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İÇİNDEKİLER / CONTENTS

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Glutatyon ile İlişkili Enzim Sistemleri Kullanılarak *Oreochromis niloticus*'ta Cıva Toksisitesi Üzerine Antioksidan Olarak Selenyum ve Mineral Olarak Zeolitin Koruyucu Etkilerinin Araştırılması

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Özet

Cıva yeryüzündeki en toksik ağır metallerden biridir. Selenyum canlılar için gerekli olan ve antioksidan özellikleri de bulunan bir elementtir. Zeolit ise sucul ortamlarda ağır metallerin uzaklaştırılmasında yaygın bir şekilde kullanılan bir mineraldir. Bu araştırmada *Oreochromis niloticus*'un dokularındaki glutatyon (GSH) ve GSH ile ilişkili enzim sistemleri üzerine cıvanın toksik etkileri ve bu biyokimyasal toksisite üzerine selenyumun ve zeolitin olası koruyucu etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla balıklar 0,01 ve 0,1 mg/L cıva; 0,01 mg/L cıva+0,1 mg/L selenyum, 0,1 mg/L cıva+1,0 mg/L selenyum ve 0,01 mg/L cıva+ 0,1 g/L zeolit, 0,1 mg/L cıva+1,0 g/L zeolit derişimlerinin etkisine 7 ve 21 gün süreler ile bırakılmış ve solungaç, karaciğer ve kas dokularındaki GSH düzeyi ve glutatyon peroksidaz (GPx), glutatyon-S-transferaz (GST) ve glutatyon redüktaz (GR) enzim aktiviteleri belirlenmiştir. Cıvanın tek başına ve cıva+selenyum ve cıva+zeolit karşımlarının etkisinde incelenen tüm biyokimyasal parametrelerde dokulara, ortam derişimlerine ve etki süresine bağlı olarak önemli değişimler saptanmıştır. Solungaç ve karaciğerde GSH düzeyi ve GR aktivitesi azalma, GPx ve GST aktiviteleri ise artış göstermiştir. Kasta ise GST dışındaki parametrelerde önemli bir değişim gözlenmemiştir. İncelenen tüm parametreler üzerine tek başına cıva etkisinin selenyum ve zeolit ile birlikte etkisine göre daha yüksek ve kimyasalların etkilerinin genel olarak Hg>Hg+Zeolit>Hg+Se şeklinde olduğu saptanmıştır. Araştırma sonuçlarımız *O. niloticus*'ta cıvanın neden olduğu toksisite üzerine selenyum ve zeolitin koruyucu bir etkiye sahip ve selenyumun zeolite oranla koruyuculuk etkisinin biraz daha fazla olduğunu göstermektedir.

Anahtar Kelimeler: *Oreochromis niloticus*, Cıva, Selenyum, Zeolit, Glutatyon

Investigation of Protective Effects of Selenium as Antioxidant and Zeolite as Mineral on Mercury Toxicity in *Oreochromis niloticus* Using Glutathione-Related Enzyme Systems

Abstract

Mercury is one of the most toxic heavy metals on earth. Selenium is an element that is essential for living things and has antioxidant properties. Zeolite is a mineral commonly used in the removal of heavy metals in aquatic environments. In this study, it was aimed to determine the toxic effects of mercury on glutathione (GSH) and GSH-related enzyme systems and the possible protective effects of selenium and zeolite on this biochemical toxicity in tissues of *Oreochromis niloticus*. For this purpose fish were exposed to 0.01 and 0.1 mg/L mercury; 0.01 mg/L mercury+0.1 mg/L selenium, 0.1 mg/L mercury+1.0 mg/L selenium and 0.01 mg/L mercury+ 0.1 g/L zeolite, 0.1 mg/L mercury+1.0 g/L zeolite for 7 and 21 days and and GSH level and activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) in gill, liver and muscle tissues were determined. Significant alterations in GSH level and GSH-related enzymes activities in the exposure of Hg alone, Hg+Se, and Hg+zeolite mixtures were observed due to tissues, medium concentrations, and exposure period. In the gill and liver tissues, GSH level and GR activity reduced while GPx and GST activities increased. In the muscle, it was not observed a significant change in other parameters except for the GST. The effect of Hg alone on analyzed all parameters were higher than in combination with Se and zeolite and the order of their effects found Hg>Hg+zeolite>Hg+Se. Our research results show that selenium and zeolite have a protective effect on the toxicity caused by mercury in *O. niloticus* and that selenium has a slightly more protective effect than zeolite.

Keywords: *Oreochromis niloticus*, Mercury, Selenium, Zeolite, Glutathione

GİRİŞ

Endüstriyel ve tarımsal faaliyetlerin artmasına bağlı olarak toksik ağır metallerin akuatik ortamlardaki konsantrasyonları da artış göstermektedir (Fırat vd, 2018). Cıva (Hg) organizmada herhangi bir biyolojik rolü bulunmayan ve çok düşük düzeylerde bile toksik etkisini gösterebilen oldukça tehlikeli bir metaldir. Hg tüm canlılar üzerine oldukça zararlı etkileri olan küresel bir kirletici olarak kabul edilmektedir (ATSDR, 2020). Küresel bir sorun olmasının ana nedenleri biyolojik bulunurluğu ve biyobirikiminin yüksek, lipofilik karakterli, hücresel toksisiteye sahip, vücutta parçalanamaması ve uzaklaştırılmasının zor olmasından kaynaklanmaktadır (Yang vd., 2010; Fırat ve Kaya, 2019).

Selenyum (Se) selenite, selenate, selenomethionin ve selenosistein gibi çeşitli biçimlerde bulunmakta ve glutatyon peroksidaz, iodothyronin 5'-deiosinaz ve thioredoksin reduktaz gibi çeşitli enzimlerin yapısında yer aldığından başta insan olmak üzere birçok canlı organizma için önemli bir element olarak düşünülmektedir (Su vd., 2008). Se insanlarda ve diğer hayvan türlerinde birçok biyolojik fonksiyona sahiptir. Se'nin bazı önemli biyolojik aktiviteleri şunlardır: organizmaların normal büyüme ve gelişimleri için bu elementin iz derişimlerine gereksinim duyulmaktadır (1), homeostatik işlevlerin korunmasında ve sürdürülebilirliğinde görevlidir (2), güçlü bir antioksidan ve antikanser özelliğe sahiptir (3), immün sistem için gereklidir (4) (Hamilton, 2004; Cogun vd., 2012).

Zeolitler çevrede düzenleyici etkisi olan minerallerden olup yüksek iyon değiştirme kapasitelerinden dolayı sucul organizmalarda ağır metal toksisitesini önlemek için geniş bir şekilde kullanılmaktadırlar (Papaioannou vd., 2005). Doğal bir mineral olup volkanik küller ve deniz suyu kombinasyonunda binlerce yılda oluşmaktadır (Mishra ve Jain, 2011). Zeolitler üç boyutlu mikro gözenekli yapısı sayesinde oluşan boşluk hacmi toplam hacmin yaklaşık %50'sine kadar tekabül edebilmekte ve boşluğun yapısına ve büyüklüğüne bağlı olarak Cs^{+}, Rb^{+}, NH_4^{+}, Sr^{+}, Mg^{+2}, Na^{+}, Al^{+3}, Ca^{+2}, Fe^{+2}, Hg^{+2}, Cd^{+2}, Pb^{+2} gibi element ve molekülleri seçici şekilde boşluklarında tutabilmektedirler (Mishra ve Jain, 2011).

Çoğu çevresel kirleticiler ve onların metabolitleri akuatik organizmalarda oksidatif stres oluşturma yeteneğindedir. Metaller, balıklarda hidrojen peroksit, hidroksil ve süperoksit radikalleri gibi serbest oksijen türlerini (ROT) oluşturarak oksidatif strese neden olabilmektedirler (Fırat vd. 2009). Balıklarda kirleticilere karşı oluşan antioksidan sistemler önemli savunma mekanizmalarıdır. Antioksidanlar hücresel homeostazinin korunmasında hayati bir rol oynamakta ve bu savunma sisteminin engellenmesi durumunda DNA hasarı, proteinlerin oksidasyonu, enzimlerin inhibisyonu ve lipid peroksidasyonu gibi hücresel hasarlara neden olan oksidatif stres oluşmaktadır. GSH ve GSH ile ilişkili enzim sistemleri, balıklardaki en önemli antioksidan sistemlerden biridir (Zirong ve Shijun, 2007). Bu sistemde, GSH bir kofaktör olarak GST ya da Se bağlı GPx yoluyla peroksitleri uzaklaştırarak, serbest radikallerin enzimatik olmayan redüksiyonu ya non-enzimatik olarak ya da GST aracılığıyla ksenobiyotiklerin konjugasyonu gibi çeşitli yollarla balıkları oksidatif hasara karşı korumaktadır (Zirong ve Shijun, 2007; Fırat vd., 2009).

GSH ve onunla ilişkili enzimler (GPx, GR, GST gibi) hücre içinde koruyucu bir rol oynamaktadır (Elia vd., 2003). Gerçekten de kendiliğinden ya da GST'nin katalizlediği reaksiyonla bir ksenobiyotiğe GSH'ın konjugasyonu ksenobiyotiğin aksiyonunu azaltabilmekte bu moleküllerin suda daha çok çözünmesini sağlayarak hızlı bir şekilde vücuttan atılmasına neden olabilmektedir. GR, GSH'ın rejenarasyonunda GPx ile iş birliği halindedir. Hücrelerde GSH'ın antioksidan rolü konsantrasyonuna, dönüşümüne ve sentezlenme oranına bağlıdır (Fırat vd., 2009). GSH'ın dönüşümü GSH'ın redoks döngüsü ile olmaktadır. Bu süreçte GPx ve GR gibi enzimler önemli rol oynamaktadır (Zirong ve Shijun, 2007).

Balıklar su ortamlarının kirlenmesine çok duyarlı canlılardır. Ağır metallerin etkisindeki balıklarda biyokimyasal parametrelerin analizi hem organizmanın genel sağlık durumu hem de hedef organda oluşturabileceği toksik etkinin belirlenmesinde yararlı olmaktadır. Hg balıklar için en tehlikeli ağır metallerden biridir. Hg'nin bu canlılarda neden olduğu toksik etkileri azaltacak ya da iyileştirecek mekanizmalar hem balıklar hem de birinci dereceden besin kaynaklarını oluşturdukları insanlar için oldukça önemlidir. Bu nedenle sunulan bu çalışmada tatlı su balığı *Oreochromis niloticus*'ta cıvanın toksik etkileri ve bu toksisite üzerine bir antioksidan olarak selenyumun ve bir mineral olarak zeolitin koruyucu/iyileştirici etkileri GSH ve GSH ile ilişkili enzim sistemleri kullanılarak araştırılmıştır.

MATERYAL ve YÖNTEM

Sunulan çalışmada araştırma materyali olarak *Oreochromis niloticus* kullanılmıştır. Balıklar, Çukurova Üniversitesi Su Ürünleri Fakültesi balık yetiştirme havuzlarından alınarak laboratuvara getirilmiş ve içerisinde 120 L bekletilmiş çeşme suyu bulunan 40x140x40 cm ebatlarındaki stok cam akvaryumlarda üç ay süre ile bekletilerek ortam koşullarına adaptasyonları sağlanmıştır. Bu süre içerisinde deneyde kullanılacak balıklar 12,07±0,21 cm boy ve 32,81±0,72 g ağırlığa ulaşmıştır. Deneyler 25±1 ^{o}C sıcaklıkta yürütülmüştür. Günde sekiz saat aydınlanma periyodu uygulanmış ve merkezi havalandırma sistemi ile akvaryumlar havalandırılmıştır. Balıklar, ticari balık yemiyle (Pınar Balık Yemi, Türkiye) beslenmiştir. Denemeler başlamadan iki gün önce yem kesilmiş, denemeler süresince balıklar vücut ağırlıklarının %2'si kadar yem ile günde iki defa beslenmiştir. Deney ortam suyunun fizikokimyasal özellikleri; toplam sertlik 335,7±2,3 mg/L $CaCO_3$, çözünmüş oksijen 7,49±0,15 mg/L, pH 8,21±0,07, sıcaklık 21,11±0,20 ^{o}C olarak ölçülmüştür.

Deneylerde kullanılan cıva çözeltileri 1M cıva klorür [$(HgCl)_2$] (SIGMA) stok çözeltisinden, selenyum çözeltileri 1M sodyum selenit [(Na_2SeO_3)] (SIGMA) stok çözeltisinden ve zeolit çözeltileri (<75 mikron çapında, İstanbul Rota Madencilik AŞ) stok çözeltisinden seri seyreltmeler yöntemi ile hazırlanmıştır. Deneyler cıva, cıva + selenyum ve cıva + zeolit karışımları dikkate alınarak üç seri olacak şekilde yürütülmüştür. Deneyler her bir seride içerisinde 12 adet balık bulunan üç cam akvaryumda yapılmıştır. Balıklar birinci seride cıvanın 0,01 mg/L ve 0,1 mg/L; ikinci seride cıva + selenyumun 0,01 mg/L Hg + 0,1 mg/L Se ve 0,1 mg/L Hg + 1,0 mg/L Se ve üçüncü seride ise cıva + zeolitin 0,01 mg/L Hg+0,1 g/L zeolit ve 0,1 mg/L Hg+1,0 g/L zeolit derişimlerinin etkisine 7 ve 21 gün sürelerle bırakılmıştır. *O. niloticus* için Hg'nin 96 saat-LC_{50} değeri 0,2 mg/L olarak saptanmıştır (Ishikawa vd., 2007). Çalışmamızda test edilen Hg'nin 0,01 ve 0,1 mg/L derişimleri, bu LC_{50} değerinin sırasıyla 1/20 ve 1/2'si baz alınarak subletal konsantrasyonlar olarak seçilmiştir.

Deneylerde birinci seride bulunan 3 akvaryumun ikisine 120 L farklı Hg çözeltileri; ikinci serideki 3 akvaryumun ikisine Hg+Se karışımları; üçüncü serideki 3 akvaryumun ikisine ise Hg+zeolit karışımları konulmuş, her serideki üçüncü akvaryuma ise 120 L dinlenmiş çeşme suyu konarak kontrol grubu olarak kullanılmıştır. Deneyler altı tekrarlı olarak ve her tekrarda bir balık kullanılarak yürütülmüştür. Deney akvaryumlarında kullanılan kimyasal çözeltilerinin derişimlerinde buharlaşma, adsorbsiyon ve akümülasyon gibi nedenlerle değişim olabileceği dikkate alınarak çözeltiler birer gün arayla taze hazırlanan stok çözeltilerden uygun seyreltmeler yapılarak değiştirilmiştir.

Denenen süreler sonunda deney akvaryumlarından rastgele seçilen balıklar, çeşme suyuyla yıkanarak temizlenmiş, yüzeylerinde bulunan su damlacıkları kurutma kağıdıyla alınmış ve boy ve ağırlıkları saptanarak diseksiyona hazır hale getirilmiştir. Balıklar diseksiyondan önce spinal yapılarak öldürülmüştür. Steril aletlerle solungaç, karaciğer ve kas dokuları buz üzerinde disekte edilmiş ve bir kısım dokular biyokimyasal analizler için % 0,59 NaCl ile yıkanarak ağırlıkları alındıktan sonra analize kadar -80'de muhafaza edilmişlerdir. Dokular 1/10 ağırlık/hacim (w/v) olacak şekilde içerisinde 0,25 M sükroz bulunan 0,05 M soğutulmuş Na-P tamponu (pH: 7,4) ile buz içerisinde ultra-turrax homojenizatörde 3 dakika süreyle 10000 rpm'de homojenize edilmiştir. Homojenatlar +4 ^{o}C sıcaklıkta 30 dakika süreyle 10000 rpm'de santrifüj edilmiş ve elde edilen süpernatantlarda GPx, GST ve GR aktivitesi ile GSH ve protein düzeyleri spektrofotometrik yöntemlerle belirlenmiştir. GPx aktivitesi Beutler (1984); GST aktivitesi Habig vd., (1974); GR aktivitesi Carlberg ve Mannervik (1975), GSH düzeyi Beutler (1984) ve toplam protein miktarı ise Lowry vd. (1951) önerdiği yöntemlere göre belirlenmiştir. Dokuların enzim aktiviteleri U/mg protein, GSH düzeyleri ise µmol/g protein olarak hesaplanmıştır.

Elde edilen verilerin istatistiksel analizi IBM SPSS Statistics 21 paket programı kullanılarak One Way-ANOVA ve takiben Student – Newman Keul's Test (SNK) ve student *t* Test kullanılarak yapılmıştır.

BULGULAR

Cıva, cıva+selenyum ve cıva+zeolit karışımlarının denenen tüm ortam derişimlerinde ve her iki etki süresinde *O. niloticus*'un dokularındaki GSH düzeyi ve GSH ile ilişkili enzim aktivitelerindeki değişimler Tablo 1-4'te verilmiştir. Denenen süreler dikkate alındığında GSH düzeyinde hem cıvanın tek başına hem de cıva+selenyum ve cıva+zeolit karışımlarının tüm ortam derişimlerinde solungaçta önemli bir azalma izlenirken (Tablo 1, $P<0,05$), karaciğerde 21. günde düşük cıva ve cıva+selenyum ve cıva+zeolit derişimleri dışındaki diğer derişimlerinde önemli bir azalma saptanmıştır (Tablo 1,

P<0,05). Kas dokusunda ise denenen tüm kimyasalların ortam derişimlerinde önemli bir değişim gözlenmemiştir (Tablo 1, P>0,05). 7 günlük süre sonunda 0,1 mg/L Hg ve 0,1 mg/L Hg +1,0 mg/L Se ve 0,1 mg/L Hg+1,0 g/L zeolit karışımının etkisinde GSH düzeyinde sırasıyla, solungaçta %53, %35 ve %40 ve karaciğerde ise %57, %40 ve %42 düzeyinde azalış görülmüştür. Bu durum, GSH düzeyinde saptanan azalmanın cıvanın selenyum ve zeolitle birlikte etkisine oranla doğrudan cıvanın etkisinde daha fazla olduğunu göstermektedir.

Tablo 1. Cıva (mg/L), cıva (mg/L)+selenyum (mg/L) ve cıva (mg/L)+zeolit (g/L) etkisine bırakılan *O. niloticus*'un dokularında GSH düzeyi (µmol/g protein)

Doku	7 gün	21 gün
Solungaç		
Kontrol	1,69±0,04 ax	1,77±0,03 ax
0,01 Hg	1,02±0,05 bx	1,30±0,02 by
0,01 Hg + 0,1 Se	1,28±0,03 cx	1,25±0,04 bx
0,01 Hg + 0,1 Zeolit	1,10±0,06 bx	1,35±0,02 by
Kontrol	1,69±0,04 ax	1,77±0,03 ax
0,1 Hg	0,80±0,02 bx	1,11±0,04 by
0,1 Hg + 1,0 Se	1,10±0,02 cx	1,19±0,02 bx
0,1 Hg + 1,0 Zeolit	1,01±0,03 cx	1,07±0,06 bx
Karaciğer		
Kontrol	1,29±0,03 ax	1,32±0,04 ax
0,01 Hg	0,69±0,04 bx	1,22±0,07 ay
0,01 Hg + 0,1 Se	0,97±0,02 cx	1,25±0,06 ay
0,01 Hg + 0,1 Zeolit	0,89±0,03 cx	1,28±0,05 ay
Kontrol	1,29±0,03 ax	1,32±0,04 ax
0,1 Hg	0,55±0,03 bx	0,82±0,03 by
0,1 Hg + 1,0 Se	0,78±0,04 cx	1,07±0,02 cy
0,1 Hg + 1,0 Zeolit	0,74±0,02 cx	1,03±0,03 cy
Kas		
Kontrol	1,96±0,05 ax	1,98±0,04 ax
0,01 Hg	1,90±0,07 ax	1,94±0,06 ax
0,01 Hg + 0,1 Se	1,93±0,06 ax	1,92±0,08 ax
0,01 Hg + 0,1 Zeolit	1,97±0,05 ax	1,93±0,06 ax
Kontrol	1,96±0,05 ax	1,98±0,04 ax
0,1 Hg	1,92±0,04 ax	1,93±0,04 ax
0,1 Hg + 1,0 Se	1,91±0,05 ax	1,95±0,07 ax
0,1 Hg + 1,0 Zeolit	1,95±0,03 ax	1,96±0,05 ax

Veriler Aritmetik ortalama±Standart hata şeklinde sunulmuştur (n=6). "a, b ve c" harfleri aynı dokudaki derişimler "x ve y" harfleri ise aynı derişimdeki etkileşim süreleri arasındaki ayırımını göstermek için kullanılmıştır. Farklı harfler, veriler arasındaki istatistiksel ayrım olduğunu göstermektedir (P<0,05).

Belirlenen etki süreleri dikkate alındığında solungaç GPx aktivitesi kontrol grubuyla karşılaştırıldığında 7 günlük süre sonunda 0,01 ve 0,1 mg/L Hg etkisinde artarken (P<0,05), cıva+selenyum ve cıva+zeolit karışımlarının etkisinde 7 ve 21 günlük süreler sonunda önemli bir değişim göstermemiştir (P>0,05) (Tablo 2). Karaciğer GPx aktivitesi her iki cıva ortam derişiminde ve denenen her iki süre sonunda önemli düzeyde artarken, cıva+selenyum karışımlarının yüksek ortam derişiminde, cıva+zeolit karışımlarının her iki derişiminde 7 günlük süre sonunda, arttığı belirlenmiştir (Tablo 2, P<0,05). 7 günlük süre sonunda Hg, Hg+Se ve Hg+zeolit gruplarının yüksek ortam derişiminin etkisinde karaciğer GPx aktivitesinde sırasıyla, %75, %29 ve %35 düzeyinde artış belirlenmiştir. Kas dokusunda ise hem cıvanın doğrudan hem de selenyum ve zeolit ile birlikte etkisinde denenen tüm ortam derişimlerinde anlamlı bir değişim gözlenmemiştir (Tablo 2, P>0.,05).

Tablo 2. Cıva (mg/L), cıva (mg/L)+selenyum (mg/L) ve cıva (mg/L)+zeolit (g/L) etkisine bırakılan *O. niloticus*'un dokularında GPx aktivitesi (U/mg protein)

Doku	7 Gün	21 Gün
Solungaç		
Kontrol	0,55±0,02 ax	0,53±0,04 ax
0,01 Hg	0,68±0,01 bx	0,52±0,03 ay
0,01 Hg + 0,1 Se	0,56±0,02 ax	0,54±0,02 ax
0,01 Hg + 0,1 Zeolit	0,57±0,01 ax	0,51±0,02 ax
Kontrol	0,55±0,02 ax	0,53±0,04 ax
0,1 Hg	0,75±0,03 bx	0,60±0,02 ay
0,1 Hg + 1,0 Se	0,54±0,03 ax	0,57±0,04 ax
0,1 Hg + 1,0 Zeolit	0,53±0,04 ax	0,56±0,05 ax
Karaciğer		
Kontrol	0,48±0,03 ax	0,49±0,02 ax
0,01 Hg	0,72±0,04 bx	0,64±0,02 bx
0,01 Hg + 0,1 Se	0,51±0,02 ax	0,50±0,01 ax
0,01 Hg + 0,1 Zeolit	0,58±0,01 cx	0,63±0,03 bx
Kontrol	0,48±0,03 ax	0,49±0,02 ax
0,1 Hg	0,84±0,03 bx	0,65±0,03 by
0,1 Hg + 1,0 Se	0,62±0,02 cx	0,47±0,03 ay
0,1 Hg + 1,0 Zeolit	0,65±0,01 cx	0,50±0,02 ay
Kas		
Kontrol	0,22±0,03 ax	0,21±0,02 ax
0,01 Hg	0,20±0,02 ax	0,21±0,03 ax
0,01 Hg + 0,1 Se	0,21±0,04 ax	0,22±0,02 ax
0,01 Hg + 0,1 Zeolit	0,20±0,04 ax	0,20±0,03 ax
Kontrol	0,22±0,03 ax	0,21±0,02 ax
0,1 Hg	0,21±0,04 ax	0,22±0,02 ax
0,1 Hg + 1,0 Se	0,21±0,02 ax	0,23±0,03 ax
0,1 Hg + 1,0 Zeolit	0,22±0,02 ax	0,20±0,03 ax

Veriler Aritmetik ortalama±Standart hata şeklinde sunulmuştur (n=6). "a, b ve c" harfleri aynı dokudaki derişimler "x ve y" harfleri ise aynı derişimdeki etkileşim süreleri arasındaki ayırımını göstermek için kullanılmıştır. Farklı harfler, veriler arasındaki istatistiksel ayrım olduğunu göstermektedir (P<0,05).

Her iki etkileşim süresi dikkate alındığında solungaç GST aktivitesi kontrol grubuyla karşılaştırıldığında 7 günlük süre sonunda tek başına cıva ile cıva+selenyum ve cıva+zeolit karışımlarının düşük ve yüksek ortam derişimlerinin etkisinde artmıştır (Tablo 3, P<0,05). Bununla birlikte 21 günlük süre sonunda 0,1 mg/L Hg ortam derişimi hariç denenen tüm ortam derişimlerinde solungaç GST aktivitesinde önemli bir değişim gözlenmemiştir (P>0,05). Karaciğer GST aktivitesi düşük ve yüksek ortam derişimlerinde cıvanın doğrudan etkisinde 7 ve 21 günlük süreler sonunda, cıva+selenyum ve cıva+zeolit karışımlarında ise 7 günlük süre sonunda önemli bir artış göstermiştir (Tablo 3, P<0,05). Kas GST aktivitesinde ise sadece 7 günlük sürenin sonunda denenen tüm kimyasal gruplarının yüksek ortam derişimlerinde önemli bir artış saptanmıştır (Tablo 3, P<0,05). 7 günlük süre sonunda Hg, Hg+Se ve Hg+zeolit gruplarının yüksek ortam derişimlerinin etkisinde GST aktivitesi sırasıyla, solungaçta %64, %35 ve %34 ile karaciğerde %61, %29 ve %36 düzeyinde artış göstermiştir.

Tablo 3. Cıva (mg/L), cıva (mg/L)+selenyum (mg/L) ve cıva (mg/L)+zeolit (g/L) etkisine bırakılan *O. niloticus*'un dokularında GST aktivitesi (U/mg protein)

Doku	7 Gün	21 Gün
Solungaç		
Kontrol	12,03±0,21 ax	12,19±0,17 ax
0,01 Hg	15,26±0,33 bx	12,56±0,24 ay
0,01 Hg + 0,1 Se	14,90±0,29 bx	11,95±0,38 ay
0,01 Hg + 0,1 Zeolit	15,11±0,43 bx	12,87±0,65 ay
Kontrol	12,03±0,21 ax	12,19±0,17 ax
0,1 Hg	19,71±0,42 bx	15,81±0,41 by
0,1 Hg + 1,0 Se	16,29±0,19 cx	12,93±0,19 ay
0,1 Hg + 1,0 Zeolit	16,17±0,24 cx	11,73±0,57 ay
Karaciğer		
Kontrol	22,15±0,44 ax	23,48±0,57 ax
0,01 Hg	28,45±0,20 bx	27,24±0,15 bx
0,01 Hg + 0,1 Se	25,19±0,31 cx	22,85±0,78 ax
0,01 Hg + 0,1 Zeolit	25,05±0,21 cx	24,11±0,48 ax
Kontrol	22,15±0,44 ax	23,48±0,57 ax
0,1 Hg	35,72±0,68 bx	30,18±0,34 bx
0,1 Hg + 1,0 Se	28,63±0,54 cx	24,09±0,17 ax
0,1 Hg + 1,0 Zeolit	30,10±0,32 cx	26,29±0,15 cx
Kas		
Kontrol	4,17±0,05 ax	3,98±0,07 ax
0,01 Hg	4,22±0,07 ax	3,83±0,09 ax
0,01 Hg + 0,1 Se	4,20±0,06 ax	3,90±0,05 ax
0,01 Hg + 0,1 Zeolit	4,25±0,08 ax	3,87±0,06 ax
Kontrol	4,17±0,05 ax	3,98±0,07 ax
0,1 Hg	4,81±0,04 bx	4,02±0,06 ay
0,1 Hg + 1,0 Se	4,59±0,03 bx	3,89±0,07 ay
0,1 Hg + 1,0 Zeolit	4,72±0,07 bx	3,92±0,09 ay

Veriler Aritmetik ortalama±Standart hata şeklinde sunulmuştur (n=6). "a, b ve c" harfleri aynı dokudaki derişimler "x ve y" harfleri ise aynı derişimdeki etkileşim süreleri arasındaki ayırımını göstermek için kullanılmıştır. Farklı harfler, veriler arasındaki istatistiksel ayrım olduğunu göstermektedir (P<0,05).

Kontrol grubuyla karşılaştırıldığında 7 günlük süre sonunda solungaç ve karaciğer GR aktiviteleri cıvanın doğrudan ve selenyum ve zeolit ile birlikte etkisinde düşük ve yüksek ortam derişimlerinin etkisinde azaldığı (P<0,05) ve bu azalışın yüksek ortam derişimlerinde cıvanın tek başına etkisinde daha fazla olduğu belirlenmiştir (P<0,05) (Tablo 4). 21 günlük süre sonunda ise 0,1 mg/L Hg etkisinde solungaç ve karaciğer dokularında GR aktivitesindeki azalış (P<0,05) dışında denenen diğer grupların ortam derişimlerinde önemli bir değişim gözlenmemiştir (Tablo 4, P>0,05). 7. gün sonunda 0,1 mg/L Hg, 0,1 mg/L Hg+1,0 mg/L Se ve ve 0,1 mg/L Hg+1,0 mg/L zeolit gruplarının etkisinde GR aktivitesi sırasıyla, solungaçta %43, %29 ve %40, karaciğerde %48, %32 ve %29 düzeyinde bir azalış göstermiştir. Kas GR aktivitesinde 7 ve 21 günlük sürelerde cıva, cıva+selenyum ve cıva+zeolit gruplarının tüm ortam derişimlerinde anlamlı bir değişim belirlenmemiştir (Tablo 4, P>0,05).

Tablo 4. Cıva (mg/L), cıva (mg/L)+selenyum (mg/L) ve cıva (mg/L)+zeolit (g/L) etkisine bırakılan *O. niloticus*'un dokularında GR aktivitesi (U/mg protein)

Doku	7 Gün	21 Gün
Solungaç		
Kontrol	0,084±0,002 ax	0,083±0,003 ax
0,01 Hg	0,065±0,003 bx	0,075±0,004 ax
0,01 Hg + 0,1 Se	0,067±0,001 bx	0,076±0,003 ax
0,01 Hg + 0,1 Zeolit	0,069±0,002 bx	0,078±0,004 ax
Kontrol	0,084±0,002 ax	0,083±0,003 ax
0,1 Hg	0,048±0,004 bx	0,063±0,002 by
0,1 Hg + 1,0 Se	0,060±0,003 cx	0,082±0,002 ay
0,1 Hg + 1,0 Zeolit	0,050±0,002 bx	0,079±0,003 ay
Karaciğer		
Kontrol	0,056±0,003 ax	0,054±0,002 ax
0,01 Hg	0,037±0,002 bx	0,052±0,003 ay
0,01 Hg + 0,1 Se	0,040±0,002 bx	0,052±0,004 ay
0,01 Hg + 0,1 Zeolit	0,038±0,003 bx	0,055±0,003 ay
Kontrol	0,056±0,003 ax	0,054±0,002 ax
0,1 Hg	0,029±0,004 bx	0,040±0,001 by
0,1 Hg + 1,0 Se	0,038±0,002 cx	0,051±0,002 ay
0,1 Hg + 1,0 Zeolit	0,040±0,004 cx	0,041±0,002 bx
Kas		
Kontrol	0,008±0,002 ax	0,009±0,002 ax
0,01 Hg	0,007±0,001 ax	0,008±0,001 ax
0,01 Hg + 0,1 Se	0,008±0,002 ax	0,009±0,001 ax
0,01 Hg + 0,1 Zeolit	0,008±0,001 ax	0,008±0,002 ax
Kontrol	0,008±0,002 ax	0,009±0,002 ax
0,1 Hg	0,008±0,002 ax	0,008±0,002 ax
0,1 Hg + 1,0 Se	0,007±0,001 ax	0,008±0,001 ax
0,1 Hg + 1,0 Zeolit	0,009±0,001 ax	0,009±0,001 ax

Veriler Aritmetik ortalama±Standart hata şeklinde sunulmuştur (n=6). "a, b ve c" harfleri aynı dokudaki derişimler "x ve y" harfleri ise aynı derişimdeki etkileşim süreleri arasındaki ayırımını göstermek için kullanılmıştır. Farklı harfler, veriler arasındaki istatistiksel ayrım olduğunu göstermektedir ($P<0,05$).

TARTIŞMA ve SONUÇ

Sunulan çalışmada, cıvanın yüksek ortam derişiminde ve 21 günlük deney süresince balıklarda ölüm gözlenmemiştir. Metallerin yüksek ortam derişimlerinin etkisinde organizmaların hayatta kalabilmeleri, metal iyonlarının hücre içi konsantrasyonlarının regüle edilebilme kapasitesiyle yakından ilişkilidir (Azevedo vd., 2007). *O. niloticus*'un güçlü immün sistemi ve koruyucu/savunma mekanizmalarına bağlı olarak mortalite gözlenmediği düşünülmektedir. Benzer şekilde başka bir laboratuvar çalışmasında da 0,1 mg/L cıva etkisine 14 gün süreyle bırakılan *O. niloticus*'ta ölüm gözlenmemiştir (Firidin vd., 2015).

Akuatik ortamlara giren kirleticiler hem sucul ekosistemlere hem de burada yaşayan canlı organizmalara ciddi zararlar verdiğinden, son yıllarda ekotoksikolojik çalışmalarda kirleticilerin indüklediği oksidatif strese karşı sucul organizmalardaki yanıt mekanizmalarının çalışılmasına büyük bir önem verilmektedir (Soares vd., 2008). Çoğu kirleticiler serbest radikaller oluşturarak veya antioksidan enzim sistemleri değiştirerek oksidatif hasara neden olmaktadır. Bununla birlikte sucul organizmalar oksidatif strese karşı hem enzimatik hem de enzimatik olmayan antioksidan mekanizmaları ile bir yanıt oluşturmaktadırlar. Bu yanıt mekanizmaların en önemlilerinden biri de GSH ve onunla ilişkili enzim sistemleridir (GPx, GR ve GST gibi).

Oreochromis niloticus dünyada yaygın şekilde kültürü yapılan tatlı su balıklarından birisidir. Ekotoksikolojik çalışmaların çoğunda bu tür, akuatik ekosistemlerdeki kirleticilerin etkilerini değerlendirmek amacıyla kullanılmaktadır. *O. niloticus* yüksek büyüme hızı, farklı diyetlere adaptasyondaki yeteneği, hastalıklara ve taşıma esnasındaki streslere direnç göstermeleri, kolay üremeleri ve ağır metal stresini de içeren çeşitli toksik maddelere karşı geniş tolerans yeteneği gibi farklı özellikleri nedeniyle toksikolojik çalışmalarda kullanılan iyi bir biyolojik modeldir (Fontainhas-Fernandes, 1998).

GSH, antioksidan ve detoksifikasyon savunma mekanizmalarının önemli proeseslerine katılan tek sistein residüsüne sahip enzimatik olmayan bir antioksidan ve tripeptidtir. GSH, indirgeyici ajan, serbest radikal temizleyici ve GSH ile ilişkili enzimler için kofaktör olarak davrandığından aktif oksijen türlerinin neden olduğu oksidatif strese karşı hücresel savunmada rol oynayan en önemli faktörlerden biri olarak ifade edilmektedir (Verma vd., 2007). Bu molekül, ilk savunma hattı olarak metallerin hücrelerin önemli moleküler yapılarına yapacağı etkiyi engellemede önemli bir tiyol kaynağı olarak rol oynamaktadır. Sunulan çalışmada *O. niloticus*'ta her iki etkileşim süresinde GSH düzeyleri cıvanın doğrudan etkisinde solungaç ve karaciğer dokularında azalırken kas dokusunda önemli bir değişim göstermemiştir. Cıva ortam derişimi arttıkça dokuların GSH düzeylerindeki azalma oranının da arttığı saptanmıştır. *O. niloticus*'un solungaç ve karaciğer GSH düzeylerindeki azalışlar cıvanın toksik etkilerinin bir sonucu olabilir. Cıva, -SH gruplarına yüksek ilgisi olan bir metaldir. Cıvanın GSH yapısında bulunan -SH gruplarına doğrudan bağlanması ya da bu tiyol molekülünün oksidasyonunu arttırmasının bir sonucu olarak dokulardaki GSH düzeylerinde azalma olabileceği düşünülmektedir. Bulduğumuz sonuçlara benzer olarak Elia vd. (2003) yaptıkları bir çalışmada 35, 70 ve 140 mg/L Hg etkisine bırakılan *Ictalurus melas*'ın karaciğer dokusunda GSH düzeylerinde derişime bağlı olarak önemli azalmaların olduğunu belirlemişlerdir. Araştırıcılar karaciğer GSH düzeyindeki bu azalmanın, cıvanın GSH'a bağlanması ya da GSH'ın GSSG'ye oksidasyonu sonucu olabileceğini belirtmişlerdir. Ağır metallerin etkisinde organizmaların dokularındaki GSH düzeylerinde önemli değişiklikler oluşmaktadır. 40 gün süreyle 3 mg/L Cd etkisine bırakılan *O. niloticus*'un karaciğer dokusundaki GSH düzeylerin azaldığı rapor edilmiştir (Zirong ve Shijun, 2007). Araştırıcılar Cd'un neden olduğu oksidatif strese karşı GSH'ın hızlı bir koruma meydana getirdiğini ancak zamanla GSH'ın GSSG'ye oksidasyonu sonucu düzeyinde azalmaların olduğunu belirtmişlerdir.

Araştırmamızda cıva+selenyum ve cıva+zeolit ortam derişimlerinin etkisinde solungaç ve karaciğer GSH düzeylerinde azalışlar gözlemlenmiştir. Ancak bu azalışların cıvanın tek başına etkisinde daha yüksek olduğu saptanmıştır. Araştırma sonuçlarımızla paralel olarak Su vd. (2008) Hg'nın neden olduğu oksidatif stres üzerine selenyum etkisini araştırdıkları çalışmalarında Hg ve Hg+Se etkisindeki sıçanların dokularındaki GSH düzeylerinde Hg'nın tek başına etkisinde görülen azalışların Se varlığında kontrol değerlerine döndüğü belirlenmiştir. Başka bir çalışmada da cıva toksitesi üzerine bir antioksidan olan vitamin C'nin koruyucu etkisinin araştırıldığı çalışmada cıvanın tek başına etkisinde *Oncorhynchus mykiss*'in karaciğer dokusundaki GSH düzeylerinin önemli ölçüde azaldığı ancak vitamin C'nin uygulanmasıyla metalin toksik etkisinin önlendiği ve cıvanın neden olduğu GSH düzeylerindeki azalışların engellendiği rapor edilmiştir (Mozhdeganloo vd., 2015).

Sunulan çalışmada cıvanın tek başına ve cıva+selenyum ve cıva+zeolit karışımlarının etkisinde *O. niloticus*'un dokularındaki GSH ile ilişkili enzimlerin (GPx, GST ve GR) aktivitelerinde dokuya, ortam derişimine ve etkide kalınan süreye bağlı olarak önemli değişimlerin meydana geldiği belirlenmiştir. *I. melas*'ın karaciğerinde GSH ve GSH ile ilişkili enzimlerin 96 saatlik farklı Hg derişimlerinden (100, 200, 400 mg/L) etkilendiği rapor edilmiştir (Elia vd., 2000). Çalışmada yüksek metal derişimlerinin enzim aktivitelerinde azalmalara neden olduğu belirtilmiştir.

GPx hem hidrojen peroksitleri hem de hidroperoksitleri indirgeyerek serbest radikaller ve oksidatif hasara karşı etkin bir koruma sağlamaktadır. Araştırmamızda cıvanın tek başına etkisinde her iki ortam derişiminde de GPx aktivitesinin solungaç dokusunda 7 günlük süre sonunda, karaciğerde ise denenen her iki sürede arttığı saptanmıştır. Bununla birlikte cıvanın selenyum ve zeolitle birlikte etkisinde ise solungaç GPx aktivitesinde önemli bir değişim saptanmamış iken karaciğer GPx aktivitesinde genel olarak bir artış saptanmıştır. Ancak karaciğer enzim aktivitesindeki bu artışın cıvanın tek başına etkisine oranla cıva+selenyum ve cıva+zeolit karışımlarının etkisinde daha az olduğu görülmüştür. Kas GPx aktivitesinde ise cıva, cıva+selenyum ve cıva+zeolitin tüm ortam derişimlerinin etkisinde ve denenen her iki sürede de önemli bir değişim saptanmamıştır.

Çalışmamızda *O. niloticus*'un dokularında artan GPx aktivitesinin cıvanın toksik etkilerine karşı bir yanıt olarak oluştuğu ve cıvanın neden olduğu oksidatif strese karşı koruyucu bir rol oynadığı düşünülmektedir. Araştırma sonuçlarımızla benzer şekilde 4 gün süre ile 35 µg/L $HgCl_2$ etkisine bırakılan *I. melas*'ın karaciğer dokusundaki GPx aktivitesinde önemli artışlar saptanmıştır (Elia vd., 2003). Berntssen vd. (2003) yaptıkları bir deneysel çalışmada 100 mg/kg cıva içeren diyetlerle beslenen *Salmo salar*'ın karaciğer ve böbrek dokularında GPx aktivitesinin artığını belirlemişlerdir Araştırıcılar bu artışın dokularda cıvanın neden olduğu oksidatif strese bir adaptasyon yanıtı olarak oluştuğunu ifade etmişlerdir.

GST, ksenobiyotiklerin GSH ile sentetik konjugasyon reaksiyonlarını katalizleyerek oksidatif strese karşı balık dokularını koruyan detoksifikasyon enzimlerinin önemli bir grubunu oluşturmaktadır (Luo vd., 2006). Sunulan çalışmada *O. niloticus*'un solungaç ve karaciğer GST aktivitelerinin cıvanın doğrudan etkisinde her iki ortam derişiminde artığı belirlenmiştir. Yine cıva+selenyum ve cıva+zeolit karışımlarının etkisinde 7 günlük süre sonunda bu dokuların GST aktivitelerinde de artış saptanmıştır. Ancak bu artışların cıvanın tek başına etkisinde cıva+selenyum ve cıva+zeolit karışımlarına oranla daha yüksek olduğu belirlenmiştir. Kas GST aktivitesi ise denenen tüm kimyasalların etkisinde yalnızca 7 günlük süre sonunda bir artış göstermiştir. Dokuların GST aktivitesinin cıvanın neden olduğu oksidatif strese bir adaptasyon yanıtı olarak metal toksisitesini nötralize etmek amacıyla artığı düşünülmektedir. Monteiro vd. (2010) yaptıkları bir çalışmada tatlı su balığı *Brycon amazonicus*'un solungaç, karaciğer, kas ve kalp dokularında GST enzim aktivitesinin cıvanın etkisinde artığını belirlemişlerdir. Araştırıcılar GST aktivitesindeki artışların metalin neden olduğu oksidatif hasarın önlenmesinde önemli bir rol oynadığını ileri sürmüşlerdir. GST'nin çok çeşitli elektrofilik bileşikleri GSH aracılığıyla konjuge ederek daha az toksik bileşiklere dönüştürebildiği ve bu aktivitesi sayesinde toksik bileşiklerin detoksifikasyonunda önemli görevler oynadığı belirtilmektedir (Van Der Oost vd., 2003). Bu nedenle *O. niloticus*'un dokularında artan GST aktivitesinin detoksifikasyon proseslerinin indüklenmesinde önemli olduğu düşünülmektedir.

GR, bir flavoprotein olup kofaktör olarak NADPH'ı kullanarak GSSG'nin GSH'a indirgenmesini katalizlemekte ve GSH düzeylerini korumaktadır (Pena-Llopis vd., 2001). Böylece GR hücre içi GSH/GSSG oranını yükselterek –SH/-SS- oranını da korumaktır. Çalışmamızda *O. niloticus*'un solungaç ve karaciğer GR aktivitesinin cıvanın tek başına etkisinde azaldığı belirlenmiştir. Benzer şekilde cıva+selenyum ve cıva+zeolit karışımlarının etkisinde de 7 günlük süre sonunda bu dokuların GR aktivitelerinde bir azalış saptanmıştır. Ancak enzim aktivitesindeki azalışlar özellikle de yüksek ortam derişimlerinde cıvanın tek başına etkisinde daha fazla olmuştur. Kas dokusunda ise GR aktivitesi denenen tüm kimyasalların etkisinde anlamlı bir değişim göstermemiştir. Sunulan araştırmada cıva toksisitesinin bir sonucu olarak doku GR aktivitelerinin azaldığı öngörülmektedir. Azalan GR aktivitesi büyük bir olasılıkla cıvanın enzim aktivitesi üzerine olan doğrudan ya da NADPH üretimi üzerine olan toksik etkisinin sonucunda bu nükleotidin içeriğinin azalmasına bağlı olarak gerçekleştiği düşünülmektedir. Eroglu vd. (2015) *O. niloticus*'un karaciğer glutatyon metabolizması üzerine Cd, Cu, Cr, Pb ve Zn'nin etkilerini araştırdıkları çalışmalarında Cr etkisinde GR enzim aktivitesinin azaldığını saptamışlardır.

GPx ve GST kofaktör olarak GSH'ı kullanarak prooksidan süreçleri etkisizleştirmek için birlikte çalışmaktadırlar (Monteiro vd., 2010). Bu enzimler tarafından GSSG'ye yükseltgenen GSH daha sonra GR'nin katalizlediği reaksiyonda NADPH kullanılarak GSSG'den tekrar oluşmaktadır. GSH sisteminin kapasitesi GPx, GR, GST ve pentoz fosfat yolu enzimlerin aktivitelerine bağlıdır (Larose vd., 2008). Bu nedenle hücre içi GSH düzeylerindeki değişimler bu enzimlerle oldukça yakın ilişkilidir. Sunulan çalışmada da cıvanın etkisinde *O. niloticus*'un dokularında azalan GSH düzeylerinin GPx, GST ve GR enzim aktiviteleriyle ilişkili olduğu düşünülmektedir. Dokuların artan GPx ve GST aktivitelerine bağlı olarak bu enzimler tarafından GSH'ın substrat olarak kullanımı GSH düzeylerini düşürmüş olabilir. Keza azalan GR aktivitesine bağlı olarak GSSG'den tekrar GSH'ın oluşturulmasının azalması da düşen GSH düzeylerini açıklayabilir. Elia vd. (2003) çalışmamızdaki sonuçlara benzer olarak *I. melas*'ın dokularında cıva etkisinde GSH düzeyinde ve GR aktivitesinde azalma, GPx ve GST aktivitesinde ise artış gözlemlemişlerdir. Araştırıcılar artan GPx ve GST aktivitesine ve azalan GR aktivitesine bağlı olarak GSH düzeylerinin azaldığını belirtmişlerdir.

Sonuç olarak sunulan çalışmada cıvanın *O. niloticus*'un dokularında GSH ve GSH ile ilişkili enzimleri etkilediği belirlenmiştir. GSH metabolizmasında cıvanın doğrudan etkisinde meydana gelen değişimlerin cıva+selenyum ve cıva+zeolit karışımlarına oranla daha yüksek olduğu görülmüştür. Selenyum veya zeolit varlığında cıvanın GSH ve GSH ile ilişkili enzimler üzerine olan olumsuz etkilerinin azaldığı ya da iyileştiği saptanmıştır. Araştırma sonuçlarımız gerek selenyum gerekse de zeolit uygulamasının cıva toksisitesi üzerine koruyucu bir rolü olduğunu göstermektedir. Selenyumun koruyucu etkisini cıvanın alınım ve atılım oranlarını etkileyerek, Hg-Se kompleksi oluşturarak hücredeki serbest cıva bulunurluğunu azaltarak ya da antioksidan kapasitesine bağlı olarak gerçekleştirdiği düşünülmektedir. Zeolitin ise kafes şeklindeki yapısı içerisinde Hg'yi tutarak suda serbest cıva bulunurluğunu azaltarak koruyucu etki gösterdiği öngörülmektedir. Genel olarak çalışılan

parametreler üzerine selenyumun koruyucu etkisinin zeolite oranla biraz daha yüksek olduğu gözlenmiştir.

TEŞEKKÜR

Bu çalışma "Cıva, Cıva-Selenyum ve Cıva-Zeolit Karışımlarına bırakılan *Oreochromis niloticus*'ta Cıvanın, Dokulardaki Birikimi ve GSH ile İlişkili Enzim Aktivitelerine Etkisi" başlıklı ve YÖK Ulusal Tez Merkezi Referans Nosu "414359" olan Doktora tez çalışmasından üretilmiştir. Bu araştırma FEF2012D1 nolu Çukurova Üniversitesi Bilimsel Araştırma Projesi ile yürütülmüştür.

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Karadeniz'in Şile Kıyılarında Yaşayan *Mesogobius batrachocephalus* (Pallas, 1814) Türünün Pul Morfolojisinde Cinsiyete Bağlı Değişimlerin Tespiti

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Özet

Bu çalışmanın amacı Karadeniz'in Şile kıyılarında yaşayan *Mesogobius batrachocephalus* türünün vücudunun farklı bölgelerinde bulunan pullarının morfolojik ve morfometrik özelliklerinin cinsiyete bağlı değişimlerini tespit etmektir. Bu çalışma kapsamında, toplam 32 birey ($n_{♀}$= 16, $n_{♂}$=16) değerlendirilmiştir. Bireylerin minimum ve maksimum total boy ve ağırlıkları sırasıyla 17,6 - 27,7 cm ve 46,6 - 231,0 g aralığındadır. Farklı vücut bölgelerindeki pullar dişi ve erkek bireyler için ayrı ayrı değerlendirilmiştir. Pul boyutu ve pul şekli gibi morfolojik özellikler hem dişi-erkek bireyler hem de incelenen vücut bölgeleri arasında ayırt edici karakterler olarak belirlenmiştir. Diğer morfolojik özellikler (pul merkezinin pozisyonu, sirkuli, radii tipi, pulun rostral ve kaudal kenarı) ise hem dişi-erkek bireylerde hem de belirlenen bölgeler arasında benzer özellikler göstermektedir. *M. batrachocephalus* bireylerinin vücut bölgelerindeki pullar karşılaştırıldığında, dişi ve erkek bireylerdeki pulların morfometrik değerleri (genişlik, alan ve çevre) arasında 1. bölge, 4. bölge ve 5. bölge için istatistiksel olarak anlamlı farklılıklar tespit edilmiştir ($P<0,05$). Bu çalışmada, *M. batrachocephalus* türünün pul morfolojisi ve morfometrisine ait bilgiler ilk defa sunulmuştur. Çalışma sonucunda dişi ve erkek bireylerin bu özellikler bakımından farklılık gösterdiği tespit edilmiştir. Bu sebeple filogenetik çalışmalarda önemli yer tutan pul morfolojisi ve morfometrisinin, *M. batrachocephalus* türünün hem dişi hem de erkek bireylerinde ayrı ayrı değerlendirilmesinin gerektiği önerilmektedir.

Anahtar Kelimeler: Mesogobius batrachocephalus, morfometri, pul morfolojisi, Karadeniz, Şile

Determination of Gender-Based Changes in Scale Morphology of *Mesogobius batrachocephalus* (Pallas, 1814) Inhabiting the coast of Şile in Black Sea.

Abstract

This study aimed to determine the gender-related changes in the morphological and morphometric properties of the scales for *Mesogobius batrachocephalus* inhabiting Şile coast of the Black Sea. In this study, a total of 32 individuals ($n_{♀}$= 16, $n_{♂}$=16) were evaluated. The minimum and maximum total length and weight of the fish samples were in the range of 17.6-27.7 cm and 46.6 - 231.0 g, respectively. The scales in different body regions were evaluated for female and male specimens separately. The morphological features such as scale size and scale shape were identified as distinguishing features between both female-male specimens and specified regions. The other morphological features (scale focus position, circuli, radii type, and the rostral and kaudal margin) of the scale were showed similar characteristics both in females-males and specified regions. When the scales in the body regions of *M. batrachocephalus* specimens were compared, the statistically significant differences were determined between the morphometric values (width, area, and perimeter) of females and males for 1., 4. and 5. Regions ($P<0.05$). In this study, data on scale morphology and morphometry of *M. batrachocephalus* was presented for the first time. As a result of the study, it was determined that female and male individuals differ in terms of these characteristics. For this reason, it is suggested that scale morphology and morphometry, which have an important place in phylogenetic studies, should be evaluated separately in both female and male individuals of *M. batrachocephalus*.

Keywords: Mesogobius batrachocephalus, morphometry, scale morphology, Black Sea, Şile

GİRİŞ

Balıkçılık biyolojisinde balıkların derileri üzerindeki sert ve yassı iskelet elemanları pul olarak adlandırılmaktadır (Bräger ve Moritz, 2016). Genel olarak pullar; plaka benzeri plakoid pullar, elmas şeklindeki ganoid pullar, ince, pürüzsüz, disk benzeri sikloid pullar ve kaudal kenarı boyunca küçük çıkıntılar taşıyan ktenoid pullar şeklinde sınıflandırılmaktadır (Casteel, 1976; Patterson vd., 2002).

Balıklarda pullar tipik olarak vücudun arkasından önüne doğru, ardından da dorsal ve ventral yönlü olarak yanlara doğru ilerleyen bir oluşum halindedir (Helfman vd., 1997; Motamedi vd., 2020).

Pullar, balığın vücudunda epidermis tabakasına gömülü halde bulunan, kalsiyum karbonat ve kolajen içeren aynı zamanda türlerin tanımlanmasında da yararlanılan yapılardır. Ktenoid ve sikloid pullar birçok çalışmada yararlı bir tayin aracı olarak kullanılmaktadır. Pullar, moleküler tekniklere kıyasla daha az maliyetli, tahribatsız, kullanıma uygun ve avcılarının sindirim sistemleri tarafından sindirilmelerinin zor olmasından dolayı tür tanımlama araçları olarak kullanılabilecek uygun kemiksi yapılardır (Ibañez vd., 2007; Farinordin vd., 2017). Pul morfolojisi ise, 1833 yılından beri taksonomi ve sınıflandırma çalışmalarında kullanılmaya başlanmış olup ontogenetik analizler (Vignon, 2012) ve morfoloji (Jawad, 2005; Esmaeili vd., 2012; Farah-Ayuni vd., 2016) gibi birçok farklı çalışma için de faydalanılan yararlı birer araç olarak değerlendirilmiştir (Jawad ve Al-Jufaili, 2007). Örneğin, pulların morfolojik ve morfometrik özellikleri balık türlerinin ve populasyonlarının tanımlanmasında ve ayrımlarının yapılmasında tercih edilen yöntemlerden biridir (Kuusipalo, 2000; Khemiri vd., 2001; Esmaeili ve Gholami, 2011; Yedier vd., 2019). Su ortamında çeşitli sebeplere bağlı olarak bulunan ağır metaller (Lake vd, 2006) gibi kirleticiler pulların yapısı içerisinde birikmektedir ve bu sayede kirlilik üzerine yapılan çalışmalarda balıkların biyoindikatör olarak kullanılmasına da olanak sağlamaktadır (Johal ve Sawhney, 1999).

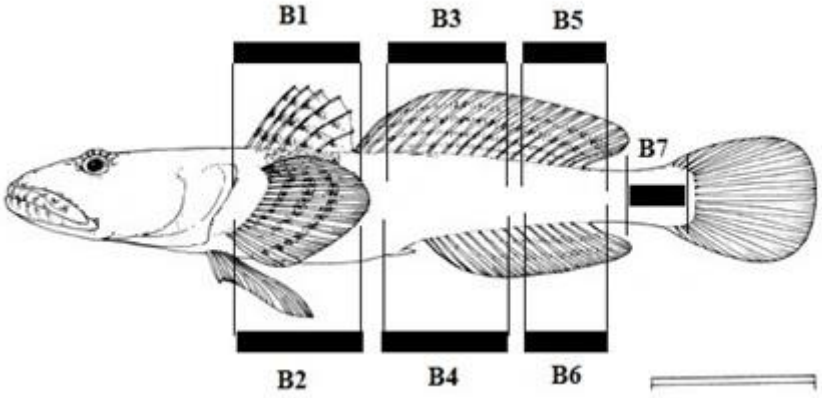
M. batrachocephalus türü Avrupa ve Asya'da Karadeniz, Azak Denizi ve Romanya kıyılarının bazı bölgelerinde yayılış göstermektedir (Froese ve Pauly, 2020). Bu tür genelde kıyı bölgelerde, haliçlerde, kum veya kayaların diplerinde acı ve tatlı su lagünleri gibi farklı habitatlarda yaşayabilmektedir. *M. batrachocephalus* türü ülkemizde yoğun olarak Karadeniz sularından rapor edilmesine rağmen türün aynı zamanda Marmara Denizi üzerindeki Haliç ve Unkapanı köprülerinin bulunduğu bölgeden avlandığı (Gökdaş, 2006) ve Marmara Denizi'nde (Bilecenoğlu vd., 2002; 2014) bulunduğu da bildirilmiştir. *M. batrachocephalus* türü ile ilgili yapılmış daha önceki çalışmalarda türün boy-ağırlık ilişkisi (Demirhan ve Can, 2007; Mihălcescu, 2011; Çalık ve Erdoğan Sağlam, 2017), beslenme ekolojisi (Rosca vd., 2010; Roşca ve Surugiu, 2010; Roşca ve Mânzu, 2011), biyokimyasal değerlendirmeleri (Sigacheva vd., 2020), enzim aktiviteleri (Rudneva vd., 2010), kan hücreleri (Soldatov ve Kukhareva, 2015), parazit-konakçı ilişkileri ve parazitolojik değerlendirmeleri (Okkay ve Özer, 2020; Okkay vd., 2020), taksonomisi (Stepien ve Tumeo, 2006) ve balıkçılıktaki durumu (Zarev vd., 2013) belirlenmiştir.

Literatür incelendiğinde, *M. batrachocephalus* türünün pul morfolojisi ile ilgili detaylı bir araştırmanın olmadığı tespit edilmiştir. Bu çalışmanın amacı, *M. batrachocephalus* türünün farklı vücut bölgelerindeki pullarının morfolojisini ayrıntılı olarak araştırmak ve cinsiyete bağlı morfolojik değişimlerin varlığını belirlemektir. Bu nedenle, bu çalışmada Şile kıyılarında yaşayan *M. batrachocephalus* türünün 7 vücut bölgesine ait pulların morfolojileri ve morfometrik özelliklerinin hem dişi hem de erkek bireylerde incelenmesi amaçlanmıştır. Ayrıca, çalışma kapsamında farklı vücut bölgelerindeki pulların 4 morfometrik ve 9 morfolojik özelliği de tespit edilmiştir.

MATERYAL ve YÖNTEM

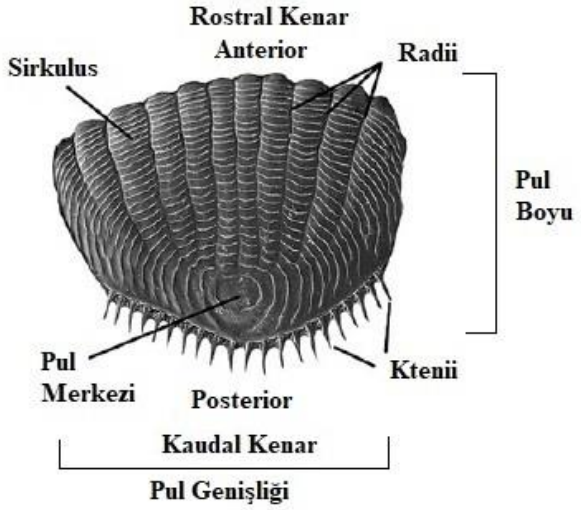
Bu çalışmada, Şile kıyılarında yayılış gösteren 32 adet *Mesogobius batrachocephalus* bireyi yerel balıkçılarla koordineli bir çalışmanın sonucunda temin edilmiştir. Laboratuvara getirilen balık örnekleri analizlere kadar dondurucuda saklanmıştır. Balık örneklerinin total (TB) ve standart (SB) boyları ± 0,1 cm, ağırlıkları ise ± 0,01 g hassasiyetle ölçülüp kataloglara kaydedilmiştir.

Örneklerin diseksiyonlarından önce *M. batrachocephalus* bireylerinin üzerinde 7 adet bölge belirlenmiş ve bu bölgelerdeki pullar değerlendirilmiştir (Şekil 1). Balık bireyleri üzerindeki bölgeler şu şekilde belirlenmiştir: 1. bölge (B1; yanal çizginin üzeri ile I. dorsal yüzgecin altında kalan bölgedir), 2. bölge (B2; yanal çizginin altında ve B1 bölgesine karşılık gelecek ventral yüzgecin gerisindeki bölgedir), 3. bölge (B3; yanal çizginin üzeri ile II. dorsal yüzgecin ilk yarısı altında kalan bölgedir), 4. bölge (B4; yanal çizginin altı ile anal yüzgecin ilk yarısı üzeri arasında kalan bölgedir), 5. bölge (B5; yanal çizginin üzeri ile II. dorsal yüzgecin son yarısı altında kalan bölgedir), 6. bölgesi (B6; yanal çizginin altı ile anal yüzgecin son yarısı üzeri arasında kalan bölgedir), 7. bölge (B7; kaudal pedünkül bölgesidir) (Şekil 1).



Şekil 1. Çalışma kapsamında *M. batrachocephalus* üzerinde belirlenen vücut bölgeleri (Froese ve Pauly, 2020' den değiştirilerek hazırlanmıştır.)

Pullar vücudun sol tarafından ve belirlenen her bir bölgeden 8 adet olacak şekilde dikkatlice ayrılmıştır (her birey için 56 pul). Her bölgeden pul örnekleri ayrı ayrı distile su içerisinde yıkanmış, ardından üzerindeki mukus ve pigmentlerden temizlenmesi amacıyla % 3'lük NaOH çözeltisinde 24 saat bekletilmiştir. Bu sürenin sonunda her bir bölgenin pulları çözeltiden çıkarılarak, tekrar distile su içerisinde yıkanmıştır. Daha sonar pullar 30 dakika % 96'lık etil alkolde bekletilmiş ve kenarları kıvrılmayacak şekilde dikkatlice lamlar üzerine dizilerek pul preparatları hazırlanmıştır (Chugunova, 1963). Her bir bireyin vücut bölgelerine ait pullar stereo mikroskop altında x1,6 büyütmede incelenerek, S8APO kamera sistemi bağlı Leica marka mikrokopta Leica Ver. 3.7.0 yazılımı kullanılarak fotoğraflanmış ve pullar üzerinde ölçümler gerçekleştirilmiştir. Jaward 2005'e göre düzenlenmiş ktenoid pul yapısı Şekil 2'de gösterilmiştir.



Şekil 2. Genel ktenoid pul yapısı

7 vücut bölgesinde ayrı ayrı incelenen pullar merkezlerinin çevresinde circuli halkalarına sahiptir. Vücut rengine göre karakteristik bir renklenme de görülmektedir. Karşılaştırmalar için, her bölgeden referans bir pul belirlenmiştir. Her pul için aşağıda belirtilen ölçümler kaydedilmiş ve analizler gerçekleştirilmiştir:

(1) morfometrik ölçümler; pul boyu (mm), pul genişliği (mm), pul alanı (mm^2) ve pul çevresi (mm);

(2) morfolojik karakterler; pul tipi, pul boyutu, pul şekli, pul merkezinin pozisyonu, sirkuli görünüşü, radii yerleşimi, pulun rostral ve kaudal kenar özellikleri.

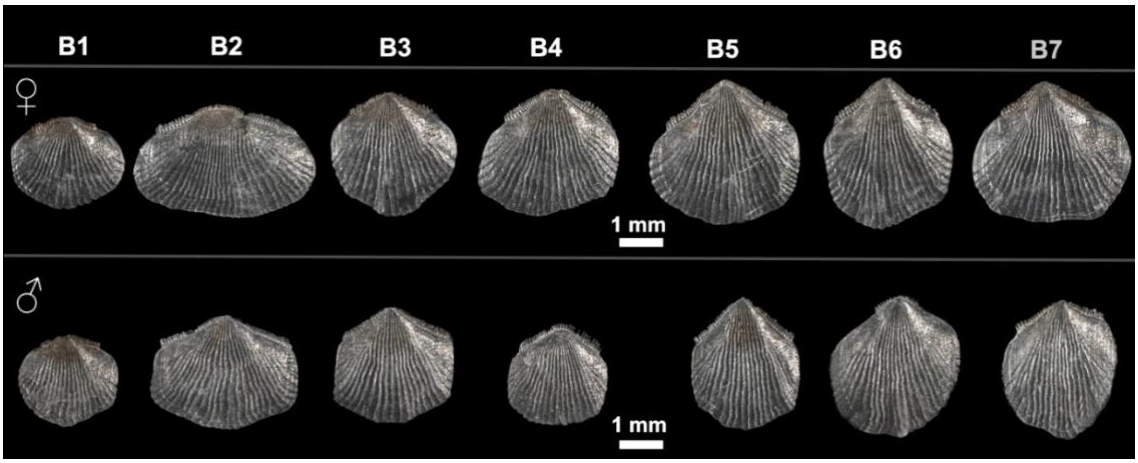
Balık örneklerinin diseksiyonları esnasında dişi ve erkek bireyler tespit edilmiştir. Dişi ve erkek bireylerin boy ve ağırlıkları arasında istatistiksel olarak fark olup olmadığı t-testi yardımıyla araştırılmıştır. Boy ve ağırlık değerleri arasında fark olduğundan ($P<0,05$) bireylere ait pulların morfolojik tayinleri ve morfometrik hesaplamaları dişi, erkek ve tüm bireyler için ayrı ayrı gerçekleştirilmiştir. Ayrıca, dişi ve erkek bireylerin aynı vücut bölgelerinde taşıdıkları pullarının morfometrik değerleri arasındaki istatistiksel farkın varlığı da t-testi kullanılarak araştırılmıştır.

BULGULAR

Bu çalışmada 32 adet *M. batrachocephalus* (♀, n = 16; ♂, n = 16) bireyi değerlendirilmiştir. Balıkların minimum ve maksimum total boyları sırasıyla dişi bireyler için 18,9-27,7 cm ve erkek bireyler için 17,6-22,2 cm; minimum ve maksimum ağırlıkları ise dişi bireyler için 61,20-231,00 g ve erkek bireyler için 46,60-70,20 g aralığında değişmektedir. Dişi ve erkek bireylerin boy ($P<0,05$) ve ağırlıkları ($P<0,05$) arasında t testi sonuçlarına göre istatistiksel olarak anlamlı bir farklılık tespit edilmiştir. Bu nedenle bireyler hem dişi ve erkek bireyler olarak ayrı ayrı hem de tüm bireyler olarak değerlendirilmiştir.

M. batrachocephalus bireyleri üzerinde belirlenen 7 vücut bölgesi Şekil 1'de gösterilmiştir. Toplamda tüm bireyler için 1792 adet pul (♀: 896 adet ve ♂: 896 adet) analiz edilmiştir. Bölgelerden seçilen pulların genel görünüşleri dişi ve erkek bireyler için ayrı ayrı Şekil 3'te sunulmuştur. *M. batrachocephalus* bireylerinin vücut bölgeleri üzerindeki pullar ktenoid tiptedir (Şekil 2; Tablo 1). Pulların bölgelere ve cinsiyete göre farklı büyüklük ve morfolojide olduğu dikkati çekmektedir. Farklı vücut bölgelerinde gözlemlenen pulların sahip olduğu şekiller diskoidal, eliptik, sirkular, kordat ve hekzagonal olarak değişiklik göstermektedir. Ayrıca dişi ve erkek bireylerde B2 ve B3 bölgeleri pullarının da birbirinden morfolojik olarak farklı olduğu göze çarpmaktadır (Şekil 3, Tablo 1). Pul merkezi tüm vücut bölgelerinde posterior kısımda olup, pullarda sirkuli hem dişi hem de erkek bireylerde belirgindir. Yalnızca B3 ve B4 bölgelerindeki bazı pullarda sirkuli düzeninin pullardaki absorbsiyon ve rejenerasyondan dolayı bozulmuş olduğu gözlemlenmiştir. Pulların radii tipi de dişi ve erkek bireylerde primer ve sekonder olarak karakterize edilmiştir (Şekil 3). Pulların rostral kenarı hem diş hem de erkek bireylerde taraklı olarak gözlenmiştir. Pulların kaudal kenarı ise tüm vücut bölgelerinde periferal kteni tiptedir (Tablo 1, Şekil 3).

Belirlenen farklı vücut bölgelerine ait pulların morfometrik karakterleri olan boy, genişlik, alan ve çevre değerleri dişi, erkek ve tüm bireyler için Tablo 2'de sunulmuştur. Dişi ve erkek bireylerin t testi ile değerlendirilen farklı vücut bölgelerine ait pulları arasında bazı vücut bölgelerinde anlamlı farklar olduğu belirlenmiştir ($P<0,05$) (Tablo 2). Buna göre, dişi ve erkek bireylerin B1 pullarının genişlik, alan ve çevre değerleri arasında ($P<0,05$); B4 pullarının boy, genişlik, alan ve çevre değerleri arasında ($P<0,05$); B5 pulların da ise genişlik, alan ve çevre değerleri arasında istatistiksel olarak anlamlı farklılıklar olduğu ($P<0,05$) tespit edilmiştir. B1 pullarının dişi ve erkek bireylerine ait ortalama genişlik değerleri sırasıyla 2,471 ± 0,074 mm ve 2,167 ± 0,064 mm; ortalama alan değerleri 3,912 ± 0,172 mm^2 ve 3,223 ± 0,172 mm^2 ve ortalama çevre değerleri 7,464 ± 0,183 mm ve 6,706 ± 0,179 mm olarak belirlenmiştir ($P<0,05$). B4 pullarının dişi ve erkek bireylerinde ölçülen ortalama boy değerleri sırasıyla 2,609±0,045 mm ve 2,383±0,055 mm; ortalama genişlik değerleri 2,939±0,157 mm ve 2,580±0,062 mm; ortalama alan değerleri 5,978±0,422 mm^2 ve 4,674±0,185 mm^2; ortalama çevre değerleri 9,106±0,324 mm ve 8,081±0,156 mm şeklindedir ($P<0,05$). B5 pullarının dişi ve erkek bireylerine ait ortalama genişlik değerleri sırasıyla 2,732±0,103 mm ve 2,392±0,056 mm; ortalama alan değerleri 5,872±0,313 mm^2 ve 4,950±0,218 mm^2 ve ortalama çevre değerleri 9,124±0,224 mm ve 8,400±0,188 mm olarak tespit edilmiştir ($P<0,05$) (Tablo 2).



Şekil 3. Dişi ve erkek bireylerin vücut bölgelerine ait pulların genel görünümleri

Tablo 1. Bölgelere göre pulların morfolojik karakterleri

Morfolojik Karakterler		**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**
Pul Tipi	♀	Ktenoid	Ktenoid	Ktenoid	Ktenoid	Ktenoid	Ktenoid	Ktenoid
	♂	Ktenoid	Ktenoid	Ktenoid	Ktenoid	Ktenoid	Ktenoid	Ktenoid
Boyut	♀	Orta	Büyük	Büyük	**Büyük**	**Büyük**	Orta	**Büyük**
	♂	Orta	Büyük	Büyük	**Orta**	**Orta**	Orta	**Orta**
Şekil	♀	Diskoidal	**Eliptik**	**Dairesel**	Kordat	Kordat	Kordat	Kordat
	♂	Diskoidal	**Kordat**	**Hekzagonal**	Kordat	Kordat	Kordat	Kordat
Pul merkezinin pozisyonu	♀	Posterior	Posterior	Posterior	Posterior	Posterior	Posterior	Posterior
	♂	Posterior	Posterior	Posterior	Posterior	Posterior	Posterior	Posterior
Sirkuli	♀	Belirgin	Belirgin	Belirgin/ Bozulmuş	Belirgin/ Bozulmuş	Belirgin	Belirgin	Belirgin
	♂	Belirgin	Belirgin	Belirgin/ Bozulmuş	Belirgin/ Bozulmuş	Belirgin	Belirgin	Belirgin
Radii Tipi	♀	Primer Sekonder	Primer Sekonder	Primer Sekonder	Primer Sekonder	Primer Sekonder	Primer Sekonder	Primer Sekonder
	♂	Primer Sekonder	Primer Sekonder	Primer Sekonder	Primer Sekonder	Primer Sekonder	Primer Sekonder	Primer Sekonder
Rostral kenar	♀	Taraklı	Taraklı	Taraklı	Taraklı	Taraklı	Taraklı	Taraklı
	♂	Taraklı	Taraklı	Taraklı	Taraklı	Taraklı	Taraklı	Taraklı
Kaudal kenar	♀	Periferal kteni	Periferal kteni	Periferal kteni	Periferal kteni	Periferal kteni	Periferal kteni	Periferal kteni
	♂	Periferal kteni	Periferal kteni	Periferal kteni	Periferal kteni	Periferal kteni	Periferal kteni	Periferal kteni

Tablo 2. *M. batrachocephalus*'un dişi, erkek ve tüm bireyleri üzerinde belirlenen 7 vücut bölgesinin pullarına ait ortalama değerler (Ort. ± SH)

Bölge	Morfometrik Değerler	♀	♂	♀ + ♂
B1	*L*, mm	2,080 ± 0,047	1,942 ± 0,059	2,011 ± 0,039
	W, mm	**2,471 ± 0,074***	**2,167 ± 0,064***	2,319 ± 0,055
	A, mm^2	**3,912 ± 0,172***	**3,223 ± 0,172***	3,567 ± 0,135
	P, mm	**7,464 ± 0,183***	**6,706 ± 0,179***	7,085 ± 0,143
B2	*L*, mm	2,156±0,083	2,331±0,084	2,243 ± 0,060
	W, mm	3,195±0,245	3,005±0,084	3,100 ± 0,128
	A, mm^2	5,417±0,589	5,245±0,326	5,331 ± 0,332
	P, mm	8,786±0,546	8,682±0,267	8,734 ± 0,299
B3	*L*, mm	2,506±0,068	2,351±0,072	2,428 ± 0,050
	W, mm	2,605±0,112	2,536±0,114	2,570 ± 0,078
	A, mm^2	4,959±0,294	4,532±0,295	4,746 ± 0,208
	P, mm	8,269±0,247	7,992±0,254	8,131 ± 0,176
B4	*L*, mm	**2,609±0,045***	**2,383±0,055***	2,496 ± 0,040
	W, mm	**2,939±0,157***	**2,580±0,062***	2,760 ± 0,089
	A, mm^2	**5,978±0,422***	**4,674±0,185***	5,326 ± 0,255
	P, mm	**9,106±0,324***	**8,081±0,156***	8,593 ± 0,199
B5	*L*, mm	2,938±0,074	2,782±0,068	2,860 ± 0,051
	W, mm	**2,732±0,103***	**2,392±0,056***	2,562 ± 0,065
	A, mm^2	**5,872±0,313***	**4,950±0,218***	5,411 ± 0,205
	P, mm	**9,124±0,224***	**8,400±0,188***	8,762 ± 0,158
B6	*L*, mm	2,918±0,100	3,033±0,071	2,975 ± 0,061
	W, mm	2,824±0,120	2,699±0,072	2,761 ± 0,069
	A, mm^2	6,265±0,399	6,199±0,267	6,232 ± 0,236
	P, mm	9,379±0,289	9,318±0,197	9,348 ± 0,172
B7	*L*, mm	2,740±0,078	2,896±0,097	2,820 ± 0,063
	W, mm	2,782±0,177	2,482±0,080	2,627 ± 0,097
	A, mm^2	5,748±0,523	5,310±0,352	5,522 ± 0,309
	P, mm	8,922±0,383	8,683±0,276	8,799 ± 0,231

*: P<0,05. A; alan, P; çevre, L; boy, W; genişlik, Ort.; ortalama, SH; standard hata.

TARTIŞMA ve SONUÇ

Bu çalışmada, pulların morfolojik ve morfometrik özelliklerini tanımlamak ve varolan farklılıkları cinsiyet düzeyinde belirlemek hedeflenmiştir. Bu kapsamda *M. batrachocephalus* türünün pul morfolojisinin ve pul morfometrisinin vücudun çeşitli bölgelerine ve cinsiyete göre değişimi incelenmiştir. Bu çalışma, Karadeniz'in Şile kıyılarında yaşayan bu türün pul morfolojisi ve pul morfometrisini belirlemeye yönelik yapılmış ilk çalışmadır. *M. batrachocephalus* türünün pulları incelendiğinde, türün pulları üzerinde "kteni" adı verilen ve ayrı ayrı dikenler şeklinde yapılar bulunduran ktenoid pullara sahip olduğu görülmektedir. Türün sahip olduğu ktenoid pulların türü ise, kaudal kenarında tek sıra veya bazen alternatif bir sıra daha küçük ikincil dikenler (ktenii) taşıyan "periferal kteni" dir (Şekil 3, Tablo 1).

Pulun yüzeyinde, anterior bölgenin kenarı rostral, posterior bölgenin kenarı ise kaudal olarak isimlendirilmektedir (Jawad, 2005). *M. batrachocephalus*'da rostral kenar her iki eşeyde de taraklı bir yapıya sahiptir. Kaudal kenar tipi periferal kteni olup *Gobius bucchichi* ve *Gobius paganellus* türleriyle benzer özellik göstermektedir (Bräger ve Moritz, 2016).

Balıkların farklı vücut bölgelerinde bulunan pulların morfometrik karakterlerinin ve morfolojilerinin belirlenmesi önemlidir. Bunun nedeni, pulların farklı vücut bölgelerinde farklı şekil ve özelliklere sahip olabilmeleri ve türler arasında taksonomik açıdan ayrımın sağlanmasında faydalı

olabilecek bazı ayırt edici karakterlere sahip olmasıdır (Kontaş vd., 2020). Bu karakterlerden bazıları türden türe değişen pulların tipleri, ölçüleri, yarıçap düzenleri, sirkuli ve merkez pozisyonlarıdır. Bu ayırt edici karakterler balık türlerini (Harabawy vd., 2012), yaşlarını (Esmaeili vd., 2007) ve cinsiyetlerini (Ganzon vd., 2012) tanımlamak için kullanılabilir (Farinordin vd., 2017). Farklı balık türlerinde pullar farklı şekil ve karakterlerde olabilirler (Jawad ve Al-Jufaili, 2007). Genel olarak *G. bucchichi* türünün pul şeklinin tam dairesel ile çanak şekline yakın diskoidal aralığında olduğu, *G. paganellus* türünün ise dairesel ve oval şekiller arasında değiştiği bildirilmiştir (Bräger ve Moritz, 2016). *M. batrachocephalus* türünün pul morfolojisi dişi ve erkek bireylerde bölgelere göre ayrı ayrı değerlendirildiğinde, B2 bölgesinde dişi bireylerde eliptik, erkek bireylerde kordat tip pul bulunmaktadır. Aynı şekilde, B3 bölgesinde dişi bireylerin dairesel, erkek bireylerin ise hekzagonal pul morfolojisine sahip olduğu belirlenmiştir (Şekil 3, Tablo 1). B1, B4, B5, B6 ve B7 bölgelerinde ise pul morfolojileri dişi ve erkek bireylerde aynı tiptedir (Şekil 3). Eliptik, dikdörtgen, beşgen, dikdörtgen, kare, üçgen ve sikloid gibi çeşitli türlerde pul morfolojilerinin olduğu Jawad (2005)'ın yaptığı bir çalışmada bildirilmiştir. Ktenoid pullara sahip bir balık türü olan *Saurida tumbil*'in pullarının da uzun, oblong, dikdörtgen ve kare olarak farklı morfolojide oldukları rapor edilmiştir (Jawad ve Al-Jufaili, 2007). Başka bir araştırıcının ktenoid pullara sahip *Upeneus vittatus* türünde her iki cinsiyet için pul tiplerini araştırdığı çalışmasında ise, bireylerin pullarının farklı vücut bölgelerinde dikdörtgen, kare, üçgen ve oblong gibi farklı morfolojilere sahip olduğu ve bu morfolojik farklılıkların dişi ve erkek bireyler arasında da gözlemlendiği tespit edilmiştir (Matondo vd., 2010). Bu çalışmada pul morfolojisi araştırılan *M. batrachocephalus* türünde ise diskoidal, eliptik, dairesel, kordat ve hekzagonal gibi farklı pul morfolojileri hem dişi hem de erkek bireylerde olduğu dikkat çekicidir. Literatürde bazı balık türlerinin vücut bölgelerindeki pul morfolojilerinde farklılıklar olabileceği bildirilmiştir (Jawad, 2005). Bu durum hem *U. vittatus*'un (Matondo vd., 2010) hem de *M. batrachocephalus*'un her iki eşeyinde de gözlemlenmiştir.

Pulun merkez noktası, ontogenez esnasında pulun ilk gelişen kısmıdır. Pul üzerindeki merkezin konumu, her bir türün yaşamı boyunca aynı kalmaktadır (Liu ve Shen, 1991; Jawad, 2005; Matondo vd., 2010). *M. batrachocephalus* türünün dişi ve erkek bireylerinde pul merkezinin konumu posterior bölgededir. Benzer şekilde *S. tumbil* bireylerinde de pul merkezi pozisyonunun posterior bölgede olduğu bildirilmiştir (Jawad ve Al-Jufaili, 2007). Yapılan bir çalışmada, *U. vittatus* türünün çeşitli vücut kısımlarındaki pullarının merkezinin genellikle ortada veya hafif posterior kısıma doğru oluştuğu ve belirlenen her bölgede her iki eşey için pul merkezinin konumunun aynı olduğu belirlenmiştir (Matondo vd., 2010). Bu durum *M. batrachocephalus* eşeylerinde de benzer şekildedir. Yani belirtilen türler için pul merkezinin pozisyonu açısından dişi ve erkek bireyler arasında belirgin bir fark bulunmamaktadır. *M. batrachocephalus* türünde tespit edilen posterior konumlu merkez noktasının sebebi, muhtemelen anterio-posterior yönlü bir pul büyümesinden ziyade pulun lateral yönde büyümesine bağlı olabileceği bildirilmiştir (Roberts, 1993; Jawad, 2005; Matondo vd., 2010).

Yaşam süreçleri içerisinde balıkların kemiksi yapılarındaki büyümeler ritmik olarak gerçekleşir ve bu durum sirkulus denilen büyüme halkalarının oluşmasına ve kemiksi yapıların dış yüzeyinde görünmesine izin verir (Ottaway, 1978). *M. batrachocephalus* bireylerinin pulları üzerinde bulunan sirkuluslar belirgin ve bozulmuş olarak iki farklı şekilde gözlemlenmiştir. 3. ve 4. Bölge pullarında ise rejenerasyon ve absorpsiyon olduğu da tespit edilmiştir. Bu durum sirkulusların devamlılığını bozmaktadır (Tablo 1). Sirkuluslarda gözlemlenen bu bozulmalar, sıcaklık değişimi, yumurtlama zamanı, göç, besinden mahrum kalma, büyüme gibi çevresel ve biyolojik faktörlere maruz kalmasından kaynaklanıyor olabilir (Bostancı ve Polat, 2008; Matondo vd., 2010).

Deniz türleri yanında tatlı su balık türleri için de pullarla ilgili yapılmış çalışmalar mevcuttur. Farinordin vd. (2017), Cyprinidae, Channidae, Nandidae familyalarına ait 17 tür için pul morfolojisini ve morfometrisini belirleyerek, türler-arası ayrımlarda kullanılabilecek bir anahtar oluşturmuşlardır. Matondo vd. (2012) *Glossogobius aureus* (Gobiidae), Yedier vd. (2016) *Garra rufa* (Cyprinidae), Farah-Ayuni vd. (2016) *Barbonymus schwanenfeldii, B. gonionotus, Barbodes binotatu, Cyclocheilichthys apagon, Cyprinus carpio, Hypsibarbus wetmorei, Hampala macrolepidota, Labeo rohita, Rasbora sumatrana* ve *Osteochilus vittatus* (Cyprinidae), Kontaş vd. (2020) *Cyprinion macrostomum* (Cyprinidae) türlerinde pul morfolojilerini araştırmışlardır.

Balıkların sınıflandırılmasında pul morfolojisinin değeri uzun zaman önce kabul edilmiş (Hughes, 1981), balıkların pul morfolojisi üzerine yapılan önceki araştırmalar, balık sistematiği için değerli bilgiler sağlamıştır (Jawad, 2005; Matondo vd., 2012; Yedier vd., 2016). *M. batrachocephalus* türü

kullanılarak yapılan bu çalışmanın, ileride diğer deniz türleriyle yapılacak çalışmalar için bir kaynak oluşturacağı düşünülmektedir. İncelenen morfolojik ve morfometrik karakterlerin sonuçları, *M. batrachocephalus* türünün sistematiği üzerine ileride çalışma yapacak araştırmacılara türe özgü ek morfolojik veriler sunmaktadır. Dişi ve erkek bireylerin pul morfolojileri ve morfometrileri arasında farklılıklar olabileceğinden dolayı, bu özelliklerin her iki eşey bakımından ayrı ayrı değerlendirilmesi gerekmektedir. Bu çalışma, pul morfolojisi ve morfometrisi üzerine ileride yapılacak araştırmalarda deniz türlerinin pul morfolojilerinin tanımlanması, morfometrik değerlerinin saptanması, Karadeniz, Marmara Denizi, Ege Denizi ve Akdeniz'deki balık türleri için bu bilgiler ışığında atlasların hazırlanarak türler hakkında daha kapsamlı bilgiler sağlanmasına katkı sağlayabilir.

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New Occurrence of Atlantic Tripletail, *Lobotes surinamensis* (Bloch, 1790) from the Turkish Part of the Aegean Sea, with Biological Notes

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Research Article

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Abstract

A new record of *Lobotes surinamensis* (Bloch, 1790) around Saros Bay, North Aegean Sea is presented, based on a single hermaphrodite specimen with detailed biological information. On 12 June 2018, the specimen was captured by fishermen with gillnets at a depth of 25 m. Sex, age, and stomach content were determined. This new record proves the Northernmost distribution of the species in the Turkish part of the Aegean Sea.

Keywords: Non-native, spread, record, population, Aegean Sea

Ege Denizi'nin Türkiye Kıyılarından Atlantik Üç Kuyruk Lobotes surinamensis (Bloch, 1790) Yeni Kaydı ve Bazı Biyolojik Özellikleri

Özet

Bu çalışmada, *Lobotes surinamensis* (Bloch, 1790)'in Saroz Körfezi'ndeki yeni kaydı tek dişi birey ile detaylı biyolojik bilgileriyle birlikte verilmiştir. Birey balıkçıların uzatma ağlarından 25 m derinlikte 12 Haziran 2018 tarihinde yakalanmıştır. Cinsiyet, yaş ve mide içeriği belirlenmiştir. Bu yeni kayıt türün Kuzey Ege'nin Türk sularındaki en kuzey dağılımını göstermektedir.

Anahtar Kelimeler: Yerel olmayan, yayılım, kayıt, popülasyon, Ege Denizi

INTRODUCTION

Atlantic tripletail, *Lobotes surinamensis* (Bloch, 1790) is a member of the family Lobotidae, is represented only single species in Turkey. Tropical and temperate waters of all oceans host Atlantic tripletail. A widespread distribution of this species only restricted around Eastern Pacific (Froese and Pauly, 2018). Although it is considered rare around the Mediterranean (Tortonese, 1975), Atlantic Tripletail continues its distribution towards the North day by day.

According to documented records, the Atlantic Triple Tail has spread from the Arabian sea and the Oman sea to the entire Mediterranean basin (Manilo and Bogorodsky, 2003; Al-Jufaili et al., 2010; Javad et al., 2015). After being identified in Sicilian waters (Doderlein, 1875), it has been reported in the Southeastern Mediterranean (Tortonese, 1975) and Israeli waters (Golani, 1996). When the records of the last 20 years were examined, many records in the Mediterranean Basin showed that the species has become more common in this region (Camilleri et al., 2005; Minos and Economidis, 2007; Zava et al., 2007; Deidun et al., 2010; Dulcic et al., 2014a; Dulcic et al., 2014b). On a large scale, Northernmost occurrance of this species were notified from Nova Scotia (Gilhen and McAllister, 1985) in the North Atlantic; from the Russian waters (Kharin et al., 2009) in the Western Pacific; Barcelona (Palom, 1991) in the Western Mediterranean and Rasa Bay (Dulcic et al., 2014b) in the Adriatic. Northern extension of Atlantic Tripletail in the Aegean Sea has been recorded by Greek and Turkish researchers. In the Aegean Sea, the first record was given by Economidis (1973). In the Turkish part, Akyol and Kara (2012) were realized first record from Izmir Bay. Up to today, the Northernmost occurrence has been identified by Tunçer and Önal (2016) from Çanakkale Strait. Also,

Gönülal and Güreşen (2014) were reported from Gökçeada Island and Bilge et al. (2017) were reported from Southern Aegean Sea.

Detailed studies were conducted on age, growth, reproductive biology, and feeding habits of the species around the Gulf of Mexico (Brown-Peterson and Franks, 2001; Franks et al., 2003; Strelcheck et al., 2004). Due to limited occurrences, no study has been found in the Mediterranean yet.

The present study aims to report the Northernmost occurrence of the *L. surinamensis*, in the Turkish part of the Aegean Sea. Beside, some biological notes were shared to increase knowledge on the biology of this species.

MATERIALS and METHODS

One hermaphrodite individual was provided by a local fisherman on 12 June 2018 from Sultaniçe shores in the Saros Bay, North Aegean Sea. A single specimen of Atlantic Tripletail was caught with gillnets at a depth of 25 m. The specimen was identified according to Tortonese (1975) and Dulcic et al. (2014a). It was photographed, some morphometric characters measured and meristic characters were counted (Figure 1).

Figure 1. Atlantic Tripletail, *Lobotes surinamensis*

The total length (TL) was measured to the nearest millimeter using a measuring board, and the total weight, gonad weight, and otolith weight were recorded to the nearest gram using precision scales. The length and width of otoliths were measured using the Q-Image digital imaging program. Sex and maturity stages were determined by macroscopic observation of male and female gonads. Age was estimated by interpreting annual growth rings on otolith according to Iglesias and Dery's (1981) method. Readings were made by three independent researchers. Otolith rings were counted under reflected light by placing the concave side of the otolith against the glass surface. The sexual maturity stage was determined with Holden and Raitt's (1974). The stomach was dried on a blotting paper, weighted with precision scales, and prey were sorted and identified to the lowest possible taxonomic level.

RESULTS

Some morphometric measurements and meristic counts of the individual are given in Table 1. The total length and weight were measured as 379 mm and 1254.6 g, respectively. The lateral line goes along nearly straight and has 56 ctenoid scales. The species has a relatively long head with a 29% of the total length. Anus is situated about 75% of the total length through the backward. Body morphology can be definable as highly compressed with a high body depth, corresponding to 48% of the total length.

Table 1. Morphometric measurements, otolith measurements, and meristic counts of the single specimen of *L. surinamensis* (Bloch, 1790) and comparison with previous data obtained from varied studies.

	In this study	**Amor et al. (2016)**		**Kechaou et al. (2018)**	**De Carlo et al. (2017)**	**Javad et al. (2015)**
Region	**North Aegean Sea**	**North-Eastern Tunisia**		**Tunisian Waters**	**Liguraın Sea**	**Oman Sea**
Sex	Hermaphrodite	♀	♀	-	Hermaphrodite	-
Morphometric measurements (cm)						
Total length (TL)	37.9	39	25	43	42.7	63.0
Standard length (SL)	33.5	33.4	19.9	36.5	-	53.0
Head length	11	5.5	5.1	12	12.4	24,5
Interorbital space	3.5	2.0	1.7	3.5	3.4	-
Predorsal length	12.5	6.7	5.1	13	12.9	17.0
Preanal length	21.5	22.5	15.8	24	27	36.5
Prepectoral length	11	-	-	-	13.3	19.0
Prepelvic length	12.5	5.7	4.7	12.5	15.6	-
Max.Body depth	18	-	-	-	15.5	34.0
Eye diameter	1.3	-	-	-	1.2	2.0
Preorbital length	2.5	-	-	-	2.4	-
Meristic counts						
Dorsal fin rays	XII + 16	XII + 16	XII + 15	XII + 16	XII + 15	-
Anal fin rays	III + 12	III + 13	III + 12	III + 12	III + 11	-
Pectoral fin rays	16	12	12	13	15	-
Caudal fin rays	16	18	18	18	16	-
Pelvic fin rays	I + 5	I + 5	I + 5	I + 5	I + 5	-
Gilrakers	12	-	-	-	-	-
Linea lateralis scal	56	-	-	-	-	-
Operculum rays	VII	-	-	-	-	-
Weight (g)						
Total weight	1254.6	1450	950	1206	1377	-
Gonad	♀2.2 – ♂4.82	7.5	6.8	-	♀1.2 – ♂0.7	-
Otolith measurements(mm)	Right / Left					
Width	9.21 / 8.99	-	-	-	-	-
Height	5.067 / 5.223	-	-	-	-	-
Weight (g)	0.0463 / 0.0455	-	-	-	-	-

Otolith length, width, and weight measured as 9.21 mm, 5.07 mm, and 0.0463 g, respectively. There were no statistical differences in morphological measurements for both right and left otoliths.

Only single growth ring was determined both right and left sagittal otoliths and age were identified as 1 (Figure 2).

Figure 2. Right sagittal otolith of *L. surinamensis*

According to macroscopic observation, sex was determined as a hermaphrodite. Female gonad was observed as small, dully transparent, and pinkish-whitish, and sexual maturity defined as 2nd stage. Also, testis was whitish to creamy and determined as 3rd stage (Figure 3a). The stomach was weighted as 12.56 g and only a single prey type was observed. Preys were poorly digested, so species of fish in the stomach content identified as *Atherina boyeri* (Figure 3b).

Figure 3. a) Hermaphrodite gonad of *L. Surinamensis* **b)** stomach content and prey type (*A. boyeri*)

DISCUSSION

Azzurro (2008) described Atlantic Tripletail as a native Mediterranean species whose range is expanding northwards. On the contrary, lots of researchers identified this species as a thermophilic non-native fish species. Tiralongo et al. (2018) were detected a well-established population around the Southern Tyrrhenian Sea, in the Central Mediterranean. In recent years, some juvenile specimens has been recorded from varied regions in the Mediterranean (Deidun et al., 2010; Tiralongo et al., 2018;

Ergüden et al., 2018). According to these results, it seems that Atlantic Tripletail can be defined as a native species of the Mediterranean.

The northernmost distribution of Atlantic Tripletail in the Mediterranean has been given in some works. Atlantic Tripletail has been reported from Barcelona shores in Western Mediterranean (Palom, 1991); from Rasa Bay, Adriatic (Dulcic et al., 2014b); from Izmir Bay, Central Aegean Sea (Akyol and Kara, 2012) and Dardanelles, North Aegean Sea (Tuncer and Onal, 2016). Minasidis et al. (2020) were lastly recorded around the Northwesternmost shores of the Aegean Sea (Strimonikos Gulf, Delta Evrou, Thermaikos Gulf, and the Thracian Sea). They were reported 5 individuals in the area between 2018 and 2019. In this study, the single hermaphrodite specimen was caught with gillnets on 12 June 2018 around Sultaniçe shores, Saros Bay, North Aegean Sea. This record constituted the Northernmost occurrence of the Atlantic Tripletail in the Turkish part of the Aegean Sea. Minasidis et al. (2020) were collected their records as part of the citizen science project. Authors stated that Atlantic Tripletail has become more common in the region. This hypothesis was supported by this simultaneous report. Atlantic Tripletail may become more common and reproduction may occur soon. Therefore, the early life stages and juveniles of the species should be closely monitored.

Morphometric measurements and meristic counts were given and compared in Table 1. Our findings on morphometric measurements were consistent with the findings of Kechaou et al. (2018) and De Carlo et al. (2017). However, some differences were observed on the morphometric measurement of 39 cm TL individual of Amor et al. (2016). These differences mostly occurred in head length, prepelvic length, and predorsal length. As can be seen in Figure 1, these measurements were relatively higher for 39 cm TL individuals. When compared the morphometric data with the findings of the study conducted in the Sea of Oman (Javad et al., 2015), relatively the same values were obtained by percentage of the total length. Information on population dynamics and reproductive biology is absent due to the lack of extensive stock in the Mediterranean. The knowledge on the biology of Atlantic Tripletail has been revealed around Mexico Gulf. Strelcheck et al. (2004) were evaluated 119 specimens, ranging in size from 293 to 763 mm TL. Sexual maturity of females was estimated as between 494 and 594 mm TL and approximately approximately 1 and 2 years old. They stated that the diet of Atlantic Tripletail mainly consists of shrimps, pelagic fish species, and crabs. Authors detected that the ratio of fish consumed as prey increased with increasing of the Atlantic Tripletail length. Besides, they argued that consumed fish constituted %31 of total prey in weight. Franks et al. (2003) were determined the feeding habits of Atlantic Tripletail around Mississippi, Gulf of Mexico. Authors were found that fishes and Crustaceans constituted total prey abundance, equally. In our study, we observed that a single hermaphrodite specimen could not reach the first maturity with its 379 mm TL according to Strelcheck's (2004) results. Synchronous hermaphroditism was determined in the studies conducted in the Ligurian Sea (De Carlo et al., 2017) and North Aegean Sea (Minos and Economidis, 2007). In both these studies, no ripe female gonads were observed. Since none of these studies had histological gonad evaluation, information about reproductive biology is insufficient.

Only a single growth ring was found, so age was determined as 1. In stomach content, a single prey type was found as small pelagic fish, *A. boyeri*. Undoubtedly, a single specimen will not give sufficient information in terms of comparison with previous studies. However, age-length key and first sexual maturity length showed close data. As stated by Strelcheck et al. (2004) and Franks et al. (2003), pelagic fish was also found as prey, in this study.

Atlantic Tripletail, *L.surinamensis* appears to expand it's spread up to the Northernmost of the Aegean Sea. It has not been reported from the Sea of Marmara and the Black Sea, yet. Besides, fewer reports have been found around the Eastern part of the North Aegean Sea, where under the influence of the Black Sea Water (BSW) inflow. BSW, which shows features of lower temperature and salinity, may create a barrier to the spread of the species via the Sea of Marmara and the Black Sea. With the global warming of the waters, we will see whether it can overcome this barrier in the forthcoming years. Beside Minos and Economidis (2007) presented a different hypothesis on the dispersal of this species. According to the authors, seasonal movements of surface currents have influenced on the dispersal of Tripletail due to its co-existence behavior with the floating–mediated passive objects. With the effect of these currents, it expands its distribution to the North in the summer months for foraging and dispersing to new areas. Although it is difficult to present an opinion on this issue with a single individual, the sampling time of individual (June) may confirm this hypothesis.

Consequently, the spread of uncommon species in a region should be monitored in terms of ecological balance. The knowledge on stock structure, reproductive capability, and feeding ecology should be increased. Potential impacts on their new habitat should be identified.

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Evaluation of the Water Quality of Karabal Stream (Gediz River, Turkey) and Comparative Performance of the Used Indices

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Abstract

We used seven biotic indices to determine the water quality of Karabal Stream (Gediz River) in West Anatolia, Turkey. The indices were based on benthic macroinvertebrate and physicochemical parameters: Saprobi Index (SI), Biological Monitoring Working Party (BMWP), Average Score per Taxon (ASPT), Family Biotic Index (FBI), Belgian Biotic Index (BBI), EPT-Taxa [%] were used as biotic indices and Shannon-Weaver index (SWDI), Simpsons index (SDI) and Margalef index (MDI) for diversity. Ten taxonomic groups were found in Karabal Stream consisting of Crustacea, Oligochaeta, Gastrapoda, Ephemeroptera, Plecoptera, Trichoptera, Odonata, Coleoptera, Diptera, and Hemiptera. The water quality along the stream varied from good class in the upstream stations, to moderate in downstream stations according to the most suitable indices. According to canonic correspondence analysis (CCA), the distributions of Diptera, Oligochaeta, and Hemiptera species are positively correlated to EC, Cl, Turbidity, Temperature, NH_4-N, NO_2-N, and NO_3-N while they are negatively correlated to DO, DOS and pH. The distributions of EPT species are positively correlated to DO, DOS and pH. According to Pearson's correlation, the BBI, BMWP (Original), BMWP (Spanish), and EPT-Taxa [%] metrics were sufficient in the estimation of water quality in the examined watercourse. Considering studies in surface waters of Turkey, the BMWP and EPT-Taxa [%] indices reflect the water quality as correctly in all studies we examined, however, BBI did not always show reliable results. Therefore there is a need for the establishment of a Turkish Biotic Index which takes into account country-specific macroinvertebrates, their abundance, biology, and ecology.

Keywords: Karabal Stream, Water Quality, Benthic Macroinvertebrates, Physicochemical Parameters, Biotic and Diversity Indices.

Karabal Çayı'nın (Gediz Nehri, Türkiye) Su Kalitesinin Değerlendirilmesi ve Kullanılan Endeks Performanslarının Karşılaştırılması

Özet

Türkiye'nin Batı Anadolu Bölgesi'ndeki Karabal Çayı'nın (Gediz nehri kolu) su kalitesini belirlemek için yedi biyotik indeks kullanılmıştır. Kullanılan indeksler bentik makroomurgasızlar ve fizikokimyasal parametreler temellidir. Biyotik indeks olarak; Saprobi İndeks (SI), Biyolojik Çalışma Grubu İndeksi (BMWP), Takson Başına Ortalama Puan (ASPT), Familya Biyotik İndeksi (FBI) ve Belçika Biyotik İndeksi (BBI), çeşitlilik indeksi olarak; Shannon-Weaver indeksi (SWDI), Simpsons indeksi (SDI) ve Margalef indeksi (MDI) kullanılmıştır. Karabal Çayı'nda Crustacea, Oligochaeta, Gastrapoda, Ephemeroptera, Plecoptera, Trichoptera, Odonata, Coleoptera, Diptera ve Hemiptera'dan oluşan 10 taksonomik grup saptanmıştır. Akarsu boyunca su kalitesi, indekslere göre akarsuyun memba kesimlerinde iyi sınıftan akarsuyun aşağı mansap kesimlerinde orta seviyeye kadar değişmektedir. Kanonik korelasyon analizi (CCA)'ne göre, Diptera, Oligochaeta ve Hemiptera türlerinin dağılımları, ÇO, ÇOS ve pH ile negatif korelasyon gösterirken EC, Cl, Turbidite, Sıcalık, NH_4-N, NO_2-N ve NO_3-N ile pozitif korelasyon göstermektedir. EPT türlerinin dağılımları ÇO, ÇOS ve pH ile pozitif korelasyon göstermiştir. Pearson korelasyon analizine göre BBI, BMWP (Orijinal), BMWP (İspanyol) ve EPT-Taksa [%] indeksleri incelenen akarsu üzerindeki su kalitesi tahmini için yeterli bulunmuştur. Türkiye yüzey sularında yapılan diğer çalışmalar da dikkate alındığında BMWP ve EPT-Taxa [%] indeksleri su kalitesi belirlemeye yönelik yapılan tüm çalışmalarda olduğu gibi bu çalışmada da su kalitesi açısından yeterli doğruluk yansıtmaktadır, ancak BBI her zaman güvenilir sonuçlar vermemiştir. Bu nedenle, ülkeye özgü makroomurgasız gruplarının bolluklarını, biyolojilerini ve ekolojilerini dikkate alan bir Türkiye Biyotik İndeksi'nin oluşturulmasına ihtiyaç vardır.

Anahtar Kelimeler: Karabal Çayı, Su Kalitesi, Bentik Makroomurgasızlar, Fizikokimyasal Parametreler, Biyotik ve Çeşitlilik İndeksleri.

INTRODUCTION

The existence of a sufficient amount of good quality water is an essential need for the future of humanity and sustainable development. Monitoring studies on freshwater resources is the first step in conservation policies. In the late 20th century, the scarcity of freshwater resources reached the point that would threaten food safety, livelihood, and health of people (Falkenmark, 1989; Kundzewicz, 1997; Vorosmarty et al., 2000). The freshwater ecosystems supply 40% of all food and agricultural crops production by irrigation, 12% of fish consumed by humans, and produces 20% of electrical energy in the World (Johnson et al., 2001). In addition to the direct effects of water scarcity, the degradation of water quality reduces its usability.

More than 3 billion people in the world can not access clean water, and this problem is of particular importance in developing countries where 90% of untreated wastewater is discharged into streams (Johnson et al., 2001). So to maintain the water quality of the existing freshwater ecosystems very important. Biological water quality monitoring in all surface water systems was obligated by the EU Water Framework Directive (WFD). According to the WFD, the bio-indicators such as benthic macroinvertebrates, phytoplankton, phytobentose (diatom), fish, aquatic macrophyte, macroalgae, and angiosperm are biological quality components in water quality monitoring. Among all these groups, benthic invertebrates are the most advantageous. Benthic macroinvertebrates live above or inside the bottom substrates of freshwater and marine ecosystems. They have limited mobility and have an important place in the food chain of freshwater ecosystems. The responses of the macroinvertebrate community to environmental changes are particularly useful in assessing the impact of every kind of pollutants in surface waters.

Many biotic indices were created according to specific geographic and climatic regions. European countries have created various indices with variable diagnosis and counting of different organisms (Korycińska and Królak, 2006; Yorulmaz et al., 2015). The Saprobi Index (SI) (Kolkwitz and Marsson, 1902) in Germany, the BMWP and ASPT (De Pauw and Hawkes, 1993) in England, the BBI (De Pauw and Heylen, 2001) in Belgium, the FBI (Hilsenhoff, 1988) in the USA, Danish Stream Fauna Index (DSFI) (Skriver et al., 2001) in Denmark, give the most reliable results specific to geographical regions. A lot of biotic indices have been used to determine water quality in Turkey (Kazancı and Dügel, 2000; Kalyoncu and Zeybek, 2009; Kazancı et al., 2010; Zeybek et al., 2014; Yorulmaz et al., 2015; Zeybek, 2017). Since the studies are conducted for 25 years, a biological water quality index specific to Turkey has not been developed.

Gediz River watercourse have been polluted due to increasing industrialization and population growth. It's aimed to investigate the level of pollution and its effect on the macroinvertebrates in the Karabal Stream. With this study, to compare the performances of biotic and diversity indices.

MATERIALS and METHODS

Study Area

This study was carried out on Karabal Stream (Gediz River, Turkey) (Figure 1). The length of the stream is 58 km. Karabal Stream is 20 km away from Uşak ctiy center. Uşak Industrial Zone was established 6 km east of the stream. Güre Plain is located at the junction of Karabal Stream and Gediz River. The Mediterranean climate prevails in the region.

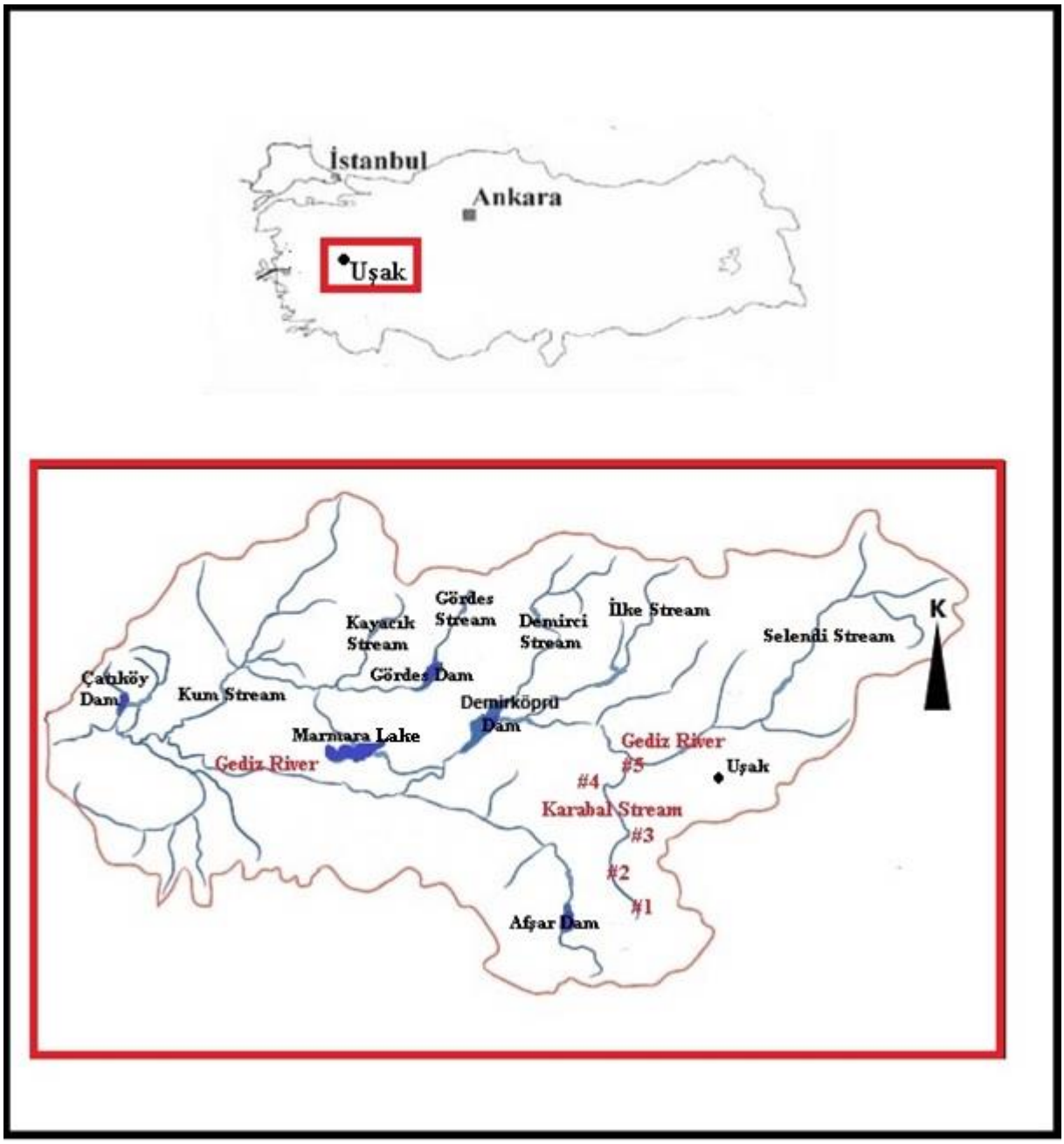

Figure 1. Map of the sampling stations in Karabal Stream.

Karabal Stream is used as an irrigation water source for the surrounding agricultural fields. The sampling stations were chosen based on stream source and domestic areas. The research was conducted between April 2019 and March 2020 at five monitoring stations that included the upstream (#1, #2, #3), and downstream (#4 and #5) sections. The sampling was carried out seasonally (April, August, November, February) over a year in the stream. Some key characteristics of sampling stations are presented in Table 1.

Table 1. Some key characteristics of sampling stations in Karabal Stream.

	Sampling Station	Coordinates (N-E)	Habitat	Stream morphology	Riparian vegetation
#1	The source point of the stream	38° 64' N 29° 34' E	Large rocks (> 80 cm) mixed with gravel and wood debris	No Macrophyte was present	It's not well developed.
#2	Karabal Stream in village Kayağıl. Agricultural areas and thermal pools are intense around the stream.	38° 63' N 29° 29' E	Large rocks, gravel, and wood debris.	Macrophyte was present	Well developed on both sides.
#3	Karabal Stream in village Eskisarat. Agricultural areas and domestic settlements are intense around the stream.	38° 64' N 29° 24' E	Sand, gravel, silt, and cobbles	Domestic wastes inflow. No Macrophyte was present	It's not well developed.
#4	Karabal Stream in village Güre. Agricultural areas and domestic settlements are intense around the stream.	38° 64' N 29° 16' E	Sand, gravel, silt, and cobbles	Domestic wastes inflow. No Macrophyte was present	It's not well developed.
#5	The point where the Karabal Stream drains into the Gediz River.	38° 67' N 29° 13' E	Cobbles, pebbles, sand, and muddy	Macrophyte present.	Well developed on both sides.

Benthic macroinvertebrates were collected from each station by using a classic 50x30 cm size with a 250 µm mesh hand net. Macroinvertebrate sampling was done from downstream towards upstream. Macroinvertebrates were obtained from the different substrate types such as rock, sand, and gravel present at the stations. Some sessile organisms adhering to the large stone, rock, or any other substrate, were collected by using forceps (AQEM Consortium, 2002). The taken organisms were stored in 70% alcohol and 4% formaldehyde and then brought to the Ege University Hydrobiology Research Laboratory. Brought samples from the stream were categorized and diagnosed to the lowest possible taxonomic level such as genus or species, under a stereomicroscope.

Physicochemical Parameters

To determine the water quality classes, 16 physicochemical parameters were monitored seasonally. Water temperature (°C), pH, electrical conductivity (EC), turbidity, oxygen saturation (DOS), and dissolved oxygen (DO) of each water sample were measured at the sampling points by oxygen meter and multiparameter. The biochemical oxygen demand (BOI_5) was assessed by using a spectrophotometer on the base of the Winkler azide method with Merck BOD Cell Test Kits. The orthophosphate (PO_4-P), ammoniacal nitrogen (NH_4-N), nitrate (NO_2-N), nitrite (NO_3-N), and chloride (Cl^-) were by using appropriate Merck kits according to manufacturer's instructions (Merck Phosphate, Ammonium, Nitrite, Nitrate, and Chloride Test Kits). Major cations such as K, Ca and Na were evaluated by flame photometry after the acid-treated on water samples, while Mg was evaluated by using the Flame Atomic Absorption Spectrometer (FAAS). All water samples were stored in an insulated cooler containing ice and taken on the same day to the laboratory and stored at 4°C until processing and analysis (APHA, 2005).

Biotic Indices

The benthic macroinvertebrates were analyzed to determine the biological quality score by using ASTERICS 4.04 software program (AQEM Consortium, 2002). With this purpose, the BBI, FBI, SI, ASPT, BMWP (Original), BMWP (Spanish) biotic indices, and SWDI, SDI, MDI diversity indices were used to determine the water quality of Karabal Stream.

Data Analysis

All statistical analyses between the physicochemical data sets, Pearson correlation analysis and biotic parameters were made using Excel 2019 (Microsoft Office®) and SPSS version 20.0. In this study, the relationship between physicochemical parameters and macroinvertebrates were determined

by using CCA based on multivariate statistical analysis (Ter Braak, 1995). Pearson correlation analysis is used to express the relationships between indices and physicochemical parameters. The faunal similarities based on benthic macroinvertebrates between the sampling stations were assessed by using the Bray-Curtis similarity index (Sommerfield, 2008; Yoshioka, 2008).

RESULTS and DISCUSSION

Physical and Chemical Parameters

The results of physicochemical variables were presented in Table 2.

Table 2. The mean and standard deviation values of physicochemical parameters in sampling stations.

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5
T	M±SD	11.4±0.21	11.8±0.28	13.0±0.67	13.9±0.85	14.4±0.62
(°C)	SE	0.062	0.081	0.185	0.228	0.163
pH	M±SD	7.13±0.04	7.03±0.04	6.70±0.08	6.60±0.08	6.95±0.05
	SE	0.014	0.015	0.030	0.031	0.018
EC	M±SD	149.2±10.6	159.7±10.7	393.2±25.8	405.0±24.5	210.2±8.22
(mS/m)	SE	0.867	0.846	1.301	1.217	0.566
Turbidity	M±SD	1.64±0.22	1.90±0.18	60.8±11.2	64.2±11.8	8.77±1.11
(NTU)	SE	0.171	0.130	1.436	1.472	0.374
DO	M±SD	11.9±0.25	11.4±0.31	10.8±0.29	10.4±0.29	10.1±0.18
(mg/l)	SE	0.072	0.091	0.088	0.089	0.056
DOS	M±SD	126.2±9.74	113.2±4.99	95.7±3.59	92.5±3.69	101.0±5.09
(%)	SE	0.867	0.469	0.367	0.383	0.506
BOI_5	M±SD	1.11±0.05	1.24±0.11	6.26±0.86	6.76±1.10	2.00± 0.11
(mg/l)	SE	0.047	0.098	0.343	0.423	0.077
NH_4-N	M±SD	0.035±0.012	0.24±0.06	7.67±2.25	7.90±2.26	1.21±0.10
(mg/L)	SE	0.064	0.122	0.812	0.804	0.090
NO_2-N	M±SD	0.06±0,01	0.13±0.035	4.94±0.68	5.10±0.36	1.95±0.17
(mg/L)	SE	0.040	0.097	0.306	0.159	0,121
NO_3-N	M±SD	1.06±0.04	1.09±0.03	5.92±0.82	6.00±0.66	1.85±0.12
(mg/L)	SE	0.038	0.028	0.337	0.269	0.088
Cl^-	M±SD	2.01±0.13	2.50±0.48	23.2±4.38	22.9±3.67	3.80±0.41
(mg/L)	SE	0.091	0.303	0.909	0.766	0.210
Ca	M±SD	21.1±2.12	24.6±5.16	56.0±8.28	54.1±8.30	30.7±4.11
(mg/L)	SE	0.461	1.040	1.106	1.128	1.347
Mg	M±SD	10.7±1.26	11.5±1.49	27.7±5.78	29.8±7.54	17.3±2.15
(mg/L)	SE	0.385	0.439	1.098	1.381	0.516
K	M±SD	12.1±0.80	12.9±1.00	27.9±3.25	29.2±3.94	15.1±0.98
(mg/L)	SE	0.230	0.278	0.425	0.729	0.252
Na	M±SD	15.9±0.40	16.4±0.49	30.9±7.56	31.9±7.04	17.5±0.86
(mg/L)	SE	0.100	0.121	1.360	1.246	0.205
PO_4	M±SD	0.025±0.019	0.032±0.017	5.43±1.10	5.34±0.86	0.77±0.17
(mg N/L)	SE	0.120	0.095	0.472	0.372	0.193

M: Mean; SD: Standard deviation; SE: Standard error

It is known that the metabolism of organisms varies with temperature. DO and DOS which is vital for aquatic organisms varies depending on the temperature (Wetzel, 2001; Tanyolaç, 2004). Benthic macroinvertebrates are sensitive to changing temperature and oxygen in the water and as temperature increases and oxygen decreases, sensitive organisms are being replaced by tolerant organisms (Wetzel, 2001; Tanyolaç, 2004; Walczyńska and Sobczyk, 2017). The Karabal Stream is suitable for organisms in terms of temperature, DO and DOS. Uyanık et al., (2005) reported the lowest DO value at the sampling point (8.3 mg/L) after the mixture of industrial and domestic wastewater in their study on Eğri Stream. Kara and Çömlekçioğlu (2004) examined the pollution of Karaçay Stream with

biological and physicochemical parameters and stated that the amount of DO was low at the sampling point after the domestic wastes mixed. Dügel (1995) and Barlas et al., (2000) reached similar results in their studies on Yuvarlakçay Stream. Kalyoncu et al., (2008) reached similar results in their studies on Aksu Stream BOI_5 is of great importance as it is a measure of organic pollution in aquatic ecosystems (WHO, 2011). In Karabal Stream, the highest BOI_5 values were measured at the sampling points (#3 and #4) affected by domestic wastes. pH, which is an indicator of the acidity of water, is one of the important factors affecting life (Wetzel, 2001; Tanyolaç, 2004). According to the pH data determined in Karabal Stream, there is no risk for organisms. The turbidity of the downstream stations of the Karabal Stream was found to be high. EC values are quite high at stations #3 and #4. At these sampling points, the accumulation of suspended solids due to the low slope and pollution pressure. This increased both density and EC values. Kalyoncu et al., (2005) and Kalyoncu and Zeybek (2009) obtained similar results in Aksu Stream and Isparta Stream, respectively. The high Cl^- values indicate that the electrical conductivity is also high. The amount of chloride in many drinking waters does not exceed 30 mg/L (Egemen and Sunlu, 1996). Cl^- values are quite high at stations #3 and #4 parallel with the turbidity and EC values. The increasing major cations such as K, Ca, Na and Mg amounts in water are due to household detergents (Tanyolaç, 2004). These major cations were found to be quite high at stations #3 and #4 due to dense domestic settlements. Elements that limit efficiency in aquatic environments are mostly PO_4, NH_4-N, NO_2-N, NO_3-N (Moss, 1987). The NO_3-N in groundwater and surface waters results from the oxidation of ammonia, which occurs as a result of the decomposition of proteins contained in vegetable and animal wastes, and nitrate fertilizers used in agricultural areas. NO_3-N is the most common form of nitrogen in freshwaters, and it is very rare in uncontaminated waters (Wetzel, 2001). It is also below the limit value reported as 50 mg/L in WHO, where the NO_3-N values determined at all stations examined in the stream are below the recommended 10 mg/L limit value in healthy waters reported by EPA. (WHO, 2011). The increase of PO_4, NH_4-N, NO_2-N, NO_3-N in stations #3 and #4 showed the richness of these two stations with nutrients caused by discharges of domestic wastes in the vicinity of Karabal Stream.

Benthic Macroinvertebrate Data

In this study, a total of 3.748 benthic macroinvertebrate samples were collected; all the specimens collected belong to nine groups: Crustacea, Oligochaeta, Gastropoda, Ephemeroptera, Plecoptera, Trichoptera, Odonata, Coleoptera, Diptera and Hemiptera. The maximum numbers of individuals were collected at station #1 (1.013 individuals), while the minimum numbers of individuals were collected at station #4 (572 individuals). The lowest number of individuals was determined in autumn while the highest number of individuals was determined in spring with the collection of all benthic invertebrate samples (Figure 2).

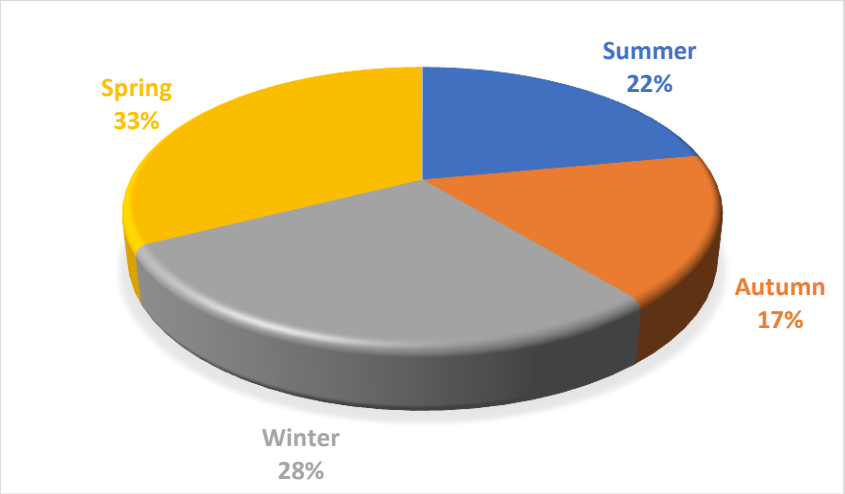

Figure 2. The total percent of benthic macroinvertebrates according to season.

As a result of this count, the most dominant group in all benthic macroinvertebrate groups was Insecta in the stream (Figure 3).

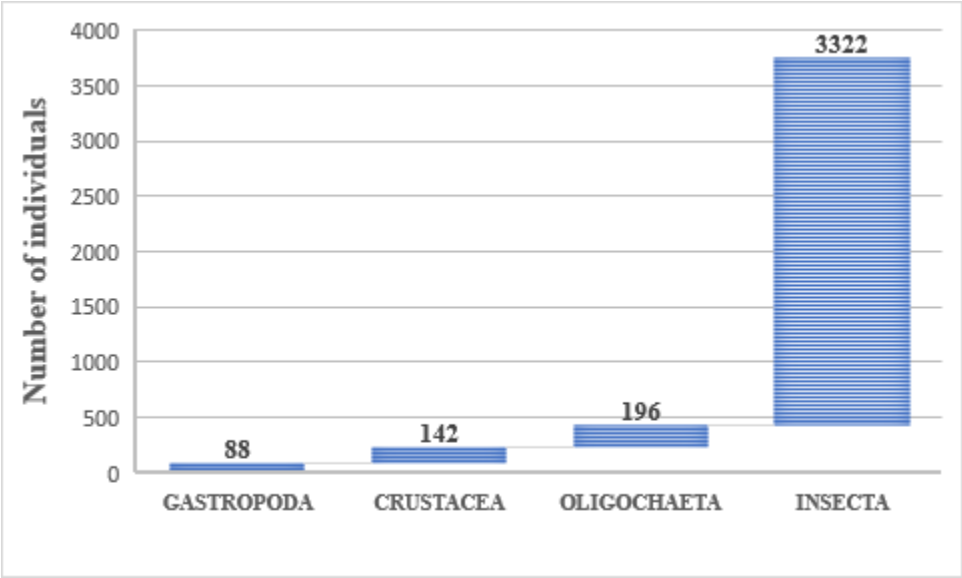

Figure 3. Benthic invertebrate groups in Karabal Stream.

Considering all taxonomic groups in Karabal Stream, Ephemeroptera was the most dominant group in station #1 (40.8%), station #2 (41%) and station #5 (31.2%); Diptera was the most dominant group in station #3 (28.6%), and station #4 (28.3%); Pleoptera was the second dominant group in station #1 (26.8%) and #2 (22.7%); Trichoptera was the second dominant group in station #3 (18.9%) and #5 (23.3%) (Figure 4).

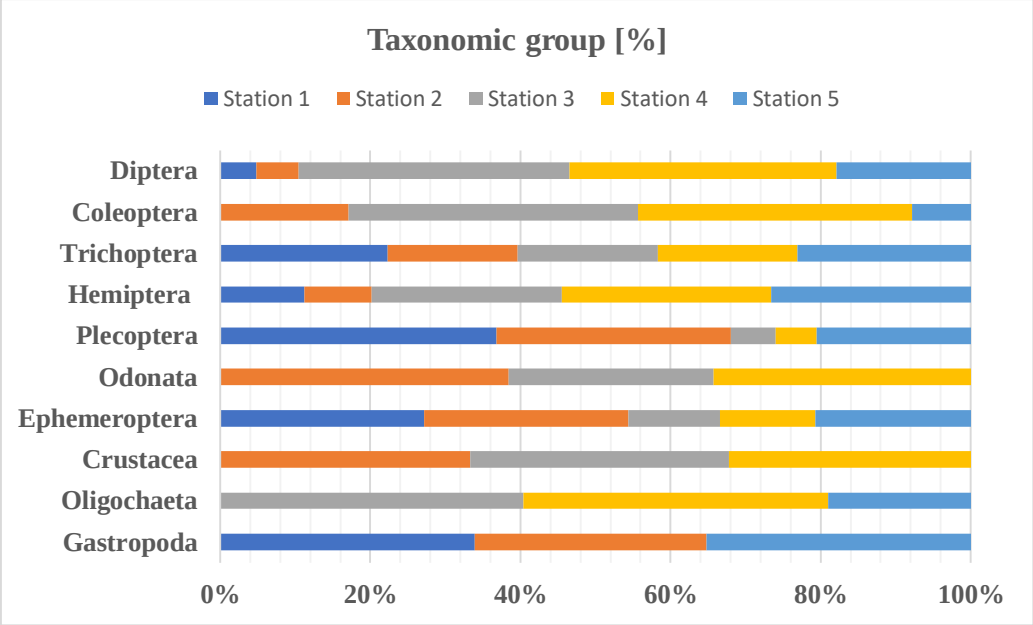

Figure 4. Distribution of taxonomic groups in Karabal Stream.

Out of 25 families identified, Insects are the richest group represented with 21 families: Ephemeroptera (6), Plecoptera (4), Trichoptera (4), Odonata (1), Coleoptera (1), Diptera (3), and Hemiptera (2) that make up 89.15% of the macroinvertebrates of the Karabal Stream Crustacea were represented with 1 family: Gammaridae, which consists 3.79% of macroinvertebrates; Oligochaeta were represented with 1 family: Tubificidae, which consists 5.23% of macroinvertebrates; Gastropoda were represented with 2 families: Bithyniidae and Lymnaeidae, which consists 2.35% of macroinvertebrates.

The dominance of benthic macroinvertebrate species according to the stations is shown in the Figure 5. As a result of the observations, *Gammarus* sp. was dominant at the station #2, #3 and #4.

Gammarus sp., which belongs to the group of Amphipoda is found in low polluted river sections (Meyer, 1987). *Chironomus* sp. and *Chironomus plumosus* were dominant species at the station #3 and #4. These species are an indicator for polysaprobic (heavily polluted) aquatic systems (Kalyoncu and Zeybek 2010; Arslan et al., 2016; Zeybek, 2017). According to Moisan and Pelletier (2008), the tolerance range of these organisms are high They can inhabit an environment with low or high DO (mg/l), DOS (%) and T (°C). The abundance of organic matter is favorable for benthic macroinvertebrates such as Diptera and Oligochaeta (Rashid and Pandit, 2014). Kalyoncu and Zeybek (2009) determined that the 6th station, which is the downstream point of the stream, has low organism diversity. On the other hand, the most dominant group was Diptera followed by Oligochaeta (*Tubifex tubifex*). *Chironomus thummi* from Diptera, and *Simulium* sp. were the most dominant taxa in Isparta Stream.

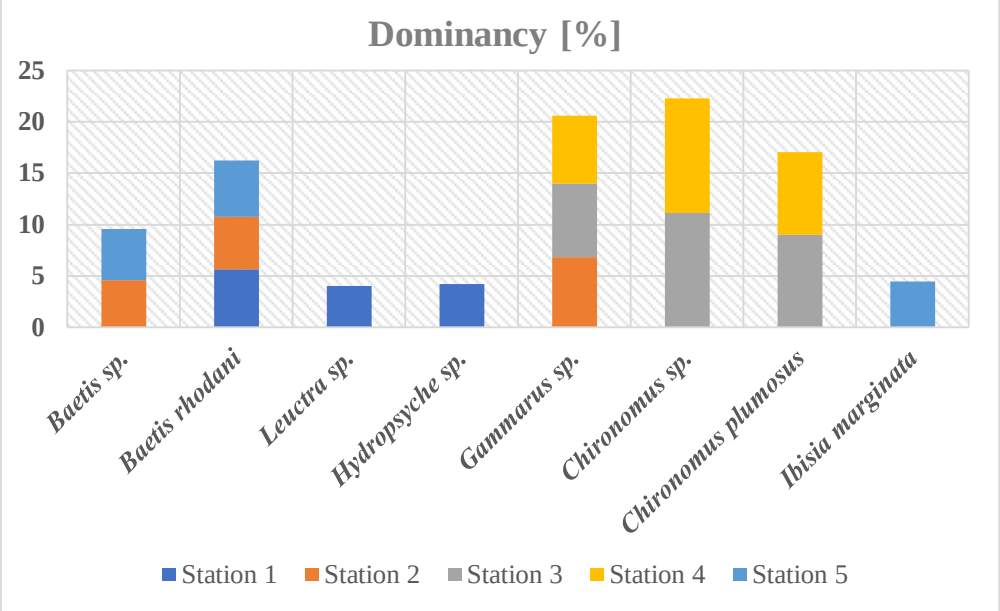

Figure 5. Dominancy (%) of taxon of benthic macroinvertebrates at the stations.

In terms of diversity, the richest were sampling station #1 and station #2 with 20 families each, 14 of them belonging to Ephemeroptera- Plecoptera- Trichoptera (EPT) group that are classified as sensitive organisms to the oxygen concentration in the water. The high percentage of EPT taxa indicates high water quality (Lenat, 1993). In stations #1 and #2, the most dominant within EPT were Ephemeroptera, with 6 families. The dominance of the orders Ephemeroptera, Plecoptera, and Trichoptera which are considered to be sensitive to environmental stress signifies relatively clean conditions (Merritt, 1978). Two Diptera, Gastropoda, and Hemiptera families composed the rest of the macroinvertebrates in these two stations. Both these stations are upstream of the Karabal Stream. According to Meyer (1987), *Baetis* sp. located in the organically less polluted stream section and included in water quality class I-II. Zeybek et al., (2014) determined most dominant taxon was Ephemeroptera (a pollution-sensitive species) in upstream sampling stations in Değirmendere Stream. Macroinvertebrate fauna consists of 19 families in stations #3 and #4. However, the number of EPT families decreased in these station.

In sampling stations #3 and #4 due to heavy pollution with domestic wastewaters, diversity of macroinvertebrates decreased and was dominated by semi tolerant and tolerant families to pollution, such as Baetidae, Chironomidae, Tubificidae, Athericidae, Dixidae, Corixidae, and Gerridae. According to Hynes (1994), the presence of highly tolerant groups of organisms in freshwater ecosystems is generally an indicator of poor water quality. In freshwater ecosystems, the number of sensitive species declines over time due to water pollution, while environmental conditions gradually change in favor of semi-tolerant and tolerant species. (Zimmerman, 1993). Going downstream, sampling stations #3, #4 and # macroinvertebrate samples consisted of Oligochaeta worms and Diptera which were present in high abundance. Oligochaeta is one of the indicator groups of poor water quality in streams and rivers, and they can tolerate heavy to extreme pollution. Many species of

Oligochaeta are tolerant of low oxygen concentration and can live in anoxic conditions (Brinkhurst and Kennedy, 1965). Oligochaeta species are also a group of organisms with high tolerance to organic pollution. (Barbour, 1996).

The increased number of species in station #5 occurred as the result of increased water level and flow velocity in this station. Due to this improvement in environmental conditions, in station #5, the number of taxa further increased. In total 22 families were present, 13 belonging to the sensitive and semi-sensitive EPT group, and the rest consisted of semi tolerant-tolerant organisms (Dytiscidae, Oligochaeta, Bithyniidae, Lymnaeidae).

The classification of the stations based on benthic macroinvertebrates composition was illustrated by using Bray-Curtis UPGMA analysis (Figure 6). As a result of the UPGMA analysis, the station #3 and #4 (96%) were the most similar to each other. The second most similar stations to each other were determined as the station #1 and #2 (86%).

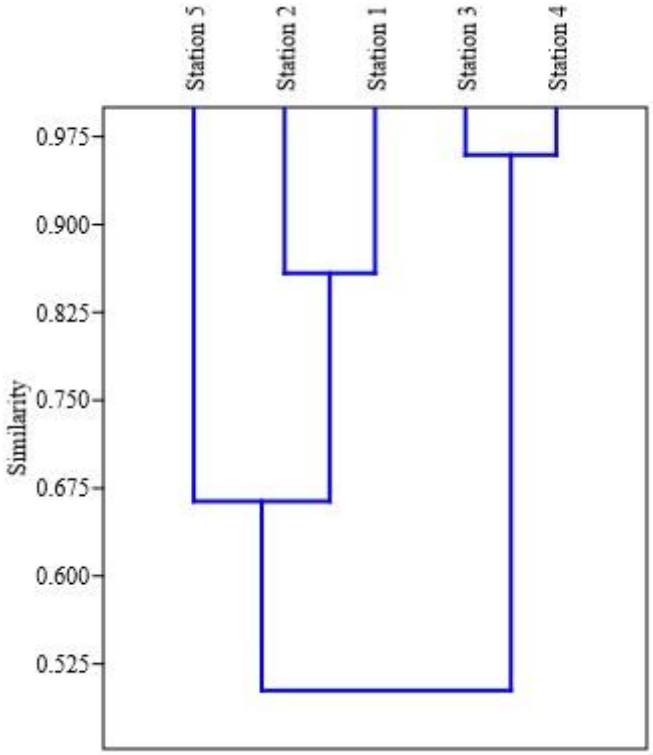

Figure 6. Classification of stations based on similarities in Karabal Stream.

The ecological conditions of Karabal Stream indicate that the stream is disturbed by anthropogenic activities. The water classification with biotic and diversity indices is shown in Table 3.

All diversity indices have shown the highest values in station #5, whereas the lowest values are registered in station #3. High species diversity at the upstream stations indicates unpolluted conditions whereas low species diversity in stations #3 and #4 indicates environmental stress. The sampling station #3 and #4 are heavily disturbed due to many domestic wastes discharged in this part of the stream. The highest BOI_5 value in these two stations indicates the presence of organic pollution in the water and the oxygen consumption for the separation of organic matter. Oxygen depletion at these stations is characterized by low species diversity. Diversity indices have proven to be useful tools for defining the structure of communities, but they do not indicate the level of pollution of water bodies. In this context, diversity indices are good for assessing organic pollution and eutrophication but are insufficient for assessing toxicity and physical changes.

The both BMWP (Original) and BMWP (Spanish) values were highest in stations #1, #2 and #5. The water is classified in Class II in these stations. The stream water quality decreased drastically and became of moderate quality at stations #3 and #4. ASPT and BBI index qualifies the water quality at all stations in Class I-High. According to SI, all stations are *Betamesosaprob*- Class II. According to FBI, the water quality is Class I in stations #1, #2, and #5 while the water quality is Class II in stations

#3 and #4 in the stream. These index scores indicate that upstream of the water body, due to the distance with inhabited areas and lack of waste discharge, the water has a minimum human impact and is of high quality. Going downstream, in urban and rural areas, human activities become more intensive and impact physical and chemical parameters of the water that is manifested with moderate water quality.

EPT-Taxa [%] was one of the metrics which gave the best response to the physicochemical variables of water. These metrics are indicated that Ephemeroptera, Plecoptera, and Trichoptera taxa are sensitive to anthropogenic effects while Oligochaeta taxa are tolerant to anthropogenic effects in aquatic ecosystems (Ode et al. 2005). In this study, the highest EPT-Taxa [%] values are obtained at the station #1 and #2. These stations are the upstream part of the water body and they are less affected by domestic wastes. On the contrary, the station #3 and #4 are downstream part of the water body. These stations are mostly affected by domestic wastes. The cause of low EPT-Taxa [%] values at the downstream stations in the stream is the pollution that accumulates in the stream as a result of the anthropogenic activities. Other factors depend on the physical properties of the stream such as high temperature, low stream slope, and reduction of streamflow.

Table 3. Average score values and water quality classes of all indices in the stream.

Metric	Station 1	Station 2	Station 3	Station 4	Station 5
SI	2.006	2.024	2.140	2.140	2,200
Water quality class	II	II	II	II	II
BMWP (Original)	133	132	98	98	131
Water quality class	II	II	III	III	II
BMWP (Spanish)	137	134	99	99	133
Water quality class	II	II	III	III	II
ASPT	7.389	7.238	6.588	6.588	6.550
Water quality class	I	I	I	I	I
BBI	10	10	9,5	9,5	10
Water quality class	I	I	I	I	I
FBI	3.270	3.340	4.270	4.270	3.730
Water quality class	I	I	II	II	I
EPT-Taxa [%]	90.227	81.264	28.667	29.783	69.507
SDI	0.971	0.971	0.954	0.955	0.973
SWDI	3,600	3.645	3.292	3.308	3.660
MDI	6.069	6.743	5.664	5.670	6.763

Our results show that there are differences between indices in water quality classification as a result of applied different indices. When similar studies using biotic and diversity indices in other countries are examined, we can see that some macro invertebrate-based indices are more sensitive, while others are less sensitive. For this reason, it is difficult to choose which index is more reliable to apply in river quality assessment in a country. (Kalyoncu and Zeybek, 2010). In our research EPT-Taxa [%], BMWP (Original), and BMWP (Spanish) seem to be more reliable and reflect the environmental situation better since they both are based on the presence of sensitive species to environmental variables. The reason why EPT-Taxa [%] shows high water quality is that Ephemeroptera, Plecoptera, and Trichoptera are considered very sensitive to pollution (Lenat, 1993). Our results show that a high number of EPT-Taxa [%] were registered upstream, in stations #1 and #2 whereas, with the increased level of pollution in station #3 and #4, the number of EPT families is reduced and was represented by semi-tolerant family Baetidae and Hydropsychidae.

In this study, the random sample cases (*10% select case*) were made on the biotic indices and physicochemical parameters to verify data sets and to determine that the data was transferred without errors in the SPSS version 20.0. Table 4 indicates the correlations of biotic and diversity indices.

As a result of the correlation analysis, the highest positive significant correlation was found between the BMWP (Original) and BMWP (Spanish) *(r-value 0.999, p<0.01)*. There was a positive significant correlation between the BMWP (Original) and BBI *(r-value 0.999, p<0.01)*. There was a positive significant correlation between the BMWP (Spanish) and BBI *(r-value 0.997, p<0.01)*. There was a positive significant correlation between the BMWP (Spanish) and EPT-Taxa [%] *(r-value 0.965, p<0.01)*. There was a positive significant correlation between the FBI and EPT-Taxa [%] *(r-value -0.987, p<0.01)*. BBI, BMWP (Original), BMWP (Spanish) and EPT-Taxa [%] are positively significant correlated with SDI and SWDI diversity indices. However, the increase in index values of BBI, BMWP (Original), BMWP (Spanish), and EPT-Taxa [%] shows good ecological quality.

Table 4. Pearson's based correlation assesment between biotic and diversity indices in the stream

	SI	BMWP (Original)	BMWP (Spanish)	ASPT	BBI	FBI	EPT-Taxa [%]	SDI	SWDI	MDI
SI	1	-0,447	-0,466	-,965**	-0,416	0,769	-0,672	-0,336	-0,331	-0,083
BMWP (Original)		1	,999**	0,659	,999**	-,915*	,956*	,992**	,987**	0,842
BMWP (Spanish)			1	0,677	,997**	-,924*	,965**	,988**	,981**	0,82
ASPT				1	0,632	-,906*	0,842	0,563	0,553	0,283
BBI					1	-,901*	,945*	,996**	,992**	0,86
FBI						1	-,987**	-0,859	-0,853	-0,633
EPT-Taxa [%]							1	,915*	,901*	0,671
SDI								1	,996**	,879*
SWDI									1	,913*
MDI										1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

CCA analysis showed 84.64% total variance between the benthic macroinvertebrate species and physicochemical parameters (Figure 7). The distributions of *Simulium* sp., *Chironomus* sp., *Chironomus plumosus*, *Tubifex tubifex*, and *Limnodrilus hoffmeisteri* are positively correlated with EC, Cl, Turbidity, T^oC, NH_4-N, NO_2-N and NO_3-N while they are negatively correlated with DO, DOS, and pH. The distributions of EPT species are positively correlated with DO, DOS, and pH.

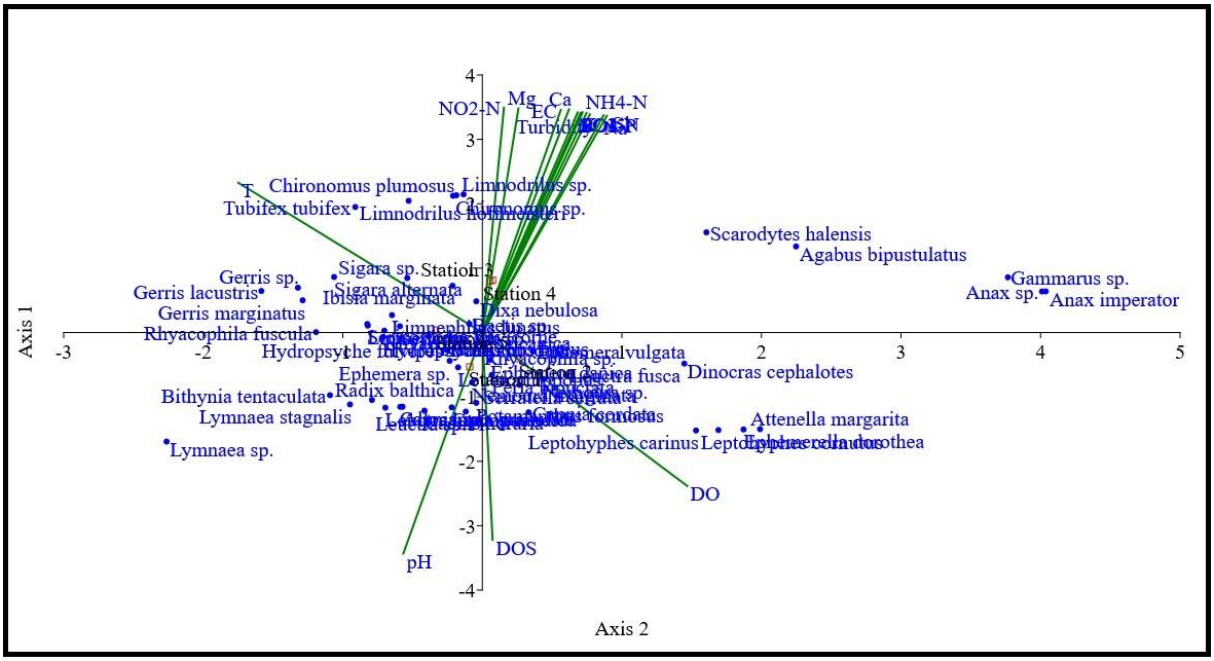

Figure 7. CCA plot of reference-, test-, and the most disturbing stations distributions with environmental variables.

Pearson correlation analyses between Biotic and diversity indices with physicochemical parameters show that all physicochemical parameters have a significant correlation with at least one biotic and diversity indices (Table 5). There is a strong positive correlation *($p<0.01$)* of temperature, DO, with SI and ASPT ($p<0.05$), which means if temperature increases, these parameters will increase too. These results indicate that the macroinvertebrate species are sensitive to increased temperature in water and as DO decreases, sensitive taxa are being replaced by highly tolerant taxa (Horrigan et al. 2005). The turbidity, EC, BOI_5, NH_4-N, NO_2-N, NO_3-N, PO_4-P, Ca, Mg, K, Na, and Cl^- concentration in the water is in negative correlation with BMWP (Original), BMWP (Spanish), BBI, FBI, EPT-Taxa [%], SDI and SWDI *(significance $p<0.01$ and $p<0.05$)* whereas pH has positive correlations *(significance $p<0.01$ and $p<0.05$)* with BMWP (Original), BMWP (Spanish), BBI, FBI, EPT-Taxa [%], SDI and SWDI. We can conclude that temperature, DO and pH have influenced the macroinvertebrate richness and abundance in Karabal Steam.

Table 5. Pearson's based correlation assessment between biotic indices and species diersity indices with physicochemical parameters of the stream.

	SI	BMWP (Original)	BMWP (Spanish)	ASPT	BBI	FBI	EPT-Taxa [%]	SDI	SWDI	MDI
T	,967**	-0,43	-0,45	-,935*	-0,399	0,743	-0,652	-0,312	-0,306	-0,056
DO	-,966**	0,446	0,47	,946*	0,414	-0,755	0,677	0,33	0,314	0,027
DOS	-0,841	0,786	0,807	,945*	0,763	-,944*	,930*	0,709	0,68	0,365
Turbidity	0,495	-,998**	-,997**	-0,697	-,995**	,935*	-,967**	-,982**	-,979**	-0,831
pH	-0,631	,954*	,962**	0,808	,943*	-,967**	,987**	,912*	,897*	0,676
EC	0,575	-,988**	-,991**	-0,763	-,983**	,964**	-,986**	-,961**	-,957*	-0,783
BOI_5	0,523	-,994**	-,995**	-0,72	-,990**	,945*	-,973**	-,973**	-,970**	-0,812
NH_4-N	0,513	-,997**	-,998**	-0,713	-,994**	,943*	-,973**	-,979**	-,975**	-0,818
NO_2-N	0,674	-,961**	-,965**	-0,837	-,952*	,989**	-,991**	-,920*	-,918*	-0,732
NO_3-N	0,525	-,996**	-,996**	-0,722	-,992**	,947*	-,975**	-,977**	-,974**	-0,818
PO_4-P	0,512	-,997**	-,997**	-0,711	-,994**	,942*	-,971**	-,981**	-,978**	-0,825
Ca	0,587	-,985**	-,989**	-0,775	-,978**	,968**	-,991**	-,958*	-,949*	-0,757
Mg	0,655	-,965**	-,969**	-0,823	-,956*	,984**	-,990**	-,924*	-,920*	-0,733
K	0,532	-,994**	-,995**	-0,729	-,989**	,949*	-,978**	-,972**	-,967**	-0,8
Na	0,477	-,999**	-,998**	-0,684	-,997**	,928*	-,964**	-,985**	-,980**	-0,829
Cl^-	0,468	-1,000**	-,999**	-0,676	-,998**	,924*	-,962**	-,989**	-,985**	-0,836

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

Kazancı and Dügel (2000) have applied BBI in their study in Turkey and they stated that BBI complied with the physicochemical parameters. Kantzaris et al., (2002) used biotic indices at two streams in Greece. The BMWP, ASPT, and Land Quality Indicators (LQI) were indicated insufficient in evaluating water quality while BBI and IBE were proper according to Kantzaris et al., (2002). Öz and Şengörür (2004) used the BBI index in their study on Melen Stream. They tried to reveal the water quality in Melen Stream. They stated that BBI was in harmony with the other indices they use. Balık et al., (2006) used the BBI in their study on the Menderes River and they stated that the water quality values of the stations determined were extremely dirty. Kalyoncu et al., (2008) stated that the BBI and physicochemical data reflect the water quality level in Aksu Stream. However, they stated that the quality values obtained from physicochemical data showed better water quality. Kazancı et al., (2010) utilized BMWP and ASPT indices in Aksu Stream. They stated that the BMWP and ASPT were adequate in assessing water quality. Ogleni and Topal (2011) applied four biotic indices in the Mudurnu River. They determined that the BMWP and ASPT were sufficient in evaluate surface water quality. Yorulmaz et al. (2015) applied five biotic indices in the Esen River. They stated that the FBI was insufficient in evaluating water quality while ASPT, BMWP, SI, and BBI were appropriate in Esen River. Zeybek et al., (2014) utilized a diverse type of BMWP and ASPT indices in Değirmendere Stream. Zeybek (2017) applied the BBI, all types of BMWP, and ASPT indices in Kargı Stream. She obtained inconsistent score values and indicated that used biotic indices don't reflect Turkish freshwater fauna and topographic characteristics as a result of her study. The results obtained according to the BBI and BMWP which are applied to the various regions in Turkey is that they reflect as well the stream quality. The fact that very few of these studies makes it difficult to determine the availability in Turkey. More studies are needed on this subject and it should be applied in streams in different regions.

DISCUSSION

The results obtained in this study show that Karabal Stream was affected by many anthropogenic activities. The main factors are industrial discharges, agricultural runoff, and land use, as well as the direct discharge of untreated wastewater into the stream. While upstream stations are less polluted as they are distant from agricultural activities, in urban areas, the stream is moderately polluted and this is reflected in the benthic macroinvertebrate community and distribution. Increased pollution at stations #3 and #4 resulted in the disappearance of sensitive species from this part of the stream, and the emergence of more pollution-tolerant species adapted to specific habitats. Our results have shown that the ecological status of the Karabal Stream is of moderate quality and urgent measures for the protection of the Gediz River Basin and other water resources in Turkey must be implemented through professional management plans for river basins.

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The determination of Water Quality in Balaban Lake (West Anatolia of Turkey)with Trophic State Indices

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Abstract

In this study, which was carried out between October 2019- August 2020, totally five stations were chosen from the research area. Water samples (for the analysis of total phosphorus and chlorophyll-*a*) were taken seasonally and also Secchi disc depth and chlorophyll-*a* were measured from these stations. Carlson's Trophic State Index, OECD criteria and value range of the Turkish Water Quality Regulation were used for the determination of the trophic status of the study area. According to obtained data, the studied area of Balaban Lake has mesotrophic character according to chlorophyll *a*, total phosphorus, and Secchi disc depth. At the end of the study, it was determined that the Balaban Lake was at the mesotrophic level according to the Carlson trophic status index, the average±1SD interval of the OECD criteria, and Turkish Water Quality Regulation.

Keywords: Balaban Lake, Carlson Trophic State Index, OECD criteria, Turkish Surface Water Quality Regulation.

Balaban Gölü (Türkiye'nin Batı Anadolu Bölgesi) Su Kalitesinin Trofik Durum İndeksleriyle Belirlenmesi

Özet

Ekim 2019-Ağustos 2020 tarihleri arasında gerçekleştirilen bu çalışmada toplam beş istasyon seçilmiştir. Bu istasyonlardan mevsimlik peryotlarla su örnekleri alınarak toplam fosfor analizleri yapılmış, ayrıca klorofil *a* ve seki diski derinliği ölçülmüştür. Çalışma alanının trofik statüsünün belirlenmesi için Carlson Trofik Durum İndeksi, OECD kriteri ve Türkiye Yerüstü Su Kalitesi Yönetmeliği değer aralıkları kullanılmıştır. Elde edilen verilere göre, Balaban Gölü'nün çalışılan bölgesi klorofil *a*, toplam fosfor ve seki diski derinliğine göre mezotrof karakterdedir. Çalışma sonucunda BalabanGölü'nün Carlson trofik durum indeksi, OECD ve Türkiye Yerüstü Su Kalitesi Yönetmeliği ortalama ±1SD değer aralığına göre mezotrofik seviyede olduğu tespit edilmiştir.

Anahtar Kelimeler: Balaban Gölü, Carlson Trofik Durum İndeksi, OECD kriteri, Türkiye Yerüstü Su Kalitesi Yönetmeliği.

INTRODUCTION

Water, which is one of the most important resources for all organisms, is essential for the continuation of life and vitality. The increasing population in the world, the development of the industry, and the need for agricultural activities increase the importance of freshwater resources (Aksungur and Firidin, 2008). Water is an indispensable natural resource for all organisms. Although a large part of the earth is covered with water, only 3% of it is freshwater. 78% of the freshwater and 3% of usable water is found in glaciers in the north and south poles. This situation limits the required drinking and utility water ratio to 22% (Gündoğdu et al., 2007).

In terms of energy production, the thermal power plants, nuclear power plants, and river resources have remained more in the background according to the dam lakes in Turkey. With this respect, 700 dams and over 500 power plants have been built recently (Küçükyılmaz et al. 2010). To determine the effective use of a water resource, it is imperative to gather information about the resource by meticulously conducting a monitoring program that will meet the anticipated expectations (Şen and Koçer, 2003). One of the most important physicochemical factors for monitoring water quality is; to determine the factors affecting the water quality by detecting the changes in the sources of pollution and thus the pollution levels (Özbay et al., 2011).

Eutrophication is one of the most important threats in freshwater systems, especially harmful algae blooms (HABs). Since eutrophic action can be seen in salty (ocean and marine), fresh (lake, reservoir, stream), fresh-salty (estuary, lagoon) ecosystems, developed and developing countries, it is a very common and still unresolved water quality problem. Although eutrophication reduces the quality of the water, it's commonly complained negative effects; bad image, odor, and excessive algae growth. All these problemscreate an increasing negative pressure on ecology. Therefore, many different methods of struggle are tried to combat eutrophication (Schindler et al., 2008; Schindler, 2012; Lurling and Tolman, 2014). To understand the results and effects of these methods; It is necessary to know the budgets and Spatio-temporal changes of the internal and external parameters of the ecosystem.

Izmir with its historical importance is localizing on the Mediterranean coast in the West of Turkey. Izmir is the third-largest city in Turkey. Izmir is the fastest-growing city with a 4.320 million population and an annual 9.5% population growth rate in Western Anatolia. Balaban Lake is determined as a case area because it is an important drinking water source for İzmir. Balaban Lake is one of the important water sources of the Tahtalı Dam Lake. Lake basin which supplies 40% of city water needs. There are many industrial establishments, agricultural lands, and animal farms around Balaban Lake. Considering these factors, the pollution situation and the trophic status of Balaban Lake have not been researched yet. In this study, it is aimed to reveal the trophic situation of Balaban Lake by using Carlson's Trophic State Index, OECD criteria, and value range of the Turkish Water Quality Regulation.

MATERIAL and METHODS

Study Area

The study was carried out seasonally at five sampling points in Balaban Lake, which were determined considering the proximity to the pollutant sources and hydrodynamic properties. Spring sampling was carried in April 2020, summer sampling in August 2020, autumn sampling in October 2019, and winter sampling in January 2020. Sampling points, coordinates, and average depths are also given in Table 1 and Figure 1.

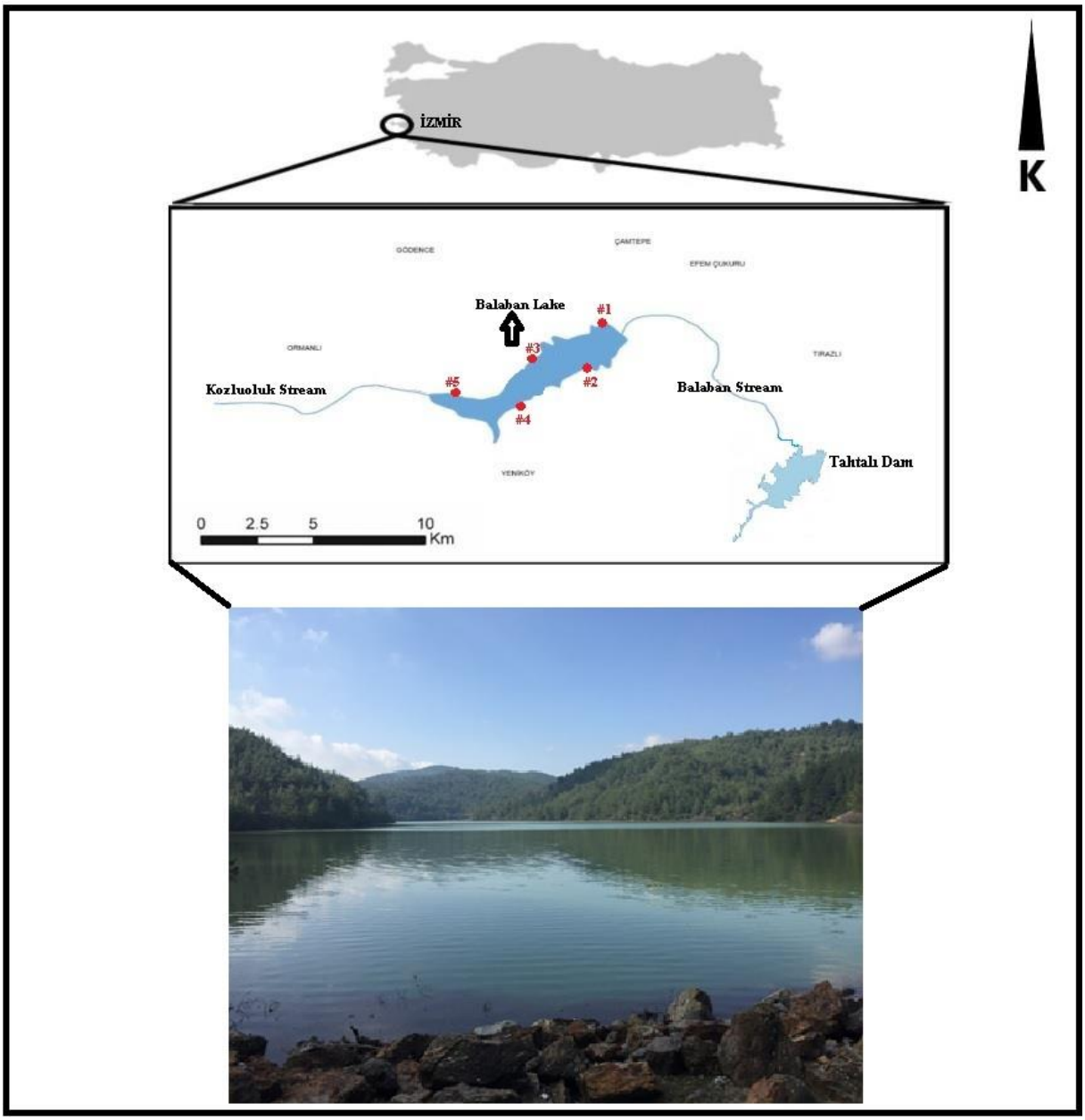

Figure 1. Study Area

Table 1. Balaban Lake sampling points, coordinates, and average depths.

Stations	Coordinates	Average depths
Station 1	38°23'19.17"N-27°02'68.31"E	6.2 m
Station 2	38°22'98.69"N-27°02'78.50"E	3.3 m
Station 3	38°22'94.14"N-27°02'25.07"E	3.0 m
Station 4	38°22'68.85"N-27°02'23.68"E	6.5 m
Station 5	38°22'69.70"N-27°01'93.42"E	2.8 m

Domestic pressures and threats arising from the settlement areas within the scope of the Balaban Lake basin can be counted as the domestic wastewater and agricultural activities of the villages on the Kozluoluk Stream, especially in the 2nd, 3rd and 5th stations.

Trophic State Indices

Chlorophyll-*a* (Chl *a*), total phosphorus (TP) and Secchi depth (SD), total nitrogen (TN), and dissolved oxygen (DO) parameters are used to determine the trophic conditions of lakes. The index variables of these parameters were associated with each other by the linear regression model. Using these variables, lake waters can be classified in terms of efficiency. For example, in the light of these

data, the nutrient level of a lake or it's status in terms of productivity can be determined in the simplest and easiest way by the trophic status index set forth by Carlson (1977), and lakes are included in a trophic class according to direct variables or indices calculated from variables.

According to the concept of trophic state, lakes are located in a series of trophic flows (oligotrophic-mesotrophic-eutrophic-hypertrophic) that progresses continuously from one to the other. If the mean values of the Carlson trophic state index (TSI), the TSI values are close to 0, the lake is closer to the oligotrophic level, closer to 100 it is accepted that the lake has a hyperutrophic structure (Carlson and Simpson, 1996) (Table 2).

Table 2. Carlson trophic state index and its associated parameters.

TSI	Trophic Level	Chl *a* (mg/m^3)	Secchi (m)	TP (µg/L)
<30	Oligotrophic	<0.95	>8	<6
40-50	Mesotrophic	2.6-7.3	4-2	12-24
50-60	Eutrophic	7.3-20	2-1	24-48
70-80	Hyperutrophic	56-155	0.25-0.5	96-192

SD, Chl *a*, and TP are used in the formulas for calculating the Carlson TSI. The index expressed by;

$$TSI = 60 - 14.43 \ln(SD)$$
$$TSI = 30.6 + 9.81 \ln(Chl\ a)$$
$$TSI = 4.14 + 14.43 \ln(TP)$$

In the OECD criteria, TP, TN, Chl *a* and SD values are used (OECD, 1982) (Table 3).

Table 3. The OECD classification of trophic status.

Trophic Level	TP (µg/L)	Chl *a* (mg/m^3)	SD (m)
Oligotrophic	8	1.7	9.9
Mesotrophic	26.7	4.7	4-2
Eutrophic	84.4	14.3	2.45

In addition to these, the DO parameter is used in the trophic state index of the Turkish Surface Water Quality Regulation (Anonymous, 2012) (Table 4).

Table 4. Lake eutrophication criteria (Anonymous, 2012)

Trophic Level	TP (µg/L)	TN (µg/L)	Chl *a* (µg/L)	SD (m)	DO (mg/L)
Oligotrophic	<10	<0.35	<3.5	>4	>7
Mesotrophic	10-30	0.35-0.65	3.5-9.0	4-2	6-4
Eutrophic	31-100	0.651-1.20	9.1-25.0	1.9-1.0	3
Hyperutrophic	>100	>1.2	>25.0	<1	<3

In the research, DO, TP, Chl *a*, SD, and TN data were examined. DO content was measured with the Oxi 315i/ SET WTW Oxygen meter. The SD was determined directly in place with the Secchi disc. TP and TN were measured by spectrophotometric technique. chl *a* was measured in the field by using the BBE Moldaenke AlgaeTorch.

Statistical evaluation of the dataset was made by using the PAST3 and Excel 2019 (Microsoft Office^R). Analysis of variance (One- Way ANOVA) was applied to all data and the differences between the group means were determined according to the Tukey test and multiple comparison test, and "$p < 0.05$" was used as the significance level.

RESULTS

The comparison of Balaban Lake DO, SD, TP, Chl *a*, and TN values over the stations is given in Table 5.

Table 5. DO, SD, TP, TN and Chl *a* values of stations in Balaban Lake.

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5
DO (mg/l)	R	13.4-14.1	12.4-14.0	11.1-13.5	12.6-13.5	10.8-12.4
	M±Sd.	**13.8**^{ab}±0.29	**13.4**^{ab}±0.71	**12.1**^{a}±1.07	**13.0**^{ab}±0.40	**11.5**^{b}±0.73
SD (m)	R	4.0-5.0	0.5-0.8	0.3-0.8	4.0-5.0	0.3-0.8
	M±Sd.	**4.75**^{ab}±0.5	**0.7**^{a}±0.14	**0.57**^{b}±0.22	**4.75**^{ab}±0.5	**0.57**^{b}±0.22
TP (µg/L)	R	4.02-4.97	8.23-10.14	11.8-13.6	3.11-4.23	10.2-12.5
	M±Sd.	**4.49**^{b}±0.38	**9.42**^{a}±0.84	**12.6**^{ab}±0.76	**3.82**^{b}±0.49	**11.6**^{ab}±0.98
TN (mg/L)	R	0.558-0.665	0.660-0.699	0.682-0.711	0.550-0.591	0.691-0.703
	M±Sd.	**0.632**^{a}±0,05	**0,681**^{ab}±0.01	**0.697**^{ab}±0.01	**0.577**^{b}±0.02	**0.698**^{ab}±0.01
Chl-*a* (mg/m^3)	R	1.67-1.94	5.42-5.67	6.32-6.97	1.78-1.99	5.71-6.03
	M±Sd.	**1.79**^{ab}±0.12	**5.55**^{a}±0.11	**6.65**^{b}±0.32	**1.89**^{ab}±0.08	**5.85**^{a}±0.14

R: Range; M: Mean; Sd: Standard deviation. *****Different characters on the same line indicate that the difference between stations is statistically significant *(p <0.05)*.

During the study, Balaban Lake DO content was determined to be between 10.8-14.1 mg/L and an average of 12.8 mg/L. Seasonal average DO contents were determined to be 13.2 mg/L in the spring, 12.5 mg/L in the summer, 12.1 mg/L in the autumn, and 13.3 mg/L in the winter (Figure 2).

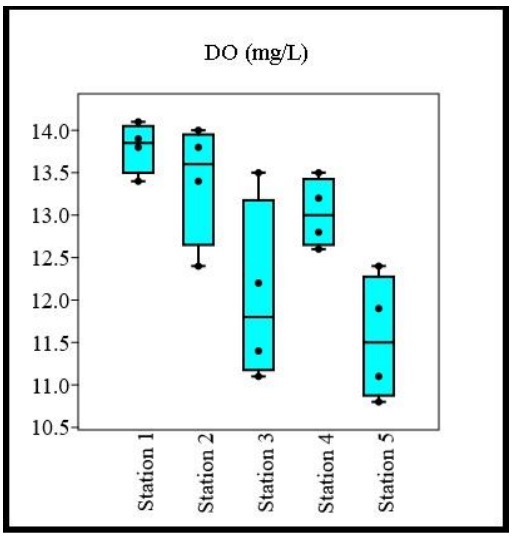

Figure 2. Balaban Lake dissolved oxygen variation graph.

SD was measured between 0.3-5.0 m and the average was determined to be 2.27 m. Seasonal average SD variations are 2.42 m in spring, 2.36 m in summer, 1.82 m in autumn, and 2.48 m in winter (Figure 3).

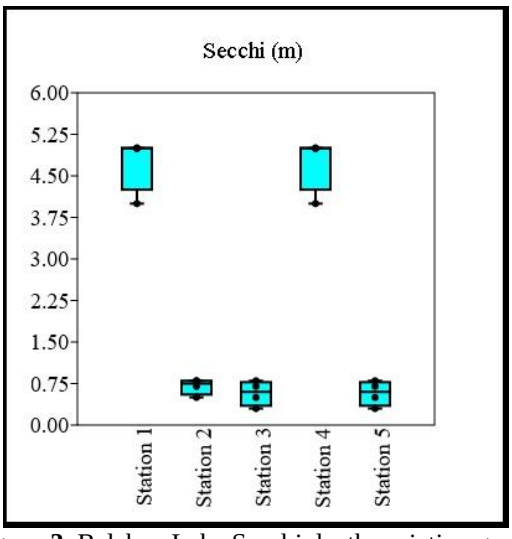

Figure 3. Balaban Lake Secchi depth variation graph.

It was observed that the differences between stations in DO and SD parameters were statistically insignificant, but seasonal variation differences in SD were statistically significant *(p <0.05)*. The effect of wind and weather conditions at the time of measurement is evident in the inter-seasonal variability of the SD measurements.

TN values were determined between 0.55-0.71 (mean: 0.66) mg/L in Balaban Lake. The lowest TN value was determined in the 4th station in the winter, while the highest TN value was determined in the 3rd station in the autumn (Figure 4). In the statistical analysis, it was seen that the difference between the stations was statistically significant (*p <0.05*).

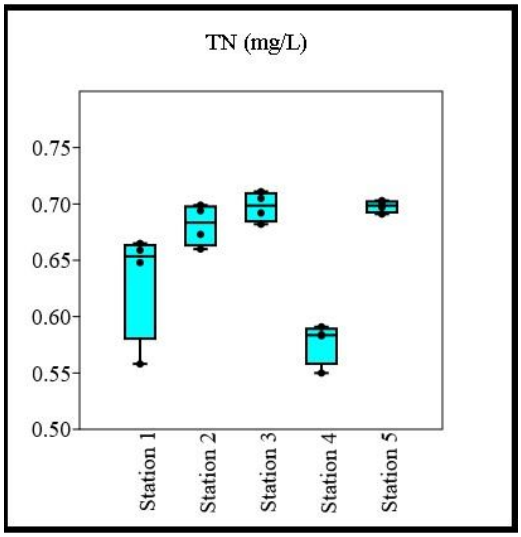

Figure 4. Balaban Lake total nitrogen variation graph.

The TP concentration of Balaban Lake varied between 3.11-12.7 (mean: 8.41) µg/L. In the statistical analysis, it was seen that the differences between the 3rd and 5th stations and all other stations were statistically significant *(p <0.05)* (Figure 5). When seasonal TP changes were examined, average TP content was determined as 8.68 µg/L in spring, 8.35 µg/L in summer, 9.08 µg/L in autumn, and 7.50 µg/L in winter. In the 3rd and 5th stations, Kozluoluk Stream, which dries up especially in summer, transports pollutant loads to the lake during rainy periods. This situation causes TP values to be higher than other stations.

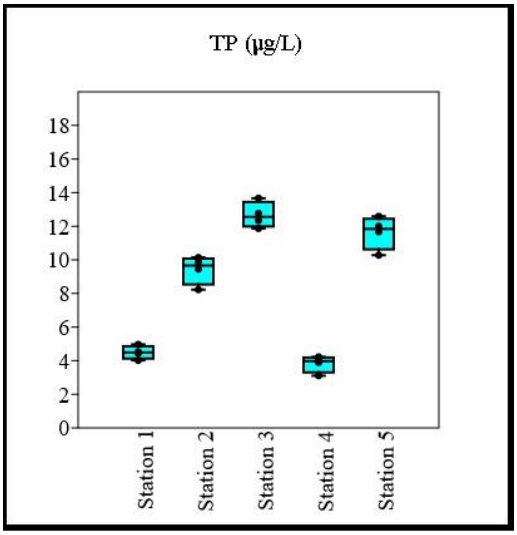

Figure 5. Balaban Lake total phosphorus variation graph.

The chl measurements of Balaban Lake varied between 1.67-6.97 (mean: 4.35) µg/L (Figure 6). In the statistical analysis, it was seen that the differences between the 2nd, 3rd and 5th stations and other two stations (1st and 4th) were statistically significant *(p <0.05)*. The seasonal chl *a* levels of Balaban Lake were determined to be 4.26 µg/L in spring, 4.45 µg/L in summer, 4.51 µg/L in autumn and 4.18

µg/L in winter. No statistically significant difference was observed between the seasons based on chl *a* value *(p <0.05)*.

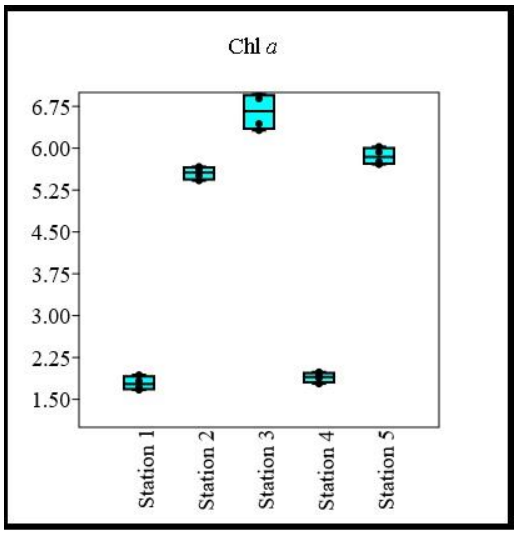

Figure 6. Balaban Lake chl *a* variation graph.

Carlson TSI (SD) values in Balaban Lake varied between 36.6-77.5. The highest TSI (SD) value was determined in the 3rd and 5th stations in the autumn, and the lowest TSI (SD) value in the 1st and 4th stations in the autumn. The difference between stations in terms of TSI (SD) values was not found to be statistically significant *(p <0.05)*.

Carlson TSI (TP) values in Balaban Lake varied between 23.7-41.8. The lowest TSI (TP) value was calculated at the 4th station in the summer and the highest TSI (TP) value at the 3rd station in the autumn. The difference between stations in terms of TSI (TP) values was not found to be statistically significant *(p <0.05)*.

Carlson TSI (Chl *a*) values varied between 35.6-49.6 in Balaban Lake. In Balaban Lake, the highest TSI (chl *a*) value was recorded at the 3rd station in the autumn, and the lowest TSI (chl *a*) value at the 1st station in the winter. The difference between stations in terms of TSI (chl *a*) values was not found to be statistically significant *(p <0.05)*.

According to the Carlson TSI, it was determined that Balaban Lake showed mesotrophic characteristics in terms of average TSI (SD), TSI (TP) and TSI (chl *a*) values. Balaban Lake was at the mesotrophic level according to the average TSI values in general (Table 6).

Table 6. Carlson trophic state index results.

TSI	Average Score	Trophic Level
TSI (Chl *a*)	43.5	Mesotrophic
TSI (SD)	50.0	Mesotrophic
TSI (TP)	33.2	Mesotrophic
TSI (average)	43.2	Mesotrophic

According to the OECD criteria, it was determined that it has mesotrophic properties in terms of TP; it has oligotrophic properties in terms of chl *a*; it has eutrophic properties in terms of SD parameter (Table 7).

Table 7. OECD trophic state index results.

Parameters	Average Score	Trophic Level
TP (µg/L)	8.4	Mesotrophic
TN (mg/L)	0.65	Oligotrophic
Chl *a* (mg/m^3)	4.34	Oligotrophic
SD (m)	2.27	Eutrophic

According to the trophic status index of the Turkish Surface Water Quality Regulation, it was determined that it has oligotrophic in terms of DO and TP values, mesotrophic in terms of TN, SD, and chl *a* (Table 8).

Table 8. Turkish Surface Water Quality Management Regulation trophic state index results.

Parameters	Average Score	Trophic Level
TP (µg/L)	8.4	Oligotrophic
TN (mg/L)	0.65	Mesotrophic
Chl *a* (mg/m^3)	4.34	Mesotrophic
SD (m)	2.27	Mesotrophic
DO (mg/L)	12.8	Oligotrophic

DISCUSSION

SD, which is an indicator of the light transmittance of aquatic environments, generally varies depending on the depth of water. However, seasonal variations are also very effective on SD. According to the OECD (1982) trophic classification, lakes with a depth of 0.8-1.5 m are classified as eutrophic, lakes between 1.4-2.4 m as mesotrophic, and lakes between 3.6-5.9 m as oligotrophic (Ryding and Rast 1989). According to OECD (1982) values, the 1st and 4th stations in Balaban Lake are mesotrophic, but the 2nd, 3rd and 5th stations exhibit a eutrophic structure. In Balaban Lake, the reason for the eutrophic situation is due to the low turbidity in these stations as a result of the growth of filamentous algae and aquatic plants. In this study carried out seasonally, the average SD value was found to be 2.27 m. Erk'akan and Bayrak (1992) reported the average SD values of Eğirdir Lake was 1.95 m. Zeybek et al., (2012) reported the average SD values of Eğirdir and Kovada Lake was 1.60 m. Yağcı et al., (2013) reported the average SD value of Eğirdir Lake was 1.77 m in the monthly study in 2016. Tanyolaç (2000) reported that many factors affect the transparency and light transmittance of water in lakes, such as plankton density, dissolved organic and inorganic substances in water, the chemical structure of the water, the angle and wavelength of light, the state of the water surface, and cloudiness.

Güneş et al., (2011) reported in their study that the TP content showed a distribution and change depending on the measurement time and points, however, the values detected were at low levels (8.41 µg/L). Researchers have reported that this situation shows meso-eutrophic properties for the lake and that it is necessary to control the use of domestic and animal wastes, especially fertilizers, and avoid mixing with the lake water as much as possible in order not to reach the further trophic level. In addition, the TP content determined in Balaban Lake provides the A1-K value in terms of Quality Standard, however, the phosphate element is extremely important for the lake water quality (in terms of algae production and eutrophication), especially with nitrogen, and it is important to control this parameter at its source absolutely. have reported. According to Zeybek et al., (2012), the average TP amount is below the lowest analysis limits in November, April, and June, highest 0.12 µg/L in July; the lowest 0.10 in October, the highest 0.72 in July on the channel between 2010 and 2011 in Eğirdir Lake Köprübaşı Region, Kovada Canal, and Kovada Lake Entrance Zone; They reported that the lowest analysis limits in Kovada Lake (February, April) was measured as 0.91 µg/L (July) (lowest analysis limits <0.05 µg/L).

Güler and Çobanoğlu (1997) reported that although phosphorus is present in many minerals, its amount in water is limited due to its low solubility in alkaline soils, it can pass through water, rocks, and soils, as well as artificial fertilizers and industrial wastes. Goldman and Horne (1983) reported that phosphorus accumulates in the embankments of deep lakes under oxygenated conditions, separates from bottom mud in O_2-free environments, and passes into the water, thus causing ferric (Fe^{+3}) ion to decrease in efficiency since it binds PO_4^{-3} in oxygenated environments. In addition, they reported that PO_4^{-3} ion is captured by Fe^{+3}, $CaCO_3$, and mud (silt) in three different ways in waters and that mud (silt) is more effective in shallow lakes.

Dodds (2002) reported that nitrogen and phosphorus are the primary limiting nutrients in terms of algal production potential in aquatic ecosystems, however, phosphorus is a more limiting element in freshwater ecosystems than nitrogen. Howarth et al., (2000) reported that the limiting nutrient is nitrogen in marine ecosystems and phosphorus in freshwater ecosystems for algae and plant growth.

Smith (1982) reported that when the TN/ TP ratio is <10, nitrogen, when the TN/ TP ratio is> 17, phosphorus is the limiting nutrient, while the TN/ TP ratio between 10-17 is balanced for the freshwater ecosystem. Varol (2013) reported that according to Carlson TSI value, Batman Dam Lake is at a mesotrophic level, and according to the average TN/ TP ratio, phytoplankton development in the lake is limited by nitrogen and phosphorus together.

Chl *a*, the essential photosynthetic pigment, is an indicator of algal biomass in water (Henderson-Sellers and Markland, 1987; Wetzel, 2001; Taş et al. 2019). The highest chl *a* value was determined at the 3rd station in autumn in Balaban Lake. Chl *a* content was measured as low in winter months and higher in warm months. The results obtained are similar to the information provided by various researchers on chl measurements made in lakes and their seasonal changes (Taş, 2003; Maraşlıoğlu, 2007; Cüce et al. 2020). Güneş et al., (2011) reported that they did not observe a significant difference between the mid-lake and surface stations in terms of chl *a* concentration and that the measured chl values indicate a significant increase in biomass compared to previous years in their study, although they do not pose a risk for lake water in the current situation. In the control of this increase, macrophytes spreading on a significant part of the lake bottom are the factors, because water quality and productivity in shallow lakes; reported that the level of nutrient salt varies depending on high aquatic plants, phytoplankton status, light transmittance, and water level. Yağcı et al., (2013) reported that the lake chl *a* content varied between 0.58-9.70 µg/L in Eğirdir Lake.

Jarosiewicz et al., (2011) determined the current trophic status of the lakes (Rybiec, Niezabyszewskie, Czarne, Chotkowskie, Obłęże, Jasień Południowy, Jasień Północny, Jeleń) located between the Pomeranian rivers Wieprza and Łupawa in the north of Poland with Carlson TSI. For this purpose, they analyzed four trophic state indexes (TSI (SD), TSI (chl *a*), TSI (TP), and TSI (TN)) and the relationship between them. As a result of the study, they reported that the trophic level of the aforementioned lakes were in mesotrophic and eutrophic conditions. They reported that the TSI (TP) values in the analyzed lakes were higher than the index values calculated based on other variables, however, the differences between indices for certain lakes indicated that phosphorus was a factor limiting algal productivity in the analyzed lakes.

Akyüz (2016) stated that the TSI (chl *a*) value has the lowest value in all seasons, indicating that there is a number of nutrients that can provide more algae growth than the existing algae growth. For this reason, he reported that the key limiting parameters for the lake that are more effective than nutrients should be evaluated. In addition, the lake has excess phosphorus and turbidity without algae; this indicates that nitrogen concentration and Secchi depth are key limiting parameters; He also reported that the average TN/ TP ratio of less than 30 in the summer and autumn seasons, when algae growth is high, supports this result.

Sömek and Ustaoğlu (2016) determined that the TSI (SD) results calculated from the average SD and chl *a* measurements in the lakes in Saklıgöl, Gökçeova Pond, Kartal Lake, and Karagöl, located in the southeast of the Aegean region, are between 47.3 and 59.3. They also reported that TSI (chl *a*) results varied between 38.1 and 45.5, and TSI (average) varied between 42.7 and 55.5.

Tepe et al., (2018) stated that the water column of Karkamış Dam Lake between 0-8 m was mesotrophic in terms of TP, TN, and chl *a* index values according to the Carlson TSI, and the TP amount according to the mean ±1 SD interval of the OECD criteria. They reported that the trophic status of the Karkamış Dam Lake showed a transition from the oligotrophic class to the mesotrophic class, and that phosphorus was the limiting nutrient in terms of TN/ TP ratio.

The conditions that slow down the pollution effect of Balaban Lake can be counted as feeding the lake water with groundwater, the lack of industrial facilities around the lake, the low population density around the lake, and the high DO content of the water. However, it is of great importance to take precautions in the streams that are connected to the lake, to discipline irrigation and spraying in agricultural lands, and to establish wastewater treatment units in settlements connected to the lake.

As a result, it should be one of our primary duties not to pollute our waters, which are very precious for our country and the world. We need to aim to create environments in a way and in such a way that the existing pollution can be cleaned in its cycle, without destroying the environment, protecting forests and other vegetation areas.

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Proximate Composition of Traditional Turkish Stuffed Meatballs Produced with Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) Mince and Determination of its Colour and Sensory Quality during Frozen Storage (-18ºC)

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Research Article

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Abstract

The nutritional value of stuffed meatballs made with minced rainbow trout rather than red meat in the stuffing was investigated, as well as color variations (L *, a *, and b *) and sensory quality, using two different cooking methods (boiling and frying) on samples stored in the freezer (-18 ^{o}C). As a result of this research, the moisture, the crude ash, the lipid, and the crude protein contents of stuffed meatballs produced with rainbow trout were found as %58.25 ± 0.93, %2.01 ± 0.05, %5.28 ± 0.29, and %7.69± 0.08, respectively. The content of total saturated fatty acids composition (∑SFA), total monounsaturated fatty acids composition (∑MUFA), and total polyunsaturated fatty acids composition (∑PUFA) was found to be 12.52 ± 0.23%, 39.11 ± 1.27%, and 43.73 ± 1.98%, respectively. The atherogenic (AI) and thrombogenic indexes (TI) for dietary factors associated with cardiovascular disease were found to be 0.10 and 0.25, respectively. In the evaluation of color (L *, a *, and b *) of frozen samples, L * (black-white) and b * (blue-yellow) values in all groups (raw, boiled, and fried) increased significantly at the end of storage compared to the initial value ($p<0.05$). Following frying and boiling, the * (green-red) values of the frozen-stored samples decreased significantly ($p<0,05$). Sensory quality was assessed in this study using two cooking methods: boiling and fried, both of which are common serving methods for stuffed meatballs. Although a significant decrease was detected in all sensory quality parameters of samples during frozen storage, it was determined that it did not reach the unacceptable limit at the end of storage.

Keywords: Proximate Composition, Frozen Storage, Stuffed Meatballs, Rainbow Trout, Color

Gökkuşağı Alabalık (*Oncorhynchus mykiss* Walbaum, 1792) Kıyması ile Üretilmiş Geleneksel Türk İçli Köftesinin Besinsel Komposizyonu ve Dondurularak Depolama Boyunca (-18ºC) Renk ve Duyusal Kalitesinin Belirlenmesi

Özet

Bu çalışmada, iç harcında kırmızı et yerine gökkuşağı alabalık kıyması kullanılarak üretilen içli köftelerin besinsel komposizyonu belirlenmiş ve dondurularak depolanan (-18 ^{o}C) örneklere iki farklı pişirme metodu uygulanarak (haşlama ve kızartma) renk (L*, a* ve b*) ve duyusal kalitesinde meydana gelen değişimler incelenmiştir. Araştırma sonucunda, üretilen gökkuşağı alabalıklı içli köftelerin nem, ham kül, lipit ve ham protein düzeyi sırasıyla %58,25±0,93, %2,01±0,05, %5,28±0,29 ve %7,69± 0,08 olarak bulunmuştur. Gökkuşağı alabalıklı içli köftelerin toplam doymuş yağ asitleri (∑SFA), toplam tekli doymamış yağ asitleri (∑MUFA) ve toplam çoklu doymamış yağ asitleri (∑PUFA) miktarları sırasıyla %12,52±0,23, %39,10±1,27 ve %43,73±1,98 olarak saptanmıştır. Kardiyovasküler hastalıkla ilişkili diyet faktörleri için Aterojenik (AI) ve trombojenik indeksler (TI) sırasıyla 0,10 ve 0,25 olarak bulunmuştur. Dondurarak depolanan örneklerin renk (L*, a* ve b*) değerlendirmesinde, tüm gruplarda (ham, haşlanmış ve kızartılmış) L* (siyah-beyaz) ve b* (mavi-sarı) değerlerinin depolama sonunda başlangıç değerine göre önemli oranda arttığı ($p<0,05$) bulunmuştur. Dondurarak depolanmış örneklerin kızartılma ve haşlama işleminden sonra a* (yeşil-kırmızı) değerlerinin ise önemli oranda azaldığı bulunmuştur ($p<0,05$). Bu çalışmada duyusal kalite, içli köftelerin geleneksel sunum formları olan kızartma ve haşlama olarak iki farklı pişirme metodu ile değerlendirilmiştir. Dondurarak depolama boyunca örneklerin pişirme öncesi, kızartma ve haşlama sonrası tüm duyusal kalite parametrelerinde önemli bir azalma saptansa da depolamanın sonunda tüketilmezlik sınırına ulaşmadığı belirlenmiştir.

Anahtar Kelimeler: Besin Komposizyonu, Dondurarak Depolama, İçli Köfte, Gökkuşağı Alabalığı, Renk

INTRODUCTION

Bulgur, which is one of the first processed foodstuffs in the world, has been the main ingredient in many dishes of the Turkish people in Anatolia for 3-4 thousand years. There are many types of dishes made using bulgur such as pilav, raw meatballs, sour meatballs, stuffed meatballs, salads, soups, and as a meat substitute in vegetarian dishes (Yaman, 1992; Yu and Kies, 1993; Işık, 2006; Dönmez et al., 2004). Now, it is widely consumed not only in Turkey but also in Greece, Cyprus, the Middle East, North Africa, and East Europe (Basaran, 1999).

Traditional Turkish stuffed meatballs are kinds of meatballs that are made from bulgur and filled in with dough. Its name is the original "Kibbe" from Arab cuisine. This dish was called Syriac Torpedo by the British on Syrian territory during World War II. This dish is made mainly in Adana, Osmaniye, Kahramanmaraş, Elazığ, Malatja, Mardin, Diyarbakır, Hatay, Gaziantep, Sanlıurfa and Adıyaman, and derived from the Persian term köfte Eren and Sezgin, 2017; Yüzgül, 2019).

Stuffed meatballs are prepared in a variety of ways around the country, with variations owing to the ingredients used in the dough and filling preparation. Ground meat with or without tail fat (depending on region), tomato paste, onion, parsley, and spices are the main filling materials for stuffed meatballs (walnuts can also be put on demand). Since it is complex and time-consuming to cook, it is no longer a regular dinner in Turkey, but rather a special occasion meal (Ballı, 2013). There are two ways to prepare stuffed meatballs. The first is the boiling process, which is commonly used in large quantities due to its lightness. It unquestionably has a spot at the table when it comes to hosting special events such as celebrations, promises, weddings, and henna evenings. The second method is frying, which is more common in big cities, especially in hotels and restaurants, due to the possibility of the meatballs being scattered in the boiling method. The second is frying. It is made in big cities, especially in hotels and restaurants. Frying is more preferred in such places because of the possibility of the meatballs being scattered in the boiling method (Arslanhan, 2014). In a study on consumer preferences of soup, appetizers, main dishes and desserts offered to consumption in local Turkish cuisine, it is stated that stuffed meatballs are the most preferred food by customers among many appetizers (Güler et al., 2016). The presence of ready-to-cook stuffed meatballs from different brands in supermarkets' prepared ready-to-eat foods category is one of the most important indicators of consumer appetite for it.

Changes in consumer behavior, heavy workload conditions, a lack of time to prepare, and a need to eat certain meals outside of season have mostly led to the widespread use of frozen ready-to-cook or ready-to-eat foods (Cuneo, 1998). As the sector's production quality has improved, frozen ready meals (cook or eat form) have started to be offered as an alternative to home meals (Sarasin, 2000).

It is stated that the most critical and frequent challenge of human beings in the developed and changing world is sufficient and malnutrition. In this regard, animal foods have an important role due to meat protein and its biological properties. According to the data of the World Health Organization (WHO), 1 gram protein for each kilogram of a healthy person's body weight per day should be consumed and 42% of it should be of animal origin (; Saygın and Demirbaş, 2018). Even though modern Turkish cuisine represents local changes, it primarily consists of dishes based on meat, vegetables, and pastries. Seafood-based meals are more common in coastal areas and in areas where freshwater supplies, such as lakes and streams, are abundant. Fresh seafood intake in Turkey is very modest as compared to red meat and cereals in other countries (Şengör and Ceylan, 2018). According to TUIK (2020) data; the annual average fish consumption per person varies around 6.3 kg. However, it is a well-known fact that fish and their products are much more important in terms of health and flavor than other animal-based foods in the current time (Çolakoğlu, 2004). Therefore, including fish-containing products in diets not only enhances food quality but also increases fish intake. Furthermore, there is a high potential for adding fish-containing items as a seasoning to ready-to-eat meals in the form of "ready to serve" or "ready to eat" that are traditionally popular (Reddy et al., 2012).

Aramouni et al. (2001) studied stuffed meatballs (named as kubbee) made from ground beef (10% fat), bulgur wheat, and spices with a filling of barbecue sauce and evaluated their shelf-life of raw and precooked vacuum packaged product during 180 days of storage at -18 ^{o}C. Khazaal (2004) patented a food described as stuffed meatballs. Güler et al. (2016) investigated stuffed meatballs in terms of consumer's preference for a favorite local food menu composed of regionally known appetizers and Yağmur et al. (2014) studied the proximate composition of stuffed meatballs produced traditionally in Adana. Although fish-containing products were studied for other dishes prepared from bulgur such as

çiğ köfte (Kaba et al., 2014; Ozturk et al., 2016), no studies have been found on the production of stuffed meatballs with fish and their quality changes during frozen storage.

The purpose of this study was to determine the proximate composition, color changes (L *, a *, and b *), and sensory quality of stuffed meatballs, a traditional Turkish cuisine, produced using rainbow trout mince as a filling ingredient, and their sensory quality during frozen storage (-18 ^{o}C).

MATERIAL and METHODS

Material

In the study, the cage-cultured rainbow trout (*Oncorhynchus mykiss*), 1.25 ± 0.09 kg in weight and 37.83 ± 1.83 cm length used as fish material, were obtained from a local company in Perşembe, Ordu. Other ingredients used in making stuffed meatballs (onion, bulgur, salt, black pepper, farina, flour, tomato paste, parsley, and sunflower oil) were obtained from a local market.

Methods

Preparing stuffed meatballs with rainbow trout mince

The stuffed meatballs with rainbow trout were developed by taking into account the various recipes used for traditionally produced stuffed meatballs, and they were boiled and introduced to six trained panelists for hedonic testing. In the hedonic test, the stuffed meatballs were evaluated using a 1–3-point scale for odor, smell, and taste. These scores are defined as (1) I don't like it at all, (2) I like it, and (3) I like it very much (Doloksaribu et al., 2015). The stuffed meatballs with rainbow trout that received the highest score in the hedonic test were formulated following the panelists' recommendations, and two formulations were made, namely stuffing materials and outer shell. (Tables 1 and 2).

Stuffing materials

The ratio (%) of stuffing materials of stuffed meatballs with rainbow trout is given in Table 1.

Table 1. The proportions of the materials used in the preparation of the stuffed meatballs with rainbow trout (%)

Stuffing materials	%
Rainbow trout mince	34.22
Onion	47.42
Salt	0.33
Black pepper	0.53
Parsley	7.47
Sunflower oil	10.03

The harvested rainbow trout from cages were beheaded, gutted, washed, filleted, and cut into small cubes immediately after killing and transferred to the Processing Technology Laboratory of the Faculty of Fatsa Marine Science in a foam box containing crushed ice within 1 hour. After the onions and parsley were picked and washed, they were minced in the Ortimax 700 W food processor. The onions were roasted in sunflower oil until they turn pink, and then the minced rainbow trout fillets were added. When the water of the mixture was absorbed, parsley, salt, and black pepper were added and cooked for a few more minutes (Figure 1).

Figure 1. Cooked stuffing materials

The outer shell of stuffed meatballs

The proportions (%) of the outer shell materials used in the preparation of stuffed meatballs with rainbow trout are shown in Table 2.

Table 2. The proportion of outer shell materials used in the preparation of stuffed meatballs with rainbow trout (%)

Materials of the outer shell	%
Bulgur	52.84
Farina	13.48
Flour	12.57
Salt	2.71
Tomato paste	18.40

Boiled water (2/3 ratio, v/w) was added to the bulgur and farina mixture, and it was kept at room temperature until the water was absorbed by the bulgur and farina. After the bulgur and farina sucked the water, the other ingredients; flour, salt, and tomato paste were added and the whole mixture was mixed homogeneously. The mixture was kneaded by hand until it was shaped (Figure 2).

Figure 2. Kneading of the outer shell of stuffed meatballs with rainbow trout

To produce stuffed meatballs in equal diameter and size, the meat grinder (EMP.12.01. P type 220W) was equipped with stuffed meatball-making apparatus. The prepared bulgur mixture was passed through a meat grinder and cut into 7 cm lengths each (Figure 3 and Figure 4).

Figure 3. Cylindrically shaped outer shell passing through stuffed meatballs apparatus.

Figure 4. Ready to fill shaped outer shell of stuffed meatballs with rainbow trout.

The previously cooked stuffed materials were filled into the outer shell that was ready to be filled, and the ends of the meatballs were covered and made ready to be frozen. The total weight of the produced stuffed meatballs was determined as 54.87 ± 2.53 g. After the stuffed meatballs were placed on metal trays and frozen at -40º C, the packaged products in refrigerator bags were stored at -18ºC, and the changes in color and sensory quality were investigated by monthly sampling (Figure 5)

Figure 5. The frozen stuffed meatballs with rainbow trout

Proximate Composition Analysis

In the analyzes, the stuffed materials and outer shell of the stuffed meatballs with rainbow trout were thoroughly homogenized in a Waring blender and each sample from the homogenate was analyzed in triplicate for the determination of moisture, crude ash, total lipid, and crude protein analysis, and duplicate for the determination of fatty acid composition.

Moisture and ash analysis was performed on all samples using AOAC (Association of Official Analytical Chemists, 1990) methods. The Kjeldahl method was used to determine the crude protein content (AOAC, 1990). To evaluate the lipid content, Bligh and Dyer (1959) method has been used. Fatty acid methyl esters were analyzed from extracted lipids according to the method of Ichihara et al. (1996). 4ml of 2M KOH and 2ml of n-heptane were added in a screw-capped glass test tube containing 25 mg extracted oil sample. The sample heptane layer, which was mixed in a vortex for 2 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm, was taken for analysis in a gas chromatography (GC) device. The fatty acid composition was determined using GC (Gas chromatography) with automatic sampling with flame ionization detector (FID) and 30m x 0.32mm ID x 0.25µm film thickness SGE column (Perkin Emler, USA). Injector and detector temperatures were set to 220 ° C and then 280 ° C respectively. Meanwhile, the oven temperature was kept at 140 ° C for 5 minutes. Then it was brought up to 200 ° C by increasing 4 ° C every minute and from 200 ° C to 220 by 1 ° C every minute. The split application was carried out at a ratio of 1:50. Fatty acids were identified by comparing the FAME mixture consisting of standard 37 components depending on their arrival times. The results of the two GC analyses performed in the same way were expressed in GC area (%) as mean values ± standard deviation.

According to De Lorenzo et al. (2001), the atherogenic (AI) and thrombogenic indexes (TI) were determined as follows:

AI= (C12:0+C14:0+C16:0/n-3PUFA+n-6 PUFA+MUFA

TI= (C14:0+C16:0+C18:0)/0.5(n-6 PUFA) +3.(n-3 PUFA)+n3/n6 PUFA)

The abbreviations MUFA and PUFA refer for monounsaturated fatty acids and polyunsaturated fatty acids, respectively.

Instrumental Color Measurement

In color measurements, The CIE $L^*a^*b^*$ values of samples were measured in the reflectance by a Chroma Meter Konica-Minolta CM-5 (Osaka, Japan) according to Calder (2003). Before starting the analysis, the instrument was calibrated with white and black plates.

Sensory Analysis

Color, odor, flavor, texture, and general acceptability parameters were tested to determine the sensory content of the stuffed meatballs with rainbow trout, according to Paulus et al (1979). The sensory assessment scheme was used, and the panelists were graded on a 9-point hedonic scale (1, dislike extremely to 9, like extremely). The samples were prepared in two separate ways, frying, and boiling, and then introduced to the six trained panelists for evaluation. Samples were allowed to dissolve for 5 hours at refrigerator condition (+ 4°C) before cooking. The frying was performed in a Teflon-coated pot for 1 minute 40 seconds in deep oil (Fig.6). The samples were cooked in boiling

water for 10 minutes during the boiling process (Fig 7). The panelists were given the samples when they were still hot.

Figure 6. Fried stuffed meatballs with rainbow trout.

Figure 7. Boiled stuffed meatballs with rainbow trout.

Statistical Analysis

For data analysis of stuffed meatballs with rainbow trout, a one-way analysis of variance (ANOVA) was applied using the SPSS version 16 software at a 5% confidence level, and Duncan's multiple range test at a p-value of < 0.05 were run to determine significant differences (Duncan, 1955).

RESULTS

Proximate Composition of Raw Stuffed Meatballs with Rainbow Trout

The amount of moisture, crude ash, lipid and crude protein of stuffed meatballs with rainbow trout were found to be 58.25 ± 0.93%, 2.01 ± 0.05% (4.81 ± 0.13 g / 100 g dry weight), 5.28 ± 0.29% (12.64 ± 0.71 g / 100 g dry weight) and 7.69 ± 0.08% (18.41 ± 0.19 g / 100 g dry weight), respectively (Figure 10).

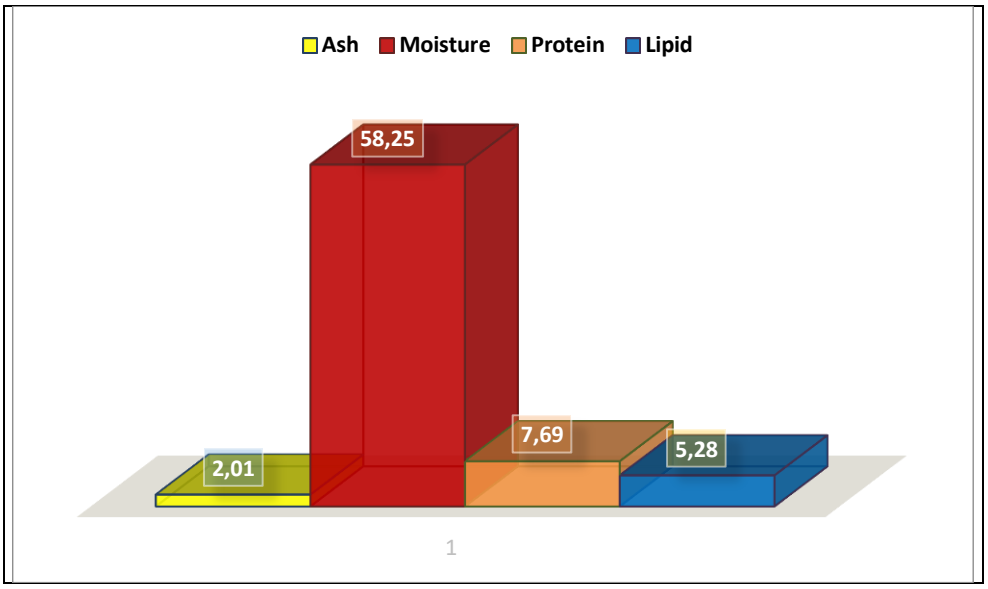

Figure 10. Proximate composition of stuffed meatballs with rainbow trout (%, wet weight basis)

In the study, the total saturated fatty acid (∑SFA), the total monounsaturated fatty acid (∑MUFA), and the total polyunsaturated fatty acid (∑PUFA) content of stuffed meatballs with rainbow trout were determined to be 12.52 ± 0.23%, 39.11 ± 1.27% and 43.73±1.98%, respectively. The highest fatty acid among the fatty acid composition of stuffed meatballs with rainbow trout was estimated as linoleic acid (C18: 2n6) with 39.70 ± 0.47%, followed by oleic acid (C18: 1n9) with 37.86 ± 1.18%. The amount of C18:2 n6, C18:3 n3, C20:3 n6, C20:5 n3, and C22:6 n3 as the PUFA was found as 39.70 ± 0.47%, 1.80 ± 0.07%, 0.11 ± 0.01%, 0.55 ± 0.01% and 1.13 ± 0.95%, respectively (Table 3). Additionally, the amounts of PUFA/SFA, n3 PUFA, n6 PUFA, n6/n3, and EPA+DHA were estimated to be 3.491.11, 4.020.34, 39.71+0.24, 10,880.29, and 1.680.48, respectively. Furthermore, the AI and TI for dietary factors associated with cardiovascular disease were found to be 0.10 and 0.25, respectively.

Table 3. Fatty acid methyl esters (FAME) composition of stuffed meatballs with rainbow trout (%)

FAME	%
C16:0	08.29±0.16
C17:0	00.06±0.00
C18:0	03.31±0.07
C20:0	00.31±0.01
C22:0	00.52±0.01
C24:0	00.03±0.01
∑SFA	**12.52±0.23**
C14:1	0.010±0.00
C16:1	00.28±0.39
C17:1	00.03±0.00
C18:1n-9	37.86±1.18
C18:1n-7	00.02±0.01
C20:1n-9	00.76±0.02
C22:1n-9	00.13±0.00
C24:1n-9	00.03±0.00
∑MUFA	**39.11±1.27**
C18:2n-6	39.70±0.47
C18:3n-3	01.80±0.07
C20:3 n-6	00.11±0.01
C20:4 n-6	00.01±0.00
C20:5n-3	00.55±0.01
C22:6 n-3	01.13±0.95
∑PUFA	**43.73±1.98**
∑PUFA/∑SFA	3.49±1.11
∑n6 PUFA	39.71+0.24
∑n3 PUFA	4.02±0.34
n6/n3	10,88±0.29
EPA+DHA	1.68±0,48
AI	0,10±0.09
TI	0,25±0.04

The Effect of Frozen Storage on Color (L *, a *, b *) Values of Stuffed Meatballs with Rainbow Trout

Table 4 shows the changes in L *, a*, and b* values of stuffed meatballs with rainbow trout during frozen storage.

Table 4. Changes in L *, a*, and b* value of stuffed meatballs with rainbow trout during frozen storage *

Months	L*	a*	b*
0	58.16 ± 0.33^{b}	17.51 ± 0.79^{b}	26.83 ± 0.55^{c}
1	57.27 ± 0.33^{a}	16.62 ± 0.91^{b}	26.03 ± 0.65^{b}
2	59.47 ± 0.67^{c}	16.56 ± 0.69^{b}	26.99 ± 0.28^{c}
3	62.85 ± 0.20^{e}	14.25 ± 0.54^{a}	24.85 ± 0.39^{a}
4	60.55 ± 0.41^{d}	14.82 ± 0.41^{a}	29.79 ± 0.53^{d}

*Different letters in the same column show the differences at a 0.05 significance level ($p < 0.05$).

A significant increase in the L * values of the stuffed meatballs with rainbow trout was found at the end of storage compared to the initial values ($p < 0.05$). Concerning the * value, while there was no significant difference in the first two months of storage ($p > 0.05$), this value decreased significantly in the 3rd month of storage ($p < 0.05$) and this decrease remained similar at the end of storage ($p > 0.05$).

The b * value of raw samples significantly increased from 26.83 ± 0.55 at the beginning of storage to 29.79 ± 0.53 at the end of storage ($p < 0.05$).

Changes in the Color (L *, a *, b *) Values of Frozen Stuffed Meatballs with Rainbow Trout After Boiling

Changes in the color (L *, a *, b *) values of frozen stuffed meatballs with rainbow trout after cooked by boiling water are shown in Table 5.

Table 5. Changes in the color (l *, a *, b *) values of frozen stuffed meatballs with rainbow trout after cooked by boiling water*

Months	L*	a*	b*
0	55.19 ± 0.51^{b}	15.21 ± 0.35^{b}	22.62 ± 0.31^{a}
1	55.14 ± 0.20^{b}	16.30 ± 0.70^{c}	24.94 ± 0.31^{b}
2	51.62 ± 0.62^{a}	19.62 ± 0.50^{d}	29.33 ± 0.88^{c}
3	55.74 ± 0.60^{b}	15.66 ± 0.44^{bc}	25.29 ± 0.79^{b}
4	58.00 ± 0.66^{c}	13.58 ± 0.62^{a}	24.90 ± 0.80^{b}

*Different letters in the same column show the differences at a 0.05 significance level ($p < 0.05$).

L * values of the samples at the beginning and the first month of storage were found to be insignificant ($p > 0.05$), while a significant decrease was found in the second month of storage ($p < 0.05$). While the L* values of samples were similar to the beginning and the 3rd month of storage ($p > 0.05$), they showed a significant increase at the end of the storage ($p < 0.05$).

In the statistical analysis performed, a significant increase was detected in the value of a* of samples in the first two months of storage ($p < 0.05$). At the end of storage, a significant decrease was found in the samples of a*values ($p < 0.05$).

In the samples, there was a significant increase in b* value of samples in the first two months of storage ($p < 0.05$), while a significant decrease was found in the third month ($p < 0.05$). In the 4th month, which is the last month of storage, the b* value in the samples was determined to be similar to the value of samples in the 1st month ($p > 0.05$).

Changes in the Color (L *, a *, b *) Values of Frozen Stuffed Meatballs with Rainbow Trout After Frying

Changes in the color (L *, a *, b *) values of frozen stuffed meatballs with rainbow trout after frying are given in Table 6.

Table 6. Changes in the color (L *, a *, b *) values of frozen stuffed meatballs with rainbow trout after frying *

Months	L*	a*	b*
0	40.20 ± 0.28^{a}	25.13 ± 0.61^{d}	30.03 ± 0.83^{c}
1	32.95 ± 0.84^{a}	19.21 ± 0.35^{c}	24.92 ± 0.76^{a}
2	43.62 ± 0.77^{b}	23.11 ± 0.13^{c}	29.82 ± 0.41^{c}
3	50.15 ± 0.82^{c}	20.32 ± 0.58^{a}	28.81 ± 0.06^{b}
4	49.23 ± 0.24^{c}	21.97 ± 0.72^{b}	31.75 ± 0.63^{d}

*Different letters in the same column show the differences at a 0.05 significance level ($p < 0.05$).

In this study, it was determined that the L* value of the samples increased significantly in the first three months of storage and did not show a significant change after this month ($p < 0.05$).

While a fluctuating change in the value of a* was observed during storage in the samples ($p < 0.05$), a significant decrease was found at the end of storage compared to the beginning value ($p < 0.05$).

The b * value of the raw samples significantly decreased in the 1st month of storage compared to the initial value ($p < 0.05$). Then, this value increased again in the 2nd month and was found to be similar to the initial value ($p > 0.05$). However, the b * value of the samples at the end of storage was found to be significantly higher than the initial value ($p < 0.05$).

Sensory Quality of Stuffed Meatballs with Rainbow Trout During Frozen Storage

Sensory Assessment after Boiling

The changes in sensory quality parameters of boiled stuffed meatballs with rainbow trout are shown in Table 7.

Table 7. The changes in sensory quality parameters of stuffed meatballs with rainbow trout cooked after boiling

Months	Color	Odor	Flavor	Texture	Overall acceptability
0	8.0±1.54^{c}	8.6±0.51^{b}	8.5±0.54^{d}	8.3±0.81^{b}	8.3±0.51^{c}
1	7.0±0.63^{bc}	8.0±0.63^{b}	8.0±0.00^{cd}	8.0±0.63^{b}	7.9±0.66^{c}
2	7.0±0.63^{bc}	6.6±0.51^{a}	7.7±0.51^{bc}	7.0±0.63^{a}	6.8±0.75^{b}
3	6.3±0.51^{ab}	6.1±0.75^{a}	7.3±0.55^{b}	6.3±0.51^{a}	6.0±0.00^{a}
4	5.6±0.51^{a}	6.2±0.98^{a}	6.5±0.23^{a}	6.1±0.75^{a}	6.1±0.40^{a}

*Different letters in the same column show the differences at a 0.05 significance level ($p < 0.05$).

According to the results of the sensory analysis, a significant decrease in the color value of boiled stuffed meatballs with rainbow trout was found at the end of storage compared to the initial value ($p < 0.05$).

The odor value in the samples evaluated by the panelists, there was no significant difference ($p > 0.05$) in the 1st month of storage ($p > 0.05$), and a significant decrease in the odor value of the samples occurred in the 2nd month of storage ($p < 0.05$). After this month, it was determined that there was no significant change until the end of storage ($p > 0.05$).

In samples cooked with boiling water, the flavor score significantly decreased at the end of storage periods ($p<0.05$).

Statistically, it was determined that there was no significant difference in the texture value of the samples in the 1st month of storage ($p > 0.05$), while there was a decrease in this value in the 2nd month ($p < 0.05$) and this decrease was not significant until the end of storage ($p > 0.05$).

In the samples, there was no significant difference in the overall acceptability value in the first month of storage ($p > 0.05$), while a significant decrease was found in the third month of storage ($p < 0.05$).

Sensory Assessment after Frying

The changes in sensory quality parameters of frozen stored rainbow trout stuffed meatballs cooked after frying are shown in Table 8.

Table 8. The changes in sensory quality parameters of frozen stored rainbow trout stuffed meatballs cooked after frying *

Months	Color	Odor	Flavor	Texture	Overall acceptability
0	8.8±0.40^{d}	8.1±0.75^{c}	8.7±0.52^{c}	8.5±0.54^{c}	8.3±0.51^{c}
1	8.1±0.75^{c}	8.0±0.63^{c}	8.2±0.^{41bc}	8.0±0.63^{bc}	7.9±0.66^{c}
2	7.6±0.51^{c}	7.5±0.54^{bc}	8.0±0.52^{b}	7.5±0.54^{b}	6.8±0.75^{b}
3	6.8±0.40^{b}	6.5±0.54^{a}	7.7±0.75^{b}	6.5±0.54^{a}	6.0±0.00^{a}
4	6.0±0.63^{a}	7.0±0.89^{ab}	6.8±0.52^{a}	6.0±0.89^{a}	6.1±0.40^{a}

*Different letters in the same column show the differences at a 0.05 significance level ($p < 0.05$).

According to the statistical analysis, there was a decrease in the color value in the 1st month of storage compared to the initial value ($p < 0.05$), but there was no change between the 1st month and the 2nd month ($p > 0.05$). A significant decrease was observed again in the 3rd and 4th months of storage ($p < 0.05$).

When the effect of frozen storage on odor value was examined, it was determined that there was a significant decrease in odor values of fried samples at the end of storage compared to the initial value ($p < 0.05$).

The sensory score for the flavor of the cooked samples by frying significantly decreased at the end of the storage time when compared to the initial value ($p<0.05$).

As a result of the statistical analysis, it was found that the texture value of the samples, which was 8.5 ± 0.54 at the beginning, significantly decreased to 6.0 ± 0.89 in the 4th month of storage ($p < 0.05$). After these reductions, no significant change was noted ($p > 0.05$).

When the general acceptability values of the samples were analyzed statistically, a significant decrease was found at the end of storage compared to the initial value ($p < 0.05$).

DISCUSSION

Proximate Composition of Stuffed Meatballs with Rainbow Trout

In this study, the amount of lipid 5.28 ± 0.29% (12.64 ± 0.71 g / 100 g dry weight). Aramouni et al. (2001) found the fat content of kibbeh (stuffed meatballs) produced from ground beef as 2.36% (7.53 g / 100 g dry matter). It appears that the researcher did not use any oil in the filling formulation and cooking. Therefore, it is thought that the reason for this difference in the lipid content of stuffed meatballs is due to the formulation. In this study, the protein content of stuffed meatballs made from rainbow trout was found to be 18.41% ± 0.19 g / 100g dry matter. Aramouni et al. (2001) found that the crude protein content of stuffed meatballs produced from ground beef was 51.21 g 100 g dry matter. They used a high percentage of ground beef (33.33% ground beef, 16.67% bulgur, and 50% water) in the outer shell of stuffed meatballs. It may explain the reason why the crude protein value was found higher than the value obtained in this study. Again, the same studies found the raw ash content of meatballs as 3.16 g / 100 g dry matter. It is seen that this value found is very close to the value found for rainbow trout stuffed meatballs (3.45 g / 100 g dry matter). Aramouni et al. (2001), the moisture content of the meatballs made by meatballs was 68.67% (31.32% dry matter) higher than our findings. This is thought to be due to the different formulations to produce stuffed meatballs. In the literature review, no scientific data was found about the production of stuffed meatballs produced from fish. In this study, it is thought that the bulgur, farina, and tomato paste used in the outer mixture have a proportional effect on the nutritional composition of the stuffed meatballs made from rainbow trout.

In the present study, the highest amount of fatty acid within the fatty acid composition was estimated as linoleic acid (C18: 2n6) with 39.70 ± 0.47%, followed by oleic acid (C18: 1n9) with 37.86 ± 1.18%. The amount of C20: 5n3 and C22: 6 n3 was found to be 0.55 ± 0.01% and 1.13 ± 0.95%, respectively. The total saturated fatty acid (∑SFA) content was 12.52 ± 0.23%, the total monounsaturated fatty acid (∑MUFA) amount was 39.10 ± 1.27% and the total polyunsaturated fatty acid (∑PUFA) amount was found to be 43.73 ± 1.98%. According to the results of this research, it is thought that the fatty acid compositions of rainbow trout, sunflower oil, and bulgur are used at high rates in the formulation of rainbow trout stuffed meatballs (depending on the amount of oil) are effective on the fatty acid composition of rainbow trout stuffed meatballs. Our findings are supported by previous studies mainly the fatty acid composition of ingredients used for stuffed meatballs made from rainbow trout. Öz (2009) estimated that ∑SFA, ∑MUFA, and ∑PUFA of farmed rainbow trout were 20.74%, 26.57%, and 51.12%, respectively. Bayrak and Bayraktar (1995) obtained 44.73% linoleic acid and 41.99% oleic acid within the fatty acid composition of the sunflower oil in their study. Dağlıoğlu et al. (2002), who studied the fatty acid composition of 13 grain-based foods produced by Turkish companies, found that bulgur, which is used as the main material in the outer shell of stuffed meatball made from rainbow trout, consists of 18: 1, 18: 2 and 18: 3 fatty acids and they found that the 18: 2 fatty acids had the highest level with 56.8%.

The fatty acid composition of dietary fats, and especially of certain specific fatty acids, is essential in human nutrition and health. A reduced saturated fat intake and an elevated PUFA-to-SFA ratio are linked to a lower risk of human coronary heart disease and increasing this ratio can be resulted in cholesterol reduction in plasma (Mcafee et al., 2010). As a result, the PUFA/SFA ratio is one of the primary criteria used to determine the nutritional content of foods' lipid fractions. This ratio was observed in this analysis to be 3,49±1.11; a ratio PUFA/SFA above 0.4 is recommended. (Wood et al. 2004). The observed AI and TI value of <3 in the present study was mainly due to the n-6 PUFA content, especially 18:2 n6. These findings for TI are significant since atherosclerosis involves thrombosis. Very low levels of the above indices are prescribed in a "Healthy" diet (Ulbricht and Southgate, 1991).

The Effect of Frozen Storage on Color (L *, a *, b *) Values of Stuffed Meatballs with Rainbow Trout

Color is defined as a characteristic of light, which is both an inherent property of *light* and an artifact of the human eye. Physically, it is measurable in terms of intensity and wavelength. However, color as a perceptual phenomenon can be changed depending on the observer and the conditions under which color is observed (Pathare et al.,2013). Researchers state that the color of material appears only when the light from a luminous object or source is visible, or its surface is illuminated. Keskin et al. (2018) stated that color measurement should be numerically expressed in food sciences and agriculture to determine the color value precisely, accurately, and repeatably. For this purpose, different color definition models (color model) or color spaces (color spaces) have been developed to define the color value.

The HunterLab L *, a *, b * and modified Commission International de l'Eclariage (CIE) system called CIELAB, are often used in the food industry. The degree of lightness (L), the degree of redness or greenness (+/-a), and the degree of yellowness or blueness (+/-b) is measured by the systems (Pathare et al., 2013).

Color is one of the most important quality characteristics affecting the purchasing behavior of the consumer. Many factors affect ultimate perceived color such as animal genetics, species, nutritional background, postmortem storage temperatures and time, postmortem changes in muscle, and a whole host of processing, packaging, and lighting and display variables (Hunt et al., 2012). Expected changes in colors can also indicate problems with processing or packaging. It is stated that the taste of food is generally affected by color. For these reasons, color measurement and analysis are very important to optimize the quality and value of foods (Pathare et al., 2013).

In this study, the L * value of the samples increased during storage ($p<0.05$). This increase in L * value indicates that the whiteness of the product increased during storage. This finding was different from the finding of Aramouni et al. (2001) who could not find a significant change in the L * value of the stuffed meatballs produced from ground beef during storage. These differences were thought to be due to the differences in the formulation of the outer shell.

In the present study, the * value of samples, which indicates the storage redness, significantly decreased ($p<0.05$). Tomatoes are especially rich in lycopene content and are among the most produced, consumed, and traded agricultural products in the world. It is stated that although lycopene is the main carotenoid compound in tomato and tomato products, β-carotene is also present in these products. Calligaris et al. (2002) suggest that the loss of red color intensity in tomato products may be due to the chemical and physical changes of the carotenoids in tomatoes, especially lycopene. Cosmai et al. (2017) stated that the red color in tomato-based products has a positive effect on the appearance of the product, and the decrease in red color density during frozen storage is due to the oxidation of carotenoids, including lycopene, and the co-oxidation of enzymatic reactions. According to these results found by the researchers, the decrease in the * value, which indicates the red color intensity of the rainbow trout meatballs, may be due to the deterioration of the carotene in the tomato paste (18.40%) in the outer shell of the stuffed meatballs during frozen storage. Similarly, a decrease in the*value of stuffed meatballs (outer shell consisting of meat and bulgur) during frozen storage was found by Aramouni et al. (2001). However, in this study, it is thought that the reason for the decrease in redness value was not due to tomato paste as in this study (the researcher did not use tomato paste in the outer shell formulation), but because the hemoglobin, which gives the red color of ground beef, is reduced during frozen storage.

In this study, a significant increase was found in b* values of frozen raw stuffed meatballs, cooked frozen raw stuffed meatballs with boiling water, and frying at the end of storage periods when compared to initial values ($p<0.05$). Unlike these findings, Aramouni et al. (2001), in their study, emphasized that there was no significant change in the b * value of meat stuffed meatballs during frozen storage. In this study, sensory quality stuffed meatballs were evaluated in two ways: boiled and fried as common consumption type of it and fried samples were preferred by the panelists according to color and flavor. Although a significant decrease was detected in the sensory quality parameters of samples during frozen storage, it was found that they did not reach the unacceptable limit at the end of storage. One of the most remarkable changes in sensory evaluation is the decrease in color, especially in fried products. This is seen from the decrease in a * value, which indicates the redness.

CONCLUSION

In comparison to stuffed meatballs made from other meats, made from rainbow trout have been nutritionally enhanced in terms of including salmon, which play an important role in human nutrition. and can consume as an alternative value-added product for people to increase fish consumption. The instrumental color values could affect frozen storage. Although the scores of sensory quality parameters of stuffed meatballs declined during frozen storage after 4 months of storage, it was determined that it did not exceed the unacceptable limit (score <5) by the panelists.

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Su Ürünleri Tüketim Tercihleri Üzerine Uşak İlinde Bir Anket Çalışması

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Özet

Bu çalışmada 2020-2021 balık avcılığı sezonunda Uşak İli merkezinde yaşayan 1000 birey ile görüşülerek anket çalışması yapılmış ve su ürünleri tüketimine yönelik davranışları araştırılmıştır. Ankete katılanların %55,5'i kadın, %44,5'i erkeklerden oluşmaktadır. Katılımcıların %59,3'ü lise mezunu, %27,6'sı öğrenci, %21,1'i işçi, %28'i ev hanımı, %19,3'u memur, %1,5'i emekli ve %2,5'i de diğer meslek mensuplarıdır. Katılımcıların tercihinde, öncelikle kırmızı et (%23,5), tavuk (%18,9) balık (%15,5) ve tüm et türlerinin (%36) tüketimi görülürken, %6'sının et tüketmediği belirlenmiştir. Ankete katılanların balık tüketim sıklıkları haftada bir %38,1 oranla birinci sıradadır. Deniz balıkları tüketimi fazla olmamakla birlikte en çok tüketilen tür hamsidir. Katılımcılar balığı büyük çoğunlukla kış aylarında (%79,1) tüketmekte ve halk pazarlarından temin etmektedirler.

Anahtar Kelimeler: Uşak,balık, anket,su ürünleri,tüketim alışkanlıkları

A Survey Study on Fisheries Consumption Preferences in Uşak Province

Abstract

In this study, it was tried to determine fish consumption behaviors by surveying 1000 people living in the Uşak city center in the 2020-2021 fishing season. The respondents are women (55.5%) and men (44.5%). Most of the respondents are high school graduates (59.3%). Considering the educational status of the participants, 27.6% are students, 21.1% workers, 28% housewives, 19.3% civil servants, 1.5% retired, and 2.5% other professionals. In the preference of the participants, it was determined that primarily red meat (23.5%), chicken (18.9%), fish (15.5%), and all meat types (36%) were consumed, while 6% did not consume meat. The fish consumption frequencies of the respondents were 38.1 percent per week. Although the consumption of sea fish is not high, the most consumed sea fish is anchovy.Participants consume fish most in winter (%79.1) and obtain them from public markets.

Keywords: Uşak, fish, survey, seafood, consumer behaviors

GİRİŞ

Ülkemizde kişi başına düşen su ürünleri tüketimi daha çok kıyı bölgelerinde yoğunlaşmıştır. Kişi başı yıllık ortalama balık tüketimi 2019 yılında 6,3 kg olup dünya ortalamasının çok altındadır (Anonim, 2020). Karadeniz Bölgesinde 25 kg, İstanbul, İzmir, Ankara gibi büyük şehirlerde 16 kg, Doğu ve Güney Doğu Anadolu Bölgesinde 0.5 kg tüketim görülmektedir (İSÜB, 2018). Gürgün (2006), Van bölgesinde yaptığı çalışmada, 262 bireyin %82,2'sinin balıkları taze tükettiği, %4,2'sinin ise hiç balık tüketmedikleri, %59,1'inin ilk sırada inci kefalini tükettiği sonucuna ulaşmıştır. Arslan ve İzci (2016), Antalya İli'nde su ürünleri tüketim alışkanlıklarını belirlemek için bir çalışma yapmış ve katılımcıların su ürünlerini %43,67'lik oranla iki haftada bir tükettiği, taze ürünlerin genelde kızartıldığı ve fiyatların ise su ürünleri tercihlerinde önemli yer tutuğunu tespit etmiştir. Bu konuda Türkiye'nin birçok bölgesinde ve Türkiye dışında yapılmış anket çalışmaları mevcuttur. Gürgün (2006) Van Gölü'ne kıyısı bulunan bazı ilçelerde, Çadır (2012) Keban Baraj Gölü Ova Bölgesinde, Güngör (2014) Erzurum ve Van'da, Çiçek vd. (2013) Elazığ'da, Odabaşı (2016) Diyarbakır'da, Menteşe (2016) Tunceli'de, Şen (2017) Erzincan'da, İbiş (2014) Sivas'ta olmak üzere Doğu ve Güney Doğu Anadolu bölgesinde benzer çalışmalar gerçekleştirmiştir. Karadeniz Bölgesi'nde ise Balık

vd.'nin (2013) Ordu'nun Fatsa ve Aybastı İlçelerinde, Aydın ve Karadurmuş (2013)'un Trabzon ve Giresun'da, Temel (2014)'in Rize'de, Uzundumlu ve Dinçel (2015)'in Trabzon'da ve Kızılaslan ve Nalinci (2013)'nin Amasya'da gerçekleştirdiği çalışmalar dikkat çekmektedir. Akdeniz ve İç Anadolu Bölgeleri'nde ise; Burdur (Orhan ve Yüksel, 2010), Antalya (Arslan ve İzci, 2016), Konya ve Mersin (Şen, 2011) ve Niğde illerinde (Bashimov, 2017) gerçekleştirilen bazı çalışmalara ulaşılmıştır. Kocaeli'nde (Akçay ve Vatansever, 2013), İzmir'de (Çaylak, 2013), Manisa'da (Çelik, 2014), Gökçeada'da (Doğan ve Gönülal, 2014) ve Çanakkale ve Ankara'da (Bayraktar, 2015) Ege ve Marmara Bölgeleri'ni içine alan benzer çalışmalara rastlanmaktadır. Ayrıca Özek'in (2008) Kırgızistan'da gerçekleştirdiği çalışma da dikkat çekmektedir.

Kocaman Balıkçılığın "Türkiye'nin Balık Tüketim Haritası" başlıklı araştırmasına göre, ülkede kişi başına yıllık tüketilen balık miktarı 7,6 kg olurken, Karadeniz Bölgesi 25 kg ile ilk sırada yer almıştır. Yani Karadeniz Bölgesi'nde yaşayan ile Türkiye'nin diğer bölgesinde yaşayanlar arasında balık tüketimi konusunda 17,4 kg'lık farkın olduğu görülmektedir. Tüketim, Doğu ve Güneydoğu Anadolu Bölgeleri'nde ulaşımın zor olduğu yıllarda su ürünlerinin bölgeye az ulaştırılması nedeniyle, tüketim alışkanlığı kazanılmadığından 1 kg'ın altına kadar inebilmektedir. İzmir, Ankara, İstanbul gibi büyük şehirlerde ise yıllık kişi başı tüketilen balık miktarı 16 kg olarak saptanmıştır. Türkiye'de en çok küçük balıklar özellikle hamsi tüketilirken, onu sardalya ve istavrit takip etmektedir. İstanbul'da lüfer, mezgit ve palamut gibi bölgesel türler tercih edilirken, yöreye özgü olan kupes ve tirsi ise en çok İzmir'de tercih edilmektedir. Türkiye'de balık tercihlerini en çok etkileyen unsurların başında tazelik ve fiyat gelirken yöresel olarak avlanan veya yetiştirilen balıklar lezzet alışkanlığı ve değişik işleme yöntemleri sebeplerinden dolayı tercih edilebilmektedir. Örneğin Göller Bölgesi'nde sazan ve sudak diğer bölgelere nazaran yaz kış tercih edilen balık türleridir. Türkiye'de işlenmiş balığın %48'lik oranının İstanbul'da yaşayanlar tarafından tüketildiği, İstanbul'dan sonra Muğla'da %38, Antalya'da %7 oranlarında tüketildiği bildirilmektedir (Deniz Haber Ajansı, 2018).

Çalışmanın amacı su ürünleri tüketimi üzerine Uşak İlindeki tüketicilerin davranışlarını belirlemektir. Böylelikle Türkiye'de tüketimi az olan su ürünlerinin tercih edilmesinde etkili olan faktörlerin Uşak İli bazında ortaya konulması amaçlanmıştır. Tüketicilerin görüşleri doğrultusunda üretim planlaması, pazarlama ve su ürünlerini tanıtımı konularında fikir oluşturulması amaçlanmıştır.

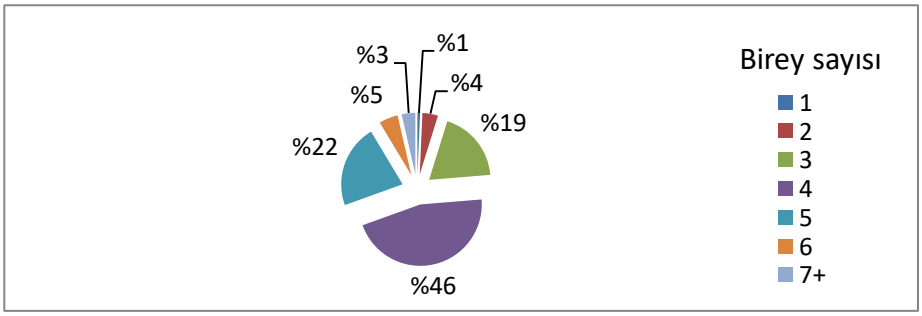
MATERYAL VE YÖNTEM

Çalışma, 2021 Ocak ve 2021 Şubat ayları arasında Uşak İl merkezinde yaşayanlar içerisinde tesadüfü olarak seçilen 1000 kişi ile 16 sorudan oluşan anket sorularının yüz yüze görüşülerek cevaplanması şeklinde gerçekleştirilmiştir. Ankete katılanların bölgede yerleşik halktan olmalarına özen gösterilmiştir. Katılımcılara sosyo-ekonomik durumları, su ürünleri tüketim davranışları, tükettiği türler, miktarları, tercih sebepleri ve ürün tedarik yerlerini belirlemeye yönelik sorular sorulmuştur.

Elde edilen veriler, MS-Excel ve aritmetik ortalamalar alınarak yorumlanmış ve değerlendirilmiştir. Uşak, Türkiye'nin Ege Bölgesinin doğusunda yer almaktadır. Ege ve İç Anadolu Bölgeleri arasında bir geçit durumundadır. Doğuda Afyonkarahisar, batıda Manisa, kuzeyde Kütahya, güneyde Denizli illeri ile komşudur.

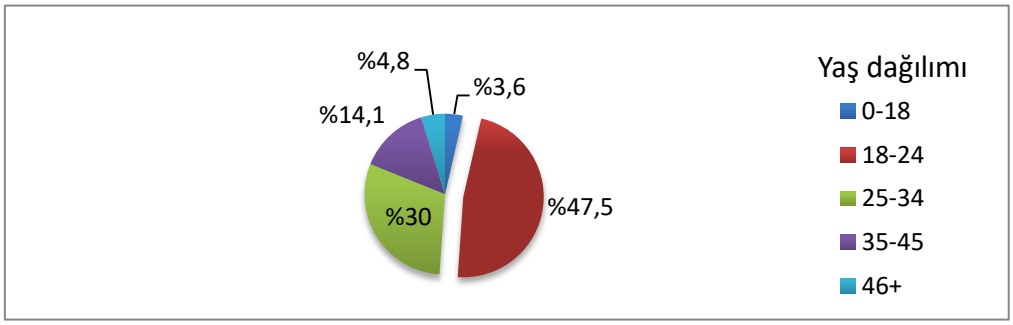
BULGULAR

Anket çalışmasında görüşülen katılımcıların 445 kişisi erkek (%44,5), 555 kişisi (%55,5) kadındır. Anket uygulamasına katılan bireylerin büyük çoğunluğu %46 oranla 4 kişilik ailelerdir. En düşük çoğunluk ise %1 oranla 1 kişi olarak yaşayanlardır (Şekil 1).



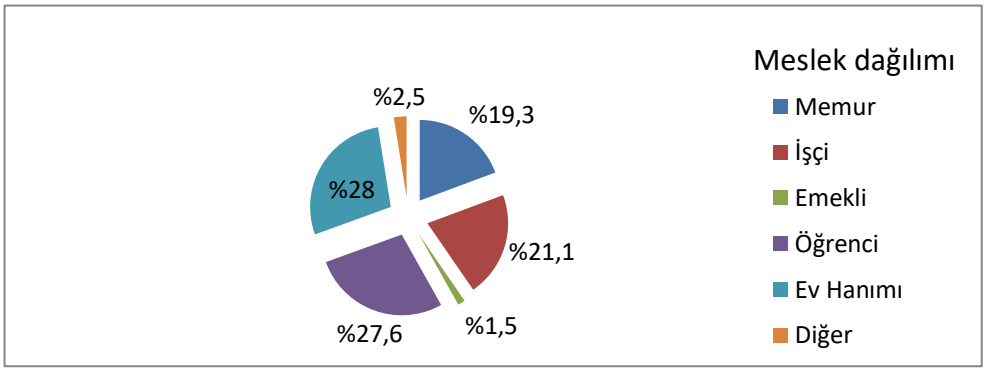
Şekil 1. Ankete katılanların aile birey sayıları

Araştırmada bireylerin yaş dağılımı incelendiğinde; 18 yaşından küçükler %3,6; 46 yaş ve daha büyükler %4,8; 35-45 yaş arası %14,1; 25-34 yaş arası %30; 18-24 yaş arası %47,5 oranında oldukları görülmüştür (Şekil 2).



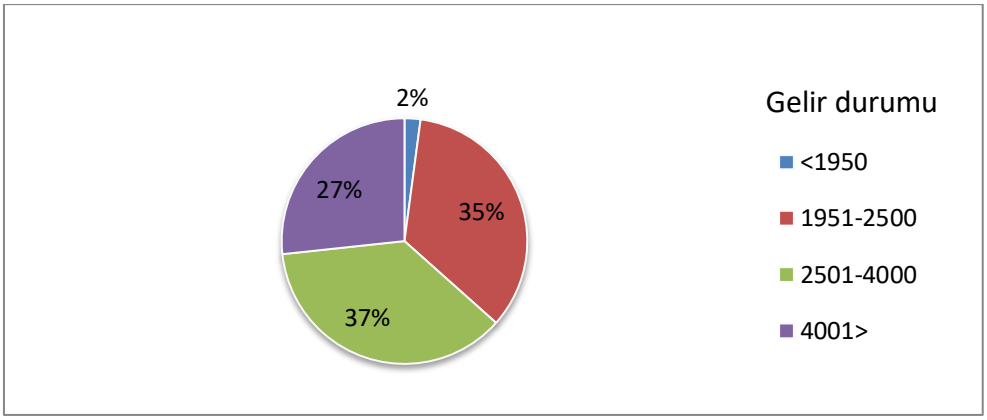
Şekil 2. Katılımcıların yaş dağılımı

Anket uygulanan bireylerin mesleklerine göre dağılımı; % 27,6'sı öğrenci, %21,1'i işçi, %28'i ev hanımı, %19,3'ü memur, %1,5 emekli ve %2,5'i diğer mesleklerdir (Şekil 3).



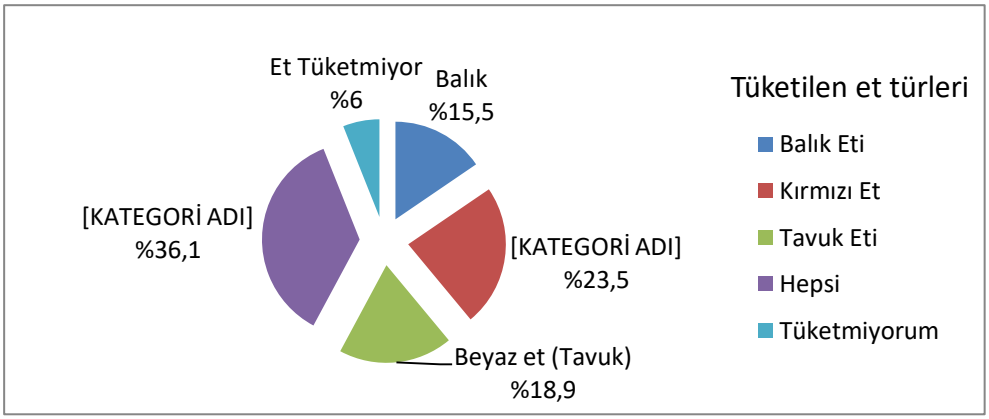
Şekil 3. Katılımcıların meslek dağılımı

Katılımcıların gelir düzeylerine bakıldığında; % 36,7'si 2501-4000 TL arasında, %34,5'i 1951-2500 TL arasında, %26,7'si 4001 TL'den fazla ve %2,1'i 1950 TL'den aşağı gelire sahip olduğu tespit edilmiştir (Şekil 4).



Şekil 4. Ankete katılanların gelir durumu

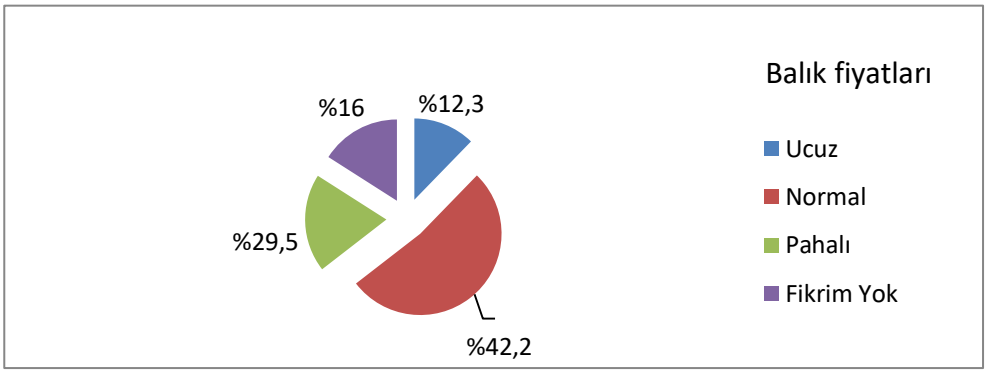
Anket uygulamasına tabi olan bireylere balığın diğer etlerle karşılaştırmaları sorulduğunda; %15,5'inin balığı severek tükettiği, %6'sının et tüketmediği, %18,9'unun beyaz et (tavuk), %23,5'inin kırmızı et ve %36,1'inin ise bütün etleri severek tükettikleri sonucu elde edilmiştir (Şekil 5).



Şekil 5. Ankete katılanların tercih ettikleri et türleri

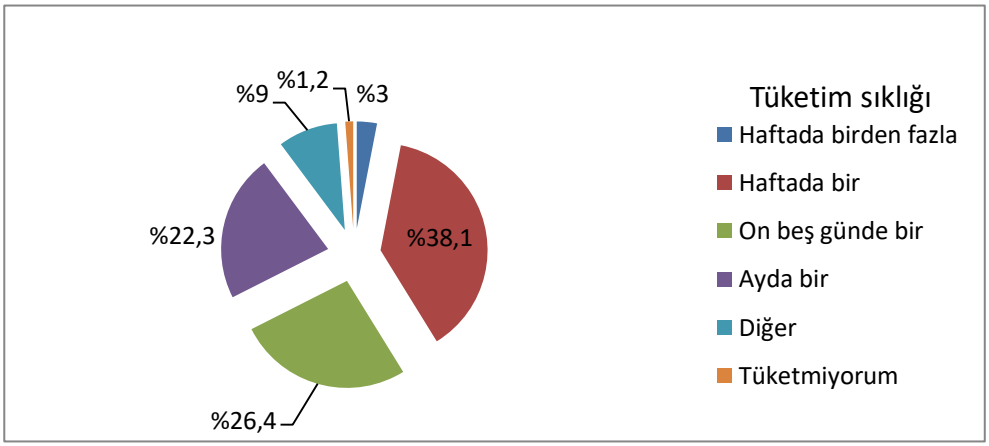
Yapılan çalışmada balığı tercih etme sebepleri sorulmuş ve balık tüketen bireylerin %82'si lezzetli ve besleyici olduğu için tercih ettiklerini bildirmişlerdir.

Anket uygulamasına katılan bireylerin balığın diğer etlere göre fiyatları karşılaştırdığında %42,2'si normal, %29,5'i pahalı ,%12,3'ü ucuz ve %16'sı da fikrinin olamadığını belirtmiştir (Şekil 6).



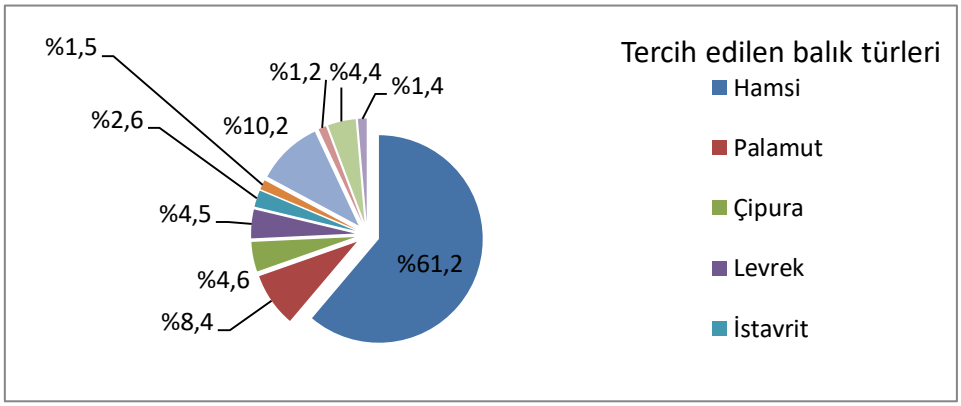
Şekil 6. Ankete katılanların balık fiyatları konusundaki değerlendirmeleri

Anket uygulamasına katılan bireylerin balık tüketim sıklığı araştırıldığında; %38,1'i haftada bir, %26,4'ü on beş günde bir, %22,3'ü ayda bir, %9'u diğer,%3'ü haftada birden fazla ve %1,2'si ise balık tüketmediği sonucu bulunmuştur (Şekil 7).



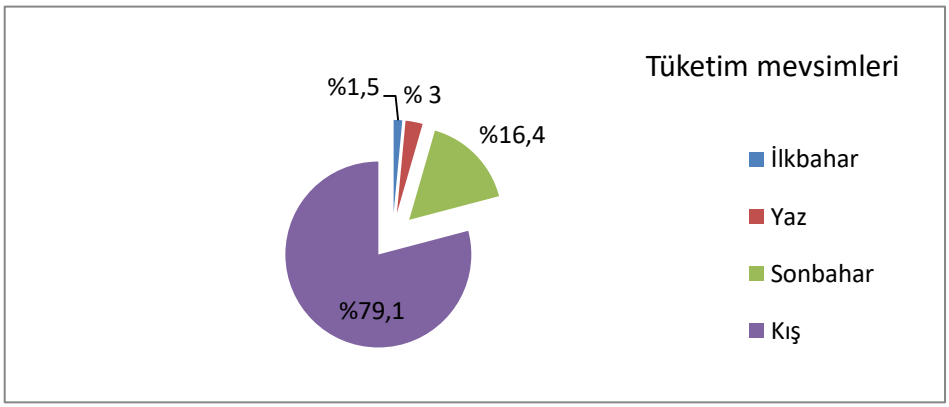
Şekil 7. Ankete katılanların balık tüketim sıklığı

Yapılan çalışmada balık tüketicilerinin %61,5'i deniz balıklarını, %5'i tatlı su balıklarını, %33,5'i ise her iki balığı tüketmeyi tercih etikleri belirlenmiştir. Tüketicilerin en fazla tercih ettiği balık türü hamsi (%61,2), en az tükettiği sazandır (%1,2) (Şekil 8). Ankete katılanların yaklaşık %70'inin ucuz balık tercih ettiği görülmektedir (hamsi, istavrit, palamut).



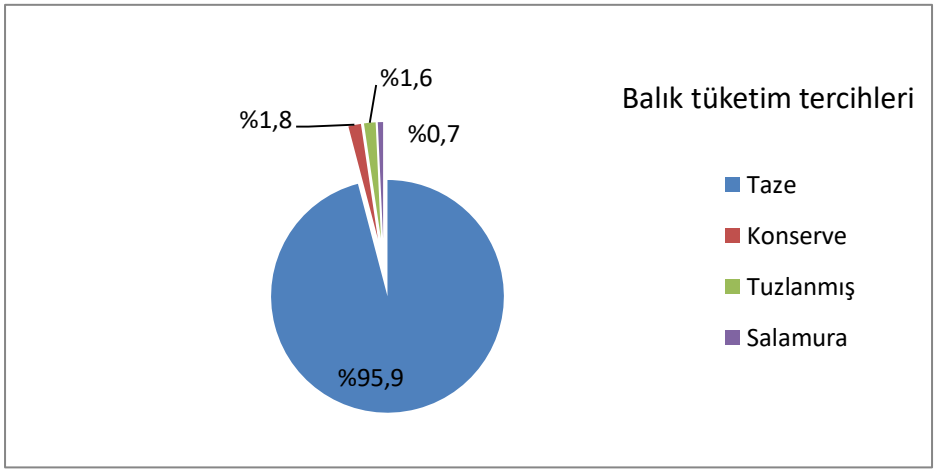
Şekil 8. Ankete katılanların tercih ettikleri balık türleri

Katılımcılara balık temin ettikleri yerler sorulduğunda %58'i pazar yeri, %33,1'i balık hali ve %8,8'i süper marketleri, satın alırken de öncelikle taze balığı (%68,6) tercih ettiklerini söylemiştir. Diğer tercihler sırasıyla türe (%11,7), fiyata (%11,3) ve satıcıya güvendir (%8,4). Ankete katılanların büyük çoğunluğu, deniz balıkları av sezonu olan kış aylarında %79,1, sonbaharda %16,4, yaz aylarında %3 ve ilkbaharda %1,5 oranında balık tükettiklerini bildirmiştir (Şekil 9).



Şekil 9. Ankete katılanların balık tüketimine, mevsimlerin etkileri

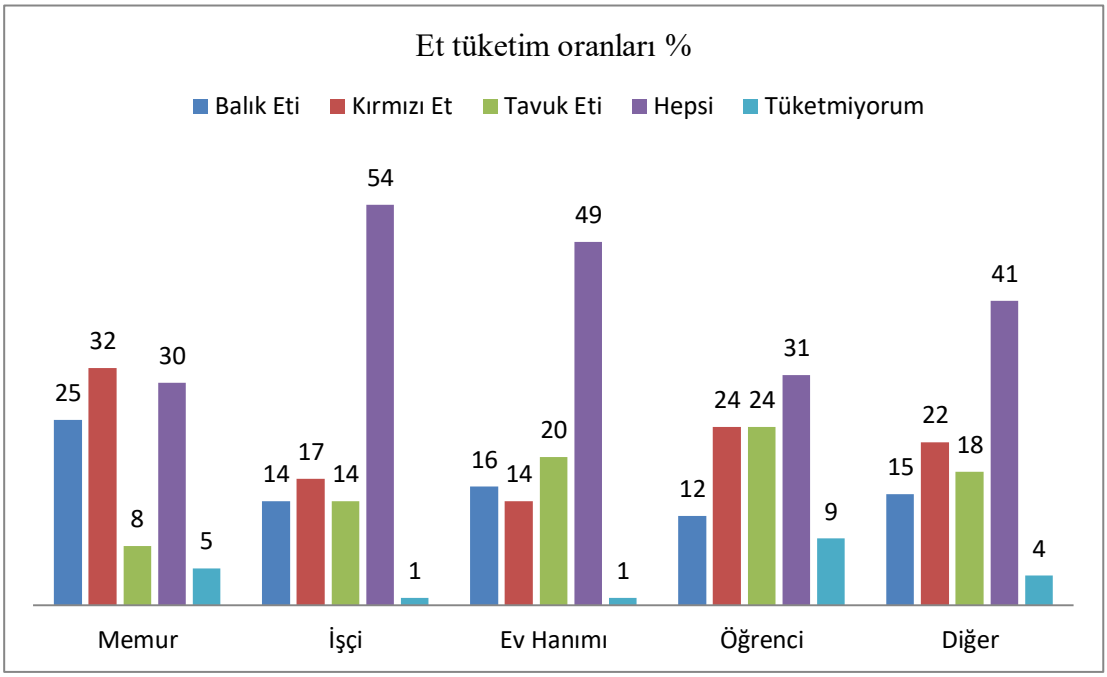
Yapmış olduğumuz çalışmada ankete katılan bireylerin %95,9'u balıkları işlenmemiş (taze), %1,8'i konserve, %1,6'sı tuzlanmış ve %0,7'si de salamura olarak tüketmektedir (Şekil 10).



Şekil 10. Ankete katılanların balık tüketim tercihleri

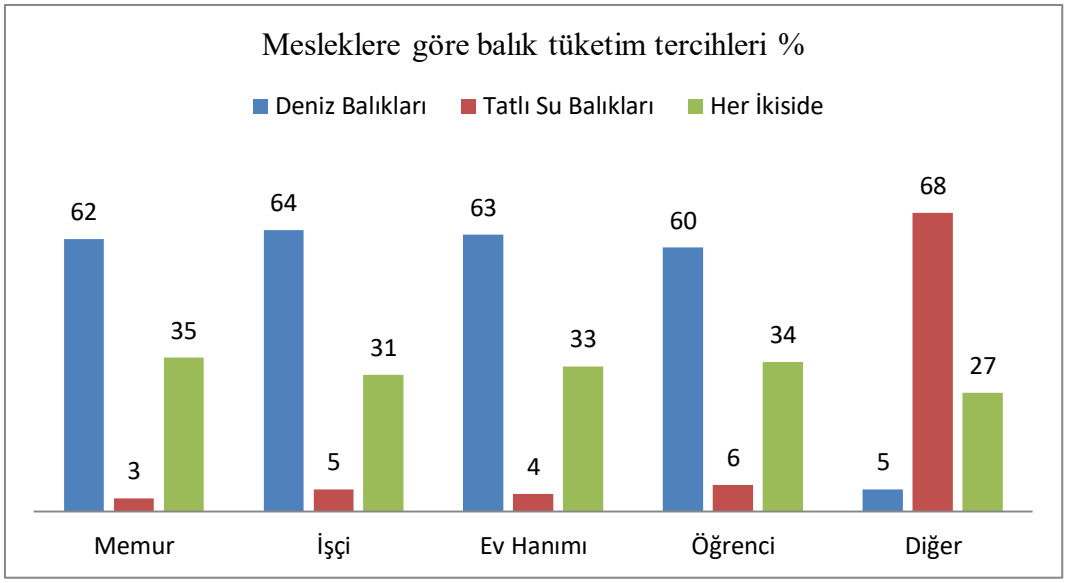
Katılımcılardan kırmızı eti memurlar %32, işçiler %17, ev hanımları %14 öğrenciler %24 ve diğer meslek mensupları %22 oranında tercih etmektedir. Balığı daha çok memurlar (%25), tavuk etini

öğrenciler (%20), et ürünlerinin hepsini işçiler (%54) tüketmektedir. Öğrenciler %9 oranında et ürünü tüketmemektedir (Şekil 11).



Şekil 11. Ankete katılanların meslek gruplarına göre et ürünü tüketim oranları (%)

Anket uygulamasına katılan memurların %62'si deniz balıklarını, %3'ü tatlı su balıklarını ve %35'i her ikisini de, işçilerin ise %64'ü deniz balıklarını, %5'i tatlı su balıklarını ve %31'i de her iki türü tercih etmektedir. Ev hanımlarının %63'ü deniz balıklarını, %4'ü tatlı su balıklarını ve %33'ü ise her ikisini, öğrencilerin ise %60'ı deniz balıklarını, %6'sı tatlı su balıklarını ve %34'ü ise her iki türü, diğer meslek mensuplarının ise %5'i deniz balıklarını, %68'i tatlı su balıklarını ve %27'si ise her iki türü de tercih etmektedir (Şekil 12).



Şekil 12. Ankete katılanların mesleklerine göre balık tercihleri (%)

TARTIŞMA VE SONUÇ

Kaliteli gıda tüketimi günümüzün en önemli konularından birisidir. Her geçen gün, bireylerin tercihlerini belirleme ve doğru besini tüketmesinde yardımcı olmak amacıyla çeşitli araştırmalar

yapılmaktadır. Su ürünlerinde tüketiminin durumunu, kişilerin taleplerini, avcılık ve yetiştiricilik pazar paylarını belirlemek için yapılan anket çalışmaları önemli bilgi kaynağı oluşturmaktadır. Bu amaçla Ege Bölgesinin küçük ve gelişmekte olan Uşak İli'nde, su ürünleri tüketim alışkanlıklarının belirlenmesi amacıyla yapılan bu çalışma ilk oluşu bakımından da önemlidir. 2020- 2021 balık avcılığı sezonunda, tesadüfen seçilen 445 erkek, 555 kadın olmak üzere toplam 1000 kişiyle soru-cevap şeklinde anket çalışması uygulanmıştır.

Tablo 1'de özetleri verilen, anket çalışmalarında erkek bireylerin çoğunlukta olduğu görülmektedir. Erkek egemen görüşün etkisi, anketlere yansımış olabilir. Balık vd. (2013) ankete katılan erkeklerin yüzdesi (%87,45) en yüksek olan çalışma ile dikkat çekmektedir. Arslan ve İzci (2016), toplam 1365 kişiyle yaptıkları çalışmada erkek ve kadın oranlarının birbirine yakın olması nedeniyle bizim sonuçlarımızla yakınlık göstermektedir. Bu konudaki diğer çalışmaların bilgileri Tablo1 de özetlenmiştir.

Tablo 1. Bazı anketlere ait katılımcı düzeyleri

Kaynak	İl	Ankete Katılan Kişi Sayısı	Erkek (%)	Kadın (%)
Bizim çalışma	Uşak	1000	44,5	55,5
Akçay ve Vatansever (2013)	Kocaeli	384	54,43	45,57
Bayraktar (2015)	Ankara, Çanakkale	200	53	47
Balık vd. (2013)	Ordu	600	87,45	12,55
Arslan ve İzci (2016)	Antalya	1365	51	49
Balcı vd. (2016)	Malatya	409	69	31
Bashimov (2017)	Niğde	150	64,67	35,33
Şen (2017)	Erzincan	213	52,58	47,42
Terin vd. (2016)	Van	260	75	25

Anket çalışmamıza katılan bireylerin %38,1'lik oranla haftada bir balık tükettiği saptanmıştır. Konuyla ilgili yapılan çalışmalarda çoğunlukla tüketimin iki haftada bir olduğu görülmüştür (Tablo 2). Akçay ve Vatansever (2013) tarafından yapılan çalışmada tespit ettikleri tüketim sıklığının bizim çalışmamızın verilerine yakındır. Doğan ve Gönülal (2014), Arslan ve İzci (2016) araştırmalarında tüketim sıklığının kayda değer oranda fazla olduğunu bildirmişlerdir. Tüketim sıklığındaki farklılığın anket çalışmalarının yapıldığı yerlerin deniz kıyısında olup olmamasının etkisi önemlidir. Bazı özet çalışmalar Tablo 2'de verilmiştir.

Tablo 2. Bireylerin balık tüketim sıklıkları aşağıda özet şeklinde verilmiştir

Kaynak	İl	Tüketim Sıklığı	%
Bizim çalışma	Uşak	Haftada bir	38,1
Arslan ve İzci (2016)	Antalya	Haftada bir	43,45
Akçay ve Vatansever (2013)	Kocaeli	Haftada bir	37,01
Kızılaslan ve Nalinci (2013)	Amasya	On beş günde bir	34,85
Balık vd.(2013)	Ordu(Fatsa-Aybastı)	On beş günde bir	36,7-36,5
Bashimov,(2017)	Niğde	On beş günde bir	36,0
Çiçek vd. (2013)	Elazığ	On beş günde bir	33,0
Doğan ve Gönülal (2014)	Gökçeada	Haftada 2-3 kez	49,6

Anket uygulamamıza katılan bireylerin; %15,5'ünün balığı severek tükettiği, %6'sının et tüketmediği, %18,9'unun beyaz et, %23,5'inin kırmızı et ve %36,1'sinin ise bütün et ürünlerini severek tükettikleri belirlenmiştir (Şekil 5).

Yapılan diğer çalışmalara bakıldığında, iç kesimlerde kırmızı et tüketimi fazladır. Balıketi tüketimi denize kıyısı olan illerde çok yüksek, iç kesimlerde düşük olduğu vurgulanmıştır. Çalışmamıza en yakın sonuçlar, kırmızı et tüketiminde Bayraktar (2015)'ın Ankara ilindeki çalışmasında (%38) ve balık tüketiminde İbiş (2014)'in Sivas ilinde yaptığı çalışmada (%16) bulunmuştur. Konuyla ilgili yapılan diğer çalışmalar incelendiğinde balık, beyaz ve kırmızı et tüketim tercihlerinin bölgesel farklılıklar oluşturduğu görülmüştür (Tablo 3).

Tablo 3. Yapılan çalışmalarda tüketilen et ürünleri

Kaynak	İl	En Fazla Tüketilen Et Ürünü(%)
Bizim çalışma	Uşak	Kırmızı Et (%23,5)
Çolakoğlu vd. (2006)	Çanakkale	Beyaz Et (%47,5)
Özek (2008)	Kırgızistan	Kırmızı Et (%69,8)
Şen (2011)	Konya / Mersin	Kırmızı Et (%47,8)/ Kırmızı Et(%47)
Balık vd. (2013)	Fatsa / Aybastı	Balık (%95,8) / Balık (%90,6)
Çaylak (2013)	İzmir	Beyaz Et (%33,8)
Çiçek vd. (2013)	Elazığ	Kırmızı Et (%41)
Doğan ve Gönülal (2014)	Gökçeada	Kümes Hayvanları (%37,3)
Güngör (2014)	Erzurum / Van	Kırmızı Et (49,8) / Beyaz Et(%39,7)
İbiş (2014)	Sivas	Beyaz Et (%49) / Balık (%16)
Odabaşı (2016)	Diyarbakır	Beyaz Et (%41,2)
Bayraktar (2015)	Ankara /Çanakkale	Kırmızı Et (%38) / Balık (%34)

Ankete katılan bireylerin tercih ettikleri balık türleri: hamsi (%61,2), alabalık (%10,2), palamut (%8,4), çipura (%4,6), levrek (%4,5), istavrit (%2,6), çinekop (%1,5), turna (%1,4), sazan (%1,2) ve %4,4 ile diğer balıklardır (Şekil 8).

Türkiye genelinde en çok avcılıktan üretilen ve tüketilen türün en başında hamsi gelmektedir. Günümüzde ülkenin artık en uzak yerleşim yerlerine kadar hamsi ve istavrit kolaylıkla ulaştırılmaktadır. Diğer çalışmalarda Çanakkale'de lüfer, Van Gölü çevresinde inci kefali ve İzmir'de çipura en fazla tüketilen balıklar olurken, diğer illerde fiyatı uygun olan hamsi ilk sırada yer almaktadır. Bu çeşitliliğin ürüne yakınlıkla doğru orantılı olduğu görülmektedir (Tablo 4).

Tablo 4. Yapılan çalışmalardaki en fazla tüketilen balık türleri

Kaynak	İl	Balık Türü
Bizim çalışma	Uşak	Hamsi
Çolakoğlu vd. (2006)	Çanakkale	Lüfer
Gürgün (2006)	Van Gölü Çevresi	İnci Kefali
Orhan ve Yüksel (2010)	Burdur	Hamsi
Şen (2011)	Mersin / Konya	Hamsi
Çaylak (2013)	İzmir	Çipura
Temel (2014)	Rize	Hamsi
Çelik (2014)	Manisa	Hamsi
Doğan ve Gönülal (2014)	Gökçeada	Lüfer
Uzundumlu ve Dinçel (2015)	Trabzon	Hamsi
Terin vd. (2016)	Van	Hamsi

Uşak halkının büyük çoğunluğu, deniz balıkları av sezonu olan kış aylarında (%71,9), balığı pazar yerinden (%58,1) tedarik ederek, satın alırken öncelikli olarak fiyatından çok balıkların tazeliğine (%68,6) bakarak tükettiklerini beyan etmişlerdir (Şekil 9). Diğer çalışmalara bakıldığında ise Kızılaslan ve Nalinci (2013), İbiş (2014) ve Terin vd. (2016)'nin araştırmalarında, balığın kış mevsiminde tüketildiğini belirlemişlerdir. Odabaşı (2016) ve Şen (2017), yapmış oldukları çalışmalarında market ve pazar yerlerinden balık ve balık ürünleri temin edildiğini; Arslan ve İzci (2016), Antalya ilinde fiyatların önemli bir fonksiyon olduğunu tespit etmişlerdir. Özek (2008) Kırgızistan halkının su ürünleri alımını etkileyen faktörlerin başında fiyat olduğunu belirlemiştir. Bunun yanında balıkların taze tüketildiği sonucuna ulaşılmış çalışmalarda dikkat çekmektedir (Şen 2011; Balık vd. 2013; Çaylak 2013; Aydın ve Karadurmuş 2013; Çelik 2014; Güngör 2014; Arslan ve İzci 2016; Odabaşı 2016; Bashimov 2017). Bahsedilen çalışmalar (Tablo 5)'de ayrıntılı bir şekilde verilmiştir.

Çalışmamızda ve konuyla ilgili başka çalışmalarda tüketim mevsiminin kış, temin yerinin pazar alanları olduğu görülürken, mevcut çalışmada satın almadaki ilk tercihin tazelik olduğu diğer araştırmalarda ise genelde fiyatın ön plana çıktığı tespit edilmiştir. Bütün çalışmalarda tüketim şeklinin genellikle işlenmemiş ürünler olduğu saptanmıştır.

Tablo 5. Bazı araştırmalarda bulunan sonuçlar

Kaynak	İl	Tüketim Mevsimi	Temin Yeri	Satın almada ilk tercih sebepleri	Tüketme yöntemi
Bizim çalışma	Uşak	Kış	Pazar yeri	Tazelik	İşlenmemiş
Özek (2008)	Bişkek	-	Pazar yeri	Fiyat	-
Arslan ve İzci (2016)	Antalya	-	Market	Fiyat	İşlenmemiş
Güngör (2014)	Erzurum / Van	Kış	-	-	İşlenmemiş
Çaylak (2013)	İzmir	-	Pazaryeri	Tazelik	İşlenmemiş
Aydın ve Karadurmuş (2013)	Trabzon / Giresun	-	Balıkçı Tezgâhı	-	İşlenmemiş
Bashimov,(2017)	Niğde	Kış	Balık Hali	Sağlık	İşlenmemiş
Doğan ve Gönülal (2014)	Gökçeada	Kış	Balık Hali	Fiyat	-
Uzundumlu ve Dinçel (2015)	Trabzon-Beşikdüzü	-	Balık Hali	Fiyat	-
Terin vd. (2016)	Van	Kış	Balıkçı Tezgâhı	Tazelik	İşlenmemiş
Kızılaslan ve Nalinci (2013)	Amasya	Kış	Balıkçı Tezgâhı	Lezzet	İşlenmemiş
İbiş (2014)	Sivas	Kış	Balık Hali	Sağlık	İşlenmemiş
Şen (2017)	Erzincan	-	Market	Sağlık	İşlenmemiş
Odabaşı (2016)	Diyarbakır	Kış	Balıkçı Tezgâhı	-	İşlenmemiş
Şen (2011)	Mersin / Konya	Kış	Balık Hali	-	İşlenmemiş
Çelik (2014)	Manisa	Kış	Pazar yeri	Tazelik	İşlenmemiş
Balık vd. (2013)	Ordu (Fatsa-Aybastı)	Kış	Balıkçı Tezgâhı	-	İşlenmemiş

Çalışmamızda ankete katılan bireylerin sorulara verdikleri cevaplardan, su ürünlerinin en fazla haftada bir tüketildiği, en çok işlenmemiş ve satın alırken taze su ürünlerinin tercih edildiği, tüketimi olumsuz etkileyen faktörlerin başında ise tüketim alışkanlığının az olması sonucuna varılmıştır. Ülkemizde üretim yıldan yıla değişkenlik gösterebilmektedir. Bunun sonucu olarak balık fiyatlarındaki değişim, bireylerin tüketim tercihlerini etkilemektedir.

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The Effects of Supplemented Diets with Turmeric Powder on Pigmentation and Growth of Yellow Tail Cichlid *Pseudotropheus acei* (Regan 1922)

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Research Article

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Abstract

There are few studies about the effect of turmeric powder on the skin pigmentation of fish in the literature. This study was conducted to research body and tail pigmentation and growth performance of *Pseudotropheus acei* (Regan 1922) fed supplemented diets with different ratio turmeric (*Curcuma longa*) powder. Five diets were prepared by adding four different ratios of turmeric powder (T1: 1%, T3: 3%, T5: 5%, and T7: 7%) to C: control diet (without turmeric powder). A total of 75 broodstock (mean weight 6g) were randomly stocked (4♀:1♂) in 15 aquariums (70x30x40cm), with three replications. The fish were fed twice daily at 8:30 and 20:30 with the diets supplemented turmeric as ad libitum during the 90 days.

At the end of the experiment, the body and tail pigmentation of *P. acei* significantly increased with increasing turmeric rate in diets ($P < 0.05$). The blue indicator –b values in male and female body color were high in all turmeric supplemented groups compared to the control group ($P<0.05$). Also, the yellow color indicator + b value in the color of the male and female tail increased with the addition of turmeric. H_{ab} values were determined in the blue area for the body and in the yellow area for the tail. However, pigmentation of all groups supplemented turmeric powder was visually not different from the color of the control group. There were no significant differences between final weights, weight gain SGR, FCR, and survival rates of *P. acei* fed diets supplemented with turmeric powder compared to the control ($P>0.05$). As a result of the study, skin and tail pigmentation and growth performance of *P. acei* were not improved by supplementing with turmeric powder to diets.

Keywords: Pseudotropheus acei, *Curcuma longa*, growth performance, pigmentation

Zerdeçal Tozu İlave Edilmiş Diyetlerin Sarı Kuyruk Çiklit *Pseudotropheus acei* (Regan 1922)'nin Vücut Pigmentasyonu ve Büyüme Performansı Üzerindeki Etkileri

Özet

Literatürde, zerdeçal tozunun balıkların vücut ve kuyruk pigmentasyonu üzerindeki etkilerinin belirlendiği az sayıda çalışma bulunmaktadır. Bu çalışma, farklı oranlarda zerdeçal (Curcuma longa) tozu ilaveli diyetlerle beslemenin *Pseudotropheus acei*'nin (Regan 1922) vücut-kuyruk pigmentasyonu ve büyüme performansına etkilerini belirlemek amacıyla oluşturuldu. Dört farklı oranda zerdeçal tozu (T1:% 1, T3:% 3, T5:% 5 ve T7:% 7) C: kontrol diyetine (zerdeçal tozu olmadan) eklenerek beş diyet hazırlanmıştır. Toplam 75 anaç (ortalama ağırlık 6g) 15 akvaryumda (70x30x40cm) üç tekerrürlü olarak rastgele stoklanmıştır (4♀: 1♂). Balıklar, 90 gün boyunca doyuncaya kadar zerdeçal ilaveli diyetler ile günde iki kez saat 8:30 ve 20:30 da beslenmişlerdir. Denemenin sonunda, diyetlerdeki zerdeçal oranının artmasıyla *P. acei*'nin vücut ve kuyruk pigmentasyonu önemli ölçüde arttığı belirlenmiştir ($P <0,05$). Erkek ve dişi vücut rengindeki mavi renk göstergesi –b değerleri tüm zerdeçal ilaveli gruplarda kontrol grubuna göre yüksek bulunmuştur ($P<0,05$). Ayrıca zerdeçal ilavesiyle erkek ve dişi kuyruk rengindeki sarı renk göstergesi + b değeri artmıştır. H_{ab} değerleri vücut için mavi, kuyruk için sarı alanda belirlenmiştir. Bununla birlikte, zerdeçal tozu ilaveli tüm grupların pigmentasyonu görsel olarak kontrol grubunun renginden farklı değildi. Kontrole kıyasla zerdeçal tozu ilaveli diyetler ile beslenen *P. acei* final ağırlık, ağırlık kazancı SGR, FCR ve yaşama oranları arasında önemli bir farklılık tespit edilmemiştir ($P <0,05$). Sonuç olarak, *P. acei*'nin diyetlerine zerdeçal tozu ilavesi vücut ve kuyruk pigmentasyonu ve büyüme performansı verilerini iyileştirmedi.

Anahtar Kelimeler: Pseudotropheus acei, *Curcuma longa*, büyüme performansı, pigmentasyon

INTRODUCTION

Ornamental fish demand increase worldwide every year (Srikrishnan et al., 2017). One of the most attractive features of ornamental fish is undisputed their colors (Kop and Durmaz, 2008). Pigmentation

of fish demonstrates its quality and wellness. Especially, the colors of male fishes become brighter (Alpbaz and Hoşsucu, 1996). The lively colors in the fish, as well as fish species, is one of the considerable determinants of the marketing of fish (Hatlen et al., 1997). Ornamental color fish production is an important issue for producers (Hekimoglu, 2005). It is known that many types of diet are marketed for the coloration of fish in different countries recently (Brineshrimp Direct, 2005).

The fish cannot synthesize carotenoids like other animals, and they must obtain from diets (Sommer et al., 1991). Both synthetic and natural carotenes are used in the coloration of fish. But the synthetic carotenoids causes some health problems, there is an increasing demand for the elimination of the use of synthetic carotenes **(**Ambati et al., 2019; Mohammed and Mohd, 2011). Natural carotenoids are derived from plants while synthetic ones are mainly by-products from coal distillation (Ambati et al., 2019; Carbonell-Capella et al., 2014). Recently, natural carotenoids were started using due to the toxic effect of synthetic carotenoid pigments (Ambati et al., 2019). Antioxidant activity is better in the case of natural carotenoids, unlike synthetic ones. (Murthy et al., 2005). In addition**,** synthetic carotenoids added to feeds increase the price of feed by 20-25% (Yesilayer et al., 2008). The cichlid fish is a take part in the species which people excessively prefer on the world. *Pseudotropheus acei* is found in shallow water on a clear sandy bottom and in bogs in Lake Malawi (Parry et al., 2005).

Turmeric is a spice that comes from the root of *Curcuma longa*, a member of the ginger family, Zingaberaceae (Chainani-Wu N., 2003). Curcumin, an active component of turmeric, is a yellow pigment that has been isolated from the ground rhizome part of the curcuma plant curcumin; has anti-inflammatory, antioxidant, anticancerogenic, and hypolipidemic properties (Tayyem et al., 2006).

A study was done on pigmentation of fish; Mukherjee et al., (2009) reported that high pigmentation was observed in caudal fin and muscle of fish (*P. reticulata*) fed with diets added turmeric powder (0.09 %). For this aim, we researched the pigmentation effect of turmeric powder in different fish *Pseudotropheus acei* (Regan 1922) that has yellowfins. For this aim, we researched the pigmentation effect of turmeric powder in the diet at the different ratios on fish *Pseudotropheus acei* (Regan 1922) pigmentation that has yellowfins.

MATERIAL and METHOD

Experimental diets

Experimental diets were isonitrogenous (37% crude protein) and isoenergetic (4400kcal). Five diets were prepared by adding four different ratios of turmeric powder (T1: 1%, T3: 3%, T5: 5%, and T7: 7%) to C: control diet (without turmeric powder). The composition of the diets is shown in Table 1. The feed ingredients were supplied from a local fish feed manufacturer. Turmeric powder was obtained from a special spice seller (Isparta, Turkey). All ingredients were ground to small particle size (0.5 mm) in a mill. Dietary ingredients were mixed in a mixer. Micro ingredients were first mixed and then slowly added to the macro ingredients to ensure a homogenous mixture. Water was added to obtain a 30% moisture level. Diets were passed through a mincer with a 1 mm sieve. The pellets were fan-dried and stored frozen at -20^{0}C until used.

Table 1. Formulation and proximate composition of experimental diets

Ingredients (%)	C	T 1	T3	T5	T7
Turmeric	0.00	1.00	3.00	5.00	7.00
Fish meal	35.00	35.00	35.00	35.00	35.00
Soybean	22	22	22	22	22
Corn starch	7.00	6.00	4.00	2.00	0.00
Wheat	26	26	26	26	26
Fish oil	7.00	7.00	7.00	7.00	7.00
Vitamin mix[1]	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	1.00	1.00	1.00	1.00	1.00
Pellet binder	1.00	1.00	1.00	1.00	1.00
Chemical Analyses					
Crude protein (%)	36.91	36.99	37.15	37.31	37.47
Crude lipid (%)	10.24	10.30	10.41	10.53	10.65
Crude fiber (%)	1.75	1.80	1.89	1.98	2.07
Crude ash (%)	9.33	9.33	9.33	9.33	9.33
Gross energy (kcal)	4426.33	4423.71	4418.47	4413.23	4407.99

Vitamin premix.[1]; per kg, 4,000,000 IU vitamin A, 480,000 IU vitamin D3, 40,000 mg vitamin E, 2400 mg vitamin K3, 4,000 mg vitamin B1, 6,000 mg vitamin B2, 40,000 mg niacin, 10,000 mg calcium D-pantothenate, 4,000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-biotin, 1,200 mg folic acid, 40,000 mg vitamin C and 60,000 mg inositol. Mineral premix.[2]; per kg 23,750 mg Mn, 75,000 mg Zn, 5,000 mg Zn, 2,000 mg Co, 2,750 mg I, 100 mg Se, 200,000 mg Mg.

Experimental setup

P. acei were provided from a local commercial aquarium (Afyonkarahisar, Turkey). Fish were then transferred to the aquaculture laboratory of Egirdir Fisheries Faculty, Suleyman Demirel University, Isparta, Turkey. A total of 75 broodstock (mean weight 6g) were randomly stocked (4♀:1♂) in 15 aquariums (70x30x40cm) for 90 days. Shelters were placed in the aquariums containing the fish, and the water was well aerated and filtered. The water temperature was maintained at a mean of 27°C. The temperature of the aquariums was maintained at a constant by a thermostat heater. The dissolved oxygen rate ranged from 5 to 7 mg L^{-1} during the experiment. Experimental groups were fed by hand, *ad libitum* twice daily (8:30 and 20:30). The aquariums were cleaned daily by siphoning out from the waste feed and feces.

Growth parameters

The growth parameters were calculated according to the following formulas;

Specific Growth Rate (SGR) (% day^{-1}) = ((ln FBW - lnIBW)/T) *100

Feed Conversion Ratio (FCR) = Feed intake/weight gain,

Survival rate (%) = Final fish number/initial fish number)*100

T time (days), FBW; Final body weight, IBW; Initial body weight

Pigmentation Analysis

Skin and tail color measurements were performed at the beginning and the end of the experiment with a colorimeter (Konica Minolta CR 300). Skin and tail color parameters of L*, a*, b* were measured from the regions close to the lateral line and dorsal section (CIE, 1986) after fish were put to sleep with an anesthetic (MS-222 75 mg/L). All of the fish were used color measuring. Chroma (Ch) and hue angle (H°) was calculated with the aid of a* and b* values. L*: (+) brightness, (−) darkness, a*: (+) redness, (−) greenness, b*: (+) yellowness, (−) blueness (Nickell and Bromage, 1998). In this system, L * value is the brightness or luminance variable and the values a * and b * are the chromaticity coordinates (a * < 0 green color, a * > 0 red color, b * < 0 blue color, b * > 0 yellow color) that were determined (Yesilayer et al., 2020). Chroma (Ch) indicates color intensity and brightness and was calculated with the equation of Ch = (a*2 + b*2) ½. The hue angle is calculated with is calculated with following formulas; if (+a*, +b*), Hab° = ArcTan(b*/a*); if (-a*, +b*) and (-a*, -b*), H°ab = 180 + ArcTan (b*/a*) ; if (+a*, -b*), Hab° = 360 + ArcTan (b*/a*) (McLellan et al. 1995). For hue (H ° ab), 0° indicates a tone of red, 90° a tone of yellow, 180° a tone of green and 270° a tone of blue (Hunt, 1977; Yeşilayer and Erdem, 2011).

Ethics statement

Suleyman Demirel University Animal Experiments Local Ethics Committee, Meeting date: 06.07.2017, Meeting number: 04, Decisions number: 02.

Statistical Analysis

The significance of differences between experimental groups was analyzed by one-way analysis of variance (ANOVA). All data were calculated by using the SPSS computer program (SPSS 20.00). The Duncan test was used to determine the differences among treatment means ($P < 0.05$).

RESULTS

Growth performance and survival rate of *P. acei* (Regan 1922)

The results on the growth performance and survival rate of *P. acei* were given in Table 2. There were no significant differences between final weight, weight gain SGR (Specific growth rate), FCR (Feed Conversion Ratio), and survival rate of *P. acei* fed with experimental diets ($P>0.05$).

Table 2. Growth performance and survival rate of *P. acei* (Regan 1922)

Growth Performance	C	T1	T3	T5	T7	df	F	P
Initial mean weight (g)	6.27± 0.43	6.30 ± 0.36	6.13 ± 0.34	6.39 ± 0.36	6.39 ± 0.22	4	0.07	0.99
Initial mean length (cm)	7.75 ± 0.16	7.65 ± 0.20	7.84 ± 0.15	7.83 ± 0.13	7.70 ± 0.11	4	0.26	0.90
Final mean weight (g)	8.65 ± 0.55	7.61 ± 0.59	8.08 ± 0.43	7.55 ± 0.45	7.52 ± 0.80	4	0.79	0.54
Final mean length (cm)	8.60 ± 0.22	8.52 ± 0.21	8.55 ± 0.21	8.60 ± 0.19	8.52 ± 0.29	4	0.03	0.99
Feed Conversion Ratio (FCR)	3.10±0.30	4.37±0.27	4.41±0.63	4.57±0.41	3.55±0.42	4	2.27	0.13
Specific growth rate (SGR; % day^{-1})	0.36±0.04	0.28±0.04	0.31±0.03	0.18±0.09	0.17±0.01	4	2.49	0.13
Weight gain (g)	2.23±0.23	0.97±0.27	1.67±0.37	1.25±0.36	0.92±0.19	4	3.47	0.05
Survival rate (%)	92.13 ± 3.10	91.44 ± 4.00	95.09 ± 2.78	91.91 ± 3.66	90.08 ± 3.46	4	0.26	0.90

Pigmentation body and tail of *P. acei* (Regan 1922)

The results on the pigmentation body and tail of *P. acei* were presented in Tables 3 and 4.

The blue indicator –b value in the body color of the female increased with the addition of turmeric, the highest –b values were found in T3 and T7 groups. At the same time, the L values increased with the addition of turmeric, namely the color opened. H_{ab} values were determined in the blue area and decreased with the addition of turmeric, It approached 270^o (blue). The highest H_{ab} value was determined in the control group (325^o) ($P<0.05$). Ch brightness value increased with the addition of turmeric. The highest Ch values were determined in the T3 and T5 groups ($P<0.05$).

The blue indicator –b values in male body color were high in all turmeric supplemented groups compared to the control group ($P<0.05$). H_{ab} values were determined in the blue area and increased with the addition of turmeric and it moved away from 270^o. The highest H_{ab} values were found in T1 (290^o) and T5 (291^o) groups.

The yellow color indicator + b value in the color of the female tail increased with the addition of turmeric. The highest + b value was determined in the T7 group ($P<0.05$). L values increased with the addition of turmeric, namely the color opened. The highest L value was found in the T1 group. Especially in the T7 group, the chroma brightness value was found to be higher than the control ($P<0.05$).

The yellow color indicator + b value in the male tail color increased with the addition of turmeric. The highest + b value was found in T3 group ($P<0.05$). H_{ab} values were determined in the yellow area and increased with the addition of turmeric and it moved away from 90^o. The highest H_{ab} value was determined in the T3 group (97.51) group ($P<0.05$). Ch brightness value increased with the addition of turmeric. The highest was detected in the T3 group ($P<0.05$).

Table 3. Tail and body L*a*b* pigmentation of *P. acei* (Regan 1922) (Mean ± SE)

Body									
		C	**T1**	**T3**	**T5**	**T7**	**df**	**F**	**P**
Female	L*initial	40.92±5.31	40.92±5.31	40.92±5.31	40.92±5.31	40.92±5.31	-	-	-
	L*90th day	34.86±1.11^{a}	39.78±1.13^{b}	39.34±1.08^{ab}	37.03±0.70^{ab}	40.54±1.68^{b}	4	4.38	0.00
	a* initial	2.15±0.57	2.15±0.57	2.15±0.57	2.15±0.57	2.15±0.57	-	-	-
	a*90th day	2.08±0.10	2.31±0.14	2.40±0.14	2.46±0.13	2.16±0.19	4	1.49	0.21
	b*initial	-2.10±0.80	-2.10±0.80	-2.10±0.80	-2.10±0.80	-2.10±0.80	-	-	-
	b*90th day	-1.49±0.17^{a}	-2.20±0.22^{ab}	- 2.61±0.25^{b}	-2.35±0.25^{ab}	-2.61±0.27^{b}	4	4.56	0.00
Male	L*initial	32.30±2.96	32.30±2.96	32.30±2.96	32.30±2.96	32.30±2.96	-	-	-
	L*90th day	30.04 ± 3.39	33.06 ± 3.04	39.14 ± 1.87	30.96 ± 1.15	34.93 ± 3.37	4	1.77	0.17
	a* initial	0.70±0.37	0.70±0.37	0.70±0.37	0.70±0.37	0.70±0.37			
	a*90th day	0.43±0.18^{b}	2.30±0.54^{a}	1.23±0.24^{ab}	1.86±0.16^{ab}	1.63±0.62^{ab}	4	3.63	0.03
	b*initial	-3.74±0.89	-3.74±0.89	-3.74±0.89	-3.74±0.89	-3.74±0.89			
	b*90th day	-3.16±0.81^{a}	-5.72±0.42^{b}	-5.25±0.34^{b}	-5.06±1.01^{b}	-5.74±0.50^{b}	4	3.12	0.04
Tail									
		C	**T1**	**T3**	**T5**	**T7**	**df**	**F**	**P**
Female	L*initial	52.04±4.66	52.04±4.66	52.04±4.66	52.04±4.66	52.04±4.66	-	-	-
	L*90th day	54.47±2.11^{c}	68.05±0.74^{a}	60.84±1.82^{cb}	62.24±1.47^{ab}	62.04±1.61^{ab}	4	9.20	0.00
	a* initial	-1.38±1.29	-1.38±1.29	-1.38±1.29	-1.38±1.29	-1.38±1.29	-	-	-
	a*90th day	-1.46±0.38^{ab}	-2.27±0.24^{b}	-0.17±0.80^{a}	-2.55±0.33^{b}	-2.54±0.31^{b}	4	4.53	0.00
	b*initial	24.44±3.32	24.44±3.32	24.44±3.32	24.44±3.32	24.44±3.32	-	-	-
	b*90th day	18.08±1.64^{c}	23.59±1.08^{ab}	23.08±1.55^{cb}	24.28±1.11^{ab}	28.92±0.76^{a}	4	7.99	0.00
Male	L*initial	52.83±4.15	52.83±4.15	52.83±4.15	52.83±4.15	52.83±4.15	-	-	-
	L*90th day	49,57±3.11^{ab}	69.04±1.01^{c}	66.31±1.65^{c}	61.49±2.98^{cb}	46.58±9.40^{a}	4	9.20	0.00
	a* initial	-1.77±2.09	-1.77±2.09	-1.77±2.09	-1.77±2.09	-1.77±2.09	-	-	-
	a*90th day	-0.75±0.19^{c}	-1.89±0.50^{cb}	-3.64±0.28^{a}	-2.43±0.22^{ba}	-2.09±0.26^{cb}	4	11.65	0.00
	b*initial	21.09±3.28	21.09±3.28	21.09±3.28	21.09±3.28	21.09±3.28	-	-	-
	b*90th day	15.41±1.82^{c}	22.34±0.88^{b}	27.62±0.43^{a}	20.85±0.25^{b}	18.87±0.99^{ab}	4	17.67	0.00

In the same line, values with different superscript letters are significantly different (P<0.05)

L*: (+) brightness, (−) darkness, a*: (+) redness, (−) greenness, b*: (+) yellowness, (−) blueness (Nickell and Bromage, 1998).

Table 4. Tail and body H^oab and Chroma (Ch) values of *P. acei* (Regan 1922) (Mean ± SE)

Body									
		C	**T1**	**T3**	**T5**	**T7**	**df**	**F**	**P**
Female	H^oab initial	315.02±3.58	315.02±3.58	315.02±3.58	315.02±3.58	315.02±3.58			
	H^oab 90th day	325.19±3.65^{b}	317.95±3.32^{ab}	315.49±3.58^{ab}	316.74±3.20^{ab}	310.71±3.33^{a}	4	2.33	0.06
	Ch initial	2.67±0.14	2.67±0.14	2.67±0.14	2.67±0.14	2.67±0.14			
	Ch 90th day	2.67±0.10^{a}	3.17±0.16^{ab}	3.68±0.21^{b}	3.45±0.20^{b}	3.31±0.26^{ab}	4	5.21	0.00
Male	H^o ab initial	279.15±1.71	279.15±1.71	279.15±1.71	279.15±1.71	279.15±1.71			
	H^oab 90th day	275.23±2.24^{a}	290.79±3.70^{b}	283.83±3.11^{ab}	291.77±3.52^{b}	285.07±5.42^{ab}	4	3.33	0.03
	Ch initial	4.10±0.26	4.10±0.26	4.10±0.26	4.10±0.26	4.10±0.26			
	Ch 90th day	4.33±0.21	5.46±0.81	5.43±0.72	4.82±1.89	5.46±0.12	4	1.28	0.32
Tail									
		C	**T1**	**T3**	**T5**	**T7**	**df**	**F**	**P**
Female	H^oab initial	93.80±0.63	93.80±0.63	93.80±0.63	93.80±0.63	93.80±0.63			
	H^oab 90th day	95.37±0.66	95.35±0.48	95.51±0.58	96.11±0.52	94.91±0.54	4	0.56	0.68
	Ch initial	23.83±0.59	23.83±0.59	23.83±0.59	23.83±0.59	23.83±0.59			
	Ch 90th day	24.24±1.40^{a}	24.25±1.00^{a}	24.88±1.28^{ab}	25.66±0.86^{ab}	29.05±0.77^{c}	4	3.35	0.02
Male	H^oab initial	96.36±1.38	96.36±1.38	96.36±1.38	96.36±1.38	96.36±1.38			
	H^oab 90th day	92.91±0.83^{a}	94.71±1.05^{ab}	97.51±0.56^{b}	96.65±0.61^{ab}	94.53±1.49^{ab}	4	5.02	0.01
	Ch initial	23.71±0.79	23.71±0.79	23.71±0.79	23.71±0.79	23.71±0.79			
	Ch 90th day	17.42±2.05^{a}	22.44±0.92^{b}	27.86±0.43^{c}	20.99±0.25^{ab}	18.95±1.03^{ab}	4	17.03	0.00

In the same line, values with different superscript letters are significantly different ($P<0.05$)

DISCUSSION

In the present study, there were no differences in growth performance, FCR, and survival rate of *P. acei* fed with diets with addition turmeric different ratios (0, 1, 3, 5, and 7%) compare to the control group. Similarly, Mahmoud et al., (2014) reported that *Oreochromis niloticus* fed diets added 0, 0.25, and 0.50 % turmeric powder had not different final weights. Ferreira et al., (2017) indicated that turmeric powder supplementation (2, 4, 6, 8, and 10 %) had not an effect on the growth of juvenile *Astyanax aff. bimaculatus*. Sahu et al., (2008) determined that growth performance was no affected in *Labeo rohita* fed the basal diet with addition turmeric powder different ratio (0.01, 0.05, 0.1, and 0.5 %), but the highest survival ratio was found in group addition turmeric 0.5 %. Hassan, (2016) pointed that there were no significant differences in body weight gain of hens fed dietary added turmeric powder (0, 2, and 4%). Alagawany et al., (2016) reported that growth performance was no affected in rabbits fed the basal diet with addition turmeric different ratios (0.2, 0.4, and 0.6 %).

However, Rebecca and Bhavan, (2014) obtained the better weight gain and survival rate in all groups compared to the control in *M. rosenbergii* fed with diets containing *A. sativum*, *Z. officinale*, and *C. longa* in three different ratios (1%, 3%, and 5%). Abdelwahab and El-Bahr, (2012) indicated the higher growth levels according to the control were obtained in Sea Bass fed diets supplemented with different ratios (0.5, 1.0%) with a mix of turmeric powder and black cumin seeds (*Nigella sativa*) (1:1 ratio). Akdemir et al., (2017) observed to increase in body weight, feed intake, and weight gain fed with curcumin-supplemented diet at 0.02 % of diet levels of high stocking density conditions in trout. Mukherjee et al., (2009) observed SGR in *Poecilia reticulata* fed supplemented diet with turmeric powder (0.09 %). Arunkumar et al., (2016) indicated that the best of FCR and weight gain were obtained in carp fed with copepod enriched with turmeric powder (0.9 g/l). Poongodi et al., (2012) found the better growth performance, SGR, feed conversion efficiency, and survival rate in all groups compared to the control in *Macrobrachium rosenbergii* postlarvae fed with diets containing (1 %) *Allium sativum*, *Zingiber officinale*, *Curcuma longa* and *Trigonella foenum-graecum* powder. Yusuf et al., (2017) determined in the 0.4 % group the highest growth in *O. niloticus* fed with a diet supplemented with different ratio turmeric powder. The reasons for the differences between the studies with the current study may be the following reasons; in the present study, the numbers of females were higher than males because the males are a fighter. *P. acei* species gives offspring every two months. Therefore, the females maybe have used for gonad development most of the nutrients obtained from feed. As results of this, the growth may be negatively affected by gonadal development. In addition, unlike most other studies, higher rates of tumeric powder were used in this study.

There were a few studies on the effects of turmeric in fish pigmentation. Mukherjee et al., (2009) reported that high pigmentation was observed in the caudal fin and muscle of fish (*P. reticulata*) fed with diets added turmeric powder (0.09 %). However, Nascimento et al., (2019) indicated that supplementing to diets of turmeric powder (0-2.5 %) no improved the orange pattern of skin pigmentation of *Trichogaster labiosa* orange thick-lipped gourami. Similarly, in the present study, the pigmentation increased statistically by increasing of turmeric powder ratio in diets. However, the pigmentation of groups supplemented turmeric powder was visually not different from the color of the control group. As a result of the study, skin and tail pigmentation and growth performance of *P. acei* were not improved by supplementing with turmeric powder to diets.

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Age, Growth, and Reproduction of Common Sole, *Solea solea* (Linnaeus, 1758) in the Sea of Marmara, Turkey

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Research Article

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Abstract

This study revealed the length-weight relationship, age, growth and mortality parameters, and reproductive biology of the common sole, *Solea solea* in the Sea of Marmara, Turkey. Samplings were conducted with bottom trawl between March 2017 and December 2018 at 34 stations. The length-weight relationship was calculated as $W=0.0082\times TL^{3.01}$. Ages were ranged between 1 and 5 years. The von Bertalanffy growth parameters were calculated as L_∞=34.56 cm, K=0.48 y^{-1}, and t_0=-0.01 y. The size at first maturity was 21.9 cm TL. The extended reproduction period was observed (from September to April). The rates of natural mortality (M), total mortality (Z), fishing mortality (F), and exploitation rate (E) were calculated to be 0.79, 2.4, 1.61, and 0.67, respectively. The biological reference points were calculated as F_{opt}=0.395; F_{lim}=0.53 and E_{opt}=0.333, respectively. The length where the maximum yield can be obtained (L_{opt}) was found as 22.3 cm TL. The results showed that *S.solea* is under the influence of excessive fishing pressure in the Sea of Marmara.

Keywords: Common sole, Length-weight relationship, Population parameters, Sexual maturity, Excessive fishing pressure

Marmara Denizi'nde Solea solea' nın (Linnaeus, 1758) Yaş, Büyüme ve Üreme Özellikleri

Özet

Bu çalışmada Dil Balığı, *Solea solea* türünün Marmara Denizi'ndeki boy-ağırlık ilişkisi, yaş, büyüme ve ölüm parametreleri ve üreme biyolojisi ele alınmıştır. Örneklemeler 34 istasyondan Mart 2017 ile Aralık 2018 arasında dip trolü ile gerçekleştirilmiştir. Boy-ağırlık ilişkisi $W=0{,}0082\times TL^{3.01}$ olarak hesaplanmıştır. Bireyler 1 ile 5 yaş aralığında dağılım göstermiştir. Von Bertalanffy büyüme parametreleri L_∞=34,56 cm, K=0,48 y^{-1}, and t_0=-0,01 y şeklinde hesaplanmıştır. İlk eşeysel olgunluk boyu 21.9 cm TL tespit edilmiştir. Eylül'den Nisan'a kadar geniş bir üreme periyodu tespit edilmiştir. Doğal ölüm oranı (M), toplam ölüm oranı (Z), balıkçılık ölümü (F) ve sömürülme oranı sırasıyla 0,79, 2,4, 1,61 ve 0,67 olarak belirlenmiştir. Biyolojik referans noktaları sırasıyla F_{opt}=0,395; F_{lim}=0.53 ve E_{opt}=0,333 olarak hesaplanmıştır. En yüksek ürünün elde edilebileceği en uygun boy (L_{opt}) 22,3 cm TL bulunmuştur. Sonuçlar Dil Balığı'nın Marmara Denizi'nde aşırı avcılık etkisinde olduğunu göstermektedir.

Anahtar Kelimeler: Dil Balığı, Boy-ağırlık ilişkisi, Populasyon Parametreleri, Eşeysel olgunluk, Aşırı av baskısı

INTRODUCTION

Common sole, *Solea solea* (Linnaeus, 1758), is one of the commercially important members of the Soleidea family. In Turkey, the species distributed in the Black Sea, Marmara Sea, Aegean Sea, and Norteastern Mediterranean coasts (Mater et al., 2003). Globally, distribution ranges from Eastern Atlantic to the western Black Sea (Froese and Pauly, 2007). It can grow up to 70 cm tall and 26 years old. It generally lives on sandy and muddy grounds and at depths of 0-150 m (Froese and Pauly, 2007).

The scientific knowledge on the common sole has been published in various aspects. The feeding ecology and diet (Molinero and Flos; 1992; Cabral, 2000; Ende et al., 2018), the early life ecology (Le Pape et al., 2007; Parma et al., 2013; Di Pane et al., 2020); culture potential (Imsland et al., 2003; Avella et al., 2011); genetic (Ferraresso et al., 2016; Deconinck et al., 2020) and physiology (Davoodi and Claireaux 2007; Frapiccini et al., 2018) of the common sole have been studied by several authors. The previous studies have been centered on the length-weight relationship of common sole (Djbali et

al., 1993; Deniel, 1990; Ramos, 1982; Girardin et al, 1986; Costa, 1990; Vianet et al., 1989; Jennings et al., 1998; Campillo, 1992; De Veen, 1976; Koutrakis and Tsikliras, 2003; Vianet et al., 1989; Duncker, 1923; Dorel, 1986; Deniel, 1984; Coull et al., 1989; Demirel and Dalkara, 2012; Hoşsucu et al.,1992; Özaydın et al., 2007; Kınacıgil et al., 2008; Gökçe et al., 2010; Türkmen, 2003; Bök et al., 2011). Also, reproduction biology was studied (Muus and Nielsen, 1999; Quéro et al., 1986; De Veen, 1976, Oral, 1996). Studies on the population parameters of the species in Turkey are insufficiant. Growth parameters were studied by Türkmen (2003) in Iskenderun Bay (Northeastern Mediterranean), Hoşsucu et al. (1999), and Cerim and Ateş (2020) in the Aegean Sea.

Previous studies on growth parameters and reproduction in the study area were limited to in a single study (Oral, 1996). To our knowledge, this is the first study on the first sexual maturity length of common sole in the Sea of Marmara. The goal of this paper is to present detailed and up-to-date information on the age, growth, mortality, and reproduction biology of the common sole in the Sea of Marmara. Due to the stock status of the economical demersal fish species which has under high fishing pressure need to be monitored continuously, we want to reveal useful data for fisheries management authority.

MATERIAL AND METHOD

The samples were collected between March 2017 and December 2018 at 34 stations located in three different depth contours (20-50, 50-100, 100-200) from the Sea of Marmara (Figure 1). Samplings were conducted with bottom trawl which has MEDIT's standards, at a speed of 3 miles and 0.5 h duration. The catch-per-unit-effort (CPUE) values (kg h^{-1}) were calculated as being the catchweight (Cw) divided by the swept area (a) and for each haul and the mean values were computed based on depths (Sparre and Venema, 1998).

$$\boldsymbol{CPUE = \frac{\sum Ci/Nh}{\sum t/Nh}}$$

where ′Ci′ is the catch amount in N or W (kg) for species i; ′Nh′, is the number of hauls, and ′t′ is haul duration in hours ′h′.

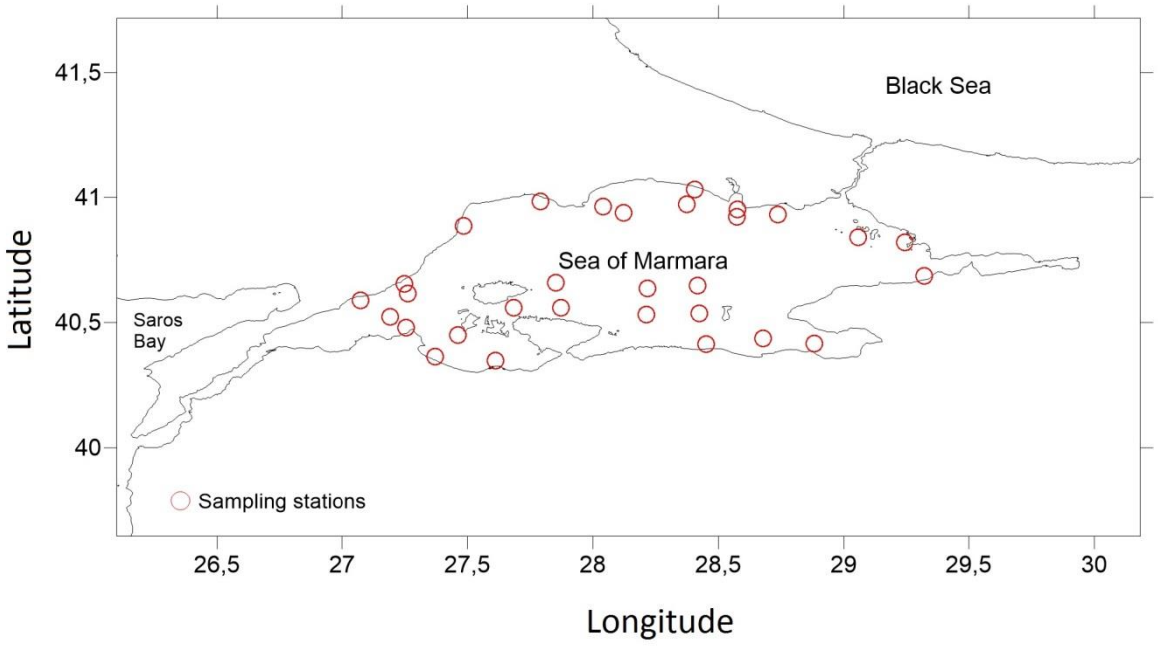

Figure 1. Sampling stations in the Sea of Marmara

The total length (TL) of the species was measured with the nearest 0.1 cm precision ruler, and total weight (W) was measured with 0.01 g precision balance. The length-weight relationship parameters were calculated using Le Cren (1951)'s formula

$W=a\times TL^{b}$ (1)

where W is the total weight (g) and TL is the total length (cm), *a* and *b* are regression parameters. The growth type was identified according to the equation (Sokal and Rohlf, 1987):

$ts=(b-3)/SE(b)$ (2)

where ts is *t*-test value, b is the slope, and SE(b) is the standard error of the slope. A significant difference of *b* values from 3, which represent isometric growth, was examined with the *t*-test (Pauly, 1993).

Sagittal otoliths were used for age determination. Growth parameters were estimated using the von Bertalanffy growth equation:

$Lt= L_{\infty}\ [\ 1-exp(\ k(t-t_0\ ))\ ]$ (3)

where L(t) is the length at age, L_{∞} is the asymptotic length, K is the growth factor, and t_0 is the theoretical age when the size of fish is zero. Growth parameters were estimated using the FISAT II program package (Gayanilo et al., 2005). The ϕ growth performance index was calculated as follows;

$\phi = logK+ 2\times log\ L_{\infty}$ (4)

Total mortality (Z) was found using the length converted catch curve (Pauly, 1984). Natural mortality (M) was determined using Pauly's (1980) formula,

$Log(M) =(-0.0066)-0.279\times log(L)+ 0.6543\times log(K\ )+ 0.4634\times log(T\ )$ (5)

Fishing mortality (F_{curr}) was calculated using the following formula

$F_{curr}=Z-M$ (6)

The exploitation rate (E_{curr}) was obtained using the formula

$E_{curr}=F_{curr}/Z$ (7)

For comparison and interpretation of calculated mortality and exploitation rates, three reference points were calculated, which were the optimum fishing mortality (F_{opt}), fishing mortality limit reference point (F_{lim}) and optimum exploitation rate (E_{opt}) according to Patterson (1992), Gulland (1971) and Frose et al. (2008), respectively.

$F_{opt}= 0.5M$

$F_{lim}= (2M)3^{-1}$ (Patterson, 1992)

$E_{opt}= F_{opt}\ .\ (M+ F_{opt})^{-1}$ (Gulland, 1971)

Besides, the length where the maximum yield can be obtained (L_{opt}) was calculated.

$L_{opt}= 3L_{\infty}\ .\ (3+ (M\ .\ K^{-1}))^{-1}$ (Frose et al., 2008)

Stages of maturity were determined by Holden and Raitt (1974): immature, maturing, ripening, ripe, and spent. The gonadosomatic index (GSI) was calculated using the formula developed by Gibson and Ezzi (1980):

$GSI= (Gonad\ weight/(Body\ weight-Gonad\ weight))\times 100$ (8)

The length at first maturity (L_{50}) was estimated by fitting a logistic function using the Newton algorithm which is defined as:

$P(1)=1/1+e-(a+b1)$ (9)

where P(1) was the proportion of mature specimens at length 1, and a and b are the parameters of the logistic equation (Piñeiro and Saínza, 2003).

RESULTS

A total of 80 *S.solea* individual was evaluated for analyses. 65 of the 80 individuals (84%) were determined as female and the remaining 15 of them were male (16 %). The sex ratio was calculated as 1:0.2 in favor of females. Total length values were varied between 9.0 and 32.0 cm TL, with a mean of 23.81 ± 4.44 cm TL. The total weight of the individuals was ranged from 7.56 to 319.62 g, with a mean of 126.02 ± 64.4 g (Table 1). The length composition and length-frequency distribution of the individuals are shown in Figures 2 and 3. The highest represented length group was determined as 22 cm TL for males and 27 cm TL for females. The mean CPUE value was calculated as 0.1 kg h^{-1}. According to depth contours (20-50 m, 50-100 m, 100-200 m) the CPUE values were determined as 0.11 kg h^{-1}, 0.09 kg h^{-1} and 0.07 kg h^{-1}, respectively.

Table 1. Length-weight parameters according to the sex of *S.solea* in the Sea of Marmara

Sex	N	Length distribution (cm)		Weight distribution (g)	
		Min-Max	Mean±se	Min-Max	Mean
Female	65	10.8-31.5	25.74 ± 3.83	11.45-300.83	157.25±67.14
Male	15	17.5-27	23.17 ± 2.77	46.94-163.30	104.92±33.5
Combined sexes	80	9-32	23.81 ± 4.44	7.56-319.62	126.02±64.4

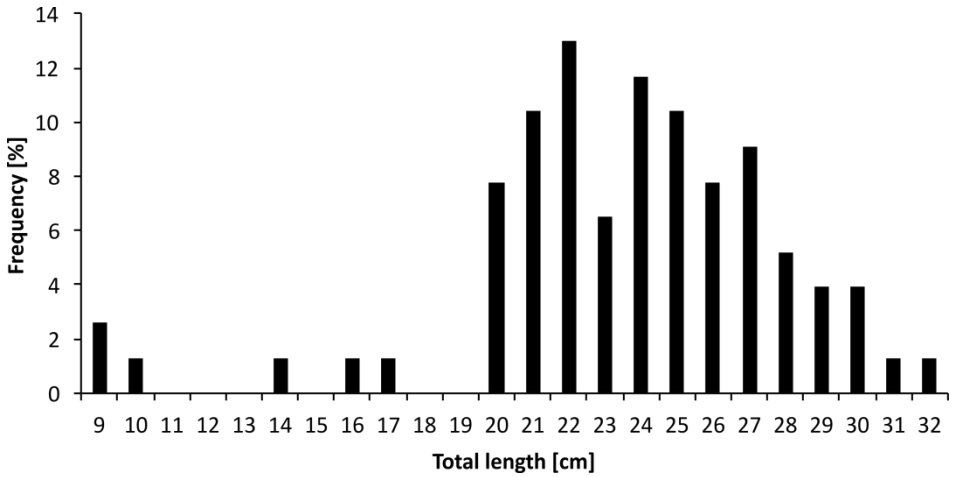

Figure 2. Length frequency distribution of *S. solea* for combined sexes

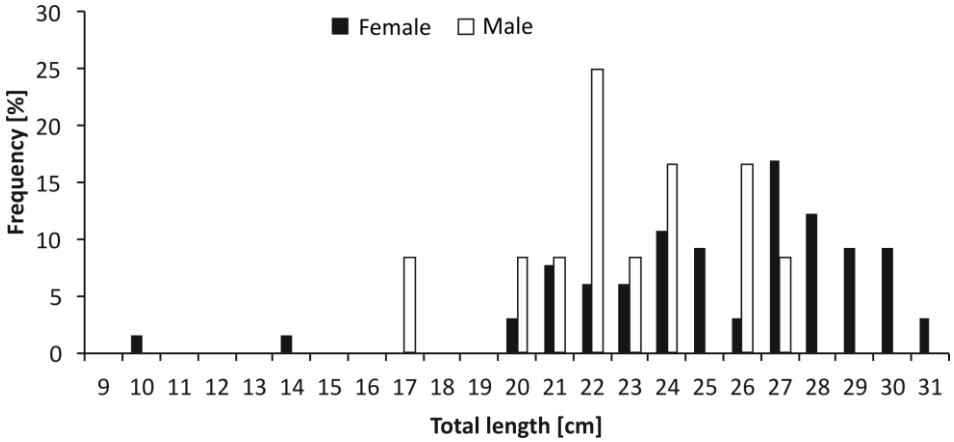

Figure 3. Length frequency distribution of female and male of *S. solea*

The relationship between the total length (L) and weight (W) of *S.solea* was calculated as $W=0.0082\times L^{3.01}$ ($R^2=0.96$) for both sexes. According to *t*-test values, common sole showed isometric growth ($p>0.05$). GSI values of the individuals were ranged from 0.01 and 2.19. The GSI values of the females were differed via months in 2017 and 2018 (Figure 4). The maximum GSI for females was determined in September and December in 2017 and April and October in 2018, and the minimum GSI was found on August for both 2017 and 2018. Mature gonads of females were encountered from September to January in 2017, April 2018, and September to December 2018. Whereas, the mature gonads of males were found only in September 2017 and April in 2018. According to GSI values and maturity stages for both years, the extended spawning period occurred from September to April. Besides, spawning was peaked in two periods, autumn (September-December) and spring (April) (Figure 4). The first sexual maturity length for female individuals was determined as L_{50} = 21.9 cm (Figure 5).

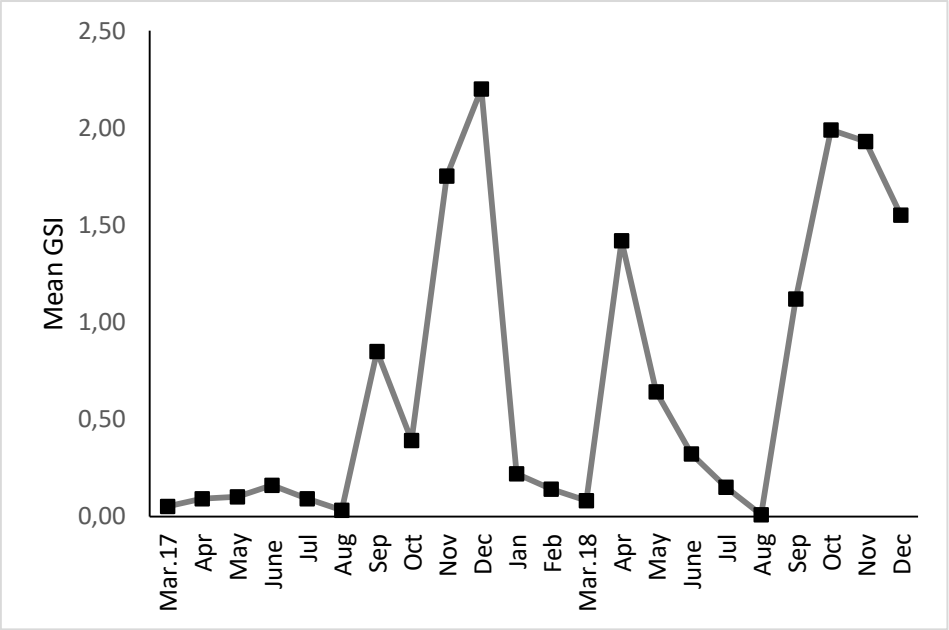

Figure 4. Monthly variation of Gonadosomatic index of female *Solea solea*

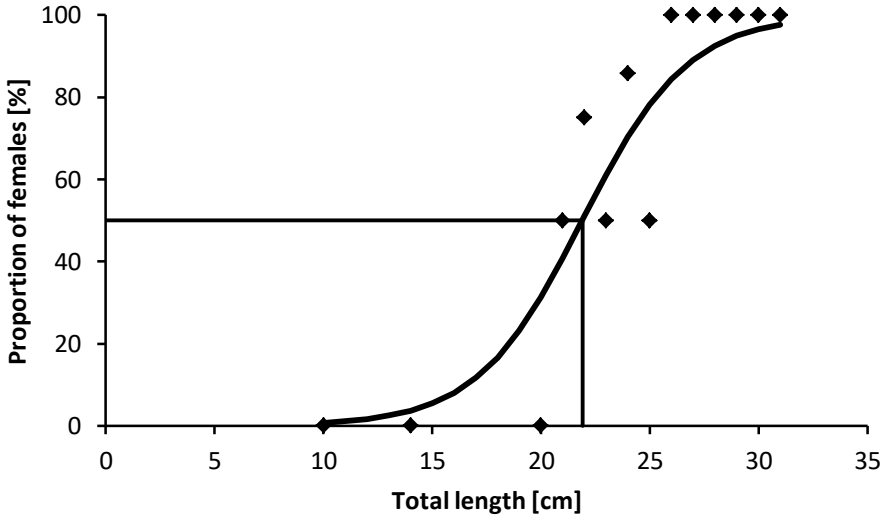

Figure 5. The first reproductive length (L_{50}) of *S.solea* female individuals.

It was determined that the age distribution was ranged from 1 and 5 (Table 3). The asymptotic length (L_∞), growth coefficient (K), and prenatal age (t_0) were calculated as 34.56 cm TL, 0.48 $y^{-1,}$ and -0.01 y, respectively. (Figure 6). The total mortality (Z) was calculated as 2.4. The natural mortality (*M*) and fishing mortality (F_{curr}) were detected as 0.79 and 1.61, respectively. The exploitation rate (E_{curr}) was found as 0.67. The biological reference points were calculated as F_{opt}=0.395; F_{lim}=0.53 and E_{opt}=0.333, respectively. The length where the maximum yield can be obtained (L_{opt}) was found as 22.3 cm TL.

Table 2. The total length-age key of *S.solea* individuals

Ages	Min- Max length	Mean length
1	9-10.8	9.6±0.6
2	17.5-24.5	22.03±0.4
3	25-29	26.54±0.39
4	28	28
5	30.5-32	31.33±0.44

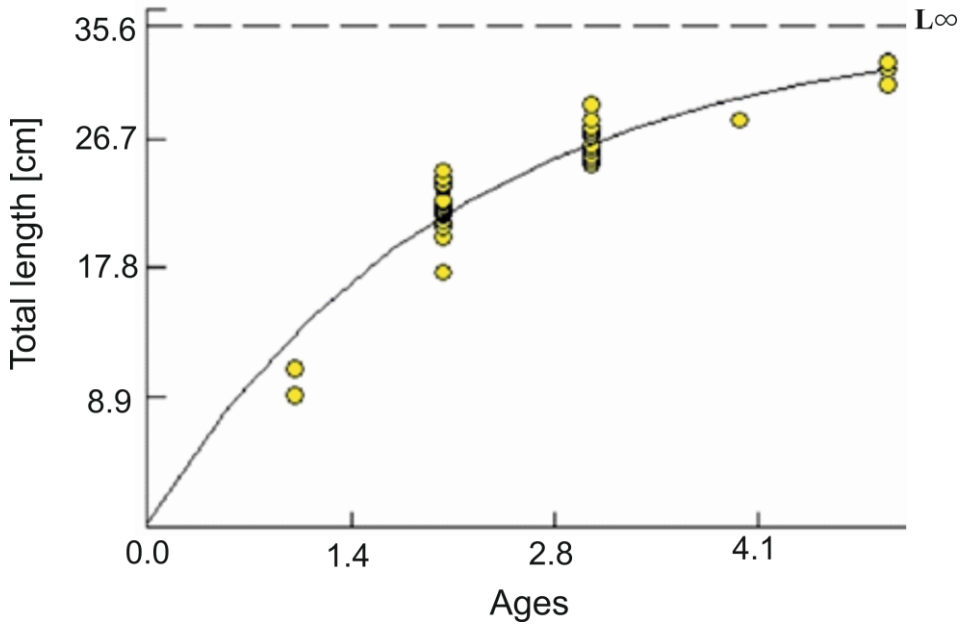

Figure 6. The von Bertalanffy growth curve of *S.solea* in the Sea of Marmara.

DISCUSSION

Although detailed and outnumbering sampling, collected individual number of common sole was observed low. On the other hand, fishing mortality was calculated as 1.61. This value was observed as the highest estimated fishing mortality value among 10 commercially important demersal fish species (*Merluccius merluccius, Merlangius merlangus, Chelidonichthys lucernus, Lophius budegassa, Zeus faber, Mullus surmuletus, Mullus barbatus, Trigla lyra, Citharus linguatula)* which undertaken in this project in the Sea of Marmara. The CPUE values showed that 74% of the total catch of common sole was sampled at the stations located lower than 100 m depths. Trawl fisheries are restricted in the Sea of Marmara. Therefore, the reason of the low CPUE can be thought of as a result of high fishing pressure that originated from beam trawls in the Sea of Marmara. According to fisheries statistics in Turkey, beam trawl vessel number has been increased from 297 to 634 in the last 8 years (TUIK, 2020). Although the target species of beam trawls in the Sea of Marmara is deep water rose shrimp (*Parapenaeus longirostris)*, it may have a large fishing pressure on other demersal fish species as well.

The sex ratio in this study was far from expected value. Higher female number may arise from the small number of sampling. According to Table 3, it can be seen that the growth type of common sole is mostly as isometric and positive allometric. In this study, an isometric growth was observed.

Table 3. The length-weight relationship parameters of *S.solea* from different regions.

Researchers	Region	Sex	*a*	*b*	R^2	*Growth type*
Duncker 1923	North Sea	F+M	0.007	3.10	0.954	
De Veen 1976	Netherlands	M	0.008	3.00		
		F	0.009	3.00		
Deniel 1984	Douarnenez Bay, Britain		0.005	3.21		
Bedford et al. 1986	England	F+M	0.008	3.07		
Dorel 1986	Biscay Bay		0.005	3.18	0.998	
	North and south Bay, France		0.004	3.26	1.000	
Coull et al. 1989	Moray Firth, Alman Bight and Clyde, Scotland		0.004	3.31		A+
Vianet et al. 1989	Lion Bay	F+M	0.006	3.04	0.980	
Hoşsucu 1992	Aegean Sea	F+M	0.005	3.14		
Campillo 1992	Lion Bay	M	0.011	2.94		
		F	0.009	2.99		
Djabali et al. 1993	Adriatic Sea		0.007	3.00		
Oral, 1996	Sea of Marmara	F+M	0.0013	3.62		
Koutrakis and Tsikliras 2003	Porto-Lagos, Aegean Sea	juvenil	0.010	3.00	0.988	
Turkmen 2003	İskenderun Bay	M	0.012	2.99	0.922	I
		F	0.009	3.08	0.947	I
Mendes et al. 2004	Nazaré to St André, Portugal	F+M	0.007	3.09	0.953	
Dulčić and Glamuzina 2006	Mirna, North Adriatic, Croatia	F+M	0.002	3.45	0.946	A+
Özaydın et al. 2007	Aegean Sea	F+M	0.002	3.20		
Kınacıgil et al. 2008	Aegean Sea	F+M	0.002	3.36		A+
Veiga et al. 2009	Algarve	F+M	0.008	3.08	0.969	I
Gökçe et al. 2010	İskenderun Bay	juvenil	0.049	2.35	0.980	
Bok et al. 2011	Sea of Marmara	juvenil	0.004	3.17	0.928	I
Demirel and Dalkara 2012	Sea of Marmara	F+M	0.006	3.06	0.853	I
Maci et al. 2012	Acquatina, Lecce,	F+M	0.011	3.06	0.981	
Crec'hriou et al. 2013	Catalan coasts, France	F+M	0.010	2.96	0.932	
Froese and Sampang 2013	North Sea	F+M	0.005	3.20	0.975	
Cerim and Ateş, 2020	Aegean Sea	F+M	0.008	3.064	0.99	A+
This study	Sea of Marmara	F+M	0.0082	3.01	0.96	I

A+ : positive allometry, A- : negative allometry, I: isometry

According to GSI values and maturity stages, an extended spawning period occurred from September to April. An extended spawning duration for common sole was observed from the studies conducted by Quéro et al. (1986) and Oral (1996). A relatively shorter spawning period was seen in some studies (Table 4). These variations may be stemmed from geographical differences, sampling times, and sampling types. On the other hand, Cerim and Ateş (2019) were found several batches in the spawning season of common sole and stated that partial spawning is a common situation. They observed different peaks in dense spawning times between years. These results coincided with ours. Although it varied by years, spawning peaked in autumn and at the end of the winter. They interpreted that this variation can be closely related to temperature variations of seawater between years. Different spawning peaks in a year were also observed by Anguis and Canavate (2005) for Senegal sole (*Solea senegalensis*) in the south Atlantic coast of Iberia. Devauchelle et al. (1987) were stated that the common sole spawns between 8 and 12.5 °C in a natural environment. According to the mean deep water temperature values measured in our study, higher temperature values were observed in 2018. Thus, it can be said that the bottom water temperature is the main determinant for the spawning duration of the common sole.

Table 4. Reproductive parameters of *S.solea* from different regions.

Author	Area	Sex	Reproductive time	Lm (cm)
De Veen 1976	Netherlands	F		27.0
		F		30.0
Dorel 1986	Bay of Biscay, France			22.0
	Bay of Biscay, France	F		31.0
	East and west channel, France			28.0
Quéro et al. 1986	Bay of Biscay		December-May	
	Netherlands		April-June	
Deniel 1990	Douarnenez Bay, France			32.0
Rijnsdorp and Vethaak 1997	North Sea, England			26.0
Rijnsdorp and Vethaak 1997	Germany		March-June	
Jennings et al. 1998	North Sea, England			24.8
Muus and Nielsen 1999	South England		May-June	
Oral 1996	Sea of Marmara		December-February	
Vasilakopoulos et al. 2011	Irish Sea	M	May-June	
	Skagerrak and Kattegat		April-June	
Froese and Sampang 2013	North Sea	M		18.8
	Trevose, England	F + M		18.8
This study	Sea of Marmara	F	Autumn- Spring	21.9

The first sexual maturity length (L_m) of the common sole was determined as 21.9 cm TL. When compared the results with the findings of studies outlined in Table 4, it can be seen that the sexual maturity length of common sole occurred at smaller lengths. The smaller L_{50} values may arise from various factors. One of the most possible explanations may be explained with the probabilistic maturation reaction norm (PMRN). According to PMRN the L_{50} can vary abide by the length interval of the sampling. As the length group gets smaller, the value of L_{50} gets smaller (Rijndorp, 1989; Horwood, 1993; Sampson and Al-Jufaily, 1999). Also, Morgan (2003) was stated that due to the high fishing pressure length range of the stock becomes smaller. A 23 cm TL mean length in our study support this hypothesis. Besides, Shuozeng (1995) stated that males can reach sexual maturity earlier than females. The study of Froese and Sampang (2013) was convinced this finding, whereas the low male individual number in our study enable us to ignore this situation. Additionally, Pauly (1994) identified that L_{50} appears to increase with latitude for many flatfish species. Contrary to this,

Horwood (1993) found that L_{50} of the common sole was higher in the southern latitude than northern. When the results of L_{50} values of the studies summarized in Table 4 were investigated, higher L_{50} values were seen although carried out in more northern areas. So our finding supported Horwood (1993)'s findings.

When the age length key of this study was examined, a great majority of individuals (85%) were formed between 0 and 3 age class. On the other hand, the maximum age of stock was observed as 5 years. The maximum age was determined as 9 years by Cerim and Ates (2020) in the southern Aegean Sea, 8 years by Turkmen (2003) in Iskenderun Bay, like 7 years by Ramos (1982) in the western Mediterranean, and also 7 years by Oral (1996) in the Sea of Marmara, as 6 years by Stergiou et al. (1997) in the Amvrakikos, Greece. Hossucu et al. (1999) found the maximum age as 5 years in the Izmir Bay, the Aegean Sea, where area of overexploited stocks. The sampling method, fishing pressure, food availability, and competition variations between the studies and areas may cause the varied age distributions.

The asymptotic length in our study was estimated as 34.56 cm TL. This result is compatible with the maximum length (32 cm TL) in the data set. Relatively lower estimated asymptotic length caused a higher K value. As can be seen in Table 5, the K values showed differences between the studies. Smaller K values were calculated in some studies as Teixeira and Cabral (2010), Turkmen (2003), and Cerim and Ateş (2020), which found the maximum age higher than 8. The lower K values of these studies were highly related to the higher age class in the data set. Besides, lower values of growth parameters in our study may arise from high fishing mortality and a limited number of individuals examined. This situation was based on fishing pressure by Nash and Geffen (2015). Due to high fishing pressure tends to higher fishing mortality rates, the older age classes disappear from the stock and the age class becomes smaller. Additionally, the selectivity of the commercial fishing nets excludes 0 age group in the data set. Hence, the age interval becomes bounded and the growth parameters may calculate smaller. As can be seen in Table 5, the ϕ growth performance index values reported by the researchers ranged between 2.03 and 3.04. It was determined that there was no statistically significant difference between the value obtained in this study and previous studies ($p>0.05$) (Table 5). Hence, it can be said that these parameters were closely related to the age length distribution of the data set.

Table 5. Von Bertalanffy growth parameters of *S.solea* from different regions.

Author	Area	Sex	L∞	K (year^{-1})	t_0(year)	Ø
Ramos 1982	Castellon coast, Spain	M	38.8	0.240	-1.09	2.56
		F	46.4	0.220	-0.75	2.68
Froglia and Giannetti 1985	Adriatic Italy		38.3	0.492	-3.57	2.86
Froglia and Giannetti 1986	Adriatic Italy	M	23.2	0.828	-1.66	2.65
		F	37.9	0.504	-5.36	2.86
Girardin et al. 1986	Lion Bay	M	53.8	0.160		2.67
		F	47.2	0.274		2.79
Wurtz and Matricardi 1986	Tiran Sea		35.8	0.406		2.72
Vianet et al. 1989	Lion Bay		48.8	0.240	-0.77	2.76
Costa 1990	Tagus Bay, Portugal		48.3	0.470		3.04
Deniel 1990	Douarnenez Bay Britain	M	42.4	0.397	0.09	2.85
		F	48.2	0.329	0.08	2.88
Erzini 1991	North Sea		37.4	0.310		2.64
Djabali et al. 1993	Adriatic Italy		40.1	0.680		3.04
Oral 1996	Sea of Marmara		37.1	0.100	-3.267	2.27
Stergiou et al. 1997	Amvrakikos Bay, Greece		35.6	0.380	-0.41	2.67
Jennings 1998	Kelt Sea, England		49.8	0.130		2.51
Hossucu et al. 1999	Aegean Sea	M	30.0	0.330	-1.04	2.50
		F	42.5	0.170	-1.96	2.49
Turkmen 2003	İskenderun Bay	M	26.0	0.221	-1.31	2.17
		F	29.9	0.181	-1.55	2.21
Teixeira and Cabral 2010	Portugal	M	45.7	0.210	-1.57	2.64
		F	52.1	0.230	-0.11	2.80
Colloca et al. 2013	Adriatic Sea, Italy		39.6	0.440	-0.46	2.84
Froese and Sampang 2013	North Sea		40.0	0.148	-3.00	2.37
Gabr 2015	Bardawil Bay, Egypt,		31.1	0.330	-1.51	2.47
Cerim and Ateş, 2020	Aegean Sea	F+M	33.95	0.208	-0.032	2.54
		F	31.98	0.236	-0.037	2.41
		M	29.11	0.324	-0.030	2.29
This study	Sea of Marmara	M+F	34.56	0.48	-0.01	2.76

Oral (1996) sampled 523 individuals between 1992 and 1994 with beam trawl and beach seine between 5 and 90 m depths at 13 stations located in the Sea of Marmara. The sampling stations of this study and Oral's study are similar. Due to CPUE was not calculated in that study, stock status compared based on the number of individuals caught. A quite low individual number in this study clearly showed that the common sole stocks under threatened in the Sea of Marmara. Overfishing, changes in the sea the physic-chemical parameters of seawater, and wrong fisheries management applications may be a result of this problem.

Biological reference points are defined as a principal tool for fishery management strategies. Due to comparison and interpretation of the calculated mortality rates, biological refecence points reveals useful information(Zhang et al. 2017; Cerim et al., 2020). The calculated fishing mortality rate (F_{curr}=1.61) in this study was relatively higher than the estimated optimum (F_{opt}=0.395) and limit (F_{lim}=0.53) fishing pressure. Current fishing mortality is higher than the reference points. Also, estimated optimum length (L_{opt}= 22.3 cm) where the maximum yield can be obtained was above both L_{50} =21.3 cm and the minimum landing length (20 cm) that was determined by the Turkish Fisheries Management Authority. Fishing pressure on low sizes should be decreased to ensure a sustainable fishery.

Consequently, after 25 years, it can be said that the stock structure has been damaged. High mortality rates and low age interval and biomass supported this result. As with many other species, the minimum landing sizes should be rearranged and fishing pressure should be decreased. Although the trawl fishery is restricted, illegal trawling is still ongoing and causes problems in the Sea of Marmara. Also, the shallower distribution of many commercial demersal fish species in the Sea of Marmara has become the target of beam trawls. The laws should be persuader and control mechanisms should be increased.

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Seasonal Variations in Fatty Acid and Nutritional Composition of Sand Smelt (*Atherina boyeri*) caught from Cekerek Dam (Yozgat, Turkey)

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Research Article

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Abstract

Seasonal variations of proximate and fatty acid compositions of the muscle tissue of sand smelt (*Atherina boyeri*) harvested in Cekerek (Sureyyabey) Dam were investigated. Seasonal nutritional composition of sand smelt muscle in October, March and July were found as 17.00%; 16.72%; 15.64% for protein, 1.95%; 2.00%; 2.86% for lipid, 79.25%; 79.17%; 78.06% for moisture and 1.80%; 2.10%; 2.49% ash, respectively. Sand smelt showed the lowest protein values in summer, while the highest protein values were observed in autumn and spring. The highest lipid content of sand smelt was found in summer ($P < 0.05$). The moisture content of sand smelt in summer was significantly ($p<0.05$) lower than those of the content in autumn and spring. The lowest ash content was observed in autumn (1.80%) whereas sand smelt gave the highest ash level was in summer. During the seasons, the proximate and FA composition values of sand smelt showed variations ($p<0.05$). Seasonal fatty acid compositions of sand smelt ranged from 24.63 to 25.69% for saturated fatty acid (SFA), from 20.75 to 26.43% for monounsaturated (MUFA), and from 28.29% to 36.11% polyunsaturated fatty acid (PUFA). Palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1ω7), oleic acid (18:1ω9), linoleic acid (LA, 18:2ω6), arachidonic acid (ARA, 20:4ω6), linolenic acid (18:3ω3), eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) were major fatty acids of sand smelt in different seasons. The highest proportion of EPA (5.59-7.22%) and DHA (12.33-19.77%) were observed in spring. The results showed that sand smelt is good source of n-3 PUFAs and is rich in EPA+DHA for human nutrition.

Keywords: Cekerek (Sureyyabey) Dam, DHA/EPA, nutritional composition, Sand smelt, seasonal variation in fatty acids.

Çekerek Barajı'ndan (Yozgat, Turkey) Avlanan Gümüş Balığının (*Atherina boyeri*) Yağ Asidi ve Besin Kompozisyonundaki Mevsimsel Değişimleri

Özet

Çekerek (Süreyyabey) Barajı'nda avlanan gümüş balığının (*Atherina boyeri*) besinsel ve yağ asidi kompozisyonunun mevsimsel değişimleri araştırılmıştır. Gümüş balığının mevsimsel besin kompozisyonu Ekim, Mart ve Temmuz aylarında sırasıyla protein için %17,00; %16,72; %15,64, lipid için %1,95; %2,00; %2,86, nem için %79,25; %79,17; %78,06 ve kül için %1,80; %2,10; %2,49 olarak bulunmuştur. Gümüş balığında en yüksek protein değerleri sonbahar ve ilkbaharda gözlenirken, en düşük protein değeri yazın gözlenmiştir. Gümüş balığının en yüksek yağ oranı yaz mevsiminde gözlenmiştir ($p<0,05$). Gümüş balığının yaz mevsimindeki nem içeriği sonbahar ve ilkbahar mevsimindeki nem içeriğinden önemli ($p<0,05$) ölçüde düşüktü. En düşük kül oranı sonbaharda görülürken, gümüş balığının en yüksek kül oranı yaz mevsiminde görülmüştür. Gümüş balığının besin ve yağ asidi kompozisyonu mevsimler boyunca değişkenlik göstermiştir ($p<0,05$). Gümüş balığının mevsimsel yağ asidi bileşimleri doymuş yağ asidi (SFA) için %24,63 ile %25,69, tekli doymamış yağ asidi (MUFA) için %20,75 ile %26,43 ve çoklu doymamış yağ asitleri için (PUFA) %28,29 ile %36,11 arasında değişim göstermiştir. Palmitik asit (16: 0), stearik asit (18: 0), palmitoleik asit (16: 1ω7), oleik asit (18: 1ω9), linoleik asit (LA, 18: 2ω6), araşidonik asit (ARA, 20: 4ω6), linolenik asit (18:3ω3), eikosapentaenoik asit (EPA, 20: 5ω3) ve dokosaheksaenoik asit (DHA, 22: 6ω3) gümüş balığının farklı mevsimlerdeki başlıca yağ asitleriydi. En yüksek EPA (%5,59-7,22) ve DHA (%12,33-19,77) oranı ilkbaharda görülmüştür. Sonuçlar, gümüş balığının iyi bir n-3 PUFA kaynağı ve insan beslenmesi için EPA + DHA açısından zengin olduğunu göstermiştir.

Anahtar Kelimeler: Çekerek (Süreyyabey) Barajı, DHA / EPA, besin kompozisyonu, Gümüş balığı, yağ asitlerinde mevsimsel değişimler.

INTRODUCTION

Fish and fish products are completely nutritious owing to their low fat, rich in high-quality proteins and contain high component of precious ω3 polyunsaturated fatty acids (PUFAs), long muscle fibers, liposoluble vitamins, essential minerals, and all essential amino acids that make up beneficial food sources (Ali et al., 2019; Fallah et al., 2013; Lauriano et al., 2018; Mahadevan et al., 2019). Recently, there has been an increasing interest in the fatty acid composition of fish and fish products due to their nutritional and numerous health benefits.

The ω3 PUFAs contain α-linolenic acid (ALA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) whereas the main ω6 PUFAs include linoleic acid (LA) and arachidonic acid (ARA), which have an important role in human health (Abedi and Sahari 2014; Robert et al. 2014). ω3 fatty acids are valuable especially EPA and DHA for humans, preventing coronary artery disease, inflammatory and autoimmune disorders, and arrhythmias (Connor 2000; Kinsella 1987; Alexander Leaf et al. 2003; Özogul et al. 2009; Schmidt et al. 2005; Simopoulos 1991) as well as other diseases such as hypertension, atherosclerosis, arthritis, asthma, psoriasis, and cancer (Fallah et al. 2013; Gogus and Smith 2010). In addition, ω3 PUFAs seem to play the main role in the improvement and function of the nervous system (brain), photoreception (vision), and reproductive system (Alasalvar et al. 2002; Prato and Biandolino 2012; Sidhu 2003).

The ω3 and ω6 polyunsaturated fatty acids are considered essential since they cannot be synthesized in the human body, thus they must be provided by food. Hence, every kind of fish should be consumed two or three times a week, advised by the Nutrition Committee of the American Heart Association (Kris-Etherton et al. 2002; Özogul et al. 2009). However, previous studies imply that nutrient contents of most fish species are more irregular in their fatty acid (FA) profiles due to environmental factors, such as feeds, temperature, salinity, and FA composition of their nutrition (Bandarra et al. 2001; Inhamuns and Franco, 2008; James Henderson and Tocher, 1987; Massresha et al. 2017; Ozogul et al. 2011).

Atherina boyeri (sand smelt) is a small, short-lived, euryhaline fish generally living in freshwater hypersaline and forming large local populations. It is spread from the south of Spain to Morocco and Madeira in the Mediterranean, Black Sea, and Atlantic, and in isolated populations on the coasts of England and the Netherlands (Chrisafi et al., 2007; Quignard & Pras, 1986). It is also found the Caspian Sea (Ekmekçi et al., 2013). The species is very well adapted to the ecosystem it enters, grows quickly, and produces large populations due to the wide range of food and habitat preferences (Ekmekçi et al., 2013). Recently, sand smelt has entered into many natural and artificial lakes in different parts of Turkey (Becer and Bilgin, 2018). The first records of *A. boyeri* in Cekerek (Süreyyabey) Dam, one of the largest dams in Turkey was given by Benzer (2018).

Sand smelt (*Atherina boyeri*) has become an important and economic species that both exports and contributes to the region's and Turkey's economy (Becer and Bilgin, 2018; Çevik et al., 2018). Sand smelt can be processed in different methods including drying (Abou-Zied et al., 2020), fish finger (Izci et al., 2011a), fish chips (Izci et al., 2011b), fried big-scale sand smelt (*Atherina boyeri*) (Dal Bosco et al., 2019). In Turkey, its catch has been reported as 4744 tons in 2019 according to the Turkish Statistical Institute. For this reason, sand smelt (*Atherina boyeri*) is among the commercially important fish species found in Cekerek (Sureyyabey) Dam (in Yozgat, Turkey). To our knowledge, no previous study exists on the effects of seasonal variations on the proximate and fatty acid composition of this important species in Cekerek (Sureyyabey) Dam. Therefore, the objective of this study was to determine the seasonal variations in proximate and fatty acid profiles muscle tissue of sand smelt (*Atherina boyeri*) caught in the Cekerek (Sureyyabey) Dam.

MATERIAL and METHODS

Fish samples

Sand smelt (*Atherina boyeri*) used in the present study were obtained from the local fisherman of Cekerek (Sureyyabey) Dam just after catching, between October 2018 and July 2019. A minimum of thirty fish from each species was collected in October, January, March, and July for each season except during the reproduction season which is in the period between April and June. After catching, fish species were immediately iced in a styrofoam box with ice and transported to the Seafood Processing Technology Laboratory of the Fisheries Faculty at Cukurova University. The duration of time between harvesting and arrival of the fish at the laboratory was 5-6 hours, where they were

always kept on ice during transportation. After that, whole fish were labeled and frozen at -24°C and kept under frozen conditions (-24 °C) until analyzed. Proximate and fatty acid composition analyses were done within 2 months after sampling. At the beginning of each analysis, the frozen fish was thawed in a refrigerator (2–4°C) overnight. After thawing, the length and weight measurements of the fish were taken. The mean length and weight values were 7.71±0.76 cm and 4.24±1.31g in October; 8.49±0.57cm and 6.34±1.52 g in March; 6.98±0.45cm and 3.11±0.56 g in July for sand smelt. Then, the fish was immediately gutted, filleted, and minced for chemical composition analyses. Triplicate analyses were made.

Proximate analysis

The contents of ash and moisture were determined according to the method in AOAC (1984). Protein content was carried out by Kjeldahl's method (AOAC 981.10, 1998). Lipid content was determined by the method of Bligh and Dyer (1959).

FAME analyses

Methyl esters were prepared by transmethylation using 2M KOH in methanol and n-heptane according to the method as described by Ichihara et al. (1996) with minor modification. Extracted lipids (10 mg) were dissolved in 2 ml heptanes followed by 4 ml of 2 M methanolic KOH. The tube was then vortexed for 2 min at room temperature. After centrifugation at 4000 rpm for 10 min, the heptanes layer was taken for GC analyses.

Gas chromatographic condition

The fatty acid composition was analyzed by GC Clarus 500 with an autosampler (Perkin Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m x 0.32 mm, ID x 0.25 lm, BP20 0.25 UM, USA). The oven temperature was 140 °C, held 5 min, raised to 200 °C at a rate of 4 °C /min and to 220°C at a rate of 1 °C /min, while the injector and the detector temperature were set at 220 °C and 280°C, respectively. The sample size was 1 μL and the carrier gas was controlled at 16 psi. The split used was1:100. Fatty acids were identified by comparing the retention times of FAME with a standard 37 component FAME mixture (Supelco). Three replicates of GC analyses were carried out and the results were expressed in GC area % as mean value ± standard deviation (SD).

Statistical Analysis

Analysis per sample was carried out in triplicate and the results are shown as the average and standard deviation. All data obtained separately for each sampling period were subjected to analysis of variance (one-way ANOVA) and the Duncan's Multiple Range Test was applied to determine significant differences at a p-value of <0.05 using the SPSS version 22 software (SPSS, Chicago, Illinois).

RESULT and DISCUSSION

Proximate composition

The seasonal variations in proximate composition values of muscle tissues of sand smelt are presented in Table 1. Protein contents of sand smelt samples in all seasons ranged from 15.64% to 17.00% and statistical differences were found in summer ($p < 0.05$). Sand smelt showed the lowest protein in summer, while the highest protein values were observed in autumn and spring. The average length and weight of sand smelt were also higher in spring and autumn than in summer (Table 1). Izci et al. (2011) and Smida et al. (2014) reported similar protein contents for sand smelt, while Dal Bosco et al. (2019) reported slightly higher protein content (18.40%) for sand smelt from Tunisian Coast. The differences in protein contents may be attributed to fish size, genetic factors, gender, and seasonal variations. Many previous studies (Köse et al. 2010; Effiong and Mohammed, 2008)) have also shown that the protein composition of fish can change depending on the fish species, size, gender, and season.

The highest lipid content of sand smelt was observed in summer as 2.86%, with significant variation amongst seasons ($p<0.05$) (Table 1). The lipid contents of sand smelt in the current study were similar to the results of previous studies (Dal Bosco et al. 2019; Prato and Biandolino 2012). Moreover, Bouriga et al. (2014) also found that the fat content of the sand smelt (*Atherina lagunae*) populations from Kerkennah Islands and Bizerta Lagoon were maximal at the end of spring – beginning of summer and minimal at the end of the winter. The increase of lipid content in summer may also be explained by an increased feeding activity and growth rate of fish because of higher water temperature (Bouriga et al., 2014). However, Yavuzer (2020) reported a higher lipid level (3.58%) for

sand smelt from Hirfanlı Dam, Turkey in November. This author also found that the size of the sand smelt was higher (between 11-18 cm) compared to the current study and its weight was similar and lower (between 1-3 g) than our research data (Table 1). The differences in fat contents may be based on regional fish diet, gender, size, and age. Moreover, it was found that the lipid content of fish is influenced by gender, species, season, diet and geographical origin (Ackman, 1989; Özogul et al., 2007; Rasoarahona et al., 2005).

The moisture content varied from 78.06% to 79.25% for sand smelt and in summer was significantly ($p<0.05$) lower than those of the content in autumn and spring (Table 1). The lowest ash content was observed in autumn whereas sand smelt gave the highest ash level was in summer (Table 1). Ash contents of sand smelt samples in all seasons were statistically different ($p<0.05$). Izci et al. (2011a) and Bilgin et al. (2011) also found similar moisture and ash contents of sand smelt. However, Yavuzer, (2020) reported lower moisture content (76.27%) and higher ash content (3.33%) for sand smelt compared to the current study.

The proximate and fatty acid composition of fish muscle tissue varies from species to species and is also affected by several factors for singular or many fish of the same species taken at different times or under different situations (James Henderson and Tocher 1987; Tanakol et al. 1999; Vasconi et al. 2015). These factors contain the fish species, size, age, and reproductive cycle of the fish, season, salinity, type of breeding and nutrition, spawning time, geographic location, and water temperature (Linhartová et al. 2018). In summary, it can be said that sand smelt is a good protein source and its nutritional composition varies according to the seasons and these specific factors.

Table 1. Proximate composition (%), average total length and weight of sand smelt in Cekerek (Süreyyabey) Dam Lake in different seasons.

Common and The species name of fish	Composite	Autumn (October)	Spring (March)	Summer (July)
Sand smelt. (*Atherina boyeri*)	Protein	17.00±0.14^{a}	16.72±0.43^{a}	15.64±0.15^{b}
	Lipid	1.95±0.50^{b}	2.00±0.20^{b}	2.86±0.27^{a}
	Moisture	79.25±0.14^{a}	79.17±0.21^{a}	78.06±0.41^{b}
	Ash	1.80±0.02^{c}	2.10±0.16^{b}	2.49±0.18^{a}
	Average fish total length (cm)	7.71±0.76	8.49±0.57	6.98±0.45
	The average fish total weight (g)	4.24±1.31	6.34±1.52	3.11±0.56

Data are shown as mean ± standard deviation (SD). Different letters (a–c) in the same row for each fish show significant differences ($p<0.05$).

Fatty acid composition

General

Fatty acid compositions of the sand smelt captured from Cekerek Dam in different seasons are given in Table 2. There were significant differences ($p < 0.05$) in the fatty acid (FA) profiles of the sand smelt among seasons. Total saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) level of sand smelt were the lowest in spring while sand smelt gave the highest level (25.69%) in autumn for SFA, and autumn (26.43%) and summer (26.30%) for MUFA. Polyunsaturated fatty acids (PUFA) ranged from 28.29% in autumn to 36.11% in spring. Sand smelt contained a higher amount of PUFAs than MUFAs and SFA throughout different seasons ($p < 0.05$). Seasonal differences in PUFA, MUFA, and SFA contents might be due to the differences in fish species, size and gender, ecological factors, feeding behavior, and food items availability.

Saturated Fatty Acids (SFA)

The main SFA were palmitic acid (C16:0, 15.76-16.19%), stearic acid (C18:0, 4.54-5.17%), followed by myristic acid (C14:0, 2.09-3.32%) in three seasons. The dominant SFA in sand smelt were palmitic acid and stearic acid, contributing approximately 63.02-64.60% and 18.24-20.99% to the total SFA content respectively throughout different seasons (Table 2). It was found that the contents of stearic acid of sand smelt changed considerably during the seasons ($p<0.05$) while no statistical differences were found for palmitic acid. Many authors have reported myristic, palmitic and stearic acid as major acids among SFAs for the same and other fish (Dal Bosco et al., 2019; Rahman et al., 1995; Tanakol et al., 1999; Yavuzer, 2020). Although, Dal Bosco et al. (2019) reported similar

results value for stearic acid, Tanakol et al. (1999) and Yavuzer (2020) found higher results values of the amounts of palmitic acid and stearic acid in sand smelt from the Marmara Sea (27.1 -7.0%) and Hirfanlı Dam, Turkey (26.85-6.40%), respectively. The fatty acid profile of fish species is not constant and it changes according to many morphological and ecological factors such as species, season, life stage, location, diet, and age of the fish, which have effects on the nutrient composition of fish and even vary broadly within species and among species (Lunn and Theobald 2006; Ozogul et al. 2018).

The total SFA content of sand smelt was the highest in autumn, while the lower level of total SFA was observed in spring. There were significant differences ($p<0.05$) among the seasons as regards total SFAs (Table 2). In general, SFA content was low (<30%), except for certain species (Ackman 1989; Guler et al. 2008). Similar results were observed in our study during different seasons (21–28.14%). Moreover, Öksüz et al. (2019), Luczynska et al. (2014) and Linhartová et al. (2018) were reported similar results for the same and other freshwater fish species.

Monosaturated Fatty Acid (MUFA)

The highest proportions of MUFA in the examined fish were oleic acid (C18:1n-9, 9.72-13.20%), palmitoleic acid (C16:1n7, 5.49-8.26%), and cis-vaccenic acid (C18:1n7, 3.43-4.29%). Another minor MUFA detected was erucic acid (C22:1n9, 0.11-0.62). In terms of seasons, there were statistical differences in the fatty acid composition of fish species ($p < 0.05$), (Table 2).

Oleic acid was the main fatty acid among the MUFAs in the lipids of fish accounting for 46.84-50.19% of the total MUFAs, during different seasons in the present study. Dal Bosco et al. (2019) reported for sand smelt from Trasimeno Lake, slightly higher values of oleic (14.06%) than those found in this study. Tanakol et al. (1999) observed for sand smelt caught in the Marmara Sea had a considerably lower value of this acid (4.6%) compared to the results of this study for sand smelt (9.72-13.20%). Bilgin et al. (2011) reported a little lower oleic acid (8.52%) for sand smelt from Egirdir Lake in Turkey.

Palmitoleic acid was the second main MUFA in the lipids of five fish species studied, constituting about 26.45-31.40% of the total MUFAs for all seasons. The highest palmitoleic acid was found in summer at 8.26%. The results showed that sand smelt is a good source of palmitoleic acid. Prato and Biandolino (2012) and Yavuzer (2020) found similar variable palmitoleic contents in sand smelt. However, Tanakol et al. (1999) found very lower (2.9%) and Dal Bosco et al. (2019) observed much higher values (10.72%) of palmitoleic oleic acid for sand smelt than those found in this study (Table 2). The differences of oleic and palmitoleic acid contents in the same fish species could be due to the geographical location, size, muscle zone analyzed, sexual maturation, and catching season (Prato and Biandolino 2012).

The total MUFA content of sand smelt was the highest in autumn and summer while the lower level of total MUFA was observed in spring ($p < 0.05$) (Table 2). The main reason for the high MUFA content in autumn and summer could be since the fish has higher palmitoleic (C16:1ω7) and oleic (C18:1ω9) acid content in these seasons. Early studies have also shown that the level of unsaturation of total lipids in fish is conversely connected with the ecological temperatures (Vasconi et al. 2015). Moreover, sand smelt exhibited a lower content of MUFA (20.75-26.43%) than the total PUFAs content (28.29-36.11%) ($p<0.05$) during all seasons, in the present study (Table 2). Also, Dal Bosco et al. (2019) and Prato and Biandolino, (2012) reported for sand smelt from Trasimeno Lake and from Mar Grande Sea had a much lower content of MUFA (31.56% and 17.82% respectively) than contents of PUFAs.

Polyunsaturated fatty acid (PUFA)

The most represented PUFA in sand smelt were decosahexaenoic acid (DHA, C22:6ω3, 12.33-19.77%), eicosapentaenoic acid (EPA, C20:5ω3, 5.59-7.22%), linoleic acid (LA, C18:2ω6, 2.85-3.05%), linolenic acid (ALA, 18:3ω3, 3.19-4.81%) and arachidonic acid (AA, C20:4ω6, 0.23-0.29%) in all seasons. It was reported that the ratio of these FAs changed significantly during the seasons ($p<0.05$), (Table 2). The percentages of dominant fatty acids found in our study were similar to the amounts reported in the literature for this species (Dal Bosco et al., 2019; Prato & Biandolino, 2012; Tanakol et al., 1999).

The proportions of total PUFA of sand smelt were found to be 28.29% in autumn and 36.11% in spring and 31.28% in summer (Table 2). Similar results were found by Prato and Biandolino (2012), Dal Bosco et al. (2019), and Yavuzer (2020). Moreover, a seasonal variation in total levels of SFA, PUFA, and DHA was reported by Yanes-Roca et al. (2009), suggesting that PUFA is functionally

important for fish's normal growth, development, and reproduction. The reproduction period of sand smelt in Cekerek Dam is in the period between April and June. In our study, the proportions of total PUFA was decreased slightly from March (36.11%) to July (31.28%) that represent before the beginning and the end of the reproduction period for sand smelt, respectively. PUFA can also be used for more reproductive purposes for this species, which is confirmed by studies by Yanes-Roca et al. (2009) and Köse et al. (2010) on the functionality of PUFA in fish.

It was very conspicuous that the higher PUFA values of sand smelt were mostly derived from the high DHA and EPA amounts in the present study. The PUFA consisted primarily of ω3 and ω 6 PUFA and the main contributor to ω3 PUFAs was DHA (C22:6ω3), followed by EPA (C20:5ω3) in all examined fish. The highest amounts of EPA and DHA were obtained from sand smelt (7.22%-19.77%) in spring while sand smelt gave the lowest proportions of EPA and DHA (5.59%-12.33%) in autumn ($p<0.05$) (Table 2). Vasconi et al. (2015) observed a similar amount of EPA (8.61%) and DHA (18.13%) in sand smelt from Subalpine Lakes in Italy. Moreover, the highest total EPA and DHA values were found for sand smelt in spring (26.99%) and followed by summer (23.86%) ($p<0.05$) whereas the lowest total EPA and DHA (17.92%) value was obtained from sand smelt in autumn. A close association between feeding activity and fatty acid composition of the muscle lipids was found by Vasconi et al. (2015). Sand smelt is a species of carnivorous or opportunistic predator; it feeds on zooplankton and benthic organisms (Bartulović et al. 2004; Benzer 2018). Moreover, many studies have shown that zooplankton abundance is the highest in spring and lowest in winter (Bulut, 2018; Bulut & Saler, 2014). In general, zooplankton provides highly unsaturated fatty acids (HUFA) that are physiologically essential, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Keva et al., 2019; Makhutova et al., 2016). This could be clarified by the fact that these fish species consumed larger amounts of freshwater phytoplankton and zooplankton, which were abundant in ω3 PUFA, in spring than in other seasons.

Generally low levels (e.g. 3.19- 4.81%) of other ω3 fatty acids were found, one of which was linolenic acid (18:3ω3), which showed significant differences ($p<0.05$) among the seasons. The highest linolenic acid was found in autumn at 4.81% while sand smelt gave the lowest amount of linolenic acid was found in summer at 3.19% (Table 2). Dal Bosco et al. (2019) observed slightly higher values (5.45%) of linolenic acid for sand smelt in autumn whereas Yavuzer (2020) observed very lower linolenic acid (0.43%) content than those found in this study. Polat and Beklevik (1999) reported that many fish species the can convert linolenic acid to other long-chain ω-3 series fatty acids such as EPA and DHA. The results showed that sand smelt is also a good source of PUFAs.

EPA and DHA, found only in fish and seafood, possess excessively beneficial effects to prevent coronary artery disease in humans (Guler et al. 2008; Leaf, A and Weber 1988). Although PUFAs (especially, EPA and DHA) are effectively synthesized only by aquatic organisms, human consumption of marine and freshwater products can provide these essential components (Özogul et al. 2007). These results indicated that the investigated all fish samples are a great source of EPA and DHA, especially in spring.

The most abundant ω6 PUFA was linoleic acid (C18:2ω6), followed by arachidonic acid (ARA; C20:4ω6). Sand smelt gave the highest linoleic acid (3.05%) content in autumn, while the highest arachidonic acid (0.29%) was in spring for sand smelt (Table 2). Yavuzer (2020) and Prato and Biandolino (2012) observed higher arachidonic acid and lower linoleic acid contents for the same species. Hoewever, Dal Bosco et al. (2019) found that higher arachidonic (5.14%) and linoleic acid (8.18%) content than our study results. These variations might be originated from the fatty acid composition of freshwater microalgae (Ahlgren et al., 1990, 1992), zooplankton feed (Caramujo et al. 2008; Desvilettes et al. 1997), and freshwater insects (Ghioni et al. 1996) that provide to the population of the lake ecosystems (Vasconi et al. 2015). Arachidonic acid like EPA and DHA is involved in maintaining cell membrane structure and function and also contributes to reproductive systems in fish (Cejas et al. 2004).

The amounts of ω3 PUFAs in sand smelt (ranging from 22.73% to 31.29%) were higher than those of ω6 PUFAs (ranging from 3.98 to 4.95%) in all seasons ($p<0.05$) (Table 2). These results are in an agreement with previous studies related to fatty acid profiles of freshwater fish species (Branciari et al., 2020; Dal Bosco et al., 2019; Yavuzer, 2020). Fish are the main nutritional source of essential unsaturated fatty acids, particularly EPA and DHA. However, they are not able to synthesize long-chain ω3 PUFAs. Fish nourish on microorganisms (for instance algae) or on smaller fish that consume

PUFA synthesizing micro-organisms, thence acquiring long-chain ω3 PUFAs (Lunn & Theobald, 2006; Prato & Biandolino, 2012). Hence, the ratios of these ω3 PUFAs in fish muscle are based on nourishment (Sargent et al., 2002) and their changes can be due to differences in the feeding habits of the fish (Norrbin et al. 1990; Prato and Biandolino 2012).

A maximum dietary ratio of ω6/ω3 has been recommended as 4.0 (HMSO 1994). Levels higher than the maximum level are dangerous to human health and cause cardiovascular diseases (Dridi et al. 2017; Köse et al. 2010). In the present study, the ω6/ω3 ratios in sand smelt were in the range of 0.15–0.22 and the ω6/ω3 ratio in this fish species during seasons did not surpass the suggested maximum level. Sand smelt showed the lowest ω6/ω3 (0.15) ratio during spring and summer whereas the highest level of ω6/ω3 (0.22) was in autumn (Table 2). The highest PUFA/SFA ratio (1.47) was obtained from sand smelt in spring although the lowest PUFA/SFA ratio was in autumn for sand smelt (Table 2). The PUFA/SFA proportion is 0.45 which is advised a minimum level of PUFA/SFA proportion (Piretti et al., 1988). Tanakol et al. (1999) reported higher than 1 for sand smelt, from the Black Sea, a PUFA/SFA. In the present study, this proportion did not surpass the suggested minimum ratio for sand smelt during seasons.

Table 2. Seasonal variation on total (%) fatty acid compositions in muscle lipids of the sand smelt from Cekerek (Süreyyabey) Dam.

Fatty acids (%)	Sand smelt (*Atherina boyeri*)		
	Autumn	**Spring**	**Summer**
C12:0	0.08±0.00^{b}	0.03±0.01^{c}	0.10±0.01^{a}
C14:0	2.45±0.09^{b}	2.09±0.19^{c}	3.32±0.11^{a}
C15:0	0.57±0.02^{a}	0.45±0.02^{b}	0.48±0.03^{b}
C16:0	16.19±0.31^{a}	15.91±0.57^{a}	15.76±0.45^{a}
C17:0	0.84±0.03^{a}	0.52±0.01^{b}	0.43±0.02^{c}
C18:0	5.03±0.09^{a}	5.17±0.09^{a}	4.54±0.40^{b}
C20:0	0.18±0.01^{a}	0.13±0.02^{b}	0.05±0.01^{c}
C22:0	0.11±0.03^{a}	0.08±0.03^{a}	-
C24:0	0.23±0.13^{a}	0.24±0.02^{a}	0.21±0.03^{a}
ΣSFA	**25.69±0.39^{a}**	**24.63±0.25^{b}**	**24.89±0.73^{ab}**
C14:1	0.64±0.05^{a}	0.47±0.06^{b}	0.62±0.06^{a}
C15:1	0.27±0.02^{a}	0.18±0.02^{b}	0.20±0.01^{b}
C16:1	7.32±0.37^{b}	5.49±0.54^{c}	8.26±0.23^{a}
C17:1	0.19±0.01^{b}	0.19±0.01^{b}	0.21±0.01^{a}
C18:1ω9	12.60±0.62^{a}	9.72±0.67^{b}	13.20±0.17^{a}
C18:1ω7	4.29±0.10^{a}	3.75±0.11^{b}	3.43±0.13^{c}
C20:1ω9	0.48±0.02^{a}	0.38±0.02^{b}	0.22±0.01^{c}
C22:1ω9	0.62±0.02^{a}	0.57±0.01^{b}	0.11±0.01^{c}
C24:1ω9	0.03±0.00^{b}	-	0.07±0.02^{a}
ΣMUFA	**26.43±0.98^{a}**	**20.75±1.30^{b}**	**26.30±0.07^{a}**
C18:2ω6	3.05±0.03^{a}	2.88±0.10^{b}	2.85±0.05^{b}
C18:3ω6	0.59±0.03^{a}	0.33±0.03^{b}	0.15±0.02^{c}
C18:3ω3	4.81±0.40^{a}	4.31±0.28^{b}	3.19±0.20^{c}
C20:2 cis	0.40±0.01^{a}	0.16±0.01^{c}	0.25±0.01^{b}
C20:3ω6	1.07±0.03^{a}	1.07±0.02^{a}	0.98±0.03^{b}
C20:4ω6	0.23±0.01^{b}	0.29±0.01^{a}	-
C20:5ω3	5.59±0.11^{c}	7.22±0.21^{a}	6.01±0.30^{b}
C22:2 cis	0.21±0.02^{a}	0.10±0.01^{b}	-
C22:6ω3	12.33±0.51^{c}	19.77±1.36^{a}	17.85±0.57^{b}
ΣPUFA	**28.29±0.20^{c}**	**36.11±1.18^{a}**	**31.28±0.27^{b}**
PUFA/SFA	1.10±0.01^{c}	1.47±0.03^{a}	1.26±0.05^{b}
Σω3	22.73±0.24^{c}	31.29±1.32^{a}	27.05±0.24^{b}
Σω6	4.95±0.06^{a}	4.56±0.15^{b}	3.98±0.08^{c}
ω6/ω3	0.22±0.00^{a}	0.15±0.01^{b}	0.15±0.00^{b}
DHA	12.33±0.51^{c}	19.77±1.36^{a}	17.85±0.57^{b}
EPA	5.59±0.11^{c}	7.22±0.21^{a}	6.01±0.30^{b}
DHA/EPA	2.20±0.05^{b}	2.74±0.11^{a}	2.98±0.24^{a}

Data are shown as mean ± standard deviation (SD). SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; DHA: docosahexaenoic acid; EPA:eicoesapentaenoic acid; Σn-6 PUFA: total n-6 polyunsaturated fatty acid; Σn-3 PUFA: total n-3 polyunsaturated fatty acid. Different letters (a-c) in the same row for each fish indicate significant differences in Table 2 ($p<0.05$).

CONCLUSION

This study represented seasonal variability in proximate and FA composition of muscle tissue from economically important sand smelt caught in Cekerek Sureyyabey Dam. To the authors' knowledge, it is the first time that an evaluation of the proximate and fatty acid composition of sand smelt from Cekerek Sureyyabey Dam has been carried out. According to our results, the proximate and FA composition values of sand smelt showed differences during seasons. These variations in fatty acids are due to environmental, other ecological, and morphological influences and also depend on their natural nutrition and feeding habits. Sand smelt gave higher lipid contents in summer. Moreover, the total EPA and DHA (%) value of the fish species was the highest in spring. In addition, sand smelt, the carnivorous fish, gave the higher PUFA value than the content of SFA and MUFA during all seasons and also had the highest total EPA+DHA value (26.99%) and total ω3 value in spring ($p<0.05$). This study exhibit that these fish species are a good source of nutrients and these species seem to be appropriate for the human diet, although there were differences in lipid and fatty acid composition during seasons.

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Acute Toxicity of Zinc on Southern Medicinal Leech, *Hirudo verbana* Carena, 1820

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Research Article

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Abstract

In the present study it was aimed to determine the acute toxicity of zinc on southern medicinal leech *Hirudo verbana* Carena, 1820 that is used in ecotoxicological studies. The experiment was conducted for 96 hours using the semi-static bioassay method with replicate two times. The leeches were exposed to zinc concentrations of 6.25 mg/L, 12.50 mg/L, 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L, respectively. As a result of the experiment, the LC_{50} values for 24, 48, 72, and 96 hours were determined 48.30 mg/L, 19.68 mg/L, 15.48 mg/L, and 14.12 mg/L, respectively. The LT_{50} values for 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L zinc concentrations were determined 38.63 hours, 24.55 hours, 14.62 hours, and 12.78 hours, respectively. Defecation, intensive mucus output, uncontrolled swimming, attempt to escape from the experiment media, reducing the cling power, coiling (buckling), vomiting the digestive blood, the body deformations, penile prolapse, hemorrhage, and abrasion in the wall of the body were the main physiological and behavioral responses in the leeches which were exposed to the different zinc solutions. The data obtained revealed that the zinc sensitivity of *H. verbana* was moderate when compared to other organisms. It is expected that the data of the present study will contribute to the management of endangered medicinal leech populations.

Keywords: heavy metal, bioassay, mortality, LC_{50}, LT_{50}.

Çinkonun Güney Tıbbi Sülüğü *Hirudo verbana* Carena, 1820 Üzerine Akut Toksisitesi

Özet

Bu çalışma ile çinkonun ekotoksikolojik çalışmalarda kullanılmakta olan güney tıbbi sülüğü *Hirudo verbana* Carena, 1820 üzerine akut toksisitesinin belirlenmesi amaçlanmıştır. Deneme 96 saat süreli yarı statik biyodeney yöntemiyle iki tekerrürlü yürütülmüştür. Bu amaçla, sülükler 6,25 mg/L, 12,50 mg/L, 25 mg/L, 50 mg/L, 100 mg/L ve 200 mg/L'lik çinko konsantrasyonlarına maruz bırakılmıştır. Deney ve hesaplamalar sonucunda LC_{50} değerleri 24, 48, 72 ve 96. saatler için sırasıyla 48,30 mg/L, 19,68 mg/L, 15,48 mg/L ve 14,12 mg/L olarak belirlenmiştir. 25 mg/L, 50 mg/L, 100 mg/L ve 200 mg/L konsantrasyonları için belirlenen LT_{50} değerleri sırasıyla 38,63 saat, 24,55 saat, 14,62 saat ve 12,78 saat olarak hesaplanmıştır. Çinkoya maruz kalmış sülüklerde dışkılama, aşırı mukus çıkışı, kontrolsüz yüzme, ortamdan uzaklaşma girişimleri, tutunma gücünün zayıflaması, kıvrılma (burkulma), sindirilmiş kanın kusulması, vücutta oluşan deformasyonlar, erkeklik organının dışa sarkması, hemoraji ve vücut duvarında aşınmalar başlıca fizyolojik ve davranışsal tepkileri oluşturmuştur. Elde edilen veriler, diğer organizmalarla kıyaslandığında *H. verbana* türünün çinko duyarlılığının orta düzeyde olduğunu ortaya koymuştur. Bu çalışmada elde edilen verilerin nesli tehlike altında olan tıbbi sülük popülasyonlarının yönetimine katkı sağlaması beklenmektedir.

Anahtar Kelimeler: ağır metal, biyodeney, mortalite, LC_{50}, LT_{50}.

INTRODUCTION

Medicinal leeches have been used to treat some diseases of both humans and animals in both traditional and modern medicine, as well as in veterinary (Gödekmerdan et al., 2011; Mumcuoglu, 2014; Sobczak and Kantyka, 2014). The enzymes secreted by medicinal leeches are used in the production of some drugs and cosmetic products (Ayhan and Mollahaliloğlu, 2018; Lemke and Vilcinskas, 2020; Shakouri and Wollina, 2021). Medicinal leeches play some important roles in aquatic ecosystems (Ceylan et al., 2015); however, because of over-collection and habitat destruction, medicinal leech populations have been weakening day by day in many wetlands (Sağlam, 2011;

Ceylan and Çetinkaya, 2017, Sağlam, 2018). It is a necessity, therefore, to take protective measures for the sustainable management of medicinal leech populations.

Heavy metals affect both environments and organisms (Mishra et al., 2019) causing toxic effects (Tchounwou et al., 2012). Aquatic toxicology has increasing importance for aquatic ecosystems as well as for all organisms. Research in this field has now become a necessity (Çetinkaya, 2005).

The heavy metals lead to an activity decreases in invertebrates (Wicklum et al., 1997). Zinc, as an essential trace element for all organisms (Eisler, 1993; Salgueiro et al., 2000), is a heavy metal found in the highest concentrations in wastewaters of the majority of the modern industrialized societies (Boardman and McGuire, 1990), and its concentration is increasing due to the effects of both natural and anthropogenic factors (Özkul, 2008). The erosion of the rocks in the earth's crust, the wastewater originated the industrial and urban discharges, and mining activities are the main sources of zinc in natural waters (Sönmez and Çetinkaya, 2003; Kori-Siakpere and Ubogu, 2008). The discharge of high zinc concentrations in aquatic ecosystems causes a decrease in the number of individuals of many species of macro-invertebrates including the leeches (Willis, 1979). Zinc leads the aquatic organisms to death with its cytotoxic effect (Kodama et al., 1982), as well as the acid-base balance and due to the negative effects on the regulation of ions (Senthil Murugan et al., 2008). Because medicinal leeches are among the endangered species (Sağlam, 2011; Sağlam et al., 2018; Ceylan, 2020; Kvist et al., 2020), determining the toxicologic limits becomes important to maintain sustainable management.

The southern medicinal leech, *Hirudo verbana* is used in various toxicological and pharmacological studies due to its sensitivity and easy housing features in laboratory conditions. It, as well as being a sensitive organism for ecotoxicological studies, is being used in the studies carried out in neurobiological, neuroethological, physiological, histological, microbiological fields and in many engineering disciplines (Petrauskienė, 2008a). The chronic lethal effects of zinc, copper, and their mixtures to medicinal leech *Hirudo verbana* were investigated by Petrauskienė (2008b). Although there is data on the chronic lethal effects, the acute toxicity of zinc and on *H. verbana* and the physiological and behavioral changes of the leeches belonging to the *H. verbana* that are exposed to the zinc is not known. It was aimed to reveal these yet unstudied data with the present study. Considering that southern medicinal leech, *H. verbana* is endangered and its populations are weakening day by day in both Europe and Turkey, it is hoped that data obtained will contribute to the management policies of the medicinal leech populations in nature and fill the gap in the literature.

MATERIALS and METHODS

Leeches

The study was conducted using a total of 112 medicinal leeches *Hirudo verbana* Carena 1820 that bred in the medicinal leech production unit of Eğirdir Fisheries Research Institute, Turkey. The bodyweight of leeches was determined as 3.01 ± 0.09 g. The leeches were assigned randomly to the experimental group, and it was confirmed that the weight of leeches in the experimental group was normally distributed ($P > 0.05$).

Toxicity test

$ZnSO_4.7H_2O$ (Merck) was used as the toxicant in the experiment. The toxicity test was conducted using the standard method that was applied by Çetinkaya (2005) and Petrauskienė (2008b). The stock solution was prepared by using distilled water. The Zn^{+2} trial concentrations were prepared in different 7 doses of 0.00 mg/L, 6.25 mg/L, 12.50 mg/L, 25.00 mg/L, 50.00 mg/L, 100.00 mg/L and 200.00 mg/L by taking from the stock solution. The leeches were placed as 8 individuals with two replications into the 5L pet jars, filled with the solutions prepared in the concentrations. The jars were closed to prevent the leeches from moving away from the test environment. The experiment was performed using the semi-static test, the groups of controls and treatment concentrations were renewed at intervals of 24 hours, and the experiment was continued for 96 hours. The reactions of the leeches and mortality were recorded (Çetinkaya, 2005).

The temperature, dissolved oxygen concentration, pH, alkalinity ($CaCO_3$), total water hardness ($CaCO_3$), and electrical conductivity were measured as 19.3 °C, 5.72 mg/L, 7.80, 242 mg/L, 350 mg/L, and 460 µS/cm, respectively.

Statistical analyses

LC_{50} values for 24 hours, 48 hours, 72 hours, and 96 hours and the LT_{50} values for each calculable concentration, within the 95% confidence limits, were determined using the Probit Regression analysis using IBM SPSS Statistics version 25.0 for Windows package software (IBM Corp., Armonk, NY, USA) (Lei and Sun, 2018; Navarrete et al., 2021).

RESULTS

Except for the defecation at the beginning of the experiment, it wasn't observed abnormal behavior, reaction, and death in the leeches of the control group and the 6.25 mg/L concentration during the study. In addition to the defecation, the common responses of the leeches in 12.50 mg/L and the higher concentrations were uncontrolled swimming, excess mucus secretion especially in the suckers (Figure 1 and Figure 2), vomiting blood (Figure 3), weakening of the grip strength in suckers, twisting, the deformations in the body (Figure 4 and Figure 5c), hemorrhage both on and surround of male genital organs and penile prolapse (Figure 6), hemorrhage in different parts of the body (Figure 5a, Figure 6a, Figure 7 and Figure 8), and wearing on the body walls (Figure 5b).

No death has been recorded in control and 6.25 mg/L groups during the experiment. It is observed that all leeches died in 25, 50, 100, and 200 mg/L concentration groups and, while 5 of 16 leeches died in the 12.50 mg/L concentration group.

LC_{50} values for 24 hours, 48 hours, 72 hours and 96 hours were 48.30 mg/L, 19.68 mg/L, 15.48 mg/L and 14.12 mg/L, respectively. It is noted a decline in the LC_{50} values as long as the zinc exposure time is extended. The LC_{50} values and 95% confidence limits determined in the zinc acute toxicity test were presented in Table 1.

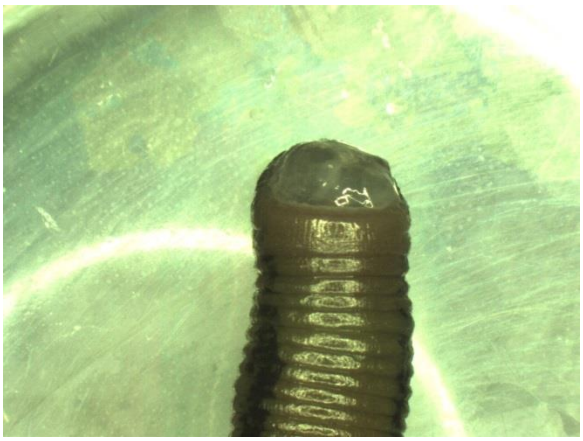

Figure 1. Excessive mucus secretion in the anterior sucker

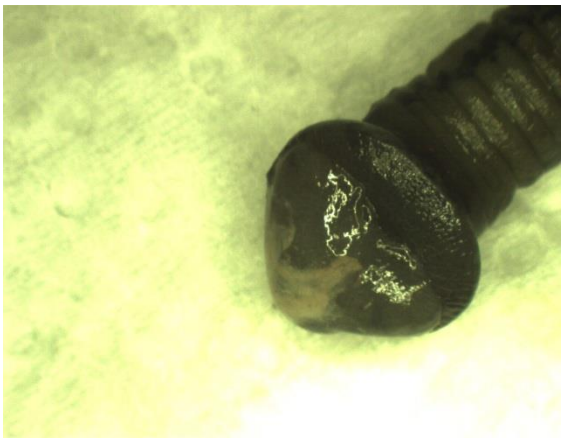

Figure 2. Excessive mucus secretion in the posterior sucker

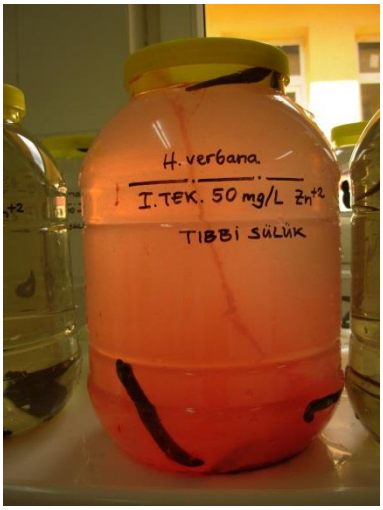

Figure 3. Blood vomiting observed in the 45th minute of the experiment in 50 mg/L Zn^{+2} concentration

Figure 4. Morphological deformation in the anterior part of the leech

Figure 5. Hemorrhage in the ventral region of the leeches (a), wearing on the body wall (b), and deformation occurring in the anterior part (c)

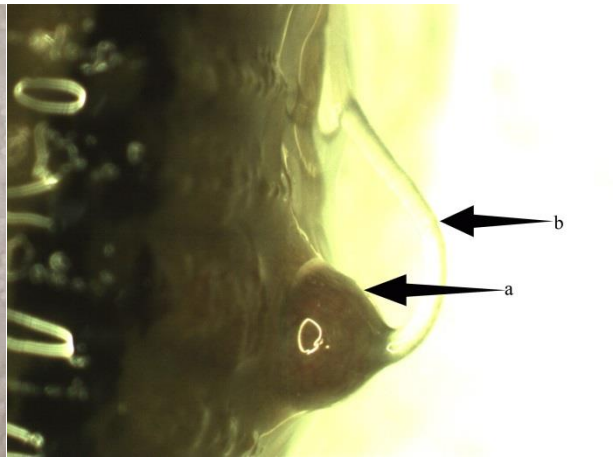

Figure 6. Hemorrhage on and around the male genital organ of *H. verbana* (a) and prolapse of the male genital organ (b)

Figure 7. Hemorrhage on anterior sucker of *H. verbana*

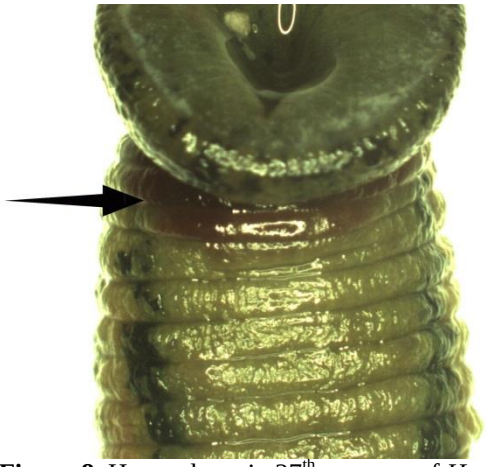

Figure 8. Hemorrhage in 27th segment of *H. verbana*

Table 1. LC_{50} values and 95% confidence limits in zinc acute toxicity test in medicinal leech, *H. verbana*.

Time (hour)	LC_{50} (mg/L)	95% confidence limits (hour)
24	48.30	14.81 - 66.92
48	19.68	14.89 - 22.19
72	15.48	12.86 - 19.15
96	14.12	11.75 - 17.66

It was observed that LT_{50} durations were shortened depending on the increased concentrations. LT_{50} values were determined for 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L as 38.63 hours 24.55 hours 14.62 hours and 12.78, respectively. LT_{50} values and 95% confidence limits for these values are given in Table 2.

Table 2. LT_{50} values and 95% confidence limits determined in zinc acute toxicity test in medicinal leech, *H. verbana.*

Concentrations (mg/L)	LT_{50} (hour)	95% confidence limits (hour)
25	38.63	36.72 - 40.87
50	24.55	23.90 - 25.23
100	14.62	13.68 - 15.78
200	12.78	12.00 - 13:80

DISCUSSION

The leeches were exposed to 0.00 mg/L, 6.25 mg/L 12.50 mg/L, 25.00 mg/L, 50.00 mg/L, 100.00 mg/L and 200.00 mg/L zinc concentrations in the present study. The observed behavioral changes and abnormalities occurring in the bodies and deaths are recorded during this experiment continued for 96 hours.

Petrauskienè (2008b) investigated the chronic effects of zinc, copper, and their mixtures on medicinal leech *H. verbana* and given the LC_{50} values for periods ranging from 48 hours to 30 days. LC_{50} values were found 19.68 mg/L and 14.12 mg/L, respectively for 48 hours and 96 hours in our study, however, in the mentioned study these values were 33.94 mg/L and 15.83 mg/L, respectively. It is thought that the differences might be caused by the water quality of the test solution (ion concentration, pH, water temperature, etc.) and the size of the leeches.

Sağlam and Şahin (2006) investigated the acute toxicity effect of copper sulfate ($CuSO_45H_2O$) on medicinal leech *H. medicinalis*. The LC_{50} values for 12 hours, 24 hours, 48 hours, 72 hours and 96 hours were found to be 0.2093 mg/L, 0.0878 mg/L, 0.0444 mg/L, 0.0261 mg/L and 0.0044 mg/L, respectively. The LT_{50} values for the 0.025, 0.05 and 0.1 mg/L concentrations were found to be 60h:10min, 34h:03min and 24h:15min, respectively. Considering the LT_{50} values in 96 hours, the toxic effects of copper sulfate for medicinal leeches seem to be higher compared to zinc.

According to the previous studies, the LC_{50} values vary over a wide range, the results of the previous acute toxicity studies on some aquatic organisms are presented in Table 3.

Table 3. LC_{50} values in some aquatic organisms regarding zinc toxicity by comparing with the present study.

Species	96 h LC_{50}	48 h LC_{50}	24 h LC_{50}	References
Salmo gairdneri	1.9 - 3.0 mg/L	-	-	Holcombe and Andrew, 1978
Mytilus edulis	20.8 mg/L	-	-	Hietanen et al., 1988
Chalcalburnus tarichi	16.02 mg/L	17.13 mg/L	17.09 mg/L	Sönmez and Çetinkaya, 2003
Capoeta fusca	102.9 mg/L	-	-	Ebrahimpour et al., 2010
Pagrus major	10.1 mg/L	4.3 mg/L	-	Huang et al., 2010
Oreochromis niloticus	72.431 mg/L	-	-	Ezeonyejiaku and Obiakor, 2011
Atherina boyeri	-	-	1.768 mg/L	Bulut et al., 2013
Acipenser transmontanus	0.150 - 0.625 mg/L	-	-	Vardy et al., 2014
Branchiura sowerbyi	0.97 ± 0.07 mg/L	-	-	Lobo et al., 2016
Tubifex tubifex	8.7 ± 0.84 mg/L	-	-	Lobo et al., 2016
Percocypris pingi	2.852 mg/L	2.933 mg/L	3.504 mg/L	Zeng et al., 2018
H. verbana	15.83 mg/L 14.12 mg/L	33.94 mg/L 19.68 mg/L	- 48.30 mg/L	Petrauskienè, 2008b The present study

Regarding the data given in Table 3, *H. verbana* is more resistant to zinc compared to silver fish, rainbow trout, Japanese seabream, white sturgeons, and tubificid worms, which to have the same sensitivity as pearl mullet and seems to be more sensitive compared to other species. This indicates that *H. verbana* has moderate sensitivity to zinc.

The initial responses of the leeches exposed to the zinc were defecation, excessive mucus secretion, and uncontrolled swimming. Defecation was the reaction that occurred in the first 10 minutes of the experiment, whereas uncontrolled swimming and excessive mucus secretion occurred in the first 20 minutes. The mucus secretion in the leeches exposed to the zinc concentration of 200 mg/L was transparent gel consistency; however, turned into red in jelly consistency when the leeches vomit digested blood. The examination was made on the dead leech from an external perspective, after removing them from the zinc solution. It was observed the presence of mucus strongly attached to the anterior and posterior suckers of some leeches (Figure 1 and Figure 2). In case of solution replacement made at 24-hour intervals, certain responses were not observed such as defecation, excessive mucus secretion, and uncontrolled swimming. Petrauskienè (2005) reported that the most sensitive responses of the leeches treated with crude oil were mobility and avoidance from the environment. Sağlam and Şahin (2006) stated that the mucus secretion was more intense in anterior and posterior suckers.

The leeches had difficulty taking hold with their suckers and then fell to the bottom of the jars and began twisting movements in 200 mg/L concentration, especially within 30 minutes at the beginning of the experiment. It was recorded that their twisting movements were intensified on the bottom of the container after 40 minutes of the experiment beginning, and in continuation of this movement the leeches have lost the integrity of their body form and certain permanent deformations have occurred (Figure 4 and Figure 5c). Petrauskienė (2005) being consistent with our observation, reported that the body form of the medicinal leech *H. medicinalis* treated with crude oil changes due to the continuous contraction and relaxation of the muscles in certain segments. The deformation on the body walls of the leeches occurred also in another toxicity study made with copper (Kutlu et al., 2010).

The incidence of hemorrhage in the experiment was listed as follows: the anterior sucker (67%), the ventral region of the body (61%), male genital organs (32%), and posterior sucker (29%). Sağlam and Şahin (2006) reported that the hemorrhage occurred in different body parts of the leeches exposed to copper sulfate.

The toxicological studies performed with leeches became restricted due to the significant decrease in natural leech populations, therefore the cultivated leeches should be used for these studies (Petrauskienė, 2004). In the toxicity studies to be done, it would not be appropriate to use the rare species and reduce the required number of the individual to the minimum level for the other species.

In conclusion, although the concerns are expressed that the short-term heavy metal toxicity experiments will be insufficient to predict the environmental impact on invertebrates (Petrauskienė, 2008b), the low concentrations to be used in the studies to investigate the long-term chronic effects of the toxicant are likely to be inadequate to determine the lethal effects of the discharges of the high concentrations into the receiving environments. It is hoped that the LC_{50} and LT_{50} values, the physiological and behavioral changes defined as toxicity indicators will contribute to the studies to be made with *H. verbana* and zinc and will be useful for establishing standards and implementation of measures for the protection of medicinal leech populations in nature.

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A multi-model analysis of growth and maturity biometrics in common Snakeheads, *Channa striata*, and *Channa punctatus* collected from Hakaluki Haor, Northeast Bangladesh

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Research Article

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Abstract

The Snakeheads are the most common fishes in freshwater wetlands of Bangladesh, but in recent years they are subjected to decline by overfishing pressure, anthropogenic factors, and climate changes. A one-year-long biometric investigation was undertaken which involve monthly random sampling of *Channa striata* and *Channa punctatus* from local wetland and measured total length, body weight data, and gonad's weight in the laboratory. Then data were treated and analyzed by following the different multi-model tools i.e., Fulton's condition factor, relative condition factor, allometric condition factor, and Pearson's correlation coefficient. The results exhibit a strong correlation between the total length and body weight of *Channa striata* and *Channa punctatus* with an r^2 value of 0.826, 0.819, respectively. Pearson's correlation showed a trend of both positive and negative correlation coefficients between different conditions factors and morphometry for both species. The peak value for gonad's weight, gonadosomatic index, and Dobriyal index was reported in the monsoon while the bottom value was in winter for both sexes of both species. The temperature, dissolved O_2, and turbidity were found as fluctuating throughout the season while dissolved NH_3 and pH remained constant. This data will help in the betterment of conservation, management, and fishing policy by the government, scientists, and fisheries industry.

Keywords: Growth biometrics, conditions factors, maturity indices, snakeheads, environmental parameters.

Kuzeydoğu Bangladeş, Hakaluki Haor'dan Toplanan Yılanbaş, Channa striata ve Channa punctatus'ta Büyüme ve Olgunluk Biyometrisinin Çok Modelli Bir Analizi

Yılanbaşlar, Bangladeş'in tatlı su sulak alanlarındaki en yaygın balıklardır, ancak son yıllarda aşırı avlanma baskısı, antropojenik faktörler ve iklim değişiklikleri nedeniyle azalmaya maruz kalmaktadırlar. Yerel sulak alanlardan Channa striata ve Channa punctatus'un aylık rastgele örneklenmesini ve laboratuvarda toplam uzunluk, vücut ağırlığı verileri ve gonad ağırlığının ölçülmesini içeren bir yıllık bir biyometrik araştırma yapılmıştır. Daha sonra veriler, Fulton'un koşul faktörü, göreceli koşul faktörü, allometrik koşul faktörü ve Pearson'ın korelasyon katsayısı gibi farklı çoklu model araçları takip edilerek işlenmiş ve analiz edilmiştir. Sonuçta, Channa striata ve Channa punctatus'un toplam uzunluğu ve vücut ağırlığı arasında sırasıyla 0.826, 0.819 r2 değeriyle güçlü bir korelasyon sergilediği görülmüştür. Pearson korelasyonu, her iki tür için farklı koşullar faktörleri ve morfometri arasında hem pozitif hem de negatif korelasyon katsayıları eğilimi gösterdi. Gonad ağırlığı, gonadosomatik indeks ve Dobriyal indeksi için en yüksek değer musonda rapor edilirken, her iki türün her iki cinsiyeti için de en düşük değer kış mevsiminde olmuştur. Çözünmüş NH3 ve pH sabit kalırken sıcaklık, çözünmüş O2 ve bulanıklık mevsim boyunca dalgalı olarak bulunmuştur. Bu veriler, hükümet, bilim adamları ve balıkçılık endüstrisi tarafından koruma, yönetim ve balıkçılık politikasının iyileştirilmesine yardımcı olacaktır.

Anahtar Kelimeler: Büyüme biyometrisi, kondisyon faktörleri, olgunluk endeksleri, yılanbaşlar, çevresel parametreler

INTRODUCTION

The snakeheads are a very common freshwater fish group that occurred in different small ponds systems, natural hollows, narrow channels, rivers, wetlands waters, and flood plains of Bangladesh (Amzad et al., 2015; Haniffa & Sridhar, 2002; Mian et al., 2020) and among them, *Channa striata* and *Channa punctatus* have drawn the commercial interest due to their good market price, nutrition value and air-breathing adaptability (Graham, 2011; Haniffa et al., 2004; Jannatul et al., 2015). Besides this

they are very precious for their medicinal properties and are believed to assist in healing wounds and compensate for stress associate with surgical pain (Jais et al., 1997; Song et al., 2013). However, their availability tends to be low in recent years due to disease, loss of habitat, and over-exploitation from natural sources (Farhana et al., 2016; Navy et al., 2017).

The Hakaluki Haor is one of the largest wetland water resource systems in Bangladesh (Mohiuddin & Uddin, 2019), which receives water flow from Kusiyara River (Chowdhury et al., 2019; Hossain et al., 2017). The Haor fisheries are considered one of the diverse freshwater resources in Bangladesh and provide a large catch of native fishes and other aquatic resources (Islam et al., 2018). The resources allocated within the Hoar wetland provide both nutritional, economic, and ecological services to the nearby community (Islam et al., 2011). The Haor fisheries are now drawing attention to policymakers and biologists due to their key role in conserving biodiversity and managing stock for the different endangered aquatic organisms (Haque & Basak, 2017).

Biometrics data carry key information regarding the growth and population structure of fish, which would be used in future conservation, management, and adopting policies for harvesting (Hossain et al., 2016; Muchlisin et al., 2010). The different qualitative and quantitative body metrics provide an actual measurement of stock assessment and recruiting success (Felizardo et al., 2015; Lucifora et al., 1999; Richter, 2007). The total length and body weight are extensively used in the growth biometry of fish species (Alves et al., 2018; Emre, 2020; Hajjej et al., 2011; Okgerman, 2005; Richter, 2007; Tharwat et al., 2018), and are drawn interest to ichthyologist for easy acquisition without losing the stock and for the different available model to analyse them (Binohlan & Froese, 2009; Le Cren, 1951). Therefore, current research work had been undertaken to investigate the length weight relationship, gonadosomatic index and Dobriyal index of C. striata and C. punctatus in the greater Sylhet region to assess their biological condition, stock status, and population structure.

MATERIALS AND METHODS

Both *C. striata* and *C. punctatus* were collected as live from artisanal fishermen on site (Figure 1) monthly and they had been bringing back to the wet laboratory of the Fish Biology and Genetics department, Sylhet Agricultural University. The environmental parameters of each site were measured by using the YSI professional multi-probe and manufactured ammonia test kit (Model HI 3824, Japan) simultaneously. However, turbidity was measured in cm using the conventional Secchi Disk method. A total of random 288 individual fish of both species were used for the current study. They were cleaned very well by using fresh tape water and then put on an anesthetics solution to slow down their locomotion and anxiety. Finally, they were placed on a paper towel to remove access to water for better weight measurement. The total length data were obtained by using a cm-scale designed in a wooden structure for quick measurement and weight data have been estimated by using an electric balance (China Electronic Analytical Balance, Model: FA2204, Made in China) with two decimals readings. Finally, the weight of gonads and sexual status was assessed as well followed by sacrificing all sampled fish. Sample from each gonad was put on glass slide and instantly stained with aceto-carmine to detect cells under microscope.

Fulton's condition factor (K_F) was calculated from the total length and weight data set by following the formula $K_F = 100 \times (W/L^3)$, cm (Htun-Han, 1978), where W is the bodyweight of fish in g, L is the total length of fish in cm. The same data set were used to measure the allometric condition factor (K_A) following the below formula $K_A = W/L^b$ *(Caspers, 1969),* where *W* is the bodyweight of fish in g, *L* is the total length of fish in cm. Again, the relative condition factor (K_R) was calculated from the above data by adopting the below measurement, $K_R = W/(a \times L^b)$ (Le Cren, 1951), where *W* is the body weight in g and *L* is the total length in cm. The values for a and b were calculated by establishing length and weight data in regression parameters. The sexual maturation status of fishes was explored by calculating the following indices: Gonadosomatic index (GSI) = (GW/BW) ×100 (Cone, 1989) and Dobriyal index (DI) = $\sqrt[3]{GW}$ (Dobriyal et al., 1999), where GW is the gonad's weight and BW is the body weight in g.

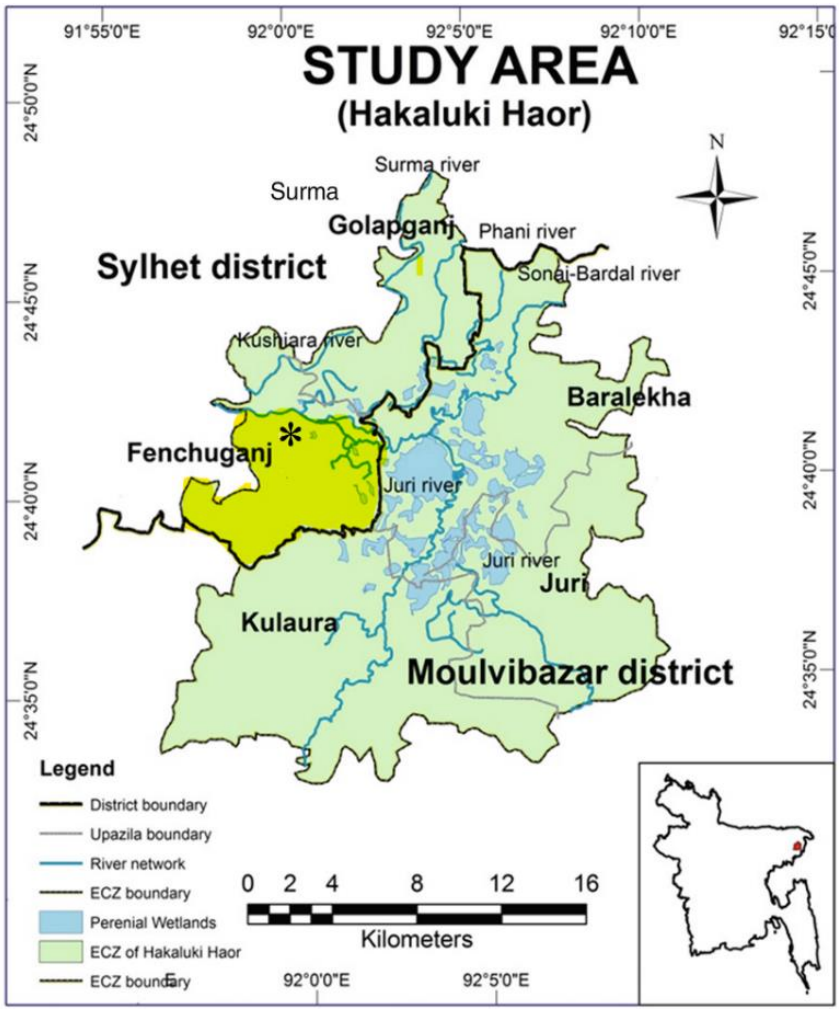


Figure 1. The location of the study site (*) at Sylhet, Northeast Bangladesh (Map modified from Islam et al., 2018)

All analysis, regression relationship, and descriptive statistics were conducted in IBM SPSS v26 at 5 % of the significant level ($P < 0.05$). The means values were justified by Duncan's analysis in comparing the subset on ANOVA at $P < 0.05$.

RESULTS

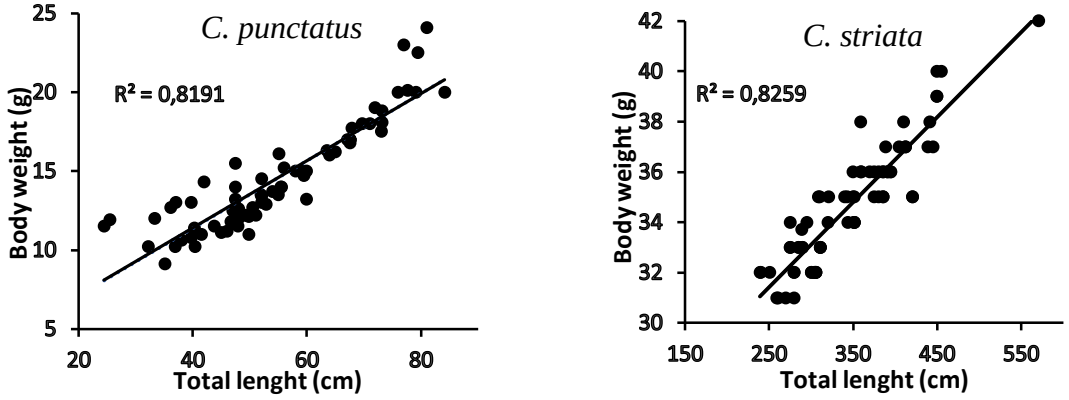
The temperature, turbidity, and dissolved O_2 were revealed as the most fluctuating environmental parameters in Halaluki Haor, while NH_3 and pH were constant over the year (Table 1). The temperature and turbidity were accounted as highest in monsoon (27.73±0.69^{0}C and 29.56±1.98 cm), while the lowest in winter (20.63±1.93^{0}C and 19.20±1.47 cm) (Table 1). The level of dissolved O_2 ranged between 5.33-7.36 mg/L with a peak on monsoon (7.36±0.41 mg/L) and a bottom in winter (5.53±0.25 mg/L) (Table 1).

Table 1. Seasonal variation on the environmental parameters at Hakaluki Haor, Northeast Bangladesh

	Water temperature (^{0}C)	Turbidity (Sechi's disk in cm)	pH	Dissolved O_2 (mg/L)	NH_3 (mg/L)
Winter	20.63±1.93^a	19.20±1.47^a	7.05±0.21^a	5.53±0.25^a	0.060±0.032^a
Pre-monsoon	24.45±1.62^b	27.87±2.04^b	7.44±0.67^a	6.00±0.33^a	0.073±0.022^a
Rainy/monsoon	27.73±0.69^b	29.56±1.98^b	7.31±0.24^a	7.36±0.41^b	0.087±0.007^a
Post-monsoon	24.90±2.18^c	18.98±2.42^a	7.20±0.05^a	5.84±0.57^a	0.083±0.010^a

The linear regression plots of length-weight data showed a positive correlation coefficient of 0.819 and 0.826 for *C. punctatus* and *C. striata* and respectively (Figure 2.A-B). The figure represented the

discrete nature of the sample's total length and body weight throughout the following season (Figure 2.A-B).



A B

Figure 2. Linear regression between and body weight (g) and total length (cm) of *C. punctatus* and *C. striata* collected from Hakaluki Haor, Northeast Bangladesh

The descriptive analysis of data showed that the body weight and total length of *C. punctatus* vary between 24.50-84.20 g and 9.10-24.20 cm ($p<0.05$) (Table 2). Again, the total length and body weight of *C. striata* ranged between 31-42 cm and with a mean of 239-571 g respectively ($p<0.05$) (Table 2). The 95 % credible interval has been calculated for each species. The length and weight metrics for both species show a significant variation between the different seasons and the highest total length and body weight for *C. punctatus* have been recorded in the rainy season as18.10±3.06 cm and 67.82±10.80 g respectively ($p<0.05$). On the other hand, the highest value for the above metrics observed in the pre-monsoon season was 366.83±54.59 g for body weight and as 35.67±2.28 cm for total length (Table 2). The lowest total length value was reported in winter as 12.36±1.68 cm for *C. punctatus* and as 34.22±3.15 cm for *C. striata* (Table 2). The lowest body weight value recorded in winter was 43.07±10.13g for *C. punctatus* while 328.00±49.79g in post-monsoon for *C. striata* (Table 2).

Table 2. Descriptive statistics for the seasonal variation in total length (cm) and body weight (g) data in *C. punctatus* and *C. striata.*

Species		Season	Mean±SD	95% Credible interval	Minimum	Maximum
Channa punctatus	Body weight (g)	Winter	43.07±10.13^{a}	38.03-48.10	24.50	67.50
		Pre-monsoon	54.09±11.70^{b}	48.28-59.91	33.30	73.10
		Rainy/monsoon	67.82±10.80^{c}	62.45-73.19	47.50	81.00
		Post-monsoon	53.99±12.11^{b}	47.97-60.01	35.10	84.20
	Total length (cm)	Winter	12.36±1.68^{a}	11.53-13.20	10.20	17.00
		Pre-monsoon	14.05±2.52^{a}	12.80-15.31	11.00	18.80
		Rainy/monsoon	18.10±3.06^{b}	16.58-19.62	13.50	24.10
		Post-monsoon	13.64±2.79^{a}	12.25-15.03	9.10	20.00
Channa striata	Body weight (g)	Winter	353.22±84.93^{c}	310.99-395.45	259.00	571.00
		Pre-monsoon	366.83±54.59^{d}	339.69-393.98	275.00	455.00
		Rainy/monsoon	341.61±60.51^{b}	311.52-371.70	239.00	450.00
		Post-monsoon	328.00±49.79^{a}	303.24-352.76	240.00	410.00
	Total length (cm)	Winter	34.22±3.15^{a}	32.65-35.79	31.00	42.00
		Pre-monsoon	35.67±2.28^{a}	34.54-36.80	32.00	40.00
		Rainy/monsoon	34.28±2.11^{a}	33.23-35.33	32.00	39.00
		Post-monsoon	34.71±1.68^{a}	33.87-35.54	32.00	38.00

The value of a parameter from total length and body weight data has been accounted for as 0.022 for *C. punctatus* in contrast to 0.031 for *C. striata*. The b value was located at 2.88 for *C. punctatus* and 2.3 for *C. striata* (Table 3).

Table 3. Descriptive statistics for a and b value from $W = a \times TL^{b}$ equation for *C. punctatus* and *C. striata* collected from natural wetlands of Sylhet, Northeast Bangladesh.

	a		b		r^2
	Value	95% Credible interval	Value	95% Credible interval	
Channa punctatus	0.022	0.09-0.02	2.88	1.71-3.08	0.819
Channa striata	0.031	0.012-0.0281	2.3	1.96-2.49	0.826

The mean value of Fulton's condition factor, reative condition facotrs and allometrics condition facotrs were accounted as 2.03 ±0.85, 0.13 ± 0.05 and 0.028± 0.01 respectively for *C. puncatus* respectively ($p<0.05$), while 0.83± 0.07, 3.28±0.25 and 0.09 ± 0.007 respectively for *C. striata* ($p<0.05$) (Table 4).

Table 4. Descriptive statistics for different condition factors in *C. punctatus* and *C. striata*.

	N	Minimum	Maximum	Mean ± SD	95% Credible interval
Channa punctatus					
Fulton's condition factor		0.578	4.66	2.03 ±0.85	1.94-2.11
Relative condition factor	144	0.039	0.27	0.13 ± 0.05	0.046-0.214
Allometric condition factor		0.008	0.061	0.028± 0.01	-0.0564-0.111
Channa striata					
Fulton's condition factor		0.654	0.981	0.83± 0.07	0.741-0.909
Relative condition factor	144	2.75	3.94	3.28±0.25	3.20-3.37
Allometric condition factor		0.083	0.118	0.09 ± 0.007	0.016-0.183

Pearson's correlation between the biometrics and condition factors was found to be significant for *C. punctatus* ($p<0.01$). The total length and body weight showed negative Pearson's correlation with condition factors accounting as r value of -0.87, -0.87, -0.87, -0.68, -0.67, and -0.67 for Fulton's condition factor, relative condition factor, and allometric condition factor respectively ($p<0.01$) (Table 5). Again, in the case of *C. striata* the total length was positively correlated with relative condition factor (0.48) and allometric condition factor (0.48), while body weight was found to be negatively correlated with Fulton's condition factor (-0.45) ($p<0.01$) (Table 5).

Table 5. Pearson's correlation between biometrics and different condition factors of *C. punctatus* and *C. striata*.

	Total length	Body weight	Fulton's condition factor	Relative condition factor	Allometric condition factor
Channa punctatus					
Total length	1	0.90**	-0.87**	-0.87**	-0.87**
Body weight	0.90**	1	-0.68**	-0.67**	-0.67**
Fulton's condition factor	-0.87**	-0.68**	1	1**	1**
Relative condition factor	-0.87**	-0.67**	1**	1	1**
Allometric condition factor	-0.87**	-0.67**	1**	1**	1
Channa striata					
Total length	1	0.90**	-0.04	0.48**	0.48**
Body weight	0.90**	1	-0.45**	0.09	0.09
Fulton's condition factor	-0.04	-0.45**	1	0.84**	0.84**
Relative condition factor	0.48**	0.09	0.84**	1	1**
Allometric condition factor	0.48**	0.09	0.84**	1**	1

** Correlation is significant at the 0.01 level (2-tailed)

The weight of gonads has been reported as highest during the monsoon for both sexes in *C. striata* and *C. punctatus*. The highest gonadal weight for male *C. punctatus* was reported as 1.02 ±0.24g while the female was 3.33 ±0.44g. On the other hand, the highest gonad's weight was 2.37 ± 0.23g in male *C. striata* and 17.70 ±0.94g in female. The winter was accounted for as the lowest stage of gonads weight for the male and female individuals of both species (P<0.05) (Figure 3).

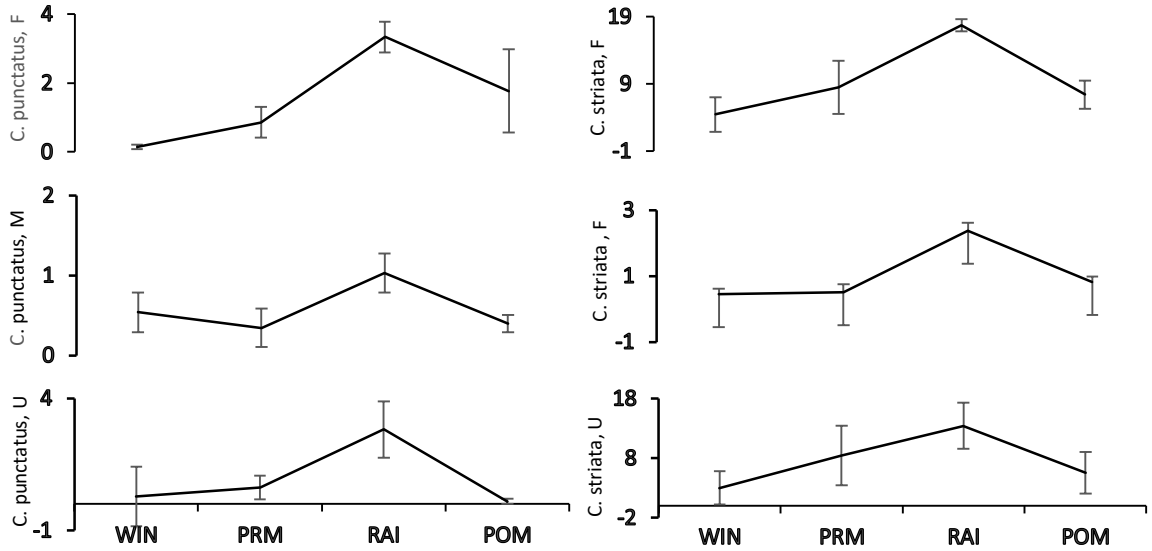


Figure 3. Monthly changes in gonad weights of *C. punctatus* and *C. striata* (M-Male, F-Female, Combined sex, WIN-winter, PRM=pre-monsoon, RAI-rainy/monsoon, and POM-post-monsoon).

Again, the value of GSI and DI were reported as maximum during the monsoon season and lowest during the winter season for the male and female individuals of both species (p<0.05) (Figure 4, 5). The trend of increasing GSI and DI indices afterward the winter indicated the maturation phases of gonads for the breeding at monsoon season.

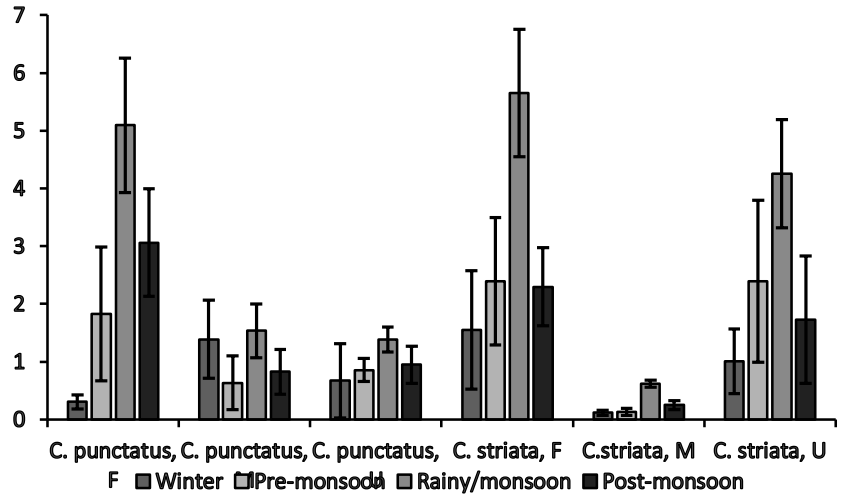


Figure 4. Monthly changes in gonadosomatic index of *C. punctatus* and *C. striata* (M-Male, F-Female, U-Combined sex).

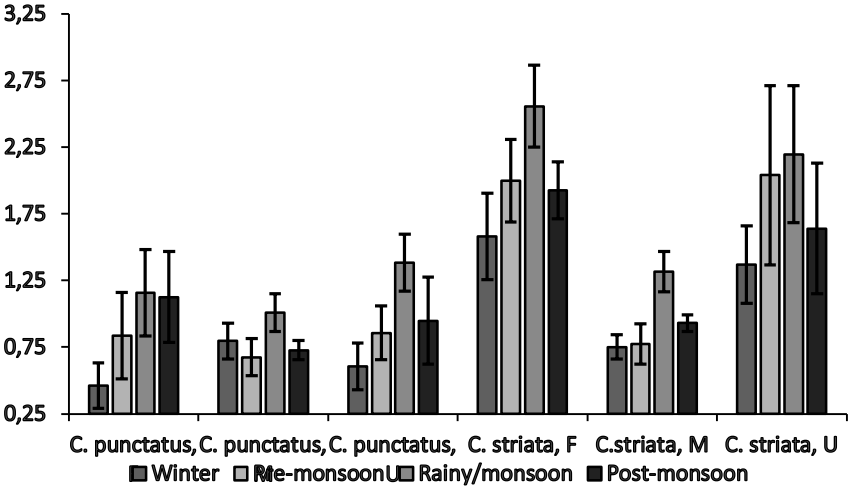


Figure 5. Monthly changes in Dobriyal index of *C. punctatus* and *C. striata* (M-Male, F-Female, U-Combined sex).

The body weight and total length of female *C. punctatus* showed a positive Pearson's correlation with gonad's weight (0.59 and 0.63) ($p<0.05$), gonadosomatic index (0.38 and 0.32) ($P<0.01$), and Dobriyal index (0.37 and 0.37) ($P<0.01$) (Table 5). Similarly, the correlation between biometrics of female *C. striata* showed a positive correlation with gonad's weight (0.48 and 0.51) ($p<0.05$), in contrast, male *C. striata* showed correlation with gonadosomatic index (0.34 and 0.38) ($P<0.01$) (Table 6). Other correlations remained non-significant at $P<0.01$.

Table 6. Pearson's Correlation of body weight and total length with the different maturity indices of *C. punctatus* and *C. striata*

	Body weight vs Gonad's weight		**Total length vs Gonad's weight**		**Body weight vs Gonado-somatic index**		**Total length vs Gonado-somatic index**		**Body weight *vs* Dobriyal index**		**Total length *vs* Dobriyal index**	
	M	F	M	F	M	F	M	F	M	F	M	F
CP	0.18	0.59*	0.19	0.63*	0.02	0.38**	0.08	0.32**	0.03	0.37**	0.07	0.37**
CS	0.15	0.48*	0.11	0.51*	0.34**	0.01	0.38**	0.11	0.09	0.00	0.11	0.15

*Correlation is significant at the 0.05 level (2-tailed) **Correlation is significant at the 0.01 level (2-tailed), CP= *C. punctatus*, CS= *C. striata*, M=Male, F=Female

DISCUSSION

The physicochemical parameters of water are key governing factors in determining optimal fish growth (Hasan et al., 2019; Martínez Cruz et al., 2012; Shahnawaz et al., 2010). Previous research on the same site recorded a mean value of water temperature between 21-28°C, Sechi's disc turbidity reading as 19-30 cm pH, dissolved O_2 and NH_3 have fluctuated as 6.99-7.48, 5.33-5.98 mg/L, and 0.009-0.016 mg/L at Hakaluki Haor, Northeast Bangladesh (Hossain & Rabby, 2020). While water temperature of 24-27^{O}C, p^{H} 5.9-7.1, dissolved O_2 4.6 - 5.6 mg/L, NH_3 0.9-2.18 mg/L have been observed by Islam et al., (2014). The t water quality attributed obtained in current research fall within the suitable range to support fish growth and support to previously available literature as well.

A strong correlation between length and weight metrics already have been well documented for several finfish species (Ferdaushy & Alam, 2015; Jabed et al., 2020; Rahman et al., 2020; Uddin et al., 2017). A study from Jannatul et al., (2015) reported r^2=0.87 for *C. striata*, Mian et al., (2020) obtinaed a r^2=0.96 for *C. punctatus* collected from wild sources of northeast Bangladesh. Additionally, different former studies on snakeheads also revealed a moderately robust correlation between their major growth metrics (Amzad et al., 2015; Haniffa & Sridhar, 2002; Kapil et al., 2011; Sohel et al., 2017).

The value of 'a' parameter from total length and body weight data has been accounted for as 0.022 for *C. punctatus* in contrast to 0.031 for *C. striata* ($p<0.05$). Parameter b (slope) determines the allometric or isometric growth rate, which depends on the genetically determined effects, and if it remains constant and supervises the assumption of values similar to or equal to 3.0, this means that the entity, along with ontogenetic growth, remains unchanged in form. The 'b' value was calculated 2.88 for *C. punctatus* and 2.3 for *C. striata* when the expected range is between 2.5 and 3.5 (Islam et al., 2017; Singh & Serajuddin, 2017). The condition factor is the perfect indicator of relations between biotic and abiotic factors and physiology of fish in a particular environment (Golam Mortuza & Al-Misned, 2013; Mozsár et al., 2015; Okgerman, 2005). The value of Fulton's condition factor was accounted for between 1.614-1.863 (Singh & Serajuddin, 2017), 1.094-1.235 (Datta et al., 2013) for *C. punctatus* in Indian water bodies. While this value for *C. striata* ranged between 1.50-1.49 in Philippines (Dumalagan et al., 2017), 1.02-1.22 in West Bengal, India (Chakraborty et al., 2017). Current findings are also aligned to the previous research, although a few deviations are arising due to geographical position and recent environmental features.

The climbed-up trend of ovarian masses during the premonsoon and monsoon season refers to the procession of physiology toward preparing for the breeding cycle. The fluctuating growth of ovarian weight throughout the different seasons for both species also revealed potentiality of several breeding peaks throughout the year (Jabed et al., 2020; Mian et al., 2017). The peak in the rainy season refers to the onset of breeding activities and sudden dropped following after this indicate spent or spawned ovary. Several previous studies reported *C. punctatus* and *C. striata* as multi-peak spawner (Amzad et al., 2015; Ghaedi et al., 2013; Haniffa & Sridhar, 2002; Jannatul et al., 2015; Mian et al., 2020).

Dobriyal (DI) indices are now being considered as a better indicator of spawning season other than using fish weight-based indices (Tahami et al., 2018). The value Dobriyal (DI) is thought to be more environmental specific which represents the health condition, availability of food, and physiological supports (Abedi et al., 2011; Faghani Langroudi & Mousavi Sabet, 2018). Again, gonadosmomatic (GSI) indices are a numerical indicator of spawning season solely based on body weight (Mian et al., 2020; Nunes et al., 2011). Both indices were quite a at peak during the rainy season for both species, which is strongly supported by the previous research for *C. punctatus* (Amzad et al., 2015; Sohel et al., 2017) and *C. punctarus* (Ghaedi et al., 2013; Jannatul et al., 2015) from tropical waters.

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İmidacloprid'e Maruz Bırakılan Sazan (*Cyprinus carpio*) Yavrularının Bazı Biyokimyasal Özelliklerindeki Değişimler

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Özet

Bu çalışmada imidacloprid'in sazan yavrularının bazı biyokimyasal parametrelerine olan toksik etkisinin belirlenmesi amaçlanmaktadır. Araştırmada ortalama ağırlığı 0,34±0,03 g ve 2,97±0,21 cm boyunda olan sazan yavruları kullanıldı. Balıklar, imidacloprid'in 2,8 ve 5,6 mg/L konsantrasyonuna 4 ve 7 gün için maruz bırakıldı. Süre sonunda balıkların tüm vücudunda lipit, protein ve glikojen içeriği belirlendi. Test gruplarında çalışma sonuna kadar ölüm gözlenmedi. İmidacloprid uygulanan balıklarda protein ve glikojen miktarlarında önemli derecede azalma ($p<0,05$) olduğu tespit edildi. Toplam lipit düzeyi, 7. günde 5,6 mg/L imidacloprid uygulanan grupta yüksek olduğu görüldü. Ancak bu değerin kontrol grubundan istatistiki olarak farklı olmadığı tespit edildi. Çalışmada elde edilen veriler, imidacloprid'in balıkların biyokimyasal parametrelerine toksik etki gösterdiğinin bir kanıtı olarak düşünülebilir.

Anahtar Kelimeler: Imidacloprid, *Cyprinus carpio*, glikojen, toplam protein, toplam lipit

Changes in the Some Biochemical Constituents in Fry of Common Carp (*Cyprinus carpio)* Exposed to Imidacloprid

Abstract

This study was conducted to investigate the possible toxic effects of imidacloprid biochemical parameters of carp. The fish (weight 0,34±0,03 g, total length 2,97±0,21 cm) were subjected to 2,8 and 5,6 mg L^{-1} of Imidacloprid concentration for 4 and 7 days. Alterations were observed in glycogene, total protein, and total fat activities in the whole body of carp. No mortalities occurred in any group during the test. The glycogene and total protein levels in imidacloprid treated fishes were significantly ($p<0,05$) lower than the control in all the concentrations. Total fat levels were higher in imidacloprid treatment groups, which were not significantly different. This study was conducted to investigate the possible toxic effects of imidacloprid biochemical parameters of carp.

Keywords: Imidacloprid, carp, *Cyprinus carpio*, glycogene, total protein, fat

GİRİŞ

Son yarım asırlık süre içerisinde özellikle modern tarımsal uygulamalarda çeşitli hastalık ve zararlılara karşı kimyasal ilaçların kullanılmaktadır. Bu ilaçlar pratik, kolay ve etkili bir yöntem olduğu için kullanımı sürekli olarak artmaktadır (Göktürk, 2007; Tiryaki vd., 2010; Gül, 2017). İstenmeyen organizmaları yok etmek için zirai mücadelede kullanılan pestisitler suda, atmosferde ve toprakta birikerek çevre kirliliğine neden olmaktadır (İnce ve Bekbölet, 1991; MacMahon, 1994).

Pestisitler tüm canlılarda özelliklede insanlar da kronik ve akut zehirlenmelere, enzim faaliyetlerinde bozulmalara, sinir sisteminde tahribata, hücre membran yapısında değişmelere neden olmaktadır (Çakır ve Yamanel, 2005). Bu tür kimyasalların bilinçsiz ve aşırı kullanımı zararlı populasyonlarında bağışıklık mekanizmasının gelişmesine veya çevreye faydalı birçok türün yok olmasına neden olmaktadır (Yıldız vd., 2005). Bunlara ilaveten üremeyle ilgili bozukluklar, enzim aktivitesi üzerindeki değişiklikler, beslenme alışkanlıkları ve beslenme ile ilgili anormallikler, davranışlarda ve algılamada eksiklik gibi birtakım değişikler görülmektedir (Kitchin, 1984).

Neonikotinoid bileşenler sınıfında olan Imidacloprid bir pestisittir (El-Gendy vd., 2010). Dünya üzerinde insektisit olarak hızla artan bir kullanıma sahiptir (Liu vd., 2010). Toprak içerisinde birikim etkisi ve hareket etme özelliğine sahip olup, 48-190 gün arasında yarılanma ömürleri vardır (Andaç, 2015). Yarılanma ömrü suyun 5,7 ve 9 pH'da 31 günden daha fazladır. İmidacloprid balıklarda toksit etkisi diğer canlılara göre kısmen düşüktür. Arılar, toprak böcekleri, beyaz sinekler, termitler gibi emicilerin kontrolü amacıyla İmidacloprid tohum, toprakta ve ağaç yapraklarında kullanılırlar (Buckingham vd., 1997).

Imidacloprid bir nörotoksin olup asetilkolin reseptörlerini bloke etme özelliğine sahiptir. Sucul ekosistemlere tarım arazilerinde akan sularla kontamine olarak kirletmektedir (Morrissey vd., 2015; Bonmatin vd., 2015; Karahan vd., 2017).

Imidacloprid ile yapılan akut ve kronik çalışmalar, bu pestisitin hayvan dokularında oksidatif ve biyokimyasal yolları etkileyebileceğini ve önemli zararlı etkileri olduğunu göstermiştir (Tomizawa ve Casida, 2005). Ancak, imidacloprid'in balıklardaki etkisi araştırılmış ve tam olarak ortaya konulamamıştır (Rajput vd., 2012; Desai ve Parikh 2013; Iturburu vd., 2017). İmidacloprid'in, sazan yavrularında lipit, protein ve glikojen düzeyine etkisi hakkında yayınlanmış herhangi bir rapor bulunmamaktadır. Bu çalışmada imidacloprid'in sazan yavrularının bazı biyokimyasal parametrelerine etkisi araştırılmıştır.

MATERYAL ve YÖNTEM

Balık ve deneysel plan

Sazan, *Cyprinus carpio,* (ortalama ağırlığı 0,34±0,03 g), Devlet Su İşleri 9. Bölge Müdürlüğü Keban Balık Üretimi Şube Müdürlüğünden temin edildi. Ortam şartlarına adaptasyonlarının sağlanması için 2 hafta beklenilmiştir. Cam akvaryumlara konan balıklara bir hava pompası vasıtasıyla sürekli olarak hava verilmiştir. Balıklar ticari bir balık yemi (% 40 protein, % 11 yağ, % 10,1 ham kül, % 1,3 ham selüloz, % 2,4 kalsiyum, % 1,5 fosfor, % 0,3 sodyum içeren 0,6 NM granül yavru yemi) ile günlük olarak beslenmiştir. Çözünmüş oksijen, sıcaklık, pH, amonyum ve nitrit düzeyleri çalışma boyunca kontrol edilerek kabul edilebilir değerler arasında olması sağlanmıştır.

Balıklar her grupta 25 balık olacak şekilde 3 gruba ayrılmıştır. Birinci gruba, kontrol grubu olarak herhangi bir işlem yapılmamıştır. Diğer iki gruba ise sırasıyla 2,8 ve 5,6 mg/L imidacloprid (Tyor ve

Harkrishan 2016) ilave edilmiştir. Balıklar 96 saat ve 168 saat (7 gün) (OECD, 2019) için bu konsantrasyonlara maruz bırakılmıştır. Deney iki tekerrür olacak şekilde yapılmıştır. Her grupta 25 balık olmak üzere toplamda 150 balık kullanılmıştır. Deney süresi sonrasında balıklar anestezik bir madde (50 mg/L, benzokain) (Hseu ve vd., 1998) ile anestezi yapılmış ve tüm vücut alüminyum folyolar içinde -20 ^{o}C'de biyokimyasal işlemler yapılana kadar muhafaza edilmiştir. Balık kullanımı ve deneysel protokol KSÜZİRHADYEK Hayvan Etik Kurulu tarafından onaylanmıştır (Protokol no: 2017 /01).

Biyokimyasal testler

Toplam protein tayini Plummer (1971)'e göre %10 trikloroasetik asitile homojenize edilen tüm vücut dokusu gerekli işlemler yapıldıktan sonra Folin-phenolreagent kullanarak Lowry vd. (1951)'nın bildirdiği metoda göre gerçekleştirilmiştir.

Tüm vücut lipit düzeyi Folch vd. (1957)'nın bildirdiği kloroform: metanol tekniğine göre yapılmıştır.

Glikojen düzeyi ise Nicholas vd. (1956)'na göre antron kullanarak bir UV-Vis spektrofotometre kullanarak 620 nm'de glukoz standardına karşı tespit edilmiştir.

İstatistik analiz

Gruplar arası farklılıkları ortaya koymak için parametrik testler kullanılmıştır. Duncan testi ve tek yönlü varyans analizi (ANOVA) ile ortalama değerler arasındaki farklılıklar karşılaştırılmıştır. Çalışma gruplarına ait verilerin ortalama ve standart hatası (Ortalama±S.Hata) belirlenmiştir.

BULGULAR

Kontrol ve deneysel imidacloprid uygulanan gruplardaki balıklarda herhangi bir fiziksel deformasyon, beslenme ve yüzme davranışlarında değişim ve ölüm kaydedilmemiştir.

İmidacloprid uygulanan sazan yavrularının tüm vücut dokusunda toplam protein aktivitesinde, doza bağlı olarak önemli derecede azalma ($p<0,05$) olduğu tespit edildi (Tablo 1 ve Tablo 2). Toplam

protein düzeyi her iki deneysel grupta uygulamanın 4. gününde kontrol grubuna göre hafif bir azalma göstermişken 7. günde bu azalmanın istatistiki olarak anlamlı olduğu görülmüştür.

Toplam lipit düzeyinde ise istatistiki bir değişimin olmadığı ortaya konulmuştur (p>0,05) (Tablo1 ve Tablo 2).

İmidacloprid'in 5,6 mg/L'lik dozunun uygulandığı grupta glikojen içeriliğinin, özellikle uygulamanın 7. gününde kontrol ve diğer imidacloprid grubuna göre önemli derecede bir azalmanın (p<0,05) olduğu tespit edilmiştir (Tablo1 ve Tablo 2).

Tablo 1. Sazan yavrularının toplam protein, lipit ve glikojen içeriğine 4. gün imidacloprid dozlarının etkisi

	4. gün		
	Kontrol	**2,8 mg/L**	**5,6 mg/L**
Protein (mg/g)	5,92±0,13[a]	5,78±0,14[a]	4,71±0,12[b]
Lipid (mg/g)	0,79±0,12	0,74±0,11	0,81±0,14
Glikojen (µg/g)	3,73±0,3	3,70±0,5	2,70±0,4

X±SH: Aritmetik ortalama; ±: Standart hatası; [a,b]: Gruplar arasındaki fark

Tablo 2. Sazan yavrularının toplam protein, lipit ve glikojen içeriğine 7. gün imidacloprid dozlarının etkisi

	7. gün		
	Kontrol	**2,8 mg/L**	**5,6 mg/L**
Protein (mg/g)	5,88±0,11[a]	4,89±0,08[b]	4,01±0,11[c]
Lipid (mg/g)	0,82±0,11	0,77±0,12	0,78±0,12
Glikojen (µg/g)	3,71±0,4[a]	3,43±0,4[ab]	2,49±0,3[b]

X±SH: Aritmetik ortalama; ±: Standart hatası; [a,b,c]: Gruplar arasındaki fark

TARTIŞMA ve SONUÇ

Bu çalışmada; İmidacloprid'in 2,8 ve 5,6 mg/L konsantrasyonuna 4 ve 7 gün süre ile maruz bırakılan sazan (*Cyprinus carpio*) yavrularının bazı biyokimyasal parametrelerine olan toksik etkisi araştırılmıştır. Toplam protein (mg/mL), lipit (mg/g) ve glikojen (µg/g) değerleri incelenmiştir.

Inyang vd. (2018) tarafında yapılan çalışmada İmidacloprid'e maruz bırakıltığı *Heterobranchus bidorsalis*'in bazı metabolitler (albümin ve toplam protein) ve elektrolitler (Na +, K +, Ca_2 +) üzerindeki etkisini araştırmıştır. Otuz beş yetişkin *Heterobranchus bidorsalis* (ortalama uzunluk, 22,43 ± 2,42cm; ortalama ağırlık, 166,70 ± 0,33 g), 14 gün boyunca 0,28, 0,42 ve 0,56 mg/L imidacloprid konsantrasyonlarına maruz bırakmıştır. Toplam protein 0.00 mg/L'de 5,00 µg/L, 0,28 mg/L'de 10,25 µg/L, 0,42 mg/L'de 20,50 µg/L ve 0,56 mg/l'de 33,50µgl-1 olarak ölçmüştür. Kontrol grubuna göre deney gruplarının karaciğerde önemli derecede toplam protein artmıştır (p<0,05). Bu çalışmada ise İmidacloprid uygulanan sazan yavrularının tüm vücut dokusunda toplam protein aktivitesinde, konsantrasyona bağlı olarak önemli derecede azalma (p<0,05) olduğu tespit edildi. Toplam protein düzeyi her iki deneysel grupta uygulamanın 4. gününde kontrol grubuna göre hafif bir azalma göstermişken 7. günde bu azalmanın istatistiki olarak anlamlı olduğu görülmüştür. Bu çalışmada ise, toplam protein seviyelerinde görülen azalmanın nedeni, serbest radikallerin proteinlerin tiyol gruplarına bağlanması ve protein yapısında farklı konfigürasyonlar geliştirmesi olabilir (Kayhan vd., 2017).

Lipitler balıklardaki başlıca enerji deposu ve mobilizasyonu yüksek enerji taleplerinin olduğunu gösterir (Fernández-Vega vd., 2015). Bu çalışmada, Toplam lipit düzeyi 4. günde 0,79±0,12 (kontrol), 0,74±0,11 (2,8 mg/L), 0,81±0,14 (5,6 mg/L); 7. günde 0,82±0,11 (kontrol), 0,77±0,12 (2,8 mg/L), 0,78±0,12 (5,6 mg/L) olarak ölçülmüştür. Toplam lipit düzeyinde ise istatistiki bir değişimin olmadığı ortaya konulmuştur (p>0,05). Yüksek hidrofobik özellikteki toksik maddeler lipit dokusu tarafından hapsedildikleri için ilgili reseptörlerle etkileşime giremezler ve organizmaya herhangi bir zarar vermeden uzun süreler boyunca vücutta kalabilirler (Landrum ve Fisher 1999).

Bu çalışmada, glikojen düzeyi 4. günde 3,73±0,3 (kontrol), 3,70±0,5 (2,8 mg/L), 2,70±0,4 (5,6 mg/L); 7. günde 3,71±0,4 (kontrol), 3,43±0,4 (2,8 mg/L), 2,49±0,3 (5,6 mg/L) olarak ölçülmüştür. İmidacloprid'in 5,6 mg/L'lik dozunun uygulandığı grupta glikojen içeriliğinin, özellikle uygulamanın 7. gününde kontrol ve diğer imidacloprid grubuna göre önemli derecede bir azalmanın (p<0,05) olduğu tespit edildi. Bu, İmidacloprid'e maruz kalan balıkların genel bir cevabı gibi görünmektedir

(Hori vd., 2006; Abdel-Hameid, 2007). Bu glikojen düşüşü İmidacloprid'in neden olduğu enerji harcamalarının bir göstergesi olabilir (Kayıs ve Emre, 2012).

Bu çalışma iki farklı konsantrasyondaki imidacloprid'in, farklı zaman periyodunda sazanların yavrularının tüm vücudunda protein, lipit ve glikojen aktivitelerinde olumsuz bir etki oluşturduğunu göstermiştir. Düşük konsantrasyonda olmasa da yüksek konsantrasyonda imidakloprid ile karşı karşıya kalan sazan yavrularında özellikle 7. günde biyokimyasal parametrelerde olumsuz etkiler gözlenmiş olması bu maddenin balıklarda toksik etki oluşturabileceğini göstermektedir. Fakat bu ifadenin doğruluğunun tam olarak ortaya konabilmesi için bu madde ile balıkların yüksek dozlarda muamele edilmesi ve uzun süre gözlenmesi gerekmektedir.

TEŞEKKÜR

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New record of *Argyropelecus gigas* Norman, 1930 and *Conocara fiolenti* Sazonov & Ivanov, 1979 from the Arabian Sea Coasts of Oman

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Abstract

Two specimens of *Argyropelecus gigas* and *Conocara fiolenti* were collected from off the city of Salalah on the Arabian Sea coasts of Oman. The specimens represent the first confirmed record of the species from the northwestern quadrat of the Indian Ocean. Meristic and morphometric characters match those described for these species.

Keywords: range extension, Salalah, New record, collection, Indian Ocean

Umman'ın Arap Denizi Kıyılarından *Argyropelecus gigas* Norman, 1930 ve *Conocara fiolenti* Sazonov & Ivanov, 1979'un için yeni kayıtlar

Özet

Umman'ın Arap Denizi kıyılarındaki Salalah kentinden *Argyropelecus gigas* ve *Conocara fiolenti* örnekleri toplandı. Örnekler, Hint Okyanusu'nun kuzeybatı kuadratından bu türler için doğrulanmış ilk kayıtları temsil etmektedir. Meristik ve morfometrik karakterler bu türler için tanımlananlarla eşleşmektedir.

Anahtar Kelimeler: Menzil genişletme, Salalah, Yeni kayıt, koleksiyon, Hint Okyanusu

INTRODUCTION

With the continuous records of fish species, it became obvious that human knowledge about marine fish diversity is far from been comprehensive and many species are waiting for records and descriptions. Research reports authenticating range extensions are often disregarded several records of fish species that are considered unimportant, but knowledge of those species coming from certain localities deliver the basic information for investigations on biogeography, speciation, ecology, fisheries, and conservation (Smith et al., 2017).

Scientific investigations on the Omani ichthyofauna started in the late 1800s with the appearance of the publication of Boulenger (1887, 1889). Since then, numerous reports were published on the fish fauna of Oman, including the Arabian Gulf and Sea of Oman (White and Barwani, 1971; Heemstra, 1973; Fischer and Bianchi, 1984; Kuronuma and Abe, 1972; Al-Abdessalaam, 1995; Jawad and Al-Mamry, 2009; Jawad et al., 2010a, b; Al-Jufaili et al., 2010; Jawad, 2011a, b).

Individuals of *Argyropelecus gigas* Norman, 1930 were recorded from the Eastern Pacific from Portugal to South Africa (Badcock, 1984) and in the Western Atlantic including the Gulf of Mexico (Rass, 1971). In the Indian Ocean, *A. gigas* was reported in the food of the longnose lancetfish (*Alepisaurus ferox*) in the Seychelles waters (Potier et al., 2007) an din the food of yellowfin tuna *Thunnus albacares* (Bonnaterre, 1788) in the eastern Arabian Sea (Varghese and Somvanshi, 2016).

Conocara fiolenti Sazonov and Ivanov, 1979 distributed in the eastern Atlantic from Azores Island and in the South Atlantic at (31°S, 3°E) and Pacific (19°49'N, 151°48'E) (Froese and Pauly, 2020). It has been reported from the eastern part of the Indian Ocean (Sazonov et al., 2009).

In the present paper, we report on two fish new records belonging to the families Sternoptychidae and Alepocephalidae**.** From the distribution point of view of the two species, the specimens of *A. gigas* and *C. fiolenti* represent new records for the North West region of the Arabian Sea and the coasts of Oman Arabian Sea coasts of Oman.

MATERIALS and METHODS

One adult specimen of each of *A. gigas* and *C. fiolenti* (Figure 1A, B) were caught off the coast of Salalah City in the Arabian Sea coast of Oman Gulf (15°30′02″N 54°37′26″E). The fishes measured 80- and 250-mm TL respectively. The sizes of the two specimens were within the reported sizes for these two species by Markle and Sazanov (1990) and Quéro et al. (1990) respectively. The fish was caught by deep fishing trawler (300 -1000 m) operating in the vicinity of Salalah City on 6 July 2011. The specimens were fixed in 10% formalin and later preserved in 70% ethanol for deposit in the fish collection of the Omani Marine Science and Fisheries Centre, Ministry of Agriculture and Fisheries, Muscat, Sultanate of Oman.

Methods for taking measurements and counts follow Schultz (1938) for *A. gigas* and Sazonov et al. (2009) for *C. fiolenti*. Taxonomy and spelling follow Eschmeyer et al. (2020).

The morphological and meristic characters of the two specimens are in general agreement with those given for the species in the literature and the proportions and meristic values are presented in Table 1.

Figure 1. A- *Argyropelecus gigas, 80 mm TL;* ***B-*** *Conocara fiolenti*, 125 mm TL collected from the Arabian Sea coasts of Oman (Photograph by Saud M. AL JUFAILI).

Table 1. Morphometric and meristic characters of *Argyropelecus gigas and Conocara fiolenti* from the Arabian Sea coasts of Oman (measurements in mm)

Morphometric characters	***A. gigas***	**C. *fiolenti***
Total length (TL)	80	250
Standard length (SL) (% in TL)	71.4 (89.4)	232.5 (93)
Head length (HL) (% in SL)	23.8 (33.3)	46.5 (20)
Snout length (% in HL)	5.7 (23.4)	12 (25.8)
Eye diameter (% in HL)	8.6 (36.1)	7.5 (16.1)
Predorsal fin length (% in SL)	38.1 (53.4)	153 (65.8)
Postdorsal fin length (% in SL)	51.4 (71.9)	180 (77.4)
Prepectoral fin length (% in SL)	19.1 (26.8)	72 (30.9)
Preanal fin length (% in SL)	45.2 (63.3)	142.5 (61.3)
Body depth (% in SL)	35.7 (50)	48 (20.7)
Caudal peduncle depth (% in SL)	8.1 (11.3)	18 (7.74)
Meristic characters		
Number of dorsal fin rays	9	19
Number of anal fin rays	13	27
Number of pectoral fin rays	11	9
Number of pelvic fin rays	6	-

RESULTS and DISCUSSION

The specimen of *A. gigas* has the following set of characters: body deep and laterally compressed; dorsal profile elevated; angular shape snout, tubular eye directed dorsally; mouth vertical; prominent post-orbital spine; absence of spine at the posterior end of the abdomen beneath the origin of pelvic fins; photophores making an almost uninterrupted series from behind pectoral to the base of caudal fin; preopercle at a lower angle with one spine, which is slightly curved outward and directed ventrally and above which is a very small one directed outward; no scales found on body. Colour in preserved specimen light brown on the upper part of body getting blackish on sides and abdomen.

The specimen of *C. fiolenti* is characterised in having an elongated torpedo-shaped body, with the deepest part located at the pelvic fins region and decreased gradually towards the tail; head conical in shape and medium size, with depth, increases posteriorly; snout long and eye small; caudal peduncle not marked; anal opening located mid-way between the origins of pelvic and anal fins; leathery keel structure present at the anterior origin of the dorsal fin, which is a vertical line with the anterior 3rd of the base of anal fin; rays in anal fin short increased posteriorly; pelvic fins located near the mid-part of the body closer to anal-fin than to the pectoral fins; pectoral fins are long situated below the imaginary horizontal line passing through the mid-part of body, they closer to the middle of the body than the ventral body profile; scales are small, head, the area around the anus, the area extending from preoperculum to base of pectoral fins are with no scales; lateral line canal well-developed.

Quite a few other fish species have lately been recognized for the first time in the waters of Oman, probable due to the increased fishing effort and monitoring (Jawad and Al Mamry, 2009; Jawad et al., 2011a, 2011b, 2015; Jawad and Pitassy, 2015; Al-Marzouqi et al., 2018). This range extension extends of both *A. gigas* and *C. fiolenti* to the Indian Ocean region is important from the zoogeography of these species and will be a significant addition to the geographical range distribution of both *A. gigas* and *C. fiolenti*. These species typically occur in relatively deep waters, and it is possible, if not probable, that it is found in the entire Northwest Indian Ocean region.

CONCLUSIONS

New records of fish species from the Omani waters regularly appear in scientific publication. The presence of *Argyropelecus gigas* and *Conocara fiolenti* in the coasts of Salalah on the Arabian Sea coasts of Oman was not unexpected, but it considered a confirmation of their presence in this part of the world. The meristic and morphometric characters showed to be matching those described for these species.

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Sample Article

A Taxonomic Study on Zooplankton Fauna of Kiğı Dam Lake (Bingöl-Turkey)

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Abstract

The present study was conducted to determine zooplankton fauna of Kiğı Dam Lake during September 2012 and August 2013 seasonally. The zooplankton samples were collected by using plankton net with the mesh size of 55µm horizontally and preserved in 4% formaldehyde. Total 22 taxa (16 Rotifera, 4 Cladocera, and 2 Copepoda) were identified in Kiğı Dam Lake.

Keywords: Kiğı Dam Lake, species distribution, zooplankton.

INTRODUCTION

The majority of zooplankton (Copepoda, Cladocera and Rotifera) transform the phytoplankton to animal protein (Cirik and Gökpınar, 1993), and they play a significant role in food chain. It was reported that some species are the indicators of water quality, and eutrophication due to their sensitivity to environmental changes and therefore zooplankton studies on lakes have acquired significant importance (Berzins and Pejler, 1987; Mikschi, 1989).

Many studies were carried on zooplankton in Turkey (Özdemir and Şen, 1994; Göksu et al., 1997, 2005; Saler and Şen, 2002; Bozkurt and Sagat, 2008; Bulut and Saler, 2013a, 2013b; 2014a, 2014b; Saler et al., 2015a, 2015b). No previous research about zooplankton of Kiğı Dam Lake has been recorded. In this study zooplankton species and their seasonal variations of Kiğı Dam Lake have been investigated.

MATERIAL and METHODS

Kiğı Dam Lake was built on Peri Stream between 1997 and 2003. The maximum water capacity is 507.55 hm3 and has surface area 8.35 km2 and maximum depth of 168 m (Şimşek, 2016) (Figure.1). The species were identified according to Edmondson (1959), Flössner (1972), Ruttner-Kolisko (1974), Kiefer (1978), Koste (1978), Negrea (1983), Segers (1995), and Einsle (1996).

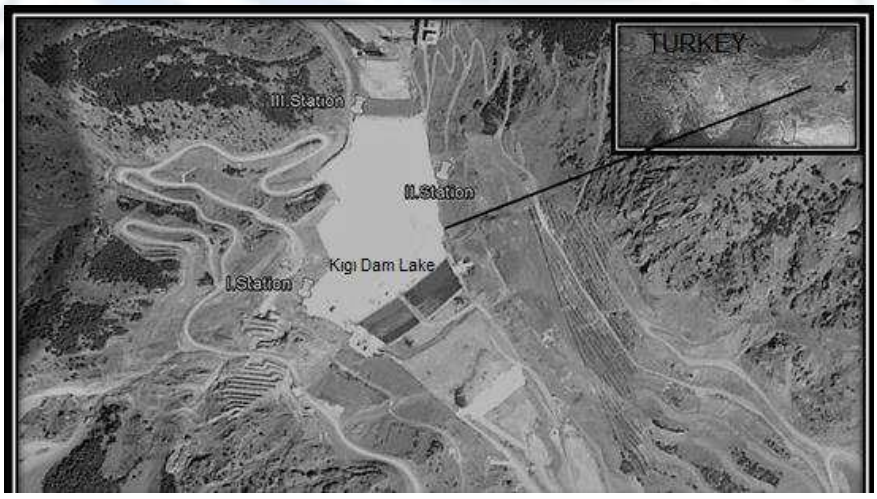

Figure1. Stations of Kiğı Dam Lake

RESULTS

A total of 22 taxa consisting of 16 Rotifera, 4 Cladocera and 2 Copepoda species were identified in the Dam Lake (Table 2).

The lowest numbers of taxa were recorded in winter at first stations (4 species). Some water quality parameters (pH, dissolved oxygen, and surface water temperature) were measured at study field (Table 3).

Table 3. Seasonal changes of water quality parameters in Kiğı Dam Lake

	Autumn	**Winter**	**Spring**	**Summer**
Water temperature (℃)	16	7.2	17.2	22.5
pH	7.0	6.8	6.9	7.3
D.O (mgL-1)	6.2	7.3	6.0	5.2

DISCUSSION

Zooplankton is known as the indicator of trophic status of aquatic habitats. They are also used to signify the water quality in freshwater systems. *K. cochlearis* and *P. dolichoptera* from Rotifera are indicators of productive habitats, while *N. acuminata* and *N. squamula* are indicators of cold waters (Kolisko, 1974). In Kiğı Dam Lake *K. cochlearis*, *P. dolichoptera* and *N. squamula* were observed.

In Murat River (Bulut and Saler, 2014a), Kalecik Dam Lake (Bulut and Saler, 2013b), Peri Stream (Saler et al., 2011), that were located in the same region with Kiğı Dam Lake, rotifers were recorded as dominant species as to number of individuals and abundance, followed by Cladocera and Copepoda species.

ACKNOWLEDGEMENT

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Ö R N E K MAKALE

Eğirdir Gölü'nden Tatlısu Istakozu (*Astacus leptodactylus* Eschscholtz, 1823)'nun Sindirim Enzim Aktivitelerinin Mevsim, Büyüklük ve Cinsiyete Bağlı Olarak Değişimi

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Özet

Bu çalışma, Eğirdir Gölü'nde yaşayan *Astacus leptodactylus* türünde mide sindirim enzim aktiviteleri üzerine (proteaz α-amilaz ve lipaz) mevsim ve eşeyin etkisini araştırmak amacıyla oluşturulmuştur. Bu amaçla kerevitler 1 yılda 4 mevsim olarak Eğirdir Gölü'nden avlandı. İlkbahar mevsiminde,kerevitlerin pinterlere girmemesi nedeniyle örnekleme yapılamadı. Bu nedenle, sonuçlar üç mevsim (sonbahar, kış, yaz) ve eşeyler üzerinden faktöriyel düzeyinde varyans analizi ile değerlendirildi. Bulgular, eşey ve mevsim faktörlerinin kerevitlerin midesindeki proteaz ve lipaz aktivitesini önemli düzeyde etkilediğini ($p<0,05$), α-amilaz aktivitesinde ise istatistiksel olarak önemli düzeyde etkili olmadığını gösterdi ($p>0,05$). Bulgular ayrıca, kerevitlerin,proteaz enzim aktivitesinin sonbahar ve kışın, lipaz enzim aktivitesinin ise kışın eşeyler arası önemli değişim gösterdiğini ortaya çıkardı ($p<0,05$).

Anahtar kelimeler: Astacus leptodactylus, sindirim enzim aktivitesi, lipaz, α-amilaz, proteaz.

Variation of Digestive Enzyme Activities Depending on Season, Size and Sex of Freshwater Crayfish (*Astacus leptodactylus* Eschscholtz, 1823) from Lake Eğirdir

Abstract

This study was conducted to search the effect of sex and season on digestive enzyme activities (protease, α-amylase, lipase) in Astacus leptodactylus species that lives in Egirdir Lake.The freshwater crayfish were caught as four seasons in a year from Egirdir Lake. The sampling was not possible for crayfish since they were not entered into trap in spring season. Therefore, the results were evaluated over three seasons and sex by factorial variance analysis. The results showed that sex and season affected to protease and lipase activities ($p<0.05$), whereas they did not affect statistically α-amylase activity in crayfish stomach ($p>0.05$). Results also indicated that protease enzyme activity in crayfish showed significant changes in autumn and winter while lipase enzyme activity showed changes in winter between sexs ($p<0.05$).

Keywords: Astacus leptodactylus, digesitve enzyme activity, α-amylase, lipase, protease.

GİRİŞ

Astacus leptodactylus, dünyada Türk ıstakozu (kerevit) olarak da bilinen (Köksal,1988) ve ülkemiz içsularında doğal olarak bulunan bir decapoda (on ayaklı) türüdür. Astacus ekonomik değeri yüksek kabuklu türlerinden biridir (Bolat, 2001). Kerevit 1986 yılı öncesi özellikle Eğirdir Gölü balıkçılarının başlıca gelir kaynağı iken bu türün daha sonra gerek aşırı avcılık ve gerekse hastalık nedeniyle popülasyonu azalmıştır (Köksal, 1988; Ackefors, 2000; Bolat, 2001; Harlıoğlu ve Aksu 2002; Harlıoğlu ve Mişe 2007; Bilgin vd., 2008).

MATERYAL ve YÖNTEM

Bu çalışmada, Ekim 2014 ile Haziran 2015 tarihleri arasında Eğirdir Gölü'nde kerevit (10-15 cm) avcılığı gerçekleştirilmiştir. Avcılıkta kerevit pinterleri kullanılmış, örneklemeler mevsimsel olarak yapılmıştır. İlkbahar mevsiminde, kerevitlerin pinterlere girmemesi nedeniyle örnekleme yapılamamıştır.

Enzim aktivite analizler

Bu çalışmada; α-amilaz, proteaz ve lipaz olmak üzere üç çeşit sindirim enzim aktivitesi araştırılmıştır.Tatlısu istakozundaki α-amilaz enzim aktivitesi, Metais ve Bieth (1968)'e göre yapılmıştır.

İstatistiksel analizler

Verilerin değerlendirilmesinde SPSS 23 istatistiki paket programından yararlanılmış ve sonuçlar, faktöriyel düzeyinde varyans analizi testi ile $P<0,05$ önem düzeyinde test edilmiştir. Faktörlerin seviye ortalamalarının arasındaki farklılıkların belirlenmesinde Tukey testi kullanılmıştır.

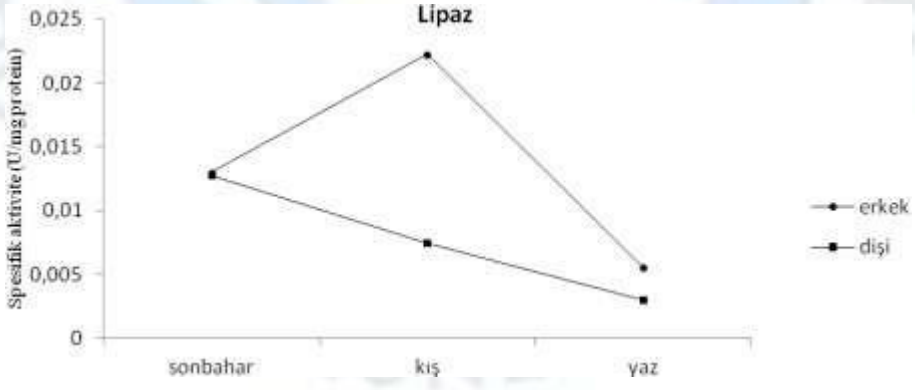
BULGULAR

Eşey ve mevsimsel farklılıkların kerevit midesindeki α-amilaz enzim aktivitesi üzerinde önemli bir etkisinin olmadığı tespit edilmiştir ($p>0,05$) (Tablo 1).

Tablo1. Kerevit midesindeki α-amilaz aktivitesinin eşey ve mevsime göre değişimi,(Ort.± S.H.) (U/mgprotein)

Eşey/Mevsim	Sonbahar	Kış	Yaz
Dişi	$^{a}0,013\pm 0,0011^{a}$	$^{b}0,007 \pm 0,0006^{b}$	$^{b}0,003 \pm 0,0010^{a}$
Erkek	$^{a}0,013 \pm 0,0035^{a}$	$^{b}0,022 \pm 0,0026a$	$^{c}0,006\pm 0,0006^{a}$

Lipaz aktivitesi erkek kerevitlerde sonbahar mevsiminden kış mevsimine kadar artan ve kış mevsiminden yaz mevsimine kadar azalan bir grafik oluşturmuş, dişi bireylerde ise sonbahar mevsiminden yaz mevsimine kadar azalan bir grafik oluşmuştur (Şekil 3).



Şekil 3. Kerevit midesinde farklı mevsimlerdeki lipaz aktivitesi (U/mg protein)

TARTIŞMA ve SONUÇ

Mevcut sindirim enzimlerinin profili ve faaliyetleri başta olmak üzere sindirim sisteminde ortaya çıkan fizyolojik süreçler, türlerin geniş besin çeşitliliğinden faydalanmasını etkilemektedir. Farklı kabuklu türleri, farklı beslenme alışkanlıklarına ve yaşam alanlarını yansıtan bir dizi sindirim enzime sahiptir (Anonim, 2017; Coccia vd., 2011)…….

Ayrıca, maksimum enzim aktivitesini belirleyebilmek için; farklı pH, sıcaklık ve reaksiyon sürelerinde denemeler yapılması da önemli taşımaktadır……

TEŞEKKÜR

Bu çalışma, yüksek lisans tezinden özetlenmiştir. (veya varsa proje desteği yazılmalı)

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