilmekte olup, yıl boyunca tezgahlara ürün sunulması kolaylaşmaktadır (Mocanu vd., 2012).

Uzun yıllardan beri yayın balığının Avrupa'da yetiştiriciliği yapılmasına rağmen ülkemizde yetiştiriciliği ile ilgili çalışmalar oldukça sınırlıdır. Bu türün yetiştiriciliği bilgi ve tecrübe gerektirdiğinden ve üreticiler risk almak istemediklerinden yetiştiriciliğine yönelmekte tereddüt etmektedirler. Bu çalışma ülkemizdeki yayın balığı yetiştiriciliğine nispeten ışık tutabilmek, üreticileri cesaretlendirmek ve ekonomik öneme sahip bu türün kontrollü şartlarda yetiştirilebilirliğini ortaya koymak için yürütülmüştür. Çalışmada, kapalı devre sistemde tatlı suda ve düşük tuzluluktaki (‰5) artezyen suyunda yetiştirilen yayın balıklarının (*Silurus glanis*) büyüme performanslarının karşılaştırılması amaçlanmıştır.

MATERYAL VE METOT

Balık

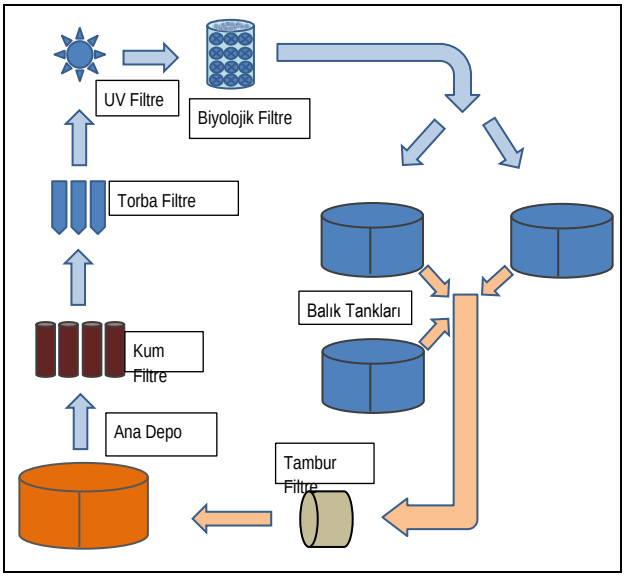
Denemede kullanılan yayın balığı (*Silurus glanis*) ticari bir işletmeden yavru iken alınıp, büyütülmüştür. Çalışmaya başlamadan önce balıklar bulundukları ortama 15 gün süre ile adapte edilmişlerdir. Ortalama ağırlığı 507±13,02 g ve ortalama total boyu 42,85±0,45 cm olan balıklar üç ay boyunca günde iki öğün ağırlıklarının %3'ü kadar (sabah 09:00, akşam 17:00) beslenerek aylık olarak büyüme, beslenme ve yaşama oranlarına bakılmıştır. Denemede %45 protein içerikli 4 mm alabalık yemi (Skretting) ile beslenmiştir (Tablo 1).

Tablo 1. Denemede kullanılan yemin kimyasal kompozisyonu
Table 1. Chemical composition of the feed used in the experiment

İçerik	Miktar
Ham protein(%)	45.0
Ham yağ (%)	20.0
Selüloz (%)	3.0
Kül (%)	6.5
Fosfor (%)	1.0
Sindirilebilir Enerji (MJ/kg)	17.8
Vitamin A (UI/kg)	4000
Vitamin D (UI/kg)	2500
Vitamin E (mg/kg)	150
Vitamin C (mg/kg)	100

Deneme düzeneği

Çalışma İzmir Kâtip Çelebi Üniversitesi Su Ürünleri Fakültesi tesisinde yürütülmüş olup, 2017 yılı Nisan – Haziran ayları arasında üç ay süreyle yürütülmüştür. Balıklar 15'er adet olarak 1000 litre hacme sahip 6 adet silindir konik tanklarda stoklanarak bakımları yapılmıştır. Deneme tatlı su (Y1, Y2, Y3) ve ‰5 tuzluluktaki artezyen suyunda (T1, T2, T3) olmak üzere üç paralel olarak tasarlanmıştır. Çalışma 2 adet farklı kapalı devre sistemden, (3 adet 1 tonluk silindir konik tank ve filtrasyon ünitelerinden (kum filtresi, mekanik filtre, torba filtre, UV lamba ve biyolojik filtre ve oksijen ünitesi)) oluşmaktadır (Şekil 1). Çalışmada ‰5 tuzluluğa, 27°C sıcaklığa sahip 80 m derinden çıkan artezyen suyu, dinlendirildikten sonra kullanılmıştır. Tatlı su olarak ise çeşme suyu dinlendirilerek kullanılmıştır. Günlük olarak su parametrelerinin ölçümü (oksijen, sıcaklık, pH) Hach Lange Multiparametre HQ40D cihazı ile suyun nitrat azot ölçümü ise Hach DR 6000 cihazı ve Hatch kitleri (Nitrat: LCK 340, Amonyum: LCK 304, Nitrit: LCK 341) ile ölçülmüştür. Kapalı devre sistemlerin günlük su değişim oranı %20 olarak ayarlanmıştır.



Şekil 1. Denemelerin yürütüldüğü kapalı devre sistemlerin genel dizaynı

Figure 1. The general design of the recirculating system in which trials are carried out

Büyüme parametreleri

Üç ay süren deneme boyunca tüm balıklardan başlangıç ve birer aylık periyodların sonunda bireysel olarak toplam boy ve ağırlık ölçümleri yapılmıştır. Ağırlık ölçümlerinde ±1 g hassasiyetli terazi kullanılmıştır. Total boy ölçümleri ise milimetrik (±1 mm) cetvelle yapılmıştır. Büyümede etkili olan parametreler aşağıda verilen formüllere göre hesaplanmıştır.

Ağırlık artışı (g) = Son ağırlık – Başlangıç Ağırlığı

Yem Dönüşüm Oranı (FCR) = Tüketilen Yem Miktarı (g) / Ağırlık Artışı (g)

Spesifik Büyüme Oranı (SGR) = 100 x [(ln Son Ağırlık - ln İlk Ağırlık) / Süre]

Yaşama Oranı (%) = (Canlı Balık Sayısı- Ölen Balık Sayısı) x 100 / Toplam Balık Sayısı

İstatistiksel analizler

Büyüme verilerinin istatistiksel olarak değerlendirilmesinde elde edilen ortalama değerler "Ortalama±Standart Sapma"

şeklinde verilmiştir. İstatistiksel analiz SPSS 22.0 Windows programı ile yapılmıştır. Gruplar arasındaki istatistiksel farklar T testi ile test edilmiştir. Tüm testlerde yanılma düzeyi P<0,05 olarak kabul edilmiştir.

BULGULAR

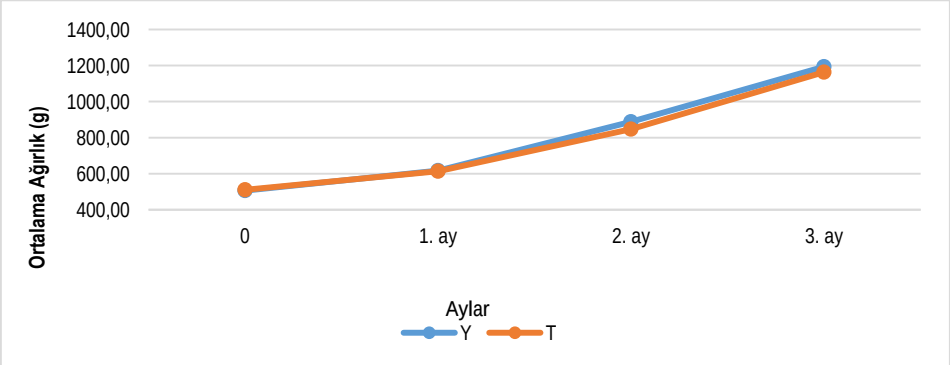
Üç ay süreden denemenin sonucunda tatlı su ve ‰5 tuzluluğa sahip ortamda büyütülen balıklara ait büyüme performansları Tablo 2'de yer almaktadır. Üç ayın sonunda toplam canlı ağırlık artışı Y grubunda daha yüksek görülürken bu durum istatistiksel açıdan önemsiz görülmüştür (P>0,05). Ortalama canlı ağırlık artışında istatistiksel açıdan fark görülmese de (P>0,05) Y grubu daha yüksek bir değer göstermiştir. Total boy artışı yine Y grubunda daha yüksek görülmekle birlikte aralarındaki fark istatiksel olarak önemsiz bulunmuştur (P>0,05). Yem tüketimi miktarı ve oranı yine Y grubunda yüksek görülmesine rağmen istatistiksel açıdan önemsiz görülmüştür (P>0,05). Spesifik büyüme oranı T grubuna nazaran Y grubunda daha yüksek görülmüş olup gruplar arasında istatistiksel farklılık ortaya çıkmıştır (P<0,05) . Yem dönüşüm oranı yine Y grubunda daha yüksek çıkmakla birlikte T grubu ile arasındaki fark istatistiksel açıdan anlamsız çıkmıştır (P>0,05). Grupların yaşama oranları arasında herhangi bir istatistiksel fark görülmemiştir (P>0,05).

90 günlük deneme süresince aylık ağırlık değişimi Şekil 2'de, aylık boy artışı ise Şekil 3'te yer almaktadır. Aylık artışlara baktığımızda değerler birbirlerine yakın olmakla birlikte Y grubundaki balıklar birinci aydan itibaren T grubuna göre daha fazla ağırlık artışı göstermiştir. Grupların total boylarındaki artışlar yakın seviyelerde olmakla birlikte en fazla boy artışı sırasıyla Y grubu balıklarında görülmüştür. Çalışma süresince takip edilen su kriterleri Tablo 3'te yer almaktadır.

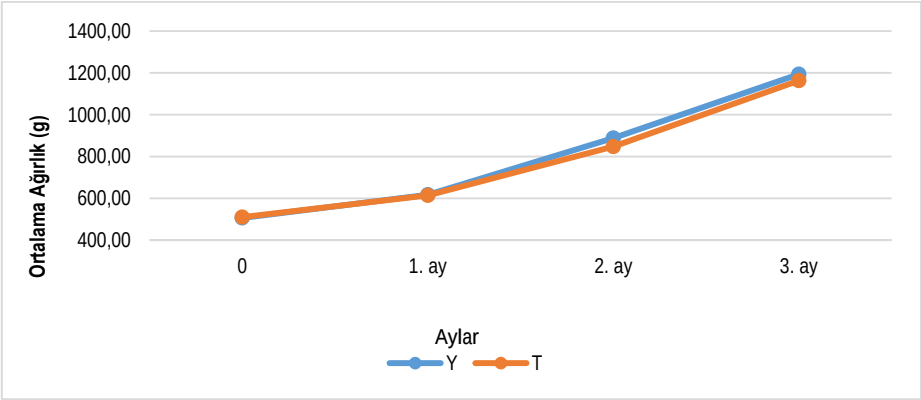
Tablo 2. Kapalı devre sistemde tatlı su ve ‰5 tuzlulukta yayın balıklarının (*Silurus glanis*) büyüme performansları
Table 2. Growth performance of catfish (*Silurus glanis*) in freshwater and ‰5 saltywater in recirculating system

DENEME GRUPLARI	Y	T
Başlangıç biomassı (g)	7584 ±239,75 [a]	7650 ±129,08 [a]
Sonuç biomassı (g)	17912 ± 566,29 [a]	17451,67 ± 300,90 [a]
Toplam canlı ağırlık artışı (g)	10328 ± 357,41 [a]	9801,67 ± 173,12 [a]
Başlangıç ortalaması (g)	505,6 ± 15,98 [a]	510 ± 8,06 [a]
Sonuç ortalaması (g)	1194,13 ± 0,00 [a]	1163,44 ± 0,00 [a]
Ortalama canlı ağırlık artışı (g)	688,5±23,83 [a]	653,4±11,54 [a]
ilk ort. total boy (cm)	42,6 ±0,50 [a]	43,1 ± 0,07 [a]
Son ort. total boy (cm)	55,90 ± 0,14 [a]	55,0 ± 1,05 [a]
Boy artışı (cm)	13,4 ± 0,80 [a]	11,9 ± 1,01 [a]
Yem tüketimi (gr)	18866,67 ± 1065,62 [a]	16681,67 ± 966,23 [a]
SGR%	0,95 ± 0,01 [a]	0,92 ± 0,02 [b]
FCR	1,83 ± 0,06 [a]	1,70 ± 0,07 [a]
Yaşam oranı %	100 ± 0,00	100 ± 0,00
Balık adedi	45	45
Deneme süresi (gün)	90	90

*Aynı satırdaki farklı harflerle gösterilen ortalamalar istatistiksel olarak farklıdır (P<0,05)



Şekil 2. Deneme gruplarının 3 ay boyunca ortalama ağırlık artışları
Figüre 2. The increasing of average weight of the experiment groups for 3 months



Şekil 3. Deneme gruplarının 3 ay boyunca ortalama boy artışları
Figure 3. The increasing of average lenght of the experiment groups for 3 months

Tablo 3. Deneme gruplarının 3 aylık dönemde ortalama su parametreleri
Table 3. Average water parameters of experimental groups in 3 months period

	Y1	Y2	Y3	T1	T2	T3
Sıcaklık	21,45±2,15	21,40±2,21	21,28±2,56	20,34±1,25	21,16±1,57	21,28±1,42
Oksijen	6,73±0,71	6,77±0,84	7,58±2,16	7,19±1,46	8,20±0,88	7,33±1,12
pH	7,76±0,31	7,63±0,42	7,67±0,39	6,94±0,55	7,14±0,27	7,36±0,48
NO_3-N	30.2±4,33	29.3 ±4,2	29,8 ±4,5	29,4±3,54	30,1±3,14	30,3±4,35
NO_2-N	0.07±0,04	0.08±0,08	0.075±0,035	0,077±0,06	0,081±0,017	0,08±0,02
NH_4-N	0.2±0,11	0.18±0,14	0.19±0,07	0,21±0,34	0,19±0,32	0,21±0,61

TARTIŞMA

Yapılan bu çalışma ile ekonomik değere sahip, birçok ülkede olduğu gibi ülkemizdeki tatlı sularda da doğal olarak bulunan yayın balıklarının 3 ay boyunca hem tatlı su, hem de ‰5 tuzluluğa sahip yeraltı suyu ile büyüme denemesi yapılmıştır. Ülkemizde kültür yoluyla yayın balığı üretimi yapan sadece bir işletme bulunmakla birlikte, şu ana kadar kayda değer bir yetiştiricilik üretimi mevcut değildir. Ancak Avrupa ve Amerika'da talep gören önemli türler arasında yer almaktadır.

Tatlı ve tuzlu su ortamlarındaki gelişimleri ile ilgili farklı türler üzerine çeşitli çalışmalar mevcut olup yayın balığının hem tatlı su, hem de tuzlu suda karşılaştırmalı büyütülmeleri üzerine ve büyük boyda yetiştiriciliği ile ilgili basılı bir makaleye ulaşılamamıştır. Mevcut olan çalışmanın sonucunda en iyi ağırlık, boy artışı ve SGR oranı fazla bir fark olmamakla birlikte tatlı suda yetişen (Y) grupta, FCR oranı ise en iyi T (‰5 tuzlu suda büyüyen) grubunda tespit edilmiştir. Ortalama ağırlığı 88,08±0,91 g ve 85,58±1,15 g olan gökkuşağı alabalığının (*Oncorhynchus mykiss*) 90 gün boyunca Karadeniz'de hem deniz suyunda hem de tatlı suda yetiştiriciliği yapılmıştır. Deniz suyunda yetişen balıkların daha iyi gelişim gösterdiği saptanmıştır (Yiğit ve Orhan, 1999). Bir başka çalışmada Mozambik tilapiası (*Oreochromis mossambicus*) ‰1, ‰10, ‰19, ‰28 ve ‰36 tuzluluktaki deniz suyunda 43 gün boyunca beslenmiş, en iyi büyüme oranı ‰36 tuzluluktaki suda, en iyi yaşama oranı ise ‰28 tuzlulukta tespit edilmiştir (Watanabe vd., 1993). Liao ve Chang (1983). Mozambik tilapiası ile tatlı, acı ve tuzlu suda yaptıkları çalışmada ortalama 105 g'lık balıkları kullanmışlar. En iyi büyüme ve yaşama oranını tatlı su, ikici sırada en iyi büyüme oranını ise acı su grubunda tespit etmişler. En iyi SGR oranını ise sırasıyla tatlı su ve acı suda tespit etmişler. Aynı araştırıcılar aynı çalışmada ortalama 18 g'lık balıklarda en iyi büyüme oranını öncelikle acı su daha sonra tatlı su grubunda, en iyi SGR oranını ise sırasıyla acı su ve tuzlu suda tespit etmişlerdir. Bu araştırmacıların ilk çalışmalarındaki sonuçları mevcut çalışmayla benzer durumları gösterirken ikinci çalışmalarının sonuçlarıyla tersi bir durum sergilemektedir. Ortalama ağırlığı 0.56–1.20 g olan kırmızı tilapia (*Oreochromis* sp.) balığı ile 22, 27 ve 32°C'de, ‰0, ‰18 ve ‰36 tuzlulukta yapılan 58 günlük çalışmada en iyi büyüme ve yem tüketimi artan sıcaklıkta yüksek olmakla birlikte en iyi yem tüketimi ve büyüme oranı ise ‰18 tuzlulukta saptanmıştır (Watanabe vd., 1993). Clay (1977) karabalıklarla (*Clarias lazera*) ‰5, ‰10 ve ‰20 tuzlulukta yaptığı denemede ‰20 tuzluluğa kadar herhangi bir stres belirtisi görülmediğini, ‰20 tuzlulukta ise 20 saat içerisinde balıkların öldüğünü belirtmiştir. Afrika karabalığı (*Clarias gariepinus*) larvaları ile ‰0, ‰2,5, ‰5, ‰7,5 ve ‰10 tuzlulukta yapılan denemenin sonucunda ‰0 ve ‰5

aralığındaki tuzluluklarda yaşama ve büyüme oranları arasında fark görülmezken ‰7,5 tuzluluktaki yaşama ve büyüme oranı daha aşağı tuzluluk değerlerine göre daha düşük çıkmış, ‰10 tuzluluk değerinde ise tüm balıklar 48 saat içerisinde ölmüştür (Britz vd., 1989). Düşük tuzlulukta gelişimin daha iyi olması mevcut çalışma ile benzerlik göstermektedir. Florczyk vd. (2014) juvenil yayın balıkları ile yaptıkları besleme çalışmasında 37/12, 45/15, 45/20 protein/yağ içeriği olan yem kullanmış, 50 günlük besleme sonucunda ortalama 4,22 - 4,25 g aralığındaki balıkların protein ve yağ oranı yüksek olan yemle en iyi büyüme performansı ve SGR oranı artışı gösterdiğini belirtmişlerdir.

Yayın balığının büyüme, yem tüketimi ve yumurtlama için ihtiyaç duyduğu optimum su sıcaklığı 25-28ºC olarak belirtilmiştir (Copp vd., 2009). Çalışma süresince kapalı devre sistemde yayın balıklarının büyütüldüğü tankların sıcaklığı 20,34±1,25 ile 21,45±2,15 ºC arasında değişim göstermiş olup, sonuçlar başka bir çalışma ile benzer çıkmıştır (Havasi vd., 2010). Mocanu vd. (2012) juvenil yayın balıklarıyla 37 gün boyunca süren kapalı devre sistemdeki stok yoğunluğu çalışmasında su sıcaklığını 26–26,5 ºC olarak saptamışlardır. Adamek vd. (1996) kapalı devre sistemde 22,5 g ve 99 g'lık yayın balıkları ile yaptıkları probiotk katkılı yem çalışmasında su sıcaklığını 22-26 ºC'ler arasında ölçmüşlerdir. Bu iki çalışmadaki sıcaklık değerlerinin mevcut çalışmanın değerlerinden farklı olduğu görülmüştür. Tanklardaki NO_3-N değerleri 29.3±4,2 ile 30,3±4,35 arasında değişirken yavru yayın balıklarının bulunduğu tankların değerleri bu çalışmanın değerlerinden daha yüksek (Mocanu vd., 2012), yavru boydaki yayın balıkları ile kapalı devre sistemde yapılan besleme denemesinde NO_3-N değeri bu çalışmadan oldukça düşük, NO_2-N değeri ise oldukça yüksek çıkmıştır (Bekcan vd., 2006). Yapılan çalışmanın NH_4-N değerleri Adamek vd. (1996) bulduğu değerlerden düşük, Mocanu vd. (2012) yaptığı çalışmanın değerlerinden yüksek çıkmıştır. Bu araştırmacıların sonuçları mevcut çalışmamın sonuçlarıyla farklılık göstermektedir. Bu çalışmadaki NH_4-N değerleri su değişimi ve biyolojik arıtmadan dolayı düşük seviyelerde çıkmıştır. NO_3-N değerlerinin yüksek olması ise balıklar açısından sorun yaratmamıştır. Deneme boyunca her iki grupta herhangi bir ölüme rastlanmamıştır. Ağırlıkları 79,64-80,09 g olan yayın balıkları ile yapılan besleme çalışmasında balık gruplarında yaşama oranı %100 çıkmıştır (Zaikov vd., 2008). Deneme boyunca aylık olarak ağırlık ve boy ölçümleri takip edilmiştir. Sonuçların istatistiksel açıdan bir fark taşımadığı saptanmıştır. Dolayısıyla hem tatlı sularda hem de acı sularda bu türün yetiştiriciliğinin yapılabileceği kanaatine varılmıştır. Yayın balığı hızlı büyüyen ve ekonomik değeri yüksek olan bir türdür. Ülkemiz sularında da doğal olarak bulunmakla birlikte yetiştiriciliğinin yapılmıyor olması eksiklik olarak değerlendirilmektedir. Yapılan çalışma sonucunda, elde edilen bilgiler ışığında üreticiler bilgilendirilerek ve teşvik edilerek bu türün yetiştiriciliğe kazandırılabileceği umulmaktadır. Bu türün yetiştiriciliği ile ilgili daha birçok çalışmaya ihtiyaç vardır. Anaçlardan yumurta elde edilmesi, larval yetiştiricilik vb. kısımları oldukça eksiktir. Çalışmaların devam ettirilmesi amacıyla yapılan bu çalışmanın sonraki çalışmalara ışık tutacağı düşünülmektedir.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Gonadal development of the holothurian *Holothuria polii* (Delle Chiaje, 1823) in spawning period at the Aegean Sea (Mediterranean Sea)

Ege Denizi'nde (Akdeniz) *Holothuria polii* (Delle Chiaje, 1823) **türü deniz hıyarının yumurtlama dönemindeki gonad gelişimi**

Mustafa Tolga Tolon[1*] ● Serhat Engin[2]

[1]Ege University, Faculty of Fisheries, Aquaculture Department, 35040, Bornova, İzmir, Turkey http://orcid.org/0000-0002-2233-0663
[2]Ege University, Faculty of Fisheries, Aquaculture Department, 35040, Bornova, İzmir, Turkey http://orcid.org/0000-0002-1663-1769

*Corresponding author: tolga.tolon@ege.edu.tr
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Abstract: The Mediterranean Sea cucumbers including *Holothuria polii* has become commercially important in international trade due to the high demand of consumers from Far East countries. Sea cucumbers fisheries is a valuable income for the regional fishermen but natural stocks endangered by overfishing in recent years. Fisheries regulations and aquaculture studies are important precautions for preserving natural stocks. All these efforts are based on reproduction biology of this species which slightly differed among regions. In this study, the reproduction biology including morphological characteristics, gonadosomatic index and gonadal development stages of *H.polii* at the eastern coast of Aegean Sea (Izmir, Turkey) were investigated during the reproduction (spawning) period from July to October 2018. Gonads and gonad sections of 60 sea cucumbers (120.60 ± 19.56 g) have been observed by macroscopic and microscopic inspections. Three gonadal development stages have been identified by histologic observations as mature (III), spawning (IV) and post-spawning stage (V) in gonadal tubules. Results indicate that both female and male gonads are at mature and spawning stage in July and all gonads are at post-spawning stage in October. The mean gonad weight was 12.53 ± 1.33 g at the beginning of the spawning period (July) and 1.87 ± 0.58 g at the end of the spawning period (October). Gonadosomatic index decreased from 17.53 ± 0.02% (July) to 3.37 ± 0.01% (October) after spawning. The gonadosomatic index, gonad weight, and spawning were related to the seawater temperature. According to this study, the spawning period of *H.polii* at the eastern coasts of the Aegean Sea starts in July and completely ends in October. As a result, the data related to the reproduction biology in spawning period of *H.polii* would guide stock management and artificial breeding of this species under controlled conditions.

Keywords: Mediterranean, sea cucumber, gonadal development stage, reproduction biology, gonadosomatic index, *Holothuria polii*

Öz: *Holothuria polii* türünün de dahil olduğu Akdeniz'de yaşayan deniz hıyarları uzak doğu ülkeleri tüketicilerinin yoğun talebi karşısında uluslararası ticarette önem kazanmışlardır. Son yıllarda deniz hıyarı avcılığı bölgedeki balıkçılar için iyi bir gelir kaynağı olmakla birlikte aşırı avcılık nedeniyle doğal stokları tehlike altına girmiştir. Bu anlamda av düzenlemeleri ve yetiştiricilik çalışmaları doğal stokların korunmasında önemli önlemlerdir. Bu girişimlerin tümü türün bölgeler arasında farklılıklar gösteren üreme biyolojisini temel almaktadır. Bu çalışmada, Ege Denizi'nin doğu kıyılarındaki (İzmir, Türkiye) *H.polii* türü deniz hıyarlarının morfolojik özellikleri, gonadosomatic indeksi ve gonad gelişim evrelerini içeren üreme biyolojisi, Temmuz-Ekim 2018 arasındaki üreme (yumurtlama) döneminde incelenmiştir. Bu kapsamda, ortalama ağırlıkları 120,60 ± 19,56 g olan 60 deniz hıyarının gonad ve gonad kesitleri makroskobik ve histolojik incelemelerle gözlenmiştir. Yumurtlama dönemindeki *H.polii* ergin bireylerinin gonad tübüllerinin histolojik incelemesinde olgun (III), yumurtlama (IV) ve yumurtlama sonrası (V) olmak üzere üç gonad gelişim evresi tanımlanmıştır. Elde edilen sonuçlar her iki cinsiyetteki deniz hıyarı gonadlarının Temmuz ayında olgun ve yumurtlama aşamasında, Ekim ayında ise yumurtlama sonrası aşamada olduğunu ortaya koymaktadır. Türün yumurtlama dönemi başında (Temmuz) ortalama gonad ağırlığı 12,53 ± 1,33 g iken, yumurtlama dönemi sonunda (Ekim) 1,87 ± 0,58 g olarak kaydedilmiştir. Gonadosomatik index ise %17,53 ± 0,02 (Temmuz)'den yumurtlama sonrası aşamada (Ekim) %3,37 ± 0,01'e düşmüştür. Gonadosomatik indeks, gonad ağırlığı ve yumurtlama döneminin deniz suyu sıcaklığı ile doğru yönlü ve önemli bir etkileşim içinde olduğu saptanmıştır. Bu çalışma sonucuna göre, *H.polii* türü deniz hıyarının Ege Denizi'nin doğu kıyılarındaki yumurtlama dönemi Temmuz ayında başlayıp, Ağustos ve Eylül aylarında yoğun olarak devam etmekte ve Ekim ayında tamamen son bulmaktadır. Sonuç olarak türün yumurtlama dönemindeki üreme biyolojisine ilişkin elde edilen veriler hem stok yönetiminde hem de yetiştiricilik çalışmalarında yol gösterici niteliktedir.

Anahtar kelimeler: Akdeniz, deniz hıyarı, üreme biyolojisi, gonad gelişim evreleri, gonadosomatik indeks, *Holothuria polii*

INTRODUCTION

Sea cucumbers, distributed in almost all seas of the world with approximately 1711 species (WoRMS, 2018). They feed on detritus such as diatoms, cyanophytes, macroalgae, crustaceans, bivalve shells, sponge ossicles and nematodes accumulated on the seafloor (Belbachir and Mezali, 2018) and transform matter and energy by processing organic nutrients in the benthic ecosystems (Purcell et al., 2016). They stimulate nutrient conversion, sediment mixing, bioturbation and microalgae growth in marine sediment due to their feeding and movement patterns (Wolkenhauer et al., 2010). In addition to their ecological importance, the body wall of cucumber is valuable seafood that is particularly demanded by Asian consumers. Moreover, bio-extracts obtained from sea cucumbers are used in the production of pharmaceutical, nutraceutical and cosmetics products (Purcell, 2014). Approximately ten thousand tons of dried sea cucumbers are subject to international trade (Purcell et al., 2013), which means that approximately 200 million sea cucumbers are collected each year from the marine ecosystem (Purcell et al., 2016). The high price and economic value of sea cucumber bring the danger of overfishing and the depletion of its stocks. Today, natural stocks of sea cucumbers are

threatened by overfishing in many parts of the world. Less known sea cucumber species that are not consumed regionally are gaining commercial importance in international trade due to the decrease in trade amount of high-value species (Sellem et al., 2017).

Holothuria polii (Delle Chiaje, 1823), a member of Holothuroidea class of Echinodermata phylum, is widely distributed in soft sandy sediments and sea meadows of the sublittoral zone at the Mediterranean coasts of Algeria, Croatia, Egypt, France, Italy, Spain, Tunisia and Turkey. *H.polii* are mainly collected by the fishermen of Turkey and the other Mediterranean countries. Mediterranean sea cucumbers, including *H.polii*, are exported in dried and frozen form to Far East countries at prices of approximately 19 to 48 USD per kg (TURKSTAT, 2019) and became an important source of income for fishermen in the region. Due to the increasing economic importance of sea cucumbers, fishing arrangements, restocking and aquaculture efforts are required to ensure the sustainability of natural stocks (Toscano et al., 2018). Researches on the breeding biology of *H. polii* could have several applications (Rakaj et al., 2019). Although some pioneering research on the aquaculture of *H. polii* has resulted successfully (Rakaj et al., 2019; Tolon, 2017) but there is still scarce information on spawning period and gonadal development which are needed for breeding under controlled conditions.

Entire studies on the reproductive biology of sea cucumber species clearly show that reproductive dynamics differ according to the geographical regions (Bulteel et al., 1992; Costelloe, 1988; Despalatovic et al., 2004; Despalatović et al., 2003; Fajardo-León et al., 2008; McEuen, 1988; Navarro et al., 2012; Tuwo and Conand, 1992; Valls, 2004). The effect of water temperature that determines the spawning period is prominent in the temperate zones like the Mediterranean, where seawater temperature significantly varies between regions (Conand, 1981; Costelloe, 1988; Despalatovic et al., 2004; Tuwo and Conand, 1992). Considering the limited mobility of sea cucumbers, it is important to define the region-specific reproductive biology including gonadal development and spawning periods required for aquaculture and stock management studies.

This study aims to determine the morphological characteristics, gonadosomatic index and gonadal development stages of *H.polii* distributed on the eastern coast of the Aegean Sea (Mediterranean) during the reproduction (spawning) period. Thereby, findings derived from the study would be a good reference in the literature concerning microscopic observations of Mediterranean sea cucumber *H.polii*'s reproduction biology. Moreover, the data related to the reproduction biology in spawning period of *H.polii* would beneficial in both stock management and artificial breeding of this species under controlled conditions.

MATERIAL AND METHOD

H. polii individuals were sampled from the Urla (38° 21'55.42 "N; 26° 46'8.88" E) coasts of the Gulf of Izmir (Turkey), at the Aegean Sea. The dense *H.polii* stocks and prohibition of fishing were the main reasons for the selection of this location (Figure 1).

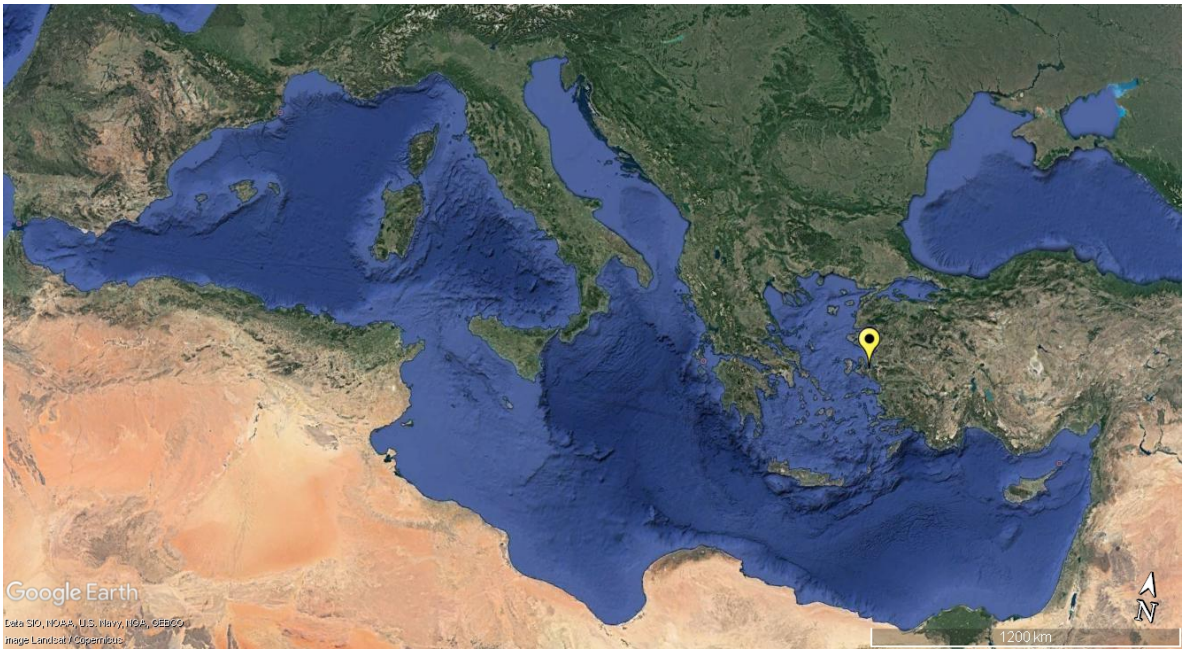

Figure 1. Sampling area in Gulf of Izmir, Aegean Sea (Mediterranean) (38°21'55.42"N; 26°46'8.88"E) (Google Earth, 2019)

Two samplings were carried out in July and October 2018, when the seawater temperature at the selected region reached their highest and the lowest level within the year. In order to determine the sampling period, 10-year monthly average seawater temperatures in Urla (İzmir) region and daily seawater temperature data covering the working period were obtained from the data of Urla Mendirek Station of General Directorate of Meteorology of Turkey (MGM, 2018). In addition, seawater temperatures were recorded with a dive type thermometer (Mares Puck Pro) in each sampling.

H.polii adults were hand-picked from a depth of 2-4 m by scuba diving. The collected sea cucumbers were immediately brought to Ege University Fisheries Faculty, Urla Research Unit Laboratories (38°21'48.46 "N; 26 ° 46'14.95" E). Samples kept in an ice-molded tank during transfer to prevent them from ejaculating viscera due to stress. Total 60 sea cucumbers, mean wet weight of 120.60 ± 19.56 g were selected from a group of adult individuals in the study. Sea cucumbers were dissected by longitudinal incision on their ventral side. Total length (TL), total weight (TW), gutted body weight, (GBW) and gonad weight (GW) were measured for each sea cucumber (Conand, 1981). The gonadosomatic index (GSI) to determine the level of gonadal development was calculated according to the following formula (Asha and Muthiah, 2008; Conand, 1981; C. J. M. B. Conand, 1993; Ramofafia et al., 1995) :

GSI= GW / GBW

where GSI=Gonadosomatic index; GW=Gonad weight and GBW= Gutted body weight

Gonads were removed and fixed in 10% buffered neutral formalin solution for 24 hours. Gonad samples were dehydrated in graded alcohol series according to Roberts and Ekman (2012). Sections from the samples embedded in paraffin were cut at 5-6 µm by Rotary Microtome Device (Leica RM2125 RTS), cleared in xylene and stained with hematoxylin-eosin. Gonad tissues inspected and pictured under a phase-contrast microscope (Olympus CX-31). The gonadal development stages were identified according to Despalatovic et al. (2004) as Stage I: recovery (resting or indeterminate tubules); Stage II: growing (increasing tubules); Stage III: mature (ripe tubules); Stage IV: spawning (partly emptied tubules); and Stage V: post-spawning (empty tubules). Sex of the individuals was determined by microscopic observation of the gonads.

Statistical analysis

Data are presented as mean ± standard error of the mean unless otherwise stated. The homogeneity of variances was tested (Levene test) and, whenever necessary, the log transformation log (x + 1) was used (Zar, 1996). Differences between TL, TW, GBW and GW values of male and female individuals were tested by ANOVA and t-test. The average values of the GSI by sex were analyzed by a Mann and Whitney test. Pearson test was used to analyze the existing correlation between the GW, GSI, and temperature. Mean GSI differences between the sampling periods were analyzed by t-test. SPSS v.24 software package was used for all statistical analysis.

RESULTS

Sex was primarily determined by macroscopic observations based on gonad color. The tubules of the female gonads are usually yellow or orange, and male gonads are pale white (Figure 2). However, to make a complete gender determination, reproductive cells in the gonads of all *H.polii* individuals were examined and the gender was confirmed by microscopic observations.

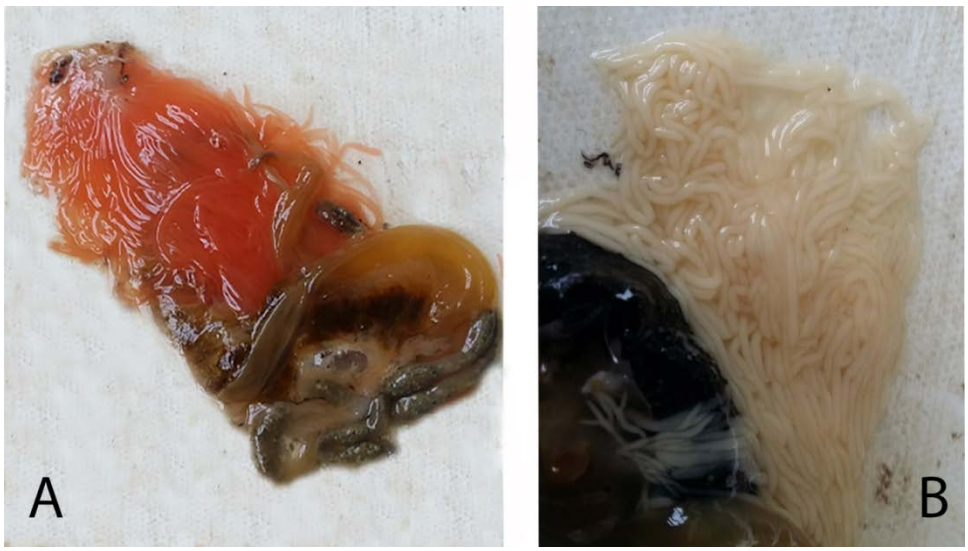

Figure 2. Mature gonads of female (A) and male (B) *H.polii* adults

Total of 60 samples (28 male and 32 female) were inspected in this study. Gender ratio did not show significant difference from 1: 1 distribution according to chi-square test results (x^2=0.133; df=1; p=0.72). Average length (TL) of sampled sea cucumbers was 14.14 ± 1.83 cm (13.99 ± 1.66 cm male; 14.29 ± 2.03 cm female). There was no significant difference between the genders in terms of mean lengths (t-test; p = 0.66; p> 0.05). The average weight (TW) of the sampled sea cucumbers was 120.60 ± 19.56 g (120.81 ± 17.30 g male; 120.38 ± 22.21 g female). There was no significant difference between female and male mean weights (t-test; p = 0.95; p> 0.05). The mean gutted body weight was 64.41 ± 12.08 g (67.30 ± 9.85 g male; 61.53 ± 13.69 g female). There was no significant difference between male and female individuals in terms of gutted body weight values (t-test; p = 0.20; p> 0.05) (Table 1).

Table 1. Morphological observations on the *H.polii* specimens

	TL (cm)	TW (g)	GBW (g)
Male	13.99 ± 1.66^{a}	120.81 ± 17.30^{b}	67.30 ± 9.85^{ab}
Female	14.29 ± 2.03^{a}	120.38 ± 22.21^{b}	61.53 ± 13.69^{ab}
Mean	14.14 ± 1.83	120.60 ± 19.56	64.41 ± 12.08

TL: Total length (cm); TW: Total wet weight (g); GBW: Gutted body weight (g); n=60
Data with different superscripts in columns indicate significant differences from each other (p< 0.05)

The mean gonad weight of all samples was 12.53 ± 1.33 g (11.81 ± 2.16 g male gonads; 13.34 ± 1.56 g female gonads) at the beginning of the spawning period (July). The mean gonad weight at the end of the spawning period (October) was determined as 1.87 ± 0.58 g (2.13 ± 0.93 g male gonads; 1.64 ± 0.76 g female gonads). There was no significant difference in gonad weights between genders in both sampling periods (ANOVA; July p=0.87; p>0.05) (ANOVA; October p=0.99; p>0.05). However, significant differences were found between the mean gonad weights of *H.polii* individuals in July and October in both genders (t-test; p=0.00; p<0.05) (Table 2).

Table 2. Mean gonad weights of *H.polii* within the reproduction period

Period	Male gonad (g)	Female gonad (g)	Mean gonad weight (g)
July 2018	11.81 ± 2.16^{a}	13.34 ± 1.56^{a}	12.53 ± 1.33^{a}
October 2018	2.13 ± 0.93^{b}	1.64 ±0.76^{b}	1.87 ± 0.58^{b}

Data with different superscripts in columns indicate significant differences from each other (p< 0.05)

The mean gonadosomatic index of all specimens was 17.53 ± 0.02% at the beginning of the spawning period (15.97 ± 0.03% male; 19.30 ± 0.02% female). It was decreased to 3.37 ± 0.01 % at the end of the spawning period (3.70 ± 0.02% male; 3.08 ± 0.01% female). There was no significant difference in gonadosomatic index between genders in both sampling periods (ANOVA; July p=0.74; p>0.05) (ANOVA; October p=0.99; p>0.05). However, a significant difference was found between the mean gonadosomatic index in July and October in both male and female individuals (t-test; p=0.00; p<0.05) (Table 3).

Table 3. Gonadosomatic index of the *H.polii* during the reproduction period

Period	Male (%)	Female (%)	Mean (%)
July 2018	15.97 ± 0.03^{a}	19.30 ± 0.02^{a}	17.53 ± 0.02^{a}
October 2018	3.70 ± 0.02^{b}	3.08 ± 0.01^{b}	3.37 ± 0.01^{b}

Data with different superscripts in columns indicate significant differences from each other (p< 0.05)

There is a significant difference between the monthly seawater temperature means of the sampling area during the study period (ANOVA; p=0.00; p<0.05). Gonadosomatic index (Pearson; p = 0.77) and gonad weights (Pearson; p = 0.92) of the sampled sea cucumbers were positively and significantly correlated with the seawater temperature at the studied location (Figure 3). Gonad weight and gonadosomatic index mean values were the highest in July parallel with the highest mean seawater temperature (25.80 ± 0.95 ºC), while they decreased significantly in October when the mean seawater temperature dropped to 19.81 ± 1.17 ºC.

Microscopic observations of the gonads

Morphological and histological examination of gonads tissues revealed three gonadal development stages in male and female individuals. Mature (III) and spawning stages (IV) were determined in gonads of female and male individuals in July samples, while gonads of both genders were in post-spawning stage (V) in October. Since the aim of the study was to determine the developmental stages of female and male *H. polii* gonads during the spawning period, Stage I (recovery: resting or indeterminate tubules) and Stage II (growing: increasing tubules) phases were not observed.

Observations on the gonads of female *H.polii* by development stages

Stage III (Mature): The gonad wall was thin in this stage. The development of oocytes was completed and the presence of previtellogenic oocytes was not observed in the germinal layer, but the number of vitellogenetic eggs increased (Figure 4A).

Stage IV (Spawning): The gonad wall was still thin in this stage. Gaps were detected in the tubules for easy ovulation (Figure 4B).

Stage V (Post-spawning): After spawning, a large migration of phagocytic hemocytes from thick-walled phagocytic gonadal tubules and gametes remaining in the resorption process observed in the gonads. Tubules were empty, flaccid and wrinkled. Large gaps are visible in the gonads (Figure 4C).

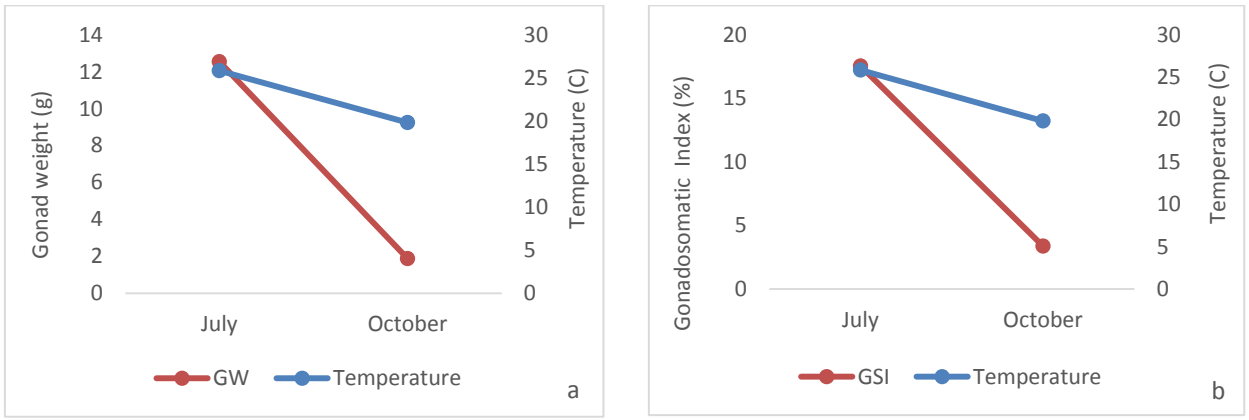

Figure 3. Mean seawater temperature versus (a) gonad weight and (b) gonadosomatic index of *H.polii*

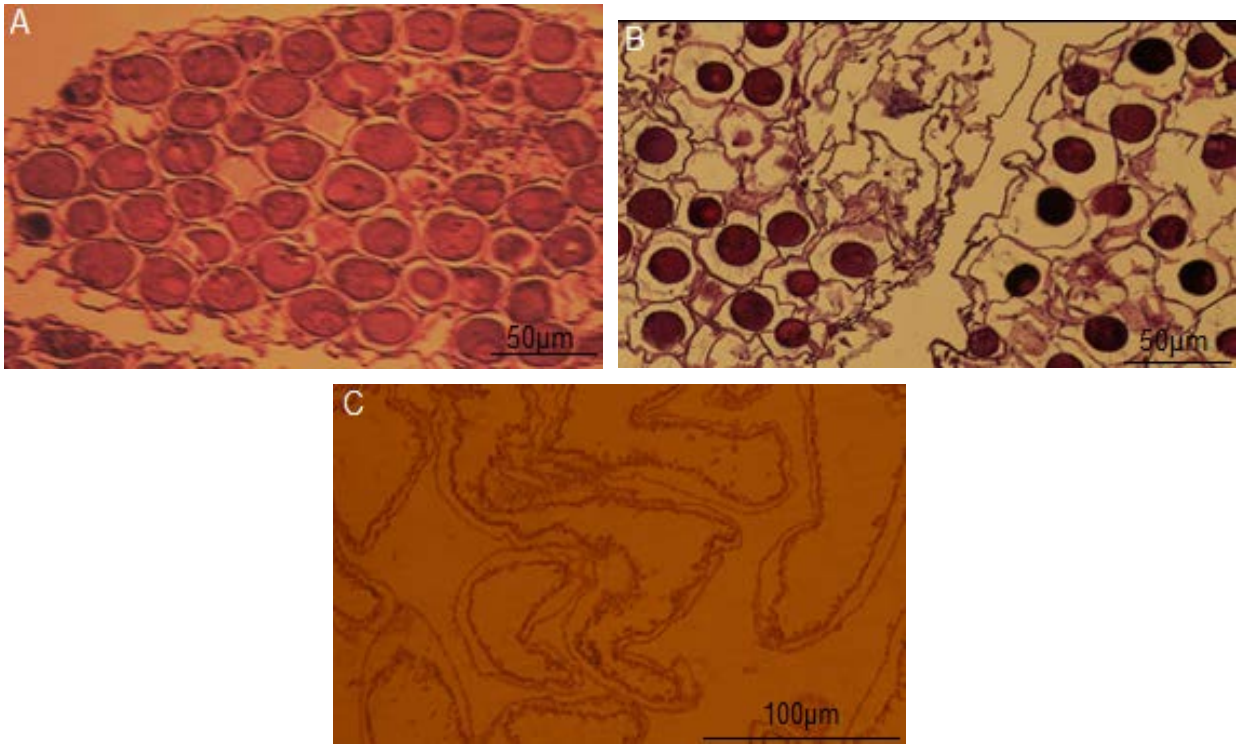

Figure 4. Microscopical characteristics of gonadal stages of the females. (A) mature stage; (B) spawning stage; (C) post-spawning stage. Scale bars: A, B, 50 µm, C, 100 µm.

Microscopic observations on gonads of male *H.polii* by development stages

Stage III (Mature): Lumens have reached their maximum diameter and were filled with seminal fluid. Histologically, the walls of the lumens were smooth and thin, with germinal folds. The lumen of the spermatozoa tubules looks like a mature testicle. Spermatogenesis formation was accelerated and apparent. Gonad wall was thin (Figure 5A).

Stage IV (Spawning): The expulsion of gametes from the gonad began, the gonad wall thickened. Histologically, the walls of the tubules are smooth and thin, with germinal folds. Cells and tubules found in early spermatogenesis stages were characterized by mature spermatozoa (Figure 5B).

Stage V (Post-spawning): After resorption gonad was almost empty (Figure 5C).

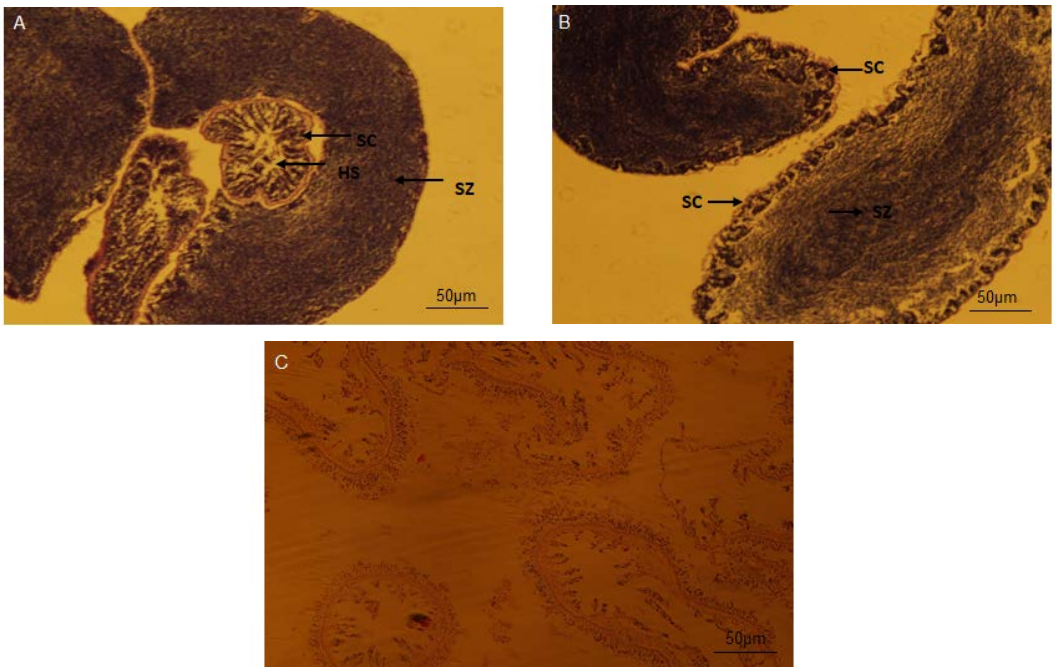

Figure 5. Microscopical characteristics of gonadal stages of the males. (A) Mature stage; (B) spawning stage; (C) Post-spawning stage. HS, haemal sinus; SC, spermatocyte columns; SZ, spermatozoa. Scale bars: 50 µm

DISCUSSION

Gonadal development stages of *H.polii* in spawning period were evaluated in this study to fully identify the gonadosomatic index, gonad size and morphological properties of both sex in a wild population from İzmir (Turkey) coasts of the Aegean Sea. Results derived from the study indicate the occurrence of three development stages in gonads. Mature and spawning stages observed at the beginning of the spawning period, and only postspawning stage at the end.

Reproduction and gonadal development in holothuroids are depending on water temperature. Gonadal development reaches to the highest level in the periods when the water temperature also reaches the highest levels, and spawning occurs (Conand, 1981; Costelloe, 1988; Tuwo and Conand, 1992). Morphological examinations of the gonads in this study revealed that gonadosomatic indexes and gonad weights of the sampled sea cucumbers were high in July when the water temperature reached the highest value of the year. Seasonal changes in water temperature by regions may cause changes in the reproduction period of sea cucumbers. In the study covering the northern part of the Aegean Sea, Bardanis and Batjakas (2018) reported that spawning for *H.polii* occurred between July and August and that the gonads had gone through the recovery stage from September. The rapid decrease in water temperature from September is effective in the early termination of the spawning period. In the southern regions of the Mediterranean, Slimane-Tamacha et al. (2019) and Sellem et al. (2017) reported that the gonad development was completed in May with the effect of earlier warming in the summer period and the ovulation started in June and ended in September. In the current study, the post-spawning stage observed in some individuals revealed that ovulation started in July, extensively continued in August and September, and ended completely in October at Eastern coasts of the Aegean Sea. However, not only the temperature but also many other environmental factors such as sediment types, feeds, water quality etc. are known to affect the sea cucumbers gonadal development in the nature. Therefore, the effects of these factors on gonad development should be examined in detail in future studies.

In terms of gonad weights and gonadosomatic indexes, there were no significant differences among the gender in both periods which means that gonad development is synchronous in *H.polii* male and female. The mean gonadosomatic index calculated in this study is 17.53%, which is above the gonadosomatic indexes calculated in previous studies during the same periods of the year. Bardanis and Batjakas (2018) observed the highest gonadosomatic index by 14% in late June and Sellem et al. (2017) observed 16% in May. The highest values of gonadosomatic index, which is an important indicator of gonad development, do not vary excessively among the regions, but the periods in which they reach these highest values may be different.

Studies on reproduction biology of economic sea cucumber species that are widely distributed in the Mediterranean like *H.polii* are important for the sustainability of natural stocks, which are endangered due to overfishing.

This study reveals that the reproductive period and gonadal development of this species differ even between the close geographic locations. The future studies on reproduction biology of sea cucumbers will guide the region-specific conservation measures and stock enrichment efforts suitable for the species.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Akut bakır konsantrasyonlarına maruz bırakılmış pangasus balıklarında (*Pangasius hypophthalmus*) saptanan hematolojik ve histolojik değişimler

Hematological and histological alterations detected in striped catfish (*Pangasius hypophthalmus*) exposed to acute copper concentrations

Semra Küçük

Aydın Adnan Menderes Üniversitesi Ziraat Fakültesi Su Ürünleri Mühendisliği Bölümü, 09100 Aydın, Türkiye http://orcid.org/0000-0002-9299-5986

skucuk@adu.edu.tr

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Öz: Bu çalışmada, hematolojik ve histolojik değişiklikleri incelemek için pangasus balıkları (*Pangasius hypophthalmus*) 0,0; 2,5; 5,0; 10,0 mg L^{-1} bakır sülfat konsantrasyonlarına 48 saat süreyle maruz bırakılmıştır. Ardından her akvaryumdan dörder balık seçilmiştir. İlk 24 saatte ve ikinci 24 saatte kan örnekleri alınmıştır. Sonrasında histolojik analiz için böbrek, karaciğer, dalak ve solungaç doku örnekleri toplanmıştır. Hematokrit, hemoglobin, eritrosit sayısı, MCHC, MCH ve MCV değerleri için kan örnekleri analiz edilmiştir. Histolojik değerlendirme içinde doku örnekleri hematoksilen ve eozin boyama yöntemi kullanılarak analiz edilmiştir. Bakır konsantrasyon artışlarına bağlı olarak pangasus balıklarında hematolojik ve histolojik bazı önemli değişiklikler saptanmıştır. ≥ 2,5 mg L^{-1} Cu konsantrasyonlarına maruz bırakılmış pangasusların hematokrit oranında, hemoglobin miktarlarında ve eritrosit sayısında 48 saat içinde önemli artışlar olmuştur. Fakat MCHC, MCH ve MCV değerlerinde istatiksel bir farklılık gözlenmemiştir. İlk 24 saatlik süreçte ≥ 2,5 mg L^{-1} Cu konsantrasyonları pangasuslarının solungaçlarında ödeme, hiperplaziye, ve füzyona yol açmıştır. 48 saat sonunda ≥ 2,5 mg L^{-1} konsantrasyonlar solungaçlarda yoğun hiperplaziye ve kıkırdak ve epitel dokuda dejenerasyonlara neden olmuştur. Karaciğer hücrelerinde piknotik nükleus ve dejenerasyonlar, dalakta nekroz ve hemosterin kümecikleri artışı, böbrek tübüllerinde dejenerasyon ve siyah lekeler görülmüştür. Sonuç olarak ≥ 2,5 mg L^{-1} akut bakır konsantrasyonları pangasus balıklarında 48 saat süre içinde yüksek mortaliteye ve histolojik bozukluklara neden olmuştur.

Anahtar kelimeler: Bakır, Hematoloji, Histoloji, *Pangasius hypophthalmus*

Abstract: In this study, to examine hematological and histological alterations, striped catfish exposed to 0.0, 2.5, 5.0, 10.0 mg L^{-1} copper concentrations for 48 hours. After that, four fish were selected per aquarium. First 24 and second 24 h, blood samples were taken. And then, their gills, liver, kidney, and spleen samples were collected. The blood samples were analyzed for hematocrit, hemoglobin, number of erythrocytes, MCHC, MCH and MCV. The tissue samples stained with standart technique of haematoxylin and eosin for histological assessment. Copper depending on concentrations increase caused some important hematological and histological alterations on striped catfish. Number of erythrocyte, hematocrit and hemoglobin of striped catfish exposed to ≥ 2.5 mg L^{-1} of Cu concentrations increased in 48 h. Within first 24 h, ≥ 2.5 mg L^{-1} of Cu caused edema, hyperplasia and fusion in gills. After 48 h, ≥ 2.5 mg L^{-1} of Cu formed very intense hyperplasia and degeneration of cartilage and epithelium tissue in the gills. Pyknotic nuclei and degenation in liver; necrosis and increased number of hemosterin clustering in spleen; degeneration of renal tubules and black spots in kidney were seen. As a result of that, ≥ 2.5 mg L^{-1} of Cu caused high mortality and histological disorders within 48 h.

Keywords: Copper, Hematology, Histology, *Pangasius hypophthalmus*

GİRİŞ

Çağımızın en önemli sorunlarından bir tanesi evsel, endüstriyel, tarımsal ve madencilik faaliyetleri sonucu oluşan atıkların tamamen arıtılmadan göl, nehir ve deniz gibi doğal sulara bırakılmasıdır (Marcussen vd., 2007; Çağdaş vd., 2017). Bu atıklar içinde ağır metallerde yer almaktadır. Ağır metaller normalde sularda iz miktarda bulunurlar. Yalnız bu miktar bölgenin endüstriyel, tarımsal ve madencilik faaliyetlerine ve jeokimyasal yapısına göre de artış ya da azalış göstermektedir.

Endüstriyel ve tarımsal faaliyetlerin atışına bağlı olarak suda yoğunlaşan ağır metallerin taşınımı ve birikimi birçok parametre değişimine paralel olarak değişim gösteren karmaşık prosesler içerir. Su içindeki ağır metallerin bir kısmı birçok fiziksel ve biyokimyasal etkileşimlere uğrar. Bir kısmı da dip sedimentinde birikir ve burada bir takım oksidasyon ve redüksiyon işlemlerine maruz kalır. Diğer bir kısmı da suda yaşayan canlıların bünyesinde birikir (Şener ve Şener, 2015).

Ağır metaller balıkların organlarında (kas, karaciğer, solungaç, böbrek, bağırsak) birikim yaparak onların vücutlarının morfolojisinde ve biyokimyasında bir takım değişimlere sebep olmaktadır (Abdel-Warith vd., 2011; Youis vd., 2013; Olsson, 1998). Besin zincirinin bir halkasından giren ağır metaller ilk girdiği miktarın kat kat üstünde dozlara ulaşarak besin halkasının en üst kısmında yer alan insanlara kadar yükselebilmekte ve onların hayatını tehdit edebilmektedir (Özkan vd., 2018; Perçin vd., 2011).

Pangasuslar (*Pangasius hypophthalmus)* bir tatlı su balığı türüdür. Uzakdoğu Asya kökenlidirler. *Chordata* şubesinin *Actinopterygii* sınıfının *Siluriformes* takımının *Pangasiidae*

familyasında yer alırlar. Bu balıkların Tayland ve Vietnam'da yetiştiriciliği yapılmaktadır. Bular pulsuz, çıplak derilidirler ve ayrıca çok yumuşak, beyaz ve tatlımsı bir etleri vardır. Bu tat bizim damak lezzetimize çok hitap etmemektedir.

Balıklar deri, solungaç ve bağırsak yoluyla aldıkları ağır metalleri çeşitli organlarında (solungaç, karaciğer, böbrek, kas, gonad, beyin, vb.) biriktirmektedirler. Bu konuda yapılmış oldukça çok çalışma bulunmaktadır (Ünlü vd., 1995; Kalay ve Canlı, 2000; Çalta vd., 2000; Mol vd., 2010; Perçin vd., 2011; Begum vd., 2013). Perçin vd (2011) Doğu Akdeniz'deki doğal ve çiftlik ortamında bulunan mavi yüzgeçli orkinos balıklarının (*Thunnus thynnus*) kas dokularındaki ağır metal (Cu, Mn, Ni, Zn ve Fe) birikimini saptamıştır. Ünlü vd.(1995) Dicle Nehri'nde *Capoeta trutta* balığında ağır metal birikimini araştırmıştır. Balık kas ve karaciğer dokusunda Co, Cd, Cu, Ni, Mo ve Zn birikimini araştırmıştır. Cu ve Zn'nin kas dokusundaki birikim değerlerinin ağır metal kabul değerlerinin üzerinde olduğunu rapor etmiştir. Fakat Co, Cd, Mo değerlerini AAS (Atomik absorpsiyon spektrofotometresi)'nin tayin sınırları içinde saptayamamıştır. Kalay ve Canlı (2000) Tilapiaları (*Tilapia zilli*) 1 mg L^{-1} derişimde Cu, Zn, Cd ve Pb metallerine 10 gün süreyle maruz bırakmış ve karaciğer, solungaç, beyin ve kas dokularında metal birikimini saptamıştır. Cd'nin 7 gün sonunda solungaç ve karaciğerde yaklaşık 80 µg g^{-1} (k.a.) civarında birikime sebep olduğunu bildirmiştir. Sonrasında bu balıklar 1, 7, 15 ve 30 gün süreyle temiz suda (metal içermeyen) bekletilerek dokularda arıtım yapılmış ve metal birikim miktarlarını AAS'de alevli tekniği ile ölçmüştür. Pb, Cd ve Cu miktarlarının solungaçlarda azaldığını; karaciğerde yükseldiğini bildirmiştir. Çalta vd. (2000) Keban Baraj Gölü'nde bulunan *Capoeta trutta* balığının kas, deri, karaciğer, gonad, böbrek ve solungaçlarında bazı metal (Cu, Fe, Mn, Zn, Co, Cr, Cd, Pb) birikim düzeylerini araştırmıştır. Cu kas, gonad, böbrek ve solungaçta; Mn, Fe ve Zn'yi tüm dokularda tespit etmiştir. Mol vd. (2010) Fırat Nehri üzerindeki Atatürk Baraj Gölü'nde yedi balık türünün Zn, Cu, As, Cd, Hg ve Pb seviyelerini saptamıştır. Metal miktarlarının her türde farklı olduğunu ve bu balıkların Zn, Cu, Cd ve As bakımından insan tüketiminde kullanılmasının bir sakınca yaratmadığını bildirmiştir. Fakat, Hg'nin yayında (*Silurus triostegus*) ve Pb'nini Dicle çipurasında (*Acanthobrama marmid*) yüksek olduğunu saptamıştır. Begum vd. (2013) *Heteropneustes fossilis* türünün kas, solungaç, mide, bağırsak ve karaciğerinde bazı ağır metal (As, Pb, Cd, Cr, Zn ve Cu) seviyelerini belirlemiştir. Ağır metal konsantrasyonlarının farklı dokularda önemli farklılık gösterirken istasyonlar arası bir farklılık göstermediğini bildirmiştir. Cd'yi solungaçlarda 2.87-4.27 µg g^{-1} ve karaciğerde 2.25-5.50 µg g^{-1} gibi en yüksek, kasta 0.30-0.40 µg g^{-1} kadar düşük değerlerde bulmuştur. Kaslarda bulunan ağır metal miktarının gıdalarda izin verilen miktarı geçmediğini açıklamıştır.

Ağır metaller canlı vücuduna girdiğinde bir takım bozukluklara neden olmaktadır. Bu nedenle vücutlarında metalloprotein (MT) adı verilen özel proteinler üreterek metalleri bağlamakta ve zararsız hale getmektedirler. MT'ler düşük moleküller ağırlıkta, tek zincirli, sistein amino asitince zengin ve sitosiklik polipeptid yapıda bulunan moleküllerdir. MT'lere ilk olarak 1957 yılında at böbreğinde rastlanmıştır (Margoshes ve Vallee, 1957). Daha sonra memeli, bitki, fungus, protozoa, balık ve diğer türlerde bulunmuştur (Hamer, 1986; Yu vd., 1998, 2000; Liu vd., 2000; Yıldız vd., 2012). MT'ler memelilerde 4 grupta temsil edilmektedir. MT-1 ve MT-2 vücutta Zn dengesinin sağlanmasında, ağır metal toksisitesinde ve oksitatif stresle baş etmede etkin kullanılırken MT-3 sinir ve sinir bağı dokularında ve MT-4 epitel dokularda yoğun bulunmuştur (Vasak, 2005). Su canlılarından pisilerde (*Plathichtys flesus*) (Overnell ve Abdullah, 1988), beyaz karideslerde (*Peneaus vannamei*) (Moksnes vd., 1995; Wu ve Chen, 2005), kahverengi alabalıklarda (*Salmo trutta*) ve yılan balıklarında (*Anguilla anguilla*) (Linde vd., 2001), deniz midyelerinde (*Mytillus sp.*) (Acker vd., 2005), tatlısu yengeçlerinde (*Sinopotamon henanense*) (Ma vd., 2008), sazanlarda (*Cyprinus carpio*) (Kovarova vd., 2009), tatlısu midyelerinde (*Anadonta woodiana*) (Li vd., 2015), kefallerde (*Leuciscus cephalus*) ve tatlısu levreklerinde (*Perca fluviatilis*) (Bayhan ve Ünübol Ayhan, 2016) MT'ler tespit edilmiştir.

Bakırın farklı balık türleri üzerine toksik etkisinin histolojik olarak incelendiği bazı çalışmalar olmasına rağmen (Wani vd., 2011; Salman vd., 2012; Velcheva vd., 2013; Atabati vd., 2015; Al-Tamimi vd., 2015; Alkobaby ve Abd El-Wahed, 2017), akut bakır toksisitesinin pangasus balıklarında üzerine histolojik etkileri hakkında henüz mevcut bir araştırma bulunmadığından bu çalışma yapılmıştır. Ayrıca altı aylık sazanlar (*Cyprinus carpio*) için 48 saat LC_{50} değeri 8,00 mg L^{-1} (Karan vd., 1998); tilapia balıkları (*Oreochromis niloticus*) için 96 saat LC_{50} değeri 25,00 mg L^{-1} (Naseem vd., 2015) ve 31,20 mg L^{-1} (Alkobaby ve Abd El-Wahed, 2017); *Labeo rohita* balığı için 96 saat LC_{50} değeri 33,41 mg L^{-1} (Kousar ve Javed, 2012) olarak bildirilmiştir. Bu sonuçlara bağlı olarak bu çalışmada LC_{50} değerine (% 50 ölümün gerçekleştiği letal konsantrasyonu) yakın konsantrasyonlar denenmek istendiğinden yüksek Cu konsantrasyonları tercih edilmiştir. Bu çalışmada, farklı bakır dozlarına (0,0; 2,5; 5,0; 10,0 mg L^{-1}) 48 saat süreyle maruz bırakılan pangasus balıklarında oluşan hematolojik ve histolojik değişimlerin incelenmesi amaçlanmıştır.

MATERYAL VE METOT

Balıkların bakır maruziyeti

Bu çalışmada, laboratuvarda yetiştirilmiş 102,13±22,59 mm'lik ve 16,21±13,47 g'lık pangasus balıkları kullanılmıştır. Balıklar 0,0; 2,5; 5,0; 10,0 mg L^{-1} bakır sülfat ($CuSO_4.5H_2O$, Merck katalog no: 1.02790.1000) konsantrasyonlarına 48 saat süreyle maruz bırakılmıştır. 15 adet balık 20 L suya stoklanmış (50x20x30 cm) ve 24 saatte bir aynı bakır

konsantrasyonlarına sahip sularla % 100 su değişimi sağlanmıştır. Çalışma iki tekrarlı (toplam 8 akvaryum) olarak yürütülmüştür. Ortam sıcaklığı 24 °C'de sabit tutulmuştur. Deneme süresince her akvaryuma ilave havalandırma yapılmıştır. Günlük kontroller yapılarak ölü balık sayıları kayıt edilmiştir. Deneme boyunca her akvaryumda sıcaklık, çözünmüş oksijen, pH gibi su kalite parametrelerinin ölçümü günde bir kez Multi Parameter Cihazı (WTW Multi 3420 set G) kullanılarak yapılmıştır. Su kaynağının toplam sertlilik, alkalinite ölçümleri bir kez deneme başlangıcında ölçülmüştür.

Balıklardan Kan Alımı

Her akvaryumdan 24 saatte bir olmak üzere dörder balık rastgele seçilip balıkların kuyruk venalarından vakumlu tüp ve iğneler kullanılarak kan örnekleri alınmış ve soğuk zincir takip edilerek +4 °C'de 1-2 gün muhafaza edilmiştir. Sonrasında Mission Ultra Hb aygıtı (Acon, Amerika) kullanılarak hematokrit ve hemoglobin miktarları ölçülmüştür. Alınan kanlardan Thoma lamı ve Natt-Herrick solüsyonu kullanılarak tüm örneklerin eritrosit sayısı belirlenmiştir. Daha sonra eritrosit indeksleri aşağıdaki formüller kullanılarak hesaplanmıştır (Alwan vd., 2009; Duman ve Şahan, 2014).

MCHC (Ortalama Eritrosit Hemoglobin Konsantrasyonu) (%) = (HB-HCT)x100

MCH (Ortalama Eritrosit Hemoglobini) (pg) = (HB-RBC)x10

MCV (Ortalama Eritrosit Hacmi) (μ^3) = (HCT/RBC)x10

HB = Hemoglobin (g L^{-1})

HCT = Hematokrit (%)

RBC = Eritrosit sayısı (10^6 mm^{-3})

Histolojik İnceleme

Her akvaryumdan rastgele seçilen dörder balıktan kan örnekleri alındıktan sonra aynı balıklardan solungaç, karaciğer, böbrek ve dalak doku örnekleri toplanmıştır. Örnekler % 10'luk tamponlu nötral formalin solüsyonuna koyularak en az 24 saat fikse edilmiştir. Fiksasyondan sonra doku örnekleri Roberts (2012)'e göre yükselen alkol serilerinde dehidre edilmiştir. Sonrasında doku örnekleri parafin içinde dondurularak Rotary Mikrotom Cihazı (Leica RM2125 RTS) kullanılarak 5-6 µm kalınlığında doku kesitleri alınmıştır. Ardından doku kesitleri ksilen ile şeffaflaştırılıp hematoksilen-eozin boyama (H&E) yöntemi ile boyanmıştır (Culling vd.,1985). Bu şekilde hazırlanan preparatlar ışık mikroskobu (Euromex-Novex B serisi) altında incelenmiş ve histopatolojik değerlendirmeleri yapılmıştır. Bulunan bulguların fotografları kamera (Novex Cmex DC 5000) ile çekilmiş ve sonuçlar dijital ortamda kayıt altına alınmıştır.

İstatiksel analizler için SPSS programı kullanılarak General Linear Model- Univariate ve Duncan testi uygulanmıştır. Konsantrasyon grupları arasındaki farklılıkların önem derecesine bakılmıştır (*P<0,05*).

BULGULAR

Akvaryumlarda su kalite parametrelerinden sıcaklık 25,4±0,15 °C, pH 8,41±0,34 ve oksijen 7,68±0,15 mg L^{-1}, toplam sertlik 670 mg L^{-1} $CaCO_3$, alkalinite 565 mg L^{-1} $CaCO_3$, olarak ölçülmüştür. Günlük su değişimi sırasında akvaryumların tabanında az miktarda mavi-beyaz kireç tarzında çökelmelerin olduğu görülmüştür.

2,5-5,0 mg L^{-1} bakıra 24 saat süreyle maruz bırakılmış balıkların solungaçlarında ödem, yoğun hiperplazi ve sekonder lamellerin kıkırdak yapısında dejenerasyon, 10,0 mg L^{-1} bakırda da yoğun hiperplazi, ödem ve dejenerasyon görülmüştür (Şekil 1). 48 saatte 2,5-10,0 mg L^{-1} akut bakır konsantrasyonlarına maruz kalan balıkların karaciğerlerinde piknotik nükleus saptanmış ve bu durum bakır konsantrasyonu artışına bağlı olarak artmıştır (Şekil 2). 2,5-10,0 mg L^{-1} akut bakır konsantrasyonlarına maruz kalmış balıkların dalaklarında vakuolleşmeler, nekroz ve hemosterin küme sayısında artış (Şekil 3), böbreklerinde tübüller dejenerasyon ve nekroz (dağılmalar) bulunmuştur (Şekil 4). Denemenin ilk 24 saatinde 5,0 ve 10,0 mg L^{-1} Cu konsantrasyonlarında ilk kayıplar gözlenirken son 24 saatte mortalite % 73,3-76,7 olmuştur (Tablo 1).

Tablo 1. 24 ve 48 saat akut bakır konsantrasyonlarına maruz bırakılan pangasusların yaşama oranı

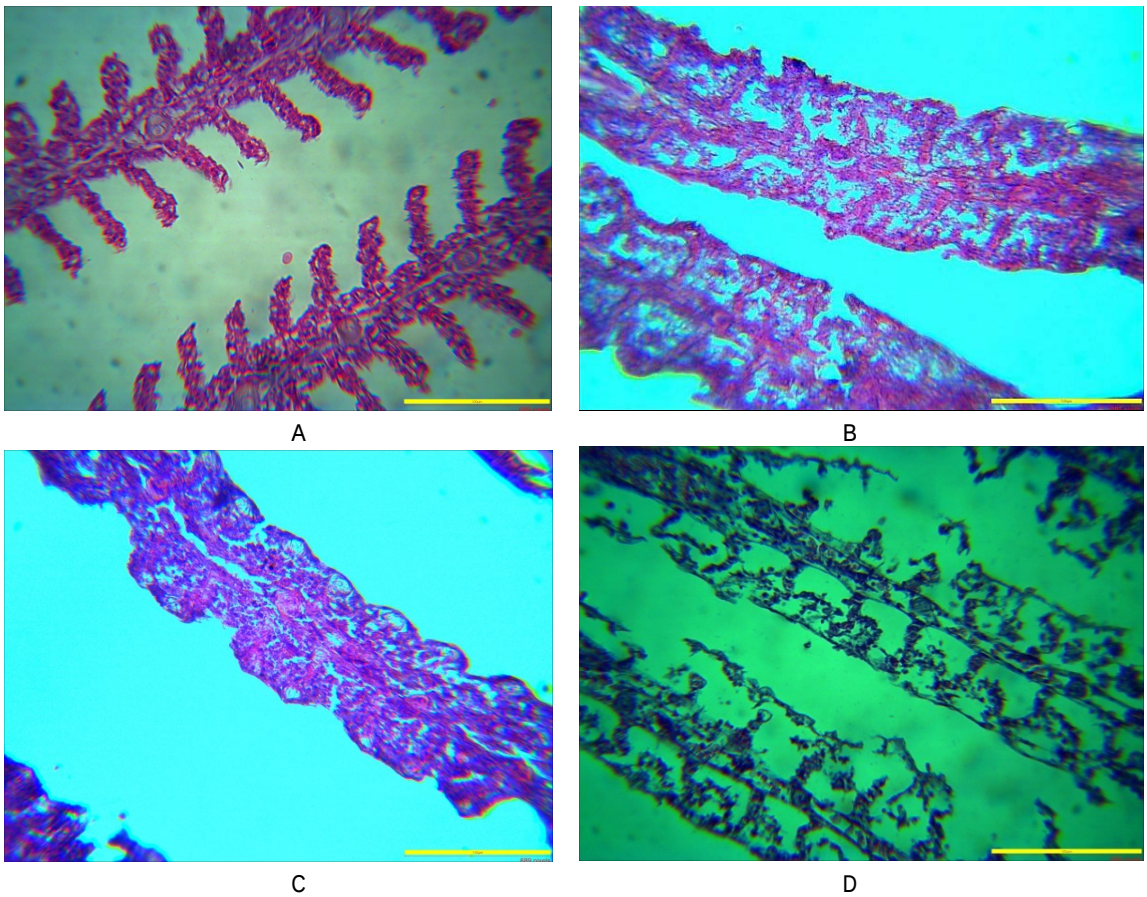
Table 1. Survival of striped catfish exposed to 24 and 48 h acute copper concentrations

Grup	24 saat	48 saat
Kontrol	100,0±0,00	100,0±0,00
2,5 mg/L	100,0±0,00	53,3±37,71
5,0 mg/L	93,3±4,71	16,7±2,36
10,0 mg/L	73,3±18,86	26,7±18,86

Akut bakır konsantrasyonları pangasus balıklarının kan parameterlerinde değişimlere neden olmuştur (Tablo 2). Hematokrit oranında, hemoglobin miktarlarında, eritrosit sayısında 2,5-10 mg L^{-1} Cu konsantrasyonları arasında ilk 24 saatte istatistiki olarak farklılık görülmemiştir ama ≥ 2.5 mg L^{-1} Cu konsantrasyonları hematokrit oranında, hemoglobin miktarlarında ve eritrosit sayısında 48 saat içinde yükselmelere neden olmuştur. Fakat MCHC, MCH ve MCV değerlerinde istatistiksel olarak bakır konsantrasyonları açısından bir farklılık olmamıştır.

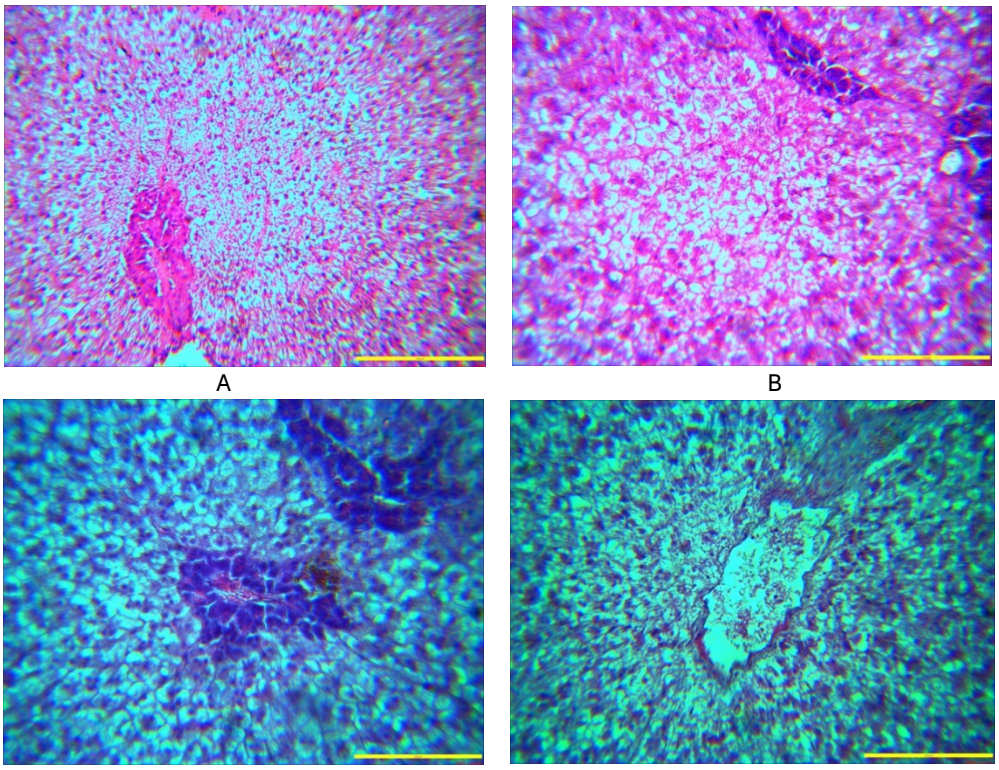
Tablo 2. 24 ve 48 saat akut bakır konsantrasyonlarına maruz bırakılan pangasusların kan parametrelerinin değişimleri
Table 2. Blood parameter changes of striped catfish exposed to acute copper concentrations for 24 and 48 h

	24 saat					
	RBC (10^6 mm^{-3})	HCT (%)	HB (g L^{-1})	MCHC (%)	MCH (pg)	MCV (μ^3)
Kontrol	100,13,3±14,03^{a}	27,60±3,50^{a}	59,25±11,81^{a}	213,09±16,73^{a}	5,93±1,09^{a}	2,78±0,37^{a}
2,5 mg/L	123,0,1±20,93^{ab}	45,50±10,44^{ab}	69,25±12,55^{ab}	160,36±53,83^{a}	5,75±1,49^{a}	3,75±0,89^{a}
5,0 mg/L	131,50±16,20^{bc}	50,95±16,87^{b}	81,00±11,52^{bc}	170,24±47,77^{a}	6,16±0,37^{a}	3,88±1,24^{a}
10,0 mg/L	149,25±9,47^{c}	60,78±14,96$_{b}$	93,00±11,40^{c}	162,55±52,30^{a}	6,24±0,75^{a}	4,08±1,02^{a}
	48 saat					
	RBC (10^6 mm^{-3})	HCT (%)	HB (g L^{-1})	MCHC (%)	MCH (pg)	MCV μ^3)
Kontrol	104,00±14,21^{a}	28,70±3,91^{a}	61,75±12,69^{a}	218,33±53,60^{a}	6,02±1,46^{a}	2,84±0,80^{a}
2,5 mg/L	148,00±25,97^{b}	58,45±16,34^{b}	72,25±26,27^{a}	143,92±102,01^{a}	5,08±2,40^{a}	3,93±0,91^{a}
5,0 mg/L	197,50±35,94^{c}	68,15±9,86^{b}	107,00±15,36^{b}	157,72±17,30^{a}	5,46±0,38^{a}	3,48±0,33^{a}
10,0 mg/L	216,25±25,62^{c}	76,10±13,99^{b}	117,00±20,56^{b}	155,01±20,36^{a}	5,40±0,57^{a}	3,52±0,52^{a}



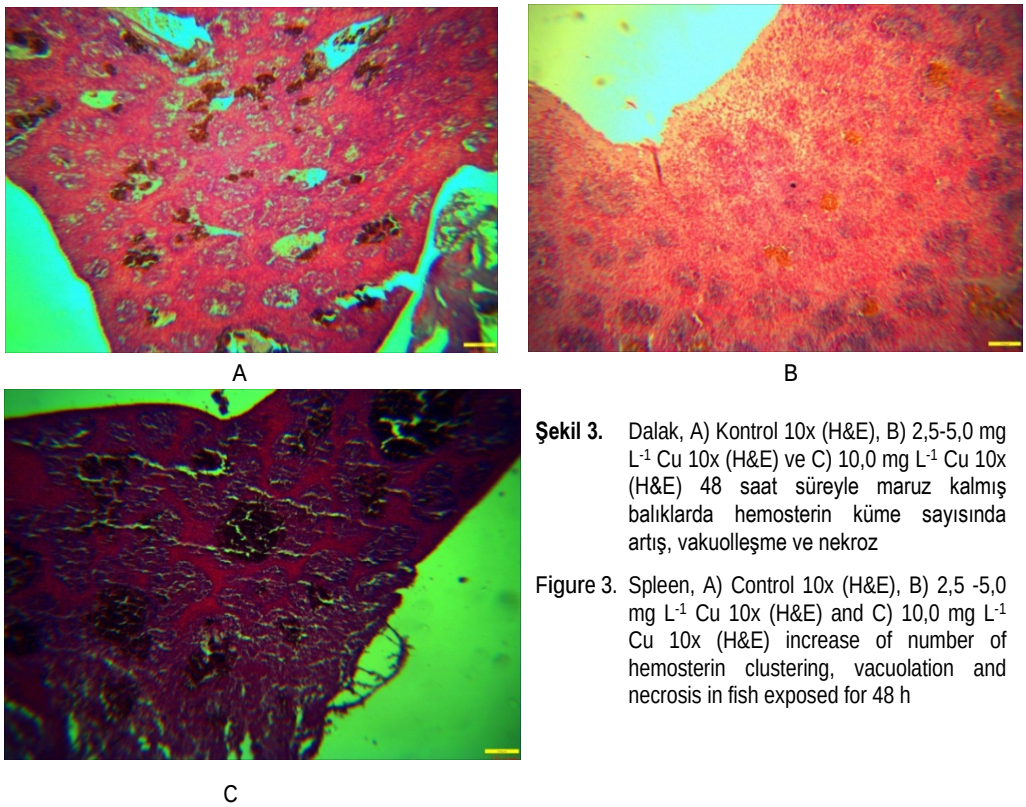
Şekil 1. Solungaç, A) Kontrol 40x (H&E), B) 2,5 mg L^{-1} Cu 24 saat 40x (H&E) ve C) 5,0 mg L^{-1} Cu 40x (H&E) 24 saat süreyle maruz bırakılmış balıklarda sekonder lamellerin kıkırdak yapısında dejenerasyon, ödem ve hiperplazi, D) 10,0 mg L^{-1} Cu 40x (H&E) 48 saat süreyle maruz kalmış balıklarda yoğun hiperplazi, ödem ve dejenerasyon

Figure 1. Gills, A) Control 40x (H&E), B) 2,5 mg L^{-1} Cu for 24 h 40x (H&E) ve C) 5,0 mg L^{-1} Cu 40x (H&E) degeneration in the seconder lamella cartilage structure of fish exposed for 24 h, edema, hyperplaia, D) 10,0 mg L^{-1} Cu 40x (H&E) intense hyperplasia, edema and degeneration in fish exposed for 48 h



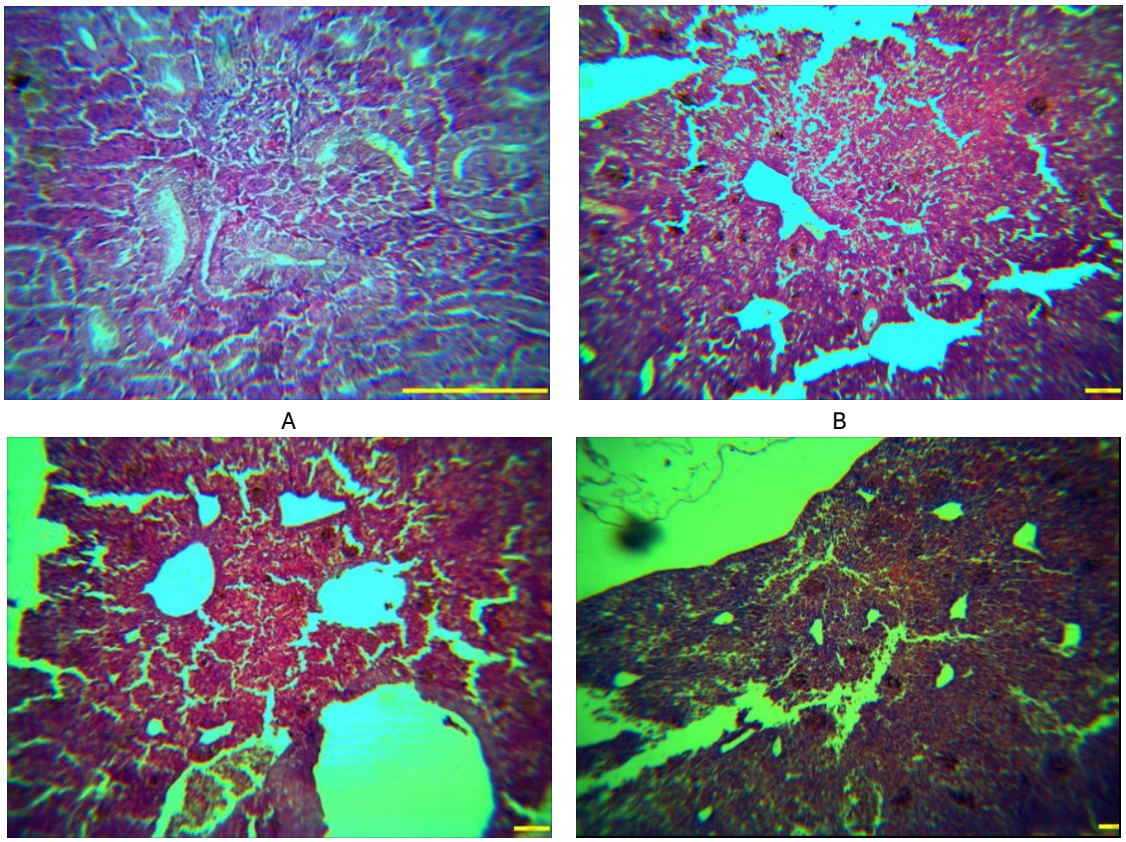
Şekil 2. Karaciğer, A) Kontrol 40x (H&E), B) 2,5 mg L^{-1} Cu 40x (H&E) 24 saat süreyle, C) 5,0 mg L^{-1} Cu 40x (H&E) ve D) 10,0 mg L^{-1} Cu 40x (H&E) 48 saat süreyle maruz kalan balıklarda piknotik nükleus

Figure 2. Liver, A) Control 40x (H&E), B) 2,5 mg L^{-1} Cu 40x (H&E) for 24 h, C) 5,0 mg L^{-1} Cu 40x (H&E) ve D) 10,0 mg L^{-1} Cu 40x (H&E) picnotic nuclei in fish exposed for 48h



Şekil 3. Dalak, A) Kontrol 10x (H&E), B) 2,5-5,0 mg L^{-1} Cu 10x (H&E) ve C) 10,0 mg L^{-1} Cu 10x (H&E) 48 saat süreyle maruz kalmış balıklarda hemosterin küme sayısında artış, vakuolleşme ve nekroz

Figure 3. Spleen, A) Control 10x (H&E), B) 2,5 -5,0 mg L^{-1} Cu 10x (H&E) and C) 10,0 mg L^{-1} Cu 10x (H&E) increase of number of hemosterin clustering, vacuolation and necrosis in fish exposed for 48 h



Şekil 4. Böbrek, A) Kontrol 10x (H&E), B) 2,5 mg L^{-1} Cu 10x (H&E) ve C) 5,0-10,0 mg L^{-1} Cu 10x (H&E) 24 saat süreyle D) 10,0 mg L^{-1} Cu 10x (H&E) 48 saat süreyle maruz kalmış balıkların böbrek tübüllerinde dejenerasyon ve nekroz

Figure 4. Kidney, A) Control 10x (H&E), B) 2,5 mg L^{-1} Cu 10x (H&E) ve C) 5,0-10,0 mg L^{-1} Cu 10x (H&E) for 24 h D) 10,0 mg L^{-1} Cu 10x (H&E) degeneration in kidney tubulles and necrosis in fish exposed for 48 h

TARTIŞMA

Bu çalışmada, ≥ 2,5 mg L^{-1} Cu konsantrasyonlarına maruz bırakılmış pangasusların hematokrit oranında, hemoglobin miktarlarında ve eritrosit sayısında 48 saat içinde artışlar olmuştur. Fakat MCHC, MCH ve MCV değerlerinde istatiksel bir farklılık gözlenmemiştir. Literatüre bakıldığında bu sonuçları destekleyici çalışmalar mevcuttur (Tablo 3).

Tablo 3. Literatürde bakır maruziyetine bırakılmış bazı balık türlerine ait kan parametre sonuçları
Table 3. Blood parameter results of some fish species exposed to copper in literature

Tür	Metal	Sonuç	Kaynak	Maruziyet Süresi
Mozambik tilapiası (*Oreochromis mossambicus*)	Cu	Hemoglobin artışı	Cyriac vd., 1989	24-168 saat
Heteropneustes fossilis	Cu	Hemoglobin miktarında artış	Singh ve Reddy, 1990	24 saat-30 gün
Karabalık (*Clarias gariepinus*)	Cu	Hemoglobin değerinde ve eritrosit sayılarında artış	Van Vuren vd., 1994	96 saat
Nil Tilapiası (*Oreochromis niloticus*)	Cu, Pb	Hematokrit sayısında artış, eritrosit sayısında azalış	Çiftçi vd., 2015	7-30 gün
Catla catla	Cu, Cd	Hematokrit, hemoglobin ve eritrosit sayısında azalma (Cd kaynaklı HCT ve HB de düşme olabilir)	Hasan vd., 2018	24-96 saat
Pangasus *(Pangasius hypophthalmus)*	Cu	Hemoglobin, hematokrit ve eritrosit sayısında artış	Bu çalışma	2 gün

Cu maruziyetinin Mozambik tilapialarında (*Oreochromis mossambicus*) hemoglobin artışına sebep olduğunu Cyriac vd. 1989'de bildirmiştir. Aynı şekilde Singh ve Reddy (1990) Cu maruziyetinin (0,25 mg L^{-1}) *Heteropneustes fossilis* balığının kanındaki hemoglobin miktarını arttırdığını bildirmiştir. Van Vuren vd. (1994) 48 saatlik akut Cu maruziyetinin *Clarias gariepinus* balıklarının hemoglobin değerinde ve eritrosit sayılarında artışlara neden olduğunu rapor etmiştir. Çiftçi vd. (2015) akut Cu konsantrasyonlarına maruz bıraktıkları Nil tilapialarının (*Oreochromis niloticus*) eritrosit sayısında azalış ve hematokrit oranlarında artış olduğunu bildirmiştir. Fakat Hasan vd. (2018) *Catla catla* balıklarında Cu ve Cd maruziyeti sonucunda hematokrit oranında, hemoglobin ve eritrosit sayısında bir azalmanın olduğunu bildirmiştir.

Akut bakır konsantrasyonlarına maruz bırakılmış pangasusların solungaçlarda, ödem, hiperplazi, epitel ve kıkırdak dokuda dejenerasyon; karaciğerde, hepatositlerde piknotik nükeus; dalak hücrelerinde hemosterin küme sayılarında artış, vakuolleşme ve nekroz; böbrek tübüllerinde dejenerasyon ve nekroz (dağılmalar) saptanmıştır. Bakırın çeşitli balık türleri üzerine histolojik etkisi bazı çalışmada incelenmiştir. Bu çalışmanın sonuçları ile önceden yapılmış çalışmaların sonuçları arasında oldukça büyük benzerlikler bulunmuştur (Tablo 4).

Tablo 4. Literatürde bakıra maruz bırakılmış bazı balık türlerinde saptanmış semptomlar
Table 4. Symptoms detected in some fish species exposed to cooper in literature

Tür	Doku	Semptomlar	Maruziyet Süresi	Kaynak
Sazan (*Cyprinus Carpio*)	Solungaç	Hiperplazi, ödem, hipertrofi, telanjiektazi, lamellerde kıvrılma	14 gün	Karan vd., 1998
Nil Tilapiası (Oreochromis niloticus)	Solungaç	Ödem, epitel dokuda döküntüler, lamellar damarlarda vazodilasyon (tıkanma), az miktarda füzyon, proliferasyon, aneurizm	21 gün	Figueiredo-Fernandes vd., 2007
Kara Balık (*Clarias gariepinus*)	Solungaç	Kısa sürede, füzyon, ödem, epitel dokuda döküntü, hiperplazi Uzun sürede, hipertrofi, aneurizm	30-60 gün	Wani vd., 2011
G.Alabalığı (*Oncorhynchus mykiss*)	Solungaç Böbrek	Hiperplazi, füzyon, hipertrofi, etipel dokuda döküntüler Tübüllerde dejenerasyon	24-96 saat	Salman vd., 2012
Havuz balığı (*Carassius gibelio*)	Solungaç	Proliferasyon, vazodilasyon, aneurizm, ödem, füzyon	21 gün	Velcheva vd., 2013
Sazan (*Cyprinus carpio*)	Solungaç Karaciğer Böbrek	Dejenerasyon, konjesyon Dejenerasyon, nekroz Dejenerasyon, konjesyon, nekroz	3-6 hafta	Al-Tamimi vd., 2015
Ot Sazanı (*Ctenopharyngodon idella*)	Solungaç	Hiperplazi, epitelial dejenerasyon	24-96 saat	Atabati vd, 2015
Asya Deniz Levreği (*Lates calcarifer*)	Solungaç Karaciğer	Epitelial nekroz, hipertrofi, Aneurizm, hemoraji, ödem, füzyon Vakuoleşme, hipertrofi	28 gün	Maharajan vd., 2016
Nil Tilapiası (*Oreochromis niloticus*)	Solungaç Karaciğer	Hiperplazi, epitel dokuda döküntüler, ödem, lamellerde kıvrılma Vakuoleşme, piknotik nüklei	96 saat	Alkobabby ve Abd El-Wahed, 2017
Pangasus *(Pangasius hypophthalmus)*	Solungaç Karaciğer Dalak Böbrek	Yoğun hiperplazi, Piknotik nükleus, Hemosterin kümelerinde artış, vakuolleşme ve nekroz Tübüllerde dejenerasyon ve nekroz	2 gün	Bu çalışma

Karan vd. (1998) Cu'a maruz kalan sazanların solungaçlarında hiperplazi, ödem, hipertrofi, telanjiektazi, lamellerde kıvrılma olduğunu saptamıştır. Figueiredo-Fernandes vd. (2007) Cu'ya maruz bırakılan Nil tilapialarının (*O. niloticus*) solungaçlarında ödem, epitel dokuda döküntüler, lamellar damarlarda vazodilasyon (tıkanma), az miktarda füzyon, proliferasyon, anörizm olduğunu bildirmiştir. Wani vd. (2011) karabalığı (*Clarias gariepinus*) 0, 2 ve 5 mg L^{-1} Cu konsantrasyonlarına 30 ve 60 gün süreyle maruz bırakmıştır. 30 günlük sürede füzyon, ödem, lamellar epiteliumda kalkmalar ve hiperplazi; 60 günlük sürede hipertrofi, anörizm (talenjiektazi) ve epitel dokularda hemoraji saptanmıştır. Salman vd. (2012) gökkuşağı alabalığı (*Oncorhynchus mykiss*) yavrularını (0,5 g) 0,0; 0,1; 0,2 ve 0,4 mg L^{-1} Cu

konsantrasyonlarına 24, 48, 72 ve 96 saat süreyle maruz bırakarak solungaç, karaciğer, böbrek ve sindirim kanalını histolojik olarak incelemiştir. Solungaçlarda, füzyon, hiperplazi, epitel hücrelerde kalkmalar (döküntüler), nekroz; karaciğer de, nükleus deformasyonları; böbrek tübüllerinde dejenerasyon; mide de vakuolleşme olduğunu bildirmiştir. Velcheva vd. (2013) *Carassius gibelio* balığını 0,00, 0,05, 0,10 mg L^{-1} Cu konsantrasyonlarına 21 gün süreyle maruz bırakıp solungaçlarındaki histolojik değişiklikleri incelemiştir. Ödem, füzyon, hiperplazi, anörizm ve vazodilasyon bulmuştur. Atabati vd. (2015) ot sazanlarını (*Ctenopharyngodon idella*) 0,0; 2,5 ve 5,0 mg L^{-1} Cu konsantrasyonlarına 96 saat boyunca maruz bırakmıştır. Primer ve sekonder lamellar epitelial hücrelerde dejenerasyon ve hiperplazi görüldüğünü bildirmiştir. Al-Tamimi vd. (2015) sazanları (*C. carpio*) 0,0; 0,5; 0,9 ve 1,2 mg L^{-1} Cu konsantrasyonlarına 3 ve 6 hafta süreyle maruz bırakarak solungaç, karaciğer, böbrek ve kas dokusunda oluşan histolojik değişiklikleri araştırmıştır. Solungaçlarda, sekonder lamellar dejenerasyon ve konjesyon; karaciğer hücrelerinde dejenerasyon ve nekroz ve inflamasyon; böbrek hücrelerinde konjesyon, hemoraji, dejenerasyon ve nekroz; kas hücrelerinde hyalizasyon ve interstitial fibrillerin kaybı, dejenerasyon ve nekroz görüldüğünü bildirmiştir. Maharajan vd. (2016) Cu'ya maruz bıraktığı Asya deniz levreği'nin (*Lates calcarifer*) solungaçlarında epitelial nekroz, hipertrofi, anörizm, hemoraji, ödem, füzyon; karaciğerinde vakuoleşme, hipertrofi görüldüğünü belirtmiştir. Alkobaby ve Abd El-Wahed (2017) Nil tilpialarını (*O. niloticus*) (2,97 g) 0, 5, 10, 15, 20, 25, 30, 35 ve 40 mg L^{-1} Cu konsantrasyonlarına 96 saat süreyle maruz bırakmıştır. Solungaçlarda, hiperplazi, lamellar epiteliumda döküntüler, füzyon, ödem, sekonder lamellerde kıvrılmalar; karaciğer de, sitoplazmik vakuolleşme ve piknotik nükleus olduğunu bildirmiştir.

Bakır toksisitesi, balıkların tüm organlarını etkileyip onlarda histolojik bozukluklara yol açabilmektedir. Zira bakır, balık kanında Ca^{+2}, Na^{+}, Cl^{-}, K^{+} seviyelerinde inişlere, kan şekeri ve Mg^{+2} seviyelerinde yükselmelere; ayrıca deri, bağırsak ve solungaç mukoz salgısında artışa, solungaçlarda klorid hücre sayısında artışa ve omurgada deformasyonlara sebep olduğu Olsson (1998) tarafından bildirilmiştir.

Bu çalışmada yüksek bakır dozları kullanılması yüksek mortaliteye yol açmıştır. Fakat bakır sudaki fazla karbonat iyonları ile etkileşime girip bakır karbonat şeklinde (suda mavi beyaz kireç) çökelmelere neden olmuştur. Çünkü suyun toplam sertlik ve alkalinite değerleri yüksektir. Sonuç olarak bu durum bakır toksisite değerini kısmen zayıflatmış olabilir (Wurts ve Perschbacher, 1994; Ebrahimpour vd., 2010).

Pangasus balıkları bakır toksisitesine çok aşırı hassasiyet göstermişlerdir. Denemenin ilk 24 saatinde 5,0 ve 10,0 mg L^{-1} Cu konsantrasyonlarında ilk kayıplar gözlenirken 48. saatte mortalite çok yükselmiştir (% 73,3-76,7). Benzer çalışmalarda da bakır toksisitesinin mortaliteyi artırdığı görülmüştür. Gökkuşağı alabalıkları 0,5 mg L^{-1} Cu konsantrasyonuna maruz bırakıldıklarında mortalite % 70 olurken bakır konsantrasyonu 2 mg L^{-1}'ye çıkarıldığında mortalite % 100'e yükselmiştir (Gündoğdu, 2008). Sazanlar 8-10 mg L^{-1} Cu konsantrasyonuna maruz bırakıldıklarında mortalite 24 saatte % 54-60'a yükselmiştir (Thangan, 2016).

SONUÇ

Bu çalışmada, bakır toksisitesini histolojik olarak incelemek için pangasus balıkları 0,0; 2,5; 5,0; 10,0 mg L^{-1} $CuSO_4.5H_2O$ konsantrasyonlarına 48 saat süreyle maruz bırakılmıştır Bu çalışmanın sonuçları kısaca şöyle özetlenebilir:

Bakırın LC_{50} değerine yakın olması amacıyla kullanılan yüksek dozlardan 2,5 mg L^{-1} pangasus balıkları için maksimum doz olmuştur. Çünkü bu doz ile daha yüksek dozların (5,0, 10,0 mg L^{-1}) oluşturduğu hematolojik (HCT, HB ve RBC artışı) ve histololojik (solungaçlarda yoğun hiperplazi; karaciğerde piknotik nükleus; dalakta hemosterin kümelerinde artış, vakuolleşme ve nekroz; böbrek tübüllerinde dejenerasyon ve nekroz) sonuçlar benzer bulunmuştur. İlerde yapılacak toksisite çalışmalarda 2,5 mg L^{-1} Cu pangasus balıkları için maksimum doz olarak kabul edilebilir. Bakır canlı metabolizmasında iz element olarak kullanılmasına rağmen yüksek dozları pangasus balıkları için toksik bir element olmuştur. Bakırın balıklar üzerinde oluşturabileceği metabolik ve histolojik bozukluklar konusunda ilerde yapılacak çalışmalarda pangasus balıkları hassas bir model balık türü olarak kullanılabilir.

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KISA ARAŞTIRMA SHORT COMMUNICATION

Lepistes (*Poecilia reticulata* **Peters, 1859)'in Türkiye içsularından ilk kaydı**

First record of the Guppy (*Poecilia reticulata* Peters, 1859) in inlandwaters of Turkey

Gürel Türkmen

Ege Üniversitesi, Su Ürünleri Fakültesi, 35100, Bornova, İzmir

http://orcid.org/0000-0002-2683-6258

gurel.turkmen@ege.edu.tr

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Öz: Giderek artan dünya akvaryum ticareti sucul ortamlardaki istilalara neden olan önemli yollardan birisidir. Son yıllarda tatlısu akvaryum balıklarının doğal tatlısu kaynaklarında varlıklarının tespit edilmesi giderek artmaktadır. Türkiye içsularında yerli olmayan türlerin görülmesinde akvaryum ticaretinin sınırlı olsa da etkisi giderek büyüyen oranda artmaktadır. Bu çalışmada, Lepistes (*Poecilia reticulata*) Türkiye'de doğal içsularında ilk kez kaydedilmiştir. *P. reticulata*'nın büyük ihtimal ile doğaya bırakıldıkları Çeşme-Ildır (İzmir) akiferine yerleşerek üreyebildikleri görülmüştür.

Anahtar kelimeler: Poecilia reticulata, yabancı tür, akvaryum balığı, Türkiye

Abstract: The ever-increasing global trade of ornamental aquarium fishes is one of the most important pathways for aquatic invasion. Occurrence of ornamental freshwater fishes in natural freshwaters has been increasing in recent years. The ornamental trade accounts for a limited but steadily growing proportion of fish introductions to Turkish inlandwaters. In this study, Guppy, *Poecilia reticulata*, is recorded for the first time from natural inlandwaters of Turkey. *P. reticulata* was probably released into the wild, but is now established in aquifer of Çeşme-Ildır (İzmir).

Keywords: Poecilia reticulata, alien species, aquarium fish, Turkey

GİRİŞ

Lepistes balığı (*Poecilia reticulata* Peters, 1859)'nın doğal yaşam alanı Venezüella-Guayre Nehri olup Güney Amerika'nın Kuzeydoğusunda Brezilya, Guyana, Barbados, Venezüella ve Trinidad ve Tobago'da tatlısu ve acısularda doğal olarak dağılım gösterirler (Rosen ve Bailey, 1963).

Yüksek ve hızlı üreyebilme kapasiteleri sayesinde yoğun seleksiyon yöntemleri ile birçok varyeteleri geliştirilmiş ve çok kısa bir sürede dünya akvaryum ticaretinde popüler olmuşlardır. Ayrıca lepistes balıkları laboratuvar ve sivrisineklere karşı biyolojik kontrol çalışmalarında da kullanılan türlerin başında gelmektedir (Magurran, 2005). Günümüzde dünyada 11 ülkede doğal olarak bulunduğu ve 74 farklı ülkede ise biyolojik kontrol amaçlı doğal su kaynaklarına aşılandığı rapor edilmektedir (CIBA, 2019). Avrupa kıtasında Arnavutluk, Çekya, Hollanda, İngiltere, İspanya, İtalya, Macaristan, Romanya, Sırbistan ve Slovakya'da doğal ortamlarda bulundukları rapor edilmiştir (Milenkovic vd., 2014). Son dönemde birçok akvaryum balık türünün doğal ortamlarda yabancı tür olarak rapor edildiği buna neden olarak da akvaryum meraklılarının bir süre sonra bu hobiden sıkıldıkları veya akvaryuma sığmayacak boyutta büyüyen balıkları doğal ortamlara bırakmalarının etkili olduğu bildirilmektedir (Sandilyan, 2016). Türkiye'de bu etkinin sınırlı olduğu ancak akvaryum ticaretinin her geçen gün büyümesi ile bu oranın arttığı vurgulanmıştır (Tarkan vd., 2015). Bu güne kadar Türkiye'de doğal habitatta 5 farklı tatlısu akvaryum balığının (*Carassius auratus*, *Pygoplichthys nattereri*, *Pterygoplichthys disjunctivus*, *Pterygoplichthys, pardalis*, *Pangasius sanitwongsei*) varlığı rapor edilmiştir (Yoğutçuoğlu ve Ekmekçi, 2018). Bu çalışma ile Türkiye'de doğal tatlısu kaynaklarında *P. reticulata* türünün varlığı ilk kez rapor edilmektedir.

MATERYAL VE METOT

Çalışmadaki *P. reticulata* örnekleri Temmuz 2019 tarihinde İzmir ili Çeşme-Ildır'da bulunan Ildır Akiferleri (TOB, 2018) yerel adı Ildır Azmağı'ndan 38° 22' 49" K, 26° 29' 15" D 0.5 mm göz açılığında kepçe kullanılarak yakalanmıştır (Şekil 1). Balıklar yakalandıktan sonra karanfil yağı ile bayıltılarak resimlenmiş ve bazı morfometrik özelliklerinin tespiti amacıyla %4'lük formaldehit solüsyonu ile tespit edilmiştir. Toplamda 7 erkek ve 5 dişi birey olmak üzere 12 birey örneklenmiştir. Tür tayininde McDowall (1999) ve Poeser vd. (2005) kaynaklarından yararlanılmıştır. Standart boy ölçümleri 0.1 mm hassasiyette kumpas ile gerçekleştirilmiş boy aralığı ve "ortalama±standart hata" olarak sunulmuştur. Baş uzunluğu ölçümü standart boy ölçümünün yüzde (%) oranı olarak ifade edilmiştir. Stereo mikroskop kullanılarak yanal çizgi pul ve yüzgeç ışınları sayıları tespit edilmiştir. Dorsal, anal, pektoral ve pelvik yüzgeç ışınlarında toplam ışın sayısı kaudal yüzgeç te ise dallanmış ışın sayısı dikkate alınmıştır (Rosso vd., 2017). Yüzgeç ışın sayıları tepe değerleri (mod) ile birlikte verilmiştir.

Şekil 1. Türkiye'de *P. reticulata*'nın ilk kaydedildiği çalışma alanını gösterir harita (Google Earth, 2019)
Figure 1. Map of the study area showing the first records of *P. reticulata* in Turkey (Google Earth, 2019)

BULGULAR VE TARTIŞMA

Yakalanan bireylerde vücut iğ şeklinde ve baş bölgesi basıktır. Baş ve vücut iri sikloyid pullar ile örtülüdür. Alt çene üst çeneden biraz uzun olup ağız küçük ve terminal konumludur. Seksüel dimorfizm yetişkin erkek bireylerde görülen gonopodium ile karakteristiktir. Vücut rengi erkek bireylerde mavimsi-yeşilimsi veya mavimsi-grimsi olup, yanal çizgi boyunca çelik-mavimsi bant ve ventral bölgelerde turuncu desenler ile farklı bölgelerde koyu renkte lekelerden oluşur (Şekil 2). Kuyruk yüzgeci farklı yapılarda görülebilmektedir (Şekil 4f). Dişilerde vücut rengi grimsi-yeşilimsi olup çelik-mavimsi yansımalar görülebilmektedir. Karın süt beyazı rengindedir (Şekil 3).

Erkek bireylerde ortalama standart boy 17,9±0,30 mm boy aralığı ise 16.8-18.9 mm arasında değişirken, dişilerde ortalama standart boyun 21,8±0,48 mm boy aralığının ise 20.4-23.2 mm arasında değiştiği görülmüştür. Yanal çizgideki pul sayısı erkeklerde 25-26, dişilerde 26-28 arasında tespit edilmiştir. *P. reticulata* bireylerinde tespit edilen bazı meristik ve morfometrik özellikler Tablo 1'de sunulmuştur. Dorsal yüzgeç dişi bireylerde anal yüzgeç hizasında başlar iken erkek bireylerde biraz arkasında başlamakta ve her iki eşeyde de toplam ışın sayısı 7 olarak tespit edilmiştir (Şekil 4a). Pektoral ışın sayısı erkeklerde 14-15 (Şekil 4b), dişilerde 14 olarak; kuyruk yüzgecindeki dallanmış ışın sayısı erkeklerde 13-14 (Şekil 4c), dişilerde 12-13 arasında değiştiği tespit edilmiştir. Pelvik yüzgeç ışın sayısı erkek ve dişi bireylerde de 6 olarak belirlenmiştir (Şekil 4d). Erkek bireylerde kopülasyon organı olarak kullanılan gonopodiumun baş boyundan daha uzun olduğu görülmüştür (Şekil 4e).

Şekil 2. Yakalanan erkek *Poecilia reticulata* birey
Figure 2. Collected male specimen of *Poecilia reticulata*

Şekil 3. Yakalanan dişi *Poecilia reticulata* birey
Figure 3. Collected female specimen of *Poecilia reticulata*

Tablo 1. Yakalanan *P. reticulata* bireylerinin bazı meristik ve morfometrik özellikleri
Table 1. Some meristic and morphometrics of collected speciemens of *P. reticulata*

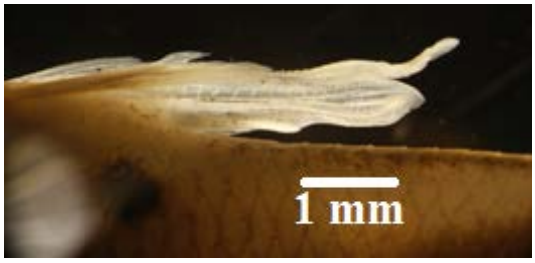
	Erkek (n=7)	**Dişi (n=5)**
Standart Boy (mm)	17,9±0,30 (16,8-18,9)	21,8±0,48 (20,4-23,2)
Baş Uzunluğu (%SB)	22,7-26,3	23,5-25,2
Dorsal Yüzgeç (toplam ışın)	7	7
Anal Yüzgeç (toplam ışın)	8	9
Pektoral Yüzgeç (toplam ışın)	14-15 (mod:14)	14
Pelvik Yüzgeç (toplam ışın)	6	6
Kaudal Yüzgeç (dallanmış ışın)	13-14 (mod:13)	12-13 (mod:12)
Yanal Çizgi (pul sayısı)	25-26 (mod:26)	26-28 (mod:28)

a. erkek dorsal-yüzgeç ışınları
a. male dorsal-fin rays

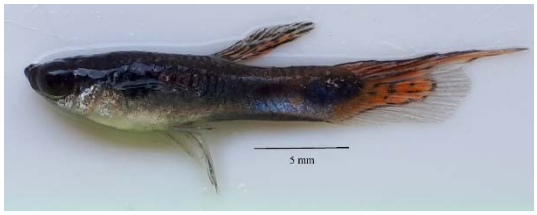
b. erkek pektoral-yüzgeç ışınları
b. male pectoral-fin rays

c. erkek kuyruk-yüzgeci ışınları
c. male caudal-fin rays

d. dişi pelvik-yüzgeç ışınları
d. female pelvic-fin rays



e. gonopodium
e. gonopodium



f. erkek *P. reticulata*
f. male of P. reticulata

Şekil 4. Yakalanan *P. reticulata* bireylerinde bazı meristik özellikler
Figure 4. Some meristics of collected specimens of *P. reticulata*

Tablo 2. Türkiye içsularında rapor edilen akvaryum balık türleri
Table 2. Ornamental fish species reported from various inlandwaters of Turkey

Tür Adı	Lokasyon	Referanslar
Carassius auratus	Birçok lokasyon -1950	İnnal, 2012
Pygocentrus nattereri	Sakarya Nehri, 2006	Tarkan, 2006
Pterygoplichthys disjunctivus	Asi Nehri, 2007	Özdilek, 2007
Pterygoplichthys pardalis	Pınarbaşı-Sakarya, 2016	Emiroğlu vd., 2016
Pangasius sanitwongsei	Sakarya Nehri, 2018	Yoğurtçuoğlu ve Ekmekçi, 2018

Yöre halkı ile yapılan sözlü görüşmede azmak alanında 10 yılı aşkın bir süre öncesinde amatör amaçlı akvaryum balıkları yetiştiriciliği yapan küçük çaplı amatörce bir işletmenin olduğu bilgisine ulaşılmıştır. Büyük olasılık ile bu işletmeden kaçan lepistes balıklarının bu alanda yaşayabildikleri ve üreyebildikleri, bu sayede süreklilik arz eden bir popülasyon oluşturabildikleri düşünülmektedir. Ildır Akiferi yıllık emniyetli yer altı suyu verimi ortalama 11 m^3/saat olup Küçük Menderes Havzasın'da yer alır. Bu havzada yıllık bazda 25 C° lik yaz günleri sayısı toplamı 155 gündür. Bu sıcaklık değerinde Türkiye ortalaması olan 112 gündür. Aynı havzada gözlenen 35 C° lik yaz günü sayısı da 20 gün ile yine ortalamanın çok üzerindedir (TOB, 2018). Bölgenin iklim özelliklerinin söz konusu popülasyonun oluşmasında ve devamında önemli rol oynadığı sanılmaktadır.

Bu güne kadar Türkiye sularında 5 farklı akvaryum balık türünün varlığı rapor edilmiştir (Tablo 2). Bu türlerden sadece *Carassius auratus* türü ve *Pterygoplichthys disjunctivus* ve *Pterygoplichthys pardalis* türlerinin doğal popülasyonlarında süreklilik sağladıkları bildirilmiştir (Yoğurtçuoğlu ve Ekmekçi, 2018). Türkmen ve Karadal (2019) Türkiye'de en çok ticareti yapılan 50 farklı tatlısu akvaryum balığı türlerini tespit etmişlerdir. Bu grupta yer alan ve Lepistes balığının da içerisinde bulunduğu 11 balık türünün Avrupa içsularında doğal ortamlarda yabancı tür olarak kaydedildiklerini bu türlerin ülkemiz sucul ekosistemi için tehdit olabileceklerini ifade etmişlerdir.

Bu çalışma ile *P. reticulata* yeni ve doğal kaynaklarda yerleşmiş olan bir tür olarak bildirilmiştir. Bu tip sucul ekosistemlerin uzun süreli ve kapsamlı çalışmalar ile takip edilmesi daha sağlıklı verilere ulaşmak için oldukça önem arz etmektedir. Ayrıca ülkemizde akvaryum kaynaklı yabancı türlerin endemik türler ve ekosistem üzerinde potansiyel baskısı gün geçtikçe artmaktadır. Bu konuda gerekli düzenlemelere ihtiyaç duyulurken akvaryum severlerin konu ile ilgili farkındalıkları arttırılmalıdır.

TEŞEKKÜR

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KAYNAKÇA

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CASE REPORT VAKA TAKDİMİ

Occurrence of an abnormal one-eyed black anglerfish *Lophius budegassa (Spinola, 1807)* from Central Aegean Sea, Turkey

Orta Ege Denizi, Türkiye'de anormal tek gözlü fener balığı *Lophius budegassa (Spinola, 1807)*'nın bulunuşu

Ahmet Mert Şenbahar[1]* ● Okan Özaydın[2]

[1] Department of Basic Sciences, Faculty of Fisheries, Ege University; Izmir, Turkey http://orcid.org/0000-0001-6613-8932
[2] Department of Basic Sciences, Faculty of Fisheries, Ege University; Izmir, Turkey http://orcid.org/0000-0003-0198-4286

*Corresponding author: a.mertsenbahar@gmail.com
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Abstract: During the seasonal trawling studies between September 2017 and August 2018, one specimen of the *Lophius budegassa* (Spinola, 1807) was found as one-eyed. This is the first record of this type, morphological abnormality of this fish species from Aegean Sea. Abnormality could be caused by environmental or genetic factors. The other reason might be a one-eyed specimen could be attacked by other carnivores when specimens at early stages. However, based on morphological investigations there were no visible assault scar or wound lesion were found on orbital skin tissue.
Keywords: One-eyed, abnormality, *Lophius budegassa*, Central Aegean Sea

Öz: Eylül 2017 – Ağustos 2018 tarihleri arasında gerçekleşen mevsimsel trol örneklemeleri sırasında bir adet *Lophius budegassa* (Spinola, 1807) bireyi tek gözlü olarak bulunmuştur. Bu tip bir bildiri Akdeniz'de morfolojik anormallik olarak bu tür için bir ilktir. Anormallik faktörü çevre ve genetik kaynaklı olabileceği gibi diğer bir sebep olarak, tek gözlü bireyin erken büyüme evrelerinde yırtıcılar tarafından saldırıya uğramış olduğudur. Ancak morfolojik incelemeler sonucunda orbital deri dokusunda görünür hiçbir yara izi ve yara lezyonu görülmemiştir.
Anahtar kelimeler: Tek gözlülük, Anormallik, *Lophius budegassa*, Orta Ege Denizi

INTRODUCTION

Black-bellied angler *Lophius budegassa* occurs shallow waters to down to depths and they feed on benthic species, fish and crustaceans because they are carnivorous (Whitehead, 1986). Black anglerfish are typical bottom living species, the former having a depth range between 70 m and 800 m and the latter extending to depths >1000 m (Dardignac, 1988). Also, these two species are important in European fisheries. Anglerfish are known to be one of the top demersal predators in European waters. However, despite their high economic value, little is known about their biology and ecology (Farina et al., 2008; Landa et al., 2001; Issac et al., 2017). Black anglerfish has a more southern distribution therefore Mediterranean and Eastern North Atlantic from British Isles to Senegal (Fishbase, 2019). Anglerfishes are gaining high economic value day by day and becoming demand species in Turkish fish markets.

Diagnosis of *Lophius budegassa*; dorsal fin ray; 8 or 9 anal fin rays; 22-26 pectoral fin rays; length of third dorsal fin spine greater than snout width but less than distance between posterior frontal spines; length of forth dorsal fin spine approximately equal to snout width; esca a simple pennant-like flap; peritoneum dark (Caruso, 1983). So far, there is no explanatory study has been made about the cause of monocularism or being one-eyed of Lophius budegassa. The aim of this study is to identify this morphological abnormality and report this case.

MATERIALS AND METHODS

During the seasonal trawling studies between September 2017 and August 2018, one specimen of *L. budegassa* specimen were captured in offshore of Karaburun – Foça side of Turkey, 38°46'58.89" N - 26°24'38.10" E (Figure 1). All the trawls occurred between 45 – 360 m. However, there is no certain data of bathymetric existence of this abnormal specimen. One-eyed individual transported from the vessel with ice filled styrofoam box and were kept in the freezer. The morphometric features of the sample were measured. The total height measurement of the sample was measured with a 1 mm precision measuring board and the weight was measured with an electronic weighting machine with 0.01 g sensitivity.

RESULTS AND DISCUSSION

Eye deformations in Lophius species generally observed as blindness and blindness has been always described in albino individuals or with pale body coloration (Alonso-Allende, 1983; Bucke et al., 1994; Landa et al., 1998;

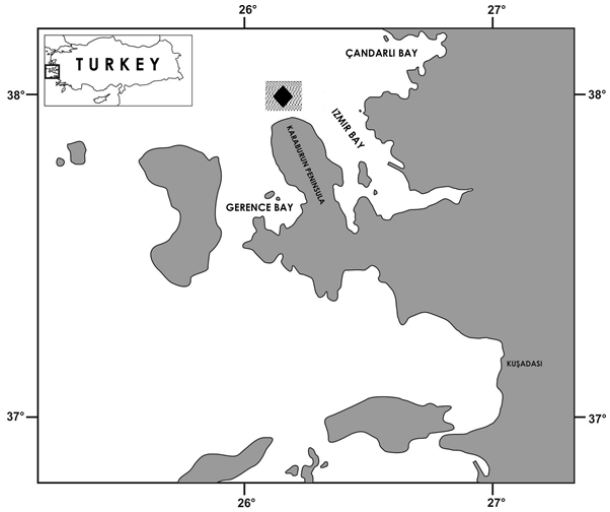

Figure 1. Sampling area of obtained one-eyed individual

Colmenero et al., 2016). One-eyed or monocularism is a very rare abnormality in *Lophius budegassa*.

Until now, there was only one report that occurred about being one-eyed and this was Monocular-leucism in SE Irish Sea, by Quigley et al. (2015). However, that species has two abnormalities. These are Monocularism and leucism. Overall, it is wrong to ignore the relations between coloration and eye deformations. However, in this report, the one specimen of *L. budegassa* has dark-brown coloration as other Lophius species. In the study of Colmenero et al. (2016), a single blind individual of *L. budegassa* was found and the coloration of that species was reported as same as in this study but in the end, they could not find the cause of blindness.

Until now, researchers focused on 4 major inducement factors that are related to eye abnormalities. These are parasitic, genetic alteration during the embryonic development of the eye structure, genetic changes, and pollution effects. According to studies of Bucke et al. (1994), there is no relationship between blindless anomaly and genetic changes or pollution effect but in the report of Colmenero et al. (2016), the microsporidian parasite *S. lophii* mentioned as could be associated with anatomical anomalies such as eye deformities. As a result of these inferences and previous reports, the cause of being one-eyed or monocularism is currently unknown. Based on the measurements and observation, it has been seen no significant differences except abnormal morphology as one-eyed. *L. budegassa* species from this research sex, length and weight measurements found as female (♀), 21.7 cm and 151.1 g respectively. Comparing the pictures of two individuals of *L. budegassa* (Figure 2), it is clearly shown that there is an orbital cavity under the skin tissue but no eyes (Figure 3). The etiology of monocularism is currently unresolved and abnormality could be caused by environmental or genetic factors.

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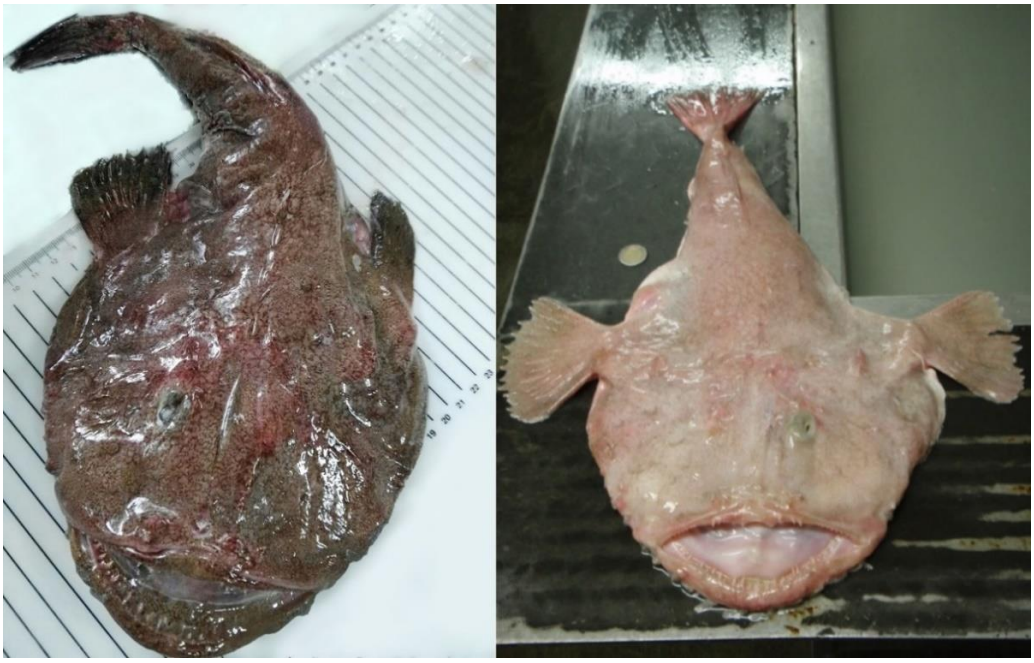

Figure 2. Left photo: Monocular abnormality in *Lophius budegasssa* (left of the eye), Central Aegean Sea, 2018. Right photo: Monocular leucism in *Lophius budegassa* (right of the eye), SE Irish Sea, January 2013. Photo: Declan Quigley

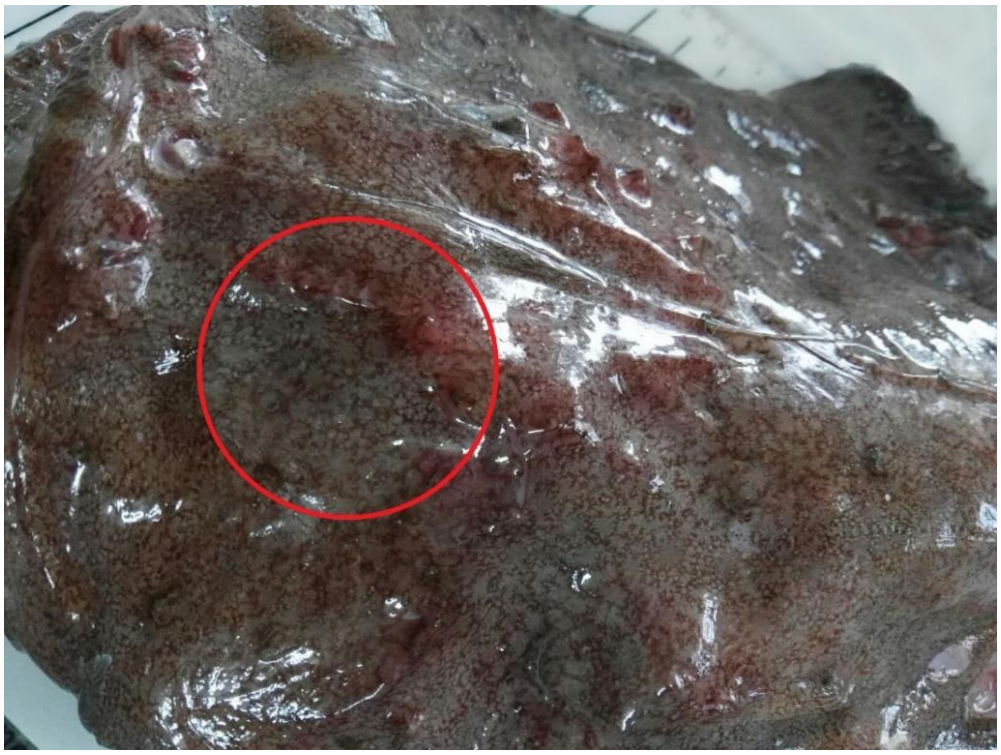

Figure 3. There is an orbital cavity under the skin tissue

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