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

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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

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Yiğitler Deresi'nde (Nif Çayı, İzmir) dağılım gösteren *Gammarus izmirensis* (Amphipoda) türünün populasyon yapısı

Population structure of *Gammarus izmirensis* (Amphipoda) inhabiting Yiğitler Brook (Nif Stream, İzmir)

Murat Özbek[1*] • Efe Ulutürk[2]

[1]Ege Üniversitesi, Su Ürünleri Fakültesi, Temel Bilimler Bölümü, 35100, Bornova, İzmir https://orcid.org/0000-0003-4607-3507
[2]Ege Üniversitesi, Su Ürünleri Fakültesi, Temel Bilimler Bölümü, 35100, Bornova, İzmir https://orcid.org/0000-0002-9246-8078
*Corresponding author: murat.ozbek@ege.edu.tr

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Öz: Nif Çayı'nın kollarından biri olan Yiğitler Deresi yıl boyu süren düzenli akış rejimine sahiptir. Nispeten kirlenmemiş oluşuyla İzmir ili ve civarının biyolojik çeşitlilik açısından önemli sucul habitatlarından biri konumundadır. Bu çalışma ile tip lokalitesi Yiğitler Deresi olan *Gammarus izmirensis* Özbek, 2007 türünün populasyon yapısı hakkında bilgi sahibi olunması amaçlanmıştır. Tür hakkında orijinal tanımının yapıldığı çalışma dışında herhangi bir bilgi bulunmamaktadır. *Gammarus* bireyleri Ekim 2015-Eylül 2016 döneminde yapılan aylık örneklemelerle toplanmıştır. Örneklemeler tek bir lokaliteden yapılmıştır. Çalışmada 1938 adet *G. izmirensis* bireyi incelenmiş olup, bunların 850 adedi dişi, 586 adedi erkek ve 502 adedi de juvenil bireylerden oluşmaktadır. Erkek bireylerde maksimum vücut boyu 14,5 mm, dişi bireylerde ise 14,0 mm olarak ölçülmüştür. Jüvenil bireylerin Mart ve Ağustos aylarında daha fazla görüldükleri dikkati çekmektedir. Bu türün, ilkbahar ve yaz olmak üzere, yıl içinde birden fazla kez ürediği tespit edilmiştir.

Anahtar Kelimeler: *Tatlısu, Gammaridae, dere, taban omurgasızları, populasyon, Türkiye*

Abstract: Yiğitler Brook, one of the branches of Nif Stream, has a regular flow regime throughout the year. The brook is one of the important aquatic habitats in terms of biological diversity by having relatively unpolluted water. The main objective of the present study is to get information about the population structure of *Gammarus izmirensis* Özbek, 2007 whose type locality is Yiğitler Brook. There is no information about the species in literature except its original description paper. *Gammarus* specimens were sampled in monthly intervals between October 2015 and November 2016. Samplings were performed at one locality. During the study, a total of 1938 *G. izmirensis* specimens were investigated. Of them; 850 were females, 586 were males and 502 were juveniles. The maximum body length was 14.5 mm in males and 14.0 mm in females. The number of juveniles is higher in March and August. The species has a multivoltine life cycle with two generations (spring and summer) throughout the year.

Keywords: *Freshwater, Gammaridae, brook, macroinvertebrates, population, Turkey*

GİRİŞ

Gelişmekte olan ülkelerde nüfusun hızla çoğalması, şehirleşme ve endüstrileşme sonucu su kaynaklarının fütursuzca kullanımı, buna karşılık arıtma tesislerinin yetersiz olması ya da bulunmaması, dolayısıyla kanalizasyon ve sanayi atık sularının akarsuya boşaltılması büyük problemdir. Tüm bu aktivitelerin sucul ekosistemlere doğrudan ve/veya dolaylı yoldan pek çok olumsuz etkisi olmaktadır. Ortamda yaşayan makrobentik organizmalar kirlilikten en çok etkilenen canlı grubu arasında yer almaktadır.

Nif Çayı, İzmir il merkezinin 14 km kadar kuzey doğusundan ve Yamanlar Dağı'nın doğu yamaçlarından doğar. Önce Kemalpaşa ovası içinde doğuya doğru akar. Sonra kuzeye yönelerek Manisa-Turgutlu karayolunu kesip, kuzeybatı istikametinde akışına devam eder. İzmir-Bursa yolu üzerindeki Gediz köprüsü yakınlarında Gediz ile birleşir. Nif Çayı, Nif Dağı eteklerinden akan ve

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yazın kuruyan birçok derecik tarafından beslenmektedir. Bu derelerin en önemlilerinden biri olan Yiğitler Deresi yıl boyu akışın olduğu, çakıllı taşlı bir dip yapısına sahip olan bir sucul ortamdır. Tipik bir potamon zonu olan Yiğitler Deresi'nin üst kısımlarında balık çiftliği yer almaktadır. Suyun berrak aktığı kısımlardan yapılan örneklemelerde zengin bir canlı çeşitliliği gözlenmiştir (Özbek vd., 2013).

Yiğitler Deresi'nde dağılım gösteren bentik makro omurgasızlar üzerine yapılmış birkaç çalışma mevcuttur. Özbek ve Ustaoğlu (1998) Yiğitler Deresi'nin üst kısmında yer alan bir lokaliteden *Gammarus uludagi* Karaman, 1975 türünü rapor etmişlerdir. Özbek, (2007) *Gammarus izmirensis* türünü Yiğitler Deresi'nden tanımlamıştır. Bir diğer çalışmada (Özbek vd., 2019), Nif Çayı ve kollarının su kalitesi fiziko-kimyasal analizler ve biyolojik indeksleme yöntemleriyle incelenmiştir.

Bu çalışmanın temel amacı, literatüre 2007 yılında kazandırılan ve Türkiye'ye endemik olan *G. izmirensis* türünün populasyon yapısı hakkında bilgi sahibi olmaktır. Türün orijinal tanımının yapıldığı yayın dışında bu türle ilgili literatürde herhangi bir bilgi bulunmamaktadır. Bu çalışma ile ülkemizin temel zenginliklerinden biri olan endemik canlılar listemizde yer alan bir tür hakkında daha fazla bilgi edinilmesi amaçlanmıştır.

MATERYAL VE METOT

Yiğitler Deresi'nde dağılım gösteren *G. izmirensis* Özbek, 2007 türünün populasyon yapısının saptanması amacıyla, Ekim 2015 ile Eylül 2016 tarihleri arasında aylık örnekleme çalışmaları yapılmıştır. Örnekleme derenin üst bölgesinde tek bir mevkiden yapılmıştır (Şekil 1).

Şekil 1. Nif Çayı haritası ve çalışma yapılan istasyon

Figure 1. The map of Nif Stream and the sampling site

Derenin üst kısmında ve 38°23'57" K - 27°36'36" D koordinatında yer alan istasyon genel olarak taşlı-çakıllı bir zemin yapısına sahiptir. Kenar kısımları vejetasyonca zengindir. İstasyonda su akışı yıl boyunca devam etmektedir (Şekil 2). Genel olarak nispeten hızlı akışlı ve berrak suya sahip olan istasyon tipik bir potamon zonu olarak adlandırılabilir.

Şekil 2. Çalışma yapılan istasyonun genel görüntüsü

Figure 2. General view of the sampling site

G. izmirensis bireyleri 180 µm göz açıklığındaki el kepçeleri (kick-net) ile alınan bentik örneklerin incelenmesiyle toplanmıştır. Toplanan bireyler saha çalışmasının sonunda %4'lük formalin çözeltisi ile sabitlenmiş ve laboratuvara taşınmıştır.

Örneklenen bireyler erkek-dişi ve juvenil olarak ayrı ayrı tüplere alınmış ve stereomikroskop altında ölçülerek incelenmişlerdir. Cinsiyet gelişimi (erkek genital organının bulunmaması ve dişide görülen yumurta kesesinin görülmemesi) olmayan bireyler jüvenil bireyler olarak dikkate alınmıştır. Ölçümlerde mikrometrik oküler kullanılmıştır. Örneklerin boylarının ölçümünde toplam boy birey düz tutulduğunda rostrum ucundan telson ucuna kadar olan mesafe olarak alınmıştır. Dişi bireylerde yumurtalı olanlar ayrıca değerlendirilmiş ve yumurta sayıları alınmıştır.

BULGULAR

Çalışma konusunu oluşturan amphipod türünün genel görünüşü (Şekil 3), sistematikteki yeri ve özellikleri aşağıda verilmiştir.

Phylum : Arthropoda
Classis : Crustacea
Subclassis : Malacostraca
Superordo : Eucaridea
Ordo : Amphipoda
Subordo : Senticaudata
Familia : Gammaridae

Gammarus izmirensis Özbek, 2007

Şekil 3. *Gammarus izmirensis* Özbek, 2007'in genel görünüşü

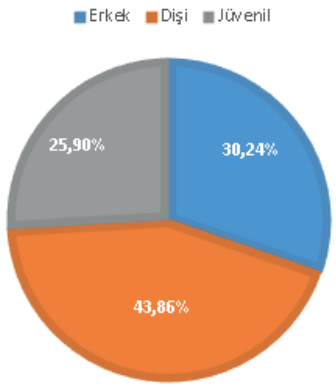
Figure 3. Habitus of *Gammarus izmirensis* Özbek, 2007

Morfoloji: Orta boylu bir türdür. *Gammarus pulex pulex* (L., 1758)'e benzer fakat erkeklerde 4. pereopodta karakteristik kısa setalar taşır. 2. antenin flagellasında bayrak şeklinde setalar mevcuttur. 5-7. pereopodların anterior kenarları boyunca uzun setalar yer almaz. Epimer plakları az veya hafif sivrilmiş yapıdadır.

Dağılım: Tür tip lokalitesi olan Nif Çayı'nda dağılım göstermektedir.

Ekoloji: Temiz ve nispeten hızlı akan bir derede yaşamaktadır.

12 aylık örnekleme dönemi sonucunda, toplamda 1938 adet *G. izmirensis* bireyi incelenmiştir. İncelenen bireylerin 850 adedi dişi, 586 adedi erkek ve 502 adedi de juvenil bireylerden oluşmaktadır (Şekil 4).



Şekil 4. Örneklenen *G. izmirensis* bireylerinin cinsiyete göre oranları

Figure 4. Sex ratio of the sampled specimens of *G. izmirensis*

Yiğitler Deresi'nden örneklenen *G. izmirensis* populasyonunda ölçülen toplam boy değerlerine ait tanımlayıcı veriler Tablo 1'de verilmiştir. İncelenen populasyonda en küçük dişi ve erkek bireyin 4,0 mm boyunda olduğu görülmüştür. En küçük jüvenil bireyin toplam boyu 1,2 mm olarak ölçülmüştür. Erkek bireylerde maksimum boy 14,5 mm iken dişi bireylerde 14,0 mm olarak ölçülmüştür. Jüvenil bireylerde maksimum boy 5,5 mm olarak tespit edilmiştir.

Tablo 1. Örneklenen *G. izmirensis* bireylerinde ölçülen toplam boy değerleri

Table 1. Total length values of the sampled specimens of *G. izmirensis*

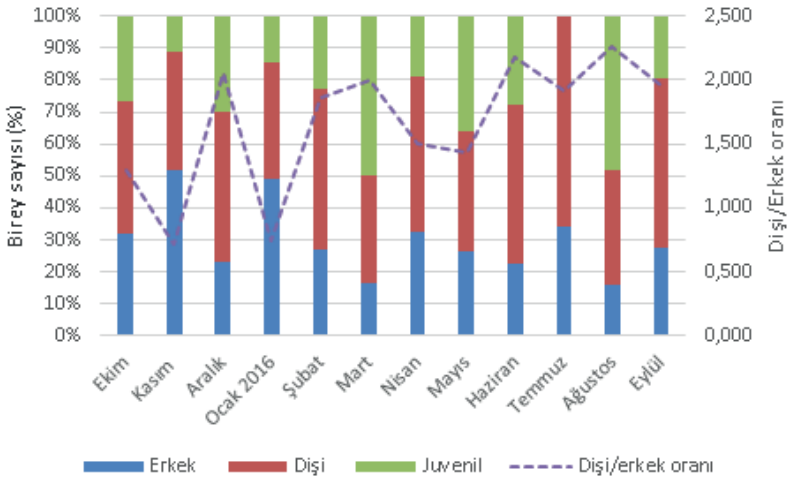
	Dişi	Erkek	Jüvenil	Genel
Min. boy (mm)	4,0 ± 1,958	4,0 ± 2,438	1,2 ± 0,784	1,2 ± 2,672
Max. boy (mm)	14,0 ± 1,958	14,5 ± 2,438	5,5 ± 0,784	14,5 ± 2,672
Birey sayısı	850	586	502	1938

G. izmirensis bireylerinin populasyon yapısının genel görünümü dikkate alındığında, Kasım ve Ocak aylarında erkek bireylerin populasyonda daha fazla bireyle temsil edildikleri buna karşın, dişi bireylerin Haziran, Ağustos ve Eylül aylarında daha fazla bireyle temsil edildiği dikkati çekmektedir. Jüvenil bireylerin Mart ve Ağustos aylarında daha fazla görüldükleri dikkati çekmektedir. Jüvenilllerin sayısında gözlenen bu artış türün özellikle ilkbahar ve yaz döneminde olmak üzere, yıl içinde birden fazla üreme periyoduna sahip olduğu konusunda ipuçları vermektedir (Şekil 5).

G. izmirensis bireylerinin toplam boya göre frekans dağılımları incelendiğinde jüvenil bireylerin bulunduğu 2,0 - 4,0 mm aralığında büyük bir yığılma olduğu gözlenmektedir. Cinsiyetin belirlenmiş olduğu diğer boy grupları dikkate alındığında 7-10 mm bandında dişi bireylerin erkeklere göre daha fazla oldukları, 10-15 mm bandında ise erkek bireylerin dişilere göre daha yoğun oldukları görülmektedir (Şekil 6).

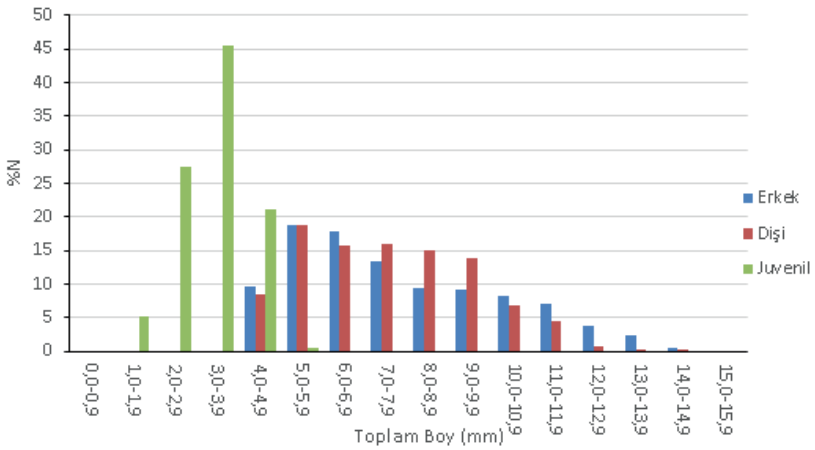
G. izmirensis bireylerinin aylık boy frekans grafikleri Şekil 7'de verilmiştir.

G. izmirensis bireylerinin total boy değerlerinin incelenmesi sırasında, bireylerin baş boyları da ölçülmüştür. Baş boyu ile total boy arasında herhangi bir ilişki olup olmadığı test edilmiştir. Yapılan ölçümler sonucunda tüm bireyler, erkek bireyler, dişi bireyler ve juveniller için elde edilen sonuçlar Şekil 8'de verilmiştir.



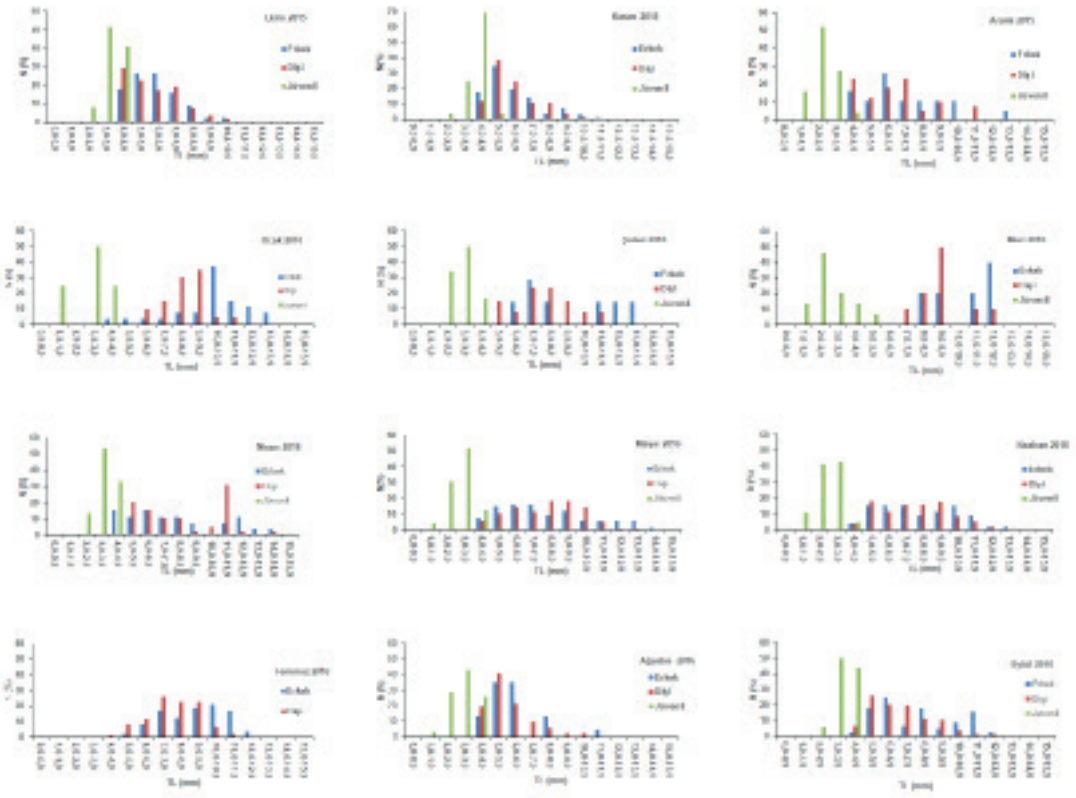
Şekil 5. *G. izmirensis* bireylerinin aylara ve cinsiyete göre yüzdelik oranları

Figure 5. Percentage ratios of *G. izmirensis* specimens according to months and sexes

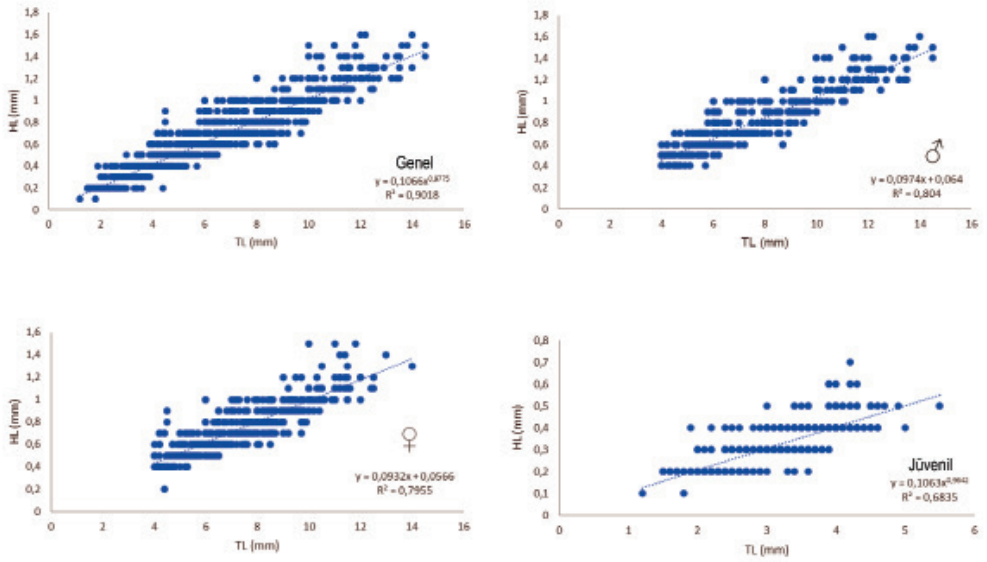


Şekil 6. *G. izmirensis* bireylerinde toplam boy frekans değerleri

Figure 6. Total length frequency of *G. izmirensis* specimens



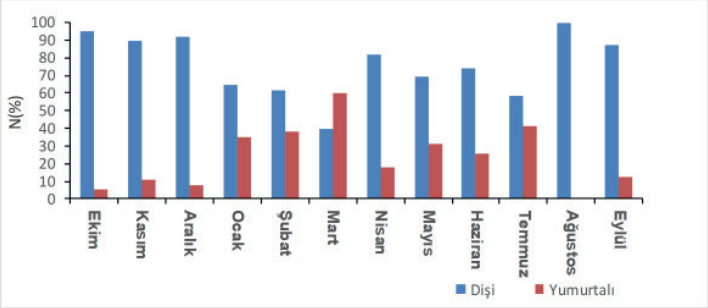
Şekil 7. *G. izmirensis* bireylerinde aylık boy frekansları (TL: toplam boy)
Figure 7. Monthly frequences of *G. izmirensis* specimens (TL: total length)



Şekil 8. *G. izmirensis* bireylerinde baş boyu ile toplam boy arasındaki ilişki (HL: baş boyu; TL: toplam boy)
Figure 8. Relations between head length and body length of *G. izmirensis* specimens (HL: head length; TL: total length)

Örneklenen tüm dişi bireyler içinde yumurtalı olanlar sayılmış ve yumurtalı dişilerin tüm dişilere oranları grafik halinde gösterilmiştir (Şekil 9). Yumurtalı dişilerin oranlarının tespiti bu türün üreme dönemleri hakkında fikir vermesi açısından önem arz etmektedir.

Bu durumda, yumurtalı dişi bireylerin yıl boyu populasyon içinde görüldükleri, buna ilaveten, Ocak-Mart dönemi ile Mayıs-Temmuz döneminde oransal dağılımlarında bir artış olduğu görülmektedir.



Şekil 9. *G. izmirensis* bireylerinde yumurtalı bireylerin aylık oranları

Figure 9. Monthly ratios of ovigerous females of *G. izmirensis* specimens

TARTIŞMA VE SONUÇ

Amphipodlar sucul ekosistemlerde yaygın ve bol bulunan organizmalar olup, ikincil üretimde önemli bir paya sahiptirler (Waters ve Hokenstrom, 1980). Bunun yanında, detritus üzerinden beslenmeleri ve besin zincirinin daha üst seviyesindeki canlıların diyetinde yer almaları sebebiyle de önemlidirler (Kaiser vd., 1992; Musko, 1992).

Gammarus cinsine ait bireylere akarsu ortamlarının temiz, sucul bitkiler bakımından zengin kısımlarında, genellikle çakıllı taşlı habitatlarda sıklıkla rastlanır. Belirli derecede organik kirliliğe tahammül edebilirler ve BMWP skor sisteminde orta derecede kirlenmiş sucul ortamların indikatörü olarak gösterilirler (Paisley vd., 2014).

Gammarus türlerinin çoğunda eşeysel dimorfizm görülür (Karaman ve Pinkster, 1977). Erkekler (genellikle) daha iri cüsseli olurlar, 2. gnathopodların yapısı, tüylenmesi ve büyüklüğü en kolay gözlenen eşeysel dimorfizm özelliklerinden biridir. Bunun yanında, cinsiyet organlarının mevcudiyeti de cinsiyet belirlenmesinde dikkate alınan özelliklerden biridir.

G. izmirensis türü hakkında literatürde çok az bilgi mevcut olup, populasyon yapısı hakkında herhangi bir bilgi mevcut değildir.

Ülkemiz içsularında dağılım gösteren *Gammarus* cinsine ait türlerin populasyon yapıları hakkında yapılmış çalışma sayısı oldukça azdır. Yeşilırmak Nehri'nde dağılım gösteren *Gammarus pulex* (L., 1758) türünün hayat siklusunun incelendiği çalışmada (Duran, 2007), genç bireylerin ilkbahar-yaz başında daha yoğun görüldüğü rapor edilmiştir. Bizim çalışmamızda jüvenil bireyler temmuz ayı hariç tüm aylarda gözlenmiş olup, Mart ve Ağustos aylarında pik yaptığı görülmektedir (Şekil 5). Jüvenillerin yoğunlukta olduğu boy grupları dikkate alındığında, 2-4 mm aralığında yoğunluk olduğu görülmektedir (Şekil 6). Costa ve Costa, (1999) Sado Haliç'inde (Portekiz) dağılım gösteren *Gammarus locusta* (L., 1758) jüvenillerin 3-5 mm bandında daha yoğun gözlendiğini belirtmektedir. Duran (2007), dişilerin erkeklere oranla yaz ayları hariç diğer tüm dönemlerde daha fazla sayıyla temsil edildikleri belirtmiş olup, bizim çalışmamızda dişi bireylerin Kasım ve Ocak ayları dışında erkek bireylere göre daha fazla sayıyla temsil edildikleri tespit edilmiştir (Şekil 5). Yumurta taşıyan bireyler bizim çalışmamızda neredeyse yıl boyu populasyonda gözlenmiş olup, Ocak-Mart dönemi ile Mayıs-Haziran döneminde daha fazla sayıya ulaşmıştır. Duran (2007) Yeşilırmak Nehri'nde dağılım gösteren *G. pulex* türünde yaz aylarında yumurtalı dişi bulunmadığını belirtmiştir. Özellikle sıcak ılıman bölgelerde dağılım gösteren *Gammarus* türlerinin birçoğunda yıl içinde birden fazla kez yumurtlama ve üreme olayı bildirilmiştir (Costa ve Costa, 1999; Subida vd., 2005). Yiğitler Deresi'nde dağılım gösteren *G. izmirensis* türünde de yıl içinde birden fazla kez üreme faaliyeti olduğu görülmektedir (Şekil, 7).

Gammarus cinsine ait olan türlerin bazıları alabalıkların temel besinleri arasında yer almaktadır (Newman ve Waters, 1984). Yüksek protein içermeleri nedeniyle akvaryum ve balık çiftliklerinde yem olarak kullanılan bu canlıların populasyon yapılarının bilinmesi, muhtemel yetiştiricilik faaliyetlerinde kullanılacak bazı temel bilgilerin elde edilmesi açısından yararlı olacaktır (Mirzajani vd., 2011)

TEŞEKKÜR

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Evaluation of DNA damage by Comet Assay in populations of endemic Beyşehir frog *Pelophylax caralitanus* (Arıkan,1988)

Endemik Beyşehir kurbağası *Pelophylax caralitanus* (Arıkan,1988) populasyonlarında Comet Analizi ile DNA hasarın değerlendirilmesi

Uğur Cengiz Erişmiş

Afyon Kocatepe University, Faculty of Sciences and Literatures, Moleculer Biology & Genetics Department, Afyonkarahisar, Turkey https://orcid.org/0000-0002-1288-3088
uerismis@aku.edu.tr

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Abstract: In study this, the level of DNA damage in three populations of endemic Beyşehir frog (*Pelophylax caralitanus*) inhabiting on Lakes of Karamık, Eber and Beyşehir with different anthropogenic pollution was assessed by using the DNA Comet Assay technique. Frog erythrocytes cells were used for the analysis. Seven adult individuals were collected from each biotope containing minimum 100 cells were analysed. While the results showing a significant increase in the DNA comet index in the contaminated zone [(Eber Lake= Idc=0.22±0.015), ($p<0.05$)], the majority of the cells did not contain any DNA damage in the clean zone (Karamık Lake, Idc=0.08±0.001). The obtained data demonstrated t that the endemic Beyşehir frog could serve as a useful flag species in an indicator of anthropogenic impact on ecosystems.

Keywords: *Pelophylax caralitanus, endemic Beyşehir frog, DNA damage, comet assay, anthropogenic impact*

Öz: Bu çalışmada, DNA Comet Assay tekniği kullanılarak, farklı antropojenik kirliliğe sahip Karamık, Eber and Beyşehir *Göllerinde yaşayan endemik* Beyşehir kurbağası (*Pelophylax caralitanus*) üç populasyonunda DNA hasar düzeyleri değerlendirilmiştir. Analizler için kurbağa eritrosit hücreleri kullanıldı ve uygun biyotoplardan toplanılan 7 ergin bireyin, her birinden minimum 100 hücre ile analiz edilmiştir. Sonuçlar DNA comet indeksinde kontamine olmuş zonda [(Eber Gölü = Idc=0.22±0.015), ($p<0.05$)] artış olduğu gösterirken, temiz zonda, hücrelerin çoğunluğunun hasarsız DNA içerdiğini (Idc = 0.08 ± 0.001) göstermiştir. Elde edilen veriler, endemik Beyşehir kurbağasının ekosistemler üzerindeki antropojenik etkinin bayrak türü göstergesi olarak kullanılma olasılığını göstermektedir.

Anahtar Kelimeler: *Pelophylax caralitanus, endemik Beyşehir kurbağası, DNA hasarı, comet assay, antropojenik etki*

INTRODUCTION

In last few decades, intensive industrial developments have led to a dramatic increase in the concentration of numerous chemicals in the aquatic and terrestrial ecosystems that pose a potential danger to the habitat of all living beings including human beings. Anthropogenic activities such as modern farming methods, urbanization, industrialization that involve the usage of different chemical pollutants and toxicants including heavy metals, biocides, pesticides, industrial effluents etc. which ultimately reach into aquatic environments and become responsible for the degradation of aquatic ecosystem (Dautremepuits et al., 2004; Marquis et al, 2009). These pollutants contaminate and accumulate in areas such as small bays, lakes, and seas by transportation via various water connections and flows at under/over ground level. Many of these pollutants contribute to aquatic environmental degradation and in turn results biomagnifications in aquatic organisms and as well as in the consumer of aquatic products like humans. Thus, they are harmful for the health of both human and other animals (e.g. Kalay et al., 1999; Ashraf, 2005).

Amphibians are good bioindicators of environmental pollution due to their susceptibility to chemicals during their freshwater cycles. This may

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be due to their sensitivity to changes of their habitat, and to the fact that their larvae inhabit aquatic environments. In addition, the presence of gills and a thin skin are both attributes of larval and adult that serve amphibians well as model organisms for study (Pollet and Bendell-Young, 2000; Huang et al., 2007; Erismis et al., 2013).

DNA damage by environmental xenobiotics is frequently assessed by single cell gel electrophoresis (SCGE) or the Comet assay (Singh et al., 1988), which detects DNA strand breakage and alkali-labile sites by measuring the migration of DNA from immobilized individual cell nuclei. In this assay, the cells are embedded in agarose gel on microscopic slides, lysed and then electrophoresed under alkaline condition. Cells with damaged DNA show increased migration of DNA fragments from the nucleus and the length of the migration indicates the amount of DNA strand breakage; the latter can be estimated by manual and computerized image scoring procedures (Kumar et al., 2013). The technique is highly sensitive for detecting DNA damage in any eukaryotic cell type and requires only a few cells. The minimum technical requirements for doing this assay in vitro and in vivo are well established (Cotelle and Férard, 1999; Hartmann et al., 2003). The Comet assay is sufficiently sensitive for detecting DNA damage in frogs (Dhawan et al., 2009). Endemic Beyşehir frogs (*Pelophylax caralitanus*) is a recently described species (Arıkan, 1988) in the Lake District of south-western Anatolia. Historically, it has been a common inhabitant of lakes and ponds at elevations of 950-1200 m (Arıkan, 1988; Budak et al., 2000; Jdeidi et al., 2001), ranging from the Konya plain to Denizli. Ongoing threats to the survival of this species include habitat loss and over-harvesting by commercial collectors, principally for the western European frogleg market. While it is remain locally abundant at some sites, overall the Beyşehir frog population is in rapid decline and is now considered an endangered species [(IUCN International Union for Conservation of Nature)-(the IUCN Red List of threatened Species - 2016)]. Although a number of studies have investigated the distribution, demographic studies, morphometry, serology, The fungal pathogen (*Batrachochytrium dendrobatidis*) and demographic data of *P. caralitanus* (e.g., Arıkan, 1988; Budak et al., 2000; Kaya et al., 2002; Erismis and Chinsamy, 2010; Erismis et al., 2013; Erismis, 2018), evaluation of DNA damage detected by micronuclei and comet assay of Turkey anurans are few (Gürkan et al., 2012; Erismis et al., 2013). The aim of the present study was to develop the first information concerning the possible biological effects of pollution from clastogenic chemicals in three lakes on endemic Beyşehir Frog (*P. caralitanus*) in Lake District of south-western Anatolia, Turkey.

MATERIALS AND METHODS

Twenty-one adults (11 males, 9 females) were collected by hand, mainly from the middle of July through the end of August 2018, which corresponds to the period of annual activity of the species studied. Blood samples from each adult frog were taken using a heparinized syringe and needle via cardiac puncture. After performing the blood sampling, the

frogs were freed to their natural habitats. Twenty-one adult endemic Beyşehir frogs were selected from three lakes of Lake District of south-western Anatolia (Figure 1) for the analysis which differed in the level of anthropogenic load.

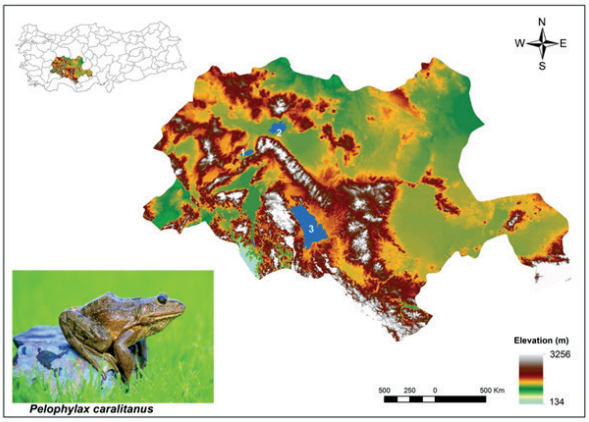

Figure 1. The map of the sampling localities; 1. Karamık Lake, 2. Eber Lake, 3. Beyşehir Lake

Characteristics of the three lakes studied are; 1. Karamık Lake (38°26'50"N 30°53'17'E; 1020 m asl) is located in the southeast of the city of Afyonkarahisar, in the west of central Anatolia region of Turkey. The wastewater of Afyonkarahisar - Çay SEKA paper factory was emptied to the lake in the past. After the year 2004, this factory was closed, and the waste water was prevented. 2. Eber Lake (38°37'53"N, 31°6'37"E; 967 asl), an "A" class wetland of south-western Anatolia, is very important for ornithology, fisheries and the native crayfish. But the structure of Eber Lake has been affected by industrial pollution from the both alkaloid and enamel factories located near it. 3. Beyşehir Lake (37°42'37"N, 31°27'10'E; 1125 m asl) is ecologically vulnerable and taken under protection as a "national park" however, domestic and industrial sewage collection and treatment is insufficient contributing to contamination of surface and ground water resource.

Single-cell electrophoresis (Comet assay) procedure and determination of DNA damage

Blood samples from each adult frog were taken using a heparinized syringe and needle via cardiac

puncture. After performing the blood sampling, the frogs were freed to their natural habitats. The samples of 5-10 µL venous blood from the frogs were collected and examined to determine if any DNA damage had occurred. Single-cell suspensions were prepared by diluting whole blood with phosphate buffered saline (PBS) (1:200, v/v) and were utilized immediately during the analyses. Then, the whole blood samples of 0.5 µL were mixed with 100 mL of 0.5% low-melting agarose in PBS at 37 °C. Subsequently, 80 mL of this mixture was layered onto a slide pre-coated with a thin layer of 1 % normal melting point agarose, covered immediately with a coverslip and stored for 5 min at 4 °C to allow the agarose to solidify. After removing the coverslips, the slides were immersed in freshly prepared cold (4°C) lysing solution (2.5 M NaCl, 100 mM EDTA-Na_2; 1 % Na-laurylsarcosine, 10 mM Tris–HCl, pH 10-10.5; 1 % Triton X-100 with 10 % DMSO being added just before use) for at least 1 h. The slides were then electrophoresed (25 V/300 mA, 25 min) after they were immersed in freshly prepared alkaline electrophoresis buffer (0.3 mol/L NaOH and 1 mmol/L EDTA-Na_2, pH 13) at 4 °C for unwinding (40 min). Experimental treatments were carried out under minimal illumination. After the electrophoresis application, the slides were neutralized (0.4 M Tris-HCl, pH 7.5) for 5 min. Each slide was stained with ethidium bromide (20 lg/mL) and covered with a coverslip. Slides were stored at 4 °C in humidified sealed containers until analysis. The nuclei were ranked by the four stages of DNA destruction. At least 100 nuclei were counted on each specimen (Fig. 2). Comets were randomly captured at a constant depth of the gel, avoiding the edges of the gel, occasional dead cells, cells near or in a trapped air bubble and superimposed comets. Results are reported as the mean ± SE. The Student-t test for paired samples was used for statistical analysis. When more than two means were compared, one-way ANOVA was performed, followed by the Duncan post-hoc test. The level of significance was set at $P<0.05$, sometimes expressed as DNA comet index (I_{dc}), using the formula: Idc = (0n0 + 1n1 + 2n2 + 3n3 + 4n4) / Σ, where n0-n4 is the number of "DNA comets" of each type, Σ is the sum of the counted "DNA comets".

RESULTS AND DISCUSSION

Blood cells with either no damage or varying degrees of damage are shown in Fig. 2. Four types of damaged DNA and non- damaged DNA in the blood cells of Beyşehir frog living in three study lakes (Fig. 2a-e) were observed. Apoptotic cells were observed (Figure 2f) but not evaluated, since they represented dead cells (Olive et al., 1993).

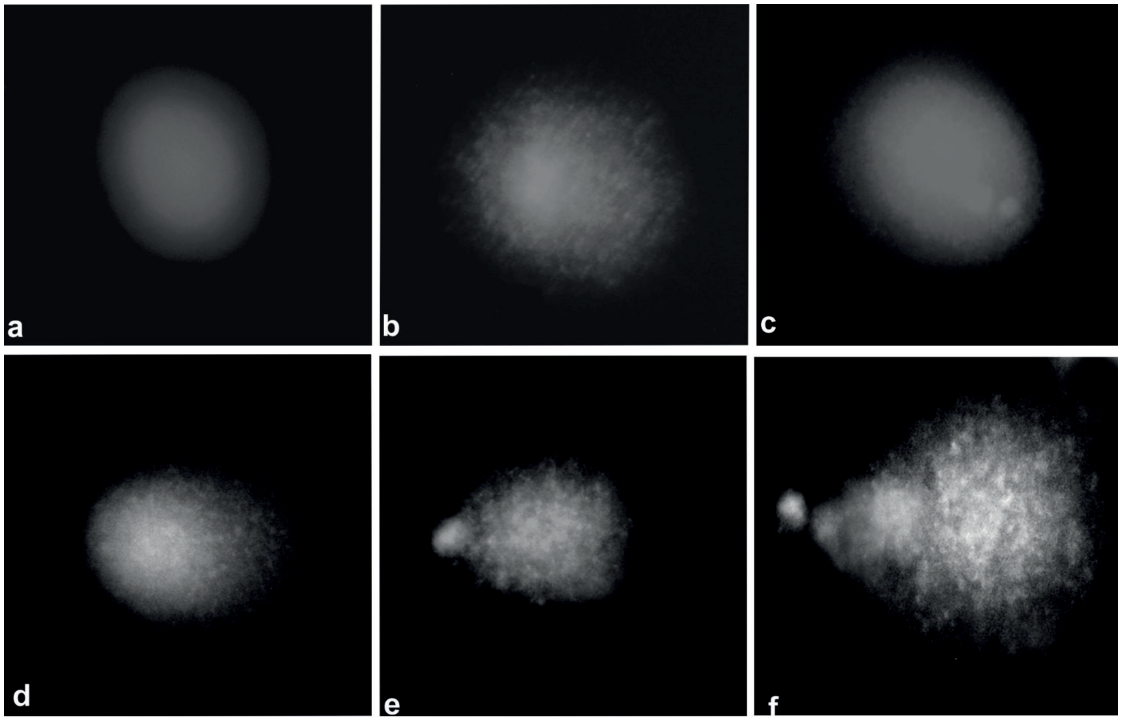

Figure 2. The classification of *Pelophylax caralitanus* blood cells by the comet assay. A: undamaged DNA; B-D: DNA damage; E: Maximum DNA damage; F: Apoptotic cell. Bar = 10µm.

In the comet assay, DNA fragments move from the nucleoid core towards the anode, resulting in 'comet' formation. The results were summarized in Table 1.

Cells sampled from Karamık Lake, in most cases, had intact nuclei and the nuclei with the third and fourth stages of DNA destruction were rarely found (Table 1). According to the information obtained in this study, due to less industrial and agricultural pollution of this lake, the level of DNA damage in the studied blood of Beyşehir frogs was considered negligible, because the average I_{dc} values reached the first stage of damage. The average value of the DNA comet index for Karamık Lake was I_{dc} = 0.089 ±0.005. In this regard, the present study indicated that the value of DNA damage was the smallest among the studied lakes, despite dwelling in an obviously polluted habitat by SEKA paper factory before 2004. This finding may indicate the absence of severe damaging factors disturbing the cytogenetic stability at the collection sites. However, it may also suggest that there are some kinds of repair mechanisms in homeostatic processes which can neutralize the detrimental effects of environmental components in the animals. Nevertheless, according to our data, certain tendencies occur towards an increase in the amount of damaged DNA. Thus, one factor ANOVA, performed using the aggregate of all studied blood of Beyşehir frog in three lakes revealed significant differences between the studied groups in the DNA comet index (ANOVA = 53.22; df = 2;18, $P_{0.00}$<0.05).

During the experiment, an increase was observed in the degree of DNA damage under the influence of industrial pollution from the alkaloid, enamel factories and weapon factories located in the vicinity of Eber Lake and Beyşehir Lake respectively. I_{dc} values of DNA damage in the collected Beyşehir frogs were analysed statistically. The blood of Beyşehir frogs in Eber Lake, which was the one of the considered areas in this study, provided the highest Idc value of DNA damage (Idc = 0.227 ±0.015). Results showed that I_{dc} values of DNA damage were statistically significant between Eber Lake and Beyşehir Lake (Student's t test = 3.83; $P_{0.002}$< 0.05). These results could be occurred due to both the alkaloid factory pollution and the enamel factory pollution. Despite the protected status of Eber Lake, fertilizers and pesticides can be drained into the surrounding lands because Eber Lake is adjacent to the agricultural fields in the Akarçay basins. A similar pattern of increasing proportion of damaged DNA was also observed in the Beyşehir Lake, probably due to the same cause.

As an environmental biomonitoring tool, the comet assay has been used increasingly by various scientists globally to assess and establish the genotoxic effects of xenobiotics on aquatic organisms (Rajaguru, 2001; Huang et al., 2007; Ismail et al., 2014). The comet technique used here, also known as single-cell gel electrophoresis (the SCGE technique), is a simple, fast, sensitive, and widespread protocol that has been used in recent years to measure and evaluate DNA damage in different cell types even with many organisms (e.g Devaux et al., 1998; Wang and Jia, 2009; Snegin, 2011a). One of the most important advantages of the technique is that it can be used with virtually all types of cells, as well as being applicable to amphibian species. Many studies carried out on amphibian species in fresh waters as well as in terrestrial can be found in the literature (e.g. Ralph and Petras, 1998; Ferreira et al., 2004; El-Zein et al., 2006; Vershinin, 2008; Wang and Jia, 2009; Dhawan et al., 2009; Zhelev et al., 2014). In this study, we found that cells from the adults of endemic Beyşehir frogs from Karamık Lake which was cleaner than the other two lakes had compressed DNA and maintained the circular form of their normal nucleus, with little or no evidence of comet formation (Fig. 2a). In contrast, blood cells from frogs living in three polluted water bodies displayed an altered appearance (Fig. 2b-e). To our knowledge, this is the first study carried out on the DNA damage in populations of Turkish endemic frog (*P. caralitanus*) in different lakes of the Lake District of south-western Anatolia. Many studies revealed that, the contaminants were the major concern in amphibians because many populations were declining dramatically and the death of entire populations increasingly occurred frequently, in some cases, due to anthropogenic changes in the environment (Pollet and Bendell-Young, 2000; Marquis et al., 2009; Erismis et al., 2013; Zhelev et al., 2014). The findings of this study are in parallel with the literature report that the amphibians are very sensitive to pollution, because they live at the interface of two environments land and water and can easily absorb pollutants through the skin. Therefore, the present study confirms that the endemic Beyşehir frogs in Lake District of south-western Anatolia, is good bio-indicators of to assess the genotoxicity and they may also serve as an early warning of natural responses to environmental contaminants

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Table 1. DNA damage indicators in the studied groups of *Pelophylax caralitanus; N:* Number of cells analysed, Na: Number of apoptotic cells

Lake	Indicators	Number of animals						
		1	2	3	4	5	6	7
Karamık Lake	N	103	106	108	114	123	102	112
	Na	0	0	0	0	0	0	0
	0 stage	97	98	103	106	118	97	108
	1 stage	3	6	3	6	2	3	2
	2 stage	1	1	-	-	2	2	1
	3 stage	2	-	2	2	-	-	-
	4 stage	-	1	-	-	1	-	1
	I_{dc}	0.106	0.113	0.083	0.105	0.081	0.068	0.071
Eber Lake	N	136	109	113	127	113	113	111
	Na	0	1	0	0	1	0	1
	0 stage	120	96	98	115	104	96	97
	1 stage	9	8	12	6	6	9	8
	2 stage	3	2	-	2	2	3	1
	3 stage	2	-	1	1	4	3	3
	4 stage	2	3	2	3	1	2	2
	I_{dc}	0.213	0.220	0.203	0.196	0.230	0.283	0.243
Beyşehir Lake	N	118	120	108	111	114	104	110
	Na	0	1	1	0	0	0	0
	0 stage	109	108	98	96	105	96	103
	1 stage	3	8	5	10	4	5	3
	2 stage	2	-	2	4	1	1	1
	3 stage	3	3	-	1	-	2	1
	4 stage	1	1	3	-	4	-	2
	I_{dc}	0.169	0.175	0.194	0.189	0.192	0.125	0.145

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Bacterial agents isolated from cultured marsh frog (*Pelophylax ridibundus*, Pallas 1771)

Yetiştiriciliği yapılan ova kurbağasından (*Pelophylax ridibundus*, Pallas 1771) izole edilen bakteriyel etkenler

Gülşah Dökenel[1] ● Selmin Özer[2]*

[1]Department of Aquaculture, Faculty of Fisheries, Mersin University, 33190 Mersin, Turkey https://orcid.org/0000-0002-9236-1334
[2]Department of Aquaculture, Faculty of Fisheries, Mersin University, 33190 Mersin, Turkey https://orcid.org/0000-0001-6283-5976
*Corresponding author: selmin.oezer@gmail.com

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Abstract: Marsh frogs (*Pelophylax ridibundus*) are preferred in European cuisine. In recent years, interest in farming of marsh frogs has increased, but little is known about their bacterial diseases. This research was carried out in a marsh frog farming operation in Mersin, Turkey, in order to determine the bacterial diversity. For this purpose, a total of 339 frog, 30 water, and 8 feed samples were collected. Isolation and identification of bacteria were carried out by conventional techniques and the VITEK-2 compact system. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method. A total of 239 isolates of 49 different species, including 31 Gram negative rod-shaped bacteria, 9 Gram positive rod-shaped sporeforming bacteria, and 9 Gram positive cocci-shaped non-sporeforming bacteria have been identified. These bacteria species were detected from 25 (83.3%) water, 5 (62.5%) feed samples, and 64 (84.2%) of 76 frog specimens. Antimicrobial susceptibility and MAR index values ranged between 1.4-95.8% and 0.13-0.73, respectively. In conclusion, the presence of opportunistic pathogenic bacteria in water, feed and frog specimens, which could pose risk for frogs and human health, have been detected in the marsh frog farm in Mersin. This study reveals, that further investigations are necessary for sustainable marsh frog breeding in Turkey.

Keywords: *Bacteria, frog disease, raniculture, marsh frog, Pelophylax ridibundus*

Öz: Ova kurbağası (*Pelophylax ridibundus*) Avrupa mutfağında tercih edilen bir türdür. Son yıllarda kurbağa yetiştiriciliğine ilgi artmış olmasına karşın bakteriyel hastalıkları hakkında çok az şey bilinmektedir. Bu araştırma, bakteri çeşitliliğini tespit etmek amacıyla Mersin'de bir ova kurbağası çiftliğinde gerçekleştirilmiştir. Bu amaçla toplam 339 kurbağa, 30 su ve 8 yem örneği toplanmıştır. Bakterilerin izolasyonu ve tanımlanması geleneksel teknikler ve VITEK-2 kompakt sistemi ile gerçekleştirilmiştir. Antimikrobiyal duyarlılık testi Kirby-Bauer disk difüzyon yöntemi ile yapılmıştır. 31 Gram negatif basil, 9 Gram pozitif basil ve 9 Gram pozitif kok içeren 49 farklı türden toplam 239 izolat tanımlanmıştır. Bu bakteri türlerinin 25'i (% 83,3) su, 5'i (% 62,5) yem örneklerinden ve 64'ü (% 84,2) 76 kurbağa numunesinden tespit edilmiştir. Antimikrobiyal duyarlılık ve MAR indeksi değerleri sırasıyla % 1,4-95,8 ve 0,13-0,73 arasında değişmektedir. Sonuç olarak, Mersin'deki ova kurbağası çiftliğinde kurbağa ve insan sağlığı açısından risk oluşturabilecek fırsatçı patojenik bakterilerin varlığı kurbağa, su ve yem örneklerinde tespit edilmiştir. Bu çalışma, Türkiye'de sürdürülebilir ova kurbağalarının yetiştirilmesi için daha ileri araştırmaların gerekli olduğunu ortaya koymaktadır.

Anahtar kelimeler: *Bakteri, kurbağa hastalığı, kurbağa yetiştiriciliği, ova kurbağası, Pelophylax ridibundus*

INTRODUCTION

Frogs, one of the delicacies of the world cuisine, are also used as pets, education and research tools, and different industries (Pasteris et al., 2006). Frogs caught from the nature till the beginning of the 20th century are now successfully cultivated in South America and some Far Eastern countries, especially the American bull frog (*Lithobates catesbeianus*, Shaw 1802) (Amborski et al., 1983). Some frog species of our country's natural habitat are an important export item for Europe. Since production through cultivation has become mandatory, because of increased demand and reduced natural resources, extensive and semi-intensive farming of marsh frog (*Pelophylax ridibundus*) has been conducted by some private and public institutions in recent years. However, intensive raniculture is still in

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experimental status in Turkey. Unfortunately, farming operations often lead to increased risk of diseases and mortality (Pasteris et al., 2006). Within the aquatic environment, frogs are in contact with a number of potentially pathogenic bacteria. Stressed conditions like crowding or unsanitary lead to overcome weakened immune barriers and cause disease (Mauel et al., 2002). Causative agents of bacterial diseases in many frog species, both wild (Schadich and Cole, 2010) and cultivated (Amborski et al., 1983; Mauel et al., 2002; Huys et al., 2003; Pasteris et al., 2006; Pasteris et al., 2009; Pasteris et al., 2011; Pilarski & Schocken-Iturrino, 2011; Jeong et al., 2014; Xiaoying et al., 2015), and in water samples (Pasteris et al., 2006; Hacioglu et al., 2015) have been reported previously. However, as far as literature reviews are concerned, no reports about bacterial agents of marsh frogs have been found. According to our knowledge, this is the first research about bacteria in cultured marsh frogs.

To detect the efficacy of drugs, antimicrobial susceptibility testing has been conducted. Different rates of antimicrobial susceptibility tests of bacterial isolates from various frog species were reported (Lee et al., 2009; Pilarski and Schocken-Iturrino, 2011; Tee and Najiah, 2011; Hacioglu et al., 2015). In order to indicate the intensity of exposure of antibacterial drugs to bacteria, some investigators have reported multiple antibiotic resistance (MAR) index values of isolates from reared American bull frogs (Lee et al., 2009; Tee and Najiah, 2011) and some wild frog species (Hacioglu and Tosunoglu, 2014; Hacioglu et al., 2015).

There is a great interest in frog farming in Turkey and a potential for development of frog farming due to expanding demand in export markets. Therefore, the aim of this study was to shed light on the emerging diseases of farmed marsh frogs, which possess a promising development potential in our country.

MATERIALS AND METHODS

Frog, water and feed samples

Samples were collected from the pilot-scale marsh frog farm in Aydıncık, Mersin, Turkey. For ungoing of the farm, full-cycle production and hatchery-rared system, and unchlorinated underground-water was used. Water temperatures ranged between 16ºC and 28ºC during the sampling dates. Frogs were fed with ground and pellet feed.

A total of 339 frog (Table 1), 30 water (8 tap and 22 pool water), and 8 pellet feed (7 farm-made frog feed and one commercial trout feed) samples were collected randomly 8 times according to metamorphic stages between 30.03.-12.10.2017. All samples were brought under adequate conditions to the laboratory and processed on same day. Whereas water and pellet feed samples were transported in aseptic and cooled conditions, all frog samples were carried alive in water included plastic containers. Life frog samples were examined for external findings; weight and length measurements before any treatment (Table 1).

Microbiological analysis

After euthanasia of frog samples by transdermal exposure of buffered MS-222 (% 1) (Hacioglu et al., 2014) and disinfection by povidin/iodin solution (400 ppm) (Brown et al., 1997), dissection has been applicated (Whitaker & Wright, 2001) and clinical signs have been recorded.

Bacteriological analysis of frog specimens were carried out on whole eggs, embryos (4 days old), and larvae; heads and bodies without internal organs of tadpoles and baby frogs; and lungs, liver, spleen, blood and lesions of juvenile frogs and adult frogs. Since similar samples were combined and homogenized, a total of 76 frog specimens of 339 samples were studied (Table 1). Isolation of bacteria have been made by conventional methods (Austin & Austin, 2007). Frog specimens were streaked directly onto Trypticase Soy Agar (TSA; Merck) and Tryptone Yeast Extract Salts Agar (TYESA) (Brown et al., 1997). For water and feed samples 0.3 mL of appropriate three-fold serial dilutions, prepared with peptone water (PW) have been used. Incubation time for TSA was 48-72 hours at 30°C and 5-7 days at 15°C for TYESA. Different colonies on TSA and yellow colonies on TYESA have been subcultured and used for identification. Isolates were stored at -20°C in Nutrient broth or TYES Broth supplemented with 15% (v/v) glycerol. Bacteria were also indentified by the VITEK-2 compact system (bioMerieux, France) (VITEK-2 GN for Gram negative rod-shaped bacteri, VITEK-2 GP for Gram positive cocci-shaped bacteria and VITEK-2 BCL ID cards for Gram positive rod-shaped bacteria) according to the manufacturer's instructions. Before carrying out the VITEK tests, some traditional identification tests such as Gram, catalase, cytochrome oxidase, motility and flexirubin (Plumb and Browser, 1983) have been performed.

Antimicrobial susceptibility testing and multiple antibiotic resistance (MAR) index

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standart Institute (CLSI, 2010). The incubation was carried out at 30°C for 24-48 hours. For this purpose, amoxycillin (AMX-10 µg), clindamycin (CC-2 µg), ofloxacin (OFX-30 µg), penicillin (P-10 µg) (BD BBL, USA), doxycycline (D-30 µg), enrofloxacin (ENR-5 µg), eritromycin (E-15 µg), florfenicol (FFC-30 µg),

Table 1. Weight and length of frog samples according to metamorphic phases

					Weight (g)		Length (cm)			
	Metamorphic				Min.-Max.	Mean±SD	Min.-Max.		Mean ±SD	
Date	Stages	n	ns	nsb			NS	NL/NT	NS	NL/NT
30.03.17	Adult frog	10	10	7	18.23-46.37	37.77±7.91	6.0-8.8	15.0-18.5	7.18±0.60	16.67±1.0
	Egg	26	1	1	NM	NM		NM		0.16
	Embryo	53	3	3	NM	NM		0.2-0.45		0.32±0.08
06.04.17	YSL-4	51	2	2	NM	NM		0.7-1.0		0.85±0.09
	YSL-8	41	2	2	NM	NM		0.9-1.0		0.97±0.05
20.04.17	Fed larvae	20	2	2	0.01-0.08	0.03±0.02		1.0-1.7		1.24±0.22
	Diseased larvae	20	2	2	NM	NM		0.6-1.6		1.02±0.24
12.06.17	Tadpole	18	2	2	0.17-0.35	0.27±0.06		1.5-4.1		2.59±0.66
30.06.17	Tadpole	13	1	1	0.34-0.90	0.53±0.15		3.5-4.7		4.09±0.39
30.06-12.07.17	T hindlimb	13	2	2	0.99-2.21	1.5±0.4		4.8-6.5		5.57±0.6
30.06.17	T four limbs	3	1	1	1.75-1.9	1.82±0.08		6.2-6.5		6.33±0.15
12.07.17		4	2	2	0.53–1.66	1.09±0.33		3.5–5.5		4.55±0.82
10.08.17		12	2	2	0.57-1.68	1.02±0.3		3.0-5.0		4.34±0.62
12.07.17	Baby frog	9	2	2	0.39-0.92	0.67±0.19	1.5-2.0	3.5-5.0	1.8±0.2	4.37±0.52
12.07.17	Diseased tadpole	6	1	1	0.66-2.60	1.64±0.89		4.0-6.5		4.88±1.18
10.08.17	Baby frog	23	23	18	0.56-2.4	1.14±0.53	1.5-3.0	3.0-7.0	2.1±0.34	4.78±1.02
12.10.17	Juvenil frog	14	14	10	4.46-40.20	16.75±9.16	3.5-7.5	9.0-16.5	5.33±0.96	12.8±2.09
	Diseased adult frog	4	4	4	24.08-39.0	33.66±6.9	6.2-7.5	15.5-18.0	6.92±0.53	17.0±1.22
	Total	**339**	**76**	**64**						

n: Number of samples, ns: Number of specimens, nsb: Number of bacteria detected specimens, NM: Not measured, NS: Nose-sacrum, NL: Nose-limb, NT: Nose-tail, YSL-4: Yolk-sac larvae, 4 days old, YSL-8: Yolk-sac larvae, 8 days old.

gentamicin (GM-10 µg), lincomycin (L-2 µg), neomycin (N-30 µg), oxytetracycline (T-30 µg), streptomycin (S-10 µg), trimethoprim-sulfamethoxazole (SXT-23,75-1,25 µg) and vankomycin (Va-30 µg) (Bioanalyse, Turkey) discs were used.

MAR index of the isolates were calculated according to Krumperman's method (Lee et al., 2009) as follows: [*MAR index = X / (Y x Z)*]

X: Total count of bacteria resistant to antibacterial drugs, Y: Total antibacterial drug count used,

Z: Total isolate count

While MAR index values higher than 0.20 indicate, that the animal was exposed to antibacterial drugs, values equal to or less than 0.20 suggests, that drugs were rarely or never used for therapeutic purposes (Lee et al., 2009). *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923) were used as reference strains.

RESULTS

External and internal symptoms have been rarely observed in frog samples. Air bubbles were detected in the abdomen of 14-21 day old larvae. At this phase, the mortality rate has exceeded 80%. External symptomes like swollen abdomen and gas bubble at the vent in tadpoles, spinal deformities, ulcerative lesions, swelling and weakness in limb muscles in

tadpoles and adult frogs were observed. Internal signs like haemorrhages and ulcerative injuries in muscles, anemia and haemorrhages in intestines and liver have been detected in adult frogs (Figure 1).

In all examined samples, a total of 239 isolates of 49 different species were identified, of which 31 (63.26%) were Gram negative rod-shaped bacteria, 9 (18.37%) were Gram positive rod-shaped sporeforming bacteria, and 9 (18.37%) were Gram positive cocci-shaped non-sporeforming bacteria. Of these isolates, 172 (72%) have been detected from 64 (84.2%) of 76 frog specimens, 60 (25.1%) from 25 (83.3%) of 30 water, and 7 (2.9%) from 5 (62.5%) of 8 feed samples. According to literature, some detected bacteria of this study are reported for the first time in frogs (Tables 2, 3, and 4).

Antimicrobial susceptibility testing was performed on 71 bacterial isolates of the 239 bacterial isolates, covering 31 different species. Antimicrobial susceptibility ratios ranged between 1.4 and 95.8% (lincomisin and enrofloxacin) (Table 5).

MAR index values of 26 of 31 isolates (83.87%) have been detected higher than 0.20. Resistance, susceptibility and MAR index values of the isolates can be seen on Table 6.

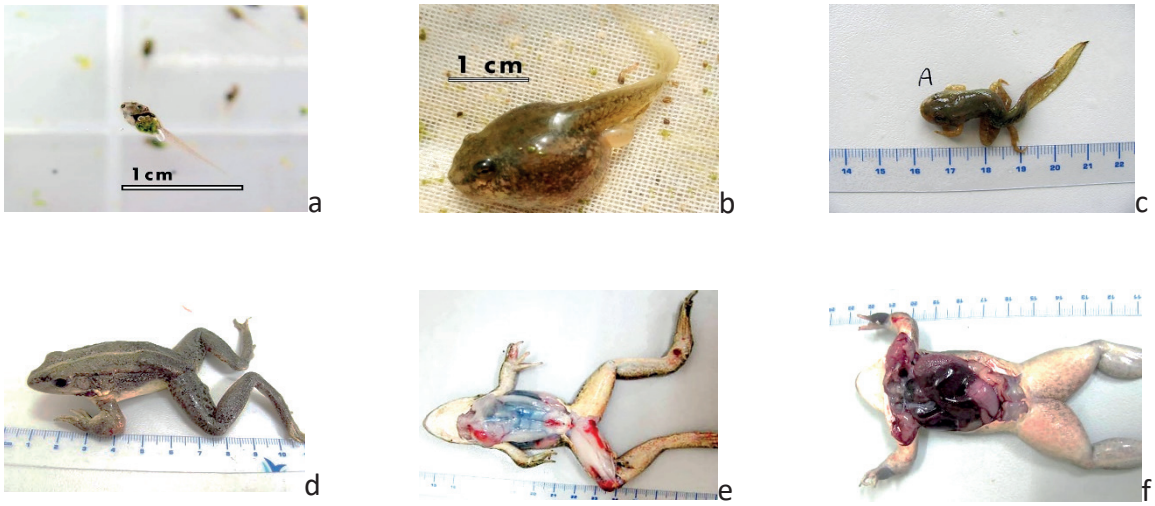

Figure 1. External and internal pathological features of marsh frog specimens

a. Fourteeen days old swimming larvae, air bubbles in abdomen

b. Tadpoles with swollen abdomen and gas bubble at the vent

c. Scoliosis in tadpole, four limbs stage

d. Spinal deformities, ulcerative lesions, swollen and weak muscles of limbs, adult stage

e. Haemorrhages and ulcerative lesions in muscles, adult stage

f. Haemorrhages in intestines and liver, adult stage

Table 2. Number of the Gram negative rod-shaped bacteria according to samples

No	Gram negative rod-shaped bacteria	Frog	Water	Feed	Total
1	*Acinetobacter haemolyticus*	3	0	0	**3**
2	*Aeromonas hydrophila/ caviae*	12	7	0	**19**
3	*Aeromonas sobria*	12	4	0	**16**
4	*Bordetella hinzii*	0	1	0	**1**
5	*Brevundimonas diminuta/ vesicularis*†	6	0	0	**6**
6	*Chryseobacterium indologenes*	2	5	0	**7**
7	*Citrobacter braakii*	0	1	0	**1**
8	*Citrobacter freundii*	26	2	0	**28**
9	*Delftia acidovorans*†	3	1	0	**4**
10	*Edwardsiella tarda*	18	2	0	**20**
11	*Elizabethkingia meningoseptica*	1	0	0	**1**
12	*Enterobacter asburiae*	0	3	0	**3**
13	*Escherichia coli*	7	0	0	**7**
14	*Klebsiella oxytoca*	1	0	0	**1**
15	*Klebsiella pneumoniae ozaenae*	1	0	1	**2**
16	*Morganella morganii*†	2	1	0	**3**
17	*Pantoea* spp.	0	1	1	**2**
18	*Plesiomonas shigelloides*	1	0	0	**1**
19	*Providencia rettgeri*†	0	1	0	**1**
20	*Pseudomonas aeruginosa*	1	2	0	**3**
21	*Pseudomonas fluorescens*	0	2	0	**2**
22	*Pseudomonas mendocina*†	0	1	0	**1**
23	*Pseudomonas putida*	1	1	0	**2**
24	*Rhizobium radiobacter*†	0	1	0	**1**
25	*Salmonella* spp.	13	1	0	**14**
26	*Serratia fonticola*†	0	1	0	**1**
27	*Serratia odorifera*†	0	1	0	**1**
28	*Shewanella putrefaciens*	11	1	0	**12**
29	*Sphingomonas paucimobilis*†	10	5	0	**15**
30	*Sphingobacterium t halpophilum*	0	1	0	**1**
31	*Stenotrophomonas maltophilia*†	0	1	0	**1**
	Total	**130**	**47**	**2**	**179**

†First isolation in frogs

Table 3. Number of the Gram positive rod-shaped sporeforming bacteria according to samples

No	Gram positive rod-shaped sporeforming bacteria	Frog	Water	Feed	Total
1	*Bacillus cereus/mycoides/ thuringiensis*	9	5	2	**16**
2	*Bacillus clausii*	1	0	1	**2**
3	*Bacillus fortis*	1	0	0	**1**
4	*Bacillus megaterium*	1	1	0	**2**
5	*Bacillus pumilus*	1	0	0	**1**
6	*Bacillus smithii*	0	1	2	**3**
7	*B.subtilis/amyloliquefaciens/atropha*†	2	2	0	**4**
8	*Brevibacillus choshinensis*	1	0	0	**1**
9	*Lysinibacillus sphaericus/ fuciformis*†	1	0	0	**1**
	Total	**17**	**9**	**5**	**31**

†First isolation in frogs

Table 4. Number of the Gram positive cocci-shaped non-sporeforming bacteria according to samples

No	Gram positive cocci-shaped non-sporeforming bacteria	Frog	Water	Feed	Total
1	*Aerococcus viridans*†	1	1	0	**2**
2	*Enterococcus gallinarum*†	2	0	0	**2**
3	*Granulicatella adiacens*†	1	0	0	**1**
4	*Kocuria kristinae*†	0	1	0	**1**
5	*Kocuria rhizophila*†	2	1	0	**3**
6	*Kocuria rosea*†	1	0	0	**1**
7	*Micrococcus luteus/lylae*†	16	0	0	**16**
8	*Staphylococcus aureus*	0	1	0	**1**
9	*Staphylococcus equorum*†	2	0	0	**2**
	Total	**25**	**4**	**0**	**29**

†First isolation in frogs

DISCUSSION

Although there were no serious clinical signs in adult frogs, high mortalities continued in 2-3 weeks old larvae from April to July 2017 and the final mortality ratio exceeded 80% during the present study. Whereas D'Silva (2015) reported an economically tolerable mortality rate in frog growth of 20% in the spawning phase, 10% in tadpole phase, 35% in baby and juvenile phase and 10% in the fattening phase. We estimate that the high mortality rate of larvae was due to gas bubble disease, because massive greening of water

Table 5. Susceptibility and resistance rates of antimicrobial drugs of the isolates

	Gr- rod-shaped bacteria		Gr+ rod-shaped bacteria		Gr+ cocci-shaped bacteria		Total	
Antimicrobial drugs	S %	R %	S %	R %	S %	R %	S %	R %
Lincomycin	0	100	6.7	93.3	0	100	1.4	98.6
Penicillin	2.2	97.8	26.7	66.7	50.0	41.7	15.3	81.9
Clindamycin	4.4	91.1	40.0	53.3	41.7	41.7	18.1	75.0
Amoxicillin	24.4	75.6	40.0	60	66.7	33.3	34.7	65.3
Vancomycin	6.7	91.1	86.7	6.7	33.3	33.3	27.8	63.9
Erythromycin	6.7	68.9	26.7	13.4	33.3	33.3	13.9	50.0
Neomycin	35.6	31.1	80.0	0	33.3	41.7	44.4	26.4
Streptomycin	44.4	31.1	73.3	20.0	58.3	8.3	52.8	25.0
SXT	75.6	22.2	66.7	33.3	83.3	16.7	75.0	23.6
Oxytetracycline	31.1	33.3	73.3	6.7	66.7	8.3	45.8	23.6
Florfenicol	68.9	26.7	93.3	6.7	75.0	16.7	75.0	20.8
Gentamycin	73.3	20.0	93.3	0	66.7	16.7	77.1	14.3
Ofloxacin	93.3	4.4	80.0	0	75.0	8.3	87.5	4.2
Doxycycline	86.7	6.6	93.3	0	100	0	90.3	4.2
Enrofloxacin	97.8	0	100	0	83.3	8.3	95.8	1.4

S: susceptibility, R: resistance, SXT: Trimethoprim-sulfamethoxazole

and, especially in the afternoons, increased air bubbles on the water surface of pools and in the abdomen of tadpoles were observed (Figure 1a). Indeed, Lutz and Avery (1999) reported that tadpoles are particularly susceptible to gas bubble disease caused by the oxygen supersaturation associated with afternoon algal photosynthesis.

The most common disease of frogs is the red-leg syndrome, also known as bacterial dermatosepticemia. External symptomes like anorexia, lethargy, discolorations, hemorrhages, ulcers, necrosis, swelling due to subcutaneous edema, focal hemorrhages in skin and skeletal muscles, especially of the limbs; assites, discoloration, and megali of liver and spleen, and other pathological findings such as hemorrhages in internal organs have been reported (Taylor et al., 2001; Pasteris et al., 2006). Although some of the symptoms detected in this study may indicate dermatosepticemia; it can be suggested that these findings could be accidental, because they were present only in a few adult frogs and point to other diseases as well.

Most of the 61 isolates of water samples were *Aeromonas hydrophila/caviae*, *Chryseobacterium indologenes*, *Sphingomonas paucimobilis*, *Bacillus cereus/mycoides/thuringiensis*, *Aeromonas sobria*, *Enterobacter asburiae* and *Citrobacter freundii,* respectively (Table 2, Table 3). Hacioglu et al. (2015) detected in water samples from the environment of wild frogs (including *P. ridibundus*) *A. hydrophila/caviae*, *Pseudomonas aeruginosa*, *Ps. fluorescens* and *Shewanella putrefaciens* similar; and *Actinobasillus* sp., *Elizabethkingia meningoseptica*, *Enterobacter gergoviae*, *Escherichia coli, Klebsiella oxytoca*, *Moroxella* sp., *Pasteurella multocida, Serratia liquefasciens*, *Ser. rubidaea* and *Vibrio carchariae* dissimilar compared to our water findings. Aeromonads, natural members of the aquatic environment (Austin and Austin, 2007), have been reported in previous studies in various diseases of frogs, especially in cases of red-leg sydrome (Mauel et al., 2002; Huys et al., 2003; Pasteris et al., 2006; Lee et al., 2009).

Previously, *Citrobacter freundii* was reported in gut microbiota of American bull frogs (Miles et al., 2004), internal organs of diseased *Rana dybowskii* (Jeong et al., 2014) and as agent of the red-leg sydrome (Pasteris et al., 2011). In this study, the presence of *Citrobacter freundii* in both frog and water samples indicates, that it can be an opportunistic pathogen for *P. ridibundus*. *Edwardsiella tarda,* the etiological agent of edwardsiellosis of catfish and eel (Austin and Austin, 2007), was detected in both frog and water samples of four-limbed tadpoles, baby and juvenile marsh frog pools. *Klebsiella pneumoniae ozaenae*, isolated in both tadpoles and feed in this study, has been reported

Table 6. Resistance, susceptibility and MAR index values of the isolates

No	Gram negative rod-shaped bacteria	n	R	I	S	R %	S %	MAR
1	*Acinetobacter haemolyticus*	1	8	1	6	53.3	40.0	0.53
2	*Aeromonas* spp.	10	67	15	68	44.7	45.3	0.45
3	*Bordetella hinzii*	1	6	1	8	40.0	53.3	0.40
4	*Chryseobacterium indologenes*	1	12	0	3	80.0	20.0	0.80
5	*Citrobacter* spp.	2	13	4	13	43.3	43.3	0.43
6	*Delftia acidovorans*	1	6	2	7	40.0	46.7	0.40
7	*Edwardsiella tarda*	1	5	1	9	33.3	60.0	0.33
8	*Elizabethkingia meningoseptica*	1	8	2	5	53.3	33.3	0.53
9	*Enterobacter asburiae*	1	6	1	8	40.0	53.3	0.40
10	*Escherichia coli*	2	12	1	17	40.0	56.7	0.40
11	*Klebsiella* spp.	2	8	3	19	26.7	63.3	0.27
12	*Morganella morganii*	1	5	2	8	33.3	53.3	0.33
13	*Pantoea* spp.	2	8	5	17	26.7	56.7	0.27
14	*Plesiomonas shigelloides*	1	7	2	6	46.7	40.0	0.47
15	*Providencia rettgeri*	1	8	1	6	53.3	40.0	0.53
16	*Pseudomonas* spp.	6	55	7	28	61.1	31.1	0.61
17	*Salmonella* spp.	5	36	11	28	48.0	37.3	0.48
18	*Serratia* spp.	2	13	2	15	43.3	50.0	0.43
19	*Shewanella putrefaciens*	1	5	1	9	33.3	60.0	0.33
20	*Sphingomonas paucimobilis*	1	8	3	4	53.3	26.7	0.53
21	*Sphingobacterium thalpophilum*	1	8	2	5	53.3	33.3	0.53
22	*Stenotrophomonas maltophilia*	1	11	0	4	73.3	26.7	0.73
	Total	**45**	**315**	**67**	**293**	**46.7**	**43.4**	**0.47**
	Escherichia coli (ATCC25922)	1	6	0	9	40	60	0.40
	Gram positive rod-shaped sporeforming bacteria							
23	*Bacillus spp.*	13	49	23	123	25.1	63.1	0.25
24	*Brevibacillus choshinensis*	1	2	1	12	13.3	80.0	0.13
25	*Lysinibacillus sphaericus/fuciformis*	1	3	0	12	20.0	80.0	0.20
	Total	15	54	24	147	24	65.3	0.24
	Gram positive cocci-shaped non-sporeforming bacteria							
26	*Aerococcus viridans*	1	3	2	10	20.0	66.7	0.20
27	*Enterococcus gallinarum*	1	3	5	7	20.0	46.7	0.20
28	*Granulicatella adiacens*	1	7	1	7	46.7	46.7	0.47
29	*Kocuria spp.*	3	18	10	17	40.0	37.8	0.40
30	*Micrococcus luteus/lylae*	4	9	7	44	15.0	73.3	0.15
31	*Staphylococcus spp.*	2	8	4	18	26.7	60.0	0.27
	Total	12	48	29	103	26.7	57.2	0.27
	Enterococcus faecalis (ATCC29212)	1	5	2	8	33.3	53.3	0.33
	Staphylococcus aureus (ATCC25923)	1	1	1	13	6.6	86.6	0.06

n: number of isolates, S: susceptibility, R: resistance, I: intermediate

to cause haemorrhage, ulcer and reddening of the abdomen in brown tree frogs (*Litoria ewingii*) (Mauel et al., 2002). As a matter of fact, these coliforms are widespread in nature, microbiota and opportunistic pathogens of humans and animals (Holt et al., 2000).

The most dominant species isolated in liver, lung and / or blood specimens of juvenile and adult frog specimens were *Salmonella* spp. They have also been detected in pool water of juvenile frogs. It is remarkable that *Salmonella* spp. appeared only in adult and juvenile frogs. Salmonellae, which have only two species and more than 2500 serotypes (LPSN, 2017), are mostly pathogenic to human and cause severe infections ranging from simple gastrointestinal disorders to death (Holt et al., 2000). Although common in nature, intestines of humans, warm-blooded and cold-blooded animals, *Salmonella* spp. are known nonpathogenic for aquatic animals (CFSPH, 2013). In fish disease analysis, bacteria can be detected in internal organs only in presence of an infection. Therefore, the relationship of *Salmonella* and frogs should be investigated, because of their presence in liver and lungs. Since only the internal organs of adult frogs were examined in this study, it would be appropriate to investigate also the meat of frogs for *Salmonella* spp. and other potentially pathogenic bacteria.

Acinetobacter haemolyticus, *A. hydrophila / caviae* (Miles et al., 2004; Pasteris et al., 2006), *C. freundii*, *E. coli*, *Klebsiella* sp., *Plesiomonas shigelloides*, *Pseudomonas aeruginosa*, *Ps. putida, Bacillus cereus / mycoides / thuringiensis* and *Micrococcus* sp. (Miles et al., 2004), reported from the intestines of the healthy American bull frogs, were also detected in this research. The findings of *A. hydrophila / caviae*, *C. freundii* (Hacioglu and Tosunoglu, 2014; Hacioglu et al., 2015), *E. coli*, *K. pneumoniae ozaenae* (Hacioglu and Tosunoglu, 2014) and *Salmonella* spp. (Hacioglu et al., 2015), isolated by mouth and cloacal swaps of wild frogs in our country (Hacioglu and Tosunoglu, 2014; Hacioglu et al., 2015), were identical to ours. Taylor et al. (2001) reported that *Enterobacter* sp., *E. coli*, *K. ozaenae*, *Plesiomonas shigelloides*, *Salmonella* spp., *B. cereus / mycoides / thuringiensis*, *B. megaterium*, *Micrococcus* sp. and *Staphylococcus* spp., also present in our findings, are usually nonpathogenic to amphibians due to their presence in their normal microbiota. Nevertheless, it has been emphasized that these bacteria should be considered as causative agents, if detected in blood or coelomic cavity or at very high rates (Taylor et al., 2001). Thus, *Acinetobacter* sp. (Jeong et al., 2014), *A. hydrophila/caviae* (Mauel et al., 2002; Huys et al., 2003), *Aeromonas* sp. (Amborski et al., 1983), *Chryseobacterium indologenes*, *Citrobacter braakii*, *E. tarda* (Tee and Najiah, 2011), *Enterobacter* sp., *E. coli* (Pasteris et al., 2009) and *Pseudomonas* sp. (Amborski et al., 1983; Tee and Najiah, 2011) were reported in diseased frogs. In contrast, Schadich and Cole (2010) found that *A. hydrophila/ caviae* was harmless to *Litoria ewingii*. Although lack of serious findings of red-leg syndrome, previous reported etiologic agents like *A. hydrophila* (Pilarski and Schocken-Iturrino, 2010; Pasteris et al., 2011; Tee and Najiah, 2011; Jeong et al., 2014; Xiaoying et al., 2015), *E. tarda*, *Chryseobacterium indolgenes*, *Pseudomonas* spp. (Tee and Najiah, 2011) and *K. pneumoniae* (Schadich and Cole, 2010) were also identified in this study. It was reported that *Staphylococcus* species, found in the pool water of tadpoles (*S. aureus*) and juvenile frogs (*S. equorum*) in the present study, cause general edema and whirling diseases in farmed bullfrogs (FAO, 2016). Nevertheless, the pathogenic effects of these bacteria on marsh frogs should be investigated. It is obvious, that most of the isolates of this investigation are natural microbiota of water and frog specimens. However, it should be taken into consideration, that many bacteria seize opportunities in stress situations. For this reason, the pathogenicity and virulence of these isolates should be determined in cultivated frog species.

Despite resistance problems, antibacterial drugs are still used extensively in the treatment of bacterial diseases. Lee et al. (2009), detected resistance to lincomycin (90-95%), amoxicillin (72.5-80%), oxytetracycline (70-75%), erythromycin (65-75%), sulfamethoxazole (47.5-42.5%), doxycycline (47.5-50%) and florfenicol (10-0%) against *Aeromonas* spp. and *Edwardsiella* spp. isolated from the internal organs of cultivated *Rana catesbeiana*, respectively. Similarly, all isolates from reared American bull frogs showed resistance to lincomycin (92%) and high sensitivity to florfenicol and doxycycline (Tee and Najiah, 2011). Our findings of high resistance (93.3-100%) to lincomycin and high sensitivity to doxycycline (90.3%) and florfenicol (75%) were close to these investigations (Table 5).

The MAR index values of all isolates were ranging from 0.13 to 0.73. These values were changing between 0.27-0.73 for Gram negative rod-shaped bacteria, 0.13-0.25 for Gram positive rod-shaped sporeforming bacteria and 0.15-0.47 for Gram positive cocci-shaped non-sporeforming bacteria (Table 6). Although, no antibacterial medication was applied during this study, except enrofloxacin once in the larval stage, the MAR index values of 26 of 31 (83.9%) isolate species were quite high, especially all Gram negative rod-shaped bacteri were higher than 0.20 (100%) (Table 6). Yet, high sentivity (97.8%) of enrofloxacin was detected for Gram negative rod-shaped bacteri (Table 5). MAR index results for *Aeromonas* spp. and *Edwardsiella* spp. (0.45 and 0.33) (Table 6) showed similarity to Lee et al. (2009) (0.27 and 0.31, respectively). Antibiogram and MAR values of Gram negative bacteria detected from wild

frogs have been studied previously in Turkey (Hacioglu and Tosunoglu, 2014; Hacioglu et al., 2015). Hacioglu and Tosunoglu (2014) reported 6.5-46.6% resistance for all Gram negative bacteria and detected 35%, 28%, 19%, and 6% resistance for erythromycin, amoxicillin, oxytetracycline and gentamicin, respectively. Hacioglu et al. (2015) determined the resistance values of the same drugs as 60-85%, 50-80%, 25-55%, and 10-20%. The results of the present study for Gram negative rod-shaped bacteri were 0-97.8% in general, and showed 68.9%, 75.6%, 33.3%, and 20% resistance of the reported drugs, respectively (Table 5). These findings were higher than the values of Hacioglu and Tosunoglu (2014), but congruent to Hacioglu et al. (2015). These inconsistencies can be caused by the variety of frog species, habitat and environment conditions. Whereas MAR index values of Gram negative bacteria of wild frogs were between 0-0.58 (Hacioglu and Tosunoglu, 2014), higher values (0.27-0.73) were detected in reared marsh frogs in our study.

It was pointed out, that reptiles and amphibians, which harbor highly antimicrobial resistant bacteria, can threaten the health of aquatic animals and humans (Hacioglu and Tosunoglu, 2014). Although there was no regular application of drugs in the marsh frog farm, the reasons for the high antibacterial resistance have to be investigated seriously.

CONCLUSION

In this study, presence of opportunistic pathogenic bacteria in frog, water and feed and specimens have been detected in the marsh frog farm in Mersin. Most of these bacteria were previously reported as agents of some diseases of vaious frog species. Some are also zoonotic and can pose risk for even human health. As far as it is known, this is the first microbiological study on farmed marsh frogs. Further investigations are necessary for the development of sustainable marsh frog culture and for protection of health, both frog and human.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Analysis of profitability of shrimp value chain in Delta State, Nigeria

Felix Odemero Achoja

Department of Agricultural Economics and Extension, Faculty of Agriculture, Delta State University, Asaba, Nigeria https://orcid.org/0000-0002-9705-4923
lixmero40@yahoo.com

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Abstract: This study investigated the profitability of shrimp value chain. Primary data were obtained using well structured questionnaire from randomly selected 240 shrimp operators (harvesters, processors and marketers). Descriptive and inferential statistical tools were used to analyze collected data. The result revealed that the mean profit realized was N70,092 N36,255 and N26,097.30 by harvesters, processors and marketers respectively. Value added at was N500 and N1000 per basket by processors and marketers respectively. Test of hypothesis indicates that quantity sold (0.218), price (0.033), bargaining power (0.002) and union dues (0.099), showed positive and significant relationship with shrimp value chain profitability. While market levy and store rent showed negative relationship with profitability in shrimp value chain. It was recommended that there should be moderate store rent. Marketers should increase their bargaining power at the point of procurement and union dues should be reduced so that shrimp value chain profitability can be enhanced.

Keywords: *Profitability, constraints, shrimps, value chain, Delta State*

INTRODUCTION

The beginning of shrimp farming is unknown. Some say shrimp farming began in China while others say it began in Japan. There are 600,000 people employed in shrimp cultivation; and industry earns $301 million per year, (GOB, 2002).

The aquaculture industry has grown significantly over the years, although its full potential has not yet been realized. More and speedy development is required to keep up with the growth in demand for shrimps in the world. Nigeria is among tropical countries endowed with rich shrimp resources. According to Dublin-Green and Tobor (1992), the coastal waters of Nigeria are characterized by abundance of important living resources including shrimps, predominantly members of the family penaeidae. With a production capacity of 12,000 metric tons (MT) per year, Nigeria's shrimps supply is presently from capture fisheries. Increasing human population and the soaring per capita demand for shrimp has created a demand-supply gap.

Nigeria is one of the countries with abundance natural supply of Shrimps with an annual production of 12,000 metric tons. However, with the decline in global wide capture there is a need to supplement production with Shrimps aquaculture and Nigeria is regarded as a potential frontier in Shrimp farming because of its numerous coastal and mangrove areas. Fish including shrimp provide 22% of the protein intake in Sub-Saharan Africa, and has been dubbed "rich food for poor people" (Bene and Heck 2005).

Shrimp value chain has been recognized to perform critical role in economic development of nations, including Nigeria; consequently, many reforms are being carried out to develop agricultural value chain in Nigeria. Such reforms, as it relates to shrimp value chain would produce or generate very little or no outcome unless an extensive empirical study is conducted to described the shrimp value chain, by identifying the relevant socio-economic characteristics of actors and actions in the chain.

As it stands, efforts must be made to analyze the profitability in the shrimp value chain. This is because profit is the reward for business activities. There is the need to find out whether or not the profit earned by actors in shrimp value chain justified the efforts and cost invested in the business.

It is a common knowledge that the shrimp value chain is operated numerous economic agents

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producers, processor, marketers etc and they earn different levels of profit in the process. Before now there is no study to the best of my knowledge that analyzed the profit differentials in the shrimp value chain in the study area. Analyzing the profit differentials in the chain will shed some light on the sustainability of the value chain over time and space. Profit is the motivation for doing business and where it is evenly distributed in a value chain system the operators, will be happy to continue to operate in their various lines of specialization.

Sustainable profit in the shrimp value chain could be subject to some exogenous factors. The assumptions underlying the relationship between profitability and the underlying factors are yet to be investigated and ascertain. The knowledge of these underlying factors could be used to predict the profitability in shrimp value chain with relative certainty.

Furthermore, the shrimp value chain like other value chain, could be impaired by some constraints. It is important to identify these constraints for the purpose of isolating the more serious ones for appropriate policy actions that can improve the shrimp value chain in the study area.

This is obvious from the fact that shrimp market holds daily in the area. The greater percentage of the local people depends on the shrimp industry for their livelihood (some as producers, marketers and transporters). The shrimp industry contributes to the quality and quantity of protein consumed by the citizenry.

Despite the significance of the shrimp industry, there is no study conducted to assess the level of profitability or otherwise of this venture. This necessitates a comprehensive study on the shrimp value chain in the study area. The empirical information on profitability and constraints is useful to investors in the shrimp value chain (shrimp harvesters, processors, marketers, credit administrators) by identifying problem areas, prospects and potential areas of improvements.

The broad objective of the study is to analyze the profitability and constraints of shrimp value chain in Delta State, Nigeria. The specific objectives of the study are to:ascertain the level of profitability in shrimp value chain

i. compare the level of profit earned by the actors in the shrimp value chain in study area

ii. Assess the value added in shrimp value chain in the study area.

iii. determine the factors that influenced profitability in shrimp value chain

The following hypotheses were formulated and tested to guide the study:

Ho_1: There is no significant difference in the profitability among actors in shrimp value chain.

Ho_2: The selected market indicators do not have significant effect on profitability in the shrimp value chain

CONCEPTUAL FRAMEWORK AND LITERATURE REVIEW

Concept of value chain

The value chain analysis involves breaking an industry business chain into its constituent parts for better understanding of its structure and functioning. "The analysis consists of identifying chain actors at each stage, their functions and relationships; determining the chain governance, to facilitate chain formation and strengthening; and identifying value adding activities in the chain and assigning costs and value added to each of those activities" (UNIDO, 2009). According to Kaplinsky and Morris (2001), "The value chain describes the full range of activities which are required to bring a product or service from conception, through the different phases of production (involving a combination of physical transformation and the input of various producer services), delivery to final consumers, and final disposal after use".

Value chains provide the framework for designing and implementing many development programs and projects. Given a multitude of different arenas of application, geographical locations, commodity types, target groups and desired outcomes, a variety of closely related conceptualizations of value chains has emerged (Stamm and Von Drachenfels 2011). For the purpose of this study, we define a value chain as "the full range of activities and services required to bring shrimp from its farmers for sale to its final markets" (Microlinks 2012). A value chain, thus, encompasses the entire network of actor's involved input supply, production, processing, marketing and consumption.

Analytical Framework

Value chains analytical framework provides a valuable visual framework for understanding the structural connective tissue linking small scale shrimp farmers with input suppliers, processors, traders and final consumers.

MATERIALS AND METHODS

Study Area

The study was conducted in Delta State, Nigeria. This study area was chosen for the study because there is a good number of people who base their

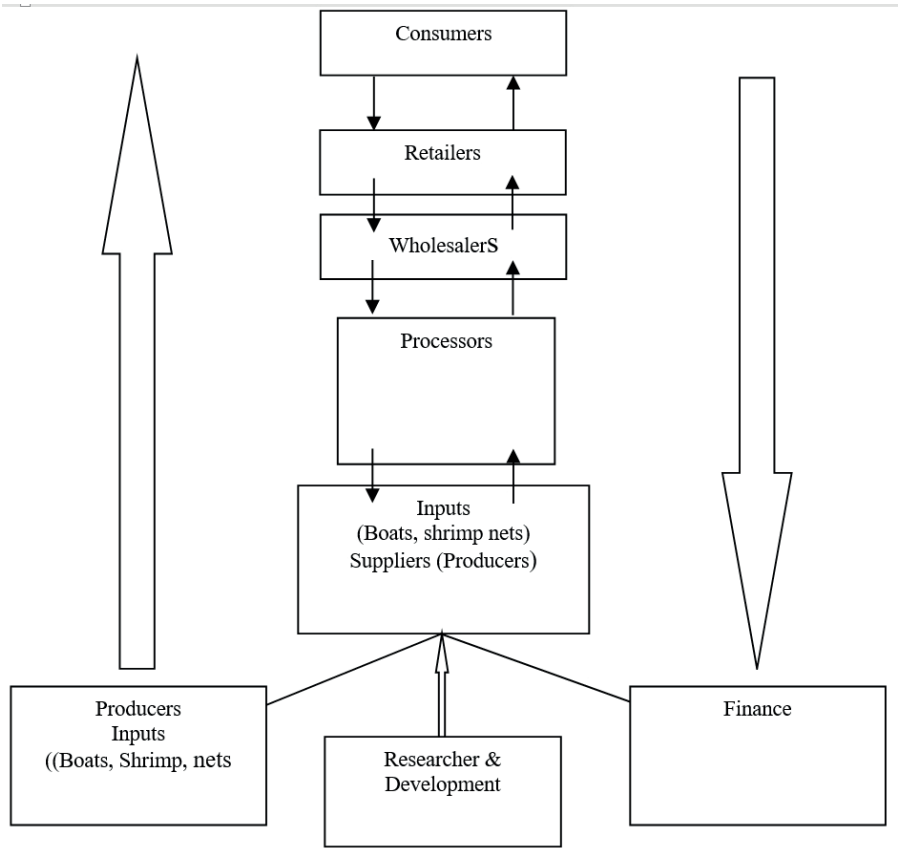

Figure 1. A value chain marketing system (Source: Research in US (RIU) undated)

livelihood on shrimp related business in the area. It has an estimated land area of 1,722 km^2 and lies between latitudes $5^0$2859.7 N and longitude $5^0$44.04.6^{0}E population of one hundred and sixteen thousand six hundred and eighty-one (116,681) from the census of 2006 (NPC 2006) but this population has grown since then. Delta State is home to the Urhobos, Isoko Warri and Itsekiri entropic group in. The Ijaw Inhabit Ogbe-Ijoh, Gbaramatu, Isaba and diebiri communities while the Itsekiris Inhabit the Ugborodo and Madangho communities. The popular languages spoken by the people are English, Urhobo, Isoko, Ijaw and Itsekiri. It is located in the mangrove swamp forest of Delta State with rainfall ranging from about 2500mm – 2800mm per annual. The Economic activities in Area are growing of crops like maize, plantain and vegetables. However, fishing activities including shrimp production is the dominant economic activity of the people.

Population of the Study

The population for the study is all the operators or actors such as shrimp producers, processors and marketers in the shrimp value chain in the study area.

Sampling Techniques and Sample Size

A multiple sampling techniques were used for study. First, snow ball technique was used to get the key informants. Key informants were interviewed such as the shrimp producers, processor and marketers. The essence is to obtain the list of registered shrimp marketers (sampling frame).

Secondly, sampling frame (a list of operators) were considered. From this list, systematic sampling technique was employed to draw the sample of 240 respondents. The respondents were sampled as follows. Six communities were involved in the study. In each community, 10shrimp producers, 10 processor and 20 marketers were sampled. This gave a total of 240 respondents.

Methods of Data Collection

Primary data were used for the study. Questionnaire was used to collect data. The questionnaire was made of

five parts; socio-economic characteristics of operators in the shrimp value chain. Level of profitability earned in the shrimp value chain, constraints by the actors in the shrimp value chain. Factor influencing the profitability in the shrimp value chain.

Methods of Data Analysis Techniques

The data for this study were analyzed using both descriptive and inferential statistics.

Objective I:Ascertain the level of profitability in shrimp value chain

This objectives was achieved using profit function as stated below

$Ni = TR - TC$ ……..(1)

Where:

Ni = Net income (naira)

TR = Total revenue (naira)

TC = Total cost (total variable cost + total fixed cost)

Objective II: Compare the level of profit earned by the actors in the shrimp value chain in study area. This objective was achieved using Analysis of variance (ANOVA) of profit.

Objective III: Determine the factors that influenced profitability in shrimp value chain. This objective will be achieved using ordinary least square technique of multiple regression.

Model Specification

The implicitly form of multiple regression model takes the form

$Y = f(X_1, X_2, \ldots, Xn) + \mu$……(2)

The explicit form of the linear function takes the form:

Linear Function

$NP = \beta_0 + \beta_1 QTY + \beta_2 TRP + \beta_3 DIS + \beta_4 PRC + \beta_5 MKL + \beta_6 STR + \beta_7 WES + \beta_8 CMM + \beta_9 COP + \beta_{10} UND + \beta_{11} BGP + \mu$ ……. (3)

Semi – log Function

$NP = \log\beta_0 + \beta_1 \log QTY + \beta_2 \log TRP + \beta_3 \log DIS + \beta_4 \log PRC + \beta_5 \log MKL + \beta_6 \log STR + \beta_7 \log WES + \beta_8 \log CMM + \beta_9 \log COP + \beta_{10} \log UND + \beta_{11} \log BGP + \mu$ ……. (4)

Double log Function

$\log NP = \log\beta_0 + \beta_1 \log QTY + \beta_2 \log TRP + \beta_3 \log DIS + \beta_4 \log PRC + \beta_5 \log MKL + \beta_6 \log STR + \beta_7 \log WES + \beta_8 \log CMM + \beta_9 \log COP + \beta_{10} \log UND + \beta_{11} \log BGP + \mu$ ……. (5)

Where:

NP = Net profit (₦)

QTY = Quantity of shrimp produced, processed, and traded (basket)

TRP = Transportation cost (₦)

DIS = Distance to the nearest buyer (km)

PRC = price of shrimp/basket (₦)

MKL = Market Levy (₦)

STR = Store rent (₦)

WGS = Wages (₦)

CMM = Communication (₦)

COP = Cost of Packaging (₦)

UND = Union Dues (₦)

BGP = Bargaining power (rating from strong to weak

μ = Stochastic Error term

β_0 = Constant or Intercept term

β_1- β_{11} = Regression co-efficient of respective variables

RESULTS AND DISCUSSION

The result showed the profitability among shrimp value chain operators . The different profit earned by the (producers, processors, marketers). The highest profit earned by the actors in the shrimp value chain is the production in Table 1.

Value added in shrimp value chain in the study area

The result showed the value addition at the different levels of the value chain the Table.2.

The analysis of variance (~~Anova~~) (ANOVA) indicates that there is significant different at the level of profit earned by the operators in the shrimp value chain (producers, processors and marketers). The null hypothesis which states that there is no significant difference in the level of profit, in the shrimp value chain is reject and the alternative hypotheses which states that there is significant difference in the level of profitability among operators in the shrimp value chain is accepted. This finding implies that different operators perform different functions and as a result profit earned vary significantly in the value chain.

Table 5 shows the result of the relationship between dependent variables. Shrimp value chain and the independent variable. Shrimp value chain. The shrimp

value chain (quality sold, price, market levy, store rent and bargaining power). The Linear model was chosen as the best model because it has highest R^2 value of 0.513 (51%). This implies that 51% of variation in profit earned by operators in shrimp value chain was explained by the joint effect of the exogenous variables in the model.

Testing of Hypothesis

H_o: *The selected social-economic variables do not have significant effect on the profitability in the shrimp value chain*.

The null hypothesis which states that the selected socio- economic variables do not have significant effect on the profitability in shrimp value chain in Delta state was rejected and the alternative accepted. It indicates that five (5) selected shrimp operators; quantity sold (.027) *** (-68992.465), price (033)*** (23439.717), store rent (015)*** (-015.931), bargaining power (002)*** (68036.162) and union dues (009)*** (42272.222) showed positive and significant relationship with profit earned by operators in the shrimp value chain in Delta state. While market levy paid by operator in the shrimp value chain showed a negative relationship with the profit earned by operators in shrimp value chain.

At this point, the results of the statistical significance of the individual explanatory variables in the model are discussed as follows.

Quantity Sold

The result of the study showed that quantity sold. (0.027)*** has a positive and a significant relationship with profitability of the shrimp value chain in Delta state. The Bata weight as seen in the Table 5 showed that quantity sold (with B = 68992.456:P < 0.001) is a positive predictor of profitability in the value chain. The positive value of beta coefficient indicates that an increase in the quality sold will also lead an increase in profitability in the value chain.

Price

The result of the study showed that price (0.033)*** has a positive and a significant relationship with the profitability of shrimp value chain in Delta state. The Beta Weight as seen in the Table 5 showed that price (with B = 23439.717:P< 0.001) is a positive predictor of profitability of the value chain. The positive value of Beta coefficient, indicate that an increase in the price, price will also lead an increase in the profit earned by operators in the value chain. Price is an important

Table 1. Distribution of Descriptive statistics of profit among shrimp value operation

Operators	Total	Mean	Min	Maximum
Producers	₦2,803,700	₦70,092.50	₦22,400	₦108,100
Processors	₦1,450,200	₦36,255.	₦22,800	₦65,700
Marketers	₦107,892	₦36,97.30	₦10,800	₦59,300

Table 2. Distribution of value Addition per basket in shrimp value chain in the study aria

Producers price/basket		Processor price/basket		Marketers price/basket
₦3,000	→	₦3,500	→	₦4,500
Value added =₦500	→	₦1000		

Table 3: ANOVA Test of Significant Difference in Profit among actors in shrimp value chain

Source of Variation	SS	df	Ms	F	P-value	F crit
Between Groups	4.42E+10	2	2.21E+10	71.10319	6.174E-21**	3.073763
Within Groups	3.64E+10	117	3.11E+08			
Total	8.06E+10	119				

Table 4: Constraints in shrimp value chain in Delta state, Nigeria

	Variables	Total	Mid point	Remark
A	Inadequate finance	55.7	4.64	Serious
B	Inadequate access to market	339	2.82	Not serious
C	Low price of shrimp	328	2.73	Not serious
D	Lack of fishing inputs	346	2.88	Not serious
E	Lack of storage processing facilities	409	3.40	Serious
F	Availability of working capital	419	3.49	Serious
G	Dictate price based on local market	429	3.59	Serious
H	Adulteration	296	2.46	Not serious
I	Inadequate government regulatory measure in the value chain	196	1.63	Not serious
J	Quality of shrimp supplied in terms of size	313	2.60	Not serious
k	Variation and adulteration	355	2.96	Not serious
i	Inadequate technology and knowledge transfer also operation	232	1.93	Not serious
m	Lack of co-ordination in the value chain	288	2.40	Not serious

a. **Inadequate Facilities:** The result indicates that inadequate facility is a serious constraint in the study area in shrimp business. This is so because mid-point (4.64) and is greater than the cut-off (3.00).

b. **Inadequate access to Market:** The finding indicates that inadequate access to market is not a serious constraint in the study area in the shrimp business. This is so because the mid-point is (2.82) and is lesser than the cut-off (3.00).

c. **Low Price of Shrimp:** The finding indicates that low price of shrimp is not a serious constraint in the study area in the shrimp business. This is so because the mid-point is (2.73) and is lesser than the cut-off (3.00).

d. **Lack of Fishing Inputs:** The result indicate that lack of fishing inputs is not a serious constraint in the study area in the mid-point is (2.88) and is less than the cut-off (3.00).

e. **Lack of Storage Processing Facilities:** The result shows that lack of storage processing facilities is a serious constraint in the study area in the shrimp business. This is so because the mid-point (3.40) and is greater than the cut-off (3.00).

f. **Availability of Working Capital:** The result indicate that availability of working capital is a serious constraint in the shrimp business. This is so because the mid-point is (3.49) and is less than the cut-off (3.00)

g. **Dictate Price based on local Market:** The result indicate that dictate price based on local market is a serious constraint in the shrimp business because the mid-point is (3.57) and is greater than (3.00).

h. **Adulteration:** The result indicates that Adulteration is not a serious constraint in the shrimp business in the study area, because the mid-point is (2.46) and less than (3.000).

i. **Inadequate government regulatory Measure in the Value Chain:** The finding indicates that inadequate government regulatory measures in the value chain is not serious constraint in the study area because the mid-point is (1.63) and is less than (3.00).

j. **Quality of Shrimp Supplied in term of size:** The result indicates that quality of shrimp supplied in terms of size is not a serious constraint because the mid-point (2.60) and is less than (3.00).

k. **Variation and Adulteration:** The finding shows that variation and adulteration is not a serious constraint because the mid-point (2.96) and is less than (3.00).

l. **Inadequate Technology and Knowledge Transfer among Operators:** The finding indicates that this constraint is not serious in the shrimp business because the mid-point is (1.93) and less than (3.00).

m. **Lack of Co-ordination in the Value Chain:** The finding indicates that lack of co-ordination is not a serious constraint in the shrimp business in the study area because the mid-point (2.40) and is less than (3.00).

Table 5. Factors that influenced profitability in shrimp value chain

Model Summary

Model	R	R^2	Adjusted R^2	Standard error	
Linear	.716a	.513	.342	12.33936	
Semi log	.696a	.484	.329	26741.82096	
Double log	.677a	.458	.296	.62505	
Linear	Sum of square	Df	Mean of square	F	Sig
Regression	142740452102	7	2039149315.74	3.007	.025
Residual	13563461575.5	20	678173078.775		
	27837506788.7	27			

Model	Unstandardized coefficients		Standardized coefficient	t-cal	Sig
	B	Std.Error	Beta		
(Constant)	-165067.824	129847.812		-1.271	.218
Quantity sold	-68992.456	28846.330	-840	-2.392	.027**
Price	23439.717	10208.634	.560	2.296	.033**
Market levy	-10191.801	41301.807	-058	-2.47	.808
Store rent	-53015.931	19994.711	-802	-2.651	.015**
Bargaining power	68036.162	19047.043	1.311	3.572	.002**
Union dues	42272.222	14489.949	.610	2.913	.009**

a. dependent variable: Net profit

b. predictors: quantity sold, price, market level

store rent, bargaining power

*** = significant at 1%.

component of a marketing plan as it determines firm's profit and survival. Consumers tend to buy more from marketers whose products are cheap. And so, if the price of the shrimp is too high, consumers tends to buy less which will turn affect the sales return of the business. The more flexible, proper and better the shrimp marketers uses pricing strategy, the higher their sales revenue.

Store Rent

The result of the study showed that store rent (0.015)*** has a negative and significant relationship with the operator's profit in the shrimp value chain in Delta state. The beta weight as seen in the Table 5 showed that store rent (With B= -53015.931:P< 0.001) is a negative predictor of profitability of actors in the value chain. The negative value of the coefficient indicates that an increase in the store rent will lead also a decrease in the profit of actors in shrimp value chain. Operators should not pay too much on store rent especially, considering the size of their businesses because high store rent tends to increase total cost of doing business thereby reducing net profit earned.

Bargaining Power

The result of the study shows that bargaining power (0.002)*** has a positive significant relationship with the profit earned by shrimp value chain operators in Delta state. The beta weight as seen in Table 5 shows that Bargaining power (With B = 36.162: P < 0.001) is a positive predictor of the profitability of actors in the value chain. The positive value of beta coefficient indicates that 36.162% increase in the bargaining power will lead also a 1% increase in the profitability in the

shrimp value chain. Bargaining power is an important economic concept that measures the ability of trading parties in negotiation to influence each other. It is an important profit predictor because trading parties with higher bargaining power enjoys better leverage.

Union Dues

The result of the study showed that union dues has a positive and significant relationship with the shrimp operators in the value chain in Delta state. The beta weight as seen in Table 5 showed that union dues (with $\beta = 72.222$, $P < 0.001$) is a positive predictor of profitability of operators in the value chain. It indicates that an increase in the union dues will also lead an increase in profit of operators in the shrimp value chain. Union dues are the cost of membership to belong to shrimp market union. The dues paid to the union by its membership help to pay for the cost of their activities, education or events engaged in by the union members. Being a member of shrimp market union protects the operator's operational existence. Human Resources Information, (2010) had earlier emphasized thatunion dues are unreimbursed employee expenses necessary for union membership maintenance.

CONCLUSION

This study analyzed shrimp value chain in the study area Delta state. The study identified three major actors in the shrimp value. They are shrimp producers/farmers, shrimp processors and shrimp marketers. Shrimp value chain producers mean profit (N70,092.50), minimum (N22,400) and maximum (N708,100) for processor mean profit (N36,255) minimum (N22,800) and the maximum (N65,700) marketer (N26,97.30) minimum (N10,800) and maximum (N59,300). The profitability of shrimp value chain was influenced by quantity sold (0.027)*** price (0.003*** store rent (0.015)*** bargaining power (0.002)*** union dues (0.007)*** if these factors are taken care of, the shrimp value chain in Delta State Nigeria will improve. The study suggests that to ensure steady improvement and survival of shrimp value chain the industries the identified constraints such as inadequate finance, lack of storage facilities and inadequate working capital should be taken care of by the relevant stakeholder in shrimp value chain.

On the basis of the findings, the following recommendations were made:

1.There should be moderate store rent, marketers should increase their bargaining power at the point of procurement and union dues should be reduced.

2.Shrimp marketers should add processing activities to their operations, so as to enhance their profit level substantially.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Detection of feeding dietary *Rhizostoma pulmo* (Macri, 1778) in Samsun coasts of the Black Sea, Turkey

Karadeniz'in Samsun kıyılarında *Rhizostoma pulmo* (Macri, 1778) türünün beslenme rejimi tespiti

Memet Ali Dönmez[1]* ● Levent Bat[2]

[1] University of Ondokuz Mayis, Faculty of Science and Letters Department of Biology TR55139 Kurupelit / Samsun, Turkey https://orcid.org/0000-0002-1795-8667
[2] University of Sinop, Fisheries Faculty, Department of Hydrobiology, TR57000 Akliman / Sinop, Turkey https://orcid.org/0000-0002-2289-6691
*Corresponding author: benefsic@gmail.com

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Abstract: In this study, gastric contents of the medusa *Rhizostoma pulmo* distributed along the Samsun coast of the Black Sea were investigated between August 2008 and January 2010. Moreover, the relationship between the umbrella diameter and prey selectivity was determined. Captured *R. pulmo* individuals during the sampling period varied from 14.5 to 42.5 cm in umbrella diameter. The largest umbrella diameter was observed in October in both periods of the present study. Gastric contents of total 231 *R. pulmo* individuals have been gathered during the sampling period from all stations. 31 taxa, 10 larvae, 2 nauplii and fish and Copepod eggs from 11 phyla have been identified in the gastric contents of *R. pulmo* individuals. It has been detected that, feeding choices of *R. pulmo* individuals have differentiated and their food count has increased due to increasing umbrella diameter (according to Spearman rank correlation, r=0.70; p<0.05). According to the gastric content analyses, the feeding dietary of this species predominantly consists of copepods and dinoflagellates. It has been observed that taxa belonging to Crustaceans (59 %; Copepods 45.9 %) and Dinoflagellates (15.4 %) were densely present in the gastric contents gathered from *R. pulmo* individuals. The Phylum Ciliophora was another abundantly observed group in the gastric content of *R. pulmo*, with a 7.2 % representation rate. It was determined that jellyfish have a wide range of nutrition from phytoplankton to fish eggs. The sort of prey and amount of nutrition in stomach contents increased in parallel with the umbrella size. It is concluded that *R. pulmo* has important effects on the pelagic zone of the Black Sea ecosystem.

Keywords: *Rhizostoma pulmo, Black Sea, Samsun coasts, jellyfish, gastric content, umbrella*

Öz: Bu çalışmada Karadeniz'in Samsun kıyıları boyunca dağılan *Rhizostoma pulmo* medüzünün mide içeriği Ağustos 2008 ile Ocak 2010 arasında araştırılmıştır. Ayrıca şemsiye çapı ve av seçiciliği arasındaki ilişki belirlenmiştir. Örnekleme dönemi boyunca yakalanan *R. pulmo* bireylerinin şemsiye çapı 14,5 ila 42,5 cm arasında değişmiştir. En büyük şemsiye çapı, bu çalışmanın her iki döneminde de Ekim ayında gözlenmiştir. Örnekleme döneminde tüm istasyonlardan toplam 231 *R. pulmo* bireylerinin mide içerikleri toplanmıştır. *R. pulmo* bireylerin mide içeriğinde 31 takson, 10 larva, 2 adet nauplii ve 11 adet filumdan balık ve Copepod yumurtaları tespit edilmiştir. *R. pulmo* bireylerin beslenme tercihlerinin farklılaştığı ve artan şemsiye çapına göre besin sayısının arttığı tespit edilmiştir (Spearman rank korelasyonuna göre, r = 0,70; p <0,05). Mide içerik analizlerine göre, bu türün beslenme rejimleri ağırlıklı olarak Kopepod ve Dinoflagellatlardan oluşmaktadır. *R. pulmo* bireylerden alınan mide içeriğinde Kabuklulara (% 59; Kopepodların payı % 45,9) ve Dinoflagellatlara (% 15,4) ait taksonların yoğun olarak bulunduğu gözlenmiştir. Ciliophora filumu, *R. Pulmo* türünün mide muhtevasında,% 7,2›lik bir temsil oranı ile bol miktarda bulunan diğer bir grubu oluşturmuştur. Denizanasının, fitoplanktondan balık yumurtasına kadar geniş bir beslenme aralığına sahip olduğu belirlenmiştir. Mide içeriğindeki av türü ve besin miktarı, şemsiye büyüklüğüne paralel olarak artmıştır. *R. pulmo* türünün Karadeniz ekosisteminin pelajik bölgesi üzerinde önemli etkileri olduğu sonucuna varılmıştır.

Anahtar kelimeler: *Rhizostoma pulmo, Karadeniz, Samsun kıyıları, denizanası, mide içeriği, şemsiye*

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INTRODUCTION

The Black Sea ecosystem is the youngest and dynamic ecosystem among the semi enclosed seas in the Atlantic basin (Tokarev and Shulman, 2007) and could be characterized with very low environmental capacity due to its very thin aerobic biotic layer (Proceedings of the 39th European Marine Biology Symposium, held in Genoa, Italy, 21-24 July 2004, 2007). The Black Sea, as being a semi-closed sea, gives the coast to six countries and has a popular status due to its commercial significance and economic potential. The main problems of the Black Sea such as anthropogenic pollution, eutrophication, overfishing and the presence of invasive species, and also climate change make this unique ecosystem unpredictable and unstable in the long term (Sezgin et al., 2010; Bat, 2014; Bat and Özkan, 2015; Bat, 2017).

While the Black Sea is characterized as a highly productive ecosystem at all trophic levels until the middle of 1970s, it was degraded to low diversity that was dominated by gelatinous species in 1990s (Shiganova, 1998; Bat et al., 2011). The number of gelatinous species in the Black Sea ecosystem was reported to be seven (Satılmış et al., 2006; Öztürk et al., 2011). However, recent studies indicate that this is eight (Isinibilir and Yılmaz, 2017; Isinibilir et al., 2017). *R. pulmo*, the biggest gelatinous species in the Black Sea (Golemansky, 2007), was observed at high numbers at the Northwestern Black Sea shores during late 1960s and early 1970s. Then, the population of *R. pulmo* decreased between 1973 and 1974 gradually. For the temporal and spatial records of *Rhizostoma* species, irregular distribution, gross aggregation or blooms have been observed (Lilley et al., 2009). It is widely distributed all over the world oceans, including the Northern and Southern Atlantic Ocean, the Mediterranean Sea, Aegean Sea, Sea of Marmara, the Black Sea, and the Red Sea ("Marine Species Identification Portal : *Rhizostoma pulmo*," 2011). *R. pulmo* is seen at all the coasts of the Mediterranean Sea and the Black Sea (Mariottini and Pane, 2010), whereas there are a few studies available on the distribution of *R. pulmo* at the Southern Black Sea. The deteriorating marine ecosystems, especially coastal waters, have favorable conditions for the excessive increase of jellyfish populations. Eutrophic processes depending on pollution, and rarity or absence of predator species feeding on jellyfishes make these species visible (Parsons and Lalli, 2002). Trophic process of jellyfishes is quite complex. Among pelagic consumer species, jellyfishes could change the food web completely when they reproduce excessively. The Black Sea is one of seas which exposed to the most dramatic changes in terms of jellyfish (Mutlu, 2001). The fall in the yield of anchovy in parallel with introducing of jellyfish to the ecosystem in 1980's, is one of the best examples of this problem. Thereafter, after introduction to the Black sea ecosystem of *Beroe ovata*, which is the other invasive species, this ctenophore has play an effective role in control of the *Mnemiopsis leidyi* populations (Svetlichny et al., 2004; Gordina et al., 2005; Anninsky et al., 2005; Finenko et al., 2006).

Determining of the status in food web comprehensively of *R. pulmo,* which is one of archaic and dominant species in the Black Sea, will contribute to understanding the presence state of the ecosystem as a whole, and to the efforts of future planning.

The main aim of the present study was to determine the possible effects of *R. pulmo* on pelagic community by identifying its stomach content.

In the present study nutrition regime of *R. pulmo* found at the Samsun coasts of the Black Sea was investigated.

MATERIAL AND METHODS

R. pulmo, commonly known as the barrel jellyfish, the dustbin-lid jellyfish or the frilly-mouthed jellyfish, is a scyphomedusae in the family Rhizostomatidae. The sampling of the jellyfish was carried out during cruises of a commercial vessel from August 2008 to January 2010 (excluding January, February, March, Aril, May and June 2009) from Samsun coasts of the Black Sea, Turkey (Figure 1). The samples were collected from 6 stations totally including Yeşilırmak and Kızılırmak catchments.

The samples were collected during daytime with 3 replicates of vertical and horizontal hauls by using plankton net (mesh size= 1 mm, mouth diameter =80 cm, length= 3 m). The samples were hauled from 1-25 m in depth by plankton net. After dissecting of mouth arm of collecting jellyfish by a blade, the umbrella was deposited on a flat lath and its diameter was measured. Samples were then preserved in borax-buffered formalin solution (final concentration 4%) until laboratory analysis. The temperature values of surface water at each station were measured by Sınar brand mercury thermometer.

Preparation of the Stomach Content: The stomach content was achieved by taking the fluid of stomach by an injector. For this aim, the oral arms were taken together with oral clubs around manubrium (oral cavity). Additionally, the fluid on gonads located around the stomach cavity and in oral arms was taken by the injector. Therefore it was implemented only on oral arms of individuals with a large umbrella in diameter (Hyslop, 1980). Collected gastric fluids of all medusa were transferred into 5% buffered formalin and then the remains in these fluids were examined under a NIKON model stereomicroscope and counted.

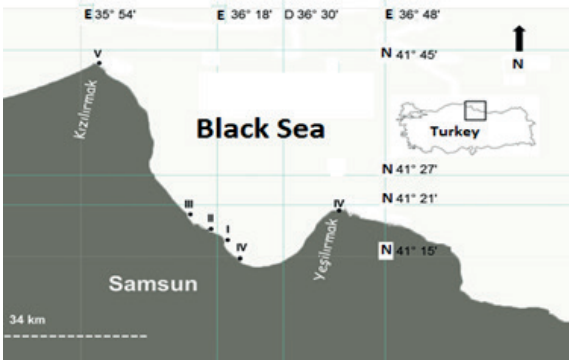

Figure 1. Location of monthly sampling stations along Samsun coasts between April 2008 and March 2010. The stations are represented with roman numbers

Statistical evaluation of morphological characters and digestive physiology data: SPSS v25 packet program was used to evaluation of morphological characters and digestive physiology data. Spearman's rank correlation and Pearson's correlation were used to compare data obtaining from *R. pulmo* samplings.

RESULTS

Hydrological results: Temperature values of all stations reached to maximum level in August. The highest sea-water temperature was determined during the sampling period was 27.1 ^{0}C in August 2009, while the lowest temperature was measured as 7.2 ^{0}C in January 2009. There was no significant differences in terms of temperature values between sampling stations with respect to months (P>0.05).

The Umbrella diameter of *Rhizostoma pulmo* by months: The umbrella diameter of 696 jellyfish trapped throughout the sampling period varied from 14.5 to 42.5 cm (Table 1 and Figure 2). The mean umbrella diameters of *R. pulmo* individuals who start appearing in the end of summer varied between months during sampling period. The mean umbrella diameters of *R. pulmo* individuals were 23 cm in August 2008, while it reached to 26 cm in October 2008. Similarly, the umbrella diameter, which averaged 21 cm in July 2009, increased to 27 cm in October 2009. The mean of umbrella diameter reached the peak level in October, and then decreased toward the winter season when they disappeared.

Table 1. The highest and lowest umbrella diameter and the weight of collected jellyfish by months

R. pulmo	The umbrella diameter (cm)			Weight (g)			The number of sampled stomach content	The number of empty stomach
	The lowest	The highest	Mean	The lowest	The highest	Mean		
Aug. ´08	14.5	29.2	22.93.1	350	2.000	1.100400	23	1
Sep. ´08	19.3	27.4	23.52.3	620	1.670	1.100300	10	0
Oct. ´08	19.7	38.6	26.14.7	710	4.370	1.600800	21	1
Nov. ´08	18.2	32.3	24.13.4	680	2.220	1.200400	15	1
Dec. ´08	18.0	29.0	23.22.9	670	2.060	1.000300	17	1
Jul. ´09	18.5	26.2	21.02.1	720	1.430	900200	21	0
Aug.´09	16.0	33.6	22.22.8	400	2.880	1.000300	23	2
Sep. ´09	19.0	37.6	25.64.5	760	3.870	1.500700	27	3
Oct. ´09	16.8	42.5	27.84.5	350	4.730	1.800800	24	0
Nov. ´09	14.9	34.6	23.93.3	380	3.010	1.200400	25	4
Dec. ´09	19.4	27.0	22.82.2	770	1.840	1.200500	17	3
Jan. ´10	21.3	31.4	25.33.4	750	2.530	1.400600	8	0

Gastric fluid results: During the study period, stomach contents of a total of 231 jellyfish were obtained. A total of 16 stomach contents couldn't be evaluated since they were empty. A total of 31 taxa belonging to 10 larvae, 2 Nauplii and fish and copepod eggs were identified in all the stomach contents of jellyfish (Table 2). The stomach content consisted of 31 taxa, larvae and eggs (Figure 3). The stomach contents were varied highly by seasons on spatial and temporal scales. Also, it was determined that the food preferences varied depending on the stomach diameter and, the number of food type increased. The total number of identified planktonic preys in the stomach of jellyfish was 30129 (Figure 4).

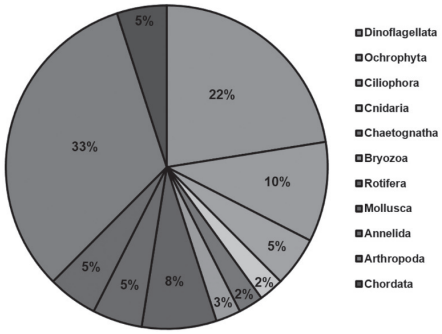

Figure 3. Percentages of Phyla identified in stomach contents of medusa

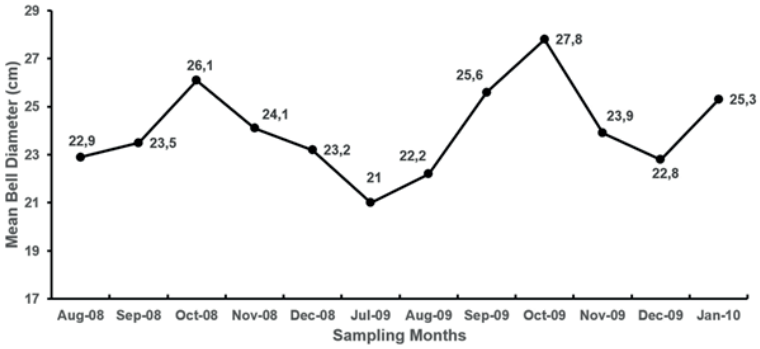

Figure 2. Mean Bell diameter frequency of captured jellyfish between August 2008 and January 2010 from Samsun coasts of the Black Sea, Turkey

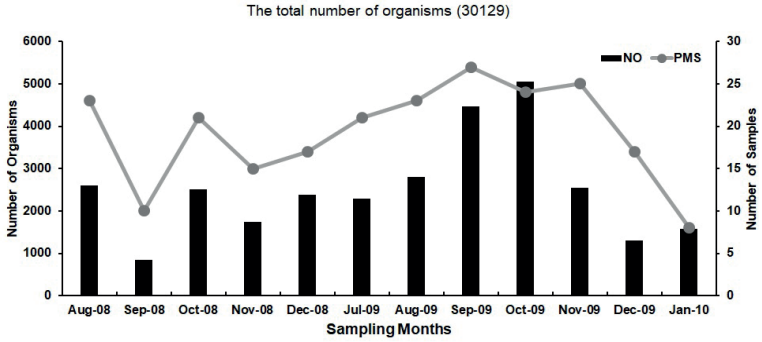

Figure 4. The total number of organisms detected in stomach contents. NO: Number of organisms, PMS: Number of medusa stomach samples

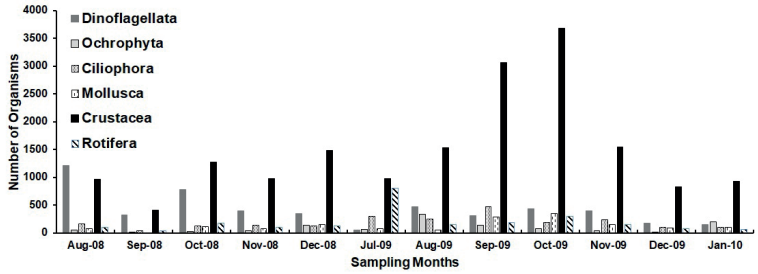

Figure 5. Monthly distribution of the most encountered food groups

Table 2. The groups determined in the stomach content of the jellyfish

Phylum: Dinoflagellata	**Phylum: Annelida**
Ceratium fusus	**Class: Polychaeta**
Ceratium tripos	Polychaete larvae
Gonyaulax sp.	Planktonic Polychaete
Neoceratium furca	**Phylum: Arthropoda**
Noctulica scintillans	**Sub phylum: Crustacea**
Prorocentrum micans	**Class: Maxillopoda**
Protoperidinium divergens	**Sub class: Copepoda**
Protoperidinium sp.	*Acartia tonsa*
Protoperidinium conicum	*Centropages ponticus*
Phylum: Ochrophyta	*Oithona brevicornis*
Achnantes sp.	Copepoda nauplii
Chaetoceros sp.	Copepodit
Conscinodiscus spp.	Copepods eggs
Pseudosolenia calcaravis	**Sub class: Thecostraca**
Phylum: Ciliophora	**Infra class: Cirripedia**
Favella spp.	Cirripedia nauplius
Tintinnopsis spp.	Cypris larvae
Phylum: Cnidaria	**Class: Malacostraca**
Actinula larvae	Shrimp larvae
Phylum: Chaetognatha	Crab zoea larvae
Sagitta sp.	**Class : Cladocera**
Phylum: Bryozoa	*Evadne spinifera*
Cyphonautes larvae	*Penilia avirostris*
Phylum: Rotifera	*Podon* sp.
Asplancha sp	**Phylum: Chordata**
Polyyartha sp.	Fish eggs
Trichocerca spp.	**Sub phylum: Tunicate**
Phylum: Mollusca	Tunicate larvae
Bivalves larvae	
Gastropod larvae	

In the analysis of stomach content, it was established that crustaceans was the most abundant group with 17.974 representative samples and 59.6% rate. The second group was dinoflagellates with a representation rate of 15.4%. *N. furca* was the most encountered species (Figure 5). *Acartia tonsa* and *Oithona brevicornis* were the most intensive species among the copepods. However, *Centropages ponticus* was the only species found in stomach of the jellyfish collected from Kızılırmak Delta (V. station).

Larvae of Bivalvia were another abundant group that was encountered throughout the study period. Shrimp larvae, tunicate and crab zoea larvae, *Oikopleura* sp., *Sagitta* sp., *Noctulica scintillans* and fish eggs were observed at a low rate. Copepod species and the individuals of *N. furca* were encountered frequently in the stomach content analysis. Copepods were mostly recorded in October, and the number of copepod individuals was 3.245 in stomach content of jellyfishes caught in October 2009.

The Relationships between the Stomach Content of *R. pulmo* and the Umbrella Diameter: The distribution of the stomach contents according to umbrella diameter was not homogenous. The total number of prey per gastric sac varied proportionally with umbrella diameter. The highest prey amount per gastric sac of jellyfish was recorded to be as 1.164 and this number was obtained from jellyfish with an umbrella diameter of 42.5 cm. This diameter was also the largest one that was observed among the sampling period. The smallest umbrella diameter found among the individuals was 16.5 cm and the total prey number in this sample was 61. Pearson Correlation showed that the relationship between the stomach content and umbrella diameter was r=0.68 and this relationship was significant statically at $p<0.0001$. According to this result of, there was a strong relation between umbrella diameter and the total number of the organisms found in the stomach content (Table 3 and Figure 6).

There was a strong correlation between umbrella diameter and species richness of the stomach content. Representing this relationship statistically, Spearman Rank Correlation was r=0.94, and it was significant at $p<0.0001$ (Table 4).

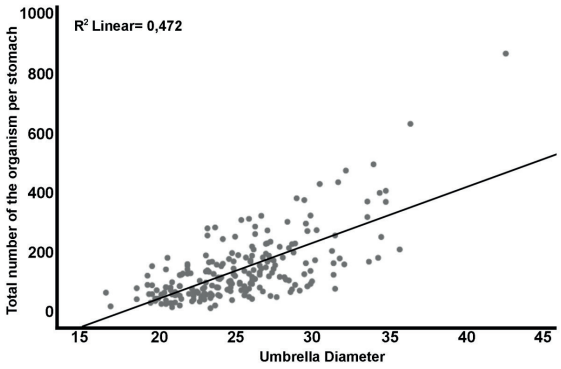

Figure 6. Relationship between umbrella diameter and total number the organisms in stomach. The regression line "fitted to" the scatterplot of values shown figure 4

Table 3. The statics relationship between umbrella diameter and the number of organisms detecting in stomach content

Correlations

		UD	TO
Umbrella Diameter	Pearson Correlation	1	.687**
	Sig. (2-tailed)		.000
	N	215	215
Total number of the organisms per stomach	Pearson Correlation	.687**	1
	Sig. (2-tailed)	.000	
	N	215	215

. Correlation is significant at the 0.01 level (2-tailed). **UD: Umbrella diameter (cm), **TO:** total number of organisms.

Table 4. The correlation between the types of groups identified in the stomach contents and the diameters of the umbrella

Correlations

			Umbrella Diameter	Species Richness
Spearman's rho	MUD	Correlation Coefficient	1.000	.946**
		Sig. (2-tailed)	.	.000
		N	19	19
	SP	Correlation Coefficient	.946**	1.000
		Sig. (2-tailed)	.000	.
		N	19	19

. Correlation is significant at the 0.01 level (2-tailed). **MUD: Mean Umbrella diameter (cm), **SP:** species richness

The species richness was 7.7 per individual having an umbrella diameter of 19 cm, while the individuals of *R. pulmo* with an umbrella diameter of 42.5 cm represented the richest group in terms of stomach content containing 28 different food groups . Although diatoms, dinoflagellates, copepods, cladocerans, rotifers and molluscs could be encountered in stomach content of the individuals with all umbrella diameters, *Oikopleura* sp. (Appendecularia) was encountered only in stomach of the specimens having a diameter ≥25 cm; *Acartia tonsa*, *Sagitta* sp. (Chaetognatha), *N. scintillans* (Dinoflagellata) and tunicate larvae were found only in stomach of the specimens having a diameter ≥26 cm; crabs and fish eggs (Chordata) were observed in diameter groups ≥27 cm whereas the shrimp larvae (Crustaceae) were found in stomach content of the jellyfish with a diameter ≥31-32 cm. *Oithona brevicornis* from Copepoda was observed in all umbrella diameters, and on the contrary *Centropages ponticus* was observed only in specimens with a diameter of 19 cm.

DISCUSSIONS AND CONCLUSIONS

The nutrition regime of *R. pulmo* is important for evaluating the effect of jellyfish on the ecosystem. Jellyfish may occupy an important trophic level in energy flow (Lilley et al., 2009). Increasing eutrophication and differentiation of nutrition levels cause increase in abundance and biomass of particularly jellyfish. A few data are available on the effect of nutrition regime of medusa on pelagic food web. The study carried out by Pérez-Ruzafa et al. (2002) on the distribution and the nutrition regime of medusa is one of the most important studies up to date. Öztürk and Topaloğlu (2011) found juvenile sardines and other fish in the stomach of medusa obtained from the Aegean Sea in 1973. According to that a total of 19 fish eggs were recorded in the stomach content of *R. pulmo* and no adult fish were found. Mayer (1910) stated that medusa cover the small animals with mucus between their oral arms and transfer the food to stomach cavity by channels of oral arms. It was observed that fish cubs with an approximate length of 4 cm were covered by mucus among the oral arms of two jellyfish at station

II in October 2008 and Station IV in December 2009, respectively and they were photographed.

Pérez-Ruzafa et al. (2002) reported in their stomach content analysis that the jellyfish fed dominantly on large diatoms (62.4%). Tintinnids were the second group in stomach contents. The other groups were veliger larvae and copepods with a representation rate of 6% and 3.8%, respectively (Pérez-Ruzafa et al., 2002). In stomach contents of jellyfish that were caught along Samsun coasts, crustaceans (59%), copepods (45.9%) and dinoflagellates (15.4%) were found to be abundant. Another frequently observed group was ciliophorans (7.2%).

Zooplankton have a vital role in network of sea (Chang et al., 2009). They are the basic consumer of primer production, and food source for organisms that are presented in higher trophic levels and having economical value (Kovalev et al., 1999). Among zooplankton, copepods are the most represented group both quantitatively and qualitatively and share an important part in holoplankton (Sever, 2009). In addition to *Acartia tonsa, Centropages ponticus* and *Oithona brevicornis,* nauplii and copepod eggs are the most abundant crustacean groups. *A. tonsa* is distributed around estuarine and coastal areas where the nutrient concentrations are high (Paffenhöfer and Stearns, 1988). Individuals of *A. tonsa* were found in stomach contents of samples obtained from station V and VI around Kızılırmak and Yeşilırmak. *Acartia* species could play a key role in the relationships of food web since they feed on ciliates and also serve as competitors to ciliates by consuming to phytoplankton (Marcus, 2004). They have a wide range of feeding regime and consume phytoplankton at first preference but in most of the studies it was reported that they also consume ciliates, dinoflagellates, rotifers, and Copepods (Gifford and Dagg, 1988; Marcus and Wilcox, 2007). *A. tonsa* which feed on organisms constituting the diet of *R. pulmo* both compete with jellyfish and serve as a nutrient for him. Another species encountered in stomach content of jellyfish was *O. brevicornis* which has been firstly recorded in Sevastopol harbor of the Northern Black Sea in 2001 (Gubanova and Altukhov, 2007). It has been estimated that this species might have come to the Sevastopol harbor by ballast water of ships (Gubanova and Altukhov, 2007; Selifonova, 2009; Zvyagintsev and Selifonova, 2010). *Oithona nana*, a settled copepod species, disappeared from the available community due to excessive grazing on plankton by *M. leidyi* which is an invasive jellyfish species and introduced to the Black Sea ecosystem after 1980's (Gubanova et al., 2014). Subsequent introduction of *B. ovata*, which is a predator of *M. leidyi* (Svetlichny et al., 2004; Anninsky et al., 2005) to the ecosystem and disappearance of *O. nana* from the community resulted in increase of *O. brevicornis*, a species which resemble *O. nana* in its ecological features (Gubanova and Altukhov, 2007). The highest abundance of *O. brevicornis* in Novorossisk harbor was observed in October 2006 (Gubanova and Altukhov, 2007; Selifonova, 2009). This result has been consistent with the data recorded in October during which the highest abundance and biomass values of jellyfish were observed. Similarly, the findings of Birinci Özdemir et al. (2018) support this. Also, the results obtained from zooplankton samplings supported this data (Üstün et al., 2018). Selifonova (2009) determined that 50% of the female individuals had egg sacs in samples of October 2006 when it was the most abundant. These findings provide opportunities to explain the causes of copepod eggs and Nauplius at the high representative level in the stomach content of jellyfish at October and November.

The eutrophication process has accelerated due to the decrease of silicon flow to the sea as a result of dams constructed on rivers which dumped into the Black Sea and the increase of nitrogen and phosphorus introduction due to anthropogenic activities (Zaitsev and Mamaev, 1995; Borysova et al., 2005; Baytut et al., 2010). Increased nitrogen (N) and decreased silicon (Si) (or both decreased) and decreased Si/N caused changes in the composition of phytoplankton species in the ecosystem. Therefore, diatoms were replaced by coccolithophores and dinoflagellates that were dominant in phytoplankton community (Humborg et al., 1997; Rabalais, 2009). The other factor which strengthened the competition success of the dinoflagellate species is that they have vertical migration ability to waters rich in nutrient values if the values decrease. Copepods prefer dinoflagellates as food rather than diatoms because they are nutritious, provide high egg production and support nest success (Kleppel et al., 1991; Vehmaa et al., 2011). During the study period, the stomach content of jellyfish was composed mostly of copepods and dinoflagellates. It was determined that dinoflagellates and zooplankton showed abundant distribution within the area where the jellyfish were recorded. The data on plankton could explain the cause of abundant presence of *N. furca* and *Ceratium* species in the stomach content of jellyfish.

Ceratium species (Dinoflagellates) could exist characteristically throughout the year. The water temperature effects the distribution and reproduction period of *Ceratium* species (Baek et al., 2008). *N. furca* was the most abundant species among Dinoflagellates. *Ceratium tripos* was the second most observed Dinoflagellate species. It was determined that the number of consumed dinoflagellate species per individuals of *R. pulmo* decreased in parallel

to decreasing water temperature. The relationship between the number of dinoflagellates in the stomach content and the water temperature for the two study periods was r=0.90, p<0.05 and r=0.7, p<0.05 (Spearman Rank Correlation), respectively, and a strong positive relation was found between these two variables.

Pérez-Ruzafa et al. (2002) determined in their study that the jellyfish fed mainly on large diatoms such as *Asterionella* and *Coscinodiscus*. Diatom species determined in the present study have a widespread distribution in the world. On the contrary of the study carried out by Pérez-Ruzafa et al. (2002), which established the representation rate of diatoms as 62.4%, the percentage of diatoms in our study was as low as 3.8% along the research period. The third food group determined in the stomach content of jellyfish was composed of the members of the phylum Ciliophora (*Favella* spp. and *Tintinnopsis* spp.). In the study of Pérez-Ruzafa et al. (2002), Tintinnids were the second important food group for jellyfish.

Identifications of large diatoms and Tintinnids in the stomach content of jellyfish supported the idea that there was a moderately eutrophic food web based on large phytoplankton and an oligotrophic food web based on small particles in Mar Menor Lagoon. It was stated that the large jellyfish controlled the food web from top to bottom their feeding habits (large diatoms, Ciliates, Veliger larvae and Copepods) in Mar Menor Lagoon, and they had a direct effect on nutrient input of the lagoon by removing the large diatoms, and they could reduce the predation pressure on small phytoplankton by removing ciliates and copepods. Also, it was presumed that the jellyfish could be a control agent on the result of eutrophication process from top to bottom controlling the food web (Pérez-Ruzafa et al., 2002).

R. pulmo could digest any organism as small as enough to pass through its mouth opening (Russel, 1970). It was determined that the food groups increased proportionally with the diameter size of the umbrella. The stomach content of medusa having an umbrella diameter of ≥25 cm contained large crab larvae, tunicate larvae, fish egg, *Sagitta* sp., *N. scintillans* and *A. tonsa*, while shrimp larvae were found only in individuals with a ≥31 cm umbrella size. In the stomach content of the medusa with a 42.5 cm umbrella diameter, there were 28 different food groups, and the individual with an umbrella diameter of 30 cm had 24 food groups. The highest number of observed food groups in jellyfishes with an umbrella diameter ranging between 16 and 20 was 15. According to Spearman Rank Correlation analysis, the relation between the umbrella diameter and the number of food groups in stomach content was r=0.94. This result shows that the medusa may consume larger food by its increasing umbrella size.

Populations of jellyfishes are characterized by their abundances and high fluctuations, and constitute an important part of biomass. They are affected the equilibrium of carbon, nitrogen and phosphorus by their high biomass and the corrupted population dynamics (Pitt, Welsh, and Condon, 2009). Pérez-Ruzafa et al. (2002) reported that the species of *R. pulmo* and *Cotylorhiza tuberculata* that have wide distribution in shores of Mar Menor would provide a positive effect on improving the nutrient balance and resolving the effects of eutrophication (Kingsford et al., 2000; Perez-Ruzafa et al., 2002). *R. pulmo* and other jellyfishes distributing in the Black Sea could be contribute to resolve of high productivity depending on the excessive nutrient input and improve the marine ecosystem.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Barotrauma treatment performance of fish release devices and its effects on fishing operations

Balık bırakma takımlarının barotravma tedavi performansı ve balıkçılık operasyonlarına etkileri

Aydın Demirci[1*] ● Ozan Bayraktar[2]

[1]İskenderun Technical University Faculty of Marine Sciences and Technology, Department of Marine Technologies, Hatay/Turkey https://orcid.org/0000-0002-7168-9904
[2]İskenderun Technical University Faculty of Marine Sciences and Technology, Department of Marine Technologies, Hatay/Turkey https://orcid.org/0000-0003-0013-5071
*Corresponding author: aydin.demirci@iste.edu.tr

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Abstract: The aim of this study is to analyze the barotrauma treatment performance of different fish release devices and its effects on different fishing operations. Experiments were carried out between September 2017 and April 2018 in the Iskenderun Bay, located in the Eastern Mediterranean. Fishing Release Hook (FRH), Fish Release Clamp (FRC) and Fish Release Basket (FRB) were tested in three different fishing activities, which are longline, hand line and bottom trawl fishery, respectively for *Epinephelus aeneus*, *Epinephelus costae* and *Nemipterus randalli*. They were released into the sea after fishing operations. Study results showed that fish release devices were very effective for barotrauma treatment. The use of FRB is suitable for bottom trawl fishing, while the use of FRH and FRC is more ergonomic in longline and hand line fishery.

Keywords: *Fish release devices, barotrauma treatment, trawl, longline, hand line*

Öz: Bu çalışmanın amacı, farklı balık bırakma takımlarının barotravma tedavi performansını ve farklı balıkçılık operasyonlarına etkilerini analiz etmektir. Çalışma, Eylül 2017 ile Nisan 2018 arasında Doğu Akdeniz›de yer alan İskenderun Körfezinde gerçekleştirilmiştir. Bu çalışmada, parakete, olta ve dip trol balıkçılığı olmak üzere üç farklı balıkçılık faaliyetinde Balık Bırakma Oltası (BBO), Balık Bırakma Kıskacı (BBK) ve Balık Bırakma Sepeti (BBS) olmak üzere üç adet balık bırakma takımı kullanılmıştır. *Epinephelus aeneus*, *Epinephelus costae* ve *Nemipterus randalli* türlerinin bireyleri balıkçılık operasyonlarından sonra denize bırakılmıştır. Çalışma sonuçları balık bırakma takımlarının barotravma tedavisi için çok etkili olduğunu göstermiştir. BBS kullanımı dip trol avcılığına uygunken BBO ve BBK ise parakete ve olta balıkçılığında daha kullanışlıdır.

Anahtar kelimeler: *Balık bırakma takımları, barotravma tedavisi,trol, parakete, olta*

INTRODUCTION

Developing fishing technology is an important issue for ecological sustainability (Prellezo and Curtin, 2015). The mitigation of non-target catch and the determination of its effects cover an important part of fishing technology researches (Cooke et al., 2016; Cook et al., 2019). The non-target catch involves the capture of fully banned by marine species and small species of economic targets that they are mostly under minimum legal catch size (Lloret et al., 2018; Şimşek and Demirci, 2018; Karp et al., 2019).

The concept of discard mitigation shows sustainability in the use of resources as ecosystem-based fisheries (Long et al., 2015; Kenny et al., 2018; Şimşek, 2018). In this regards, Many studies have been carried out to reduce the capture of unwanted species during fishing operations (Şimşek and Demirci, 2016; Demirci and Ulaş, 2017; Ulaş et al., 2017; Şimşek, 2018; Şimşek and Demirci, 2018). However, nowadays there are catching of non-target catches in both recreational and commercial fishing (Pauly and Zeller, 2016; Zeller et al., 2018). The amount of these species is estimated to be 7 million tons (Kelleher, 2005). It has been reported that the discoloration of trawl fishing in Iskenderun Bay varies between 30% and 70% (Demirci, 2003). it was thought that these discarded species could survive

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depending on region, depth, duration, etc. (Saygu, 2011; Demirci et al., 2012; Şimşek, 2012; Saygu and Deval, 2014; Şimşek, 2018; Şimşek and Demirci, 2018).

Release of non-target species on board leads to some controversy between managers and researchers (Johnsen and Eliasen, 2011; Condie et al., 2014; Lloret et al., 2018; Karp et al., 2019). The European Union has started to implement a discard management plan, which will limit the release of sea creatures into the sea by identifying non-target catch composition (Damalas, 2015: Uhlmann et al., 2019). This implement does not want the discarded catch species into the sea after commercial fishing activities; instead, it is recommended that fishermen use more selective catching means. Although selective fishing gears have used in all fisheries, non-target catch will always be present, therefore, the live return of these discarded marine individuals to the sea seen as the most plausible method, especially fragile fish stock structures for species (Koslow et al., 2000; Mace, 2001; Gislason, 2003).

When considering the stress effect of the fishing operation on discarded fish species, it has necessary to reduce the effect as much as possible in the sea. Thus, the survival rates for many discarded fish species are over 50% (Şimşek, 2012; Demirci and Şimşek, 2018). However, for some fish species, additional applications are required especially for the demersal fish species with enlarged swim bladder (Şimşek and Demirci, 2018). These fish species passed quickly from the natural depth to the surface of the water. Therefore, these fish species have exposed to barotrauma.

Barotrauma is caused by the expansion of gas bubbles in the fish body due to the sudden pressure drop during the fishing operations. Depending on the severity of this trauma, bleeding in the internal organs of the body, the outflow of the eyes and pushing the stomach towards the mouth or even exiting for enlarged swim bladder. In this case even if a fish is released into the sea, it cannot move normally and cannot get deep and stays on the surface of the sea like a balloon (Uluç, 2014).

Barotrauma is intended to help fish return to normal swimming ability as soon as possible. Two methods have been used so far; (1) removal of excess gas pressure from the fish swim bladder and (2) releasing the external environment of the fish by re-pressurization by dipping.

In recent years, there have been some examples of application in the field of barotrauma treatment in fisheries (Şimşek, 2018). The most common and old method is to remove the gas y puncturing the fish from the outside of the swim bladder with a pointed needle. However, this application not only provides rapid recovery of the air sac of the fish but also does not produce the desired result in the eyes and other body cavities. In addition, it is suggested that a surgical application may cause secondary problems.

A hollow needle has the most commonly used method to sink into the swim bladder from the back muscles to expel the expanded gases, which are grown up as a result of the involuntary and rapid coming to the sea surface. This method was controversial because it was a surgical procedure and the achievements have been different from the species (Wilde, 2009). Moreover, the studies conducted by Campbell et al. 2014 and Wilde (2009) have formed discussions about the implementation of this method. Wilde (2009) argues that the potential for damaging organs and the likelihood of further tearing of the tissue associated with perforation from the edge of the fish are more severe than the positive effects. However, he showed that the death caused by the use of this method was low and suggested that there was a healing method (Wilson and Burns, 1996; Nguyen et al., 2009; McLennan et al., 2014).

Fish release devices are much more effective than venting needle because the only evacuation did not complete the gas in the fish-swimming bladder, and quickly returned the gases to the appropriate pressure levels (Butcher et al., 2012). Hyperbaric artificial recompression and field research, which form the basis of this method, has been shown to be more effective in the treatment of barotrauma symptoms (Roach et al., 2011; Pribyl et al., 2012; Drumhiller et al., 2014). The use of fish release devices eliminates the possibility of unwanted organ puncture and tissue damage in barotrauma treatment. These devices are generally composed of reverse basket, weighted clamp with clamp, grooved fishing rod or weighted cages with pressure increase. These methods are summarized, the fish placed in systems are reduced to the appropriate depth and they are provided to exit from these cages. In another way, the fish is lowered to the required depth by means of the grapple or needle from the chin section and this point also recovers the fish jaw that regains its ability to swim again (Stephensona et al., 2010; Demirci et al., 2013; Uluç, 2014; Brownscombe et al., 2017).

Iskenderun Bay has an important fishing area in the Mediterranean, where many commercial fishing methods take place. In addition, intensive recreational fishing activities have carried out in the region with angler fishing (Demirci and Arslantaş, 2018). In this region, the release of individuals in the form of barotrauma is necessary for both commercial fishing and recreational fishing activities on the sea. Depending on species diversity, there are a lot of unwanted species in the Iskenderun Bay fishery (Mavruk and Avşar, 2008).

White grouper *Epinephelus aeneus* and Dusky grouper *Epinephelus marginatus* which have been protected until 2020, are unintentionally caught in many fishing methods. It is a legal obligation to release these species into the sea after the fishing operation. However, these and other grouper species are deeply caught and most barotrauma when they come to the surface (Runde and Buckel, 2018). Due to the sudden pressure drop, barotrauma of these species shows the signs of swelling of the air sac, coming into the mouth and swelling of the eyeballs (Şimşek and Demirci, 2018). When a fish in this state is released to the sea, it cannot be immersed in water and cannot sustain its vitality (Demirci et al., 2013; Uluç, 2014).

In this study, three different release devices were analyzed by field trials and presented to fishermen. For these analyzes, the fish exposed to barotrauma releasing experiments were carried out with long line, hand line and trawl fishery in the Iskenderun Bay. At the end of this presentation and applications, fishingreturns were evaluated.

MATERIALS AND METHODS

In this study, release trails were made for Randall's threadfin bream, White grouper and Gold blotch grouper species with Fishing Release Hook (FRH) (Figure 1), Fish Release Clamp (FRC) (Figure 2) and Fish Release Basket (FRB) Figure 3.

FRH includes two systems for release more than one fish at the same time, by using hook or multiple hooks. The tool consists of two sections, which have a straight flexible, robust, curved hook and the main stem and lead weight. This tool components made of simple, cost-effective and easily available.

The FRC can be made of rigid plastic or metal products, a set of clamps on the main body, and the other part of this clamp is movable. These pincers were attached to the fish in the barotrauma by fish chins in the release deeper. The deeper layer weight required to squeeze the fish's chin was adjusted to the size of the fish.

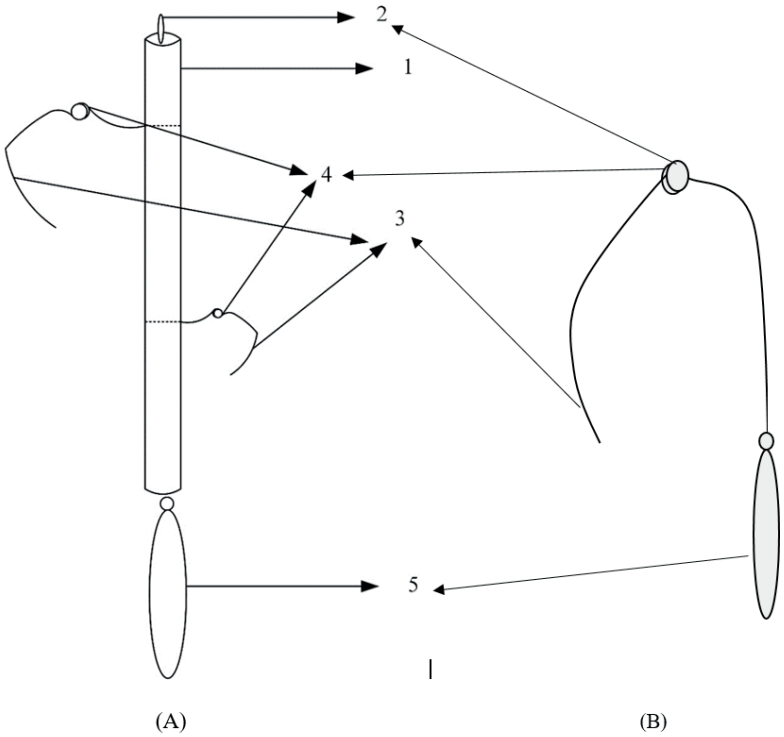

Figure 1. The Scheme of Fish Release Hook (FRH) (A: Use in releasing several fishes at the same time, B; Tool used in releasing a fish, 1: main body, 2: body-lashing ring, 3: needles of different size, 4: spring crimp, 5: lead)

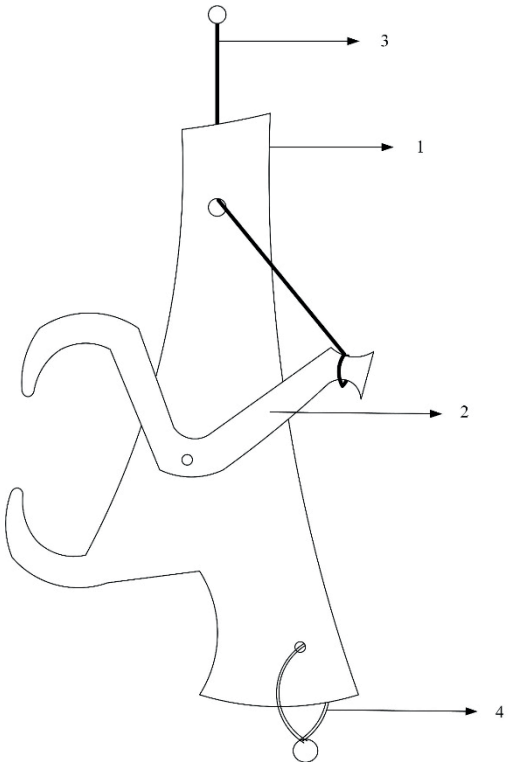

Figure 2. The Scheme of Fish Release Clamp (FRC) (1; main body, 2; movable clamp arm mounted on the main body, 3; steel rope, 4; lead)

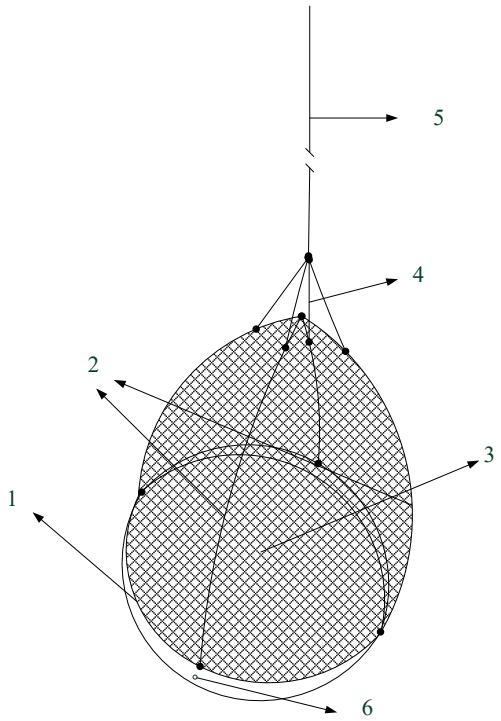

Figure 3. The Scheme of Fish Release Basket (FRB) (1; chrome steel frame, 2; connecting ropes, 3; part, 4; body connecting ropes, 5; main size, 6; extra weight for attaching holes)

The FRB, originally developed by nets as a mesh bag or fish bag, was dropped into the sea. It is important to select the mesh material, size and design, in order to prevent from damage fishes or escapees

It was tried to leave these fish in the form of barotrauma to a depth of 30 meters with this equipment after were caught fish by trawl, long line and hand line in the İskenderun Bay. Because, it is reported that the increase in the pressure at this depth of barotrauma treatment has rapid and effective results (Şimşek, 2018; Demirci et al., 2018; Şimşek and Demirci, 2018).

While the fishes were released with these devices, video recording with the underwater cameras were done during the operations on the ship. According to fish species and sizes, these records were evaluated and compared as a Positive and negative aspects at six points;

- Cost and feasibility of the team (C; Cost and Feasibility)
- Team portability on board (T; Transport)
- Attaching the release device to fish in barotrauma (F; Fitting)
- Hanging the fish from the boat to the water (RP; Releasing Procedures)
- Fish exposed to barotrauma immersing to depth (I; Immerse)
- Fish at the bottom of the water barotrauma recovered from the release device (FE; Fish Escaping)

RESULT AND DISCUSSION

Six attempts were made on the two sea expeditions in trawl fishing. In these experiments 30 individual of Randall's threadfin bream, 8 individual of white grouper and 12 individual of gold blotch groupers were successfully release to 28 meters deep with 30 FRBs, and all were recorded on video with in a healthy swim. The images of these release trials were shown in Figure 4. The FRB was not considered for the trawl-fishing vessel as the deck level of the ship was not suitable for its height above the sea surface.

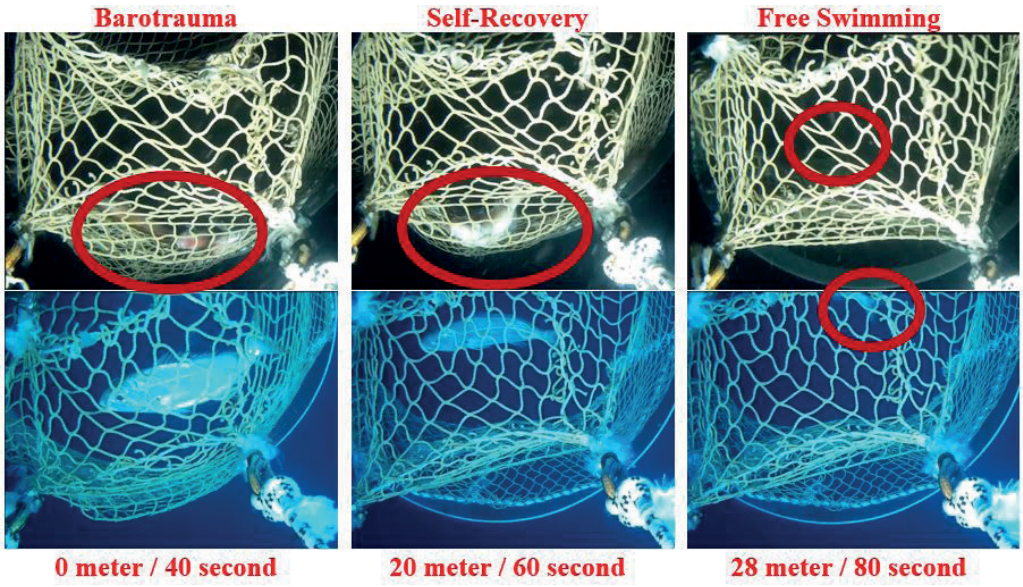

Figure 4. Images of the experimental of the white groupers and Randall's threadfin bream individuals with barotrauma after the trawl fishing with the FRB

Table 1. The FRB with different species and sizes in the form of barotrauma after trawl fishing

Fish Species	Number	Mean Length (cm)	Release Deeper (meter)	Release Hauling (second)	Result (%)
Randall's threadfin bream	30	15.05	28	100	100
White grouper	8	23.98	28	120	100
Gold blotch grouper	12	20.68	28	120	100

FRH and FRC experimental releasing were conducted in the fish caught by long line and the hand line. In these FRH trials, 10 individual of Randall's threadfin bream, 5 individual of white grouper and 6 individual of gold blotch grouper were releasing, while in FRC trials 12 individual of Randall's threadfin bream, 4 individual of white grouper and 5 individual of gold blotch grouper were releasing in the dip sea (30 m). It was not possible to get rid of the fish fishing with FRH. However, when the lead weight was used more with the FRC, the fish could not be recovered from the device even though the fish body form had recovered from barotrauma. In this unfavorable case, the fish sample was back to the surface again when it returned to the devices. Therefore; it was concluded that appropriate weight selection is required for fish species and size when using FRC for barotrauma fish releasing. In these FRC trials, it was found that a maximum weight of half kg was sufficient for the evaluation between the fish size and the release weight. Results of FRC is given in Table 3. The maximum released fish weight in these trials is 700 g, in this context: the weight of the fish should be more than half weight of the fish, but not exceed the weight of the fish. In other words, it is not possible to get rid of 500 g lead weight, which is widely used for 200 g fish. The appearance of White grouper and Gold blotch grouper during releasing with FRC is given in Figure 5 and Figure 6, respectively.

Another point to be considered in the use of FRC is the fish was attached to the devices. In this study, claws were attached to the upper and lower chin. Attachment of the chin to the uppers gave more positive results than the lover chin. Other important considerations were the sharpness and suitability of the size of the clamp to the fish mouth.

Figure 5. Release of the white grouper in the form of barotravma deeper, recovery and self-recovery of fish (a: Lowering, b: Barotrauma recovery, c: rescue effort and d: rescue)

Table 2. The FRH with different species and sizes in the form of barotrauma after the longline and hand line fishing

Number	Species	Length (cm)	Release Depth (meter)	Release Hauling (second)	Result
1	Randall's threadfin bream	17.6	20	135	+
2		15.4	18	140	+
3		18.1	25	155	+
4		12.3	20	210	+
5		10.8	16	150	+
6		14.8	22	145	+
7		13.9	25	150	+
8		18.4	28	170	+
9		16.2	28	125	+
10		11.7	18	150	+
11	White grouper	19.6	25	125	+
12		15.4	25	90	+
13		22.9	25	75	+
14		28.2	35	70	+
15		24,8	30	70	+
16	Gold blotch grouper	18.4	25	100	+
17		16.3	25	90	+
18		17.5	20	90	+
19		18.2	25	85	+
20		17.9	25	90	+
21		18.8	20	60	+

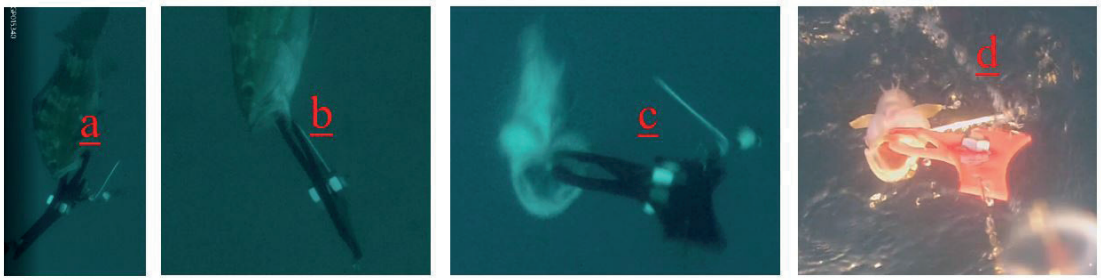

Figure 6. Images of Gold blotch groupers with the release clamp to the bottom in the form of barotrauma, the recovery and recovery of the fish itself cannot recover from the clamp (a: immerse, b: barotravma recovery, c: escape effort and d: return to the surface)

Table 3. The FRC with different species and sizes in the form of barotrauma after the longline and hand line fishing

Number	Fish Species	Length (cm)	Release Depth (meter)	Release Hauling (second)	Result
1	Randall's threadfin bream	15.2	30	-	-
2		13.7	18	140	+
3		15.8	25	155	+
4		14.2	32	210	+
5		12.9	16	150	+
6		16.1	24	145	+
7		12.4	25	150	+
8		13.5	28	170	+
9		13.6	28	125	+
10		16.8	25	150	+
11		17.5	23	135	+
12		16.4	22	120	+
13	White grouper	19.7	35	125	+
14		16.9	25	90	-
15		18.4	25	75	+
16		18.3	25	70	+
17	Gold blotch grouper	15.5	25	100	-
18		16.4	25	90	+
19		15.9	20	90	+
20		18.2	25	85	+
21		17.6	25	90	+

There is no scientific study related on fish release device. Therefore, a comparative evaluation could not be made. There is only one field study on commercial longline fishery in the Eastern Mediterranean (Gökçe et al., 2018). In this study, Grouper species were dropped to the bottom with fish release hooks.

As a result of this study, the obtained performances of the fish release devices are shown in Figure 7. In this figure, a scale from red to blue was created because of the more comprehensible. The red color on this scale represents the negative state while the blue represents the positive state. In this assessment, fish release devices were considered into five issues. While the FRB was negative in terms of cost and move, the use of FRC and FRH requires experience. it can also be said that the use of FRB is suitable for bottom trawl fishing, while the use of FRH and FRC is more ergonomic in longline and hand line fishery.

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	FRB	FRC	FRH
C	Connecting ropes requires experience. If the diverter frame can chose to be stainless, the cost increases.	The material must be prepared from a hard material. Requires experience and knowledge.	Low cost and easy to supply.
T	Difficult to transport on small ships due to the size.	Easy	Easy
F	Easy	Requires experience	Requires experience easy than other.
RP	Requires experience. However, more than one fish can be release at the same time.	It is necessary to adjust the clamp mouth and weight according to the size of the fish.	Due to the height of the ship deck, the fish may fall from the needle without entering the water. Needle length and suspension distance must be set.
I	Fish cannot be damaged.	The speed must be checked. It is very fast when the fish is injured or the desired depth before it comes out.	The speed must be checked. Injury of the fish when it is too fast.
FE	Easy escaping	If the weight is too much, it is very difficult.	The ability of the fish to swim and the length of the needle require a wait. However, when the devices is lifting, the fish is easy escaping.
Color scale			
Positive			Negative

Figure 7. The analyzes with use of different release devices for barotrauma fish (**C;** Cost and Feasibility, **T;** Transport, **F;** Fitting, **RP;** Releasing Procedures, **I;** Immerse, **FE;** Fish Escaping)

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Nutritive value and safety aspects of acidified mantis shrimp during ambient storage

Asitlendirilmiş mantis karidesinin besin değeri ve ortam sıcaklığında depolanması süresince güvenlik değerlendirilmesi

Gülsün Özyurt[1] ● Caner Enver Özyurt[2] ● Elif Tuğçe Aksun[3] ● Ali Serhat Özkütük[4*]

[1]Department Seafood Processing Technology, Faculty of Fisheries, Çukurova University, 01330, Adana, Turkey https://orcid.org/0000-0003-1073-115X
[2]Department Seafood Processing Technology, Faculty of Fisheries, Çukurova University, 01330, Adana, Turkey https://orcid.org/0000-0002-6502-982X
[3]Department Seafood Processing Technology, Faculty of Fisheries, Çukurova University, 01330, Adana, Turkey https://orcid.org/0000-0003-1993-0569
[4]Department of Fisheries, Yumurtalık Vocational School, Çukurova University, 01680, Yumurtalık, Adana, Turkey https://orcid.org/0000-0001-7464-3015
*Corresponding author: aliserhat@cu.edu.tr

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Abstract: In this study effects of acidification with an organic acid (3 %, formic acid - FA) and an organic-inorganic acid mixture (1.5 % FA + 1.5 % sulphuric acid - FASA) were evaluated on a non-target species (mantis shrimp - *Erugosquilla massavensis*). Nutritional composition (proximate analysis and fatty acid composition), chemical (biogenic amine concentrations, non-protein nitrogen -NPN and pH) and microbiological assessments (total viable counts -TVC and lactic acid bacteria counts -LAB) were conducted under 27-28 °C. The analysis conducted for a 60 day period at 1st, 7th, 14th, 30th, 42nd and 60th day intervals. Moisture contents of acidified shrimp were significantly lower than the raw material. No changes in protein contents and an increase in lipid content only in FASA group were observed. Palmitic acid, stearic acid, palmitoleic acid, oleic acid and docosahexaenoic acid (DHA) were observed to be the dominant fatty acids in raw and acidified mantis shrimp. The initial pH value of mantis shrimp was 7.71 and became stable (4.14-3.97) throughout the storage period. An increase was observed in NPN contents and FA and FASA were 0.56 and 0.51 g 100 g^{-1}, respectively at the end of the storage. Putrescine (3.00 mg 100 g^{-1}), tyramine (2.94 mg 100 g^{-1}) and serotonin (2.71 mg 100 g^{-1}) were found to be the dominant biogenic amines in raw mantis shrimp. No significant changes in biogenic amine concentrations were observed in general during the storage period. TVC was found as 4.16 log cfu g^{-1} at the beginning of the storage period. Bacterial load was decreased after the addition of acids and stayed low throughout the storage period. Increases were observed in LAB and this value were 4.50 and 5.68 log cfu g^{-1} for FA and FASA group, respectively at the end of the storage period. The results showed acid treated mantis shrimp could be considered potential feed component due to its high nutritional value and safe in regards of biogenic amines.

Keywords: *Mantis shrimp, fatty acid composition, biogenic amines, acidification, total viable count, lactic acid bacteria*

Öz: Bu çalışmada organik asit (% 3, formik asit - FA) ve bir organik-inorganik asit karışımı (% 1.5 FA + % 1.5 sülfirik asit - FASA) ile asitleşmenin etkileri, hedef dışı bir tür (mantis karidesi - *Erugosquilla massavensis*) üzerinde değerlendirildi. Besin kompozisyonu (besin içeriği ve yağ asidi kompozisyonu), kimyasal (biyojenik amin konsantrasyonları, protein olmayan azot -NPN ve pH) ve mikrobiyolojik değerlendirmeler (toplam bakteri sayımı - TVC ve laktik asit bakteri sayısı - LAB) 27-28 °C'de yapılmıştır. Analizler 1., 7., 14., 30., 42. ve 60. günlerde 60 günlük bir süre boyunca yapıldı. Asitlenmiş karideslerin nem içeriği hammaddeden önemli derecede düşük bulunmuştur. Protein içeriğinde herhangi bir değişiklik gözlenmezken sadece FASA grubunda lipit içeriğinde bir artış gözlendi. Çiğ ve asitlendirilmiş mantis karidesinde palmitik asit, stearik asit, palmitoleik asit, oleik asit ve dokosaheksaenoik asit (DHA) dominant yağ asitleri olarak gözlenmiştir. Mantis karidesinin başlangıç pH değeri 7.71 olurken, depolama süresi boyunca kararlı hale gelmiştir (4.14 - 3.97). NPN içeriklerinde bir artış gözlenmiş ve depolamanın sonunda FA ve FASA sırasıyla 0.56 ve 0.51 g 100 g^{-1} olmuştur. Çiğ mantis karidesinde baskın biyojen aminler olarak, putresin (3.00 mg 100 g^{-1}), tiramin (2.94 mg 100 g^{-1}) ve serotonin (2.71 mg 100 g^{-1}) bulunmuştur. Depolama süresi boyunca genel olarak biyojenik amin konsantrasyonlarında belirgin bir değişiklik gözlenmemiştir. TVC, depolama periyodunun başlangıcında 4.16 log kob g^{-1} olarak bulunmuştur. Asit ilavesi yapıldıktan sonra bakteri yükü azalmış ve depolama süresince düşük kalmıştır. LAB'da artışlar gözlenmiş ve bu değerler depolama periyodunun sonunda sırasıyla FA ve FASA grubu için 4.50 ve 5.68 log kob g^{-1} olmuştur. Elde edilen sonuçlar, asitle işleme tabi tutulan mantis karidesinin yüksek besin değeri nedeniyle potansiyel besleme bileşeni olarak kabul edilebileceğini ve biyojenik aminler açısından güvenli olduğunu göstermiştir.

Anahtar kelimeler: *Mantis karidesi, yağ asidi kompozisyonu, biyojenik aminler, asidifikasyon, toplam bakteri sayımı, laktik asit bakterileri*

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INTRODUCTION

Incorporation of non-targeted species into the economy of the fishing industry has a great potential to become an important source of income for fishermen. The utilization of seafood processing waste or discard species enhances the efficiency of animal protein usage, minimizes the environmental problems, and adds a nutritional benefit to diets prepared from such materials. Mantis shrimp (*Erugosquilla massavensis*) which migrated by the Suez Channel is in great numbers in the Mediterranean Sea. However, its consumption as a food source is refused because of its small size. Therefore it may be use for feed source for animal nutrition.

It is stated that the composition of the acidified seafood products is very similar to the raw materials in general (Mach and Nortvedt, 2009; Arruda et al., 2007). Acidification of seafoods for use as animal feed has many advantages like easy production, low investment, energy and labour. Acidification can be done either by organic and inorganic acid. The selection of acid type interconnected with the cost and availability of the acids. For fatty and bony fish, 3-6% of concentrated sulphuric acid is recommended, while for organic acids such as formic acid and propionic acid recommended value is 2-3%. Although sulphuric acid is cheaper than organic acid, it needs to be used in larger quantities and must be neutralized prior to use on the feed (Rurangwa et al., 2014). For this reason, organic and inorganic acids can be mixed and should be considered to be used for researches.

Biogenic amines may be a potential risk in acidified fish feed because of its low pH and chemical properties, and they may reduce the biological value of the feed because of toxicity. The amount of biogenic amines formed are influenced by factors such as fish species, the presence of decarboxylase enzymes, microbial growth, availability of free amino acids, water activity, pH and elevated temperature conditions. In spite of the broadly acknowledged association between histamine and scombroid fish poisoning, histamine seems to be inadequate to cause toxicity single-handedly. Putrescine and cadaverine have also been known to strengthen histamin's toxic activity by limiting the intestinal histamine-metabolizing enzymes, diamine oxidase (Hungerford and Arefyev, 1992) and histamine N-methyltranferase (Stratton et al., 1991). For this reason, it is important to investigate the safety of acidified feed stuffs in regards of biogenic amines. In order to research the possibilities of using as an animal feed, mantis shrimps were stabilized through ensilage by direct acidification with 3% formic acid (FA) and 1.5 % formic acid + 1.5 % sulphuric acid (FASA) in this study. Chemical, microbiological and nutritional evaluations and also biogenic amines concentrations were performed for determining the safety and nutritive values of acidified mantis shrimp.

MATERIALS AND METHODS

Acidification of mantis shrimp

Average weight and length of mantis shrimp (*Erugosquilla massavensis*) were 11.93±1.37 g and 19.87±5.68 cm, respectively (n=40). All samples that were provided by local fisherman were minced by a grinder and divided into two equal groups. The first group (FA) was mixed with 3 % formic acid (v/w) and the second group (FASA) was mixed with 1.5% formic acid and 1.5 % sulphuric acid (v/w). After that, 250 mg kg^{-1} butylated hydroxytoluene (BHT) as an antioxidant and 2.2 g kg^{-1} potassium sorbate as fungicide were added. Both groups (FA and FASA) were stored at 27-28 °C in plastic jars with caps and stirred daily until ripening. Each group was managed as triplicates, stored 60 days and sampled for analyses at 0^{th}, 7^{th}, 30^{th} and 60^{th} day.

Nutritional composition analyses of acidified mantis shrimp

The moisture content and crude ash of acidified mantis shrimps were detected in an oven at 103 and 550 °C, respectively until the weight of samples became stable. The crude protein and lipid were analysed according to AOAC (1999, 981.10) and Bligh and Dyer (1959) procedure, respectively. Lipid samples were turned into their constituent fatty acid methyl esters (FAMEs) by using the method of Ichihara et al., (1996). Gas chromatography (Clarus 500, Perkin Elmer, Shelton, CT, USA) was used for the separation and quantification of the fatty acid methyl esters.

Chemical analyses of acidified mantis shrimp

pH was measured using a digital pH metre (WTW 315i, Germany). Non-protein nitrogen (NPN) analysis was used to estimate the protein autolysis. Samples (40 g) were stirred with TCA (60 mL, 20 % thrichloracetic acid) and filtered. After that, Kjeldahl's procedure was used for determining the nitrogen content and the results were expressed as NPN (AOAC 2002; method 991.21). Trimethylamine (TMA) and biogenic amine (BA) analysis were completed using a rapid HPLC (high-performance liquid chromatography) method (Özogul et al., 2002). A Shimadzu Prominence HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector, two binary gradient pumps (Shimadzu LC-10AT), auto sampler (SIL 20AC), column oven (CTO-20AC) and valve unit FCV-11AL with a communication bus module (CBM-20A) was used. The column was a reverse-phase, ODS Hypersil, 5µ,

250x4.6mm (Phenomenex, Macclesfield, Cheshire, UK). Oven temperature was 30 °C and mobile phase was acetonitrile and HPLC grade water.

Microbiological analyses of acidified mantis shrimp

10 g of acidified mantis shrimp from three different jars for each group were randomly sampled for total aerobic bacteria counts (TVC) and lactic acid bacteria analysis. TVC were incubated on PCA (Fluka 70152, Switzerland) plates for 2 days at 30ºC. LAB counts were carried out by the pour plate method and they were grown on MRS (Fluka 69964 Steinheim, Spain) agar at 30°C for 5 days.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). All analyses were performed at least triplicate and differences between means were analysed by one-way analysis of variance (ANOVA) and mean comparisons were carried out using Duncan's (Duncan, 1955) multiple range tests and t-test. Data were expressed as mean ± standard deviation (SD) and significant differences were defined as $P < 0.05$.

RESULTS AND DISCUSSION

Nutritional composition of acidified mantis shrimp

The moisture, ash, protein and lipid content of raw material were found 74.19 %, 7.30 %, 15.99 % and 1.11 %, respectively (Table 1). Moisture contents of acidified shrimps were significantly less than the raw material ($P<0.05$). Ash contents also increased in both groups significantly ($P<0.05$). However, other than the lipid content of FASA group, no significant changes were observed in lipid and protein contents of acidified shrimps. It can be concluded that the acidification process for preserving mantis shrimp caused minor variations in protein and lipid contents for the raw mantis shrimp. Among fatty acids, palmitic acid, stearic acid, palmitoleic acid, oleic acid and docosahexaenoic acid (DHA) were the dominant fatty acids in raw and acidified mantis shrimp (Table 1). Generally, there were no significant changes in fatty acid contents after acidification treatment. DHA was determined as the dominating fatty acid within PUFA in both groups, even after the acidification process.

Chemical assessment

The initial pH value of mantis shrimp was 7.71 and the pH value in both groups showed significant decrease (4.14 - 3.97) during the storage period (Figure 1). During storage period, pH of the acidified shrimps remained stable under 4.5 which was the recommended pH value for acidified fish (Espe and Lied, 1999). The non-protein nitrogen (NPN) contents which were known as protein solubilisation values for the acidified shrimps are presented in Figure 1. The increase of NPN was likely occurred because of the release of NPN components either by the breakdown of compounds like trimethylamine oxide (TMAO) or protein hydrolysis by enzymes. Non-protein nitrogen (NPN) contents were found as 0.56 and 0.51 g $100g^{-1}$ for FA and FASA, respectively at the end of the storage periods.

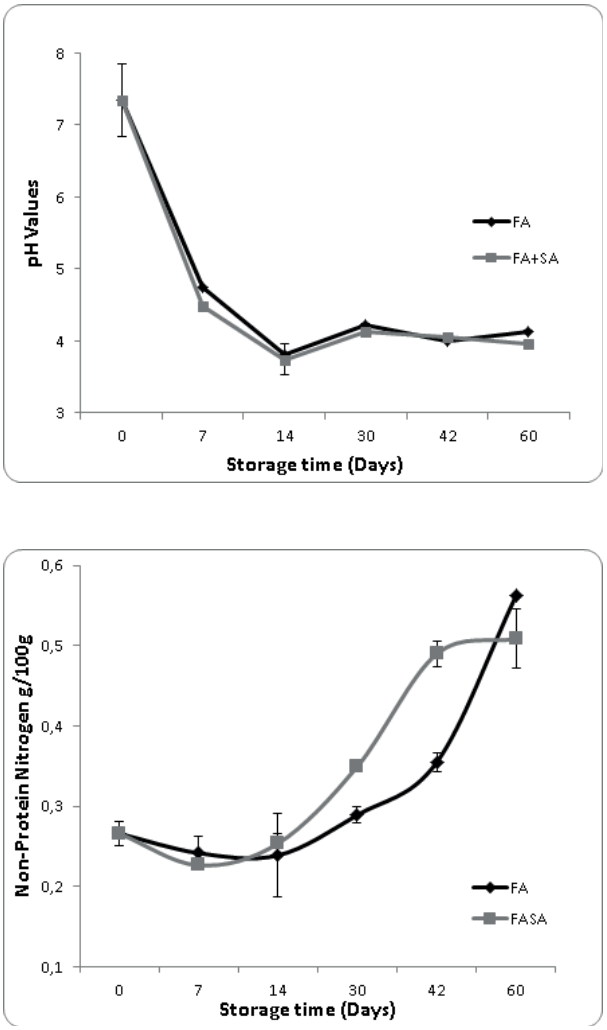

Figure 1. The changes of pH, Non-protein nitrogen values, in acidified mantis shrimps during storage period. Means values of three (n=3) independent determinations. Standard deviations are indicated by bars

Table 1. Proximate and fatty acid compositions of acidified mantis shrimps

		Raw *E. massavensis*	FA	FA+SA
Proximate composition (%)	Moisture	74.19±0.14[c]	72.63±0.12[b]	71.45±0.46[a]
	Ash	7.30±0.33[a]	8.57±0.26[b]	9.07±0.23[b]
	Protein	15.99±0.23[a]	15.98±0.20[a]	15.61±0.51[a]
	Lipid	1.11±0.03[a]	1.20±0.05[a]	1.89±0.19[b]
Fatty acid composition (% of total fatty acids)	C12:0	3.77±0.27[b]	2.02±0.22[a]	3.93±0.01[b]
	C14:0	4.16±0.06[b]	3.82±0.13[a]	4.18±0.08[b]
	C15:0	0.32±0.03[a]	0.25±0.01[a]	0.32±0.04[a]
	C16:0	18.14±0.72[a]	21.75±2.08[b]	18.32±0.91[a]
	C17:0	0.49±0.01[b]	0.31±0.04[a]	0.49±0.01[b]
	C18:0	8.82±0.17[a]	10.86±2.84[a]	8.73±0.06[a]
	C20:0	0.27±0.01[a]	0.29±0.06[a]	0.27±0.01[a]
	C22:0	0.04±0.01[a]	0.14±0.06[b]	0.04±0.01[a]
	C14:1	0.45±0.05[b]	0.26±0.00[a]	0.46±0.06[b]
	C15:1	0.04±0.01[a]	-	0.04±0.01[a]
	C16:1	5.83±0.28[a]	6.95±0.19[b]	5.93±0.32[a]
	C17:1	0.12±0.00	0.10±0.00	0.12±0.00
	C18:1 n9	7.39±0.39[a]	8.23±0.66[a]	7.52±0.47[a]
	C18:1n7	4.26±0.02[a]	4.71±0.95[a]	4.25±0.01[a]
	C20:1	0.84±0.03[b]	0.61±0.07[a]	0.82±0.00[b]
	C22:1n9	0.15±0.00	0.63±0.00	0.15±0.00
	C18:2n6	1.37±0.23[a]	1.41±0.13[a]	1.27±0.21[a]
	C18:3n3	0.25±0.04[a]	0.28±0.02[a]	0.27±0.01[a]
	C20:2 cis	5.87±0.56[a]	4.55±0.70[a]	5.71±0.69[a]
	C20:4 n6	0.34±0.06[a]	0.23±0.08[a]	0.31±0.01[a]
	C20:5n3	1.20±0.08[b]	0.47±0.06[a]	1.24±0.07[b]
	C22:2 cis	1.34±0.06[a]	1.25±0.13[a]	1.32±0.04[a]
	C22:6 n3	8.14±0.31[a]	8.79±0.64[a]	8.17±0.43[a]

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

[a-c] Values in a same line followed by different letters indicate significant differences (P<0.05)

As a result of the activities of assorted endogenous and bacterial decarboxylase enzymes, it was observed that acidified mantis shrimp had significant levels of free amino acids which were the precursors for biogenic amines. The initial TMA level in raw mantis shrimp was 30.39 mg $100g^{-1}$ (Table 2). After formic and sulfuric acid addition, the TMA value considerable decreased during 30 days (16.88-24.27 mg $100g^{-1}$) and then increased in 60th day (44.42 mg $100g^{-1}$). However there were no significant differences between FA group and FASA group at 60 day of storage in regards of TMA value. Similarly, high TMA value in acidified fish products had been also observed in other studies (Jiang et al 2007; Achinewhu and Oboh 2002).

The production of 10 biogenic amines was found in raw and acidifed mantis shrimps (Table 2). Putrescine (3.00 mg $100g^{-1}$), tyramine (2.94 mg $100g^{-1}$) and seratonine (2.71 mg $100g^{-1}$) were the major biogenic amines in the raw mantis shrimp. The presence of histamine, spermidine, tryptamine, cadaverine, agmatine, 2-phenylethylamine and spermine was found to be in lower concentrations (0.08-1.27 mg$100g^{-1}$)

Table 2. TMA and biogenic amine concentrations of acidified mantis shrimp groups (FA and FASA, mg 100g^{-1})

Days	0	7		30		60	
		FA	FASA	FA	FASA	FA	FASA
TMA	30.39±17.73	52.18±0.57^{a}	16.88±0.03^{b}	46.79±1.67^{a}	24.27±1.88^{b}	44.30±13.31^{a}	44.42±1.58^{a}
HIS	0.22±0.00	0.94±0.48^{a}	0.22±0.01^{a}	0.32±0.08^{a}	3.71±2.96^{a}	0.2±0.00^{a}	1.24±1.29^{a}
PUT	3.00±0.70	2.29±0.23^{a}	1.02±0.68^{a}	3.09±0.62^{a}	2.61±0.22^{a}	2.19±0.19^{a}	3.00±0.41^{a}
CAD	1.27±0.19	1.04±0.14^{a}	0.45±0.21^{a}	1.33±0.34^{a}	1.36±0.19^{a}	1.03±0.05^{a}	1.32±0.18^{a}
TYR	2.94±0.09	6.44±0.16^{a}	9.72±6.34^{a}	5.82±1.82^{a}	7.16±1.56^{a}	4.53±1.28^{a}	8.69±3.11^{a}
SPD	0.11±0.02	0.07±0.01^{a}	0.17±0.12^{a}	0.09±0.02^{a}	0.31±0.22^{a}	0.07±0.00^{a}	0.30±0.22^{a}
SPN	0.11±0.00	0.10±0.00	-	0.34±0.03^{a}	0.47±0.05^{a}	0.11±0.00^{a}	0.59±0.57^{a}
TRP	0.08±0.05	0.15±0.13^{a}	0.06±0.00^{a}	0.07±0.04^{a}	0.15±0.17^{a}	0.43±0.37^{a}	0.15±0.16^{a}
PHEN	0.21±0.00	0.19±0.08^{a}	0.24±0.06^{a}	0.29±0.15^{a}	0.45±0.4^{a}	0.14±0.04^{a}	0.33±0.23^{a}
AGM	0.99±0.55	4.82±0.03^{a}	7.29±0.01^{b}	7.71±4.46^{a}	13.64±0.05^{a}	8.64±3.75^{a}	11.35±2.61^{a}
SER	2.71±0.92	4.99±1.58^{a}	8.45±0.42^{a}	4.33±0.34^{a}	7.52±2.39^{a}	4.37±0.38^{a}	9.25±1.31^{a}

TMA= Trimethylamine, PUT=Putrescine, CAD=Cadaverine, SPD=Spermidine, TRP=Tryptamine, PHEN= 2-Phenylethylamine, SPN=Spermine, HIS=Histamine, SER= Seratonine, TYR=Tyramine, AGM= Agmatine

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

a-b Values in a same column followed by different letters indicate significant differences of the parameter with respect to the kind of groups at same storage days

than putrescine, spermidine and tryptamine. High concentrations of histamine resulted in a reduction in weight gain and feed consumption, therefore present a eventual long-term health hazard for poultry and other animals. No regulation for histamine content in animal food can be found European Union (EU Directive 32/2002; Macan et al., 2006). High contents of histamine (50- 510 mg 100 g^{-1}) were reported in fish meals by some researchers (Pike 1991; Macan et al 2006). The initial histamine content in this study was 0.22 mg 100g^{-1}. The histamine concentrations were found considerably low level in both groups. During 60 days storage, maximum histamine content was 3.71 mg 100g^{-1} and there were no significant differences in FA and FASA groups (P>0.05). On the other hand, the legal limits for histamine content for human consumption regarded by the EU (EEC 1991) as less than 10 mg 100 g^{-1} have not been reached by both groups during storage period. Cadaverine and putrescine contents of acidifed mantis shrimps (FA, FASA) were not considerably changed during storage. However, they were no significant difrences were observed between groups at sampling days. At the 60th day of storage, cadaverine and putrescine concentrations were 1.03 and 2.19 mg 100g^{-1} for FA group and 1.32 and 3.00 mg 100 g^{-1} for FASA group, respectively. Considerably high putrescine (9.20 - 24.1 mg 100g^{-1}) and cadaverine (48.0 - 120.5 mg 100g^{-1}) concentaration were reported for some fish products (Mah et al., 2002; Mohamed et al 2009). In the present study, maximum putrescine and cadaverine contents was found as 3 mg 100g^{-1}. The initial tyramine content was 2.94 mg 100 g^{-1} in raw mantis shrimp and then considerably increased in both groups. However there were no significant difrences between FA ans FASA group at the same storage days. Kuley et al., (2011) reported that quantitatively the most common biogenic amine in fermented meat products was tyramine.

In this study, it was also observed that tyramine was found to be abundant in acidified mantis shrimp. On the other hand, the tyramine concentrations in this study were fairly lower than reported for limit of human consumption (80 mg 100g^{-1}) by Ten Brink et al., (1990).

Microbiological assessment

Figure 2 shows total viable counts (TVC) in acidified shrimps. Initial bacterial load was 4.16 log cfu g^{-1}. Bacterial growths sharply decreased after addition of acids. Initial lactic acid bacteria count was as low as 2.77 log cfu g^{-1}. The increase in lactic acid bacteria counts were observed in groups FA and FASA during storage. Jini et al., (2011) reported that lactic acid bacteria counts in visceral wastes of different freshwater fishes were found in the range of 4.22-5.88 log cfu g^{-1}. Similarly, lactic acid bacteria counts were found in range of 4.50-5.68 log cfu g^{-1} in this study (Figure 2).

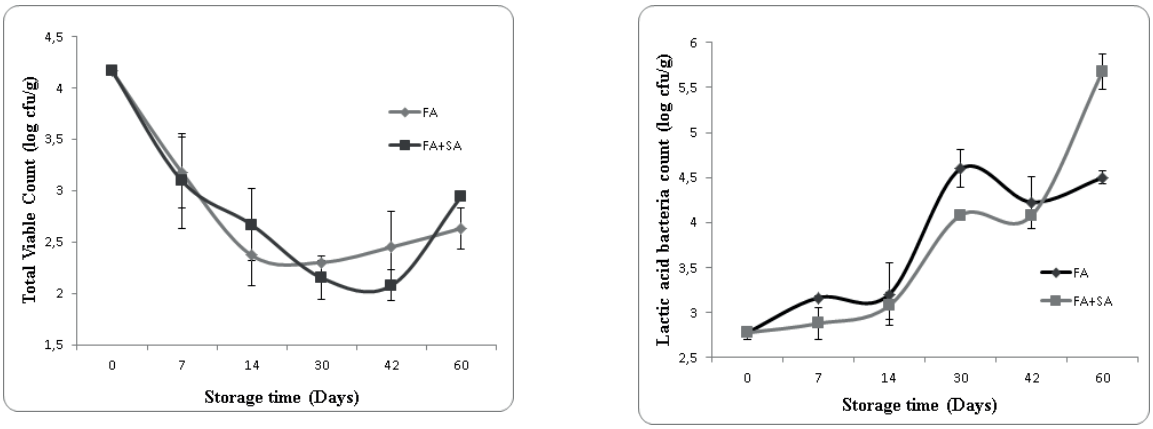

Figure 2. The changes of Total viable and Lactic acid bacteria counts in acidified mantis shrimps during storage period. Means values of three (n=3) independent determinations. Standard deviations are indicated by bars

CONCLUSIONS

According to chemical, microbiological and nutritional evaluation, acidified mantis shrimp has great potential as a feed component because of its nutritious components and storage stability. In general, the range of the biogenic amine concentrations detected in this study can be called as safe as were reported by many studies for fish products. It can be concluded that acid type used for preparation of acidification did not differently effected on biogenic amine formation. Therefore, formic acid or mixture of formic and sulfiric acid can be used for acidification of mantis shrimp without no adverse effect to animal health in regards of biogenic amines.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Comparative *Diplectanum aequans* (Monogenea) infestations in cultured European seabass (*Dicentrarchus labrax*) in the Black Sea and the Aegean Sea

Karadeniz ve Ege Denizi'ndeki kültür levrek balıklarında (*Dicentrarchus labrax*) karşılaştırmalı *Diplectanum aequans* (Monogenea) enfestasyonları

Ahmet Özer[1]* ● Sevilay Okkay[2] ● Türkay Öztürk[3] ● Birol Baki[4] ● Gökçe Acar[5] ● Arzu Güven[6] ● Dilara Kaya Öztürk[7]

[1] Faculty of Fisheries and Aquatic Sciences, Sinop University, 57000, Sinop, Turkey https://orcid.org/0000-0002-2890-6766
[2] Kocaeli University, Faculty of Agriculture and Natural Sciences, 41285, Kocaeli, Turkey https://orcid.org/0000-0003-4440-3525
[3] Faculty of Fisheries and Aquatic Sciences, Sinop University, 57000, Sinop, Turkey https://orcid.org/0000-0001-5568-3214
[4] Faculty of Fisheries and Aquatic Sciences, Sinop University, 57000, Sinop, Turkey https://orcid.org/0000-0002-2414-1145
[5] Faculty of Fisheries and Aquatic Sciences, Sinop University, 57000, Sinop, Turkey https://orcid.org/0000-0002-0127-6602
[6] Faculty of Fisheries and Aquatic Sciences, Sinop University, 57000, Sinop, Turkey https://orcid.org/0000-0002-8220-5282
[7] Faculty of Fisheries and Aquatic Sciences, Sinop University, 57000, Sinop, Turkey https://orcid.org/0000-0003-2505-231X
*Corresponding author: aozer@sinop.edu.tr

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Abstract: In the present study, monogenean infestations were investigated on the gills of the European seabass *Dicentrarchus labrax* grown in culture cages located at Yakakent coast of the Black Sea and Milas coast of the Aegean Sea. Parasitological investigations were conducted simultaneously at both localities during September 2014, February and April 2015. While a total of 100 fish specimens were examined from Yakakent samples whose average length was 19.37 cm, a total of 88 fish specimens were examined from Milas samples whose length was 22.67 cm in average. Only one monogenean species *Diplectanum aequans* was recovered from investigated fish specimens at both sampling localities. Infestation prevalence (%) and mean intensity values of *D. aequans* were 89.0% and 9.6 ± 0.8 in Yakakent samples while those infestation indices were 90.9% and 13.3 ± 1.2 in Milas samples, respectively. The difference between the infestation prevalence (%) values of *D. aequans* at both sampling localities was not statistically significant, on the other hand, the difference was statistically significant in the mean intensity values at both locations. *Diplectanum aequans* showed a homogeneous distribution in all gill arches of fish without any statistically significant difference. This is the first comparative study on the infestation of *D. aequans* on the gills of European sea bass *D. labrax* cultured in the Black Sea and the Aegean Sea.

Keywords: *Diplectanum aequans, Dicentrarchus labrax, Black Sea, Aegean Sea*

Öz: Bu araştırmada, Karadeniz'in Yakakent ve Ege Denizi'nin Milas kıyılarındaki ağ kafeslerde yetiştiriciliği yapılan Avrupa levrek balığının (*Dicentrarchus labrax*) solungaçlarındaki monogen parazitlerin neden olduğu enfestasyonlar araştırıldı. Parazitolojik inceleme eş zamanlı olarak Eylül 2014, Şubat ve Nisan 2015 tarihlerinde alınan balık örneklerinde gerçekleştirildi. Yakakent'teki kafeslerden ortalama boyları 19.37 cm olan toplam 100 adet balık incelenirken, Milas'taki kafeslerden ortalama boyları 22.67 cm olan 88 adet balık incelendi. Her iki örneklem alanından incelenen balık türünde sadece bir monogen parazit olan *Diplectanum aequans* türünün varlığı belirlendi. *Diplectanum aequans* türü monogen parazitin enfestasyon oranı (%) ve enfeste balık başına ortalama sayıları Yakakent örneklerinde sırasıyla %89.0 ve 9.6 ± 0.8 olarak belirlenmişken, enfestasyon parametreleri Milas örneklerinde sırasıyla %90.9 ve 13.3 ± 1.2 olarak belirlendi. Her iki örneklem alanından yakalanan balıklarda belirlenen *D. aequans* enfestasyon oranları (%) arasındaki fark istatistiki olarak önemli bulunmazken, her iki örneklem alanındaki balıklardaki enfeste balık başına ortalama *D. aequans* değerleri arasındaki fark istatistiki olarak önemli bulundu. *Diplectanum aequans* türü parazit incelenen balıkların tüm solungaç yapraklarında homojen bir dağılım gösterdi ve enfestasyon parametrelerinde istatistiki bir fark belirlenmedi. Bu araştırma Karadeniz ve Ege Denizi'nde kültürü yapılan Avrupa levrek balığının (*D. labrax*) solungaçlarındaki *D. aequans* türü monegen parazit enfestasyonları üzerine gerçekleştirilen ilk karşılaştırma çalışmasıdır.

Anahtar kelimeler: *Diplectanum aequans*, *Dicentrarchus labrax*, *Karadeniz, Ege Denizi*

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INTRODUCTION

Monogeneans are widespread parasites throughout freshwater, marine and brackish habitats and commonly found on fishes and lower aquatic invertebrates (Özer and Öztürk, 2005). These parasites are located on the gills, fins and skin of their hosts and feed on mucus and epithelial cells of the skin and gills. This nutritional activity is irritant and stimulates productions of excessive mucus, epithelial hyperplasia, haemorrhage, focal redness or deep maturation and, moreover, create openings for bacterial invasion (Noga, 1995, Reed et al., 2009). The Diplectanidae Monticelli, 1903 is a family comprising approximately 48 genera and approximately 523 described species of a wide diversity of marine and freshwater fishes. The genus *Diplectanum* Diesing 1858 is the largest in the family Diplectanidae. *Diplectanum aequans* (Wagener, 1857) is considered a generalist parasite and has been reported from the gills of either cultured or wild *Dicentrarchus labrax* (European seabass), *Dicentrarchus punctatus* (spotted seabass) and *Sparus aurata* (gilthead seabream) from the Aegean Sea, the Mediterranean Sea, the Adriatic Sea and the Black Sea (Oliver, 1987; Tokşen, 1999, 2007; Cecchini et al., 2001; Mladineo, 2006; Akmırza, 2010; Antonelli and Marchand, 2012; Yardımcı and Pekmezci, 2012; Öğüt and Uzun, 2014).

This parasite can lead to diseases such as Diplectanosis and it is considered to be the one of the most significant ectoparasitic diseases of European seabass *Dicentrarchus labrax* (Whittington and Chisholm, 2008). Silan and Maillard (1989) reported mortalities in seabass with a possible cause by the thousands of *Diplectanum aequans* individuals and they mentioned that the parasite became more proliferated at the beginning of warming in water temperature. Dezfuli et al. (2007) reported annual stock losses of juvenile *D. labrax* caused by *D. aequans* range between 5 – 10% in Italy. Öğüt and Uzun (2014) also reported negative influence of *D. aequans* on the fitness of cultured juvenile seabass *D. labrax* in the central Black Sea. *Diplectanum* parasites are oviparous and produce eggs on the gill of seabass and then, followed by a larval stage (oncomiracidium), post larval stage, II. post-larval stage, intermediate stage and adult stage in its life cycle (Silan and Maillard, 1989) and host switch by this monogenean parasite may occur at any stage of this developmental cycle. All developmental stages of this parasite are also temperature driven (Cecchini et al., 2001). Thus, considering wild fish and culture fish as vectors for disease propagation, it is important to investigate and determine their current status in some species of fish such as seabass which is an intensively cultured species in Turkey. Moreover, histopathological gill damages caused by Diplectanid parasites mainly consist of hyperplasia of the epithelium, fusion of branchial lamellae and the presence of haemorrhagic and inflammatory foci with leucocytic infiltration and these pathological negative impacts on fish hosts indicate their restricting importance in the cultivation of seabass (Gonzalez-Lanza et al., 1991).

Dicentrarchus labrax has been an important species in the Mediterranean, the Aegean and the Black Sea fish culture in sea cages and Turkey is one of the largest *D. labrax* producers in Europe (FAO, 2016). The present study aimed to investigate infestations by a monogenean parasite *Diplectanum aequans* on the gills of *D. labrax* collected simultaneously in the culture cages located in the Black Sea and the Aegean Sea for the first time.

MATERIALS AND METHODS

Monogenean parasites were investigated on the gills of the European seabass *Dicentrarchus labrax* grown in culture cages located at Yakakent coast of the Black Sea (41°40'53.12"N 35°28'22.48"E) and Milas coast of the Aegean Sea (37°17'32"N 27°26'43"E) (Figure 1). Parasitological investigations were conducted simultaneously at both sampling locations during September 2014, February and April 2015. A total of 100 from Yakakent samples and 88 from Milas samples were examined at the Faculty of Fisheries and Aquatic Sciences in Sinop according to the methods specified by Öztürk and Özer (2014). Fish samples from both localities were transferred in boxes containing dried ice and then gills were examined using a light microscope at x100 and x200 magnification. Each gill arches from both sides of all fish samples were carefully examined to determine their exact locations on the gills and total number of parasite individuals per gill arch were counted and noted. Calculation of infection prevalence (%) and mean intensity follow the definitions by Bush et al. (1997). Parasite species was identified using a phase contrast Olympus microscope (BX53) equipped with a digital camera (DP50) and drawing attachment. Morphological identification was conducted with the definitions by Oliver (1968) and Mounira (2008). Measurements were made on 30 fresh parasite individuals and are given in micrometers as mean and range. Some water parameters were measured at a depth of 5 meters using YSI 556 MPS model field type multiparameter device.

The Kruskal-Wallis test (nonparametric ANOVA) was performed to find out the preference of *D. aequans* for particular gill arches of host fish as well as seasons. The differences in parasite loading on the combined 4 left and 4 right gill arches were tested statistically by the Mann-Whitney U test. All statistical analyses were performed at the significance level of 5% using the program GraphPad InStat 3.00.

Figure 1. Map of sampling locations around the Yakakent coasts of the Black Sea and the Milas coasts of the Aegean Sea. * indicates exact location of sampling localities Yakakent and Milas.

RESULTS

Diplectanum aequans (Wagener, 1857) (Figure 2) was the only monogenean species identified on the gills of *Dicentrarchus labrax* in the present study. Morphometric data of this parasite species are as follows; total length of body 1250 µm (1200-1280 µm), width of body 350 µ (310-380 µm), diameter of haptor 196 µm (110-300 µm) and length of marginal hooks 11 µm (11-12 µm).

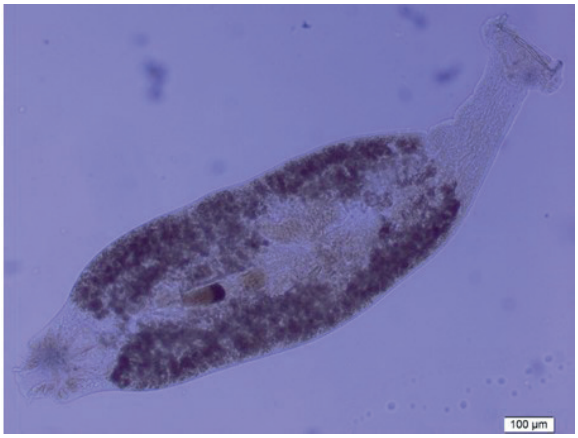

Figure 2. An individual of *Diplectanum aequans* (Wagener, 1857)

The infestation prevalence (%) and mean intensity values of each sampling months from both localities were calculated and presented in Table 1.

The difference between overall mean intensity values of *D. aequans* from both sampling localities was statistically significant ($p<0.05$). On the other hand, *D. aequans* individuals showed a homogeneous distribution on all gill arches of examined fish without any statistically significant difference in the infestation indices ($p>0.05$). Some water parameters at Milas and Yakakent farming sites were measured during the sampling months and presented in Table 2.

DISCUSSION

Various species of Monogenea have been identified to be pathogens that can cause diseases in fishes either directly or by secondary infection (Thoney and Hargis, 1991). *Diplectanum* spp. are the gill parasites which have been associated with high mortalities of host fish as a result of Diplectanosis, the name of the disease they cause. These parasites can be transmitted from fish to fish in wild and in captivity, and from wild to captive fish in culture cages (Özer et al., 2015). During the investigation period in the present study, *Diplectanum aequans* was the only monogenean species identified on the gills of European seabass *Dicentrarchus labrax* and this parasite species has already been reported from this host fish in wild and culture conditions from the Aegean Sea, the Mediterranean Sea, the Adriatic Sea, the Black Sea and Portuguese coasts of the Atlantic Ocean (Oliver, 1987; Silan and Maillard, 1989; Cognetti-Varialle et al., 1992; Tokşen, 1999, 2007; Santos, 1996; Mladineo, 2006; Whittington and Chisholm, 2008; Akmırza, 2010; Antonelli and Marchand, 2012; Yardımcı and Pekmezci, 2012; Öğüt and Uzun, 2014). However, this is the first simultaneous study comparing *D. aequans* infestations at two distinct sampling localities at the Black Sea and the Aegean Sea. Prevalence of infestation (%) found in the present study is quite similar (between 50 - 99%) to that reported by the above mentioned authors for the Mediterranean Sea, the Baltic Sea and the Black Sea.

Table 1. Infestation prevalence (%) and mean intensity values of *Diplectanum aequans* determined from European seabass (*Dicentrarachus labrax*) collected from culture cages in two sampling localities in the Black Sea and Aegean Sea and its distribution on the gill arches of the host fish

	Fish Length ± S.E	Infestation Prevalence (%)	Mean Intensity ± S.E
Sampling months			
Yakakent (Black Sea)			
September 2014 (n=39)	16.9 ± 0.2	87.2	6.3 ± 0.9
February 2015 (n=31)	22.4 ± 0.2	96.8	16.4 ± 1.5
April 2015 (n=30)	22.5 ± 0.2	83.3	5.8 ± 0.8
Overall (n=100)		**89.0**	**9.6 ± 0.8[a]**
Milas (Aegean Sea)			
September 2014 (n=30)	16.0 ± 0.2	96.7	6.9 ± 0.8
February 2015 (n=36)	25.4 ± 0.3	91.7	17.5 ± 1.6
April 2015 (n=22)	26.7 ± 0.5	81.8	15.9 ± 3.6
Overall (n=88)		**90.9**	**13.3 ± 1.2[b]**
Gill arches			
Yakakent (Black Sea)			
Left 1		54.0	2.1 ± 0.2
Left 2		56.0	2.0 ± 0.2
Left 3		46.0	2.3 ± 0.2
Left 4		44.0	2.0 ± 0.2
Total left		**82.0**	**5.1 ± 0.5[a]**
Right 1		45.0	2.1 ± 0.2
Right 2		56.0	2.3 ± 0.2
Right 3		45.0	2.3 ± 0.2
Right 4		51.0	2.1 ± 0.2
Total right		**84.0**	**5.3 ± 0.5[a]**
Milas (Aegean Sea)			
Left 1		51.1	2.8 ± 0.4
Left 2		54.5	2.8 ± 0.3
Left 3		56.8	2.6 ± 0.3
Left 4		46.6	2.9 ± 0.3
Total left		**84.1**	**6.9 ± 0.8[a]**
Right 1		62.5	2.8 ± 0.3
Right 2		59.1	3.0 ± 0.3
Right 3		54.5	2.5 ± 0.3
Right 4		59.1	2.5 ± 0.2
Total right		**78.4**	**8.1 ± 0.7[a]**

*Different superscript letters in any statistical comparison show significant differences ($p<0.05$)

Table 2. Water temperature (ºC), dissolved oxygen (mg/lt), salinity (ppt) and pH values measured at both sampling localities and three sampling months

Environmental parameters	September		February		April	
	Milas	Yakakent	Milas	Yakakent	Milas	Yakakent
Temperature	25.40	23.00	16.54	15.00	16.90	9.90
Oxygen	9.23	7.80	8.77	8.70	9.99	9.00
Salinity	35.00	16.31	35.50	16.97	35.00	15.90
Ph	8.55	8.20	8.41	8.00	8.50	8.63

Moreover, monthly prevalence (%) of infestation at both sampling localities showed similar pattern at both sampling localities without any significant difference ($p>0.05$), even though there were fluctuations in the infestation indices. Environmental temperature is known to be one of the important variable both for the host and the parasite (McCarty, 2001; Xenopoulos et al., 2005). Higher values of both infestation prevalence (%) and mean intensities in February at both sampling localities may reflect the stimulating impact of temperature on the spawning of *D. aequans* along with the decrease in fish resistance to parasitic infestations in lower temperatures. Moreover, Cecchini et al. (2001) showed experimentally that eggs of *D. aequans* were able to survive at 5℃ and egg hatching could be observed for temperatures ranging from 10℃ to 30℃. Similar to our results, Gonzalez-Lanza et al. (1991) also reported a seasonal variations, with maximal infection levels of *D. aequans* occurring in winter, suggesting continuous parasites recruitment in cultured seabass from the Spanish Mediterranean coasts. Thus, following the coincidence of above mentioned both host and parasite factors, we can assume that European seabass exposed to increased *D. aequans* infestations when they are more susceptible in winter. Antonelli and Marchand (2012) indicated that significantly higher *D. aequans* infestations in winter along with negligible infestation levels in summer on the host fish *D. labrax* were resulted from the adaptation of this parasite to cold temperature. Further to this indication, Lambert and Maillard (1974) and Gonzalez-Lanza et al. (1991) also suggested continuous recruitment and the persistence of this parasite species throughout the year.

In the present study, the overall mean intensity values of *D. aequans* at the Aegean Sea samples (13.3 ± 1.2) is higher than that of the Black Sea samples (9.6 ± 0.8) with statistically significant difference ($p<0.05$). However, our data on intensity values were always lower than those reported from Coscica coasts of the Mediterranean Sea (Antonelli and Marchand, 2012), Tuscany coasts of the Mediterranean Sea (Cognetti-Varriale et al. 1992), Spanish Mediterranean Sea (Gonzalez-Lanza et al. 1991), the northern coast of the Adriatic Sea (Dezfuli et al. 2007), Lion Gulf of the Mediterranean Sea (Oliver, 1987), Yardımcı and Pekmezci (2012), but higher than those of the eastern Black Sea (Öğüt and Uzun, 2014) and the eastern Adriatic Sea (Mladineo, 2006). Above mentioned sampling areas for *D. aequans* infestations on *D. labrax* have different water salinity values ranging from 17‰ to 38‰ and it is clear that *D. aequans* has the ability of survival as well as hatching success within this range of salinity. Seasonal intensity values at both sampling localities in the present study revealed seasonal fluctuations, winter having the highest infestation values. According to Antonelli and Marchand (2012) these variations in rates of infestation through the seasons were attributed to the changes in the fish behaviour and peak abundances recorded in winter in the present study coincided with an immunodeficiency of fish linked to brutal changes in temperature during the transition from autumn to winter as was determined by Faliex et al. (2008). Moreover, these periods of decreasing water temperature resulted in a stress response of the European seabass (Hadj Kacem et al., 1987) reflecting a weakened immune system of fish, and therefore a greater vulnerability, leaving the opportunity for parasites to increase their populations rapidly (Oliver, 1982).

Host and, to some extent, site specificity is a well-known phenomenon in monogenean parasites of fish. This study provided a comparative data on the distribution of *D. aequans* on the gill arches of host fish *D. labrax* collected from two distinct sampling localities with different salinities, 17‰ in the Black Sea and 35‰ in the Aegean Sea. A homogenous distribution of this parasite without any significant difference ($p>0.05$) over the gill arches of fish at both sampling localities were determined in the present study. However, some contradictory results on the distribution of *D. aequans* on the gill arches of host fish *D. labrax* have been reported in a limited number of previous studies.

Cognetti-Varielle et al. (1992) reported a non-significant distribution of this parasite's adult and juvenile forms over the gill arches at both left and right side of host fish but, noted that the location of juvenile and adult forms of this parasite at the posterior or anterior parts of each hemibranch of any given gill arches may be different. They attributed these results to the difference in the inlet-outlet water flow through the branchial chambers. Özer and Öztürk (2005) reported no significant preference of another monogenean *Dactylogyrus cornu* for the left and right sets of the gills of host fish *Vimba vimba tenella* although, statistically significant numbers occurred on the filaments of the second gill arches of both sides. Several authors suggested that monogenean distribution to be influenced by the hydrostatic pressure of the branchial pump, coughing action and water current over the gill surface during the respiratory cycle (Hughes and Shelton, 1958; Paling, 1968; Wootten, 1974).

In conclusion, the present study provided the first simultaneous and comparable infestation details of *D. aequans,* a previously known monogenean parasite of *D. labrax* collected from two distinct localities in the Black Sea and the Aegean Sea, and results provided here are of practical importance on the determination of control strategies to be developed against this significant pathogen.

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Author contributions

All authors conceived, designed and conducted field and laboratory work. A.Ö. analysed the data and wrote the initial version of the manuscript; all other authors provided editorial advice.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Dalyan balıkçılığının iş sağlığı ve güvenliği açısından değerlendirilmesi

Evaluation of the lagoon fishery on occupational health and safety

Seda Köken[1*] ● Tevfik Ceyhan[2] ● Zafer Tosunoğlu[2]

[1]Ege Üniversitesi, Fen Bilimleri Enstitüsü, İş Güvenliği Anabilim Dalı, Bornova, İzmirr https://orcid.org/0000-0002-6758-7024
[2]Ege Üniversitesi, Su Ürünleri Fakültesi, Bornova, İzmir https://orcid.org/0000-0002-4799-5709
[3]Ege Üniversitesi, Su Ürünleri Fakültesi, Bornova, İzmir https://orcid.org/0000-0002-1168-9611
*Corresponding author: seda_koken@hotmail.com

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Öz: Dalyanlar kıyı lagünlerinin su giriş çıkışlarının kontrollü bir şekilde kullanıldığı balıkçılık sektörünün önemli bir parçasıdır. Günümüzde dalyan alanlarındaki tehlike ve risklere karşı iş sağlığı ve güvenliği kapsamında koruyucu ve önleyici tedbirler uygulanmaya başlanmıştır. Dalyan balıkçılığı, iş sağlığı ve güvenliğine ilişkin işyeri tehlike sınıfları tebliğinde; 03.11.01 NACE kodu ile deniz ve kıyı sularında yapılan balıkçılık (gırgır balıkçılığı, dalyancılık dahil) faaliyetleri arasında tehlikeli sınıfta yer almaktadır. Bu çalışmada iş sağlığı ve güvenliği açısından dalyan balıkçılığının incelenmesi amaçlanmıştır. Bu sebeple, Ege Denizi kıyılarında aktif faaliyetine devam eden 6 dalyan arasından rastgele üç dalyan belirlenmiştir ve Ekim 2017 – Nisan 2018 tarihleri arasında 92 dalyan çalışanı ile yüz yüze anket çalışmaları yapılmıştır. Ankete katılan balıkçıların %97'si erkek, %3'ü bayan çalışanlardan oluşmaktadır. Bir numaralı dalyan çalışma ortamında (D1) %90, iki numaralı dalyan çalışma bölgesinde (D2) %74 ve üç numaralı dalyan alanının (D3) tamamında ilkokul mezunu çalışan bulunmaktadır. Çalışanların işe girişlerde D1 (%29), D2 (97) ve D3 (%17) sağlık muayeneleri yapılmıştır. Mesleki iş kazaları ve meslek hastalıkları hakkında çalışanların D1'de %25'i, D2'de %29'u ve D3'de %26'sı haberdar olmaktadır. Dalyanlarda yaşanan iş kazalarının sıklığı sıra ile %50 kesikler, %19 ezikler, %16 kırık-çıkık, %9 boğulma ve %6 baş bölgesi yaralanmaları olarak tespit edilmiştir. Dalyan kuzuluklarında iş kazalarının en fazla yaşandığı alan olan iskele platformlarını çalışanların D1'de %44'ü, D2'de %11'i ve D3'de %35'i güvenli bulmamaktadır.

Anahtar sözcükler : *Dalyan balıkçılığı, iş sağlığı, iş güvenliği, kuzuluklar, av aracı*

Abstract: Lagoons are important parts of the fishery sector as the areas where the entry and exit of waters are used under controlled conditions. At the present time, the protective and preventive measures have been started to be applied against the hazards and risks in the fields of occupational health and safety. Lagoon fisheries are among the fishery activities (including purse seine fishing, scuba fishing) in marine and coastal waters that are coded as 03.11.01 in the NACE code in occupational health and safety in the workplace hazard classes declaration and are classified as dangerous. In this study, it is aimed to examine the fishery in terms of occupational health and safety. For this reason, randomly 3 lagoons were determined among the 6 lagoons that continue their active activities on the coasts of the Aegean Sea between October 2017 and April 2018, 92 surveys were interviewed face to face. The 97% of the fishermen surveyed were male and 3% female employees. 90% of D1, 74% of D2 and all D3 employees are primary school graduates. The rate of health examinations of employees before work is 29% in D1, 97% in D2 and 17% in D3. The 25% of the employees in D1, 29% in D2 and 26% in D3 have knowledge about occupational accidents and occupational diseases. In terms of occupational accidents in lagoon, cutting, crushing, fracture-dislocation, suffocation and head injuries were frequently determined as 50%, 19%, 9% and 6%, respectively. The pier platforms where occupational accidents are most prevalent in lagoon barrier trap are not safe by employees, 44% in D1, 11% in D2 and 35% in D3.

Keywords: *Occupational health, occupational safety, barrier trap, lagoon fisheries, fishing gear*

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GİRİŞ

İş sağlığı ve güvenliği hakkında eski yaklaşımlar reaktif yaklaşım olarak isimlendirilmekte olup, bu yaklaşım olay sonrası inceleme, yani olmuş olan olaylar ile uğraşır, tazmin edici yaklaşımlar içerir ve güvensiz koşullar ihmal edilebilmektedir. Günümüzdeki iş sağlığı ve güvenliğine ait proaktif yaklaşım iş akışındaki güvensiz koşulları belirleyerek daha çağdaş bir yaklaşım ile tehlike oluşturan faktörlerin riske dönüşmemesi için önleyici faaliyetler geliştirir, risk değerlendirmesi ve güvenlik kültürüne odaklanılır. Türkiye'de iş sağlığı ve güvenliği uygulamaları 6331 sayılı İş Sağlığı ve Güvenliği Kanunu (T.C. Resmi Gazete, 2012) doğrultusunda T.C. Aile, Çalışma ve Sosyal Hizmetler Bakanlığı kontrolünde teftiş ve denetimler ile sürdürülmektedir.

ILO (2019) dünyada yılda yaklaşık 340 milyon iş kazası meydana geldiğini bildirmektedir. Bunlardan 357.948'i ölümle sonuçlanan iş kazaları olmaktadır. Türkiye'de yaşanan iş kazalarına bağlı ölümlerde ise Dünya ve Avrupa Birliği ortalamalarının çok üzerindedir. 2016 yılı verilerinde ölümlü iş kazası sıralamasında Türkiye Avrupa'da birinci sırada, dünyada üçüncü sırada yer almaktadır (Öçal ve Çiçek, 2017). Türkiye'de 2007 ile 2016 yılları arasındaki ortalama iş kazası sayısı 295,078'dir. En çok kaza 2012 yılında 1.647.127'dir. Erginel ve Toptancı (2017) 2012 yılında ve sonrasında kaza bildirim sayısında artış yaşanması 6331 sayılı kanun ile getirilen zorunluluk ve yaptırımların uygulanmasıyla ilişkilendirmiştir.

Lagünler genellikle nehir, kaynak veya yeraltı suları tarafından beslenen, denize karışmayan, sığ göllerdir. Lagün kıyı seti, akarsuların getirdiği kumları, kıyıya yığması veya denizin sürükleyip getirdiği çakıl, kum ve molozların bir koy veya körfez önüne yığması ile meydana gelmektedir (Tosunoğlu vd., 2017). Özel ekosistemler olan sulak alanlar ve lagünler birçok görev üstlenmektedir. Kara ve deniz arasında yer alan lagünler hem karasal hem de denizel faktörlerin etkisinde deniz suyu ve tatlı su ortamları arasında geçiş bölgeleridir (Acarlı vd., 2006; Balık vd., 2008). Dalyanların yapısal özelliğinden kaynaklı akıntı, rüzgâr, su hareketleri ve su sıcaklığı çalışanları tehlikeye sokan durumlardandır. Dalyanlarda su bitkilerinin yoğunluğu oldukça fazladır ve bu durum av araçlarına oldukça zarar vermektedir.

Lagün ekosistemleri, çok sayıda balık türüne larva ve yetişkin aşamalarında yiyecek ve barınak sağlayan sığ ve acı sular olup besleyici açıdan zengin habitatlardır. Bu sahalara beslenme amacıyla giren balıklar belli bir süre sonra girişler kapatılarak hapsedilmektedir (Kınacıgil ve İlkyaz, 1997). Lagünde geleneksel olarak kuzuluk, uzatma ağları, kargılı ağlar ve pinter gibi av araçları kullanılmaktadır (Acarlı vd., 2006). Dalyan balıkçılığının temel av aracı sabit bariyer tuzaklarıdır. Bu tuzaklar kargı, plastik veya demir çubuklardan yapılmakta olup kuzuluk olarak tanımlanmaktadır (Gökçe ve Tosunoğlu, 2016).

Genel olarak ülkemiz lagünlerinde ilk hareketi kol gücü ile verilen pancar motorlu tekneler kullanılmaktadır. Teknelerin manevra kabiliyetini ve hızlarını artıran şanzıman ve motorun çalışmasını hızlandıran "Lombardini" ve "Katana" marka motorlar kullanılmaktadır (Demirol ve Yüksel, 2014). Ayrıca bu tekneler pancar motorlara göre daha az gürültülü çalıştığından son zamanlarda daha çok tercih edilmektedir. Dalyanlarda motorsuz tekne diye adlandırdığımız altı düz yapıdaki kuritalar günümüzde de sığ sularda kullanılmaya devam edilmektedir. Bu teknelerde motor olmadığından tekne üzerindeki insanlar tarafından el gücü ile teknenin gidişi yönlendirilmektedir.

Dalyanda çalışan balıkçılar kooperatif elemanı olarak işçi statüsünde taşeron firmalara bağlı olarak çalışmaktadır. Çalışma alanı bakımından dalyanlar yerleşim yerlerinden uzakta yer almaktadır. Dalyanların kurulum zamanında oldukça fazla sayıda taşeron işçi çalışmakta, diğer zamanlarda bu sayı oldukça azalmaktadır. Ayrıca bunların dışında dalyan sahası içinde pasif av araçları (uzatma ağı, pinter) ile yıl boyu dalyanı kiralayan kooperatife üye balıkçılar avcılık yapmaktadır.

Avrupa Birliği ekonomik faaliyetlerin istatistiki sınıflandırılması kısaca NACE olarak adlandırılmaktadır. Ekonomik Faaliyetlerin Uluslararası Standart Sanayi Sınıflaması (ISIC) ile ilişkisinden dolayı NACE, ekonomik faaliyetlere ilişkin istatistiki verileri dünya düzeyinde karşılaştırma açısından oldukça önemli bir araçtır (Anonim, 2019). Türkiye'de 6331 sayılı iş sağlığı ve güvenliği kanununa göre sektörlere ilişkin işyerlerinin tehlike sınıfları verilmektedir. T.C. Resmi Gazete (2017) de balıkçılık ve su ürünleri yetiştiriciliği 03 ana başlığı altında; Balıkçılık ve Su ürünleri yetiştiriciliği sırasıyla 03.1 ve 03.2 NACE kodu ile iki sınıfa ayrılmıştır. Deniz ve kıyı sularında yapılan balıkçılık (gırgır balıkçılığı, dalyancılık dahil) 03.11.01 NACE kodu ile tehlikeli sınıfta, deniz kabuklularının (midye, ıstakoz vb.), yumuşakçaların, diğer deniz canlıları ve ürünlerinin toplanması (sedef, doğal inci, sünger, mercan, deniz yosunu vb.) 03.11.02 NACE kodu ile çok tehlikeli sınıfta deniz balıkçılığı faaliyetleri kapsamında iki kısma ayrılarak sınıflandırılmıştır. NACE kodu 03.12.01 olan tatlı sularda (ırmak, göl) yapılan balıkçılık (alabalık, sazan, yayın vb.) tehlikeli sınıftadır. Denizde yapılan balık yetiştiriciliği 03.21.01 ve denizde yapılan diğer su ürünleri yetiştiriciliği 03.21.02 NACE kodlarıyla beraber tehlikeli sınıfta, tatlı sularda yapılan balık yetiştiriciliği 03.22.01 NACE kodu ile az tehlikeli ve tatlı su ürünleri yetiştiriciliği 03.22.02 NACE koduyla tehlikeli sınıfta yer almaktadır.

Dünyada iş sağlığı ve güvenliği alanında su ürünleri sektörü kapsamında araştırmalar (Moreau ve Neis,

2009; Norwegian Labour Inspection Authority, 2001; Myers ve Durborow, 2012; EL-Saadawy vd., 2014; Guertler vd., 2016) bulunmakta iken Türkiye'de yapılan araştırmalar yetiştiricilik tesisleri (Atayeter ve Atar, 2013; Özönel, 2015; Uyumsal, 2017; Perçin, 2018a, 2018b), balıkçılık (Ulukan, 2016), küçük ölçekli balıkçılık (Perçin vd., 2011; Perçin, 2017) ve büyük ölçekli balıkçılık (Perçin, 2017), balık halleri (Akyol vd., 2016), balıkçı barınakları ve balıkçı limanları (Perçin, 2018c), su ürünleri işleme tesisleri (Atayeter ve Terzioğlu, 2009; Mert ve Ercan, 2014) ve genel (Şık, 2017) alt başlıkları altında toplanabilir.

Dünyada ve Türkiye'de iş sağlığı ve güvenliği konusunda dalyan balıkçılığında yapılan bir çalışmaya rastlanılmamıştır. Balıkçılık sektörü içerisinde çalışan personel sayısı bakımından dalyan balıkçılığı önemli bir yer tutmaktadır ve çalışma koşulları oldukça ağır, iş sağlığı ve güvenliği açısından tehlikeler içermektedir. Bu çalışmada, dalyanlarda çalışanların iş sağlığı ve güvenliği açısından karşılaştığı tehlikeler, mesleki risk bilinci ve iş sağlığı ve güvenliği konusunda ne derece bilgili oldukları ölçülmeye çalışılmıştır. Ayrıca mesleki risk faktörleri tanımlanarak bu tehlikeleri azaltacak ve ortadan kaldıracak önlemlerin ortaya konması amaçlanmıştır.

MATERYAL ve METOT

Türkiye'nin Ege Denizi kıyılarında avcılığın aktif olarak sürdürüldüğü 6 adet dalyan içinden rastgele 3 dalyan seçilmiştir ve Dalyan 1 (D1), Dalyan 2 (D2), Dalyan 3 (D3) olarak isimlendirilmiştir.

Dalyanların çalışma prensibi

Dalyanlarda avcılık işlemleri, pinter ve uzatma ağları ile yapılırken, kuzuluklarda da hasat işlemi yapılmaktadır. Kuzuluklar kargı-saz gibi malzemelerin eşit uzunlukta ve deniz dibine rahat batması sağlanacak şekilde kesilerek hazırlanmaktadır. Kuzulukların oluşturulmasında maket bıçağı gibi pek çok kesici alet, tokmaklar ve çiviler kullanılarak el gücüne dayalı iş yapılmaktadır. Kargı-sazların ve ağaç kazıkların taşınma işlemlerinde motorsuz el gücüne dayalı altı düz kurita denilen tekneler kullanılmaktadır. Rüzgâr ve fırtına sonucunda kargı setleri zarara uğrayabilmektedir. Bu tehlikeler sonucunda setin yıkılması, balığın kaçması ve tüm ürünün kaybolması riski oluşabilmektedir. Bu tür tehlike ve risklerin oluşması iş kazalarını da beraberinde getirmektedir. Balıkların göç davranışına uygun olarak yapılan ve yapay olarak hazırlanan kuzulukların açılma ve kapanma zamanları farklılık gösterse de, Ege dalyanlarında genellikle dalyanın kapatılması haziran başında, açılması da aralık-ocak aylarında olmaktadır.

Ekim 2017 – Nisan 2018 tarihleri arasında su ürünleri sektörü içinde en çok görülen iş kazaları ve ölümlü kazalar göz önünde bulundurularak kullanılan av araçlarından kaynaklı kaza yaralanmaları araştırılmış ve dalyan çalışma sahaları incelenmiştir. Toplamda 92 dalyan balıkçısı ile yüz yüze görüşülmüştür ve D1ve D2'de çalışan balıkçıların %33,7'si D3'de çalışan balıkçıların ise %32,6 ile dalyan balıkçılığı konusunda iş sağlığı ve güvenliği çerçevesinde anketler yapılmıştır.

İstatistiki analizler

Elde edilen veriler tek yönlü varyans analizi ve χ^2 testleri kullanılarak istatiksel olarak incelenmiştir İstatistiki analizlerde SPSS 20.0 programı kullanılmış ve yanılma olasılığı 0,05 olarak kabul edilmiştir.

BULGULAR

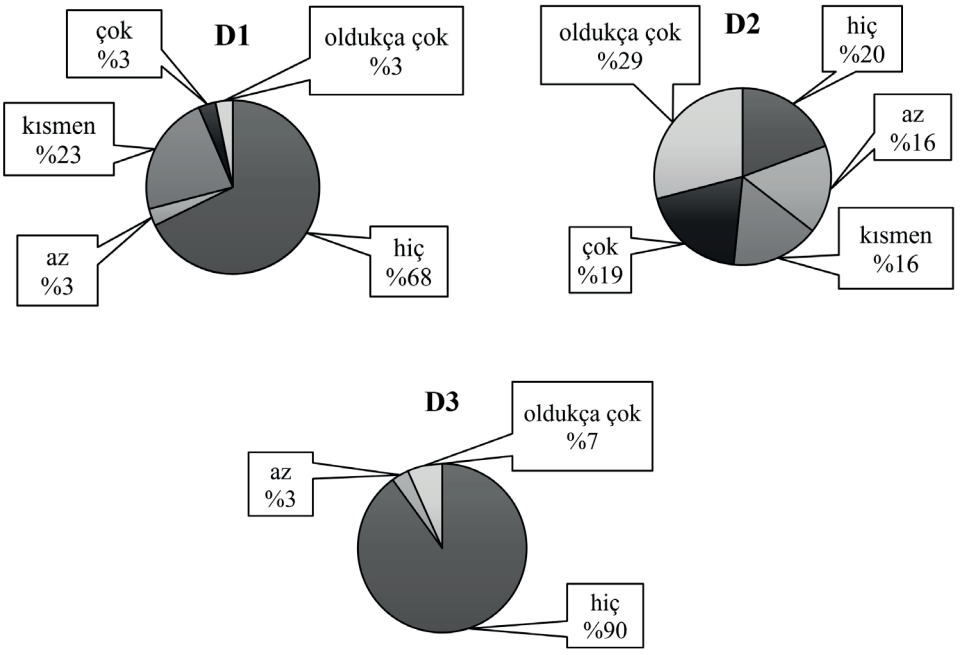
Ege Bölgesi'nde faaliyet gösteren D1 ve D3 dalyanlarında çalışanların tamamı erkek bireylerden, D2'de çalışanların ise %10'u kadınlardan oluşmaktadır. D1'de çalışanların %90'ı, D2'de %74'ü ve D3'de ankete katılanların tamamı ilköğretim mezunudur. Dalyanlar arasında çalışanların eğitim durumları karşılaştırıldığında istatistiki açıdan anlamlı bir farklılık çıkmamıştır ($p>0.05$). Dalyan çalışanları 23 ile 74 yaş aralığındadır. D1, D2, D3'de çalışanların yaş ortalaması sırasıyla 53, 39 ve 55'dir. D1 - D2 ve D3 – D2 çalışanlarının yaşları arasında anlamlı derecede farklılık olduğu ($p<0.05$), D1 – D3 arasında ise fark olmadığı sonucu çıkmıştır ($p>0.05$).

Günlük çalışma saati (8 saat) ve haftalık toplam çalışma saati (45 saat) incelendiğinde D1'de %13, D2'de %84'ü, D3'de çalışanların %44'ü uygun sürelerde çalışmaktadır. Günlük çalışma saatleri karşılaştırıldığında D1 – D2 ve D3 – D2 arasında istatistiki açıdan fark olduğu ($p<0.05$), D1 – D3 arasında farklılık olmadığı görülmektedir ($p>0.05$).

D1 ve D2'de çalışanlar arasında hiç kimseye meslek hastalığı tanısı konulmamıştır. D3'de ise bir kişi meslek hastalığını bel fıtığı olarak kendisi belirtmiştir fakat bu durum meslek hastalıkları birimi tarafından raporlanmamıştır. Çalışanların meslek hastalığı teşhisinin konulmasına dair dalyanlar arasında istatistiki açıdan anlamlı bir farklılık bulunmamıştır ($p>0.05$).

İş kazaları açısından incelendiğinde, D2'de hiç iş kazası yaşanmadığı, D1 ve D3'de ise %2 oranında olmak üzere toplamda %4 oranında iş kazası yaşandığı saptanmıştır. D1'de tekneden suya düşme ve zeminde kayma-düşme kazaları yaşandığı, D3'de ise uzuv kopması ve göz bölgesine gelen sivri cisim sonucu iş kazası olarak tespit edilmiştir. Dalyanlar da meydana gelen iş kazaları karşılaştırıldığında istatistiki olarak anlamlı fark tespit edilememiştir ($p>0.05$).

Dalyanlar arasında çalışanların iş sağlığı ve güvenliği kanunu hakkında ne derece bilgi sahibi oldukları Şekil 1'de verilmiştir. Konu hakkında D1-D3 arasında istatistiksel açıdan anlamlı bir fark yok iken ($p>0.05$), D1-D2 ve D3-D2 arasında anlamlı fark saptanmıştır ($p<0.05$).



Şekil 1. Dalyan çalışanlarının iş sağlığı ve güvenliğine dair bilgi oranları

Figure 1. The rates of knowledge on the occupational health and safety of lagoon employees

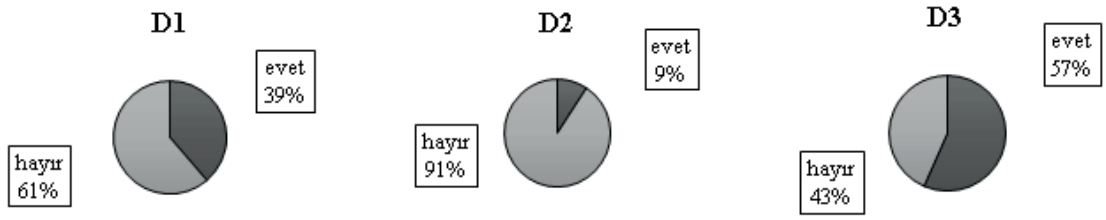
İş sağlığı ve güvenliğine ilişkin uzman kişiler tarafından bilgilendirme toplantıları D2'de %70, D3'de %10 ve D1'de %7 oranında yapılmaktadır. D1-D2 ve D3-D2 arasında çalışanların bilgilendirilmesinde istatistiki açıdan fark çıkmış ($p<0.05$), D1-D3 arasında ise fark bulunamamıştır ($p>0.05$).

İş sağlığı ve güvenliğinin benimsenmesi ile birlikte çalışma alanlarında uyarı levhalarının bulundurulduğu tespit edilmiştir. D2'de çalışanların sadece %62'si uyarı levhalarının bulunduğunu belirtmesine D2'de çalışma alanı ziyaret edildiğinde uyarı levhalarına rastlanılmıştır. D1'de çalışanların %32'si uyarı levhalarının bulunduğunu belirtse de bu dalyan ziyaretinde uyarı levhalarına rastlanılamamış olup, uyarı levhaları olarak kara yolu levhalarını göstermişlerdir. D3'de ise hiç uyarı levhası bulunmamaktadır. Bu konuda dalyanlar arasındaki karşılaştırmada istatistiki açıdan fark bulunmuştur ($p<0.05$).

Dalyan çalışanları kış aylarında kişisel koruyucu donanımları (KKD) daha yoğun kullanılmaktadır. Tüm dalyanlarda çalışanların %53'ü sürekli KKD kullandıklarını, %39'unun sıklıkla kullandıklarını belirtmişlerdir. Dalyan çalışanlarından KKD kullanımı kendi içlerinde değerlendirildiğinde ise D1-D2 ve D2-D3 dalyanları arasında istatistiki açıdan fark bulunmamış ($p>0.05$) iken D1-D3 dalyanları arasındaki fark istatiksel olarak önemli bulunmuştur ($p<0.05$).

Balıkçılık faaliyetlerinin tehlikeli sınıfta yer aldığı bilgisi D1 dalyanı çalışanlarının %62'si, D2 dalyanı çalışanlarının %73'ü ve D3 dalyanı çalışanlarının %43'ü tarafından bilinmektedir ($p>0.05$). D1'deki balıkçıların %45'i, D2 de balıkçıların %42'si ve D3 deki balıkçıların %62'si dalyan sahasında kayma-düşme yaşamıştır. Ancak bu olay balıkçı sayısı incelendiğinde istatistiki olarak önemli olarak bulunmamıştır ($p>0,05$).

Balıkçılıkta sık karşılaşılan kazalardan biri de tekneden suya düşmek olarak bilinmektedir. Tekneden suya düşmenin iş kazası olduğu belirtildiğinde, çalışanların bu durumu iş kazası olarak görmedikleri, bunu işlerinin bir parçası olarak benimsediklerini bildirmektedirler (Şekil 2). D1-D3 dalyanları arasında istatistiki açıdan fark çıkmaz iken ($p>0.05$), D2-D3 ve D1-D2 dalyanları arasında tekneden suya düşme oranlarının istatistiki açıdan farkı önemlidir ($p<0.05$).



Şekil 2. D1, D2 ve D3'de çalışanların tekneden suya düşme oranları
Figure 2. The rates of falling of employees from boat to water in D1, D2 and D3

Dalyanlarda iş sağlığı ve güvenliği bakımından saha çalışması yapıldığında gerek ortam şartları, gerekse çalışanlardan kaynaklı tehlikelerin çalışanları tehdit ettiği gözlemlenmiştir. Bu tehlikeler ortam şartlarının yetersizliği, teknik yatırımların azlığı, iyileştirmelerin yapılmaması, teknik eleman eksikliği ve bilgi eksikliği olarak tespit edilmiştir. Teknik eleman eksikliği D1 ve D2'de, yetersiz teknik yatırımlar D1 ve D3'de yüksek oranda tespit edilmiştir (Tablo 1).

Dalyanlarda yaşanan kazaların %50'si kesikler, %19'u burkulma - ezilmeler, %16'sı kırık-çıkıklar, %9'u boğulma ve %6 baş bölgesi yaralanmalarıdır (Tablo 2).

D1'de %44, D2'de %11 ve D3'de ise çalışanların %35'i iskele platformlarını güvenli bulmadıklarını dile getirmişlerdir. Balıkçılar üç dalyanda da iskele platformlarını güvensiz olarak tanımlamışlardır ($p>0.05$). İskele platformlarında tahtalar arasında boşlukların bulunması, çıkıntıların olması, çivi, cıvata benzeri malzemeler bulunması ve zeminin ıslak olması tehlike oluşturan etmenler olarak bildirilmiştir (Tablo 3).

Pinter ile avcılık yapan D2'deki balıkçıların %67'sinin tetanos aşısı yaptırdığı, D1'deki balıkçıların %19'unun, D3'de çalışanların ise %50'sinin tetanos aşısı olmadığı tespit edilmiştir. D1 ve D2' dalyanında pinter avcılığı yapan balıkçılarda sadece takılma ve düşme, D3 dalyanında ise en fazla takılma olayı (%67) görülmekte olup bunu düşme (%50), incinme (%33) ve burkulma (%16) olaylarının takip ettiği tespit edilmiştir.

Tablo 1. Dalyanlarda işin seyrini aksatan durumlar
Table 1. Conditions that disrupt the work processes in lagoons

Dalyan	Bakım Şartlarının Yetersizliği	Yetersiz Teknik Yatırım	İyileştirme Çalışmalarının Yapılmaması	Teknik Eleman Eksikliği	Bilgi Eksikliği
D1	%72,2	%77,8	%33,3	%77,8	%38,9
D2	%40,0	%40,0	%20,0	%80,0	%60,0
D3	%74,1	%96,3	%81,5	%66,7	%63,0

Tablo 2. D1, D2 ve D3'de yaşanan iş kazaları
Table 2. Work accidents in D1, D2 and D3

Dalyan	Boğulma	Baş bölgesi	Burkulma - Ezilme	Kırık- Çıkık	Kesikler
D1	%8,3	%8,3	%16,7	%25,0	%41,7
D2	%11,1	%11,1	%22,2	%22,2	%33,3
D3	%9,1	%0,0	%18,2	%0,0	%72,7

Tablo 3. Dalyan iskele platformlarındaki tehlikeler
Table 3. Dangers in lagoon pier platforms

Dalyan	Tahtalar arasında boşlukların bulunması	Tahtalar arası çıkıntılar	Çivi, cıvata benzeri malzemelerin batması	Zeminin ıslak olması
D1	%33,3	%83,3	%25,0	%33,3
D2	%66,7	%66,7	%50,0	%83,3
D3	%92,3	%100,0	%100,0	%100,0

TARTIŞMA VE SONUÇ

Balıkçılık ve su ürünleri yetiştiriciliği faaliyetleri İş Sağlığı ve Güvenliğine İlişkin İşyeri Tehlike Sınıfları Tebliğ'inde tehlikeli sınıfta 031101 NACE kodu ile yer almaktadır (T.C. Resmi Gazete, 2017). Bu çalışmada D1'de çalışan balıkçıların %62'si, D2'de %73'ü, D3'de %43'ünün bu sınıflamadan haberinin olmadığı tespit edilmiştir. Akyol vd. (2016), iş sağlığı ve güvenliği hakkında iş kazaları üzerine eğitim ve bilgilendirme toplantılarının İzmir balık halinde yapıldığını, Mert ve Ercan (2014) ise su ürünleri tesislerinde 14 işletmenin 8'inde eğitimlerin verildiğini bildirmişlerdir. Bu çalışmada sadece D2 çalışanlarının %70'i iş güvenliği uzmanları tarafından eğitimlerin verildiğini bildirmişlerdir. Dalyanlar arasında iş sağlığı ve güvenliği eğitimlerinin yapılmasında fark bulunması, D2'nin ortak sağlık ve güvenlik birimi (OSGB) tarafından hizmet alması ile açıklanabilir. İş kazalarının %90'ı insan hatasından kaynaklanmaktadır. Eğitim eksikliği ve bilgisizlik oluşan kazaların en önemli sebeplerinden biridir (Ceylan, 2012). Çalışanların tamamının bilinçlenmesi amacıyla eğitim verilmesi gereklidir. Bu bilgilendirmeler sonucunda oluşabilecek tehlikelere karşı önlem alıp uygulamak iş kazalarının azalmasında önemli bir rol oynamaktadır.

Perçin vd. (2011), küçük ölçekli balıkçılık yapan çalışanların %43'ünün, Akyol vd. (2016) ise İzmir balık halinde çalışanların %37'sinin ilkokul mezunu olduğunun belirtilmesi ile birlikte Perçin (2018b) İzmir ilinde kuluçkahane ve kafeslerde çalışanların %25'inin üniversite mezunu olduğunu tespit etmişlerdir. Bu çalışmada D3'de çalışanların tamamının, D1'de %90'ı, D2'de çalışanların ise %74'ünün ilkokul mezunu olduğu tespit edilmiştir. Limanda ve balık halinde çalışanların, dalyanlarda çalışanlara göre eğitim durumlarının daha yüksek olduğu söylenebilir. İnsan kaynaklı iş kazalarına sebep olarak fiziksel, duygusal ve çevresel faktörlerin yanı sıra işçi özelliklerinin de direk veya indirek olarak etken olduğu bilinmektedir (Arashpour ve Arashpour, 2010; Martins vd., 2011). Rahmani vd. (2013), elektrik endüstrisinde düşük eğitim seviyesine sahip işçilerin daha çok iş kazasına uğradıklarını bildirmiştir. Özönel (2015), su ürünleri işletmelerinde iş kazası geçiren çalışanların %23'ünün ilkokul mezunu olduğunu belirtmiştir. İş kazalarının sayısı eğitim seviyesinin yükselmesi ile azalabileceği söylenebilir.

Akyol vd. (2016) İzmir balık hali çalışanlarına ilişkin yapılan araştırmada en az 6 saat en fazla 12 saat çalıştırılan personelin günlük çalışma süresinin ortalama 8,5 saat olduğunu tespit etmişlerdir. Uyumsal (2017), su ürünleri kuluçkahanelerinde görev alan personellerin %92'sinin günde 7-8 saat çalıştığını belirtmiştir. Bu çalışmada D1'de %13'ü, D2'de %84'ü, D3'de çalışanların %44'ü günde 7-8 saat aralığında çalışmaktadır. D1 çalışanlarının %58'i günlük 10 saatten fazla çalışmaktadır. T.C. Resmi Gazete (2004) tarafından haftalık çalışma süresi en fazla 45 saat olarak tespit edilmiştir. Bu sürenin haftanın çalışılan günlerine eşit olarak uygulanacağı yine aynı kanunda belirtilmektedir. Bu bilgiler ışığında dalyanlarda çalışma saatlerine uyulmadığı tespit edilmiştir. Hava durumu, iş yoğunluğu ve çalışan azlığı gibi sebeplerle dalyan balıkçılarının çalışma saatlerinin fazla olabilmesine karşın Salminen (2010) tarafından fazla mesai yapılan çalışmalarda, haftada 45 saat yapılan çalışmalara göre iş kazalarının iki kat arttığı belirtilmiştir.

Norveç İş Teftiş Kurumu (Norwegian Labor Inspection Authority, 2001) verilerine göre, su ürünleri sektöründe kas- iskelet sistemi bozuklukları, cilt alerjileri, astım, işitme kayıpları en sık karşılaşılan meslek hastalıklarıdır. Perçin vd. (2011) balıkçıların %85'inin deniz yüzeyinden gelen ışık yansımasından dolayı gözlerinde sağlık sorunu yaşadıklarını ve

%84'ünde kas-iskelet sistemi rahatsızlıklarının olduğunu belirtmişlerdir. Perçin (2017), küçük ölçekli ve büyük ölçekli balıkçılarda meslek hastalıklarını; kas iskelet sistemi, bel fıtığı ve romatizma, solunum sistemi hastalıkları ve astım, deri ve cilt hastalıkları ve egzema, ağız ve diş sorunları, çürük ve yaralar, madde bağımlılığı, sigara, alkol, uyuşturucu kullanımlarının rastlanma sıklıklarının yoğun olarak görüldüğünü tespit etmiştir. Perçin (2018b) kuluçkahane çalışanlarının kas-iskelet sistemi rahatsızlıkları yaşadıklarını, kırık, çıkık ve çarpma (%90), cilt problemleri ve alerji (%86), göz ve kulak problemleri (%85) ile karşılaştığını belirtmiştir. Bu çalışmada sadece dalyan çalışanlarının %1'lik kısmının meslek hastalığına (sadece bel fıtığı) yakalandığı tespit edilmiştir. Fakat bu hastalığın meslek hastalığı olarak sağlık kuruluşlarında raporlanmadığı belirtilmiştir.

Sağlam vd. (2015), Güllük dalyanında çalışan balıkçıların yaş ortalamasının 48 olduğunu belirtmişlerdir. Özönel (2015), su ürünleri sektöründe 0-1 yıl arasında tecrübeye sahip çalışanların daha fazla oranda iş kazası geçirdiğini vurgulamıştır. SGK (2016), tarafından iş kazalarının en fazla yaşandığı yaş aralığı 22 ile 29 olduğu belirtilmiştir. Çalışmada D1, D2 ve D3'de çalışanların yaş ortalaması sırasıyla 53, 39 ve 55'dir. Burt (2015) genç çalışanların yaşadığı iş kazalarının yaşları ile birlikte gelen özelliklerinden(fiziksel, psikolojik vb) değil tecrübelerinden kaynaklandığını belirtmiştir. Bu çalışmada dalyanlarda iş kazası oranının % 4 çıkması yaş ortalamasının yüksek olması yani tecrübe sahibi insanların çalışıyor olması ile ilişkilendirilebilir.

Mert ve Ercan (2014) 14 su ürünleri işletmesinden 8 inde KKD kullanılmadığını tespit etmişlerdir. Atayeter ve Atar (2013) ise soğuk, nem ve kasların tekrarlayan bir şekilde gerilerek zorlanması gibi faktörlerin kombinasyonu olarak romatizmal şikâyetin her zaman olabileceğini belirtmişlerdir. Perçin (2018a), üretim tesislerinde formaldehit ve sodyum hipoklorit gibi kimyasal kullanımında kişisel koruyucu donanım olarak eldiven ve çizme kullanmadıklarını belirtmiştir. Bu çalışmada tüm dalyanlarda çalışanların %53'ü sürekli KKD kullandıklarını, %39'unun sıklıkla kullandığı bilgisi elde edilmiştir. D2'de kişisel koruyucu donanımlar hakkında pek çok uyarıcı işaretler bulunmakta ve diğer dalyanlara oranla kişisel koruyucu donanıma daha fazla önem verilmektedir. İş kazası riskini en aza indirmek için koruyucu giysiler ya da yeterince sıcak tutan giysiler temin edilmeli ve balıkçılar tarafından giyilmelidir.

Pinter ile avcılık, uzatma ağları ile yapılan avcılıktan daha zor ve yorucudur (Demirol ve Yüksel, 2014; Dereli vd., 2018). Guertler vd. (2016) çalışanların %90'ı fiziksel iş yükünden, Perçin (2018b) ise çalışanlarının %80'i ağır çalışma koşullarından şikâyet ettiğini bildirmişlerdir. Guertler vd. (2016), istiridye kafeslerinin denizden çekilmesinin zor iş olduğunu, rüzgârlı ve fırtınalı zamanlarda bu işlemlerin daha da zorlaştığını bildirmektedir. Bu çalışmada pinter avcılığı yapan balıkçılarda takılma, düşme, incinme ve burkulma olayları tespit edilmiştir. Pinter ile avcılık esnasında fırtına, akıntı, çamur-bataklık zemin ve rüzgâr gibi çevresel faktörlerin av aracını denizden almayı zorlaştırmasından kaynaklanan takılma ve düşme sonucu iş kazalarını oluşturabilmektedir.

Atayeter ve Atar (2013), yüksekten düşme veya suya düşmeye karşı kafeslerin açık kenarları boyunca korkulukların sağlanması gerektiğini bildirmişlerdir. Perçin (2017), küçük ölçekli ve büyük ölçekli balıkçılıkta yoğun yaşanan; çalışma alanında ip, halat, kabloya takılma, merdivenlerden kayma-düşme, malzeme, makine, teçhizat veya ekipmanlara vurma, çarpma veya hafif burkulma vakalarını hafif yaralanmalı kazalar olarak tanımlamıştır. Perçin (2018c), balıkçı barınaklarında; çalışma alanındaki düzensizlik, çevre kirliliği, kişisel koruyucu donanımların kullanılmaması, kolay alevlenen kimyasal malzemelerin bir arada bulunmasından dolayı yangına sebep olabilecek tehlike ve risklerden bahsetmiştir. Perçin (2018a), balık tanklarında ve kafeslerde; yüksek alanlar, ıslak kaygan zeminlerin çalışanlar için risk oluşturduğunu belirtmiştir. Bu çalışmada ortam şartları bakımından kuzuluklardaki iskele platformlarında; tahtaların arasındaki boşluklar, çivi, cıvata benzeri malzemelerin bulunması, kıymıklar ve zeminin ıslak olması gibi tehlikeler tespit edilmiştir. Ayrıca platformlarda herhangi bir korkuluk ya da tutunacak bir yer bulunmamaktadır. Çalışanların yürüyeceği zemin ve diğer yüzeylerin kaymaz, yeterince sağlam, kırıksız, malzemelerin taşınmasına ve güvenli yürüyüşe imkân sağlayacak şekilde olması gerekir. Yüzeyler engel, çıkıntılar, çıkıntı yapan çiviler ve cıvatalardan arındırılmış olmalıdır.

Su ürünleri sektöründe meydana gelen iş kazaları ıslak, kaygan ve buzlu yüzeylerde kayma-düşme, kimyasallar, makinalar, kesici aletlerden kaynaklı yaralanmalar, gemi ve teknede yüksekten düşme, denize düşme, soğukta çalışma, boğulma ve soğuk depolama gibi çalışma ortamlarından oluşmaktadır (Norwegian Labor Inspection Authority, 2001; Myers ve Durborow, 2012; Moreau ve Neis, 2009). Şık (2017), fiziksel tehlikeler, kimyasal tehlikeler ve sudan geçebilecek hastalıkların su ürünleri sektöründe kaza ve hastalıklara neden olabileceğini belirtmiştir. Ulukan (2016) ise su ürünleri sektöründe ağ çekme esnasında ağlarda bulunan kurşunların güvertede çalışanların kafalarına düşmesi sonucunda açık yaraya neden olması, kaygan güverte zemini nedeniyle düşme sonucu gerçekleşen incinme ve burkulmalar sık karşılaşılan durumlar olmasına rağmen çalışanlar arasında iş kazası olarak görülmediğini belirtmiştir. Özönel (2015), iş kazaları sonucunda yaralanma ve yüzeysel yaralanma (%38), kemik kırıkları (%13), çıkıklar ve incinmeler (%29), uzuv kaybı (%10), beyin

sarsıntısı ve iç yaralanmalar (%6), suda boğulma (%4) ve birden fazla yaralanma (%16) olduğunu bildirmiştir. Perçin vd. (2011) balıkçılarda iş kazası olarak küçük yaralanmaları (%64), kemik kırıklarını (%28), uzuv kopmasını, kas yırtılmasını ve diğer yaralanmaları (%8) tespit etmişlerdir. Bu çalışmada ise dalyanlarda toplam çalışanların %4'ü iş kazası geçirmiş olup bu kazalar kayma, düşme ve yaralanma olduğu tespit edilmiştir. Ayrıca dalyanda kayma-düşme oranı %51dir. Dalyan çalışanlarının %63'ü tekneden suya düştüğünü, %29'u iskele platformlarını güvenli bulmadığını ifade etmekle birlikte %4'ü iskele platformlarından kaynaklı iş kazası yaşamıştır. Çalışmada bu yaşanan kazaların Ulukan (2016)'ında belirttiği gibi çalışanlar tarafından iş kazası olarak görülmediği gözlemlenmiştir.

Bu çalışmada D1'de çalışan balıkçıların %71'i, D2'de çalışanların %80'inin iş sağlığı ve güvenliği hakkında bilgilendirilmediği tespit edilmiştir. Dalyan çalışanlarının iş güvenliği kültürü ve mesleki risk bilinci hakkında yetersiz bilgiye sahip oldukları anlaşılmaktadır. Uyumsal (2017), mesleki risk bilinci ve iş sağlığı ve güvenliği kültürünün Türkiye'de çalışanlar için hem yeni hem de idraki zor olduğunu belirtmiştir. Bunun nedeninin ise toplum arasında benimsenmesi için çıkan yasanın çok yeni olması ve çalışanların bu zamana kadar yetersiz tedbir alarak çalışmayı yeterli bulmaları ile açıklamıştır. İş sağlığı ve güvenliği hakkında; iş kazalarını önlemenin, çalışma ortamında oluşabilecek tehlikeleri azaltmak, güvenli ve sağlıklı çalışma sahalarını oluşturmak için iş güvenliği kültürünün yaygınlaşması gerekmektedir (Erginel ve Toptancı 2017). Bu amaçla 'İş Sağlığı ve Güvenliği' kültürünün yayılması ve toplum içinde olgunlaşabilmesi için eğitimin başlangıcından itibaren bu konuda çalışmaların yapılması gerektiği araştırmacılar tarafından vurgulanmaktadır (Perçin ve Haydan, 2017).

Sonuç olarak, su ürünleri sektörünün bir parçası olan dalyanlar iş sağlığı ve güvenliğine ilişkin tehlike sınıfta yer alan balıkçılık sektörünün dalı olan dalyanlarda iş sağlığı ve güvenliği açısından çalışanlara temel iş sağlığı ve güvenliği eğitimleri verilmeli, sağlık muayeneleri yapılmalı ve çalışma saatlerine dikkat edilmelidir. Dalyan sahaları içinde gerçekleştirilen balıkçılık faaliyetleri için ramak kala olaylarının tespiti üzerine araştırmalar yapılması, çalışma alanlarında oluşabilecek tehlikeler için kontrol listelerin hazırlanması, risk analizi ve acil durum eylem planı çalışmalarının yapılması iş sağlığı ve güvenliği açısından dalyan balıkçılığının yönetilmesine ışık tutacaktır.

TEŞEKKÜRLER

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SHORT COMMUNICATION **KISA ARAŞTIRMA**

Presence of the *Mesopodopsis slabberi* (Van Beneden, 1861) (Crustacea: Mysida) from the Mediterranean Sea coast of Turkey

Türkiye'nin Akdeniz Kıyılarında *Mesopodopsis slabberi* (Van Beneden, 1861) (Crustacea: Mysida)'nin varlığı

Tahir Özcan[1*] • A. Suat Ateş[2] • Seçil Acar[3]

[1]İskenderun Technical University, Faculty of Marine Sciences and Technology 31200 Iskenderun, Hatay, Turkey https://orcid.org/0000-0002-9278-4150
[2]Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology 17100 Çanakkale, Turkey https://orcid.org/0000-0002-4682-1926
[3]Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology 17100 Çanakkale, Turkey https://orcid.org/0000-0002-6426-8095
*Corresponding author: tahozcan@yahoo.com

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Abstract: Mysid, *Mesopodopsis slabberi* (Van Beneden, 1861) was for the first time recorded in İskenderun Bay (eastern Mediterranean). A total of 10 specimens belong to *M. slabberi* was collected at the depths between 0 and 2 m. This paper is on the first record of *M. slabberi* from the Levantine coast of Turkey.

Keywords: *Mesopodopsis slabberi, Mysida, Crustacea, İskenderun Bay, eastern Mediterranean*

Öz: Misid, *Mesopodopsis slabberi* (Van Beneden, 1861) İskenderun Körfezi'nde ilk kez kaydedilmiştir. *Mesopodopsis slabberi*'nin toplam 10 bireyi 0-2 m arasındaki derinliklerden toplanmıştır. Bu makale *M. slabberi*'nin Türkiye'nin Levantin kıyılarından ilk kayıtı üzerinedir.

Anahtar kelimeler: *Mesopodopsis slabberi, Mysidaa, Crustacea, İskenderun Körfezi, doğu Akdeniz*

INTRODUCTION

Mysids are common motile peracarid crustaceans at shallow coastal waters and estuaries and play an important role as a food resource for many organisms that use these areas as nurseries (Mees and Jones, 1997; Dauvin and Desroy, 2005; Buji and Panampunnayıl, 2011). Mysid, *Mesopodopsis slabberi* (Van Beneden, 1861) is classified in the genus, *Mesopodopsis* Czerniavsky, 1882 (Sardo et al. 2005). The taxonomy of the genus *Mesopodopsis* Czerniavsky, 1882 and of the species *slabberi* in particular, has been a matter of controversy. Suprabenthic crustaceans are not easily sampled with conventional samplers like van Veen grab. *M. slabberi* was for the first time recorded in the Bosphorus and the Sea of Marmara for Turkish coasts (Demir, 1952). Then, it was found in Izmir and Sigacik Bay (the eastern Aegean Sea) by Katağan 1985 and Katağan and Ledoyer 1979. However, we present herein the local record of *M. slabberi* for the Turkish Mediterranean coast.

MATERIAL AND METHODS

Sampling area was İskenderun Bay (the eastern Mediterranean) (Figure 1). Specimens were obtained by means of a Van veen Grab with the surface area of 0.1 m^{-2}. Samples were collected in three replicates from sandy-muddy bottoms between 0 and 2 m depths. A total of 10 specimens was collected (12.08.2015) at the 1 and 2 stations (Ceyhan River, eastern Mediterranean) (Figure 1). The species was identified based on Wittmann (1992). The nomenclature for this species follows Worms (2018). Sample specimen was photographed, preserved in formalin of 4%, and deposited in the Museum of the Faculty of Marine Sciences and Technology, Iskenderun Technical University, Iskenderun-Hatay, Turkey (Collection of Dr. T. Özcan).

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RESULTS AND REMARKS

M. slabberi is easily distinguished and has a slender transparent body. Its eyes are of an uncommon length-twice longer than the diameter of the carapace in the gastric region (Wittmann 1992, Sardo et al. 2005) (Figure 2).

Suprabenthic mysid, *M. slabberi* was found in coastal areas where there is input of freshwater. The species was observed only in summer samples in spite of seasonal sampling strategy. Total body length of specimens (from the tip of the rostrum to the tip of the telson) obtained varied between 2.2 and 8.4 mm. Mysid, *M. slabberi* is common in oligohaline, brackish, coastal marine, and weakly metahaline waters in the salinity range of 1.3-43‰ salinity without unit (Wittmann, 1992; Mees and Jones, 1977; Mouny et al. 2000).

M. slabberi, plays an important role in the trophic food web as it is consumed by many fish species (e.g. sand smelt, common goby, mullet and sea bass) (Greenwood et al. 1989; Delgado et al. 1997; Bartulovic et al. 2004) and therefore it can be considered as an important prey for fishes in the study area. Bartulović et al. (2004) mentioned that *M. slabberi* is an important prey item for fish, *Atherina boyeri* Risso, 1810 on the Crotia coast (the east Adriatic Sea).

According to previous references, approximately 32 mysid species (Turkish Black Sea: 5, the Turkish Strait System: 14, Turkish Aegean Sea: 26, Turkish Mediterranean Sea: 3) are known from the Turkish coast and among these 3 species occurred on the Levantine Sea coast Turkey (Bakır et al. 2014). With this new record, the number of mysid species known from the Levantine Sea coast of Turkey increased to 4. Consequently, this study focuses on the first presence of *M. slabberi* from the Levantine coast of Turkey.

Figure 1. Map showing the sampling location (1: plus, +; 2: inverted triangle,▼)

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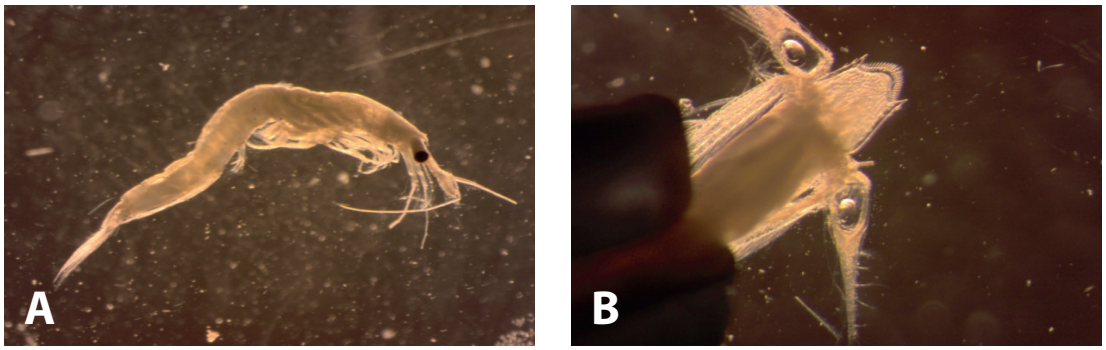

Figure 2. *Mesopodopsis slabberi* (Van Beneden, 1861), A; Dorsal view B; Telson view, (photographed by Dr. T. Özcan).

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KISA ARAŞTIRMA **SHORT COMMUNICATION**

Midye (*Mytilus galloprovincialis* Lamarck, 1819) türünde çinko pritiyonun toplam hemosit sayıları üzerine etkileri

The effects of zinc pyrithione on total hemocyte counts of mussel (*Mytilus galloprovincialis* Lamarck, 1819)

Selma Katalay[1]* ● Melike Merve Ayhan[2] ● A. Çağlan Günal[3]

[1]Manisa Celal Bayar Üniversitesi Fen-Edebiyat Fakültesi Biyoloji Bölümü Şehit Prof. Dr. İlhan Varank Kampüsü, Manisa https://orcid.org/0000-0001-7024-5730
[2]Manisa Celal Bayar Üniversitesi Fen-Edebiyat Fakültesi Biyoloji Bölümü Şehit Prof. Dr. İlhan Varank Kampüsü, Manisa https://orcid.org/0000-0002-3696-486X
[3]Gazi Üniversitesi Fen Bilimleri Enstitüsü Çevre Bilimleri A.D, Ankara https://orcid.org/0000-0002-9072-543X
*Corresponding author: katalayselma@gmail.com

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Öz: Bu çalışmada, 48 ve 96 saat süre ile 20 ve 40 µg/L subletal çinko pritiyona (ZnPT) maruz kalan denizel ortamdaki kontaminantları yüksek seviyede biriktiren Akdeniz midyelerinin (*Mytilus galloprovincialis*) toplam hemosit sayıları üzerindeki etkisi belirlenmiştir. Toplam hemosit sayımı, çevresel kirleticilerin etkilerini incelemede iyi bir biyoindikatördür. Çinko pritiyona maruz kalan midyelerde toplam hemosit sayılarının kontrol grubuna göre artış gösterdiği belirlenmiştir ($p<0,05$). Farklı kullanım alanları olan ve sucul ortama karışan bu maddenin düşük konsantrasyonlarının bile midyeleri olumsuz etkilediği saptanmıştır.

Anahtar kelimeler: *Çinko pritiyon, antifouling, Mytilus galloprovincialis, hemosit*

Abstract: In this study, the effect of Mediterranean mussels (*Mytilus galloprovincialis*) which accumulate contaminants in the marine environment exposed to 20 and 40 µg/L sublethal zinc pyrithion (ZnPT) at high levels for 48 and 96 hours on total hemocyte counts was determined. Total hemocyte count is a good bioindicator to assess the effects of environmental pollutants . It was determined that total hemocyte counts were increased in mussels exposed to zinc pyrithione when compared to the control group ($p<0.05$). It has been found that this substance, which has different usage areas and contaminate aquatic environment, negatively effect the mussels even in low concentrations.

Keywords: *Zinc pyrithion, antifouling, Mytilus galloprovincialis, hemocyte*

GİRİŞ

Yirminci yüzyılın ikinci yarısında, nüfus artışındaki hızlanmaya bağlı olarak artan çevre kirliliği, yaşam kaynaklarının daha fazla kirlenmesine neden olmuş ve sonuçta ekosistemin bozulması giderek çok daha ciddi bir hal almıştır. İnsan sağlığı açısından önemli ve çevresel etkilere sahip olan kimyasal maddelerin etkilerinin belirlenmesi, ekotoksikoloji ve çevre sağlığı açısından önemli bir araştırma alanıdır.

Endüstriyel kaynaklı kirlilik, populasyon artışı ve bunun sonucunda antropojenik aktivitenin artışı, akuatik ekosistemde dünya çapında bir problem haline gelmiştir. 1970 ve 1980'lerin sonlarında antifouling olarak organo kalay bileşikler, gemicilik ve yat aktivitelerinin yüksek olduğu bölgelerde deniz organizmalarının dokularında, sedimentte ve su kolonunda yüksek konsantrasyonlarda saptanmıştır. Deniz suyu ile temas eden teknenin yüzeyi, deniz organizmaları ve diğer deniz canlıları tarafından kaplanmaya açıktır. Antifouling boyaların kullanılmasıyla teknelere zarar veren ve maddi sorunlarla birlikte zaman kayıplarına neden olan deniz canlılarının gemi karinalarına yapışması önlenebilmektedir. Antifouling kaplama günümüze kadar özellikle tribütilin (TBT) gibi aktif ajanlarla yapılmış olup ve dünya çapında da tehdit oluşturmuştur (Duydu,1993).

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1990'lardan beri çeşitli biyositler, organotinlerin yerine geçmiştir. Bu bileşikler kalaylı organik bileşikler kadar etkin ancak onlar kadar doğaya ve organizmaya zararlı olmayan antifouling bileşiklerin geliştirilmesi için yoğun araştırmalar yapılmaktadır Irgarol, diuron, çinko pritiyon ve bakır pritiyon ve Sea-Nine gibi çok çeşitli antifouling maddeler piyasaya sunulmuştur. Bu biyositlerin çevresel olarak organotin bileşiklere göre daha az zararlı olacağı düşünülmüştür. Bununla birlikte biyositlerin çevresel etkileri hakkında çok az bilgi vardır. Pek çoğunun toksik etkileri belirlenmiştir ancak çoğu hakkında kıyısal suları kirletme düzeyleri, organizmaya etkileri ve deniz ortamlarında yaratacakları potansiyel riskler hakkında yeterli bilgi yoktur (Harino,2004).

Çinko pritiyon organotin biyositlerin yerine altenatif olarak İlk defa 1990'larda Arch Chemicals (Norwalk ,CT USA) tarafından piyasaya sunulmuştur. Günümüzde antifouling boyalarda organokalaylı bileşiklerin yerine en büyük umut olarak görülmüştür (Dahllof vd., 2005).

Çinko pritiyon (ZnPT), tıp alanında (deri hastalıkları tedavisinde, kozmetik sektöründe kepek önleyici şampuan yapımında); boya endüstrisinde (antifouling madde olarak yaygın biçimde uygulanan) kullanımı çok yaygındır. Bununla beraber sucul çevrede oluşturabileceği olumsuz toksik etkiler, deniz trafiğinin çok yoğun olduğu denizel ekosistemlerde toksisitesinin belirlenmesi açısından büyük önem taşımaktadır (Marcheselli vd., 2010).

Çinko pritiyon bazlı antifouling boyalar özellikle Avrupa ve Kuzey Kore' de yaygın bir şekilde dünya çapında uygulanmaktadır. Tribütilinin (TBT) yerine çinko pritiyon bazlı antifouling ürünler Avrupa da yat ve gemilerde sıklıkla kullanılmaktadır. Çinko pritiyon batılı ülkelerde en yaygın kullanılan biyosittir. Çinko pritiyon toksisitesinin denizel türlerde ultra trace konsantrasyonlarda bile toksik etkisi olduğu gösterilmiş olmasına rağmen uluslararası kommunite tarafından kıyısal çevre ve biyotada birikip kalıcı olduğu keşfedildikten sonra ancak çinko piritiyonun çevresel kaderine yönelik çalışmalara son zamanlarda odaklanılmıştır (Marcheselli vd., 2010; Marcheselli vd., 2011; Dahllof vd.,2005).

Son 10-15 yıldır kalaylı organikler kadar etkin ancak onlar kadar doğaya ve organizmalara zararlı olmayan antifouling bileşiklerinin geliştirilmesi için yoğun araştırma yapılmaktadır. Irgarol 1051, Sea Nine 211, bakır pritiyon ve çinko pritiyon gibi piyasaya sunulan çok çeşitli antifouling maddelerin pek çoğunun toksik etkileri belirlenmiştir. Ancak çoğu hakkında kıyısal sularda meydana getireceği toksik etki düzeyleri, organizmalara etkileri ve deniz ortamlarında yaratacakları potansiyel riskler konusunda yeterli bilgi yoktur (Okamura vd., 2004).

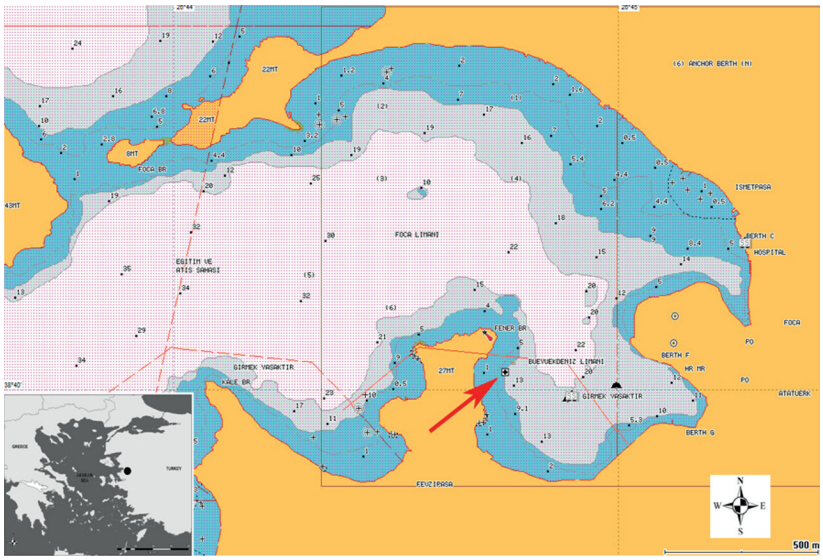
Çinko pritiyon, çok düşük konsantrasyonlarda dahi deniz canlılarına zararlı olabilir. Deniz canlılarının hayatta kalma, üreme, populasyon durumuna dair parametreler üzerine olan etkileri tanımlanmıştır. Çinko pritiyonun subletal konsantrasyonlarına maruz kalan deniz midyelerinin dokularında birikebildiği belirtilmiştir. Bu bileşiklerin deteksiyon limitleri marina içinden alınan midyelerde tanımlanmıştır (Marcheselli vd., 2010).

Bivalve mollusklar dünya çapında yaygın bir dağılışa sahip olmaları ve sedenter yaşam sürdürüp filtre ederek beslenme şekillerinden dolayı, besin kaynakları olan kimyasal kirleticileri ve bakterileri akümüle ederler ve bunun sonucunda bağışıklık sistemi olumsuz yönde etkilenir (Fisher vd.,2000).

Midyeler kontaminantların da dahil olduğu değişen çevresel faktörlere maruz kalan açık dolaşım sistemine sahiptir. Bivalvelerin immün defansında hemositler, amaboid lokomasyon yeteneğine sahiptirler ve bağışıklık savunmasında anahtar rol oynamaktadırlar (Cheng, 1981). Çalışmamızda bu bilgilerin ışığında sucul canlılara toksisitesi ve hedef olmayan canlılar üzerine muhtemel olumsuz etkileri nedeniyle, Çinko pritiyonun, *Mytilus galloprovincialis* türü midyelerde akut toksik etkileri araştırılmıştır ve bu kimyasalın midyeler üzerindeki olası toksik etkileri immünolojik yönden değerlendirilmeye çalışılmıştır.

MATERYAL VE METOT

Bu çalışmada kullanılan Akdeniz midyesi (*Mytilus galloprovincialis*) İzmir İli, Foça (N38°40.037 E26°44.748') ilçesinden toplanmış (Şekil 1), deniz suyu içerisinde laboratuvar ortamına ulaştırılmıştır. Deney öncesi bir hafta süre ile akvaryumlarda uyum periyoduna (pH:7.88, Sıcaklık: 17.5 ± 1°C) tabi tutulmuşlardır. Yapay deniz suyu Coral Pro Salt (Red Sea Europe, France) kullanılarak hazırlanmıştır. Deneylerde 15 L'lik akvaryumlar kullanılmış, 15'er midye (ort. boy 4.18 cm, $n = 90$) stoklanmıştır. Midyeler, 96 saatlik süre ile 20 ve 40 µg/L çinko pritiyona ZnPT (CAS no: 13463-41-7 Sigma Aldrich) maruz bırakılmıştır. Zn PT medikal firmadan temin edilmiştir. 48 ve 96 saat sonunda addüktör kası uyarılarak insülin enjektörü yardımıyla hemolenf örnekleri 1 ml olarak alınmıştır Hemolenf örnekleri 1:1 oranında hemolenf ve %4 lük formalin olacak şekilde 2.5' luk enjektörlerle çekilmiştir. Toplam hemosit sayımı Thoma lamı kullanılarak ışık mikroskobu altında yapılmıştır. (Günal vd. 2018). İstatistik değerlendirmede Kruskall Wallis testi uygulanmıştır.



Şekil 1. Örnekleme istasyonu
Figure 1. The sampling station

BULGULAR

96 saatlik süre ile ön denemelerle belirlenen 20 ve 40 µg/L Çinko pritiyona maruz bırakılan midyelerde, 48. saatte 40 µg/L ile, 96. saatte hem 20 hem de 40 µg/L Çinko pritiyona konsantrasyonuna maruz kalan midyelerde toplam hemosit sayılarının kontrol grubuna göre artış gösterdiği belirlenmiştir (Şekil 2). Denemelerde her akvaryumda 15'er adet midye olacak şekilde (toplam n=90) midye kullanılmıştır.

TARTIŞMA VE SONUÇ

Kan Parametreleri, organizmanın fizyolojik koşulları ve onların ortamlarındaki fizikokimyasal değişikliklere cevabın değerlendirilmesinde önemli bir araç olarak tanımlanmıştır (Ittoop vd., 2009).

Yüksek seviyedeki kontaminantlar, estuarin havza ve kıyılarda yaşayan sucul canlıları hastalıklara daha yatkın hale getirmektedir. Kronik maruziyet sonucu larval anomallikler, doku bozuklukları, kabuklu iskelet deformitelerine sebep olmaktadır. İmmun sistemdeki herhangi bir değişiklik organizmanın çevresel stresten ne kadar etkilendiğini göstermektedir (Ittoop vd., 2009).

Bivalvlerde hücresel ve ve humoral komponentler koordineli bir şekilde yürümektedir. Hücresel cevaplar, fagositoz ve sitotoksik reaksiyonlarla mikroplarla savaşan hemositlerle gerçekleşmektedir. Dolaşım hücreleri veya hemositler öncelikle immün defanstan sorumludur. Molusklar da immün cevap, bakteri, fungus ve parazitlere karşı önemli bir defans fonksiyonudur. İnvertebratlarda sağlıklı bir immün sistem, organizmanın büyüme, gelişme ve hastalıklara yakalanma riskinin belirlenmesinde önemli role sahiptir. Organizmanın hücresel immün sisteminin araştırılması, kontaminantlara kronik,subletal maruziyet tayinininde çok yararlı bilgiler edinmemizi sağlamaktadır. (Chu ve Hale, 1994; Pipe ve Coles vd., 1995). Kontaminantlara maruziyetin, immün cevabın yükselmesine neden olduğu belirtilmiştir. Kirleticilere maruz kalan organizmaların hemosit sayısında ve fagositoz oranında artış olduğu belirtilmiştir (Fisher vd., 2000). Kontaminant etkisi sonucusirküle olmayan hemositlerden dolayı sirkülatory sistemin duvarına yapışması gereken hemositler, kontaminant maruziyetini takiben dolaşım sistemine doğru hareket ettiği için hemosit sayısında artış gözlenmektedir (Baier-Anderson ve Anderson, 2000).

İnvertebrat immün sisteminde hücresel bağışıklık, hemosit fonksiyonu ile humoral bağışıklık ise serum faktörleriyle sağlanmaktadır. Bununla birlikte her iki sistem birbirleriyle koordineli olarak çalışmaktadır . Mollusklarda çevresel koşullardaki değişiklik hem hücresel hemde humoral bağışıklık mekanizmasını etkilemektedir. (Cheng, 1981).

İmmün sistemin hücresel ve humoral komponenlerinin çevresel kirleticiler ile interaksiyonu sonucu immün sistem ya baskılanır ya da stimüle olur (Chima vd., 1999). Stresli koşullar altında ya da kısa sürede düşük seviyede kontaminatlara maruziyette

hemosit sayısında önemli artış olduğu gözlenmektedir (Anderson,1981; Moore, 1988; Renwrantz, 1990).

Daha önceki çalışmalarda ağır metal kirliliğin etkisiyle total hemosit sayısında artış olduğu belirlenmiştir (Pickwell ve Steinert, 1984; Pipe vd.,1995, 1999; Fisher vd.,2000). Gastropod abalone, *Haliotis diversicolor* üzerinde TBT ve benzo[a]pyrene'nin (BaP) immün sistemine etkisinin incelendiği bir başka çalışmada, subletal BaP konsantrasyonuna uzun süreli kronik maruz kalmanın bağışık ilişkili parametrelerin aktivitesinde değişim olarak immüno kompetansını modüle ettiği gözlenmiştir (Gopalakrishnan vd., 2011).

$CdCl_2$ bileşiğine maruz kalan istiridyelerde doza bağlı olarak total hemosit sayısında artış olduğunu gözlemiştir (Hannam vd., 2010). Çalışmamızda çinko pritiyona (ZnPT) maruz kalan midyelerde (*Mytilus galloprovincialis*) total hemosit sayılarının kontrol grubuna göre artış gösterdiği belirlenmiştir. Bu bulgulara benzer olarak çinko pritiyonun yarattığı strese bağlı olarak toplam hemosit sayısında artış gözlenmiştir. Günal vd, (2018) 24 ve 96 saat süre ile 10 ve 30 µg/L subletal bakır pritiyona maruz kalan midyelerde toplam hemosit sayıları kontrol grubuna göre azaldığı belirlenmiştir. Bununla beraber çalışmamızda çinko pritiyona maruz kalan midyelerin toplam hemosit sayısında arttığı gözlemlenmiştir. Canlılar toksik maddeyle karşılaştıkları zaman immunolojik yanıt olarak total hemosit sayılarında artma ya da azalma şeklinde verebildiği görülmektedir.

Total hemosit sayısındaki artışın çevresel stresörlere en yaygın cevap olduğu belirlenmiştir (Coles vd.,1994). Sonuç olarak çinko piritiyonun total hemosit sayısındaki değişikliğin antifouling ve diğer denizel ekosisteme bulaşan kirleticilerin etkilerinin incelenmesinde uygun bir biyobelirteç olarak kullanılabileceği belirlenmiştir.

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Tablo 1. Çinko pritiyona maruz kalan midyelerin toplam hemosit sayılarındaki (THS) değişim

Table 1. Changes in total hemocyte counts (THC) of mussels exposed to zinc pyrithione

Konsantrasyon	48 saat	96 saat
Kontrol	398064,5±39712,0a*	328387,1±20230,0a
20 µg/L ZnpT	357419,4±20567,0a	813548,4±27923,0c
40 µg/L ZnpT	698709,7±27962,0b	928387,1±16817,0c

*Aynı satır ve sütunlarda küçük harfler arasındaki fark istatistik olarak önemlidir (P<0,05)

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DERLEME **REVIEW**

Su ürünlerinde *Aeromonas* ve *Plesiomonas* cinsi mikroorganizmalar ve etkileri

Aeromonas and *Plesiomonas* spp. in fishery products and their effects

Berna Kılınç

Ege Üniversitesi Su Ürünleri Fakültesi, Avlama ve İşleme Teknolojisi Bölümü, 35100, Bornova-İzmir https://orcid.org/0000-0002-4663-5082
berna.kilinc@ege.edu.tr

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Öz: *Aeromonas* ve *Plesiomonas* cinsi bakteriler su ürünlerinde yaygın bulunan bakteri grubudur. Bu mikroorganizmaların patojen türleri insanlarda hastalık ve enfeksiyonlara neden olabilmektedir. Bu nedenle yapılan bu derleme çalışmasında; *Aeromonas* ve *Plesiomonas* cinsi mikroorganizmaların bulaşma kaynakları, bu mikroorganizmaların sularda, su ürünlerinde ve depolanan su ürünlerinde bulunmasının tanıtılması amaçlanmıştır. Ayrıca *Aeromonas* ve *Plesiomonas* cinsi mikroorganizmaların neden olduğu hastalıklar ve bu mikroorganizmaların kontrolü için uygulanan yöntemler üzerine yapılan çalışmalar incelenmiştir.

Anahtar kelimeler: *Aeromonas, Plesiomonas, su ürünleri*

Abstract: *Aeromonas* and *Plesiomonas* genus of bacteria have been frequently found in fishery products. The pathogen species of these genus can be caused infections and diseases in humans. For this reason the purpose of this review is to recognise the genus of *Aeromonas* and *Plesiomonas,* the sources of contamination, the incidence of these bacteria in waters, fishery products and stored fishery products were reviewed. In addition to this; the diseases and infections of *Aeromonas* and *Plesiomonas* spp. and the prevention methods for being controlled of these genus of bacteria in fishery products were examined.

Keywords: *Aeromonas, Plesiomonas, fishery products*

GİRİŞ

Aeromonas ve *Plesiomonas* cinsi bakteriler *Vibrionanceae* familyasında yer almakta olup, su ortamında bulunmaktadır (Perales, 2003). Bu familyanın türleri gram negatif, aerobik ve fakültatif anaerobik, polar flagellayla hareketli, oksidaz pozitif, bazıları kavisli morfolojik yapıya sahiptir. Doğada yaygın olarak tatlı su, deniz ve sıklıkla sucul organizmalar da bulunmaktadır (Carter, 1990). *Aeromonas* ve *Plesiomonas* cinsi bakteriler insan enteropatojeni olarakta tanınmaktadır. *Aeromonas* türlerinin sucul ortam yanısıra topraktan, yetiştiriciliği yapılan hayvansal ürünlerden, beyaz ve kırmızı renktekiçeşitli et ve ızgara ürünlerinden, yumurta, süt, sebze ve meyvelerden izole edildiği bildirilirken, *Plesiomonas shigelloides* türünün çoğunlukla su ürünleri kaynaklı ürünlerden izole edildiği belirtilmiştir (Levin 2014; Wadström ve Ljungh, 1991). *Aeromonas* türleri 4°C'de hızlı bir şekilde çoğalmaktadır bu nedenle buzdolabında depolanan ürünler *Aeromonas* türleri açısından önemli risk oluşturmaktadır. Şimdiye kadar *Aeromonas* türleri ile ilgili geniş çaplı gıda ve su kaynaklı salgınların rapor edilmediği bildirilmiştir (Wadström ve Ljungh, 1991). Meksika'da kültür gökkuşağı alabalıklarına (*Onchorhyncus mykiss*) farklı *Aeromonas* türlerinin deneysel olarak infekte edilmesi üzerine yapılan çalışmada gökkuşağı alabalıklarında önemli ölüm oranına neden olan izolatların *A. bestiarum* (%8,8), *A. hydrophila* (%12,2), *A. salmonicida* (18,8) ve *A veronii* (%8,8) olduğu bildirilmiştir (Zepeda-Valenquez vd., 2017). *Aeromonas* cinsi bakterilerin ilk olarak balık patojeni olduğu bildirilmesine karşın, bu bakteri türlerinin hayvanlarda ve insanlarda da yaygın olarak bulunduğu belirtilmektedir (Martino vd., 2016). *Aeromonas* cinsine ait 25 tür bulunmasına

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karşın, bunlardan 14'ünün insanlarda hastalık meydana getirdiği bildirilmektedir. Bu türlerden *A. hydrophila, A. caviae, A. veronii ve A. trota* türlerinin ise insanlarda en fazla hastalığa neden oldukları ve klinik izolasyonlarının da % 85'in üzerinde olduğu bildirilmiştir (Liu, 2015). *Aeromonas* cinsinin birçok türü olduğu belirtilmesine karşın (Perales, 2003), *Plesiomonas* cinsinin sadece bir türü (*Plesiomonas shigelloides*) bulunmaktadır. *P. shigelloides* gram-negatif basildir. Su kaynaklı mikroorganizma olup, insanlarda ve hayvanlarda patojenik olan bir mikroorganizmadır. Bağırsak hastalıklarına neden olmaktadır (Santos vd., 2014). *P. shigelloides* tatlı su yetiştiriciğinde kültür gümüş sazan (*Hypophthalmichthys molitrix*) türlerinde en yaygın ölüme sebep olan mikroorganizmadır (Behera vd., 2018). Türün gelişmesi için gerekli en düşük sıcaklık 8-10°C, en yüksek ise 42-45°C'dir. Çoğu izolatları pH 4,0-9,0 arasında gelişim gösterir (Levin, 2014).

Yukarıda belirtildiği üzere *Aeromonas* ve *Plesiomonas* türlerinin su ürünlerinde varlığı, balıklarda ve insanlarda hastalıklara ve enfeksiyonlara neden olması, bu mikroorganizmaların su ürünlerinde kontrolü üzerine çok sayıda çalışmanın yapılmış olması nedeniyle, bu derlemede yapılan çalışmaların incelenerek sunulması amaçlanmıştır.

Aeromonas ve *Plesiomonas* cinsi mikroorganizmaların bulaşma kaynakları

Aeromonas ve *Plesiomonas* cinsi mikroorganizmaların bulaşma kaynaklarının sucul, karasal ve hem sucul hemde karasal organizmaların yaşadıkları ortamların olduğu bildirilmiştir (Janda, 2002). Yapılan bir çalışmada balık patojeni olan *A. salmonicida* türünün yayılmasında denizlerde bulunan mikroplastiklerinde vektör olduğu belirtilmiştir (Virsek vd., 2017). *Aeromonas* ve *Plesiomonas* cinsi bakteriler doğal olarak çeşitli su kaynaklarında bulunabilmesinden dolayı, balık ve su ürünleri gibi su ile ilgili gıdaların kontaminasyonuna neden olmaktadır (Grim, 2013). Bu bakterilerle kontamine olan su ve gıdalar insanlarda enfeksiyon riskini oluşturmaktadır (Rodruguez-Morales ve Castaneda-Hernandez, 2019). İnsan tüketiminde kullanılan buzların ve buzdolabında depolanan gıdalarında insanlarda enfeksiyon hastalıkları açısından risk oluşturabileceği belirtilmiştir (Falcao vd., 2002). *Aeromonas* türleri ile kontamine çiğ ve pişirilmiş gıdaların insanlardaki enfeksiyonlar için potansiyel kaynak olduğu belirtilerek, *A. hydrophila*'nın ve *A.sobria* izolatlarının yaklaşık %50'sinin sitotoksin ürettiği belirtilmiştir (Fricker ve Tompsett, 1989). Yapılan bir çalışmada gıda ve su örneklerinde baskın olan *Aeromonas* türünün *A. hydrophila* olduğu belirtilerek, 51 izolatın 37'sinin hemolisin ürettiği belirtilmiştir (Knochel ve Jeppesen, 1990). Suların, çiğ ve uygun şekilde pişirilmemiş su ürünlerinin *Aeromonas* ve *Plesiomonas* cinsi mikroorganizmaların yayılmasında araç olabileceği vurgulanmıştır (Ward ve Hackney, 1991). Balıkların yaşadığı kanal ve akıntı sularında *Aeromonas* türlerinin varlığının incelendiği çalışmada *A. hydrophila* türünün yaz aylarında kanalizasyon ve kanala taşınan akıntı suyunda artış gösterdiği bildirilmiştir. Çalışmada aynı bölgede baharda kanala taşınan akıntı suyundaki balıklarda *A. salmonicida* ve *A. bestiarum* türleri saptanırken, yaz aylarında ise çoğunlukla balıklardan *A. veronii* türü izole edilmiştir. *Aeromonas* türlerinin balıklarda ve balıkların avlandığı sularda bulunması balıklarla temasta bulunan balıkçıların ve kanalizasyon işleminde çalışan işçilerin potansiyel sağlığını etkileyebileceği bildirilmiştir (Popovic vd., 2015).

Aeromonas ve *Plesiomonas* cinsi mikroorganizmaların sularda ve su ürünlerinde bulunması

İsveç'te üç farklı belediye içme suyu dağıtım sistemi *Aeromonas* türlerinin varlığı açısından incelenmiştir. Üç dağıtım sisteminden incelenen 27 içme su örneğinin 23'ünde (%85) *Aeromonas* pozitif olarak saptanmıştır. Rastgele seçilen 61 *Aeromonas* izolatının 41'i *A. hydrophila*, 20'sinin ise *A.sobria* olduğu bildirilmiştir. Bu çalışmada *Aeromonas* türlerinin içme suyunda varlığı tüketicilerin yüksek sayıda *Aeromonas* türlerine maruz kalabileceğini gösterdiği vurgulanmıştır (Krovacek vd, 1992). İsveç'te iki göl ve nehirden *P. shigelloides* izole edilmiştir. *P.shigelloides*'in bütün izolatlarının O_{18},O_{23},O_{26},O_{58} ve O_{60} farklı serotipte olduğu belirtilmiştir. Bu çalışmada *P. shigelloides*' in soğuk iklim su ortamlarında da olabileceği ve benzer iklim koşullarına sahip doğal alanlarda su kaynaklı patojenlerin insan enfeksiyonlarına sebep olabileceği bildirilmiştir (Krovacek vd., 2000). *P. shigelloides*'in su ortamından 25 izolatının, hasta insanların dışkılarından 10 ve hayvanlardan 5 izolatının polimeraz zincir reaksiyonu (PZR) cihazı ile tanımlandığı belirtilmiştir (Gonzalez-Rey, 2000). Yapılan diğer bir çalışmada toplam olarak 73 adet *P. shigelloides*' in izole edildiği çalışmada insanlardan 24, hayvanlardan 21 ve su ortamından ise 28 izolatın izole edildiği bildirilmiştir (Gonzalez-Rey vd., 2004).

Tatlı su ve deniz balıklarından yılan (*Anguilla anguilla*), kalkan (*Psetta maxima*) ve gökkuşağı alabalıklarından *(Oncorhynchus mykiss)* izole edilen bakteri türlerinin *A. jandaei, A. hydrophila, A. caviae, A. encheleia, A. allosaccharophila, A. salnonicida, A. sobria, A. veroi biovar veronii, A. media, V. anguillarum, V. furmissii ve P. shigelloides* oldukları bildirilmiştir (Esteve, 1995). Balıklardan 208 izolatın izole edilerek tanımlandığı çalışmada *A. hydrophia*'nın en baskın olan tür (%43,8)

olduğu, bu türü sırasıyla *A. veronii* biyotipi *sobria* (%26,9), *A. caviae* biyotip I (%16,3), *A. salmonicida* (%6.7), *A. sobria* (%2,9), *A. cavia* biyotip II (%2,4) ve *A. veronii* biyotip *veronii* (%1) türlerinin takip ettiği bildirilmiştir (Noterdaeme vd., 1996). Yapılan diğer bir çalışmada *A. hydrophila* türünün varlığı 66 adet istiridye, karides, balık ve surimi örneğinde incelenmiştir. *A. hydrophila* türü en yüksek %50 oranında pozitif olarak istiridyelerde belirlenirken, incelenen surimi örneklerinin hiç birinde *A. hydrophila* türünün saptanmadığı belirtilmiştir (Tsai ve Chen, 1996). Türkiye'nin Çanakkale ilinde bazı otel mutfaklarından, balık marketlerinden toplanan 127 kabuklu su ürünleri patojenik bakteri türleri açısından incelenmiştir. 127 örneğin 84'ünün *Vibrio* ve *Aeromonas* cinsine ait olduğu saptanan çalışmada örneklerin % 29,1'inde *A. hydrophila* olduğu bildirilmiştir (Çolakoglu vd, 2006). İtalya'nın Adriyatik Denizi'nden toplanan 144 midye (*Mytilus galloprovincialis*) örneğinde patojenik *Aeromonas* türlerinin incelendiği çalışmada 32 *Aeromonas* türünün izole edildiği bunların 12'sinin hastalık yapma özelliğine sahip olduğu ve enteropatojenik olduğu belirtilmiştir. Çalışmada patojenik *Aeromonas* türlerinin yüksek sayıda varlığının, çiğ veya az pişmiş midyelerin tüketilmesi sonucunda insanlarda enfeksiyon gelişiminde risk oluşturacağı öngörülmüştür (Ottaviani vd.,2006). Tatlı su balığı olan inci balığının (*Alburnus alburnus*) ilkbahar aylarında Ankara'da Mogan Gölü'nden yakalanarak, balıkların mukusundan bakterilerin izole edildiği bildirilen çalışmada, PZR cihazı ile içerisinde *Aeromonas* cinsi bakterilerinde bulunduğu 12 farklı cinste bakterilerin tanımlandığı belirtilmiştir (Ozaktas vd., 2012). Diğer bir çalışmada marketlerden 203 adet yemeğe hazır halde gıda ürünü (fermente, tütsülenmiş ve pişirilmiş) satın alınarak incelenmiştir. İncelenen örneklerin % 23,2'si *Aeromonas* cinsi açısından pozitif olarak belirlenmiştir. Çalışmada fermente gıdalardan *Aeromonas* cinsi bakterilerin daha az izole edildiği bildirilirken, tütsülenmiş ve pişirilmiş gıdalardan daha fazla izole edildiği vurgulanmıştır (Hudson vd., 1992). Ticari aşamadaki vakum paketlenmiş soğuk tütsülenmiş gökkuşağı alabalığındaki (*Onchorhyncus mykiss*) *Aeromonas* cinsi bakterilerin toplam floranın %17'sinden daha azını oluşturduğu belirtilmiştir (Gonzalez-Rodruguez vd., 2002). 6 büyük parekende satış yapan marketten içerisinde balık ve salatalarında bulunduğu farklı çeşitlerde toplam 520 adet gıda örneğinin *A. hydrophila* açısından incelendiği çalışmada incelenen örneklerin %10,8'inden *Aeromonas* cinsi bakteriler izole edilmiştir. *Aeromonas* türlerinin çoğunlukla >%10 balık ve taze salatalardan izole edildiği belirtilmiştir (Walker ve Brooks, 1993). Akivades (*Protothaca staminea*) ve istiridyelerde (*Crassostrea gigas*) *Plesiomonas shigelloides* türünün varlığının PZR cihazı ile sayısal olarak saptandığı çalışmada akivades dokularında *P. shigelloides* varlığının 60 ile $2,0x10^4$ kob/g, istiridye dokularında ise $2,0x10^2$ kob/g ile $6,0x10^4$ kob/g aralığında değişim gösterdiği belirtilmiştir (Gu ve Levin, 2006).

Aeromonas ve *Plesiomonas* cinsi mikroorganizmaların depolanan su ürünlerinde bulunması

Aeromonas türlerinin yaygın olarak kırmızı et, tavuk ve su ürünleri gibi ürünlerde varlığı ve 4°C'de gelişebilme yeteneği nedeniyle bu ürünlerin tüketimi ile ilgili olarak halk sağlığı açısından risk oluşturabileceği belirtilmiştir (Beuchat 1991). *Aeromonas* türleri düşük sıcaklıklarda buzdolabı koşullarında (4-10°C) gelişmekle kalmayıp aynı zamanda *A. hydrophila* ve *A. sobria* türlerinin sitotoksin, hemolisin ve enterotoksin üretebileceği bildirilmiştir (Krovacek vd., 1991). *A. hydrophila* türünün gıda patojeni olduğu ve bu mikroorganizmanın bazı izolatlarının insanlarda hastalığa neden olabildiği vurgulanmıştır. Özellikle 5 yaşının altında ve bağışıklık sistemi baskılanmış hastalarda bağırsak hastalıklarında önemli rol oynadığı belirtilmiştir. Hastalıkların çoğunun su ürünleri ve yemeğe hazır gıdaların uzun süre buzdolabında depolanması ile ilgili olduğu belirtilmektedir. Psikrotrofik bakteri olan *A. hydrophila* buzdolabı sıcaklıklarınında üreyebilmektedir. Bu nedenle gıda güvenliği açısından önemli bir mikroorganizmadır (Daskalov, 2006). *Aeromonas* türlerinin çiğ ve pişirilmiş gıdaların potansiyel kaynağı olduğu ve insanlara bulaştığı belirtilmiştir (Fricker ve Tompsett, 1989). Soğukta depolama esnasında *Aeromonas* türlerinin mikrobiyolojik tehlike oluşturabileceğinin bildirildiği çalışmada, sushi ürünlerde pirincin asitlendirilmesinin düşük sıcaklıkla birlikte <4°C'de kısa süreli depolama esnasında patojenik *Aeromonas* türlerinin gelişimini önlemek için ön koşul olduğu vurgulanmıştır (Hoel vd., 2018). Taze karides (*Litopenaeus vannamei*)'in bozulmasına sıcaklığın etkisinin belirlenmesi üzerine yapılan çalışmada taze karidesler tropikal çiftlikten hasat edilerek farklı sıcaklıklarda (30°C, 4°C ve 1°C) depolanmıştır. 30°C'de baskın bakterilerin *Enterobacter* ve *Acinetobacter* olduğu bildirilirken, 4°C' de *Pseudomonas* ve *Aeromonas* ve 1°C' de *Aeromonas* ve *Enterococcus* olduğu bildirilmiştir (Don vd., 2018). Çipura (*Sparus aurata*) filetolarına *A. hyrophila* inoküle edilerek buzdolabı koşullarında farklı gaz kombinasyonlarıyla paketleme yaparak farklı sıcaklıklarda depolama yapıldığı çalışmada sıcaklığın mikrobiyal gelişimin azaltılmasında en iyi faktör olduğu bildirilmiştir. Ayrıca *A. hydrophila*'nın 4°C'de önemli gelişim gösterdiği ve mikrobiyal inaktivasyonun ise 0°C'de olduğu belirtilmiştir (Provincial vd., 2013). Yapılan diğer bir çalışmada ise *Aeromonas*'ın sazan balıklarında (*Cyprinus carpio*) yaygın populasyona sahip olduğu

ve soğukta depolama esnasında da diğer bozulma yapan mikroorganizmalardan nispeten daha yüksek mikrobiyal sayıda saptandığı bildirilmiştir (Zhang vd., 2017). %2 kuru tuzlamış ve tuzlanmamış sazan (*Aristichthys nobilis*) filetolarının 4°C'de depolanması esnasında balıkların başlangıç florasında 11 cins bakteri bulunduğu *Aeromonas* cinsinininde baskın bakteri grubu olduğu belirtilmiştir. Tuzlanmış ve tuzlanmamış gruplar arasında mikrobiyal florada farklılıkların gözlendiği fakat tuzlanmamış sazan filetolarında depolamanın sonunda da baskın bakteri grubunun *Aeromonas* cinsi olduğu bildirilmiştir (Liu vd., 2017). Marketlerde önceden paketlenerek taze olarak satışa sunulan gökkuşağı alabalığı (*Onchorhyncus mykiss*) ve somonlar (*Salmo salar*) 3°C'de depolanması esnasında gökkuşağı alabalık filetolarındaki *Aeromonas* türlerinin somon dilimlerindekine kıyasla daha az olduğu fakat her iki grupta da bozulmaya bağlı olarak *Aeromonas* türlerinin önemli şekilde artış gösterdiği bildirilmiştir (Gonzalez-Rodruguez vd., 2002).

Pseudomonas shigelloides türü bakterinin histidini dekarboksile eden bakteriler arasında önemli role sahip olduğu bildirilen çalışmada bu türün su ürünleri ile ilgili sıklıkla izole edilen histamin üreticisi olduğu vurgulanmıştır (Lopez-Sabater, 1996). Yapılan bir çalışmada iki supermarkettte oksijen geçiren film ile paketlenmiş (-2°C ile +1°C) arasındaki sıcaklıklardaki soğutma dolaplarında tutulan toplam 52 deniz balığı (11 adet yılan, 9 adet kılıç, 13 adet dil, 13 adet orfoz, 5 adet mezgit, 1 adet trança) *P. shigelloides* türünün varlığı açısından incelenmiştir. Çalışmada incelenen deniz balıklarının %23'ünden (orfoz filetolarının 11'inden, trança filetolarının 1'inden) *P. shigelloides* türünün PZR cihazı ile izole edildiği bildirilmiştir. (Herrera vd., 2006).

Aeromonas ve *Plesiomonas* cinsi mikroorganizmaların neden olduğu hastalıklar

Aeromonas türlerinin içme suyu, nehir ve diğer su kaynaklarında ve su ürünlerinde bulunması, bu bakterinin insanlardaki gastroenterit hastalıkların görülmesinde önemli rol oynadığını göstermektedir (Beuchat 1991). Gıda kaynaklı hastalıklar gelişmekte olan dünyanın en yaygın problemlerinden biridir. Bu nedenle HACCP programı ile birlikte uygulanacak hızlı mikrobiyolojik metotların başarılı bir şekilde uygulanmasının gıda güvenliğinin sağlanmasında endüstriye yarar sağlayabileceği belirtilmektedir (Vanne vd., 1996). Su ürünleri çabuk bozulan gıdalar olduğu için avcılıktan tüketime kadar ki aşamada yüksek standartlarda hijyen, iyi taşıma ve kalite sistemlerinin gerekli olduğu belirtilmektedir (Jack ve Read, 2008). *Aeromonas* cinsinin *A. sobria*, *A. hydrophila* ve *A. veronii* 3 türü tüm klinik *Aeromonas* izolatlarının yaklaşık %85'ininin oluşturduğu vurgulanmıştır. Bağışıklık sistemi baskılanmış hastalarda ciddi *Aeromonas* septisemi durumları meydana geldiği belirtilmiştir. Sistemik enfeksiyonla birlikte gelişen diğer durumlar arasında hepatik nekroz ve habis tümör de olduğu bildirilmiştir (Levin, 2014). *A. hydrophila* türünün sularda, hayvanlarda ve gıdalarda (su ürünleri, tavuk ve kırmızı et) bulunduğu bildirilerek, bu patojen toksinleride içeren farklı hastalık yapma faktörü üretebildiği belirtilmiştir. Psikrotrofik *A. hydrophila* buzdolabında depolanan gıdalarda da ürebildiğinin üzerinde durularak, bu mikroorganizmanın insanlarda gastroenterit, septisemi, taravmatik ve su kaynaklı yara enfeksiyonları, medikal sülük tedavisi sonrası enfeksiyonları içeren hastalıklarla ilişkili olduğu vurgulanmıştır (Daskalov, 2006). Kontamine olmuş suların tüketimi veya teması özellikle yaz aylarında *Aeromonas* türleri ile ilgili gastroenterit hastalıkların ortaya çıkmasında büyük risk oluşturabileceğine değinilmiştir. Ayrıca *Aeromonas* ile kontamine olmuş gıdalarda enfeksiyonun yayılmasında araç olabileceği belirtilmiştir (Kirov, 1993). *Aeromonas* türlerinin insanlarda bağırsak ve bakterinin neden olduğu yara enfeksiyonları gibi hastalıklara neden olabildiği vurgulanmıştır. *Aeromonas* türlerinin çoğunlukla tatlı su balıkları olan sazan ve alabalıkların, memelilerin, sürüngenlerin, hem karada hem suda yaşayan hayvanların ve kuşların bireysel ve yaygın hastalıklarına ve ölümlerine sebep olabildiği bildirilmiştir. *Aeromonas*'ın hasta kişilerde septisemi yoluyla hematolojik ve karaciğerle ilgili hastalıklara da sebep olduğu belirtilmiştir (Janda, 2002). *A. hydrophila* türü ile kontamine olmuş sularla temas edildiğinde deri ve yumuşak doku enfeksiyonlarına sebep olduğu belirtilen çalışmada bu mikroorganizmanın yumuşak doku travmasına ve iltihabına neden olduğu bildirilmiştir. *A. hydrophila* nedeniyle hastada yaranın 30 saat içinde septisemi yoluyla yayıldığı elde ve ayaklarda ciddi bakteriyel doku enfeksiyonuna neden olduğunu bildiren durum rapor edilmiştir (Yang vd., 2004). Yapılan bir çalışmada *A. hydrophila* mikroorganizmasının sirozla ölümcül septisemiye sebep olduğu bildirilmiştir (Koravacek vd., 1993). Teropatik balık (*Garra rufa*) üzerine yapılan çalışmada *A. sobria* türü mikroorganizmanın *G. rufa* için patojen olduğu ve bu balıklarla tedavi uygulanan bağışıklık sistemi baskılanmış hastalarda potansiyel risk oluşturabileceğine değinilmiştir (Majtan vd., 2012). *A. salmonicida* türünün balık patojeni olduğu belirtilmesine karşın, mezofilik *A. salmonicida* türünün insan enfeksiyonlarına neden olduğu bazı vakalar rapor edilmiştir (Vincent vd., 2019). Yapılan diğer bir çalışmada ishale neden olan veya olmayan *A. veronii* türünün insanlardan izole edilen baskın tür olduğu bildirilmiştir (Chen vd., 2015). *Plesiomonas* ve *Aeromonas* cinsi mikroorganizmaların neden olduğu gastroenterit hastalıkların genellikle hafif, asemptomatik fakat yaygın olduğu bildirilmiştir. Bu mikroorganizmaların bağışıklık sistemi baskılanmış kişilerde septisemi, bağırsak

rahatsızlıkları dışında da diğer enfeksiyonlara; karaciğer safra yolları ve kanser hastalıklarına neden olabildiği belirtilmiştir. Bu hastalıkların en büyük risk faktörleri arasında; taze ve az pişirilmiş su ürünlerinin tüketimi, özellikle gelişmiş ülkelerden hijyen ve sanitasyon uygulamaları yetersiz geri kalmış ülkelere yapılan seyahatler sayılmaktadır (Grim, 2013). Pişirilmemiş kabukluların tüketimi sonucundan en sıklıkla görülen semptomun hafiften ağıra doğru gelişen ishal olduğu bildirilmiştir. Buna karşın, bağışıklık sistemi baskılanmış kişilerde ve çocuklar da diğer enfeksiyonların gelişimi ile ölümlerin meydana geldiği de vurgulanmıştır (Levin, 2014). *P. shigelloides* türünün kafa yarası ile ilgili olarak derialtı dokusunun yaygın iltihabına neden olduğuna dair vakaların yanısıra bu patojen mikroorganizmanın yaygın olarak gastroenterit hastalıklara ve nadir olarak ta salgın hastalıklara neden olabileceği belirtilmiştir. Daha az sıklıkla da insanlarda septisemi ile bir veya daha fazla komplikasyona neden olduğuna değinilmiştir (Janda, 2002). *P. shigelloides* türünün çoğu infekte kişide kolera benzeri ishale sebep olduğu bildirilmiştir. Ayrıca bu mikroorganizmanın bağışıklık sistemi baskılanmış hastalarda zatürreye sebep olduğunu bildiren ilk çalışma olduğu belirtilmiştir (Schneider vd., 2009). Diğer bir çalışmada bulaşma kaynağı tesbit edilememesine rağmen, yenidoğan 2,5 günlük bebekte *P. shigelloides* türünün septisemi ve menenjite neden olduğu saptanmıştır (Ozdemir vd., 2010). *P. shigelloides* türünün tatlı su ve denizler de özelliklede ılık ve tropikal iklim sularında bulunduğuna değinilerek, insanların tropikal bölgelere ziyareti veya pişirilmemiş su ürünlerinin tüketimi sonucununda ishal görüldüğü vurgulanmıştır. Bağırsak hastalıkları dışında rahatsızlıkların çok nadir görüldüğü yeni doğan ve bağışıklık sistemi baskılanmış hastalarda ölümcül olabildiği bildirilmiştir. Çalışmada *P. shigelloides*'le kontamine olmuş sularda yüzen kadın hastanın uterus ile over arasındaki tüplerde tuba iltihabına neden olduğu bildirilmiştir (Roth vd., 2002).

Aeromonas ve *Plesiomonas* cinsi mikroorganizmaların kontrolü için uygulanan yöntemler

Aeromonas ve *Plesiomonas* cinsi mikroorganizmaların insanlarda hastalıklara neden olmasının önlenmesi ve mikroorganizmaların kontrolünün daha iyi hijyenik koşulların sağlanmasına, içme suyu kaynaklarının sanitasyon uygulamalarına ve kabuklu su ürünlerinin uygun şekilde pişirilmesine bağlı olduğu bildirilmiştir (Janda, 2002). Su ve gıdalar için alınması gereken önlemler ve hijyen kurallarının farkındalığı tropikal bölgelere seyahat eden turistler içinde önemli olduğuna değinilen çalışmada bazı durumlarda hastalıkları önleyici ilaçlar ve antibiyotiklerin kullanımı gerekebileceği, su kaybını önlemek içinde ağız yoluyla alınan rehidrasyon solüsyonlarının gerekli olduğu bildirilmiştir (Leggat ve Goldsmid, 2002). Gıda güvenliğinin sağlanmasında *A. hydrophila* türünün sıcaklık, pH, NaCl, oksijen, fosfat gibi birçok faktöre oldukça duyarlı olduğu bildirilmiştir (Daskalov, 2006). Soğuğa toleranslı *A. hydrophila*'nın soğuk tütsülenmiş somon balıklarına inoküle edilerek, 5 ve 10°C iki farklı sıcaklıkta aerobik ve vakum paket koşulları altında depolandığı çalışmada *A. hydrophila*'nın sadece 10°C'de depolanan örneklerde üreyebildiği belirtilmiştir (Hudson ve Mott, 1993). Ozon gazı ile birlikte soğutma işleminin uygulamalarının farklı çiğ balıkların kalitesi ve raf ömrü üzerine etkilerinin incelendiği çalışmada birlikte kullanımının balıkların raf ömürlerini arttırmada etkili olduğu belirtilmiştir. İçerisinde *Aeromonas* cinsi bakterilerinde bulunduğu mikrobiyal kontrol üzerine en iyi etkinin *Arnoglossus laterna* balığı ile *Eledone moschata* ahtapotu üzerine olduğu belirtilmiştir. Çalışmada muamele koşullarının ve oranlarının her su ürünü için ayarlanmasına ihtiyaç olduğu vurgulanmıştır (Aponte vd., 2018). Yapılan bir çalışmada soğuk tütsüleme uygulanan gökkuşağı alabalıkları (*Oncorhynchus mykiss*) vakum paketlenerek buzdolabında 4°C'de 21 gün depolanmıştır. *A. hydrophila* üzerine tütsünün antimikrobiyal etkisinin incelendiği çalışmada kullanılan bütün tütsü ekstraktlarının *A. hydrophila*'ya karşı etkili olduğu belirtilmiştir (Sunen vd., 2003). Yapılan diğer bir çalışmada tütsülenmiş somonda gıda kaynaklı patojenlerin (*A. hydrophila*, *P. shigelloides* gibi) kontrolü için ultra viole lambası ve non termal atmosferik plazma tek ve birlikte kombinasyonlarının incelendiği çalışmada tütsülenmiş somonların kalitesi üzerine birlikte uygulandığında bakteri yüklerinde 0,1-1,57 log kob/g azalmaya neden olduğu bildirilmiştir (Colejo vd, 2018). Somon filetolarında *A. hydrophila*'nın kontrolüne yönelik yapılan diğer bir çalışmada *Lactobacillus pentosus* 39 koruyucu kültürü ve onun bakteriyosininin kontrol grubu ile karşılaştırıldığında *A. hydrophila* sayısında sırasıyla (2,1 ve 1,4 log kob/g) azalmaya neden olduğu belirtilmiştir. *A. hydrophila* gelişimini kontrol için *Lactobacillus pentosus* 39 türü mikroorganizmanın etkisini gösteren çalışmada gıdalarda laktik asit bakteri starter kültürlerinin kullanımı gıda güvenliğinin sağlanması ve yemeğe hazır gıdaların depolanması esnasında raf ömrünün arttırılmasında yarar sağlayacağı vurgulanmıştır (Anacarso vd., 2014). Panga balığında (*Pangasius pangasius*) patojenlerin kontrolü için biyobozunur gıda paketlerinin antimikrobiyal etkisinin incelendiği çalışmada bakteriosin 7293 içeren antimikrobiyal biyobozunur gıda paketlerinin panga balık filetoları için iyi antimikrobiyal paketleme olduğu söylenmiştir. Bu bakteriyosin 7293 içeren biyobozunur gıda paketlerinin balıkların ret edilmesine sebep olan *A. hydrophila* gibi patojen mikroorganizmaları etkili bir şekilde inhibe ettiği (2-5 log kob/cm^2) bildirilmiştir (Woraprayote vd., 2018). Yapılan diğer bir çalışmada protein hidrolizatı ve karanfil esensiyel yağının agar film özellikleri ve pisi

balığı (*Paralichthys orbignyanus*) filetolarının raf ömrü üzerine etkisi incelendiği çalışmada Karanfil esansiyel yağının pisi balığının raf ömrünün arttırılmasında doğal koruyucu olarak kullanılabileceği saptanmıştır. Bu çalışmada kontrol filmlerle karşılaştırıldığında karanfil esensiyel yağı içeren filmlerin içerisinde *A. hydrophila*'nın da bulunduğu bakterilerin gelişimini önemli bir şekilde geciktirdiği belirtilmiştir (Rocha vd., 2018). Sazan balıklarına (*Cyprinus carpio*) tarçın esensiyel yağı uygulanması işleminin kontrol grubu ile karşılaştırıldığında balıklardaki *Aeromonas* türlerinin gelişimini inhibe ettiği saptanmıştır (Zhang vd., 2017). 4°C'de depolanan gümüş sazan filetolarının (*Hypophthalmichthys molitrix*) mikrobiyolojisi ve kalitesi üzerine kitosan oligosakkaritlerinin etkisinin incelendiği çalışmada %1 kitosan oligosakkarit uygulamasının *Aeromonas* türlerinin gelişimini önemli ölçüde inhibe ettiği belirtilmiştir (Jia vd., 2018). 4°C'de depolanan sazan filetolarında (*Chenopharyngodon idellus*) en yüksek antimikrobiyal aktivitenin tarçın kabuğı yağında saptandığı ve tarçın kabuğu yağının *Aeromonas* türlerini tamamen inhibe ettiği bildirilmiştir (Huang vd., 2017). Yapılan bir çalışmada ay çiçeği protein ile karanfil esensiyel yağı içeren filmlerin buzdolabında depolanan sardalya köftelerine uygulandığında oksidasyonu engellediği ve toplam mezofil bakterilerin gelişimini geciktirdiği bildirilmiştir (Salgado vd., 2013). Yapılan diğer bir çalışmada da minimal olarak işlenmiş balık ürünlerinin güvenliği ve korunmasında doğal ekstraktların antimikrobiyal etkisinin bozulma yapan ve patojen bakteriler üzerine (*P. fluorescens*, *A hydrophila* ve *L. innocua*) etkisi incelenmiştir. Çalışmada yenilebilir filmlerin balık filetolarının korunmasında potansiyel olarak kullanılabileceği bildirilmiştir (Iturriaga vd., 2012). Su ürünlerinin tüketilmeden önce uygun şekilde pişirilmesinin enfeksiyonların önlenmesinde etkili olduğu ve su ürünlerinin pişirildikten sonra da çapraz bulaşmanın önlenmesi gerektiği vurgulanmıştır. Pişirilen su ürünlerinin mikroorganizmaların çoğalmasını önleyecek derecelerde soğuk (<4°C) ve sıcak (>60°C) tutulması gerektiği belirtilmiştir (Ward ve Hackney, 1991). 2-7 kGy ışınlama dozlarının gıda patojenlerini azaltabileceği ve balıkta bozulma yapan bakterilerin sayısını önemli şekilde düşürebileceği vurgulanmıştır (Arvanitoyannis ve Stratakos, 2010). Yemeğe hazır bir gıda olan somon carpaccio'nun stabilitesini ve kalitesini yükseltmenin etkili yolunun jelatin- kitosan-karanfil esensiyel yağı filmi ve yüksek basıncın birlikte kullanılmasının olduğu belirtilmiştir (Gomez-Estaca vd., 2018). Panga balığının (*Pangasius hypophthalmus*) işleme esnasında işleme zinciri boyunca toplam 174 izolat, 20 cins ve 38 tür bakteri tanımlanmıştır. Bütün işleme hattında çeşitli işleme basamaklarında baskın olarak *Aeromonas, Acinetobacter, Lactobacillus* ve *Enterococcus* cinsi bakterilerin saptandığı bildirilmiştir. Çözündürülmüş panga filetolarının raf ömürlerinin arttırılmasında etkili ölçüm metotlarının seçilmesi ve panga filetolarının işlenmesinde kullanılan iyi üretim uygulamalarının geliştirilebileceği vurgulanmıştır (Thi vd., 2013). *A.hydrophila* türü mikroorganizmanın çeşitli gıda substratlarında ve çeşitli işleme tesislerinde farklı depolama sıcaklıklarında üreme, ölüm ve transmisyonunun tahmin modellerinin yapılmasının gerekli ve acil olduğu belirtilmektedir (Yang vd., 2016). Gelecekte moleküler test metotları ile yapılan çalışmalarla genotipleri, antimikrobiyallere karşı direnç mekanizmaları gibi konularda bilgilerin sağlanması gerektiği bildirilmiştir. Mikrobiyoloji laboratuvarları ve araştırma merkezlerinin klinik, gıda ve su kaynaklarında bu mikroroganizmaların insan sağlığında oluşturduğu risklerin daha iyi anlaşılmasının sağlanarak araştırma yapmalarının teşvik edilmesi gerektiği vurgulanmaktadır (Ghenghesh vd., 2015). Gıdaların uygun işleme, satış, depolama ve tüketime hazırlama aşamalarında hijyen ve sanitasyon kurallarına uyulmasını takiben bütün aşamalarda takibinin yapılmasının (kaynağından, üretici, işleme, dağıtım, satış ve tüketici) gıdaların raf ömürlerinin arttırılması ve gıdaların güvenliğinin sağlanmasında etkili olacağı bildirilmiştir (Sofos, 2014).

SONUÇ

Patojen *Aeromonas* ve *Plesiomonas* cinsi mikroorganizmalar su ürünlerinin yakalandığı veya hasat edildiği çevrede bulunmaktadır. Bu mikroorganizmalar sulardan, denizlerden ve su ürünlerinden temas ve tüketim sonucu bulaşmaktadır. *A. hydrophila*'nın buzdolabı sıcaklıklarında gelişebilen bir bakteri olması nedeniyle bu bakterinin su ürünlerinden elemine edilmesi için ilave koruyucu yöntemlerinde kullanılması gerektiği daima göz önünde bulundurulmalıdır. Su ürünlerinin tüketimi nedeniyle *Aeromonas* ve *Plesiomonas* kaynaklı enfeksiyonların önlenmesinde bilinçlendirme ve eğitim çalışmalarının yapılmasının etkili olacağı tahmin edilmektedir. Sürekli gelişmekte olan teknoloji ile birlikte gelişen etkin metotlar sayesinde patojen mikroorganizmaların sularda ve su ürünlerinde saptanmasının giderek kolaylaşacağı ve gerekli önlemlerin alınacağı düşünülmektedir.

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