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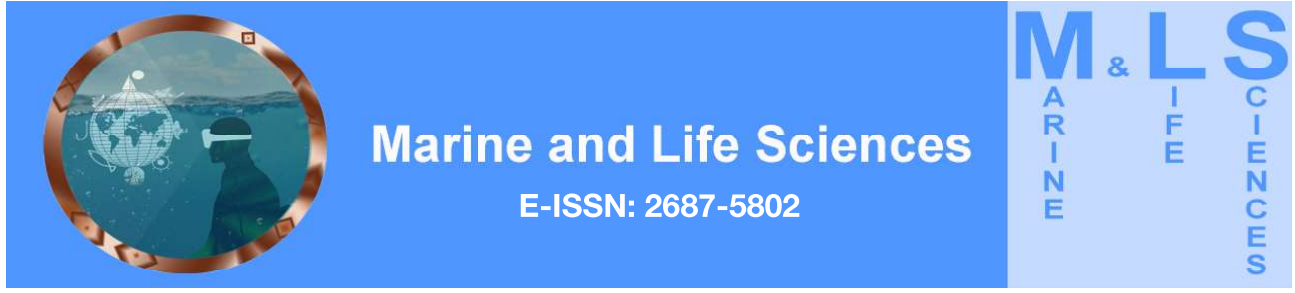
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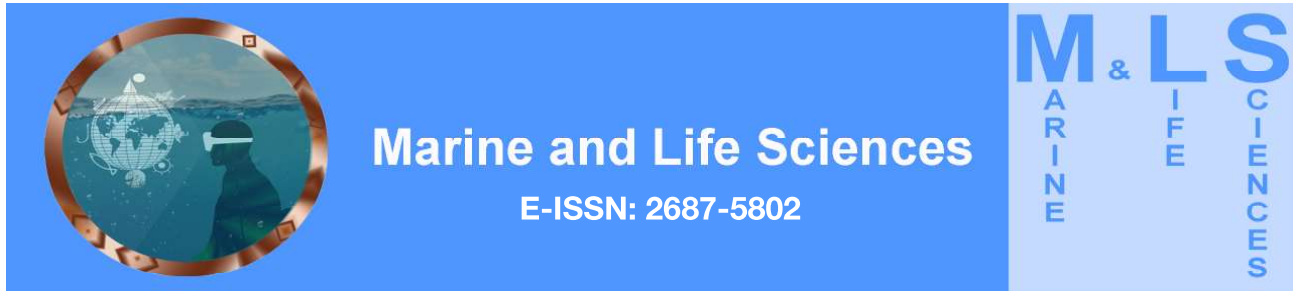
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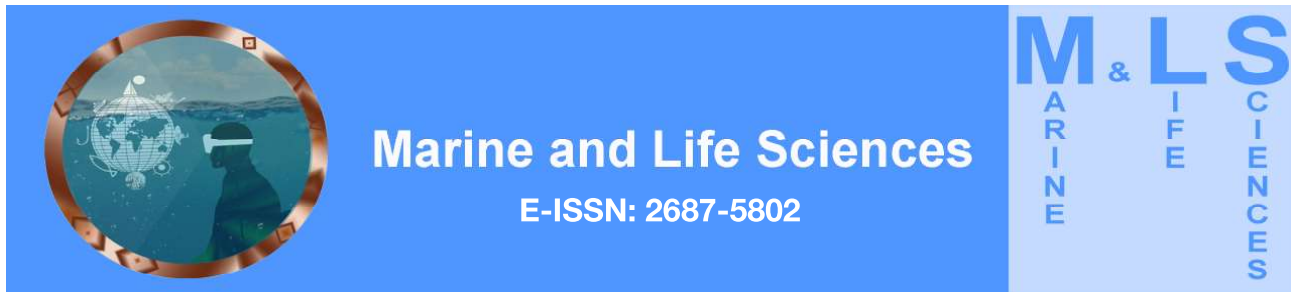
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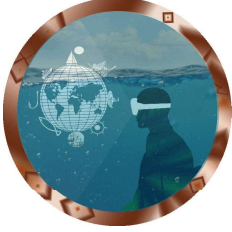
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Contents

Title	Type	DOI	Pages
<p>Maximum size record of Bogue (<i>Boops boops</i> Linnaeus, 1758) caught around Gökçeada Island (Northern Aegean Sea, Turkey)</p> <p>Özgür CENGİZ</p>	<p><i>Research Article</i></p>	<p>10.51756/marlife.732491</p>	<p>1-6</p>
<p>Investigation of bacterial pollution in Ceyhan River (Turkey) and the resistance levels of gram (+) and gram (-) bacteria to antibiotics</p> <p>Esra BIÇKICI, Meltem EKEN</p>	<p><i>Research Article</i></p>	<p>10.51756/marlife.913566</p>	<p>7-14</p>
<p>Selectivity of 40 mm Square and 90° turned mesh codend for the European hake (<i>Merluccius merluccius</i> Linnaeus, 1758) and Blue whiting (<i>Micromesistius poutassou</i> Risso, 1827)</p> <p>Celalettin AYDIN, Mehmet CİLBİZ</p>	<p><i>Research Article</i></p>	<p>10.51756/marlife.920464</p>	<p>15-23</p>
<p>Assessment of some heavy metal accumulation and potential health risk for three fish species from three consecutive bay in North-Eastern Mediterranean Seack Sea</p> <p>Ece Kılıç, Mehmet Fatih CAN, Alper YANAR</p>	<p><i>Research Article</i></p>	<p>10.51756/marlife.938938</p>	<p>24-38</p>
<p>Intra-and inter-specific competition effects on survival and growth of juvenile <i>Procambarus acutus acutus</i> and <i>Procambarus clarkii</i></p> <p>Yavuz MAZLUM</p>	<p><i>Research Article</i></p>	<p>10.51756/marlife.949292</p>	<p>39-43</p>
<p>Biological traits of a data deficient species in the Asi River: <i>Barbus lorteti</i> (Sauvage, 1882)</p> <p>Sevil DEMİRCİ, Şükran YALÇIN ÖZDİLEK</p>	<p><i>Research Article</i></p>	<p>10.51756/marlife.944696</p>	<p>44-49</p>



Maximum size record of Bogue (*Boops boops* Linnaeus, 1758) caught around Gökçeada Island (Northern Aegean Sea, Turkey)

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ABSTRACT

The information on maximum length, weight, and age of living things within an ecosystem are necessary for population dynamics and stock assessment studies. In this connection, a single specimen of *Boops boops* with 32.6 cm in total length and 375.00 g in total weight was caught off Gökçeada Island (Northern Aegean Sea, Turkey) with gill nets by commercial fisherman on 20 May 2017. This size has been recorded as the maximum length of the species for the Northern Aegean Coast of Turkey.

Gökçeada civarında (Kuzey Ege Denizi, Türkiye) yakalanan Kupes Balığının (*Boops boops* Linnaeus, 1758) maksimum boy kaydı

ÖZET

Bir ekosistem içindeki canlıların maksimum boy, ağırlık ve yaş ile ilgili bilgileri popülasyon dinamiği ve stok değerlendirme çalışmaları için gereklidir. Bu bağlamda, 32,6 cm total boya ve 375,00 gr ağırlığa sahip bir adet kupes balığı (*Boops boops* Linnaeus, 1758) 20 Mayıs 2017 tarihinde Gökçeada açıklarında ticari balıkçılar tarafından uzatma ağıları ile yakalanmıştır. Bu boy, Türkiye'nin Kuzey Ege kıyıları için türün maksimum uzunluğu olarak kaydedilmiştir.

Anahtar Kelimeler

Kupes
Boops boops
Maksimum boy
Gökçeada
Türkiye

Giriş

Sparidae familyasına ait olan kupes balığı (*Boops boops* Linnaeus, 1758) Akdeniz havzasının tüm kıyılarında dağılım gösteren, çeşitli dip yapısına sahip yerlerde yaşayan, semipelajik veya demersal bir türdür (Bauchot ve Hureau, 1986). Juvenil bireylerinin genellikle karnivor, ergin bireylerinin ise herbivor oldukları belirtilmekle beraber kupes balığının protogynous özelliğe sahip olduğu ve Akdeniz havzası için dişilerin 1 yaşında eşeyssel olgunluğa ulaştığı ifade edilmektedir (Manaşırılı ve ark., 2006). Tüm denizlerimizde dağılım gösterdiği bilinen (Fricke ve ark., 2007) bu tür, bilhassa, kış aylarında Yunanistan ve İtalya gibi Avrupa ülkelerine ihraç edildiği için ekonomik öneme sahip

olup Kuzey Ege kıyılarında olta, uzatma ağı ve gırgır ağılarıyla avcılığı yapılmaktadır (Cengiz ve ark., 2013). Ticari açıdan oldukça önemli olmasından dolayı, hem ülkemiz sularında (Manaşırılı ve ark., 2006; Bilge, 2008; Karakulak ve Erk, 2008; Cengiz ve ark., 2013; Cengiz ve ark., 2014; Andsoy, 2015; Kara ve Bayhan, 2015; Soykan ve ark., 2015; İlkyaz ve ark., 2017; Cengiz ve ark., 2019a) hem de dünyada (Gordo, 1996; Alegría Hernandez, 1989; Tsangridis ve Filippousis, 1991; Kallianiotis, 1992; Allam, 2003; Khemiri ve ark., 2005; Bottari ve ark., 2014; Layachi ve ark., 2015; Kherraz ve ark., 2016) türün popülasyon yapısı ve avcılığı ile ilgili çok sayıda araştırma mevcuttur.

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Maksimum boy ve ağırlık değerleri, balıkçılık yönetimi açısından oldukça önemli parametrelerdir (Dulčić ve Soldo, 2005). Bu parametreler, doğrudan ya da dolaylı olarak, stok tayini çalışmalarının çoğunda kullanıldıkları için, özellikle, ticari olarak sömürülen balık türlerinde maksimum boy ve ağırlık değerlerinin güncellenmesi her geçen gün önem kazanmaktadır (Borges, 2001). Bu sebeplerden dolayı, biyolojik fonksiyonlar büyüklüğe özgü (Peters, 1983) olduğu için bir popülasyondaki balıkların maksimum büyüklüğünün doğru ölçümü bu çeşit çalışmalar yapanlar için son derece önem taşımaktadır. Örneğin, bir canlının metabolik hızı vücut büyüklüğü ile ters orantılı olmasına rağmen toplam gıda tüketimi vücut büyüklüğü ile doğru orantılıdır. İlk yumurtadan çıkma boyu, ilk üreme boyu ve yaşam süresi balıkların maksimum boyuyla doğrudan ilişkilidir (Freedman ve Noakes, 2002; Van der Veer ve ark., 2003). Bu bilgilere ilaveten, maksimum boy ve ağırlık ile ilgili değerler, Von Bertalanffy ve Gompertz büyüme modelleri gibi birçok balıkçılık modeli için önemli bir bileşendir (Quinn ve Deriso, 1999). Kanıtlanan boy, Türkiye'nin Kuzey Ege kıyıları için kupes balığının en büyük boy kayıdır.

Materyal ve Yöntem

Türkiye'nin Kuzey Ege kıyıları Saros Körfezi, Gelibolu Yarımadası, Gökçeada, Bozcaada ve Edremit Körfezi olmak üzere 5 alt bölgeye ayrılmaktadır (Cengiz ve Paruğ, 2020). Çanakkale Boğazı'ndan yüzey akıntı yardımıyla Ege Denizi'ne akan ve besleyici elementler, oksijen ve plankton açısından zengin olan Karadeniz suları, özellikle, Kuzey Ege Denizi'ndeki balık faunasını olumlu yönde etkileyen etmenlerden birisidir (Cengiz ve ark., 2012). Kuzey Ege Denizi uzun bir kıta sahanlığına, çamurlu ve kumlu düz bir dip yapısına ve daha fazla miktarda besleyici elementlere sahiptir (Maravelias ve Papaconstantinou, 2006) ve Güney Ege Denizi ile kıyaslandığında fitoplankton



Şekil 1. Türkiye'nin Kuzey Ege kıyıları ve Gökçeada

ve zooplankton açısından çok daha zengindir (Theocharis ve ark., 1999). Bu sebeplerden dolayı Gökçeada (Şekil 1) tür açısından çeşitlilik sergilediği için (Keskin ve Ünsal, 1998; Karakulak ve ark., 2006; Altın ve ark., 2015) önemli bir balıkçılık sahası olarak kabul edilebilir.

Birey, 20 Mayıs 2017 tarihinde Gökçeada açıklarında (Şekil 1) ticari balıkçılar tarafından uzatma ağları ile yakalanmış, Mater ve ark. (2009) göre tanımlandıktan sonra bilimsel ismi FishBase'de (Froese ve Pauly, 2019) kontrol edilmiştir. Toplam uzunluk, ağız kapatıldığında balık kafasının ön ucu ile kuyruk yüzgecinin en uzun ışınının uç noktası arasındaki uzunluk olarak ifade edilir (Anderson ve Gutreuter, 1983). Elde edilen bireyin boyu ± 1 mm, vücut ağırlığı ± 0.01 g hassasiyette ölçülmüştür.

Sonuç

Gökçeada açıklarından avlanan kupes balığı 32,6 cm total boya ve 375,00 gr ağırlığa sahip olup (Şekil 2), Türkiye'nin Kuzey Ege kıyıları için kupes balığının maksimum boy ve ağırlık değerlerinin karşılaştırılması Tablo 1'de sunulmaktadır.



Şekil 2. 32,6 cm total boya ve 375,00 gr toplam ağırlığa sahip kupes balığı

Herhangi bir ekosistem içindeki bir balık popülasyonu aşırı avcılığa maruz kalırsa, balık boyları zaman içerisinde kademeli olarak azalır. Bundan dolayı, ancak aşırı avcılığa maruz kalmayan bireyler bu çeşit bir boya ulaşabilir. İlave olarak, balıkların beslenme faaliyetleri ve buna bağlı olarak ortamdaki besin bolluğu; sıcaklık, oksijen, tuzluluk, kirlilik gibi parametre değerleri; predatörlerin varlığı ve türler arasındaki av-avcı ilişkisinin rolü bu çeşit boya ulaşmayı etkileyen, diğer bir önemli unsurlardır (Helfman ve ark. 2009; Acarli ve ark., 2018). Bu bilgiler ışığında bu değerlerin aşırı avcılık faaliyetlerine ve çevresel şartlara bağlı olduğu sonucu ortaya çıkmaktadır.

Tablo 1. Türkiye'nin Kuzey Ege kıyıları için kupes balığının maksimum boy ve ağırlık değerlerinin karşılaştırılması

Araştırmacı(lar)	Bölge	N	L _{mak} (cm)	W _{mak} (gr)
Karakulak ve ark. (2006)	Gökçeada	518	32,1	-
İşmen ve ark. (2007)	Saros Körfezi	189	22,0	91,00
Bilge (2008)	Edremit Körfezi	1150	28,1	237,55
Çakır ve ark. (2008)	Edremit Körfezi	1231	22,1	111,60
Karakulak ve Erk (2008)	Gökçeada	428	26,3	-
Cengiz ve ark. (2013)	Gelibolu Yarımadası	504	27,0	-
Andsoy (2015)	Edremit Körfezi	389	23,9	154,39
Cengiz ve ark. (2019a)	Saros Körfezi	968	27,6	259,63
Bu çalışma	Gökçeada	1	32,6	375,00

Ülkemiz sularında bu çeşit çalışmaların her geçen gün sayısının artması [(*Alectis alexandrina* (Akyol ve Çoker, 2019); *Argyrosomus regius* (Tokaç ve ark., 2017); *Balistes capriscus* (Cerim ve ark., 2021); *Belone belone* (Acarlı ve ark., 2018); *Boops boops* (Ceyhan ve ark., 2018); *Chelidonichthys lucerna* (Akyol, 2013; Hasimoğlu ve ark., 2016; Özdemir ve ark., 2019); *Diplodus annularis* (Cengiz ve ark., 2019b); *Diplodus puntazzo* (Aydın, 2019; Cengiz, 2019a); *Diplodus sargus* (Paruğ ve Cengiz, 2020a); *Diplodus vulgaris* (Cengiz ve ark., 2019c); *Gonostoma denudatum* (Ayas ve ark., 2020); *Fistularia commersonii* (Koç ve ark., 2019); *Lithognathus mormyrus* (Aydın, 2018a; Cengiz, 2019b); *Mullus barbatus* (Filiz, 2011); *Mullus surmuletus* (Cengiz, 2019c); *Oblada melanura* (Akyol ve ark., 2014; Cengiz, 2020a); *Pagellus bogaraveo* (Paruğ ve Cengiz, 2020b); *Phycis*

(Filiz ve Sevingel, 2014); *Pomatomus saltatrix* (Cengiz, 2014; Bal ve ark., 2018); *Sardina pilchardus* (Cengiz ve Sepil, 2018); *Sarpa salpa* (Cengiz, 2020b); *Sciaena umbra* (Cengiz ve ark., 2019d); *Scomber japonicus* (Cengiz, 2020c); *Scomber scombrus* (Cengiz, 2020d); *Siganus rivulatus* (Soykan ve ark., 2021); *Solea solea* (Cengiz, 2018a); *Sparisoma cretense* (Filiz ve Sevingel, 2015); *Sparus aurata* (Aydın, 2018b; Cengiz, 2018b); *Spicara maena* (Cengiz, 2020e); *Spondyllosoma cantharus* (Cengiz, 2018c); *Stephanolepis diaspros* (Akyol ve ark., 2018; Metin ve Akyol, 2021); *Symphodus melops* (Aydın, 2020); *Umbrina cirrosa* (Aydın ve Sözer, 2020; Aydın, 2021; Cengiz ve Paruğ, 2021) konunun son derece önemli olduğunu ortaya koymaktadır.

Sonuç

Sonuç olarak, bu çalışmada Türkiye'nin Kuzey Ege kıyıları için kupes balığının yeni maksimum boy ve ağırlık değerleri ile ilgili veriler literatüre kazandırılmış olmakla beraber, türle alakalı çalışmalarda bilim insanlarının bu verilerden faydalanabileceği umulmaktadır.

Teşekkür

Yazar yardımlarından dolayı ticari balıkçılara teşekkürü borç bilir.

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Çıkar Çatışması

Yazar herhangi bir çıkar çatışması olmadığını deklare etmektedir.

Etik Onay

Yazar bu tür bir çalışma için resmi etik kurul onayının gerekli olmadığını bildirmektedir.

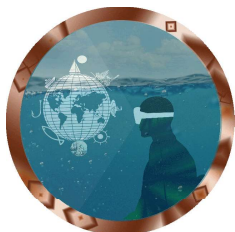
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Investigation of bacterial pollution in Ceyhan River (Turkey) and the resistance levels of Gram (+) and Gram (-) bacteria to antibiotics

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ABSTRACT

In this study, bacteriological pollution of Ceyhan River flowing into Iskenderun Bay (Northeast Mediterranean) and antibiotic resistance of Gram (+) and Gram (-) bacteria were investigated. Water samples were collected from May 2014 to April 2015 in monthly periods and 222 Gram (-) and 74 Gram (+) bacteria were isolated from collected water samples. The isolates were contained 8 different species (*Acinetobacter baumannii*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) which have been identified with Vitek II automated culture system. Microorganism susceptibility tests were performed in accordance with CLSI (Clinical and Laboratory Standards Institute, 2015) criteria. Resistance of the isolates to 15 different antibiotics (Amikacin, Meropenem, Levofloxacin, Imipenem, Piperacillin, Gentamycin, Cefepime, Ceftazidime Penicillin, Oxacillin, Clindamycin, Erythromycin, Ciprofloxacin, Vancomycin, Rifampin) was investigated. The highest antibiotic resistance was found in *E. faecalis* with 37% against Penicillin antibiotics. No resistance to vancomycin antibiotics has been observed. It was concluded that the Ceyhan River was exposed to fecal bacterial contamination, and it was revealed that this situation would adversely affect both the ecosystem and human health. Measures to protect and improve the ecological and microbiological qualities of rivers and lakes are key to preserving the quality and quantity of water resources for the future.

Keywords

Ceyhan River
Bacterial pollution
Antibiotic resistance

Introduction

Streams are ecosystems that are primarily affected by environmental pollution. Pollutants originating from agricultural, domestic and industrial activities are first introduced into surface waters. When human populations were low, waste materials mixed with streams could be diluted and disintegrated naturally in a short distance. However, with the industrialization and rapid increase in the population that came with development, industrial and domestic wastes also increased and rivers became unable to clean themselves. Quality of surface waters such as lakes, streams, dams and agricultural waters is very important for the continuity of the aquatic

ecosystem and agricultural activities as well as public health (Noori et al., 2018; Gümüş et al., 2021). Water resources used for drinking and household usage should be adequate amount with appropriate chemical and bacteriological characteristics (Gümüş et al., 2013).

A bacteriological analysis is based on the presence of coliform bacteria. For example, *E. coli*, a coliform bacterium, is used as an indicator in the bacteriological analysis. Although *E. coli* is not a pathogenic microorganism, its presence is important for health as it may represent presence of fecal or orally transmitted disease agents. If sewage water is dumped into lakes or rivers without any prior treatment, it causes the

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transmission of pathogenic microbes to humans (Unat, 1997).

According to the reports from relevant organizations such as the United Nations and UNESCO, approximately eighty countries, including forty percent of the world's population, are already suffering from water shortages. For this reason, it is vital to evaluate the water resources very well, to prevent the pollution of the river resources by wastewater, and conscious water management and precautions to be taken without disturbing the quality of life.

The Ceyhan River is an important stream that is exposed to wastes from industrial activities, septic tanks from rural areas, domestic wastewaters from settlements, pesticides and artificial fertilizers used in agricultural areas, leachate from solid waste storage facilities, wastes from cattle, and ovine livestock. Wastewater from the settlements is mostly collected by the sewerage system and discharged without adequate treatment. In this study, bacterial species causing microbiological pollution (along with antibiotic resistance of these bacteria) were investigated.

Material and Methods

Ceyhan River is one of the largest rivers of Turkey. The first source of Ceyhan River is in the mountains surrounding the Elbistan lowland. The length of the Ceyhan River is 509 km and the rainfall area is 20,000 km². The river comes out under the name Söğütlü Creek, grows on various sources, and is named as Ceyhan River with joining the Göksun and Hurman streams. After passing through the straits in the Ahır and Engizek Mountains, it enters the northeastern part of the Çukurova plain, after crossing the Misis Mountains, it flows in its wide delta and flows into the Gulf of İskenderun (Figure 1).

During the bacterial isolation and identification phase, water samples were brought to the Bacteriology Laboratory of the Department of Microbiology under the cold chain by taking 100 ml sterile containers from the water surface monthly. Samples were planted on Blood Agar for Gram (+) bacteria isolation and on EMB (Eosin Methylene Blue) agar for Gram (-) bacteria isolation. For transplantation, 1 ml of water sample was placed in plates and spread with a sterile drumstick. In the incubator, the Petri plates were incubated in a flat position for 1 hour for the plates to absorb the liquid material. Then, the incubation phase was started for at least 72 hours. At the same time, water samples were

planted in Mueller-Hinton Broth medium as 10% of the medium and left to incubate at 37°C for 72 hours with agar smears. Microbial isolations were performed by making bacterial passages from Mueller-Hinton broth into solid media when it is needed. The bacterial identification stage was started by applying Gram staining, catalase test, plasma coagulase test, oxidase test, sugar tests and advanced biochemical tests from the microorganism colonies that appeared at the end of the incubation. The gram staining method is used in the first step for the identification of microorganisms grown on solid media. A clean slide of microorganism colonies produced in blood and EMB agar was suspended with 1 drop of saline solution and the bacterial colony spread was homogenized with the help of a loop and then allowed to dry in air. The preparations dried in the air were treated with Crystal Violet (2 min), Lugol (2 min) after the flame fixation process, followed by 1 % aqueous acid fuchsin (30 sec) after the alcohol decolorization process and dyeing process was carried out. Then, the preparations were examined with immersion oil used with the 100x objective and Gram (+) Gram (-) bacteria distinction was made, which is the first step of the identification. The antimicrobial susceptibilities of the microorganisms identified with conventional methods were also examined by verifying their species identification with automated systems. The identified isolates were stored at -70°C in storage media containing 20% glycerol until the study was completed.

Bacterial identification (with bacteria identification kits; Biomerieux, France) was performed by microbiological analysis of water samples. Gram(-) and Gram (+) antibiogram susceptibility tests were performed according to the type of bacteria identified. In the study, for Gram (-) bacterial origins, Amikacin, Levofloxacin, Gentamicin, Cefepime, Meropenem, Ciprofloxacin, Imipenem, Piperacillin, Ceftazidim susceptibility with Gram (+) cocci (staphylococci), Oxacillin, Clindamycin,



Figure 1. A view from Ceyhan River (original)

Ciprofloxacin, Penicillin, Erythromycin, susceptibility were investigated. Microbial identifications were evaluated simultaneously with conventional methods and automated culture systems. Verification of microorganisms identified by the conventional method was also performed by automated culture systems. Antimicrobial susceptibility tests were performed with the help of Vitek II (Biomérieux, France) automated culture system. Microorganism susceptibility tests were performed according to CLSI (Clinical delta and flows into the Gulf of Iskenderun (Figure 1).

In this study, water samples were taken from the sampling location (37°01.696'N-35°48.669'E), which is under intense pollution. The study was conducted between May 2014 and April 2015. Water samples were taken in monthly periods and studied in 3 parallel (Figure 2).

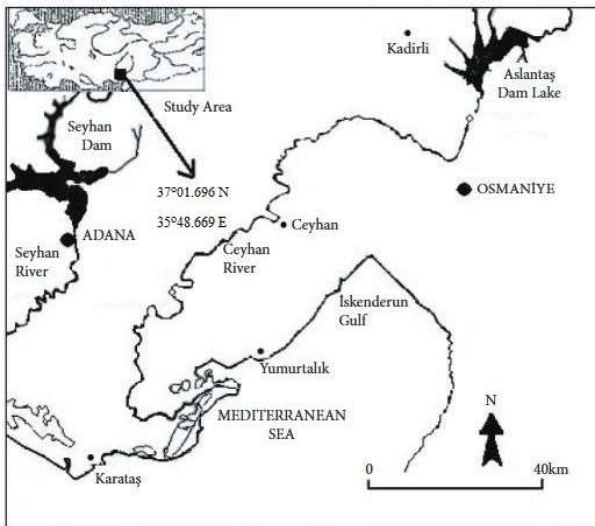


Figure 2. Study area (modified from Kurutaş Belge et al., 2009)

During the bacterial isolation and identification phase, water samples were brought to the Bacteriology Laboratory of the Department of Microbiology under the cold chain by taking 100 ml sterile containers from the water surface monthly. Samples were planted on Blood Agar for Gram (+) bacteria isolation and on EMB (Eosin Methylene Blue) agar for Gram (-) bacteria isolation. For transplantation, 1 ml of water sample was placed in plates and spread with a sterile drumstick. In the incubator, the Petri plates were incubated in a flat position for 1 hour for the plates to absorb the liquid material. Then, the incubation phase was started for at least 72 hours. At the same time, water samples were planted in Mueller-Hinton Broth medium as 10% of the medium and left to incubate at 37°C for

72 hours with agar smears. Microbial isolations were performed by making bacterial passages from Mueller-Hinton broth into solid media when it is needed. The bacterial identification stage was started by applying Gram staining, catalase test, plasma coagulase test, oxidase test, sugar tests and advanced biochemical tests from the microorganism colonies that appeared at the end of the incubation. The gram staining method is used in the first step for the identification of microorganisms grown on solid media. A clean slide of microorganism colonies produced in blood and EMB agar was suspended with 1 drop of saline solution and the bacterial colony spread was homogenized with the help of a loop and then allowed to dry in air. The preparations dried in the air were treated with Crystal Violet (2 min), Lugol (2 min) after the flame fixation process, followed by 1% aqueous acid fuchsin (30 sec) after the alcohol decolorization process and dyeing process was carried out. Then, the preparations were examined with immersion oil used with the 100x objective and Gram (+) Gram (-) bacteria distinction was made, which is the first step of the identification. The antimicrobial susceptibilities of the microorganisms identified with conventional methods were also examined by verifying their species identification with automated systems. The identified isolates were stored at -70°C in storage media containing 20% glycerol until the study was completed.

Bacterial identification (with bacteria identification kits; Biomérieux, France) was performed by microbiological analysis of water samples. Gram(-) and Gram (+) antibiogram susceptibility tests were performed according to the type of bacteria identified. In the study, for Gram (-) bacterial origins, Amikacin, Levofloxacin, Gentamicin, Cefepime, Meropenem, Ciprofloxacin, Imipenem, Piperacillin, Ceftazidim susceptibility with Gram (+) cocci (staphylococci), Oxacillin, Clindamycin, Ciprofloxacin, Penicillin, Erythromycin, susceptibility were investigated. Microbial identifications were evaluated simultaneously with conventional methods and automated culture systems. Verification of microorganisms identified by the conventional method was also performed by automated culture systems. Antimicrobial susceptibility tests were performed with the help of Vitek II (Biomérieux, France) automated culture system. Microorganism susceptibility tests were performed according to CLSI (Clinical and Laboratory Standards Institute) criteria (CLSI, 2015).

Table 1. Bacterial species isolated from Ceyhan River (Jan.: January, Feb.: February, Mar.: March, Apr.: April, Jun.: June, Jul.: July, Aug.: August, Sep.: September, Oct.: October, Nov.: November, Dec.: December, A.B.: *Acinetobacter baumannii*, E.C.: *Escherichia coli*, E.F.: *Enterococcus faecalis*, K.P.: *Klebsiella pneumoniae*, P.A.: *Pseudomonas aeruginosa*, P.V.: *Proteus vulgaris*, S.A.: *Staphylococcus aureus*, S.E.: *Staphylococcus epidermidis*)

MONTHS	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Ceyhan River	A.B.	A.B.	A.B.	A.B.	A.B.	A.B.	A.B.	A.B.	A.B.	A.B.	A.B.	A.B.
	P.A.	P.A.	P.A.	P.A.	P.A.	P.A.	P.A.	P.A.	P.A.	P.A.	E.F.	E.F.
	S.E.	S.E.	S.E.	S.A.	E.F.	S.A.	E.F.	S.E.	S.E.	S.E.	K.P.	K.P.
	E.F.	E.F.	E.F.	E.F.	K.P.	E.F.	K.P.	E.F.	E.F.	E.F.	P.V.	P.V.
	K.P.	K.P.	K.P.	K.P.	P.V.	K.P.	P.V.	K.P.	K.P.	K.P.	E.C.	E.C.
	P.V.	P.V.	P.V.	P.V.	E.C.	P.V.	E.C.	P.V.	P.V.	P.V.		
	E.C.	E.C.	E.C.	E.C.		E.C.		E.C.	E.C.	E.C.		

Results

Studies on microbiological contamination in aquatic environments have always been important research issues of scientists. Also, most of the studies on antibiotic resistance in aquatic environments deal with fecal-derived bacteria, which are both indicators of pollution and may related to infectious diseases. Recently, the emergence of antibiotic resistance of pathogenic bacteria in clinical environments has caused serious problems in all over the world.

When all water samples in our study were examined, pathogenic microorganisms for human health were found (*S. aureus*, *S. epidermidis*, *P. aeruginosa*). In addition, intestinal bacteria (*E. coli*, *K. pneumoniae*, *A. baumannii*, *E. faecalis*, *P. vulgaris*) was detected.

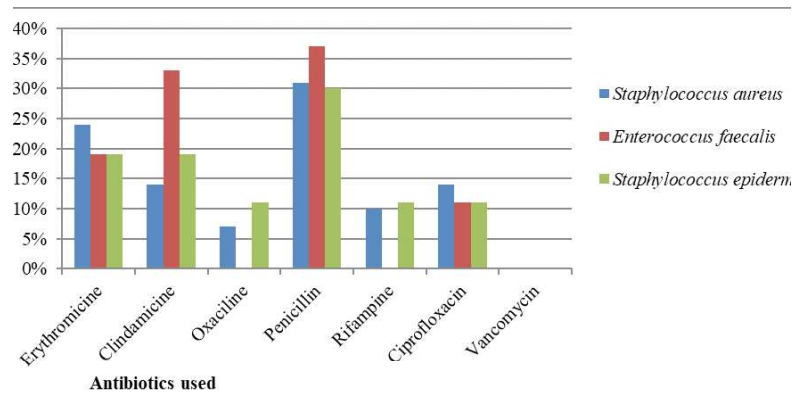
Looking at the months; *A. baumannii*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, and *P. vulgaris* species were detected in all months of the year; while, *S. aureus* was detected in April, June, and *S. epidermidis* was detected in all months except May, July, November, December. When the species are considered as variety, high diversity in the Ceyhan River was observed. The reason for this is that the Ceyhan River is exposed to a wide range of wastes such as domestic, industrial, slaughterhouse wastes, and the wastewater

supplied to the river is mostly collected by the sewage system and discharged without adequate treatment.

Microorganisms, which are pathogenic for human health, were found in all reproductive samples. Antimicrobial susceptibility tests are applied to determine the in-vitro activity of an antimicrobial agent against a particular bacterial species. In this study, resistance to Penicillin, Oxacillin, Clindamycin, Erythromycin, Ciprofloxacin, Vancomycin and Rifampin antibiotics were evaluated with antibiogram kits (Biomérieux, France) in gram-positive bacteria isolated from water samples. In the study, the lowest resistance rate was found to be 7% against Oxacillin followed by 10% Rifampin (Figure 3).

In the study, susceptibility to Amikacin, Meropenem, Ciprofloxacin, Levofloxacin, Imipenem, Piperacillin, Gentamicin, Cefepime and Ceftazidime was investigated with antibiogram kits (Biomérieux, France) in Gram-negative bacterial strains. The lowest resistance rate was found as Amikacin with 2.85%, followed by Meropenem with 3.09% and Imipenem with 4.12% (Figure 4).

Figure 3. Antibiotic resistance rates of Gram (+) bacteria



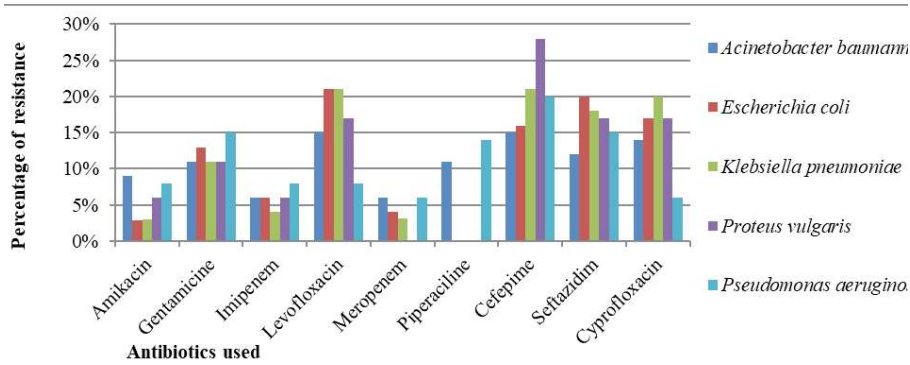


Figure 4. Antibiotic resistance rates of Gram (-) bacteria

Discussion

Eight different types of bacteria have been identified after the identification of the isolates isolated from the Ceyhan River which was poured into the Iskenderun Bay. When the identification results are examined, it is seen that 70.7% of the total isolates were *E. coli*. It is known as fecal pollution indicator in aquatic environments, and high abundance (70.7%) of *E. coli* indicates disposal of large amounts of contaminated sewage wastes directly or indirectly in water environments (Toroğlu et al., 2008). In the study conducted by Böger et al. (2021), they detected antibiotic-resistant bacteria in all water samples and found that *E. coli* and *Enterococcus* species of these bacteria were resistant to Cyprofloxacine in some rivers in Brazil. According to this study, they concluded that antibiotics are likely to come from household waste and may contribute to the spread of bacterial resistance. In our study, it was found that *E. coli* and *Enterococcus* species are resistant to Cyprofloxacine. Yerlikaya et al. (2021) found that *E. coli* strains were resistant to Amikacin 2.3% and Meropenem 4.6%. In our study, it was found that *E. coli* was 2.8% resistant to Amikacin and 4% Meropenem. Tahri et al. (2021) tested the resistance of *E. coli* strains to 16 antibiotics in Moroccan groundwater and found the highest resistance to Cef tazidime. In our study, *E. coli* was found resistant to Cef tazidime. Most of the genus *Pseudomonas* bacteria, which account for 56% of the isolates, are found extensively in nature, soil and water. It has a disease-causing feature for some of the plants, animals, and humans. Since it is a bacteria that can be found widely in nature, it can survive in organic substances and water for a long time. The presence of this bacteria in aquatic environments has been reported to pose a risk to human health (Mena and Gerba, 2009). In our study, *P. aeruginosa* was highly isolated (approximately 56%), which is important in terms

of the ability of bacteria to spread between aquatic ecosystems and humans and animals and to have high antibiotic resistance potential.

K. pneumoniae is commonly found bacteria in nature. This bacterium is found in the upper respiratory tract and fecal flora of humans, and become an opportunistic pathogen in the face of unfavorable conditions. Therefore, it is responsible for hospital infections (Çetinkaya et al., 2005). The isolation of *K. pneumoniae* from the studied water sources is important, because it represents that sewage and hospital waste water are mixed discharged in water resources. In the study, *K. pneumoniae* showed high resistance to Cefepime and Cyprofloxacine antibiotics. Similarly, in the study conducted by Bircan Yıldırım and Vurmay, in 2017, it was reported that *K. pneumoniae* showed high resistance to Cefepime (52.9%) and Ciprofloxacine (47.1%). *Acinetobacter* species, which are opportunistic pathogens, can settle in the hospital environment and cause serious nosocomial infections in hospitalized patients and patients with the suppressed immune system. *A. baumannii* is the most isolated species from clinical samples, especially in hospital-acquired infections (Koneman et al., 1992). In the study, *A. baumannii* was found in most isolates. *P. vulgaris*, one of the bacterial species isolated in the study, is found in human feces as a normal flora element. For this reason, it is often found in sewage waters. When find suitable conditions in humans, it causes infections. It is especially found in wound infections and urinary tract, which are hospital infections. *E. faecalis* is also isolated in our study which is found at a higher rate in the stool compared to other enterococci species. Morinigo et al. (1990) studied the isolation of *E. faecalis* in water samples taken from seashore and river settlements. As a result of their study,

they isolated *E. faecalis* at a level that could pose a risk to human health, especially in areas with a risk of contamination with faecal wastes. Although *S. epidermidis*, one of the bacteria isolated in our study, is not usually a pathogen, it is a big risk for patients with an insufficient immune system and patients with permanent catheters. Since it is part of the normal flora of humans, it has developed resistance to many common antibiotics such as methicillin, novobiocin, clindamycin and benzyl penicillin (Nilsson et al., 1998). *S. aureus*, one of the bacteria isolated in our study, is densely found in the feces of humans and animals. *S. aureus* is a highly virulent microorganism that is common in humans as a disease agent. In the 1950s, it gained resistance to many antibiotics, and in 1961, the problem of multiple antibiotic resistance emerged in methicillin-resistant *S. aureus* strains and staphylococci became one of the "problematic" microorganisms (Tambic et al., 1997; Günaydın et al., 2002). Penicillin resistance has gradually increased in staphylococci since 1944. In addition to penicillin, resistance to antibiotics such as erythromycin, tetracycline, and streptomycin was also developed in the 1950s (Haznedaroğlu, 2008). Yerlikaya et al. (2021) found erythromycin resistance as 23.3% and the highest antibiotic resistance 98.5% against penicillin in the study of *S. aureus* strains. Similarly, in our study, it was found that *S. aureus* was resistant to erythromycin by 24% and showed the highest antibiotic resistance to penicillin (31%). In many studies, vancomycin resistance was not found as in our study (Yerlikaya et al., 2021; Sümer et al., 2001; Zer et al., 2002; Yurtsever et al., 2009).

Differences in the pollution between the months were considered as total coliforms related since due to variation human-animal wastes and contamination. Spot pollution sources disrupt the ecological balance of the environment due to continuous waste inputs and therefore constantly change the environment of competition between microorganisms. Therefore, there are no expected changes from environmental factors in the areas where there are point sources of pollution (Clark, 1989).

Resistance percentages of *E. coli* bacteria were found to be between 0-39% against Amikacin and 1-54% against Gentamicin (Can et al., 2005; Çiçek, et al., 2006). The percentages of resistance in our study also fall within this range. In Yugoslavia, Mirovic et al. (2000) found that penicillin resistance rate was 0.9% in *E. faecalis* strains; Toutouza et al. (2001) found that penicillin resistance rate was 75.8% in *E. faecalis* strains isolated.

In recent years, the problem of increasing resistance to antibiotics has become a threat to the whole world (Akçam et al., 2004). Antibiotic resistance rates in this study are from the years of our study and are likely to change in the following years.

Antibiotics have a high water solubility and are mixed with aquatic environments following human activities through sewer systems and as a result of a farm, slaughterhouse, and land uplifting studies (Daughton and Ternes, 1999). Domestic wastes contain bacteria carrying R-plasmids, most of them originating from the human intestinal flora. Since R-plasmids, which give resistance to antibiotics, are widespread in these bacteria, the discharge of wastewater into environmental waters causes the spread of such resistant bacteria to the environment (Karayakar et al., 2004). Recent studies have shown that non-therapeutic antibiotic use plays an important role in increasing antibiotic resistance in surface waters (Kümmerer, 2009). Since domestic wastewater is the most important source of antibiotic resistance, excessive and misuse of antibiotics should be avoided by following the rational antibiotic usage policy. It can be thought that the emergence of resistance is due to the intensive and unconscious use of antibiotics and the addition of resistant bacteria in waste to water environments without being purified from city sewers. Antibiotic use is a common practice in animal nutrition. Resistant bacteria, which are transferred to the receiving environment (as fertilizer) by animal faeces, often cause epidemic infections in humans through the food chain and ultimately contribute to the spread of antibiotic resistance.

Conclusion

When the results of the study are examined, it is concluded of the river water flowing into Iskenderun Bay has a potential risk in terms of public health. It will be appropriate to prevent sewage and other domestic and similar wastes flowing into these waters, to ensure the control and operation of the treatment system, and to examine the parts where rivers pass through cities in terms of microbiological and physicochemical aspects. Suitability of the water quality is important for the use of water for a specific purpose. It is important to speed up water quality monitoring and evaluation studies in order to improve water resources polluted by industrial and agricultural reasons and to protect our natural resources.

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COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

Authors contributed equally to this paper.

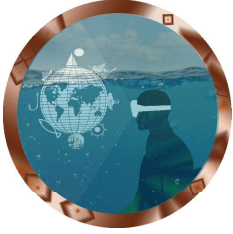
Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Selectivity of 40 mm square and 90° turned mesh codend for the European hake (*Merluccius merluccius* Linnaeus, 1758) and Blue whiting (*Micromesistius poutassou* Risso, 1827) in trawl fisheries

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Trawl

Selectivity

Aegean Sea

Merluccius merluccius

Micromesistius poutassou

ABSTRACT

In this study, it was investigated that to determine the selectivity of 40 mm square (40S) and 90° turned mesh (40T90) codend for the european hake (*Merluccius merluccius*) and blue whiting (*Micromesistius poutassou*). Fishing experiments were conducted on the international waters of the Aegean Sea by using 1200 mesh tailored trawl. Codends have knotted polyethylene (PE) 380d/21 no rope thickness and 5 m in lengths. The hooped covered codend technique was employed for the estimated codend selectivity. The selectivity parameters were estimated by using SELNET software. The plots were made with R (v.4.0.3) based RStudio (v.1.4.1106) software using the "ggplot2 (v.3.3.3)" package. Nineteen successful hauls, 11 with 40S and 8 with 40T90 codends, were performed. The mean L_{50} values (50% retention length) of 40S and 40T90 were found to be a 13.23 cm and 12.55 cm total lengths for the european hake and 23.73 cm and 19.91 cm total lengths for the blue whiting, respectively.

Trol balıkçılığında 40 mm göz açıklığındaki kare ve 90° döndürülmüş torbaların bakalyaro (*Merluccius merluccius*, Linnaeus, 1758) ve derinsu mezgiti (*Micromesistius poutassou* Risso, 1827) seçiciliği

ÖZET

Bu çalışmada, bakalyaro (*Merluccius merluccius*) ve derinsu mezgiti'nin (*Micromesistius poutassou*) 40 mm ağ göz açıklığına sahip kare (40S) ve döndürülmüş (40T90) torbalardaki seçicilik parametreleri araştırılmıştır. Denemeler 1200 gözlü dip trolü kullanarak Ege Denizi'nin uluslararası sularında gerçekleştirilmiştir. Torbalar düğümlü polietilen (PE)380d/21 no ip kalınlığında ve 5 m uzunluğundadır. Torba seçiciliğinin ölçümünde çemberli örtü torba tekniğinden yararlanılmıştır. Boy seçiciliği analizi için SELNET programı kullanılmıştır. Grafikler, "ggplot2 (v.3.3.3)" paketi kullanılarak RStudio (v.1.4.1106) yazılımı ile yapılmıştır. Toplam 19 geçerli çekim yapılmıştır. Bu çekimlerin 11'i 40S ve 8'i ise 40T90 ile gerçekleştirilmiştir. Bakalyaro için 40S ve 40T90 torbandan elde edilen, ortalama L_{50} değerleri sırası ile 13,23 ve 12,55 cm'dir. Mezgiti için L_{50} değeri 40S torbada ortalama 23,73; 40T90 torbada ise 19,91 cm olarak bulunmuştur.

Anahtar Kelimeler

Trol

Seçicilik

Ege Denizi

Merluccius merluccius

Micromesistius poutassou

Giriş

Trol balıkçılığının sürdürülebilirlik hedefleri balıkçılık efor, kota kontrolü ile donam ve dizayn değişimleri gibi düzenlemelerle yapılabilir de en

etkili yöntemin seçicilik olduğu bildirilmektedir (Hall ve ark., 2000). Genel olarak trollerde seçicilik tür ve boy seçiciliği olmak üzere ikiye ayrılmakla beraber, temel yaklaşım bireylerin

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hayat döngüleri içerisinde en az bir defa üredikten sonra yakalanmasına dayanmaktadır. Bu da belirli bir boyun üzerindeki bireylerin yakalanması, altındakilerin ise avcılık esnasında kaçması veya tasfiye edilmesi ile sağlanabilmektedir (Armstrong ve ark., 1990). Trol ağının kanatlarından itibaren başlayan seçicilik %90'dan fazla oranla son bölümde gerçekleşmektedir (Wileman ve ark., 1996). Bu nedenle seçicilik çalışmaları daha çok trolün son bölümü olan torba kısmında yoğunlaşmıştır.

Türkiye'de trollerle ilgili yasal düzenleme Ticari Amaçlı Su Ürünleri Avcılığını Düzenleyen Tebliğ (Tebliğ No:5/1) ile yürütülmektedir (Anonim, 2020). Bu tebliğde trol balıkçılığına ilişkin zaman, bölge kısıtlamalarının yanında donam ile ilgili olarak Ege ve Akdeniz'de trol torba kısmında kullanılan ağ gözü açıklığı 44 mm baklava gözlü veya buna alternatif olarak 40 mm kare gözlü ağların kullanımına müsaade edilmiştir. Diğer taraftan son yıllarda baklava gözlü torbanın düğüm yönünün 90° döndürülmesiyle (*T90*) elde edilen torba tasarımı ile de çalışmaları yürütülmektedir (ICES, 2011; Wienbeck ve ark., 2011; Tokaç ve ark., 2014). Doksan derece döndürülmüş bir torbanın kullanımı aynı materyalden yapılmış standart baklava gözlü bir torbanın kullanımıyla karşılaştırıldığında bazı türlerde daha iyi bir seçicilik gösterdiği ortaya konmuştur (Moderhak, 1997; Madsen, 2007; Madsen ve ark., 2012). *T90* torbanın Baltık Denizi'nde *Gadus morhua* hedefli trol balıkçılığında mevcut torbaya alternatif olarak kullanılabilirliği belirtilmiştir (Anonim, 2005).

Türkiye'de trol seçiciliği üzerine çok sayıda çalışma mevcuttur (Tokaç ve ark., 1998; Stewart, 2002; Tosunoğlu ve ark., 2003; Özbilgin ve ark., 2012). Kare gözlü torbaların yuvarlak (fusiform) balıkların aynı boyuttaki baklava gözlü torbalara nazaran daha iyi sonuç verdiği ortaya konmuştur (Aydın ve Tosunoğlu, 2010; Dereli ve Aydın, 2016). Ancak uzun çekim süresince torbanın dolması ile düğüm kaymalarına neden olabilmekte bu da kare göz formunun yitirilmesine neden olabilmektedir (Herrmann ve ark., 2007). *T90* torba ile türlerin seçicilikleri üzerine yapılan çalışmalar; *Aristaeomorpha foliacea* (Deval ve ark., 2016), *Aristeus antennatus* (Deval ve ark., 2016), *Boops boops* (İlkyaz ve ark. (2017), *Dentex moroccanus* (Dereli ve Aydın, 2016), *Diplodus annularis* (Tokaç ve ark., 2014), *Mullus barbatus* (Tokaç ve ark., 2014; Dereli ve Aydın, 2016), *Pagellus erythrinus* (Tokaç ve ark., 2014), *Parapenaeus longirostris* (Aydın ve Tokaç, 2015; Deval ve ark., 2016; Şensurat-Genç ve ark., 2018), *Phycis blennoides* (Aydın ve Tokaç, 2015), *Plesionika martia*, (Deval

ve ark., 2016), *Trachurus trachurus* (Dereli ve Aydın, 2016) Şensurat-Genç ve ark., 2018)'dir. Diğer taraftan *T90* torba ile bakalyaro üzerine iki (Dereli ve Aydın, 2016; Şensurat-Genç ve ark., 2018), derin su mezgiti üzerine yürütülmüş bir (Tokaç ve ark., 2010) çalışma mevcuttur.

Bu çalışmada, trol balıkçılığında 40 mm ağ göz açıklığına sahip kare (*40S*) ve 90° döndürülmüş (*40T90*) polietilen (*PE*) materyale sahip torbaların bakalyaro ve derinsu mezgiti avcılığındaki seçicilik parametreleri araştırılmıştır.

Materyal ve Yöntem

Denemeler, 24 Ağustos-13 Eylül 2012 tarihleri arasında ticari trol teknesi "Hapuloğlu (23,83 m uzunluk ve 522 kW ana motor) ile Ege Denizi Kuşadası Körfezi uluslararası sularında gerçekleştirilmiştir. Trol sahalarının derinliği 280 ile 470 m (ortalama 373 m) arasında değişmektedir. Ortalama çekim süresi ve hızı sırasıyla 250 dakika ve 2,4 deniz mili arasındadır. Ahşap ve çelikten yapılmış her biri 160 kg ağırlığında 190×90 cm ebatlarında geleneksel trol kapıları kullanılmıştır. Denemelerde Aydın ve Tokaç (2015)'te kullanılan 1200 gözlü aynı dip trolü kullanılmıştır. Aynı materyale sahip 40 mm nominal ağ gözü boyutunda kare (*40S*) ve döndürülmüş (*40T90*) torba test edilmiştir. Torbalar düğümlü polietilen (*PE*)380d/21 no ip kalınlığında ve 5 m uzunluğundadır. Torbaların birleştiği tünel bölümü 44 mm göz açıklığı 300 çevre göz sayısına sahiptir (44×300=13200 mm). Tünel sonundaki çevre göz açılımını sağlamak amacıyla döndürülmüş torba (13200 mm/40 mm) 330 ağ gözü olarak hesaplanmıştır. Kare gözlü torba ise Avrupa Birliği'nde uygulanan yönetmeliğe göre yapılmıştır. Yönetmelikte tünel kısmının çevresi torbanın 2-4 katı şeklinde olmalıdır ifadesi vardır (Anonim, 2006). Bu bağlamda kare gözlü torba çevresi (13200 mm/40 mm)/2) 165 göz (bar) olarak yapılmıştır. Polipropilen (*PP*) malzemeden yapılmış 5,0 mm ip çapında, çevre göz sayısı 65 olan ve 5 m uzunluğundaki muhafaza ağı kullanılmıştır. Torba ve muhafaza ağlar birbirine bağlanarak birleştirilmiştir. Ortalama ağ gözü açıklıklarını tespit etmek için 4 kg ağırlık ile hazırlanmış dijital kumpastan yararlanılmıştır. Üç farklı yerden olmak üzere birbirine ardışık 20 ağ gözü ölçülmüştür. Torbalara ilişkin özellikler Tablo 1' de verilmiştir.

Torbaların seçiciliğinin ölçümünde çemberli örtü torba tekniğinden yararlanılmıştır (Wileman ve ark., 1996). Örtü torba 7,5 m uzunluğunda, düğümsüz, poliamid (*PA*) malzemeye sahip ve 24 mm göz açıklığındadır. Örtü torbanın örnekleme torbalarına maskeleme etkisini azaltmak amacıyla 1,6 m çapa (R) sahip iki adet çember, trol torbasının 2,5 ve 5,0

Tablo 1. Denemelerde kullanılan torbaların bazı teknik özellikleri

Parametreler	Test torbaları		Muhafaza torba
	40S	40T90	
Nominal [mm]	40	40	88
Ölçülen	40,6±0,1	40,6±0,1	115,2
Ölçülen ağ göz sayısı	60	60	60
İp kalınlığı	380d/21	380d/21	5 mm Ø
Materyal	PE (dügümlü)	PE (dügümlü)	PP (el örgüsü)
Torba boyutları			
Çevre göz sayısı	165 göz/bar	330 göz	65 göz
Uzunluk	125 göz	125 göz	50 göz

40S: 40 mm kare gözlü torba, 40T90: 40 mm ve 90° döndürülmüş torba.

metresinde olacak şekilde donatılmıştır. Çember yapımında kullanılan yüksek yoğunluklu polietilen (HDPE) malzemenin çapı 40 mm'dir.

Her çekimden sonra türler torba ve örtü olarak ayrılmış, tür bazında sayı ve ağırlıkları alınmıştır. Türlerin total boyları 0,5 cm hassasiyetli ölçüm tahtası ile ölçülmüştür.

Bakalyaro için yasal yakalanma boyutuna (20 cm) göre yapılan değerlendirmeler Tarım ve Orman Bakanlığının yayınladığı 5/1 Numaralı Ticari Amaçlı Su Ürünleri Avcılığını Düzenleyen Tebliğde belirtilen boy yasağı sınırlamasına göre yapılmıştır (Anonim, 2020). Bu tebliğde derinsu mezgiti için herhangi bir boy sınırlaması yoktur. Bu tür için yapılan değerlendirmelerde Mir-Arguimbau (2020) tarafından 18 cm total boy olarak bildirilen L_{50} ilk üreme boyu esas alınmıştır.

Bu çalışmada, uygulanan deneysel tasarım torba ve örtüde yakalanan bireylerin torbaların boy seçiciliğini tahmin etmede binomial veri olarak analizini mümkün kılmaktadır. Deney torbası için her bir çekim arasında da seçicilik parametrelerinde fark beklenmektedir (Fryer, 1991). Sonuçların balıkçılığa uygulama aşamasında belirlenmiş olan ortalama L_{50} değerleri kullanılabilir.

Çalışmada boy seçiciliği için farklı parametrik modeller $r_{codend}(l, v_{codend})$ test edilmiştir. v_{codend} modelin parametrelerinden oluşan bir vektördür. Analizin amacı, deneysel verileri (tüm çekimlerin ortalaması) en çok gözlemlenen v_{codend} parametre değerinin tahminini sağlamaktır. Bu amaçla gözlemlenen deneysel verilerin maksimum olasılığını sağlamak için aşağıdaki 1 no'lu denklem kullanılmıştır.

Eşitlik 1'de ifade edilen belirli bir torba ile

$$\sum_{i=1}^I \{n C_{ij} \times \ln(r_{codend}(l, v_{codend})) + n C C_{ij} \times \ln(1.0 - r_{codend}(l, v_{codend}))\}$$

gerçekleştirilen denemeler için veriler boy gruplarını (l) içermektedir. Çalışmada Logit, Probit, Gompertz ve Richard olarak 4 farklı model test edilmiştir. İlk üç model, L_{50} (% 50 yakalanma olasılığı olan balık boyu) ve seçicilik aralığı (SR ; L_{75} ve L_{25} arasındaki fark) olarak tanımlanmaktadır. Richard model ise bunlara ilaveten eğrinin asimetrisi olarak tanımlanan ve $(1/\delta)$ olarak bilinen parametreye ihtiyaç duymaktadır. Uygulanan seçicilik parametrelerinin denklemleri eşitlik 2'de verilmiştir.

Bir modelin verileri yeterince tanımlayabilme ve temsiline değerlendirilmesi, p değerinin hesaplanmasına dayanır; p değeri, uygulanan model ile gözlemlenen deneysel veriler arasındaki maksimum olasılığı sağlamak için farklılıkların elde edilme olasılığını ifade eder. Bu nedenle, uygulanan modelin verileri modellemede kullanılabilmesi için p değerinin 0,05'in altında olmamalıdır (Wileman ve ark., 1996).

İstatistiğin zayıf olması durumunda ($p < 0,05$); gözlemlenen ya da ölçülen bir değer ile bir veri modeli oluşturulduktan sonra bu modele göre değer arasındaki farkın seçicilik eğrileri kullanılarak deneysel verileri model yapısından mı yoksa verilerdeki aşırı dağılımdan mı kaynaklandığını belirlemek için hesaplanmaktadır (Wileman ve ark., 1996). iki no'lu eşitlikte de ele alınan dört model arasından en iyi modelin seçimi için AIC değerlerinin karşılaştırılmasına dayanmaktadır. En iyi model en düşük AIC değerine sahip olandır (Akaike, 1974). Torbalar için en iyi model tanımlandıktan sonra ortalama boy seçiciliğinin güven aralığının hesaplanması için örnekleme doğallık gibi varsayımlar karşılanmadığında ya da standart hata kestirimleri elde bulunmadığında, ampirik verilerden yola çıkarak yapay örneklem alt

$r_{codend}(l, v_{codend})$

$$= \left\{ \begin{array}{l} \text{Logit}(l, L_{50}, SR) = \frac{\exp\left(\frac{\ln(9)}{SR} \times (l - L_{50})\right)}{1.0 + \exp\left(\frac{\ln(9)}{SR} \times (l - L_{50})\right)} \\ \text{Probit}(l, L_{50}, SR) \approx \Phi\left(\frac{1.349}{SR} \times (l - L_{50})\right) \\ \text{Gompertz}(l, L_{50}, SR) \approx \exp\left(-\exp\left(-\left(0.365 + \frac{1.573}{SR} \times (l - L_{50})\right)\right)\right) \\ \text{Richards}\left(l, L_{50}, SR, \frac{1}{\delta}\right) = \frac{\exp\left(\text{logit}(0.5^\delta) + \frac{\text{logit}(0.75^\delta) - \text{logit}(0.25^\delta)}{SR} \times (l - L_{50})\right)}{1 + \exp\left(\text{logit}(0.5^\delta) + \frac{\text{logit}(0.75^\delta) - \text{logit}(0.25^\delta)}{SR} \times (l - L_{50})\right)} \end{array} \right. \quad 2$$

Φ normal dağılım için kümülatif yoğunluk fonksiyonudur.

kümeleri seçip bu örneklem değerlerinden standart hataların ve güven aralıklarının hesaplanması yöntemi olan tekrarlı (bootstrap) uygulanmıştır.

Boy seçiciliği analizi için SELNET programı (Herrmann ve ark., 2012) kullanılmıştır. Seçicilik eğrisi ile parametrelerin güvenlik sınırlarını elde etmek için çift tekrarlı (double bootstrap) yönteminden yararlanılmıştır. Millar (1993)'de tanımlanan bu yöntem hem çekimlerde hem de çekimler arası varyasyonları göz önünde bulundurmaktadır. Çekimler arası varyasyonun ve bootstrap yöntemi ile yeniden örneklenen çekimlerin gruplarının hesaplanması bu prosedüre dahil edilmiştir. Her boy sınıfı için yeniden örneklenen veriler, çekimler arası varyasyonunu hesaplamak için kendi arasında bootstrap yapılmıştır.

Her boot strap, tanımlanan seçim modeli kullanılarak analiz edilmiş birleştirilmiş (havuzlanmış) bir veri kümesiyle sonuçlandırılmıştır. Bu sayede her bir bootstrap ortalama bir seçicilik eğrisiyle sonuçlandırılmıştır. Analiz edilen her tür için, 1000 tekrar yapılmış ve Efron (1982) %95 güven sınırları tahmin edilmiştir.

Boy seçiciliği için torbalar arasındaki farkın belirlenmesi için 3 numaralı eşitlikteki genel delta eğrisi ($\Delta r(l)$) kullanılmıştır.

$r_{test}(l)$, her bir torba için temel tasarımda yapılan değişiklik yeni dizayndan elde edilen torba için elde edilen yakalanma oranını, $r_{baseline}(l)$ her bir temel dizayn ile yapılan ikili karşılaştırmadan elde

$$\Delta r(l) = r_{test}(l) - r_{baseline}(l) \quad 3$$

edilen yakalanma oranını, $\Delta r(l)$ ise 1000 tekrardan sonra elde edilen Efron %95 güven aralığını ifade etmektedir.

Yeniden elde edilen örneklem iki grup içinde rastgele ve bağımsız olduğundan, sonuçları, bootstrap kullanarak elde edilen verilere bağlıdır (Herrmann ve ark., 2018) (eşitlik 4):

$$r_{test}(l)_i - r_{baseline}(l)_i \quad i \in [1 \dots 1000],$$

i : bootstrap tekrar indeksi. Torbalar arasındaki farkın önemli olup olmadığı boy gruplarının delta eğrilerinin %95 güven aralıklarında 0,0'la çakışmaması gerekmektedir. Grafikler, "ggplot2 (v.3.3.3)" paketi (Wickham, 2016) kullanılarak R (v.4.0.3) tabanlı RStudio (v. 1.4.1106) yazılımı ile yapılmıştır.

Bulgular ve Tartışma

Çalışmada 11'i 40S (Toplam 50,41 saat) ve 8'i 40T90 ile (Toplam 28,75 saat) toplam 19 geçerli çekim gerçekleştirilmiştir. *Parapenaeus longirostris*, *Illex coindettii*, *M. merluccius*, *Phycis blennoides*, *Lophius piscatorius* ve *M. poutassou* türleri 40S'de avın %61,9'unu ve 40T90'da %69,5'ini oluşturmaktadırlar. Geri kalan diğer türler (balık ve omurgasızlar) ve tanımlanamayanlar 40S'de 38.1% 40T90'da ise %30,5'dir. Av kompozisyonuna ilişkin veriler Tablo 2'de verilmiştir.

Tablo 2. Denemelerden elde edilen toplam av ve türlerin torbalardaki yakalanma oranı (%)

Parametreler	40S			40T90		
	Toplam	Torba	Örtü	Toplam	Torba	Örtü
Ağırlık (kg)	2423,1	1903,6	519,6	1847,1	1495,4	351,7
<i>Parepeaneus longirostris</i> (%)	29,8	34,1	13,9	31,2	33,0	23,6
<i>Illex coindetii</i> (%)	14,9	19,0	0,0	19,7	23,1	5,0
<i>Merluccius merluccius</i> (%)	4,9	4,9	5,0	2,5	3,0	0,5
<i>Phycis blennoides</i> (%)	5,3	4,5	8,5	3,6	3,5	4,0
<i>Lophius piscatorius</i> (%)	4,5	5,7	0,0	3,9	4,8	0,0
<i>Micromesistius poutassou</i> (%)	2,5	0,5	9,8	8,6	6,0	20,0
Diğer (%)	38,1	31,3	62,8	30,5	26,6	46,9

Tablo 3. Torbalar için farklı modellerden elde edilen AIC değerleri

Tür	Torba	Logit	Probit	Gompertz	Richard
Bakalyaro	40S	3781,69	3794,59	3822,16	3782,85
	40T90	619,11	617,75	619,98	621,09
Mezgit	40S	689,05	689,58	689,02	691,16
	40T90	1766,10	1766,05	1765,91	1767,93

En iyi modelin uygulanması için farklı modellerden elde edilen AIC değerleri Tablo 3’de verilmiştir. En düşük AIC değerini bakalyaro için 40S’de Logit model (3781,69), 40T90’da Probit model (617,75) vermiştir. Mezgit için 40S(689,02) ve 40T90’da (1765,91) Gompertz model en düşük AIC değerini vermiştir.

Ortalama L_{50} ve SR değerleri ile onların güven aralıkları Tablo 4’te ve ortalama seçicilik eğrileri bakalyaro için Şekil 1, derinsu mezgiti için de Şekil 2’de verilmiştir. Bakalyaro için 40S torbadan elde edilen, ortalama L_{50} değeri 13,23 cm, 40T90 torbadan elde edilen ise 12,70 cm’dir. Bakalyaro için 40S torbadan elde edilen SR değeri 3,30 iken

için (Şekil 3 sağ kısım) her boy sınıfı için yakalama oranları arasında istatistiki farklılık belirlenmemiştir (max CI değerleri tüm boy sınıflarında 0.0 değerinin üzerinde bulunmuştur). Derinsu mezgiti’nde ise yakalama oranları (Şekil 4 sağ kısım) 13,00-17,00 cm boy aralığında fark önemli, diğer boy sınıflarında ise önemsiz bulunmuştur. Değerlendirmelerde kullanılan referans boylar baz alındığında (bakalyaro için 20 cm asgari av boyu ve derinsu mezgiti için 10 cm ilk üreme boyu) her iki tür içinde 40S ve 40T90 torbaların seçicilikleri arasında istatistiksel fark bulunmamıştır.

Bu çalışmada, bakalyaro ve derinsu mezgiti’nin 40S ve 40T90 torbalarda seçicilik parametreleri araştırılmıştır. 40S torba 40T90 torbaya göre

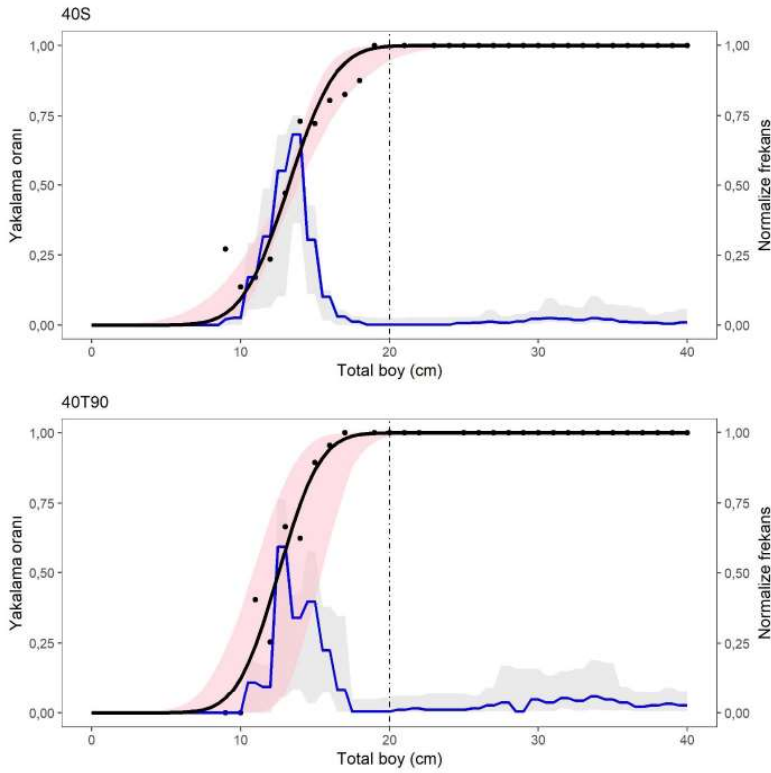
Tablo 4. Denemelerde bakalyaro ve derinsu mezgiti için elde edilen seçicilik parametreleri (L_{50} : %50 yakalanma boyu, SR: Seçicilik aralığı, df: serbestlik derecesi)

Tür	Torba	L_{50} (cm)	SR (cm)	p-value	Deviance	df
Bakalyaro	40S	13,23 (12,97-13,87)	3,30 (2,58-5,3)	0,0528	93,94	41
	40T90	12,70 (10,83-14,81)	2,96 (1,97-3,65)	0,3665	47,16	32
Mezgit	40S	23,73 (17,96-112,94)	9,34 (1,87-100,0)	0,0001	41,40	13
	40T90	19,91 (14,46-57,60)	18,14 (4,75-100,00)	0,0324	27,91	16

40T90’den elde edilen SR değeri 2,96’dır. Derinsu mezgiti için 40S torbada ortalama L_{50} değeri 23,73 cm, 40T90 torbada 19,91 cm, SR değeri 40S torbada 9,34 ve 18,14 cm olarak bulunmuştur. Seçicilik eğrilerinin birebir karşılaştırılmasında Delta yakalanma olasılığı grafiklerinden faydalanılmıştır (Şekil 3). Bu çerçevede, bakalyaro

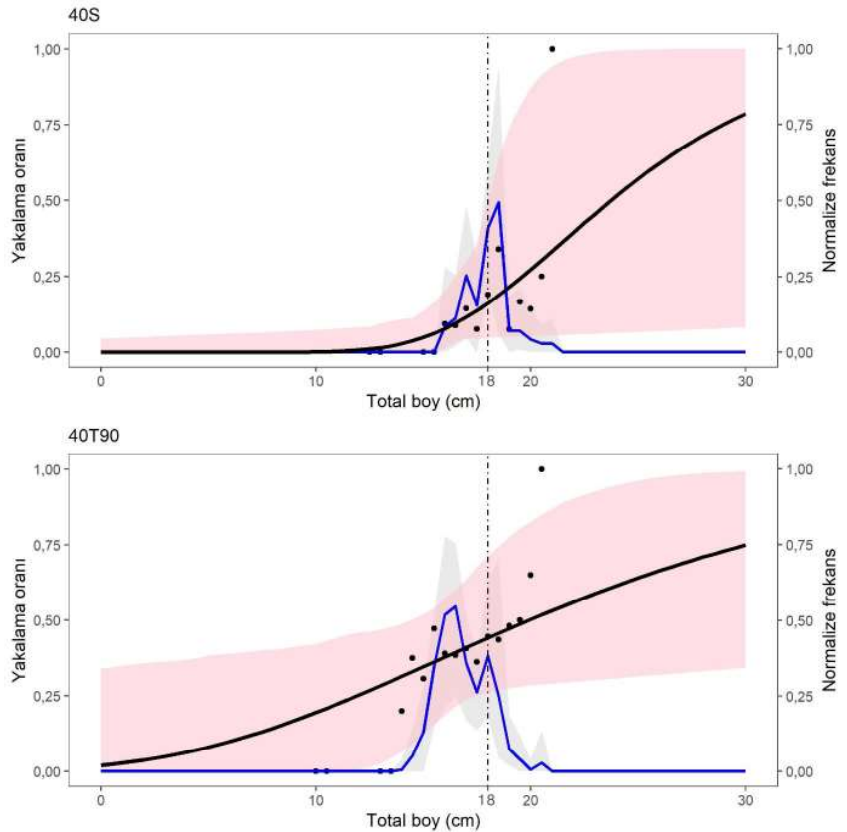
bakalyaro için %4, mezgit için ise %19 daha yüksek L_{50} değeri üretmiştir.

40S ve 40T90 torbalarından elde edilen L_{50} değerleri, ticari balıkçılıkta kullanılan 44 mm baklava gözlü ağlar ile karşılaştırıldığında her iki türün boy seçiciliği geliştirilmiştir. Özbilgin ve ark. (2005),



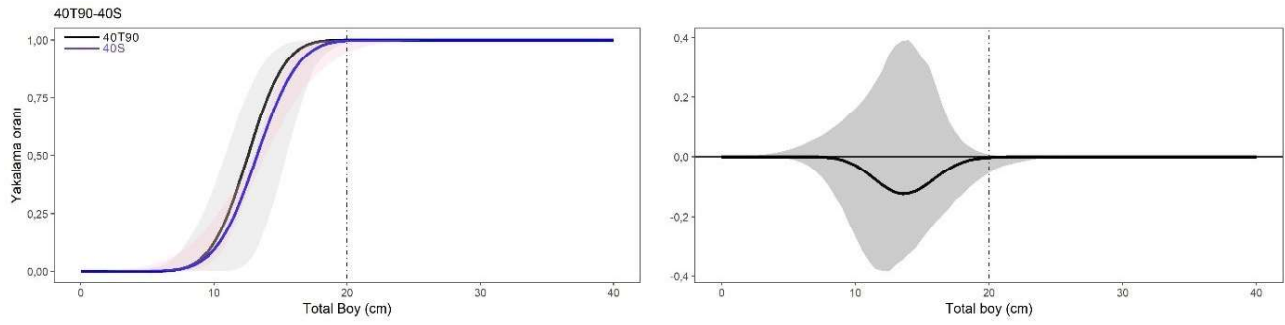
Şekil 1. Bakalyaro'ya ilişkin 40S ve 40T90 torbadan elde edilen ortalama seçicilik eğrileri ve popülasyonun boy dağılımı

Şekil 2. Derinsu mezgiti'ne ilişkin 40S ve 40T90 torbadan elde edilen ortalama seçicilik eğrileri ve popülasyonun boy dağılımı

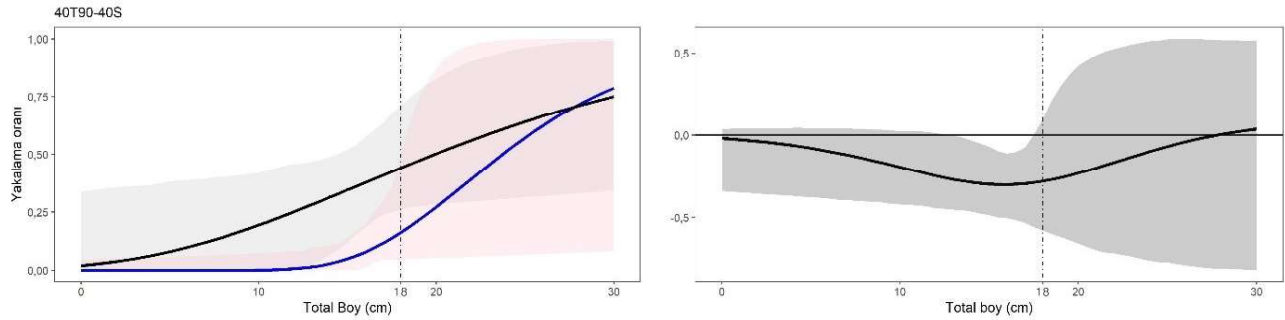


Ege Denizi'nde bakalyaro için standart torbada (40 mm nominal *PE*), örtüye hiçbir bireyin geçmediği için herhangi bir seçicilik sonucuna ulaşamazken, Aydın ve Tosunoğlu (2010), 44 mm baklava gözlü torba ile 10,4 cm L_{50} değerine ulaşmışlardır. Yapılan diğer araştırmalarda da bakalyaro için kare gözlü torbaların seçiciliğinin baklava gözlü torbalara

nazaran daha yüksek olduğu ortaya konulmuştur (Campos ve Fonseca, 2013, Campos ve ark., 2003; Dereli ve Aydın, 2016). Özbilgin ve ark. (2005), kare gözlü üst panel (üst panelde 50 bar kare, alt panelde 100 baklava göz) torba ile L_{50} değerini 15,25 cm, Aydın ve Tosunoğlu (2010) 40 mm kare gözlü torba için 14,4 cm olarak bildirmiştir. Bu



Şekil 3. Bakalyaro için 40S ve 40T90 torbalardan elde edilen L_{50} sonuçlarının karşılaştırılması



Şekil 4. Derinsu mezgiti için 40S ve 40T90 torbalardan elde edilen L_{50} sonuçlarının karşılaştırılması

çalışmada ise, 40S torba için bulunan 13.23 cm L_{50} değeri bakalyaro için kare gözlü ve döndürülmüş torbadan elde edilen 12.70 cm L_{50} değerleri ile karşılaştırılabilir niteliktedir. Diğer taraftan 40T90 torbadan tespit edilen L_{50} değeri ile 40S arasında sadece %4 gibi bir farkın ve bu farkın istatistiksel olarak önemsiz olması 40S torbaya bir alternatif olabileceği düşünülmektedir. Bununla birlikte, yapılan çalışmalarda bulunan L_{50} değerleri, 20 cm yasal asgari av boyu ile kıyaslandığında halen düşük olduğu görülmektedir (Anonim, 2020). Çevre göz sayısı düşürülmüş daha büyük ağ göz açıklığına sahip torbalar ile bu türün seçiciliğin geliştirilebileceği düşünülmektedir. Kaldı ki çevre göz sayısının düşürülmesi L_{50} değerini anlamlı bir şekilde yükseltmektedir (Sala ve Lucchetti 2011; Şensurat-Genç ve ark., 2018).

Kıta yamacı ve sahanlığından 1000 m derinlere kadaryayılım gösteren derinsu mezgiti yaygın olarak 300-400 m'lerde bulunmaktadır (Cohen, 1990). Bu derinlikler Türkiye sularında genellikle uluslararası sular niteliğinde değerlendirilmektedir ve her çekimden seçicilik parametrelerini hesaplamada kullanılabilecek birey yakalanmadığı için derinsu mezgiti üzerine seçicilik çalışmaları nispeten daha azdır. Tokaç ve ark. (2010), 300 göz çevresine sahip 42,42 mm göz açıklığındaki baklava gözlü torba ile L_{50} değerini 18,75 cm, üst paneli kare göz (75 bar) ve alt paneli baklava (150 göz) olan ve 41,65 mm kare gözlü torbada ise 19,42 cm

bulmuştur. Kaykaç (2010) nominal 40 mm göz açıklığında, 300 göz çevre göz sayısına sahip baklava gözlü torba ile L_{50} değerini 16,98 bulurken, 48 mm göz açıklığında kare gözlü üst panel ile sahip (40 mm göz açıklığında 150 göz çevre göz sayısına sahip) 22,84 cm L_{50} değeri elde etmiştir. Bu çalışmada 40.6 mm göz açıklığına sahip torbadan 40S için 23,73 cm L_{50} değeri elde edilmiştir ki bu değer Tokaç ve ark. (2010) ve Kaykaç (2010)' dan daha yüksek bir değerdir. 40T90 için elde edilen 19,91 cm L_{50} değeri ise Kaykaç (2010) ve Tokaç ve ark. (2010)'nin baklava gözlü torbadan elde ettiklerinden daha yüksektir. Derinsu mezgiti için yapılan seçicilik çalışmalarından tür için bildirilen 18 cm ilk üreme boyunun üzerinde değer elde edilmiştir.

Sonuç

Sonuç olarak 40 mm ağ göz açıklığına sahip torbalarla yapılan bu çalışmada, kare gözlü torba döndürülmüş torbaya göre her iki tür içinde seçiciliği geliştirmiştir. Yasal yakalanma boyu dikkate alındığında bakalyaro için her iki torbada 20 cm değerinin altında sonuç vermiştir. Bu nedenle bakalyaro seçiciliğinin geliştirilmesi için çevre göz sayısı düşürülmüş veya büyük göz açıklığındaki torbalarla denemelerin gerçekleştirilmesi gerekmektedir. Her iki torbadan mezgit için elde edilen L_{50} değerleri 18 cm olarak bilinen ilk üreme boyuna göre yeterli bulunmuştur.

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ETİK STANDARTLARA UYUM

Yazarların Katkısı

Tüm yazarların makaleye katkısı eşittir.

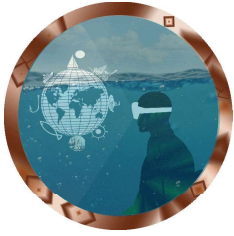
Çıkar Çatışması

Yazarlar herhangi bir çıkar çatışması olmadığını deklare etmektedir.

Etik Onay

Yazarlar bu tür bir çalışma için resmi etik kurul onayının gerekli olmadığını bildirmektedir.

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Assessment of some heavy metal accumulation and potential health risk for three fish species from three consecutive bay in North-Eastern Mediterranean Sea

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Keywords

Heavy metal accumulation
Fish
Human health
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Mullus barbatus
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Siganus rivulatus

ABSTRACT

Due to rapid urbanization and industrialization especially near water resources, heavy metal pollution in both water and inland environments have been studied all around the world. In this study, some selected heavy metals (Al, Sr, Cd, Co, Ni and, Pb) and fish species (*Mullus barbatus*, *Solea solea*, and *Siganus rivulatus*) from three Bays (İskenderun, Mersin, and Antalya from North-Eastern Mediterranean Sea) were used for heavy metal accumulation level evaluation and health risk assessment on both general and fish populations. A variety of accumulation patterns for considered metals were observed in tissues. Significant ($p<0.05$) inter- and intraspecies/tissues/bays differences were detected. The most stable tissue in terms of Al and Sr accumulation was determined as muscle. For Cd, Co and Ni accumulation the most stable tissue was found as liver. Lastly, for Ni accumulation skin was found to be the most stable tissue. The Target Hazard Quotients (THQ) and Total Target Hazard Quotients (TTHQ) values based on muscle were not exceeded 1.00. Therefore, these results suggest that both general and fish populations are not subjected to the significant potential health risk from those bays.

Introduction

In the last century, due to the industrialization and urbanization in the world, aquatic environments have been seriously threatened by pollutants. Heavy metals are one of these anthropogenic pollutants and they are considered as dangerous for the aquatic environment because of their toxicity, high persistence, non- biodegradability, and tendency to accumulate in organisms (Çoğun et al., 2017). The accumulation rate and amount of heavy metals may vary depending on the fish species, quality of some environmental parameters, such as salinity, temperature, pH, hardness, heavy metal concentration, exposure period, sex and size of fish (Yılmaz et al., 2010). Thus, metal accumulation ratios in fish tissues may show fluctuations at different locations, even for the same fish species (Yılmaz, 2003).

Since fisheries products have many polyunsaturated fatty acids, liposoluble vitamins, minerals and essential proteins, they are important for human consumption in terms of nutritional value (Mohanty et al., 2019; Kiliç et al., 2019). For those reasons, there have been growing interest in "food safety" and keeping food quality at acceptable levels on aquatic products for human beings worldwide. The Target Hazard Quotients (THQ) and Total Target Hazard Quotients (TTHQ) values have been used for assessing potential health risks of individual and total effects of heavy metals, respectively. However, TTHQ has been expected to be much more reliably helpful to assess and compare their combined risks and therefore have been widely employed in recent literature (Yi et al., 2011; Korkmaz et al., 2017; Rajan and Ishak, 2017).

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İskenderun Bay, Mersin Bay, and Antalya Bay have rapid and intense urbanization, industrialization along with marine traffic issues, ship accidents (Can et al., 2020). All these have been resulting in the intensity of anthropogenic pollutants on these three bays from the North-Eastern Mediterranean. Red mullet (*Mullus barbatus*), Common sole (*Solea solea*), and Marbled spine foot (*Siganus rivulatus*) are fish species that exist in these three bays. While red mullet and common sole are sharing similar habitats in terms of depth, seabed and feeding types, marbled spine foot found on shore and feeds on algae. Therefore, in this study, accumulation rates of some selected heavy metals (Al, Sr, Cd, Co, Ni, and Pb) in the different tissues (muscle, liver, skin, and intestine) of Red mullet (*M. barbatus*), Common sole (*S. solea*), and Marbled spine foot (*S. rivulatus*) were determined and compared according to both inter- and intra-species/ tissues/bays.

Also, the accumulation stability of these metals in different tissues was examined. Target Hazard Quotients (THQ) and Total Target Hazard Quotients (TTHQ) values (both general and fish population) of these heavy metals accumulation in muscle tissue as the edible part of fish were assessed.

Material and Methods

Fish Species

Solea solea, *Mullus barbatus*, and *Siganus rivulatus* were studied. Fish species were confirmed according to Froese and Pauly (2020).

Common sole (*S. solea*) is demersal marine species living on sandy or muddy bottoms, ranging from nearshore to 200 m of depth. Adults feed mainly on polychaete worms, mollusks, and small crustaceans.

Red mullet (*M. barbatus*) is benthic species on muddy bottoms of the continental shelf between

5 and 300 m. They are also found on gravels and sandy bottoms and it feeds on benthic invertebrates (crustaceans, worms, mollusks).

Marbled spine foot (*S. rivulatus*) occurs in shallow waters over substrates clothed with algae, including rocky and sandy areas at depths of less than 15 m. They are herbivorous, feeding mainly on algae.

Studied Areas

Fish samples were taken from local fishermen in the İskenderun, Mersin, and Antalya Bay, in April 2016. These three consecutive bays are located in the Northern East coast of the Mediterranean Sea (Figure 1). All bays have intensive marine traffic, tourism activities and shelf regions are surrounded by domestic areas. Moreover, many heavy industrial facilities have been established around İskenderun and Mersin bays which may cause an increase in heavy metal pollution risk for aquatic life. They interact with each other through current systems (Hamad et al., 2005).

Sampling, Preparation and Metal Analysis

Sampling

Fish samples (n=15 specimens for each species) were taken from local fishermen in İskenderun, Mersin, and Antalya Bays in April 2016 (Figure 1). The samples were brought to the laboratory on ice immediately and then frozen at -25°C until dissection. Total fish length and weight were measured to the nearest millimeter and gram before dissection. The mean length and weight of the *S. solea*, *M. barbatus*, and *S. rivulatus* were 25.22±2.20 cm and 135.90±43.11 g, 19.64±4.07 cm and 158.31±123.61 g and 17.29±2.08 cm and 108.34±72.52 g, respectively. The mean body length of each species from three bays were not significantly different (p>0.05).

Figure 1. Map of Studied Areas (İskenderun, Mersin, and Antalya Bay) from North Eastern Mediterranean Sea



Preparation

Fish samples were dissected to get tissue samples (epaxial muscle, intestine, skin, and liver). Studied tissue from each fish was transferred to a petri dish after being wet weighed and 2 mL nitric acid (HNO₃, 65%, S.W.: 1.40, Merck) and 1 mL perchloric acid (HClO₄, 60%, S.W.: 1.53, Merck) mixture were added in the sample which is located in the experimental tube. Then, tissue samples were wet digested on a hotplate at 120°C for 8 h. They were transferred to polyethylene tubes and volume were set up to 10 mL using deionized water. Samples were passed through a 0.45-µm membrane filter before analysis.

Analysis

All analyses were carried out in triplicate by using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Perkin Elmer Nexion 350 X). Blanks were carried out in the same manner as samples and concentrations were determined using standard solutions prepared in the same acid matrix. Calibration standarts were prepared from a multi-element ICP Standard (Merck). The quality of data was checked against the analysis of standard reference material DORM-2 (National Research Council of Canada; dogfish muscle and liver MA-A-2/TM Fish Flesh). The recovery values for Al, Sr, Cd, Co, Ni, and Pb were measured as 99.98, 93.25, 94.16, 96.57, 91.22 and 97.12%, respectively. Metal concentrations were calculated in micrograms per gram wet weight (µg metal g⁻¹ w.wt.).

Target Hazard Quotients (THQ) and Total Target Hazard Quotients (TTHQ)

Estimated weekly intake (EWI) amount were calculated by multiplying the mean concentrations of each metal and the weight (g) of weekly consumed fish. In Turkey, the weekly fish consumption amount is 105.42 g (TUIK, 2018). The average weight of a person was considered as 72.8 kg (TUIK, 2016) and multiplied by provisional tolerable weekly intakes (PTWI) values, proposed for each element. Then the percent PTWI was calculated.

In order to evaluate the potential health risk of *S. solea*, *M. barbatus*, and *S. rivulatus* consumption, Target Hazard Quotients (THQ) which is indication of heavy metal exposure risk was calculated. THQ calculation formula is given below (Han et al., 1998; Chien et al., 2002; Storelli, 2008).

$$THQ = [(E_f \times E_D \times F_{IR} \times C) / (R_{FD} \times W_{AB} \times T_A)] \times 10^{-3}$$

where, E_f is the exposure frequency: 365 days/year, E_D is the exposure duration: the average lifetime is assumed as 70 years according to (Bennett et al., 1999). F_{IR} is the food ingestion rate: 15.06 g/day for Turkish consumers, according to TUIK (2018). C is the determined metal concentration in muscle tissue (mg/kg).

R_{FD} is the oral reference dose (mg/kg/day): Al, Sr, Cd, Co, Ni, and Pb have been suggested as 1 (EFSA, 2008), 0.6 (US EPA, 2009), 0.0001 (ATSDR, 2012), 0.0003 (CDEP, 2008), 0.02 (US EPA, 2009) and 0.00357 (FAO/WHO, 2004) respectively.

W_{AB} is the average body weight: 72.8 kg, according to TUIK (2016). T_A is the average exposure time for non-carcinogens (365 days/year \times E_D , assuming 70 years in this study). In this study, the total THQ (TTHQ) is treated as the arithmetic sum of the individual metal THQ values (Yi et al., 2011):

$$TTHQ = THQ (\text{toxicant 1}) + THQ (\text{toxicant 2}) + \dots + THQ (\text{toxicant n})$$

THQ and TTHQ values were estimated for the general population (THQ_{gp}) and fishermen (THQ_f) separately to compare the risk of heavy metals from different consumers. In this study, FIR was assumed for Turkish fishermen to be two times higher than the general population as 30.12 g/day. The THQ and TTHQ \geq 1.0 refers to people may experience significant health risk from the intake of individual metals through fish consumption (Yi et al., 2011).

Statistical analyses

All data were checked for outliers and then descriptive statistics and Box-Whisker plots were calculated and drawn, respectively. Both inter- and intra-species/ tissues/bays differences were assessed using by one-way PERMANOVA (Permutational multivariate analysis of variance) test.

The stability (variability) of heavy metal accumulation in different tissues was evaluated by coefficient of variation (Cv, %), i.e. high variability indicates low stability and low variability indicates high stability. All computations and statistical analyses were carried out using Microsoft Excel and Past software (V. 3.23) (Hammer et al., 2001).

Results and Discussion

Heavy metals were widely studied pollutants in marine ecosystems. Some of them (iron, chromium, manganese, cobalt, selenium) are essential in trace amounts for aquatic life; while others were

harmful to aquatic life (Kalay and Canlı, 2000). Results of essential metal accumulation of *S. solea*, *M. barbatus*, and *S. rivulatus* can be found at Can et al. (2020).

It is well known that cadmium and lead are harmful metals for any biological process. Although aluminum, cobalt, nickel are considered as non-essential, these are playing important role in bioactivities at trace amounts. Sr mainly accumulates in bony tissues of fish and results in the development of scoliosis and osteoporosis. At high concentrations, Sr also is accumulated in soft tissues (Neff, 2002).

The mean values ($\mu\text{g g}^{-1}$ w.wt.) with standard deviation ($\bar{x}\pm\text{sd}$) and coefficient of variation (Cv, %) of measured heavy metals (Al, Sr, Cd, Co, Ni, and Pb) in the tissues muscle (M), intestine (I), skin (S), and liver (L) of *S. solea*, *M. barbatus*, and *S. rivulatus* by studied locations are given in Table 1-6 and Table 7, respectively. Also, mean and standard deviations of heavy metal concentration in muscle tissues of studied species and the estimated Target Hazard Quotients (THQ) in Table 8, respectively.

Aluminum (Al)

Aluminum, one of the most abundant metal in the Earth (7.5-8.1%), has very active ion (Al^{3+}) and bonds covalently with some compounds. Although it is not classified as heavy metal, it has toxic effects. Al toxicity and bioavailability to aquatic biota largely depends on its solubility and represents inverse relationship. Aluminum toxicity is reported to be among the factors lead to Alzheimer's disease, dementia, and Parkinson's disease (Chin-Chan et al., 2015).

There were no significant differences detected among the tissues for three species in each bays, except *S. rivulatus* (skin and muscle, $p<0.05$) from Antalya Bay (Table 1, denoted as A, B, C). Mean Al concentrations in the tissues of *S. solea* had same pattern for three bays as $I>S>L>M$. Al concentration for *M. barbatus* was ranked as $I>L>S>M$ for İskenderun, Mersin Bays and $I>S>L>M$ for Antalya Bay. Mean Al level ranking of *S. rivulatus* for İskenderun Bay was ordered as $I>S>L>M$; on the other hand, the ranking for Mersin and Antalya Bays were as $I>M>S>L$ and $S>I>L>M$, respectively.

The mean Al concentrations in the muscle tissue of *S. solea* among the three locations were not significantly different ($p>0.05$), but a significant difference was found in the muscle of *M. barbatus*

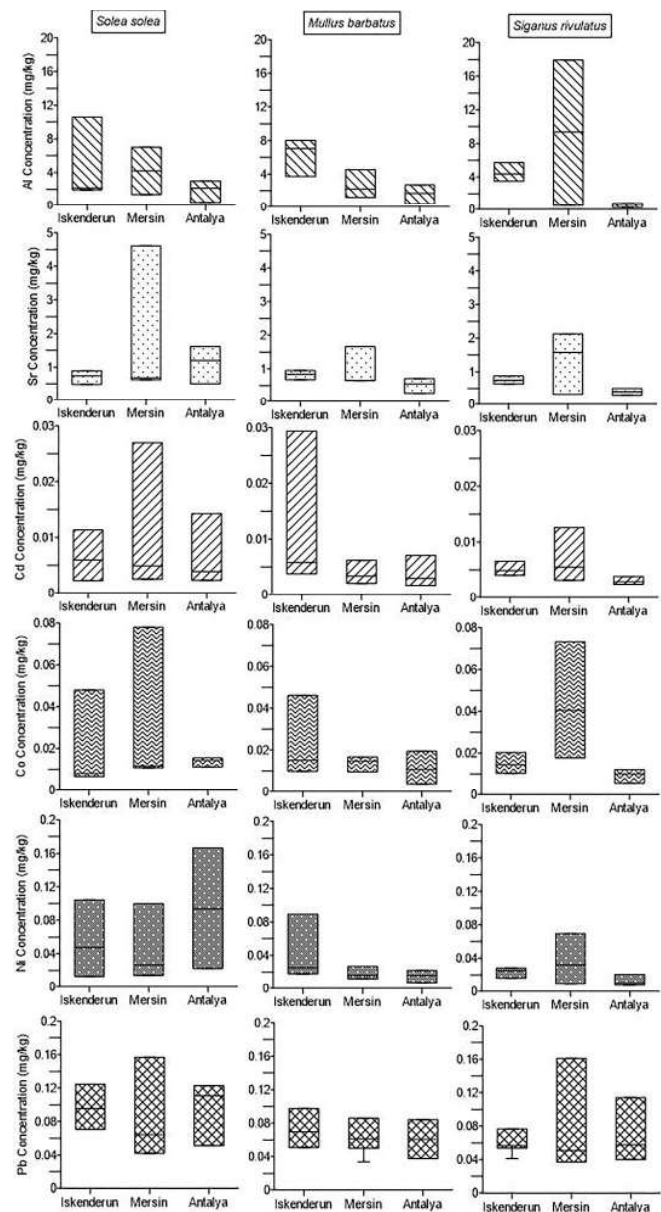


Figure 2. Box and whisker plots indicating heavy metal concentration in muscle tissues of *Solea solea*, *Mullus barbatus*, *Siganus rivulatus*

and *S. rivulatus* among locations ($p<0.05$, Table 1 denoted as x, y). There were no significant differences among the same tissues of different species at the same locations of all samples ($p>0.05$, Table 1, denoted as a).

The highest mean Al accumulation in muscle tissues was observed in *S. rivulatus* at Mersin Bay ($7.62 \pm 9.40 \mu\text{g g}^{-1}$ w.wt. Figure 2a). Also, in general, Al accumulation stability was somehow higher in muscle than that of the other tissues for all species and bays (Table 7).

Table 1. Mean Al concentration ($\mu\text{g metal g}^{-1}$ wet weight [w.wt.]) with standard deviation in the tissues of *Solea solea*, *Mullus barbatus*, *Siganus rivulatus* with respect to studied locations

	Location		Intestine	Skin	Liver	Muscle
<i>S. solea</i>	İskenderun	\bar{x}	49.57 ^{A;a;x}	15.28 ^{A;a;x}	5.55 ^{A;a;x}	7.49 ^{A;a;x}
		$\pm s$	40.20	9.82	3.55	8.15
	Mersin	\bar{x}	34.14 ^{A;a;x}	5.34 ^{A;a;x}	4.49 ^{A;a;x}	3.61 ^{A;a;x}
		$\pm s$	28.20	3.62	6.68	2.58
	Antalya	\bar{x}	5.32 ^{A;a;x}	3.79 ^{A;a;x}	3.83 ^{A;a;x}	1.83 ^{A;a;x}
		$\pm s$	2.16	3.03	3.22	1.34
<i>M. barbatus</i>	İskenderun	\bar{x}	91.65 ^{A;a;x}	13.32 ^{A;a;x}	25.89 ^{A;a;x}	6.46 ^{A;a;y}
		$\pm s$	88.47	7.26	11.68	1.99
	Mersin	\bar{x}	19.75 ^{A;a;x}	4.11 ^{A;a;x}	9.56 ^{A;a;x}	2.38 ^{A;a;x}
		$\pm s$	9.16	3.12	9.39	1.29
	Antalya	\bar{x}	125.90 ^{A;a;x}	15.81 ^{A;a;x}	3.99 ^{A;a;x}	1.63 ^{A;a;x}
		$\pm s$	70.21	15.56	4.01	1.14
<i>S. rivulatus</i>	İskenderun	\bar{x}	28.31 ^{A;a;x}	27.80 ^{A;a;y}	4.73 ^{A;a;y}	4.50 ^{A;a;y}
		$\pm s$	13.91	14.99	1.01	1.08
	Mersin	\bar{x}	29.06 ^{A;a;x}	6.33 ^{A;a;xy}	2.54 ^{A;a;xy}	7.62 ^{A;a;xy}
		$\pm s$	39.40	6.91	1.53	9.40
	Antalya	\bar{x}	2.04 ^{A;a;x}	2.46 ^{AB;a;x}	1.91 ^{AB;a;x}	0.56 ^{B;a;x}
		$\pm s$	0.39	2.25	1.31	0.27

A,B,C denotes differences among tissues in the same species at the same location
a,b,c denotes differences among same tissues of different species at same locations
x,y,z denotes differences among locations in the same tissues of same species

The range of mean Al accumulation in muscle tissues of *M. barbatus* (1.63-6.46) in our study was higher than that of previous studies (0.45-1.68). No data was available for the Al accumulation in *S. rivulatus* (0.56-7.62) and *S. solea* (1.83- 7.49) on the same species in same bays (Table 1, Table 9). But, Yılmaz et al., (2010) reported the Al accumulation in the muscle tissue of *T. lucerna*, *L. budegassa* and *S. lascaris* from İskenderun Bay with the range of 2.23 to 4.93 $\mu\text{g g}^{-1}$ w.wt.

Strontium (Sr)

Strontium is involved in bone and cartilage metabolic processes in combination with calcium. Strontium is rarely distributed in the environment, thus, limited number of studies conducted about its accumulation on the aquatic organisms (Carvalho et al., 2005; Yılmaz et al., 2018).

The following differences were detected among tissues (Table 2, denoted as A, B, C); *S. solea* (skin and liver from Mersin) and *S. rivulatus* (skin, liver, and muscle from İskenderun and Mersin). With regard to the results given in Table 2, the patterns of Sr occurrence in the selected tissues can be listed as follows in descending order: *S. solea* (İskenderun), *M. barbatus* (İskenderun and Antalya

Bays); $I > S > L > M$ and for *S. rivulatus* (İskenderun and Antalya Bays); $S > I > L > M$.

The mean Sr concentrations in the muscle tissue of *S. solea* and *M. barbatus* among the three locations were not significantly different ($p > 0.05$), except for *S. rivulatus* from İskenderun and Mersin Bays ($p < 0.05$, Table 2; denoted as x, y, z). There were no significant differences among the same tissues of different species at the same locations of all samples ($p > 0.05$, Table 2; denoted as a).

Strontium levels ($\mu\text{g g}^{-1}$ w.wt.) of muscle ranged from 0.74 to 2.25 for *S. solea*, 0.51 to 0.90 for *M. barbatus* and 0.40 to 1.23 for *S. rivulatus* from bays (Figure 2b).

There has been no research conducted on the Sr accumulation in the studied fishes from three bays, except the current one. The maximum variability in Sr accumulation was detected in the intestine, the minimum variability was detected in the muscle. These results show that in terms of Sr accumulation stability in tissues, the muscle was much more stable than other tissues (Table 7).

Table 2. Mean Sr concentration ($\mu\text{g metal g}^{-1}$ wet weight [w.wt.]) with standard deviation in the tissues of *Solea solea*, *Mullus barbatus*, and *Siganus rivulatus* with respect to studied locations.

	Location		Intestine	Skin	Liver	Muscle
<i>S. solea</i>	İskenderun	\bar{x}	9.92 ^{A,a,x}	8.59 ^{A,a,x}	1.54 ^{A,a,x}	0.74 ^{A,a,x}
		$\pm s$	6.84	4.41	0.66	0.18
	Mersin	\bar{x}	10.32 ^{A,a,x}	91.6 ^{AB,b,x}	0.96 ^{AC,a,x}	2.25 ^{A,a,x}
		$\pm s$	5.54	3.44	0.33	1.95
	Antalya	\bar{x}	1.32 ^{A,a,x}	13.76 ^{A,a,x}	2.72 ^{A,a,x}	1.11 ^{A,a,x}
		$\pm s$	0.82	10.45	2.20	0.57
<i>M. barbatus</i>	İskenderun	\bar{x}	13.19 ^{A,a,x}	9.38 ^{A,a,x}	3.14 ^{A,a,x}	0.88 ^{A,a,x}
		$\pm s$	10.87	15.49	0.95	0.17
	Mersin	\bar{x}	3.69 ^{A,a,x}	0.76 ^{A,a,x}	0.84 ^{A,a,x}	0.90 ^{A,a,x}
		$\pm s$	2.64	0.60	0.51	0.47
	Antalya	\bar{x}	14.44 ^{A,a,x}	3.34 ^{A,a,x}	1.25 ^{A,a,x}	0.51 ^{A,a,x}
		$\pm s$	7.12	1.91	0.26	0.22
<i>S. rivulatus</i>	İskenderun	\bar{x}	11.97 ^{A,a,x}	14.51 ^{AB,a,y}	0.88 ^{AC,a,x}	0.72 ^{AC,a,y}
		$\pm s$	10.84	3.82	0.23	0.11
	Mersin	\bar{x}	11.22 ^{A,a,x}	7.75 ^{AB,b,x}	0.84 ^{AC,a,x}	1.23 ^{AC,a,y}
		$\pm s$	16.16	3.26	0.40	0.81
	Antalya	\bar{x}	1.46 ^{A,a,x}	5.68 ^{A,a,x}	1.01 ^{A,a,x}	0.40 ^{A,a,x}
		$\pm s$	0.84	1.64	0.68	0.09

A,B,C denotes differences among tissues in the same species at the same location
a,b,c denotes differences among same tissues of different species at same locations
x,y,z denotes differences among locations in the same tissues of same species

For *L. budegassa* and *S. lascaris* from İskenderun Bay, significant differences in Sr concentrations in the liver were observed; however, there was no such difference in skin and muscle of the same species. Also in the same study, Sr levels ranged from 0.78 $\mu\text{g g}^{-1}$ w.wt. in *L. budegassa* to 1.58 ($\mu\text{g g}^{-1}$ w.wt. in *S. lascaris* for muscles, and from 2.03 in *L. budegassa* to 2.46 $\mu\text{g g}^{-1}$ w.wt. in *S. lascaris* for livers (Yılmaz et al., 2010).

Carvalho et al. (2005) found that Sr concentrations were significantly different between demersal and pelagic species, tend to higher concentration for demersal species. Our results on muscle were consistent with that pattern (Figure 2b).

Cadmium (Cd)

Cadmium and its chloride and sulfate salts are freely soluble and all of them are poisonous for living organisms and it may cause enzyme inhibition in high concentrations. It could be taken up through Ca channels in the gills of fish and mollusks (Galvez et al., 2006) and also could get from food (McRae et al., 2018).

Mean cadmium concentrations of fish tissues from three bay are shown in Table 3 ($\mu\text{g g}^{-1}$ w.wt.). No significantly differences were detected among the tissues of each location for *S. solea* (Table 3,

denoted as A, B, C) and the accumulation rate in the tissues of *S. solea* was the same for all stations and ranked as $L > I > S > M$. Except İskenderun Bay ($p < 0.05$, denoted as A, B, C), there was no differences among the tissues of *M. barbatus* and Cd concentration was ranked as $L > I > S > M$ for three Bays. Some differences were observed among tissues of *S. rivulatus* from Mersin and Antalya Bays (Table 3, denoted as A, B, C) and it was ranked as $L > I > S > M$ except Antalya Bay where it follows $I > L > S > M$.

The mean Cd concentrations in the muscle tissue of *S. solea* and *M. barbatus* among the three locations were not significantly different ($p > 0.05$), except for *S. rivulatus* from İskenderun Bay ($p < 0.05$, Table 3; denoted as x, y).

There were no significant differences among same tissues of different species at same locations of all samples ($p > 0.05$, Table 3; denoted as a), except in the liver tissue of *S. solea* and *S. rivulatus* from İskenderun Bay ($p < 0.05$, Table 3; denoted as b).

Maximum Cd concentrations in the muscle tissue ($\mu\text{g g}^{-1}$ w.wt.) of *S. solea*, *M. barbatus*, and *S. rivulatus* were found as 0.01, 0.03 and, 0.01 (İskenderun Bay), 0.03, 0.01 and, 0.01 (Mersin Bay) and 0.01, 0.01 and, 0.003 (Antalya Bay), respectively (Figure 2c).

Table 3. Mean Cd concentration ($\mu\text{g metal g}^{-1}$ wet weight [w.wt.]) with standard deviation in the tissues of *Solea solea*, *Mullus barbatus*, *Siganus rivulatus* with respect to studied locations

Location		Intestine	Skin	Liver	Muscle	
<i>S. solea</i>	İskenderun	\bar{x}	0.01 ^{A;a;x}	0.008 ^{A;a;x}	0.04 ^{A;b;x}	0.004 ^{A;a;x}
		$\pm s$	0.00	0.01	0.03	0.01
	Mersin	\bar{x}	0.01 ^{A;a;x}	0.04 ^{A;a;x}	0.02 ^{A;a;x}	0.01 ^{A;a;x}
		$\pm s$	0.00	0.01	0.03	0.01
	Antalya	\bar{x}	0.02 ^{A;a;x}	0.02 ^{A;a;x}	0.06 ^{A;b;x}	0.002 ^{A;a;x}
		$\pm s$	0.02	0.02	0.05	0.01
<i>M. barbatus</i>	İskenderun	\bar{x}	0.20 ^{A;a;x}	0.007 ^{AB;a;x}	0.11 ^{AC;a;x}	0.01 ^{AB;a;x}
		$\pm s$	0.12	0.01	0.01	0.01
	Mersin	\bar{x}	0.03 ^{A;a;x}	0.004 ^{A;a;x}	0.004 ^{A;a;y}	0.002 ^{A;a;x}
		$\pm s$	0.03	0.01	0.03	0.00
	Antalya	\bar{x}	0.08 ^{A;a;x}	0.01 ^{A;a;x}	0.16 ^{A;a;z}	0.003 ^{A;a;x}
		$\pm s$	0.10	0.01	0.01	0.01
<i>S. rivulatus</i>	İskenderun	\bar{x}	0.02 ^{A;a;x}	0.02 ^{A;a;x}	0.04 ^{A;b;y}	0.004 ^{A;a;x}
		$\pm s$	0.01	0.01	0.03	0.01
	Mersin	\bar{x}	0.06 ^{A;a;x}	0.004 ^{AB;a;x}	0.10 ^{AC;a;y}	0.004 ^{AB;a;x}
		$\pm s$	0.06	0.01	0.04	0.01
	Antalya	\bar{x}	0.12 ^{A;a;x}	0.004 ^{AB;a;x}	0.01 ^{AC;a;x}	0.003 ^{A;a;x}
		$\pm s$	0.03	0.01	0.03	0.001

A,B,C denotes differences among tissues in the same species at the same location
a,b,c denotes differences among same tissues of different species at same locations
x,y,z denotes differences among locations in the same tissues of same species

Table 4. Mean Co concentration ($\mu\text{g metal g}^{-1}$ wet weight [w.wt.]) with standard deviation in the tissues of *Solea solea*, *Mullus barbatus*, *Siganus rivulatus* with respect to studied locations.

Location		Intestine	Skin	Liver	Muscle	
<i>S. solea</i>	İskenderun	\bar{x}	0.17 ^{A;a;x}	0.03 ^{A;a;x}	0.033 ^{A;a;x}	0.02 ^{A;a;x}
		$\pm s$	0.19	0.01	0.02	0.02
	Mersin	\bar{x}	0.08 ^{A;a;x}	0.02 ^{A;a;x}	0.09 ^{A;a;x}	0.03 ^{A;a;x}
		$\pm s$	0.06	0.01	0.12	0.04
	Antalya	\bar{x}	0.08 ^{A;a;x}	0.13 ^{A;a;x}	0.08 ^{A;b;x}	0.01 ^{A;a;x}
		$\pm s$	0.08	0.15	0.08	0.01
<i>M. barbatus</i>	İskenderun	\bar{x}	0.31 ^{A;a;x}	0.03 ^{A;a;x}	0.11 ^{A;a;x}	0.02 ^{A;a;x}
		$\pm s$	0.30	0.02	0.07	0.02
	Mersin	\bar{x}	0.07 ^{A;a;x}	0.02 ^{B;a;x}	0.10 ^{AB;a;y}	0.01 ^{B;a;x}
		$\pm s$	0.03	0.01	0.04	0.01
	Antalya	\bar{x}	0.15 ^{A;a;x}	0.05 ^{AB;a;x}	0.33 ^{AC;a;x}	0.01 ^{AB;a;x}
		$\pm s$	0.05	0.03	0.01	0.01
<i>S. rivulatus</i>	İskenderun	\bar{x}	0.18 ^{A;a;x}	0.09 ^{A;b;y}	0.13 ^{AC;a;y}	0.01 ^{B;a;x}
		$\pm s$	0.12	0.03	0.06	0.01
	Mersin	\bar{x}	0.32 ^{A;a;x}	0.07 ^{AB;a;y}	0.23 ^{AC;a;x}	0.04 ^{AB;a;x}
		$\pm s$	0.37	0.04	0.08	0.02
	Antalya	\bar{x}	0.08 ^{A;a;x}	0.02 ^{A;a;x}	0.04 ^{A;a;x}	0.01 ^{A;a;x}
		$\pm s$	0.06	0.01	0.03	0.00

A,B,C denotes differences among tissues in the same species at the same location
a,b,c denotes differences among same tissues of different species at same locations
x,y,z denotes differences among locations in the same tissues of same species

The maximum variability in Cd accumulation was detected in the muscle, the minimum variability was detected in the liver (Table 7). These results show that in terms of Cd accumulation stability in tissues, the liver was much more stable than other tissues.

The mean Cd concentrations in the muscle tissues of ($\mu\text{g metal g}^{-1}$ w.wt.) *S. solea* (0.004-0.01), *M. barbatus* (0.003-0.01) and *S. rivulatus* (0.003-0.004) were very lower than previous studies from same bays as 0.11-0.38, 0.01-2.04 and, 0.06-0.24, respectively (Table 3, Table 9).

Cobalt (Co)

Although cobalt is interpreted as a non-essential metal, it is essential in very small amounts for all living organisms. Co is the active center of coenzymes called cobalamins which is found in vitamin B12 and it has a role in blood pressure regulation and thyroid function. Its inorganic form is also a micronutrient for bacteria, algae, and fungi (Lison, 2015).

There were no significant difference ($p > 0.05$) detected among the tissues of *S. solea* in all locations. But the differences were detected for *M. barbatus* (Mersin and Antalya Bays) and *S. rivulatus* (İskenderun and Mersin Bays), (Table 4, denoted as A, B, C). Mean Co concentrations in the tissues of *M. barbatus* and *S. rivulatus* had the same pattern for three bays as $I > L > S > M$. But there was no regular pattern observed for *S. solea* from three bays (Table 4). The mean Co concentrations in the muscle tissue of *S. solea* and *M. barbatus* among the three locations were not significantly different ($p > 0.05$, Table 4 denoted as x). But a significant difference was found in the muscle of *S. rivulatus* among locations ($p < 0.05$, Table 4 denoted as x, y). There were no significant difference detected among muscle tissues of different species at the same locations of all samples, except *S. rivulatus* (İskenderun and Mersin Bays: skin and liver, $p > 0.05$, Table 4; denoted as a, b, c).

Maximum Co concentrations in the muscle tissue ($\mu\text{g g}^{-1}$ w.wt.) of *S. solea*, *M. barbatus*, and *S. rivulatus* were found as 0.05, 0.05 and, 0.02 in İskenderun Bay, 0.08, 0.02 and, 0.07 in Mersin Bay and 0.02, 0.02 and, 0.01 in Antalya Bay, respectively (Figure 2d).

The maximum variability in Co accumulation was detected in the intestine and the minimum variability was detected in the liver. These results show that in terms of Co accumulation stability in

tissues, the liver was much more stable than other tissues (Table 7).

The mean Co concentrations in the muscle tissues of ($\mu\text{g metal g}^{-1}$ w.wt.) *S. solea* (0.01- 0.03), *M. barbatus* (0.01-0.02) and *S. rivulatus* (0.01-0.04) (Table 4) were found remarkable smaller than previous studies from same bays as 0.17-0.43, 0.02-0.44 and, 0.08-0.39 respectively (Table 9).

Nickel (Ni)

Nickel and certain nickel compounds, which are essential for human beings, are listed as carcinogens (ATSDR, 2011). Nickel is rarely found in fish, plants, and animals and its accumulation in aquatic life is very rare (Yılmaz et al., 2018).

There were no significant differences among the tissues for *S. solea* in each location. The accumulation rate in the tissues of *S. solea* had different patterns and muscle showed the lowest rate in all locations. (Table 5, denoted as A, B, C). Except for Antalya Bay ($p < 0.05$, denoted as A, B, C), there were no difference detected among the tissues of *M. barbatus*. Ni concentration was ranked as $I > S > L > M$ for Mersin and Antalya Bays, $I > L > S > M$ for İskenderun Bay. Significant differences were not detected among the tissues of *S. rivulatus* for each bays ($p > 0.05$, Table 5; denoted as A, B, C) and it was ranked as $I > S > L > M$ except from Antalya Bay where it followed $S > I > L > M$ pattern (Table 5). The mean Ni concentrations in the muscle tissue of *S. solea* and *M. barbatus* among the three locations were not significantly different ($p > 0.05$, Table 5 denoted as x). But a significant difference was found in the muscle of *S. rivulatus* among locations ($p < 0.05$, Table 5 denoted as x, y). There were no significant differences among the same tissues of different species at the same locations of all samples ($p > 0.05$, Table 5 denoted as a).

The maximum variability in Ni accumulation was detected in the intestine, the minimum variability was detected in the skin. These results show that in terms of Ni accumulation stability in tissues, the skin was much more stable than other tissues (Table 7).

Ni concentrations in the muscle tissue ($\mu\text{g g}^{-1}$ w.wt.) of *S. solea*, *M. barbatus* and *S. rivulatus* were varied from 0.01-0.10, 0.02-0.09, 0.02-0.03 in İskenderun Bay; 0.01-0.10, 0.01-0.06, 0.01-0.07 in Mersin Bay; and 0.02-0.17, 0.01-0.02, 0.01-0.02 in Antalya Bay, respectively (Figure 2e).

The range of mean Ni accumulation in muscle tissues of *S. solea*, (0.04-0.05), *M. barbatus*

Table 5. Mean Ni concentration ($\mu\text{g metal g}^{-1}$ wet weight [w.wt.]) with standard deviation in the tissues of *Solea solea*, *Mullus barbatus*, *Siganus rivulatus* with respect to studied locations.

Location		Intestine	Skin	Liver	Muscle	
<i>S. solea</i>	İskenderun	\bar{x}	0.19 ^{A;ax}	0.07 ^{A;ax}	0.05 ^{A;ax}	0.05 ^{A;ax}
		$\pm s$	0.15	0.03	0.02	0.04
	Mersin	\bar{x}	0.10 ^{A;ax}	0.04 ^{A;ax}	0.09 ^{A;ax}	0.04 ^{A;ax}
		$\pm s$	0.08	0.01	0.12	0.04
	Antalya	\bar{x}	0.08 ^{A;ax}	0.21 ^{A;ax}	0.09 ^{A;bx}	0.04 ^{A;ax}
		$\pm s$	0.09	0.13	0.10	0.08
<i>M. barbatus</i>	İskenderun	\bar{x}	0.35 ^{A;xy}	0.06 ^{A;ax}	0.09 ^{A;ax}	0.04 ^{A;ax}
		$\pm s$	0.30	0.04	0.01	0.03
	Mersin	\bar{x}	0.06 ^{A;ay}	0.05 ^{A;ax}	0.03 ^{A;ay}	0.03 ^{A;ax}
		$\pm s$	0.03	0.05	0.02	0.02
	Antalya	\bar{x}	0.38 ^{A;ax}	0.10 ^{B;ax}	0.03 ^{BC;ax}	0.02 ^{C;ax}
		$\pm s$	0.40	0.04	0.01	0.006
<i>S. rivulatus</i>	İskenderun	\bar{x}	0.25 ^{A;ax}	0.15 ^{A;ax}	0.03 ^{A;ax}	0.02 ^{A;ay}
		$\pm s$	0.23	0.08	0.01	0.005
	Mersin	\bar{x}	0.18 ^{A;ax}	0.04 ^{A;ax}	0.03 ^{A;ax}	0.03 ^{A;ay}
		$\pm s$	0.25	0.02	0.008	0.02
	Antalya	\bar{x}	0.04 ^{A;bx}	0.08 ^{A;ax}	0.02 ^{A;ax}	0.01 ^{A;ax}
		$\pm s$	0.03	0.09	0.008	0.005

A,B,C denotes differences among tissues in the same species at the same location
a,b,c denotes differences among same tissues of different species at same locations
x,y,z denotes differences among locations in the same tissues of same species

Table 6. Mean Pb concentration ($\mu\text{g metal g}^{-1}$ wet weight [w.wt.]) with standard deviation in the tissues of *Solea solea*, *Mullus barbatus*, *Siganus rivulatus* with respect to studied locations

Location		Intestine	Skin	Liver	Muscle	
<i>S. solea</i>	İskenderun	\bar{x}	0.21 ^{A;ax}	0.18 ^{A;bx}	0.22 ^{A;ax}	0.10 ^{A;ax}
		$\pm s$	0.10	0.06	0.09	0.02
	Mersin	\bar{x}	0.01 ^{A;ax}	0.24 ^{A;ax}	0.05 ^{A;ax}	0.08 ^{A;ax}
		$\pm s$	0.10	0.34	0.04	0.05
	Antalya	\bar{x}	0.10 ^{A;ax}	0.63 ^{A;ax}	0.37 ^{A;ax}	0.09 ^{A;ax}
		$\pm s$	0.06	0.88	0.48	0.03
<i>M. barbatus</i>	İskenderun	\bar{x}	0.47 ^{A;ax}	0.21 ^{A;ax}	0.49 ^{A;ax}	0.24 ^{A;ax}
		$\pm s$	0.23	0.22	0.40	0.38
	Mersin	\bar{x}	0.43 ^{A;ax}	0.20 ^{A;ax}	0.47 ^{A;ax}	0.06 ^{A;ax}
		$\pm s$	0.59	0.29	0.50	0.02
	Antalya	\bar{x}	0.35 ^{A;ax}	0.29 ^{A;ax}	0.20 ^{A;ax}	0.06 ^{A;ax}
		$\pm s$	0.13	0.17	0.04	0.02
<i>S. rivulatus</i>	İskenderun	\bar{x}	0.33 ^{A;ax}	0.37 ^{AB;acx}	0.15 ^{A;ax}	0.06 ^{AC;ax}
		$\pm s$	0.15	0.11	0.07	0.01
	Mersin	\bar{x}	2.03 ^{A;ax}	0.34 ^{A;ay}	0.52 ^{A;ax}	0.23 ^{AB;ax}
		$\pm s$	2.62	0.09	0.36	0.35
	Antalya	\bar{x}	0.56 ^{A;ax}	0.17 ^{A;ax}	0.27 ^{A;ax}	0.06 ^{A;ax}
		$\pm s$	0.55	0.07	0.12	0.03

A,B,C denotes differences among tissues in the same species at the same location
a,b,c denotes differences among same tissues of different species at same locations
x,y,z denotes differences among locations in the same tissues of same species

(0.006-0.03), and *S. rivulatus* (0.005-0.02) in our study (Table 5) were lower than that of previous studies (0.14-3.27), (0.13-4.22), and (0.69-3.43), respectively (Table 9).

Lead (Pb)

The inorganic lead salts are considered to be moderately toxic to marine organisms because of their low solubility. Lead binds to enzymes and hormones in live cells, it can cause in fish to deficit or decrease in survival, growth, development, and metabolism, to increase mucus formation (Burger et al., 2002).

There were no significant differences ($p>0.05$) among the tissues for studied fish in each location, except *S. rivulatus* from İskenderun and Mersin Bays and no regular pattern on accumulation rates was observed in among studied tissues. (Table 6, denoted as A, B, C). The mean Pb concentrations among the muscle tissue of all species from three bays were not significantly different ($p>0.05$, Table 6; denoted as x). Also, there were no significant differences among muscle tissues of different species at the same locations of all samples ($p>0.05$, Table 6; denoted as a).

Maximum Pb concentrations in the muscle tissue ($\mu\text{g g}^{-1}$ w.wt.) of *S. solea*, *M. barbatus*, and *S. rivulatus* were found as 0.12, 0.91 and, 0.08 in İskenderun Bay, 0.16, 0.09 and, 0.84 in Mersin

Bay and 0.12, 0.08 and, 0.11 in Antalya Bay, respectively (Figure 2f).

The maximum variability in Pb accumulation was detected in the intestine, the minimum variability was detected in the liver. These results show that in terms of Pb accumulation stability in tissues, the liver was much more stable than other tissues (Table 7).

The range of mean Pb accumulation in muscle tissues of *S. solea* (0.08-0.10) and *M. barbatus* (0.006-0.24) (Table 6) were smaller than of previous studies (0.037-1.31) and (0.03-5.94), respectively (Table 9). There was no conducted study on Pb accumulation for *S. rivulatus* in the same bays.

THQ and TTHQ

The highest levels of THQ_{gp} and THQ_f for *S. solea*, *M. barbatus* and *S. rivulatus* in İskenderun, Mersin and Antalya Bays were determined for AI (Table 8). The TTHQ_{gp} and TTHQ_f for *S. solea*, *M. barbatus* and *S. rivulatus* in İskenderun, Mersin, and Antalya Bays were calculated as (0.257 and 0.514), (0.479 and 0.958), and (0.149 and 0.298); (0.178 and 0.356), (0.103 and 0.206), (0.153 and 0.306); (0.028 and 0.056), (0.655 and 1.310), and (0.013 and 0.026), respectively. Among these values, only TTHQ for fishermen (1.310) is exceeded the value of 1 (reference value). But it does not mean that fishermen are under the risk.

Table 7. Coefficient of variation (%CV) of heavy metals in the tissues of *Solea solea*, *Mullus barbatus*, *Siganus rivulatus* with respect to studied locations.

Station	Species	Metal	Muscle	Intes-tine	Skin	Liver	Metal	Muscle	Intes-tine	Skin	Liver	Metal	Muscle	Intes-tine	Skin	Liver
iskenderun	<i>S. solea</i>	Al	108.86	81.08	64.23	63.91	Ni	82.54	78.33	34.48	36.51	Pb	21.91	48.72	34.23	42.20
Mersin			71.56	82.61	67.87	148.74		94.85	80.92	31.67	146.78		65.92	72.60	137.29	75.77
Antalya			73.08	40.53	79.94	84.13		85.29	116.81	63.78	110.16		40.56	56.97	140.55	129.99
iskenderun	<i>M. barbatus</i>		30.85	96.52	54.52	45.11		84.71	85.58	62.36	15.71		156.26	49.19	105.04	83.10
Mersin			54.20	46.36	75.78	98.21		79.75	47.84	117.67	85.92		104.55	136.07	144.17	104.55
Antalya			69.75	55.76	98.40	100.35		28.57	105.06	39.46	47.14		38.49	35.94	59.43	21.21
iskenderun	<i>S. rivulatus</i>		24.05	49.14	53.91	21.36		22.82	90.74	56.17	33.33		25.57	45.43	30.07	46.42
Mersin			123.24	135.61	109.10	60.32		84.98	135.22	57.60	29.88		151.59	129.07	27.19	69.04
Antalya			48.68	19.01	90.97	68.20		40.00	75.90	113.73	46.48		47.83	96.45	42.82	44.83
iskenderun	<i>S. solea</i>	Co	81.22	111.15	34.81	52.54	Cd	136.93	-	104.58	61.88	Sr	23.88	68.95	51.39	42.74
Mersin			127.27	67.39	60.85	139.77		141.42	-	136.93	111.11		86.62	53.72	37.51	34.67
Antalya			43.30	100.51	117.83	100.51		200.00	66.66	158.69	6.73		51.12	61.82	75.94	81.06
iskenderun	<i>M. barbatus</i>		86.60	95.94	52.54	64.28		122.47	61.51	66.66	6.73		18.90	82.41	165.16	30.17
Mersin			39.12	38.06	46.48	36.51		223.60	94.28	136.93	61.88		52.53	71.62	79.52	60.88
Antalya			81.64	32.17	63.19	4.28		200.00	125.41	91.28	4.56		43.66	49.28	57.15	21.01
iskenderun	<i>S. rivulatus</i>		39.12	69.05	32.96	42.31		136.93	46.48	55.90	69.72		15.15	90.56	26.34	25.96
Mersin			57.05	115.78	47.39	35.83		136.93	106.71	223.60	45.50		66.13	144.04	41.98	47.81
Antalya				86.06	50.00	57.04			23.00	136.93	30.01			57.25	28.87	66.77

Table 8. The estimated weekly intakes (EWI), established provisional tolerable weekly intake (PTWI) and percent PTWI (%) of the muscle tissue of *S. solea*, *M. barbatus* and *S. rivulatus* from three bays of Northern East Mediterranean Sea consumed by adult people in Turkey.

Station	Heavy Metal	PTWI	PTWI*	<i>S. solea</i>			<i>M. barbatus</i>			<i>S. rivulatus</i>		
				EWI*	PTWI (%)	THQ (unitless)	EWI*	PTWI (%)	THQ (unitless)	EWI*	PTWI (%)	THQ (unitless)
İskenderun	Al	7000	509600	5239.55	1.0	0.256	9687.41	1.901	0.4740	2992.37	0.6	0.15
	Sr	4200	305760	1048.54	0.34	0.014	1394.18	0.5	0.0018	1265.23	0.4	0.002
	Cd	0.7	510	1.06	2.0	0.0001	20.88	41.0	0.0029	1.90	3.7	0.0003
	Co	2.1	153	17.97	11.8	0.0001	32.77	21.4	0.0001	19.03	12.4	0.0001
	Ni	140	10192	20.08	0.2	0.0001	37.00	0.4	0.0002	26.43	0.3	0.0002
	Pb	25	1820	22.20	1.2	0.0001	49.68	2.7	0.0003	34.88	1.9	0.0002
Mersin	Al	7000	509600	3607.54	0.708	0.18	2087.58	0.41	0.10	3070.59	0.60	0.0015
	Sr	4200	305760	1048.54	0.3	0.0014	387.92	0.1	0.0005	1185.95	0.4	0.004
	Cd	0.7	510	1.06	2.0	0.0001	3.17	6.2	0.0004	6.34	12.4	0.0009
	Co	2.1	153	8.46	5.5	0.0000	7.19	4.7	0.0000	33.82	22.1	0.0001
	Ni	140	10192	10.25	0.1	0.0001	6.34	0.06	0.0000	19.03	0.2	0.0001
	Pb	25	1820	14.80	0.8	0.0001	45.45	2.5	0.0003	214.57	11.8	0.0014
Antalya	Al	7000	509600	562.32	0.11	0.028	13307.63	2.61	0.6511	215.63	0.04	0.0106
	Sr	4200	305760	139.52	0.0	0.0002	1527.37	0.5	0.002	154.32	0.1	0.0002
	Cd	0.7	510	2.33	4.5	0.0003	8.46	16.6	0.0012	12.16	23.8	0.0017
	Co	2.1	153	8.46	5.5	0.0000	15.86	10.4	0.0000	39.11	25.5	0.0001
	Ni	140	10192	8.46	0.08	0.0001	41.22	0.4	0.0003	4.76	0.0	0.0000
		25	1820	102.21	5.6	0.0007	37.00	2.0	0.0002	60.25	3.3	0.0004

EWI : estimated weekly intake in µg/week
PTWI: Established Provisional Tolerable Weekly Intake in (µg/week/kg body weight)
*PTWI for average Turkish adult (body weight is taken as 72.8 kg based on TÜİK (2016) data (µg/week/72.8 kg body weight)

Conclusion

This study was conducted to evaluate heavy metal accumulation (Al, Sr, Cd, Co, Ni and, Pb) in the tissues of (intestine, skin, liver, and muscle) selected fish species (*M. barbatus*, *S. rivulatus*, and *S. solea*) and to access health risk potential for both general and fish populations. Results showed different accumulation patterns among tissues for all species. Significant ($p < 0.05$) inter- and intraspecies/tissues/bays differences were detected. The most stable tissue in terms of Al, Sr, Cd, Co, Ni and, Pb accumulation was determined as muscle, muscle, liver, liver, skin and, liver, respectively.

Considering edible part of fish which is mainly muscle tissue, THQ and TTHQ values were calculated and results did not exceeded by 1.00. Therefore, these results suggest that both general and fish populations have not subjected to the significant potential health risk from these bays, yet. Our findings on heavy metal accumulation in fish were mostly consistent with previous studies conducted in the same areas while monitoring

programs should be continued to keep protecting the environment and human health in the future.

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COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

Authors contributed equally to this paper.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Table 9. Heavy metal accumulation ($\mu\text{g g}^{-1}$ w.wt.) and THQ values of muscle tissue of fish from previous studies evaluated in Iskenderun, Mersin and Antalya Bays.

Species	Metal	Bay	w.wt*	THQ	Reference
<i>M. barbatus</i>	Al	İskenderun	1.33	0.007	(Dural et al., 2010)
<i>M. barbatus</i>	Al	İskenderun	1.68	0.009	(Turan et al., 2009)
<i>M. barbatus</i>	Al	İskenderun	0.45	0.002	(Türkmen et al., 2005)
<i>S. solea</i>	Cd	İskenderun	0.20	0.001	(Türkmen, 2011)
<i>S. solea</i>	Cd	İskenderun	0.11	0.001	(Ersoy and Çelik, 2010)
<i>S. solea</i>	Cd	Mersin	nd	nd	(Korkmaz et al., 2017)
<i>S. solea</i>	Cd	Mersin	0.38	0.002	(Türkmen, 2011)
<i>M. barbatus</i>	Cd	İskenderun	0.10	0.001	(Turan et al., 2009)
<i>M. barbatus</i>	Cd	İskenderun	0.17	0.001	(Türkmen et al., 2005)
<i>M. barbatus</i>	Cd	İskenderun	0.62	0.003	(Çoğun et al., 2005)
<i>M. barbatus</i>	Cd	İskenderun	0.29	0.001	(Kalay et al., 1999)
<i>M. barbatus</i>	Cd	İskenderun	2.04	0.011	(Kargin, 1996)
<i>M. barbatus</i>	Cd	Mersin	nd	nd	(Korkmaz et al., 2017)
<i>M. barbatus</i>	Cd	Mersin	0.21	0.001	(Kalay et al., 1999)
<i>M. barbatus</i>	Cd	Antalya	0.01	<0.001	(Yipel and Yarsan, 2014)
<i>M. barbatus</i>	Cd	Antalya	0.02	<0.001	(Türkmen & Pınar, 2018)
<i>S. rivulatus</i>	Cd	İskenderun	0.24	0.001	(Ateş et al., 2015)
<i>S. rivulatus</i>	Cd	Antalya	0.06	<0.001	(Ateş et al., 2015)
<i>S. solea</i>	Co	İskenderun	0.17	0.001	(Türkmen, 2011)
<i>S. solea</i>	Co	Mersin	0.43	0.002	(Türkmen, 2011)
<i>M. barbatus</i>	Co	İskenderun	0.19	0.001	(Türkmen et al., 2005)
<i>M. barbatus</i>	Co	İskenderun	0.06	<0.001	(Tepe et al., 2008)
<i>M. barbatus</i>	Co	Mersin	0.44	0.002	(Tepe et al., 2008)
<i>M. barbatus</i>	Co	Antalya	0.02	<0.001	(Türkmen and Pınar, 2018)
<i>M. barbatus</i>	Co	Antalya	0.05	<0.001	(Tepe et al., 2008)
<i>S. rivulatus</i>	Co	İskenderun	0.39	0.002	(Ateş et al., 2015)
<i>S. rivulatus</i>	Co	Antalya	0.08	<0.001	(Ateş et al., 2015)
<i>S. solea</i>	Ni	İskenderun	0.22	0.001	(Ersoy and Çelik, 2010)
<i>S. solea</i>	Ni	İskenderun	0.14	0.001	(Kaya and Turkoglu, 2017)
<i>S. solea</i>	Ni	İskenderun	0.83	0.004	(Türkmen, 2011)
<i>S. solea</i>	Ni	Mersin	3.27	0.017	(Türkmen, 2011)
<i>S. solea</i>	Ni	Mersin	0.26	0.001	(Korkmaz et al., 2017)
<i>M. barbatus</i>	Ni	İskenderun	0.13	0.001	(Turan et al., 2009)
<i>M. barbatus</i>	Ni	İskenderun	0.27	0.001	(Türkmen et al., 2005)
<i>M. barbatus</i>	Ni	İskenderun	1.21	0.006	(Kalay et al., 1999)
<i>M. barbatus</i>	Ni	İskenderun	0.92	0.005	(Tepe et al., 2008)
<i>M. barbatus</i>	Ni	Mersin	0.61	0.003	(Kalay et al., 1999)
<i>M. barbatus</i>	Ni	Mersin	0.14	0.001	(Korkmaz et al., 2017)
<i>M. barbatus</i>	Ni	Mersin	4.22	0.022	(Tepe et al., 2008)
<i>M. barbatus</i>	Ni	Antalya	0.42	0.002	(Türkmen and Pınar, 2018)
<i>M. barbatus</i>	Ni	Antalya	0.96	0.005	(Tepe et al., 2008)
<i>S. rivulatus</i>	Ni	Antalya	0.69	0.004	(Ateş et al., 2015)
<i>S. rivulatus</i>	Ni	İskenderun	3.43	0.018	(Ateş et al., 2015)
<i>S. solea</i>	Pb	Mersin	0.48	0.002	(Korkmaz et al., 2017)
<i>S. solea</i>	Pb	İskenderun	0.38	0.002	(Ersoy & Çelik, 2010)

<i>S. solea</i>	Pb	İskenderun	1.13	0.006	(Türkmen, 2011)
<i>S. solea</i>	Pb	Mersin	1.31	0.007	(Külcü et al., 2014)
<i>S. solea</i>	Pb	Mersin	0.37	0.002	(Türkmen, 2011)
<i>M. barbatus</i>	Pb	İskenderun	0.45	0.002	(Dural et al., 2010)
<i>M. barbatus</i>	Pb	İskenderun	0.11	0.001	(Turan et al., 2009)
<i>M. barbatus</i>	Pb	İskenderun	0.82	0.004	(Türkmen et al., 2005)
<i>M. barbatus</i>	Pb	İskenderun	0.04	<0.001	(Tepe et al., 2008)
<i>M. barbatus</i>	Pb	İskenderun	1.88	0.010	(Çoğun et al, 2006)
<i>M. barbatus</i>	Pb	İskenderun	1.82	0.009	(Kalay et al., 1999)
<i>M. barbatus</i>	Pb	İskenderun	0.50	0.003	(Tepe et al., 2008)
<i>M. barbatus</i>	Pb	Mersin	5.94	0.031	(Kalay et al., 1999)
<i>M. barbatus</i>	Pb	Mersin	1.27	0.007	(Külcü et al., 2014)
<i>M. barbatus</i>	Pb	Mersin	0.40	0.002	(Tepe et al., 2008)
<i>M. barbatus</i>	Pb	Mersin	0.16	0.001	(Korkmaz et al., 2017)
<i>M. barbatus</i>	Pb	Mersin	0.89	0.005	(Tepe et al., 2008)
<i>M. barbatus</i>	Pb	Antalya	0.03	<0.001	(Türkmen and Pınar, 2018)
<i>M. barbatus</i>	Pb	Antalya	0.32	0.002	(Tepe et al., 2008)
<i>M. barbatus</i>	Pb	Antalya	0.22	0.001	(Tepe et al., 2008)
<i>M. barbatus</i>	Pb	İskenderun	0.39	0.002	(Ateş et al., 2015)
<i>M. barbatus</i>	Pb	Antalya	0.13	0.001	(Ateş et al., 2015)

Dry weight values were converted to wet wt. dividing by 5 [According to Yılmaz (2010)]

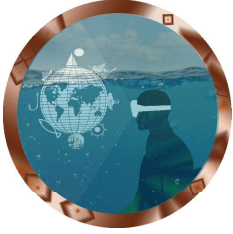
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Intra-and inter-specific competition effects on survival and growth of juvenile *Procambarus acutus acutus* and *Procambarus clarkii*

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ABSTRACT

Four similar sizes eastern white river crayfish (*Procambarus acutus acutus*) and red swamp crayfish (*Procambarus clarkii*) were stocked separately to determine intra and inter-specific-competition. Three treatment levels were used three treatment levels, with six replicates tanks each with stand green rice (representing a stocking density of approximately 21 individuals per m²). This study was carried out during 90 days to determine growth and survival rate. Results showed that the growth of *P. acutus acutus* in the interspecific-treatment tank was found higher than those grown in the intraspecific-treatment tanks. For *P. clarkii*, no significant differences in growth (estimated from the mean Total length, TL) were detected between treatments. The survival of the two species was the same up to the first 60 days of the experiment, while the difference in survival was only noticeable at 90 days. The survival rate of *P. clarkii* in the intraspecific-treatment tanks (55.0%) was higher than in the interspecific tanks (26.7%) treatment, while the survival of *P. acutus acutus* was found similar in two treatments. It was achieved higher survival, faster growth and larger size in *P. a. acutus* than *P. clarkii* when grown in the tank study. Size of body was important in determining competitive interactions between the two species.

Keywords

Procambarus acutus acutus
Procambarus clarkii
 Interactions
 Competition

Introduction

Crayfish culture has developed rapidly in recent years and has become one of the most important contributors to the nutritional supply of human demands (Yazıcı and Mazlum, 2019; Mazlum et al., 2019; Mazlum et al., 2020). Its annual production, which was 63.750 million kg in the U.S.A (Lutz, 2019). Presently there are more than 95% of the production comes from Louisiana (Huner, 1995; Eversole and McClain, 2000; Lutz, 2019). Red swamp crayfish and white river crayfish production are the most important commercial crayfish species in North America and are successful in commercial crayfish ponds. In addition both of them may thrive in the low-energy-input, extensive aquaculture systems used in Louisiana and other southern states.

P. clarkii can be easily distinguished from the *P. acutus acutus*. The color of red swamp varies from light olive green to reddish black depending on maturity stage. *P. a. acutus* are sandy white or dark brown color depending on molting stage. *P. a. acutus* chelae are narrower and longer than those *P. clarkii* crayfish. Crayfish are classified according to their feeding habits as herbivores, detritivores, omnivores and sometimes obligate carnivores (Correia, 2005; Nystrom, 2002; Mazlum and Şirin, 2020). They are capable of living in many different habitats in terms of physiological, morphological and behavioral characteristics. Crayfish are found abundant and predominantly among all invertebrates. This organism play an important role in the freshwater food chain by

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feeding on the residues and detritus of thousands of animals, from living and rotten plants, cereals, algae and vertebrates to smaller vertebrates such as small fish species (Lodge et al., 2012; Twardochleb et al., 2013).

P. clarkii and *P. acutus acutus* are cultivated simultaneously in various proportions in Louisiana culture ponds, with the *P. clarkii* dominated catch (Huner and Barr, 1991). However, many ponds were stocked with *P. clarkii* in South Carolina eventually became dominated by *P. acutus acutus* (Mazlum and Eversole, 2000; Mazlum, 2003). Moreover, approximately two years of supplementation, stocking 59-114kg/ha of *P. clarkii* in 1996 and 1997, increased the average proportion of *P. clarkii* in the harvest to only 2% (Eversole et al., 1999). *P. clarkii* was not found in the harvest from these ponds in 2000, 2001 and 2002. *P. clarkii* and *P. acutus acutus* are frequently found in the same habitat and biological requirements are similar between two species (Huner and Barr, 1991; Mazlum and Eversole, 2004, 2005). The red swamp crayfish appears to tolerate warmer water temperatures and poor water quality than white river crayfish. *P. clarkii* is the preferred species in culture because it has a higher fecundity and tolerance to low dissolved oxygen (DO) and great market appeal. *P. acutus acutus* seem to prefer cooler water temperatures and flowing water where oxygen levels are higher (Mazlum and Eversole, 2004, 2005; Mazlum et al., 2021).

Competitive interactions between two (or more) co-occurring crayfish species when they highly overlap ecological niches and use similar resources can have long-term adverse effects on species (Söderback, 1991; Gherardi, 2002). Our results clearly demonstrate the competitive advantage of *P. acutus acutus* over *P. clarkii*. Gherardi (2004) reported that larger chelae crayfish species provide more fight-winning advantages over similar length and shorter chelae species. Our previous results indicated that *P. acutus acutus* had longer chelae and longer hatchlings than *P. clarkii*, but this difference was not significant (Eversole and Mazlum, 2002; Eversole et al., 2006) for proving that size confers advantages in conspecific interactions. Several studies have been designed to better understanding *P. clarkii* and *P. acutus acutus* interactions. The aim of this was to determine the effects of competitive interactions on the growth and survival of *P. clarkii* and *P. acutus acutus*.

Material and Methods

The experiment was conducted in 18 rectangular

plastic 57 L tanks located at the Aquaculture Research Facility, Clemson University, Clemson, South Carolina. Prior to experiment, the eighteen tanks were planted with rice (Figure 1). Four *P. clarkii* and *P. acutus acutus* of the same size were stocked separately for intraspecific treatments or in combination in interspecific-treatment with two individuals of each species. Three treatment levels were used in the experiment, with six replicates tanks each (representing a stocking density of approximately 21 individuals per m²) with stand green rice. Juvenile crayfish were randomly placed to each of eighteen trial tanks (n=4).



Figure 1. View of experimental tanks from top indicating the position of the standpipe. Rice stand was not fully grown at the time of the photograph.

Flowing pond water with a flow rate of approximately 10 L/h was used in the present study. Dissolved oxygen and temperature were determined daily in the early morning with a 55 YSI oxygen meter. Crayfish were held at 12L:12D cycle. Crayfish were fed daily ad libitum in a 5 cm glass petri dish placed at the bottom of the tank. Uneaten feed was removed from the tank before the next feeding. We measured total length (length from the tip of rostrum to telson, TL) to the nearest millimeter using a digital caliper at the beginning of the experiment and then crayfish were measured monthly interval to determine growth over the 3 month study.

At the end of the experiment, crayfish were counted and measured individually. Growth was evaluated by change in total length (TL) over 90 days and survival (%) calculated according the following equations:

$$\text{Survival (\%)} = (\text{final number of crayfish} / \text{initial number of crayfish}) \times 100.$$

All data were analyzed by using SPSS software (Version 16.0; SPSS; Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare mean differences growth and survival between treatments. A post hoc Duncan's multiple range

test was used to test for differences between the treatments. Differences were considered significant at the 95% confidence level. All means were presented with \pm standard deviation (SD).

Results

Water temperatures and dissolved oxygen (DO) during the experiment were within acceptable ranges for cambarid crayfish growth (Huner, 1990; Mazlum and Eversole, 2005). There were no differences between the treatments for the mean water temperature of 18.2°C and the mean DO of 5.8 mg/L during the study. The rice feed was decayed and consumed for crayfish throughout the experiment so that very little vegetation and the noxious matter remained in the tanks at the end of the study.

The mean starting of sizes of the two species was similar in both competition treatments. At the end of the experiment, the growth of *P. a. acutus* was found to be significant between treatments (Table 1). *P. a. acutus* which grew significantly faster in the interspecific-treatment tanks than in the intraspecific-treatment tanks. It was observed that no difference was detected in the growth between the treatments for *P. clarkii* (Table 1). After 90 days, the growth rate in the inter specific-treatment tanks was 76.2 \pm 10.3 mm TL in compared to 54.6 \pm 8.6 mm TL in the intraspecific-treatment tanks.

Table 1. Mean and standard deviation of the total length (TL) of *P. a. acutus* and *P. clarkii* in the intraspecific- and interspecific-treatment tanks.

Sample Days	<i>P. a. acutus</i> TL (mm)		<i>P. clarkii</i> TL (mm)	
	Intraspecific	Interspecific	Intraspecific	Interspecific
30 days	24.9 \pm 0.4 ^a	25.2 \pm 0.4 ^a	24.3 \pm 0.5 ^a	24.0 \pm 0.5 ^a
60 days	50.6 \pm 7.2 ^a	54.0 \pm 5.8 ^a	53.0 \pm 7.8 ^a	55.6 \pm 5.5 ^a
90 days	54.6 \pm 8.6 ^a	76.2 \pm 10.3 ^b	63.1 \pm 9.5 ^a	62.6 \pm 4.3 ^a

Different superscript letters in each line represent significant differences ($P < 0.05$).

At the 90 days, survival of the both species was lower in the interspecific-treatment tanks (50.0%) than in the intraspecific-treatment (26.7%) tanks. *P. clarkii* survival in the interspecific-treatment tanks (26.7%) was also lower than in the intraspecific tanks (55.0%) (Table 2). *P. acutus acutus* survived at a similar rate in the interspecific-treatment tanks (50.0%) and intraspecific-treatment tanks (63.7%) whereas *P. a. acutus* survival was similar in the two treatments.

Table 2. Mean and standard deviation of the survival (%) of *P. a. acutus* and *P. clarkii* in intraspecific and interspecific-treatment tanks.

Sample Days	<i>P. acutus acutus</i> Survival (%)		<i>P. clarkii</i> Survival (%)	
	Intraspecific	Interspecific	Intraspecific	Interspecific
30 days	24.9 \pm 0.4 ^a	25.2 \pm 0.4 ^a	24.3 \pm 0.5 ^a	24.0 \pm 0.5 ^a
60 days	50.6 \pm 7.2 ^a	54.0 \pm 5.8 ^a	53.0 \pm 7.8 ^a	55.6 \pm 5.5 ^a
90 days	54.6 \pm 8.6 ^a	76.2 \pm 10.3 ^b	63.1 \pm 9.5 ^a	62.6 \pm 4.3 ^a

Means within a species that share different superscripts are significantly different ($P < 0.05$).

Discussion

Sympatric crayfish species compete for food resources (Bulter and Stein, 1985), and aggression and competition for these food sources play an important role in crayfish interactions (Soderback, 1991). In this study, it was revealed that the competition between the two species is more intense than the competition between the species. For example, the survival of two species in the interspecific treatment tanks was lower than in the intraspecific treatment tanks. The growth (TL) of both species was found to be higher in the interspecies treatment than in the intraspecies treatment, which supports previous studies (Kozak et al., 2007). Compared to interspecific pairs, individuals of *P. acutus acutus* initiated and gained greater numbers of interactions. Because of this, he was able to dominate agonistic interactions against his opponents without getting into more or longer fights and thus getting into more fights. While these values did not change for *P. clarkii*, the unilateral results of the fights reveal the competitive advantage of *P. acutus acutus* as *P. clarkii* individuals did not win the competitions. As in other similar studies, crayfish length, weight and claw length had effects on dominance, and as a result, the dominance of *P. acutus acutus* over *P. clarkii* was determined by (Eversole et al., 2006; Fero et al., 2007; Martin and Moore, 2007; Mazlum and Eversole, 2005). Eversole et al. (2006) reported that the aggressive behavior of *P. acutus acutus* individuals is the most important factor that provides an important advantage and determines the outcome. It is true that where aggressive behavior leads to death, competing crayfish have the potential to grow faster depending on the feed source. Therefore, the highest growth is expected to occur in tanks with the highest mortality (ie, interspecies tanks) (Table 2). This indicates that larger crayfish continue competitively over smaller crayfish (Momot, 1984). In environments with low stocking density, mortality is higher in tanks with high stocking (McClain, 1995). Eversole et

al. (2006) indicated that male *P. a. acutus* had significantly longer chelae than male *P. clarkii* while female *P. a. acutus* chelae were longer than female *P. clarkii*. They suggested that the body size of *P. a. acutus* was important in defining the interaction between the two species. This situation has been noted in previous studies (Eversole et al., 2006; Mazlum et al., 2007). This may be one reason why the survival of red swamp crayfish in interspecies tanks is lower than in intraspecies tanks for white river crayfish. Eversole et al. (1999) showed in their previous studies that the percentage of *P. clarkii* in the harvest of these two species decreased gradually from 97% in 1991 to 45%, 33%, 4% and 1% in 1992, 1993, 1994 and 1995, respectively. Considering the previous results, it is thought that body size, fecundity and egg diameter are effective as well as abiotic and biotic factors in the decrease of *P. clarkii* in culture population (Eversole and Mazlum, 2002).

Conclusion

P. a. acutus achieved higher survival, faster growth and larger size than *P. clarkii* when grown in the tank study. The size of the body was significant in describing competitive interactions between two species.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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Biological traits of a data deficient species in the Asi River: *Barbus lorteti* (Sauvage, 1882)

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Keywords

Population dynamics
Freshwater fish
Growth parameters

ABSTRACT

This study was conducted using 201 *Barbus lorteti* (Sauvage, 1882) individuals, obtained in 1997 from Asi River. In this study, the length-weight and age-weight parameters were estimated using the von Bertalanffy growth equation. The total length for the individuals was varied between 7.4 and 31.0 cm with an average of 18.37 cm. The distribution of weight was fixed as 3.8 and 274.9 g and 82.57 ± 4.3 g in average. The length-weight relationship was estimated as $W = 0.013 * L^{2.97}$. In the age based scale readings-of the individuals in the sampling the age classes were ranging between I-IV. For this species, von Bertalanffy equation in length growth were estimated as $L_t = 28.45 [1 - e^{-0.43(t+(-0.23))}]$, and the equation in weight growth was estimated as $W_t = 382.76 * [1 - e^{-0.34(t+(-0.19))}]^{2.97}$. The growth performance index (Φ) and Condition factor was estimated as 2.56 and 1.11, respectively.

Introduction

Turkey, with its special geographical location and freshwater potential, contains many endemic and cosmopolitan fish species. (Innal and Erk'akan, 2006). Approximately 387 freshwater fish species were determined as endemic (Froese and Pauly, 2010).

Barbus lorteti is an endemic species, belonging to the family of Cyprinidae, mainly distributed in the lower parts of Asi River from Syria to Turkey. IUCN assessed this species as data deficient (DD) because of the absence of reliable data on distribution, abundance, trend and threat of this species (Crivelli, 2018). The population of this species has decreased after collection of the specimens, and various attempts to collect them species is wasted in last decade. The population of this species has decreased dramatically in the last 25 years (Karataş et al., 2021). The changes and deteriorations occurred in the freshwater ecosystems, emerged more devastatingly such

as the extinction of the biological diversity (Sala et al., 2000). The excessive use of the water, agricultural irrigation and extreme low tide as a result of dam and set applications change the habitat characteristics of the river (Crivelli, 1995). It is asserted that destruction in Asi River caused the deterioration of the species as bringing over exploitation with it. According to the commercial fishing activities, the existence of this species could be mentioned especially as being in the lower parts of Asi River till the years of 1990s (Demirci and Demirci, 2009). After this term, following the negativities (partial water reduction, drying, domestic waste, industrial pollution, agricultural activities and irrigation) occurred in Asi River, no *B. lorteti* individuals were encountered in the main river bed (Yalçın, 1999).

Although little is known about concerning the checklist and systematic features of this species (Çiçek et al., 2018), the little was known about the population structure, habitat and ecological features of this species. Age-length and age-

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weight features belonging to this species were given for the first time. In addition, in this study, the reasons for the deterioration of the *B. lorteti* population are tried to be explained by comparing the biological characteristics of *Barbus luteus* which is another endemic specie still existing in the region (Yalcin et al., 2004; Gokçek and Akyurt, 2008).

Material and Methods

The Asi River, also named as Orontes, was located in both the borders of Syria and Turkey. The river rose from the Bekaa Valley in Lebanon and reached after in Syria. 94 km of its water were located in the borders of Turkey (Yalçın Özdilek et al., 2006; Demirci et al., 2020). In the last set, it flowed into Mediterranean from Samandag (Yalcin, 1999; Demirci et al., 2012; Demirci et al., 2016). There are many fish passages in the Asi River (Demirci et al., 2018). The research area, representing four different capture areas, is shown in Figure 1.

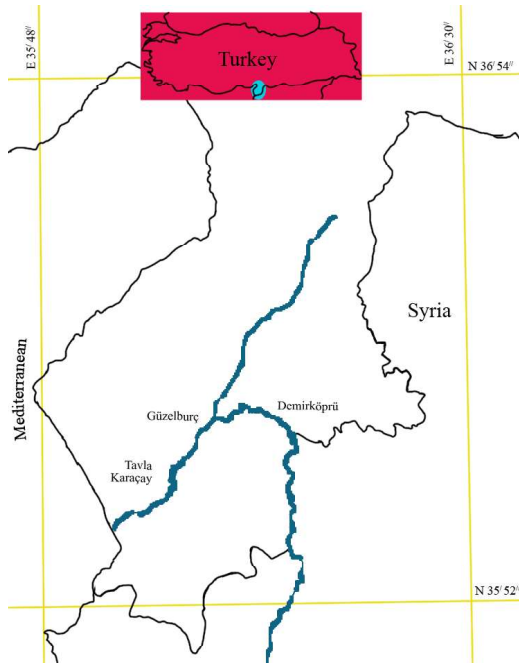


Figure 1. Map of the research area with four different catching (Demirköprü, Güzelburç, Tavla and Karaçay) region.

B. lorteti samples were obtained from the local fishers who threw fyke net, electro-shocker and trammel net. These catching gears were thrown in the evening and then collected in the next morning. 201 individuals were sampled and brought to the laboratory by being fixed in the 4% of formalin solution following the preliminary studies. The length measurements of the fish samples were realized by fish measuring scale with 1 mm interval and the weight measurements were done

with digital scale with 0.1 g. In age determination, scales were used. For this purpose, the scales taken from the left anterior-dorsal region of the fish were washed with distilled water and kept in 3% NaOH solution for 3-6 hours, dipped in 96% ethyl alcohol and kept for 3-6 hours. After washing with distilled water for 30 minutes, it was dried and fixed between two slides and became ready for examination (Lagler, 1966). These preparations were aged in a small magnifying binocular microwave. In the estimation of the length-weight relationship, the regression analysis method was used (Ricker, 1975).

$$W = a L^b$$

Here the 'W' indicated the total weight of the fish in gram; 'L' indicated the total length of the fish in cm, 'a' and 'b' coefficients indicated the regression parameters, which vary according to the species. von Bertalanffy growth equation was estimated by the least squares method based on the lengths and weights observed in every age groups (Beverton and Holt, 1957).

$$L_t = L_{\infty} * [1 - e^{-K(t-t_0)}]$$

$$W_t = W_{\infty} * [1 - e^{-K(t-t_0)}]^b$$

The " L_t ", the fish length in t age, " L_{∞} ", the asymptotic length based on the growth gradient in fish, " K^{-1} " the growth coefficient in length and the " t_0 " the hypothetical age, as based upon the weight is zero. Similarly " W_t ", the fish weight in t age, " W_{∞} ", the asymptotic weight based on the growth gradient in fish, " K^{-1} " indicates the growth coefficient in weight and the " t_0 " indicates the hypothetical age, as based upon the weight is zero.

The growth performance index used to compare the growth rate in fish was fixed as taking advantage of the formula of $\Phi' = \log K^{-1} + 2 \log L_{\infty}$ (Sparre and Venema, 1998). In this equation; Φ = shows the growth performance index, L_{∞} = asymptotic length (cm), K^{-1} = Brody's growth coefficient (year^{-1}).

Fulton's Condition Factor was estimated and the arithmetical average of these were taken. The Fulton Condition Factor was stated in the below formula (Holden and Raitt, 1974).

$$K = (W * 100) / L^b$$

As the species has currently not observed in nature, the genetic analyses provided that the samples obtained in the research belonged to the species of *B. lorteti*. In the genetic structure analysis, from the preferred molecular methods,

the mtDNA-RFLP analysis method was used (Watanabe, 2018).

Results

The age distribution of 201 *B. lorteti* species which were obtained from four different regions of Asi River was ranged between I-VI. The majority of the population in the region was constituted by the individuals in the age intervals of II and III (73 and 65). Respectively, the number of species and age; there were 43 items of *B. lorteti* samples in I. age, 14 of them in IV. age, 6 of them in V. age, 4 of them in VI. age. The number of individuals based on the ages were shown in Table 1.

Table1. Age- Length size distributions of *Barbus lorteti* from Asi River.

Length Class (cm)	Age Groups (year)						Total
	I	II	III	IV	V	VI	
8	2						2
10	16						16
12	19	13					32
14	6	6	5				17
16		23	12				35
18		11	11				22
20		15	8	1			24
22		5	11	2			18
24			14	10	2		26
26				1	3	2	6
28						2	2
30					1		1
Total	43	73	61	14	6	4	201
Mean	12.37	17.62	20.67	24.14	27.00	28.35	18.37
Standard Deviation	1.52	2.97	3.20	1.36	1.82	0.74	4.80

It was determined that the length distribution of the samples was between 7.4 and 31.0 cm. When all data are taken into account, the average total length of this population starting from the age of I to VI, were found to be 12.37 cm, 17.62 cm, 20.67 cm, 24.14 cm, 24.55 cm, and 28.35 cm, respectively. When the length distribution of the sampled population was examined, the majority of the stock was constituted by the individuals being 17-20 cm total length interval.

The weight distributions of the sampled population by age were found as 109, 19.90 g, 52.13 g, 117.69 g, 175.69 g, 203.55 g starting from the first age to last age, respectively. The weight of VI age group was found to be 268.95 g in average (Table 2).

According to the measured weights, the minimum and the maximum values were measured as respectively 3.8 and 299.0 g, respectively.

Table 2. Weight distributions of *Barbus lorteti* from Asi River.

Weight Class (g)	Age Groups (year)						Total
	I	II	III	IV	V	VI	
25	36	1					37
50	7	33					40
75		34	2				36
100		5	17				22
125			21				21
150			11				11
175			6	11	1		18
200			3	1			4
225			1	1	4		6
250				1	1	1	3
275						2	2
300						1	1
Total	43	73	61	14	6	4	201
Means	19.90	52.13	117.69	175.69	203.55	268.95	82.57
Standard Deviation	5.21	14.15	31.65	22.69	17.69	21.58	60.99

The mean weight value of the population was estimated as 82.57 g. According to the 201 items obtained from the Asi River *B. lorteti* population, the total length (cm) and weight (g) regression parameters were determined. At the end of this regression analysis, the relationship was found as $W=0.013 L^{2.94}$. According to the b coefficient estimated in this formula, the weight and the length regarding this species revealed an isometric increase (Figure 2).

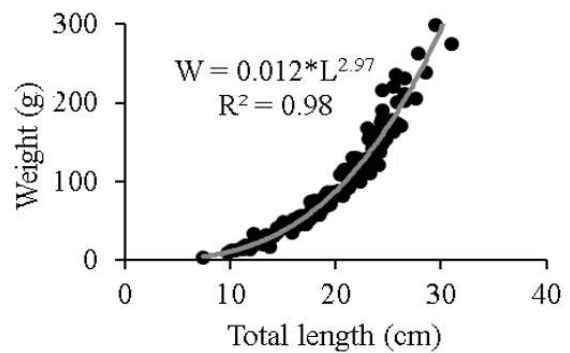


Figure 2. The relationship between length and weight of *Barbus lorteti* from the Asi River.

According to the von Bertalanffy equation, the growth model of the mentioned population in age and length was found as: $L_t=28.45*[1-e^{-0.43(t+(-0.23))}]$ (Figure 3). The growth performance index for the fish species was estimated as $\Phi=2.56$. Likewise, the growth model in weight estimated in accordance to von Bertalanffy equation was fixed as $W_t= 501.04 [1-e^{-0.22(t+(-1.05)]}2.97$ (Figure 4).

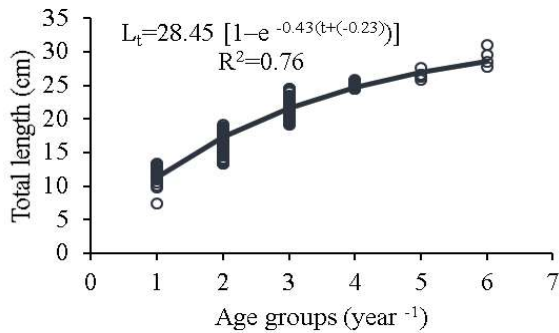


Figure 3. Length size and age von-Bertalanfy growth parameter of *Barbus lorteti* from Asi River.

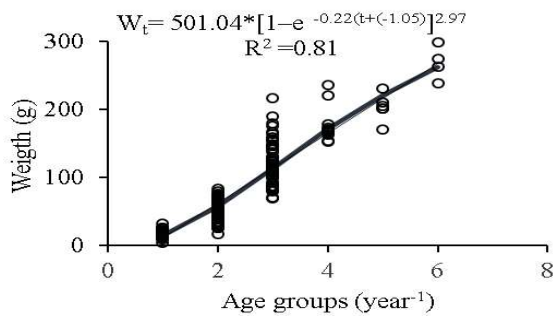


Figure 4. Age and weight von-Bertalanfy growth parameter of *Barbus lorteti* from Asi River.

The condition factor estimated from the *B. lorteti* population is shown in Figure 5. While the mean condition factor was estimated as 1.11 the maximum and the minimum condition factor was found as 1.82 and 0.68, respectively.

Statistical difference between the measured length and weight values were investigated by Khi Square (X^2) Test.

Discussion

In this study, biology of the *B. lorteti*, the reasons of the stock's precipitation in Asi River where their population were about to dissappear were tried to be determined. On the other hand the *B. luteus* population, which was sampled in Asi River in a similar way in the same and near period, was continuing its stock (Yalçın Ozdilek et al., 2004). In this context, it would be beneficial to discuss some of the biological and population features of these two species in this part of the article to make comparison. Also, as there was not adequate information concerning the age and length features of *B. lorteti*, the comparisons were made referring to *B. luteus*, which lived in the same river ecosystem and was caught in the same period.

In the *B. lorteti* population in Asi River, individuals

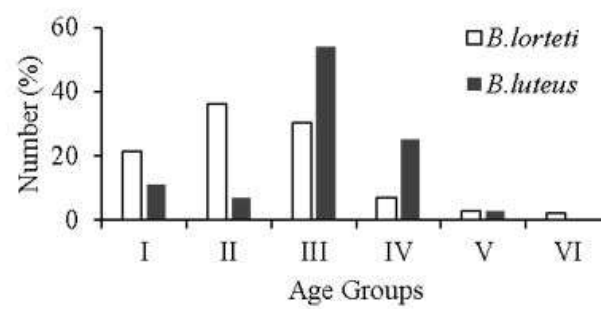


Figure 5. The comparison of condition factor as per the ages of *Barbus lorteti* and *Barbus luteus* in the Asi River (Gökçek and Akyurt 2008).

were encountered between the ages of I-VI. In the same period, especially in the research that was made by Yalçın Ozdilek et al. (2004), the age distribution of the *B. luteus* population, changed between the ages of I-V. Gokçek and Akyurt (2008) encountered to *B. luteus* individuals up to the 9 age group. In this case, when the environmental conditions were convenient, they showed a longer length of time compared to *B. lorteti*. As this species involved in the group living long, they continued its population density in our day. As in both of the studies one group of individuals were more dominant which was age 3 for *B. luteus* and age 2 for *B. lorteti*. Mean individual sizes in age groups were higher in length and weight for *B. lorteti*. Especially the individual weights as per the age, was nearly twice as much compared to the *B. luteus*. This comparison was presented in Figure 5. When the growth features of both species were compared, no significant difference was noticed. However, Φ prime index of *B. lorteti* (2.56) was found be lower than *B. luteus* (2.65).

In this context, though the age distribution of the *B. lorteti* population was low in the Asi River environment which had negative habitat conditions during the process of time. It was thought that it entered a deterioration process as giving negative reaction due to its relatively high weight. As an example to negative conditions, reduction of the average rainfall in Asi River and the increase of the agricultural irrigation in summer months, could be given. As the reduction in water level made catching in this region easy, it was thought that the population might have limited the age composition (Yalcin, 1997).

In the samples done throughout one year, the individuals in the length group of 7.4 and 31.0 cm, were caught by using the fyke net, electroshocker, trammel net and throw net. Yalcin Ozdilek et al. (2004) obtained the *B. lorteti* in the length group

of 5.1 and 24.7 cm in the same region with fyke net, electroshocker and a 17*17-30*30 mm of throw nets. Gokçek and Akyurt (2008) caught the individuals in the length group of 7.5 and 38.4 cm, with 12 -34 mm of gillnet, 12- 22 mm of cast net. In *B. lorteti* population, no 0 age group individuals were encountered. It was thought that this case was resulted from the catching tool selectivity occurred basing on the mesh openness in the fyke and trammel net. In both of the studies made in the same region in different times, this case was observed in *B. luteus* population, as well.

In the length-weight relationship which was formed by *B. lorteti* individuals in Asi River, *b* value was found as 2.97. Yalcin Ozdilek et al. (2004) estimated the *b* value as 3.08 in the same river system. Gokcek and Akyurt (2008) found *b* value as 2.97 for female and 3.0 for male individuals seperately. It could be said that when the *b* value was taken into consideration, the *B. lorteti* individuals were achieved isometric growth in Asi River. The sample population obtained from Asi River, was fixed to be ranged between 3.8 g and 299.0 g.

Yalcin Ozdilek et al. (2004) found that the *B. luteus* population in this region varied between 2.1 g and 187 g. In *B. lorteti* population, L_{∞} value was found as 28.45 cm. In *B. luteus* population which was caught from the same environment, L_{∞} value was found as 25.89 cm by Yalcin Ozdilek et al. (2004), 38.77 cm for female and 40.32 cm for male individuals by Gokçek and Akyurt (2008). When the K^{-1} values were examined in the length growth, it was found as 0.43 in *B. lorteti* population; while in *B. luteus* population the values of 0.23, 0.30 were found.

W_{∞} value in the *B. lorteti* population was determined as 501.04 g. *B. luteus* individuals revealed the 318.53 g W_{∞} value (Yalcin Ozdilek et al., 2004). Gokçek and Akyurt (2008) were found *B. luteus* W_{∞} value as 750.40 g. When the condition factor was examined as per the ages, it was seen that there was significant difference (Figure 5).

Studying the population dynamic parameters of *B. lorteti* living in Asi River, has provided the exposure of the growth features. Moreover this may contribute to the explanation of the deterioration of this species. Especially, the determination of growth and other biological features of the species living in this type of river systems, could be beneficial for both the sustainability of the natural populations and the fishing management studies. This species might have been subjected to over

catching in that period unlike from *B. luteus*. As it is a hardly encounter species, it is difficult to get information about the minimum legal catching size due to its first reproduction size. Particularly, determining the growth and other biological features of the populations which faced with the danger of extinction, would be beneficial in respect to take the protective measures of the stock.

Conclusion

As a result, this study is very important as it was the first data giving the growth features of the rarely encountered species while determining the history of the river ecosystem at the same time. In addition, this paper confirms that the population of *B. lorteti* is rarely encountered species in the Asi River and this species might be represented by small isolated populations or really extinct in the river. Asi River have ben exposed to various anthropogenic threats such as pollution, water restrictions, barriers, invasives, overexploitation etc. (Yalçın Özdilek et al., 2004). These adverse conditions might be responsible for decreasing populations not only for *B. lorteti*, but also for decreasing other endemic/native fish species. We suggest further detailed survey for determining living populations along the river and its tributaries. We also suggest to be arranged an effective management plan for sustainable use of freshwater fish species for river basin.

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COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions

Authors contributed equally to this paper.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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