Ege Journal of
Fisheries and Aquatic Sciences

www.egejfas.org

E-ISSN 2418-3140

EgeJFAS

Su Ürünleri Dergisi

Volume 37 Number 4 2020

Ege University Faculty of Fisheries

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Corresponding Address	**E- ISSN 2148-3140**
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	

Su Ürünleri Dergisi

Ege Journal of Fisheries and Aquatic Sciences

Volume 37 Number 4 **E-ISSN 2418-3140**

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Published by

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Su Ürünleri Dergisi
Ege Journal of Fisheries and Aquatic Sciences

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

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

Yayımlanma Tarihi **Publishing Date**
15 Aralık December 15th, 2020

İletişim **Contact**
Ege Üni. Su Ürünleri Fakültesi, 35100, Bornova, İzmir Ege Uni. Faculty of Fisheries, 35100, Bornova, Izmir, Turkey
Tel: +90 232 311 3838 Fax: +90 232 388 3685 http://www.egejfas.org info@egejfas.org

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 325-328 (2020)
Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.01
RESEARCH ARTICLE
ARAŞTIRMA MAKALESİ

New record of three freshwater fish species from a western drainage of Lake Urmia for the Turkish fauna

Urmia Gölü'nün batı drenajından Türkiye faunası için üç yeni tatlısu balık türü kaydı

Cüneyt Kaya

Recep Tayyip Erdogan University, Faculty of Fisheries and Aquatic Sciences, 53100 Rize, Turkey
https://orcid.org/0000-0002-4531-798X

cnytkaya@yahoo.com

Received date: 11.03.2020 *Accepted date: 13.05.2020*

How to cite this paper:
Kaya, C. (2020). New record of three freshwater fish species from a western drainage of Lake Urmia for the Turkish fauna. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 325-328. DOI: 10.12714/egejfas.37.4.01

Abstract: In the scope of this study, three freshwater fish species were newly recorded for Turkey from a western drainage of Lake Urmia: *Alburnoides petrubanarescui, Alburnus atropatenae* and *Oxynoemacheilus elsae*. All of them were found in headwaters of Nazli-chay River in the basin of the hypersaline Lake Urmia. The Lake is fed by many small springs and thirteen permanent rivers. However, it is still seriously threatened and drying up rapidly. In the previous studies, the existence of a stream in the western part of the Urmia Lake within border of Turkey was not mentioned.

Keywords: Anatolia, Nemacheilidae, Leuciscidae, first record

Öz: Bu çalışma kapsamında, Urmia Gölü'nün batı drenajından Türkiye için üç yeni tatlı su balığı türü bildirilmişir: *Alburnoides petrubanarescui*, *Alburnus atropatenae* ve *Oxynoemacheilus elsae*. Bu türlerin hepsi yüksek tuzluluğa sahip Urmia Gölü havzasındaki Nazlı Çay'ın membalarında tespit edilmiştir. Bu göl, birçok küçük su kaynağı ve 13 devamlı nehir ile beslenmektedir. Ama yine de ciddi tehdit altındadır ve hızla kurumaktadır. Daha önceki çalışmalarda, Urmiye Gölü havzasının batı kesiminde Türkiye sınırları içerisinde ki akarsuların mevcudiyetinden bahsedilmemiştir.

Anahtar kelimeler: Anadolu, Nemacheilidae, Leuciscidae, yeni kayıt

INTRODUCTION

Lake Urmia (also known as Orumiyeh) is located in northwest Iran. The lake is one of the largest permanent hypersaline lakes in the world and has an importance on biodiversity in the area (Kelts and Shahrabi, 1986) but the lake level has fallen dramatically during the last decades, and the salinity of the lake has strongly increased due to human activities and poor management. Meanwhile, the lake is almost dried out (Jörg Freyhof, pers. comm, 2018).

The ichthyofauna of Lake Urmia basin was reviewed by Ghasemi et al. (2015) and they listed 29 fish species, five of which are endemic to the lake basin. These are; *Acanthobrama urmianus* (Günther, 1899), *Alburnoides petrubanarescui* (Bogutskaya and Coad, 2009), *Alburnus atropatenae* (Berg, 1925), *Petroleuciscus ulanus* (Günther, 1899) and *Romanogobio persus* (Günther, 1899). Recently, an additional endemic species (*Oxynoemacheilus elsae*) has been described from the Zarineh-Simineh, Sofi and Mahabad rivers draining to Lake Urmia (Eagderi et al., 2018). On the other hand, eleven exotic species inhabit the Lake basin: *Carassius auratus* (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782), *Ctenopharyngodon idella* (Valenciennes, 1844), *Cyprinus carpio* Linnaeus, 1758, *Hemiculter leucisculus* (Basilewsky, 1855), *Hypophthalmichthys molitrix* (Valenciennes, 1844), *Pseudorasbora parva* (Temminck & Schlegel, 1846), *Oncorhynchus mykiss* (Walbaum, 1792), *Gambusia holbrooki* (Girard, 1859), *Sander lucioperca* (Linnaeus, 1758), *Rhinogobius similis* (Gill, 1859). There are thirteen permanent rivers and many small springs in Lake Urmia basin, all of them within the borders of Iran (Eimanifar and Mohebbi, 2007; Stevens et al., 2012) except one small stream, which has its upper parts in Turkey. This drainage of Lake Urmia (headwater of Nazli-chay River) originates from the Mor Mountain (about 25 km inside from the Esendere customs) which is located near Kısıklı village, Turkey. The second stream source from eastern Yüksekova drains to Nazli-chay River in Iran. The presence of these streams in Turkish boundaries had never been mentioned by the researchers who conducted taxonomic studies in the upper Great Zap River which is geographically very close to the area (Kaya et al., 2016; Kelle, 1978; Kuru, 1975). Ghasemi et al. (2015) recognized four species in Nazli-chay River: *Alburnus atropatenae, Capoeta capoeta*, *Barbus cyri* and *Oxynoemacheilus brandtii*. Here, I have attempted to determine the fish species inhabit this stream, because of the possibility to occurrences of potential native fish records for Turkish freshwaters.

MATERIALS AND METHODS

This survey was conducted on streams Esendere (headwater of Nazli-chay River) and Onbaşılar (a drainage of Nazli-chay River). Esendere Stream is about 25km in Turkey. After leave the Turkish boundaries, it flows about 60 km to the east towards the Lake Urmia. Onbaşılar Stream source from 20 km east of Yüksekova and it is about 15 km Turkey.

Fish samples were caught with pulsed DC electro-fishing equipment at five sampling sites in September 2019 in Esendere and Onbaşılar streams, southeast of Anatolia (Table 1; Figure 1). After anaesthesia using MS-222, the collected materials were fixed in 5% formaldehyde solution and transferred to the laboratory for morphological investigation. Bogutskaya and Coad (2009), Eagderi et al. (2018) and Khaefi et al. (2017) were followed to identify the fishes. The map (Figure 1) was created using the Qgis v. 2.6.1-Brighton software.

Abbreviations: SL: standard length; FFR: Zoology Museum of the Faculty of Fisheries, Recep Tayyip Erdogan University, Rize, Turkey.

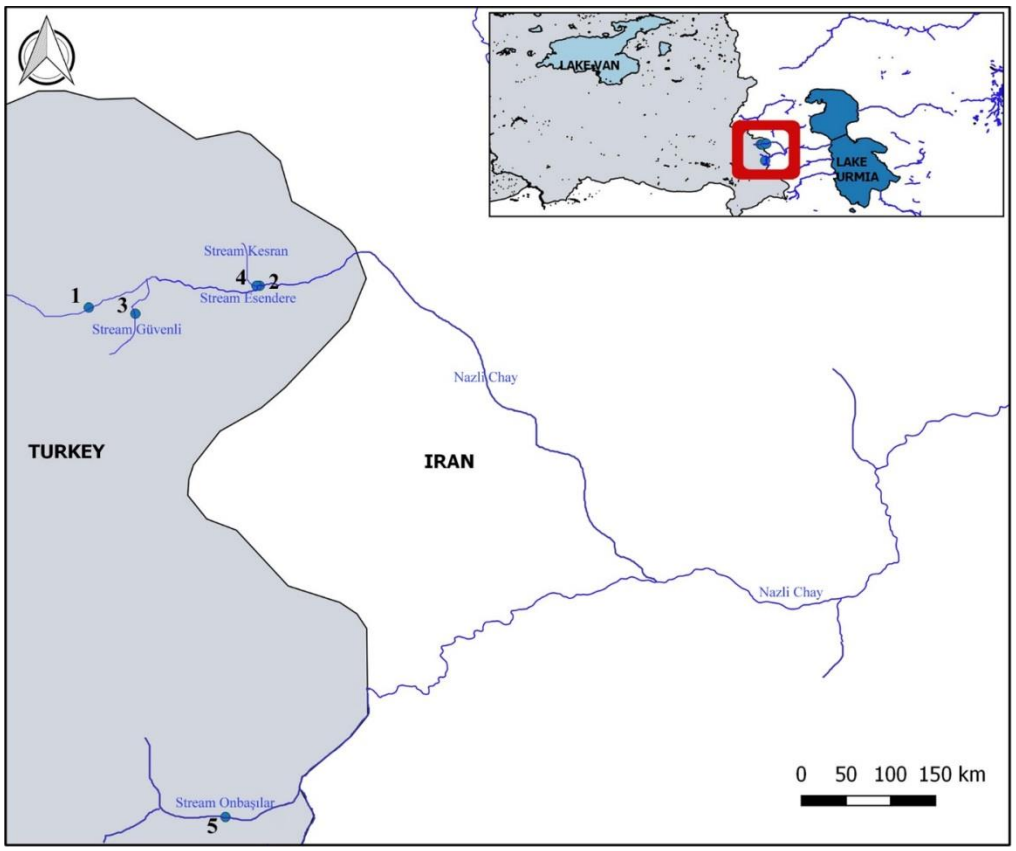

Figure 1. Sampling sites in the survey area

Table 1. Sampling sites in the area and the species obtained during the survey

	Stream	Coordinates	Species
1	Esendere (upper part)	37.707891N 44.549040E	*Barbus cyri*, *Alburnoides petrubanarescui*
2	Esendere (lower part)	37.714213N 44.604656E	*Barbus cyri*, *Alburnoides petrubanarescui*
3	Güvenli	37.705966N 44.564413E	*Barbus cyri*, *Alburnoides petrubanarescui*
4	Kesran	37.714035N 44.603367E	*Oxynoemacheilus elsae*
5	Onbaşılar	37.544105N 44.593580E	*Alburnus atropatenae, Barbus cyri*, *Alburnoides petrubanarescui*

RESULTS

In the present study, four species were found in the Esendere and Onbaşılar streams. Three of them new record for fish fauna of Turkey (*Alburnoides petrubanarescui, Alburnus atropatenae* and *Oxynoemacheilus elsae*). Fourth species is *Barbus cyri* which occurs also in Turkish Kura-Aras drainages (Kaya et al. 2020).

***Alburnoides petrubanarescui* Bogutskaya & Coad, 2009** (Figure 2)

Figure 2. *Alburnoides petrubanarescui,* FFR 7016, 73 mm SL, Esendere Stream

Common name. Urmia spirlin
Type locality. Qasemlou Chay, Urmia Lake basin
Distribution. The species is known from only in Lake Urmia basin. Here, the species was found in Esendere and Onbaşılar streams.

***Alburnus atropatenae* Berg, 1925** (Figure 3)

Figure 3. *Alburnus atropatenae,* FFR 8814, 125 mm SL, Onbaşılar Stream

Common name. Urmia bleak
Type locality. Qasemlou Chay, Urmia Lake basin
Distribution. The species is known from only in Lake Urmia basin. Here, the species was found in Onbaşılar Stream.

***Oxynoemacheilus elsae* Eagderi, Jalili & Çiçek, 2018** (Figure 4)

Figure 4. *Oxynoemacheilus elsae,* FFR 15536, 53 mm SL, Kesran Stream

Common names. Urmia loach

Type locality. Zarineh River, near Shahin-Dej city, Urmia Lake basin
Distribution. *Oxynoemacheilus elsae* described from Zarineh-Simineh, Sofi and Mahabad rivers drainages, Lake Urmia basin, Iran. Here, the species was found in Kesran Stream where is the draining to Esendere Stream (Figures 1 and 5).

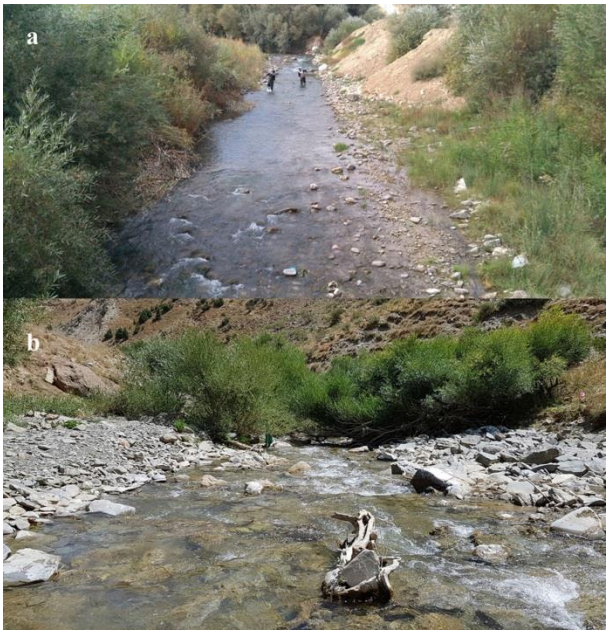

Figure 5. View of sampling sites; a, Esendere Stream; b, Onbaşılar Stream

DISCUSSION

Before the visit the Esendere and Onbaşılar streams I had expected to found more fish species in the area. Despite the intensive sampling of the appropriate habitats of the *Oxynoemacheilus elsae*, only one individual of the species was found. In Esendere Stream, which has a generally shallow and fast flowing structure, no *Alburnus atropatenae* was found. In Onbaşılar Stream, six adult samples of the species was found where the stream is deeper and flowing slower. Many *B. cyri* and *A. petrubanarescui* juveniles were observed in both Esendere and Onbaşılar drainages. Adult specimens of both species were found in Onbaşılar Stream. Probably, Esendere Stream mostly is preferring for spawning for both *B. cyri* and *A. petrubanarescui*. On the other hand, it was not found other species such as *Capoeta capoeta* and *Oxynoemacheilus bergianus* both of which are very widespread in the Lake Urmia drainages in Iran.

A. petrubanarescui, A. atropatenae and *O. elsae* were listed in Endemic fishes of Iran (Eagderi et al., 2018). This study provided an evidence the presence of these three species in Turkey. In this case, these species should be excluded from endemic fishes of Iran. None of these species have not been yet evaluated against IUCN criteria, therefore

their current statues are "Not Evaluated". I strongly recommend that the conservation status of endemic fish species of Urmia Basin should be assessed against IUCN criteria. Because, day by day the problems growing and the lake is going to die. Fortunately, neither during the survey in this study nor by Ghasemi et al. (2015) any exotic species were found in Nazli-chay River and its drainages. However, as mentioned above, eleven exotic species inhabit the Lake basin. Some species among these, such as *Carassius gibelio* and *Pseudorasbora parva*, have a high invasiveness potential and threat on native species. Therefore, all endemic fishes of Lake Urmia seem in threat.

The type specimens of *Alburnoides petrubanarescui* collected by V.D. Vladykov in 1962 from Qasemlou Chay (37°21′N, 45°09′E), Urmia basin (Bogutskaya and Coad, 2009). The species has not been found again after it was described by Bogutskaya and Coad (2009), despite its type locality and other drainages of the Lake Urmia have been searched several times. Even Iranian researchers speculate that may the species have never been there and it was described by the materials mislabelled (Jörg Freyhof, pers. comm., 2019). Recently, Jouladeh-Roudbar et al. (2020) have confirmed that the species cannot be found in the area even though extensive effort sampling the type locality and adjacent area by many researchers. Even, they emphasised the species possibly extinct and encouraged further surveys in the area. Fortunately, with the results of this study, the presence of the species in Lake Urmia basin has been confirmed. The threats on *A. petrubanarescui* populations seem greater. Populations of *A. petrubanarescui* may be restricted with Turkish part, and probably it is absent or very restricted in Iranian part.

Recently, a new barbel, *Barbus urmianus*, has been described in Mahabad-Chai River (36°29'55.14"N 45°33'54.26"E) a southern drainage of Lake Urmia (Eagderi et al., 2019). The *Barbus* specimens collected in Esendere and Onbaşılar streams in this study, morphologically identical with *B. cyri*. Therefore, these barbel population were acknowledged as *B. cyri*.

ACKNOWLEDGEMENTS

I would like to thank Cevdet Kaya and Safter Demir (Bitlis) for their help in the fieldwork, Esra Bayçelebi (Rize) for her help in laboratory and Hazel Baytaşoğlu (Rize) for producing the map. Many tanks to Jörg Freyhof (Berlin) and Davut Turan (Rize) for their remarkable comments to the earlier version of the manuscript. I also would like to thank to Ayşe Demirbaş and Utku Avcı (Rize) for improving the language of the manuscript, and Baran Yoğurtçuoğlu (Ankara) editing the pictures.

Fish collections were approved and granted by the Ministry of Food, Agriculture and Livestock, General Directorate of Fisheries and Aquaculture (codes for the protocols: 67852565-140.03.03-E.4052273 and 76000869-804.01-00000919222). All applicable international, national or institutional guidelines for the care and use of animals were followed.

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Ege Journal of Fisheries and Aquatic Sciences, 37(4), 329-334 (2020)
Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.02
ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Pullu sazan (*Cyprinus carpio* L)'da paraoksonaz ve arilesteraz enzim aktivitelerine curcuminin etkisi

The effect of curcumin on paraoxonase and arylesterase enzyme activities in scaly carp (*Cyprinus carpio* L)

Selman Akoğul[1] ● Serpil Mişe Yonar[2]*

[1] Diyarbakır Ergani Tarım ve Orman Müdürlüğü, Ergani, Diyarbakır
[2] Fırat Üniversitesi Su Ürünleri Fakültesi, 23119, Elâzığ

https://orcid.org/0000-0003-4524-6437
https://orcid.org/0000-0003-2736-5731

Corresponding author: serpilmise@gmail.com
Received date: 25.12.2019 *Accepted date: 16.05.2020*

How to cite this paper:
Akoğul, S. & Mişe Yonar, S. (2020). The effect of curcumin on paraoxonase and arylesterase enzyme activities in scaly carp (*Cyprinus carpio* L). *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 329-334. DOI: 10.12714/egejfas.37.4.02

Öz: Bu araştırmada; pullu sazanda (*Cyprinus carpio*) paraoksonaz ve arilesteraz enzim aktivitelerine curcuminin etkisi incelenmiştir. Bu amaçla curcumin 10, 20 ve 40 mg/kg yem dozlarında 21 gün süreyle balık yemlerinde verilmiştir. Bu periyodun sonunda balıklardan serum, karaciğer ve böbrek örnekleri alınmış ve paraoksonaz ve arilesteraz enzim aktivitelerindeki değişimler araştırılmıştır.

Curcumin uygulanan grupların serum ve karaciğer paraoksonaz ve arilesteraz enzim aktiviteleri kontrol grubuna göre istatistiksel olarak önemli düzeyde artmıştır. Böbrek paraoksonaz ve arilesteraz enzim aktivitelerinde belirlenen artış ise istatistiksel olarak önemsiz bulunmuştur.

Anahtar kelimeler: Arilesteraz, Balık, Curcumin, *Cyprinus carpio*, Enzim, Paraoksonaz

Abstract: In this study, the effect of curcumin on paraoxonase and arylesterase enzyme activities in scaly carp (*Cyprinus carpio*) were examined. For this purpose, curcumin was added into the feed during 21 days with 10, 20 and 40 mg/kg doses. At the end of this period, serum, liver and kidney samples were taken from the fish and changes in paraoxonase and arylesterase enzyme activities were investigated.

The serum and liver paraoxonase and arylesterase enzyme activities of the curcumin treated groups showed significant increases compared to the control group. The increase in kidney paraoxonase and arylesterase enzyme activities was statistically insignificant.

Keywords: Arylesterase, Fish, Curcumin, *Cyprinus carpio*, Enzyme, Paraoxonase

GİRİŞ

Balıklarda görülen hastalıkların tedavisinde kemoterapötik maddeler kullanılmaktadır. Ancak balıkların karaciğer, böbrek, bağırsak, deri gibi organlarına zarar vermesi, kas dokusunda birikerek insanlara geçmesi, bakterilerin kemoterapötik ilaçlara direnç kazanması, su zeminine çökerek sedimentasyon oluşturması, bağışıklık sistemini olumsuz yönde etkilemesi, etkisinin kısa süreli olması, oksidatif strese neden olması ve antioksidan mekanizmayı baskılaması, tüm enfeksiyonlara karşı etkili olmaması kemoterapötik ilaçların kullanımını sınırlandırmaktadır (Arda vd., 2005; Sağlam ve Yonar, 2009). Bu nedenle enfeksiyöz hastalıkların kimyasal maddeler kullanılarak kontrol altına alınmasında önemli problemlerle karşılaşılmaktadır. Son zamanlarda hastalığın çıkmasını engelleyecek korunma önlemlerinin alınması, aşılama, doğal ya da sentetik immunostimulanlar ile balıkların direncini azaltarak hastalıkların oluşumuna sebep olan stres faktörlerine karşı antioksidanların kullanılabilirliği konusu oldukça önem kazanmıştır. Diğer taraftan su kalitesi kriterlerindeki değişim, yem kalitesinin düşük olması, aşırı stoklama, havuz temizliğine yeterince önem verilmemesi, gerekli hijyen koşullarına dikkat edilmemesi gibi yetiştiricilik koşullarının zaman zaman yetersizliği balıklarda strese neden olmaktadır. Bu da bağışıklık sisteminin etkinliğini azaltabilmektedir (Karaca vd., 2014). Bu nedenlerden dolayı hastalık oluşmadan alınacak önlemler büyük önem taşımaktadır.

Bu önlemlerin alınmasında immunostimulan ve antioksidanların kullanılması önemli bir yer tutmaktadır. Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6 heptadiene-3,5-dione]; *Zingiberaceae* (Zencefilgiller) familyasına ait *Curcuma longa* (Turmerik, Zerdeçal, Zerdeçöp) bitkisinin rizomlarında bulunan, sarı-turuncu renkli biyoaktif bir maddedir. Uzak doğu ülkelerinde özellikle de Çin ve Hindistan' da yaygın olarak bulunan ve kullanılan *Curcuma longa* bitkisinin köklerinden elde edilen turmerik bir diğer ifadeyle zerdeçal, toprak altında yumrularıyla bir metreyi geçecek kadar büyüyen çok yıllık (yaşam süresi iki yıldan fazla olan) bir bitkidir. Bu bölgelerde baharat, gıdalarda bozulmayı önleyici ve boya maddesi olarak tercih edilmektedirler. Kullanımı oldukça eskiye dayanan zerdeçaldan geleneksel tedavide kullanılan bir ilaç olarak safra bozuklukları, anoreksiya, öksürük, diyabetik yaralar, karaciğer bozuklukları, romatizma ve sinüzit gibi farklı

hastalıkların iyileştirilmesinde de faydalanıldığı bildirilmiştir (Jagetia ve Aggarwal 2007; Chattopadhyay vd., 2004; Maheshwari vd., 2006). Curcumin halen kozmetik ve ilaçlarda olduğu kadar baharat, köri (hint baharatı), hardal, patetes cipsleri gibi çok sayıda gıdada renk verici ajan olarak yaygın bir şekilde kullanım alanına sahiptir (Joe vd., 2004; Okada vd., 2001). Curcuminin kimyasal yapısı incelendiğinde benzen halkaları üzerinde fenolik ve metoksi grupları bulunduğu, yine β pozisyonunda bağlı 2 keton grubu içerdiği görülmektedir. Curcuminin bu yapısı kendisine antioksidan özellik kazandırmaktadır (İşitez, 2014). Bu özelliğinin yanı sıra son yıllardaki çalışmalarla curcuminin birçok farklı farmakolojik aktivite gösterdiği belirlenmiştir. Antikanserojen, antiinflamatuar, antitümor ve antioksidan özeliklere sahip (Huminiecki vd., 2017) curcuminin, kemoproventif, antiproliferatif, nöroprotektif, antimutajenik ve antimikrobiyal gibi önemli aktiviteler gösterdiği de belirtilmiştir (da Silva vd., 2018). Curcuminin önemli bir hormon düzenleyicisi olduğu, kardiovasküler hastalıkların yanı sıra aterosikleroz ve otoimmun hastalıkları engellediği ifade edilmiştir (Huminiecki vd., 2017).

Abraham Mazur tarafından ilk olarak 1946 yılında keşfedilen paraoksonaz enziminin hem paraoksonaz hem de arilesteraz enzim aktivitesi gösterdiği belirlenmiştir (Mazur, 1946; Mackness vd., 1987). Üç formdan oluşan (paraoksonaz 1, paraoksonaz 2, paraoksonaz 3) paraoksonaz/ arilesteraz enzim ailesinin son yıllardaki çalışmalarda yüksek bir antioksidan aktiviteye sahip olduğu, yüksek dansiteli lipoprotein (HDL) ile düşük dansiteli lipoproteini (LDL) ve makrofajları oksidasyondan koruyarak antioksidan aktivite gösterdiği ifade edilmiştir (Gan, 1991; Li vd., 1993; Mackness vd., 1996; Mackness vd., 1997; Azarsız ve Sönmez, 2000; Gürsu ve Özdin, 2002).

Diğer taraftan curcuminin balıklar üzerindeki antioksidan etkisi, farklı parametreler kullanılarak farklı çalışmalarda ortaya konulmasına rağmen paraoksonaz ve arilesteraz enzim aktivitesine etkisini araştıran herhangi bir çalışmaya rastlanılmamıştır. Paraoksanaz ve arilesteraz enzimlerinin aktivitesi curcumin kullanılarak arttırılabilir ve bu sayede balıklar stres faktörlerine karşı daha güçlü hale getirilerek hastalıklara karşı daha dayanıklı bireyler elde edilebilir. Bu çalışmada paraoksonaz ve arilesteraz enzim aktivitelerindeki değişimler incelenerek curcuminin pullu sazan (*Cyprinus carpio*)'daki muhtemel antioksidan etkilerinin ortaya çıkarılması amaçlanmıştır.

MATERYAL VE METOT

Çalışmada kullanılan ve ortalama ağırlığı 50 ± 10 g olan pullu sazanlar (*Cyprinus carpio*) DSİ IX. Bölge Müdürlüğü Keban Su Ürünleri Şube Müdürlüğü'nden canlı olarak temin edildi ve Fırat Üniversitesi Su Ürünleri Fakültesi' ne getirildi.

Çalışmada 33 x 100 x 60 cm ebatlarındaki 12 farklı cam akvaryum kullanıldı. Çalışmaya başlamadan önce dezenfekte edilen akvaryumların üstü balıkların kaçmasını önlemek için balık ağı kullanılarak örtüldü. Akvaryumlar hava kompresörü yardımıyla sürekli havalandırıldı. Canlı olarak getirilen ve sağlıklı olup olmadıklarını belirlemek için makroskobik analizleri yapılan balıklar 33 x 100 x 60 cm ebatlarındaki ve ayarlanabilen termostatlı ısıtıcılarla su sıcaklığı 23 ± 1°C'ye ayarlanmış 12 farklı cam akvaryumun her birinde 10 adet olacak şekilde yerleştirildi. Balıklar on beş gün süreyle ortama adapte edildi. Adaptasyon sırasında günde iki kez olmak üzere balıklara alabildikleri kadar ticari balık yemi verildi. 3 tekrarlı olarak yürütülen çalışmada her bir tekrar için 40 toplamda 120 balık kullanıldı.

Araştırmada, Sigma-Aldrich' den temin edilen curcumin (*Curcuma longa*; katalog no: C1386; kimyasal formülü: (E,E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Diferuloylmethane, Diferulylmethane, Natural Yellow 3**)** kullanıldı. Curcumin 10, 20 ve 40 mg/kg yem düzeyinde özel bir firmadan temin edilerek toz haline getirilmiş pelet yemlerle karıştırıldı. Çeşme suyu yardımıyla hamur haline dönüştürülen yem kıyma makinesinden geçirildi ve böylece tekrar pelet haline getirildi. Pelet haline getirilen yemler tepsilere bırakıldı ve yem fırınında kurutuldu. Kuruyan yemler kullanılana kadar koyu renkteki cam şişeler içinde 4°C'de saklandı. Balıklara uygulanan curcuminin oranları Mişe Yonar vd. (2013) ve Mişe Yonar vd. (2014)'e göre seçildi. Araştırma Fırat Üniversitesi Hayvan Deneyleri Etik Kurulu Başkanlığı'nca onaylandı (Protokol No: 2018/20).

Adaptasyonun sağlanmasından sonra balıklar aşağıdaki gibi biri kontrol üçü deneme olmak üzere dört grubu ayrıldı.

K: Curcumin içermeyen yem verilen grup (kontrol grubu)

CUR-10: 10 mg/kg yem dozunda curcuminin 21 gün süreyle verildiği grup,

CUR-20: 20 mg/kg yem dozunda curcuminin 21 gün süreyle verildiği grup,

CUR-40: 40 mg/kg yem dozunda curcuminin 21 gün süreyle verildiği grup.

Curcuminin 21 gün süreyle uygulanmasından sonra 22. günde her bir akvaryumdaki 10 balık (her bir grup için örnek sayısı 30) benzokainin 25 mg/L'lik konsantrasyonuyla anestezi edildi (San ve Yonar, 2017). Serum ve doku örneklerinin alınması için öncelikle anestezi edilen balıkların kavdal venasından kan örnekleri antikoagülant içermeyen jelli tüplere dolduruldu. Kan örneklerinin 3500rpm'de 10 dakika santrifüj edilmesiyle serumlar çıkarıldı.

Kan örneklerinin alınmasının ardından balıklar tekrar makroskobik olarak muayene edildi. Makroskobik muayeneyi takiben usulüne uygun şekilde otopsi edilen (Arda vd., 2005) balıklardan karaciğer ve böbrek örnekleri ayrılarak çıkarıldı. Karaciğer ve böbrekten homojenat hazırlamak için bu dokular 0,5 gram ağırlığında tartıldıktan sonra %1,15'lik potasyum klorür (KCl) ile 1/10 oranında dilüe edilerek homojenizatör yardımıyla ezilip homojenize edildi. Homojenatların 10 dakika boyunca +4 ºC'de, 3200 rpm' deki santrifüjünü takiben çıkarılan süpernatantlar enzim aktivitelerini ölçmek için kullanıldı (Mişe Yonar vd., 2014).

Serum ile karaciğer ve böbrek homojenatlarında paraoksonaz ve arilesteraz enzim aktivitesi spektrofotometrik olarak ölçüldü. 850 µl Tris-HCl tamponu (100 mM, pH:8) içerisine 100 µl substrat çözeltisi (2 mM paraokson + 2mM koenzim $CaCl_2$) ile 100 µl örnek eklenerek 37 ˚C'de absorbansta 1 dakikada oluşan değişim 412 nm'de okundu. Böylelikle paraoksanın p-nitrofenole enzimatik dönüşüm hızı belirlenerek paraoksonaz enzim aktivitesi ölçüldü. Aynı prensiple arilesteraz enzim aktivitesi de belirlendi fakat substrat olarak fenilasetat kullanıldı (Dubravka vd., 2001). Enzim aktivitelerinin hesaplanması için hazırlanılan standart grafikler kullanıldı.

İstatistiksel analizler için SPSS 21.0 istatistik paket programı kullanıldı. Kontrol ve deneme grubu balıklarının serum, karaciğer ve böbreğindeki paraoksonaz ve arilesteraz enzim aktivitelerinde belirlenen değişimler tek yönlü varyans analizi (ONEWAY – ANOVA) ile test edildi. Gruplar arasındaki farklılığın tespitinde ise Tukey testinden yararlanıldı (Sümbüloğlu, 1998; Kocaçalışkan ve Bingöl, 2008; Kalaycı, 2010).

BULGULAR

Adaptasyon ve deneme süresince balıkların yem alımlarında herhangi bir problem yaşanmazken balıklarda herhangi bir ölüm de gerçekleşmedi. Çalışmaya başlamadan önce makroskobik olarak muayene edilen ve yine kan alımını takiben otopsi edilen balıkların klinik muayenesinde herhangi bir bulguyla karşılaşılmadı.

Araştırma süresince sıcaklık, oksijen düzeyi ve pH'da önemli değişiklikler oluşmadı. Bu değerler sırasıyla 23±1 ˚C, 7,2±0,2 ve 8,15±0,13 mg/L olarak belirlendi.

Farklı oranlarda curcumin verilen CUR-10, CUR-20 ve CUR-40 deneme gruplarının serum, karaciğer ve böbreğindeki paraoksonaz enzim aktivitesinde kontrol grubuna göre belirlenen değişimler Tablo 1' de gösterilmiştir.

Curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme gruplarında serum paraoksonaz enzim aktivitesinin kontrol grubuna göre istatistiksel olarak önemli düzeyde arttığı görüldü ($p<0.05$). Bu artış kontrol grubuna göre CUR-10, CUR-20 ve CUR-40 deneme gruplarında sırasıyla %13,80, %24,38 ve %29,39 olarak gerçekleşti. Curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme grupları kendi içinde karşılaştırıldığında ise CUR-20 ve CUR-40 gruplarının serum paraoksonaz enzim aktivitesinin CUR-10 grubundan farklı olduğu ($p<0.05$), CUR-40 grubunun serum paraoksonaz enzim aktivitesinin CUR-20 grubundan herhangi bir farklılık göstermediği belirlendi ($p>0.05$).

Tablo 1. Kontrol grubu ile curcumin uygulanan grupların serum, karaciğer ve böbreğindeki paraoksonaz enzim aktivitesi (ortalama ± standart hata)
Table 1. Paraoxonase enzyme activity in serum, liver and kidney of control group and curcumin treated groups (mean ± standard error)

Doku	K	CUR-10	CUR-20	CUR-40
Serum (U/mL)	33,75±4,07^{a}	38,41±5,22 ^{b}	41,98±4,79^{c}	43,67 ± 3,92^{c}
Karaciğer (U/g)	36,86±5,21^{a}	44,48±4,73^{b}	49,20±4,11^{c}	53,76 ± 4,62 ^{d}
Böbrek (U/g)	23,04±3,16^{a}	23,47±2,10^{a}	23,88±3,02^{a}	23,95 ± 2,80^{a}

^{a,b,c,d}: Aynı satırda yer alan farklı harfler taşıyan değerler arasındaki fark istatistiksel olarak önemli bulunmuştur ($p<0.05$)

Kontrol grubuna göre farklı dozlarda curcuminin verildiği CUR-10, CUR-20 ve CUR-40 deneme gruplarında karaciğer paraoksonaz enzim aktivitesinin istatistiksel olarak önemli düzeyde arttığı belirlendi ($p<0.05$). Bu artış kontrol grubuna göre CUR-10, CUR-20 ve CUR-40 deneme gruplarında sırasıyla %20,67, %33,47 ve %45,84 olarak tespit edildi. Curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme grupları kendi içinde karşılaştırıldığında ise her üç grubun da karaciğer paraoksonaz enzim aktivitesinin birbirinden farklı olduğu görüldü ($p<0.05$).

Farklı oranlarda curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme gruplarının böbrek paraoksonaz enzim aktivitesinde kontrol grubuna göre istatistiksel olarak önemli olmayan bir artış görüldü ($p>0.05$). Bu artış kontrol grubuna göre CUR-10, CUR-20 ve CUR-40 deneme gruplarında sırasıyla %1,86, %3,64 ve %3,94 olarak gerçekleşti. Yine curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme grupları kendi içinde karşılaştırıldığında her üç grubun böbrek paraoksonaz enzim aktivitesinin de birbirinden farklı olmadığı saptandı ($p>0.05$).

Farklı oranlarda curcumin verilen CUR-10, CUR-20 ve CUR-40 deneme gruplarının serum, karaciğer ve böbreğindeki arilesteraz enzim aktivitesinde kontrol grubuna göre belirlenen değişimler Tablo 2'de gösterilmiştir.

Curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme gruplarında serum arilesteraz enzim aktivitesinin kontrol grubuna göre istatistiksel olarak önemli düzeyde arttığı görüldü ($p<0.05$). Bu artış kontrol grubuna göre CUR-10, CUR-20 ve CUR-40 deneme gruplarında sırasıyla %10,78, %21,83 ve %24,12 olarak saptandı. Curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme grupları kendi içinde karşılaştırıldığında ise CUR-20 ve CUR-40 gruplarının serum arilesteraz enzim aktivitesinin CUR-10 grubundan farklı olduğu ($p<0.05$), CUR-40 grubunun serum arilesteraz enzim aktivitesinin CUR-20 grubundan herhangi bir farklılık göstermediği belirlendi ($p>0.05$).

Tablo 2. Kontrol grubu ile curcumin uygulanan grupların serum, karaciğer ve böbreğindeki arilesteraz enzim aktivitesi (ortalama ± standart hata)
Table 2. Arylesterase enzyme activity in serum, liver and kidney of control group and curcumin treated groups (mean ± standard error)

Doku	K	CUR-10	CUR-20	CUR-40
Serum (U/mL)	122,49±11,30[a]	135,70±14,83[b]	149,23±17,21[c]	152,04±16,09[c]
Karaciğer (U/g)	147,33±15,75[a]	164,43±18,13[b]	185,47±20,58[c]	182,68±14,92[c]
Böbrek (U/g)	96,22±12,01[a]	98,28±10,05[a]	99,01±14,62[a]	98,36±10,29[a]

[a,b,c,d]: Aynı satırda yer alan farklı harfler taşıyan değerler arasındaki fark istatistiksel olarak önemli bulunmuştur ($p < 0.05$)

Kontrol grubuna göre farklı dozlarda curcuminin verildiği CUR-10, CUR-20 ve CUR-40 deneme gruplarında karaciğer arilesteraz enzim aktivitesinin istatistiksel olarak önemli düzeyde arttığı belirlendi ($p<0.05$). Bu artış kontrol grubuna göre CUR-10, CUR-20 ve CUR-40 deneme gruplarında sırasıyla %11,60, %25,88 ve %23,99 olarak tespit edildi. Curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme grupları kendi içinde karşılaştırıldığında ise CUR-20 ve CUR-40 gruplarının karaciğer arilesteraz enzim aktivitesinin CUR-10 grubundan farklı olduğu ($p<0.05$), CUR-40 grubunun karaciğer arilesteraz aktivitesinin CUR-20 grubundan herhangi bir farklılık göstermediği belirlendi ($p>0.05$).

Farklı oranlarda curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme gruplarının böbrek arilesteraz enzim aktivitesinde kontrol grubuna göre istatistiksel olarak önemli olmayan bir artış görüldü ($p>0.05$). Bu artış kontrol grubuna göre CUR-10, CUR-20 ve CUR-40 deneme gruplarında sırasıyla %2.14, %2,89 ve %2,22 olarak gerçekleşti. Yine curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme grupları kendi içinde karşılaştırıldığında her üç grubun böbrek arilesteraz enzim aktivitesinin de birbirinden farklı olmadığı saptandı ($p>0.05$).

TARTIŞMA

Araştırma boyunca kontrol ve farklı oranlarda curcumin verilen deneme grubu balıklarında ölüm olayıyla karşılaşılmamıştır. Balıkların curcumin içeren yemleri aldıkları görülmüştür. Çalışma öncesindeki adaptasyon sırasında ve curcuminin uygulandığı 21 günlük süre zarfında hem kontrol hem de deneme grubu balıklarında yapılan makroskobik muayene sonucunda klinik herhangi bir bulguya rastlanılmamıştır. Yine her iki süre zarfında bu balıklar rutin davranışlar göstermiştir. Bu bulgular 21 gün için uygulanan farklı dozlardaki curcuminin balıklarda herhangi bir olumsuz etki göstermediğini, güvenilir bir şekilde belirtilen doz ve sürelerde balıklara verilebileceğini açığa çıkarmıştır.

Hem yetiştiriciliğinin yapılması hem de doğal ortamda geniş dağılım göstermesi nedeniyle ülkemiz için önemli bir balık türü olan pullu sazan laboratuvar ortamına kolayca uyum gösterebilmektedir. Bu türün değişik ortamlara kolayca uyum sağlaması, beslenme ve yetiştirilmesindeki kolaylık, doğal sulardan bol ve kolayca elde edilebilmeleri ve ayrıca ekonomik değerlerinin yüksekliği gibi önemli özellikleri yüzünden akuatik ve toksikolojik çalışmalarda oldukça fazla tercih edilmektedir. Bu araştırmada da paraoksonaz ve arilesteraz enzim aktivitelerindeki değişimlerin incelenmesiyle elde edilen sonuçlar kullanılarak curcuminin antioksidan etkisinin ortaya çıkarılması için *Cyprinidae* familyasına ait pullu sazan (*Cyprinus carpio*) kullanılmıştır.

Curcuminin farklı dozlarının uygulandığı bu tez çalışmasında paraoksonaz ve arilesteraz enzim aktivitesinin belirlenmesinde serum, karaciğer ve böbrek kullanılmıştır. Balıklarda immunostimulan ve antioksidan karaktere sahip maddelerin etkilerinin belirlenmesi amacıyla en fazla tercih edilen organlar kan ve karaciğerdir (Çağdaş vd., 2017; Yonar vd., 2019). Çünkü diğer omurgalılarda olduğu gibi balıklarda da primer metabolik organ karaciğerdir. Diğer taraftan karaciğerde meydana gelen aktivitelerdeki değişimlerin yansıması kan dokusunda da görülebilmektedir (Percin ve Konyalioglu, 2008). Ayrıca paraoksonaz enzimi memelilerde karaciğerde sentezlenmekte, HDL' ye bağlı olarak serumda bulunmaktadır. Açıklanan bu nedenlerden dolayı bu çalışmada da curcumin uygulandıktan sonra paraoksonaz ve arilesteraz enzim aktivitesinde oluşan değişimler serum ile karaciğer ve böbrekte incelenmiştir.

Balıklarda paraoksonaz ve arilesteraz enzimi aktiviteleri ile ilgili çalışmalar son yıllarda bir hayli artmıştır. Folly vd. (2001) paraoksonaz enzim aktivitesinin varlığını ve bu enziminin HDL ile ilişkisini *Piaractus mesopotamicus* türü balıklarda, Bastos vd. (2004) ise *Hypostomus punctatus*, *Piaractus mesopotamicus*, *Brycon cephalus* ve *Salminus brasiliensis* türü neotropikal balıklarda paraoksonaz enzim aktivitesinin varlığını göstermişlerdir. Doğadan yakalananlara göre kültür şartlarında yetiştirilen gökkuşağı alabalığında (*Oncorhynchus mykiss*) (Karataş ve Kocaman, 2012) ve kültür altındaki kaynak alabalığında (*Salvelinus fontinalis*) (Karataş ve Kocaman, 2014) serum paraoksonaz enzim aktivitesi daha yüksek bulunmuştur. Yöntürk ve Yonar (2018) antioksidan özelliğe sahip polenin 21 gün süreyle %1, %2 ve %4 oranında uygulandığı alabalıkların (*Oncorhynchus mykiss*) serum ve karaciğer paraoksonaz ve arilesteraz enzim aktivitesinde istatistiksel herhangi bir farklılık belirleyememişlerdir. Bu çalışmada ise farklı oranlarda curcumin içeren yemlerle beslenen pullu sazanda paraoksonaz ve arilesteraz enzim aktiviteleri serum, karaciğer ve böbrekte araştırılmış, curcumin uygulamasıyla bu aktivitelerin arttığı belirlenmiştir. Ayrıca paraoksonaz ve arilesteraz enzim aktivitesinin dokular arasında farklılık gösterdiği saptanmıştır.

Diğer taraftan balıklar için toksik olan metal ve pestisitlerin paraoksonaz ve arilesteraz enzim aktivitesine etkisini araştıran çalışmalar da yapılmıştır. Bu çalışmalarda

paraoksonaz ve arilesteraz enzim aktivitesindeki değişiklikler incelenerek toksik maddelerin vücutta oluşturduğu negatif etkiler belirlenmeye çalışılmıştır. Bakır, civa, kadmiyum ve kobalt metallerinin sazanlarda (Beyaztaş vd.,2007), (Ni^{2+}), (Cd^{2+}), (Hg^{2+}) ve (Cu^{2+}) metallerinin *Scyliorhinus canicula* balıklarında (Sayın vd., 2012) paraoksonaz enzim aktivitesini inhibe ettiği belirlenmiştir. Çinko sülfat ($ZnSO_4$) formunda 5 ve 10 mg/L konsantrasyonlarındaki çinkonun 10 gün boyunca uygulandığı *Capoeta capoeta*' da plazma paraoksonaz-1 enzim aktivitesinin düştüğü ve bu aktivitenin metallere karşı çok duyarlı olduğu ifade edilmiştir (Deveci vd., 2015). Benzer bir sonuç yine sazanlarda yapılan bir çalışmadan elde edilmiş, 28. gün boyunca 15, 30 ve 60 ppb konsantrasyonlarında uygulanan kromun serum paraoksonaz ve arilesteraz enzim aktivitesini düşürdüğü tespit edilmiştir (Yonar vd., 2012). Gökkuşağı alabalığı (*Oncorhynchus mykiss*)' nda karbosulfanın mutajenik, genotoksik ve enzim inhibitör etkisinin araştırıldığı bir çalışmada, paraoksonaz enzim aktivitesinde istatistiki olarak önemli olmayan bir inhibisyonun gerçekleştiği belirlenmiştir (Altinok vd., 2012). Yapılan başka bir araştırmada ise paraoksonaz ve arilesteraz enzim aktivitelerinin 0,25, 0,5 ve 1 mg/L konsantrasyonundaki malathion uygulamasıyla azaldığı ancak bu aktivitelerin uygulama sonunda kontrol grubuna yaklaştığı görülmüştür (Kılıç ve Yonar, 2017). Bu çalışmada ise 10, 20 ve 40 mg/kg yem oranında curcumin uygulanan CUR-10, CUR-20 ve CUR-40 deneme gruplarında paraoksonaz ve arilesteraz enzim aktivitelerinin arttığı saptanmıştır. Toksik maddelerin aksine antioksidan ve immunostimulan özelliklere sahip curcuminin özellikle serum ve karaciğer paraoksonaz ve arilesteraz enzim aktivitelerinde kontrole göre istatistiksel olarak önemli bir artışa yol açmıştır. Bu sonuca göre curcuminin pullu sazanda herhangi bir toksik etki göstermediği ve stres oluşturmadığı dolayısıyla güvenli bir şekilde kullanılabileceği görülmektedir.

Curcuminin balıklardaki antioksidan etkisi farklı parametreler kullanılarak yapılan çalışmalarda da gösterilmiştir. Mişe Yonar vd. (2014) tarafından gökkuşağı alabalığında yapılan bir çalışmada, kontrol grubuna kıyasla curcuminin 10 mg/kg yem, 20 mg/kg yem ve 40 mg/kg yem oranında uygulandığı grupların karaciğer, böbrek ve dalak dokusunda malondialdehit (MDA) düzeylerinin düştüğü, glutatyon peroksidaz (GSH-Px), glutatyon redüktaz (GR) ve glutatyon S-transferaz (GST) enzim aktiviteleri ile redükte glutatyon (GSH) düzeyinin arttığı ifade edilmiştir. Benzer bir sonuç Manju vd. (2012) tarafından elde edilmiş %0,5 ve 1 düzeyinde yeme katılan curcuminin, 2. hafta sonunda *Anabas testudineus* türü balıklarda karaciğer lipit peroksidasyon düzeyini önemli oranda azalttığı fakat GSH-Px ve GR enzim aktivitelerinde herhangi bir değişime neden olmadığı tespit edilmiştir. Bu çalışmada ise curcumin uygulanan her üç deneme grubunda da paraoksonaz ve arilesteraz enzim aktivitelerinin kontrol grubuna göre istatistiksel olarak önemli düzeyde arttığı, yine curcuminin artan dozuna bağlı olarak paraoksonaz ve arilesteraz enzim aktivitelerindeki artışın daha fazla olduğu belirlenmiştir. Bu sonuç yukarıdaki araştırmacıların bulgularıyla paralel olarak curcuminin antioksidan kapasiteyi arttırmasıyla açıklanabilir. Nitekim deney fareleri ve insanlar üzerinde yapılan çalışmalarda da paraoksonaz enzim aktivitesiyle oksidatif stres arasında karşılıklı bir ilişki olduğu, paraoksonaz enziminin diğer özelliklerinin yanı sıra antioksidan özellik de gösterdiği ifade edilmiştir (Aviram vd., 1999).

Sonuç olarak; curcuminin pullu sazanın farklı dokularında paraoksonaz ve arilesteraz enzim aktivitelerini olumlu yönde etkilediği tespit edilmiştir. Bu sonuç curcuminin balıklarda antioksidan savunmayı güçlendirdiğini buna bağlı olarak da balıklara antioksidan olarak uygulanabileceğini göstermektedir. Ayrıca bu çalışmadan elde edilen sonuçlar antioksidanların etkilerinin veya kullanılabilirliğinin belirlenmesinde paraoksonaz ve arilesteraz enzim aktivitelerinin ölçülmesinin bir biyobelirteç olarak kabul edilebileceğini göstermektedir.

TEŞEKKÜR

Bu çalışma; Yüksek Mühendis Selman AKOĞUL'un yüksek lisans tezinden özetlenmiş ve Bilimsel Araştırma Projeleri (BAP) Yönetim Birimi tarafından SÜF.18.01. Nolu proje ile desteklenmiştir.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 335-342 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.03

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Can the early stage copepoda (Copepodites and Naupliies) abundance play important role on the fatty acid composition of *Sagitta setosa* (Chaetognatha) in the Southeastern Black Sea?

Güneydoğu Karadeniz'de erken aşamadaki kopepod (Kopepoditler ve Naupliler) bolluğu *Sagitta setosa* (Chaetognatha) yağ asiti kompozisyonu üzerinde önemli rol oynayabilir mi?

Nurgül Şen Özdemir[1]* ● Ali Muzaffer Feyzioğlu[2] ● Fatma Caf[3] ● İlknur Yıldız[4]

[1] Department of Veterinary Medicine, Vocational School of Technical Sciences, Bingöl University, 12000, Turkey https://orcid.org/0000-0001-6656-822X
[2] Department of Marine Sciences and Technology Engineering, Sürmene Faculty of Marine Sciences, Karadeniz Technical University, 61530, Trabzon Turkey https://orcid.org/0000-0003-1171-5493
[3] Department of Veterinary Medicine, Vocational School of Technical Sciences, Bingöl University, 12000, Turkey https://orcid.org/0000-0002-0363-4848
[4] Marine Sciences and Technology Institute, Karadeniz Technical University, Trabzon, 61000, Turkey https://orcid.org/0000-0003-2424-8644

Corresponding author: nsozdemir@bingol.edu.tr
Received date: 12.12.2019 *Accepted date: 01.06.2020*

How to cite this paper:
Şen Özdemir, N., Feyzioğlu A.M., Caf, F. & Yıldız, İ. (2020). Can the early stage copepoda (Copepodites and Naupliies) abundance play important role on the fatty acid composition of *Sagitta setosa* (Chaetognatha) in the Southeastern Black Sea? *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 335-342. DOI:10.12714/egejfas.37.4.03

Abstract: In this study, the relationship between the fatty acid composition, abundance of carnivore *Sagitta setosa* (*S. setosa*) and total copepod abundance (copepodites and nauplii) which are the main diets of *S. setosa* was investigated. Sampling was conducted monthly during a year. During the sampling period, *S. setosa* and the total the copepod abundance showed a parallel distribution until November. Average total lipid was at the highest with 3% in spring and autumn, while the lowest was 2% in winter and summer. While DHA/EPA ratio (2.23) as carnivory index in *S. setosa* and the total copepod (copepodites and nauplii) abundance (1375 ind/m^3) were at the highest in winter, but another carnivory index, 18:1ω9/18:1ω7 ratio did not show a parallel increase with copepod abundance. However, 18:1ω9 which increases the carnivory index, is among the main fatty acids in *S. setosa*. This might be indicated that *S. setosa* does not related to predation on the copepods, but it shows that feeding in a carnivorous style. Additionally, the herbivory index ∑ n-3/∑ n-6 ratio was detected in spring and autumn at the highest level. Consequently, although there is no direct effect between the copepod abundance and *S. setosa* fatty acids, it is possible an indirect effect.

Keywords: Carnivory index, fatty acids, *Sagitta setosa*, Southeastern Black Sea

Öz: Bu calışmada karnivor bir tür olan *Sagitta setosa*'nın yağ asiti kompozisyonu ve bolluğu ile üzerinden yoğun bir şekilde beslendigi kopepodların (kopepoditler ve kopepot nauplileri) bolluğu arasındaki ilişki incelenmistir. Örneklemeler bir yıl boyunca aylık periyotlarda yapılmıştır. Örnekleme dönemi boyunca *S. setosa* ve toplam copepod bolluğu, kasım ayına kadar parallel bir dağılım göstermiştir. Ortalama toplam lipit miktarı %3 ile en yüksek ilkbahar ve sonbahar, en düşük ise %2 ile kış ve yaz aylarında belirlenmiştir. *S. setosa*'da karnivor indeksi olarak DHA/EPA oranı (2,23) ve kopepod bolluğu (1375 birey/m^3) en yüksek değerlerini kış döneminde almışlardır. Bu dönemdeki yüksek kopepod bolluğunun *S. setosa*'daki karnivor indeksi yağ asitlerini artırmaya katkı sagladığı söylenebilir. Fakat diğer bir karnivor indeksi olan 18:1ω9/18:1ω7 oranı kopepod bolluğu ile papalel bir artış gostermemiştir. Ancak, karnivor indeksini artıran 18:1ω9 un *S. Setosa'*daki temel yağ asitleri arasında olması, *S. setosa*'nın her zaman kopepodlar üzerinden bir predasyon sergilemese de karnivor beslendiğine kanıt olarak gösterilebilir. Bunun yanında, herbivor indeksi olan ∑ω3/∑ω6 oranı en yüksek ilkbaharda ve sonabaharda belirlenmiştir. Sonuç olarak, kopepod bolluğu ile *S. setosa*'nın yağ asitleri arasında direkt bir etki olmasa da indirekt bir etkinin varlığından bahsedilebilir.

Anahtar kelimeler: Karnivor indeksi, yağ asitleri, *Sagitta setosa*, Güneydoğu Karadeniz

INTRODUCTION

Chaetognaths are important zooplankton predators in marine environments (Reeve, 1980; Feigenbaum and Maris, 1984; Baier and Purcell, 1997). Their main diet consists of copepods (Feigenbaum and Maris, 1984; Feigenbaum, 1991; Duró and Saiz, 2000). On the other hand, they are noteworthily a link between the phytoplankton and many fish species, especially including commercially important fish species (Vinogradov et al., 1992; Kovalev et al., 1998).

Sagitta setosa (*S. setosa*) which is a chaetognath species, is commonly found in the Black Sea Sea (Zenkevitch, 1963; Vinogradov et al., 1990; 1992; Niermann and Greve, 1997). It accumulates more along rim current than shelf areas and in the central gyres of the Black Sea (Niermann et al., 1997). The most crucial factors affecting the growth of *S. setosa* in the Black Sea are temperature and food supply. (Beşiktepe and Ünsal, 2000; Yıldız and

Feyzioğlu, 2014). Additionally, the abundance of *S. setosa* was affected by the existence of appropriate food intensity during the previous month (about a month ago). Therefore, in this study, the purpose was to determine the relation between the seasonal fatty acid composition of *S. setosa*, which is one of the important zooplankton predators in the Black Sea ecosystem, and the seasonal abundance distribution of copepodites and copepod nauplii, which are the main diet of *S. setosa*. It was investigated in a different perspective on which periods/which fatty acids of *S. setosa* have a high level and whether or not there is a relationship between *S. setosa* fatty acids and copepod (copepodites and nauplii) abundance. Because, fatty acids (FA) are among the most important molecules transferred from plant to animal in aquatic food webs. Certain classes of FA, such as the omega-3 (ω3; n-3), highly unsaturated fatty acids (HUFA) which are available in limited quantities and very important for herbivore zooplankton are transferred from phytoplanktonic organisms to upper trophic levels (Müller-Navarra, 1995; Müler-Navarra et al., 2000; Ravet et al., 2003). FA have three characteristics and storage patterns. These special features make them useful tracers of diets and aquatic food webs. First, organisms can establish biosynthesize, modify chain length, and introduce double bonds in FA. However, they are subject to biochemical limitations in these processes depending on the phylogenetic group and even species (Cook, 1996). Second, unlike other dietary nutrients (e.g., proteins and carbohydrates) which digestion is completely degraded, FA are released from the digestive amounts of digested lipid molecules, but are generally not deformed and taken up in basic forms by tissues. The important consequences of these restrictions within plants, bacteria, and animals, and the uptake of intact FA by consumer tissues, is that individual isomers as well as "families" of FA bioaccumulate through food chains, and they can be traced back to specific food web origins. Third, unlike most other nutrients, fat is stored as reservoirs in animal bodies. These often-substantial stores can later be mobilized to provide fuel for short or long-term energy demands (Pond, 1998). Therefore, FA accumulate over time and represent an integration of dietary intake over days, weeks, or months, depending on the organism and its energy intake and storage rates. In addition, it is important to know FA composition of the zooplankton which by taxonomic affiliation, changed by diet and modified by starvations or temperature (Arts et al., 2009).

MATERIALS AND METHODS

Location of expedition and sampling

R/V YAKAMOZ research boat, which belonged to Faculty of Marine Science, KTU was used in samplings. The zooplankton samples were collected monthly from the Southeastern Black Sea Sürmene Bay between March 2012-February 2013. The station was 3 sea miles far (40° 57' 12" N-40° 09' 30" E) from the coast, and has a depth of 400 meters. The Exgolabur 7 GPS was used in determining of the station. The zooplankton samples were collected vertically with Hansen plankton net having 110cm mouth diameter and 200 µm mesh widthr from 130m depth to the surface in determination of the abundance and fatty acid composition (Harris et al., 2000). A conductivity- temperature-depth-oxygen CTD profiler (CTD, General Oceanic Idranaut 316) was used in determination of temperature values.

Determination of abundance for the zooplankton species

The zooplankton samples were preserved in a 4% formaldehyde seawater solution buffered with sodium borate. Countings were done under stereomicroscopes (Olympus BH2 and Nikon) using 4× and 10× objectives and a Bogorov-Rass counting chamber. Quantitative analyses of species were performed by using 3 ml subsamples. Countings were repeated on 4 subsamples (Harris et al., 2000). The copepods (copepodites, copepod nauplii) were identified to species or genus level (Mauchline et al., 1998; Johnson and Allen, 2005). The zooplankton abundance was expressed as the number of the individuals in m^3 (ind/m^3). Zooplankton abundance was performed with monthly intervals, copepodites and nauplii of 5 species from copepoda were detected (*Calanus euxinus, Pseudocalanus elongatus, Acartia clausi, Paracalanus parvus, Centropages ponticus*): 1 species of cyclopoid copepod was detected (*Oithona similis*): and 1 species was detected from Chaetognatha (*S. setosa*).

Total lipid and fatty acid analysis

Lipids were quantitatively extracted from the samples using chloroform/methanol (2:1) (Folch et al., 1957). To determine the fatty acids methyl esters (FAME) is used 2 ml chloroform and 1 ml 0.21 N NaOH in methanol solution were used. Then, 0.5 N acetic acid solution was added. Lower phase was evaporated and 2 ml hexane was added on the lipid and the sample was transferred to a vial (Kates, 1986). The FAME were detected by Shimadzu GC-17 gas chromatograph (GC). Capillary columns with a length of 25 m, inner diameter of 0.25 µm, and with a thickness of 25 µ (Permabond) were used (Macherey-Nagel). Column temperature was set to 120-220 °C, with the increment of 5 °C/min until 200 °C and 4 °C/min to 220 °C. The column was kept for 8 min at 220 °C and the total time was determined as 35 minutes. Injection temperature is set to 240 °C and detector temperature to 280 °C. Nitrogen was used as the carrier gas.

Statistical analysis

STATISTICA 8.0 was used in statistical analysis. The datas were analyzed using analysis of variance (one way ANOVA) method and comparisons among averages were carried out by using Tukey Test. Tukey test created by Post-

hoc, homogenous groups ($p<0.05$). In definitions of the statistical differences, the Spearman Rank Correlation was applied ($p < 0.05$).

RESULTS AND DISCUSSION

Fatty acids have often been used to track energy transfer, as well as to study predator-prey relationships (Falk-Peterson et al., 1990: 2000; Litzow et al., 2006). In this study, the relation between *S. setosa* abundance and fatty acids and the total copepod abundance was examined in the sampling period to reveal the prey-predator relation. A constant increase was observed from Spring to Winter (Spring: 268 ind/m^3, Summer: 398 ind/m^3, Autumn: 814 ind/m^3, Winter: 1375 ind/m^3) in the total copepod (copepod nauplii and copepodite) abundance. While copepod nauplii reached the highest abundance in February (457 ind/m^3), copepodites reached the highest abundance in January (1668 ind/m^3). Although *S. setosa* and the total copepod abundance showed a parallel distribution until November, the total copepod abundance increased *S. setosa* abundance decreased when compared to the previous month in November and February. In December, the total copepod abundance decreased *S. setosa* abundance increased compared to the previous month. (Figure 1). This situation is common only in late summer and autumn." (Øresland, 1983, 1985; Yıldız and Feyzioğlu, 2014). Breeding and growth of *S. setosa* accur after the copepods reached the highest abundance (Niermann et al., 1997) because, copepods are the primary food source of *S. setosa* (Feigenbaum, 1991). Food and temperature are major factors affecting the growth of *S. setosa* in the Black Sea (Beşiktepe and Ünsal, 2000). We determined that the average water temperature (°C) in the 100 m water column was based during the sampling period. The lowest average water temperature was determined in March (8 ºC) and the highest average water temperature was determined in August (14 ºC) during the sampling period.

It was reported that breeding of *S. setosa* was probably most intensive from June to November (indicated by the frequency of small individuals) in 1994-1996 in the different stations by Beşiktepe and Ünsal (2000). Øresland (1987) observed small individuals of *S. setosa*, indicating intensive spawning, from July to early October in the western English Channel. Niermann and Greve (2007) observed the replacement of the entire population of *S. setosa* by a new generation in August in the Black Sea. Microscopic observation suggested that two broods were produced abundance the breeding period, as most of the adults belonging to the new generation had eggs in their gonads in June (Beşiktepe and Ünsal, 2000). In this study, too, it is observed that based on the average weight per individual of *S. setosa*, the biggest individuals are in May (10 mg/ind) and June (9 mg/ind), and the smallest individuals are in November and February (2 mg/ind). *S. setosa* abundance and individual weights showed a parallel change in August, (Figure 2). It was found that the relationship between temperature and abundance and average individual weight of *S. setosa* was not significant ($r^2 = 0.5$; $r^2 = -0.5$, respectively) ($p < 0.05$). However, the relationship between the total copepod abundance and individual weight of *S. setosa* was significant ($r^2 = 0.8$) ($p < 0.05$).

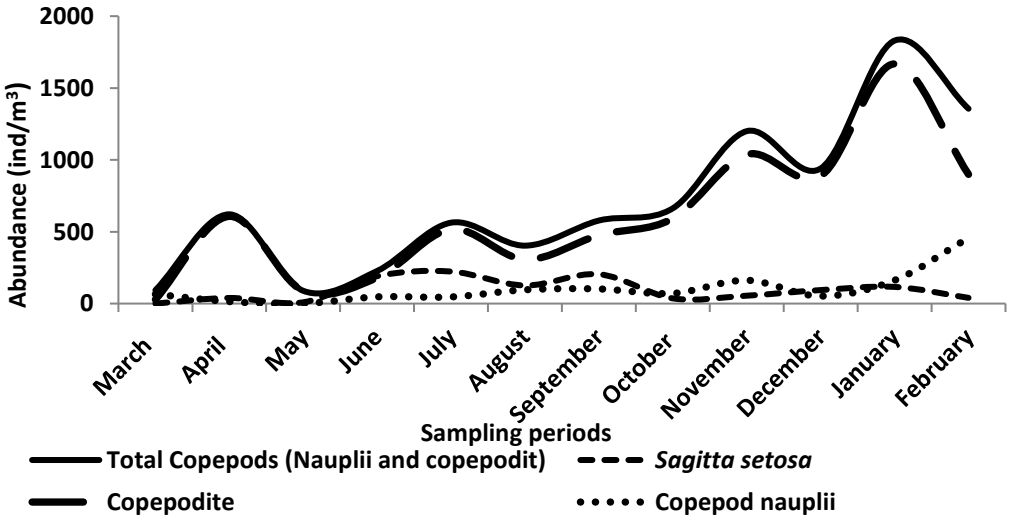

Figure 1. Abundance of the total copepods and *S. setosa* during the sampling period

In the study, the total lipid was at the highest in Spring and Autumn with 3%, and at the lowest in winter and summer with 2% during the sampling period. The average lipid per individual was at the highest in spring (0.21 mg/ind) and in summer (0.17 mg/ind), and at the lowest in autumn (0.16 mg/ind) especially in winter (0.07 mg/ind). The average

individual weight showed parallelism to ∑lipid (mg/ind) level, and there is a linear relation between them (R^2=0.9). The average individual weight was determined at the highest in spring (6.4 mg) and in summer (6.7 mg), and at the lowest in autumn (4.7 mg) and winter (3.2) (Figure 3). When the fact that small individuals enter the population in periods when the individual weight was low in *S. setosa* is considered, we may claim that this period continues during autumn when the onset of this period was observed. This shows that in *S. setosa*, there is a relation between the time-dependent change of the lipid level and the development periods. It is observed that the lipid levels are high in adulthood periods. Choe et al. (2003) found that chaetognath *Parasagitta elagans* lipid levels were relatively high in spring and summer and low in the fall and winter from the hyperbenthic zone of Conception Bay, Newfoundland. Their results showed that *P. elegans* (Copepoda) had rich lipid while maturing abundance spring and summer. This increase in lipid when mature copepods increased in the spring and summer rather than when total abundance of copepods increased in the fall (Choe et al., 2003). However, it was unlike this situation in our study. And it was determined that the abundance was low in the copepod individuls at all stages in periods when total lipid was high. This made us consider that in these periods, *S. setosa* feed on other zooplanktonic species, which are abundant in this period, rather than copepods. Şen Özdemir (2013) reported in Southeastern Black Sea Region that the most abundant zooplankton group was the cladocerans in 2012-2013 period in June (581 ind/m^3) and in July (1748 ind/m^3: 52% of the total zooplankton).

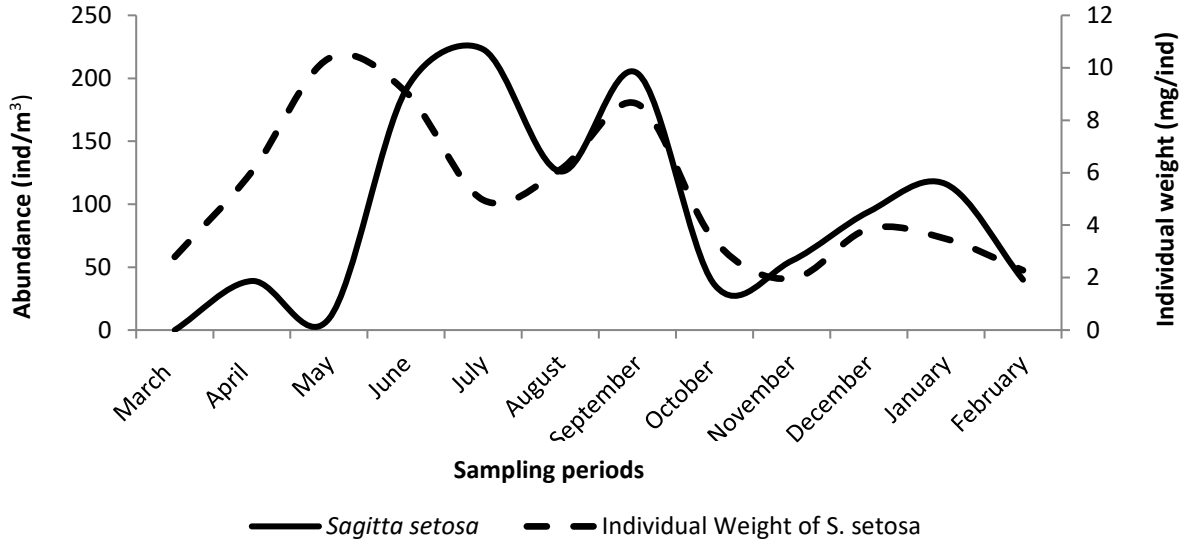

Figure 2. The changes in the average individual weight (mg/ind) and abundance of *S. setosa* during the sampling period

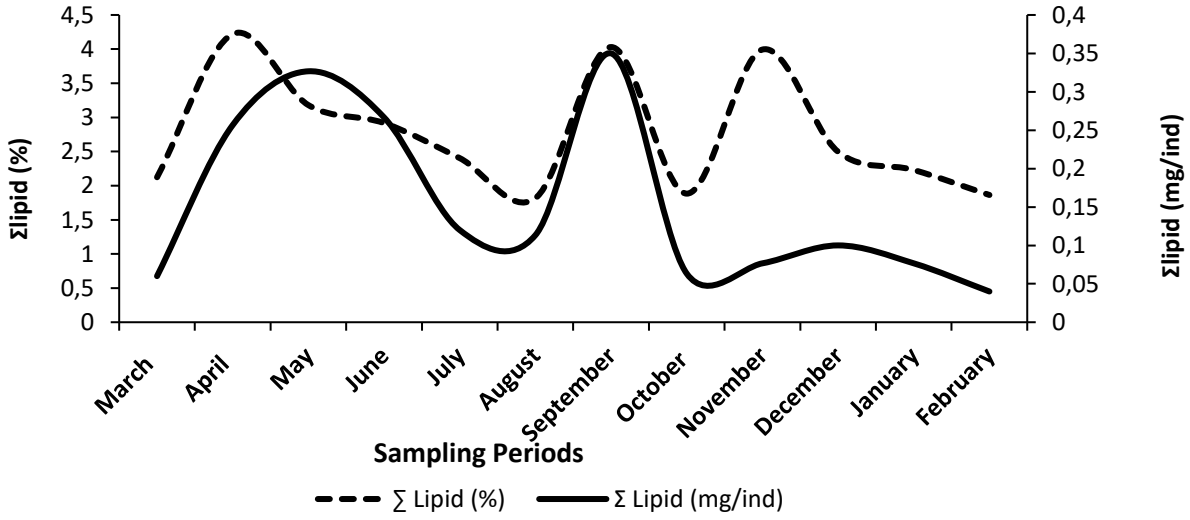

Figure 3. Changes of total lipid (Σlipid) (%: mg/ind) of *Sagitta setosa* during the sampling period

Beşiktepe and Ünsal (2000) observed the highest abundance in September. Similar observations were made in Crimea coastal area in the Black Sea. It was reported that in periods when *S. setosa* is abundant at the highest level, the copepods, which are their primary feed, are also abundant (Feigenbaum, 1991). Large numbers of copepods appear in June. This period of high copepod density and higher temperature is followed by the growth and maturity of *S. setosa*, suggesting that food and temperature are major factors affecting the growth of *S. setosa* in the Black Sea (Beşiktepe and Ünsal, 2000). Ünal (2002) determined that the *S. Setosa* abundance increased together with the increase in copepods in Summer and early Autumn in the coastal and open stations. A similar situation was also determined by Yıldız and Feyzioğlu (2014).

Öztürk (2002) determined the highest abundance in November in 1999, and reported that the highest values were determined in summer months in 2000-2001. It was reported in this study that the highest abundance between 1999-2001 (4.451 ind/m^2) in 2000 July. Yıldız and Feyzioğlu (2014), reported that *S. setosa* reached its highest abundance in October in 1999 (82.618 ind/m^2), in May in 2000 (93.768 ind/m^2), in summer months in 2001, in August in 2002, (9.713 ind/m^2), in June in 2005 (3.699 ind/m^2) and in 2006, in September (17.752 ind/m^2). The findings that were obtained about the *S. setosa,* which was reported to have been determined with the highest abundance level in summer, are in agreement with the data obtained by by Beşiktepe and Ünsal (2000), Öztürk (2002) and Yıldız and Feyzioğlu (2014). However, when the changing environmental conditions in time are considered in sampling periods and according to stations, it is normal that there are several differences.

In this study, during the sampling periods, the following values were determined in *S. setosa*, which is a carnivore species: ΣSFA 21.65%, ΣMUFA 19.87%, ΣPUFA 58.47% . 18:0 is a major fatty acid in animals and some fungi, and a minor component in most plants (Rustan and Drevon, 2005). Saturated fatty acids (SFA) including 16:0, 18:0 and 14:0 are typical calanoid copepods (Prahl et al., 1984: Harvey 1987: Sargent and Falk-Peterson, 1988; Veloza et al., 2006). In this study, 18:0, 14:0 and 16:0 were among the most important SFA (Table 1). When the copepod abundance is considered along the sampling period, it is observed that 16:0, which is at the highest level in March in *S. setosa*. Therefore, it is considered that the species that might be preferred as nutrient by copepods are these, because the fatty acid composition is affected by the changes in nutrient sources and variety along the year (Morris, 1971; Ackman et al., 1974; Lee et al., 1971). Veloza et al. (2006) indicated that 16:0 was one of most abundant SFA in *A. tonsa*. Similarly, Şen Özdemir et al. (2017) 16:0 was the most abundant fatty acid in *C. euxinus* of the Eastern Black Sea. In this study, all the copepod species that might be preferred by *S. setosa* except for *O. similis* (Cyclopoid) are being calanoid copepods might indicate the predation of *S. setosa* over these copepod species in this intensity.

Table 1. SFA composition of *Sagitta setosa* during the sampling season (% determined total FAME)

FA	March	April	May	June	July	August	September	October	November	December	January	February
14:0	-	1.21±0.06^{b}	1.73±0.35^{b}	2.62±0.09^{ab}	2.75±0.10^{ab}	1.86±0.08^{b}	3.01±0.99^{ab}	-	4.52±2.51^{a}	1.75±0.03^{c}	1.34±0.08^{c}	1.00±0.21^{e}
15:0	-	0.42±0.03^{b}	-	0.66±0.04^{a}	-	-	-	-	-	-	-	-
16:0	18.87±0.56^{a}	16..54±0.24^{b}	15.14±0.62^{bc}	14.58±0.07^{cd}	13.88±0.36^{cd}	11.85±0.09^{c}	14.99±0.29^{bcd}	14.52±0.64^{cd}	12.93±1.02^{ac}	13.97±0.28^{cd}	13.89±0.64^{cd}	14.12±0.16^{c}
17:0	-	1.36±0.05^{ab}	-	0.60±0.02^{c}	1.12±0.32^{b}	1.48±0.03^{a}	-	-	1.27±0.04^{ab}	1.49±0.02^{a}	-	1.13±0.13^{cd}
18:0	-	3.40±0.03^{d}	3.93±0.13^{defg}	3.87±0.06^{dfg}	3.78±0.04^{dg}	4.70±0.03^{abde}	4.38±0.25^{bdef}	5.59±0.38^{a}	4.74±0.74^{abd}	5.20±0.02^{abc}	5.22±0.06^{ab}	4.30±0.09^{efg}
20:0	-	0.83±0.01	-	-	-	-	-	-	-	-	-	-
22:0	-	0.52±0.09	-	-	-	-	-	-	-	-	-	-
24:0	-	0.99±0.03^{a}	-	-	-	-	-	-	-	1.25±0.39^{a}	-	0.96±0.08^{a}
∑SFA	18.87±0.53^{g}	25.27±0.04^{a}	20.80±0.84^{dfg}	22.34±0.14^{bcf}	21.53±0.63^{dfg}	19.89±0.0^{eg}	22.38±0.46^{bcf}	20.10±1.01^{eg}	23.75±0.78^{ab}	23.67±0.19^{abc}	20.45±0.61^{deg}	21.91±0.65^{cdf}

Values in the same line followes by different letters are significantly different (P<0.05), n=3, mean ±SD

PUFA are synthesized by primary producers and then consumed and incorporated into the tissues of grazers and secondary consumers (Dalsgaard et al., 2003). In particular, the PUFA such as 20:4 n-6 (ARA: Arachidonic acid), EPA and DHA cannot be synthesized by most heterotrophs, but are essential components of membranes, and as such are termed essential fatty acids (EFA). The source of these EFA are of significant interest in aquatic food web studies and can provide fundamental information about plankton condition and trophodynamics. Furthermore, the relative quantities of marker fatty acids in consumers, including 22:6 n-3, 20:5 n-3 (EPA), 18:1 n-9, and long chain monounsaturated fatty acids (MUFA), are often used as indicators of specific food sources (Falk-Petersen et al., 2000; Dalsgaard et al., 2003). For example: ratios 18:1 n-9/18:1 n-7, DHA/EPA and 16 carbon/18 carbon fatty acids (∑16C/∑18C) are also useful when determining the relative contributions of animal prey or different phytoplankton groups (e.g. dinoflagellate versus diatom food) to consumer diets (Dalsgaard et al., 2003). ∑n-3/∑n-6 ratio represents an herbivory index (Sargent and Falk-

Petersen, 1981), and the ratios 18:1 n-9/18:1 n-7 and DHA/EPA are carnivory indixes (Cripps and Atkinson, 2000; Dalsgaard et al., 2003). ∑PUFA/∑SFA ratio can be a useful freshness (Derieux et al., 1998) or diatom index for suspended particulates (Claustre et al., 1989), or a potential carnivory index in some consumers (Cripps and Atkinson, 2000). In this manner, fatty acid profiles can inform us about the predominant food and feeding plasticity of herbivorous and omnivorous zooplankton. Alterations in fatty acids occur from one trophic level to the next, but valuable dietary information can be retained despite these metabolic modifications (Dalsgaard et al., 2003). In the study, DHA/EPA changed between 1.62-2.49 as carnivory index, and winter is seen that it is the period with highest values with an average of 2.29 (Table 2). When the fact that the total copepod abundance is at the peak level in winter (2549 ind/m^3) is considered, it might be thought that the carnivory index of *S. setosa* being high may stem from the copepods it feds on. As a result of the statistical analyses, it was observed that the EPA level (whose increase decreases carnivore index) was at the lowest level in *S. setosa* in autumn (9.15%), which is different from the other seasons ($p<0.05$). 18:1 n-7 is not considered as the dominant MUFA during the sampling period in *S. setosa*. 18:1 n-9 is considered as the most dominant MUFA (8.57-12.20) in *S. setosa* (Table 3). However, unlike DHA/EPA, 18:1 n-9/18:1 n-7 ratio had its lowest average value (8) in winter, and had its highest average value (11) in summer.

However, the fact that the 18:1 n-7, which will decrease the carnivore index, was not detected in the fatty acids during the sampling period. 18:1 n-9 being among the most important fatty acids of *S. setosa* may be shown as the proof that although *S. setosa* does not always show a predation on copepods, it feeds in a carnivorous manner. In addition, the ∑n-3/∑n-6 ratio, which is the herbivore index, was determined in spring and autumn at the highest levels.

Table 2. PUFA composition of *S. setosa* during the sampling period (% determined total FAME)

FA	March	April	May	June	July	August	September	October	November	December	January	February
18:2n6c	-	1.92±0.04^{d}	2.41±0.02^{bc}	2.19±0.06^{cd}	2.25±0.09^{cd}	3.69±0.26^{a}	2.23±0.02^{cd}	3.48±0.20^{a}	2.28±0.44^{cd}	2.10±0.08^{cd}	2.82±0.24^{b}	1.42±0.02^{e}
18:3n3c	-	1.70±0.02^{bc}	1.37±0.08^{cef}	1.13±0.04^{efg}	0.96±0.01^{g}	2.44±0.42^{a}	-	-	1.12±0.012^{efg}	1.97±0.52^{b}	1.44±0.33^{ce}	1.05±0.02^{fg}
20:2n6	-	2.51±0.04^{a}	1.40±0.01^{d}	-	-	-	-	-	-	1.64±0.01^{b}	2.15±0.05^{b}	1.29±0.07^{d}
20:3n6	-	0.25±0.03^{c}	-	-	-	2.77±0.33^{a}	-	-	-	0.70±0.03^{b}	-	-
20:4n3	-	1.79±0.01^{c}	1.53±0.01^{ce}	-	-	2.49±0.16d	-	3.29±0.01^{e}	-	1.41±0.13^{de}	1.76±0.24^{c}	1.13±0.03^{d}
20:4n6	-	0.53±0.01^{d}	-	0.75±0.02^{b}	0.88±0.02^{b}	-	-	-	1.10±0.02^{a}	0.77±0.02^{c}	-	0.58±0.04^{d}
22:2	-	0.51±0.01^{a}	-	-	-	1.57±0.04^{a}	-	-	-	-	-	-
20:5n3(EPA)	19.26±0.34^{a}	16.45±0.12^{de}	16.90±0.30^{a}	17.92±0.14^{abc}	17.42±0.17^{bcd}	15.62±0.39^{ef}	18.68±0.33^{ab}	16.82±0.69^{cde}	14.43±0.43^{f}	15.98±0.08^{de}	16.99±0.40^{cde}	18.75±0.27^{ab}
22:5n3	-	0.57±0.01^{b}	-	-	-	-	-	-	-	0.68±0.05^{a}	-	-
22:6n3(DHA)	39.24±1.23^{a}	26.61±0.18^{d}	32.45±0.84^{b}	34.99±0.28^{ab}	35.41±0.86^{ab}	32.39±0.47^{b}	37.75±0.91^{ac}	39.30±1.34^{a}	32.38±2.52^{b}	38.20±0.41^{ac}	39.94±1.32^{a}	37.23±0.15^{ac}
∑PUFA	58.49±1.57^{dc}	52.84±0.29^{ef}	56.06±1.22^{de}	56.98±0.36^{d}	56.91±0.93^{d}	60.97±0.52^{bc}	58.65±0.57dc	63.41±1.57^{ab}	51.31±2.55^{f}	63.47±0.48^{ab}	65.45±1.12^{a}	63.01±0.34^{ab}
DHA/EPA	2.04±0.03^{cd}	1.62±0.01^{e}	1.92±0.04^{d}	1.95±0.01^{d}	2.03±0.03^{cd}	2.07±0.03^{cd}	2.02±0.06^{cd}	2.34±0.02^{ab}	2.24±0.10^{bc}	2.39±0.01^{ab}	2.49±0.11^{a}	1.99±0.02^{d}

Values in the same line followes by different letters are significantly different ($P<0.05$), n=3, mean ±SD

Table 3. MUFA composition of *S. setosa* during the sampling period (% determined total FAME)

FA	March	April	May	June	July	August	September	October	November	December	January	February
15:1	-	-	-	-	-	-	-	-	-	-	-	1.16±0.05
16:1n7	10.74±0.63^{a}	7.35±0.17^{bc}	6.28±0.01^{fg}	5.51±0.09^{deg}	6.61±0.20^{cf}	6.23±0.29^{fg}	6.11±1.00^{fg}	7.73±0.64^{b}	5.19±0.01^{de}	4.97±0.25^{e}	5.09±0.08^{e}	6.33±0.18^{fg}
18:1n7	-	3.51±0.07^{a}	2.86±0.12^{b}	-	-	-	-	-	-	1.83±0.02^{c}	1.67±0.04^{c}	1.61±0.08^{c}
18:1n9*t*	-	-	1.04±1.02^{c}	1.02±0.01^{c}	1.07±0.01^{c}	-	-	-	1.61±0.05^{a}	-	-	1.23±0.08^{b}
18:1n9c	11.90±0.42^{a}	9.98±0.15^{bc}	9.30±0.36^{c}	10.90±0.58^{ab}	11.13±0.5^{ab}	8.57±0.09^{cd}	10.93±0.22^{ab}	9.28±0.24^{c}	7.67±1.37^{d}	7.30±0.27^{d}	8.77±0.57^{cd}	7.36±0.03^{d}
20:1 n9	-	0.43±0.01^{d}	1.21±0.01^{bc}	1.25±0.07^{b}	0.90±0.01^{c}	-	1.94±0.06^{a}	-	1.79±0.13^{a}	-	-	-
20:1nX	-	-	2.45±0.03^{b}	1.39±0.06^{c}	0.95±0.01^{d}	-	-	-	3.18±0.12^{a}	-	-	-
22:1n9	-	0.63±0.04^{cd}	-	0.62±0.02^{cd}	0.91±0.02^{c}	2.64±0.19^{b}	-	-	6.62±0.19^{a}	0.73±0.04^{cd}	-	-
24:1	-	-	-	-	-	1.70±0.16^{a}	-	-	-	-	-	-
∑MUFA	22.64±1.00^{b}	21.89±0.26^{def}	23.13±0.38^{b}	20.69±0.49^{bcd}	21.57±0.30^{bcd}	19.14±0.59^{cde}	18.97±0.11^{de}	17.01±0.81^{efg}	26.06±1.77^{a}	14.83±0.43^{g}	15.54±0.84^{fg}	17.69±0.05^{e}

Values in the same line followes by different letters are significantly different ($P<0.05$), n=3, mean ±SD

Seas and oceans that are located in temperate zones are characterized with two seasonal peaks, which are spring and autumn, in terms of phytoplankton concentrations (Lalli and Parsons, 2004). Chlorophyll-a (Chl-a) concentration decreases in winter in the coastal areas of Trabzon in Southeastern Black Sea Region, and increases in autumn. Two main peaks are observed, which are late winter-early spring, and early summer (mostly June) (Alkan et al., 2013). Şen Özdemir et al. (2017) indicated that average chl-a concentration increased in spring (May-late spring) and winter

(December-early winter). Chl-a is used as the indicator of the phytoplankton abundance. This shows that the herbivory index being high in *S. setosa* in this period stems from the herbivorous nutrients that are consumed due to high chl-a levels. In addition, it is clear that the reproduction cycles in chaetognaths are synchronized with the intense energy input that occurs with the spring phytoplankton blooms (Choe et al., 2003).

As a conclusion, we may say that although there is no direct relation between the copepod abundance and the fatty acids of *S. setosa*, it has an indirect effect. As a result, we can say that copepod abundance and S. setosa have an indirect effect, although there is no direct relationship between fatty acids. This indirect effect can be defined as contributing to the higher trophic levels, especially when copepods are abundant, S. setosa receives herbivory index fatty acids by feeding on copepods. Choe et al. (2003) indicated that the temporal variation in the abundance of adult copepods was highly correlated with the biochemical levels of chaetognath species (*Parasagitta elegans*). In aquatic food webs, FAs are synthesized by phytoplankton and bacteria, and then transferred via zooplankton to higher trophic levels (Parrish, 2009). Additionally, Choe et al. (2003) suggested that that there was tight coupling of energy transfer from primary producers to carnivorous hyperbenthic chaetognaths following the spring phytoplankton bloom in Newfoundland coastal waters. Therefore, if we consider the food chain as a whole, not only copepods, but also the abundance changes of the main nutrient sources (phytoplankton) of the copepods can be said to have an effect on the fatty acid composition of *S. setosa.*

ACKNOWLEDGEMENTS

We thank Research Assistant Ümit DOKUZPARMAK and the crew of KTU YAKAMOZ for help in collection of the samples, and Prof. Dr. Ökkeş YILMAZ for his help with GC analyses. This work was a part of PhD study supported by KTU BAP, 2010.117.001.9.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 343-351 (2020)
Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.04
RESEARCH ARTICLE
ARAŞTIRMA MAKALESİ

Growth, nutrient utilization, body composition, hematology and histopathology of the liver of *Clarias gariepinus* fed cooked sunflower based diets

Wasiu Adeyemi Jimoh

Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, PMB 1515, Ilorin, Nigeria https://orcid.org/0000-0003-0174-301X

jimoh.wa@unilorin.edu.ng

Received date: 27.12.2019 *Accepted date: 09.06.2020*

How to cite this paper:
Jimoh, W.A. (2020). Growth, nutrient utilization, body composition, hematology and histopathology of the liver of *Clarias gariepinus* fed cooked sunflower based diets. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 343-351. DOI: 10.12714/egejfas.37.4.04

Abstract: This study investigated the use of cooked sunflower seed meal as soybean meal replacer in *Clarias gariepinus* diet in a 56-day feeding trial using growth performance, nutrient utilization, body composition, digestibility, hematology and liver histology as indices of assessment. Sunflower seed meal was cooked for 10, 20 and 30 minutes. Each of the differently cooked sunflower seed meal replaced soybean meal portion of control diet at 15, 30 and 45% to produce nine 40% crude protein, 18kJ/g test diets. A diet without sunflower meal served as the control. Triplicate groups of fish in 70-litre capacity aerated rectangular plastic tanks were alloted to each dietary treatment at a stocking rate of 15 fingerlings (3.72±0.22g average weight) per tank in a completely randomized design. Fish were fed to satiation. Data obtained from the experiment were subjected to statistical analysis. The results of the experiment revealed that up to 30% replacement level of 10- and 20-minutes cooked sunflower produced a statistically similar results with soybean-based control diets.

Keywords: sunflower, *Clarias gariepinus,* haematology, histopathology, digestibility

INTRODUCTION

The trend of growth in aquaculture production witnessed in the recent years all over the world (Troell et al., 2014) calls for the need to develop economical feed that will support the growth of fish and sustainable aquaculture production (Azaza et al., 2009). Soybean has been a conventional protein source feed ingredient for aquaculture species but its competitive use among other livestock users and even human being is on the rise and this could be a threat to the sustainability of aquaculture production (Azaza et al., 2009; Tacon and Metian, 2008). A lot of researchers have ventured into prospecting the economically viable and nutritionally comparable plant protein source to ease the problem of high cost of conventional protein feedstuffs and their availability question in fish feed manufacture (Hassaan et al., 2017; Hassaan et al., 2015; Jimoh et al., 2013a; Jimoh and Aroyehun, 2011; Jimoh et al., 2014b; Jimoh et al., 2013b; Kumar et al., 2010; Saha and Ghosh, 2013). Sunflower seedmeal has been identified as a good source of especially sulphur-containing amino acids (Gohl, 1991). More so, like all other unconventional protein source, it is a low-cost plant protein source when compared to soybean meal (Hassaan et al., 2015; Köprücü and Sertel, 2012) and readily available in the market (Lozano et al., 2007). As a result of its good nutrient profile, research directions have been made on its use in fish feed. Sunflower seedmeal has been included in the diet *Oreochromis mossambicus* (Jackson et al., 1982); *Onchorhynchus mykiss* (Sanz et al., 1994; Tacon et al., 1984); *Oreochromis niloticus* (Sintayehu et al., 1996); *Onchorhynchus mykiss* (Stickney et al., 1996); *Anguilla Anguilla* (Garcia-Gallego et al., 1998); *Tilapia rendalli* (Olvera-Novoa et al., 2002); *Salmo salar* (Gill et al., 2006); *Sparus aurata* (Lozano et al., 2007); *Acanthopagrus schlegelii* (Hassaan et al., 2018). Work on inclusion of sunflower seed meal in the diet of *Clarias gariepinus* were Akintayo et al. (2008) and Fagbenro et al. (2010). However, a major setback to the use of plant protein sources lie in their deficiency in some essential amino acids primarily lysine, higher fibre content, the presence of anti-nutritional factors (Alarcón et al., 1999; Francis et al., 2001; Gaylord et al., 2004; Hertrampf and Piedad-Pascual, 2012).

Processing of plant protein sources is necessary in order to improve their nutritive value (Soltan, 2005). It leads to little or no antinutrient in plant protein sources thereby increase their utilization. Adeparusi and Jimoh (2002) reported that thermal processing of plant protein sources and their inclusion level significantly affect their digestibility not only in fish but also in other farm animals. Paucity of information exists on the use of sunflower in *Clarias gariepinus* diet. Akintayo et al. (2008) fed toasted sunflower meal to *Clarias gariepinus* while Fagbenro et al. (2010) included unprocessed sunflower seedmeal in *Clarias gariepinus* diet. An attempt is therefore being made in this study to investigate the inclusions of differently cooked sunflower seedmeal on the nutrient utilization, growth performance, digestibility, body composition, hematology and histopathology of the liver of *Clarias gariepinus.*

MATERIALS AND METHODS

Feed ingredients' processing and diet preparation

Three batches of sunflower seed were put in boiling water (100°C) for 10, 20 and 30 minutes to serve as processing time interval. The seedmeal were dried, ground, and locally made screw press was used to mechanically defat the sample The three samples were designated as C10, C20 and C30 respectively according to their time of processing. Nine iso-nitrogenous (30% crude protein) and isocaloric diet having fishmeal, soybean meal, sunflower meal, cassava flour, fish and vegetable oil (1:1) and vitamin – mineral premix as the ingredients. Each batch of sunflower were included at 15, 30 and 45% replacement level with soybean meal (Table 1) following the recommendation of Hertrampf and Piedad-Pascual (2012) that 20% sunflower meal should be included in the omnivorous species. The feedstuff that has been pulverized with hot water added to aid binding was fed into a Hobart - 200T pelleting and mixing machine to produce pellets which were sun –dried (30-32°C) then kept frozen in a refrigerator. Proximate composition of the diets prepared were carried out using the methods of AOAC (1990). physiological value of 5.61Kcal/g protein, 9.50 Kcal/g lipid and 4.11Kcal/g carbohydrate (Tacon, 1995) was used in determining the gross energy content of samples . The method of Spackman et al. (1958) was followed in amino acid analysis of differently-processed sunflower seed meals using the ion exchange chromatography (IEC). The amino acid analysis was carried out in the Department of Zoology, University of Jos, Nigeria using Automatic Technicon Sequential Multi-sample Amino Acid Analyzer (Model No 0209, Technicon, Ireland).

Table 1. Proximate composition and essential amino acid profile of differently processed sunflower seed meal

Proximate	Processed Sunflower		
	C10	C20	C30
Moisture	9.1	8.97	9.28
Crude Protein	40.39	38.36	35.83
Crude Fibre	11.90	12.83	12.58
Crude Lipid	5.38	6.22	5.41
Ash	11.28	10.38	12.28
NFE	22.02	22.02	24.62
Amino Acid			
Lysine	3.68	3.36	3.20
Histidine	2.00	3.56	2.92
Arginine	8.97	8.95	9.04
Threonine	3.13	3.34	3.41
Valine	3.97	4.03	4.07
Methionine	1.03	1.40	1.79
Isoleucine	2.91	3.22	4.95
Leucine	3.72	5.92	6.29
Phenylalanine	5.71	5.04	5.07

Experimental system and fish

Fingerlings of *Clarias gariepinus* (<5g) were obtained from Ondo state Government Fish Farm, Alagbaka, Akure, Nigeria and transported life to the laboratory of the Department of Fisheries and Aquaculture inside aerated polythene bags. The fish was acclimated in glass tanks on commercial pelleted diet for seven days. Fifteen fingerlings were stocked into each tank randomly with three replications per treatment. Experimental diet was allotted randomly to the tanks and each group of fish was fed to apparent satiation two times in a day at 9:00-10:00 hrs and 16:00-17:00 hours for 56 days. Fish was removed from each tank every 14 days and batch-weighed with the weight in each dietary group recorded accordingly. Siphoning during the first 3 weeks of the experiment served to collect faecal samples. The faecal samples were oven – dried at 45°C and was analyzed for its proximate composition. Daily mortality was monitored and recorded. At the beginning of the feeding trial and at the end, composite whole fish was sacrificed for carcass analysis of its crude protein, crude Ash fibre, and lipid. Dissolved Oxygen and water temperature were monitored three times in each week using a combined digital YSI Do Meter (YSI, Model 57) electronic pH meter (Metter Toledo, Model 320) was used in monitoring pH.

Acid insoluble ash (AIA) analysis

The AIA was calculated based on the procedures explained in Adeparusi and Jimoh (2002). AIA in faeces and feed was gotten by adding 25ml of 10% HCl to the ash content that has been previously weighed with a water glass serving as cover. The resulting mixture was boiled gently over a low flame for 5 minutes after which it was filtered using ashless filter and washed with hot distilled water, the residue from the filter was returned into the crucible and then ignited until it is carbon – free and it was weighed.

$$\% \text{ AIA} = \frac{\text{weight of AIA}}{\text{weight of ash}} \times 100$$

Digestibility Coefficient

The value obtained for AIA in different faecal samples and diets were used as indicator in the calculation of digestibility coefficient as described in Jimoh et al. (2014a); Jimoh et al. (2010).

Organic Matter Digestibility (AOMD) was calculated as follow

$$\% \text{ AOMD} = 100 - \left[100 \frac{(\% \text{ AIA in Feed})}{((\% \text{ AIA in Faeces})}\right]$$

$$\% \text{ Digestibility} = 100 - \left[100 \frac{(\% \text{ AIA in Feed})}{((\% \text{ AIA in Faeces})} \times \frac{(\% \text{ Nutrients in Faeces})}{(\% \text{ Nutrients in Feed})}\right]$$

Table 2. Gross, proximate composition (g/100g dry matter), acid insoluble ash and energy content of experimental diets at varying replacement levels of differently cooked sunflower seedmeals

	CTR	CSF115	CSF130	CSF145	CSF215	CSF230	CSF245	CSF315	CSF330	CSF345
Fishmeal	27.24	27.24	27.24	27.24	27.24	27.24	27.24	27.24	27.24	27.24
Soybean Meal	46.71	39.71	32.70	25.70	39.71	32.70	25.70	39.71	32.70	25.70
Cooked Sunflower	-	7.62	15.24	22.87	7.92	15.84	23.75	8.34	16.68	25.01
Corn Meal	11.25	11.25	11.25	11.25	11.25	11.25	11.25	11.25	11.25	11.25
Fish Oil	5.09	5.09	5.09	5.09	5.09	5.09	5.09	5.09	5.09	5.09
*Vit/Min Premix	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Starch	4.71	4.09	3.48	2.85	3.79	2.88	1.97	3.37	2.04	0.71
Total	100	100	100	100	100	100	100	100	100	100
Proximate Composition										
Moisture	9.72± 0.48	9.28±0.10	9.31±0.04	9.77±0.46	9.28±0.03	9.29±0.03	9.27±0.04	9.38±0.03	9.25±0.05	9.36±0.06
Crude Protein	40.19±0.08	40.30±0.08	40.29±0.07	40.27±0.11	40.22±0.16	40.29±0.09	40.27±0.04	40.24±0.07	40.28±0.07	40.27±0.01
Crude Lipid	12.16±0.02	12.14±0.03	12.13±0.05	12.14±0.03	12.15±0.05	12.10±0.03	12.17±0.05	12.17±0.06	12.17±0.06	12.12±0.04
Crude fibre	9.72±0.04^{a}	9.72±0.15^{a}	9.69±0.16^{a}	9.78±0.10^{a}	9.71±0.17^{a}	9.70±0.13^{a}	9.58±0.14^{ab}	9.56±0.23^{ab}	9.36±0.09^{b}	9.34±0.08^{b}
Ash	5.87±0.05	5.84±0.12	6.00±0.14	5.60±0.55	5.86±0.20	5.99±0.73	5.85±0.45	5.71±0.42	5.77±0.49	5.79±0.47
NFE	22.33±0.51^{b}	22.72±0.05^{a}	22.58±0.22^{a}	21.00±2.17^{b}	22.78±0.15^{a}	22.62±0.82^{a}	22.85±0.50^{a}	22.95±0.21^{a}	23.17±0.40^{a}	23.11±0.58^{a}
AIA	0.75±0.05	0.80±0.02	0.77±0.04	0.78±0.05	0.78±0.07	0.77±0.03	0.77±0.04	0.82±0.07	0.82±0.07	0.79±0.07
Energy (KJ/g)	18.08±2.05^{ab}	18.17±0.48^{a}	18.13±1.13^{a}	18.00±1.26^{b}	18.16±1.83^{a}	18.13±3.40^{a}	18.19±2.31^{a}	18.20±1.15^{a}	18.25±2.22^{a}	18.22±2.02^{a}

Row means with different superscripts are significantly different (p<0.05) from each other
NFE: Nitrogen free Extract AIA: Acid insoluble Ash
* *Specification: each kg contains: Vitamin A , 4,000,000IU; Vitamin B, 800,000IU; Vitamin E, 16,000mg, Vitamin K_3, 800mg; Vitamin B_1, 600mg; Vitamin B_2, 2,000mg; Vitamin B_6, 1,600mg, Vitamin B_{12},8mg; Niacin,16,000mg; Caplan, 4,000mg; Folic Acid, 400mg; Biotin, 40mg; Antioxidant 40,000mg; Chlorine chloride, 120,000mg; Manganese, 32,000mg; Iron 16,000mg; Zinc, 24,000mg; Copper 32,000mg; Iodine 320mg; Cobalt,120mg; Selenium, 800mg manufactured by DSM Nutritional products Europe Limited, Basle, Switzerland*

Diet Performance Evaluation

Growth performance and nutrient utilization of fish was determined following the methods described in Jimoh et al., (2019) for weight gain, feed conversion ratio, (FCR), Survival (%), protein efficiency ratio (PER), specific growth rate (SGR %/ day), and, net protein utilization (NPU) responses was calculated as

$$Weight\ Gain\ (\%) = \frac{Final\ weight - Initial\ Weight}{\text{Initial weight}} \times 100$$

$$SGR\ (\%/day) = \frac{ln\ (final\ body\ weight) - ln\ (initial\ body\ weight)}{\text{Time (in days)}} \times 100$$

$$FCR = \frac{dry\ weight\ of\ feed\ fed}{\text{Fish weight Gain}}$$

$$PER = \frac{Fish\ weight\ gain}{\text{Protein Fed}}$$

$$NPU = \frac{Net\ protein\ in\ Carcass}{Protein\ Fed}$$

Hematological examination of the fish

The blood of fish that has been previously euthanized in clove oil (100 ml/l) was obtained using heparinized syringe into a EDTA smeared sampling bottle. The procedure of Svobodova et al. (1991) was followed in the blood analysis.

Histological examination of test organ

At the end of the experiment, three fish per treatment was sampled for histological analysis; the test organisms that have been euthanized were cut open to excise the liver. After which the excised livers were fixed in 10% formalin and dehydrated in graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, with the use of a rotatory microtome and staining with Harris haematoxyllin-eosin (H&E) stain for ten minutes (Bancroft and Cook, 1994). The stained slide was observed under a light microscope and snapped using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P).

Statistical analysis

All data were expressed as mean ± standard deviation and were analyzed using one-way analysis of variance (ANOVA) test using SPSS 17.0 version. Duncan's multiple – range test (Zar, 1996) was used to separate the treatment means where ANOVA revealed significant difference (P<0.05),

RESULTS

Whole body composition

The whole-body composition of fish at the beginning and at the end of the experiment is presented in Table 3. Significant variations (P< 0.05) existed between the final and initial body composition of fish used during the experiments with respect to proximate analysis. Carcass crude protein reduced with increase in cooking time and inclusion level. No significant variations (p>0.05) was observed in the carcass protein of fish fed CTR, CSF115, CSF130, CSF215, CSF315 while the lowest value was recorded for fish fed CSF345.

Table 3. Proximate composition of carcass of *Clarias gariepinus* fed varying levels varying replacement levels of cooked samples of sunflower seedmeal-based diets

	Initial	Experimental Diets									
		CTR	CSF115	CSF130	CSF145	CSF215	CSF230	CSF245	CSF315	CSF330	CSF345
Moisture	76.62±0.14^{a}	72.44±0.28^{e}	72.51±0.19^{de}	72.60±0.25^{cde}	72.76±0.09^{cd}	72.56±0.21^{cde}	72.62±0.19^{cde}	72.72±0.02^{cde}	72.62±0.03^{cde}	72.86±0.01^{ab}	73.10±0.04^{b}
C. Protein	15.17±0.05^{e}	17.83±0.12^{a}	17.72±0.14^{ab}	17.68±0.19^{abc}	17.58±0.09^{bc}	17.74±0.07^{ab}	17.58±0.05^{bc}	17.51±0.05^{c}	17.71±0.08^{ab}	17.53±0.06^{c}	17.32±0.04^{d}
.C. Lipid	5.28±0.04^{f}	6.39±0.03^{a}	6.37±0.02^{ab}	6.35±0.03^{abc}	6.30±0.03^{de}	6.37±0.02^{ab}	6.35±0.02^{abc}	6.33±0.01^{bcd}	6.35±0.02^{abc}	6.32±0.01^{cde}	6.29±0.02^{e}
Ash	2.93±0.07^{g}	3.53±0.04^{a}	3.49±0.04^{ab}	3.44±0.03^{bc}	3.36±0.02^{def}	3.45±0.05^{bc}	3.43±0.03^{bcd}	3.38±0.03^{cde}	3.43±0.04^{cde}	3.34±0.06^{ef}	3.29±0.03^{f}

Row means with different superscripts are significantly different (p<0.05) C. Crude

Table 4. Growth performance and nutrients utilisation of *Clarias gariepinus* fed cooked sunflower meal-based diets

Parameters	Experimental Diets									
	CTR	CSF115	CSF130	CSF145	CSF215	CSF230	CSF245	CSF315	CSF330	CSF345
Ini. Weight	3.45±0.05^{a}	3.69±0.32^{a}	3.36 ±0.52^{a}	3.92 ±0.37^{a}	3.80 ± 0.20^{a}	3.45 ± 0.29^{a}	4.02±0.06^{a}	4.00 ±0.31^{a}	3.77± 0.49^{a}	3.78±0.55^{a}
Fin.Weight	12.43±0.08^{ab}	12.32±0.14^{abc}	11.87±0.49^{abcd}	11.51±0.58^{cd}	12.66±0.59^{a}	11.62±0.52^{bcd}	9.51±0.52^{f}	11.85±0.60^{abcd}	11.26±0.42^{de}	10.53±0.30^{e}
[1]M W Gain	8.98± 0.09^{a}	8.63± 0.19^{ab}	8.51± 0.62^{abc}	7.59± 0.22^{cde}	8.86 ± 0.69^{a}	8.18±0.80^{abcd}	5.49±0.58^{f}	7.85 ± 0.34^{bcd}	7.49 ± 0.71^{de}	6.75± 0.26^{e}
[2]% W.Gain	259.94±5.57^{a}	235.45±25.73^{ab}	258.88±55.82^{a}	194.36±13.54^{b}	233.80±27.32^{ab}	239.42±42.07^{ab}	136±6.17^{c}	196.62±10.46^{b}	202.39±40.21^{ab}	191.84±35.68^{bc}
[3]SGR	2.29±0.03^{a}	2.16±0.14^{abc}	2.27±0.27^{a}	1.93±0.80^{c}	2.15 ±0.15^{abc}	2.17 ±0.22^{abc}	1.53±0.13^{d}	1.94±0.06^{bc}	1.97 ±0.25^{abc}	1.84 ±0.22^{c}
[4]FCR	1.21±0.02^{d}	1.22 ±0.02^{cd}	1.23 ±0.02^{bcd}	1.25 ±0.04^{abc}	1.22 ±0.02^{cd}	1.24 ±0.01^{bcd}	1.28±0.03^{a}	1.24 ±0.02^{bcd}	1.24 ±0.02^{bcd}	1.27±0.04^{ab}
[5]PER	2.07 ±0.04^{a}	2.05 ±0.03^{ab}	2.03 ±0.04^{abc}	1.97±0.06^{bcd}	2.05 ±0.03^{ab}	2.02 ±0.02^{abc}	1.94±0.04^{bc}	2.02±0.04^{abc}	2.01±0.04^{abcd}	1.97±0.06^{cd}
[6]NPU	61.39 ±2.23^{b}	60.53±4.49^{b}	60.04±5.66^{b}	63.39 ±5.21^{b}	59.47±5.08^{b}	59.77±6.60^{b}	83.33±8.92^{a}	65.25±0.54^{b}	63.91 ±8.78^{b}	62.64±2.75^{b}
[7]%Survival	97.78±3.85^{ab}	97.78±3.85^{ab}	86.67±3.34^{ab}	82.22 ±3.85^{a}	88.89±3.88^{ab}	97.78 ±3.85^{ab}	91.11±1.18^{ab}	91.11 ±3.85^{ab}	86.67±3.35^{ab}	86.67±3.84^{ab}

Row means with different superscripts are significantly different (p<0.05).

[1] Mean weight gain= final mean weight –initial mean weight
[2] Percentage weight gain= [final weight-initial weight /initial weight] X 100
[3] Specific growth rate= [In final weight-In initial weight] X 100
[4] Feed conversion ratio=dry weight of feed fed /Weight gain (g)
[5] Protein efficiency ratio=fish body weight (g)/ Protein fed
[6]Net protein utilization= [protein gain/protein fed] X 100
[7] Percentage survival = {(total number of fish- mortality)/total number of fish] X 100
Ini. : Initial Fin. : Final

Survival, growth performance and nutrient utilization

Growth performance and nutrient utilization by *Clarias gariepinus* fed varying levels of cooked sunflower meal based experimental diets are expressed in Table 4. Percentage survival was very high (>80%) across all dietary treatment groups and no significant difference (p>0.05) was recorded in percentage survival of all the fish fed the dietary treatment except the fish fed CSF145. The control diets trated group had the best growth performance. However, the mean weight gain, specific growth rate, % weight gain, and feed intake were statistically similar (p>0.05) among the fish fed control diet and diets CSF115, CSF130, CSF215 and CSF230.

Faecal sample proximate composition

The differently fed *Clarias gariepinus* faecal samples' proximate compositions are presented in Table 5. A reduced nutrient contents of the faecal samples tested was observed when compared with that of the feed. The protein content of the faecal samples of fish fed diet CTR, CSF115, CSF130, CSF230, CSF315 and CSF330 were statistically similar (p>0.05). No significant variation (p>0.05) was observed in the crude lipid content of the faecal samples of fish fed diet CTR and that of fish fed other test diets except CSF245. The crude fibre content of the faecal output of fish fed diet CTR and fish fed other test diets except diets CSF130 CSF230 and CSF315 were statistically indifferent (p>0.05).

The faecal AIA of the differently fed fish did not significantly vary (P>0.05) except that of the fish fed CSF245 and CSF345. Similarly, no statistical variation (P>0.05) was observed in the energy value of the faecal samples of fish fed all the dietary treatments.

Apparent nutrient digestibility coefficients in each diet

The apparent nutrient digestibility coefficient of cooked sunflower-meal based diets fed to *Clarias gariepinus* fingerlings is given in Table 6. Significant variations (p<0.05) was observed in the nutrient digestibility's values of fish fed various dietary treatments. However, no significant difference (p>0.05) was recorded in the AOMD of control diet fed group and test diets fed groups except that of the fish fed CSF245 and CSF345. Similarly, there was no significant variations (p>0.05) in the APD of control diet fed fishand the fish fed other test diets except those fed diets CSF145, CSF245 and CSF345. Same trend of results as recorded for APD was observed in the ALD of the fish fed dietary treatments except that significant variation (p<0.05) was recorded between the fish fed control diets and the fish fed CSF245, CSF330, and CSF345 diets. The AED value of the fish fed control diets were not significantly different (p>0.05) from the fish fed CSF115, CSF130, CSF215 and CSF230 diets. So also, no significant difference (p>0.05) was recorded in the AED values of the fish fed diets CSF145, CSF315, and CSF330.

Fish fed control diets were not significantly different (p>0.05) from the fish fed the test diets except the fish groups fed diets CSF245, CSF330, and CSF345. Apparent ash digestibility and apparent carbohydrate digestibility coefficient in all fish fed the test diets did not significantly vary (p>0.05) from control except that of the CSF345.

Hematological profile

The hematological parameters of *Clarias gariepinus* fed differently cooked sunflower meal-based diets replacing soybean meal is presented in Table 7. There existed significant variations (p<0.05) in the haemoglobin content of the fish fed various dietary treatment. However, was no significant variations (p>0.05) in the haemoglobin content of fish fed control diets and test diets CSF315, CSF115, CSF215, CSF130. Similar pattern as observed above was also recorded for the PCV of the fish fed different dietary treatment except that there was significant difference(p<0.05) between the control diets and diet CSF130. There was no significant difference in the white blood cell counts of fish group fed the control diet and those fed test diets; CSF115, CSF130, CSF145, CSF215, CSF230, CSF315, and CSF330. RBC decreases with increase in levels of inclusion of differently cooked sunflower. No significant variation (p>0.05) existed in the MCHC of fish fed differently cooked sunflower meal-based diets and control diets except in fish fed test diets CSF345. The MCV follows the same pattern as recorded for MCHC except that fish fed diets CSF245 and CSF345 showed significant variation (p<0.05) in MCV from other dietary treatments. The MCH of fish fed the different dietary treatments were statistically similar (p>0.05). The trend of results of the ESR of fish fed differently cooked sunflower meal-based diets were similar to the pattern of results of the RBC recorded above

Histopathology

Different dietary treatments exhibit different changes in the liver of *Clarias gariepinus.* Diffuse hepatic vacuolation was recorded in control diet. Other test diets exhibit diffuse to severe fatty infiltration of hepatocytes with diffuse to severe, diffuse vacuolar degeneration of hepatocytes. Severe fatty infiltration of hepatocytes was recorded in fish fed CSF115, CSF215,230 and CSF345. Severe hepatic vacuolation were recorded in fish fed CSF130,145, CSF245, CSF315 and CSF345.

Table 5. Proximate composition (g/100g dry matter), acid insoluble ash and energy contents of faecal samples of *Clarias gariepinus* fed varying replacement levels of cooked samples of sunflower seedmeal-based diets

Parameter	Experimental Diets									
	CTR	CSF115	CSF130	CSF145	CSF215	CSF230	CSF245	CSF315	CSF330	CSF345
Moisture	10.63±0.81^{a}	10.00±1.42^{ab}	9.62±0.26^{b}	10.10±0.23^{ab}	10.07±0.45^{ab}	9.87±0.39^{ab}	9.84±0.46^{ab}	9.99±0.60^{ab}	9.93±0.47^{sb}	10.06±0.38^{ab}
Crude Protein	15.73±0.71^{c}	15.24±.31^{c}	16.98±1.80^{abc}	20.17±1.33^{ab}	20.53±1.04^{a}	16.21±3.79^{bc}	16.90±1.83^{abc}	15.51±2.28^{c}	16.09±3.66^{bc}	16.84±1.02^{abc}
Crude Lipid	6.15±0.10^{b}	6.18±0.69^{b}	6.89±0.76^{ab}	6.77±1.09^{ab}	6.20±0.31^{b}	7.19±0.99^{ab}	7.91±0.87^{a}	5.94±0.74^{b}	6.72±0.65^{ab}	6.50±0.31^{b}
Ash	9.65±0.41^{b}	10.07±1.21^{b}	10.44±.51^{b}	10.82±2.43^{b}	10.62±0.56^{b}	10.99±.23^{b}	11.37±1.71^{b}	12.26±2.05^{b}	12.43±2.86^{b}	15.48±1.81^{a}
Crude fibre	11.98±0.62^{ab}	11.35±1.35^{ab}	12.44±1.20^{a}	11.17±0.27^{ab}	11.11±1.29^{ab}	12.77±1.08^{a}	11.92±0.99^{ab}	12.43±0.89^{a}	11.83±0.26^{ab}	10.31±1.15^{b}
NFE	45.86±1.30^{ab}	47.17±1.83^{a}	43.63±3.45^{abc}	40.97±0.48^{c}	41.46±1.29^{c}	42.97±3.54^{bc}	42.07±0.48^{bc}	43.87±3.21^{abc}	43.00±1.13^{bc}	40.80±0.53^{c}
AIA	3.29±0.38^{ab}	3.34±0.40^{ab}	3.40±0.27^{ab}	3.42±0.21^{ab}	3.44±0.43^{ab}	3.38±0.05^{ab}	3.06±0.20^{c}	3.64±0.09^{a}	3.35±0.40^{ab}	2.67±0.13^{c}
Energy (kcal/100g)	334.52±4.12	337.44±3.29	339.49±2.36	345.19±19.59	343.92±6.70	335.29±15.24	342.23±5.41	323.16±14.14	330.26±21.93	323.32±3.30

Row means with different superscripts are significantly different (p<0.05)
NFE: Nitrogen free Extract AIA: Acid insoluble Ash

Table 6. Apparent digestibility coefficient of nutrients of cooked sunflower meal-based diets fed to *Clarias gariepinus*

Parameters	Experimental Diets									
	CTR	CSF115	CSF130	CSF145	CSF215	CSF230	CSF245	CSF315	CSF330	CSF345
AOMD	76.99±1.69^{ab}	75.85±0.53^{ab}	77.19±1.03^{a}	77.09 ±0.27^{a}	77.25±1.01^{a}	77.38±0.69^{a}	74.70±0.49^{b}	77.47±1.31	75.31±0.99^{ab}	70.26±1.11^{c}
APD	91.01±0.42^{a}	90.92 ±0 .36^{a}	90.41 ±0.72^{ab}	88.53±0.62^{bc}	88.37±1.00^{bc}	90.92±1.98^{a}	89.39 ±1.11^{abc}	91.36 ±0.82^{a}	90.19 ±2.00^{ab}	87.58 ±0.52^{c}
ALD	88.36±0.92^{ab}	87.76 ±1.32^{ab}	87.08 ±0.79^{ab}	87.24 ±1.97^{ab}	88.38±0.99^{ab}	86.59 ±1.49^{ab}	83.56±2.05^{c}	89.05±0.82^{a}	86.39 ±0.79^{b}	84.04±1.40^{c}
AED	82.19±1.23^{ab}	81.24±0 .77^{ab}	82.14±0.67^{ab}	81.48±0.68^{bc}	81.97±0.87^{ab}	82.51±0.46^{ab}	80.09±0.56^{c}	83.29±0.63^{a}	81.33±0.67^{ab}	77.92±0 .70^{d}
AAD	77.13±2.29^{a}	74.76±5.74^{a}	75.42±1.71^{a}	74.58±6.13^{a}	75.15±0.96^{a}	74.39±0.13^{a}	69.91±5.09^{a}	70.98±6.00^{a}	67.09±8.45^{a}	50.54±7.59^{b}
AFD	53.19±1.46^{ab}	53.48±0.43^{ab}	52.83±3.16^{abc}	53.89±5.58^{ab}	56.71±6.60^{a}	51.68±1.88^{abc}	48.49±0 .74^{bc}	50.97±0.69^{abc}	49.25±1.33^{bc}	47.14±1.24^{c}
ACD	52.68±0.59^{abc}	49.75±7.16^{bc}	55.84±5.16^{abc}	54.95±5.08^{abc}	58.63±0.33^{a}	56.81±6.32^{ab}	53.43±0.62^{abc}	56.85±5.17^{ab}	54.11±3.45^{abc}	47.51±1.11^{c}

Row means with different superscripts are significantly different (p<0.05)
AOMD Apparent Organic Matter Digestibility
APD Apparent Protein Digestibility
ALD Apparent Lipid Digestibility
AED Apparent Energy Digestibility
AAD Apparent Ash Digestibility
AF D Apparent Fibre Digestibility
ACD Apparent Carbohydrate Digestibility

Table 7. Hematological profile of blood of *Clarias gariepinus* fed cooked sunflower meal-based diets

	Experimental Diets									
	CTR	**CSF115**	**CSF130**	**CSF145**	**CSF215**	**CSF230**	**CSF245**	**CSF315**	**CSF330**	**CSF345**
Hb	10.19±0.06^{a}	10.10±0.12^{a}	9.86±0.18^{ab}	9.15±0.23^{c}	10.08±0.07^{a}	9.65±0.15^{b}	8.79±0.01^{d}	10.20±0.18^{a}	9.62±0.13^{b}	8.69±0.22^{d}
PCV	30.33±0.13^{ab}	30.36±0.40^{ab}	29.37±0.39^{bc}	27.14±0.84^{d}	30.44±0.21^{ab}	29.57±0.30^{abc}	27.03±0.65^{d}	30.74±0.16^{a}	28.81±0.82^{c}	27.01±0.32^{d}
WBC (x10^{3})	6.50±0.142^{abc}	6.59±0.12^{abc}	6.43±0.04^{bc}	6.65±0.07^{abc}	6.50±0.14^{abc}	6.40±0.21^{bc}	6.68±0.04^{abc}	6.45±0.14^{bc}	6.378±0.04^{c}	6.74±0.05^{a}
RBC	3.31±0.50^{ab}	3.35±0.04^{a}	3.23±0.08^{abc}	2.93±0.07^{d}	3.34±0.04^{a}	3.19±0.06^{bc}	2.87±0.11^{d}	3.37±0.06^{a}	3.12±0.01^{c}	2.81±0.02^{d}
MCHC	33.58±0.35^{a}	33.25±0.04^{ab}	33.56±0.16^{a}	33.70±0.19^{a}	33.12±0.01^{ab}	32.62±0.18^{ab}	32.51±0.81^{ab}	33.18±0.42^{ab}	33.42±1.39^{ab}	32.15±0.43^{b}
MCV	91.78±0.99^{c}	90.63±0.04^{c}	90.93±1.19^{c}	92.60±0.64^{bc}	91.14±0.52^{c}	92.71±0.71^{bc}	94.37±1.22^{ab}	91.23±1.07^{c}	92.34±2.21^{bc}	96.28±0.40^{a}
MCH	30.82±0.65^{a}	30.14±0.02^{a}	30.51±0.26^{a}	31.21±0.04^{a}	30.18±0.17^{a}	30.24±0.07^{a}	30.69±1.17^{a}	30.27±0.04^{a}	30.84±0.55^{a}	30.96±0.55
ESR	3.67±0.02^{a}	3.63±0.04^{a}	3.55±0.06^{ab}	3.29±0.09^{c}	3.63±0.02^{a}	3.47±0.06^{b}	3.16±0.04^{d}	3.67±0.07^{a}	3.46±0.04^{b}	3.13±0.08^{d}

Row means with different superscripts are significantly different (p<0.05).

Hb: Haemoglobin content (gm/100ml) PCV: Packed Cell Volume (%) WBC: White Blood Cell Count (10^{4}mm^{3})

RBC: Red Blood Cell Count (10^{6}mm^{3}) MCHC: Mean Corpuscular Haemoglobin Concentration (%)

MCV: Mean Corpuscular Volume (u^{3}) MCH: Mean Corpuscular Haemoglobin (pg)

ESR: Erythrocyte Sedimentation Rate (mm/hr)

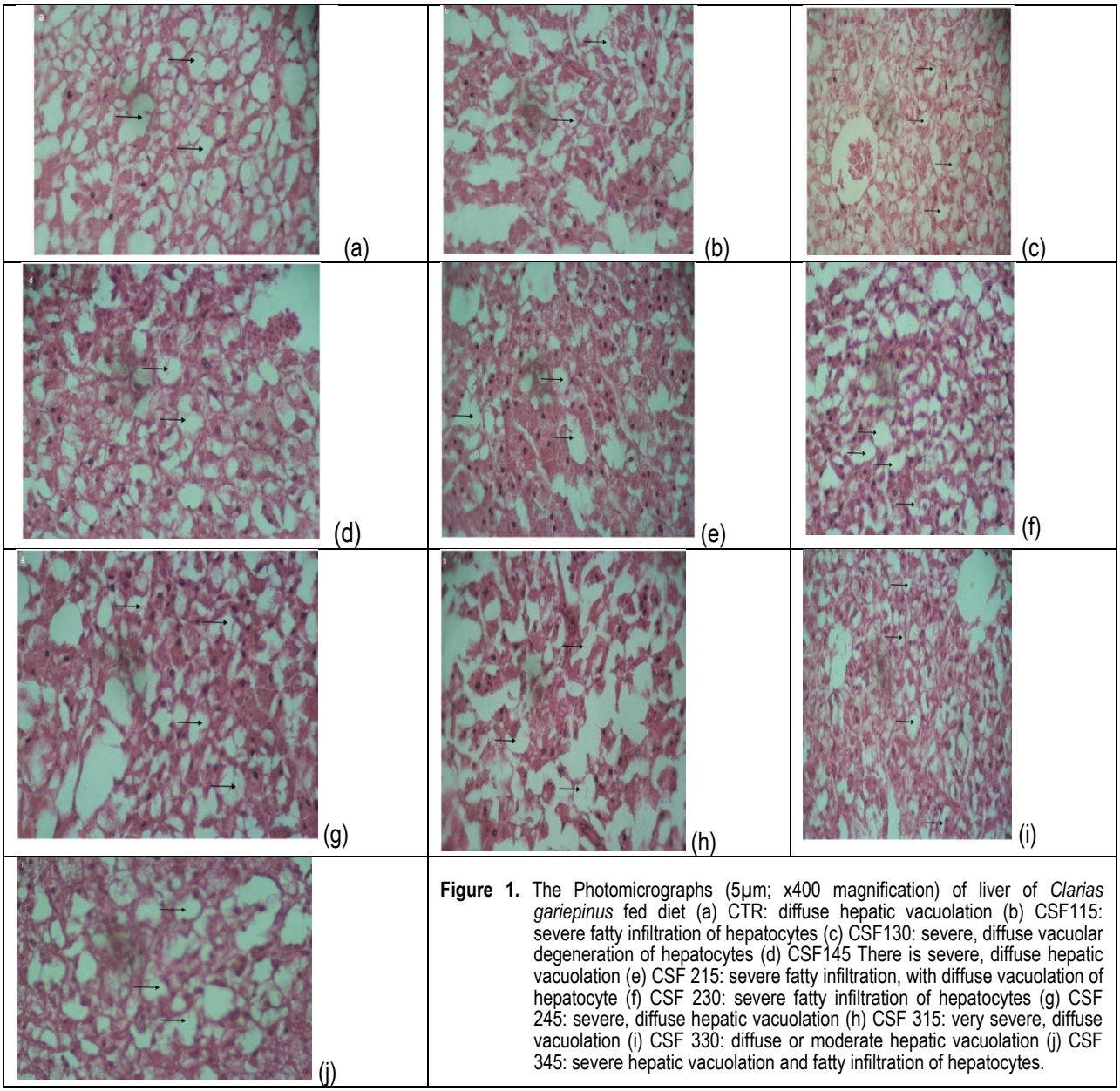

Figure 1. The Photomicrographs (5µm; x400 magnification) of liver of *Clarias gariepinus* fed diet (a) CTR: diffuse hepatic vacuolation (b) CSF115: severe fatty infiltration of hepatocytes (c) CSF130: severe, diffuse vacuolar degeneration of hepatocytes (d) CSF145 There is severe, diffuse hepatic vacuolation (e) CSF 215: severe fatty infiltration, with diffuse vacuolation of hepatocyte (f) CSF 230: severe fatty infiltration of hepatocytes (g) CSF 245: severe, diffuse hepatic vacuolation (h) CSF 315: very severe, diffuse vacuolation (i) CSF 330: diffuse or moderate hepatic vacuolation (j) CSF 345: severe hepatic vacuolation and fatty infiltration of hepatocytes.

DISCUSSION

The best growth performance was recorded in fish fed the soybean-based control diets which was not statistically different from the fish exposed to diets CSF115, CSF130, CSF215 and CSF230. Lower cooking time and inclusion level of sunflower seed meal had a comparable performance with control diets. Our result is in consonance with the observation of Köprücü and Sertel (2012) who also reported the superior growth performance by carp, *Ctenopharyngodon Idella*, fed soybean based control diet over that of fish fed sunflower based diets. The nutrient profile of soybean seed meal is superior to that other plant protein sources (Storebakken, 2000). The reduction in growth performance by *Clarias gariepinus* at higher inclusion level might not be unconnected to higher crude fibre content of sunflower seedmeal. Higher content of crude fibre has been reported to limit its use in aquaculture diets (Hertrampf and Piedad-Pascual, 2012). Other plausible reason could be the presence of chlorogenic acid and caffeic acid; the phenolic compounds that have capacity to reduce protein solubility (González-Pérez and Vereijken, 2007). Crude fibre and these phenolic compounds are present at lower inclusion level but their presence could still be contained by the physiological mechanisms of fish. There is tolerable level of these compounds in fish within which the physiological mechanism of fish will not be impaired (Francis et al., 2001). Other studies that establish lower growth performance at higher inclusion of sunflower seedmeal are Jackson et al. (1982); Tacon et al. (1984); Stickney et al. (1996); Sintayehu et al. (1996); Olvera-Novoa et al. (2002).

Carcass crude protein and carcass crude lipid followed the same trend as observed with growth performance. They reduced with increase in cooking time and inclusion level. No significant variation was recorded in the carcass protein of fish fed CTR, CSF115, CSF130, CSF215, CSF315. This result is in consonance with the finding of Hassaan et al. (2015) who reported decrease in carcass protein with increase inclusion level of fermented soybean meal. Our results are in tandem with the observation of Saha and Ghosh (2013) for rohu, *Labeo rohita* fed *Jatropha curcas* seedmeal and Hassaan et al. (2017) for fermented, de-oiled *Jatropha curcas* fed to *Oreochromis niloticus*. The reduction in carcass lipid content at higher inclusion level observed in this study could be traceable to phenolic compounds, chlorogenic acid, an anti-oxidant that has capacity to reduce deposition of lipid in the carcass (Sun et al., 2017). Similar observation was made by Hassaan et al. (2017). The reduction in lipid level with increasing inclusion level recorded in this study is in consonance with the observation of Zhou et al. (2011). Jimoh et al. (2019) reported similar trends of results when hybrid lemon fin barb (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) was fed diets containing selected leaf meals of dietary high fibre.

The non-significant difference recorded in the apparent protein and lipid digestibility of control diet fed fish group and other test diets' fed groups except those fed diets CSF145, CSF245 and CSF345 gave a better picture of the growth performance trend, the higher the amount of input to metabolism as a result of digestibility, the higher the anabolism activity for protein accretion (Lim et al., 2004). The lower digestibility coefficient recorded for protein and lipid in this study at higher inclusion level irrespective of the processing time employed was in tandem with the report of Hassaan et al. (2017). At higher inclusion level, the non-digestible cellulose content increases, the anti-nutrient component increases making the amino acids in the diet become imbalanced (Eusebio et al., 2004; Zhou et al., 2011) which explains why the digestibility coefficient reduced when compared with control.

No significant differences were recorded in the haemoglobin content, RBC and PCV contents control diet fed group and fish fed test diets; CSF315, CSF115, CSF215, CSF130. Similarly, there was no significant difference in the white blood cell counts of fish group fed the control diet and those fed test diets except CSF345. These are primary indicator of fish physiology; good for knowing the health status of fish (Bahmani et al., 2001). Diet composition can change blood profile (Feist and Longshaw, 2000). The trend of results obtained in this study agrees with Aderolu et al. (2015) who fed degraded rice husk to Nile tilapia. Blom et al. (2001) and Rinchard et al. (2003) also reported a decrease in primary haematological parameters with inclusion of plant protein sources. Possible reason for the trend of haematological parameters recorded in this study might be attributed to the stress created as a result of anti-nutrient, higher fibre content and quality of amino acid content of the diet (Zhou et al., 2011). Although contrast is the report of Jimoh et al. (2015a) who fed *Citrullus lanatus* to *Oreochromis niloticus* that higher profile of haematological parameters were recorded.

Diffuse to severe fatty infiltration of hepatocytes with diffuse to severe vacuolar degeneration of hepatocytes. Severe hepatic vacuolation were recorded in fish fed CSF130,145, CSF245, CSF315 and CSF345. Jimoh et al. (2015b) reported similar trends of results when *Citrullus lanatus* was fed to Nile tilapia (*Oreochromis niloticus*). The pathological condition recorded in this study might be attributable to the presence of anti-nutrients in the feedstuff. Gatta et al. (2011) gave plausible reason to the high vacuolation of the hepatocytes as dietary lipid induced. The presence of high vacuolation of the liver was reported also reported by Valente et al. (2011). Liver is susceptible to damage as a result of its metabolic function of detoxifying xenobiotic compounds. (Nero et al., 2006).

CONCLUSION

The replacement level of soybean meal by sunflower seedmeal that supports optimum performance by *Clarias gariepinus* appear from this study to be up to 30% for 10 and 20 minutes cooked sunflower seedmeal or 15% for 30 minutes cooked sunflower seedmeal.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 353-356 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.05

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Species diversity and dominancy indexes in Izmir Bay (Aegean Sea) purse seine fishery

İzmir Körfezi (Ege Denizi) gırgır balıkçılığında tür çeşitliliği ve baskınlık indeksleri

Ahmet Mert Şenbahar[1]* ● Özlem Güleç[2] ● Zafer Tosunoğlu[3]

[1] Ege University, Faculty of Fisheries, Department of Marine-Inland Waters Sciences and Technology, Izmir, Turkey https://orcid.org/0000-0001-6613-8932
[2] Ege University, Faculty of Fisheries, Department of Fishing and Processing Technology, Izmir, Turkey https://orcid.org/0000-0003-2217-2316
[3] Ege University, Faculty of Fisheries, Department of Fishing and Processing Technology, Izmir, Turkey https://orcid.org/0000-0002-1168-9611

Corresponding author: a.mertsenbahar@gmail.com
Received date: 11.03.2020 *Accepted date: 09.05.2020*

How to cite this paper:
Şenbahar, A.M., Güleç, Ö. & Tosunoğlu, Z. (2020). Species diversity and dominancy indexes in Izmir Bay (Aegean Sea) purse seine fishery. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 353-356. DOI: 10.12714/egejfas.37.4.05

Abstract: Purse seine fishery is known with its importance in Aegean Sea to catch pelagic species. In this study, to determine the diversity index values of species caught by purse seine, all samplings were carried out between September 2, 2017, and April 6, 2018 in Izmir Bay. As a result, a total of 17 fish species (Osteichthyes) belonging to 11 families and also 2 species from invertebrates (Cephalopoda and Arthropoda) were determined. Bony fishes and invertebrates consist of 99.9% and 0.1% of the total biomass, respectively. *Sardina pilchardus* was the most dominated species that occupied as 80.2% of the overall bony fishes followed by *Engraulis encrasicolus* (14.6%) and *Sardinella aurita* (1.5%). Diversity index values of species were found-1.026 by Shannon-Weaver and 0.63 by Simpsons, respectively. The highest dominancy was found for *S. pilchardus* with 71.1%. Overall final results indicate that the diversity of species in Izmir Bay purse seine fishery is very low and also *S. pilchardus* is the most over-dominant species.

Keywords: Izmir Bay, purse seine fishery, diversity, dominancy

Öz: Gırgır balıkçılığı, Ege Deniz'ndeki pelajik türlerin yakalanmasındaki önemiyle bilinmektedir. Bu çalışmada, İzmir Körfezi gırgır balıkçılığında, türlerin çeşitlilik indeks değerlerini belirlemek için 2 Eylül 2017 ile 6 Nisan 2018 tarihleri arasında örneklemeler yapılmıştır. Çalışmada, 11 familya'ya ait toplam 17 balık türü (Osteichthyes) ve ayrıca omurgasızlardan (Cephalopoda ve Arthropoda) 2 adet tür belirlenmiştir. Toplam biyokütlenin %99,9'u kemikli balıklardan, %0,1'i de omurgasızlardan oluştuğu tespit edilmiştir. Toplam av kompozisyonu içinde *Sardina pilchardus*, kemikli balıkların %80,2'sini, bunu sırasıyla %14,6 ile *Engraulis encrasicolus* ve %1,5 ile *Sardinella aurita* izlemiştir. Türlerin çeşitlilik indeks değerleri Shannon-Weaver -1,026 ve Simpsons 0,63 olarak bulunmuştur. %71,1 ile en yüksek baskınlık, *S. pilchardus*'da tespit edilmiştir. Tüm bu sonuçlar, İzmir Körfezi gırgır balıkçılığında tür çeşitliliğinin çok düşük olduğunu, ayrıca *S. pilchardus* türünün de aşırı baskın olduğunu göstermektedir.

Anahtar kelimeler: İzmir Körfezi, gırgır balıkçılığı, çeşitlilik, baskınlık

INTRODUCTION

Izmir Bay is situated at the western coast of the Anatolian peninsula, and is connected to the Aegean Sea. The bay is roughly "L" shaped (Sayın, 2003) and also, one of the important fishing areas for Turkey due to the diversity and abundance of fish with high commercial value (Metin et al., 2000; Cihangir et al., 2001; Cihangir et al., 2004). Due to its nature, Izmir Bay constitutes one of the rich areas of the Aegean Sea in terms of nutrients leading to rich and abundance of the living resources (Ünlüoğlu et al., 2017). Purse seine fishery is not only important for Aegean Sea but also for the Black Sea to catch small pelagic species, especially European anchovy, sardines, Atlantic bonito, bogue, etc. as well as big pelagics such as tunas. In addition, the catch quantity of a purse seiner is too much to compare with other fishing gears (e.g. trawls, seines). Vast majority marine fish landing (approximately 60-70%) supplied by purse seine in 2018 fishing season (Turkstat, 2019). In 2018, 373 purse seine vessels worked on all Turkish seas, but the number of these fishing vessels are between 3-5 for the Izmir Bay. Also, Izmir Bay is also known as an important spawning and nursery ground for many pelagic fish species (Ünlüoğlu et al., 2017).

Species diversity is considered to be main index of the species structure in a community that can be evaluated by information indices and it has been established that the more equal the distribution of species according to their relative abundance in a fish community, the higher is the species diversity in it (Ziliukas, 2005; Korkmaz and Zencir, 2009). However, studies with purse seine fishery are scarce and require much more studies to established sustainable purse seine fishery in all Turkish Seas. For these reasons and necessities, this study aims to reveal diversity and dominancy results of the Izmir Bay purse seine fishery.

MATERIAL AND METHODS

In this study, samplings have been performed 11 times between September 2, 2017, and April 6, 2018, mostly occurred in four locations of Izmir Bay (Figure 1) in depths

between 26 and 60m As a clarification note, sampling was made only for eight months (three seasons) due to the 4/1 notification regulates commercial fishery by the Ministry of Agriculture and Forestry of Turkey. According to the regulation, there is a closed season for purse seine fishery between 15th April and 31st August in all Turkish Seas. The purse seine net used by the commercial purse seiner Afala (24m LOA) is overall 750m in length, 164m net in height and 14mm stretched mesh size.

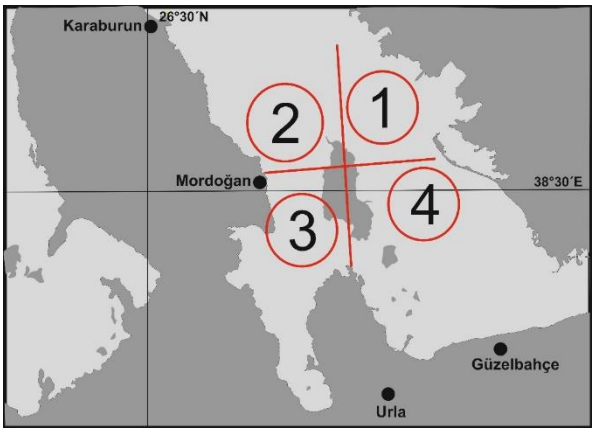

Figure 1. Purse seine fishing cites in Izmir Bay

The relationship between landing and surface water temperature data which are obtained from the global sea temperature website (Seatemp, 2018) was investigated by a linear equation. The index calculations were calculated according to Shannon and Weaver (1949) and Simpson (1949). In determining the diversity index, the basic equation of "$H = -\Sigma P_i \log_2 P_i$" was used, in the estimations; $P_i = n_i . q_i$ (kg) / $\Sigma n_i.q_i$, where (n_i) is the number of individual of i, (q_i) is average weight of i and ($_i$) are numerical codes of species. In Simpson's index equation "$Is = \sum N_i (N_i-1) / N(N-1)$", where ($N_i$) is the number of individuals of i and (N) is total individuals. In the general dominancy equation, which is $D = (n_i / N) *100$, the letter values represent the same as above. The total number of each fish species was calculated by the dividing of number of measured fish by the sampling ratio (sampled weight / total weight).

RESULTS

During the fishing period, surface water temperature (Seatemp, 2018) which is directly related to the obtained species quantity were investigated to find a relationship (Figure 2). It has been clearly shown that this relation decreased in the month of January and February and it completely deteriorated in March and April. However, as a weak linear relationship (0.38) between the water temperature and the landing of species was found (Figure 3). On the other hand, the relationship between working days in seasons, surface water temperature and landed fish has been shown parallelism naturally. As a result of annual landing report, a total of 17 fish species (Osteichthyes) belonging to 11 families and also 2 species from invertebrates (Cephalopoda and Arthropoda) were obtained (Table 1).

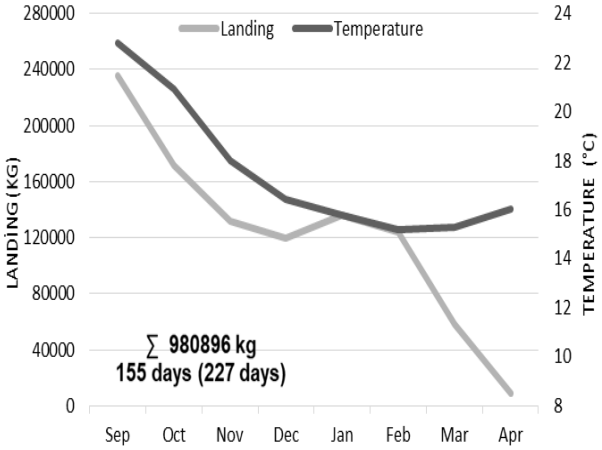

Figure 2. Relationship between monthly landing and water surface temperature in Izmir Bay

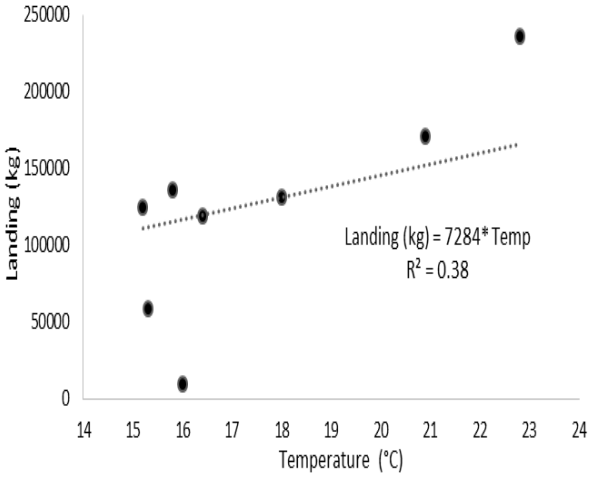

Figure 3. The linear relationship between the amount of landed fish and sea surface temperature

Table of index values of Izmir Bay has been obtained in Table 2 and Shannon-Weaver and Simpson's index values were found as -1.026 and 0.63, respectively. As a dominancy result, *S. pilchardus* landing compose of the vast majority with 71.1% and this is indicating that one of the most landed species and there is no other such species richness in the fishing area. Additionally, the percentage of the species proves that fact 99.9% of the total biomass has belonged to bony fishes and 0.1% belong to invertebrates. Within the data, *S. pilchardus* compose of 80.2% of the overall bony fishes followed by *E. encrasicolus* 14.6% and *S. aurita* as 1.5%, respectively. Considering the data, the abundance of clupeid and engraulid species is remarkable other than the rest of the marine specimens (Table 2).

Table 1. Annual landing data of the specimens

Species	Family	Average box weight (kg)	Average number of fish in a box	Annual ∑ kg	Percent
Pisces					
Osteichthyes					
Sardina pilchardus	Clupeidae	14 kg	1105	786731	80.205
Sardinella aurita	Clupeidae	14 kg	229	15302	1.560
Engraulis encarasicolus	Engraulidae	15 kg	2563	143947	14.675
Sarda sarda	Scombridae	10 kg	12	7370	0.751
Scomber scombrus	Scombridae	13 kg	67	10073	1,027
Scomber japonicus	Scombridae	13 kg	52	518	0.053
Auxis rochei	Scombridae	8 kg	12	8	0.001
Belone belone	Belonidae	12 kg	228	960	0.098
Sarpa salpa	Sparidae	13 kg	13	456	0.046
Sparus aurata	Sparidae	14 kg	75	1603	0.163
Boops boops	Sparidae	12 kg	532	5694	0.580
Coryphaena hippurus	Coryphaenidae	10 kg	5	20	0.002
Pomatomus saltatrix	Pomatomidae	12 kg	20	226	0.023
Sphyraena sphyraena	Sphyraenidae	12 kg	35	180	0.018
Liza aurata	Mugilidae	13 kg	15	5642	0.575
Dicentrarchus labrax	Moronidae	13 kg	13	173	0.018
Trachurus trachurus	Carangidae	12 kg	233	966	0.098
Cephalapoda					
Loligo vulgaris	Loliginidae	15 kg	30	153	0.016
Arthropoda					
Penaeus kerathurus	Penaeidae	8 kg	280	875	0.089
∑				**980896**	**100**

Table 2. Diversity and dominancy index values of species in Izmir Bay purse seine fishery

Species	Family	n_i	q_i (kg)	$n_i.q_i$	p_i	$p_i \log p_i$	Shannon - Weaver Index	Simpson's Index	Dominancy
Pisces									
Osteichthyes						$P_i = n_i \cdot q_i$ (kg) / $\Sigma n_i.q_i$	$H = -\Sigma P_i \log_2 P_i$	$/s = \sum N_i (N_i-1) / N(N-1)$	$D = (n_i / N) * 100$
Sardina pilchardus	Clupeidae	62095530	0.013	786730.697	0.802	0.077			71.095
Sardinella aurita	Clupeidae	250297	0.061	15302.000	0.016	0.028			0.287
Engraulis encarasicolus	Engraulidae	24595795	0.006	143947.298	0.147	0.122			28.161
Sarda sarda	Scombridae	8844	0.833	7370.000	0.008	0.016			0.010
Scomber scombrus	Scombridae	51915	0.194	10073.060	0.010	0.020			0.059
Scomber japonicus	Scombridae	2072	0.250	518.000	0.001	0.002			0.002
Auxis rochei	Scombridae	12	0.667	8.000	0.000	0.000			0.000
Belone belone	Belonidae	18240	0.053	960.000	0.001	0.003			0.021
Sarpa salpa	Sparidae	456	1.000	456.000	0.000	0.002			0.001
Sparus aurata	Sparidae	8588	0.187	1603.093	0.002	0.005			0.010
Boops boops	Sparidae	252434	0.023	5694.000	0.006	0.013			0.289
Coryphaena hippurus	Coryphaenidae	10	2.000	20.000	0.000	0.000			0.000
Pomatomus saltatrix	Pomatomidae	376	0.600	225.600	0.000	0.001			0.000
Sphyraena sphyraena	Sphyraenidae	525	0.343	180.000	0.000	0.001	**-1.026**	**0.631**	0.001
Liza aurata	Mugilidae	6510	0.867	5642.000	0.006	0.013			0.007
Dicentrarchus labrax	Moronidae	173	1.000	173.000	0.000	0.001			0.000
Trachurus mediterraneus	Carangidae	18757	0.052	966.026	0.001	0.003			0.021
Cephalapoda									
Loligo vulgaris	Loliginidae	305	0.500	152.500	0.000	0.001			0.000
Arthropoda									
Penaeus kerathurus	Penaeidae	30625	0.029	875.000	0.001	0.003			0.035

DISCUSSION

The overall result of the diversity index value is generally expected to be between 0 and 5 (Balık et al., 2011). If the value increases, it is concluded that there is a species richness in the sampled environment. For this reason, dominancy is directly depended on the species diversity resulted in higher landing. So as to diversity index value was found weak in the Izmir Bay purse seine fishery, Simpson's index value was determined as 0.63 in accordance with the crosscheck of the fishery. All these results showed that the diversity of species is low in Izmir Bay purse seine fishery, consequently single species *S. pilchardus* is dominant.

The linear relationship between water temperature and landed species did not reveal a solid correlation. In addition, a consistent relationship for diversity index has been not found. However, Izmir Bay exchanges water with the Aegean Sea almost the whole year long (Sayın et al., 2006) and it would be wrong to conclude that this situation is the same for the throughout the whole Aegean Sea.

During the sampling, it has been reported of some species such as *Dentex gibbosus*, *Pagellus erythrinus*, *Thunnus thynnus*, *Mustelus mustelus*, *Xiphias gladius* and *Octopus vulgaris* obtained during some fishing operations but the quantity of the specimens was not quite abundant (almost 1 or 2 individual for the whole year long) in the field. Therefore, it was not important to add to the estimations of the diversity index for the Izmir Bay. Besides, sampling methodology is another substantial factor to detect/obtain the specimens which are local and abundant animals within the sea. Thereto, purse seine nets are creating a limitation for sampling while trawl net and also other fishing gears independently cause different results which are depending on sampled specimens. Purse seine fishing tends to capture pelagic marine species in worldwide and purse seine nets are very important fishing gear used for capturing pelagic fishes (Demirci and Demirci, 2006). In this present study, all the identified and captured individuals are belonging upper pelagic zone community except *Penaeus kerathurus*. Apart from this, due to the inability to capture demersal and mesopelagic specimens are the main obstacle to unveil for all trophic levels of Izmir Bay. Thus, the expected studies of diversity index mainly tend to be established in minor fields or smaller areas. Mainly, it is a difficult task to gather all the data and agglomerate the fish quantity of the specimens per year. As a descriptive conclusion within the major field such as this study, we have done the calculations with only one annual landing report (∑kg) and converted it to approximately average fish quantity in a single styrofoam box. Eventually, the fisher's reports/observations and our estimations have been shown parallelism on the diversity, abundance and dominance for the Izmir Bay.

ACKNOWLEDGEMENTS

We would like to thank Ege University Scientific Research Project Coordination Unit (Project No.2017/SUF/002), project researcher and Afala purse seiner staff.

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Ege Journal of Fisheries and Aquatic Sciences, 37(4), 357-361 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.06

ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Ege Üniversitesi Su Ürünleri Fakültesi Müzesi (ESFM)'nin cephalopod envanteri

Ege University Faculty of Fisheries Museum (ESFM) cephalopod inventory

Alp Salman[1]* ● Cem İzmirli[2]

[1] Ege Üniversitesi Su Ürünleri Fakültesi Bornova İzmir
[2] Ege Üniversitesi Su Ürünleri Fakültesi Bornova İzmir

https://orcid.org/0000-0002-2853-6507
https://orcid.org/0000-0003-1362-8991

Corresponding author: alp.salman@ege.edu.tr

Received date: 13.03.2020 *Accepted date: 15.05.2020*

How to cite this paper:
Salman, A. & İzmirli, C. (2020). Ege Üniversitesi Su Ürünleri Fakültesi Müzesi (ESFM)'nin cephalopod envanteri. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 357-361. DOI: 10.12714/egejfas.37.4.06

Öz: Bu çalışmada 1988 yılından 2015 yılına kadar yapılan bilimsel çalışmalarda örneklenen cephalopod bireyleri Ege Üniversitesi Su Ürünleri Fakültesi Müzesi (ESFM) ne kaydedilmiş ve listeleri çıkarılmıştır. Müzede incelenen cephalopod türleri Marmara denizi, Ege Denizi, Akdeniz ve Kuzey Kıbrıs sularından elde edilmiştir. Bu çalışmaların sonucunda Türkiye'yi çevreleyen denizlerden 46 türe ait Sepiida ordosundan 58 adet, Sepiolida ordosundan 230 adet, Teuthida ordosundan 106 adet ve Octopoda ordosundan 146 adet saklama kavanozu olmak üzere toplamda 46 cephalopod türü örnek kavanozunda saklanmaktadır. Müzede bulunan cephalopod türleri öncelikle temizlenerek, bilinen 4 adet fiksasyon yöntemleri arasında en uygun olan formalinle fiksasyon yöntemi kullanılarak kavanozlara fikse edilmişlerdir. Daha sonra materyallerin bulunduğu kavanozların üzerine örneklemelere ait gerekli bilgiler etiketlenip ESFM müze kaydı gerçekleştirilmiştir ve müzede uygun koşullarda muhafaza edilip sergilenmektedir.

***Anahtar kelimeler*:** Cephalopoda, fiksasyon yöntemleri, ESFM, Ege Üniversitesi

Abstract: In this study, cephalopod individuals exemplified in scientific studies from 1988 to 2015 were registered and listed in the Ege University Faculty of Fisheries Museum (ESFM). Cephalopod species examined in the museum were obtained from the Marmara Sea, the Aegean Sea, the Mediterranean and Northern Cyprus waters. In this study, cephalopod individuals exemplified in scientific studies from 1988 to 2015 were registered and listed in the Ege University Faculty of Fisheries Museum (ESFM). These studies showed that Turkey seas surrounding the 46 species of sepiida orders from 58 pieces, the orders in Sepiolida 230, Teuthida orders of 106 pieces and order Octopoda total 146 storage in a jar. As a result of this study surrounding Turkey seas it has been identified 46 species of cephalopods.

The cephalopod types found in the museum were first cleaned and fixed to the jars using the formalin fixation method, which is the most suitable of the 4 known fixation methods, and then the necessary information regarding the sampling was labeled on the jars and the ESFM museum registration was performed.

***Keywords*:** Cephalopods, preserved methods, ESFM, Ege University

GİRİŞ

Günümüzde birçok müze hem turizm amaçlı hem de bilimsel kaynak oluşturması adına önemli yerler olarak görülmektedir. Bu müzeler arasında, üniversitelere ait müzeler de hem eğitim amaçlı hem de gelecekte yapılacakçalışmalar için materyallerin korunması amaçlı önemli bir yer tutmaktadır. Ege Üniversitesi bünyesinde bulunan, Su Ürünleri Fakültesi Müzesi (ESFM) de bu amaçla resmi olarak 19.03.2007'de kurulmuştur. ESFM Müzesi materyalleri, 1965 yılında Prof. Dr. Remzi GELDİAY öncülüğünde, deniz biyolojisi araştırma ve uygulama laboratuvarının kurulmasıyla oluşturulmuştur. Müzede koruma altına alınan bireyler 1930 yılından günümüze kadar uzanan, ülkemizin ve Kuzey Kıbrıs'ın iç sularından ve kıyılarında yapılan bilimsel seferlerde toplanan daha sonra tayinleri yapılıp etiketlenmiştir (ESFM, 2016).

Ege Su Ürünleri Fakültesi Müzesi (ESFM), envanter zenginliği açısından ülkemizin en büyük müzesidir ve uluslararası öneme sahiptir. ESFM materyalleri kullanılarak toplamda 75 uluslararası makale üretilmiştir ve bunların 55 tanesi Science Citation Index kapsamındaki dergilerde yayınlanmıştır. Ayrıca E.Ü Fen Bilimleri Enstitüsü Temel Bilimlere kayıtlı olan yüksek lisans ve doktora yapan öğrencilerin hazırladığı tezlerin biyolojik materyalleri de müzede kayıtlı olarak saklanmaktadır (ESFM 2016). Ege Üniversitesi Su Ürünleri Fakültesi'ne bağlı ESFM' nde ise; 120 deniz balığı türü, 154 içsu balığı türü, 1220 eklem bacaklılar (Arthropoda), 1010 halkalı solucanlar (Annelida), 900 yumuşakçalar (Mollusca), 300 fitoplankton, 50 sünger türü (Porifera) ve 300 diğer omurgasız türü (Cnidaria, Nemertini, Echinodermata, Bryozoa vb.) olmak üzere yaklaşık 4100 türe ait bireyler bulunmaktadır (ESFM, 2016). Ülkemizde yapılan araştırma ve proje çalışmalarının sonucunda toplam 51 cephalopod türü elde edilmiştir (Öztürk vd. 2014). ESFM müzesinde ise bu türlere ait 46 tür bulunmaktadır. Bu türler içerisinde Bello ve Salman (2015) tarafından dünya cephalopod faunasına yeni tür olarak katılan *Sepiola boletzkyi* türü ise tip örnek olarak ESFM müzesinde bulunmaktadır.

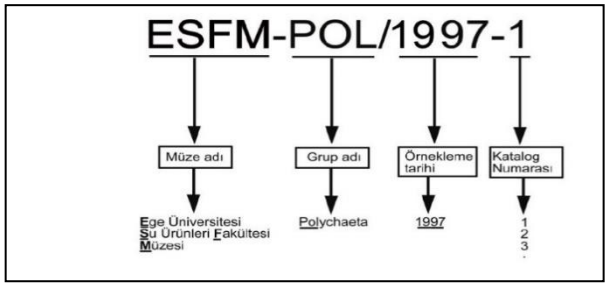
Bu çalışma Ege Üniversitesi Su Ürünleri Müzesinde bulunan cephalopod türleri üzerine yapılmıştır. Müzede bulunan cephalopod türleri temizlenip, yeniden fiksasyon solüsyonu değiştirilmiş ve müze kurallarına göre etiketlenerek bilgisayar ortamında kaydı alınıp uygun koşullar altında saklanmıştır. ESFM Müzesinde bulunan bu materyallerin toplanmasının amacı, dergilerde yayınlanmıştır. Ayrıca E.Ü Fen Bilimleri Enstitüsü Temel Bilimlere kayıtlı olan yüksek lisans ve doktora yapan öğrencilerin hazırladığı tezlerin biyolojik materyalleri de müzede kayıtlı olarak saklanmaktadır (ESFM 2016). Ege Üniversitesi Su Ürünleri Fakültesi'ne bağlı ESFM' nde ise; 120 deniz balığı türü, 154 içsu balığı türü, 1220 eklem bacaklılar (Arthropoda), 1010 halkalı solucanlar (Annelida), 900 yumuşakçalar (Mollusca), 300 fitoplankton, 50 sünger türü (Porifera) ve 300 diğer omurgasız türü (Cnidaria, Nemertini, Echinodermata, Bryozoa vb.) olmak üzere yaklaşık 4100 türe ait bireyler bulunmaktadır (ESFM, 2016). Ülkemizde yapılan araştırma ve proje çalışmalarının sonucunda toplam 51 cephalopod türü elde edilmiştir (Öztürk vd. 2014). ESFM müzesinde ise bu türlere ait 46 tür bulunmaktadır. Bu türler içerisinde Bello ve Salman (2015) tarafından dünya cephalopod faunasına yeni tür olarak katılan *Sepiola boletzkyi* türü ise tip örnek olarak ESFM müzesinde bulunmaktadır.

Bu çalışma Ege Üniversitesi Su Ürünleri Müzesinde bulunan cephalopod türleri üzerine yapılmıştır. Müzede bulunan cephalopod türleri temizlenip, yeniden fiksasyon solüsyonu değiştirilmiş ve müze kurallarına göre etiketlenerek bilgisayar ortamında kaydı alınıp uygun koşullar altında saklanmıştır. ESFM Müzesinde bulunan bu materyallerin toplanmasının amacı, bilimsel çalışmalarla denizlerimizden çıkan cephalopod türlerinin bir haritasını çıkarmak ve cephalopod faunasına ait bireylerin ileride yapılacak olan çalışmalar için karşılaştırma materyali olmasını sağlamaktır. Bu nedenle müze materyalinin gelecekte yapılacak çalışmalara da ışık tutacağı düşünülmüştür.

MATERYAL VE METOT

Formalin (HCHO); Ticari şekli %37'lik saf Formaldehit çözeltisi olarak satılmaktadır. Formalinin fiksatiflik özelliği yakın protein zincirleri arasında çapraz bağlar oluşturmasından (denaturizasyon) kaynaklanır (Roper ve Sweeney, 1983). Kullanılacak fiksatifin yoğunluğu fikse edilecek örneğin doku sıklığına bağlıdır. Cephalopodların vücut yapıları yüksek derecede protein içerdiklerinden %10'luk formalin çözeltisi ile fikse edilmeleri daha uygun olmaktadır. Bu işlem %37'lik saf formaldehite 1:9 oranında suyla karıştırılmasıyla %10'luk formalin çözeltisi elde edilerek gerçekleştirilir. Gerçekleştirilen ilk fiksasyon işleminin ardından örneklerin saklama işleminde de %10'luk formalin çözeltisi kullanılmaktadır. Bu çalışmada elde edilen cephalopod materyallerinin önce üzerleri temizlenerek eğer var ise çamurlu yapılardan arındırıldıktan sonra uygun biçimde kavanozlara yerleştirilmiştir. Saklama amacı ile kullanılacak formalin öncelikle tamponlanmalıdır. Aksi takdirde formalin çözeltisi sarı-kahve renkte formik aside dönüşerek örnek üzerindeki kitinize veya kalsifiye özellikteki sistematik karakterleri aşındırarak veya eriterek bozulmalarına sebep olmaktadır (Roper ve Sweeney, 1983). Bunun için formalin çözeltinin içine Na-Borax, $CaCO_3$ veya Hexamin gibi tampon maddeler eklenir. Uygun koşullarda saklanmasında oksidasyon ve asidifikasyonun önüne geçilebilmesi için formalinin hava ile temasını kesmek için tüm kapların çok sıkı bir şekilde kapatılması gerekmektedir.

ESFM'de bulunan cephalopod türleri örnekleme sonrasında %10'luk formalin çözeltisinde fikse edilmişler ve sonrasında ise yenilenen formalin çözeltisinde saklanmaya devam edilmişlerdir. Formalin ile saklama uzun süreli saklamalarda büyük bir avantaja sahip olmasa da ucuz ve kullanışlı bir yöntemdir. Her bir örnek kabının üzerine ESFM'ye ait kod yazılıp (Şekil 1) örnek kabına yapıştırıldıktan sonra kabın içerisine plastik-kâğıt özelliğinde etiket kullanmak sureti ile örnekleme bilgileri yazılmıştır.



Şekil 1. ESFM örnekleme etiketi
Figure 1. ESFM sampling label

BULGULAR

Türkiye'yi çevreleyen denizlerden daha önceki çalışmalarda rapor edilen 51 cephalopod türü tablo 1'de verilmiş olup bu türler içerisinde *Alloteuthis subulata* türü ESFM de bulunmamaktadır. *A. subulata* türünün dışında kalan diğer dört tür ise (*Brachioteuthis riisei, Chtenopteryx sicula, Onychoteuthis banksi, Thysanoteuthis rhombus*) paralarval safhada bireyler olarak tespit edilmiş olup Salman vd. (2003) tarafından rapor edilmiş fakat ESFM müzesine henüz kayıtlanmamıştır. Diğer türlerin ise müzede kayıtlı olduğu bölgeler (*) ile işaretlenerek Tablo 1'de belirtilmiştir.

ESFM'de bulunan ordolara ait örneklerin yıllara göre dağılımına baktığımızda Ege Denizi'nde Katağan ve Kocataş (1990) tarafından başlatılan ilk ulusal cephalopod çalışmasındaki örneklerden itibaren Türkiye'yi çevreleyen denizlerde günümüze kadar geçen süreç içerisinde çeşitli projelerde ve araştırma seferlerinde toplanış olan cephalopod türleri müze materyali olarak kayıtlanmıştır. ESFM'de kayıtlı olan cephalopod ordolarına ait örneklerin yıllara göre dağılımları Tablo 2,3,4,5'te verilmiştir. Müze genelinde cephalopod ordolarına göre toplam 540 adet kavanoz örnek bulunmaktadır.

ESFM'de kayıtlı olan *Semirossia patagonica* türü ise doktora tezi çalışmasının bir bölümünü yurt dışında yapan Önsoy (2007) tarafından E.Ü. Su Ürünleri Fakültesi'ne getirilmiş ve daha sonra müze kayıtlarına geçirilmiş Güney Atlantik okyanusunda dağılım gösteren cephalopod türlerinden birisidir.

Tablo 1. Türkiye denizlerinde dağılım gösteren Cephalopod türleri (Öztürk vd. 2014)
Table 1. Cephalopod species distributions in Turkey seas (Öztürk et al., 2014)

Fam Sepiidae	Marmara	Ege	Akdeniz
Sepia officinalis Linnaeus, 1758	+	+*	+*
Sepia elegans de Blainville, 1827	+*	+*	+*
Sepia orbignyana Férrussac, 1826	+*	+*	+*
Fam Sepiolidae			
Sepiola rondeletii Leach, 1817	+	+*	
Sepiola intermedia Naef, 1912		+*	
Sepiola ligulata Naef, 1912		+*	
Sepiola robusta Naef, 1912		+*	
Sepiola steenstrupiana Lévy, 1912			+*
Sepiola boletzkyi Bello & Salman 2015		+*	
Rondeletiola minor (Naef, 1912)	+	+*	+*
Sepietta oweniana Naef, 1916	+*	+*	+*
Sepietta neglecta Naef, 1916	+*	+*	+*
Sepietta obscura Naef, 1916	+*	+*	
Rossia macrosoma (Delle Chiaje, 1830)		+*	+*
Neorossia caroli (Joubin, 1902)		+*	
Heteroteuthis dispar (Rüppell, 1844)		+*	
Fam Loliginidae			
Loligo vulgaris Lamarck, 1798	+	+*	+*
Loligo forbesi Steenstrup, 1856		+*	+*
Alloteuthis media (Linnaeus, 1758)	+*	+*	+*
Alloteuthis subulata (Lamarck, 1798)		+	+
Sepioteuthis lessoniana Lesson, 1830			+*
Fam Ancistrocheiridae			
Ancistrocheirus lesueurii (d'Orbigny, 1842)		+*	
Fam Brachioteuthidae			
Brachioteuthis riisei (Steenstrup, 1882)		+	
FamChiroteuthidae			
Chiroteuthis veranii (Férussac, 1835)	+	+*	
Fam Chtenopterygidae			
Chtenopteryx sicula (Vérany, 1851)		+	
Fam Enoploteuthidae			
Abralia veranyi (Rüppell, 1844)		+*	+*
Abraliopsis morisii (Vérany, 1839)		+*	
Fam Histioteuthidae			
Histioteuthis bonnellii (Férrussac, 1835)		+*	+*
Histioteuthis reversa (Vérrill, 1880)		+*	
Fam Octopoteuthidae			
Octopoteuthis sicula Rüppell, 1848		+*	+
Fam Ommastrephidae			
Illex coindetii (Vérany, 1839)	+*	+*	+*
Todaropsis eblanae (Ball, 1841)	+*	+*	+*
Todarodes sagittatus (Lamarck, 1798)	+	+*	+*
Ommastrephes bartramii (Lesueur, 1821)		+*	
Fam Onychoteuthidae			
Onychoteuthis banksi (Leach, 1817)		+	
Ancistroteuthis lihctensteini (d'Orbigny, 1839)		+*	
Fam Pyroteuthidae			
Pyroteuthis margaritifera (Rüppell, 1844)		+*	+
Fam Thysanoteuthidae			
Thysanoteuthis rhombus Troschel, 1857		+	
Fam Octopodidae			
Amphioctopus aegina (Gray, 1849)			+*
Callistoctopus macropus (Risso, 1826)	+*	+*	+*
Macrotritopus defilippi (Vérany, 1851)			+*
Octopus vulgaris Cuvier, 1797	+*	+*	+*
Octopus salutii Vérany, 1837		+*	
Scaeurgus unicirrhus (Delle Chiaje in de Férussac & d'Orbigny, 1841)		+*	+*
Eledone moschata (Lamarck, 1799)	+*	+*	+*
Pteroctopus tetracirrhus (Delle Chiaje, 1830)		+*	+*
Eledone cirrhosa (Lamarck, 1798)	+	+*	+*
Bathypolypus sponsalis (Fischer, P. & Fischer, H. 1892)		+*	
Fam Tremoctopodidae			
Tremoctopus violaceus Delle Chiaje, 1830		+*	
Fam Argonautidae			
Argonauta argo Linnaeus, 1758		+*	
Fam Ocythoidae			
Ocythoe tuberculata Rafinesque, 1814		+*	

ESFM müzesinde bulunan ergin bireylere ait örnekler (*) ile işaretlenmiştir.

Tablo 2. ESFM Müzesine bulunan Sepiida ordosuna ait örneklerin yıllara göre dağılımı
Table 2. Distribution of samples of order Sepiida found in ESFM Museum by years

SEPIIDA	1988	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2002	2003	2004	2006	2007	2008	2009	2011	2012	2015	Toplam
Sepia officinalis	-	-	1	-	-	-	-	-	4	1	-	2	-	-	-	-	3	2	1	-	-	14
Sepia elegans	-	1	3	4	1	-	-	-	6	6	-	1	1	-	-	-	1	2	-	-	-	26
Sepia orbignyana	-	-	1	8	1	-	-	-	3	4	-	1	-	-	-	-	-	-	-	-	-	18
Toplam		**1**	**5**	**12**	**2**				**13**	**11**		**4**	**1**				**4**	**4**	**1**			**58**

Tablo 3. ESFM Müzesine bulunan Sepiolida ordosuna ait örneklerin Yıllara göre dağılımı
Table 3. Distribution of samples of order Sepiolida found in ESFM Museum by Years

SEPIOLIDA	1988	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2002	2003	2004	2006	2007	2008	2009	2011	2012	2015	Toplam
Sepiola rondeletii	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	2
Sepiola intermedia	1	-	1	-	-	-	-	1	1	-	-	-	-	-	-	3	3	5	-	-	-	15
Sepiola ligulata	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Sepiola robusta	-	-	1	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	-	-	-	4
Sepiola steenstrupiana	-	-	-	-	-	-	1	-	2	1	-	-	-	-	-	-	-	-	-	-	-	4
Sepiola boletzkyi	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Rondeletiola minor	-	1	14	11	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	27
Sepietta oweniana	-	3	28	42	3	-	1	2	-	-	-	-	1	-	-	2	4	2	-	-	-	88
Sepietta neglecta	-	1	7	10	2	-	-	1	-	-	-	-	-	-	-	1	1	-	-	-	-	23
Sepietta obscura	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	3
Rossia macrosoma	-	-	7	19	3	-	-	-	-	-	-	-	-	-	-	1	9	3	-	-	-	42
Neorossia caroli	-	-	3	1	-	-	-	-	-	-	-	-	-	-	-	-	10	3	-	-	-	17
Heteroteuthis dispar	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Semirossia patagonica	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Toplam	**1**	**5**	**62**	**84**	**9**	**2**	**2**	**7**	**3**	**2**			**2**	**1**		**8**	**29**	**13**				**230**

Tablo 4. ESFM Müzesine bulunan Teuthida ordosuna ait örneklerin yıllara göre dağılımı
Table 4. Distribution of samples of order Teuthida found in ESFM Museum by years

TEUTHIDA	1988	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2002	2003	2004	2006	2007	2008	2009	2011	2012	2015	Toplam
Loligo vulgaris	-	2	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	2	2	-	-	8
Loligo forbesii	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	3	-	-	-	-	5
Alloteuthis media	-	2	4	5	2	-	1	3	3	2	-	-	1	-	-	-	-	2	2	-	-	27
Sepioteuthis lessoniana	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	2
Ancistrocheirus lesueuri	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Chiroteuthis veranyi	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Abralia veranyi	-	-	5	4	-	-	-	-	-	-	-	-	1	-	-	-	4	4	-	1	-	19
Abraliopsis morisii	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Histioteuthis bonnellii	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2
Histioteuthis reversa	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Octopoteuthis sicula	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Illex coindetii	-	2	1	2	-	-	-	-	4	1	-	-	-	-	-	-	1	-	-	-	-	11
Todaropsis eblanae	-	1	1	6	1	-	-	1	1	-	-	-	1	-	-	-	-	-	-	-	-	12
Todarodes sagittatus	-	1	1	2	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	1	7
Ommastrephes bartramii	-	4	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	5
Ancistroteuthis lichtensteini	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Pyroteuthis margaritifera	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Toplam		**1**	**13**	**16**	**19**	**5**		**1**	**5**	**9**	**4**		**3**	**6**		**1**		**8**	**9**	**4**	**1**	**1**	**106**

Tablo 5: ESFM Müzesine bulunan Octopoda ordosuna ait örneklerin Yıllara göre dağılımı
Table 5. Distribution of samples of order Octopoda found in ESFM Museum by Years

OCTOPODA	1988	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2002	2003	2004	2006	2007	2008	2009	2011	2012	2015	Toplam
Amphioctopus aegina	-	-	1	4	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	6
Callisoctopus macropus	-	-	-	2	1	-	2	-	2	-	-	-	2	-	-	-	-	-	-	-	-	9
Macrotritopus defilippi	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Octopus vulgaris	-	-	3	2	-	-	-	-	1	3	-	1	-	-	-	1	3	2	-	-	-	16
Octopus salutii	-	-	4	3	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-	10
Scaeurgus unicirrhus	-	-	7	8	1	-	-	-	1	1	-	1	1	-	-	1	6	1	-	-	-	28
Eledone moschata	-	-	3	2	2	-	-	-	-	-	-	2	-	-	-	1	4	1	-	-	-	15
Pteroctopus tetracirrhus	-	-	2	1	1	-	-	1	-	-	-	-	-	-	-	1	9	3	-	-	-	18
Eledone cirrhosa	-	-	7	6	2	-	-	-	-	-	-	-	-	-	2	-	7	3	-	-	-	27
Bathypolypus sponsalis	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Tremoctopus violaceus	-	1	1	1	-	-	-	1	-	-	-	1	1	-	1	-	-	-	-	-	-	7
Argonauta argo	-	1	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Ocythoe tuberculata	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	2
Toplam		**1**	**31**	**30**	**7**		**2**	**2**	**4**	**5**	**1**	**4**	**4**		**2**	**5**	**31**	**10**				**146**

SONUÇ

Türkiye Denizlerinde günümüze dek yapılan çalışmalar sonrasında elde edilen Cephalopod türlerinin bölgelere göre dağılımları Akdeniz'de 27 adet, Ege Denizi'nde 47 adet ve Marmara Denizi'nde ise 18 adet olmak üzere toplamda 51 Cephalopod türü gözlemlenmiştir. (Öztürk vd, 2014). Karadeniz'den hiçbir örnekleme yapılamamıştır, bunun sebebi ise Karadeniz'deki tuzluluğun Cephalopod türlerinin yaşayabileceği orandan daha az olmasından kaynaklanmaktadır (Jereb ve Roper, 2005).

Yukarıdaki söz konusu araştırmaların sonucunda Ege Üniversitesi Su Ürünleri Fakültesi Müzesi'nde (ESFM) toplanan Cephalopod türlerine ait bireyler Akdeniz'den 25 tür, Ege Denizi'nden 41 tür ve Marmara Denizi'nden 11 tür olmak üzere toplamda 46 tür %10 formalin solüsyonunda fikse edilip özenle saklanmaktadır (Tablo 1).

2017 verilerine göre ülkemizde bulunan yaklaşık 19 adet Su Ürünleri Fakülteleri ve Yüksekokullarına ait müzelerde de birçok denizel canlı türleri barındırılmaktadır. Yapılan bu çalışmanın sonuçlarına göre ülkemizdeki üniversitelerin veya yüksekokulların büyük çoğunluğunda müze yoktur veya var olan müzelerin de bir kısmı kapatılmıştır (İstanbul Üniversitesi Fen Fakültesi Hidrobiyoloji Araştırma Enstitüsü Müzesi). Müzesi olan üniversitelerde ise yeterli sayıda cephalopod türü örnekleri bulunmamaktadır. Bunun sebebi ise ülkemizde müze anlayışının yeni yeni oturması, denizlerimizde yapılan bilimsel çalışmaların azlığı ve yapılacak seferler için ayrılan bütçenin azlığından kaynaklanmaktadır. Müzedeki eksiklerin giderilmesi öğrencilerin eğitimi açısından ve gelecekte yapılacak olan bilimsel çalışmalara temel olabilmesi ve günümüz faunasının belirlenebilmesi açısından çok önemlidir.

Müze envanterlerinin ortaya çıkarılması, müzelerin eğitim ve öğretime, yapılacak bilimsel çalışmalara, faunanın belirlenmesine, denizlerimizdeki mevcut türlerin karşılaştırılmasına (taksonomik, anatomik vb.), ulusal ve uluslararası araştırmacıların mevcut türleri inceleyebilmeleri bakımından çok büyük önem arz etmektedir. Bu çalışma bu açıdan irdelendiğinde bir ilk oluşturmakta ve önem kazanmaktadır.

Sonuç olarak farklı kurumlara ait olan müzelerin bulundurduğu fauna ve flora ya ait türlerin bilgisayar ortamına girdikten sonra ortak bir veri tabanı altında birleştirilmeleri ülkemizin biyolojik çeşitliliğinin tespit edilebilmesi için yararlı olacağı düşüncesindeyiz.

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Ege Journal of Fisheries and Aquatic Sciences, 37(4), 363-371 (2020)

Su Ürünleri Dergisi (2020)
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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The impact of high-pressure processing on the growth of *Photobacterium phosphoreum* and biogenic amine formation in marinated herring

Yüksek basınç işleminin ringa marinatında *Photobacterium phosphoreum* gelişimi ve biyojen amin üretimi üzerine etkisi

İlknur Uçak[1]* ● Nalan Gökoğlu[2]

[1] Nigde Omer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Nigde, Turkey
[2] Akdeniz University, Fisheries Faculty, Antalya, Turkey

https://orcid.org/0000-0002-9701-0824
https://orcid.org/0000-0002-7868-1972

Corresponding author: ilknurucak@ohu.edu.tr

Received date: 16.03.2020 *Accepted date: 08.05.2020*

How to cite this paper:
Ucak, İ. & Gokoglu, N. (2020). The impact of high-pressure processing on the growth of *Photobacterium phosphoreum* and biogenic amine formation in marinated herring. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 363-371. DOI: 10.12714/egejfas.37.4.07

Abstract: The effects of high-pressure processing (HPP) on *Photobacterium phosphoreum* growth and biogenic amine formation were evaluated in marinated herring (prepared with 2% acetic acid+8% NaCl; or 4% acetic acid+8% NaCl solutions). Marinated fish fillets were inoculated with *P. phosphoreum*, vaccum packaged and treated with HPP in different pressure levels (100, 300, and 500 MPa) and pressure holding times (5 and 10 min). Control was left as untreated for both marination group. All batches were stored at 4±1°C up to 3 months. The results showed that combined effect of HPP and 4% acetic acid had much more inhibitory effect on the growth of *P. phosphoreum*, especially pressure levels 300 and 500 MPa. During the storage period, H_2S-producing bacteria growth was not observed in the groups subjected to 500 MPa pressure. Total psychrophilic bacteria did not grow in 500 MPa pressure treated group and 300 MPa 10 min pressure treated group prepared with 2% acetic acid during the storage period. Histamine was detected insignificant levels in the fillets marinated with 4% acetic acid and treated with HPP. Except for the control group tyramine formation was not found in the samples prepared with 4% acetic acid. Similarly, putrescine was not found in the samples prepared with 2% acetic acid and subjected to HPP treatment at the beginning of the storage. Cadaverine levels were found insignificant amount and 300 and 500 MPa pressure treatments suppressed the formation in 4% acetic acid treated groups compared with 2% acetic acid treated groups. The results of this study revealed that HPP in combination with 4% acetic acid had inhibitory effect on *P. phosphoreum* growth and suppressed the formation of histamine, tyramine, putrescine and cadaverine.

***Keywords*:** High pressure treatment, Photobacterium phosphoreum, herring, histamine, biogenic amine

Öz: Yüksek hidrostatik basınç işleminin (HPP) ringa marinatında (%2 asetik asit+%8 NaCl ve %4 asetik asit+%8 NaCl solüsyonları ile hazırlanan) *Photobacterium phosphoreum* gelişimi ve biyojen amin oluşumu üzerine etkileri değerlendirilmiştir. *P. phosphoreum* ile inoküle edilmiş marine edilmiş balık filetolarına, farklı sürelerde (5 ve 10 dk) ve farklı düzeylerde (100, 300 ve 500 MPa) basınç uygulanmıştır. Her iki marinasyon grubunda da kontrol basınç uygulanmadan bırakılmıştır. Tüm örnekler 4±1°C'de 3 ay depolanmışlardır. Sonuçlar, özellikle 300 ve 500 MPa basınç düzeyleri olmak üzere HPP ve %4 asetik asitin kombine etkisinin *P. phosphoreum* gelişimi üzerine daha fazla inhibitor etkisi olduğunu göstermiştir. Depolama boyunca 500 MPa basınç uygulanan gruplarda H_2S üreten bakteri gelişiminin olmadığını gözlenmiştir. %2 asetik asit ile hazırlanarak 300 MPa 10dk ve 500 MPa basınç uygulanan gruplarda toplam psikrofilik bakterilere depolama süresince gelişememiştir. %4 asetik asit ile hazırlanan ve HPP uygulanan gruplarda histamine düzeyi önemsiz seviyelerde bulunmuştur. %4 asetik asitle marine edilen gruplarda kontrol grubu dışında tiramin oluşumu gözlenmemiştir. Benzer şekilde %2 asetik asitle hazırlanan ve HPP uygulanan gruplarda da depolama başlangıcında putresin bulunmamıştır. Kadaverin miktarı önemsiz düzeylerde bulunmuş ve %2 asetik asit uygulanan gruplara kıyasla %4 asetik uygulanan gruplarda 300, 500 MPa basınç uygulaması kadaverin oluşumunu baskılamıştır. Bu çalışmanın sonuçları, HPP uygulaması ile %4 asetik asitin kombine bir şekilde kullanımının *P. phosphoreum* gelişimi ile histamin, tiramin, putresin ve kadaverin oluşumunu baskıladığını göstermektedir.

Anahtar kelimeler Yüksek basınç uygulaması, *Photobacterium phosphoreum*, ringa, histamine, biyojen amin

INTRODUCTION

Biogenic amines (BAs) such as histamine (HIM), cadaverine (CAD), putrescine (PUT), tyramine (TYM), spermidine (SPD) and spermine (SPM) are low-molecular-weight nitrogenous compounds. BAs are formed by means of decarboxylation of corresponding free amino acids by microorganisms which possess decarboxylase activity. Many bacteria species including enteric bacteria such as *Proteus vulgaris, Enterobacter aerogenes, Enterobacter cloacae, Serratia fonticola, Serratia liquefaciens* and *Citrobacter freundii* (Kim et al., 2003; Tsai et al., 2005) are responsible for BAs formation in seafood. In addition to them, *Morganella morganii, Klebsiella pneumoniae, Hafnia alvei* and *Photobacterium phosphoreum* have strong decarboxylase activity.

P. phosphoreum is a psychrotrophic and halophilic histamine producing bacteria which has high CO_2 resistance (Dalgaard, 2000). BAs formation and spoilage reactions in seafood can be prevented by conventional preservation techniques. However, these techniques such as chilling of seafood to 0-5°C are not sufficient alone to inhibit these

reactions. Therefore, additional preservation methods are required. High pressure processing (HPP) is minimal processing food preservation technologies that depending on the pressure, pressure holding time/temperature and product characteristics allows microbial inactivation at low temperatures with fewer changes in texture, colour and flavour of the product as than the conventional technologies (Ucak et al., 2018). HPP has been employed as a gentle pasteurization technique with generating high quality and microbiologically safe foods. The inactivation mechanism of microorganisms under HPP is based on destruction of membranes and cell walls, denaturation of proteins and enzymes in the cell membrane.

Despite its nutritional value such as high biological value proteins and lipids, fish is highly perishable due to high water activity, high level of unsaturated fatty acids and neutral pH (Lougovois and Kyrana 2005). The safety consumption of seafood is an important issue which can not be ignored by consumers and there has been increasing interest to extend the shelf-life and improving the microbiological quality.

Previous studies have shown that HPP can inhibit the microbial growth (Reyes et al., 2015; Gudbjornsdottir et al., 2010; Mengden et al., 2015; Kural and Chen, 2008a; Kural et al., 2008b; Kim et al., 2013) and can suppress the BAs formation (Matejkova et al., 2013; Krizek et al., 2014) in fish and fish products. Nevertheless, there are very limited reports on the effects of HPP on inhibition of *P. phosphoreum* and formation of BAs in fish product. Thus, this study was performed to determine the inhibitory effects of HPP on microbial growth and BAs formation in marinated herring storage at 4±1°C for 3 months.

MATERIALS AND METHODS

Bacterial strain

Photobacterium phosphoreum (DSM, 15556) were cultured in histidine broth (TSB supplemented with 0.5% L-histidine and 2.5% NaCl) at 20°C for 2-3 days. Early stationary phase cells were used and 10^6 CFU/mL bacteria cultures were prepared for the inoculation.

Preparation of fish marinade

Herring (*Clupea harengus*) fillets were purchased from fish market in Germany (Quakenbrück) and transported in ice boxes to the laboratory of German Institute of Food Technologies. Then fillets were put into polyethylene bags and stored at -20°C until using. For the marination two different solutions were prepared (2% acetic acid (v/v)+8% NaCl (w/v) and 4% acetic acid (v/v)+8% NaCl (w/v)) in the glass jars. The skins of thawed fish fillets were removed aseptically and rinsed with distilled water. Fish were placed into glass jars as 1:1.5 (w/v) fish-to-solution ratio. The ripening process was performed 4°C for 3 days. Marinated fish were removed from the solutions and drained for 30 min on a sterile bench.

Bacteria inoculation and HPP treatment

Marinated fillets were dipped into the *P. phosphoreum* culture solution for 5 min. The fish and bacteria solution ratio were 100g/mL. The inoculated fillets were vacuum packaged and kept at 2-4°C to prevent the temperature effects until the HPP treatment. The vacuum-packed marinated fish were treated with a high-pressure test system (WAVE 6000/55HT; NC Hyperbaric, Burgos, Spain) possessing a 55-L chamber and a maximum pressure level of 600 MPa. The pressure-transmitting medium was cold water (10°C) to maintain temperature conditions at room temperature during HPP treatment. For every 100 MPa increase in the pressure, the adiabatic heating of pressure transmitting fluid was 3-4°C. The compression and decompression times were not included in the treatment time. 100, 300 and 500 MPa pressure levels were applied for 5 and 10-min. Control was left as untreated for each marination group. All samples were stored at 4°C for 3 months and periodically evaluated.

Microbiological analysis

The microbial analyses were performed after HPP treatment and 15, 30, 45, 60, 75 and 90th days of the storage. 10g of fish were in a lab blender containing 90 ml pre-chilled sterile peptone physiological saline solution (0.1% peptone (w/v) + 0.85% NaCl (w/v)) for 60s. Further decimal serial dilutions were prepared from this homogenate in the same chilled sterile diluent. *P. phosphoreum* counts were enumerated by spreading of 0.1mL of the sample homogenate onto Long and Hammer agar. Then plates were incubated for 5 days at 15°C. H_2S-producing bacteria counts were determined by spreading of 0.1 mL of the sample homogenate onto Iron agar Lyngby (IA, Atlas 1997). Incubation period was at 15°C for 7 days. Black colonies were counted for enumeration. Total pscyhrophilic bacteria enumeration was conducted in Plate Count Agar (PCA) and plates were incubated at 7°C for 10 days (ICMSF, 1982).

Biogenic amine analysis

Ultra Performance Liquid Chromatography (UPLC-Thermo Scientific, Photodiode Array Detector) was used for the determination of histamine, tyramine, putrescine and cadaverine were conducted according to method of Eerola et al. (1993) with slight modifications. 15 mL 0.4 mol/L perchloric acid was added to 5.0 g of fish meat prior to homogenization for 1 min using an Ultra Turrax T25 (IKA-Labortechnik, Staufen, Germany). The homogenate was centrifuged (10 min, 2250 x g) and the supernatant passed through a 0.45 µm filter. After, 1 mL of sample extract was made alkaline by adding 200 µL 2 mol/L sodium hydroxide (NaOH) and buffered with 300 µL saturated sodium bicarbonate ($NaHCO_3$). Then, 1 mL of dansyl chloride ($C_{12}H_{12}ClNO_2S$) solution was added and the reaction mixture was incubated at 40°C for 45 min. Residual dansyl chloride was removed by adding 100 µL ammonia. After 30 min, mixture was adjusted to 5 mL with acetonitrile, filtered (0.45 µm, PTFE, MS Springer filter) and analyzed.

Statistical analysis

All measurements were carried out in triplicate and data were subjected to variance (ANOVA) analysis and Duncan's multiple range tests using the SPSS Version 18.0 statistical package (SPSS Inc., Chicago, IL, USA). A difference was regarded statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Effects of HPP on growth of *P. phosphoreum*

The inhibitory effects of HPP on the viable cell counts of *P. phosphoreum* in marinated herring are given in Figure 1-2. At the beginning the viable cell counts were found as 2.64, 2.56 and 1.82 log CFU/g in control, 100 MPa 5 min and 100 MPa 10 min pressure treated samples marinated with 2% acetic acid, respectively. *P. phosphoreum* growth was not observed in 300 MPa 10 min and 500 MPa 5, 10 min pressure treated group untill 60th day of the storage, while bacteria growth was detected at 45th day of the storage in 300 MPa 5 min pressure treated group. Significantly lowest ($p<0.05$) cell counts were determined in 500 MPa 5, 10 min pressure treated groups (4.08 and 2.54 log CFU/g) followed by 300 MPa 10 min pressure treated samples (5.48 log CFU/g) at the end of the storage period.

In herring fillets marinated with 4% acetic acid, *P. phosphoreum* could not grow at the beginning of the storage. However, on the 15th day bacteria growth was observed in the control and 100 MPa 5, 10 min pressure treated groups and at the end reached 6.48, 6.05 and 5.97 log CFU/g, respectively. Bacteria population did not exceed 7 log CFU/g, which considered as limit value for fish species during the storage period in these groups. Until at the end of the storage period, viable cell counts were not detected in 300 MPa and 500 MPa pressure treated groups. Ruiz-Capillas et al. (2007) reported that HPP treatment does not always inactivate microorganisms completely but may injure a proportion of the population, and the recovery of the injured cells depends on the subsequent conditions.

The findings of present study are consistent with the Kim et al. (2013), reported that *P. phosphoreum* growth was not observed in 300 and 400 MPa pressure treated mackerel muscle. Uçak et al. (2019) found that *Morganella psychrotolerans* growth was inhibited by 300 MPa and 500 MPa pressure treatment in marinated herring. The observation of present study pointed out that HPP and 4% acetic acid combination had more inhibitory effect on the growth of *P. phosphoreum*.

Effects of HPP on H_2S-producing bacteria

Total counts of H_2S-producing bacteria in marinated herring under HPP are represented in Figure 3-4. Initially, viable counts were 2.46 and 2.37 log CFU/g in control and 100 MPa 5 min pressure treated herring fillets marinated with 2% acetic acid, respectively. Bacteria growth was detected in 100 MPa 10 min, 300 MPa 5 min and 300 MPa 10 min pressure treated groups on the 15th, 45th and 60th day of the storage, respectively. Nevertheless, H_2S-producing bacteria growth was not recorded in 500 MPa pressure treated groups.

In the fillets marinated with 4% acetic acid, H_2S-producing bacteria growth was inhibited in 300 MPa 10 min and 500 MPa pressure treated groups. At the end of the storage period, highest value was observed in control (6.91 log CFU/g), while the lowest ($p<0.05$) value was found in fillets subjected to 300 MPa 5 min pressure level (5.25 log CFU/g). H_2S-producing bacteria are responsible for the main deterioration in fish and fish products stored at anaerobic conditions. Dalgaard (1993) reported that H_2S-producing bacteria growth is inhibited by low pH. Herland et al. (2008) determined the H_2S-producing bacteria in ice stored cod fillet after 9th day of the storage and bacteria cells reached 3.97 log CFU/g on the 15th day of the storage.

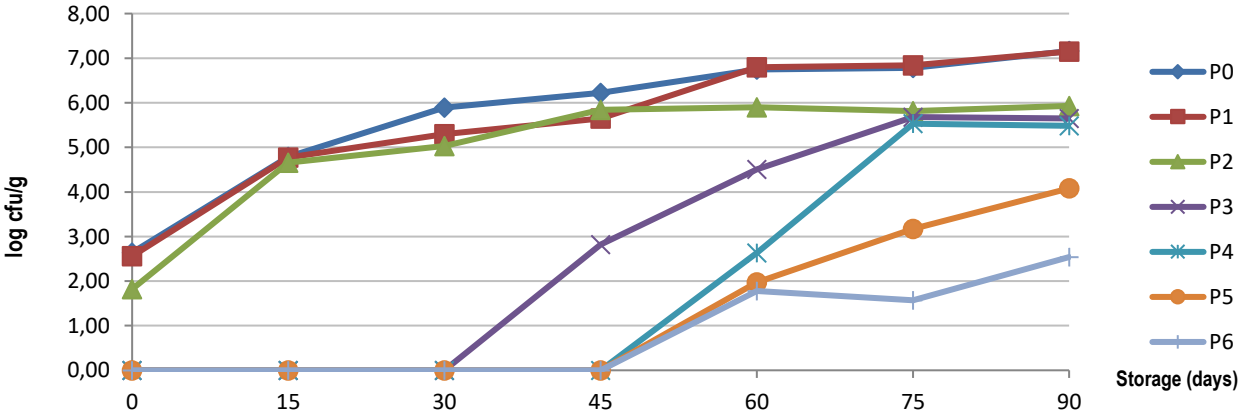

Figure. 1. Effect of HPP on the growth of *P. phosphoreum* in marinated herring prepared with 2% acetic acid and 8% NaCl. P0 (no HPP treatment), P1 (100 MPa 5 min), P2 (100 MPa 10 min), P3 (300 MPa 5 min), P4 (300 MPa 10 min), P5 (500 MPa 5 min), P6 (500 MPa 10 min)

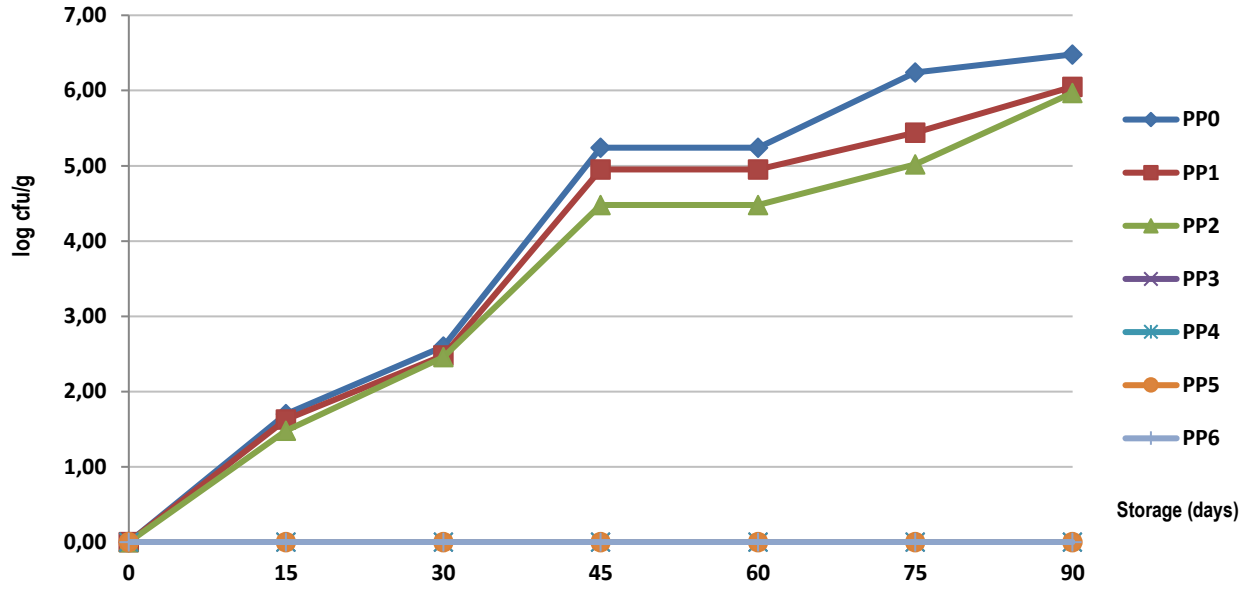

Figure 2. Effect of HPP on the growth of *P. phosphoreum* in marinated herring prepared with 4% acetic acid and 8% NaCl. PP0 (no HPP treatment), PP1 (100 MPa 5 min), PP2 (100 MPa 10 min), PP3 (300 MPa 5 min), PP4 (300 MPa 10 min), PP5 (500 MPa 5 min), PP6 (500 MPa 10 min)

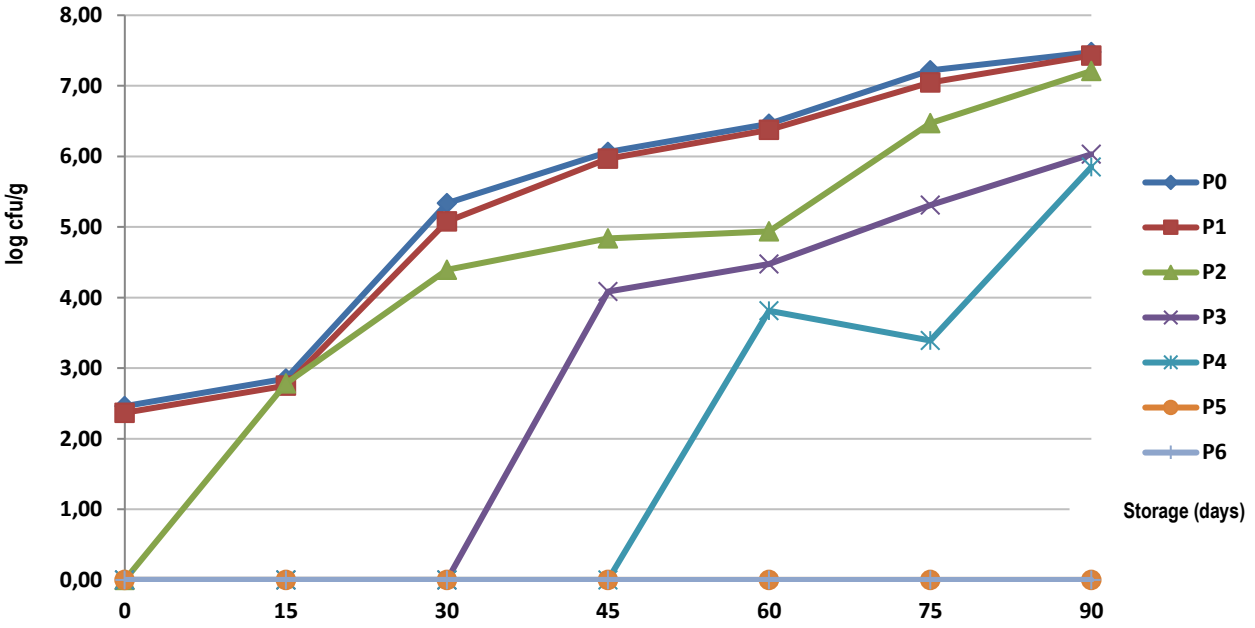

Figure 3. Effect of HPP on H_2S-producing bacteria growth in marinated herring prepared with 2% acetic acid and 8% NaCl. P0 (no HPP treatment), P1 (100 MPa 5 min), P2 (100 MPa 10 min), P3 (300 MPa 5 min), P4 (300 MPa 10 min), P5 (500 MPa 5 min), P6 (500 MPa 10 min)

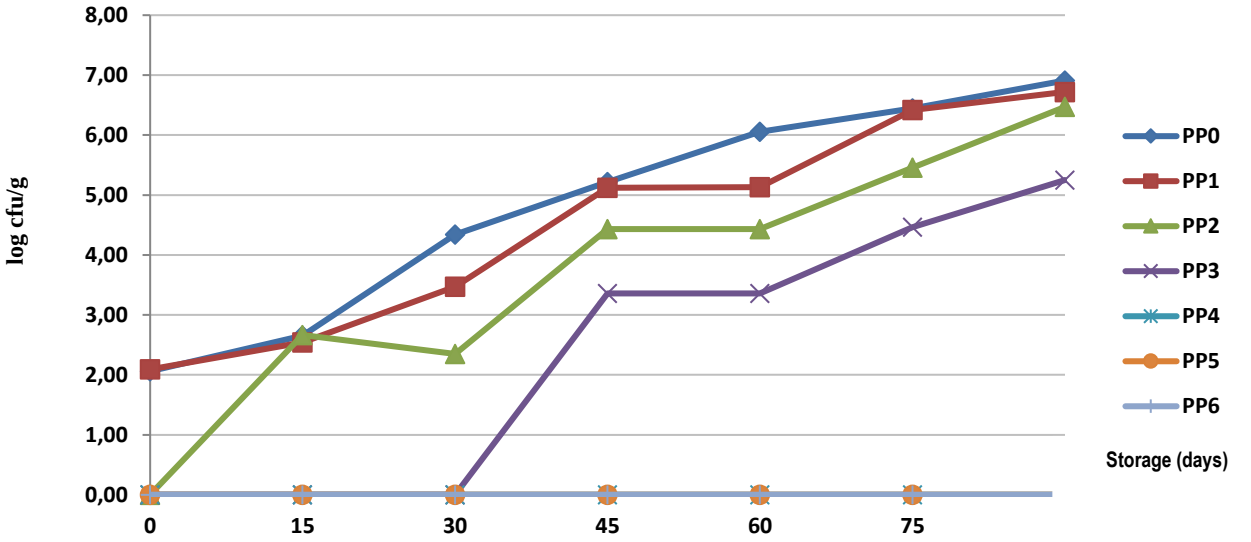

Figure 4. Effect of HPP on H_2S-producing bacteria growth in marinated herring prepared with 4% acetic acid and 8% NaCl. PP0 (no HPP treatment), PP1 (100 MPa 5 min), PP2 (100 MPa 10 min), PP3 (300 MPa 5 min), PP4 (300 MPa 10 min), PP5 (500 MPa 5 min), PP6 (500 MPa 10 min)

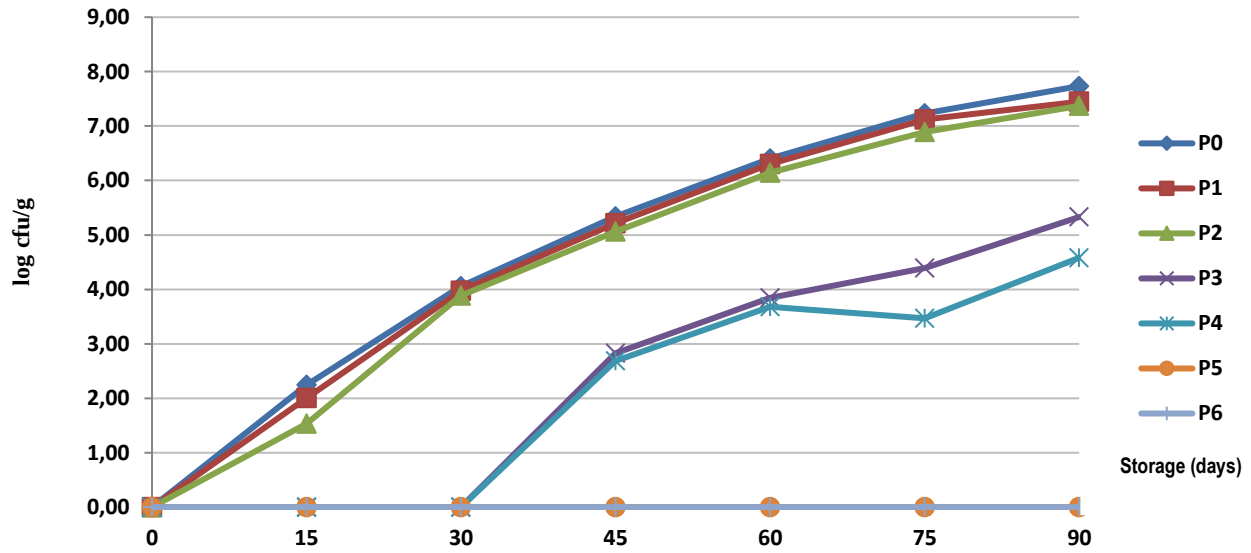

Figure 5. Effect of HPP on total psychrophilic bacteria growth in marinated herring prepared with 2% acetic acid and 8% NaCl. P0 (no HPP treatment), P1 (100 MPa 5 min), P2 (100 MPa 10 min), P3 (300 MPa 5 min), P4 (300 MPa 10 min), P5 (500 MPa 5 min), P6 (500 MPa 10 min)

Effects of HPP on total psychrophilic bacteria

Growth of total psychrophilic bacteria counts in marinated herring subjected to HPP are presented in Figure 5-6. In the aerobically stored fresh fish, gram-negative psychrotrophic bacteria are the main groups, causing the spoilage (Ibrahim Sallam, 2007). The initial total psychrophilic bacteria counts were found as 2.46 and 2.37 log CFU/g in control and 100 MPa 5 min pressure treated groups marinated with 2%, while bacteria growth was not observed in 100 MPa 10 min, 500 MPa 5 min and 300 MPa 10 min pressure applied groups until 15th, 45th and 60th days, respectively. Highest ($p<0.05$) viable counts were detected in control and 100 MPa pressure treated groups. In the groups subjected to 500 MPa HPP,

total psychrophilic bacteria growth inhibited during the storage period.

At the beginning of the storage, viable cells were not found in the groups prepared with 4% acetic acid and subjected to HPP. However, bacteria growth was observed in the control and 100 MPa pressure treated groups. During the storage period, total psychrophilic bacteria growth inhibited in the marinated herring treated with 300 and 500 MPa pressure level. Erkan et al. (2010) reported that total psychrotrophic bacteria count reached at 10^6 log CFU/g at 11 days in the red mullet fillets, while 330 MPa 5 min and 220 MPa 5 min HPP treated fillets reached this value at 17 and 15 days, respectively. Karim et al. (2011) found the initial total total psychrophilic bacteria count as 10^4 CFU/g in herring fillets and it was reported that pressure levels above 200 MPa had inhibitory effects during the storage. In another study Uçak et al. (2019) noticed that 500 MPa pressure treatment inhibited the total psychrophilic bacteria growth in marinated herring inoculated with *M. pychrotolerans*.

Effects of HPP on the biogenic amine formation

Histamine (HIM), tyramine (TYM), tryptamine (TRM), putrescine (PUT), and cadaverine (CAD) are the most important BAs in seafood associated with spoilage. Among them HIM and TYM are the most biologically active amines (Shalaby, 1996; Onal, 2007). Table 1-2 represented the effect of HPP treatment on the BAs formation in marinated herring. Initially, HIM level of control sample marinated with 2% acetic acid was 10.81 mg/kg and significantly ($p<0.05$) increased to 207.36 mg/kg at the end of the storage. In the HPP treated groups significantly highest ($p<0.05$) HIM level was observed in 100 MPa pressure treated fillets, while the lowest values were found in the groups subjected to 500 MPa HPP treatment. 4% acetic acid and HPP combination was more efficient in suppressing the HIM formation. HIM content exceeded the recommended level of 50 mg/kg by the Food Drug Administration (FDA, 2011) in control and 100 MPa 5 min pressure treated fillets marinated with 2% acetic acid after 30 days. Whereas, HIM was detected insignificant levels in herring fillets marinated with 4% acetic acid and treated with HPP.

At the beginning TYM levels were 2.27, 1.88 and 0.47 mg/kg in control, 100 MPa 5 min and 100 MPa 10 min pressure treated fillets and marinated with 2% acetic acid, respectively. TYM level exceeded the suggested acceptable limit for adults (100-800 mg/kg) by Ten Brink et al. (1990) in those groups at 30thday. TYM formation was not observed in 300 MPa and 500 MPa HPP treated fillets until 30th and 60th days, respectively. Significantly lowest ($p<0.05$) TYM values were detected in the groups treated with 500 MPa pressure level, while the highest values were observed in the control followed by 100 MPa and 300 MPa pressure treated samples. Except for the control group TYM formation was not found in the samples prepared with 4% acetic acid. This situation explains that all pressure levels succeded to suppress TYM formation.

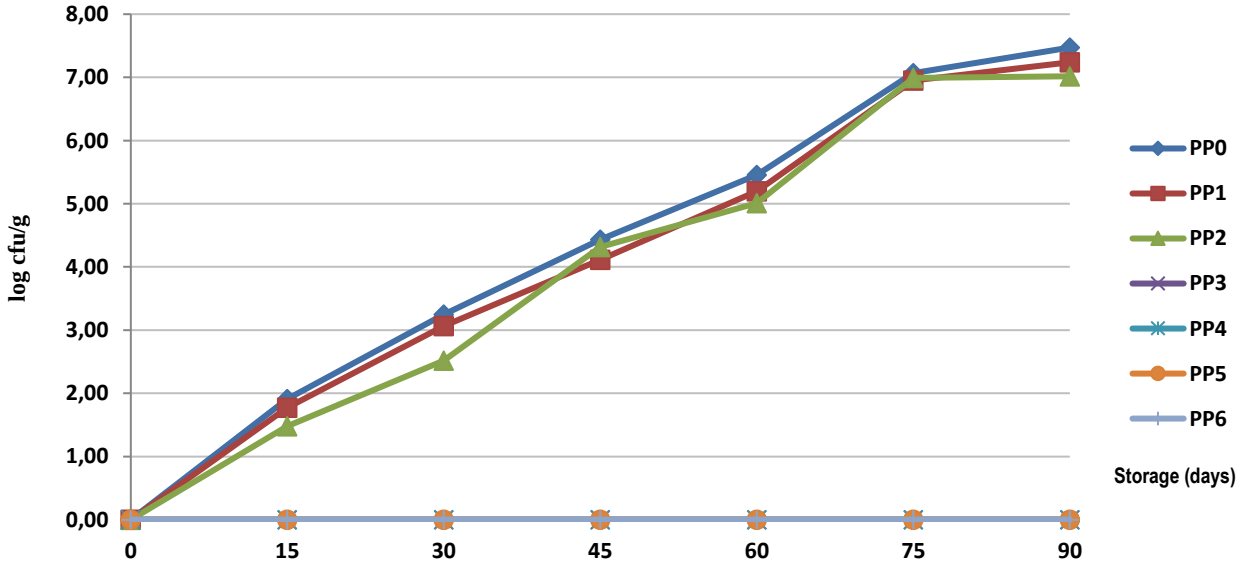

Figure 6. Effect of HPP on total psychrophilic bacteria growth in marinated herring prepared with 4% acetic acid and 8% NaCl. PP0 (no HPP treatment), PP1 (100 MPa 5 min), PP2 (100 MPa 10 min), PP3 (300 MPa 5 min), PP4 (300 MPa 10 min), PP5 (500 MPa 5 min), PP6 (500 MPa 10 min)

Table 1. Effect of HPP treatment on histamine and tyramine formation in marinated herring during storage (mg/100g)

Biogenic amines	Marination treatment	HPP treatment	Storage time (days)			
			0	30	60	90
Histamine	2% acetic acid	P0	10.81±0.02^{aD}	25.63±0.00^{aC}	116.41±0.07^{aB}	207.36±0.00^{aA}
		P1	8.54±0.04^{bC}	4.05±0.01^{bD}	110.99±0.05^{bB}	163.73±0.02^{bA}
		P2	7.71±0.03^{cC}	5.96±0.07^{cD}	9.51±0.14^{cB}	144.41±0.05^{cA}
		P3	4.63±0.00^{cC}	3.97±0.00^{dD}	7.19±0.10^{dB}	22.36±0.17^{bA}
		P4	6.43±0.01^{fA}	3.70±0.04^{eB}	3.68±0.09^{eC}	2.10±0.08^{eD}
		P5	4.73±0.06^{dA}	3.10±0.11^{fB}	1.90±0.27^{fC}	0.49±0.10^{fD}
		P6	4.63±0.13^{eA}	1.48±0.13^{gB}	0.94±0.01^{gC}	0.45±0.01^{gD}
	4% acetic acid	PP0	7.50±0.14^{aB}	4.74±0.04^{aD}	6.58±0.11^{aC}	8.36±0.03^{eA}
		PP1	4.71±0.05^{bC}	3.39±0.21^{bD}	5.87±0.05^{bB}	6.41±0.01^{eA}
		PP2	2.82±0.02^{cD}	2.89±0.06^{cC}	3.75±0.07^{cB}	4.82±0.16^{dA}
		PP3	2.36±0.01^{dA}	2.07±0.03^{dB}	ND	0.71±0.11^{dC}
		PP4	1.95±0.00^{eA}	1.58±0.15^{eB}	ND	0.71±0.09^{cC}
		PP5	1.10±0.06^{fB}	1.21±0.12^{fA}	ND	ND
		PP6	1.11±0.03^{gA}	0.62±0.09^{gB}	ND	ND
Tyramine	2% acetic acid	P0	0.47±0.05^{cD}	465.53±0.17^{bA}	462.65±0.12^{dB}	382.13±0.01^{eC}
		P1	2.27±0.07^{aD}	450.87±0.08^{cB}	508.98±0.37^{aA}	410.57±0.08^{cC}
		P2	1.88±0.27^{bD}	469.23±0.12^{aA}	437.72±0.01^{eB}	391.08±0.13^{dC}
		P3	ND	393.65±0.20^{dC}	541.81±0.08^{bA}	480.47±0.24^{bB}
		P4	ND	3.65±0.13^{eC}	535.63±0.03^{cA}	509.93±0.27^{aB}
		P5	ND	ND	1.49±0.23^{gB}	4.63±0.05^{fA}
		P6	ND	ND	3.20±0.32^{fA}	0.87±0.19^{gB}
	4% acetic acid	PP0	ND	4.48±0.04^{C}	23.36±0.16^{A}	20.46±0.11^{B}
		PP1	ND	ND	ND	ND
		PP2	ND	ND	ND	ND
		PP3	ND	ND	ND	ND
		PP4	ND	ND	ND	ND
		PP5	ND	ND	ND	ND
		PP6	ND	ND	ND	ND

ND: Not detected; Means indicated by di ferent lowercase letters in the same column differ significantly ($p<0.05$). Means indicated by different capital letters in the same row differ significantly ($p<0.05$). Each acetic acid group (2% and 4%) evaluated in itself.

Table 2. Effect of HPP on putrescine and cadaverine formation in marinated herring during storage (mg/100g)

Biogenic amines	Marination treatment	HPP treatment	Storage time (days)			
			0	30	60	90
Putrescine	2% acetic acid	P0	ND	4.78±0.17^{cC}	12.76±0.23^{bB}	13.31±0.01^{aA}
		P1	ND	3.88±0.22^{dC}	8.73±0.11^{cB}	9.61±0.08^{cA}
		P2	ND	6.31±0.34^{aC}	18.02±0.32^{aA}	11.79±0.18^{bB}
		P3	ND	5.65±0.28^{bA}	4.97±0.02d^{B}	2.48±0.29^{dC}
		P4	ND	ND	4.43±0.08^{eA}	1.89±0.36^{eB}
		P5	ND	ND	ND	ND
		P6	ND	ND	ND	ND
	4% acetic acid	PP0	ND	ND	ND	ND
		PP1	ND	ND	ND	ND
		PP2	ND	ND	ND	ND
		PP3	ND	ND	ND	ND
		PP4	ND	ND	ND	ND
		PP5	ND	ND	ND	ND
		PP6	ND	ND	ND	ND
Cadaverine	2% acetic acid	P0	13.40±0.15^{bB}	7.12±0.01^{bD}	10.38±0.17^{cC}	18.14±0.05^{aA}
		P1	13.05±0.33^{cB}	10.82±0.36^{aC}	14.81±0.25^{aA}	8.23±0.07^{bD}
		P2	13.45±0.08^{aA}	6.00±0.07^{fB}	2.19±0.28^{fC}	1.29±0.14^{cD}
		P3	11.37±0.17^{dA}	3.99±0.02^{eC}	10.98±0.31^{bB}	ND
		P4	10.91±0.02^{eA}	2.77±0.03^{gB}	1.62±0.07^{gC}	ND
		P5	9.15±0.06^{fA}	6.57±0.09^{dB}	5.45±0.09^{dC}	ND
		P6	9.00^{gA}	6.36±0.15^{cB}	2.09±0.04^{eC}	ND
	4% acetic acid	PP0	11.56±0.37^{aA}	8.70±0.05^{aB}	4.97±0.46^{aC}	3.54±0.02^{aD}
		PP1	7.41±0.26^{dA}	7.19±0.28^{dB}	4.52±0.15^{cC}	2.31±0.06^{fD}
		PP2	10.92±0.18^{bA}	8.46±0.16^{cB}	2.35±0.27^{fD}	3.13±0.01^{bC}
		PP3	6.35±0.06^{gA}	5.56±0.02^{fB}	2.31±0.02^{gD}	3.55±0.14^{aC}
		PP4	9.31±0.12^{cA}	8.49±0.09^{bB}	4.37±0.09^{dC}	2.52±0.22^{eD}
		PP5	6.74±0.03^{eA}	5.86±0.32^{eB}	4.91±0.17^{bC}	2.67±0.37^{cD}
		PP6	6.48±0.42^{fA}	4.79±0.252^{gB}	4.05±0.13^{eC}	2.64±0.12^{dD}

ND: Not detected; Means indicated by different lowercase letters in the same column differ significantly ($p<0.05$). Means indicated by different capital letters in the same row differ significantly ($p<0.05$). Each acetic acid group (2% and 4%) evaluated in itself.

The inhibitory effects of both 4% acid concentration and HPP treatment on the PUT formation were clearly visible, since there was no PUT formation in those groups (Table 2). Similarly, PUT was not detected in the samples prepared with 2% acetic acid and subjected to HPP treatment at the beginning of the storage, however, except for 500 MPa pressure treated groups PUT was observed after 0 day and reached the highest value in control.

CAD formation was found insignificant ($p>0.05$) levels in all groups during the storage period. The highest CAD level was recorded in both 4% and 2% acetic acid treated controls compared with the HPP treated marinated herring fillets, whereas the lowest values were determined in 300 and 500 MPa pressure treated groups. According to results, 100 MPa HPP treatment did not significantly affected the CAD formation in marinated herring fillets.

The results of the present study are consistent with other studies who reported that 500 MPa pressure treatment is more effective to suppress the PUT and TYM formation (Matejkova et al., 2013; Krizek et al., 2014). Ucak et al. (2019) reported significantly lower HIM formation in the marinated herring and treated with 300 and 500 MPa preesure compared with the control samples. According to another study HPP application significantly inhibited the HDC activity (Kim et al., 2013).

CONCLUSIONS

BAs can serve as indicators of fish spoilage since their presence in fresh fish is very low. *P. phosphoreum*, which is an important histamine producing bacteria, was inhibited in 300 and 500 MPa pressure treatment. Especially, the high acid concentration and HPP application combination was very efficient in inhibition of bacteria growth. The results of present study showed that the BAs content of marinated herring can noticeably reduced by the application of HPP. 4% acetic acid and HPP combination was more effective in suppressing the HIM, TYM, PUT and CAD formation.

ACKNOWLEDGEMENTS

This research was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), Deutscher Akademischer Austauschdienst (DAAD), The Scientific Research Projects Administration Unit of Akdeniz University (project no. FDK-2015-273), management and staff of Deutsches Institut für Lebensmitteltechnik (German Institute of Food Technologies).

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 373-378 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.08

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The occurrence of *Ammothella longioculata* (Faraggiana, 1940) (Arthropoda, Pycnogonida) in İzmir Bay (Aegean Sea, Turkey) and reported species from the bay

Ammothella longioculata (Faraggiana, 1940) (Arthropoda, Pycnogonida)'nın İzmir Körfezi'nde (Ege Denizi, Türkiye) bulunuşu ve körfezden rapor edilmiş türler

Cengiz Koçak

Department of Hydrobiology, Fisheries Faculty, Ege University, TR 35100, Bornova-İzmir, Turkey https://orcid.org/0000-0003-3030-7882

kocakcengiz@gmail.com

Received date: 18.02.2020 *Accepted date: 19.05.2020*

How to cite this paper:
Koçak, C. (2020). The occurrence of *Ammothella longioculata* (Faraggiana, 1940) (Arthropoda, Pycnogonida) in İzmir Bay (Aegean Sea, Turkey) and reported species from the bay. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 373-378. DOI: 10.12714/egejfas.37.4.08

Abstract: Sampling studies in İzmir Bay revealed the occurrence of a pycnogonid species, *Ammothella longioculata* (Faraggiana, 1940). *A. longioculata* is reported only one time from the Turkish waters, up to now. The presence of this rare species is reported herein for the first time from the İzmir Bay, and also second time from the Turkish waters. The distribution map of the species in the Mediterranean Sea is provided, together with photographs and line drawing of the species. Moreover, all of the early studies were reviewed on the pycnogonid fauna of İzmir Bay, distribution of each species, depth range, and type of substrate are given.

Keywords: *Ammothella longioculata*, Pycnogonida, İzmir Bay, Turkey, Mediterranean Sea

Öz: İzmir Körfezi'nde yapılan örnekleme çalışmaları, bir piknogonid tür olan *Ammothella longioculata* (Faraggiana, 1940)'nın varlığını ortaya koymuştur. *A. longioculata* bugüne kadar Türkiye sularından yalnızca bir kez rapor edilmiştir. Bu nadir türün varlığı İzmir Körfezi'nden ilk kez, Türkiye sularından ise ikinci kez bu çalışmada rapor edilmektedir. Türün Akdeniz'deki dağılımı, fotoğraf ve çizimi çalışmada sunulmuştur. Ayrıca, İzmir Körfezi'nin piknogonid faunası üzerine daha önce yapılmış çalışmaların tümü gözden geçirilmiş olup, her bir türün dağılımı, derinlik aralığı ve subsratum tipi verilmiştir.

***Anahtar kelimeler*:** *Ammothella longioculata*, Pycnogonida, İzmir Körfezi, Türkiye, Akdeniz

INTRODUCTION

Ammothella longioculata (Faraggiana, 1940) is endemic to the Mediterranean Sea. This species had been reported eleven times before from the region (Faraggiana, 1940; Stock, 1958; Krapp, 1973; Arnaud, 1987; Schüller, 1989; Chimenz et al., 1993; Munilla and Nieto, 1999; Vignoli et al., 2006; Kocak and Katagan, 2007; Krapp et al., 2008, who reported for the first time this species from the Turkish waters). The present study provides a new locality for *A. longioculata* in the Aegean Sea, information about its distribution in the Mediterranean Sea, and reported pycnogoid species from the İzmir Bay.

MATERIALS AND METHODS

One ovigerous male specimen of the species was collected by snorkeling from *Cystoseira mediterranea* facies at one station in the upper infralittoral zone (1m depth) of Urla, İzmir Bay (Figure 1). The sample was fixed in 5% formaldehyde and later rinsed with fresh water and then preserved in 70% ethanol. The specimen sampled was examined under a stereomicroscope. The drawing was made with the aid of a drawing tube mounted on an Olympus CX31 compound microscope. The following papers were used for species identification: Faraggiana (1940), Krapp (1973), and Kocak and Katagan (2007). The specimen was stored in the ESFM (Museum of the Faculty of Fisheries, Ege University, İzmir).

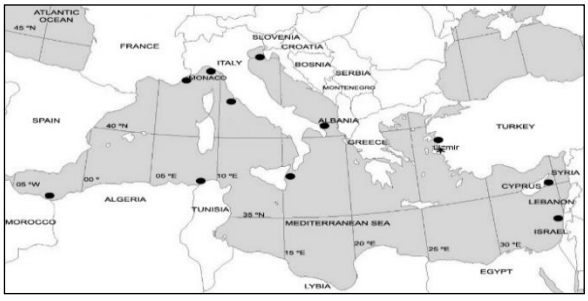

Figure 1. Distribution of *Ammothella longioculata* (Faraggiana, 1940) in the Mediterranean Sea (●), including the sampling area (∗)

RESULTS

Systematics

Class PYCNOGONIDA Latreille, 1810

Order PANTOPODA Gerstaecker, 1863

Family AMMOTHEIDAE Dohrn,1881

Genus *Ammothella* Verrill, 1900

Ammothella longioculata (Faraggiana, 1940) (Figures 2-3)

Material examined: 1 ovigerous ♂, (EFSM-PYC/2007-1), Urla (İzmir Bay, Aegean Sea), 38°22'27"N, 26°47'13"E, *Cystoseira mediterranea* Sauvageau, 1912, 1 m, 04 July 2007.

Measurements (mm): Length of the trunk (frontal margin of the cephalic segment to tip of 4th lateral process), 0.87; trunk width (across second lateral processes), 0.62; abdomen length, 0.35.

Remarks: The present specimen agrees well with the specimen given by Krapp (1973) and by Kocak and Katagan (2007). I noted only that the trunk length of the present specimen is slightly larger than those in Krapp's (1973) sample (0.84mm in the male) and Kocak and Katagan's (2007) sample (0.83mm in the male).

Worldwide Distribution: Mediterranean Sea.

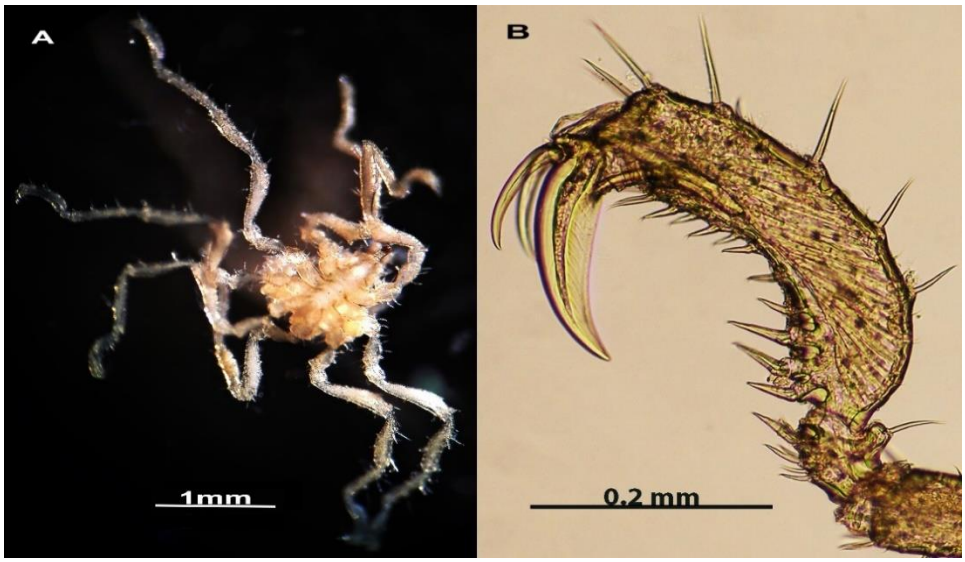

Figure 2. *Ammothella longioculata* (Faraggiana, 1940), ovigerous ♂, from İzmir Bay. A-Dorsal view; B- Tarsus and propodus of right leg 3

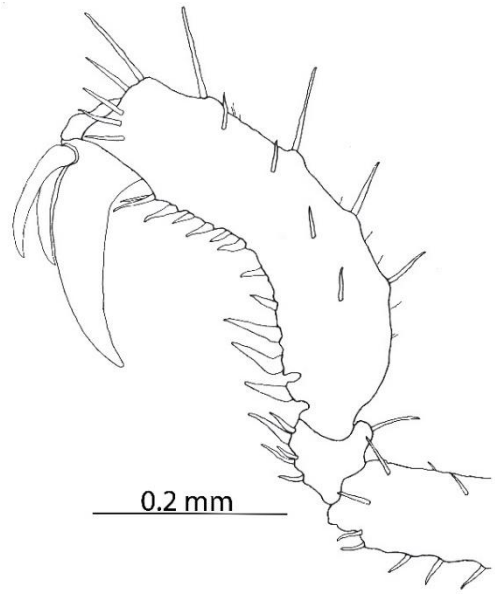

Figure 3. *Ammothella longioculata* (Faraggiana, 1940), ovigerous ♂, from İzmir Bay. Line drawing of tarsus and propodus of right leg 3

As a result of studies carried out to review the Pycnogonid fauna of the İzmir Bay, indicating the presence of 18 species inhabiting the Bay, belonging to 6 families and 9 genera:

SUPERFAMILY: ASCORHYNCHOIDEA POCOCK, 1904

FAMILY: AMMOTHEIDAE DOHRN, 1881

GENUS: *AMMOTHELLA* VERRILL, 1900

***A. appendiculata* (Dohrn, 1881)**

Synonyms: *Ammothea appendiculata* Dohrn, 1881; *Ammothea rugulosa* Verrill, 1900

This species was reported by Çinar et al. (2008) from Alsancak Harbour, Pasaport Harbour, and İnciraltı.

Depth range: 0.2m.

Substrate: *Mytilus galloprovincialis* Lamarck, 1819.

Worldwide Distribution: Cosmopolitan (Soler-Membrives and Munilla, 2015).

***A. longipes* (Hodge, 1864)**

Synonyms: *Achelia longipes* (Hodge, 1864); *Ammothea longipes* Hodge, 1864; *Ammothea magnirostris* Dohrn, 1881; *Ammothella magnirostris* (Dohrn, 1881); *Achelia hispida* King, 1974

The species was reported by Arnaud (1976) from Kalabak, Mordoğan, and Urla.

Depth range: 0.5-1.5m.

Substrate: *Cystoseira crinita* Duby, 1830; *Ellisolandia elongata* (J. Ellis & Solander) K.R. Hind & G.W. Saunders, 2013 (as *Corallina mediterranea* J.E. Areschoug, 1852).

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

***A. uniunguiculata* (Dohrn, 1881)**

Synonyms: *Ammothea uni-unguiculata* Dohrn, 1881

This species was reported by Arnaud (1976) from Kalabak, Foça and Narlıdere, and Krapp et al. (2008) from Foça.

Depth range: 0.5-1.5m.

Substrate: *C. crinata*; *Padina* sp.; *Ulva lactuca* Linnaeus, 1753; *Halopteris scoparia f. hiemalis* J.Agardh.

Worldwide Distribution: Endemic (Mediterranean) (Soler-Membrives and Munilla, 2015).

GENUS: *ACHELIA* HODGE, 1864

***A. echinata* Hodge, 1864**

Synonyms: *Ammothea brevipes* Hodge, 1864; *Ammothea echinata* (Hodge, 1864); *Achelia fibulifera* (Dohrn, 1881)

This species was reported by Arnaud (1976) from Kalabak (as *Achelia* cf. *echinata*).

Depth range: 0.5m.

Substrate: *E. elongata* (as *C. mediterranea*).

Worldwide Distribution: Cosmopolitan (Soler-Membrives and Munilla, 2015).

***A. langi* (Dohrn, 1881)**

Synonyms: *Ammothea langi* Dohrn, 1881

The species was reported by Krapp et al. (2008) from Foça.

Depth range: 0.5m.

Substrate: *C. crinita*.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

GENUS: *TANYSTYLUM* MIERS, 1879

***T. conirostre* (Dohrn, 1881)**

Synonyms: *Clotenia conirostris* Dohrn, 1881

The species was reported by Arnaud (1976) from Kalabak, Narlıdere and Foça, Krapp et al. (2008) from Foça, and Çinar et al. (2008) Alsancak Harbour, Pasaport Harbour and İnciraltı.

Depth range: 0.2-1.5m.

Substrate: *C. crinata*; *U. lactuca*; *E. elongata* (as *C. mediterranea*); *M. galloprovincialis*.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

***T. orbiculare* Wilson, 1878**

Synonyms: *Clotenia orbiculare* (Wilson, 1878)

This species was recorded by Arnaud (1976) from Kalabak, and Krapp et al. (2008) from Foça.

Depth range: 0.5-1m.

Substrate: *E. elongata* (as *C. mediterranea*); *C. crinita; H. scoparia*.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

GENUS: *TRYGAEUS* DOHRN, 1881

***T. communis* Dohrn, 1881**

The species was reported by Kocak (2019) from Mordoğan.

Depth range: 0.5m.

Substrate: *Cystoseira mediterranea* Sauvageau, 1912.

Worldwide Distribution: Mediterranean (Soler-Membrives and Munilla, 2015).

FAMILY: ASCORHYNCHIDAE HOEK, 1881

GENUS: *ASCORHYNCHUS* G.O.SARS, 1877

***A. castelli* (Dohrn, 1881)**

Synonyms: *Barana castelli* Dohrn, 1881

This species was reported by Kocak (2012) from Mordoğan.

Depth range: 10m.

Substrate: *Posidonia oceanica* (Linnaeus) Delile, 1813.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

SUPERFAMILY: PHOXICHILIDOIDEA G.O. SARS, 1891

FAMILY: PHOXICHILIDIIDAE G.O. SARS, 1891

GENUS: *ANOPLODACTYLUS* WILSON, 1878

***A. petiolatus* (Kroyer, 1844)**

Synonyms: *Phoxichilidium petiolatum* Kroyer, 1844; *Pallene attenuata* Hodge, 1863; *Phoxichilidium attennuata* (Hodge, 1863); *Phoxichilidium longicolle* Dohrn, 1881; *Phoxichilidium pygmaeum* Hoek, 1881; *Anoplodactylus longicollis* (Dohrn, 1881); *Anoplodactylus pygmaeus* (Hoek, 1881); *Anoplodactylus hedgpethi* Bacescu, 1959; *Anoplodactylus guyanensis* Child, 1977.

This species was reported by Arnaud (1976) from Kalabak.

Depth range: 0.5m.

Substrate: *E. elongata* (as *C. mediterranea*).

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

***A. pygmaeus* (Hodge, 1864)**

Synonyms: *Pallene pygmaea* Hodge, 1864; *Phoxichilidium pygmaeum* (Hodge, 1864); *Anoplodactylus exiguus* (Dohrn, 1881); *Phoxichilidium exiguum* Dohrn, 1881; *Halosoma derjugini* Losina-Losinsky, 1929; *Anoplodactylus derjugini* (Losina-Losinsky, 1929)

This species was reported by Arnaud (1976) from Tuzla, Krapp et al. (2008) from Balıklıova, and Çinar et al. (2008) from Alsancak Harbour, Pasaport Harbour, and İnciraltı.

Depth range: 0.2-8m.

Substrate: *M. galloprovincialis*; *P. oceanica*.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

***A. virescens* (Hodge, 1864)**

Synonyms: *Orithyia globosa* Goodsir, 1842; *Phoxichilidium globosum* Goodsir, 1842; *Phoxichilidium virescens* Hodge, 1864

This species was reported by Arnaud (1976) from Kalabak and Foça.

Depth range: 0.3-1m.

Substrate: *C. crinata*; *Padina pavonia* (Linnaeus) J.V. Lamouroux, 1816; *E. elongata* (as *C. mediterranea*).

Worldwide Distribution: Disrupted distribution (St. Paul I., Amsterdam, Mediterranean Sea including eastern and western basins) (Soler-Membrives and Munilla, 2015).

FAMILY: ENDEIDAE NORMAN, 1908

GENUS: *ENDEIS* PHILIPPI, 1843

***E. spinosa* (Montagu, 1808)**

Synonyms: *Chilophoxus spinosus* Montagu, 1808; *Endeis gracilis* Philippi, 1843; *Endeis laevis* (Grube, 1871); *Endeis vulgaris* (Dohrn, 1881)

This species was reported by Krapp et al. (2008) from Foça (one juvenile specimen as *E.* cf. *spinosa*).

Depth range: 0.5m.

Substrate: *C. crinita*.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

SUPERFAMILY: NYMPHONOIDEA POCOCK, 1904

FAMILY: CALLIPALLENIDAE HILTON, 1942

GENUS: *CALLIPALLENE* FLYNN, 1929

***C. emaciata* (Dohrn, 1881)**

Synonyms: *Pallene emaciata* Dohrn, 1881; *Callipallene emaciata* (Dohrn, 1881)

This species was reported by Arnaud (1976) and Krapp et al. (2008) from Foça.

Depth range: 0.5m.

Substrate: *E. elongata* (as *C. mediterranea*); *C. crinita*.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

***C. phantoma* (Dohrn, 1881)**

Synonyms: *Pallene phantoma* Dohrn, 1881; *Pallene phantopa* Norman, 1908; *Callipallene phantoma crinita* Stock, 1952.

This species was reported by Arnaud (1976) from Urla.

Depth range: 0.7-1m.

Substrate: *P. pavonia*.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

***C. spectrum* (Dohrn, 1881)**

Synonyms: *Pallene spectrum* Dohrn, 1881

The species was reported by Krapp et al. (2008) from Urla.

Depth Range: 0-1m.

Substrate: Stones; *Cladocora cespitosa* (Linnaeus, 1767).

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

***C. tiberi* (Dohrn, 1881)**

Synonyms: *Pallene tiberii* Dohrn, 1881; *Callipallene emaciata tiberii* (Dohrn, 1881)

This species was reported by Koçak (2016) from Urla.

Depth range: 0.5m

Substrate: *C. mediterranea*.

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

FAMILY: NYMPHONIDAE WILSON, 1878

GENUS: *NYMPHON* FABRICIUS, 1794

***Nymphon gracile* Leach, 1814**

Synonyms: *Nymphon gallicum* Hoek, 1881

This species was reported by Arnaud (1976) from Alsancak Harbour, and Çinar et al. (2008) from Pasaport Harbour and Alsancak Harbour.

Depth range: 0.2m.

Substrate: Boat hulls; *M. galloprovincialis.*

Worldwide Distribution: Atlantic-Mediterranean (Soler-Membrives and Munilla, 2015).

DISCUSSION

Ammothella longioculata is only known from the Mediterranean Sea. Spanish coast: Chafarinas Islands, Alboran Sea (Munilla & Nieto, 1999). French coast: Nice (Arnaud, 1987). Tunisian coast: Tabarka (Arnaud, 1987). Italian coast: Levanto, Ligurian Sea (Faraggiana, 1940); Isola Lachea (Krapp, 1973); Apulian coast (Chimenz, et al., 1993); Costa d'Argento (Vignoli et al., 2006). Croatian coast: Rovinj, North Adriatic (Schüller, 1989). Israel coast: Tantura (Stock, 1958). Northern Cypriot coast: Famagusta Bay (Kocak and Katagan, 2007); Turkish coast: Gencelli cove (Krapp et al., 2008) (Figure 1).

A. longioculata is reported only one time from the Turkish waters up to date. The presence of this rare species is confirmed for the first time from the İzmir Bay, and the second time from Turkey in the present study.

With *A. longioculata*, the total number of species is raised to 19 in the İzmir Bay. These 19 different species of pycnogonids, out of a total number of 29 species in Turkey (Kocak, 2019), represent 65.5% of all known pycnogonid species. To date, a total number of 25 pycnogonid species are found in the Turkish Aegean Sea. Thus, the reported 19 species in the İzmir Bay represent 76.0% of the actual Turkish Aegean Sea species.

The family Ammotheidae is dominant with 9 species, followed by Callipallenidae (4 species), Phoxichilidiidae (3 species). *Ammothella* and *Callipallene* are the major genera (4 species), followed by *Anoplodactylus* (3 species).

All of the known pycnogonid species in İzmir Bay were reported in shallow water. Consequently, the studies conducted along the İzmir Bay coasts were based mainly on inshore samplings. Therefore, it is believed that deep-sea samplings in İzmir Bay and more detailed examinations along the İzmir Bay coasts will result in a more accurate count of pycnogonid species.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 379-387 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.09

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Characterization and antioxidant capacity of anchovy by-product protein films enriched with rosemary and laurel essential oils

Biberiye ve defne uçucu yağları ile zenginleştirilmiş hamsi atık protein filmlerin karakterizasyonu ve antioksidan kapasitesi

Serpil Tural[1] ● Sadettin Turhan[2]* ● Fatih Öz[3]

[1] Department of Food Engineering, Ondokuz Mayis University, 55139 Samsun, Turkey
[2] Department of Food Engineering, Ondokuz Mayis University, 55139 Samsun, Turkey
[3] Department of Food Engineering, Ataturk University, 25240 Erzurum, Turkey

https://orcid.org/0000-0002-9360-3446
https://orcid.org/0000-0002-3510-4382
https://orcid.org/0000-0002-5300-7519

Corresponding author: sturhan@omu.edu.tr

Received date: 25.01.2020 *Accepted date: 31.05.2020*

How to cite this paper:
Tural, S., Turhan, S. & Öz, F., (2020). Characterization and antioxidant capacity of anchovy by-product protein films enriched with rosemary and laurel essential oils. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 379-387. DOI: 10.12714/egejfas.37.4.09

Abstract: In this study, characterization and antioxidant capacity of anchovy by-product protein (ABP) films with 0.5, 1.0 and 1.5% rosemary (REO) and laurel essential oils (LEO) were investigated. The films with REO and LEO showed higher elongation at break and water vapor permeability (WVP), but lower elastic modulus, transparency, and tensile strength. *L** and *b** values decreased as a function of essential oil (EO) amount and films became darker and slightly yellowish. The solubility of films with REO and LEO decreased by 10.00-16.05% and 13.84-18.20%, respectively. Intermolecular interaction and molecular organization in the polymer matrix were changed by EO incorporation. Films with EOs showed a nonhomogeneous surface and comparatively smooth cross-section structure providing easy permeation. The antioxidant properties of films were enriched by addition EO and the highest antioxidant capacity was determined in 1.5% LEO film. As a result, although ABP films enriched with EOs have a high WVP, they can be used as packaging material for food products that are susceptible to lipid oxidation.

Keywords: Anchovy processing by-product, protein film, antioxidant capacity, rosemary essential oil, laurel essential oil

Öz: Bu çalışmada %0,5, 1,0 ve 1,5 oranlarında biberiye (BUY) ve defne uçucu yağlarını (DUY) içeren hamsi atık protein (HAP) filmlerin karakterizasyonu ve antioksidan kapasitesi incelenmiştir. BUY veya DUY içeren filmler daha yüksek gerilme ve su buharı geçirgenliği (SBG), fakat daha düşük elastik modül, saydamlık ve kopma uzaması göstermiştir. Uçucu yağ (UY) miktarının bir fonksiyonu olarak *L** ve *b** değerleri azalmış ve filmler daha koyu ve hafif sarımsı hale gelmiştir. BUY ve DUY içeren filmlerin çözünürlüğü sırasıyla %10,00-16,05 ve %13,84-18,20 düzeyinde azalmıştır. Polimer matristeki moleküller arası etkileşim ve moleküler organizasyon, UY ilavesi ile değiştirilmiştir. UY içeren filmler homojen olmayan bir yüzey ve kolay geçirgenlik sağlayan nispeten pürüzsüz bir kesit yapısı göstermiştir. Filmlerin antioksidan özellikleri UY ilavesiyle zenginleştirilmiş ve en yüksek antioksidan kapasite %1,5 DUY içeren filmde belirlenmiştir. Sonuç olarak, UY içeren HAP filmler yüksek bir SBG' ye sahip olmalarına rağmen, lipit oksidasyonuna duyarlı gıda maddeleri için ambalaj malzemesi olarak kullanılabilirler.

Anahtar kelimeler: Hamsi işleme atığı, protein film, antioksidan kapasite, biberiye uçucu yağı, defne uçucu yağı

INTRODUCTION

Recently, many researchers have started to conduct researches on biodegradable and/or edible films and coatings produced from natural polymers, which are generally comprised of waste products of fishing, agriculture or livestock raising (Gomez-Estaca et al., 2009). This is thanks to various advantages of these films and coatings such as biodegradability, edibility, barrier properties, biocompatibility and their non-polluting and nontoxic properties (Alparslan et al., 2014). Moreover, they may be used as carriers for antioxidants, antimicrobial agents, color, nutrients, spices and herbs, and may generate localized or delayed activity when needed (Alparslan et al., 2014; Tural and Turhan, 2017). Such polymers can be based on lipid, protein, or polysaccharide: the physical properties of these films may be highly variable, depending on the kind of polymer employed (Gomez-Estaca et al., 2009; Pires et al., 2011). Many researchers have stated that in the development of biodegradable films, proteins extracted from fish by-products have been successfully used among these materials (Pires et al., 2011; Teixeira et al., 2014; Zavareze et al., 2014). Moreover, it is also vital that fish-processing by-products are effectively evaluated for the prevention of environmental pollution as well as achieving high added value products by increasing the range of products (Zavareze et al., 2014).

Generally, fish protein films are considered as having limited resistance to water vapor transmission due to being highly polar polymers and also due to their high level of hydrogen bonding and hydroxyl groups (Pires et al., 2011; Rocha et al., 2014). Therefore, researchers have tested many essential oils and extracts obtained from some plants to improve these films' barrier and functional properties. Pires et

al. (2011) studied biodegradable films, which they prepared thyme essential oil (TEO) and hake proteins: as a result, they found that TEO oil reduced the water vapor permeability and enriched the antioxidant activity. Arfat et al. (2014) observed improved water vapor permeability after adding basil leaf essential oil into composite films of fish skin gelatin (FSG) blend and fish protein isolate. According to Teixeira et al. (2014), the addition of origanum, garlic and clove essential oils to hake by-product protein films decreased water vapor permeability and broke force and elongation, while increased their free radical scavenging activity.

Rosemary (*Rosmarinus officinalis* L.) and laurel (*Lauris nobilis* L.), which have a wide area of usage such as traditional medicines, are naturally grown in Turkey (Alparslan et al., 2014; Turhan et al., 2009). Various researchers have mentioned that essential oils and extracts obtained from rosemary and laurel leaves are effective antioxidants (Alparslan et al., 2014; El et al., 2014; Gomez-Estaca et al., 2009; Turhan et al., 2009). The antioxidant capacity of rosemary comes from its epirosmanol, carnosol, rosmanol, carnosic acid, rosmaridiphenol, rosmadial, rosmarinic acid, isorosmanol and rosmariquinone contents (Turhan et al., 2009), while that of laurel essential oil is related to its eugenol and methyl eugenol contents (El et al., 2014). The addition of rosemary (REO) and laurel essential oils (LEO) into anchovy by-product (ABP) films is expected to give its antioxidant capacity and also enhance its physicochemical properties.

To our knowledge, there is no study on the characterization and antioxidant capacity of ABP films enriched with REO and LEO in the literature. Hence, this study aimed to characterize the ABP films enriched with REO and LEO and to determine their antioxidant capacities.

MATERIALS AND METHODS

Materials

Anchovy (*Engraulis encrasicolus*) processing by-products including head, frame and viscera were obtained from Sastaş A.Ş (Samsun, Turkey) and proteins in by-products were extracted using the method described by Tural and Turhan (2017). The obtained anchovy by-product protein (ABP) powder (10.41±0.09% water, 79.17±1.25% protein, 2.11±0.22% lipid and 5.15±0.03% ash (AOAC, 1990)) was stored in glass jars at 4°C until film preparation. Dried rosemary (*Rosmarinus officinalis* L.) and laurel (*Lauris nobilis* L.) leaves collected from different geographical regions in Turkey were purchased from a local market (Samsun, Turkey) and authenticated by the experts at the Department of Biology (Ondokuz Mayis University, Samsun, Turkey). The steam distillation method was used to obtain the essential oils of rosemary and laurel using a Clevenger apparatus (Sesim Kimya Laboratuvar, Turkey) as mentioned Tural and Turhan (2017). Tween 80 and glycerol were purchased from Merck (Germany).

Preparation of films

The technique described by Limpan et al. (2010) was applied by practicing several modifications to prepare the ABP films. 4.0g ABP was blended with 100mL distilled water, and the solution pH was calibrated to 11.5 with 5M NaOH. Then, glycerol was included up to 40% (w/w of ABP) as a plasticizer, and the solution was homogenized for 5 min at 10,000rpm. Subsequently, it was slowly stirred for 60 min at 85°C to allow film formation, centrifuged for 10 min at 5,000 rpm, filtered to remove undissolved residuals and cooled to 40±2°C. Tween 80 at 0.05% (v/v) of essential oil was added as an emulsifier to assist essential oil dispersion in the film-forming solution, then essential oils from rosemary (REO) and laurel (LEO) at 0 (as control), 0.5, 1.0 and 1.5% (v/v) ratios of film solution were added to the film-forming solution. These rates were selected based on the results of the study by Tural and Turhan (2017). The solution was emulsified for 3 min at 10,000rpm. Finally, the film-forming emulsion (50 g) was put into acrylic plates (15cm diameter) and left for drying for 24h at 40°C. Then, the samples of dried films were taken out of the plates and adjusted to 54% relative humidity for 3 days in a desiccator at room temperature (approx. 25°C).

Characterization of films

Film thicknesses were measured using a digital micrometer (Insize, 3101-25A model, China) with a precision of 0.001 mm. The results were expressed as the mean of ten measurements on each triplicate film sample at different locations.

The color parameters were measured with Hunter Lab system using a calibrated Minolta CR 400 chromameter (Japan) with a standard illuminant D65 and a 10° observer of 2.54 cm aperture size. Hunter *L* (lightness: 0 = black and 100 = white), *a* (redness: +*a* = red and –*a* = green) and *b* (yellowness: +*b* = yellow and –*b* = blue) values were recorded. The results were expressed as the mean of five measurements on each triplicate film sample at different locations.

The film transparency values were calculated with the method of Kurt and Kahyaoglu (2014) and the film absorbance was measured at 600 nm using a UV spectrophotometer (Helios Gama, England). The samples were cut into rectangular pieces based on the lateral area of the spectrophotometer test cell and placed in the test cell. The reference value was determined from an empty test cell. The transparency value of the film was calculated by:

$$\text{Transparency value} = \frac{\text{Abs}_{600}}{\text{x}}$$

where Abs_{600} is the absorbance at 600 nm, and x is the mean film thickness (mm). According to this equation, higher transparency values imply lower transparency. All

measurements were performed on each triplicate film sample at five different locations.

The water vapor permeability (WVP) values were obtained by the ASTM E96-05 gravimetric method (ASTM, 2005). The films (14 mm diameter) were sealed in glass cups containing silica gels (0% RH). They were stored at 25°C in desiccators containing distilled water (100% RH). The glass cups were weighed every 1h for 8h and WVP of the films was calculated by:

$$\mathrm{SBG} = \frac{\mathrm{w}}{\mathrm{t}} \times \frac{\mathrm{x}}{\Delta P \times \mathrm{A}}$$

where w/t is determined according to linear regression ($R^2 > 0.99$) from the water absorbed by the system until the steady-state was reached, x is the mean sample thickness. A is the film area exposed to moisture transfer (1.539×10^{-4} m^2), and ΔP is the partial pressure difference of the film at 25°C (kPa). All the measurements for each of the films were completed in triplicates.

The method described by Gennadios et al. (1998) was used to measure the solubility in water of the different ABP films. The initial dry matter of the preconditioned film pieces (20mm x 20mm) was determined by drying in an air-circulating oven at 105°C for 24h. Afterward, the film pieces were immersed in 50mL distilled water containing sodium azide (0.1%, w/v) and stored for 24h at room temperature (approx. 25°C) under periodic agitation. To determine the final dry weight, insoluble matter was separated carefully and left for drying for 24h at 105°C. Solubility was determined as the percent weight loss of the film pieces from the immersing. The tests were performed in triplicate.

Tensile strength (TS), elongation at break (EAB) and elastic modulus (EM) were determined using a texture analyzer (TA-XT2 Texture Analyzer, UK) according to the ASTM standard method D882-09 (ASTM, 2009). The films were cut into strips (1cm x 4cm) and conditioned for 3 days (54% RH). The distance and force were measured during the extension of the strips mounted between the grips at 1.5mm/s until break. TS was calculated by dividing the load at break by the original cross-sectional area (mm^2) of the film. Before testing, the strip thicknesses were recorded at ten points, and the cross-sectional area of the film samples was estimated based on the average values. EAB (%) was calculated by dividing the elongation at the moment of rupture by the initial gauge length and then multiplying by 100. Moreover, EM was calculated by drawing a tangent to the initial linear portion of the stress-strain curve, selecting any point on this tangent, and dividing the tensile stress by the corresponding strain. The results are expressed as the mean of five samples for each type of film.

The FTIR spectra of the films were recorded at wavenumber range 650 and 4000cm^{-1} using a FTIR spectrometer (Perkin Elmer, Model Spectrum Two, USA). Totally 32 scans were carried out at 4cm^{-1} resolution. Before analysis, a desiccator containing silica gel was used to condition the film samples at room temperature (approx. 25°C) for 2 weeks to achieve the maximum dehydration of the films.

Cross-section and surface characteristics of the film samples were visualized with a scanning electron microscope (JEOL, JSM-7001F model, Japan) at 10 kV increasing voltage. Before the visualization process, the samples were coated with gold-palladium palladium (Quorum SC7620, Laughton, UK), and photographs were taken at 1500x magnification.

The calorimetric analysis was performed using a differential scanning calorimeter (Perkin Elmer, DSC 4000 model, USA). Before analysis, film samples were kept for 2 weeks at room temperature (approx. 25°C) in a desiccator containing silica gel for dehydrating the films. A sample, weighing 5mg was put into aluminum pans, sealed, and scanned under nitrogen at 10 °C/min over the range of -50/+120°C. An empty aluminum pan was used as a reference.

Antioxidant capacity of films

The antioxidant capacity of ABP films enriched with REO and LEO was determined with methods of ferric reducing antioxidant power (FRAP) (Gao et al. 2000) and 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity (Nakajima et al. 2004). For the FRAP determination, 50 µL film solution containing 0.15 g film and 1.5 mL methanol was mixed with 0.95mL ferric-2,4,6-tripyridil-s-triazine (TPTZ) reagent (which was done by mixing 300mM acetate buffer, pH 3.6, 10mM TPTZ in 40mM HCl and 20mM $FeCl_3$ at the ratio 10:1:1). Then, the sample absorbance was read at 593nm after 30 min incubation at room temperature (approx. 25°C) using a UV-Vis spectrophotometer (Helios Gama, England). FRAP values were determined according to Trolox standard curves and expressed as mg Trolox/mL.

For the DPPH scavenging activity determination, 0.15g of ABP film sample was dissolved in 1.5mL methanol. Afterward, 10µL of film solution was mixed with 40µL of methanol and 1mL of DPPH methanol solution (100µM). Following vigorous shaking, the mixture was left at room temperature (approx. 25°C) for 30 min and then, the sample absorbance was recorded at 517nm with a UV-Vis spectrophotometer (Helios Gama, England). DPPH scavenging activity was calculated by:

$$\text{DPPH scavenging activity (\%)} \ \frac{\mathrm{A_{blank} - A_{sample}}}{\mathrm{A_{blank}}} \ \mathrm{x}\ 100$$

where A_{blank} is the absorbance of the control, and A_{sample} is the absorbance of the test sample.

Statistical analysis

Experiments were conducted as triplicates and expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used to obtain the data and Duncan's multiple range test

was used to compare the main differences among the means. SPSS statistical package program (SPSS 17.0 for windows, SPSS Inc., Chicago, IL, USA) was preferred for the analysis with a significance level of 0.05.

RESULTS AND DISCUSSION

Characterization of films

The thickness values of films varied from 0.196 mm to 0.205 mm, and the addition of different essential oils had no notable effect ($p>0.05$) (Table 1). According to Tural and Turhan (2017), the film thickness is influenced by the solid content of the film-forming solution. The addition of essential oils to the film-forming solution at low concentrations probably did not affect film thicknesses. These results were in line with the findings of Zinoviadou et al. (2009), showing that the addition of oregano oil at 0.5, 1.0 and 1.5% has no significant impact on the thickness of whey protein isolate (WPI) films. Similar results were also detected by Tural and Turhan (2017) in ABP films containing TEO. However, Jouki et al. (2014) reported that the addition of thyme essential oil to the film-forming emulsion led to an increase in the thickness of quince seed mucilage films, but this effect was only significant at the highest level of TEO used (2%).

In this study, lightness, redness and yellowness values of control film, measured as *L**, *a** and *b**, were determined to be 33.38, 33.66 and 54.15, respectively. Addition of REO and LEO significantly ($p<0.05$) affected *L**, *a** and *b** values of ABP films and in general, *L** and *b** values decreased as a function of essential oil amount. Hence, films containing essential oil at higher amounts became darker and slightly yellowish as indicated by lower *L** and *b** values. The films enriched with both REO and LEO showed higher *a** values when compared to the control film (Table 1). The color differences produced by REO and LEO are probably caused by the high-level presence of the phenolic compounds in essential oils. Similar changes in film color were also observed by Shojaee-Aliabadi et al. (2014) and Baek et al. (2018) in κ-carrageenan films at different levels of *Zataria multiflora Boiss* and *Mentha pulegium* essential oils and alginate films containing various concentrations (0%, 0.4%, 0.7%, and 1.0%) of cinnamon essential oils, respectively.

Table 1. Thickness, color and transparency values of anchovy by-product protein films enriched with rosemary and laurel essential oils

Films	Thickness, mm	Color properties			Transparency
		*L**	*a**	*b**	
Control	0.205±0.010^{a}	33.38±1.19^{d}	33.66±1.26^{c}	54.15±1.51^{c}	1.28±0.01^{d}
0.5% REO	0.199±0.003^{a}	38.36±0.10^{b}	38.71±0.36^{a}	60.54±2.06^{b}	1.35±0.14^{d}
1.0% REO	0.201±0.002^{a}	34.97±0.81^{c}	38.67±0.09^{a}	52.49±2.42^{cd}	1.63±0.29^{c}
1.5% REO	0.197±0.004^{a}	32.70±0.75^{d}	38.32±0.38^{a}	49.18±0.76^{e}	1.92±0.07^{ab}
0.5% LEO	0.198±0.003^{a}	40.32±0.43^{a}	35.73±0.10^{b}	66.62±1.71^{a}	1.70±0.04^{bc}
1.0% LEO	0.196±0.004^{a}	38.34±0.15^{b}	35.38±0.10^{b}	52.25±0.53^{cd}	1.76±0.10^{bc}
1.5% LEO	0.197±0.002^{a}	37.97±0.66^{b}	35.16±0.38^{b}	50.36±1.41^{de}	2.10±0.05^{a}

The results represent means ± SD of three replicates. Means in same column with different superscripts are significantly different ($p<0.05$). *L** = lightness/brightness (100: white, 0: black); *a** = redness/greenness (+, red; -, green); *b** = yellowness/blueness (+, yellow; -, blue); REO = rosemary essential oil; LEO = laurel essential oil.

While ABP films that have no essential oil appeared transparent, the addition and amount of REO and LEO significantly affect ($p<0.05$) the transparency values of the films. The films enriched with both REO and LEO exhibited higher transparency values compared to the control and in general, an increase in REO and LEO amounts significantly ($p<0.05$) raised the transparency values of the ABP films (Table 1). Higher transparency values show lower transparency. Therefore, transparency of the films decreased as the amount of the essential oils increased, probably because of an increase in light-scattering caused by oil droplets in the film network. Generally, the level and particle size of the dispersed phase affect the light-scattering: with more droplets, greater intensity in light-scattering and lower transparency is obtained (Shojaee-Aliabadi et al., 2014). The results of this study show that films enriched and un-enriched with essential oils were colored, but they have suitable transparencies for food packaging. Shojaee-Aliabadi et al. (2013, 2014) and Tural and Turhan (2017) reported similar results in κ-carrageenan films containing plant essential oils and in ABP films treated with TEO, respectively. Water vapor permeability (WVP) values of films changed between 1.54 and 2.08 g.mm/m^2.h.kPa, and the addition of REO and LEO significantly ($p<0.05$) increased it (Table 2). This increase was higher in films enriched with REO. Several factors may affect the WVP of films such as film thickness, the hydrophilic-hydrophobic ratio of the film components, water sensitivity and crystallinity (Jouki et al., 2014). The differences in the hygroscopic nature of the oils might lead to an increase in the WVP values of the film treated with essential oils causing different abilities to attract water to the film network. Additionally, results obtained from SEM images (Figure 2) and DSC thermograms (Figure 3) showed that REO and LEO presence led to less crystalline films, causing an increase in WVP. As known, high crystallinity, polymers are generally less permeable because of their ordered structure (Jouki et al., 2014). Results found by Mei et al. (2013) and Tural and Turhan (2017) showed very good similarity in water chestnut starch-chitosan films treated with pine needle essential oil (PNEO) and in ABP films with TEO, respectively. However,

Zinoviadou et al. (2009) mentioned that the addition of oregano essential oil (0.5, 1.0 and 1.5%) into the WPI film matrix did not affect significantly the WVP.

The highest solubility with the percentage of 63.41 was observed in the control film, whereas films with REO and LEO showed lower solubility (51.87-57.07%) (Table 2). The solubility values of ABP films treated with REO and LEO decreased by 10.00-16.05% and 13.84-18.20%, respectively, compared to the control. Additive effects on the solubility of films usually depend on the sort of compounds and concentrations as well as their natural hydrophilicity and hydrophobicity indices (Ghasemlou et al., 2013). The decrease in solubility might be due to the decrease in the hydrophilic nature of the films and the interaction between the components of REO and LEO, as well as the hydroxyl groups of ABP. Similar results were found by Ghasemlou et al. (2013) in corn starch films incorporated with plant essential oils and by Shojaee-Aliabadi et al. (2013) in κ-carrageenan films containing *Saturaje hortensis* essential oil.

Table 2. WVP, solubility and mechanical properties of anchovy by-product protein films enriched with rosemary and laurel essential oils

Films	WVP, g.mm/m^2.h.kPa	Solubility, %	TS, MPa	EAB, %	EM, MPa
Control	1.54±0.10^{d}	63.41±0.44^{a}	1.46±0.04^{a}	51.50±2.15^{e}	10.77±1.15^{a}
0.5% REO	2.04±0.04^{a}	57.07±3.42^{b}	0.62±0.01^{b}	97.57±4.34^{b}	2.77±0.25^{bc}
1.0% REO	2.08±0.11^{a}	54.99±4.26^{bc}	0.60±0.01^{b}	99.16±3.44^{ab}	3.27±0.42^{b}
1.5% REO	1.90±0.08^{b}	53.23±2.97^{bc}	0.58±0.05^{bc}	105.40±4.54^{a}	2.67±0.06^{bc}
0.5% LEO	1.75±0.02^{c}	54.63±0.74^{bc}	0.53±0.02^{c}	64.94±3.72^{d}	3.20±0.10^{b}
1.0% LEO	1.79±0.08^{bc}	52.86±1.35^{bc}	0.47±0.04^{d}	72.40±2.09^{c}	1.87±0.12^{c}
1.5% LEO	1.80±0.02^{bc}	51.87±0.21^{c}	0.47±0.01^{d}	78.76±4.87^{c}	2.20±0.10^{c}

The results represent means ± SD of three replicates. Means in same column with different superscripts are significantly different ($p<0.05$). WVP = water vapor permeability; TS = tensile strength; EAB = elongation at break; EM = elastic modulus; REO = rosemary essential oil; LEO = laurel essential oil.

Mechanical features of the film were obtained by using the values of TS, EAB, and EM. The ABP film without essential oils had a tensile strength of 1.46 MPa, which agrees with that found by Tural and Turhan (2017). The addition of REO or LEO into the films led to a decrease in TS and EM values, but an increase in EAB values. TS and EM values of the samples enriched with REO did not importantly change after the increase of the essential oil level from 0.5 to 1.5%, but TS and EM values of films treated with LEO significantly changed after the increase in the level from 0.5 to 1.0% (Table 2). Thus, essential oils led the films to become weaker, and less resistant to break, depending on the essential oil level. The partial replacement of stronger polymer-polymer interactions by weaker polymer-oil interactions in the film network in the presence of the essential oil may primarily explain this effect, which, in turn, may lead to the weakening of the network structure, and hence the TS of the ABP film. Moreover, a plasticizing effect was caused by the changes in the interaction balances, even at small concentrations of essential oils, making the film more stretchable (high EAB value) (Shojaee-Aliabadi et al., 2013; Tural and Turhan, 2017). Rojas-Grau et al. (2007) found a decrease in TS and EM values, and an increase in EAB values of alginate-apple puree edible films treated with essential oils (oregano, lemongrass and cinnamon), and this finding complies with the results of this study. Other researchers (Ghasemlou et al., 2013; Shojaee-Aliabadi et al., 2013, 2014; Tural and Turhan, 2017) also obtained similar results in edible films combined with plant essential oils.

Generally, FTIR spectra of all film samples had similar major peaks, but the peak amplitudes changed based on the level of the added essential oils (Figure 1). In all samples, the band was found at the 1043 cm^{-1} wavenumber, matching to the OH group, mostly due to the glycerol added as a plasticizer (Hosseini et al., 2015; Tongnuanchan et al., 2013; Tural and Turhan, 2017). The amplitude of this peak decreased with an increase in the level of added essential oils and this might be attributed to the dilution effect of the essential oils. A similar peak at the wavenumber of 1042-1045 cm^{-1} for fish gelatin-chitosan biocomposite films treated with different concentrations of *Origanum vulgare* L. essential oil was reported by Hosseini et al. (2015) and at the wavenumber of 1043 cm^{-1} for ABP films enriched with different concentrations of TEO was reported by Tural and Turhan (2017). All films showed major peaks at 1540-1547 cm^{-1} (amide-II, arising from bending vibration of N-H groups and stretching vibrations of C-N groups), 1624-1628 cm^{-1} (amide-I, presenting C=O stretching/hydrogen bonding coupled with COO) and 1235-1237 cm^{-1} (amide-III, displaying the vibrations in plane of C-N and N-H groups of bound amide or vibrations of CH_2 groups of glycine) wavenumbers (Arfat et al., 2014; Tongnuanchan et al., 2013). In general, the amplitudes of films decreased as the essential oil level increased and the highest amplitudes for amide-I, II and III were observed in control films. This result could be attributed to the highest protein content in the control.

In all samples, amide-A peak was observed at 3273-3276 cm^{-1} wavenumber and amide-B peak at 2918-2924 cm^{-1}. Amide-A peak shows the NH-stretching accompanied by hydrogen bonding, while amide-B peak shows the asymmetric stretching vibration of CH and NH_3^+ (Arfat et al., 2014). The amplitude of amide-A peak decreased when the films were treated with REO and LEO, especially at 1.5% level. Lower interaction between ABP confirmed this phenomenon, as expressed by lower TS with increasing EAB when essential oils were incorporated at higher levels. Peaks with

wavenumber of 2851 cm^{-1} (methylene symmetric C-H stretching) and 2871 cm^{-1} (methyl asymmetric C-H stretching) were also obtained in all film samples. The amplitude of peak at wavenumber 2851 cm^{-1} increased, while the amplitude of peak at wavenumber 2871 cm^{-1} decreased when essential oils were added to the films. It was observed that methylene symmetric stretching bands at approximately 2851 cm^{-1} were present in most lipids, while methyl asymmetric stretching bands at approximately 2871 cm^{-1} were present in most proteins (Tongnuanchan et al., 2013). Furthermore, a peak with 1737 cm^{-1} wavenumber was observed in the films with essential oils and the amplitude of this peak increased with the essential oil amount. This probably presented the C=O stretching vibration of aldehyde or ester carbonyl groups. Ketone, ester, and aldehyde are the primary chemical groups in essential oils (Arfat et al., 2014). However, no peak at 1737 cm^{-1} wavenumber was observed in the control. Similar peaks at 1733-1743 wavenumbers were found by Tongnuanchan et al. (2014) in fish gelatin films with citronella and basil essential oils. The FTIR spectra results showed that REO and LEO addition changed the molecular organization and intermolecular interaction in the polymer matrix

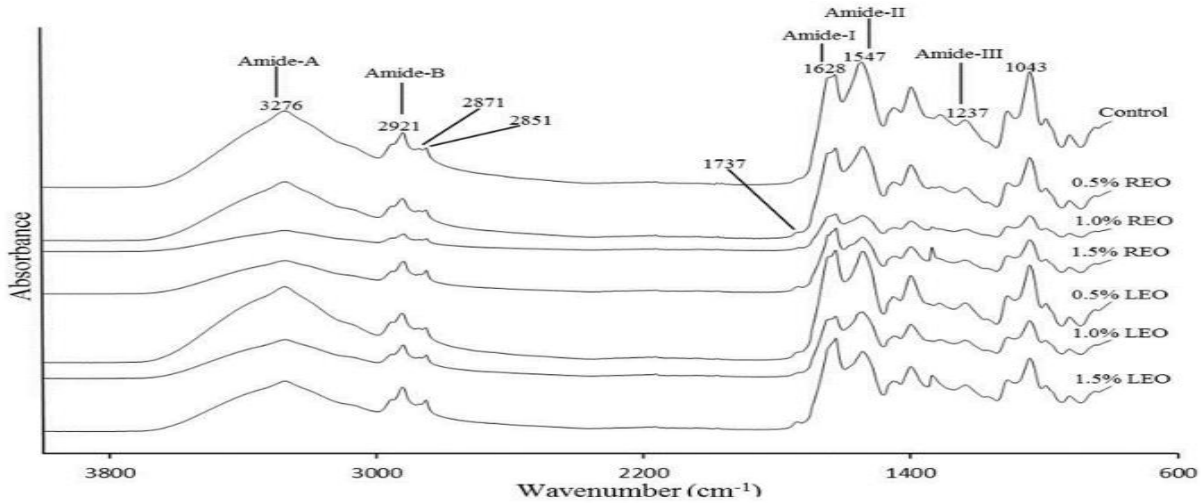

Figure 1. FTIR spectra of anchovy by-product protein films incorporated with rosemary and laurel essential oils

The addition and amount of REO and LEO affected the film structures (Figure 2). The control film gave a homogeneous and continuous surface, whereas ABP films enriched with REO and LEO showed a heterogeneous surface. Also, a great number of granules were observed in the surface of ABP films with REO and LEO, and in general, the size of granules increased as the essential oil level increased. Hereby, the permeability of surface containing granules facilitated and resulted in an increase in WVP of films treated with EO. Similar surface images were observed by Tongnuanchan et al. (2013) in the FSG films with root essential oils and by Tural and Turhan (2017) in ABP films treated with TEO. The control film showed a nonhomogeneous, compact and rough cross-section structure, while films enriched with REO and LEO showed a relatively smooth cross-section structure. The presence of REO and LEO probably led to discontinuance with lipid droplets or holes present in the protein network and this may be the reason for the higher WVP values of the films treated with EOs. Mei et al. (2013) also observed similar results after adding PNEO to the water chestnut starch-chitosan edible films. DSC thermograms of all films showed similar behaviour and exhibited clearly endothermic peaks at the phase-transition temperature (T*m*) ranges of 53.53-56.37 °C. (Figure 3).

These peaks were most probably related to the denaturation temperature of myosin and actin as observed by Rocha et al. (2013). These researchers in their studies with Argentine anchovy (*Engraulis encrasicholus*) protein isolate observed the endothermic peak at 62.2 °C. Temperature differences for endothermic peaks could be attributed to fish species, kind of muscle and heating conditions. Except films containing 1.5% LEO, ABP films enriched with REO and LEO showed slightly higher T*m* compared to control film containing 4% ABP and 40% glycerol, which may be by the reason of more hydrophobic nature and the larger molecular weight of REO and LEO compared to glycerol. Jouki et al. (2014) observed similar results in QSM films with thyme and Tongnuanchan et al. (2014) in fish gelatin films with basil and citronella. The highest glass-transition temperature (T*g*) was seen in the control film as -24.27 °C and this temperature was likely associated with T*g* of the glycerol-rich phase. The addition of 0.5% REO and LEO into the ABP films resulted in lower T*g* (-37.08 and -33.82 °C, respectively) (data not shown).

However, T*g* of films enriched with 1.0 and 1.5% REO and LEO most likely became too low to be observed in the tested temperature range. For film flexibility, a lower T*g* is better. In general, T*g* of the protein films increases with an increase in the chain stiffness assisted by inter/intra-molecular attractive forces; therefore, the addition of essential oils improves the weak structure of films (Tongnuanchan et al., 2014). In general, the DSC results were also in line with the mechanical properties of films (Table 2). An increase in T*g* of edible films with increasing essential oil levels was also observed by Jouki et al. (2014).

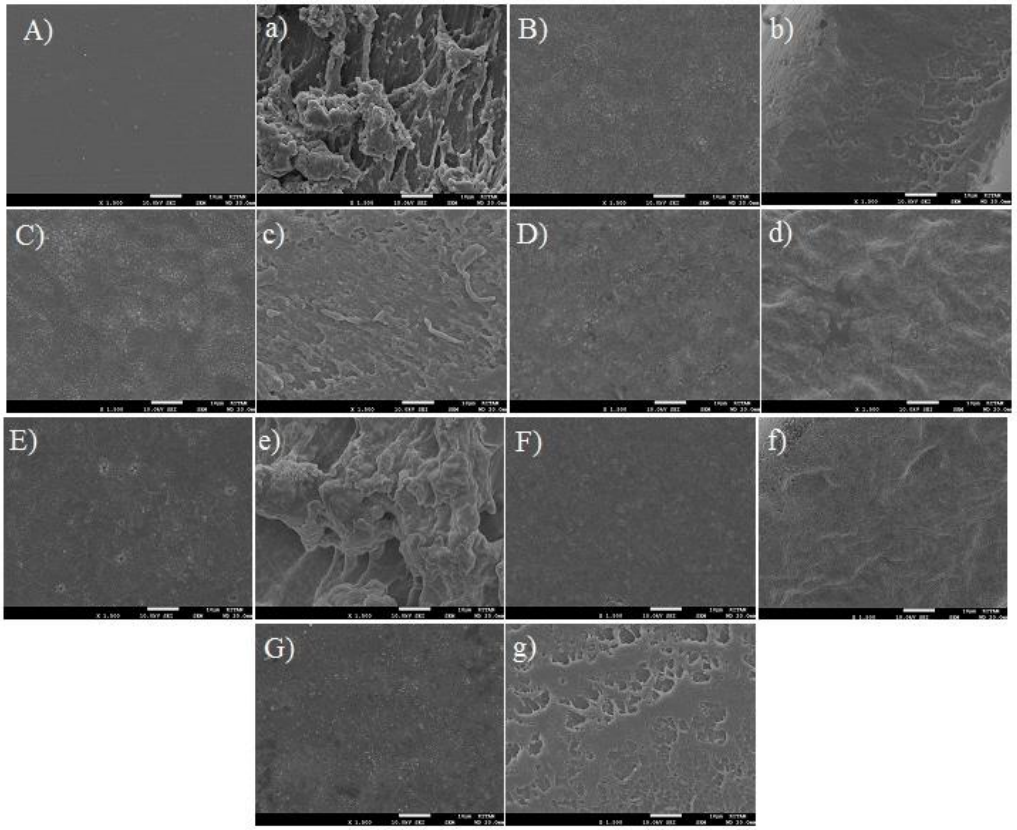

Figure 2. SEM images of the surface [A) control, B) 0.5% REO, C) 1.0%REO, D) 1.5% REO, E) 0.5% LEO, F) 1.0%LEO, G) 1.5% LEO] and cross-section [a) control, b) 0.5% REO, c) 1.0%REO, d) 1.5% REO, e) 0.5% LEO, f) 1.0%LEO, g) 1.5% LEO] of anchovy by-product protein films incorporated with rosemary and laurel essential oils

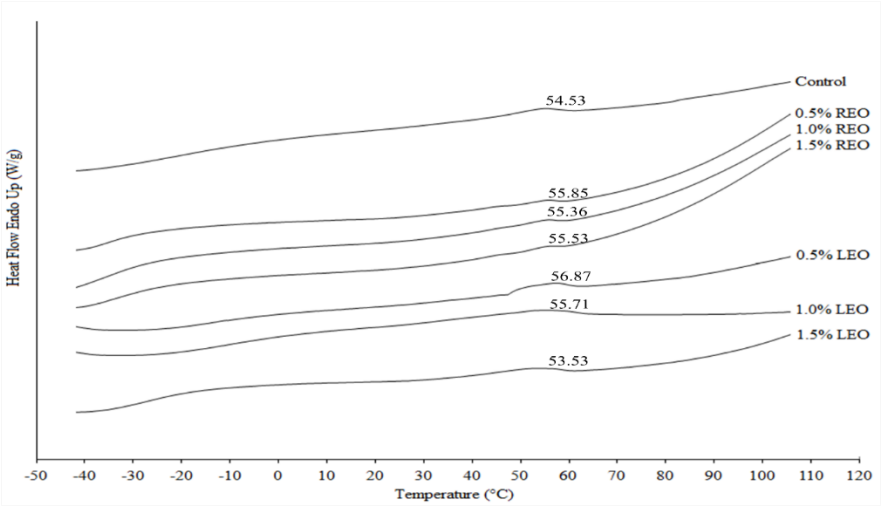

Figure 3. DSC thermograms of anchovy by-product protein films incorporated with rosemary and laurel essential oils

Antioxidant capacity of films

Experimental results pointed out that the addition of REO and LEO significantly ($p<0.05$) affected FRAP and DPPH scavenging activity values (Table 3), and the control film exhibited slight antioxidant capacity (0.91 mg Trolox/mL FRAP and 0.06% DPPH), probably due to the presence of free sulfhydryl groups and amino acids such as Trp, Met and Tyr on ABP as reported by Gencbay and Turhan (2016).

The antioxidant capacity of control ABP film was considerably improved by incorporating both REO and LEO, and in general, an increase in REO and LEO levels increased the antioxidant capacity values of films. Hake protein films treated with TEO (Pires et al., 2011) and FSG films formulated with root essential oils (Tongnuanchan et al., 2013) showed similar results. However, films with LEO exhibited significantly ($p<0.05$) higher FRAP and DPPH scavenging activity compared to the films with REO at the same level. This could be due to various major components of essential oils, the intensity of possible interactions between the constituents of film and their phenolic compounds, which is greater in the samples with REO.

Table 3. Antioxidant capacity of anchovy by-product protein films enriched with rosemary and laurel essential oils

Films	FRAP, mg Trolox/mL	DPPH scavenging activity, %
Control	0.91 ± 0.01^{e}	0.06 ± 0.03^{d}
0.5% REO	1.99 ± 0.13^{d}	9.12 ± 0.56^{c}
1.0% REO	2.09 ± 0.03^{cd}	9.48 ± 0.79^{c}
1.5% REO	2.13 ± 0.06^{bcd}	10.05 ± 0.89^{bc}
0.5% LEO	2.19 ± 0.13^{bc}	10.56 ± 0.01^{b}
1.0% LEO	2.27 ± 0.04^{b}	10.71 ± 0.24^{b}
1.5% LEO	2.37 ± 0.06^{a}	11.63 ± 0.18^{a}

The results represent means ± SD of three replicates. Means in same column with different superscripts are significantly different ($p<0.05$). FRAP = ferric reducing antioxidant power; DPPH = 2,2-diphenyl-2-picrylhydrazyl; REO = rosemary essential oil; LEO = laurel essential oil.

The highest FRAP and DPPH scavenging activity values were determined in films treated with 1.5% LEO as 2.37 mg Trolox/mL and 11.63%, respectively. Alparslan et al. (2014) presented that gelatin films enriched with LEO had the high antioxidant capacity and inhibited the lipid oxidation of trout fillets. It is considered that the antioxidant capacity of LEO related to its eugenol and methyl eugenol content (El et al., 2014). In line with our results, numerous authors have observed that extracts and essential oils of rosemary and laurel have the strong antioxidant capacity (El et al., 2014; Gomez-Estaca et al., 2009; Turhan et al., 2009).

CONCLUSION

Our findings in the present study show that *L** and *b** values decreased as a function of essential oil level and films became darker and yellowish. The incorporation of REO or LEO into the films caused to a decrease in transparency, solubility, TS and EM values, but an increase in *a** value, WVP, and EAB value, depending on the essential oil type and level. The FTIR spectra results demonstrated that the addition of REO and LEO changed the molecular organization and intermolecular interaction in the polymer matrix. The control film showed a homogeneous and continuous surface, while the films enriched with REO and LEO showed a heterogeneous. Slightly higher phase-transition and lower glass-transition temperatures were obtained in films treated with EOs. The antioxidant capacity of the film was considerably improved by incorporating both REO and LEO. These results suggested that ABP films enriched with REO and LEO could be suitable for the packaging of food products that are susceptible to lipid oxidation.

ACKNOWLEDGEMENTS

Some part of this study was taken from PhD thesis of Serpil Tural and supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (Project No. 114O854). The authors would like to thank TUBITAK for support.

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Ege Journal of Fisheries and Aquatic Sciences, 37(4), 389-396 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.10

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of GroBiotic®-A supplementation on growth performance, body composition and liver and intestine histological changes in European Seabass (*Dicentrarchus labrax*) juveniles

Grobiyotik A ilavesinin levrek (*Dicentrarchus labrax*) juvenillerinde büyüme performansı, vücut kompozisyonu, karaciğer ve bağırsak histolojik değişimleri üzerine etkileri

Metin Yazıcı[1]* ● Yavuz Mazlum[2] ● Mehmet Naz[3] ● Selin Sayın[4] ● Çiğdem Ürkü[5] Tülay Akaylı[6]

[1] Iskenderun Technical University, Faculty of Marine Sciences and Technology, 31200, Hatay, Turkey https://orcid.org/0000-0002-7011-886X
[2] Iskenderun Technical University, Faculty of Marine Sciences and Technology, 31200, Hatay, Turkey https://orcid.org/0000-0002-9547-0966
[3] Iskenderun Technical University, Faculty of Marine Sciences and Technology, 31200, Hatay, Turkey https://orcid.org/0000-0002-5129-8498
[4] Iskenderun Technical University, Faculty of Marine Sciences and Technology, 31200, Hatay, Turkey https://orcid.org/0000-0002-7497-388X
[5] Istanbul University Faculty of Aquatic Sciences, 34134, İstanbul, Turkey https://orcid.org/0000-0003-0381-9321
[6] Istanbul University Faculty of Aquatic Sciences, 34134, İstanbul, Turkey https://orcid.org/0000-0003-2375-2224

Corresponding author: metin.yazici@iste.edu.tr
Received date: 10.01.2020 *Accepted date: 09.06.2020*

How to cite this paper:
Yazıcı, M., Mazlum, Y., Naz, M., Sayın, S., Ürkü, Ç. & Akaylı, T. (2020). Effects of GroBiotic®-A supplementation on growth performance, body composition and liver and intestine histological changes in European Seabass (*Dicentrarchus labrax*) juveniles. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 389-396. DOI: 10.12714/egejfas.37.4.10

Abstract: The effects of GroBiotic®-A supplementation on growth performance, body composition, liver and intestine histology in European seabass (*Dicentrarchus labrax*) juveniles were evaluated. The commercial GroBiotic®-A was added to diets at four different levels (0, 1, 2 and 3%), three replicates and fed 4 times a day (9:00, 11:30, 14:00, 16: 30 hours) for 60 days as ad libitum. Total 480 European seabass juveniles with a starting weight of 1.40±0.08 g were randomly stocked into 12 tanks with a volume of 1 m^3. At the end of the study, the changes observed in weight, feed conversion ratios (FCR) and survival rates were calculated as 6.69 ± 5.35-7.40 ± 5.47, 0.80 ± 0.18-0.88 ± 0.20 and 96.6 ± 1.51-100 ± 0.0, respectively. When the body composition of the control and treatment groups were compared, no statistically significant differences were observed between the protein and lipid values ($p>0.05$), except ash ($p<0.05$). Histological sections of intestinal tissue; the number of goblet cells was higher than that of the control group. The highest values were determined in the group supplemented 2% GroBiotic®-A. The highest microvillus length was found in the group added 1% GroBiotic®-A. It was detected an inverse relationship between microvillus length and contribution rates as the the GroBiotic®-A additive levels increased. Also, degeneration and necrosis was detected in hepatocyte cells of seabass juvenile fed with diets supplemented 2% and 3% GroBiotic®-A as well as increase of the number of fatty vacuoles in liver tissue due to the increase in the amount of GroBiotic®-A. In conclusion, when the growth parameters, body composition and histological data were evaluated together, the feeding group supplemented 1% GroBiotic®-A performed the best.

Keywords: GroBiotic®-A, European seabass, growth, histology, aquaculture

Öz: Ticari bir prebiyotik olan Grobiyotik-A ilavesinin levrek (*Dicentrarchus labrax*) juvenillerinde büyüme performansı, vücut kompozisyonu, karaciğer ve bağırsak histolojik değişimleri üzerine etkileri değerlendirilmiştir. Grobiyotik-A, dört farklı seviyede (%0, 1, 2 ve 3) yemlere eklenmiştir ve günde 4 kere (9:00, 11:30, 14:00, 16: 30 saatlerinde) doyana kadar 60 gün boyunca besleme yapılmıştır. Çalışma 3 tekrarlı olarak yürütüldü. Başlangıç ağırlıkları 1,40±0.08 g olan kırk levrek rastgele 1 m3 hacimli 12 tanka stoklandı. Çalışma sonunda ağırlıkta gözlenen değişiklikler, yem dönüşüm oranları (FCR) ve yaşama oranları sırasıyla 6.69 ± 5.35-7.40 ± 5.47, 0.80 ± 0.18-0.88 ± 0.20 ve 96.6 ± 1.51-100 ± 0.0. olarak ölçülmüştür. Kontrol ve deneme gruplarının vücut kompozisyonları karşılaştırıldığında protein ve lipit değerleri arasında istatistiksel olarak anlamlı bir fark gözlenmezken, kül değerlerinde gözlenmiştir ($p<0.05$). Bağırsak dokusunun histolojik kesitlerinde; goblet hücrelerinin sayısı kontrol grubuna göre daha yüksek bulunmuştur. En yüksek değerler %2 Grobiyotik-A eklenen grupta tespit edilmiştir. Mikrovillus uzunluğu, en yüksek %1 Grobiyotik-A eklenmiş grupta bulunmuştur. Grobiyotik-A katkı miktarı arttıkça, mikrovillus uzunluğu ile katkı oranları arasında ters bir ilişki olduğu tespit edilmiştir. İlave olarak, %2 ve %3 yemle beslenen balıkların hepatosit hücrelerinde dejenerasyon ve nekrozun yanı sıra, Grobiyotik-A miktarındaki artış nedeniyle karaciğer dokusunda yağ vakuol sayısında artış tespit edilmiştir. Sonuç olarak, büyüme parametreleri, vücut kompozisyonu ve histolojik veriler birlikte değerlendirildiğinde, %1 Grobiyotik-A takviyesi deneme sonunda en iyi büyüme performansını göstermiştir.

Anahtar kelimeler: Grobiyotik A, levrek, büyüme, histoloji, prebiyotik

INTRODUCTION

Aquaculture is the fastest growing animal farming sector in the last 30 years, providing food to the world and contributing increasingly to sustainable economic growth (Bjørndal et al., 2019). In commercial facilities, the need to produce more in the culture system leads to undesirable consequences for the fish, which weakens the immune

system of the fish and eventually leads to disease outbreaks (Kurt et al., 2019). In commercial aquaculture, different antibiotics were used together with feeds for the prevention and treatment of bacterial diseases of aquatic animals (Vechklang et al., 2012). The use of uncontrolled and excessive antibiotics in aquaculture to prevent or treat bacterial diseases can lead to the development of bacterial resistant strains that may be a threat to the environment and human (Mancuso, 2019). The use of antibiotics extensively in animal production as growth promoters is banned in EU countries. Subsequently, various measures have been taken to reduce or even stop antibiotic use in aquaculture (Yazıcı, 2017; Mancuso, 2019).

In this regard, meeting the requirements of environmentally friendly aquaculture according to consumer demand and food safety several functional feed additives such as prebiotics, probiotics, plant extracts, immunostimulants etc. as alternative to antibiotics have been used to improve growth performance and animal health (Suzer et al., 2008; Dimitroglou et al., 2009; Vechklang et al., 2012; Yu et al., 2019). The main aims of commercial aquaculture are to increase the growth of culture organisms and to control the diseases that may occur (Adel et al., 2016). Proper nutrition has long been recognized as a vital factor in promoting normal growth and maintaining health of fish. Prepared diets provide essential nutrients necessary for normal physiological functionality, as well as other components that may protect their health (Li & Gatlin, 2004; Adel et al., 2016).

Prebiotics are defined as indigestible food components that beneficially affect the host by stimulating growth or activity of a limited number of health-promoting bacteria in the intestine while potentially limiting pathogenic bacteria (Ringø et al., 2010). Torrecillas et al., (2011) showed that prebiotics can improve feed utilization and growth positively in many different fish species. The researches of prebiotics in finfish and crustacean have mainly focused on: the effects of growth performance, feed conversion, gut microbiota, gut and liver histology, resistance against pathogenic bacteria and innate immune parameters (Ringø et al., 2010; Yu et al., 2019).

Mannan oligosaccharide (MOS) as a prebiotic has been shown to increase nutrient absorption by increasing villus height and number in the intestine, and some benefits in improving health by maintaining intestinal integrity (Dimitroglou et al., 2009). Another prebiotic used in aquaculture is also commercial GroBiotic®-A that contains a combination of partially autolyzed brewer's yeast, dairy components, and dried fermentation products (Li & Gatlin, 2004; Adel et al., 2016). The benefits of this GroBiotic®-A prebiotic have been reported in many fish to promote growth, food intake, survival, improve the immune system, and disease resistance (Li & Gatlin, 2005; Burr et al., 2009; Buentello et al., 2010; Zheng et al., 2011; Adel et al., 2016).

In recent years, European seabass (*Dicentrarchus labrax*) has become one of the most cultivated and valuable commercial fish in Mediterranean aquaculture (Carbone and Faggio, 2016). European seabass market size was valued at $1082 million in the world 2016. In the last decade, Turkey has a 43% share of world production in 2016 European seabass. This is followed by Greece (23%), Egypt (13%), Spain (12%) and Italy (4%) (Bjørndal et al., 2019). In addition, 37% of total cultured fish production in Turkey was provided from European seabass. Seabass is a species with high tolerance and high growth potential against water quality parameters. However, it is very sensitive to some stress factors caused great losses under aquaculture conditions (Carbone and Faggio, 2016). Studies on the effects of prebiotics have been limited to Mannan Oligosaccarides (MOS), Fructo Oligosaccarides (FOS), Short Chain Fructo Oligosaccarides (ScFOS) and Xylo Oligosaccarides (XOS) prebiotics (Guerreiro et al., 2015; Guerreiro et al., 2017; Yazıcı, 2017). Although many studies have investigated the documented benefits of GroBiotic®-A on different fish species by adding various ratios, there was no study investigating the effect of GroBiotic®-A on economically important European seabass. Hence, the aim of present study was to reveal the effects of GroBiotic®-A on growth parameters, body composition, intestine, and liver histology in European seabass.

MATERIALS AND METHODS

In a total of 600 (0.2-0.3± 0,08g) seabass fry were obtained from a commercial fish farm (Kılıç Seafood Corporation) at Muğla, Turkey. Before starting the study, they were kept in two circular tanks with a volume of 1 m^3 and fish at Marine Science and Technology Faculty, Aquaculture Research Facilities at Iskenderun Technical University were fed a commercial feed (Kılıç Seafood Corporation) with 63.78 % crude protein and 9.78% crude lipid for 4 weeks.

Experiments were conducted in 1m^3 cylindrical fiberglass tanks (n =12). A 20% water exchanged of the each tank was performed daily using filtered seawater. Forty European sea bass (*Dicentrarchus labrax*) juvenile (mean±standard deviation) body weight 1.43±0.08 g per fish were randomly stocked into 12, 1m^3 cylindrical fiberglass tanks filled with 0.8 m^3 of filtered seawater (40 fish/tank). Each treatment tank was supplied with aeration by using a 0.55-Greenco blower (Greenco, Model 7RB 310-7AA01, Zeguo Wenling Zhejiang, China) and air stones. Siphoning was carried out daily in the tanks with its own water inlet and outlet. Photoperiod application was set to 12 hours light and 12 hours dark. Abiotic measurements such as dissolved oxygen (DO, mg/L), temperature (°C), salinity (g/L) and pH were measured daily with a multifunction oxygen meter (YSI, Model Y85). DO, water temperature, salinity and pH were determined as 4.45±0.55mg/L, 25.75±1.25°C, 35.65±0.34g/L and 7.85±0.15, respectively.

Experimental diets and feeding

The prebiotic used in the study is commercially known as GroBiotic®-A (International Ingredient Corporation, St Louis, MO, USA) consisting of partially autolyzed brewer's yeast, dairy ingredient components and dried fermentation products (Table 1).

Table 1. GroBiotic®-A product analysis (International Ingredient Corporation, St Louis, MO, USA)

Proximate composition	Percent (%) value
Crude Protein	30.0-32%
Crude Fat	0.1-2%
Crude Fiber	2-3.0%
Carbohydrate	53.0%
Ash	6.0%
Moisture	5.0%
ME (calculated)	3,580 kcal/kg

Experimental design was arranged in triplicate by 4*3 factorial. All diets were prepared at the same time and kept in sterile plastic bags at 4°C until used. The commercial Grobiotic-A was added to diets at four different levels as a control 0, 1 (GBA1), 2 (GBA2) and 3 (GBA3) %), and fed 4 times a day (9:00, 11:30, 14:00, 16: 30 hours) for 60 days as *ad libitum*. The size and amount of diets offered the fish according to growth performance of experimental groups was readjusted every 15 days (Table 2).

Commercial feeds were placed into the mixer chamber of Alphie1 (Hexagon Product Development Pvt. Ltd. India) with GroBiotic®-A 3-D mixing feature and 25 min (1000 µ), 20 min (1200µ), 15 min. min (1500 µ) at 80 rpm with stirring. Feed sizes were adjusted according to fish measurements in 20-day periods. Alphie1 used in the study, mixing at low speed, the integrity of the feed was not disturbed, and because of the multi-dimensional mixing feature, it was ensured that GroBiotic®-A was added to the feeds homogeneously. Prepared feeds were stored at +4°C until used in plastic containers.

Growth parameters and proximate composition

Sampling strategies

Fish were weighed at the beginning and end of the trial, and survival was monitored daily. No feed was given 24 hr prior to weighing and sampling the fish. Fish were anesthetized with clove oil (5mg/L). The growth performance parameters of the fish were carried out on day 0th, 20th, 40th and 60th. The following formulas were used to calculate the growth parameters and feed consumption of fish: final weight (FW, g), weight gain (WG, g) = (final weight − initial weight), specific growth rate (SGR, % day^{-1}) = (ln final weight − ln initial weight)/times (days) × 100, weight gain (WG, %) = [(final weight − initial weight)/initial weight] × 100, feed conversion ratio (FCR) = weight gain/feed intake and survival (%) = (final animal × 100)/ initial animal (González-Félix et al., 2018).

Proximate analysis of experimental fish and feed

At the end of the experiment, standard AOAC (1997) procedures were used for the crude protein content of fish carcass samples and experimental feeds from each treatment group, Bligh and Dyer (1959) method for crude lipid content, and Vollenweider et al., (2011) method for raw ash content. Proximate analysis of fish and experimental feeds were performed in triplicate.

Histological analysis

At the end of the study, five fish randomly selected from each experimental group were autopsied and tissue samples taken from the digestive tract and liver were fixed in 10% phosphate buffered formaldehyde. After fixation, the manually processed tissue samples were coated with embedding material and embedded in paraffin blocks. 4-5 µm thick tissue samples were stained with hematoxylen-eosin (HE) staining method and examined under light microscope (Bullock, 1978).

Statistical analysis

SPSS package program was used in statistical calculations. The homogeneity of the variances was tested before comparisons between treatment groups were made. One-way ANOVA was used for statistical comparisons among the treatment groups and then the mean and standart deviation (±SD) of initial weight, weight gain, SGR, FCR, and survival of different levels of GroBiotic-A on growth performance of European seabass was compared with Duncan's multiple comparison tests to compute the 95% confidence interval.

RESULTS

Growth performance

At the end of the study, it was observed that weight gain, feed rate, specific growth rate and survival rates were statistically similar and there were no significant differences among the treatments groups (p> 0.05) (Table 2).

Table 2. Mean and standart deviation (±SD) of initial weight, weight gain, SGR, FCR, and survival of different levels of GroBiotic®-A on growth performance of European seabass fry (*Dicentrarchus labrax*)

Parameter	Treatment Groups			
	Control	GBA1	GBA2	GBA3
Initial weight (g)	1.40±0.07	1.46±0.08	1.41±0.09	1.45±0.12
Final weight (g)	14.75±0.69	15.12±0.59	14.08±1.16	14.08±0.73
Weight gain (g)	13.35±0.76	13.66±0.60	12.67±1.09	12.64±0.66
Weight gain (%)	952.48	933.19	896.52	873.56
Feed Conversion Ratio (FCR %)	0.80±0.18	0.82±0.18	0.86±0.26	0.88±0.20
Specific Growth Rate (SGR %)	3.92±0.77	3.89±0.60	3.83±1.09	3.79±0.67
Survival Rate (SR %)	97.5	100	96.6	98.5

Biochemical composition of fish

At the end of the study, ten fish randomly sampled and pooled for biochemical composition. When the body composition of the control and treatment groups were compared, the differences between protein and lipid values were not statistically significant (p>0.05), except ash (p <0.05) (Table 3). The highest protein, lipid and ash values were found as 24.28±0.29 (GBA3), 2.12±0.53 (GBA1) and 3.23±0.13 (GBA2), respectively.

Table 3. Mean and standard deviation (±SD) of protein, lipid, and ash body composition of European seabass fry (*Dicentrarchus labrax*) fed on diets containing (Control 0), 1, 2, and 3% GroBiotic®-A for 60 days. (%)

Treatments	Protein	Lipid	Ash
Control	22.07±0.75^{a}	1.73±0.87^{a}	2.33±0.16^{a}
GBA1	22.84±0.62^{a}	2.12±0.53^{a}	2.95±0.37^{b}
GBA2	24.01±1.63^{a}	1.81±0.86^{a}	3.23±0.13^{b}
GBA3	24.28±0.29^{a}	1.31±0.35^{a}	3.20±0.03^{b}

Biochemical composition of feeds

The differences between lipid and ash values of feeds used in the current study were not statistically significant (p> 0.05) except protein (p <0.05) (Table 4). The highest protein, lipid and ash values were determined as 63.78±0.20 (Control), 10.99±0.46 (GBA3) and 10.63±0.18 (Control), respectively.

Table 4. Mean and standard deviation (±SD) of protein, lipid, and ash belong to commercial feed diets of European seabass fry (*Dicentrarchus labrax*) fed on diets containing (Control 0), 1, 2, and 3% GroBiotic®-A for 60 days. (%)

Treatments	Protein	Lipid	Ash
Control	63.78±0.20^{a}	9.78±0.92^{a}	10.63±0.18^{a}
GBA1	63.50±0.51^{a}	10.22±1.02^{a}	10.46±0.07^{a}
GBA2	62.03±0.60^{b}	9.90±0.37^{a}	10.36±0.03^{a}
GBA3	61.77±0.62^{b}	10.99±0.46^{a}	10.51±0.03^{a}

Histological results

In liver tissue sections, the mean number of fat vacuoles observed in the liver tissue of the control (Figure 1a) and experimental group of 1% GBA was found to be moderate (Figure 1b). In other groups (2% GBA and 3% GBA), there was a significant increase in the number of fat vacuoles due to the increase in the additive. In addition, degeneration and necrosis of hepatocyte cells of the liver were observed in the experimental group of 2% GBA and 3% GBA (Figure 1c, d).

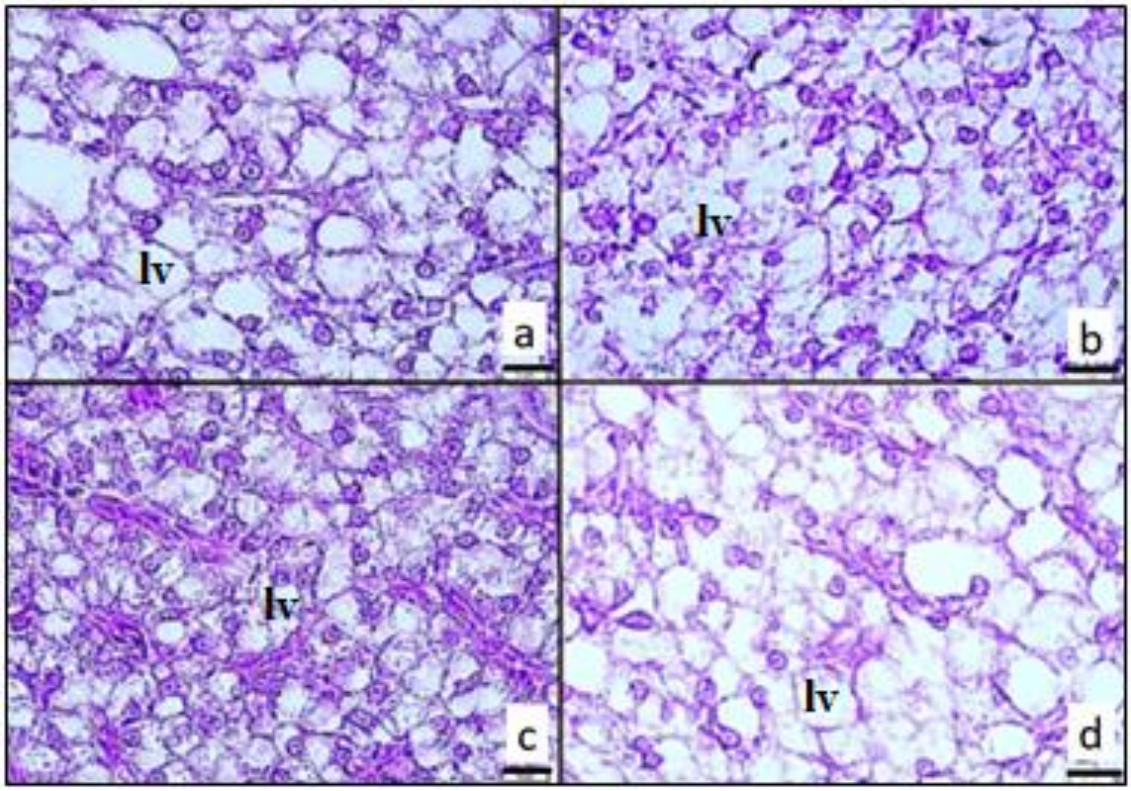

Figure 1. Light photomicrograph of liver of European seabass showing increase diffuse macro-vesicular lipid accumulation in liver tissue (a: control group; b: 1% group; c: 2% group; d: 3% group) a,b: Moderate lipid vacuoles (lv) c,d: Excessive increase in lipid vacuoles (lv), necrosis in hepatocytes (bar: 200 µm, H&E)

In intestinal tissue sections, of midgut intestinal diameter, villus length and villus width of all fish were measured (Table 5). Light photomicrograph of intestine sections of European seabass villus structure in intestine in all treatments group (Figure 2; a,b,c,d).

While the increase in the GBA2 feeding group was remarkable compared to the control group, it was found that there was an inverse relationship between villus length and contribution rates in the GBA3 feeding group (Figure 2c,d).

Table 5. The villus lengths and gut diameters measured in the intestine of European seabass fry (n=5) treatment groups

Parameters	Treatments			
	Control	GBA1	GBA2	GBA3
Intestinal diameter (µm)	1253	1790	1863	1662
Villus length (µm)	420	550	480	380
Villus width (µm)	101	115	111	99

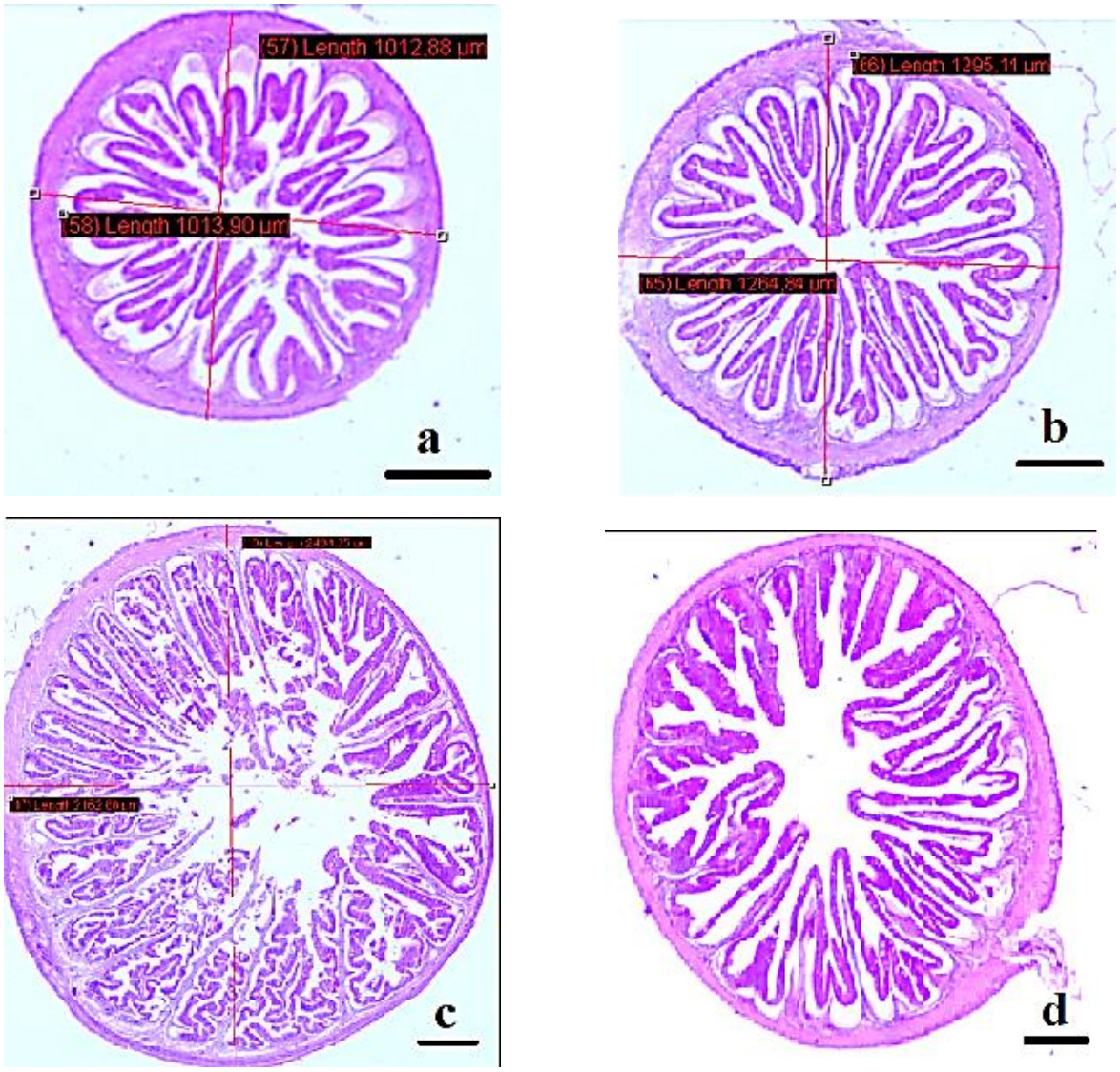

Figure 2. Light photomicrograph of intestine sections of seabass showing normal villus structure in intestine in all treatments; **a:** control group; **b:** 1% group; **c:** 2% group; **d:** 3% group (bar: 200 µm, H&E)

In the detailed microscopic examination of the intestine sections, it was observed that the structure of enterocyte cells and the number of goblet cells were normal in the control and 1% GBA group, but there was an excessive increase in the number of goblet cells (Figure 3b). However, high villi length was observed in the GBA2 group (Figure 3c) and enlargement of the lamina propria in the GBA3 group. Among the enterocyte cells, goblet cells increased compared to the control group (Figure 3d). In addition, no pathological picture was observed in all intestinal preparations examined.

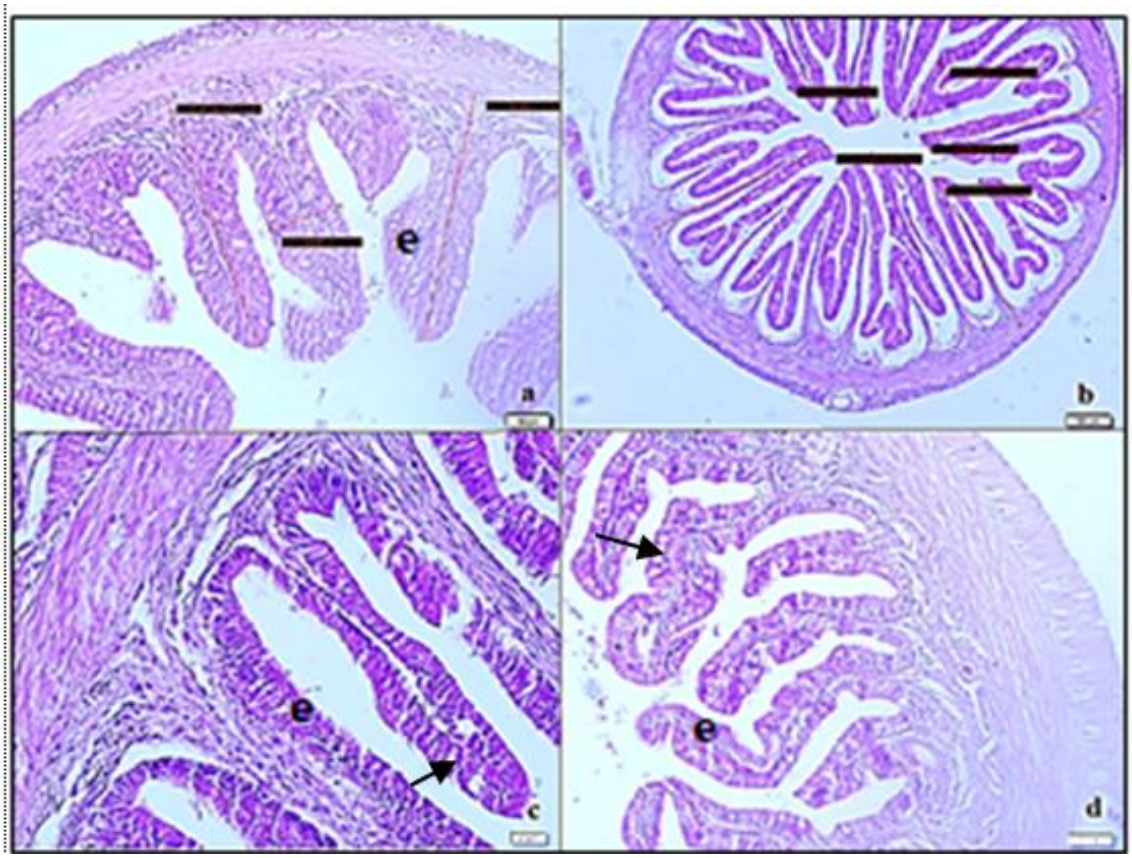

Figure 3. High magnification of histological sections for treatment groups and structure of enterocyte (e) and goblet cells (arrowed); a: control; b: GBA1; c: GBA2 ; d: GBA3 (bar: 200 µm, H&E)

DISCUSSION

In the current study, it was investigated that the addition of GroBiotic®-A affect on the growth performance, body composition, gut and liver histology of European seabass. There were no significant effects of supplementing the basal diet with GroBiotic®-A (GBA) within the range of 1–3 % on Weight gain, FCR and SGR of the European seabass (P> 0.05). Hoseinifar et al., (2016) indicated that effects of prebiotic on growth performance on fish are inconsistence. Li & Gatlin, (2004) indicated that the addition of GBA to the juvenile Hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) diet improved growth performance compared to fish fed a basal diet, while Burr et al. (2010), working on the same species in adult size noted that no change was observed in growth performance.

The lack of growth enhancement in European seabass with the addition of prebiotics was consistent with previous studies on trout (*Oncorhynchus mykiss*) (Sealey et al., 2007), Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) (Sealey et al., 2015), red drum (*Sciaenops ocellatus*) (Burr et al., 2009), Caspian kutum, (*Rutilus frisii kutum*) (Yousefian et al., 2012) and Nile tilapia (*Oreochromis niloticus*) (Vechklang et al., 2012; Peredo et al., 2015). In contrast to these studies such as tilapia (Zheng et al., 2011), rainbow trout fry (*Oncorhynchus mykiss*), (Azari et al., 2013), juvenile starry flounder (*Platichthys stellatus*), (Wang et al., 2014), rainbow trout (Staykov et al., 2007; Yilmaz et al., 2007), largemouth bass (*Micropterus salmoides*) (Yu et al., 2019) and beluga sturgeon juvenile (*Huso huso*) (Adel et al., 2016; 2017) indicated diffeences in growth performance. Peredo et al., (2015) showed that differences in the size or age of the fish used in prebiotic studies may have different effects depending on the microbiota. In addition, such intraspecific, as well as interspecific differences are quite common in prebiotic studies and may be likely attributed to initial differences in the composition of intestinal microbiota, although this has not been studied in most studies.

The body composition, including protein and lipid content, is of vital importance as it affects the growth and survival of cultivated species as it generally reflects the state of nutrition and the health of aquatic species (Hoang, 2019). Dietary prebiotic inclusion affects the protein content in the tissues of culture animals, and may also vary depending on the species. (Burr et al., 2010; Genc et al., 2007; Yilmaz et al., 2007) In this study, prebiotic supplementation on the body composition of fish did not affect protein and lipid content compared to the control group.

This situation is similar to the studies of some researchers (Adel et al., 2017, 2016; Vechklang et al., 2012; Wang et al., 2014; Zheng et al., 2011), but it shows difference from some of the studies (Burr et al., 2010; Sealey et al., 2007). They were indicated that there is a significant increase in protein. Unlike other researchers who reported no change in ash content, it was significantly higher. Survival rate at the end of the trial was higher than 97.5% in all treatments, and no significant differences were observed among treatment groups ($P > 0.05$). Results of Peredo et al. (2015) revealed that tilapia fed the diet containing 2% GBA had significantly higher survival than that of the other treatment groups. FCR of juvenile European seabass significantly tended to decrease with the supplemented GroBiotic®-A (1%,), but no significant difference was observed among the treatment groups. The results belong to the basal group of Wang et al. (2014) supported the results of current study.

The studies have shown that some prebiotics supplementation in the diets may cause significant differences in gastrointestinal morphology in some fish (Anguiano et al., 2013). Changes to the morphology of the intestine may be attributed to the production of short-chain fatty acids through the microbial fermentation of prebiotic substances. Peredo et al. (2015) GBA an improvement in gut morphology ensure benefit feed utilization, but the maintenance of an intact, healthy mucosal epithelium may help to prevent opportunistic indigenous bacterial infections. Dimitroglou et al. (2009) reported that the results of the histological studies may help to explain the improved growth performance, feed utilization, and survival of fish. In the current study, histological examinations demonstrated that the villus length and the number of goblet cells of the fish in the GBA1 feeding group increased significantly compared to the other groups in the intestinal tissue. Studies on rainbow trout and sea bream (*Sparus aurata*) with different prebiotics are consistent with the present study (Dimitroglou et al., 2009; Yilmaz et al., 2007), gilthead sea bream (Eryalçin et al., 2017). Similar to the results of the current study, previous investigations with red drum (Zhou et al., 2010), hybrid seabass (Anguiano et al., 2013) showed that GBA supplementation improved gut morphology. In this study, it was observed that prebiotic level had a positive effect on fish intestine and liver when 1% was added to the diet, but it had a negative effect due to the increase in GBA level. It was reported that the addition of 2% GBA in striped bass was effective on intestinal structures at week 4 but not at week 8 (Anguiano et al., 2013). In the present study villus lengths were found to be longer in the treatment groups supplemented with GroBiotic®-A than in the control group. The highest villus length has been determined as GBA2 (Table 5). Differences between the results of studies on the effects of prebiotics on the villus structure, using different dose levels, studying with different species, the presence of different intestinal microbiota in these species, has been reported to be caused by reasons such as the use of different culture conditions (Adel et al., 2016; Anguiano et al., 2013; Dimitroglou et al., 2009).

In conclusion, the findings of the present study indicated that weight gain, feed conversion ratio, survival, and whole body proximate composition of European seabass following 8 weeks of feeding were not significantly affected by dietary supplementation of 1% and 2% %3 GroBiotic®-A. Addition of 1% GBA to the feed showed a positive effect on the liver and intestine tissues of the seabass. However, an increase in the amount of Grobiotic-A (2% and 3%) was found to increase the number of fatty vacuoles in the liver tissue as well as degeneration and necrosis in the hepatocyte cells of the fish. Prebiotic Grobiotic®-A (1%) could be a potential dietary supplement for seabass juveniles. In particular, dietary content appears to improve the growth performance and Gastrointestinal tract GIT of juveniles. However, Further studies should be designed to investigate the effects of GroBiotic®-A on immune response and disease resistance applying challenge studies in European seabass.

ACKNOWLEDGEMENT

We would like to thank Prof. Dr. Delbert M. Gatlin and International Ingredient Corporation (St. Louis, MO) for kindly providing GroBiotic®-A. We also express our gratitude to Kılıç Seafood Corporation for providing European seabass fish used in this study.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 397-407 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.11

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Distribution of Aquatic Diptera larvae of Yeşilırmak River (Turkey) and ecological characteristics

Yeşilırmak Nehri'ndeki Sucul Diptera (Insecta) larvalarının dağılımı ve ekolojik özellikleri

Özge Başoren[1]* ● Nilgün Kazancı[2]

[1]Department of Biology, Faculty of Science, Hacettepe University, 06800, Ankara, Turkey https://orcid.org/0000-0003-3424-6423
[2]Department of Biology, Faculty of Science, Hacettepe University, 06800, Ankara, Turkey https://orcid.org/0000-0002-2554-7999

Corresponding author: ozzzge@gmail.com
Received date: 22.03.2020 *Accepted date: 18.06.2020*

How to cite this paper:
Başoren, Ö. & Kazancı, N. (2020). Distribution of Aquatic Diptera larvae of Yeşilırmak River (Turkey) and ecological characteristics. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 397-407. DOI: 10.12714/egejfas.37.4.11

Abstract: Yeşilırmak River is one of the most important running waters of Turkey, but the water quality of this river has been affected by agricultural and domestic pollution. Dams and hydroelectric power plants also threaten the habitat quality and biodiversity of the river. This research contains investigation of Diptera fauna in Yeşilırmak River and tributaries, determination ecological characteristics of the collecting sites according to System A and System B Classification of Water Framework Directive (WFD), assessment of water quality of the studied sites by measuring the physicochemical variables (water temperature, pH, electrical conductivity, dissolved oxygen, NO_2-N, NO_3-N, NH_4-N, PO_4-P) and using some metrics (abundance, number of taxa, Simpson Diversity Index, Shannon-Wiener Diversity Index, Margalef Diversity Index, Evenness). Thirty-three (33) sites were sampled from Yeşilırmak River and its tributaries in June 2010. Diptera individuals were detected in 20 of them. Two thousand four hundred forty-five (2445) individuals belonging to 12 families and 16 taxa were identified in 20 sampling sites. The water quality classes of the studied sites were Class III (moderate pollution) and Class IV (heavily pollution) according to values of physicochemical variables.

Keywords: Habitat degradation, physicochemical variables, true flies, water quality, water pollution

Öz: Yeşilırmak Nehri, Türkiye'nin en önemli nehirlerinden biridir. Ancak nehrin su kalitesi tarımsal ve evsel kirlilikten etkilenmektedir. Ayrıca, barajlar ve hidroelektrik santralleri de nehrin habitat kalitesini ve biyoçeşitliliğini tehdit etmektedir. Bu çalışmayla, Yeşilırmak Nehri ve kollarındaki Diptera komunitesi belirlenmiş, Su Çerçeve Direktifi (SÇD)'nin Sistem Ave B sınıflandırmasına göre çalışılan istasyonların ekolojileri tespit edilmiş, fizikokimyasal değişkenler (su sıcaklığı, pH, elektriksel iletkenlik, çözünmüş oksijen, NO_2-N, NO_3-N, NH_4-N, PO_4-P) ve bazı metrikler (bolluk, taksa sayısı, Simpson Çeşitlilik İndeksi, Shannon-Wiener Çeşitlilik İndeksi, Margalef Çeşitlilik İndeksi) kullanılarak istasyonların su kaliteleri değerlendirilmiştir. Yeşilırmak Nehri ve kollarında, Haziran 2010 tarihinde otuz üç (33) istasyon örneklenmiş, bu istasyonlardan 20 tanesinde Diptera bireylerine rastlanmıştır. Bu 20 örnekleme istasyonundan Diptera takımına ait 16 taksa, 12 familya, 2445 birey tanımlanmıştır. İstasyonların su kaliteleri, fizikokimyasal değişkenlere göre III. Sınıf (orta kirli) ve IV. Sınıf (çok kirli) olarak belirlenmiştir.

Anahtar kelimeler: Fizikokimyasal değişkenler, habitat bozulması, sinekler, su kalitesi, su kirliliği

INTRODUCTION

Diptera have a worldwide distribution and comprise nearly 46.000 described aquatic species (Adler and Courtney, 2019). Diptera larvae are among the most abundant members in almost all aquatic ecosystems. They are found in a variety of aquatic habitats (springs, streams, rivers, lakes) (Adler and Courtney, 2019). Aquatic flies are important in aquatic food webs and play a significant role in the processing and cycling of nutrients in lentic and lotic environments (Wotton and Malmqvist, 2001).

The European Water Framework Directive (WFD), which is accepted by the European Union Member States in 2000 (Council of European Communities, 2000), aims to prevent and improve aquatic ecosystems deterioration, to protect and restore all water bodies and to conserve water resources in Europe. Biological assessment and monitoring of water quality is very important for WFD studies. Benthic macroinvertebrates are the most commonly used organisms for biomonitoring the quality of aquatic habitats (Johnson et al., 2006; Rosenberg and Resh, 1993). In Turkey, WFD studies using macroinvertebrates has increased in last 10 years (Akay and Dalkıran, 2019; Arslan, Salur, et al., 2016; Arslan, Kökçü, et al., 2016; Başören and Kazancı, 2016; Bolat et al., 2016; Duran and Akyildiz, 2011; Ekingen and Kazancı, 2012; Kazanci, Başören, et al., 2013; Kazanci, Ekingen, et al., 2010; Kazanci, Türkmen, et al., 2010, 2013, 2015, 2017; Kazanci and Ertunç, 2010; Türkmen and Kazancı, 2015, 2016; Zeybek, 2017; Zeybek et al., 2014).

Diptera taxa are the most widely used like the other benthic macroinvertebrates in freshwater biomonitoring studies (Courtney et al., 2017; Paine et al., 1956). Some taxa are very sensitive and cannot tolerate water pollution, while some taxa are extremely tolerant and survive in heavily

polluted water (Adler and Courtney, 2019; Bouchard, 2004; Kazancı and Ertunç, 2010; Luoto, 2011). However, there are limited studies about the larval ecology of most Diptera species in the world (Wagner et al., 2008).

Yeşilırmak River is one of the important rivers in Turkey. But the habitat quality of this river has been threatened with increasing pollutant loads due to contamination from agricultural, industrial, and domestic waste in recent years. There are 19 dams and hydroelectric power plants on the river basin (Kazanci, Türkmen, et al., 2010). Deterioration of the riverbed, habitat loss, change in water quality, temperature and flow regime caused by the dams are the other important problems in the Yeşilırmak River and its tributaries. These impacts are the main causes of loss of biodiversity in freshwater habitats. All these negative activities in Yeşilırmak River Basin also negatively affect the community structure and habitats of Diptera.

The purpose of this study is to investigate the Diptera fauna of the Yeşilırmak River and tributaries, to determine ecological characteristics of the collecting sites according to System A and System B Classification of WFD, to assess the water quality of the collecting sites by measuring the physicochemical variables and using some metrics (abundance, number of taxa, Simpson Diversity Index, Shannon-Wiener Diversity Index, Margalef Diversity Index, Evenness).

MATERIAL AND METHODS

Yeşilırmak River is located in the northeastern Turkey and it is the second longest river. The catchment area of Yeşilırmak River is 38,730 km^2, which is about 5% of Turkey's surface area. It rises from Köse Mountain in the north of Sivas, flows approximately 519 km and reaches to the Black Sea. Çekerek, Kelkit, Tersakan and Salhan are the tributaries of the Yeşilırmak River.

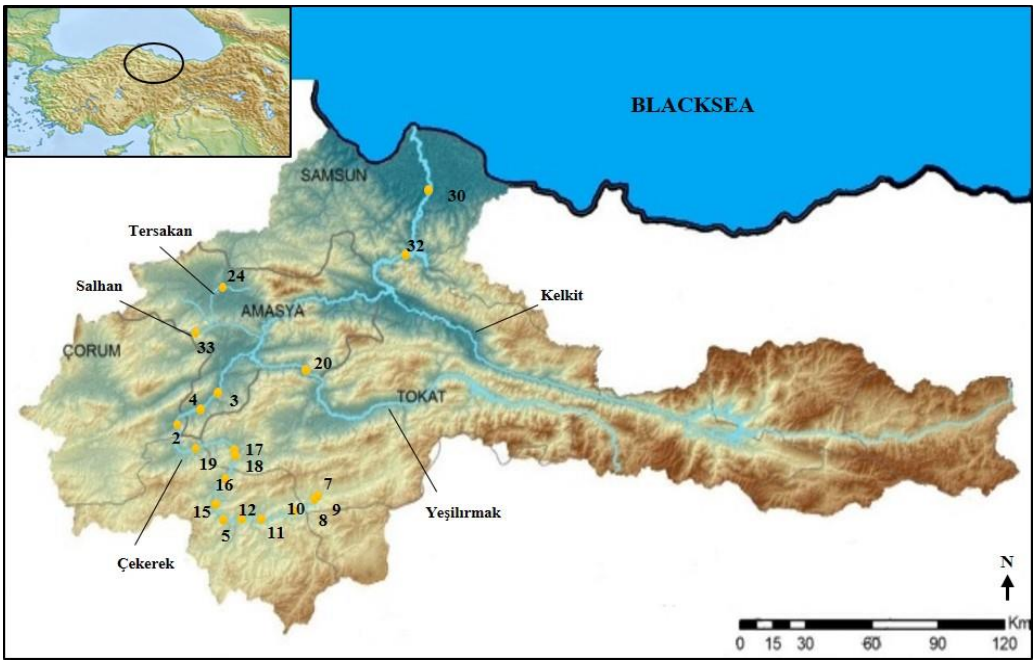

Figure 1. Sampling sites of Yeşilırmak River and its tributaries

Thirty-three (33) sites were sampled from Yeşilırmak River and its tributaries in June 2010 (Figure 1). Diptera individuals were detected in 20 of them. Geographical coordinates of these sites were given in Table 4. Some physicochemical variables and geological characteristics were recorded in collecting sites. Water temperature, pH, electrical conductivity, and dissolved oxygen were measured in the field by using an YSI 556 multi-probe system. In addition, NO_2-N, NO_3-N, NH_4-N, and PO_4-P were measured using a Hach DR/890 Datalogging Colorimeter (HACH, 2005). The water quality classes of the studied sites were evaluated by using the Surface Water Quality Regulation Annex-5 (Anonymous, 2015, 2016) (Table 3).

Diptera samples were collected by a standard pond net. Samples were preserved in 80% ethyl alcohol. Leica MZ75 stereomicroscope and Olympus CX21FS1 binocular microscope were used for identifications. Three diversity indices (Simpson Diversity Index, Shannon-Wiener Diversity Index and Margalef Diversity Index) were also used to determine water quality. Biological data were analyzed by using ASTERICS (AQEM/STAR Ecological River Classification System) software (AQEM Consortium, 2006) and all samples (including 1 individual) were taken into account for this analysis.

Certain geological and physical characteristics of sampling sites required by System A and System B classifications of WFD were given in Table 4.

RESULTS

Two thousand four hundred forty-five (2445) individuals belonging to 12 families and 16 taxa were identified in 20 sampling sites (Table 1). According to this table, the most common families of Diptera were Chironomidae, Pediciidae, Simuliidae and Tipuliidae with a wide range of tolerance.

The abundance values and number of taxa of 20 sites were given in Table 2. The highest abundance value (439) was found in Site 3 while the lowest abundance value (2) was found in Site 5, 16 and 20. The highest taxa number (7) was found in Site 8, 15 and 18 while the lowest taxa number (1) was found in Site 4, 5, 16 and 20. According to Table 2, the values of Simpson Diversity Index, Shannon-Wiener Diversity Index and Margalef Diversity Index varied between 0 and 0.689; 0 and 1.341; 0 and 1.377, respectively.

The results of the physicochemical variables (water temperature, pH, electrical conductivity, dissolved oxygen concentration, NO_2-N, NO_3-N, NH_4-N, and PO_4-P) and the water quality classes of the 20 sites were given in Figure 2, Figure 3 and Table 3.

Table 1. List of taxa in the studied sites

		2	3	4	5	7	8	9	10	11	12	15	16	17	18	19	20	24	30	32	33
Chironomidae	Gen. sp.	*	*			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Blephariceridae	*Liponeura*							*													
Dixidae	*Dixa*					*															
Dolicopodidae	Gen. sp.		*								*							*	*		*
Empididae	*Clinocera*						*	*		*	*										
Limoniidae	Gen. sp.								*	*					*	*					
	Erioptera											*									
	Hexatoma						*		*	*		*				*					
Muscidae	*Limnophora*	*																			
	Lispe																		*		
Pediciidae	*Dicranota*				*	*	*	*	*		*	*		*	*						
	Pedicia														*						
Simuliidae	*Simulium*	*	*			*	*	*	*	*	*	*		*	*			*	*		*
Stratiomyidae	*Oxycera*						*					*									
Tabanidae	*Tabanus*			*			*					*			*						
Tipulidae	*Tipula*	*									*				*			*	*	*	*

Table 2. Values of indices of the sites at Yeşilırmak River

Metric	2	3	4	5	7	8	9	10	11	12
Abundance [ind/m²]	325	439	3	2	32	78	157	73	232	136
Number of Taxa	4	3	1	1	4	7	5	5	5	6
Simpson Diversity Index	0.326	0.398	0	0	0.629	0.689	0.527	0.578	0.58	0.598
Shannon-Wiener Diversity Index	0.554	0.627	0	0	1.125	1.341	0.82	1.125	0.988	1.129
Margalef Diversity Index	0.519	0.329	0	0	0.866	1.377	0.791	0.932	0.734	1.018
Pielou's Evenness Index	0.399	0.571	NC*	NC*	0.811	0.689	0.509	0.699	0.614	0.63
Metric	15	16	17	18	19	20	24	30	32	33
Abundance [ind/m²]	30	2	5	357	14	2	134	313	17	94
Number of Taxa	7	1	3	7	3	1	4	5	2	4
Simpson Diversity Index	0.508	0	0.67	0.274	0.275	0	0.115	0.439	0.118	0.36
Shannon-Wiener Diversity Index	1.114	0	0.95	0.564	0.509	0	0.27	0.726	0.224	0.688
Margalef Diversity Index	1.302	0	1.243	1.021	0.758	0	0.613	0.696	0.353	0.66
Pielou's Evenness Index	0.572	NC*	0.865	0.29	0.463	NC*	0.195	0.451	0.323	0.496

* NC: Not calculated

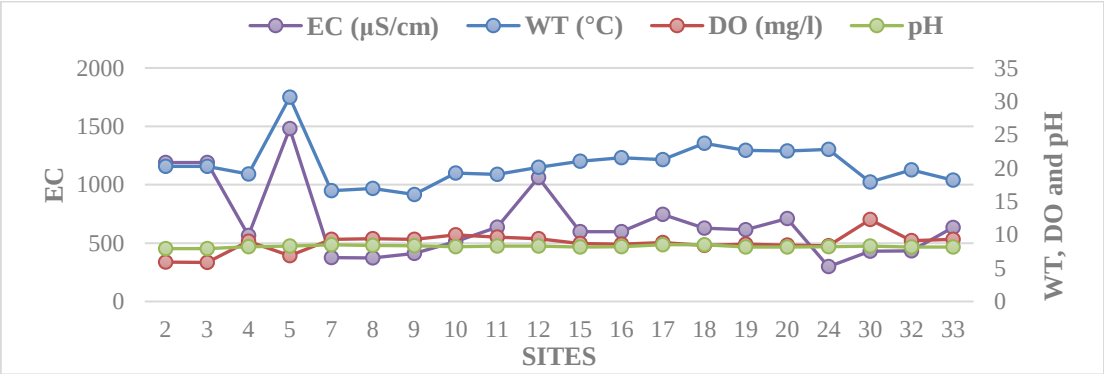

Figure 2. Values of water temperature (°C), dissolved oxygen (mg/L), pH and electrical conductivity (µS/cm)

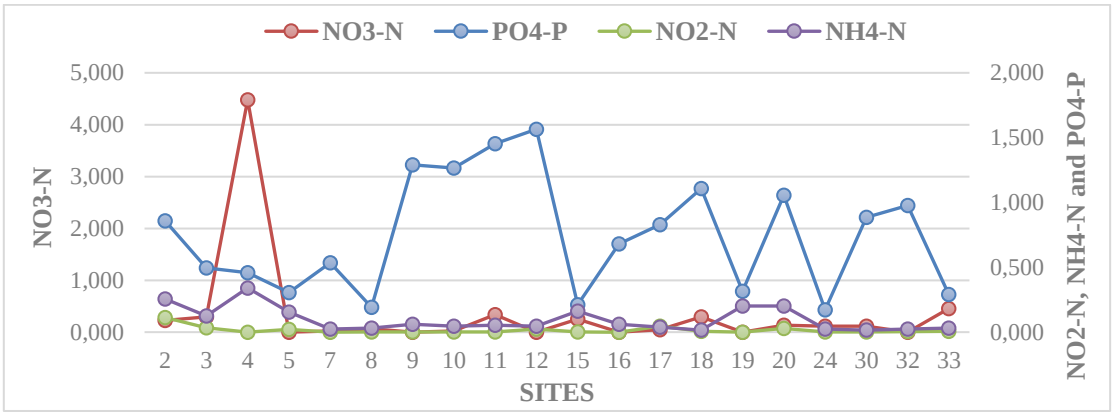

Figure 3. Values of NO_2-N (mg/L), NO_3-N (mg/L), NH_4-N (mg/L), and PO_4-P (mg/L)

Table 3. Water quality classes of the studied sites according to the physicochemical variables (Anonymous, 2015; 2016)

Site	Site name	WT (°C)	DO (mg/l)	pH	EC (µS/cm)	PO_4-P (mg/l)	NO_3-N (mg/l)	NO_2-N (mg/l)	NH_4-N (mg/l)	Final Water Quality Class
2	Çekerek-2	I	III	I	III	IV	I	III	II	IV
3	Çekerek-3	I	III	I	III	III	I	II	I	III
4	Çekerek-4	I	I	I	II	III	II	I	II	III
5	Çekerek-5	IV	II	I	III	III	I	II	I	IV
7	Çekerek-7	I	I	I	I	III	I	I	I	III
8	Çekerek-8	I	I	I	I	III	I	I	I	III
9	Çekerek-9	I	I	I	II	IV	I	I	I	III
10	Çekerek-10	I	I	I	II	IV	I	I	I	III
11	Çekerek-11	I	I	I	II	IV	I	I	I	IV
12	Çekerek-12	I	I	I	III	IV	I	II	I	IV
15	Çekerek-15	I	I	I	II	III	I	I	I	III
16	Çekerek-16	I	I	I	II	IV	I	I	I	IV
17	Çekerek-17	I	I	I	II	IV	I	II	I	IV
18	Çekerek-18	I	I	I	II	IV	I	I	I	IV
19	Çekerek-19	I	I	I	II	III	I	I	II	III
20	Yeşilırmak-1	I	I	I	II	IV	I	II	II	IV
24	Tersakan Çayı - 1	I	I	I	I	III	I	I	I	II
30	Yeşilırmak-2	I	I	I	II	IV	I	I	I	IV
32	Yeşilırmak-4	I	I	I	II	IV	I	I	I	IV
33	Salhan Çayı - 1	I	I	I	II	III	I	I	I	III

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

Yeşilırmak River and its tributaries were alkaline, with pH values between 7.92 and 8.51. The water temperature values recorded were between 16.04 and 30.6°C. The dissolved oxygen values recorded were between 5.85 and 12.27 mg/l. The electrical conductivity values recorded were between 298 and 1480 µS/cm. Nitrite nitrogen values ranged between 0 and 0.113 mg/l. Nitrate nitrogen values ranged between 0 and 4.480 mg/l. Ammonium nitrogen values ranged between 0.016 and 0.341 mg/l. Orthophosphate phosphorus values ranged between 0 and 1.565 mg/l. According to nitrogen and phosphorus concentrations, studied sites were impacted by agricultural activities.

Some geological and physical characteristics of the 20 sites, required by System A and System B of WFD were given in Table 4.

Table 4. Geological and physical characteristics of sampling sites according to System A and System B Classification of WFD

Sites	**2**	**3**	**4**	**5**	**7**	**8**	**9**
Ecoregion (System A)	Y	Y	Y	Y	Y	Y	Y
Altitude (System A)	Medium (200m-800m)	Medium (200m-800m)	Medium (200m-800m)	Medium (200m-800m)	High (>800m)	High (>800m)	High (>800m)
Altitude (System B)	526m	576m	427m	580m	1206m	1239m	1227m
Catchment Area (System A)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)
Geology (System A and System B)	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous
Latitude (System B)	40° 27' 18.20'' N	40° 33' 47.87'' N	40° 32' 16.36'' N	39° 50' 45.96'' N	40° 06' 38.11'' N	40° 06' 44.41'' N	40° 06' 39.06'' N
Longitude (System B)	35° 26' 59.18'' E	35° 30' 34.89'' E	35° 39' 55.86'' E	35° 27' 00.00'' E	36° 34' 53.52'' E	36° 35' 03.53'' E	36° 35' 00.42'' E
Substratum	%50 rock, %30 stone, %15 gravel, %5 sand	10% rock, 25% stone, 15% gravel, 50% sand	10% rock, 15% stone, 20% gravel, 5% sand, 50% clay	40% stone, 40% gravel, 5% sand, 15% clay	20% rock, 40% stone, 30% gravel, 10% sand	20% rock, 40% stone, 30% gravel, 10% sand	40% rock, 35% stone, 20% gravel, 5% sand
Stream Zone	Epirhithron	Epirhithron	Metarhithron	Metarhithron	Epirhithron	Epirhithron	Epirhithron
Riparian vegetation	100%	80%	90%	50%	90%	100%	100%
Stream width in dry period	5m	1,5m	25m	5m	50cm	1,5m	2m
Stream width in wet period	15m	1,5m	40m	7m	1m	2m	2m
Sites	**10**	**11**	**12**	**15**	**16**	**17**	**18**
Ecoregion (System A)	Y	Y	Y	Y	Y	Y	Y
Altitude (System A)	High (>800m)	High (>800m)	High (>800m)	Medium (200m-800m)	Medium (200m-800m)	High (>800m)	High (>800m)
Altitude (System B)	1153m	1054m	1117m	777m	756m	869m	874m
Catchment Area (System A)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)
Geology (System A and System B)	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous
Latitude (System B)	40° 05' 42.73'' N	40° 00' 36.82'' N	40° 01' 27.53'' N	40° 05' 17.43'' N	40° 09' 44.71'' N	40° 20' 40.79'' N	40° 20' 39.51'' N
Longitude (System B)	36° 32' 23.63'' E	36° 10' 29.57'' E	35° 48' 39.04'' E	35° 35' 14.64'' E	35° 38' 08.86'' E	35° 41' 18.32'' E	35° 41' 19.73'' E
Substratum	5% stone, 60% gravel, 25% sand, 10% clay	15% rock, 45% stone, 25% gravel, 10% sand, 5% clay	50% rock, 15% stone, 15% gravel, 10% sand, 10% clay	15% stone, 30% gravel, 30% sand, 25% clay	20% rock, 30% stone, 30% gravel, 10% sand, 10% clay	50% rock, 15% stone, 15% gravel, 10% sand, 10% clay	50% rock, 15% stone, 15% gravel, 10% sand, 10% clay
Stream Zone	Epirhithron	Metarhithron	Epirhithron	Metarhithron	Metarhithron	Epirhithron	Epirhithron
Riparian vegetation	100%	70%	100%	100%	90%	100%	100%
Stream width in dry period	2m	4m	50cm	10m	30m	50cm	60cm
Stream width in wet period	12m	12m	50cm	30m	30m	50cm	60cm

Table 4. continued

Sites	19	20	24	30	32	33
Ecoregion (System A)	Y	Y	Y	Y	Y	Y
Altitude (System A)	Medium (200m-800m)	Medium (200m-800m)	High (>800m)	Lowland (<200m)	Lowland (<200m)	Medium (200m-800m)
Altitude (System B)	476m	285m	841m	22m	77m	520m
Catchment Area (System A)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km^2)	Medium (100-1000km2)
Geology (System A and System B)	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous
Latitude (System B)	40° 23' 44.44'' N	40° 44' 52.12'' N	40° 57' 06.36'' N	41° 06' 49.76'' N	40° 56' 33.44'' N	40° 45' 18.72'' N
Longitude (System B)	35° 32' 09.75'' E	36° 08' 42.72'' E	35° 57' 54.46'' E	36° 42' 51.42'' E	36° 38' 51.98'' E	35° 29' 29.76'' E
Substratum	10% rock, 30% stone, 20% gravel, 20% sand, 20% clay	30% rock, 30% stone, 20% gravel, 10% sand, 10% clay	10% rock, 40% stone, 40% gravel, 10% sand	10% rock, 40% stone, 40% gravel, 10% sand	10% rock, 35% stone, 30% gravel, 30% sand	5% rock, 30% stone, 50% gravel, 15% sand
Stream Zone	Metarhithron	Hiporhithron	Metarhithron	Epipotamon	Hipopotamon	Epirhithron
Riparian vegetation	70%	100%	80%	60%	60%	90%
Stream width in dry period	10m	30m	5m	80m	17m	2m
Stream width in wet period	20m	40m	5m	100m	25m	7m

DISCUSSION AND CONCLUSION

Yeşilırmak is one of the 25 major basins in Turkey but the water quality of the river has been affected by various anthropogenic activities. The most important pollution sources of the Yeşilırmak River are agricultural activities, urban waste waters and sewage. Moreover, physical destruction, dams and hydroelectric power plants also threaten to habitat quality of the river. The values of physicochemical variables were negatively affected by all these activities. The water quality classes of the studied sites were Class III (9 sites) and Class IV (11 sites) according to values of physicochemical variables.

Benthic macroinvertebrates are most useful bioindicators in monitoring studies because they are sensitive to changes in the ecosystem and reflect the aquatic habitat quality (Johnson et al., 2006; Rosenberg and Resh, 1993). Aquatic Diptera is an important group of the benthic macroinvertebrate fauna (Adler and Courtney, 2019).

The families Chironomidae, Pediciidae, Simuliidae and Tipulidae are the most frequently collected families in the studied sites. These families with a high tolerance range could spread over a larger area. Some genera and species of the family Chironomidae prefer in oligosaprobic sites with high water quality but they are mostly found in betamezosaprobic and alfamezosaprobic habitats with low water quality (Epler, 2001; Gültutan and Kazancı, 2009). They can be used as bioindicators to assess changes in aquatic ecosystems and habitat quality because they are abundant, species-diverse insect group and show different level of sensitivity to habitat degradation (Armitage et al., 1995; Ferrington, 2008). The family Chironomidae was found in almost all sites in this study. This family was found extensively in Site 2, 3, 11, 24 and 30. Site 2, 11 and 30 have Class IV water quality and were heavily polluted. Site 3 and 24 have Class III water quality and were moderately polluted. All these sites were surrounded by agricultural areas. Habitat degradation was also detected in stream bed in Site 11 and 30.

The family Simuliidae is widespread in freshwater habitats. The composition of black fly larvae and pupae is affected by various environmental variables (riparian vegetation, substrate types, current velocity, dissolved oxygen, temperature, etc.) of streams (Lautenschläger and Kiel, 2005; Malmqvist et al., 1999). This family is also commonly used as biological indicators, together with Chironomidae (Adriaenssens et al., 2004; Feld et al., 2002; Kazancı and Ertunç, 2010). In this study, only *Simulium* sp. was identified as belonging to this family. Larvae and pupae of *Simulium* sp. (Simuliidae) are generally found in betamesosaprobic habitats, but they can be inhabited in oligosaprobic and alphamesosaprobic habitats (CSN 75 7716, 1998; Kazancı and Ertunç, 2008; Lechthaler et al., 2017). Stream zonation preferences of this genus are hyporhithron, epipotamon and metapotamon zone of streams (Car et al., 1995). Simuliidae was the second common family in the study area. *Simulium* sp. was found extensively in Site 3 and 18 in this study. Site 3 has Class III water quality and was moderately polluted. Habitat degradation was detected in the stream beds of this site and it affected by nutrient inputs from agricultural areas. Site 18 has Class IV water quality and was

heavily polluted. This site was located far from agricultural and urban areas. A periodic situation may have caused the low water quality.

The ecology and distribution of Tipulidae are poorly known. Most of them live in aquatic (bottom of streams) or semiaquatic habitats (margins of small rivers, wet mosses) (Gelhaus, 1986). In this study, only *Tipula* sp. was identified as belonging to this family. *Tipula* sp. prefers mainly alphamesosaprobic habitats but it is also found in betamesosaprobic and polysaprobic habitats (CSN 75 7716, 1998). Stream zonation preferences of this genus are epirhithron, metarhithron and hyporhithron but it also occur in littoral zone of streams (AQEM Consortium, 2002). This genus was more common in Site 30. This site has Class IV water quality and was heavily polluted. Site 30 is the closest site to the river mouth. This site was surrounded by agricultural areas that contribute large nutrient loads to running water.

The families Pediciidae and Limoniidae prefer similar environmental conditions (Reusch and Oosterbroek, 1997). Larvae of these families are found a wide variety of habitats (rapidly flowing streams, brackish water, intertidal zones) and they can tolerate environmental changes (Bulankova, 2003; Reusch and Oosterbroek, 1997). In this study, *Dicranota* sp. and *Pedicia* sp. were identified as belonging to family Pediciidae. *Dicranota* sp. prefers generally oligosaprobic and betamesosaprobic habitats but it also found in xenosaprobic and alphamesosaprobic habitats (Šporka, 2003). Stream zonation preferences of this genus are metarhithron and hyporhithron but it also occur in epirhithron and epipotamon zone of streams (AQEM Consortium, 2002). This genus was more common in Site 8 and 12. Site 8 has Class III water quality and was moderately polluted. This site was situated close to the source of Çekerek Stream. Site 12 has Class IV water quality and was heavily polluted. Although these sites were located far from agricultural, urban and industrial influences, they have low water quality. Another genus belonging to Pediciidae was *Pedicia* sp. in this study. There is little information about the ecology of *Pedicia* sp. in the literature. Some larval specimens of this genus were recorded from springs and headwater with low temperatures (Ujvárosi & Bálint, 2012; Ujvárosi et al., 2010). In this study, one individual of this genus was found only in Site 18. This site has Class IV water quality and was heavily polluted. Site 18 was not affected by agricultural and urban pollution like Site 8 and 12. A periodic situation may have caused the low water quality.

In this study, *Erioptera* sp. and *Hexatoma* sp. were identified as belonging to family Limoniidae. Despite that, *Hexatoma* sp. is one of the largest and also most widely distributed genus of this family, the information about preimaginal stages is inadequate (Podeniene and Gelhaus, 2015). *Hexatoma* sp. prefers oligosaprobic and betamesosaprobic habitats (CSN 75 7716, 1998). This genus was more common in Site 10. Another genus belonging to Limoniidae was *Erioptera* sp. in this study. Ecological information of this genus is poorly known. Larvae of Erioptera can live in a wide variety of substrates such as sand, moss, mud at the water margin (Kolcsár et al., 2017). In this study, one individual of this genus was found only in Site 15. Site 10 has Class IV water quality and was heavily polluted. Site 15 has Class III water quality and were moderately polluted. These sites were affected by agricultural and domestic activities. They were also situated after dams and physical destructions were detected in the stream beds. Releasing water from dams affected values of physicochemical variables and benthic macroinvertebrate community. Therefore, *Erioptera* sp. was probably drifted from the upstream parts of the stream to this site.

The larvae of Blephariceridae with ventral suction disc can inhabit in fast flowing water (Courtney and Merritt, 2008). Because larval blepharicerids are important component of stream habitat and they mostly found in clean, cold streams with high dissolved oxygen, they can be used as bioindicators for assessing the habitat quality of aquatic ecosystems (Courtney et al., 2017; Frutiger and Niederhauser, 2000; Zwick, 1977). In this study, only *Liponeura* sp. was identified as belonging to this family. *Liponeura* sp. prefers mainly oligosaprobic habitats but it is also found in xenosaprobic habitats (CSN 75 7716, 1998). Stream zonation preference of this genus is epirhithron (Schmedtje and Colling, 1996). One individual of this genus was found only in Site 9. This site has Class IV water quality and was heavily polluted. Site 9 was situated close to the source of Çekerek Stream. The reason for the low water quality is probably a periodic and temporary situation.

The family Dixidae with only two genera, *Dixa* and *Dixella*, is one of the smallest families of Diptera in Palearctic Region. They predominate in clean waters and headwater sites (Ivković and Ivanković, 2019; Wagner et al., 2008). In this study, *Dixa* sp. was identified as belonging to this family. It prefers generally oligosaprobic and betamesosaprobic habitats but it also found in xenosaprobic and alphamesosaprobic habitats (CSN 75 7716, 1998). Stream zonation preferences of this genus are crenon, epirhithron and metarhithron zone of streams (Tachet et al., 2010). This genus was found only in Site 7. This site has Class III water quality and was moderately polluted. Although Site 7 was situated near the source of Çekerek stream, it has low the water quality. This situation is probably seasonal.

The larvae of Dolichopodidae occur in a wide variety of stream habitats. They prefer mostly betamesosaprobic habitats, but they also found in oligosaprobic and alphamesosaprobic habitats. The family Dolichopodidae has wide range of stream zonation preferences from crenon to metapotamon (Tachet et al., 2010; Wagner et al., 2008). The individuals of this family were more common in Site 3 and 33. These sites have Class III water quality and were moderately polluted. Habitat deterioration was detected in the stream beds of these two sites and they were affected by nutrient inputs from agricultural areas.

The larvae of Empididae are abundant in running water habitats and have been found in a variety of habitats (Thirion, 2016). In this study, only *Clinocera* sp. was identified as belonging to this family. It prefers xenosaprobic and oligosaprobic habitats and stream zonation preference of this genus is hyporhithron but it also occur in metarhithron and epipotamon (Schmedtje and Colling, 1996). This genus was more common in Site 12. Although this site is not close to agricultural or urban areas, it has Class IV water quality and was heavily polluted. This is probably a periodic and temporary situation.

The family Muscidae is one of the largest groups of Diptera. Members of this family are found in all zoogeographic regions but aquatic larvae of Muscidae are poorly known (Hilsenhoff, 2001; Wagner et al., 2008). In this study, *Limnophora* sp. and *Lispe* sp. were identified as belonging to this family. *Limnophora* sp. prefers mainly oligosaprobic and betamesosaprobic habitats but it is also found in xenosaprobic habitats (CSN 75 7716, 1998). This genus was found only in Site 2. Another genus belonging to Muscidae was *Lispe* sp. in this study. There is no information about the ecology of *Lispe* sp. in the literature. This genus was found only in Site 30. Site 2 and 30 have Class IV water quality and were heavily polluted. These sites were affected by the agricultural run-off from the surrounding areas and habitat destruction were detected in the stream beds. In site 2, sampling was carried out after rainfalls. Therefore, *Limnophora* sp. may be drifted to Site 2, which did not have suitable conditions for this species. Site 30 was situated on the main branch of Yeşilırmak River and it is the closest site to the river mouth. It could be said that *Lispe* sp. can survive in organically polluted water and prefers potamon zone of streams.

The family Stratiomyidae has a worldwide distribution but ecological information about aquatic larvae of this family are quite limited (Hilsenhoff, 2001). In this study, *Oxycera* sp. was identified as belonging to this family. This genus occurs in clean running waters and it prefers hypocrenon zone of streams (AQEM Consortium, 2002; Kovac and Rozkošný, 2005). It was found in Site 8 and 15. These sites have Class III water quality and were moderately polluted. Site 8 was situated close to the source of Çekerek Stream. Although the physical conditions of this site are suitable for *Oxycera* sp., the water quality is low according to physicochemical variables. This is probably temporary situation. Site 15 after the dam was affected by agricultural and domestic pollutants. *Oxycera* sp. was probably drifted from upstream to this site because of releasing water from the dam.

The larvae of Tabanidae are found in a wide variety of aquatic environments (Middlekauff and Lane, 1980). In this study, *Tabanus* sp. was identified as belonging to this family. This genus prefers mainly betamesosaprobic habitats but it is also found in oligosaprobic and alphamesosaprobic habitats (CSN 75 7716, 1998). *Tabanus* sp. was found in Site 4, 8, 15 and 18. Site 4, 8 and 15 have Class III water quality and were moderately polluted. Site 18 has Class IV water quality and was heavily polluted. Site 8 was situated close to the source of Çekerek Stream. Site 8 and 18 were located far from agricultural and urban areas. A temporary situation may have caused the low water quality. Site 4 was situated close to the agricultural areas and the sampling was carried out after heavy rainfalls. This led to increased nutrient pollution. The values of nitrate nitrogen (4.480 mg/l) and ammonium nitrogen (0.341 mg/l) were the highest in this site. Site 15 were situated after dam and habitat degradation was detected in the stream beds. In addition, settlement was observed around this site.

As mentioned before, although some sites (Site 7, 8, 9, 12, 17 and 18) were situated close to the source of Çekerek Stream and far from agricultural and urban areas, the final water quality of these sites were Class III and IV. The reason for this situation was high PO_4-P value. The main sources of phosphorus in freshwater and groundwater systems are agricultural fertilizer, domestic and animal waste. Also, phosphorus in groundwater originates from dissolution of minerals that contain phosphate in aquifer sediments (Domagalski and Johnson, 2012; Fuhrer et al., 1999; Holman et al., 2008). According to Dubrovsky et al. (2010), transport of nutrients to streams and groundwaters varies seasonally. The frequent flood events, melting snow during spring and summer months (May – July) accelerates the transport of phosphorus from the soil to the stream by erosion (Dubrovsky et al., 2010; Rekolainen, 1989). Hatch et al. (1999) reported that snowmelt during June caused fluctuations of phosphate concentration in a mountain stream. Probably, the reason for the high PO_4-P value of Site 7, 8, 9, 12, 17 and 18 were a seasonal situation. Therefore, some of Diptera species may have left these sites and drifted to downstream. Consequently, the information about Diptera fauna and values of physicochemical variables of these sites can be misleading.

Multimetric indices derived from biological data are increasingly used to evaluate the habitat quality and monitor the habitat changes caused by anthropogenic effects (Buss et al., 2015; De Oliveira et al., 2019; Rosenberg and Resh, 1993). Diversity indices are also used for the evaluation of ecological health of streams and the distribution of benthic macroinvertebrates related to habitat quality. Godfrey (1978) reported that these indices are being widely used for assessment of stream pollution research. Biodiversity is defined and measured as an attribute that has two components (richness-number of existing species and evenness-distribution of individuals equally). Biodiversity can serve as an effective indicator of habitat health. Degradation and pollution of natural habitats is strongly associated with decrease in the species richness and evenness of freshwater habitats. In other words, values of the diversity indices decrease with environmental degradation (Godfrey, 1978; Ravera, 2001).

Simpson Diversity Index, Shannon-Wiener Diversity Index and Margalef Diversity Index were used in this study. Simpson Diversity Index values range between 0 and 1. The high index value (>0.6) indicates stable communities, while low index value indicates communities under stress conditions (Dash, 2003). The values of Simpson Diversity Index were between 0- 0.689 in this study. According to this index, the highest value was found in Site 8 and the lowest value was found in Site 4, 5, 16 and 20 (Table 2).

Shannon-Wiener Diversity Index is widely used in ecological studies. This index values range between 0 and 5 (Kocataş, 2006). The values above 3.0 mean that the habitat structure is stable and balanced; the values under 1.0 mean that habitat structure is degraded and polluted (Mason, 2002). The values of Shannon-Wiener Diversity Index were between 0-1.341 in this study. According to this index, the highest value was found in Site 8 and the lowest value was found in Site 4, 5, 16 and 20 (Table 2).

The Margalef Diversity Index is more sensitive to changes in the number of species than number of individuals. Thus, this biodiversity index is different from other biodiversity indices. It has no limit value. The higher species richness values reflect the stability of habitat (Margalef, 1958). The values of Margalef Diversity Index were between 0-1.377 in this study. According to this index, the highest value was found in Site 8 and the lowest value was found in Site 4, 5, 16 and 20 (Table 2).

In these three diversity indices, the highest value belonged to Site 8, the lowest value belonged to Site 4, 5, 16 and 20. Site 8 was situated close to the source of Çekerek Stream and this is one of the sites with the highest number of genera (7). Habitat degradation was not observed in this site. It has Class III water quality and was moderately polluted, but this situation is probably seasonal and temporary. Site 4, 5, 16 and 20 were surrounded by agricultural and urban areas. Site 4 has Class III water quality and was moderately polluted. The sampling was carried out after heavy rainfalls causing an increase in nutrient loading in this site. Site 5, 16 and 20 have Class IV water quality and were heavily polluted. Site 16 and 20 were situated after dam and regulator respectively. Habitat degradation was also detected in the stream beds of these two sites. These four sites have the least number of genera (1). Chironomidae gen sp, *Dicranota* sp. and *Tabanus* sp. were collected from these sites. The conditions of Site 4, 5, 16 and 20 are suitable for these three taxa. The results of biodiversity indices reflected the degradation in habitats correctly in this study.

According to the results of the study, it was determined that Yeşilırmak River and its tributaries were affected by urbanization, organic pollution draining from agricultural areas and domestic wastewaters. In addition, presence of dams, water regulators and hydroelectric power plant on the river caused changes in channel structure, temperature regime and sediment loading in Yeşilırmak River. All these changes negatively affected the water quality and Diptera community structure in almost all studied sites.

The one of the most important threat to aquatic ecosystems is anthropogenic activities in recent years. Due to these activities, Yeşilırmak River has also been heavily damaged. If these destructions continue and the necessary precautions are not taken, there will be irretrievable effects on the water quality and biodiversity of Yeşilırmak River and tributaries. Therefore, protecting water resources, preventing, and controlling the pollution, physicochemical and biological monitoring of the water quality are very important. In addition, much more research is needed to get more detailed information about the Diptera fauna of Yeşilırmak River.

ACKNOWLEDGEMENTS

This research was supported by Hacettepe University Scientific Research Projects Coordination Unit (Project title: “Constitution of biotic index for long-term biomonitoring of water quality for Yeşilırmak River by using benthic macroinvertebrates”, Project leader: Prof. Dr. Nilgün Kazancı and Project no: 07 01 601 005).

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 409-414 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.12

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The length and weight relationships and feeding ecology of knout goby, *Mesogobius batrachocephalus* (Pallas, 1814) from Southern Black Sea

Güney Karadeniz'den kayabalığı *Mesogobius batrachocephalus* (Pallas, 1814) türünün boy-ağırlık ilişkileri ve beslenme ekolojisi

Elizabeth Grace Tunka Bengil[1]* ● Mehmet Aydın[2]

[1] Girne American University, Marine School, Girne, TRNC via Turkey
[2] Fatsa Faculty of Marine Sciences, Ordu University, Ordu, Turkey

https://orcid.org/0000-0002-0071-3786
https://orcid.org/0000-0003-1163-6461

Corresponding author: tunkaeronat@hotmail.com

Received date: 09.04.2020 *Accepted date: 26.06.2020*

How to cite this paper:
Bengil, E.G.T. & Aydın, M. (2020). The length and weight relationships and feeding ecology of knout goby, *Mesogobius batrachocephalus (*Pallas, 1814) from Southern Black Sea? *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 409-414. DOI: 10.12714/egejfas.37.4.12

Abstract: Among ecologically diverse gobies species, knout goby, *Mesogobius batrachocephalus* (Pallas, 1814), or previously known as *Gobius batrachocephalus*, is a Black Sea endemic species. There are studies on this species biological features along the Black Sea but there are only studies on its length and weight relationship along the Turkish coasts of the Black Sea. This study aims to contribute to the lack of knowledge on knout goby length and weight relationship and feeding ecology inhabiting Southern Black Sea. Total of 470 individual of knout goby was collected and it was previously reported that knout goby shows negative (-) allometry though within this study it was found that it only shows negative (-) allometry in spring and positive (+) allometry in other seasons. The diet was composed of crustaceans, teleost fishes and gastropods. According to the relative importance analysis, teleost fishes are the main food item for all and male individuals but for female crustaceans are the main food item. Trophic level results show that for all individuals trophic levels is 4.34, and when sexes are compared females have higher trophic level than males. While both sexes only consume teleost during summer, in other seasons females prefer more crustacean in their diet compared to males. Niche breadth index results indicated that when all individuals diet was compared among seasons in winter the niche breadth was the broader and summer was the narrower, in case of females the broader was fall and for males it was winter. In conclusion, feeding ecology of knout goby changes between seasons and sexes but general prey groups remain the same

Keywords: Ecology, Length and Weight Relationships, Knout Goby, Mesogobius batrachocephalus, Southern Black Sea

Öz: Ekolojik olarak çok çeşitlilik gösteren kayabalığı türlerinden, daha önce *Gobius batrachocephalus* olarak bilinen kayabalığı *Mesogobius batrachocephalus* (Pallas, 1814) türü Karadeniz endemiği bir türdür. Karadeniz'de türün biyolojik özellikleri üzerine çalışmalar bulunmasına karşın Karadeniz'in Türkiye kıyılarından sadece boy-ağırlık ilişkilerine dair çalışmalar bulunmaktadır. Bu çalışma Karadeniz'de türün boy-ağırlık ilişkisi ve beslenme ekolojisi üzerine olan bilgi eksikliğine katkı sağlamayı hedelemektedir. Toplamda 470 birey toplanmış olup daha önce negatif (-) allometri gösterdiği rapor edilmiş olan kayabalığının bu çalışmada bahar döneminde negatif (-) ve diğer mevsimlerde positif (+) allometri gösterdiği tespit edilmiştir. Besinini krustaseler, teleost balıklar ve gastropodlar oluşturmaktadır. Göreceli önemlilik indeksi analizine göre tüm bireyler ve erkekler için teleost balıklar ve dişiler için krustaseler ana besin grubunu oluşturmaktadır. Tüm bireyler için trofik seviye 4.34 bulunmuş olup dişilerin erkeklerden daha yüksek trofik seviyeye sahip olduğu gözlemlenmiştir. Yazın her iki cinsiyette sadece teleost balıkları tercih etmelerine karşın diğer mevsimlerde dişiler erkeklerden daha çok krustaseleri tercih etmektedir. Niş genişliği indeks sonuçlarına göre mevsimler karşılaştırıldığında kışın en geniş ve yazın en dar olduğu, dişilerin sonbaharda ve erkeklerin kışın en geniş sonuçlara sahip olduğu bulunmuştur. Sonuç olarak, türün beslenme ekolojisi mevsimsel ve eşeyler arası değişiklik göstermekte ama genel besin grupları aynı kalmaktadır.

Anahtar kelimeler: Beslenme Ekolojisi, Boy-Ağırlık İlişkileri, kayabalığı, Mesogobius batrachocephalus, Güney Karadeniz

INTRODUCTION

Paratethyan gobies are restricted to the branches of the Marmara, Black and Caspian Seas and none permanently inhabits marine waters (Freyhof, 2011). Among this ecologically diverse species group, knout goby, *Mesogobius batrachocephalus* (Pallas, 1814), or previously known as *Gobius batrachocephalus* (Froese and Pauly 2019), is a Black Sea endemic found on sand or rock bottom of inshore habitats, estuaries, brackish- and freshwater lagoons (Freyhof, 2011). The species is commercially valuable in Turkish waters. According to Turkish Fishery Statics the fishery production of goby species is 63.3 tons. There are studies on this species biological features but there are only studies on its length and weight relationship along the coasts of Turkish coasts of the Black Sea (Demirhan and Can, 2007; Ak et al. 2009; Çalık and Erdoğan-Sağlam, 2017).

In fisheries management, knowledge on basic biology of a species is essential for its sustainable management. Length and weight relationships (LWRs) is therefore standard practice for any such management plan (Kohler et al. 1996; Schneider et al. 2000). LWRs results provide information on the species population dynamics in addition to a baseline for further studies and management plans. Additionally, in general, fish have the potential to integrate different characteristics of their habitats at spatial/or temporal scales, especially if they have a generalist feeding strategy, in which knout goby is (Rosca and Manzu, 2011), thus, the diet reflects

the prey availability and can be considered as a "sampling tool" representing the prey items available in its environment (Wootton, 1990). By examining diet composition of generalist feeders also makes it possible to monitor ecological changes due to outside factors such as climate change or other stressors in the habitat.

Aim of this study is to contribute to the study areas limited length and weight relationship knowledge and as a first for Southern Black Sea provide information on the feeding ecology of knout goby.

MATERIALS AND METHODS

470 individuals of knout goby were collected monthly with a trammel net with different mesh sizes (mesh sizes ranging between 17-24 mm) between April 2017 and March 2018 from the Southern Black Sea (Ordu province, 41°10'95.39'' N 37°17'24.78 E – 40°57'01.91'' N 38°18'59.73 E) (Figure1). Samples were brought to the laboratory fresh and morphological measurements were conducted. Total length measurements were made using a measuring board with a sensitivity of 1 mm, and weight measurements were taken with an electronic scale with a sensitivity of 0.01 g. After measurements, the individuals were dissected, the individual was cut from anus towards the head and the body cavity was exposed. Sex determinations were made through macroscopic observation of the gonad. Stomach contents were identified, separated, counted, and weighed. For identification of the stomach contents Fischer et al. (1987) and Aydın et al. (2013) were used.

Figure 1. Map of the study area

Each prey item was weighed and recorded to the nearest 0.01 g using an electronic scale. The LWRs were calculated by using power relationship in the following equation:

$$W = aL^b$$

Where W is the total weight (g); L is the total length (cm), while a and b are constants for each species or population (Schneider et al., 2000; Karachle and Stergiou, 2012). The constants were estimated by using the logarithm transformation of LWR dataset. The LWR were estimated for all, each sex and season. The b value, which indicates growth tendency, was tested with t-test (Zar, 1996) to verify whether it differs from the isometry at a 0.05 significance level.

All prey items found in the stomach were identified to the lowest possible taxonomic level. Analyses on diet comparison were made between sexes. To evaluate the importance of each prey item, percentage by number (N%), percentage by weight (W%), frequency of occurrence (FO%) and percentage index of relative importance (IRI%) were calculated (Hyslop, 1980). For each species, vacuity indices were calculated from the ratio of number of stomachs with prey items and total examined individuals.

Smith's, (1982) index was chosen to assess the niche breadth for two main reasons. Firstly, this method takes into account the availability of prey groups, and secondly it is less sensitive to selectivity of the prey groups that are of lower importance (Krebs, 2009).

$$FT = \Sigma(\sqrt{a_i p_i})$$

where FT is Smith's measure of niche breadth; pi is the proportion of individuals using prey category i; ai is IRI% of prey category i to the total prey composition.

Morisita index was chosen to calculate niche overlap between each sex and seasons.

$$C = \frac{2\sum_i^n p_{ij}p_{ik}}{\sum_i^n p_{ij}\left[{}^{(n_{ij}-1)}/_{(N_j-1)}\right] + \sum_i^n p_{ik}\left[{}^{(n_{ik}-1)}/_{(N_k-1)}\right]}$$

where C is Morisita's index of niche overlap between j and k; pij is proportion of prey category i to total prey composition used by a group j; pik is proportion of prey category i to total prey composition used by a group k; nij is number of individuals of group j that used prey category i; nik is number of individuals of group k that used prey category i; Nj and Nk are total number of species group j and k, respectively.

Trophic levels of all individuals as well as for both sexes, all and each season were estimated. All taxa found in the stomachs of examined individuals were classed under the prey categories as Crustacean, Teleost and Gastropod for easy comparison. Trophic level of identified groups and species were taken from FishBase (http://www.fishbase.org) (Froese and Pauly, 2019). IRI% of each taxon was used to calculate the proportional contribution of each taxon in a group. The contribution of each taxon and their trophic levels were then used to calculate weighted average trophic level of each prey group (Table 1). Afterwards, trophic levels of examined species were calculated by;

$$\mathrm{TL} = 1 + \left(\sum_{j=1}^{n} (IRI\%)_j * TL_j\right)$$

Where TLj is the trophic level of each prey category j; Pj is IRI% of prey category j (Pauly et al., 2000).

Table 1. Trophic level of identified groups from FishBase

Group code	Description	Trophic level
Gastropoda	Gastropods and unidentified crustaceans	2.1
Crustacea	Crustaceans and unidentified crustaceans	2.6
Teleostei	Teleost and unidentified crustaceans	3.5

All statistical analyses were performed by using Windows Office Excel software.

RESULTS

Total of 470 individual, 232 females and 238 males, of knout goby was collected. Length of all individuals ranged from 12.60-31.80 cm and weight ranged from 12.62-377.54 g (Table 2). The LWRs parameters for all individuals and both sexes by seasons are given in Table 3.

Table 2. Descriptive statistics of all, female, and male for overall and by seasons (O: overall; Sp: spring; Su: summer; F: fall; W: winter; ♀: females; ♂: males; Min: Minimum; Max: Maximum; SD: Standart deviation)

		All (470 individual)		♀ *(232 individual)*		♂ *(238 individual)*	
		TL (cm)	**W (g)**	**TL (cm)**	**W (g)**	**TL (cm)**	**W (g)**
O	**Min-Max**	12.60-31.80	12.62-377.54	13.50-31.80	27.77-357.2	12.60-31.80	12.62-377.54
	Mean±SD	23.12±4.69	129.31±75.89	23.02±4.44	128.54±73.03	23.22±3.97	130.07±77.18
Sp	**Min-Max**	13.5-31.75	28.01-30.88	13.5-31.20	29.8-305.8	14.10-31.70	28.01-302.76
	Mean±SD	25.37±4.07	164.63±64.54	25.65±3.14	172.47±56.76	24.93±5.23	152.29±74.21
Su	**Min-Max**	13.00-31.80	20.28-272.64	21.00-30.30	72.52-272.64	13.00-31.80	20.28-270.61
	Mean±SD	23.61±6.11	136.49±85.57	26.88±2.65	179.2±54.75	21.19±6.83	104.86±91.22
F	**Min-Max**	15.10-31.8	25.07-208.10	15.10-31.80	27.77-288.10	15.50-31.50	25.07-262.03
	Mean±SD	21.55±4.31	98.34±62.99	21.27±4.77	97.57±74.05	21.76±2.44	98.92±44.66
W	**Min-Max**	12.60-31.60	12.62-377.54	13.50-30.50	30.59-357.2	12.60-31.60	12.62-377.54
	Mean±SD	23.29±4.32	141.14±81.35	21.34±3.49	106.67±64.72	25.34±4.55	177.23±86.01

In total only 22% of the stomachs were full (spring 44%, summer 6%, fall 22% and winter 24%). The diet was composed of crustaceans *[(Brachynotus sexdentatus* (Risso, 1827), *Eriphia verrucosa* (Forskål, 1775)], Isopoda, *Liocarcinus navigator* [(Herbst, 1794), *Palaemon elegans* Rathke, 1837, *Palaemon serratus* (Pennant, 1777), *Upogebia pusilla* (Petagna, 1792), *Xantho poressa* (Olivi, 1792)), teleost fishes (*M. batrachocephalus*, *Neogobius melanostomus* (Pallas, 1814*), Gobius cruentatus* Gmelin, 1789, *G. niger* Linnaeus, 1758, *Merlangius merlangus* (Linnaeus, 1758), *Mullus barbatus barbatus* Linnaeus, 1758, *Symphodus melops* (Linnaeus, 1758), *Trachurus mediterraneus* (Steindachner, 1868)) and gastropods (*Tritia neritea* (Linnaeus, 1758)). According to the relative importance analysis, teleost fishes are the main food item for all and male individuals but for female crustaceans are the main prey (Table 4).

When seasons were compared, teleost fishes are the primary item and crustaceans are secondary, except summer where only teleost fishes were consumed. Crustacean consumption is highest in spring and lowest in summer. In case of gastropods, they were only consumed in fall, additionally, is the only season where all three groups were consumed. Trophic level results show that for all individuals it is 4.34, and when sexes are compared females have higher trophic level than males (Table 4).

Table 3. Length-weight relationships parameters of all, female, and male for overall and by seasons (♂: male; ♀: female; N: number of individuals; a: and b: population constants; r2: Regression coefficient; SE of b: Standard error of b; O; overall; Sp: spring; Su: summer; F: fall; W: winter)

		All	♀	♂
O	N	470	232	238
	a	0.0062	0.0062	0.0061
	b	3.13	3.13	3.12
	r^2	0.9606	0.9589	0.9633
	SE of b	0.0293	0.0428	0.0397
	Allometry	positive (+)	positive (+)	positive (+)
Sp	N	108	66	42
	a	0.0138	0.0179	0.0137
	b	2.88	2.81	2.86
	r^2	0.9223	0.8874	0.9503
	SE of b	0.0812	0.1253	0.1034
	Allometry	negative (-)	negative (-)	negative (-)
Su	N	47	20	27
	a	0.0076	0.0023	0.0076
	b	3.04	3.04	3.04
	r^2	0.9875	0.9473	0.9875
	SE of b	0.0510	0.1894	0.0607
	Allometry	positive (+)	positive (+)	positive (+)
F	N	184	79	105
	a	0.0065	0.0065	0.0065
	b	3.09	3.10	3.09
	r^2	0.9689	0.9772	0.9598
	SE of b	0.0410	0.0538	0.0623
	Allometry	positive (+)	positive (+)	positive (+)
W	N	131	67	64
	a	0.0044	0.0039	0.0037
	b	3.26	3.31	3.30
	r^2	0.9696	0.964	0.9643
	SE of b	0.0507	0.0792	0.0807
	Allometry	positive (+)	positive (+)	positive (+)

Table 4. Trophic levels and IRI % values of all, female and male individuals

	Trophic level	Taxon	Crustacea	Teleostei	Gastropoda
All	4.34	Overall	29.94	69.70	0.36
		Spring	67.66	32.34	0.00
		Summer	0.00	100.00	0.00
		Fall	22.21	75.79	2.01
		Winter	4.58	95.42	0.00
Female	4.47	Overall	51.62	47.41	0.98
		Spring	73.56	26.44	0.00
		Summer	0.00	100.00	0.00
		Fall	51.62	40.21	8.17
		Winter	2.81	97.19	0.00
Male	4.19	overall	11.06	88.94	0.00
		Spring	48.50	51.50	0.00
		Summer	0.00	100.00	0.00
		Fall	3.89	96.11	0.00
		Winter	9.35	90.65	0.00

Smith's (1982) niche breadth index results indicated that when all individuals diet was compared among seasons, in winter the niche breadth was broader and summer was narrower (spring 0.29, summer 0.14, fall 0.31, winter 0.32). In case of females, fall was broader with 0.32 (spring 0.26, summer 0.14 and winter 0.27) and for males with 0.27 it was winter (spring 0.24, summer 0.10 and fall 0.23). Results of Morisita's niche overlap analysis among all, both sexes and seasons showed that maximum overlap was observed between all individuals and females (0.96). The highest niche overlaps among seasons for all and females was fall and for males was winter (Table 5). Additionally, the result indicate that females have a broader diet than males, and female diet is the one that determines the overlap ratio between sexes.

Table 5. Morisita's niche overlap values among all, each sex and seasons

		All	Female	Male	Spring	Summer	Fall	Winter
All			0.96	0.92	0.72	0.16	0.95	0.91
Female		0.96		0.73	0.83	0.19	0.91	0.80
Male		0.92	0.73		0.36	0.12	0.92	0.93
All	**Spring**	0.72	0.77	0.54		0.06	0.49	0.40
	Summer	0.16	0.15	0.17	0.06		0.12	0.15
	Fall	0.95	0.88	0.91	0.49	0.12		0.93
	Winter	0.91	0.77	0.93	0.40	0.15	0.93	
Female	**Spring**	0.74	0.83	0.52		0.00	0.64	0.46
	Summer	0.19	0.19	0.17	0.00		0.17	0.26
	Fall	0.83	0.91	0.61	0.64	0.17		0.69
	Winter	0.84	0.80	0.76	0.46	0.26	0.69	
Male	**Spring**	0.33	0.28	0.36		0.16	0.06	0.14
	Summer	0.09	0.06	0.12	0.16		0.00	0.12
	Fall	0.81	0.66	0.92	0.06	0.00		0.87
	Winter	0.77	0.55	0.93	0.14	0.12	0.87	

DISCUSSION

When compared with studies previously conducted in the southeastern Black Sea by Demirhan and Can (2007), in the eastern Black Sea by Ak et al. (2009) and in the south-central Black Sea by Çalık and Erdoğan-Sağlam (2017) all reported that the species show negative (-) allometry and parameter b was 2.75, 2.74 and 2.78, respectively. In contrast to Demirhan and Can (2007) in this study value of b was over 3 (all 3.13, females 3.13 males 3.12) and shown positive (+) allometry, except during spring (all 2.88, females 2.81 males 2.86). But since Demirhan and Can (2007) sampling period was between January to June 2002 and which corresponds to end of winter, whole spring and early summer the negative (-) allometry results show parallelism with this study. Additionally, the negative (-) allometry during spring could be explain by species reproductive season being during spring (Roşca and Mânzu, 2011). Before the breeding period the feeding process is very intensive but during reproduction season the energy is directed to reproduction and feeding priority falls behind hence resulting innegative (-) allometry. But in knout goby within this study picks it up again during summer and parameter b values increase, and species start to show positive (+) allometry. In case of Ak et al. (2009) one-year sampling, and Çalık and Erdoğan-Sağlam (2017) sampling between September and April, negative (-) allometry results show contrast with this study. The differences in b values compare to these two studies might be the result of differences in length distribution in case of Ak et al. (2009) (184 individuals, length ranged between 5.5-18.0 cm), or in Çalık and Erdoğan-Sağlam (2017) case small sample size (37 individuals).

Knout goby is reported to feed mostly on bivalves, gastropods, amphipods, isopods, decapods, fishes and algae by previous studies (Roşca and Mânzu, 2011). According to Roşca and Mânzu (2011) species diet was composed of bivalves [*Mytilus galloprovincialis* Lamarck, 1819, *Mytilaster lineatus* (Gmelin, 1791)], gastropods [*Setia valvatoides* (Milaschewitsch, 1909), *Hydrobia sp., Bittium sp.*], amphipods, isopods [(*Idotea balthica* (Pallas, 1772)], decapods (*X. poressa*), fishes (*Mullus barbatus ponticus* Essipov 1927, gobiids), chironomid larvae and algae. However, in this study even though there are bivalves like *M. galloprovincialis* in the Turkish coasts of Black Sea no bivalve was found in the diet composition, but as a mollusk gastropod were present. Additionally, the teleost predation of knout goby was more diverse compare to teleost species reported by Roşca and Mânzu (2011). As these result show knout goby feeds mainly on mollusk, crustacean and teleost fishes but diet composition of prey species (not taxa) changes according to prey availability as previously reported and also between sexes and seasons as well. Additionally, the trophic levels of all, female and males were found to be 4.34, 4.47, and 4.19, respectively. Previously reported tentative trophic level of the species was 4.20 (Froese and Pauly, 2019) which is little lower than what has been estimated within. Higher results obtained here could be related to the species preference of teleostei species where some are located in high trophic levels (such as *M. merlangus euxinus* and *T. mediterraneus*)

Generally small value for the niche breadth shows prey specialization of a species for a small number of prey (Roşca and Mânzu, 2011). Though Roşca and Manzu (2011) estimated niche breath from Levin's Index and only studied individuals for three seasons (spring, summer and fall) they reported niche breath as 0.115 during the fall of 2008 and 0.588 during the summer of 2009. Minimal Levin's index value indicates that the species diet is more specialise, and when it is maximum it is broadest. Keeping this on mind, Roşca and Manzu (2011) index values imply that during fall the diet of knout goby is more specialise and according to the diet composition from 2008, it is mainly bivalve, *M. galloprovincialis*, and in fall 2009 it is mainly isopods, *I. balthica*. Unlike Roşca and Manzu (2011), in this study, the results show that (even though the Smith (1982) niche breath index was used) knout goby diet is more specialised during summer and broader in fall and values are the highest in winter. In conclusion, feeding ecology of knout goby changes between seasons and sexes but general prey groups remain the same.

ACKNOWLEDGEMENTS

This study was supported by Ordu University Research Fund Project No. AP-1735.

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Ege Journal of Fisheries and Aquatic Sciences, 37(4), 415-422 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.13

ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Soğukta depolanan (4±1°C) alabalık burgerlerde nar kabuğu ekstraktının antioksidan ve antimikrobiyal etkilerinin belirlenmesi

Determination of antioxidant and antimicrobial effects of pomegranate peel extract in trout burgers stored at cold temperatures (4±1˚C)

İlknur Uçak*

Niğde Ömer Halisdemir Üniversitesi, Tarım Bilimleri ve Teknolojileri Fakültesi, Niğde, Türkiye https://orcid.org/0000-0002-9701-0824

ilknurucak@ohu.edu.tr

Received date: 12.04.2020 *Accepted date: 09.07.2020*

How to cite this paper:
Uçak, İ. (2020). Determination of antioxidant and antimicrobial effects of pomegranate peel extract in trout burgers stored at cold temperatures (4±1°C). *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 415-422. DOI: 10.12714/egejfas.37.4.13

Öz: Son zamanlarda gıda endüstrisi işleme atıklarının antioksidan ve antimikrobiyal aktivitelerinin değerlendirilerek doğal katkı maddesi olarak gıdalara eklenmesi oldukça yaygın bir uygulama haline gelmiştir. Nar kabuğu da nar işleme endüstrisinde yer alan ve güçlü antioksidan ve antimikrobiyal özelliklere sahip önemli bir yan üründür. Bu nedenle bu çalışmada nar kabuğu ekstraktı ile hazırlanan alabalık burgerlerinin buzdolabında (4±1°C) depolanması süresince kalitesinde meydana gelen kalite değişimlerinin incelenmesi hedeflenmiştir. Bu amaçla alabalık burgerlerde peroksit değeri, tiyobarbitürik asit (TBARs) sayısı, toplam aerobik mezofilik bakteri (TAMB), toplam psikrofilik bakteri (TPB), toplam koliform, toplam maya-küf ve toplam laktik asit (LAB) bakteri sayıları ile duyusal kalite parametreleri değerlendirilmiştir. Depolama başında peroksit değeri 2,75 meq O_2/kg olarak bulunmuş ve depolama boyunca tüm gruplarda artarak nar kabuğu ekstraktı ile hazırlanan gruplarda kontrol (24,85 meq O_2/kg) grubuna göre önemli derecede daha düşük bulunmuştur. Alabalık etinde TBARs değeri 0,07 mg MDA/kg olarak bulunmuş ve depolama sonuna kadar tüm gruplarda artmıştır. Kontrol grubuna göre önemli derecede en düşük değerler %1 nar kabuğu ekstraktı ile hazırlanan grupta bulunmuştur. Depolamanın sonunda kontrol, %0,5 ve %1 nar kabuğu ekstraktı ile zenginleştirilen gruplarının TBARs değerleri sırası ile 2,78, 2,32 ve 2,25 mg MDA/kg'a ulaşmıştır. Nar kabuğu ekstraktının alabalık burgerlerinde TAMB, TPB, toplam koliform, toplam maya-küf ve LAB gelişimi üzerine baskılayıcı etkisi olduğu belirlenmiştir. Yapılan duyusal değerlendirmeler sonucunda nar kabuğu ekstraktının alabalık burgerlerinin raf ömrünü kontrol grubuna göre 6 gün uzattığı gözlenmiştir. Tüm bu veriler, nar kabuğu ekstraktının alabalık burgerlerinde alternatif doğal bir katkı maddesi olarak kullanılabileceği sonucunu ortaya koymuştur.

Anahtar kelimeler: Nar kabuğu ekstraktı, alabalık burger, lipit oksidasyonu, mikrobiyal kalite, raf ömrü

Abstract: Recently, it has become a common practice to utilize food processing waste as natural additives in food products by evaluating their antioxidant and antimicrobial activities. Pomegranate peel is also an important by-product involved in the pomegranate processing industry and has strong antioxidant and antimicrobial properties. Therefore, the aim of this study is to examine the quality changes of trout burgers prepared with pomegranate peel extract during the refrigerated (4±1°C) storage. For this purpose, peroxide value, thiobarturic acid (TBARs), total aerobic mesophilic bacteria (TAMB) count, total psychophilis bacteria (TPB) count, total coliform bacteria, total yeast-mold, total lactic acid bacteria (LAB) count and sensory quality parameters were evaluated in trout burgers. Peoxide value was found to be 2.75 meq O_2/kg at the beginning with increasing in all groups and were observed significantly lower in the groups prepared with pomegranate peel extract than the control group (24.85 meq O_2/kg). TBARs value of trout fillet was 0.07 mg MDA/kg and increased in all groups until the end of storage. The lowest values compared to the control group were found in the group prepared with 1% pomegranate peel extract. At the end of storage, TBARs values of the control, groups enriched with 0.5% and 1% pomegranate peel extract reached 2.78, 2.32 and 2.25 mg MDA/kg, respectively. It has been determined that pomegranate peel extract has a suppressive effect on TAMB, TPB, total coliform, total yeast-mold and LAB growth in trout burgers. As a result of sensory evaluations, pomegranate peel extract has been observed to extend the shelf life of trout burgers by 6 days compared to the control group. All these data revealed that pomegranate peel extract can be used as an alternative natural additive in trout burgers.

Keywords: Pomegranate peel extract, trout burger, lipid oxidation, microbial quality, shelf life

GİRİŞ

Son yıllarda besinsel farkındalık ve yaşam tarzında olan değişiklikler burger, sosis, kroket ve balık köftesi gibi hazır gıda tüketiminin artmasına neden olmaktadır (Cakli vd., 2005). Hazır gıda, tüketime doğrudan hazır çiğ veya pişirilmiş, başka işlemlere gerek duyulmadan tüketilebilen gıdalar olarak tanımlanmaktadır. Balık ve ürünleri depolama sırasında raf ömrünü sınırlayan bazı istenmeyen değişimlere uğrayabilmektedir. Bu istenmeyen değişimlerin başında ürünün koku, lezzet, tekstür, görünüş ve besin değeri gibi özelliklerini etkileyen lipit oksidasyonu ve mikrobiyal bozulma gelmektedir. Su ürünlerinde kalitenin korunması ve raf ömrünün arttırılması amacıyla çeşitli antioksidan ve antimikrobiyal maddelerin kullanımı oldukça yaygın bir uygulama olup son yıllarda özellikle gıda atıklarından yan ürün değerlendirme ve bu ürünlerin endüstriyel olarak kullanımları gittikçe artan bir önem kazanmaktadır.

Nar (*Punica granatum L.*) kabukları, tüm meyvenin yaklaşık %40'ını oluşturan nar suyu işleme endüstrisinin yan ürünleri olup (Çam ve Hışıl, 2010) yenilebilir kısımlardan daha yüksek miktarda fenolik madde bulundurmaktadır. Nar

kabuğunda güçlü antimutajenik, antioksidan ve antimikrobiyal özellik gösteren antosiyaninler, gallotaninler, ellagitaninler, gallagil esterler, hidroksibenzoik asitler, hidroksi sinnamik asitler ve dihidroflavonol gibi önemli miktarlarda çeşitli polifenoller tanımlanmıştır (Akhtar vd., 2015). Ayrıca nar kabuğu ekstraktında bulunan fenoller, taninler ve flavonoidler *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhimurium, Aspergillus niger, Mucor indicus, Penicillium citrinum* gibi bakteri ve mantarlara karşı antibakteriyel ve antifungal aktivite göstermektedir (Khan ve Hanee 2011; Supayang vd., 2005).

Nar kabuğu ekstraktlarının antioksidan ve antimikrobiyal etkilerinin taze balık filetosu ürünlerine etkileri hakkında çeşitli çalışmalar bulunmaktadır (Berizi vd., 2018; Berizi vd., 2016; Alsaggaf vd., 2017; Shinde vd., 2015; Zhuang vd., 2019). Ancak yapılan literatür araştırmaları sonucunda işlenmiş bir ürün olan balık burgerlerin kalitesi ve raf ömrü üzerine nar kabuğu ekstraktlarının etkileri hakkında herhangi bir çalışmaya rastlanmamıştır. Bu bilgiler doğrultusunda, önemli bir antioksidan ve fenolik madde kaynağı olarak bilinen nar kabuğu ekstraktının alabalık burgerlerde doğal bir katkı maddesi olarak kullanılabileceği düşünülmüştür. Bu çalışmanın temel hedefi önemli bir hazır gıda olan balık burgerlerin duyusal, kimyasal ve mikrobiyal kalitesinin nar kabuğu ekstraktları kullanılarak korunması ve raf ömrünün arttırılmasıdır. Bu amaçla nar kabuğu ekstraktları ile zenginleştirilen balık burgerler soğukta (4±1°C) 15 gün boyunca depolanarak periyodik olarak kalite değişimleri incelenmiştir.

MATERYAL VE METOT

Materyal

Çalışmada yerel bir marketten temin edilen narlardan (*Punica granatum*) elde edilen kabuklar kullanılmıştır. Yıkananan narların suyu sıkılarak kabukları soyulmuş ve çalışmada kullanılmak üzere ayrılmıştır.

Çalışmada ortalama ağırlıkları ve boyları sırası ile 188,52±9,46 g ve 16,82±2,37 cm olan alabalıklar (*Oncorhynchus mykiss*) kullanılmıştır. Balıklar Niğde bölgesindeki bir alabalık çiftliğinden fileto olarak satın alınmıştır. Balık filetoları buz dolu strafor kutulara koyularak 1 saat içerisinde laboratuvara ulaştırılmış ve daha sonra kıyma haline getirilmiştir.

Metot

Nar kabuklarının ekstraksiyonu

Elde edilen nar kabukları tekrar yıkanarak etüvde 45°C'de 48 saat kurutulmuştur. Kurutulan nar kabukları blendır ile öğütülerek toz haline getirilmiştir. 10 g nar kabuğu ile 100 mL (%80) etanol karıştırılarak ultrasonik su banyosunda oda sıcaklığında (25±1°C) 1 saat boyunca çözdürülmüştür (Ifesan vd., 2014). Daha sonra ekstraktlar kaba filtre kâğıdı yardımıyla süzülmüş içerisinde bulunan etanol ve rotary evaporatörde 45°C'de uçurulmuştur. Elde edilen nar kabuğu ekstraktları çalışmada kullanılıncaya kadar -80°C'de depolanmıştır.

Balık burger yapımı

Tokur vd. (2004) yöntemine göre, elde edilen balık kıymasına çeşitli katkılar ilave edilerek balık burgerler yapılmıştır. Örnekler üç gruba ayrılmış ve ekstrakt ilavesi olmadan hazırlanan grup K (kontrol), %0,5 konsantrasyonda nar kabuğu ekstraktı ile hazırlanan grup NK5 ve %1 konsantrasyonda nar kabuğu ekstraktı ile hazırlanan grup NK10 olarak adlandırılmıştır. Burgerler 50 g olacak şekilde hazırlanmış ve strafor tabaklara koyulmuştur. Daha sonra üzerleri streç filmle kaplanarak +4°C±1'de 15 gün süresince depolanarak periyodik analizler yapılmıştır. Tablo 1 balık burgerlerin hazırlanmasında kullanılan maddeleri göstermektedir.

Tablo 1. Balık burger yapımında kullanılan maddeler
Table 1. Ingredients of fish burger

	K (%)	NK5 (%)	NK10 (%)
Balık eti	87,2	86,7	86,2
Mısır unu	6	6	6
Buğday unu	4	4	4
Sarımsak tozu	0,2	0,2	0,2
Soğan tozu	0,2	0,2	0,2
Tuz	1,2	1,2	1,2
Şeker	1,2	1,2	1,2
Nar kabuğu ekstraktı	-	0,5	1

Peroksit analizi

Peroksit analizi AOAC (1990) yöntemine göre yapılmış ve aşağıdaki formüle göre hesaplanarak meq/kg cinsinden ifade edilmiştir.

PV (meq/ kg) = K x (V-V0) x 12,69 x 78,8 / w

K titrasyonda harcanan $Na_2S_2O_3$'ün konsantrasyonu (mol/lt), V titrasyonda harcanan $Na_2S_2O_3$'ün miktarı (mL), w örnek ağırlığı (g)

Tiyobarbitürikasit (TBARs) sayısı analizi

AOCS (1998) tarafından belirtilen metoda göre yapılan TBARs analizinde balık burgerden çıkarılan yağ örneği n-bütanol içerisinde çözdürülmüş ve 5 mL alınarak üzerine aynı miktarda TBA reaktifi eklenmiştir. Örnekler 120 dakika 95°C su banyosunda tutulduktan sonra soğutularak 530 nm'de spektrofotometrede okunmuş ve aşağıda verilen formüle göre hesaplanarak mg malondialdehit/kg yağ olarak ifade edilmiştir.

TBA = 50 x (Yağ örneğinin absorbansı- Blank absorbansı) / örnek ağırlığı (mg)

Mikrobiyolojik analizler

Analiz için 10 g örnek 90 mL ringer solüsyonu içerisine koyularak laboratuvar tipi blendır ile homojenize edilmiştir. Balık burger örneklerinde toplam aerobik mezofilik bakteri, toplam psikrofilik bakteri, toplam maya-küf, toplam koliform

bakteri ve toplam laktik asit bakteri sayıları belirlenmiştir. Toplam mezofilik ve psikrofilik bakteri sayımlarında yayma kültür yöntemi ile Plate Count Agar (PCA) besiyerine ekim yapılmış ve petriler sırası ile 37°C'de 24-48 ve 8°C'de 7 gün inkübasyona bırakılmıştır (Anonymous, 1998). Toplam maya-küf sayımı için yayma ekim yöntemi kullanılarak Potato Dextrose Agar (PDA) (pH'sı 3,5'e ayarlanmış) besiyerine ekim yapılmış ve petriler 25±1°C'de 5 gün inkübe edilmiştir (Anonymous, 1976). Toplam koliform sayımında Violet Red Bile Agar (VRBA) besiyerine dökme ekim yapılmış ve 37°C'de 24-48 saat inkübe edilmiştir (Anonymous, 1998). de Man Rogosa ve Shape (MRS Agar) agara yayma ekim yapılarak belirlenen laktik asit bakterileri anaerob jarlarda 30°C'de 48 saat inkübasyona bırakılmıştır (de Man vd., 1960).

Duyusal analiz

Paulus vd. (1979) tarafından belirlenen metoduna göre balık burgerler 8 panelist tarafından değerlendirilmiş ve 9 dan 1 e kadar olan hedonik skala (9: çok iyi, 8: oldukça iyi, 7:iyi, 6:biraz iyi, 5:yorumsuz, 4:biraz kötü, 3:kötü, 2:oldukça kötü, 1:çok kötü) kullanılmıştır. Panelistler tarafından hazırlanan burgerlerin renk, koku, lezzet, doku yapısı, genel beğeni kriterlerine göre değerlendirilme yapılabilmesi için ızgarada 350°C'de 5 dakika pişirildikten sonra panelistlere sunulmuştur.

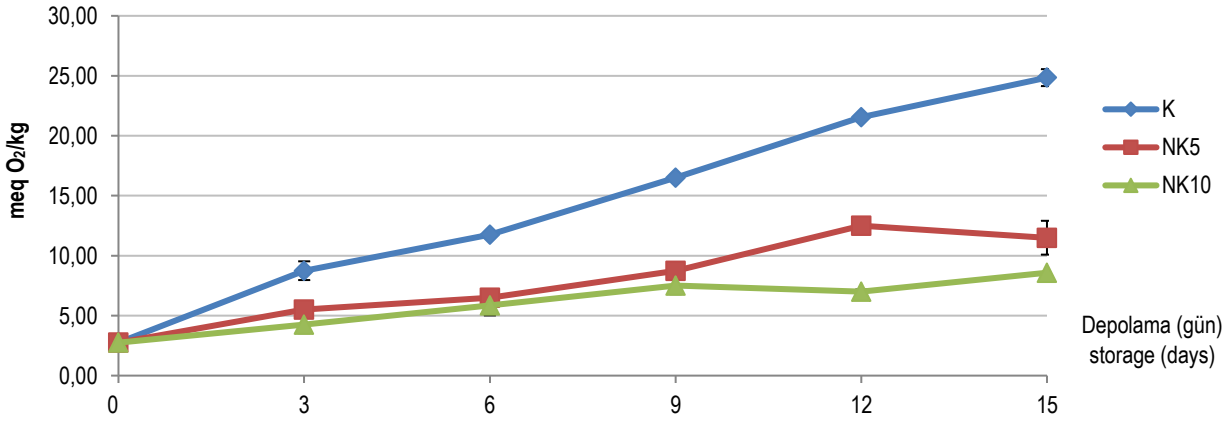
İstatistiksel analiz

Balık burgerlerin depolanması sonucu elde edilen verilerin istatistiksel olarak değerlendirilmesinde SPSS yazılımı (Version 18.0, Statistical Analysis System, Cary, NC, USA) kullanılarak verilere %5 önem düzeyinde ($p<0,05$) varyans analizi uygulanmış (One-way anova) ve duncan çoklu karşılaştırma testine tabi tutulmuştur.

BULGULAR VE TARTIŞMA

Oksidatif değişimler

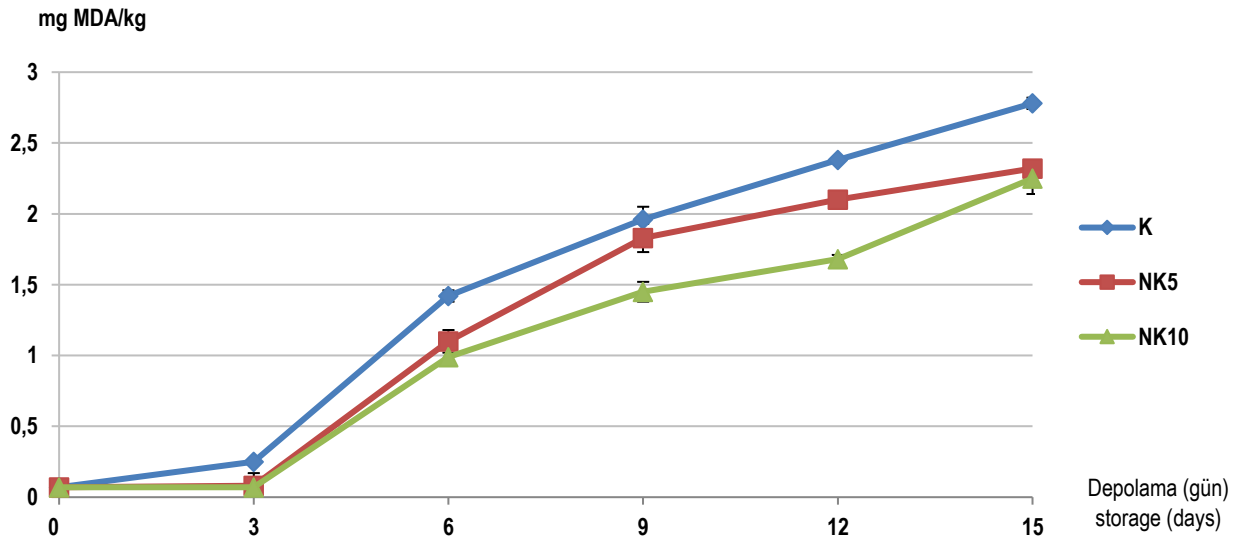
Lipit oksidasyonu su ürünlerinde başlıca bozulma nedenlerinden olup balıkta kalite kayıplarına neden olan faktörlerin başında gelmektedir. Lipit oksidasyonunun birinci aşamasında peroksit ve hidroperoksitlerin oluşumu gerçekleşmektedir. Peroksit değeri de başlangıçta oluşan birincil oksidasyon ürünlerinin ölçülmesinde kullanılmaktadır. Depolama başında alabalık etinde peroksit değeri 2,75 meq O_2/kg iken kontrol grubunda önemli derecede ($p<0,05$) artış göstererek depolama sonunda 24,85 meq O_2/kg'a ulaşmıştır (Şekil 1). Depolama boyunca tüm gruplarda artış gösteren peroksit değerleri nar kabuğu ekstraktı ile hazırlanan gruplarda kontrol grubuna göre önemli derecede ($p<0,05$) daha düşük bulunmuş, NK5 ve NK10 gruplarında depolama sonunda sırası ile 11,50 ve 8,58 meq O_2/kg değerlerine ulaşmıştır. Uçak vd. (2011) yaptıkları çalışmada biberiye ekstraktı ilavesinin uskumru burgerlerde kontrol grubuna göre daha düşük peroksit değerleri oluşmasını sağladığını bildirmişlerdir. Bir başka çalışmada da %1 konsantrasyonunda nar kabuğu ekstraktı eklenen et burgerlerin de peroksit değerlerinin kontrol grubuna göre daha düşük olduğu rapor edilmiştir (Turgut vd., 2017).



Şekil 1. Nar kabuğu ekstraktı ile zenginleştirilen alabalık burgerlerinin peroksit değerlerinde meydana gelen değişimler
Figure 1. Changes in the peroxide values of trout burgers supplemented with pomegranate peel extract

Tiyobarbitürik asit (TBARs) değeri ikincil oksidasyon ürünlerinin belirlenmesinde sıklıkla kullanılan bir yöntemdir. Nar kabuğu ekstraktı ilaveli alabalık burgerlerinin TBARs değerlerindeki değişimler Şekil 2'de verilmiştir. Alabalık etinde TBARs değeri 0,07 mg MDA/kg yağ olarak bulunmuş ve depolama sonuna kadar tüm burger örneklerinde artmıştır. Kontrol grubuna göre önemli derecede ($p<0,05$) en düşük değerler NK10 grubunda bulunmuştur.

Depolamanın sonunda K, NK5 ve NK10 gruplarının TBARs değerleri sırası ile 2,78, 2,32 ve 2,25 mg MDA/kg yağ değerlerine ulaşmıştır. Benzer şekilde, yapılan diğer çalışmalarda da farklı bitkisel ekstraktların balık burger ve balık köftesi gibi ürünlerde lipit oksidasyonunu kontrol altına aldığı ve TBARs değerlerini düşürdüğü gözlenmiştir (Özoğul ve Uçar, 2013; Guan vd., 2019; Fernandes vd., 2017).



Şekil 2. Nar kabuğu ekstraktı ile zenginleştirilen alabalık burgerlerinin TBARs değerlerinde meydana gelen değişimler
Figure 2. Changes in the TBARs values of trout burgers supplemented with pomegranate peel extract

Mikrobiyolojik değişimler

Bakteriyel gelişim balık ve balık ürünlerinin temel bozulma nedenlerindendir. Farklı konsantrasyonlarda nar kabuğu ekstraktının alabalık burgerlerinin mikrobiyal kalitesi üzerine olan etkileri Tablo 2 ve Tablo 3'te verilmiştir. Toplam aerobik mezofilik bakteri sayısı (TAMB) depolama başında 2,92 log kob/g olarak belirlenmiş ve depolama boyunca tüm gruplarda artmıştır. Nar kabuğu ekstraktı ilave edilerek hazırlanan balık burgerlerde TAMB sayısının depolama süresince kontrol grubundan önemli derecede ($p<0,05$) düşük olduğu gözlenmiştir. Depolamanın 15. gününde kontrol grubunun TAMB sayısı 7,42 log kob/g iken, NK5 ve NK10 gruplarında sırası ile 7,20 ve 7,12 log kob/g olmuştur. Depolama boyunca en düşük TAMB sayısı değerleri %1 konsantrasyonda nar kabuğu ekstraktı ile hazırlanan balık burger örneklerinde gözlenmiştir. Uçak vd. (2018) alabalık filetolarının başlangıçta toplam canlı sayısını 1,48 log kob/g bulurken, benzer şekilde Öz (2018) yaptığı çalışmada alabalık etinde başlangıç toplam canlı sayısını 2,80 log kob/g olarak tespit etmiştir.

Uskumru burgerlere farklı konsantrasyonlarda (%0,4 ve %0,8) biberiye ekstraktının eklendiği bir çalışmada depolama boyunca toplam canlı sayısının kontrol grubundan daha düşük olduğu rapor edilmiştir (Uçak vd., 2011). Keser ve İzci (2020) tarafından yapılan çalışmada, defne ve biberiye uçucu yağları ile hazırlanan alabalık köftelerinin başlangıçta toplam bakteri sayısı mevcut çalışmadan oldukça yüksek (5,24 log kob/g) bulunmuştur. Ayrıca defne ve biberiye uçucu yağlarının bakteri gelişimini yavaşlattığını bildirmişlerdir.

Nar kabuğu ekstraktı ile zenginleştirilen alabalık burgerlerinin toplam psikrofilik bakteri (TPB) sayısı depolama başında 2,65 log kob/g olarak bulunmuştur. Tüm gruplarda bakteri sayısı artış göstererek depolamanın 15. gününde kontrol grubunda en yüksek ($p<0,05$) değere (7,15 log kob/g) ulaşmıştır. Nar kabuğu ekstraktı ilave edilen gruplar ile kontrol grubu arasında önemli farklılıklar ($p<0,05$) bulunmuş ve depolama sonunda NK5 ve NK10 gruplarında TPB sayısı sırası ile 6,76 ve 6,04 log kob/g değerlerine ulaşmıştır. Singh vd. (2018) nar kabuğu ekstraktının antimikrobiyal etkisinin kafeik, siringik, sinapik, p-kumarik, ferulik, elagik, gallik ve sinamik asit gibi ana bileşiklerin varlığından kaynaklandığını bildirmişlerdir. Keser ve İzci (2020) alabalık etinde başlangıç psikrofil bakteri sayısını 4,22 log kob/g bulmuşlardır. Farklı antioksidanlar (kekik, adaçayı, defne, yeşil çay) ile hazırlanan balık burgerlerin başlangıçta psikrofil bakteri sayısının 4,90 log kob/g olarak bulunduğu çalışmada dondurularak depolama boyunca antioksidan ilave edilen gruplarda TPB sayısının kontrol grubundan daha düşük olduğu rapor edilmiştir (Özoğul ve Uçar, 2013). Mevcut çalışmada nar kabuğu ekstraktının alabalık burgerlerinde bakteri gelişimi üzerine baskılayıcı etkisi olduğu belirlenmiştir.

Toplam maya-küf sayısı alabalık etinde başlangıçta 1,45 log kob/g olarak belirlenmiş ve tüm burger örneklerinde artış göstermiştir. Nar kabuğu ekstraktı ile zenginleştirilmiş burger örneklerinin maya-küf sayısı kontrol grubuna göre önemli derecede ($p<0,05$) daha düşük düzeylerde bulunmuştur. Depolama sonunda kontrol, NK5 ve NK10 gruplarının toplam maya-küf sayıları sırası ile 6,09, 5,99 ve 5,61 log kob/g olmuştur. Kaba vd. (2013) yaptıkları çalışmada palamut balığı köftesinin başlangıç maya-küf değerinin 4,08 log kob/g olduğunu ve depolama süresince bu değerin artış göstererek depolama sonunda (10. gün) 6,32 log kob/g değerine ulaştığını bildirmiştir. Bir başka çalışmada kadife balığı köftesinin maya-küf değerleri depolama başında 3,6 log kob/g olarak bulunmuştur (Çapkın, 2008).

Tablo 2. Nar kabuğu ekstraktının alabalık burgerlerinin mikrobiyolojik kalitesi üzerine etkileri (kob/g)
Table 2. Effects of pomegranate peel extract on microbiological quality of trout burgers (cfu/g)

	Depolama (gün)	K	NK5	NK10
Toplam mezofilik bakteri sayısı	**0**	2,92±0,34^{dA}	2,92±0,34^{dA}	2,92±0,34^{dA}
	3	3,96±0,07^{cA}	3,81±0,15^{cAB}	3,47±0,15^{cB}
	6	5,44±0,01^{bA}	4,41±0,27^{cA}	4,47±0,15^{bA}
	9	5,75±0,06^{bA}	5,40±0,05b^{AB}	4,56±0,08^{bC}
	12	7,23±0,10^{aA}	7,07±0,04^{aAB}	6,93±0,01^{aB}
	15	7,42±0,00^{aA}	7,20±0,04^{aB}	7,12±0,00^{aC}
Toplam psikrofilik bakteri sayısı	**0**	2,65±0,16^{eA}	2,65±0,16^{eA}	2,65±0,16^{cA}
	3	3,03±0,10^{dA}	2,99±0,04^{dA}	2,54±0,09^{cB}
	6	4,31±0,01^{cB}	4,48±0,00^{cA}	4,46±0,00^{bA}
	9	6,48±0,00^{bA}	6,40±0,07^{bA}	5,88±0,01^{aB}
	12	7,07±0,16^{aA}	6,48±0,00^{bB}	6,04±0,03^{aC}
	15	7,15±0,03^{aA}	6,76±0,10^{aB}	6,04±0,03^{aC}
Toplam maya-küf sayısı	**0**	1,45±0,02^{dA}	1,45±0,02^{eA}	1,45±0,02^{dA}
	3	1,71±0,01^{dA}	1,61±0,21^{eA}	1,68±0,19^{dA}
	6	4,30±0,03^{cA}	3,30±0,06^{dB}	2,88±0,06^{cC}
	9	5,17±0,21^{bA}	4,90±0,00^{cB}	4,50±0,07^{bB}
	12	6,30±0,18^{aA}	5,68±0,02^{bB}	5,46±0,09^{aB}
	15	6,09±0,10^{aA}	5,99±0,03^{aB}	5,61±0,08^{aB}

Aynı satırdaki büyük harfler gruplar arası istatistiksel farkı, aynı sütundaki küçük harfler grup içi istatistiksel farkı belirtmektedir (P<0,05)
Means indicated by different capital letters in the same row differ significantly, means indicated by different lowercase letters in the same column differ significantly (P<0,05)

Tablo 3. Nar kabuğu ekstraktının alabalık burgerlerinin mikrobiyolojik kalitesi üzerine etkileri (kob/g)
Table 3. Effects of pomegranate peel extract on microbiological quality of trout burgers (cfu/g)

	Depolama (gün)	K	NK5	NK10
Toplam koliform bakteri sayısı	**0**	1,81±0,01^{eA}	1,81±0,01^{eA}	1,81±0,01^{eA}
	3	1,93±0,05^{eA}	1,92±0,10^{eA}	1,84±0,08^{eA}
	6	4,48±0,00^{dA}	3,84±0,00^{dB}	2,40±0,11^{dC}
	9	5,59±0,05^{cA}	5,04±0,01^{cB}	3,84±0,03^{cC}
	12	6,33±0,16^{bA}	6,22±0,03^{bA}	5,67±0,09^{bB}
	15	7,29±0,02^{aA}	6,92±6,92^{aB}	6,75±0,04^{aC}
Laktik asit bakteri sayısı	**0**	1,78±0,11^{eA}	1,78±0,11^{eA}	1,78±0,11^{eA}
	3	1,91±0,09^{eA}	1,87±0,05^{eA}	1,81±0,07^{eA}
	6	2,83±0,04^{dA}	2,77±0,04^{dA}	2,73±0,12^{dA}
	9	4,24±0,03^{cA}	4,08±0,01^{cA}	3,77±0,34^{cA}
	12	5,48±0,00^{bA}	5,42±0,05^{bA}	4,78±0,01^{bB}
	15	5,94±0,04^{aA}	5,85±0,08^{aA}	5,79±0,00^{aA}

Aynı satırdaki büyük harfler gruplar arası istatistiksel farkı, aynı sütundaki küçük harfler grup içi istatistiksel farkı belirtmektedir (p<0,05)
Means indicated by different capital letters in the same row differ significantly, means indicated by different lowercase letters in the same column differ significantly (p<0,05)

Toplam koliform bakteri sayısı balıkta hijyen göstergesi olarak kabul edilmektedir. Alabalık etinin başlangıç koliform bakteri sayısı 1,81 log kob/g olarak bulunmuştur. Depolamanın sonuna kadar artış gösteren koliform bakteri gelişimi %1 nar kabuğu ekstraktı ilave edilen gruplarda önemli derecede (p<0,05) baskılanmıştır. 15. günde toplam koliform bakteri sayısı kontrol grubunda 7,29 log kob/g değerine ulaşırken NK5 ve NK10 gruplarında sırası ile 6,92 ve 6,75 log kob/g olarak belirlenmiştir. Yapılan benzer çalışmalar da doğal ekstraktların balık ve ürünlerinde toplam koliform bakteri gelişimi üzerinde etkili olduğunu göstermektedir (Uçak vd., 2018; Frangos vd., 2010; Mexis vd., 2009).

Hem anaerobik hemde aerobik koşullarda gelişebilen ve fakültatif anaerobik bakteriler olan laktik asit bakteri (LAB) sayısı alabalık etinde başlangıçta 1,78 log kob/g bulunurken tüm burger örneklerinde artış göstermiştir. Ancak bu artış nar kabuğu ekstraktı eklenen gruplarda kontrol grubuna göre daha düşük düzeylerde olmuştur. Nar kabuğu ekstraktı ile hazırlanan burger örnekleri ile kontrol grubu arasında LAB gelişimi bakımından önemli faklılıklar (p>0,05) bulunmamış ancak en düşük değerler depolama sonunda yine NK5 (5,85 log kob/g) ve NK10 (5,79 log kob/g) gruplarında tespit edilmiştir. Kuş (2012) altınotu ve ökseotu ekstraktlarının alabalık filetosunda LAB gelişimini baskıladığını bildirmiştir. Benzer şekilde Frangos vd. (2010)'de yaptıkları çalışmada

kekik yağının alabalık filetosunda LAB gelişimini önemli derecede düşürdüğünü rapor etmişlerdir.

Duyusal değişimler

Balık ve ürünlerinin duyusal olarak kabul edilebilirlikleri depolanmaları süresince duyusal özelliklerinde meydana gelen değişikliklere bağlıdır. Nar kabuğu ekstraktı ile hazırlanan alabalık burgerler koku, tekstür, lezzet, görünüş ve genel beğeni parametreleri ile değerlendirilmiştir (Tablo 4).

Elde edilen veriler sonucunda kontrol grubuna ait burgerlerin 6. günde reddedildiği ve genel beğeni puanının 3,00 olduğu gözlenmiştir. NK5 ve NK10 grubu burger örneklerinin ise depolamanın 12. gününde reddedildiği ve genel beğeni puanlarının sırası 1,80 ve 3,00 olduğu bulunmuştur. Yapılan duyusal değerlendirmeler sonucunda nar kabuğu ekstraktının alabalık burgerlerinde duyusal özelliklere katkı sağladığı ve raf ömrünü kontrol grubuna göre 6 gün uzattığı gözlenmiştir.

Tablo 4. Nar kabuğu ekstraktının alabalık burgerlerinin duyusal kalite değişimleri üzerine etkileri
Table 4. Effects of pomegranate peel extract on sensorial quality changes of trout burgers

	Depolama (gün)	K	NK5	NK10
Koku	**0**	9,00± 0,00^{aA}	9,00±0,00^{aA}	9,00±0,00^{aA}
	3	6,50±0,55^{bA}	7,00±1,00^{bA}	7,00±1,00^{bA}
	6	3,40±0,55^{cC}	6,40±0,55^{bA}	5,60±0,55^{cB}
	9	0,00±0,00^{dC}	6,40±0,55^{bA}	4,80±0,45^{dB}
	12	0,00±0,00^{dC}	1,60±0,55^{cB}	3,00±0,71^{eA}
	15	0,00±0,00^{d}	0,00±0,00^{d}	0,00±0,00^{f}
Tekstür	**0**	9,00±0,00^{aA}	9,00±0,00^{aA}	9,00±0,00^{aA}
	3	6,70±0,45^{bA}	7,40±0,55^{bA}	7,20±0,84^{bA}
	6	4,70±0,45^{cA}	5,40±0,55^{dA}	4,80±0,84^{cA}
	9	0,00±0,00^{dC}	6,60±0,55^{cA}	5,40±0,55^{cB}
	12	0,00±0,00^{dC}	2,00±0,00^{eB}	3,00±0,71^{dA}
	15	0,00±0,00^{d}	0,00±0,00^{f}	0,00±0,00^{e}
Lezzet	**0**	9,00±0,00^{aA}	9,00±0,00^{aA}	9,00±0,00^{aA}
	3	6,40±0,55^{bA}	6,60±0,55^{bA}	6,40±0,55^{bA}
	6	2,20±0,45^{cC}	6,60±0,55^{bA}	4,60±0,89^{cB}
	9	0,00±0,00^{dC}	5,60±0,55^{cA}	4,60±0,55^{cB}
	12	0,00±0,00^{dC}	1,60±0,55^{dB}	2,20±0,45^{dA}
	15	0,00±0,00^{d}	0,00±0,00^{e}	0,00±0,00^{e}
Görünüş	**0**	9,00±0,00^{aA}	9,00±0,00^{aA}	9,00±0,00^{aA}
	3	6,50±0,55^{bA}	6,60±0,55^{bA}	6,80±0,45^{bA}
	6	5,50±0,55^{cA}	5,40±0,55^{cA}	4,40±0,55^{cB}
	9	0,00±0,00^{dB}	5,20±0,45^{cA}	5,00±0,00^{dA}
	12	0,00±0,00^{dC}	1,80±0,45^{dB}	2,60±0,55^{eA}
	15	0,00±0,00^{d}	0,00±0,00^{e}	0,00±0,00^{f}
Genel beğeni	**0**	9,00±0,00^{aA}	9,00±0,00^{aA}	9,00±0,00^{aA}
	3	6,30±0,55^{bA}	6,80±0,45^{bA}	6,80±0,45^{bA}
	6	3,00±0,85^{cC}	6,20±0,45^{cA}	4,80±0,45^{cB}
	9	0,00±0,00^{dC}	5,80±0,45^{cA}	4,80±0,45^{cB}
	12	0,00±0,00^{dC}	1,80±0,45^{dB}	3,00±0,00^{dA}
	15	0,00±0,00^{d}	0,00±0,00^{e}	0,00±0,00^{e}

Aynı satırdaki büyük harfler gruplar arası istatistiksel farkı, aynı sütundaki küçük harfler grup içi istatistiksel farkı belirtmektedir ($p<0,05$)
Means indicated by different capital letters in the same row differ significantly, means indicated by different lowercase letters in the same column differ significantly ($p<0,05$)

Aref vd. (2018) transglutaminaz enzimi, kitosan ve biberiye ekstraktı ile hazırladıkları balık burgerlerin raf ömrünün kontrol grubuna göre daha uzun olduğunu bulmuşlardır. Benzer bir çalışmada Özoğul ve Uçar (2013) kekik, yeşil çay, adaçayı ve defne ekstraktları ile hazırlanan balık burgerlerin duyusal olarak kontrol grubuna göre daha kabul edilebilir olduğunu ve raf ömürlerinin kontrol grubu örneklerinden 1-2 ay daha uzun oluğunu bildirmiştir. Bazı doğal bitki ekstraktlarının balık burgerlerin duyusal özellikleri üzerinde olumlu etkileri olduğu ve raf ömrünün uzadığı birçok çalışmada gözlenmiştir (Corbo vd., 2009; Uçak vd., 2011; Shinde vd., 2015).

SONUÇ

Son yıllarda endüstriyel gıda atıklarının değerlendirilmesi ve bu atıklardan antioksidan ve antimikrobiyal maddelerin elde edilerek gıdalara eklenmesi oldukça önemli konulardan biri haline gelmiştir. Nar kabuğu da meyvenin büyük bir kısmını oluşturan ve güçlü antioksidan olan yan ürünler arasındadır.

Bu çalışmadan elde edilen veriler nar kabuğu ekstraktının alabalık burgerlerinin kalite parametreleri üzerinde olumlu etkileri olduğunu göstermektedir. Balık burgerlerde mikrobiyal bozulma ve lipit oksidasyonu geciktirilmiş, ürünün raf ömrü kontrol grubuna göre 6 gün uzatılarak tüketici beğenisini kazanan bir ürün elde edilmiştir.

TEŞEKKÜR

Bu çalışma Niğde Ömer Halisdemir Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğü tarafından TGT 2019/9-BAGEP proje numarası ile desteklenmiştir.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 423-425 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.14

CASE REPORT VAKA TAKDİMİ

The first report on the phenomenon of *Capoeta aydinensis* (Cyprinidae), occurring in Gökova Bay, Aegean Sea

Gökova Körfezi'nde (Ege Denizi) *Capoeta aydinensis* (Cyprinidae) olgusu hakkında ilk rapor

Okan Akyol[1]* ● Vahdet Ünal[2] ● Hasan M. Sarı[3]

[1] Ege University Faculty of Fisheries, 35440 Urla, Izmir, Turkey
[2] Ege University Faculty of Fisheries, 35440 Urla, Izmir, Turkey
[3] Ege University Faculty of Fisheries, 35440 Urla, Izmir, Turkey

https://orcid.org/0000-0001-7738-2156
https://orcid.org/0000-0001-6157-0590
https://orcid.org/0000-0003-1000-514X

Corresponding author: okan.akyol@ege.edu.tr

Received date: 02.06.2020 *Accepted date: 14.07.2020*

How to cite this paper:
Akyol, O., Ünal, V. & Sarı, H.M., (2020). The first report on the phenomenon of *Capoeta aydinensis* (Cyprinidae), occurring in Gökova Bay, Aegean Sea. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 423-425. DOI: 10.12714/egejfas.37.4.14

Abstract: A big specimen of *Capoeta aydinensis* (435 mm TL, 1040 g) was caught on 22 March 2020 by an angler from the Gökova Bay, Muğla as an unusual habitat. This case report presents occurring of an endemic freshwater fish in the marine waters and this specimen has the maximum size throughout the Anatolia.

Keywords: Size, freshwater fish, measurement, Akçapınar stream delta, Muğla

Öz: 22 Mart 2020'de büyük bir *Capoeta aydinensis* örneği (435 mm TL, 1040 g) olağandışı bir yaşam alanı olarak Muğla Gökova Körfezi'nden bir oltacı tarafından yakalanmıştır. Bu olgu sunumu, deniz sularında endemik bir tatlı su balığının bulunuşunu göstermektedir ve bu örnek Anadolu genelinde maksimum büyüklüğe sahiptir.

Anahtar kelimeler: Boyut, tatlı su balığı, ölçüm, Akçapınar ırmak deltası, Muğla

INTRODUCTION

As a new endemic freshwater species, *Capoeta aydinensis* Turan, Küçük, Kaya, Güçlü & Bektaş, 2017 was described from the Büyük Menderes River and Tersakan, Dalaman, and Namnam streams in southwestern Turkey (Turan et al., 2017). *C. aydinensis* can be diagnosed from its congeners in the Mediterranean and in Anatolia by a combination of characters (Froese and Pauly, 2019). It is distinguished from the other *Capoeta* species by the following characters: one pair of barbel; a plain brownish body coloration; a well-developed keel in front of the dorsal-fin origin; a slightly arched mouth; a slightly convex lower jaw with a well-developed keratinized edge; a weakly ossified last simple dorsal-fin ray, serrated along about 60%–70% of its length, with 14–20 serrae along its posterior edge; 58–71 total lateral line scales; 11–12 scale rows between lateral line and dorsal-fin origin; 7–9 scale rows between lateral line and anal-fin origin (Turan et al., 2017).

The recent study reported that maximum age of *C. aydinensis* was 8 years; and its habitat preference is shady areas in spring, generally blurry waters in summer, high vegetation cover and blurry waters in autumn and high vegetation cover in winter (Akbaş et al., 2019). As an unusual habitat, this case report presents occurrence of an endemic fresh water fish, *Capoeta aydinensis* in marine waters of Gökova Bay, Aegean Sea.

MATERIAL AND METHODS

On 22 March 2020, one specimen of *C. aydinensis* with total length of 435 mm and 1040 g weight (Figure 1) was captured by an angler, used fishing pole (0.30 mm PA) on a boat off 40-50 m far from Akçapınar Stream delta, Gökova Bay at a depth of 1.5 m (Coordinates:37°02'00" N - 28°19'45", Figure 2). The bait was rock shrimp (*Palaemon* sp.). The rainy days had passed some days before, and the weather during fishing was sunny. The alive specimen was too strong and was very fluttering (M. Güven, pers. comm.).

Figure 1. *Capoeta aydinensis*, captured from Gökova Bay, Aegean Sea (A) whole body, (B) view of the shape of mouth, (C) view of head

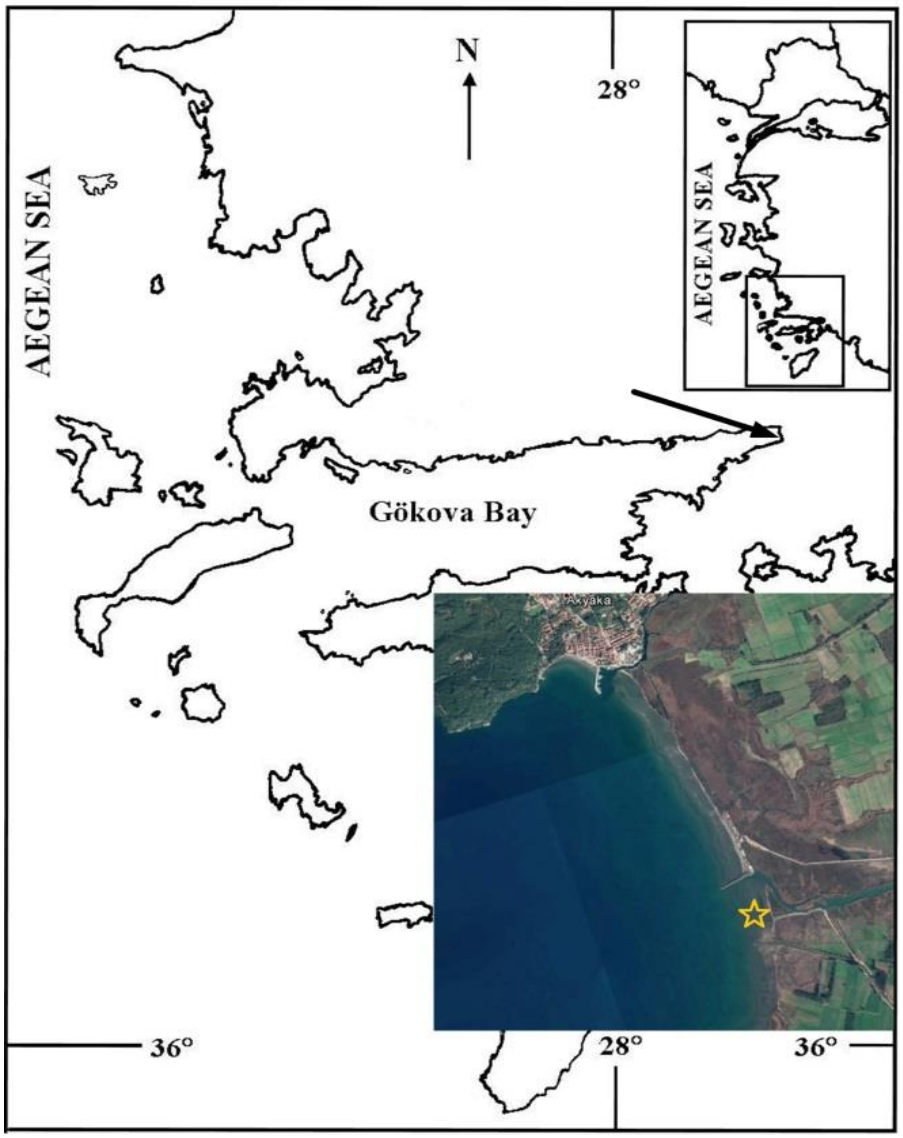

Figure 2. Sampling area (black arrow and yellow star indicate sampling location)

RESULTS AND DISCUSSION

The specimen was measured to the nearest millimeter (Table 1). All measurements, counts, proportions and color patterns determined are in accordance with the descriptions of Turan et al. (2017) and Froese and Pauly (2019).

In previous studies, Akbaş et al. (2019) reported 364 specimens (TL range: 58-348 mm) of *C. aydinensis* from Tersakan Stream (Muğla) during June 2013 – June 2014. Thereafter, 150 specimens (FL range: 130-312 mm) of *C. aydinensis* [as *C. bergamae*, it had been name used before new species definition according to Akbaş et al. (2019)] were captured from Topçam Dam Lake in Aydın province (Şaşı, 2009). Turan et al. (2017) also reported 30 specimens (SL range: 117-179 mm) in their systematic study. As seen that this paper presents the unique largest size of *C. aydinensis* throughout the Anatolia. However, the occurrence of *C. aydinensis* in a stream delta has not been astonishing since the occurred of *C. capoeta bergamea* in Köyceğiz Lagoon system (Akın et al., 2005). Bohlen (1999) expressed that several freshwater fishes migrates into brackish water for feeding and growth but have to return into waters of lower salinity for spawning. So, this case report presents not only the biggest size but also the unusual habitat of *C. aydinensis* for the Turkish fauna.

ACKNOWLEDGEMENT

The authors thank angler Mr. Mutlu Güven for bringing the fish to our attention.

Table 1. Morphometric measurements and its percentages in the standard length and in the head length and meristic counts recorded in *Capoeta aydinensis*, captured from Gökova Bay, Aegean Sea

Morphometric characters	Size (mm)	
Total length (TL)	435	
Fork length (FL)	400	
Standard length (SL)	370	
		In percentage of standard length
Head length (HL)	70	18.9
Maximum body depth	92	24.9
Pectoral fin length	65.1	17.6
Anal fin length	28.4	7.7
Anal fin height	49.5	13.4
Pelvic fin length	54.6	14.8
Dorsal fin length	55.6	15.0
Dorsal fin height	62.9	17.0
Pre-dorsal fin length	171	46.2
Pre-pectoral fin length	72.7	19.6
Pre-anal fin length	277	74.9
Pre-pelvic length	200	54.1
Upper caudal fin lobe	80	21.6
Middle causal fin lobe	30	8.1
Caudal peduncle length	63.9	17.3
Caudal peduncle depth	37	10.0
		In percentage of head length
Eye diameter	9	12.9
Head width at anterior eye margin	35.4	50.6
Head width at posterior eye margin	43.9	62.7
Head depth through eye	39.5	56.4
Head depth at snout	22.6	32.3
Inter-orbitary length	35.4	50.6
Pre-orbitary length	25.6	36.6
Post-orbitary length	41.6	59.4
Meristic characters		
Dorsal fin rays		III 8
Anal fin rays		III 5
Pectoral fin rays		I 13
Ventral fin rays		I 6
Ligne Lateral		60
Ligne transversal (dorsal fin origin)		10
Ligne transversal (anal fin origin)		7
Gill rakers		23

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Ege Journal of Fisheries and Aquatic Sciences, 37(4), 427-436 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.15

REVIEW DERLEME

Marine derived tyrosinase inhibitors

Deniz kaynaklı tirozinaz inhibitörleri

Amine Dilara Pilevneli[1]* ● Belma Konuklugil[2]

[1] Ankara University Faculty of Pharmacy, Department of Pharmacognosy, 06560 Ankara, Turkey https://orcid.org/0000-0001-8573-2718
[2] Ankara University Faculty of Pharmacy, Department of Pharmacognosy, 06560 Ankara, Turkey https://orcid.org/0000-0002-4753-0450

Corresponding author: dilarapilevneli@hotmail.com

Received date: 20.01.2020 *Accepted date: 06.05.2020*

How to cite this paper:
Pilevneli A.D. & Konuklugil, B. (2020). Marine derived tyrosinase inhibitors. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 427-436. DOI: 10.12714/egejfas.37.4.15

Abstract: The cosmetics industry has gained strong momentum all over the world in recent years and has become a growing and promising sector**.** As it is known, as in the pharmaceutical industry, the cosmetic industry has also turned into becoming marine resources by seeking new materials for its continuation to be more productive for the field. To serve this purpose, marine-derived substances are highly claimed to be an interesting as well as a fruitful source for the benefits of the cosmetics industry. In this respect, as known globally, anti-tyrosinase inhibitors used in skin whitening are obtained from a considerable number of marine organisms. In this regard, the main objective of this article is to summarize a highly significant number of natural products derived from marine sources such as algae, fungi, seaweeds and bacteria which are known to have shown anti-tyrosinase activity.

Keywords: anti-tyrosinase, skin whitening, cosmetic, marine-derived organisms

Öz: Kozmetik sektörü son yıllarda çok güçlü bir ivme kazanmış ve tüm dünyada gelecek vaad eden bir sektör haline gelmiştir. İlaç endüstrisinde olduğu gibi, kozmetik endüstrisinde de yeni maddeler arama çabasıyla denizel kaynaklara yönelinmiştir. Bu nedenle, deniz kaynaklı kimyasalların kozmetik endüstrisinin yararı için ilginç ve verimli bir kaynak olduğu düşünülmektedir. Bilindiği gibi, cilt beyazlatma amacıyla kullanılan anti-tirozinaz inhibitörleri, önemli sayıda deniz organizmasından elde edilmektedir. Bu bağlamda, bu makalenin ana amacı, anti-tirozinaz aktivitesi gösterdiği bilinen alg, mantar, deniz yosunu ve bakteri gibi deniz kaynaklarından elde edilen çok sayıda doğal ürün hakkında bilgi vermektedir.

Anahtar kelimeler: anti-tirozinaz, cilt beyazlatma, kozmetik, deniz kaynaklı organizmalar

INTRODUCTION

As it is highly and widely known, cosmetic products containing biologically active compounds are believed to be used to improve the appearance of the skin as well as improving the physiological effects of the cells. For the continuation of cosmetic developments, it is highly important and an undeniable fact to discover new bioactive substances from natural sources which are regarded as resourceful, safe, and stable (Uppala, 2015). Consumers have been demanding the use of more natural ingredients due to some negative ideas regarding chemicals and toxic substances over the past years. (Muda et al., 2017). More recently, the cosmetic industry has combined creams, lotions, and ointments with bioactive substances and it is classified as a special class of products. More interestingly, the number of cosmetic companies which are highly interested in incorporating extracts from coastal plants, marine algae, seaweeds and sea minerals into cosmetic components is obviously on the rise.

To continue with a considerable amount of background information as to the field, it may be stated that some studies carried out globally have shown that, in recent years, the cosmetics industry has been focusing on and paying more and more attention to obtain new molecules from marine sources like the pharmaceutical industry (Sumathy and Kim, 2011).

On the one hand, marine-based natural products have turned out to be a crucial field of research with great amount of economic returns attracting a large number of scientists all over the world. According to the Euro Ocean, there are around 600 European marine projects funded by both FP6 and FP7 actions, which is known to contribute to the introduction of more marine bioactive products for the market.

The marine environment is claimed to be so productive that it may provide a great amount as well as numerous resources for the discovery of new active substances to be used in the industry effectively. Despite the chemical and biological diversity of marine environment, very little has been known to be discovered until now. The desire to have a protection against ultraviolet rays, to improve the appearance and anti-aging demands has expanded the market for new cosmetic components. More natural products have been created upon the current demands in the industry. An increasing number of new molecules from the marine environment reveals a number of strong and effective activities on the skin. Secondary metabolites isolated from macrophages showed various activities (antioxidant, anti-aging, and anti-inflammatory activities) (Brunt and Burgess, 2018). Throughout this review, the main purpose of marine-derived tyrosinase inhibitors has been introduced in a detailed way.

Cosmetic/s

Cosmetics is known to be used as a term that originally comes from the Greek word kos-metikos which means to a master in fix. For thousands of years, men and women have been known to use cosmetics for the following purposes: ointment, lotions, perfumes, and so on in order to be more become beautiful, to look much younger, to hide wrinkles, scars, pimples or for a variety of scars on their faces, to protect their skin from the sun, wind and cold, to remove the unwanted hair from their bodies, and to prevent hair loss (Çomoglu, 2012).

The cosmetic sector is considered to be a rapidly developing and a promising sector and the revenues are expected to increase in the next decade. Many years after Shu Uemura firm combined deep seawater with skincare and makeup products, his profound interest in the marine ingredients of the cosmetic industry is believed to have increased continuously by making a considerable amount of progresses in the industry (Martins et al., 2014).

Asian women are known to prefer white skin and due to that reason, over the last decade, skin whitening products have had the largest share in the skincare market in Asia (Boonme et al., 2009). Almost 15% of the world's population, especially in Asian countries, are believed to use skin whitening products (Pillaiyar et al., 2017).

Skin and melanin

Skin is highly known to be a physiological barrier that protects the body against harmful factors. Keratinocytes and melanocytes continuosly function to form the outermost layer of the epidermis where melanin is known to be synthesized. Melanin is considered to play an important role in defending against harmful UV rays and reactive oxygen species. Melanin is also known to determine the color of hair, skin, and eyes depending on quantity, quality and distribution. Moreover, melanin synthesis disorders are believed to cause problems in skin pigmentation (Alğın Yapar, 2016).

Two forms of melanin are believed to be as follows; pheomelanin (yellow/red) and eumelanin (brown/ black). They are regarded as the main cause of ethnic skin color differences in the world. Melanin is synthesized in lysosome-like organelles called melanosomes within melanocyte cells. Melanogenesis is the production of melanin and is regulated by the activity of three enzymes, tyrosinase, Tyrosinase-related protein1 (TRP1), and Tyrosinase-related protein2 (TRP2). Of these, tyrosinase is the one known to be the fundamentally main determinant of melanogenesis. The first stage in melanin synthesis is known to be highly critical with tyrosinase transformations leading to the formation of eumelanin and the formation of pheomelanin in the presence of cysteine and glutathione, while the other stages are proceed spontaneously at physiological pH. Hence, genetic factors are known to play an essential role in melanogenesis, whereas hormones proopiomelanocortin-derived peptides stimulate pigmentation, particularly in the sun-exposed areas, and other paracrine factors that are known to determine skin color include endothelin-1, stem cell factor, prostaglandins, and catecholamines (Alğın Yapar, 2016).

Some compounds such as hydroquinone, arbutin and kojic acids have been used for skin whitening; however, symptoms such as contact dermatitis and skin irritation may occur after a prolonged use of these compounds. Therefore, new, active and safe hypopigmenting agents are being investigated. Under such conditions, nature is regarded as the most important and productive resource for these researches, and previous studies demonstrate this (Kim et al., 2017).

An increase in melanin production and accumulation may result in increased skin alignments and may cause hyperpigmentations such as melism, proinflammatory melanoderma, solar lentigo, etc. Hyperpigmentation of the epidermis and dermis are believed to depend on the increased number of melanocytes or enzyme activity.

Pigmentation of the skin

Pigmentation of the skin is considered to occur as a consequence of a variety of physiological processes. These; i. development of melanocytes, ii. density of melanocytes, iii. structural and enzymatic formations in melanosomes, iv. melanin synthesis, v. transfer of melanosomes to dendrites, vi. transfer of melanosomes to keratinocytes; and vii. melanin is known to be an important process of the distribution of the suprabasal layers of the skin. The first three are purely genetic, while the last four are targeted by skin whiteners (Alğın Yapar, 2016).

Tyrosinase

Tyrosinase (EC 1.14.18.1) is also regarded as polyphenol oxidase, monophenol oxidase, phenolase or catecholase and that contains copper. Its function is known to catalyze the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and the subsequent oxidation of L-DOPA to dopaquinone (Figure 1) (Vamos-Vigyazo, 1981). The name tyrosinase is used because of the specificity of the enzyme against tyrosine (monohydroxyphenylalanine) and dihydroxyphylalanine as the substrate (Whitaker, 1994).

Tyrosinase is highly believed to be very common in nature (Cooksey et al., 1997). It is widely distributed in plants, microorganisms and usually found in fungi and some animal organs (Parvez et al., 2007). Tyrosine is known to be hydroxylated with monophenolase. 3,4-dihydroxyphenylalanine (L-DOPA) is oxidized with diphenolase and converted into o-dopakinone. O-dopakinone is unstable in aqueous solutions and gives a rapid non-enzymatic reaction (Rodriguez-López J. et al., 1991).

The function of tyrosinase is believed to catalyze melanin in melanocytes through three different reactions in the biosynthetic pathway: First pathway; hydroxylation of tyrosine to L-DOPA. The second way; Oxidation of L-DOPA to dopakinone. In addition, the third way is the conversion of dopakinone to melanin in a series of complex reactions including cyclization and oxidative polymerization (Sugumaran, 1991).

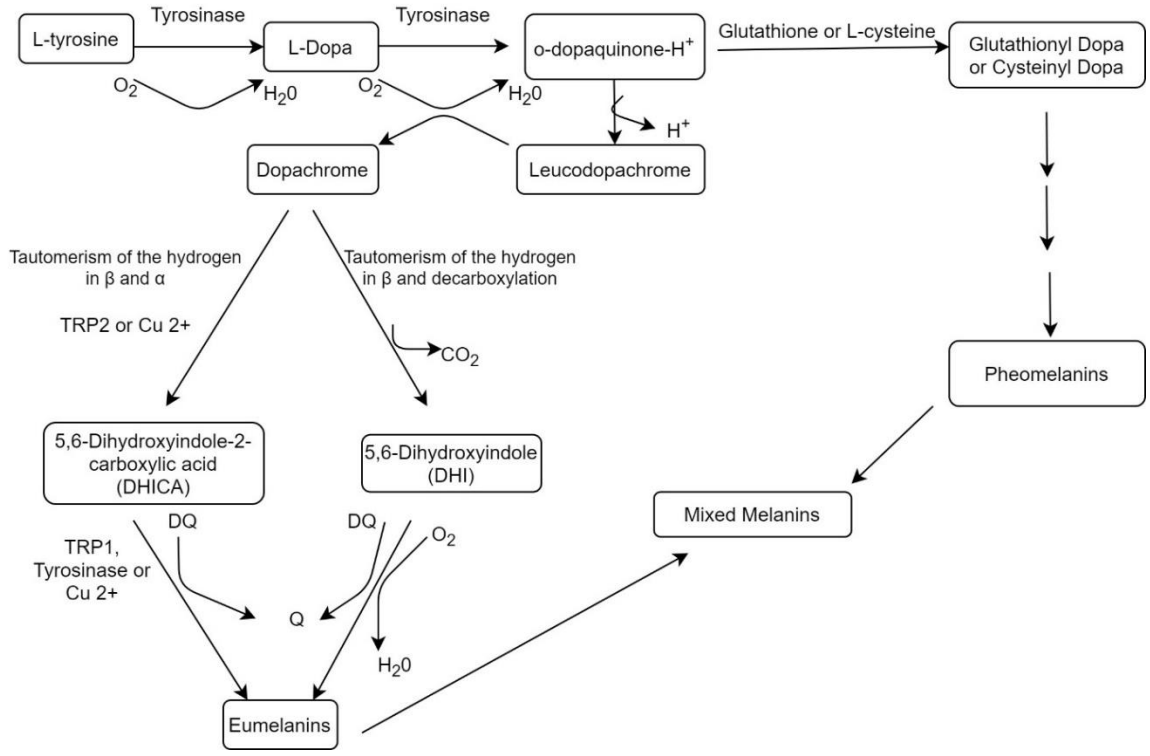


Figure 1. Scheme of the biosynthetic pathway of eumelanins and pheomelanins (Zolghadri et al., 2019)

Melanin biosynthesis is believed to be inhibited by tyrosinase inhibition. Therefore, tyrosinase inhibitors may be useful in clinical applications for the treatment of certain dermatological diseases associated with melanin hyperpigmentation. It is used in the cosmetics industry for whitening and skin whitening after the sunburn (Abd El Hady et al., 2014).

Tyrosinase is highly known to play a primordial role in the biosynthesis of melanin as well as microorganisms that are believed to affect skin color and pigmentation. Considering the medical and cosmetic sector, it may be stated that there are many studies focusing on isolation of the tyrosinase inhibitor compounds made of terrestrial and the marine environment which belong to chemical classes such as phenolic compounds (flavonoids, ligands, stilbenes, coumarins, arylbenzofurans, tannins), megastigmines (nor-isoprenoid), terpenes, cyclic peptides, alkaloids, and steroids.

The previous studies have obviously indicated that flavonoids are found to act as cofactors or substrates as well as their ability to chelate the active site of tyrosinase and thus it inhibits its effects (Kubo et al., 2000; No et al., 1999). Furthermore, flavonoids with a 4-substituted resorcinol unit in ring B are considered to be potent tyrosinase inhibitors (Shimizu et al., 2000). The compounds made of the terpene group have shown tyrosinase inhibitory activities due to its position and relative configuration of the hydroxyl groups as well as the nature and number of sugar units like in the cycloartane glycosides (Wu and Naranmadura, 2014). In addition to the previous studies, it could also be said that an important amount of consideration of some alkaloids is that tyrosinase inhibitor capacities may be due to the allosteric effects on the enzyme (Lo et al., 2009; Wu and Naranmadura, 2014).

Anti tyrosinase activity from marine sources

Depending on the studies carried out widely, it may be stated that there are more than 250,000 species described in the oceans up to date, whereas thousands are still waiting to be discovered (Corinaldesi et al., 2017; Mora et al., 2011). More than 25,000 new biologically active compounds have been identified from marine sources (Blunt et al., 2015). Bacteria and algae are the major sources of active ingredients. Furthermore, bacteria and algae are commonly known and regarded as the most important sources of biologically active substances. Recently, the focus has been on the discovery of skin whitening compounds from many marine microorganisms. Among them, zeaxanthin appears to be of particular interest and can be obtained from *Nannochloropsis oculata* (Guillerme et al., 2017; Shen et al., 2011).

A considerable number of recent studies have revealed that marine-derived fungi (MDF) from sponges are a new productive and fruitful source of bioactive metabolites with a high potential for the pharmaceutical industry. Thus, by considering the high species diversity and distribution of sponges over large areas, it may be pointed out that they are expected to host marine fungi that can produce a wide variety

of secondary metabolites. Those associated with sponges have higher species diversity and are highly effective in the production of secondary metabolites than fungi from other marine sources. Fungi symbiotes of sponges have recently attracted a promising amount of particular attention due to their capacity to produce new bioactive metabolites effectively. Marine fungi associated with sponges produce new bioactive metabolites with antibiotic, antitumor, antioxidant, antifungal, antialgal, anti-insect and acetylcholinesterase inhibitory effects (Abd El Hady et al., 2014; Almeida et al., 2011; Kjer et al., 2010; Lee et al., 2011; Mostafa et al., 2010; Thirunavukkarasu et al., 2012).

Table 1. Examples of marine active substances whose effects have been seen as a result of studies (D'Orazio et al., 2012; Fiorucci et al., 2012; Liu et al., 2006; Nastrucci et al., 2012).

Compound name	Organism	Mode of action
Cytarabin, ARA-C	Sponge: *Cryptotethia crypta*	DNA Polymerase Inhibitor
Trabectedin (ET-743), Yondelis®	Tunicate: *Ecteinascidia turbinate*	Binding to minor groove DNA alkilating Guanine at N2
Eribulin Mesylate (E7389), Halaven®	Sponge: *Halichondria okaday, Axinella carteri* (Halichondrin B)	Microtubule interfering agent
Bromophenol	Red alga: *Polysiphonia urceolata*	Protein tyrosine phosphatase 1B inhibitor
Theonellasterol	Theonella swinhoei	Selective Farnesoid X receptor antagonist
Pacifenol	Seaweeds of the marine alga *Laurencia claviformis*	COX Inhibitors

Marine fungi living in challenging conditions are believed to be shown a great interest and they are paid a great amount of attention; thefore, they are highly regarded as a source of new and promising as well as effective bioactive products. Since marine organisms live in biologically competitive environments with different pH, temperature, pressure, oxygen, light, nutrients and salinity conditions, the chemical diversity of the secondary metabolites obtained from them is quite high.

It is inevitably thought that the fungi living in the terrestrial environment have been transported to the seas by rivers, rain or some other reasons. Also, they usually live in seawater or settle in sediments with a dense population. Some have gradually adapted to the conditions of the marine environment and have been successful to survive. Cuomo et al. (1995) reported that the percentage of antibiotic production from sea fungi was higher than terrestrial fungi. This suggests that not only antibiotics but also many bioactive substances derived from marine fungi can have a much higher bioactivity than terrestrial ones (Tsuchiya et al., 2008). In this respect, many microorganisms have been isolated from the marine environment to obtain new substances. Many compounds are known to have been identified as tyrosinase inhibitors from plants, microorganisms and, synthetic compounds. In recent years, marine microorganisms that produce bioactive compounds have been noted and several new compounds have been isolated.

Sea sponges: Sea sponges are regarded as a renewable natural resource and they are considered to be highly absorbent, foam-forming, to have a soft texture and they are believed to be rather suitable for the use of the most sensitive skin.

Natural marine sponges are widely known to contain enzymes and to prevent the formation of mold and bacteria. On the other hand, natural sea sponges have been developed for bathing and cleaning in that they absorb and retain a lot of water. Even though sponges are mainly used to clean the body and face, they are also known to be used to clean the skin of babies. Furthermore, they are effectively used as a skin whitening compound (Uppala, 2015).

In a different study conducted by Lee et al. (2016), they isolated oxygenated diterpenoid Gagunin D (GD) from the sea sponge *Phorbas* sp. In this study, they found out that GD inhibits tyrosinase enzymatic activity as well as cytotoxicity activity against human leukemia cells. In addition, they reported that GD expresses proteins associated with melanosomal transfer. Because of these distinguishing properties, GD has been reported to be a potential candidate for cosmetic formulations.

In another study on sponges, Geoditin A was isolated from the *Geodia japonica*. This substance has isobarbaric triterpene structure and exhibits melanogenesis inhibition (Chang, 2012). Studies show that Geoditin A induces apoptosis through oxidative stress in leukemia HL60 cells and human colon HT29 cancer cells. The strong anti-melanogenic activity and the relatively low cytotoxicity of Geoditin A showed its therapeutic potential as a skin lightening agent (Cheung et al., 2012).

In another study conducted for marine fungi obtained from *Haliclona fascigera* sponge collected from the South Coast of West Sumatera, ethyl acetate extracts were taken and found to act as tyrosinase inhibitors. Four out of a total of 20 extracts, WR3, WR4, WR9, and WR13 showed the highest inhibition. These fungi are examined and identified as Penicillium sp. (WR3), *Aspergillus niger* (WR4), *Penicillium* sp.3 (WR9) and *Trichophyton megninii* (WR13) (Handayani et al., 2019).

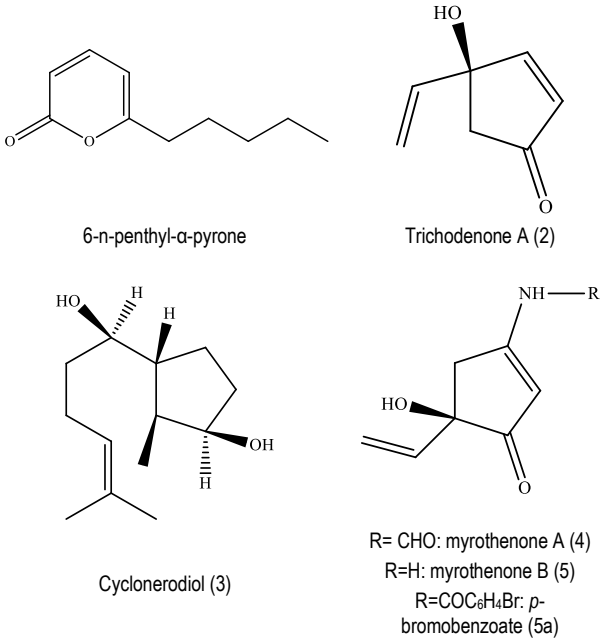
Marine fungus: Marine derived fungi have been reported to produce a wide range of active compounds which have a certain amount of effects on central nervous system, respiratory system, neuromuscular system, autonomic nervous system, cardiovascular system and gastrointestinal

system Furthermore, these compounds have been shown to be effective for antibacterial, antifungal, antidiabetic, anti-inflammatory, antiprotozoal, anti-tuberculosis, antiviral, antitumor, and cytotoxic activities (Abd El Hady et al., 2014; Christophersen et al., 1998; Hasan et al., 2015; Luo et al., 2017; Moghadamtousi et al., 2015; Pontius et al., 2008; Ramos et al., 2015; Zolghadri et al., 2019).

Most fungi are found with algae. As such in a later study carried out to serve for the same purpose, *Botrytis* sp. was collected from the red algae *Hyalosiphonia caespitosa* and the extract of this algae showed tyrosinase inhibitory activity (Sumathy and Kim, 2011; Zhang et al., 2007)

In another study, two fungi (FS1 and FS3) were isolated from sponges; *Amphimed onviridis* and *Agelas* sp. Tyrosinase inhibiting activities were evaluated and it revealed that FS3 has an important activity (Abd El Hady et al., 2014).

In the study of Li et al. (2005), new myrothenones A (4) and B (5), were isolated together with known 6-*n*-pentyl-a-pyrone (1), trichodenone A (2), and cyclonerodiol (3) from the marine derived fungus *Myrothecium* (Figure 2). It was isolated from the surface of the sea-green algae *Enteromorpha compressa* collected by Busan Baegunpo, Busan in 2002 (Li et al., 2005).



6-n-penthyl-α-pyrone

Trichodenone A (2)

Cyclonerodiol (3)

R= CHO: myrothenone A (4)
R=H: myrothenone B (5)
R=COC_6H_4Br: *p*-bromobenzoate (5a)

Figure 2. The structure of defined substances

Tsuchiya and colleagues have worked on the Trichoderma viride strain H1-7 isolated from the 100-meter deep-sea sedimentation of the Izu Islands and found that this strain exhibited tyrosinase inhibitory activity. Four types of fungal tyrosinase inhibitors were obtained from this sediment (Figure 2). In addition, strain H1-7 has been shown to produce melanogenesis inhibitors. Three of these compounds are completely different from fungal tyrosinase inhibitors (Tsuchiya et al., 2008).

In the mentioned study, they purified the tyrosinase inhibitor produced by *Trichoderma viride* strain H1-7 from a marine environment. The purified inhibitor's chemical structure was determined and it is found to be the same as homothallin II isolated from *Trichoderma koningii* and *T. harzianum* as an antibiotic (Figure 3). However, it may be stated that this compound has not been reported to have tyrosinase inhibitory activity in the literature (Tsuchiya et al., 2008).

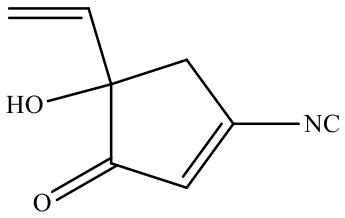


Figure 3. Chemical Structure of the Tyrosinase Inhibitor Produced by *Tricoderma viride* Strain H1-7

In another study, two new tyrosinase inhibitors were obtained from *Pestalotiopsis* sp.Z233 exhibiting a considerable amount of strong tyrosinase inhibitory activities. The inhibitors produced under abiotic stress with $CuCl_2$ (Wu et al., 2013).

Trianto et al. (2017) were studied on *Aspergillus sydowii* fungi that are considered to be a symbiotic fungus of marine sponge *Axinella* sp. and identified to have antibacterial effects. It is also known that *A. sydowii* has activities such as tyrosinase inhibitor, antimicrobial, anti-tuberculosis and acetylcholinesterase inhibitor.

Seaweed: Seaweed is known to be classified as brown, red, and green. They are believed to be commonly used as food in East Asian countries. Furthermore, they are claimed to have a different promising class of bioactive secondary metabolites such as; terpenoids, polyphenols, peptides, carotenoids, fatty acids, and phytohormones. In this respect, additionally, fluorotannins are known to be lipolytic agents that inhibit melanogenesis effectively (Pereira, 2015; Wijesinghe and Jeon, 2011)

Seaweed has been known to be used for skin care products for hundreds of years for circulation and balance of natural moisture levels. The main uses and benefits of seaweed extract are highly believed to be anti-acne, to have positive and promising effects on blood circulation activating the cell renewal, the metabolism, anti-aging, anti-inflammatory and skin whitening. In a study conducted in the related field, it is examined and the result is that the French Silab company has launched a new bleaching product (Whitonyl®) from *Palmaria palmata* (red algae) of which the main compounds are known to be oligosaccharides and the product is proved to lighten deep wrinkles, age spots and brown spots caused by exposure to chronic sunlight giving the skin a more homogenous and opal appearance (Pereira, 2018).

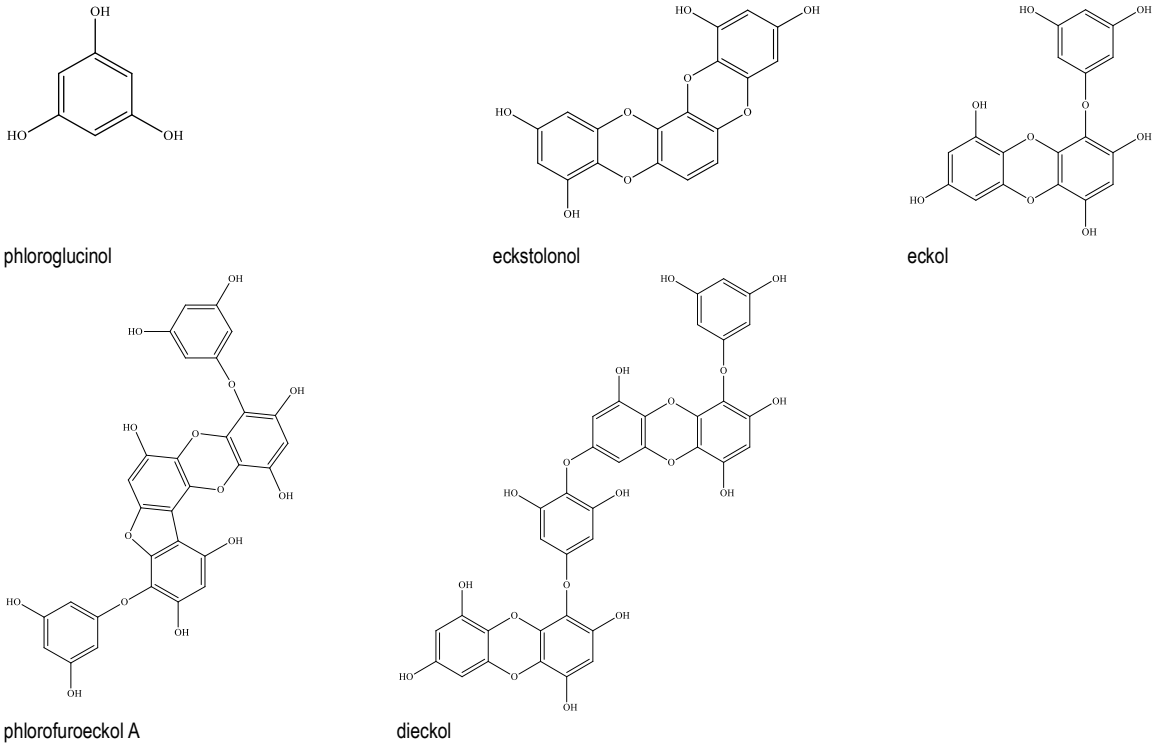


Figure 4. The Structures of the Phlorotannins from *E. stolonifera*

Ecklonia stolonifera is widely known to be a perennial brown seaweed that grows at a depth of 2-10 meters. Moreover, it is generally found in Korea and Japan and is often used as a foodstuff together with *Laminaria japonica* and *Undaria pinnatifida*. Phloroglucinol and its derivatives, ecklonia lactones were isolated from *E. stolonifera*. In addition, a certain and promising amount of studies have shown that algae have a skin-opening effect as well as an appetite suppression, antioxidant and antimutagenic activity.

In order to explore the sources with tyrosinase inhibitory activity in seaweeds, the inhibitory effects of 17 seaweed extracts, i.e. 2 Chlorophyta, 5 Phaeophyta and 10 Rhodophyta branches on fungal tyrosinase activity as substrate using L-tyrosine were investigated. Of these, only *E. stolonifera* exhibited an inhibitory activity with an IC50 value of 345 microgram / mL. Others showed the inhibitory activity of less than 50% at the highest concentration of 500 micrograms/ml. The bioassay-guided fraction of the active ethyl acetate soluble fraction from the methanolic extract of *E. stolonifera* has led to the isolation of phloroglucinol derivatives (Figure 4) (Kang et al., 2004).

Wachi et al. (1995) carried out a study on 127 marine microalgae isolates using both water (saline/buffer) and organic solvent to find out marine tyrosinase inhibitors secondary metabolites. The results suggest that marine cyanobacteria, especially members of the genus Synechococcus, may be used for photosynthetic production of tyrosinase inhibitors for biomedical applications. Cha et al. (2011) carried out a study on 43 seaweed to investigate tyrosinase inhibitory activity. Some of them (*Endarachne binghamiae*, *Ecklonia cava*, *Schizymenia dubyi* and *Sargassum silquastrum*) showed potent tyrosinase-inhibiting activity like kojic acid (Matsukawa et al., 1997).

A surprisingly interesting fungi strain was collected from the surface of the edible red algae *Gracilaria verrucosa* and the brown algae *Sargassum horneri*, from the Yokji Island of Gyeongnam. The cyclopentenone structure, myrotenone A, was isolated from this fungi identified as *Trichoderma viride*. This compound showed a strong tyrosinase inhibitory activity with an ED50 value of 6.6μM, which is more active than a positive standard kojic acid (ED50, 7.7M) (Wu and Naranmadura, 2014).

Symphyocladia latiuscula (Harvey) Yamada (Rhodomelaceae), and sea-red alga *which* contain bromophenol exhibiting a wide range of biological activities. Paudel et al. (2019) investigated the anti-tyrosinase activity of the alga and from three bromophenols isolated from the alga two of them showed an activity against L-tyrosine substrates.

In another research, six known bromophenol dimers were purified from the extract of red algae *Odonthalia corymbifera*. Both symmetric and asymmetric bromophenols showed tyrosinase inhibitory activities. Of these, asymmetric dimer 6 and symmetric dimer 1 and 2 exhibited a very strong tyrosinase inhibition (Figure 5) (Mr et al., 2017).

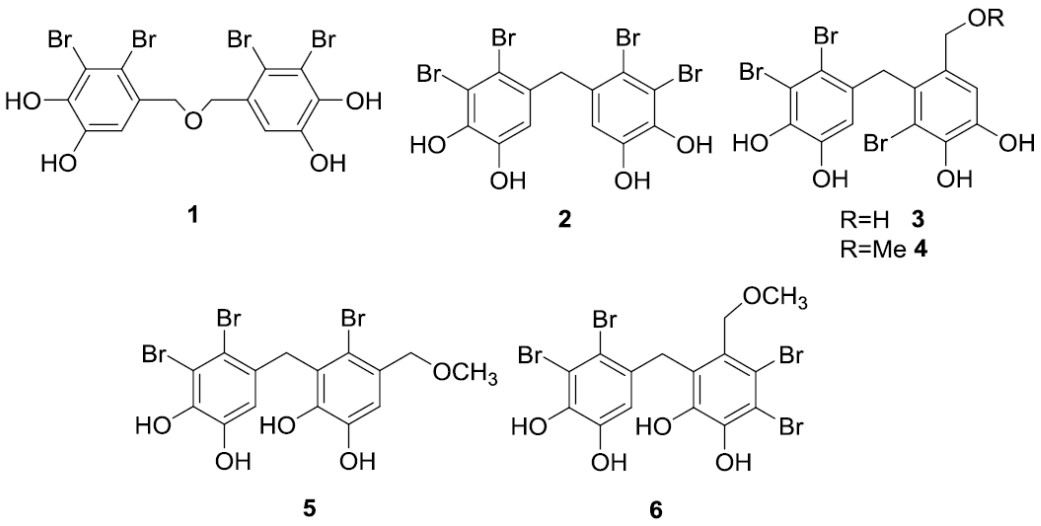


Figure 5. The structure of defined

Sargassum plagyophyllum obtained from Pasauran coastal waters is highly believed to contain quite influential as well as effective bioactive compounds such as alkaloids, steroids, flavonoids, saponins and tannins, whereas *Eucheuma cottonii* that obtained from the results of community cultivation in Lontar village, Serang, Banten (Indonesia's Pasauran coast) contains alkaloids and terpenoids. Active compounds like flavonoids are known to have tyrosinase inhibitory activities. In a study, S. *plagyophyllum* and *E. cottonii* methanol extract have shown to be more effective in the diphenolase reaction than kojic acid by inhibiting the oxidation of L-DOPA to DOPAquinone. *S. plagyophyllum* and *E. cottonii* extracts could be used in cosmetics as skin lightening whitening (Dolorosa et al., 2019).

Phloroglucinol derivatives, a secondary metabolite of brown algae, showed tyrosinase-inhibiting activity due to having copper chelating in the tyrosinase enzyme (Kang et al., 2004). In addition, β-carotene in seaweed species is known to be an active compound that directly inhibits tyrosinase. Fucoxanthin, a carotenoid found in seaweed *Laminaria japonica*, inhibited tyrosinase activity, melanogenesis and skin pigmentation due to UV-B (Sumathy and Kim, 2011).

A few studies are known to have been conducted on the skin whitening effects of marine bacteria; however, in opposition to common belief, it has been discovered that Pseudomonas produces methylene chloride that shows tyrosinase inhibitory activity (Kang et al., 2011). Moreover, some tyrosinase inhibitors (N-acyl dehydrotyrosine derivatives) have been reported from a gram-negative marine bacterium *Thalassotalea* sp. PP2-459 (Deering et al., 2016; Guillerme et al., 2017; Zolghadri et al., 2019).

During the investigation of new hypopigmenting agents from marine microbial natural products, *Bacillus* sp. (SCO-147) was found in Gwangyang Bay in South Korea and showed hypopigmenting activity. In this study, they isolated (-) - 4-hydroxysattabacin (1, 4OH-ST) and (-) - sattabacin (2) (Figure 6). 4OH-ST and sattabacin were first obtained from a *Bacillus* sp. It has been found to show antiviral activities against *Herpes simplex* virus types 1 and 2 (Mancha et al., 2013; Lampis et al., 1995). The anti-melanogenic activity of these natural products, in particular (-) - 4OH-ST and (-) - sattabacins, has not been reported yet. The efficacy of (-)-4OH-ST (1) as an anti-melanogenic agent was discovered in Kim, Leutou, Jeong, Kim, & Seong's study (Kim et al., 2017).

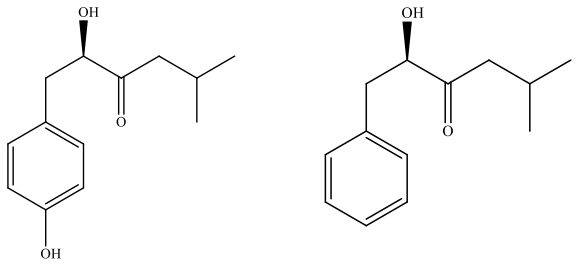


Figure 6. The molecules from *Bacillus* sp.

CONCLUSION

The marine environment is highly regarded as a rich source of both biological and chemical diversity. In this regard, marine organisms are believed to produce unique and promising compounds. Therefore, as a consequence of such kind of a rich source as mentioned above, in recent years, marine-derived natural products have gained a great importance as well as a momentum for the cosmetics industry, which seems to require continuous innovations in the field. Although marine resources have not yet been fully

explored, it is highly claimed that it has an important market in the cosmetic sector all over the world. Therefore, gaining access to marine chemical diversity including deep-sea sources is highly needed to address the huge need for new cosmetically active molecules.

As it is widely known, the cosmetics industry is considered to be a growing as well as an effectively promising market in the world. The awareness of the use of natural products instead of synthetic products seems to have increased significantly; hence, the less-discovered marine world is of interest to the cosmetics industry.

Skin whitening active ingredients, formulations, products and ongoing research, and development are known to be increasing day by day. An investigation of skin whitening mechanisms and innovative molecules involved in melanin synthesis and research on natural compounds may play an important role and may have a significant impant on the related industry for more developments as well as for further studies. In addition, the use of marine-derived active substances and extracts in the formulations of skin bleaching agents will become even more important if taken into consideration with a significant amount of care to support new studies.

Since it is scientifically known that tyrosinase plays a negative role in enzymatic depigmentation disorders for humans, these inhibitors have been a major concern and focus for the researcher throughout this study. In this context, as is known, natural resources and their active compounds are believed to have promising and great potential as tyrosinase inhibitors, primarily for the cosmetics and also food industries.

These compounds were previously isolated from plants. However, as a result of recent studies, algae, sponges, fungi and bacteria have been found to be a rich source of tyrosinase inhibitors. In this article, it can be understood that numerous inhibitors derived from marine sources have been investigated in detail and summarized accordingly. The main purpose of this review is to emphasize the importance of marine tyrosinase inhibitors for the cosmetics industry, a large market.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 37(4), 437-443 (2020)

Su Ürünleri Dergisi (2020)
DOI: 10.12714/egejfas.37.4.16

DERLEME REVIEW

Plastik ve mikroplastiklerin su canlıları ve insan sağlığı üzerindeki etkileri

Effects of plastics and microplastics on aquatic organisms and human health

Fevziye Nihan Bulat[1]* ● Berna Kılınç[2]

[1] Ege Üniversitesi Su Ürünleri Fakültesi, Avlama ve İşleme Teknolojisi Bölümü, 35100, Bornova-İzmir https://orcid.org/0000-0001-5165-3632
[2] Ege Üniversitesi Su Ürünleri Fakültesi, Avlama ve İşleme Teknolojisi Bölümü, 35100, Bornova-İzmir https://orcid.org/0000-0002-4663-5082

Corresponding author: nihanbulat@gmail.com
Received date: 13.01.2020 *Accepted date: 17.04.2020*

How to cite this paper:
Bulat, F.N. & Kılınç, B., (2020). Effects of plastics and microplastics on aquatic organisms and human health. *Ege Journal of Fisheries and Aquatic Sciences,* 37(4), 437-443. DOI: 10.12714/egejfas.37.4.16

Öz: Günümüzde plastikler birçok alanda kullanılmakta ve gün geçtikçe de kullanım alanları artmaktadır. Plastik kullanımındaki bu artış çevre kirliliğine neden olmasının yanı sıra ortamda bulunan canlıları ve dolayısıyla insan sağlığını olumsuz olarak etkilemektedir. Plastikler farklı taşınım yolu ile su ortamına kadar ulaşmaktadır. Su ortamında ulaşmış olan mikroplastikler, ortamda bulunan canlılar tarafından tüketilmektedir. Mikroplastiklerin su ortamında yaşayan türlerde (balık, midye, karides, fok vb.) bulunduğu birçok çalışmada vurgulanmaktadır. Su canlıları tarafından tüketilen mikroplastikler besin ağına dahil olarak insan tüketimine kadar ulaşmaktadır. Bu nedenle konunun öneminin vurgulanarak, bu konuda bilinçlendirmenin ve gerekli önlemlerin alınmasının sağlanması amacıyla yapılan bu derleme çalışmasında; mikroplastik üzerine yapılan çalışmaların değerlendirilmesi ve oluşturduğu riskler incelenmiştir.

Anahtar kelimeler: Plastik, mikroplastik, su ürünleri, insan sağlığı

Abstract: Today, plastics have been used in many areas and their use have been increasing day by day. This increase in the use of plastic causes environmental pollution as well as negatively affects organisms in the environment and therefore human health. Plastics reach to the water environment through different transport routes. Microplastics that have reached to the water environment are consumed by aquatic organisms. Microplastics in aquatic species (fish, mussels, shrimp, seals, etc.) were highlighted in many studies. Microplastics consumed by aquatic organisms are included in the food network, reaching as far as human consumption. Therefore, the importance of the subject have been emphasized, the evaluation of the studies on microplastics and the risks it poses have been examined in this compilation study which was carried out in order to raise awareness about this issue and to ensure that the necessary measures will be taken.

Keywords: Plastic, microplastic, seafood, human health

GİRİŞ

Plastiklerin; organik polimer bileşiklerden ve oksijen, hidrojen, kükürt, azot, karbonun da içinde bulunduğu çeşitli elementlerin bir araya gelmesiyle oluşan, sentetik malzeme olduğu belirtilmiştir (American Chemistry, 2005). Ayrıca ucuz, hafif, dayanıklı ve yeniden kullanılabilir olduğu çeşitli çalışmalarda bildirilmiştir (Laist, 1987; Andrady ve Neal, 2009). Buna ilave olarak plastiklerin hafif olması nedeniyle, rüzgâr yolu ile taşınımı olduğuna Singh ve Sharma (2008) tarafından dikkat çekilmiştir. Plastikler kullanım kolaylığı açısından da günümüzde çok sık kullanılmaktadır; buna bağlı olarakta üretimi giderek artmaktadır. Örneğin; 2014 yılında 311 milyon ton plastik üretilmiştir. Kullanılan plastiklerin 2013 yılında %72'lik kısmı deniz ortamına ve çöp alanlarına bırakılmıştır (World Economic Forum, 2016). Plastikler çevresel bir sorundur (Zeri vd., 2018). Plastik kirliliğinin; sosyo-ekonomik (Brouwer vd., 2017), turizm (Jang vd., 2014; Munari vd., 2016), insan sağlığına (Rochman vd., 2013a), deniz biyotasına (Cheshire vd., 2009) ve su ürünlerine (Vlachogianni, 2017) olumsuz etkileri olduğu bilinmektedir. Plastiklerin %70-80 oranında nehirlerin okyanuslara taşınımında etkili olduğu Horton vd., (2017) tarafından belirtilmiştir. Plastiklerin okyanus ve denizde birikmesi küresel bir sorun olmaktadır (Jahnke vd., 2017). Plastikler çeşitli etkilerle parçalanarak doğaya karışmakta ve canlılar tarafından tüketilmektedir. National Oceanic and Atmospheric Administration (NOAA) tarafından 5 mm'den küçük olan plastikler mikroplastik olarak adlandırılmaktadır (NOAA, 2008). Mikroplastiklerin renkli olmadıkları, şeffaf formda ve çok küçük boyutta oldukları bildirilmektedir. Bu özellikleri nedeniyle de su kaynaklarına geçişleri ile ciddi sorunlara yol açtıkları vurgulanmıştır (Yurtsever, 2015).

Günümüzde kullanımı yüksek olan plastiklerin su ve deniz ortamına girişi sonucunda su ürünlerine olan etkilerinin değerlendirildiği çalışmalar bulunmaktadır (Browne vd., 2008; Talsness vd., 2009; Rochman vd., 2013b; Wright vd., 2013; Rochman vd., 2014; Gassel vd., 2013; Gall ve Thompson 2015; GESAMP, 2016; Rummel vd., 2016; Clark vd., 2016). Yapılan bu çalışmalar ışığında konunun öneminin vurgulanarak gerekli önlemlerin alınması konusunda farkındalık yaratılması amacıyla mikroplastik konusunda ülkemizde ve dünyada yapılan çalışmaların derlenmesi hedeflenmiştir.

Plastiklerin ve mikroplastiklerin kullanım alanları ve su ortamlarına kontaminasyonu

Plastiklerin günümüzde birçok alanda (ambalajlama, kozmetik, tekstil gibi) kullanıldığı bildirilmiştir (Lefebvre vd., 2019). Plastikler çevreye atıldıktan sonra ise; küçük parçalara bölünerek mikroplastikleri oluşturmaktadır (Duis ve Coors, 2016). Mikroplastiklerin kişisel bakım ve kozmetik (yüz temizleme jeli, şampuan, diş macunu, deodorant vb. ürünlerde) (Yurtsever, 2018; Fendall ve Sewell, 2009; Pettipas vd., 2016), giyim ürünlerinde (Browne, 2015; Peng vd., 2017) ve çamaşır makinesi deterjanlarında (Hernandez vd., 2017) kullanıldığı belirtilmiştir. Mikroplastiklerin çeşitli kozmetik ve kişisel bakım ürünlerinden deniz ortamına geçtiğinin düşünüldüğü (Praveena vd., 2018) tarafından bildirilmiştir. Kişisel bakım ürünlerinin tatlı su ortamındaki mikroplastik artışına neden olduğu düşünülmektedir (Carr vd., 2016). Ayrıca hafif oldukları için su yüzeyinde ilerleyerek birçok alana yayıldıkları, akıntılarla birlikte sedimentlerde biriktikleri ve daha sonra toprağa da karıştıkları bildirilmiştir (Yurtsever, 2018). Evsel atık sularında, sentetik elyaf ve temizlik amaçlı kullanıma dayalı mikroplastiklerin bulunduğu (Browne vd., 2011) tarafından vurgulamıştır.

Mikroplastiklerin yüzey suları, plajlar vb. gibi alanlardan da deniz ve çeşitli su ortamlarına girişinin olduğuna değinilerek (Lusher vd., 2017); nehirlerin de mikroplastiklerin taşındığı en önemli kaynaklardan biri olduğu (Lechner vd., 2014) belirtilmiştir. Tuna Nehri'nde endüstriyel atıkların neden olduğu mikroplastiklerin bulunduğunu Lechner vd., (2014) bildirmişlerdir. Durgun su olarak bilinen göllerde, nehirlere nazaran daha fazla mikroplastik bulunduğu da yapılan çalışmalarda (Imhof vd., 2013; Free vd., 2014) belirlenmiştir. Zeri vd., (2018) atık su arıtma tesislerinin, mikroplastiklerin taşınımı için kaynak olabileceğini yaptıkları çalışmada vurgulanmıştır. Denizlerdeki plastik kirliliğinin nedenlerinden birisinin de balıkçılık olduğu bildirilmiştir (Lusher vd., 2017; Ostle vd., 2013). Balıkçılıkta kullanılan malzemelerden bazılarının (plastik ağlar, balık saklama kutuları, eldivenler, misina vb.) bırakılmış, kaybolmuş ve unutulmuş birçok av araçlarının plastik olması nedeniyle kirliliğe neden olduğu çeşitli çalışmalarda (Andrady, 2011; Lusher vd., 2017) vurgulanmıştır. Birleşmiş Milletler Çevre Raporu'nda; balıkçılık ve eğlence amaçlı kullanılan teknelerin de plastik atıklara neden olduğu belirtilmektedir (UNEP, 2009; Vlachogianni vd., 2017). Balast suları ile de mikroplastiklerin taşınabildiği vurgulanmıştır (Naik vd., 2019). Su ürünleri yetiştiriciliğinin de mikroplastik kirliliğine neden olduğu Hinojosa ve Thiel (2009) belirtilmektedir. Yapılan tüm tarımsal çalışmalar gibi, su ürünleri üretiminin de ekolojik çevreye etkisi olduğu bildirilmektedir (Yavuzcan vd., 2019).

Plastik ve mikroplastiklerin su canlılarına kontaminasyonu

Ulusal Singapur Üniversitesinde yapılan bir çalışmada; bir mikrometreden küçük olan plastik parçalarının (nanoplastik) larva aşamasından yetişkin hale gelene kadar birçok su ürününde birikerek besin zincirine dahil olduğunu belirtilmiştir (Anonim 1, 2019). EFSA (2016) ve FAO (2017) raporlarına göre; deniz ürünlerinde mikroplastikler bulunmaktadır. Mikroplastiklerin yosunlar, omurgasızlar ve bakteriler ile uzun mesafelere taşınabildiği belirtilmektedir (Kiessling vd., 2015). Denizlerdeki plastik kirliliğinin denizlerde yaşamını sürdüren organizmalar tarafından yutulduğunda fiziksel yaralanmalara (Gassel vd., 2013) sindirim sistemlerinde tıkanma ve hasarlara neden olduğu ayrıca üreme faaliyetlerini de olumsuz etkilediği üzerinde durulmuştur (GESAMP, 2016; Rummel vd., 2016). Clark vd., (2016) ve Wright vd., (2013), deniz canlıları tarafından tüketilen mikroplastiklerin, sağlık sorunları ve ölümcül durumlara yol açabileceği bildirilmiştir. Bağırsak sisteminde tıkanmaya neden olan mikroplastikler, sindirilmediği için canlılarda tokluk hissi oluşturarak besin alınmasına engel olduğu yapılan çalışmalarda vurgulanmıştır (Browne vd., 2008; Wright vd., 2013). Mikroplastiklerin beslenme yolu ve solungaçlarla canlıların vücuduna girdiği farklı çalışmalarda (Watt vd., 2016; Smith vd., 2018; Carbery vd., 2018) bildirilmiştir.

Su ortamında bulunan mikroplastikler, su ürünleri ve insanlara kontaminasyon kaynağı olarak görülmektedir. Mikroplastikler, zehirli ve zararlı maddelerin taşımasını da sağlamaktadır (Rochman vd., 2013b). Taşınan bu maddeler, mikroplastiklerin deniz canlılarının dokularında birikerek; karaciğer toksisitesine ve lezyonlara neden olduğu belirtilmektedir (Rochman vd., 2013b). Deniz canlılarının vücuduna giren mikroplastiklerin içerdiği maddelerin; karaciğer ve endokrin sisteme yaptıları olumsuz etkiler bildirilmiştir (Talsness vd., 2009; Rochman vd., 2013b; Rochman vd., 2014). Ayrıca açık denizlerde bulunan balıklarda mikro ve makro plastiklere daha az rastlandığı, kıyı türlerinde ise daha yüksek miktarda bulunduğu bildirilmiştir (Murphy vd., 2017). Yapılan bir çalışmada kral yengecinin (*Lithodes santolla) mide içeriğinde mikroplastiklerin bulunduğu bildirilmiştir (*Andrade ve Ovando, 2017*).* Deniz canlılarında saptanan mikroplastikler ve makroplastikler ile ilgili çalışmalarda; *Electrona subaspera* (Eriksson ve Burton, 2003), *Boops boops* (Nadal vd., 2016), *Triglops nybelini* (Morgana vd., 2018), *Boreogadus saida (*Kühn vd., 2018; Morgana vd., 2018), *Myripristis spp., Siganus spp., Epinephelus merra, Cheilopogon simus* (Garnier vd., 2019), karides (*Crangon crangon*) (Devriese vd., 2015; OSPAR, 2015), kalamarda (*Moroteuthis ingens*) (Philips vd., 2001), deniz kaplumbağasında (*Caretta caretta*) (Pham vd., 2017), deniz kuşları (Cadee, 2002; Rios vd., 2007; Mallory, 2008; Provencher vd., 2010; Trevail vd., 2015; Amélineau vd., 2016), kambur balina (*Megaptera novaeangliae*) (Besseling vd., 2015) ve Güney Amerika'da bulunan fok türünde (*Arctocephalus australis)* (Perez-Venegas vd., 2018), Kuzey kürklü fokunda (*Callorhinus ursinus*) (Donohue vd., 2019) pek çok olumsuz bulgu ile karşılaşılmıştır. Plankton ile beslenen Scombridae, Atherinopsidae, Engraulidae ve Clupeidae familyalarına ait olan 7 farklı balık türünde (*Odontesthes regia, Strangomera bentincki, Sardinops sagax, Opisthonema libertate, Cetengraulis mysticetus, Engraulis ringens* ve *Scomber japonicus*) mikroplastik tespit edilmiştir

(Ory vd., 2018). Sardalya (*Sardina pilchardus)* ve hamsi (*Engraulis encrasicolus*) doğal lifler ve mikroplastik tüketmektedir (Compa vd., 2018). Ekonomik değere sahip barbun (*Mullus barbatus*), sardalya (*Sardina pilchardus*), mercan (*Pagellus erythrinus*) ve kara midye (*Mytilus galloprovincialis)* mikroplastik bulunmuştur (Digka vd., 2018). Farklı midye türlerinde mikroplastik saptanmıştır (Webb vd., 2019; Browne vd., 2008).

Denizlerde ve tatlı sularda bulunan kuşların, memelilerin, kaplumbağalar ve omurgasız türlerin plastik atıklara dolandıkları için yaşamlarını yitirebilmektedirler (Laist, 1987; Gall ve Thompson, 2015). Deniz kirliliğinden (plastik, makroplastik, mikroplastik) 693 organizma yutma ve vücutlarına dolaşma sonucunda etkilenmişlerdir (Gall ve Thompson, 2015). Farklı beslenme tiplerine (omnivor, herbivor, etçil) sahip balıklardaki mikroplastik varlığı incelenmiştir. Omnivor beslenme tipine sahip balıklarda mikroplastik içeriğinin yüksek olduğu belirlenmiştir (Mizraji vd., 2017). *Pseudopleuronectes americanus*, *Roccus americanus*, *Myoxocephalus aenus*, *Menidia menidia* balık türleri ile *Sagitta elegans* ve deniz halkalı solucanının mide içeriğinde mikroplastik (Carpenter vd., 1972) ve midyelerin bağırsaklarında plastik (Browne vd., 2008) kalıntıları bulunmuştur.

Plastik ve Mikroplastiklerin Bakteri Taşınımına Etkileri

Gram negatif bakterilerin plastik atıklarda koloni oluşturmaktadırlar. Carpenter vd., (1972) tarafından yapılan bu çalışma plastikler üzerinde bakterilerin koloni oluşturduğunu bildirilen ilk yayın olmuştur (Wu vd., 2019). Mikroplastikler bakterilerin taşınımında rol almaktadırlar (Virsek vd., 2017). Bakteriler mikroplastiklerin yüzeyinde kolonileştiğini vurgulayan çalışmalar yapılmıştır (Harrison vd., 2011; Harrison vd., 2014; McCormick vd., 2014; Xu vd., 2019). Naik vd., (2019) tarafından yapılan çalışmada *V. cholerae*'nın mikroplastikler üzerinde biyofilm oluşturduğu belirtilirken, Kirstein vd., (2016) *Vibrio* spp.'nin De Tender vd., (2015) ise Vibrionaceae türlerinin mikroplastiklerde koloniler oluşturduğunu vurgulmışlardır. Zararlı alg tomurcukları (Harmful Algal Blooms-HAB) türlerinin balast suları ile mikroplastiklerin üzerinde taşındığının düşünüldüğü belirtilmektedir (Naik vd., 2019). Mikroplastiklerin üzerinde bulunan bakterilerin tanımlandığı bir çalışmada *Aeromonas salmonicida* ile 28 bakteri türünün olduğu ve *Aeromonas salmonicida* türünün balıklarda hastalığa neden olduğu belirtilmiştir (Virsek vd., 2017).

Plastik ve mikroplastiklerin insan sağlığı üzerindeki etkileri

Makroplastikler gıda ambalajlamada kullanılmakta ve zaman içerisinde küçük parçalara bölünmektedir (Scheirs, 2000; Bhunia vd., 2013). Mikroplastikler küçük boyutta oldukları için su kaynaklarına karışarak suda yaşayan canlılar tarafından sindirim yolu ile besin zincirine dahil olmaktadır (Yurtsever, 2018). Su ürünlerinde bulunan mikroplastikler, tüketildiklerinde gıda güvenliği açısından uygun değildir (Van Cauwenberghe ve Janssen, 2014). Denizdeki canlıların vücuduna giren mikroplastikler besin zincirine katılarak gıda yoluyla insanlara kadar ulaşabilmektedir (Setala vd., 2013; Romeo vd., 2005; Akarsu vd., 2017). İnsanlar ve çocuklar tarafından plastik parçalarının yanlışlıkla yutulması sonucu özofagus delinmesi yıltılması (Guirgis vd., 2011), gastrointestinal sistemde kanama (Rubin vd., 1998) ve incebağırsak delinmesi (Newell vd., 2000) gibi sağlık sorunlarına neden olmaktadır. Bunların yanı sıra su ürünlerinin işlenmesi ve paketlenmesi sırasında da mikroplastikler bulaşarak besin zincirine katılabilmektedir (Cole vd., 2013; EFSA, 2016; Carbery vd., 2018). Mikroplastiklere insan akciğerinde (Prata, 2018) ve insan dışkısında (Liebmann vd., 2018) rastlanılmıştır. Ayrıca plastiklerde bulunan maddelerin insanlarda kronik ve akut rahatsızlıklara neden olabileceği belirtilmiştir (Agency for Toxic Substances and Disease Registry (ATSDR, 2010). İnsan tüketimine sunulan tuzlarda mikroplastikler bulunmuştur (Karami vd., 2017b; Barboza vd., 2018; Seth ve Shriwastav, 2018). Mikroplastiklerin insanlara balık tüketimine bağlı olarak (Carbery vd., 2018) geçtiği ve yapılan bir çalışmada balığın etinde (Karami vd., 2017a) mikroplastiğin bulunduğu bildirilmiştir. Mikroplastiklerin insan sağlığına kanser, obezite gibi olumsuz etkilerinin olduğu bilinmektedir (Sharma ve Chatterjee, 2017). Mikroplastiklerin küçük parküllerden oluşmaları besin zinciri dışında havada sürüklenme yolu ile (Allen vd., 2019) insan vücuduna doğrudan ve dolaylı olarak solunarak sağlık riski oluşturmaktadır (Gasperi vd., 2018; Prata, 2018). Havada bulunan mikroplastikler akciğerlerde solunum yolu ile birikerek kronik ve akut iltihaplanmalara neden olmaktadır (Liu vd., 2019; Pauly vd., 1998).

SONUÇ

Günümüzde plastikler birçok alanda kullanılmakta ve gün geçtikçe de kullanım alanları artmaktadır. Plastik kullanımındaki bu artış çevre kirliliğine neden olması yanı sıra ortamda bulunan canlıları ve dolayısıyla da insan sağlığını da olumsuz olarak etkilemektedir. Plastikler farklı taşınım yolu ile çeşitli su ortamlarına ulaşmaktadır. Çeşitli su ortamlarına ulaşmış olan plastik ve mikroplastikler ortamda bulunan çeşitli canlılarda hasarlara ve hatta ölümlere bile neden olabilmektedir. Su canlıları tarafından tüketilen mikroplastiklerin besin ağına dahil olarak insan tüketimine kadar ulaştığı ve insan sağlığını da olumsuz olarak etkilediği çeşitli çalışmalarda belirtilmektedir. Bu nedenle bir an önce bu konuda bilinçlendirme çalışmalarının yapılması ve gerekli önlemlerin alınmasının sağlanması gerekmektedir. Konunun önemi üzerine farkındalık yaratılmasının sağlanarak plastik üretimi ve kullanımının çevreye, çeşitli canlılara ve insanlara verdikleri zararların önlenmesi amacıyla engellenmesi gerekmektedir. Kullanılan (temizlik malzemesi, kişisel bakım ürünleri, su şişeleri, gıda ambalaj materyalleri vb.) plastik materyallerin yerine çevre dostu ve insan sağlığına zararlı olmayan biyobozunur özellikte materyallerin yer almasının gerekli olduğu düşünülmektedir.

Gıdaların paketlenmesinde plastik ambalaj materyalleri yerine yenilebilir veya biyobozunur olan ambalajlama materyallerinin kullanılmasının olumlu etkilerinin olacağı düşünülmektedir. Çevrede oluşan plastik kirliliğinin önlenmesi amacıyla halkın bilinçlendirilmesinin sağlanması yanı sıra plastik atıkların toplanması için çeşitli (belediyeler, çevre koruma dernekleri, projeler vb.) faaliyetlerin arttırılmasının yararlı olacağı ön görülmektedir.

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