



Farklı Protein Kaynaklarının Çuka Mersini, *Acipenser ruthenus* Linnaeus 1758, Juvenillerinde Proteaz Aktivitesi Üzerine Etkileri

Recep KURT¹ , Kaya GÖKÇEK^{2*}

¹İskenderun Teknik Üniversitesi Deniz Bilimleri ve Teknolojisi Fakültesi, 31200 İskenderun, Hatay, Türkiye

²Mustafa Kemal Üniversitesi Ziraat Fakültesi Zootehni Bölümü 31000 Antakya, Hatay, Türkiye

ÖZ

Bu çalışmada, çuka balığı, *Acipenser ruthenus*, larvaları yumurtadan çıkış anından itibaren 29 gün boyunca standart besleme protokolü ile beslenmiştir. Karma yeme geçiş yapılan 29. gündeki proteolitik aktivite ve farklı protein kaynaklarının proteaz aktivite üzerine inhibisyon etkileri ölçülmüştür. Çalışmadan elde edilen bilgiler ışığında, çuka balığı juvenillerinin karma yemlerinde protein kaynağı olarak soya protein konsantresi (%38,57±10,44) ve mısır gluten (%43,07±1,72) kullanmanın, diğer yem hammaddeleri ile karşılaştırıldığında, proteolitik enzim aktivitesini istatistik açıdan daha olumlu etki yaptığı görülmüştür ($P<0,05$). Yem hammaddelerinin ikili kombinasyonları incelendiğinde ise, en düşük inhibisyon oranı balık unu: soya protein konsantresinin 1:1 oranda kullanılması (%47,93±1,72) ile elde edilmiş ve bu değer soya protein konsantresi ve mısır gluteninin tek başlarına kullanılması ile elde edilen etki ile aynı seviyede olduğu tespit edilmiştir ($P>0,05$). En yüksek inhibe edici etki ise, buğday ile pirinç kepeğinin 3:1 oranda kullanılması ile oluşmuştur (%82,39±7,23).

Anahtar kelimeler: *Acipenser ruthenus*, çuka balığı, proteaz aktivitesi, protein kaynakları, inhibisyon etkisi

MAKALE BİLGİSİ

ARAŞTIRMA MAKALESİ

Geliş : 03.07.2017

Düzeltilme : 22.11.2017

Kabul : 24.11.2017

Yayın : 27.04.2018



DOI:10.17216/LimnoFish.325726

* SORUMLU YAZAR

kayagokcek@yahoo.com

Tel : +90 534 415 07 16

Effects of Different Protein Sources on Proteolytic Activity in Juveniles of Sterlet, *Acipenser ruthenus* Linnaeus 1758

Abstract: In this trial, Sterlet sturgeon, *Acipenser ruthenus*, larvae were fed by a standart feeding protocol from hatching until 29th day, and the proteolytic activity and the inhibition effect of different portein sources on protease activity were also measured. The data obtained from study showed that using soybean protein consantrate (38.57±10.44%) and corn gluten (43.07±1.72%) as protein source in mixed feeds of dulcite juvenile strains compared to other feed raw materials, was found to have a more positive effect on proteolytic enzyme activity in terms of statistics ($P<0.05$). When the binary combinations of feedstuffs were examined, he lowest inhibition rate was obtained using 1: 1 (47.93 ± 1.72%) fish meal: soy protein concentrate, and this value was similar to that of soybean protein consantrate and corn gluten as a single protien sources in the diets ($P>0.05$). The highest inhibitory effect was obtained by using 3: 1 ratio of wheat and brass bran (82.39±7.23%).

Keywords: *Acipenser ruthenus*, sterlet, protease activity, protein sources, inhibition effect

Alıntılama

Kurt R, Gökçek K. 2018. Farklı Protein Kaynaklarının Çuka Mersini, *Acipenser ruthenus* Linnaeus 1758, Juvenillerinde Proteaz Aktivitesi Üzerine Etkileri LimnoFish. 4(1): 1-5. doi: 10.17216/LimnoFish.325726

Giriş

Mersin balıkları dünyada yaşayan en eski omurgalılar gruplarından birini oluşturmaktadır. Mersin balıkları yetiştiriciliği temel olarak havyar, tütsülenmiş balık, çorba, yapıştırıcı ve deri üzerine odaklanmıştır. Özellikle, siyah havyarının kilogramı birkaç bin dolara alıcı bulabilmektedir. Eti için üretiminden ziyade, esas hedef “ siyah altın” diye bilinen havyarın üretimidir. Tüm Mersin balığı türleri

aşırı avcılık baskısı, barajlar ve sulak alanlarda yapılan derinleştirme gibi düzenlemeler dolayısıyla yok olma tehlikesi ile yüz yüzedirler (Napora-Rutkowski vd. 2009).

Kültüre alınması kararlaştırılan bir türün temel yetiştiricilik uygulamalarının hayata geçirilebilmesi için yapılması gereken bazı temel araştırmalar bulunmaktadır. Öncelikli olarak, suni tohumlamanın yapılabilirliği,

sağlıklı bir inkübasyon sürecinin hangi optimum şartlarda olması gerektiği, yumurta açılımı sonrası pre ve post-larval aşamadaki gelişimlerin hem histolojik hem de fizyolojik olarak tespiti, larval dönemde optimum protein ve enerji oranının tespiti yapılmalıdır.

Bazı mersin balığı türlerine ait yapay dölleme, larva besleme ve sindirim enzimleri ile ilgili birçok çalışma hali hazırda mevcut olup (Willot vd. 2001; Gawlicka vd. 2002; Furne vd. 2005; Napora-Rutkowski vd. 2009; Babaei vd. 2011; Sanz vd. 2011; Noori vd. 2012), bu türler için ticari yemler üretilmiştir. Ancak, karma yemlerde kullanılan yem hammaddelerinin proteaz aktivitesi üzerine etkisi halen net bir şekilde bilinmemektedir. Yukarıda bahsedilen noktalar göz önüne alındığında çuka balığı, *Acipenser ruthenus*, alternatif tatlı su balığı olma potansiyeli çok iyi olan bir türdür. Bu çalışmada hedeflenen amaç; besin kesesinin tükenmesine müteakiben *Artemia*, *Tubifex*, *Chironomid* larvaları ve alabalık mikro diyet yem ile beslenen çuka balığı post-larvalarının, proteaz aktivitesi üzerine karma yemlerde kullanılan yem hammaddelerinin ve bunların ; ikili kombinasyonlarının etkilerini tespit etmektir. Elde edilen sonuçların ışığında, larvanın büyüme ve yaşama gücünü arttırmak amacı ile bu tür için hazırlanacak karma yemlerde hangi yem hammaddelerinin tercih edilmesi gerektiği belirlenmeye çalışılmıştır.

Materyal ve Metot

Yavru temini

Deneme, Macaristan'da bulunan bir ticari balık işletmesinde yapılmıştır (Riged&Riged Ltd., Homogmegy, Macaristan). Döllenen yumurtalar Rusya'dan ithal edilmiş ve inkübasyon McDonald şişelerinde gerçekleştirilmiştir. Deneme boyunca su sıcaklığı 17°C'de tutulmuştur. Yumurtadan çıkan besin keseli larvalar kapalı devre sisteme transfer edilmiş ve burada 29 gün boyunca ticari besleme protokolü ile beslenmiştir. Boy-Ağırlık ilişkisinin hesaplanabilmesi için günlük olarak örnekleme yapılmış (en az 10 adet birey) ve %4'lük formalin içinde muhafaza edilmiştir.

Yemleme protokolü

Çuka balıklarının pre-post larva ve juvenillerin beslenmesinde aşağıdaki ticari besleme protokolü kullanılmıştır (Tablo 1).

Ekstraksiyon prosedürü ve proteaz aktivitesinin ölçümü

Proteaz aktivitesi ve inhibisyon analizleri için balık stoğundan 29. günde tesadüfen 1g örnek alınmış, distile suda yıkanmış ve 3 ml'lik

ependorf tüplerde analizler yapılana kadar 80°C'de saklanmıştır. Sindirim enzimlerinin ekstraksiyonunda tüm larva vücudu homojenize edilmiş (35 mg ml⁻¹) ve ekstraktlar santrifüjle elde edilmiştir (12000 g, 20 dk, 4°C).

Araştırmanın 29. gününde örneklenen çuka balığı örneklerinin toplam proteaz enzim aktivitesi Walter (1984)'e göre yapılmıştır. Analizde, substrat olarak kazein (10 mg ml⁻¹ pH=9), kullanılmıştır. Substrata, larvadan elde edilen sindirim enzimine ek olarak Tris-HCl (50 mM) eklenmiş ve karışım 37°C'de 1 saat inkübe edilmiştir. Bu sürenin sonunda, reaksiyonun kontrollü bir şekilde durdurulabilmesi için karışıma Trikloroasetikasit (TCA) (500 µl, 120 g l⁻¹) ilave edilmiştir. Absorbans değeri, spektrofotometrede 280 nm'de ölçülmüştür. Juvenillerin çözünebilir protein konsantrasyonlarının ölçümü ise Bradford (1976)'ya göre yapılmıştır.

İnhibisyon analizleri

Bu çalışmada, ticari yem rasyonlarında protein kaynağı olarak kullanılan balık unu (BU), soya unu (SU), kan unu (KU), soya protein konsantrasyonu (SPK), mısır gluteni (MG), pirinç kepeği (PK), buğday unu (Buğ.U), soya küspesi (SK) ve BU: SK (1:3, 1:1, 3:1), SU:MG (1:3, 1:1, 3:1), BU:SPK (1:3, 1:1, 3:1), Buğ.U:PK (1:3, 1:1, 3:1) kombinasyonları kullanılmıştır. Bu yem hammaddelerinden elde edilen ekstraktlar, karma yeme geçiş yapmış juvenillerden elde edilen sindirim enzimleri ile reaksiyona sokulmuştur. Bu amaçla, Garcia-Carreno (1996)'nın modifiye edilmiş metodu kullanılmıştır. İlk önce, karma yeme geçiş günü olan 29. günde örneklenen larvaların enzim ekstraktları (20 µl) ile protein kaynakları (20 µl) 500 µl Tris-HCl (pH 9,0) inkübe edilmiştir (25°C'de 60 dk). Kontrol grubunda ise, protein kaynağı yerine aynı miktarda saf su kullanılmıştır. Daha sonra, karışıma 100 µl kazein eklenmiş ve 120 dk daha inkübasyona devam edilmiştir. Sonuçta, karışıma 500 µl TCA (120 g L⁻¹) eklenerek reaksiyon durdurulmuş ve absorbans değeri 280 nm'de ölçülmüştür (Alarcon vd. 1999).

İstatistik analizler

Analizler sonucunda elde edilen verilerden boy-ağırlık ilişkisi regresyon analizi ile, proteaz aktivitesi ve inhibisyon değerleri ise SPSS (15.0) paket programı kullanılarak analiz edilmiştir. Normalite testi olarak Kolmogorov-Simironov testi ve varyansın homojenitesi ise Bartlett's testi uygulanmıştır. Varyans analizi için ise One-way ANOVA testi

uygulanmıştır. Gruplar arasındaki farklılığın tespitinde ise, Duncan çoklu karşılaştırma testi

yapılmıştır. Farklar arasındaki önem derecesi $P < 0,05$ olarak değerlendirilmiştir.

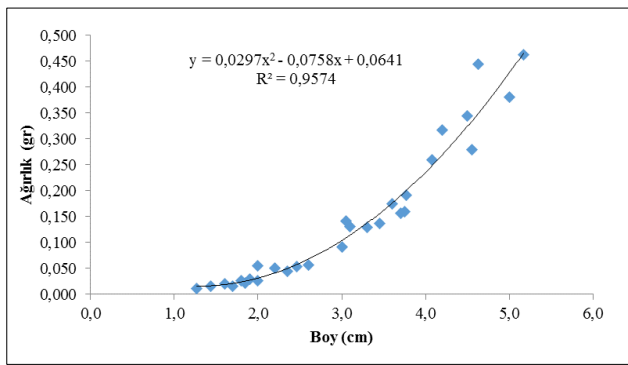
Tablo 1. Çuka mersin balığı ticari besleme protokolü

1-6. Günler	7-14. Günler	15-20. Günler	21-24. Günler	24. ve Sonra
Besin Kesesi	Artemia	Tubifex+ M.D	Chironomid +M.D.	Mikrodiyet

*M.D. :Mikrodiyet yem

Bulgular

Araştırmanın örnekleme süresince elde edilen boy-ağırlık ilişkisi ve derecesi aşağıdaki Şekil 1' de verilmiştir.



Şekil 1. Çuka balığının erken dönem boy-ağırlık değişim grafiği

Karma yeme geçiş günü olan 29. günde çuka balığının proteaz aktivitesi $311,29 \pm 0,27$ U/mg protein bulunmuştur. Ticari olarak kullanılan protein kaynaklarının çuka balığı juvenillerinin proteaz aktivitesi üzerine inhibe etme oranları aşağıdaki Tablo 2'de verilmiştir. Yem hammaddelerinin tek tek test edilmesi ile elde edilen verilerin ışığında, soya protein konsantresi ve mısır glutenin proteaz aktivitesi üzerine en az etki gösteren iki yem hammaddesi olduğu ortaya çıkmıştır ve bu iki yem hammaddesinin etki açısından aralarındaki fark istatistiki açıdan önemsiz bulunmuştur ($P > 0,05$). Bunları sırasıyla balık unu, pirinç kepeği, kan unu ve soya takip etmiştir. Yine, balık unu-pirinç kepeği ve kan unu ile soya arasındaki farklar istatistik açıdan önemsiz bulunmuştur ($P > 0,05$). Yem hammaddelerinin ikili kombinasyonları incelendiğinde ise, en düşük inhibisyon oranı BU:SPK'nın 1:1 oranda kullanılması ($47,93 \pm 1,72$) ile elde edilmiş ve bu değer SPK ve MG'nin tek başlarına kullanılması ile elde edilen etki ile aynı seviyede olduğu tespit edilmiştir ($P > 0,05$). En yüksek inhibe edici etki ise, buğday ile pirinç kepeğinin 3:1 oranda kullanılması ile oluşmuştur ($82,39 \pm 7,23$).

Tablo 2. Yem hammaddelerinin proteaz aktivitesini inhibe etme oranları (%)

Yem Hammaddesi	Ortalama (%)±S.D.
Balık unu (69 % Ham Protein) (BU)	$57,68 \pm 4,54^c$
Kan unu (75,5 % Ham Protein) (KU)	$69,66 \pm 2,97^d$
Mısır gluten (60 % Ham Protein) (MG)	$43,07 \pm 1,72^a$
Soya (48 % Ham Protein) (S)	$73,78 \pm 3,61^d$
Buğday Unu (12 % Ham Protein)	$59,04 \pm 2,22^c$
Soya Protein Kon. (58 % Ham Protein) (SPK)	$38,57 \pm 10,44^a$
Pirinç kepeği (PK)	$59,17 \pm 2,34^c$
S/MG (1:3)	$62,91 \pm 3,89^c$
S/MG (1:1)	$68,16 \pm 5,55^d$
S/MG (3:1)	$69,66 \pm 5,62^d$
BU/SPK (1:3)	$58,05 \pm 2,34^c$
BU/SPK (1:1)	$47,93 \pm 1,72^a$
BU/SPK (3:1)	$51,68 \pm 1,95^b$
Buğday/PK (1:3)	$68,53 \pm 1,13^d$
Buğday/PK (1:1)	$73,03 \pm 3,00^d$
Buğday/PK (3:1)	$82,39 \pm 7,23^e$
BU/S (1:3)	$65,17 \pm 7,94^c$
BU/S (1:1)	$69,66 \pm 3,65^d$
BU/S (3:1)	$71,91 \pm 4,05^d$

Tartışma ve Sonuç

Günümüzde, ticari olarak üretilen ve balık yemlerinde kullanılan protein kaynaklarının balıkların sağlıklı bir şekilde büyüme ve gelişmelerine olan etkisi halen tartışma konusudur. Töre vd. (2014), bazı ticari yem hammaddelerinin 30 günlük (proteaz aktivite değeri: $489,75 \pm 34,86$ U/mg) Turna, *Esox lucius*, balığı larvalarının proteaz aktivitesi üzerine yaptığı inhibe edici etkileri belirlemişlerdir. Bu çalışmada, krill $2,30 \pm 1,26$, balık hidrolizati $4,89 \pm 2,41$, balık unu $15,69 \pm 0,84$, soya unu $15,54 \pm 4,29$, havyar unu $22,72 \pm 1,36$ ve mısır gluten $32,24 \pm 3,61$ oranlarında proteaz aktivitesini inhibe ettiği ortaya konulmuştur. Bizim çalışmamızda elde edilen veriler ile karşılaştırıldığında, balık unu ve soya unu çuka balığının juvenil döneminde, turna balığından daha yüksek inhibe edici etki göstermiştir. Buna karşın, mısır glutenin gösterdiği etki açısından aradaki fark nispeten az görünse de fark istatistiki açıdan önemlidir ($P < 0,05$). Gökçek vd. (2016), 30 günlük Rus mersini, *Acipenser gueldenstaedtii*, larvaları ile yaptıkları çalışmalarında (proteaz aktivitesi: $592,74 \pm 47,61$ U/mg), ticari yem hammaddelerinin

proteaz aktivitesini inhibe edici etkilerini incelemişlerdir. Araştırmacılar, en düşük inhibisyon değerini balık unundan elde etmiştir (%15,44±5,59). Diğer protein kaynakları göreceli olarak oldukça yüksek inhibisyon değerleri sergilemiştir (SPK: %63,55±5,46; Soya unu: %71,81±3,82; Mısır gluten: %72,24±4,17; Kan unu: %97,28±2,31). Buna karşın, balık unu ve soya ununun 3:1 ikili kompozisyonunda %26,38±2,02 inhibisyon değeri elde edilmiştir. Alptekin ve Gökçek (2016), Sibirya mersin balığı (*Acipenser baerii*) juvenilleri ile yaptıkları çalışmalarında ise, en düşük inhibisyon değerini balık unu ve soya protein konsantresinden elde etmiştir (sırasıyla, %15,34±3,85 ve %14,45±1,58). Diğer protein kaynakları göreceli olarak oldukça yüksek inhibisyon değerleri sergilemişlerdir (Soya unu: %63,33±4,71; Mısır gluten: %25,56±7,86; Kan unu: %66,67±8,02). Bizim çalışmamızda ise, soya protein konsantresi ve mısır gluten maddelerinden en düşük inhibisyon elde edilmesine rağmen, değerler Alptekin ve Gökçek'in elde ettiği değerlerden oldukça yüksek bulunmuştur ($P<0,05$). Burada, türün beslenme fizyolojisinin çok önemli olduğu, yem hammaddelerinin proteaz aktivitesine bu nedenle farklı etkiler yaptığı düşünülmektedir.

Karşılaştırılan üç çalışmada, balık ununun Turna ve Rus Mersini'nin erken larval dönemlerinde hemen hemen aynı sonuçları vermesine rağmen, çuka balığında oldukça farklı bir sonuç sergilemiştir. Öte yandan, özellikle bitkisel kökenli protein kaynaklarının proteaz aktivitesi üzerine farklı etkileri olmuştur ($P<0,05$). Bunun yanında, yem hammaddelerinin ikili kombinasyonlarının proteaz aktivitesi üzerine etkisi olumsuz denebilecek seviyede yüksek bulunmuştur. Bu durum, yem hammaddelerinin kimyasal kompozisyonundan (özellikle farklı aminoasit içeriği) ve larvaların türe özgü gelişimlerine bağlı olarak farklı dönemlerde farklı sindirim enzimlerini salgılamalarından kaynaklanmış olabilir. Ayrıca, yem hammaddelerinin sahip olduğu aminoasit içeriklerinin birbirine antagonist ilişki oluşturacak seviyede olması da ayrı bir olasılıktır.

Çuka balığının erken larval döneminde karma yem rasyonlarında kullanılması en uygun protein kaynağı %58 ham protein oranına sahip soya protein konsantresi ve %60 ham protein oranına sahip mısır gluteni olduğu söylenebilir. Soya protein konsantresi, özellikle son yıllarda Kuzey Amerika kıtasında yaygın olarak üretilen ve aminoasit kompozisyonu soya unu ile karşılaştırıldığında balık yetiştiriciliği açısından çok daha iyi olduğu iddia edilen bir üründür (Kotzamanis vd. 2012). Öte yandan, tüm balık yemlerinde temel protein kaynağı olarak kullanılan balık unun en düşük inhibisyon oranına

sahip olmaması ise beklenmeyen bir sonuçtur. Bu konu ile ilgili olarak, farklı kökenli ve farklı türlere ait balık unlarının etkisi üzerinde de çalışmaların yürütülmesi gerekmektedir.

Bu çalışmada yapılan analizler ve elde edilen sonuçlar, karma yeme tamamen geçiş yapmış larvaların yemlerinde kullanılan protein kaynaklarının, larvalar tarafından salgılanan proteaz enzimini hangi oranda inhibe ettiğini bulma amaçlı olarak tasarlanmıştır. Ancak görüldüğü üzere, elde edilen veriler çuka balığı larvalarının karma yem rasyonunda hangi protein kaynağının tercih edilmesi gerektiği açısından tavsiye niteliği taşımaktadır. Daha ayrıntılı sonuçların elde edilmesi için, protein hidroliz oranlarının pH stat analizi ve aminoasit kompozisyonlarının ayrıntılı bir şekilde ortaya konulması gerekmektedir.

Teşekkür

Bu çalışmanın gerçekleştirilmesinde, Macaristan'ın Szent Istvan Üniversitesi Balık Yetiştiriciliği Bölümü'ne ve çalışmanın gerçekleştirildiği Rigid&Rigid Ltd. şirketine teşekkürlerimizi sunarız. Ayrıca, analizlerin yürütülmesinde katkıda bulunan Yük.Müh.Cemal ALPTEKİN'e teşekkür ederiz.

Kaynaklar

- Alarcon FJ, Moyano FJ, Diaz M, Fernandez-Diaz C, Yufera M. 1999. Optimization of the protein fraction of microcapsules used in feeding of marine fish larvae using *in vitro* digestibility techniques. *Aquac Nutr.* 5(2):107-113.
doi: 10.1046/j.1365-2095.1999.00093.x
- Alptekin C, Gökçek K. 2016. Balık yemlerinde kullanılan farklı protein kaynaklarının Sibirya mersini, *Acipenser baerii* Brandt 1869, juvenillerinin proteaz aktivitesi üzerine etkileri. *Yunus Araştırma Bülteni.* 16(3):201-207. [in Turkish]
doi: 10.17683/yunus.32866
- Babaei SS, Kenari AA, Nazari R, Gisbert E. 2011. Developmental changes of digestive enzymes in Persian Sturgeon (*Acipenser persicus*) during larval ontogeny. *Aquaculture.* 318(1-2):138-144.
doi: 10.1016/j.aquaculture.2011.04.032
- Bradford MM. 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dry binding. *Anal Biochem.* 72:248-254.
- Furne M, Hidalgo MC, Lopez A, Garcia-Gallego M, Morales AE, Domezain A, Domezaine J, Sanz A. 2005. Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. *Aquaculture.* 250(1-2):391-398.
doi: 10.1016/j.aquaculture.2005.05.017
- Garcia-Carreno FL. 1996. Proteinase inhibitors. *Trends Food Sci Technol.* 7(6):197-204.
doi: 10.1016/0924-2244(96)10023-6

- Gawlicka A, Herold MA, Barrows FT, de le Noue J, Hung SSO. 2002. Effect of dietary lipids on growth, fatty acid composition, intestinal absorption and hepatic storage in white sturgeon (*Acipenser transmontanus*) larvae. J Appl Ichthyol. 18(4-6):673-681.
[doi: 10.1046/j.1439-0426.2002.00371.x](https://doi.org/10.1046/j.1439-0426.2002.00371.x)
- Gökçek K, Szabo T, Alptekin C, Kurt R, Töre Y, Urbanyi B. 2016. The effect of feeding regime and inhibitory of different feed ingredients on proteases of Russian sturgeon, *Acipenser gueldenstaedtii* Brandt&Ratzenburg, 1833, at early life stages. Turkish J of Fisheries and Aquatic Sciences. 16(4): 1025-1029.
[doi: 10.4194/1303-2712-v16_4_29](https://doi.org/10.4194/1303-2712-v16_4_29)
- Kotzamanis YP, Vatsos IN, Cremer M, Illia V, Pyrenis G, Zonaras V, Buentello A, Van Eys J, 2012. Effects of high dietary inclusion of soybean meal and Navita 3010 soy supplemented with taurine on growth performance and muscle composition of European seabass (*Dicentrarchus labrax*). HCMR, Greece. (Technical Report)
- Napora-Rutkowski L, Kamaszewski M, Bielawski W, Ostaszewska T, Wegner A. 2009. Effects of starter diets on pancreatic enzyme activity in juvenile starlet (*Acipenser ruthenus*). The Israeli J Aquaculture-Bamidgeh, 61 (2): 143-150.
- Noori F, Gilbert VS, Sorgeloos P. 2012. Preliminary study on the activity of protease enzymes in Persian sturgeon (*Acipenser persicus* Borodin, 1897) larvae in response to different diets: effects on growth and survival. Aquac Res. 43(2):198-207.
[doi: 10.1111/j.1365-2109.2011.02816.x](https://doi.org/10.1111/j.1365-2109.2011.02816.x)
- Sanz A, Llorente JL, Furne M, Ostos-Garrido V, Carmona R, Domezain A, Hidalgo MC. 2011. Digestive enzymes during ontogeny of the sturgeon *Acipenser naccarii*: intestine and pancreas development. J Appl Ichthyol. 27(5):1139-1146.
[doi: 10.1111/j.1439-0426.2011.01864.x](https://doi.org/10.1111/j.1439-0426.2011.01864.x)
- Töre Y, Gökçek K, Alptekin C, Szabo T, Urbanyi B. 2014. Turna, *Esox lucius* Linnaeus 1758, balığı larvalarının proteaz aktivitesi üzerine ticari yem hammaddelerinin inhibisyon etkilerinin tespiti. Doğu Anadolu 5. Su Ürünleri Sempozyumu; Elazığ, Türkiye.
- Walter HE. 1984. Proteinases: methods with hemoglobin, casein and azocoll as sub-strates, In: Methods of Enzymatic Analysis, Vol. 5. Weinheim: Verlag Chemie Press. s. 270-277.
- Willot P, Sabeau L, Gessner J, Arlati G, Bronzi P, Gulyas T, Berni P. 2001. Sturgeon farming in Western Europe: recent developments and perspectives. Aquat Living Resourc. 14(6):367-374.
[doi: 10.1016/S0990-7440\(01\)01136-6](https://doi.org/10.1016/S0990-7440(01)01136-6)



Investigating the Effect of Different Growth Media on Biomass Production of *Pseudopediastrum boryanum* (Turpin) E. Hegewald Isolates

Dilek YALÇIN DUYGU^{1*} , İlkey AÇIKGÖZ ERKAYA² , Tülay ÖZER³ 

¹Gazi University, Faculty of Biology Education, Department of Biology, Ankara, Turkey

²Ahi Evran University, Faculty of Architecture and Engineering, Department of Environmental Engineering, Kırşehir, Turkey

³Ahi Evran University, Kaman High of Applied Science, Food Technology Department, Kırşehir, Turkey

ABSTRACT

Microalgae *Pseudopediastrum boryanum* (Turpin) E. Hegewald was chosen as a subject for the present research due to its potential uses of wastewater treatment and biodiesel production. In the present study, we investigated the growth and biomass production of *P. boryanum* through use of semi-continuous cultures employing two growth media (Allen and BG-11). In our previous study, *P. boryanum* was isolated from different freshwater reservoir through the dilution technique. The isolated *P. boryanum* strain was inoculated with 270 mL of medium + 30 mL of suspension culture and the 16:8 light/dark photoperiod was applied. Optical density was recorded by using UV-Visible spectrophotometer at 670 nm, and cell count examination was performed through drop count method. Besides, dry weight and chlorophyll-a concentration of strain were determined. The highest cell density (3.67×10^6 cells/mL), dry weight (0.032 g/mL) and chlorophyll-a ($16.39 \mu\text{g L}^{-1}$) production were observed in the Allen medium. Growth rates of *P. boryanum* were found to be 0.6676 d^{-1} in the Allen and 0.6021 d^{-1} in the BG-11 medium.

Keywords: *Pseudopediastrum boryanum*, optical density, cell density, growth parameters, culture conditions.

ARTICLE INFO

RESEARCH ARTICLE

Received : 06.11.2017

Revised : 02.01.2018

Accepted : 15.01.2018

Published : 27.04.2018



DOI:10.17216/LimnoFish.348198

* CORRESPONDING AUTHOR

dilekduygu06@hotmail.com

Tel : +90 532 328 21 47

Farklı Besi Ortamlarının *Pseudopediastrum boryanum* (Turpin) E. Hegewald İzolatlarının Biyokütle Üretimine Etkileri Üzerine Araştırma

Öz: Mikroalg, *Pseudopediastrum boryanum* (Turpin) E. Hegewald, atık su arıtımı ve biyodizel üretim potansiyelinden dolayı araştırma konusu olarak seçilmiştir. Bu çalışmada, iki besiy ortamı (Allen ve BG-11) kullanılarak, kesikli kültür sisteminde *P. boryanum*'un büyüme ve biyokütle üretimi araştırılmıştır. Önceki çalışmalarımızda, *P. boryanum* dilüsyon tekniği kullanılarak, farklı tatlısu birikintilerinden izole edilmiştir. İzole edilen *P. boryanum* suşu, 270 mL besiy ortamı + 30 mL süspansiyon kültür ile aşılanmış ve 16:8 aydınlık/karanlık fotoperiyodu uygulanmıştır. Optik yoğunluk 670 nm'de UV-Visible spektrofotometre kullanılarak tespit edilmiş ve suşların hücre sayımı damla sayım metodu kullanılarak yapılmıştır. Kültürlerin kuru ağırlık ve klorofil-a tayinleri de gerçekleştirilmiştir. Allen ortamında en yüksek hücre yoğunluğu ($3,67 \times 10^6$ hücre/mL), kuru ağırlık (0,032 g/mL) ve klorofil-a ($16,39 \mu\text{g L}^{-1}$) olarak tespit edilmiştir. *P. boryanum*'un büyüme oranları Allen besiy ortamında $0,6676 \text{ d}^{-1}$ ve BG-11 ortamında $0,6021 \text{ d}^{-1}$ olarak bulunmuştur.

Anahtar kelimeler: *Pseudopediastrum boryanum*, optik yoğunluk, hücre yoğunluğu, üreme parametreleri, kültür koşulları.

How To Cite

Yalçın Duygu D, Açıkgöz Erkaya İ, Özer T. 2018. Investigating the Effect of Different Growth Media on Biomass Production of *Pseudopediastrum boryanum* (Turpin) E. Hegewald Isolates. LimnoFish. 4(1): 6-12. doi: 10.17216/LimnoFish.348198

Introduction

The recent developments in industrialization coupled with the increase in human population have led to exhaustion of the available natural resources within a short period of time. Besides, the increase in expectations in various industrial fields and the environmental pollution has paved the way for the

research on the use of non-familiar resources for balanced and sufficient nutrients to feed the increasing population. As a solution to those problems, algae show itself as the light of hope (Sasson 1988). It is a known fact that algae contain rich nutrients; therefore, making them plays a vital role in the food chain. Along with the

use of biomass obtained from algae in food, cosmetics, chemistry, pharmacy etc., algae are also used in waste water treatment because of their ability to adhere to heavy metals (Spolaore et al. 2006). Also, as an alternative energy source, suitable algal species are used in the production of biodiesel (Brennan and Owende 2010).

Microalgae are single-cell, colony or filamentous forms of microorganisms convert solar energy into chemical energy through photosynthesis (Hosikian et al. 2010). While algae play a key role in the food chain, they convert inorganic molecules (such as carbon, nitrogen and phosphorus) into organic molecules in aquatic ecosystems (Murdock and Wetzel 2009). The microalgae are the complex heterogeneous microorganisms involving phylum with different physiological properties. As a result of that large diversity, different algae species have different growth requirements. The research into habitat of the microalgae revealed precisely that those microorganisms need certain nutrients. Along with the nutrients, temperature, intensity of light, the amount and type of nutrients, the amount of CO₂ and pH are primary factors affecting the growth of the algae (Kumar and Das 2012; Idenyi et al. 2016).

The genus *P. boryanum* is colony forms of the green algae occurring commonly in natural freshwater environments. Recently, it has become a topic of interest for the researchers. The algal species *P. boryanum* has beneficial attributes for wastewater treatment, particularly due to its high productivity and efficient removal by simple gravity sedimentation (Park et al. 2014). A pilot Cr (VI) biosorption study was carried out with the *P. boryanum* strain that we had isolated and used in the present study and the result was successful (Baykal Ozer et al. 2012). Microalgae *P. boryanum* was chosen as a subject for this research due to its potential uses of waste water treatment and biodiesel production. In this study, it is aimed to determine the characteristics of biomass production in different growth media of *P. boryanum*.

Material and Methods

Isolation

In our previous study, the samples, were collected from different freshwater reservoir, were brought to incubation at room temperature after inoculation at pre-enrichment nutrition media (MgSO₄·7H₂O-2.50 g, KNO₃-5.0 g, KH₂PO₄-1.25 g, FeSO₄·7H₂O-0.009 g and distilled water 1000 mL). *P. boryanum* was identified at species base with

microscopic examination after incubation (Bourrelly 1972; Prescott 1975). From the mixed species in the pre-enrichment medium, *P. boryanum* was isolated through dilution technique (CSIRO 2017).

Culture Conditions

In this study, semi-continuous culture system was utilized in reproduction of the cultures. Allen and BG-11 media were used for experiments (Table 1). The isolated *P. boryanum* strain was inoculated with 270 ml of medium + 30 ml of suspension culture. The pH of nutrient media was adjusted as 6.5-7. The implementation of 16:8 light/dark photoperiod (50 µmol photons m⁻²s⁻¹) was applied on cultures and they were cultivated under at 22-25°C room temperature. All tests were carried out in triplets.

Determination of Cell Density

Total cell counts were microscopically determined through drop count method. In the course of cell count, a 0.03 mL sample was dropped on the slide and lamella was covered. When the microscope objective lens was at 10x40, 36 views were identified. The calculation was made by counting at least six views and proportioning into 36 transect views.

Determination of Dry Weight

The 50 mL samples were filtered via Whatman GF/C filter papers and dried at 60°C incubator and they were weighed (Chia et al. 2013). Dry weight determination was made on 14th day following the cultivation process.

Chlorophyll-a Determination

The biomass of microalgae sample was estimated from their chlorophyll-a content measured through use of methanol method (Youngman 1978).

Maximum Absorbance Determination

The maximum absorbance was inspected by scanning sample cultures between 550 and 800 nm, using a UV-visible spectrophotometer (Biochrom Libra S22). The maximum absorbance value for microalgae was used to perform the growth curve by optical density (OD) (Santos-Ballardo et al. 2015). Optical density was recorded as 670 nm for *P. boryanum*. A linear regression equation was derived in order to describe the relationship between optical density and cell density.

Cell Growth Efficiency

By using the growth kinetics, specific growth rate and duplication time were calculated (Godoy-Hernández and Vázquez-Flota 2006). Specific

growth rate and duplication time are presented as Eq. (1 and 2):

$$\mu = \frac{\ln X_2 - \ln X_1}{t} \quad (1)$$

μ : Specific growth rate

X1 and X2 = Biomass concentration at t_1 and t_2

$$DT = \frac{\ln 2}{\mu} \quad (2)$$

Table 1. Chemical composition of the culture medium

Macroelements	Culture broth composition		
	BG-11 (g/L)	ALLEN (g/L)	(ml)
NaNO ₃	1.5	1.5 g	
K ₂ HPO ₄	0.04	-	
K ₂ HPO ₄ ·7H ₂ O	-	6 g/L	5 mL
MgSO ₄ ·7H ₂ O	0.075	6 g/L	5 mL
CaCl ₂ ·2H ₂ O	0.036	2.5 g/L	10 mL
Citric acid	0.006	4.8 g/L	1 mL
Ferric ammonium citrate	0.006	-	
EDTA (disodium salt)	0.001	-	
Na ₂ CO ₃	0.02	4 g/L	5 mL
Na ₂ SiO ₃ ·9H ₂ O	-	4.64 g/L	10 mL
Trace metal mix A5	1.0 mL	-	
P-IV metal solution	-	1 mL	
Distilled water	1.0 L	200 mL	
Trace Metal mix A5	P-IV metal solution		
H ₃ BO ₃	2.86	Na ₂ EDTA·2H ₂ O	0.75 g
MnCl ₂ ·4H ₂ O	1.81	MnCl ₂ ·4H ₂ O	0.041 g
ZnSO ₄ ·7H ₂ O	0.222	ZnCl ₂	0.005 g
NaMoO ₄ ·2H ₂ O	0.39	FeCl ₃ ·6H ₂ O	0.097 g
CuSO ₄ ·5H ₂ O	0.079	Na ₂ MoO ₄ ·2H ₂ O	0.004 g
Co(NO ₃) ₂ ·6H ₂ O	49.4	CoCl ₂ ·6H ₂ O	0.002 g
Distilled water	1.0 L	Distilled water	1.0 L

Results

The growth of microalgae *P. boryanum* in different culture media was primarily followed by counting algal cells under the microscope. Two growth media use for *P. boryanum* cultivation with varying chemical composition were studied. Both Allen and BG-11 medium were found to enhance the growth of *P. boryanum* at different densities. The biomass results obtained through two media of *P. boryanum* are presented in Fig.1. The highest cell density (3.67×10^6 cells/mL), dry weight (0.032 g/mL) and chlorophyll-a ($16.39 \mu\text{g L}^{-1}$) production were observed in the Allen medium (Fig. 1a, b and c)

The calibrated data between optical density and cell density are displayed in Figure (2). The specific growth rate was calculated and presented in Figure (3). The duplication time was found as (0.0586) for Allen medium and (0.0577) for BG-11. Growth rates of *P. boryanum* were found to be 0.6676 d^{-1} in

the Allen medium and 0.6021 d^{-1} in the BG-11 medium. Figure (4) indicates the effect of different medium on biomass productivity in mL/L/day. It is clear that the maximum biomass productivity was obtained in the Allen medium and the lowest in the BG-11 medium.

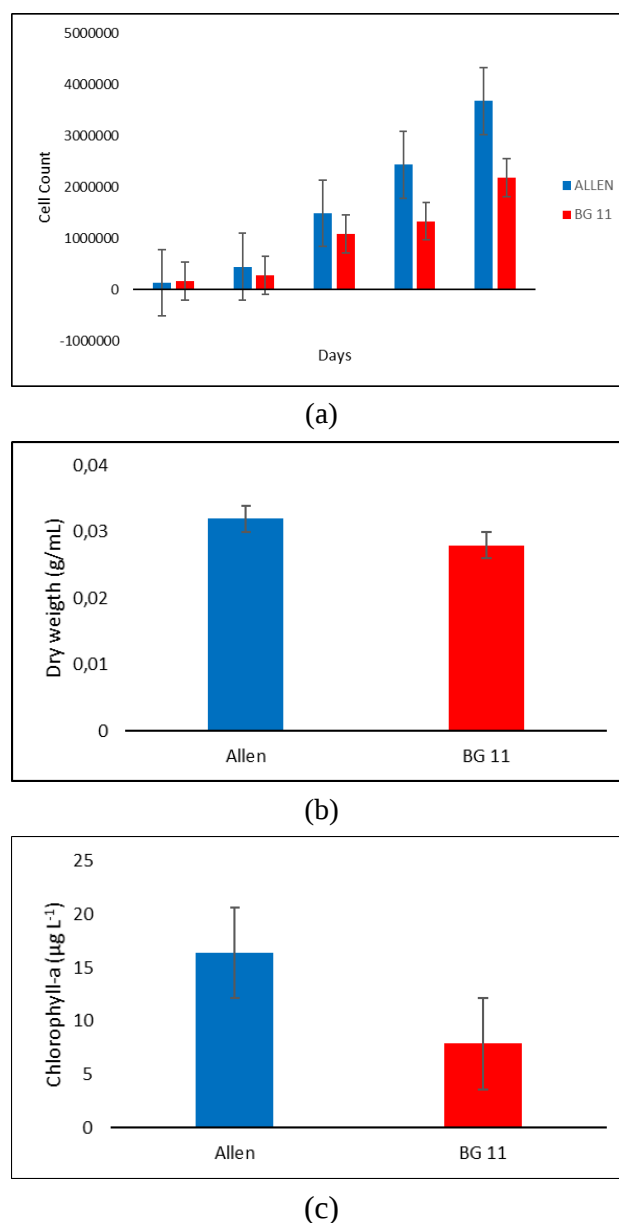


Figure 1. (a) Cell density (cells/mL); (b) dry weight (g/mL); (c) chlorophyll-a ($\mu\text{g L}^{-1}$) production. Error bars represent standard deviation for $n=3$.

Discussion

There are differences among microalgae species in terms of cell growth and different microalgae groups have different physiological requirements in their natural habitats or under culture conditions (Falkowski 1984). Environmental and culture condition parameters such as light, photoperiod, medium and temperature influence the growth of microalgae (Ak et al. 2008; Fakhri et al. 2015).

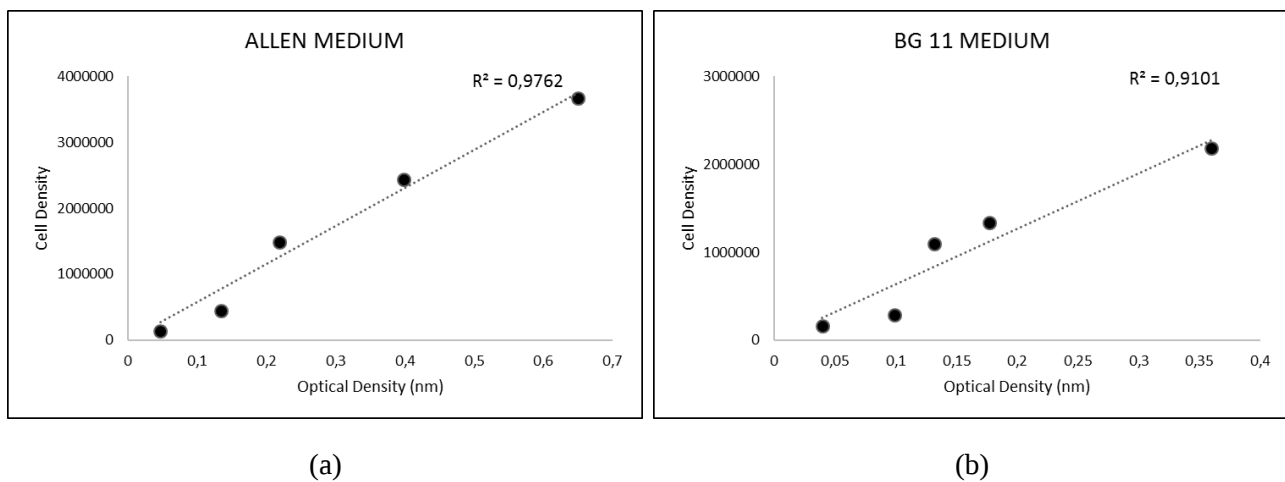


Figure 2. Calibration curve for the relationship between optical density and cell density.

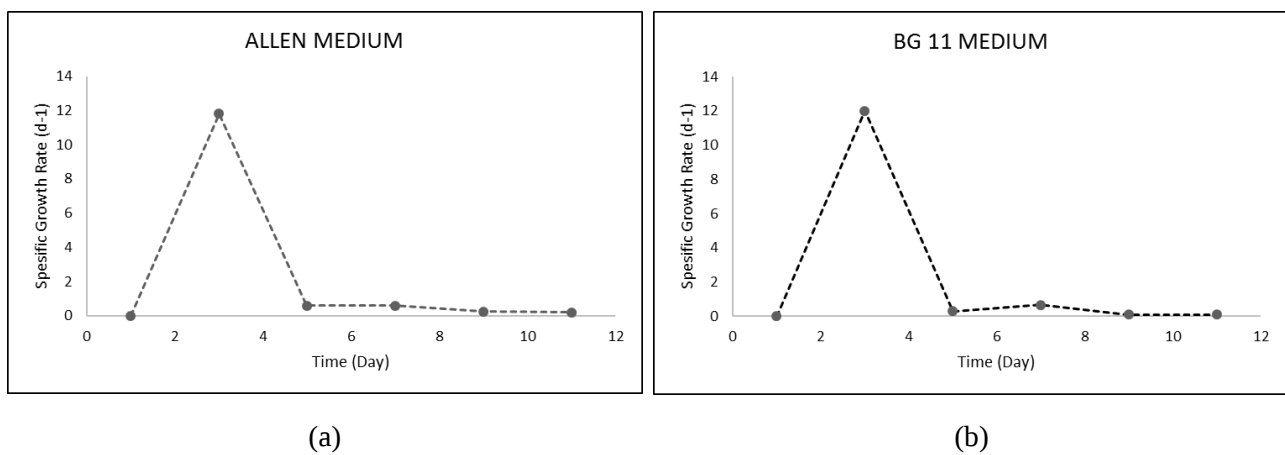


Figure 3. Specific growth rate of *P. boryanum* microalgae at different nutrients.

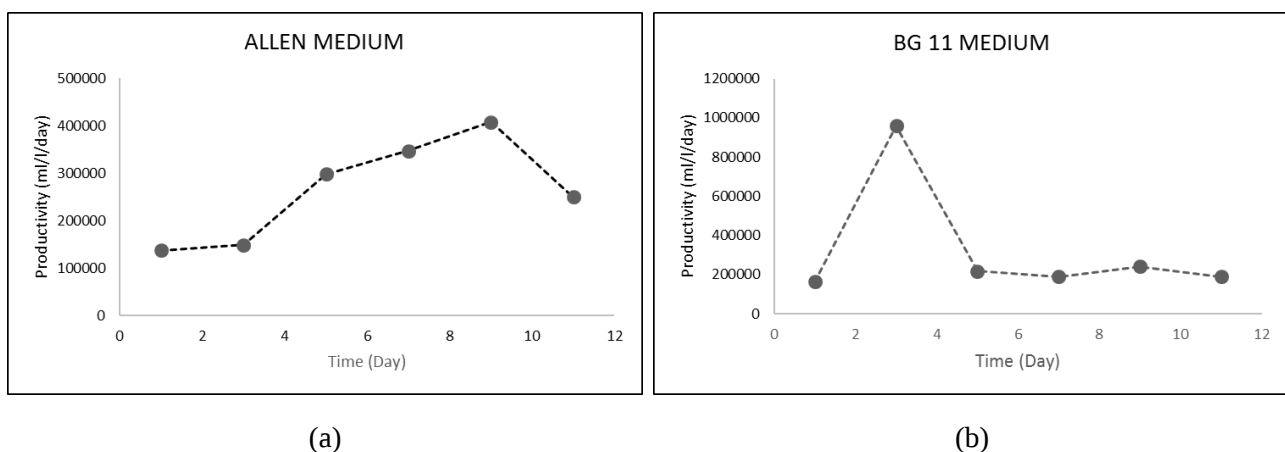


Figure 4. Biomass productivity at different media.

The compositions and the amount of nutrients have great effect on reproduction of the microalgae. The lack of those substances may result in physiological and morphological changes in the microalgae. Especially, microalgae need the macronutrient elements (i.e. carbon, nitrogen, phosphorus), basic ions (i.e. Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , SO_4^{2-}) and micronutrient metals (i.e. iron,

manganese, zinc, cobalt, copper, molybdenum, nickel and cadmium) in their habitats (Duygu Yalçın 2017).

Phosphorus is the most needed macronutrient by the microalgae after nitrogen. Phosphorus is necessary for many phosphorylation syntheses and the Calvin cycle. Therefore, the lack of phosphorus affects not only the synthesis of chlorophyll but also

the growth and metabolism of cells (Liang et al. 2013). The KH_2PO_4 and K_2HPO_4 are the sources of phosphate for algal growth in the present study. Nitrogen is a significant substance for synthesis of protein, nucleic acids and chlorophyll molecules (Lourenço et al. 2004). NaNO_3 and EDTA are the nitrogen sources of both media which were used in this study. The magnesium plays a significant role in the growth of microalgae as a cofactor of some key enzymes in the metabolic pathway (Esakkimuthu et al. 2016). Magnesium source of growth media used in this study was MgSO_4 . MgSO_4 was used in this study as Magnesium source of growth media. Iron is a vital element in algal growth and ferric ammonium citrate and FeCl_3 in the composition of the nutrients serving as the iron source for the culture in this study.

A series of measurements were performed in order to estimate growth rate and to calculate the rate of change occurring in biomass concentration. The cell number was determined through use of light microscope and the linear correlation between those measurements and cell number was determined through optical density.

Both Allen and BG-11 growth media use for *P. boryanum* cultivation with different chemical compositions were studied. Cell density which is an important biomass parameter varied significantly from medium to medium. The present study mainly dealt with the evaluation of growth rate (cell density) of *P. boryanum* which showed variations in their growth pattern in the two-growth media. The growth of *P. boryanum* in different culture media was evaluated by microscopic cell counting value. In general, Allen Medium was found to greatly influence the growth of *P. boryanum* than BG-11 medium. Based on the density concentration measurement, it was observed that the growth of *P. boryanum* was highly favoured by Allen Medium (Figure 1a). For ($n=3$), the cell density was found to be (3.67×10^6 cells/mL) in Allen Medium and (2.19×10^6 cells/mL) in BG-11 Medium at the end of 11th day. The results of this study are similar to those of Park et al. (2014) investigating the growth of *P. boryanum* under controlled conditions. The dry weight and the amount of chlorophyll-a were measured as (0.032 g/mL) and (16.39 $\mu\text{g L}^{-1}$), respectively in Allen Medium and they were found as (0.028 g/mL) and (7.854 $\mu\text{g L}^{-1}$) in BG-11 Medium in paralel with the cell number (Fig. 1b and 1c).

The optical density is an indirect method which is commonly used while measuring biomass of the microalgae, observing and controlling their growth. Through that method, the cell number can be correlated and adapted easily to the automatic

measurement systems (Ribeiro-Rodrigues et al. 2011). In general, 660-690 nm is suggested for standard tests carried out while measuring microalgal growth by spectrometer (Bricaud et al. 1998). Wavelength was scanned from 550 to 800 nm and maximum absorbance was observed at 670 nm for the analysed microalgae in different media. A linear dependence between absorbance and cell counting is assumed when absorbance is measured at the same wavelength (670 nm) for *P. boryanum* (Santos-Ballardo et al. 2015). Growth performance was influenced by media type, which supports higher growth rate (0.6676 d^{-1}) for *P. boryanum* in the Allen medium in comparison with that (0.6021 d^{-1}) in BG-11 culture media tested. That growth rate is among the values stated in the literature for *P. boryanum* (Park and Craggs 2011; Park et al. 2013). Park et al. (2014) found out the maximum growth rate of colony (h^{-1}) 0.097 ± 0.023 at 20°C and (h^{-1}) 0.060 ± 0.002 at 10°C . In that study, a very good positive correlation in both culture media was obtained for *P. boryanum* ($r=0.9762$ and $r=0.9101$, respectively). The fastest doubling time resulting from *P. boryanum* was Allen medium (0.0586) and BG-11 (0.0577), respectively.

Al-Shatri et al. (2014) carried out a study by evaluating the effects of different algal nutrient medium constituents in order to obtain cell number of optimised *Scenedesmus dimorphus*. They stated that they could obtain the highest yield through BG-11 nutrient media after Bold's Basal Medium (BBM). Besides, a study was conducted to determine the most suitable nutrient media with the aim of obtaining high biomass production rate of *Chlorella minutissima* (Singh et al. 2014). Growth measurements revealed that BG-11 medium enhanced biomass production. In the study conducted upon *Lyngbya bipunctata*, the effect of different nutrient medium was examined and it was found that the nutrient medium of BG-11 and Allen increased carotenoid amount with their wet and dry weight (Nehul 2014). The studies conducted upon different microalgae species revealed that BG-11 and Allen nutrient medium were effective in culturing of the microalgae.

The temperature in culture media in the present study was determined to be between $22\text{--}25^\circ\text{C}$ and *P. boryanum* cultures showed good development under that temperature. The light is the energy source of photosynthesis and a necessary factor to transform inorganic carbon into organic molecules and for growth and to obtain energy. Therefore, the cultures were applied 16:8 h light/dark cycles (Falkowski 1984; Wahidin et al. 2013).

In summary, this study mainly focused upon the effect of Allen Medium and BG-11 Medium on the

growth of fresh water microalgae, that is, *P. boryanum*. It was clearly observed that Allen Medium had a greater influence on the growth of *P. boryanum* when compared with BG-11 medium.

Acknowledgements

This study was presented as a paper at “International Symposium on Limnology and Freshwater Fisheries” in October 04-06, 2017.

References

- Ak I, Cirik S, Goksan T. 2008. Effect of light intensity, salinity and temperature on growth in Camalt strain of *Dunaliella viridis* Teodoresco from Turkey. *J Biol Sci.* 8(8): 1356-1359.
doi: 10.3923/jbs.2008.1356.1359
- Al-Shatri AHA, Ali E, Al-Shorgani NKN, Kalil MS. 2014. Growth of *Scenedesmus dimorphus* in different algal media and pH profile due to secreted metabolites. *Afr J Biotechnol.* 13(16):1714-1720.
doi:10.5897/AJB2013.13455
- Baykal Ozer T, Acikgoz Erkaya. I, Udo Udoh A, Yalcın Duygu D, Akbulut A, Bayramoglu G, Arica MY. 2012. Biosorption of Cr (VI) by free and immobilized *Pediastrum boryanum* biomass: equilibrium, kinetic, and thermodynamic studies. *Environ Sci Pollut Res.* 19(7): 2983-2993.
doi: 10.1007/s11356-012-0809-0
- Bourrelly P. 1972. Les Algues D'eau Douce, Tome I: Les Algues Vertes E'ditions N. Boubee & Cie 3, France: Place Saint Andre Des Arts 572p.
- Brennan L, Owende P. 2010. Biofuels from microalgae - a review of technologies for production, processing and extractions of biofuels and co-products. *Renew Sust Energ Rev.* 14(2): 557-577.
doi: 10.1016/j.rser.2009.10.009
- Bricaud A, Morel A, Babin M, Allali K, Claustre H. 1998. Variations of light absorption by suspended particles with chlorophyll a concentration in oceanic (case 1) waters: analysis and implication for bio-optical models. *J Geophys Res.* 103(13): 31033-31044.
doi: 10.1029/98JC02712
- Chia MA, Lombardi AT, Melao MGG. 2013. Growth and biochemical composition of *Chlorella vulgaris* in different growth media. *Annals of the Brazilian Academy of Sciences* 85(4): 1427-1438.
doi: 10.1590/0001-3765201393312
- CSIRO 2017. Commonwealth Scientific and Industrial Research Organization. [Cited 2017 July]. Available from <http://www.csiro.au/>.
- Duygu Yalçın D. 2017. Increasing the protein amount of *Chlorella vulgaris* [Chlorophyta] strains isolated from different fresh water ponds. *Journal of Environmental Science and Engineering B*(6): 201-208.
doi:10.17265/2162-5263/2017.04.003
- Esakkimuthu S, Krishnamurthy V, Govindarajan R, Swaminathan K. 2016. Augmentation and starvation of calcium, magnesium, phosphate on lipid production of *Scenedesmus obliquus*. *Biomass Bioenerg.* 88: 126-134.
doi: 10.1016/j.biombioe.2016.03.019
- Fakhri M, Arifin NB, Budianto B, Yuniarti A, Hariati AM. 2015. Effect of salinity and photoperiod on growth of microalgae *Nannochloropsis* sp. and *Tetraselmis* sp. *Nature Environment and Pollution Technology* 14(3): 563-566.
- Falkowski PG. 1984. Physiological responses of phytoplankton to natural light regimes. *J Plankton Res.* 6(2): 295-307.
doi: 10.1093/plankt/6.2.295
- Godoy-Hernández G, Vázquez-Flota FA. 2006. Growth measurements: estimation of cell division and cell expansion. In: Loyola-Vargas VM, Vázquez-Flota F, editor. *Plant cell culture protocols*. New Jersey (USA): Humana Press Inc. p. 51-58.
- Hosikian A, Lim S, Halim R, Danquah KM. 2010. Chlorophyll extraction from microalgae: A review on the process engineering aspects. *International Journal of Chemical Engineering*. ID 391632: 1-11.
doi:10.1155/2010/391632
- Idenyi JN, Ebenyi LN, Ogah O, Nwali BU, Ogbanshi ME. 2016. Effect of different growth media on the cell densities of freshwater microalgae isolates. *IOSR-JPBS.* 11(3): 24-28.
doi: 10.9790/3008-1103042428
- Kumar K, Das D. 2012. Growth characteristics of *Chlorella sorokiniana* in airlift and bubble column photo-bioreactors. *Bioresour Technol.* 116: 307-313.
doi: 10.1016/j.biortech.2012.03.074
- Liang K, Zhang Q, Gu M, Cong W. 2013. Effect of phosphorus on lipid accumulation in freshwater microalgae *Chlorella* sp. *J Appl Phycol.* 25(1): 311-318.
doi: 10.1007/s10811-012-9865-6
- Lourenço SO, Barbarino E, Lavin PL, Lanfer Marquez UM, Aida E. 2004. Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. *Eur J Phycol.* 39(1): 17-32.
doi: 10.1080/0967026032000157156
- Murdock JN, Wetzel DL. 2009. FT-IR microspectroscopy enhances biological and ecological analysis of algae. *Appl Spectroscopy Rev.* 44(4): 335-361.
doi: 10.1080/05704920902907440
- Nehul JN. 2014. Influence of various culture media on growth and production of carotenoids in a cyanobacterium *Lyngbya bipunctata* Lemm. *Bioscience Discovery* 5(1):60-63.
- Park JBK, Craggs RJ. 2011. Nutrient removal and nitrogen balances in high rate algal ponds with carbon dioxide addition. *Water Sci Technol.* 63(8): 1758-1764.
doi: 10.2166/wst.2011.114
- Park JBK, Craggs RJ, Shilton AN. 2013. Enhancing biomass energy yield from pilot-scale high rate algal ponds with recycling. *Water Res.* 47(13): 4422-4432.
doi: 10.1016/j.watres.2013.04.001
- Park JBK, Craggs RJ, Shilton AN. 2014. Investigating the life-cycle and growth rate of

- Pediastrum boryanum* and the implication for wastewater treatment high rate algal ponds. Water Res. 60: 130-140.
doi: [10.1016/j.watres.2014.04.028](https://doi.org/10.1016/j.watres.2014.04.028)
- Prescott GW. 1975. Algae of the western great lakes area. Michigan: W.M.C. Brown Company Publishers 977p.
- Ribeiro-Rodrigues LH, Arenzon A, Raya-Rodriguez MT, Fontoura NF. 2011. Algal density assessed by spectrophotometry: a calibration curve for the unicellular algae *Pseudokirchneriella subcapitata*. J Environ Chem Ecotoxicol. 3(8): 225-228.
- Santos-Ballardo DU, Rossi S, Hernández V, Gómez RV, Rendón-Unceta MC, Caro-Corrales J, Valdez-Ortiz A. 2015. A simple spectrophotometric method for biomass measurement of important microalgae species in aquaculture. Aquaculture 448: 87-92.
doi: [10.1016/j.aquaculture.2015.05.044](https://doi.org/10.1016/j.aquaculture.2015.05.044)
- Sasson A. 1988. Biotechnologies and development. France: UNESCO Technical Center for Agricultural and Rural Cooperation (CTA) 361p.
- Singh UB, Sharma C. 2014. Determination of suitable growth medium for *Chlorella minutissima*. Inter J of Eng Science & Research 3(8):481-485.
- Spolaore P, Joannis-Cassan C, Duran E, Isambet A. 2006. Commercial applications of microalgae. J Biosci Bioeng. 101(2): 87-96.
doi: [org/10.1263/jbb.101.87](https://doi.org/10.1263/jbb.101.87)
- Wahidin S, Idris A, Shaleh SRM. 2013. The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. Bioresource Technol. 129: 7-11.
doi: [10.1016/j.biortech.2012.11.032](https://doi.org/10.1016/j.biortech.2012.11.032)
- Youngman RE. 1978. Measurement of chlorophyll-a. New York: Water Research Centre Technical Report. Report No.: 82.



Detection of Zooplankton Fauna in Downstream of Euphrates

Ahmet BOZKURT* , M. Ayçe GENÇ 

İskenderun Technical University, Marine Sciences and Technology Faculty, 31200, İskenderun, Hatay, Turkey

ABSTRACT

Zooplankton samples were taken for determination of the zooplankton fauna of the lower Euphrates basin (between Birecik Dam Lake and Karkamış Dam Lake). In the study, 10 families from Rotifera, 6 families from Cladocera and 4 families from Copepoda, totally 20 family were found. A total of 41 zooplankton species were identified. From Rotifera, Lepadellidae was the most species rich family with 4 species, from Cladocera, Daphnidae was the most species rich family with 4 species and from Copepoda, Cyclopoidae was the most species rich family with 7 species.

Keywords: Down the Euphrates, Birecik Dam Lake, Karkamış Dam Lake, zooplankton

ARTICLE INFO

RESEARCH ARTICLE

Received : 14.11.2017

Revised : 06.02.2018

Accepted : 08.02.2018

Published : 27.04.2018



DOI:10.17216/LimnoFish.352108

* CORRESPONDING AUTHOR

ahmet.bozkurt@iste.edu.tr

Tel : +90 326 614 16 93 (3405)

Aşağı Fırat Nehri'nin Zooplankton Faunasının Tespiti

Öz: Aşağı Fırat havzasının (Birecik Baraj Gölü ile Karkamış Baraj Gölü arasındaki) zooplankton faunasının tespiti için Zooplankton örnekleri toplanmıştır. Çalışmada, Rotifera'dan 10, Cladocera'dan 6 ve Copepoda'dan 4 familya olmak üzere toplam 20 familya bulunmuştur. Toplam 41 zooplankton türü tespit edilmiştir. Rotifera'dan Lepadellidae 4 türle, Cladocera'dan Daphnidae 4 tür ile Copepoda'dan Cyclopoidae 7 tür ile en zengin familyaları oluşturmuşlardır.

Anahtar kelimeler: Aşağı Fırat Nehri, Birecik Baraj Gölü, Karkamış Baraj Gölü, zooplankton

Alıntılama

Bozkurt A, Genç MA, 2018. Detection of Zooplankton Fauna in Downstream of Euphrates. LimnoFish. 4(1): 13-16. doi: 10.17216/LimnoFish.352108

Introduction

Wetlands that are regarded as natural resources of the world because of their biodiversity; are the most important ecosystems of the earth with their natural functions and economic values. They regulate the water regime of the region by feeding or discharging underground waters, storing flood waters, controlling floods, and preventing the entry of sea water on coasts. They have a positive effect on the local climatic factors, mainly rainfall and temperature, by raising the humidity in the region.

The primary function of freshwater zooplankton is an important component in aquatic ecosystems, which act as primary and secondary links in the food chain. Zooplankton community structure is affected by physical and chemical environment. These communities are also affected by biological interactions, predation and their competition for food

resources (Neves et al. 2003). Most groups of zooplankton have been used as a bioindicator for monitoring aquatic ecosystems and the integrity of water. Zooplankton community may be considered as a bioindicators of eutrophication, because they are coupled to environmental conditions, responding more rapidly to changes than do fishes, and are easier to identify than phytoplankton. Therefore, they are potential value as water quality indicators (Sládeček 1983; Murugan et al. 1998).

This study was carried out to determine the zooplankton fauna of the lower Euphrates basin, where no studies have been conducted on zooplankton until now.

Materials and Methods

The samples of zooplankton were collected from 3 stations in lower Euphrates basin

(First station 37° 01'11" N, 37° 58' 16" E; second station 36° 57' 44" N, 38° 00' 23" E; third station 36° 53' 52" N, 38° 01' 48" E) (Figure 1) by using a plankton net with 60 µm mesh size. The net was hauled horizontally during 20 minutes in July and September 2015, during routine survey cruises and then samples were replaced into glass jar. The samples were fixed with 4% buffered formaldehyde. The zooplankton species examination was done using an Olympus CH40 microscope. To identify the species, the works of Ruttner-Kolisko (1974), Koste (1978), Segers (1995), Scourfield and Harding (1966), Smirnov (1974), Negrea (1983), Korinek (1987), Pennak (1989), Borutsky (1964), Dussart (1969), Damian-Georgescu (1970), and Kiefer and Fryer (1978) were reviewed.



Figure 1. Study area and sampling stations

Results

In the study a total of 41 species were found, including 19, 12 and 10 taxa belonging to Rotifera, Cladocera and Copepoda, respectively.

Table 1. Zooplankton species in the study area

Species	Stations		
ROTIFERA			
Lepadellidae	1	2	3
<i>Colurella colurus</i> (Ehrenberg, 1830)	-	+	+
<i>Lepadella ovalis</i> (Müller, 1786)	+	+	+
<i>Lepadella patella</i> (Müller, 1773)	+	+	+
<i>Lepadella quadricarinata</i> (Stenroos, 1898)	+	-	+
Lecanidae			
<i>Lecane closterocerca</i> (Schmarda, 1859)	+	+	+
<i>Lecane luna</i> (Müller, 1776)	+	+	+
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	-	-	+
Brachionidae			
<i>Euchlanis dilatata</i> Ehrenberg, 1832	-	+	+
<i>Keratella cochlearis</i> (Gosse, 1851)	+	+	+
<i>Keratella tropica</i> (Apstein, 1907)	+	+	+
Synchaetidae			
<i>Polyarthra dolichoptera</i> Idelson, 1925	+	+	+
<i>Synchaeta stylata</i> Wierzejski, 1893	-	-	+
Trichocercidae			
<i>Trichocerca capucina</i> (Wierzejski & Zacharias, 1893)	-	+	+
<i>Trichocerca elongata</i> (Gosse, 1886)	-	-	+
Dichranophoridae			
<i>Dicranophorus epicharis</i> Harring & Myers, 1928	-	-	+
Asplanchnidae			
<i>Asplanchna priodonta</i> Gosse, 1850	-	+	+
Mytilinidae			
<i>Lophocharis salpina</i> (Ehrenberg, 1834)	-	+	+
Testudinellidae			
<i>Testudinella patina</i> (Hermann, 1783)	+	+	+
Notommatidae			
<i>Cephalodella gibba</i> (Ehrenberg, 1830)	+	+	+
CLADOCERA			
Daphnidae			
<i>Ceriodaphnia pulchella</i> Sars, 1862	+	+	+
<i>Daphnia cucullata</i> Sars, 1862	-	-	+
<i>Daphnia longispina</i> (Mueller, 1785)	+	-	+
<i>Simocephalus expinosus</i> (Koch, 1841)	-	+	+
Chydoridae			
<i>Alona guttata</i> Sars, 1862	+	+	+
<i>Chydorus sphaericus</i> (Müller, 1776)	+	+	+
<i>Grabtoleberis testudinaria</i> (Fischer, 1851)	-	+	+
Eurycercidae			
<i>Camptocercus uncinatus</i> Smirnov 1971	-	+	+
<i>Pleuroxus laevis</i> Sars, 1861	+	-	+
Porcellionidae			
<i>Eurycerus lamellatus</i> (Müller, 1776)	-	+	+
Bosminidae			
<i>Bosmina longirostris</i> (Müller, 1785)	+	+	+
Sididae			
<i>Diaphanosoma birgei</i> Korinek, 1981	-	-	+
COPEPODA			
Cyclopoidae			
<i>Acanthocyclops robustus</i> (Sars, 1863)	-	-	+
<i>Cyclops vicinus</i> Ulyanin, 1875	+	+	+
<i>Diacyclops bicuspidatus</i> (Claus, 1857)	-	+	+
<i>Eucyclops serrulatus</i> (Fischer, 1851)	-	+	+
<i>Macrocyclops albidus</i> (Jurine, 1820)	-	-	+
<i>Megacyclops viridis</i> (Jurine, 1820)	-	+	+
<i>Thermocyclops dybowskii</i> (Landé, 1890)	-	+	+
Diaptomidae			
<i>Acanthodiaptomus denticornis</i> (Wierzejski, 1887)	-	-	+
Ameiridae			
<i>Nitocra hibernica</i> (Brady, 1880)	+	+	+
Canthocamptidae			
<i>Bryocamptus zschokkei</i> (Schmeil, 1893)	-	-	+

Detected ten families from Rotifera, Lepadellidae was the most species rich family with 4 species followed by Lecanidae and Brachionidae with 3 species each one. While Synchaetidae and Trichocercidae were represented by two species, Dichranophoridae, Asplanchnidae, Mytilinidae, Testudinellidae and Notommatidae were represented one species.

Six families were detected from Cladocera, Daphnidae was the most species rich family with 4 species followed by Chydoridae with 3 species. Porcellionidae, Bosminidae and Sididae had the least species followed by Eurycercidae with 2 species. In the Copepoda with four families, Cyclopoidae had 7 species and others, Diaptomidae, Ameiridae and Canthocamptidae had 1 species each one. It was determined that some species with wide spread from Rotifera, *Cephalodella gibba* (Ehrenberg, 1838), *Keratella cochlearis* (Gosse, 1851), *K. tropica* (Apstein, 1907), *Lecane closteroerca* (Schmarda, 1859), *L. luna* (Müller, 1776), *Lepadella ovalis* (Müller, 1786), *L. patella* (Müller, 1786), *Testudinella patina* (Hermann, 1783), from Cladocera *Bosmina longirostris* (Müller, 1785), *Ceriodaphnia pulchella* Sars, 1862, *Alona guttata* Sars, 1862, *Chydorus sphaericus* (Müller, 1776), from Copepoda *Cyclops vicinus* Uljanin, 1875, *Nitocra hibernica* (Brady, 1880) were present at all sampling stations. At the same time some species were found only one station and a few amount. These species from Rotifera *Dichranophorus epicharis*, *Lecane quadridentata*, *Synchaeta stylata*, *Trichocerca elongata*, from Cladocera *Daphnia cucullata*, *Diaphanosoma birgei*, from Copepoda *Acanthocyclops robustus*, *Macrocyclops albidus*, *Acanthodiaptomus denticornis* and *Bryocamptus zschokkei* were found in only one station.

Discussion

No studies have been found on zooplankton in the Downstream of Euphrates. But some zooplankton studies found belong to the upper parts of the Euphrates River. These studies; Saler et al. (2015) reported that they found 32 species from Rotifera, 5 species from Cladocera and 2 species from Copepoda. Rabee (2010) reported that 32 taxa belonged to Rotifera, 12 to the Cladocera and 7 to the Copepoda in Euphrates River in the North part of Iraq. Saler et al. (2014) declared that they found 15 species from Rotifera, 6 species from Cladocera and 2 species from Copepoda were identified in Uzunçayır Dam Lake in the North part of the Euphrates River. Bulut and Saler (2014) declared that they found in 25 species from Rotifera, 6 species from Cladocera and 2 species from Copepoda in Murat River (between Elazığ and Palu). It is seen that

there are significant differences in species diversity between our study and other studies. The reason of this, Euphrates system is a very large river system. In order to it passes through two geographical regions, it is under the influence of different climate zone. Thus its zooplankton content varies considerably in terms of quality and quantity.

Rotifera is the dominant group among all zooplankton groups qualitatively and quantitatively in freshwater ecosystem (Saksena 1987). The result obtained in the study was accordance with results of Saksena (1987).

It was reported that almost all species found in the study wide spread, common, cosmopolitan (Eldredge and Evenhuis 2003; Hutchinson 1967; Ruttner-Kolisko 1974; Braioni and Gelmini 1983; Ramdani et al. 2001) and they were reported from lots of study inland waters of Turkey (Ustaoğlu et al. 2004).

The presence of identified species in the study seems to be compatible with their ecological characters.

References

- Borutsky EV. 1964. Freshwater Harpacticoida. Fauna of U.S.S.R. (Crustacea), Vol. 3. Jerusalem: Israel Program for Scientific Translations 607 p.
- Braioni MG, Gelmini D. 1983. Guide per il riconoscimento delle specie animali delle acque interne Italiane: Rotiferi monogononti. Italy: Consiglio Nazionale delle Ricerche, 181p.
- Bulut H, Saler S. 2014. Murat Nehri'nin (Elazığ-Palu ilçe merkezi sınırları içindeki bölümün'de) zooplanktonu ve değişimi. Türk Tar Gıda Bil ve Tek Derg. (TURJAF). 2(1): 13-17.
[doi: 10.24925/turjaf.v2i1.13-17.32](https://doi.org/10.24925/turjaf.v2i1.13-17.32)
- Damian-Georgescu A. 1970. Fauna republicii socialiste Romania, crustacea. Vol. IV. 11 Copepoda, harpacticoida. Bucharest: Academiei Republicii socialiste Romania 249 p.
- Dussart BH. 1969. Les copépodes des eaux continentales d'europe occidentale, 2: Cyclopoides et biologie. Paris: N. Boubée et Cie. 500 p.
- Eldredge LG, Evenhuis NL. 2003. Hawaii's biodiversity: a detailed assessment of the numbers of species in the Hawaiian Islands. Bishop Mus Occas Pap. 76:1-28
- Hutchinson GE. 1967. A treatise on limnology. Vol. 2: Introduction to lake biology and the limnoplankton. New York: Wiley 1115 p.
- Kiefer F, Fryer G. 1978. Das Zooplankton der Binnengewässer. Teil 2. Stuttgart: E. Schweizerbartsche Verlag, 380 p.
- Korinek V. 1987. Revision of three species of the genus *Diaphanosoma* Fischer, 1850. Hydrobiologia 145 (1): 35-45.
- Koste W. 1978. Rotatoria. Die radertiere mittel-europas, 2nd ed. Berlin and Stuttgart: Gebruder borntraeger 673p.

- Murugan N, Murugavel P, Kodarkar MS. 1998. Cladocera: The biology, classification, identification and ecology. Indian Association of Aquatic Biologists (IAAB), Hyderabad.
- Negrea ST. 1983. Fauna republicii socialiste Romania. vol. 4, 12. Crustacea Cladocera. Bucharest: Academia Republicii Socialiste Romania 399 p.
- Neves IF, Rocha D, Roche KF, Pinto AA. 2003. Zooplankton community structure of two marginal lake of river (Cuiaba) (Mato, Grosso, Brazil) with analysis of rotifer and Cladocera diversity. *Braz J Biol.* 63 (2): 329–343.
[doi:10.1590/S1519-69842003000200018](https://doi.org/10.1590/S1519-69842003000200018)
- Pennak RW. 1989. Coelentera. In: fresh-water invertebrates of the United States: Protozoa to mollusca, 3rd edition. New York: John Wiley & Sons p. 110–127.
- Rabee AM. 2010. The effect of al-tharthar-euphrates canal on the quantitative and qualitative composition of zooplankton in euphrates River. *Journal of Al-Nahrain University.* 13 (3): 120-128
- Ramdani M, Flower RJ, Elkhiahi N. 2001. Zooplankton (Cladocera, Ostracoda), chironomidae and benthic fauna remains in sediment cores from nine North African wetland lakes: The CASSARINA Project. *Aquat Ecol.* 35 (3-4): 389–403.
[doi:10.1023/A:1011965226399](https://doi.org/10.1023/A:1011965226399)
- Ruttner-Kolisko A. 1974. Plankton rotifers. biology and taxonomy. *Die Binnengewässer* V. 26, Part 1. Stuttgart: Schweizerbart. 146 p.
- Saksena ND. 1987. Rotifers as indicator of water quality. *Acta Hydrochimica et Hydrobiologica.* 15(5): 481-485.
[doi: 10.1002/aheh.19870150507](https://doi.org/10.1002/aheh.19870150507)
- Saler S, Bulut H, Birici N, Tepe R, Alpaslan K. 2015. Karasu Nehri (Erzincan)'nin zooplanktonu. *Eğirdir Su Ürün Fak Derg.* 11(1):10-16.
[doi: 10.22392/egirdir.246346](https://doi.org/10.22392/egirdir.246346)
- Saler S, Haykır H, Baysal N. 2014. Zooplankton of Uzunçayır Dam Lake. *J FisheriesSciences.com.* 8(1):1-7.
[doi: 10.3153/jfsc.com.2014001](https://doi.org/10.3153/jfsc.com.2014001)
- Scourfield DJ, Harding JP. 1966. A key to the british freshwater cladocera. England: Fresh Biol. Ass.Sci. Publ. No. 5. 61 p.
- Segers H. 1995. “World records of Lecanidae (Rotifera: Monogononta).” *Studiedocumenten Van Het Koninklijk Belgisch Instituut Voor Natuurwetenschappen*, 81, 114 p.
- Sládeček V. 1983. Rotifers as indicators of water quality. *Hydrobiologia.* 100 (1): 169-201.
[doi: 10.1007/BF00027429](https://doi.org/10.1007/BF00027429)
- Smirnov NN. 1974. Fauna of the U.S.S.R. Crustacea. Vol. 1. No. 2, Chydoridae, Jerusalem: I.P.S.T. 644 pp.
- Ustaoglu MR, Balık S, Özdemir Mis, D. 2004. The rotifer fauna of Lake Sazlıgöl (Menemen, İzmir). *Turk J Zool.* 28:267-272.



The Relationships Between Otolith Dimensions-Total Length of Chub (*Squalius cephalus*, L.1758) Sampled from Some Inland Waters of the Middle Black Sea Region

Melek ZENGİN ÖZPİÇAK*^{ID}, Semra SAYGIN^{ID}, Aykut AYDIN^{ID}, Enes HANÇER^{ID}, Savaş YILMAZ^{ID}, Nazmi POLAT^{ID}

Ondokuz Mayıs University, Faculty of Art and Sciences, Biology Department, 55139 Atakum, Samsun, Turkey

ABSTRACT

In this study, the relationships between otolith dimensions and total length of chub (*Squalius cephalus*, L.1758) sampled from a few inland waters of Black Sea was researched. Chub samples were obtained from the four different localities (Abdal, Akçay, Terme and Yedikır). Power models were applied to estimate the relationships between the otolith measurements and total length (TL). Paired t-test, independent t test and ANOVA were done to test the data statistically. The average total length of individuals sampled from Abdal, Akçay and Terme Streams, Yedikır Dam Lake varies between 11.49 ± 0.79 cm, 10.31 ± 0.52 cm, 10.33 ± 0.29 cm and 11.11 ± 0.33 cm, respectively. When all the data were evaluated according to localities, it was found that there was no difference in terms of otolith breadth (OB) and otolith length (OL) for asteriscus and lapillus otoliths. There were differences in terms of otolith weight of lapillus in localities. There were no differences of asteriscus otolith weight of chub between localities. The relationships between TL and OB, OL and OW were determined using the power regression equation and best fit was obtained between TL and OW for Terme ($r^2=0.936$) and Yedikır ($r^2=0.912$), OL for Akçay and Abdal Streams ($r^2=0.973$).

Keywords: Chub, otolith morphometrics, total length, population, Black Sea

ARTICLE INFO

RESEARCH ARTICLE

Received : 09.10.2017

Revised : 19.03.2018

Accepted : 26.03.2018

Published : 27.04.2018

DOI:10.17216/LimnoFish.342524



* CORRESPONDING AUTHOR

melek.zengin@omu.edu.tr

Tel : +90 362 312 19 19

Orta Karadeniz Bölgesi'ndeki Bazı Akarsulardan Örneklenen Tatlısu Kefali (*Squalius cephalus*, L. 1758)'nin Otolit Özellikleri-Total Boy Arasındaki İlişkiler

Öz: Bu çalışmada Karadeniz Bölgesi'ndeki bazı içsularda yaşayan tatlısu kefalinin (*Squalius cephalus*, L.1758) total boy ile otolit özellikleri arasındaki ilişkiler araştırılmıştır. Tatlısu kefalii örnekleri 4 farklı lokaliteden (Abdal Çayı, Akçay, Terme Çayı ve Yedikır Baraj Gölü) yakalanmıştır. Otolit ölçümleri ile total boy arasındaki ilişkilerin hesaplanması için power model kullanılmıştır. İstatistiksel analizler Paired t-test, Independent t test, ANOVA ile test edilmiştir. Abdal, Akçay, Terme Çayı ve Yedikır Baraj Gölü'nden elde edilen bireylerin ortalama total boyları sırası ile $11,49 \pm 0,79$ cm, $10,31 \pm 0,52$ cm, $10,33 \pm 0,29$ cm ve $11,11 \pm 0,33$ cm arasında değişmektedir. Lokalitelere göre tüm veriler birlikte değerlendirildiğinde asteriskus ve lapillus otolitlerinin otolit enleri (OB) ve otolit boyları (OL) arasında bir farklılık bulunmamaktadır. Fakat dört lokalite birlikte değerlendirildiğinde lapillus otolit ağırlıkları arasında fark mevcuttur. Lokalitelere göre asteriskus otolit ağırlıkları bakımından bir farklılık mevcut değildir. TL ve OB, OL ile OW arasındaki ilişkiler power regresyon modeli kullanılarak belirlenmiştir. Terme ($r^2=0,936$) ve Yedikır için OW ($r^2=0,912$), Akçay ve Abdal Çayları için OL ($r^2=0,973$) değerlerinin TB ile en kuvvetli ilişkileri gösterdiği belirlenmiştir.

Anahtar kelimeler: Tatlısu kefalii, otolit özellikleri, total boy, popülasyon, Karadeniz

How To Cite

Zengin Özpiçak M, Saygin S, Aydın A, Hançer E, Yılmaz S, Polat N. 2018. The Relationships Between Otolith Dimensions-Total Length of Chub *Squalius cephalus*, L.1758) Sampled from Some Inland Waters of the Middle Black Sea Region. LimnoFish. 4 (1): 17-24. doi: 10.17216/LimnoFish.342524

Introduction

Cyprinidae is one of the widespread fish family all over the world (Bănărescu and Coad 1991; Kottelat and Freyhof 2007). *Squalius cephalus*

(chub) (L., 1758) is one of the most common freshwater fish species in Cyprinidae. The chub is distributed in the whole of Europe, the Black Sea, the Azov Sea, the Caspian Sea, and Anatolian inland

waters (Bulut et al. 2012). *S. cephalus* is an opportunistic species and common in almost all running waters in Turkey (Bogutskaya 1997). There are 21 species belongs to genus *Squalius* in freshwaters of Turkey (Froese and Pauly 2017). Chub prefers fresh, clean, and fast-flowing waters, as well as dam lakes (Kottelat and Freyhof 2007). *S. cephalus* have fusiform body covered with large-sized cyloid scales and larger mouth. The lips are weakly developed and not keratinized. Ventral and anal fins are orange-yellow color and pigment-free (Polat and Uğurlu 2011). There is a row of black pigments along the free margin of each flank scale and no or very few pigments on central parts of scales (Kottelat and Freyhof 2007). There are lots of studies about genetic, biological characteristic, population parameters and systematics of chub (Altındağ 1996; Laroche et al. 1999; Arlinghaus and Wolter 2003; Vlach et al. 2005; Koç et al. 2007; Turan et al. 2007; Bostancı and Polat 2009; Dehais et al. 2010; Innal 2010; Bulut et al. 2012; Demiroğlu et al. 2016; Cejko and Krejszef 2016; Gouskov 2016; Kılıç and Becer 2016; Özcan et al. 2017). However, studies about the otolith features of chub are limited (Tarkan et al. 2007; Bostancı 2009).

Otoliths continuously accumulate layers of calcium carbonate and trace elements, creating daily and seasonal records of age (Campana 1999; Hart et al. 2015). Morphological and morphometric characteristics of otoliths comprise an important instrument for species identification (Tuset et al. 2008). The particularity about the fish otoliths was first observed by Aristotle in the third century (Stinton 1975), their taxonomic utility was recognized by Cuvier (Cuvier and Valenciennes 1836). Otolith morphology varies between species, however separate stocks of the same species, often identical physically can sometimes be discriminated through subtle differences in otolith morphometrics (Bolles and Begg 2000; Tuset et al. 2003; Zengin et al. 2015; Ibáñez et al. 2017; Mapp et al. 2017). Furthermore, the relationship between the fish size and otolith dimensions have several benefits in estimating the size of the prey. Fish size and/or weight can be functionally related to an appropriate otolith measurement (width, length, or weight) and the resulting relationships can subsequently be used for size estimation (Nolf 1985; Pierce et al. 1991; Tollit et al. 1997; Granadeiro and Silva 2000). When the relationships between otolith dimensions and total length in a species is determined, the total length or standard length of a fish from its otolith dimensions can be estimated, or vice versa (Sen et al. 2001; Battaglia et al. 2010; Başusta et al. 2013; Yılmaz et al. 2014; Saygin et al. 2017; Yazicioğlu et al. 2017; Zengin et al. 2017).

The aim of this study was to detect the relationships between the total length and otolith characteristics of chub sampled from the four different localities (Abdal Stream, Akçay Stream, Terme Stream and Yedikır Dam Lake) along inland waters of the Middle Black Sea Region. This study is first study that examined the relationships between total length and otolith morphometrics of *S. cephalus* sampled from Abdal, Akçay, Terme Streams and Yedikır Dam Lake.

Materials and Methods

S. cephalus samples obtained from the four different localities (Abdal Stream (n=44), Akçay Stream (n=57), Terme Stream (n=55) and Yedikır Dam Lake n=62) (Figure 1). SAMUS 725 MP shocker were used to capture fish samples. Samples were collected between October 2015-April 2017.

The systematic positions of the samples were determined using various identification keys (Geldiay and Balık 2007; Kottelat and Freyhof 2007; Polat and Uğurlu 2011). All captured fish were measured to the nearest 0.1 cm for total length (TL) and weighted to the nearest 0.01 g. The sex was determined by macroscopic examination of the gonads. Utricular (lapillus) and lagenar (asteriscus) otoliths were removed by making left and right distinctions. Otoliths were weighted using Precisa precision scales (OW) (± 0.0001 g). All otoliths were photographed on the distal side with a Leica DFC295 digital camera. Otolith breadth (OB) and length (OL) (± 0.001 mm) were determined by Leica Application Suit Ver. 3.8 Imaging Software. OL was defined as the greatest distance between anterior and posterior edges, and OB was defined as the greatest distance from dorsal to ventral edges (Battaglia et al. 2010) (Figure 2).

Linear and nonlinear models were applied to estimate the relationships between the otolith measurements (OL, OB, OW) and TL.

$$y=ab^x \text{ and } y=a+bx$$

where y is otolith measurement and x is fish length (Zar 1999).

However, in evaluating the relationships between TL and otoliths dimensions, the power model is preferred because of its higher r^2 values. All data were tested by Kolmogorov-Smirnov if the data is normally distributed ($P>0.05$). Data were analysed statistically by Paired t-test, Wilcoxon test, Independent Two Sample t test, Mann-Whitney U test and ANOVA-Tukey test. SPSS 20, Minitab 17.0 and the Excel software were utilized in the evaluation of data.

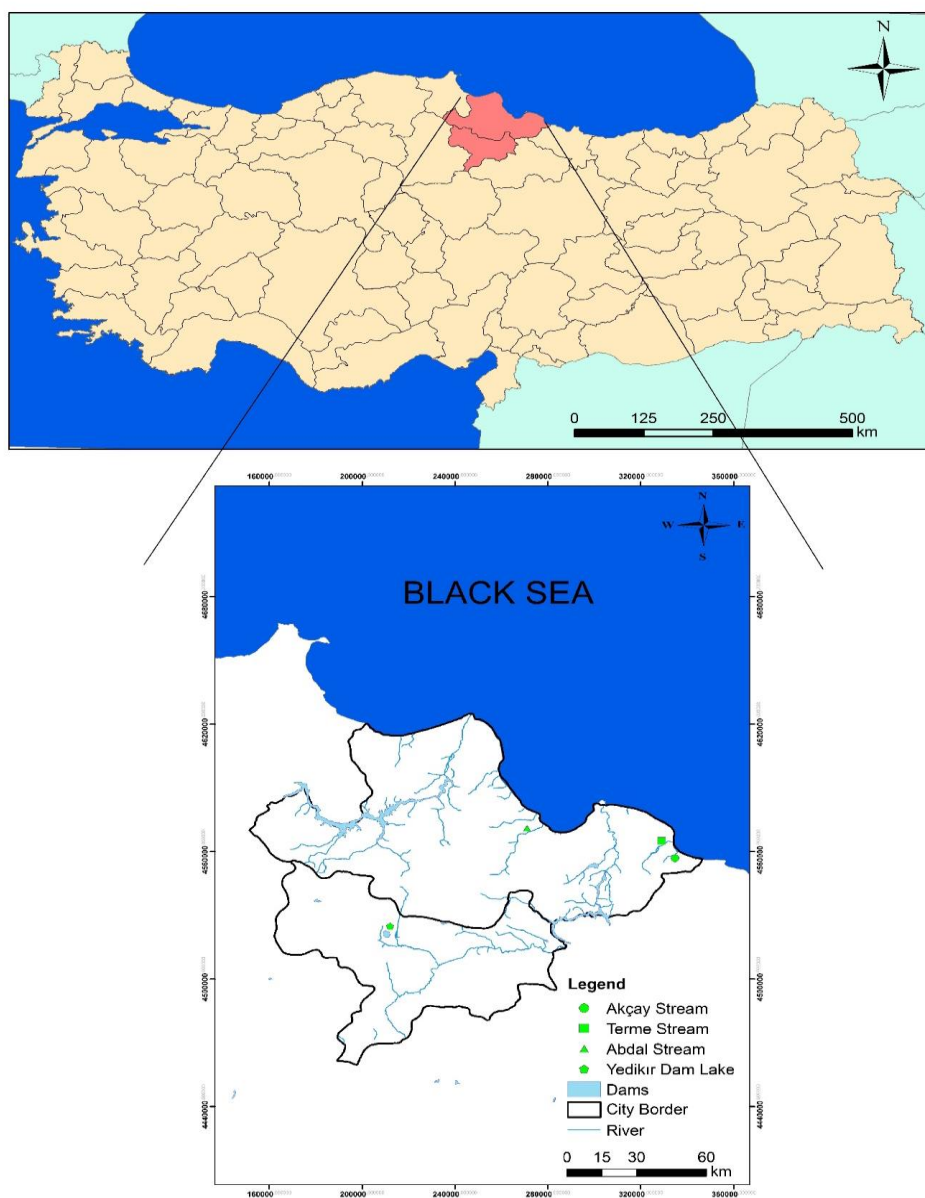


Figure 1. The map of sampling area

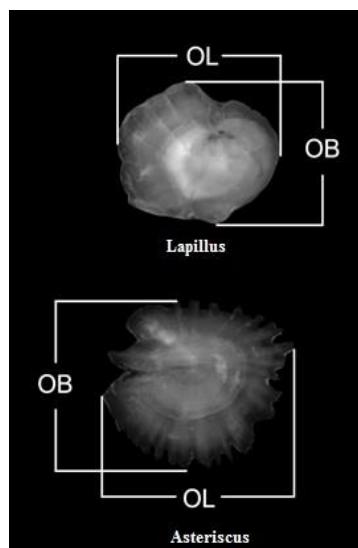


Figure 2. OB and OL measurements of lapillus and asteriscus otoliths.

Results

The descriptive statistics of chub samples from four locality indicated in Table 1. There is no differences in terms of *TL* or *W* between localities ($P>0.05$).

When the asteriscus otoliths of female and male were compared there is no difference in terms of *OB*, *OL* and *OW* in Abdal, Akçay and Terme samples ($P>0.05$). However, *OL* and *OB* of Yedikır samples were different from each other ($P<0.05$) for asteriscus otoliths of female and male. In the same way, no difference could be determined *OB*, *OL* and *OW* for Abdal and Terme whereas the *OL* of Akçay samples and *OL* and *OB* of Yedikır samples were different in

the lapillus otoliths between female and male individuals (Table 2).

The differences between otolith parameters between the right and left otolith pairs of asteriscus and lapillus were determined using appropriate tests. Wilcoxon test were used for this comparisons. Right and left asteriscus otoliths were compared there was no significant differences in terms of otolith length, breadth and weight for all localities ($P>0.05$) (Figure 3). But when right and left lapillus otoliths were compared, there was a statistically significant difference in terms of otolith length of Abdal, Akçay and Terme Streams and otolith breadth and weight of Yedikır Dam Lake Samples (Table 2).

Table 1. Descriptive statistics of *S. cephalus*

Locality	Coordinates	Parameter	N	Mean	S.d	Min.	Max.	S.e.
Abdal Stream	41°08'59.56"N 36°39'34.88" E	Total Length	44	11.49	5.26	5.60	29.40	0.79
		Weight	44	33.59	64.36	1.82	328.1	9.70
Akçay Stream	41°05'30.99"N 37°07'20.89" E	Total Length	57	10.31	3.91	5.80	18.00	0.52
		Weight	57	18.81	19.37	1.79	67.57	2.57
Terme Stream	41°09'34.03" N 36°53'28.48" E	Total Length	55	10.33	2.15	6.40	15.60	0.29
		Weight	55	13.18	8.61	2.39	46.99	1.16
Yedikır Dam Lake	40°47'11.00" N 35°33'47.55" E	Total Length	62	11.11	2.58	6.70	17.70	0.33
		Weight	62	20.18	16.75	3.39	71.30	2.13

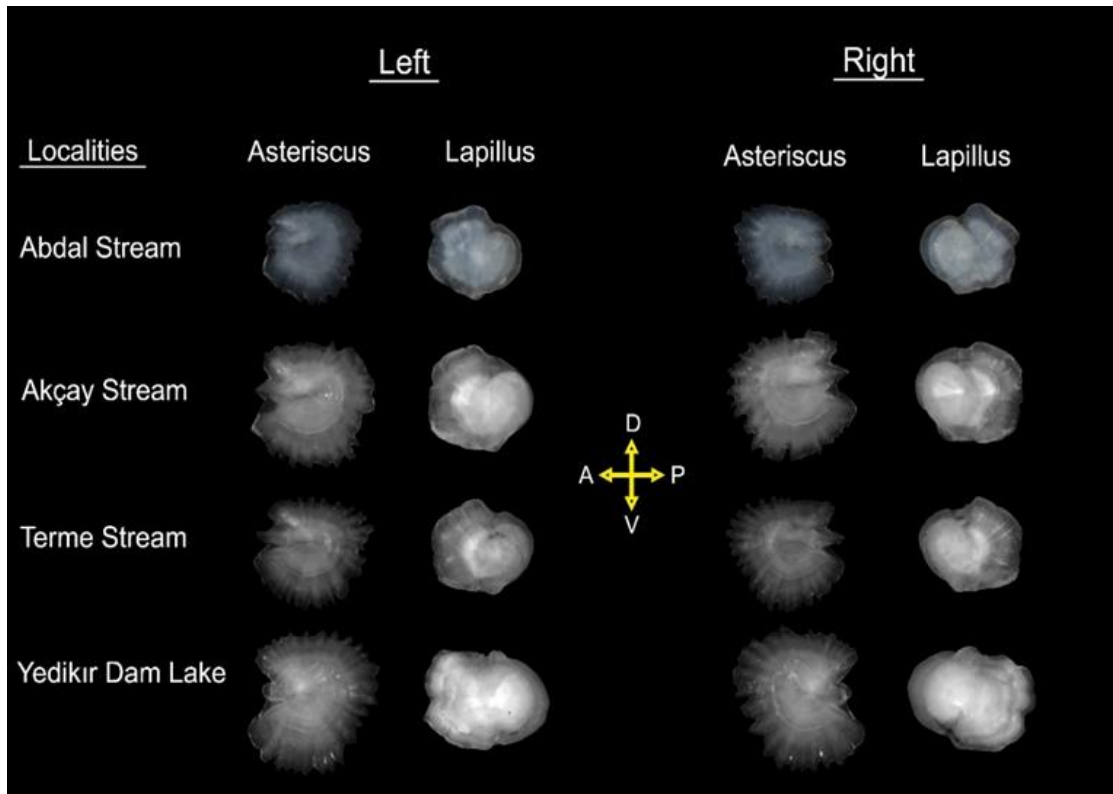


Figure 3. Right and left otolith pairs of *S. cephalus* from different localities (D: Dorsal, V: Ventral, P: Posterior, A: Anterior)

Table 2. Statistical comparisons between right and left otolith pairs of asteriscus and lapillus

Localite	Variable	Mean±S.e	Significant
Abdal Stream	Right-left asteriscus otolith length	1.60±0.61	P> 0.05
	Right-left asteriscus otolith breadth	1.49±0.51	P> 0.05
	Right-left asteriscus otolith weight	0.0023±0.002	P> 0.05
	Right-left lapillus otolith length	1.83±0.53	P<0.05*
	Right-left lapillus otolith breadth	1.24±0.041	P> 0.05
	Right-left lapillus otolith weight	0.0036±0.004	P> 0.05
Akçay Stream	Right-left asteriscus otolith length	1.53±0.58	P> 0.05
	Right-left asteriscus otolith breadth	1.43±0.50	P> 0.05
	Right-left asteriscus otolith weight	0.0019±0.002	P> 0.05
	Right-left lapillus otolith length	1.35±0.46	P<0.05*
	Right-left lapillus otolith breadth	1.14±0.46	P> 0.05
	Right-left lapillus otolith weight	0.0030±0.002	P> 0.05
Terme Stream	Right-left asteriscus otolith length	1.44±0.31	P> 0.05
	Right-left asteriscus otolith breadth	1.36±0.28	P> 0.05
	Right-left asteriscus otolith weight	0.0016±0.0008	P> 0.05
	Right-left lapillus otolith length	1.32±0.27	P<0.05*
	Right-left lapillus otolith breadth	1.14±0.14	P> 0.05
	Right-left lapillus otolith weight	0.0023±0.0012	P> 0.05
Yedikır Dam Lake	Right-left asteriscus otolith length	1.55±0.36	P> 0.05
	Right-left asteriscus otolith breadth	1.46±0.32	P> 0.05
	Right-left asteriscus otolith weight	0.0016±0.0001	P> 0.05
	Right-left lapillus otolith length	1.37±0.33	P> 0.05
	Right-left lapillus otolith breadth	1.10±0.26	P<0.05*
	Right-left lapillus otolith weight	0.0022±0.0014	P<0.05*

*Statistically different

Table 3. Equations of relationships between TL and otolith characteristics of *S. cephalus*

Locality	Asteriscus			Lapillus		
	Formula	r ²	P	Formula	r ²	P
Abdal Stream	$OL=0.188TL^{0.882}$	0.973	<0.001	$OL=0.210TL^{0.819}$	0.966	<0.001
	$OB=0.212TL^{0.806}$	0.965	<0.001	$OB=0.184TL^{0.788}$	0.929	<0.001
	$OW=1.238E-0.05TL^{2.061}$	0.945	<0.001	$OW=1.841E-0.05TL^{2.078}$	0.948	<0.001
Akçay Stream	$OL=0.157TL^{0.974}$	0.975	<0.001	$OL=0.171TL^{0.887}$	0.955	<0.001
	$OB=0.180TL^{0.893}$	0.971	<0.001	$OB=0.114TL^{0.987}$	0.946	<0.001
	$OW=7E-0.06TL^{2.279}$	0.940	<0.001	$OW=7E-0.06TL^{2.409}$	0.963	<0.001
Terme Stream	$OL=0.231TL^{0.784}$	0.609	<0.001	$OL=0.249TL^{0.715}$	0.562	<0.001
	$OB=0.249TL^{0.727}$	0.584	<0.001	$OB=0.166TL^{0.818}$	0.652	<0.001
	$OW=4E-0.06TL^{2.557}$	0.936	<0.001	$OW=7E-0.06TL^{2.501}$	0.9222	<0.001
Yedikır Dam Lake	$OL=0.278TL^{0.719}$	0.526	<0.001	$OL=0.216TL^{0.760}$	0.722	<0.001
	$OB=0.237TL^{0.746}$	0.597	<0.001	$OB=0.152TL^{0.823}$	0.843	<0.001
	$OW=6E-0.06TL^{2.320}$	0.912	<0.001	$OW=9E-0.06TL^{2.234}$	0.933	<0.001

Discussion

Many studies have been carried out in order to separate the stocks of the same species living in different localities using various methods. In recent years, studies using phenotypic, genetic methods and various bony structures for

stock separations have increased (Renán et al. 2004; Ibáñez et al. 2007; Ramírez- Pérez et al. 2010; Kohestan-Eskandari et al. 2013; Verma et al. 2014; Renán et al. 2016; Saygin et al. 2017; Ibáñez et al. 2017). The shape and morphometrics of otoliths reflects phenotype and development stage and is

influenced by factors such as sex, body condition, age, year-class, and stock as well as local environmental conditions (Mérigot et al. 2007; Vignon and Morat 2010).

Otoliths are an indirect method for studying fish populations and assessing the relationship between the environment and organisms. Relationships between bony structure dimensions and fish length are commonly used in fisheries science. These mathematical associations enable the back-calculation of fish length in previous ages (Casselman 1990). In addition, otolith studies particularly have a very important place in species identification from the discovery of fossiliferous layers in archaeological sites and prey-predator relations (Tuset et al. 2008).

Chub belongs to Cyprinidae family and prefers fresh, clean, and fast-flowing waters, as well as dam lakes (Kottelat and Freyhof 2007). This fish species were especially preferred for sport fishing. There are some studies about genetics, age, growth, feeding, length-weight relationships and reproduction features of the chub inhabiting European and Turkish waters (Turan et al. 2007; Sen and Saygın 2008; Stefanova et al. 2008; Innal 2010; Özuluğ and Freyhof 2011; Cejko and Krejszeff 2016; Özcan et al. 2017). It is a widely distributed fish species in Turkey. Particularly fish length-otolith biometry studies are important evidence that can be used to determine the size distributions of fish consumed by predators. In this study, otolith dimensions and total length relationships of chub from four different localities were investigated. Linear and nonlinear functions are preferred to describe otolith size-fish size relationships (Şen et al. 2001; Morley and Belchier 2002; Waessle et al. 2003; Tarkan et al. 2007; Battaglia et al. 2010; Kumar et al. 2012; Škeljo and Ferri 2012; Basusta et al. 2013; Felix et al. 2013; Yilmaz et al. 2014; Jawad et al. 2017). In this study, nonlinear equation was preferred for total length and otolith dimensions relationships because of higher r^2 values.

Fish size-otolith size relationships will be useful for researchers examining food habits of piscivores and size of fish in archaeological samples (Harvey et al. 2000). Bostancı (2009) was investigated relationships between fork length and otolith characteristics of chub and found strong relationships, too. In conclusion, when the relationships between fish length and otolith measurements were evaluated, otolith weight for Terme and Yedikır and otolith length for Abdal and Akçay Streams were found to be the best indicator for estimating the length of fish.

References

- Altındağ A. 1996. Some population characteristics growth and condition of chub (*Leuciscus cephalus*, Linne 1758) in Akşehir Lake. Tr J of Zool. 20(1):53-65.
- Arlinghaus R, Wolter C. 2003. Amplitude of ecological potential: chub *Leuciscus cephalus* (L.) spawning in an artificial lowland canal. J Appl Ichthyol. 19(1): 52-54. doi: 10.1046/j.1439-0426.2003.00343.x
- Bănărescu P, Coad, BW. 1991. Cyprinids of Eurasia. in: Cyprinid fishes. Systematics, biology and exploitation. London: Chapman and Hall. p. 127-155.
- Başusta A, Bal H, Aslan E. 2013. Otolith biometry-total length relationships in the population of Hazar Bleak, *Alburnus heckeli* (Battalgil, 1943) inhabiting Lake Hazar, Elazığ, Turkey. Pak J Zool. 45(1): 1180-1182.
- Battaglia P, Malara D, Romeo T, Andaloro F. 2010. Relationship between otolith size and fish size in some mesopelagic and bathypelagic species from the Mediterranean Sea (Strait of Messina), Ital Sci Mar. 74(1): 605-612. doi: 10.3989/scimar.2010.74n3605
- Bogutskaya NG. 1997. Contribution to the knowledge of leuciscine fishes of Asia Minor. Part 2. An annotated checklist of Leuciscine fishes (Leuciscinae, Cyprinidae) of Turkey with descriptions of a new species and two new subspecies. Mitt. Hamburg Zool. Inst. 94(1): 161-186.
- Bolles KL, Begg GA. 2000. Distinction between silver hake (*Merluccius bilinearis*) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometrics. Fish Bull. 98(1): 451-462.
- Bostancı D. 2009. Otolith biometry-body length relationships in four fish species (chub, pikeperch, crucian carp, and common carp). J Freshwater Ecol. 24(4): 619-624. doi: 10.1080/02705060.2009.9664339
- Bostancı D, Polat N. 2009. Age determination and some population characteristics of chub (*Squalius cephalus* L. 1758) in the Çamlıdere Dam Lake (Ankara, Turkey). Turk J Sci & Technol. 4(1): 25-30.
- Bulut S, Mert R, Konuk M, Algan B, Alaş A, Solak K. 2012. The variation of several biological characteristics of the chub, *Squalius cephalus* (L., 1758), in the Örenler Dam Lake, Northwest Anatolia, Turkey. Not Scientia Biol. 4(3):27-32.
- Campana SE. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Progr Ser. 188(1): 263-297.
- Casselman JM. 1990. Growth and relative size of calcified structures of fish. Trans of the Am Fish S. 119: 673-688. doi: 10.1577/1548-8659(1990)
- Cejko BI, Krejszeff S. 2016. Sperm characteristics of chub *Leuciscus cephalus* (L.) collected in artificial condition after Ovopel and Ovaprim treatment. Aqua Res. 47(3):1-10. doi: 10.1111/are.12544
- Cuvier G, Valenciennes A. 1836. Histoire naturelle des poissons. Tome cinquième. Livre cinquième. Des Sciénoïdes, 5(1):1-499.

- Dehais C, Eudeline R, Berrebi P, Argillier C. 2010. Microgeographic genetic isolation in chub (Cyprinidae: *Squalius cephalus*) population of the Durance River: estimating fragmentation by dams. *Ecol Freshw F.* 19(1):267-278.
doi: 10.1111/j.1600-0633.2010.00411.x
- Demirel F, Gündüz F, Yıldız N, Kurtoglu M, Çoban MZ, Yüksel F. 2016. Some growth parameters of chub (*Squalius cephalus*) living in Uzunçayır Dam Lake (Tunceli-Turkey). *LimnoFish.* 2(2): 67-76 [in Turkish].
doi: 10.17216/LimnoFish-5000125245
- Felix VR, Martinez-Perez JA, Molina JR, Zuniga REQ, Lopez JF. 2013. Morphology and morphometric relationships of the sagitta of *Diapterus auratus* (Perciformes: Gerreidae) from Veracruz, Mexico. *Rev Biol Trop.* 61(1): 139-147.
doi: 10.15517/rbt.v61i1.10938.
- Froese RD, Pauly D. 2017. Editors. 2015. FishBase. [cited 2016 Jan 25]. Available from www.fishbase.org, version (10/2015).
- Geldiay R, Balık S. 2007. Türkiye tatlısu balıkları. Bornova: Ege Üniversitesi Basımevi. 644p. [in Turkish].
- Gouskov A. 2016. Impacts of river fragmentation on the genetic population structure of the chub (*Squalius cephalus*) [PhD Thesis]. ETH Zurich. 131 p. [in English].
- Granadeiro JP, Silva MA. 2000. The use of otoliths and vertebrae in the identification and size-estimation of fish in predator-prey studies. *Cybiuim*, 24(4):383-393.
- Hart LM, Bond MH, May-McNally SL, Miller JA, Quinn TP. 2015. Use of otolith microchemistry and stable isotopes to investigate the ecology and anadromous migrations of Northern Dolly Varden from the Egegik River, Bristol Bay, Alaska. *Environ Biol Fish.* 98(1):1633-1643.
doi: 10.1007/s10641-015-0389-1
- Harvey JT, Loughlin TR, Perez MA, Oxman DS. 2000. Relationship between fish size and otolith length for 63 species of fishes from the eastern North Pacific Ocean. NOAA. Tech. Rep. NMFS 150.
- Ibáñez AL, Cowx IG, O'Higgins P. 2007. Geometric morphometric analysis of fish scales for identifying genera, species and local populations within the Mugilidae. *Can J Fish Aquat Sci.* 64(1):1091-1100.
- Ibáñez AL, Hernández-Fraga K, Alvarez-Hernández S. 2017. Discrimination analysis of phenotypic stocks comparing fish otolith and scale shapes. *Fish Res.* 185(1):6-13.
doi: 10.1016/j.fishres.2016.09.025
- Innal D. 2010. Growth properties of three Cyprinid species [*Squalius cephalus* (Linnaeus, 1758); *Tinca tinca* (Linnaeus, 1758) and *Alburnus escherichii* Steindachner, 1897] living in Camkoru Pond (Ankara-Turkey). *Kafkas Univ J Vet Fac.* 16 (Suppl-B): 297-304.
doi: 10.9775/kvfd.2010.2346
- Jawad LA, Sabatino G, Ibáñez AL, Andoloro F, Battaglia P. 2017. Morphology and ontogenetic changes in otoliths of the mesopelagic fishes *Ceratoscopelus maderensis* (Myctophidae), *Vinciguerria attenuata* and *V. poweriae* (Phosichthyidae) from the Strait of Messina (Mediterranean Sea). *Acta Zool-Stockholm.* 1-17.
doi: 10.1111/azo.12197
- Kılıç S, Becer ZA. 2016. Growth and Reproduction of Chub (*Squalius cephalus*) in Lake Yeniçağa, Bolu, Turkey. *International Journal of Agriculture & Biology.* 18(2): 419-424.
doi: 10.17957/IJAB/15.0106
- Koç HT, Erdogan Z, Tinkci M, Treer T. 2007. Age, growth and reproductive characteristics of chub, *Leuciscus cephalus* (L., 1758) in İkizcetepeler Dam Lake (Balıkesir). *Turk J Appl Ichthyol* 23(1):19-24.
- Kohestan-Eskandari S, Anvarifar H, Mousavi-Sabet H. 2013. Detection of morphometric differentiation of *Liza aurata* (Pisces: Mugilidae) in Southeastern of the Caspian Sea, Iran. *Our Nature.* 11(2):126-137.
doi: 10.3126/on.v11i2.9595
- Kottelat M, Freyhof, J. 2007. Handbook of European Freshwater Fishes. Berlin: 646 p.
- Kumar K, Chakraborty SK, Jaiswar AK, 2012. Comparative otolith morphology of sciaenids occurring along the north-west coast of India. *Indian J Fish.* 59(1):19-27.
- Laroche J, Durand JD, Bouvet Y, Guinand B, Brohon, B. 1999. Genetic structure and differentiation among populations of two cyprinids, *Leuciscus cephalus* and *Rutilus rutilus*, in a large European river. *Can J of Fish and Aqu Sci.* 56(1):1659-1667.
- Mapp J, Hunter E, Van Der Kooij J, Songer S, Fisher M. 2017. Otolith shape and size: the importance of age when determining indices for fish-stock separation. *Fish Res.* 190: 43-52.
doi: 10.1016/j.fishres.2017.01.017
- Mérigot B, Letourneur Y, Lecomte-Finiger R. 2007. Characterization of local populations of the Common Sole, *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. *Mar Biol.* 151(1): 997-1008.
doi: 10.1007/s00227-006-0549-0
- Morley S, Belchier M. 2002. Otolith and body size relationships in bigeye grenadier (*Macrourus holotrachys*) in Ccamlr subarea 48.3. *CCAMLR Sci.* 9(1): 133-143.
- Nolf D. 1985. Otolithi piscium. In: Schultze L, Kuhn O, editors. Handbook of Paleichthyology, Stuttgart: Gustav Fischer Verlag Press. p. 1-26.
- Özcan Eİ, Serdar O, Aydın R. 2017. Length-weight and length-length relationships of *Squalius cephalus* (L., 1758) in Karasu River (Erzincan-Erzurum). *Yunus Araştırma Bülteni.* 1(1):109-114. [in Turkish].
doi: 10.17693/yunusae.v1i26557.284938
- Özuluğ M, Freyhof J. 2011. Revision of the genus *Squalius* in Western and Central Anatolia, with description of four new species (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters* 22(2):107-148.
- Pierce GJ, Boyle PR, Diack JSW. 1991. Identification of fish otoliths and bones in faeces and digestive tracts of seals. *J of Zool.* 224(2):320-328.
doi: 10.1111/j.1469-7998.1991.tb04810.x

- Polat N, Uğurlu, S. 2011. Samsun İli Tatlı Su Balık Faunası. Samsun: 272 p.[in Turkish]
- Ramírez- Pérez JS, Quinonez-Velazquez C, Garcia-Rodriguez FJ, Felix-Uraga R, Melo-Barrera FN. 2010. Using the shape of sagittae otoliths in the discrimination of phenotypic stocks in *Scomberomorus sierra* (Jordan and Starks, 1895). *J Fish Aquat Sci.* 5(1):82-93.
- Renán X, Lecomte-Finiger R, Brulé T. 2004. Addressing recruitment in *Mycteroperca microlepis* populations of the north coast of Yucatan Peninsula: an otolith aging approach. *Gulf and Caribbean Fisheries Institute.* 55(1): 881–889.
- Renán X, Montero-Muñoz J, Garza-Pérez JR, Brulé T. 2016. Age and stock analysis using otolith shape in Gags from the Southern Gulf of Mexico. *T Am Fish Soc.* 145(6): 1252-1265.
[doi: 10.1080/00028487.2016.1217928](https://doi.org/10.1080/00028487.2016.1217928)
- Saygın S, Özpiçak M, Elp M, Polat N, Atıcı AA, Akçanal Ödün N. 2017. Comparative analysis of the otolith features of tarek (*Alburnus tarichi* (Güldenstädt, 1814)) from different lakes across Van Basin (Van, Erçek, Nazik, Aygır) (Turkey). *LimnoFish*, 3(2): 91-99.
[doi: 10.17216/Limnofish-130312](https://doi.org/10.17216/Limnofish-130312)
- Sen D, Aydın R, Çatla M. 2001. Relationships between fish length and otolith length in the population of *Capoeta capoeta umbla* (Heckel, 1843) inhabiting Hazar Lake, Elazığ, Turkey. *Arch Pol Fish.* 9(2):267-272.
- Sen F, Saygın F. 2008. Biological properties of chub (*Leuciscus cephalus* L., 1758) in Karasu Stream (Muş/Turkey). *J Anim Vet Adv.* 7(8): 1034-1037.
[doi: 10.1111/j.1439-0426.2011.01925.x](https://doi.org/10.1111/j.1439-0426.2011.01925.x)
- Škeljo F, Ferri, J. 2012. The use of otolith shape and morphometry for identification and size-estimation of five wrasse species in predator-prey studies. *J. Appl. Ichthyol.* 28(1): 524-530.
[doi: 10.1111/j.1439-0426.2011.01925.x](https://doi.org/10.1111/j.1439-0426.2011.01925.x)
- Stefanova E, Uzunova E, Hubenova T, Vasileva P, Terziyski D, Iliev I. 2008. Age and growth of the chub *Leuciscus cephalus* L., from the Maritz River (South Bulgaria). *Bulg J Agric Sci.* 14(2): 214-220.
- Stinton F. 1975. Fish otoliths from the english eocene. Part 1. *Palaeontol. Soc. Monogr.*, 129(1): 1-56.
- Tarkan AS, Gürsoy Gaygusuz A, Gaygusuz Ö, Acıpinar H. 2007. Use of bone and otolith measures for size-estimation of in predator- prey selection. *Folia Zool.* 56(3): 328-336.
- Tollit DJ, Steward PM, Thompson PM, Pierce GJ, Santos MB, Hughes S. 1997. Species and size differences in the digestion of otoliths and beaks: Implications for estimates of pinniped diet composition. *Can J Fish Aquat Sci* 54: 105-119.
[doi: 10.1139/f96-264](https://doi.org/10.1139/f96-264)
- Turan D, Tomovic L, Peši V. 2007. Morphological variation in a common Turkish cyprinid, *Squalius cephalus*, across Turkish water catchment areas. *J of Zool in the Middle East.* 40(1): 63-70.
[doi: 10.1080/09397140.2007.10638205](https://doi.org/10.1080/09397140.2007.10638205)
- Tuset VM, Lombarte A, Assis CA. 2008. Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Sci. Mar.* 72(S1): 7-198.
[doi: 10.3989/scimar.2008.72s17](https://doi.org/10.3989/scimar.2008.72s17)
- Tuset VM, Lombarte A, González, JA, Pertusa JF, Lorente MJ. 2003. Comparative morphology of the sagittal otolith in *Serranus* spp. *J Fish Biol.* 6(1):1491-1504.
- Verma J, Kashyap A, Serajuddin M. 2014. Phylogeny based on truss analysis in five populations of freshwater catfish: *Clupisoma garua*. *International Journal of Science and Research.* ISSN (Online): 2319-7064.
- Vignon M, Morat F. 2010. Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. *Mar Ecol Prog Ser.* 411(1): 231-241.
- Vlach P, Dusek J, Svatora M, Moravec P. 2005. Growth analysis of chub, *Leuciscus cephalus* (L.), and dace, *Leuciscus leuciscus* (L.), in the Upor stream using growth data of recaptured marked fish. *Czech J Animal Sci.* 50(1): 329-39.
- Waessle JA, Lasta CA, Favero M. 2003. Otolith morphology and body size relationships for juvenile Sciaenidae in the Río de la Plata estuary (35-36°). *Sci Mar.* 67(2): 233-240.
- Yazıcıoğlu O, Yilmaz S, Erbaşaran M, Uğurlu S, Polat N. 2017. Bony structure dimensions-fish length relationships of pike (*Esox lucius* L., 1758) in Lake Ladik (Samsun, Turkey). *North-West J Zool.* 13(1):149-153.
- Yilmaz S, Yazıcıoğlu O, Saygın (Ayaydin) S, Polat N. 2014. Relationships of otolith dimensions with body length of European perch, *Perca fluviatilis* L., 1758 from Lake Ladik, Turkey. *Pak J Zool.* 46(1): 1231-1238.
- Zar JH. 1999. Biostatistical analysis. New Jersey, Prentice-Hall.
- Zengin M, Saygın S, Polat N. 2015. Otolith shape analysis and dimensions of the anchovy *Engraulis encrasicolus* L. in the Black and Marmara Seas. *Sains Malays.* 44(1):657-662.
[doi: 10.17576/jsm-2015-4405-03](https://doi.org/10.17576/jsm-2015-4405-03)
- Zengin M, Saygın S, Polat N. 2017. Relationships between otolith size and total length of bluefish, *Pomatomus saltatrix* (Linnaeus, 1766) in Black Sea (Turkey). *North-West J Zool.* 13(1):169-171.



Hereke Deresi Alg Florası (Kocaeli-Türkiye)

Arzu MORKOYUNLU YÜCE^{1*}, Arif GÖNÜLOL², Ömer Osman ERTAN³, Şebnem ERKEBAY⁴

¹Kocaeli Üniversitesi, Hereke Ömer İsmet Uzunyol MYO, 41800, Hereke, Kocaeli

²Ondokuz Mayıs Üniversitesi, Fen-Edebiyat Fakültesi, 55105, Atakum, Samsun

³Süleyman Demirel Üniversitesi, Eğirdir Su Ürünleri Fakültesi, 32260, Isparta

⁴Kocaeli Üniversitesi, Karamürsel MYO, 41500, Karamürsel, Kocaeli

Ö Z

Akuatik ekosistemlerin besin zincirinde algler oldukça önemlidir. Lotik ve lentic ekosistemlerin biyoçeşitliliğinin belirlenmesinde incelenmeleri gerekmektedir. Hereke Deresi'nin epilitik ve planktonik florasını tespit etmek amacıyla 4 istasyon seçilmiştir. Bu çalışma Ocak 2015 – Kasım 2015 tarihleri arasında mevsimsel olarak gerçekleştirilmiştir. Hereke Deresinde yapılan bu çalışmada, 20 takson Bacillariophyta, 2 takson Chlorophyta, 2 takson Cyanobacteria (= Cyanophyta) ve 1 takson Charophyta bölümlerine ait olmak üzere toplam 25 takson tespit edilmiştir. Alg florasının % 80 Bacillariophyta grubu üyelerinden oluşmaktadır. Su sıcaklığı 8,10 – 17,1°C, çözülmüş oksijen 8,2 – 9,4 mg/L, pH 6,95 – 8,10, elektriksel iletkenliği 382,09 - 447 µS/cm olarak tespit edilmiştir. Az sayıda alg taksonunun bulunmasında derenin akış hızının etkili olduğu düşünülmektedir.

Anahtar kelimeler: Epilitik alg, Planktonik alg, Hereke Deresi, Türkiye

MAKALE BİLGİSİ

ARAŞTIRMA MAKALESİ

Geliş : 15.12.2017

Düzeltilme : 26.03.2018

Kabul : 27.03.2018

Yayın : 27.04.2018



DOI:10.17216/LimnoFish.366830

* SORUMLU YAZAR

arzu.yuce@kocaeli.edu.tr

Tel : +90 262 511 30 21

The Algae Flora of Hereke Stream (Kocaeli -Turkey)

Abstract: Algae are very important for the nutritional chain for aquatic ecosystems. They should also be examined to determine biodiversity in lotic and lentic ecosystems. In order to study the epilithic and planktonic algal flora of Hereke Stream, four stations were selected. The study was carried out seasonally between January 2015 and November 2015. In the algological study conducted at Hereke Stream; A total of 25 taxa were identified, 20 of which belong to Bacillariophyta, 2 belong to Chlorophyta, 2 belong to Cyanobacteria (= Cyanophyta) and 1 belong to Charophyta. 80 % of the total algal flora of was represented by members of Bacillariophyta. Water temperature were measured as 8.10 – 17.1 °C, dissolved oxygen 8.2-9.4 mg/L, pH 6.95-8.10, and electrical conductivity 382 - 447 µS / cm. It is thought that the flow rate of the stream is effective in the presence of a few algal taxa.

Keywords: Epilithic algae, Planktonic algae, Hereke Stream, Turkey

Alıntılama

Morkoyunlu Yüce A, Gönülool A, Ertan ÖO, Erkebay Ş. 2018. Hereke Deresi Alg Florası (Kocaeli-Türkiye) LimnoFish. 4(1): 25-29 doi: 10.17216/LimnoFish.366830

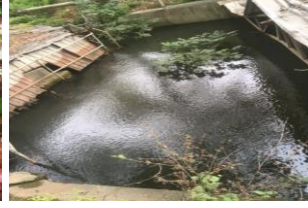
Giriş

Algler, son derece zengin karbonhidrat, protein ve özellikle yağ asidi içeriğine sahip organizmalardır. Besin değeri yüksek olan mikroalgler sudaki canlılar için besin maddeleri, vitamin ve iz elementlerin en önemli kaynağını oluşturmaktadır (Ulusoy 2017). Sucul ekosistemler için, besin zinciri açısından oldukça önemli bir yere sahip olan mikroalgler, ekosistemlerde biyoçeşitliliğin belirlenmesi amacıyla incelenmesi gereken gruplardan birini oluşturmaktadır. Dünyada oldukça farklı ekosistemlerde (su, toprak, kar,vb.) yayılış gösteren algler, bulundukları ortamın yapısını belirten

indikatör türleri de içermektedir. Ülkemiz sucul sistemlerinde (göl, gölet, baraj gölü ve akarsular) alg türlerinin belirlenmesine yönelik çalışmalar 1980'li yıllarda yoğun bir şekilde başlamış olup bu konuda gerçekleştirilmiş çok sayıda çalışma mevcuttur (Yıldız 1984; Altuner veGürbüz 1991; Gönülool ve Arslan 1992; Şen ve ark. 1996; Kolaylı ve ark. 1998; Ertan ve Morkoyunlu 1998; Kara ve Şahin 2001; Dere ve ark. 2002; Atıcı ve ark. 2003; Kalyoncu ve ark. 2009; Tokatlı ve Dayıoğlu 2011; Solak ve ark. 2012; Varol ve Şen 2014; Çiçek ve Ertan 2015). Lotik ekosistemler ve bu konuda benzer çalışmalar halen devam etmektedir. Ayrıca, Bakanlıklar (Orman



Şekil 2. Birinci istasyon



Şekil 3. İkinci istasyon



Şekil 4. Üçüncü istasyon

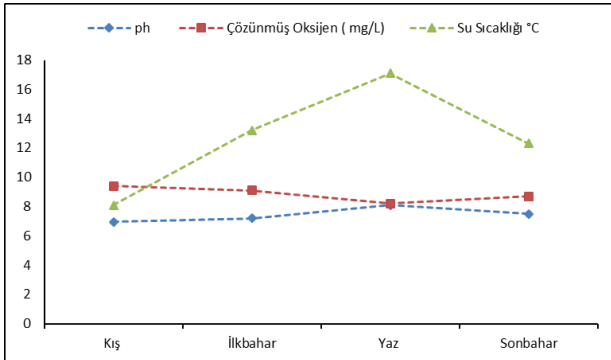


Şekil 5. Dördüncü istasyon

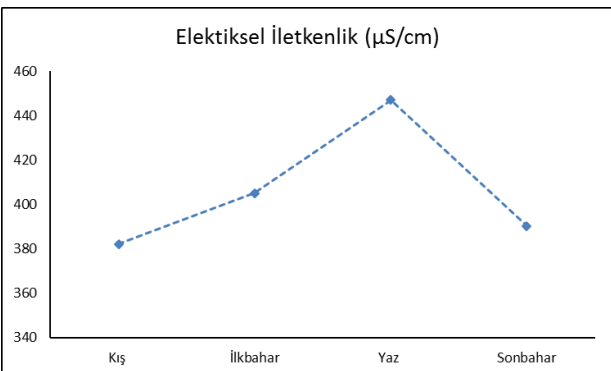
Bulgular

Hereke Deresi'nin Bazı Fiziksel ve Kimyasal Özellikleri

Hereke Deresi'nde alınan sularda yapılan analizler sonucunda; su sıcaklığının 8,1 – 17,1 °C, pH 6,95 – 8,1, elektriksel iletkenliği 382,09 - 447 µS/cm, çözülmüş oksijen 8,2 – 9,4 mg/L arasında olduğu tespit edilmiştir. Mevsimsel olarak ölçülen değerler şekil 6 ve 7' de verilmiştir. Tespit edilen değerlerin Su Kirliliği Kontrolü Yönetmeliği, kıta içi su kaynakları sınıflarına göre kalite kriterlerinde I. sınıf olduğu belirlenmiştir (SKKY 2004). Dere'ye ait bazı ağır metal değerleri Tablo 1'de verilmiştir.



Şekil 6. Hereke Deresi bazı su kalitesi parametrelerinin mevsimsel değişimi



Şekil 7. Hereke Deresi elektiksel iletkenlik değerlerinin mevsimsel değişimi

Tablo 1. Hereke Deresi bazı ağır metal değerleri (µg/L)

Su Kirliliği Kontrolü Yönetmeliği		
	SKKY- Su kalite sınıfı (I)	Hereke Deresi
İnorganik kirlenme parametreleri		
Civa (µg /L)	0,1	<0,000
Kadmiyum (µg/L)	3	<0,000
Kurşun (µg /L)	10	<0,000
Arsenik (µg/L)	20	0,03
Bakır (µg /L)	20	<0,000
Krom (toplam) (µg /L)	20	<0,000
Kobalt (µg /L)	10	<0,000
Nikel (µg /L)	20	0,02
Çinko (µg/L)	200	<0,000
Demir (µg/L)	300	3,23
Mangan (µg/L)	100	0,12
Baryum (µg/L)	1000	17,16
Alüminyum (mg/L)	0,3	<0,000

Hereke Deresi (Ulupınar) Alg Florası

Hereke Deresi'nin epilitik ve planktonik alg florasında, 20 takson Bacillariophyta, 2 takson Chlorophyta, 2 takson Cyanobacteria (= Cyanophyta) ve 1 takson Charophyta bölümüne ait olmak üzere toplam 25 takson tespit edilmiştir. Tespit edilen taksonların listesi Tablo 2'de verilmiştir.

Tartışma ve Sonuç

Önceki yıllarda içme suyu olarak kullanılan Hereke Deresi'nin algal yapısını belirlemek amacıyla yapılan bu çalışmada, epilitik ve planktonik alg florasında, 20 takson Bacillariophyta, 2 takson Chlorophyta, 2 takson Cyanobacteria (= Cyanophyta) ve 1 takson Charophyta bölümüne ait olmak üzere toplam 25 takson tespit edilmiştir. Hereke Deresi alg florasında tespit edilen taksonlardan *Gyrosigma attenuatum* (Kütz.) Rabenh. dışında diğerleri plankton örneklerinde de belirlenmiştir. Diatomelerden *Achnanthes*, *Melosira*, *Navicula*, *Craticula* ve *Ulnaria* türleri yurdumuzda araştırılan diğer akarsuların fitoplanktonunda genel olarak yaygın olarak bulunmuştur. *C. pediculus*, *C. placentula* ve *U. ulna* taksonları gerçek planktonik tür olmamalarına rağmen plankton örneklerinde tespit edilmiştir. Kıyı bölgesinde bulunan sedimanlar ve taşlar üzerinde bulunan alglerin dalga hareketleri ile kıyı bölgesinden fitoplanktona karışabileceği yapılan birçok çalışmada rapor edilmiştir (Gönülol ve Arslan 1992; Aysel 2005; Kalyoncu ve ark. 2009; Tokatlı ve Dayıoğlu 2011; Solak ve ark. 2012; Varol ve Şen 2014; Çiçek ve Ertan 2015). Hereke Deresi alg florasını belirlemek amacıyla Ocak 2015 – Kasım 2015 tarihlerinde yapılan bu çalışmada, alg florasının % 80'inin Bacillariophyta üyelerine ait olduğu belirlenmiştir.

Tablo 2. Hereke Deresi'nde tespit edilen taksonlar

Hereke Deresi Algleri	Epilitik	Planktonik
BACILLARIOPHYTA		
<i>Planothidium lanceolatum</i> (Bréb. Ex Kütz.) Lange-Bert.	-	+
<i>Achnanthis minutissimum</i> (Kützing) Czarnecki	-	+
<i>Amphora ovalis</i> (Kütz.) Kütz.	-	+
<i>Cocconeis pediculus</i> Ehrenb.	+	+
<i>Cocconeis placentula</i> Ehrenb.	+	+
<i>Craticula cuspidata</i> (Kütz.) D.G.Mann	+	+
<i>Cymbella affinis</i> Kütz.	+	+
<i>Cymbella cymbiformis</i> C.Agardh	+	+
<i>Cymbella</i> sp.	-	+
<i>Diatoma vulgare</i> Bory	+	+
<i>Encyonema ventricosum</i> (C.Agardh) Grunow	+	+
<i>Gomphonema olivaceum</i> (Hornem.) Bréb.	+	+
<i>Gomphonema parvulum</i> (Kütz.) Kütz.	-	+
<i>Gyrosigma attenuatum</i> (Kütz.) Rabenh.	+	-
<i>Melosira varians</i> C.Agardh	-	+
<i>Navicula cryptocephala</i> Kütz.	+	+
<i>Navicula radiosa</i> Kütz.	+	+
<i>Navicula rhynchocephala</i> Kütz.	+	+
<i>Navicula</i> sp.	-	+
<i>Ulnaria ulna</i> (Nitzsch) Compère	+	+
CHLOROPHYTA		
<i>Ankistrodesmus falcatus</i> (Corda) Ralfs	-	+
<i>Cladophora glomerata</i> (L.) Kütz.	+	+
CHAROPHYTA		
<i>Cosmarium granatum</i> Brébisson ex Ralfs	-	+
CYANOBACTERIA = CYANOPHYTA		
<i>Kamptomena formosum</i> (Bory ex Gomont) Strunecký, Komárek & J.Smarda	-	+
<i>Merismopedia punctata</i> Meyen	-	+

Hereke Deresi fitoplanktonunda tespit edilen 24 taksonun 14'üne epilitik kommunitede de rastlanmıştır. Bacillariophyta üyeleri her mevsim tespit edilmiş olup, ilkbahar ve sonbahar dönemlerinde sayısal olarak hafif artışlar gözlenmiştir. Araştırmada, *Cocconeis pediculus*, *Cocconeis placentula*, *Encyonema ventricosum*, *Navicula rhynchocephala* taksonları diğer taksonlara göre kantitatif olarak daha fazla tespit edilmiştir. Derede ki suyun devamlı akış halinde olması ve diğer alglerin gelişmemesi nedeni ile akışa dayanıklı olan diyatome türlerinin çoğunlukta olduğu bir alg florası hakim olmuştur. Özellikle lotik ekosistemlerde *C. pediculus*, *C. placentula* ve *E. ventricosum* gibi türler taş ve bitki yüzeylerine tutunabilme kabiliyetine sahiptirler (Yıldız 1985; Çiçek ve Ertan 2015). Chlorophyta ve Cyanophyta bölümüne ait türlerin akış nedeni ile akarsuda gelişemediği ancak mevsim sıcaklığının yükseldiği ve ortam şartlarının uygun olduğu çok kısa bir sürede az sayıda türün gelişebildiği tespit edilmiştir. Yurdumuzun diğer akarsularında yapılan araştırmalarda da buna benzer sonuçlar elde edilmiştir (Yıldız 1984; Atıcı ve ark. 2003). Akarsuların sığ kıyı bölgelerinde iplikli alglerin bol

olarak bulunduğu ve bunlardan Hereke Deresi'nde de tespit edilen *C. glomerata*'nın akarsuların temiz bölgelerinde geliştiği bildirilmiştir (Yıldız 1985). Dere suyunun hafif bazik karakterde olduğu, su sıcaklığına bağlı olarak oksijen seviyesinde mevsimsel değişimlerin olduğu tespit edilmiştir. Elektriksel iletkenlik değerinin, çevresel etkenlere bağlı bir yapı sergilediği sonucuna varılmıştır. Hereke Dere'sinde ölçülen fiziko-kimyasal parametreler ve ağır metal analizlerine göre derenin Su Kirliliği Kontrolü Yönetmeliği (SKKY 2004)'ne göre, kıta içi su kaynakları sınıflarına göre kalite kriterlerinde I. sınıf olduğu belirlenmiştir. Literatürlerde, temiz sularda yayılışının bulunduğu belirtilen türlerden *C. affinis*, *C. placentula*, *A. minutissimum* türleri Hereke Deresi'nde dört mevsimde de kaydedilmiştir ki, bu türlerin temiz sularda yayılışının bulunduğu belirtilmiştir (Tornés ve ark. 2007; Omar 2010).

Teşekkür

Bu çalışma 04-06 Ekim 2017 tarihlerinde gerçekleştirilen 1. Uluslararası Limnoloji ve Tatlısu Balıkçılığı Sempozyumu (LimnoFish)'nda sözlü sunum olarak sunulmuştur.

Kaynaklar

- Anonim. 2017a. Çevre ve Şehircilik Bakanlığı, Coğrafi Bilgi sistemleri Genel Müdürlüğü; [Erişim tarihi Eylül 2017]. Erişim Adresi: <https://www.csb.gov.tr/gm/cbs>
- Anonim. 2017b. Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü; [Erişim tarihi: Ekim 2017]. Erişim adresi: <http://suyonetimi.ormansu.gov.tr>
- Altuner Z, Gürbüz H. 1991. Karasu (Fırat) Nehri epipelik alg florası üzerinde bir araştırma. *Doğa -Turk J Bot.* 15(1991): 253-267.
- Atıcı T, Yılmaz M, Gül A, Kuru M. 2003. Delice ırmağı algleri. *G Ü Fen Bilimleri Dergisi.* 16(1):9-17.
- Aysel V. 2005. Check-list of the freshwater algae of Turkey. *J Black Sea / Mediterranean Environment.* 11(1): 1-124.
- Brummit RK, Powell CE. 1992. A list of authors of scientific names of plants, with recommended standard forms of their names, including abbreviations. Royal Botanic Gardens. Kew. 732 p.
- Çiçek NL, Ertan ÖO. 2015. Köprüçay Nehri (Antalya) su kalitesinin epilithic diatomelerle belirlenmesi. *Ege J Fish Aquat Sci.* 32(2): 65-78. doi: [10.12714/egejfas.2015.32.2.03](https://doi.org/10.12714/egejfas.2015.32.2.03)
- Dere Ş, Karacaoğlu D, Dalkıran N. 2002. Nilüfer Çayı (Bursa) epifitik algleri üzerinde bir araştırma. *Turk J Bot.* 26(4): 219-234.
- Ertan OÖ, Morkoyunlu A. 1998. Aksu Deresi'nin alg florası (Isparta-Türkiye). *Turk J Bot.* 22(4): 239-256.
- Gönüloğlu A, Arslan N. 1992. Samsun-İncesu Deresi'nin alg florası üzerinde araştırmalar. *Doğa - Turk J Bot.* 16(1992): 311-334.
- Guiry MD, Guiry GM. 2017. Algae base. World-wide electronic publication, National University of Ireland, Galway. [Erişim tarihi 3 Kasım 2017]. Erişim Adresi: <http://www.algaebase.org>.
- Husted F. 1985. The pennat diatoms, translation of husted 'DieKieselalgen, 2.Teil' with supplement by Norman G. Jensen, Koenigstein:Koeltz Scientific Books 918p.
- Kara H, Şahin B. 2001. Epipellic and Epilithic algae of Değirmendere River (Trabzon-Turkey). *Turk J Bot.* 25(4): 177-186.
- Kalyoncu H, Barlas M, Ertan ÖO. 2009. Aksu Çayı' nın su kalitesinin biotik indekslere (diatomlara ve omurgasızlara göre) ve fizikokimyasal parametrelere göre incelenmesi, organizmaların su kalitesi ile ilişkileri. *TÜBAV Bilim Dergisi.* 2(1): 46-57.
- Kolaylı S, Baysal A, Şahin B. 1998. Şana Deresi (Trabzon/Türkiye) epipelik ve epilithic algleri üzerinde bir araştırma. *Turk J Bot.* 22(3): 163-170.
- Krammer K, Lange-Bertalot H. 1991a. Süßwasserflora von Mitteleuropa. Bacillariophyceae, band 2/3, 3. Teil: Centrales, Fragillariaceae, Eunoticeae, Stuttgart :Gustav Fischer Verlag 576 p.
- Krammer K, Lange-Bertalot H. 1991b. Süßwasserflora von Mitteleuropa. Bacillariophyceae, band 2/4, 4. Teil: Achnanthaceae Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis, Stuttgart:Gustav Fischer Verlag 436 p.
- Krammer K, Lange-Bertalot H. 1999a. Süßwasserflora von Mitteleuropa. Bacillariophyceae, band 2/1, 1. Teil: Naviculaceae , Berlin:Spectrum Academicher Verlag 876 p.
- Krammer K, Lange-Bertalot H. 1999b. Süßwasserflora von Mitteleuropa. Bacillariophyceae, band 2/2, 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae, Berlin:Spectrum Academicher Verlag. 610 p.
- Omar WM. 2010. Perspectives on the use of algae as biological indicators for monitoring and protecting aquatic environments, with special reference to Malaysian freshwater ecosystems. *Trop Life Sci Res.* 21(2): 51-67.
- Özler HM, Karaman S. 2011. Gebze ve Dilovası'nın (Kocaeli) hidrojeolojik incelenmesi. Fiziki coğrafya araştırmaları: sistematik ve bölgesel. İstanbul:Türk Coğrafya Kurumu Yayınları. s.131-180.
- Patrick R, Reimer CW. 1966. The diatoms of the United States I. Philadelphia:Academy of Natural Sciences 688 p.
- Patrick R, Reimer CW. 1975. The diatoms of the United States II. Philadelphia:Academy of Natural Sciences 213 p.
- Prescott GW. 1973. Algae of the Western Great Lakes area. Dubuque Iowa:VM. C. Brown, Company, Publishers 977 p.
- Solak CN, Barinova S, Acs E, Dayıoğlu H. 2012. Diversity and ecology of diatoms from Felent creek (Sakarya River Basin) Turkey. *Turk J Bot.* 36(2): 191-203. doi: [10.3906/bot-1102-16](https://doi.org/10.3906/bot-1102-16)
- SKKY (Su Kirliliği Kontrol Yönetmeliği). 2004. Su kirliliği kontrolü yönetmeliği. Ankara. Resmi Gazete Tarihi: 31.12.2004, Resmi Gazete Sayısı: 25687
- Şen B, Toprak G, Alp M, Özrenk F. 1996. A Study on Epiphytes of *Cladophora glomerata* (L.) Kütz. 1st. European Phycological Congress; Cologne, Germany.
- Tokatlı C, Dayıoğlu H. 2011. Use of epilithic diatoms to evaluate water quality of Murat Stream (Sakarya River basin, Kütahya): different saprobity level sand ph status. *JABS.* 5(2): 55-60.
- Tornés E, Cambra J, Gomà J, Leira M, Ortiz R, Sabater S. 2007. Indicator taxa of benthic diatom communities: a case study in Mediterranean streams, *Ann Limnol. - Int J Lim.* 43 (1): 1-11. doi:[10.1051/limn/2007023](https://doi.org/10.1051/limn/2007023)
- Ulusoy Y. 2017. Enerji ve algler (mikro ve makro algler). Uludağ Üniversitesi, Teknik Bilimler MYO. Powerpoint sunum. [Erişim tarihi: Eylül 2017]. Erişim Adresi: <http://yahyau.home.uludag.edu.tr>.
- Varol M, Şen B. 2014. Dicle Nehri'nin planktonik alg florası. *Journal of Fisheries Sciences.com,* 8(4): 252-264. doi: [10.3153/jfsc.201431](https://doi.org/10.3153/jfsc.201431)
- Yıldız K. 1984. Meraç Çayı alg toplulukları üzerinde araştırmalar Kısım I. Fitoplankton topluluğu. *S.Ü. Fen Fak Fen Dergisi.* 3(1): 213-217.
- Yıldız K. 1985. Meraç çayı alg toplulukları üzerinde araştırmalar, Kısım-III. Sedimanlar üzerinde yaşayan algler. *Doğa Bilim Dergisi.* 9(2): 428-434.



The Aquatic Oligochaeta (Annelida) Fauna of the Karasu Stream

Melek ZEYBEK^{1*}  Serap KOŞAL ŞAHİN²  Seray YILDIZ³ 

¹Süleyman Demirel University, Faculty of Arts and Sciences Department of Biology, 32260, Isparta, Turkey

²Istanbul University, Faculty of Fisheries, 34134, Laleli, İstanbul, Turkey

³Ege University, Faculty of Fisheries, Department of Hydrobiology, 35100, Bornova, İzmir, Turkey

ABSTRACT

Sakarya River is defined hydrologically in three parts: upper, middle and lower Sakarya River Basin. This study was carried out in lower Sakarya River (Karasu). Aquatic Oligochaeta samples were collected from four chosen stations between January 2008 and October 2008 seasonally. In total, eight Oligochaeta species were identified during the study period. These species belong to Naididae family and to Tubificinae subfamily (Tubificoid Naidid) [*Limnodrilus hoffmeisteri* Claparède, 1862; *Limnodrilus udekemianus* Claparède, 1862; *Limnodrilus claparedeianus* Ratzel, 1868; *Potamothenix hammoniensis* (Michaelsen, 1901); *Psammoryctides deserticola* (Grimm, 1877); *Tubifex blanchardi* Vejdovsky, 1891; *Tubifex ignotus* (Stolc, 1886); *Tubifex tubifex* (Müller, 1774)]. Also, it was observed that *Limnodrilus hoffmeisteri* and *Tubifex tubifex*, were found to be dominant, and thus indicator species for pollution in the river.

Keywords: Oligochaeta, Karasu, Sakarya River

ARTICLE INFO

RESEARCH ARTICLE

Received : 08.12.2017

Revised : 20.03.2018

Accepted : 30.03.2018

Published : 27.04.2018

DOI:10.17216/LimnoFish.363933



* CORRESPONDING AUTHOR

melekzeybek@sdu.edu.tr

Tel : +90 246 211 40 75

Karasu Nehri'nin Sucul Oligochaeta (Annelida) Faunası

Öz: Sakarya Nehir havzası; yukarı, orta ve aşağı (Karasu) olmak üzere üç bölüme ayrılır. Bu çalışma Aşağı Sakarya (Karasu) Nehri'nde gerçekleştirilmiştir. Akarsu üzerinde dört istasyon seçilmiş ve bu istasyonlardan Ocak 2008-Ekim 2008 tarihleri arasında mevsimsel periyotlarla Oligochaeta örnekleri toplanmıştır. Çalışma sonunda, Oligochaeta sınıfına ait sekiz tür bulunmuştur. Bu türler Naididae familyasından ve Tubificinae (Tubificoid Naidid) alt familyasıdır [*Limnodrilus hoffmeisteri* Claparède, 1862; *Limnodrilus udekemianus* Claparède, 1862; *Limnodrilus claparedeianus* Ratzel, 1868; *Potamothenix hammoniensis* (Michaelsen, 1901); *Psammoryctides deserticola* (Grimm, 1877); *Tubifex blanchardi* Vejdovsky, 1891; *Tubifex ignotus* (Stolc, 1886); *Tubifex tubifex* (Müller, 1774)]. Ayrıca çalışmada, kirlilik indikatörü olarak bilinen *Limnodrilus hoffmeisteri* ve *Tubifex tubifex* türlerinin nehirde baskın olduğu gözlenmiştir.

Anahtar kelimeler: Oligochaeta, Karasu, Sakarya Nehri

How To Cite

Zeybek M, Koşal Şahin S, Yıldız S. 2018. The Aquatic Oligochaeta (Annelida) Fauna of the Karasu Stream. LimnoFish. 4(1): 30-35. doi: 10.17216/LimnoFish.363933

Introduction

Sakarya River is the third largest river in Turkey pouring into the Black Sea. Sakarya River basin, which is the most important river in the region, is an important source of natural energy. With the dams built on the area it provides drinking and irrigation water. The length of this river is approximately 810 km and its width 60-150 m. The river is divided into three sections in terms of hydrology: upper, middle and lower Sakarya (Karasu) River Basin (DSI 1992; Anonymous 2015). This river is used for purposes

such as usage water, recreation, agricultural irrigation, and wastewater discharge of residential and industrial establishments. There have been many water pollution problems, due to increase in population and industrialization, and it was determined that some branches of river were significantly contaminated by waste discharges of industrial plants (Dündar 2008; Balçioğlu and Öztürk 2009). Moreover, the oil pollution level was found higher than the limit value given by UNESCO in Sakarya River (Balçioğlu and Öztürk 2009).

There are various studies to determine the Oligochaeta fauna in both the lotic and lentic areas of the Sakarya River Basin (Arslan and Şahin 2003, 2004; Arslan et al. 2007; Yıldız et al. 2008). The number of studies is very limited in Karasu Stream on this subject (Arslan and Şahin 2004).

Karasu Stream, which is chosen as a research area in this study, is one of the water sources of the lower Sakarya basin. However, this stream has not been thoroughly investigated in terms of Oligochaeta fauna except a few studies in lentic habitats (Yıldız et al. 2008). Therefore, in this study, it was aimed to determine the Oligochaeta fauna of Karasu River.

Material and Methods

This study was carried out on Karasu River located in the lower part of Sakarya River basin (Turkey). This river is originated from Çataltepe near Hendek. It first flows to north with the name Kabalak Stream. After it merges with Yayla Stream, it takes the name of Karasu and it is poured from Karasu district to the Black Sea. The length of study area is approximately 30 km. Four stations were chosen on this river and samples were taken seasonally between January 2008 - October 2008 (Figure 1). Totally, 16 samples had been performed per site/single sampling from four stations at four seasons.

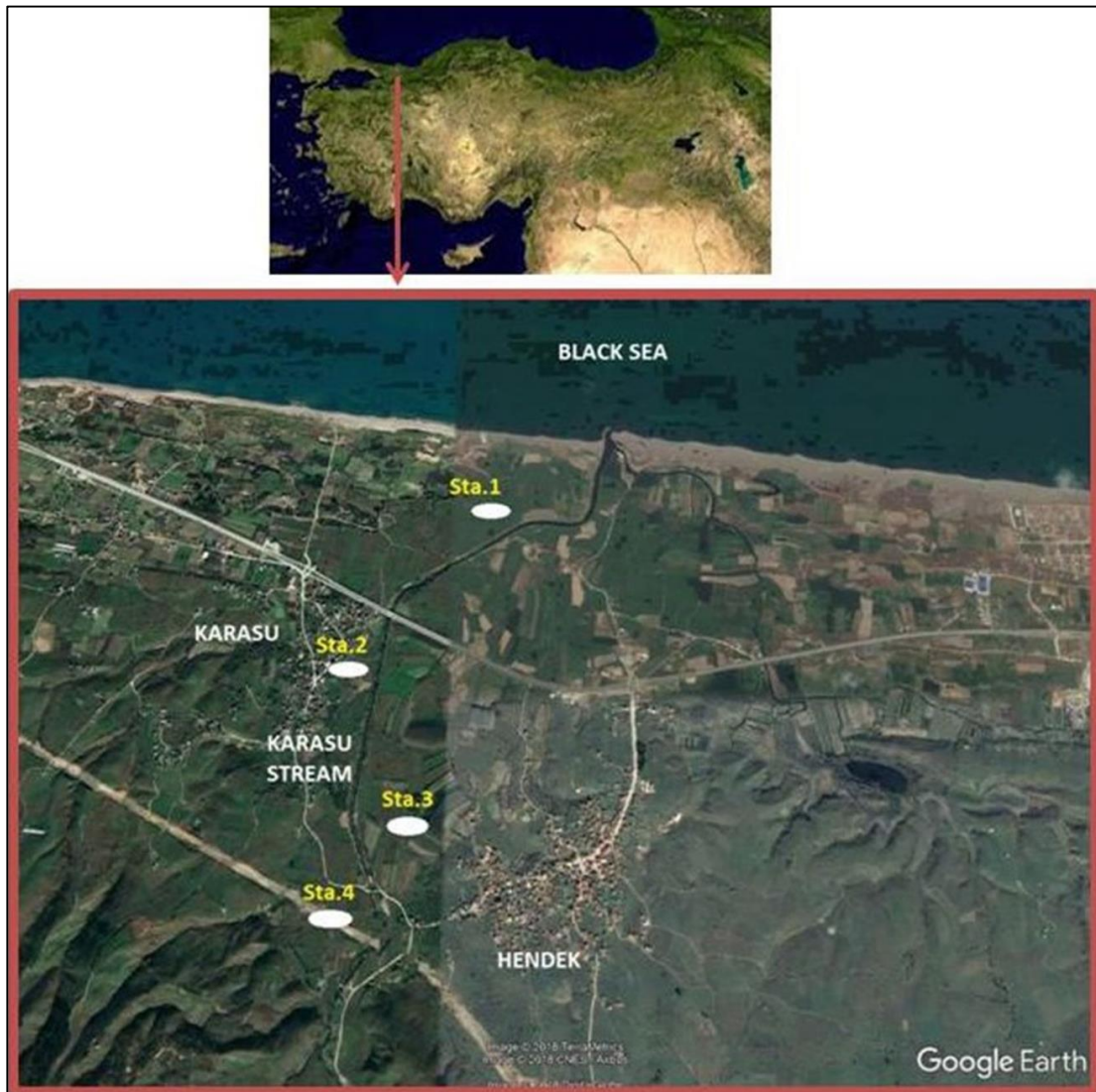


Figure 1. The study area and stations

Oligochaeta specimens were collected twice from each station by using Ekman-Birge grab (15x15 cm) and a hand-net with a mesh size (with sweeping method) 180µm. Then, they were sieved through a mesh of 500µm. The samples

were preserved in the field with 4% formaldehyde solution and preserved in 70 % alcohol until identification after washing in the laboratory. After the permanent preparation of sorted Oligochaeta specimens with CMCP-10

(polyvinyl lactophenol), samples were identified in species level under stereomicroscope and binocular microscopes. Samples were identified according to Brinkhurst and Jamieson (1971), Kathmanand Brinkhurst (1998), Timm (1999),

Timm and Veldhuijzen van Zanten (2002). Shannon-Weaver (1963) and Simpson (1949) indices were applied to detect the species diversity of the stations by using MVSP version 3.1 (Kovach 1998).

Table 1. The Secchi depth values (meter) of the stations.

		Station 1	Station 2	Station 3	Station 4
Secchi Depth (m)	Winter'08	2.3	2.5	4	3.2
	Spring'08	2.3	1.3	4	4
	Summer'08	2.3	1.5	3.8	4
	Autumn'08	2.3	2.5	4	3.2

Results

In total, eight taxa were determined from Naididae family and Tubificinae subfamily (Tubificoid Naidid) at the end of the study.

The list and density of Oligochaeta species at the stations were shown in Table 2. *Limnodrilus hoffmeisteri* was the only taxon seen in all seasons and *L. hoffmeisteri* and *Tubifex tubifex*

were found at the all stations. The species diversity values ranged from 1.27 to 1.5 for Shannon Weaver index and between 0.43-0.6 for Simpson index. In addition, evenness value of the second station was calculated rather low compared to the other stations. Additionally, the lowest diversity value was observed in the autumn and there was only one taxon (Table 2).

Table 2. Seasonal distribution and the density values of the determined taxa.

		Sta 1.	Sta 2.	Sta 3.	Sta.4
OLIGOCHAETA					
Naididae					
Tubificinae					
Winter'08	<i>Limnodrilus hoffmeisteri</i> Claparède, 1862	889	4444	1778	222
	<i>Limnodrilus claparedeianus</i> Ratzel, 1868		222		
	<i>Limnodrilus udekemianus</i> Claparède, 1862		222	2444	
	<i>Tubifex ignotus</i> (Stolc, 1886)		1110		
	<i>Tubifex tubifex</i> (Müller, 1774)	444		2889	889
	<i>Tubifex blanchardi</i> Vejdovsky, 1891				444
Spring'08	<i>Limnodrilus hoffmeisteri</i> Claparède, 1862			1110	
	<i>Potamotheix hammoniensis</i> (Michaelsen, 1901)			222	
	<i>Tubifex tubifex</i> (Müller, 1774)			2223	
Summer'08	<i>Limnodrilus hoffmeisteri</i> Claparède, 1862		2000		
	<i>Tubifex tubifex</i> (Müller, 1774)	889	444		
	<i>Tubifex blanchardi</i> Vejdovsky, 1891	222			
	<i>Psammoryctides deserticola</i> (Grimm, 1877)	222			
Autumn'08	<i>Limnodrilus hoffmeisteri</i> Claparède, 1862		444		
Species Diversity Indices					
Shannon-Weaver		1.424	1.268	1.502	1.378
Simpson		0.539	0.431	0.59	0.571
Evenness		0.719	0.538	0.786	0.857

The total densities of Oligochaets in each station showed considerable differences during the study period; the highest total density of specimens (7111 ind m⁻²) was recorded at the station 3 in winter period. Oligochaeta species were detected at the third station in the spring while at the second station in the autumn (Fig 2). Most of the determined species, especially the Tubificid ones, are characteristic for eutrophic lakes. In particular, *T. tubifex* and *L. hoffmeisteri* together with *Potamothrix hammoniensis* have high

densities throughout the river. Table 2 clearly shows the eutrophic structure of the river. Also, low Shannon-Weaver values are due to the influence of pollution according to Orclari and Hummon (1975) from high relative intensities of some species according to Statzner 1981; Sang, 1987. In our study, the presence of *L. hoffmeisteri* as the only and dominant species in the second station reduced the value of the diversity index. This information supports the study results.

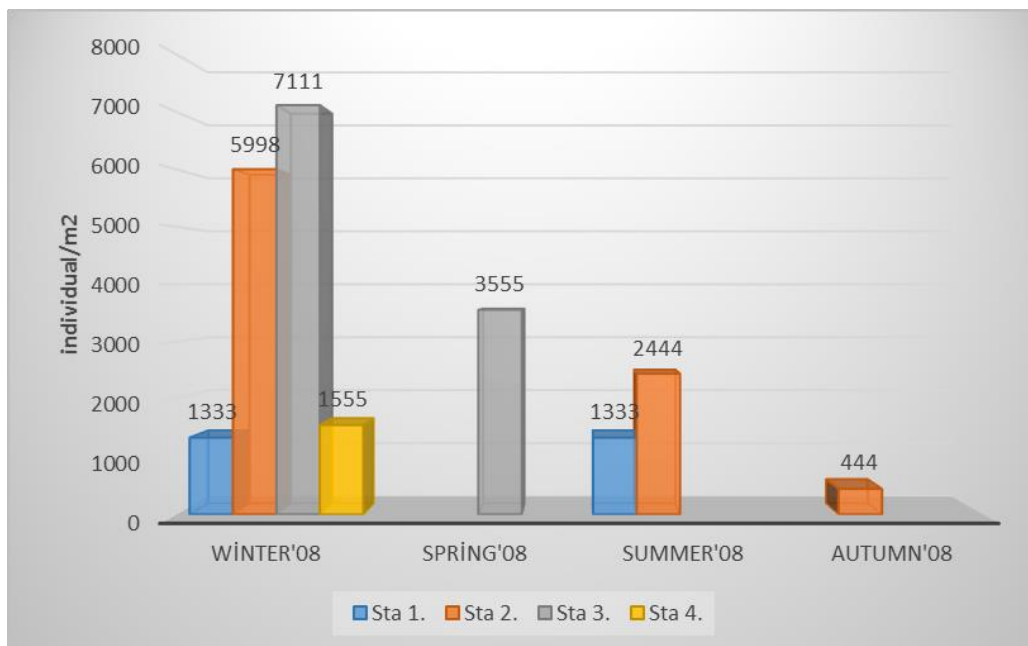


Figure 2. Seasonal abundances of the total Oligochaeta population at the stations during the study.

Discussion

This study was carried out on the Karasu Stream, one of the important branches of the Sakarya River and eight taxa were determined belong to Naididae family and Tubificinae subfamily. Naididae is one of the most important groups of aquatic Oligochaeta and they are cosmopolitan and occur throughout the world (Martin et al. 2008). Especially, taxa in subfamily Tubificinae (Tubificoid Naidid newly) are commonly associated with sediments in aquatic habitats (Yıldız et al. 2012).

Arslan and Şahin (2003) collected Oligochaeta samples from 79 stations on the Sakarya River. Five taxa were determined on Karasu Stream in their work. There are some differences in terms of species composition between those reported by Arslan and Şahin (2003) and the present study. *Ophidonais serpentina*, *Nais pardalis*, *Pristinella jenkiniae*, *Pristina aequisetata*, and *Pristina foreli* were reported from the Karasu Stream. Also, several studies have been carried out to determine the Oligochaeta fauna in several branches of the Sakarya River and lentic

areas of this river basin (Arslan and Şahin 2003, 2004; Arslan et al. 2007; Yıldız et al. 2008). In this study, *L. hoffmeisteri* and *T. tubifex* were found at the all stations.

L. hoffmeisteri is a very common, pollution-tolerant species in freshwaters and it is found in large numbers in densely polluted waters (Brinkhurst and Jamieson 1971; Mason 1996). It happens densely populated in polluted habitats, often together with *T. tubifex*.

T. tubifex is a cosmopolitan species and it is tolerant against organic pollution and lack of oxygen (Klemm 1985; Timm 1999; Yıldız and Balık 2005). According to Milbrink (1983), this species occurs in those environments where competition or predation is weak.

L. hoffmeisteri and *T. tubifex* have poly-sabrobic character (Johnson et al. 1993). These species are the typical species of heavily polluted waters in several indices running on benthic macroinvertebrates (Armitage et al. 1983).

L. udekemianus is a cosmopolitan species and is mostly found in organically polluted waters as well

as oligotrophic habitats (Klemm 1985). This species was found in winter at the second and third stations in this study. This species is often found together with *L. hoffmeisteri* but less in number (Timm 1970).

P. hammoniensis, is a reliable an indicator for eutrophy and it has a wide distribution pattern and can be found in brackish waters occasionally (Milbrink 1980; Lang 1984). In addition, it is known to be extremely tolerant to anoxic conditions in brackish or moderately alkaline bottom water (Milbrink 1980). This species was found only at the third station and had a low dominance in this study. Milbrink (1973) found this species generally associated with pollution and most often together with *T. ignotus* (Stolc 1886), which has Palaearctic distribution in Swedish waters after (Milbrink 1980), is found great rivers and deep lakes.

T. blanchardi is a freshwater tubificid, often occurring together with *T. tubifex* (Marotta et al. 2009). It has a large tolerance range and can live in various habitats (Yıldız 2003). In certain conditions, pH, salinity, mercury and hardness concentrations make stress on *T. tubifex* specimens and induce a gradial loss of hair and pectinate setae thus generating individuals similar to “blanchi” form (Marotta et al. 2009).

L. claparedeianus Ratzel, 1868, which is freshwater species with tolerance for slightly brackish water, and *Psammoryctides deserticola* (Grimm 1877), which is common, euryhalin and Ponto-Caspian immigrant species have the least dominance value in that study. *L. claparedeianus* and *P. deserticola* were observed only at stations 2 and 3, respectively.

Findings from this preliminary study on the taxonomic determination of the Karasu Stream Oligochaeta fauna suggest that the species forming the river fauna are generally cosmopolitan, or at least species with broad distribution in the Palearctic region (Timm, 1980). The results show that the river does not have a high diversity in terms of the Oligochaeta fauna.

Although there is no measurement of water quality and pollutants in the study, in the molluscan study conducted in the same region; according to the results of the seasonal measurements of nitrite, nitrate nitrogen, water temperature and pH measurements of the Lower Sakarya River where the survey was conducted according to the Water Quality and Control Regulation (SKKY) (2004), it falls into the category 3 (contaminated water) (Koşal-Şahin 2013). The pollution indicators Oligochaets like *L. hoffmeisteri* and *T. tubifex* were observed in high numbers and have a great

dominancy especially at stations 2 and 3 where pollution level was high.

References

- Anonymous 2015. The environmental situation report of Sakarya city. Sakarya: Sakarya Governor Environmental and Citizenship Province Directorate 116 p. [in Turkish]
- Armitage PD, Moss D, Wright JF, Furse MT. 1983. The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Res.* 17(3): 333-347.
doi: 10.1016/0043-1354(83)90188-4
- Arslan N, Şahin Y. 2003. Nine New Naididae (Oligochaeta) Species for Sakarya River, Turkey. *Türk J Zool.* 27(1): 27-38.
- Arslan N, Şahin Y. 2004. First records of some Oligochaeta (Naididae) species for Turkey and Sakarya River Fauna. *Türk J Zool.* 28(1): 7-18.
- Arslan N, Timm T, Erséus C. 2007. Aquatic Oligochaeta (Annelida) of Balıkdami wetland (Turkey), with description of two new species of Phallodrilinae. *Biologia.* 62(3): 323-334.
doi: 10.2478/s11756-007-0055-y
- Balcıoğlu EB, Öztürk B. 2009. Oil pollution in the surface water of Sakarya River. *J. Black Sea/Mediterranean Environment.* 15(2): 99-108.
- Brinkhurst RO, Jamieson BGM. 1971. Aquatic Oligochaeta of the World. Edinburgh: Oliver & Boyd 860 p.
- DSI (General Directorate of the State Water) 1992. General directorate of the state water, Investigation of pollution aspects of Sakarya-Seyhan Basins and report of determination of quality classes of these basins, 5, Ankara. [in Turkish]
- Dündar MŞ. 2008. Aşağı Sakarya Nehri Su ve Sediment Kalitesinin Belirlenmesi. Türkiye Bilimsel ve Teknolojik Araştırma Kurumu. Proje No: 106Y037. [in Turkish]
- Johnson RK, Wiederholm T, Rosenberg DM. 1993. Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. In: Rosenberg DM, Resh VH. (Eds.), *Freshwater Biomonitoring and Benthic Macroinvertebrates*. New York : Chapman and Hall p. 40-158.
- Kathman RD, Brinkhurst RO. 1998. Guide to the Freshwater Oligochaetes of North America. Tennessee, USA: Aquatic Resources Center, College Grove 264 p.
- Klemm DJ. 1985. A Guide to the Freshwater Annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirudinea) of North America. Dubuque, Iowa: Kendall/Hunt Publishing Company 198 p.
- Koşal Şahin S. 2013. Mollusca Species of Down Sakarya River (Karasu) and Some Physico-Chemical Variables Effecting Their Abundance. *Yunus Research Bulletin* 2013 (3): 11-19.
doi: 10.17693/yunusae.v2013i21904.235413

- Kovach W. 1998. Multi-Variate statistical package. Ver. 3.0. Pentraeth, Wales: Kovach Computer Services.
- Lang C. 1984. Eutrophication of Lakes Léman and Neuchâtel (Switzerland) indicated by oligochaete communities. In: Bonomi G, Erséus C, editors. Aquatic Oligochaeta. Developments in Hydrobiology. Dordrecht : Springer. p. 131-138.
[doi: 10.1007/978-94-009-6563-8_23](https://doi.org/10.1007/978-94-009-6563-8_23)
- Marotta R, Crottini A, Prada V, Ferraguti M. 2009. A morphological reappraisal of *Tubifex blanchardi* Vejvodský, 1891 (Clitellata: Tubificidae). Acta Zool. 90 (2): 179-188.
[doi: 10.1111/j.1463-6395.2008.00368.x](https://doi.org/10.1111/j.1463-6395.2008.00368.x)
- Martin P, Martínez-Ansemil E, Pinder A, Timm T, Wetzel MJ. 2008. Global diversity of oligochaetous clitellates (Oligochaeta; Clitellata) in freshwater. Hydrobiologia 595 (1): 117-127.
[doi: 10.1007/s10750-007-9009-1](https://doi.org/10.1007/s10750-007-9009-1)
- Mason CF. 1996. Organic pollution. In: Mason C, editor. Biology of Freshwater Pollution, 3rd ed. Essex: Longman Scientific & Technical. p. 82-88.
- Milbrink G. 1973. On the use of indicator communities of Tubificidae and some Lumbriculidae in the assessment of water pollution in Swedish lakes. Zoon 1(2): 125-139.
- Milbrink G. 1980. Oligochaeta Communities in Pollution Biology: The European Situation with Special Reference to Lakes in Scandinavia. In: Brinkhurst RO, Cook DG, editors. Aquatic Oligochaeta Biology. New York: Plenum Press. p. 433-455.
- Milbrink G. 1983. An improved environmental index based on the relative abundance of oligochaete species. Hydrobiologia 102(2): 89-97.
- Orclari, RD., Hummon WD. 1975. A comparison of benthic oligochaete populations in acid and neutral lentic environments in Southeastern Ohio. Ohio J. Sci. 75 (1): 46-49.
- Sang Q. 1987. Some ecological aspects of aquatic oligochaetes in the Lower Pearl River (People's Republic of China). Hydrobiologia 155 (1): 199-208.
[doi:10.1007/978-94-009-3091-9_27](https://doi.org/10.1007/978-94-009-3091-9_27)
- Shannon CE, Wiener W. 1963. The mathematical theory of Communication University. Urbana: Illinois Press. 125 p.
- SKKY. 2004. Su Kirliliği Kontrol Yönetmeliği 25687 Sayılı resmi Gazete.
- Simpson EH. 1949. Measurement of diversity. Nature. 163, 688.
[doi:10.1038/163688a0](https://doi.org/10.1038/163688a0)
- Statzner B. 1981. Shannon-Weaver diversity of the macrobenthos in the Schierensee brooks (North Germany) and problems of its use for the interpretation of the community structure. Verh int Verein Limnol. 21(2): 782-786.
[doi:10.1080/03680770.1980.11897087](https://doi.org/10.1080/03680770.1980.11897087)
- Timm T, Veldhuijzen van Zanten HH. 2002. Freshwater Oligochaeta of North-West Europe. World Biodiversity Database, CD-ROM Series. University of Amsterdam: Expert Center for Taxonomic Identification.
- Timm T. 1970. On the Fauna of the Estonian Oligochaeta. Pedobiologia. 10 (1):52-78.
- Timm T. 1980. Distribution of Aquatic Oligochaetes. In: Brinkhurst RO, Cook DG, editors. Aquatic Oligochaeta Biology. New York: Plenum Press. p. 55-77.
- Timm T. 1999. A Guide to the Estonian Annelida. Naturalist's Handbooks 1. Tartu-Tallinn: Estonian Academy Publishers 208 p.
- Yıldız S, Balık S. 2005. The Oligochaeta (Annelida) fauna of the inland waters in the Lake District (Turkey). Ege J Fas. 22 (1-2): 165-172.
- Yıldız S, Özbek M, Ustaoglu MR, Sömek H. 2012. Distribution of aquatic oligochaetes (Annelida, Clitellata) of high-elevation lakes in the Eastern Black Sea Range of Turkey. Turk J Zool. 36(1):59-74.
[doi:10.3906/zoo-1002-39](https://doi.org/10.3906/zoo-1002-39)
- Yıldız S, Ustaoglu MR, Balık S, Sarı HM. 2008. Contributions to the Knowledge of Oligochaeta (Annelida) Fauna of Some Lakes in the West Black Sea Region. J. Black Sea/Mediterranean Environment. 14 (3):193-204.
- Yıldız S. 2003. Taxonomical and ecological investigation of inland waters Oligochaeta (Annelida) fauna in the Lakes district [PhD Thesis]. Ege University 216 p. [in Turkish]



Immunostimulant Effects of Geophyte Plant Extract on Non-specific Defence Mechanisms of Rainbow Trout (*Oncorhynchus mykiss*)

Gülşen ULUKÖY^{1*}, Ayşegül KUBİLAY², Behire Işıl DİDİNEN², Seçil METİN², Soner ALTUN³, Öznur DİLER², Ramazan MAMMADOV⁴, Arife DULLUÇ⁵

¹Muğla Sıtkı Kocman University Fisheries Faculty, Department of Aquaculture, 48000 Muğla-Turkey

²Suleyman Demirel University Egirdir Fisheries Faculty, Department of Aquaculture, 32000 Isparta-Turkey

³Uludağ University, Veterinary Faculty, 16059 Bursa-Turkey

⁴Pamukkale University, Faculty of Arts and Sciences, Department of Biological Sciences 20000 Denizli-Turkey

⁵Isparta Directorate of Provincial Food Agriculture and Livestock 32200 Isparta-Turkey

ABSTRACT

The aim of this study was to determine the immunostimulant effects of geophyte plant extract on non-specific defence mechanisms of rainbow trout (*Oncorhynchus mykiss*). For this purpose, *Muscari comosum* was collected in Muğla region and extracted in ethanol. Then, The plant extract applied into fish by intraperitoneal injection in two different concentrations (0.5mg/ fish and 2.0 mg/fish). The average fish weight was 140 g. Following the injection on the 1st, 7th, 14th, 21st, 28th days the blood and serum samples were collected from fish in each group and examined for various parameters including percentage of hematocrit, the counts of nitroblue tetrazolium (NBT) positive neutrophils, total leukocyte counts, percentage of white blood cells and serum lysozyme activity. The results indicated that the counts of NBT (+) neutrophils, percentage of monocyte and neutrophil and total leukocyte counts increased in the group with injected 0.5mg plant extract /fish compared to control group (P<0.05). It has been revealed that this dose of *M. comosum* can be suggested to use to enhance non-specific immune system for rainbow trout aquaculture. However, immunostimulant effects of oral administration of this plant extract in rainbow trout are needed to be determine in future studies.

Keywords: Fish, immune system, *Muscari comosum*, Tassel hyacinth, blood

ARTICLE INFO

RESEARCH ARTICLE

Received : 09.01.2018

Revised : 07.03.2018

Accepted : 09.03.2018

Published : 27.04.2018

DOI:10.17216/LimnoFish.376404



* CORRESPONDING AUTHOR

gulukoy@mu.edu.tr

Tel : +90 252 211 15 19

Gökkuşluğu Alabalığı (*Oncorhynchus mykiss*)'nın Spesifik Olmayan Savunma Mekanizması Üzerine Geofit Bitki Ekstraktının İmmunostimulant Etkisi

Öz: Bu çalışmada amacımız, gökkuşluğu alabalığının spesifik olmayan savunma mekanizması üzerine geofit bitki ekstraktının immunostimulant etkisinin belirlenmesidir. Bu amaçla geofit bir bitki olan *M. comosum* Muğla bölgesinden toplandı ve etanolde ekstrakte edildi. Daha sonra farklı dozlarında (0,5 mg/balık ve 2,0 mg/balık) balıklara intraperitoneal olarak uygulandı. Balıklar ortalama 140 g ağırlığa sahipti. Deneme süresince uygulamayı takiben 1, 7, 14, 21, 28. günlerde her bir gruptaki balıktan kan ve serum örnekleri alınarak farklı parametreler (hematokrit yüzdesi, nitroblue tetrazolium (NBT) pozitif nötrofil sayısı, toplam lökosit sayısı, beyaz kan hücrelerinin yüzdesi ve serum lizozim aktivitesi) açısından incelendi. Bu çalışmanın sonucunda NBT pozitif nötrofillerin sayısı, monosit ve nötrofil yüzdeleri ve toplam lökosit sayısı 0,5 mg/balık dozunda enjeksiyon yapılan grupta kontrol grubuna kıyasla artış gösterdiği (P<0,05) belirlendi. Bu nedenle gökkuşluğu alabalığı yetiştiriciliğinde spesifik olmayan immun sistemi uyarmak için bu dozdaki *M. comosum*'un kullanılması önerilebilir. Ancak, bitki ekstraktının oral yolla yeme ilave edilerek balıklara uygulanması ile immunostimulant etkisinin belirlenmesi için gelecekte yeni çalışmalara ihtiyaç duyulmaktadır.

Anahtar kelimeler: Balık, immun sistem, *Muscari comosum*, arap sümbülü, kan

How To Cite

Uluköy G, Kubilay A, Didinen BI, Metin S, Altun S, Diler Ö, Mammadov R, Dulluç A, 2018. Immunostimulant Effects of Geophyte Plant Extract on Non-specific Defence Mechanisms of Rainbow Trout (*Oncorhynchus mykiss*). LimnoFish. 4(1): 36-41. doi: 10.17216/LimnoFish.376404

Introduction

Fish production has increased significantly over the past decades which has given rise to practices

such as over crowding, transport, handling and grading in aquacultural systems. These practices are the major factors that make the fish susceptible

against pathogens. Diseases are major constraints to aquaculture production (Plumb and Hanson 2011)

Fish disease is rarely a simple association between pathogen, a host fish and environmental problems, such as poor water quality or other stressors often contribute to the outbreak of disease. Fish disease, caused by pathogenic organisms present in the environment, they are mostly contagious and treatment may be necessary to control the disease outbreak. Therefore, intensive farming practices and infectious diseases induced major problems in aquaculture industry causing heavy loss to farmers. Several studies have been conducted on the modulation of fish immune system in order to prevent the outbreaks. Disease outbreaks are increasingly being recognized as a potential constraint on aquaculture production and trade and cause massive financial loss either through mortality or reduced meat quality, resulting in reduced profit margins (Plumb and Hanson 2011; Mehana et al. 2015).

The use of antibiotics and chemotherapeutics to combat fish diseases has the risk of generating resistant pathogens, bioaccumulation and environmental pollution. Commercial vaccines are expensive for fish farmers and are specific against particular pathogens (Christyapita et al. 2007). One of the most promising methods of controlling diseases in aquaculture is strengthening the defence mechanisms of fish through prophylactic administration of immunostimulants (Deivasigamani and Subramanian 2016; Christyapita et al. 2007). The different types of IS (e.g. glucan, chitin, lactoferrin, levamisole, vitamin B, C, E and growth hormone and prolactin) mainly facilitate the function of phagocytic cells. Several immunostimulants also stimulate the natural killer cells (NK), complement, lysozyme and antibody responses of fish (Düğenci et al. 2003; Mehana et al. 2015). Up to now, several plants which have been used as therapeutic in the control of pathogens which cause diseases in fish. (Awad and Awaad 2017). Geophyte plant, *M. comosum* (L.) Mill.1768, has medicinal properties (Villa et al. 2012; Nasrabadi et al. 2013) such as diuretic, anti-inflammatory, hypoglycemic activities (Loizzo et al. 2010) and antioxidant activity (Pieroni et al. 2002). Further, *M. comosum* had been determined immunostimulant effects on gilthead seabream (*Sparus aurata*) by Baba et al. (2014).

The aim of the present study was to determine the immunostimulant effects of *M. comosum* extract on the nonspecific immune responses of rainbow trout.

Materials and Methods

Plant extraction

The geophyte plant, Tassel hyacinth (*Muscari comosum*), was gathered from Muğla region of Turkey. Plant brought to laboratory for the extraction. Its bulbs were cleaned and chopped into small pieces (Lee et al. 2000; Tanker and Tanker 1991). The extraction process was continue in ethyl alcohol at 50°C in a water bath for 24 h. The obtained solution was filtered and then remained mass was run again with applying same process as before. This step was repeated three times. The obtained extracts combined and lyophilized. The extract was stored in dark at 4°C.

Experimental design

Healthy rainbow trout (mean weight of 140±10g) were taken from a private fish farm in Isparta and acclimated for 2 weeks and held in 600 liters tanks filled with freshwater at 12°C, with a flow rate of 1-1.5 L min⁻¹ with continuous aeration. Fish were fed with commercial pellets at 2% body weight and water quality parameters were monitored daily. The experimental fish were randomly divided into 3 groups, 50 fish/group in duplicate. The obtained lyophilized *M.comosum* extract was dissolved it in sterile phosphate-buffered saline (PBS) solution and then applied to fish with an intraperitoneal injection (i.p.) as 0.1 mL of two different concentrations as 0.5 mg/fish and 2 mg/fish. The same volume of PBS i.p. injected into fish of control group.

Sampling

The each sampling day (1st, 7th, 14th, 21st, and 28th days), randomly selected total five fish from each group were taken and anesthetized by using phenoxyethanol. After anesthetizing fish, blood samples were drawn from the caudal vein and then fish sacrificed. A part of blood was put into an Eppendorf tube and then left at 4°C overnight and centrifuged at 3500 g for 15 min and then serum collected. The serum samples were stored at -20°C until assayed.

Determination of NBT-positive cells

For the determination of the respiratory burst activity in the blood, Nitroblue tetrazolium (NBT) (Sigma No: N-6876) was used by following a modified method described by Anderson et al. (1992). A drop of blood placed onto a microscope coverslip and left in a petri dish which had humid atmosphere for 30 min at 25°C. After incubation, the coverslip washed with 0.067 mM sodium phosphate buffer (pH 6.4) to remove unwanted cells and then a drop of 0.2% NBT solution

(freshly prepared) was put on to slide and the coverslip with cells was turned cell face down on the NBT solution and incubated again in a petri dish for 30 min at 25°C. The slides were examined under microscope (40x magnification). The dark blue staining cells were counted as positive. From each fish, five coverslips were prepared and five random microscopic fields were counted on each slide. Then the total twenty-five different fields were averaged and the mean and standard error of values per field for fish were calculated.

Assay of lysozyme activity

2 mg lyophilized *Micrococcus lysodeikticus* cells (Sigma, M 3770, ATCC No. 4698) was suspended with 10 ml 0.05 M sodium phosphate buffer (pH 6.5). The 3 ml of from the solution was taken the spectrophotometer cells and than added onto 50 µl fish serum. Following this process after 30 seconds and 4.5 minutes was performed two measurements with Shimadzu (UV-120-02) spectrophotometer at 540 nm. One unit of lysozyme activity was defined as a reduction of absorbance of 0.001 / min (Engstad et al. 1992).

Counting of total leukocyte

In the blood samples, the total leukocyte were counted from sampled fish. For this purpose, a Neubauer counting chamber was used and method was followed according to by Schaperclaus et al. (1991). After collection of blood from fish, the certain amount of blood was combined with Natt–Herrick solution in a leukocyte pipe. The leukocytes were counted in duplicate samples twice from each fish.

Differential leukocyte count

The method of Steinhagen et al. (1990) and Schaperclaus et al. (1991) were followed with slight modification to obtain the percentage of cell types of leukocytes. The double stains; May–Grunwald and Giemsa were used to stain of the prepared blood smears. These staining procedure provided that the specific types of the leukocytes was seen under the light microscope. A total of 100 leukocytes in each slide were counted by using a Neubauer chamber. The percentage of the cell types were calculated.

Hematocrit level

During the sampling days, blood samples were collected from fish by using heparinized capillary tubes. From each fish, two heparinized capillary tubes full with blood were obtained. Then, the hematocrit centrifuge (worked at 10,500 g for 5 min) was used to read the hematocrit level of sampled blood. By following Steinhagen et al. 1990 and

Schaperclaus et al. 1991, the percentage hematocrit value of blood samples was determined by using a special scale.

Statistics

All data were analyzed by one-way analysis of variance using the general linear model. Duncan's Multiple Range test was used to compare treatment means. Differences were considered significant at the 0.05 probability level. All analysis was performed using the SPSS program.

Results

It has been suggested that hematological parameters are useful indicators for monitoring fish health and especially immune response. In this study, the mean number of NBT-positive cells in a microscopic field was presented in Table1. NBT-positive cells in the fish group which received 0.5 mg *M. comosum* were significantly increased ($P < 0.05$) from the other groups on 21 and 28th days. This showed that giving dose increased phagocytic activity of neutrophils.

Total leukocyte counts in *M. comosum* (0.5 mg/fish) were determined significantly higher than other groups in 1 and 7 th days ($P < 0.05$) (Table 1). While the first day after injection total leukocyte count was $0.69 \times 10^5/\mu\text{l}$ in control group fish blood, 0.5 mg/fish plant extract received fish groups showed highest cell count in 1st ($1.26 \times 10^5/\mu\text{l}$) and 7th days ($1.14 \times 10^5/\mu\text{l}$). The other samplings days the high values seen in the plant extract received fish groups compare to control group.

The percentage of neutrophils and monocytes also significantly ($P < 0.05$) increased in blood of the 0.5 mg/fish plant extract received fish group (Table 2) compare to control and other group. The highest values of neutrophils and monocytes percentage were obtained in 0.5 mg/fish plant extract received fish group in each sampling days (Table 2). The following group of high values was determined on percentage of neutrophils and monocytes in blood of 2 mg/fish plant extract received fish group. The different concentration of plant extract injected fish groups expressed stimulated activity on neutrophils and monocyte cells.

The serum lysozyme activities values was highest levels at the 21st and 28th days in the 0.5 mg/fish plant extract received fish group however, haematocrit levels in rainbow trout blood didn't change after injection of *M. comosum* geophyte plant ($P > 0.05$) into all fish groups.

Our results revealed that, *M.comosum* stimulated nonspecific immune parameters in rainbow trout.

Table 1. Serum lysozyme activity (unit/ml), Hematocrit Level (%), NBT(+) neutrophil counts/microscopic field, Total leukocyte counts ($\times 10^5/\mu\text{l}$) in fish blood after i.p. injection of geophyte plant extract.

Days	Groups	Serum lysozyme activity (unit/ml)	Hematocrit level (%)	NBT(+) Neutrophil counts	Total leukocyte counts ($\times 10^5/\mu\text{l}$)
1	0.5mg	55.25 $\pm$ 25.16 ^a	38.87 $\pm$ 4.09 ^{ab}	12.29 $\pm$ 7.67 ^{bc}	1.26 $\pm$ 0.24 ^f
	2mg	146.66 $\pm$ 98.65 ^{abcd}	37.21 $\pm$ 5.36 ^{ab}	15.13 $\pm$ 8.63 ^c	0.76 $\pm$ 0.17 ^{bcd}
	control	180 $\pm$ 67.33 ^{abcd}	39.76 $\pm$ 5.03 ^{ab}	4.24 $\pm$ 3.37 ^{ab}	0.69 $\pm$ 0.69 ^{bcd}
7	0.5mg	284 $\pm$ 116.96 ^d	39.10 $\pm$ 3.97 ^{ab}	4.61 $\pm$ 4.18 ^{ab}	1.14 $\pm$ 0.22 ^{ef}
	2mg	228 $\pm$ 164.07 ^{cd}	41.05 $\pm$ 2.57 ^{ab}	3.21 $\pm$ 1.32 ^{ab}	0.70 $\pm$ 0.14 ^{bcd}
	control	144 $\pm$ 62.18 ^{abcd}	37.19 $\pm$ 3.84 ^{ab}	3.38 $\pm$ 1.95 ^{ab}	0.78 $\pm$ 0.78 ^{bcd}
14	0.5mg	60 $\pm$ 43.20 ^a	35.49 $\pm$ 6.25 ^a	2.63 $\pm$ 1.89 ^a	0.64 $\pm$ 0.13 ^{abc}
	2mg	110 $\pm$ 62.18 ^{abc}	39.64 $\pm$ 5.70 ^{ab}	2.34 $\pm$ 1.64 ^a	0.66 $\pm$ 0.12 ^{abcd}
	control	125 $\pm$ 66.08 ^{abc}	36.04 $\pm$ 5.23 ^a	2.42 $\pm$ 1.94 ^a	0.66 $\pm$ 0.15 ^{abcd}
21	0.5mg	195 $\pm$ 99.83 ^{abcd}	39.30 $\pm$ 3.49 ^{ab}	14.53 $\pm$ 12.88 ^c	0.54 $\pm$ 0.05 ^{ab}
	2mg	220 $\pm$ 160 ^{bcd}	43.13 $\pm$ 2.82 ^b	2.82 $\pm$ 1.16 ^a	0.81 $\pm$ 0.16 ^{cd}
	control	164 $\pm$ 58.99 ^{abcd}	38.17 $\pm$ 3.07 ^{ab}	3.97 $\pm$ 3.02 ^{ab}	0.91 $\pm$ 0.29 ^{de}
28	0.5mg	152 $\pm$ 109.17 ^{abcd}	41.01 $\pm$ 3.75 ^{ab}	13.80 $\pm$ 8.18 ^c	0.63 $\pm$ 0.15 ^{abc}
	2mg	66.66 $\pm$ 30.55 ^{ab}	35.04 $\pm$ 1.61 ^a	7.19 $\pm$ 6.99 ^{abc}	0.42 $\pm$ 0.10 ^a
	control	120 $\pm$ 20.00 ^{abc}	38.65 $\pm$ 4.42 ^{ab}	4.16 $\pm$ 2.09 ^{ab}	0.60 $\pm$ 0.19 ^{abc}

Data are represented as mean $\pm$ SE (n = 10/group). Different letters represent the significant differences at $P < 0.05$

Table 2. The percentage of the leukocyte cells in fish blood after ip injection of geophyte plant extract.

Days	Groups	Leukocytes Percentage (%)		
		Lymphocyte	Monocyte	Neutrophil
1	0.5mg	83.6 $\pm$ 2.07 ^{bcd}	7.8 $\pm$ 1.48 ^b	8.6 $\pm$ 2.19 ^{abc}
	2mg	70.8 $\pm$ 6.41 ^a	6.8 $\pm$ 2.68 ^b	22.4 $\pm$ 5.36 ^d
	control	87.2 $\pm$ 3.11 ^{bcde}	2 $\pm$ 0.7 ^a	10.80 $\pm$ 3.19 ^{bc}
7	0.5mg	82.80 $\pm$ 3.11 ^{bcd}	11.6 $\pm$ 2.19 ^c	6 $\pm$ 1.73 ^{ab}
	2mg	84 $\pm$ 5.47 ^{bcd}	7.2 $\pm$ 3.11 ^b	8.8 $\pm$ 2.68 ^{abc}
	control	94.6 $\pm$ 2.6 ^{ef}	1.8 $\pm$ 0.83 ^a	3.8 $\pm$ 2.49 ^a
14	0.5mg	85.40 $\pm$ 3.91 ^{bcd}	6.8 $\pm$ 1.09 ^b	7.8 $\pm$ 3.11 ^{abc}
	2mg	88.6 $\pm$ 4.21 ^{cdef}	3.8 $\pm$ 1.64 ^a	7.6 $\pm$ 3.13 ^{ab}
	control	95 $\pm$ 1.22 ^{ef}	1.8 $\pm$ 0.44 ^a	3.4 $\pm$ 0.54 ^a
21	0.5mg	79.40 $\pm$ 10.52 ^b	6.8 $\pm$ 3.27 ^b	13.80 $\pm$ 8.92 ^c
	2mg	89.2 $\pm$ 6.14 ^{def}	3.2 $\pm$ 1.92 ^a	5.6 $\pm$ 1.81 ^{ab}
	control	95.6 $\pm$ 1.81 ^f	1.6 $\pm$ 0.54 ^a	2.8 $\pm$ 1.48 ^a
28	0.5mg	70 $\pm$ 5.61 ^a	7 $\pm$ 2.44 ^b	23 $\pm$ 2.44 ^d
	2mg	80.80 $\pm$ 11.14 ^{bc}	3.2 $\pm$ 1.92 ^a	10.2 $\pm$ 7.52 ^{bc}
	control	93.6 $\pm$ 3.36 ^{ef}	1.5 $\pm$ 0.57 ^a	3.4 $\pm$ 1.67 ^a

Data are represented as mean $\pm$ SE (n = 10/group). Different letters represent the significant differences at $P < 0.05$

Discussion

Immunostimulants, influence growth performance and health condition in aquatic species by inducing a strong defence response against pathogens while minimizing the use of antibiotics. The immunostimulatory effects of plant extracts might vary by fish species, route of administration, dose, duration. Immunostimulants are critical in activate the immune responses capable of providing complete protection against certain pathogens.

The results of the present study indicate that there is a increasing number of NBT-positive cells after i.p.injection of *M. comosum* geophyte plant extract in rainbow trout (0.5mg/fish). Similarly, there have also been reported related results in various fish species, such as in gilthead sea bream (*Sparus aurata*) for *M. comosum* (Baba et al. 2014); in tilapia *Oreochromis mossambicus* for leaf extracts of *Ocimum sanctum* (Logambal et al. 2000) and *Tinospora cordifolia* (Sudhakaran et al. 2006); in rohu (*Labeo rohita*) for *Withania somnifera* (Sharma et al. 2010); Nile tilapia (*Oreochromis niloticus*) for *Echinacea purpurea* and *Allium sativum* (Aly and Mohamed 2010); in common carp (*Cyprinus carpio*) for *Aegle marmelos* (Pratheepa et al. 2010).

Lysozyme is a fish defence element, which causes lysis of bacteria and activation of the complement system and phagocytes by acting as opsonin (Magnadottir 2006). Christyapita et al. (2007) was reported that enhanced serum lysozyme activity in *Oreochromis mossambicus* of *Eclipta alba* leaf extracts. Baba et al. (2014) also mentioned that the lysozyme activity of *Sparus aurata* serum well-enhanced after applying of i.p. injection of *M. comosum* plant extract. Similar elevated lysozyme activity in *Oreochromis mossambicus* serum was reported to acetone extract (1% w/w) from four medicinal plants (Bermuda grass, *Cynodon dactylon*; beal *Aegle marmelos*; winter cherry, *Withania somnifera* and ginger, *Zingiber officinale* (Immanuel et al. 2009). In addition, Awad et al. (2013) notify that they found increased levels of lysozyme in rainbow trout serum which fed with black cumin seed oil and nettle extract. In another study (Baba et al. 2016), all concentrations of oat extract-added (*Avena sativa*) diet fed fish serum lysozyme activity, significantly increased in the oat extract supplemented diet fed groups at all concentrations in *C. carpio*. However, in the present study, all the doses of *M. comosum* did not increase the lysozyme activity in rainbow trout. Differences may be due to different fish species, plant extraction method, route of administration and time used in these studies.

In the present study, the percentage of neutrophils and monocytes were significantly elevated ($P<0.05$) that resulted in a significant increase in the total

leukocyte counts in *M. comosum* (0.5mg/fish) applied fish. Similarly, Baba et al. (2014) also noted increased total leukocyte count, neutrophils and monocytes in *Sparus aurata* applied with *M. comosum* plant extract. In the other earlier study, the lymphocyte counts and total leukocyte counts also increased in *Oreochromis niloticus* given *Echinacea purpurea* supplemented diet with for 1 and 2 months feeding period (Aly and Mohamed 2010).

In conclusion, *M. comosum* geophyte plant extract which was given i.p. route into fish, improved and enhanced some of the nonspecific immune defense in rainbow trout. *M. comosum* could be suggested to be used for rainbow trout culture to strengthen the nonspecific immune system of fish. Thus, if it will be applied before outbreaks of disease, high numbers of mortalities might be avoided. Also, there is a need to further studies especially on the long-term feeding trials to determine the immunostimulant effects of this geophyte plant extract.

Acknowledgements

This work is a part of the research project number 104V126 financially supported by The Scientific and Technological Research Council of Turkey (TUBİTAK). This study was presented in EAFP 2007 in Grado, Italy as oral presentation.

References

- Aly SM, Mohamed MF. 2010. *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*). J Anim Physiol Anim Nutr (Berl). 94 (5), e31-e39
doi: 10.1111/j.1439-0396.2009.00971.x
- Anderson DP, Moritomo T, Grooth RD. 1992. Neutrophile, glass-adherent, nitroblue tetrazolium assay gives early indication of immunization effectiveness in rainbow trout. Vet. Immunol.Immunopathol.30(4):419-429.
doi:10.1016/0165-2427(92)90110-C
- Awad E, Awaad A. 2017. Role of medicinal plants on growth performance and immune status in fish. Fish Shellfish Immunol. 67(1):40-54.
doi: 10.1016/j.fsi.2017.05.034
- Awad E, Austin D, Lyndon AR. 2013. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture 388-391(1):193-197
doi: 10.1016/j.aquaculture.2013.01.008
- Baba E, Acar Ü, Öntaş C, Kesbiç OS, Yilmaz S. 2016. The use of *Avena sativa* extract against *Aeromonas hydrophila* and its effect on growth performance, hematological and immunological parameters in common carp (*Cyprinus carpio*). Ital. J. Anim. Sci. 15 (2): 325-333.
doi: 10.1080/1828051X.2016.1185977
- Baba E, Uluköy G, Mammadov R. 2014. Effects of *Muscari comosum* Extract on Nonspecific Immune

- Parameters in Gilthead Seabream, *Sparus aurata* (L. 1758). J World Aquacult. Soc. 45 (2): 173-182
doi: 10.1111/jwas.12100
- Christyapita D, Divyagnaneswari M, Michael RD. 2007. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. Fish Shellfish Immunol. 23(4):840-852
doi: 10.1016/j.fsi.2007.03.010
- Deivasigamani B, Subramanian V. 2016. Applications of Immunostimulants in Aquaculture: A Review. Int. J. Curr. Microbiol. App. Sci. 5(9): 447-453.
doi: 10.20546/ijcmas.2016.509.048
- Düğenci SK, Arda N, Candan A. 2003. Some medicinal plants as immunostimulant for fish. J Ethnopharmacol. 88 (1), 99-106
doi: 10.1016/S0378-8741(03)00182-X
- Engstad RE, Robertsen B, Frivold E. 1992. Yeast glucan induces increase in lysozyme and complement-mediated haemolytic activity in Atlantic salmon blood. Fish Shellfish Immunol. 2 (4): 287-297
doi: 10.1016/S1050-4648(06)80033-1
- Immanue G, Uma RP, Iyapparaj P, Citarasu T, Punitha Peter SM, Michael Babu M, Palavesam A. 2009. Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*. J Fish Biol 74 (7): 1462-1475
doi: 10.1111/j.1095-8649.2009.02212.x
- Lee TH, Qiu F, Waller GR, Chou CH. 2000. Three new flavonol galloylglycosides from leaves of *Acacia confusa*. J. Nat. Prod. 63(5): 710-712
doi: 10.1021/np990482w
- Logambal SM, Venkatalakshmi S, Michael RD. 2000. Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). Hydrobiologia, 430 (1-3):113-120.
doi: 10.1023/A:1004029332114
- Loizzo MR, Tundis R, Menichini F. 2010. Chelating, antioxidant and hypoglycaemic potential of *Muscari comosum* (L.) Mill. bulb extracts. Int J Food Sci Nutr. 61(8):780-791.
doi: 10.3109/09637486.2010.482521
- Magnadottir B. 2006. Innate immunity of fish (Overview). Fish Shellfish Immunol. 20 (2): 137-151.
doi: 10.1016/j.fsi.2004.09.006
- Mehana EE, Rahmani AH, Aly SM. 2015. Immunostimulants and Fish Culture: An Overview. Annu Res Rev Biol. 5 (6):477-489
doi: 10.9734/ARRB/2015/9558
- Nasrabadi, M, Halimi M, Nadaf M. 2013. Phytochemical screening and chemical composition of extract of *Muscari neglectum*. Middle-East J Sci Res 14(4):566–569.
doi: 10.5829/idosi.mejsr.2013.14.4.71191
- Pieroni, A, Janiak V, Durr CM, Ludeke S, Trachsel E, Heinrich M. 2002. In vitro antioxidant activity of non-cultivated vegetables of ethnic Albanians in southern Italy. Phytother. Res. 16 (5):467–473.
doi: 10.1002/ptr.1243
- Plumb, J. A., and Hanson, LA. 2011. Health Maintenance and Principal Microbial Diseases of Cultured Fishes. Hoboken, NJ: Wiley-Blackwell.
doi: 10.1002/9780470958353
- Pratheepa V, Ramesh S, Sukumaran N. 2010. Immunomodulatory effect of *Aegle marmelos* leaf extract on freshwater fish *Cyprinus carpio* infected by bacterial pathogen *Aeromonas hydrophila*. Pharm. Biol. 48 (11): 1224-1239
doi: 10.3109/13880201003713598
- Schaperclaus W, Kulow H, Schreckenbach K. (1991) Hematological and serological technique. In: *Fish Disease*, Vol. 1, 2nd edn, ed. by V.S.Kothekar), pp. 71–108. Gulab primlani, Oxonian Press, New Delhi, India.
- Steinhagen D, Kruse P, Körting W. 1990. Some hematological observations on carp, *Cyprinus carpio* L. experimentally infected with *Trypanoplasma borreli* Laveron. J Fish Dis. 13(2) :157–162
doi: 10.1111/j.1365-2761.1990.tb00768.x
- Sudhakaran DS, Sirekha P, Devasree LD, Prem Singh S, Michael RD. 2006. Immunostimulatory effect of *Tinospora cordifolia* Miers leaf extract in *Oreochromis mossambicus*. Indian J Exp Biol. 44 (9):726-732
- Sharma A, Deo AD, Riteshkumar ST, Chanu TI, Das A. 2010. Effect of *Withania somnifera* (L. Dunal) root as a feed additive on immunological parameters and disease resistance to *Aeromonas hydrophila* in *Labeo rohita* (Hamilton) fingerlings. Fish Shellfish Immunol. 29 (3):508-512
doi: 10.1016/j.fsi.2010.05.005
- Tanker M, Tanker N. 1991. Farmakognozi Cilt 1. Ankara Üniversitesi, Eczacılık Fakültesi yayını, No:66, Ankara. (In Turkish) [cited 2017 November 11] Available from <http://kitaplar.ankara.edu.tr/dosyalar/pdf/313.pdf>
- Villa F, Borgonovo G, Cappitelli F, Giussani B, Bassoli A. 2012. Sub-lethal concentrations of *Muscari comosum* bulb extract suppress adhesion and induce detachment of sessile yeast cells. Biofiling 28(10):1107–1117.
doi: 10.1080/08927014.2012.734811



Intestine Villi Morphology of Black Sea Trout (*Salmo labrax* Pallas, 1814)

Osman Tolga ÖZEL^{1*} , İsa COŞKUN² , Eyüp ÇAKMAK¹

¹Central Fisheries Research Institute, 61250, Trabzon, Turkey

²Ahi Evran University, Faculty of Agriculture, Department of Animal Science, 40200, Kırşehir, Turkey

ABSTRACT

This study was conducted to determine the effect of age, sex and weight on the middle intestine villi morphology of fifth-generation Black Sea trout (*Salmo labrax* Pallas, 1814). The fish were kept in the freshwater ponds (June-October) and marine cages (November-May) to provide the life cycle of species. Intestine tissue samples were taken during period when fish were kept in marine cages. Our results showed that, middle intestine villi morphology changed depending on fish age (I, II, III, IV and V years old) and different live weight (low weighted and high weighted) of similar ages (16 months old). The lowest intestine villi development was obtained in I years old fish. This was followed by II years old fish. The highest intestine villi development was obtained in III years old fish. Besides, intestine villi development of high weighted fish was higher than those of low weighted fish. However, it was found that middle intestine villi morphology did not changed depending on sex.

Keywords: Trout, age, sex, weight, histology

ARTICLE INFO

SHORT COMMUNICATION

Received : 13.12.2017

Revised : 05.03.2018

Accepted : 08.03.2018

Published : 27.04.2018

DOI:10.17216/LimnoFish.365434



* CORRESPONDING AUTHOR

osmantolga.ozel@tarim.gov.tr

Tel : +90 462 341 10 53

Karadeniz Alabalığı (*Salmo labrax* Pallas, 1814)'nın Bağırsak Villi Morfolojisi

Öz: Bu çalışma, 5. (F5) nesil Karadeniz alabalığı (*Salmo labrax* Pallas, 1814)'nın bağırsak villi morfolojisi üzerine yaş, cinsiyet ve ağırlığın etkisini belirlemek için yapılmıştır. Türün doğal yaşam döngüsünü sağlamak için, balıklar tatlısu havuzlarında (Haziran-Ekim) ve deniz kafeslerinde (Kasım-Mayıs) tutulmuştur. Bağırsak doku örnekleri, balıkların deniz kafeslerinde tutulduğu dönemde alınmıştır. Çalışmada elde edilen sonuçlara göre, orta bağırsak villi morfolojisi balığın yaşına (I, II, III, IV ve V yaş) ve aynı yaştaki balığın (16 aylık yaş) farklı canlı ağırlığına (düşük ve yüksek ağırlıklı) bağlı olarak değişmiştir. En düşük bağırsak villi gelişimi I yaşlı balıklarda görülmüş, bunu II yaşlı balıklar takip etmiştir. En yüksek bağırsak villi gelişimi ise III yaşlı balıklarda görülmüştür. Ayrıca yüksek ağırlığa sahip balıkların bağırsak villi gelişimi düşük ağırlıklı balıklardan daha yüksek bulunmuştur. Ancak orta bağırsak villi morfolojisi üzerine cinsiyetin etkisinin olmadığı tespit edilmiştir.

Anahtar kelimeler: Alabalık, yaş, cinsiyet, ağırlık, histoloji

How To Cite

Özel OT, Coşkun İ, Çakmak E. 2018. Intestine Villi Morphology of Black Sea Trout (*Salmo labrax* Pallas, 1814) LimnoFish. 4(1): 42-46. doi: 10.17216/LimnoFish.365434

Introduction

Black Sea trout as a subspecies of the brown trout is distributed at the Eastern Black Sea. This is an endemic species for Turkey. This species has three different ecotypes in the natural environment: sea ecotype, stream ecotype and lake ecotype. These ecotypes have great variations in terms of color (Tabak et al. 2002). In Black Sea condition, Black Sea trout are grown in freshwater ponds until smolt stage, and then smoltified in seawater when they reach nearly 12 cm of length and 15 g of weight. In marine cages, fish are grown during the periods

(November-May) allowed by the water temperature. At the end of the season, when the temperature of sea water reached critical value (18 °C) for Black Sea trout aquaculture, the fish are harvested or transferred to freshwater ponds for brood maintenance (SUMAE 2010).

The digestive canal of teleost fish is divided into headgut, foregut, midgut and hindgut. The headgut is composed of the mouth and gill cavities. The foregut is comprised of the esophagus, stomach and pylorus. The midgut contains the intestine and a variable number of pyloric caecae, and is the greatest part of the gastrointestinal tract. The hindgut begins by an

increase in diameter of the gastrointestinal tract and ends with the anus (Floris 2010). The gastrointestinal tract are responsible to storing food and water, processing ingested food and water and absorbing digested food and water, and excreting wastes (Jutfelt 2006). Intestine has very important roles such as to complete the digestive process started in the stomach, and to absorb the nutrients from ingested food (Nasruddin et al. 2014). Thus, histologically examination of the digestive system (especially intestine) is quite important. With this way, the knowledge related to nutritional status of fish is can be obtained (Chowdhary et al. 2013; Yadav et al. 2014).

The histological features of intestine may be influenced by abiotic and biotic factors (Khojasteh 2012) such as species and feed (Raskovic et al. 2011), feeding habit, age, and body shape (Cao et al. 2011). Intestine of fish is composed of mucosa, submucosa, muscularis and serosa layers (Mokhtar et al. 2015). Mucosa layer is composed of epithelium, lamina propria and muscularis mucosa (Mumford et al. 2007). Intestine villi is located in enterocyte cells of epithelium.

Intestine villi play an important role both in the absorption of nutrients and in the pumping of absorbed nutrients into the blood or lymph (Karabulut 1991). Moreover, knowledge to be obtained about the intestine villi will contribute on the nutritional studies to be carried out. Thus, the evaluation of intestine villi morphology of Black Sea trout is aimed in this study.

Material and Methods

Experimental Design

The study was conducted as three groups at Central Fisheries Research Institute (Trabzon, Turkey): Age (I, II, III, IV and V years old), sex (adult fish; between III and IV years old) and different weight of similar ages (16 months old). In the study, fifth-generation Black Sea trout were used. For age and sex studies, the fish were kept in the freshwater ponds (0.5-18.5 °C) fed by stream between May-November 2016. After that, the fish were transferred to the sea cages (6.9-7.6 °C) in November 2016. Fish were selected as five groups (I, II, III, IV and V years old) according to their age. For different weight study, the fish (from larvae to smolt stage) kept in the freshwater ponds (10.1-12.5 °C) fed by spring water between January-November 2016. After that, the fish (18.63±3.84 g) were transferred in seawater (6.9-16.6 °C) in November 2016 where they were kept for 6 months. Fish, 16 months old, were selected as two groups according to their live weight: Low weighted group (LW) and high

weighted group (HW). Fish were fed at ad-libitum feeding level during experiment. Water temperature was recorded daily. Seawater salinity was recorded as 0.18‰.

Intestine Sampling

Intestine samplings for age and sex studies were taken in January 2017 in seawater (7.6 °C), while sampling for different weight study was taken in May 2017 in seawater (16.6 °C). Twenty of fish from each group for every three experiments, middle intestine tissues were taken, and analyzed by placing the samples into 10% formalin then into the tissue cassettes for dehydration process and embedded in paraffin blocks, and subsequently cut 5-µm thickness and placed on a slide. Each sample was stained with hematoxylin and eosin. After staining, villi length (VL), villi width (VW) and villi length to villi width (VL/VW) were evaluated by using an image processing and analysis system (ZEN 2012 SP2) (Xu et al. 2003).

Statistical Analysis

Data of age were analyzed by one-way analysis of variance. Differences between means were compared using Duncan's multiple range test. Data of sex and different weight were analyzed by independent samples t test. Statistical analyses were computed using SPSS 15.0.

Results

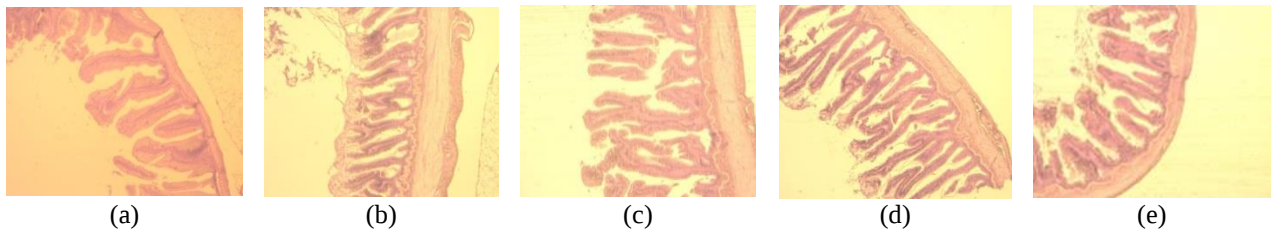
There was significantly difference between groups in terms of both fish weight and middle intestine villi morphology ($P < 0.05$). Weights of the I, II, III, IV and V years old fish were recorded as 80.83±9.09 g, 819.82±189.59 g, 2548.5±681.14 g, 3064.65±979.33 g and 3598.88±928.85 g respectively. The lowest VL, VW and VL/VW were obtained in I years old fish. This was followed by II years old fish. The highest VL and VW were obtained in III years old fish. VL of the IV and V years old fish were similar to each other, respectively. Also, VW of the II, IV and V years old fish were similar to each other, respectively. Furthermore, VL/VW of the 3, 4 and 5 years old fish were similar to each other, respectively (Table 1).

There wasn't significantly difference between groups (female and male) in terms of both fish weight and middle intestine villi morphology ($P > 0.05$). Weights of female and male fish were recorded as 2763.44 ±1111.39 g and 2826.81±711.77 g, respectively. VL, VW and VL/VW of male and female fish were 1100.86±145.92 µm and 1090.72±107.98 µm, 158.94±38.22 µm and 168.44±41.65 µm, 7.38±2.22 and 6.86±1.81, respectively (Table 2).

Table 1. Age-dependent middle intestine villi morphology of Black Sea trout.

Age	Weight (g)	VL (μm)	VW (μm)	VL/VW
I	80.83 $\pm$ 9.09 ^e	315.39 $\pm$ 92.62 ^d	65.18 $\pm$ 13.40 ^c	4.94 $\pm$ 1.42 ^c
II	819.82 $\pm$ 189.59 ^d	713.98 $\pm$ 73.44 ^c	147.06 $\pm$ 35.54 ^b	5.20 $\pm$ 1.60 ^b
III	2548.50 $\pm$ 681.14 ^c	1160.52 $\pm$ 90.74 ^a	174.76 $\pm$ 36.56 ^a	6.92 $\pm$ 1.51 ^a
IV	3064.65 $\pm$ 979.33 ^b	1092.50 $\pm$ 121.29 ^b	152.64 $\pm$ 47.89 ^b	7.80 $\pm$ 2.39 ^a
V	3598.88 $\pm$ 928.85 ^a	1046.11 $\pm$ 140.00 ^b	157.59 $\pm$ 33.07 ^b	6.99 $\pm$ 2.06 ^a

Means with different superscript letters in a column are significantly different ($P < 0.05$)

**Figure 1.** Middle intestine villi histology of Black Sea trout. One to five years old: (a,I; b,II; c,III; d,IV; e,V years old) (4x, H&E).**Table 2.** Sex-dependent middle intestine villi morphology of adult Black Sea trout.

Sex	Weight (g)	VL (μm)	VW (μm)	VL/VW
Female	2763.44 $\pm$ 1111.39	1090.72 $\pm$ 107.98	168.44 $\pm$ 41.65	6.86 $\pm$ 1.81
Male	2826.81 $\pm$ 711.77	1100.86 $\pm$ 145.92	158.94 $\pm$ 38.22	7.38 $\pm$ 2.22

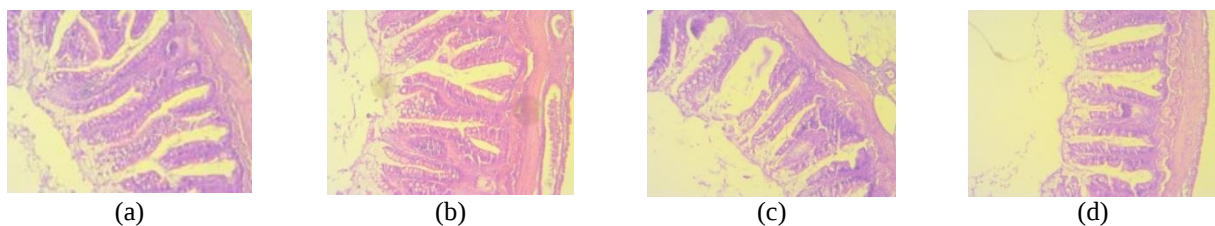
There was significantly difference between groups (LW and HW) in terms of both fish weight and middle intestine villi morphology (VL, VW and VL/VW). Weights of low weighted fish and high weighted fish were recorded as 151.86 $\pm$ 12.65 g and 372.83 $\pm$ 15.92 g,

respectively. While VL, VW and VL/VW of low weighted group were 473.38 $\pm$ 76.07 μm , 114.18 $\pm$ 22.36 μm and 4.29 $\pm$ 1.05, VL, VW and VL/VW of high weighted group were 605.96 $\pm$ 97.54 μm , 130.28 $\pm$ 22.76 μm and 4.78 $\pm$ 1.10, respectively (Table 3).

Table 3. Different weight-depend middle intestine villi morphology of Black Sea trout.

Groups	Weight (g)	VL (μm)	VW (μm)	VL/VW
Low weighted (LW)	151.86 $\pm$ 12.65 ^b	473.38 $\pm$ 76.07 ^b	114.18 $\pm$ 22.36 ^b	4.29 $\pm$ 1.05 ^b
High weighted (HW)	372.83 $\pm$ 15.92 ^a	605.96 $\pm$ 97.54 ^a	130.28 $\pm$ 22.76 ^a	4.78 $\pm$ 1.10 ^a

Means with different superscript letters in a column are significantly different ($P < 0.05$)

**Figure 2.** Middle intestine villi histology of Black Sea trout. a-b: high weighted, c-d: low weighted. (4x, H&E).

Discussion

Growth is expressed as gradually the improvement in a living organism depending on time. In fish, growth varies depending on feed consumption and quality, stocking density, sex, age, genetic variance, water chemistry, temperature, photoperiod and oxygen level (Lugert et al. 2000). Age and growth are in a close relationship (Bhatt and Jahan 2015). Growth rate can be affected fish size,

and generally tends to decrease with the increase in the size of organisms (Jonsson and Jonsson 2007). Our study demonstrated that as fish get older, its body weight increased, but growth rate were not. Fish growth rate was increasingly increased from I to III years old, but were diminished after III years old. This may be related to the completion of some physiological developments such as sexual maturity of this species. Stream and sea ecotypes of Black Sea

trout reach the sexual maturity in 22 and 34 months old, respectively (Unpublished). According to Anonymous (2012), early sexual maturation reduces growth rate in farmed Atlantic Salmon. Similarly, Jonsson and Jonsson (2007) stated that body size of Atlantic salmon changes continuously among populations, and this seems to be related to growth rate and sexual maturity age, as late maturing fish tend to be more fast growing than mature ones. Similar to the growth rate, middle intestine villi morphology also changed depending on age of fish in our study. Villi length and width were increasingly increased from I to III years old, but were diminished after III years old. The intestine villi structure developed until the fish completed sexual maturity, but then showed tend to weaken. This may arise from growth rate of fish species. Michel-Parra et al (2007) found that jejenum intestine villi length changed depending on age (10, 20, 30, 40 and 60 days) in Tilapia (*Oreochromis aureus*). It is clear fact that, studies carried out on this subject are insufficient.

Black Sea trout is an opportunistic ecotype (Tabak et al. 2002) and there is competition among their individuals in terms of nutrition. This can lead to different weight in the fish in the similar ages. In this study, although the fish were kept same environmental conditions and were fed with the same feed, fish with different weights were observed. This is reflected in the middle intestine villi morphology. Coskun and Bat (2015) found that the difference in the live weights of broiler chickens kept under the same environmental factors and fed on the same feed had no significant effect on histomorphological parameters. This may arise from the weight averages of broiler chickens are close to each other. Discussion was made on other monogastric species due to lack of knowledge with fish species.

We may say that middle intestine villi development (VL, VW and VL/VW) were proportional to the growth rate, and increased depending on live weight gain, and also changed depending on age. For better understanding of intestine villi morphology of this species, other sections (proximal and distal) of the intestine should be examined, and villi feeding studies should be carried out. In addition, special feeding (feed additives etc.) programs can be applied to improve the intestine villi morphology of low weighted fish.

Acknowledgements

This research was done as a preliminary study for the project named "Possibilities of using some phytobiotic added diets in feeding of Black Sea trout (*Salmo trutta labrax* Pallas, 1814)" supported by General Directorate of Agricultural Research and Policies. Part of the research was presented as oral

and poster presentations at the International Symposium on Limnology and Freshwater Fisheries.

References

- Anonymous 2012. The Effect of photoperiod on growth and maturation of Atlantic salmon (*Salmo salar*) in the bay of fundy. Aquaculture science branch fisheries and oceans Canada. Issue 14.
- Bhatt BJ, Jahan N. 2015. Determination of age and growth rate of fresh water fish *Labeo rohita* (Ham. 1822) by using cycloid scales. Int J Pure App Biosci. 3 (3): 189-200.
- Cao XJ, Wang WM, Song F. 2011. Anatomical and Histological Characteristics of the Intestine of the Topmouth Culter (*Culter alburnus*). Anat Histol Embryol. 40(4):292-298.
doi: 10.1111/j.1439-0264.2011.01069.x
- Chowdhary S, Srivastava PP, Jena J, Yadav AK, Dayal R, Mishra S, Srivastava M. 2013. Histological studies of the intestine in threatened Asian catfish (*Clarias batrachus*) fingerlings fed with animal or plant origin protein blended with glucosamine. Int J Fish Aquat Stu. 1(2):50-55.
- Coskun I, Bat G. 2015. Etlik piliçlerin ileum histolojisi ve goblet hücre sayısı üzerine farklı kesim canlı ağırlığının etkisi. Tavukçuluk Araştırma Dergisi 13 (2): 1-4. [in Turkish]
- Floris R. 2010. Microbial ecology of the intestinal tract of gilthead sea bream (*Sparus aurata* Linnaeus, 1758). [PhD Thesis]. University of Sassari. 133 p.
- Jonsson N, Jonsson B. 2007. Sea growth, smolt age and age at sexual maturation in Atlantic salmon. J Fish Biol. 71 (1): 245-252.
doi:10.1111/j.1095-8649.2007.01488.x
- Jutfelt F. 2006. The Intestinal epithelium of Salmonids. [PhD Thesis]. University of Gothenborg. 73 p.
- Karabulut A. 1991. Hayvan besleme fizyolojisi ve metabolizması ders notu. Uludağ Ünivesitesi, Ziraat Fakültesi, Bursa, Türkiye. [in Turkish]
- Khojasteh SMB. 2012. The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. International Journal of Aquatic Science. 3 (2): 71-88.
- Lugert V, Thaller G, Tetens J, Schulz C, Krieter J. 2000. A review on fish growth calculation: multiple functions infish production and their specific application. Rev Aquacult. 8 (1): 30-42.
doi: 10.1111/raq.12071
- Michel-Parra JG, Hernadez JRO, Garcia IJR, Valdez ODM. 2007. Effect of protein level on alevins growth and intestinal villi development of Tilapia (*Oreochromis aureus*). J Fisheries Int. 2 (3): 211-213.
- Mokhtar DM, Abd-Elhafez EA, Hassan AH. 2015 Light and scanning electron microscopic studies on the intestine of Grass carp (*Ctenopharyngodon idella*): I- Anterior intestine. J Aquac Res Development 6(11): 374.
doi:10.4172/2155-9546.1000374
- Mumford S, Heidel J, Smith C, Morrison, J, MacConnell B, Blazer V. 2007. Fish histology and histopathology. [cited from:22/04/2017] Available from: https://training.fws.gov/resources/course-resources /fish-histology/Fish-Histology_Manual_v4.pdf

- Nasruddin NS, Azmai MNA, Ismail A, Saad MZ, Daud HM, Zulkifli SZ. 2014. Histological features of the gastrointestinal tract of wild Indonesian shortfin Eel, *Anguilla bicolor bicolor* (McClelland, 1844), captured in peninsular Malaysia. Sci World J. Article ID 312670, 8 pages.
[doi:10.1155/2014/312670](https://doi.org/10.1155/2014/312670)
- Raskovic BS, Stankovic M.B, Markovic ZZ, Poleksic VD. 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. J Agricult Sci. 56 (1): 87-100.
[doi: 10.2298/JAS1101087R](https://doi.org/10.2298/JAS1101087R)
- SUMAE. 2010. Karadeniz alabalığı (*Salmo trutta labrax*) yetiştiriciliği el kitabı, E. Çakmak, M. Aksungur (Ed.), Su Ürünleri Merkez Araştırma Enstitüsü Müdürlüğü, Yayın No: 2010-2 Trabzon, 58 s. [in Turkish]
- Tabak I, Aksungur M, Zengin M, Yılmaz C, Aksungur N, Alkan A, Zengin B, Mısır DS. 2002. Karadeniz alabalığı (*Salmo trutta labrax*, Pallas 1811)'nın biyoteknolojik özelliklerinin tespiti ve kültüre alınabilirliğinin araştırılması projesi, TAGEM/HAYSUD/98/12/01/2007. [in Turkish]
- Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci. 82 (6): 1030-1036.
[doi: 10.1093/ps/82.6.1030](https://doi.org/10.1093/ps/82.6.1030)
- Yadav AK, Srivastava PP, Chowdhary S, Lakra WS, Shrivastava P, Dayal R. 2014. Histological alterations in the intestine of threatened Asian catfish, *Clarias batrachus* fed with different types of fats through semi-purified diets. Adv Appl Sci Res. 5(2):35-41.



Su Ürünleri İşleme ve Muhafazasında Yüksek Hidrostatik Basınç Kullanımı

İlknur UÇAK* 

Niğde Ömer Halisdemir Üniversitesi, Tarım Bilimleri ve Teknolojileri Fakültesi, 51240, Niğde, Türkiye

Ö Z

Su ürünlerinin yüksek hidrostatik basınç (YHB) ile işlenmesi fizikokimyasal, mikrobiyal ve duyu kalitenin gelişmesinde oldukça önem göstermektedir. Geleneksel yöntemlerle karşılaştırıldığında YHB düşük sıcaklıklarda ürün tazeliğinde çok az değişikliklerle mikroorganizmaları inaktive edebilmekte ve raf ömrünün uzamasını sağlamaktadır. Su ürünlerinde YHB' nin etkileri üzerine yapılan son çalışmalar, bu yeni teknolojinin hem faydalarını hem de eksik yanlarını ortaya koymaktadır. İşlem görmemiş su ürünleri ile kıyaslandığında, YHB su ürünlerinde depolama süresince bozulmanın azalmasını ve organoleptik özelliklerin korunmasını sağlamaktadır. Ancak renk bozulması, pişmiş görünüm ve lipid oksidasyonu YHB' nin su ürünlerinde kullanımını sınırlandıran dezavantajlardır. Ayrıca balık kasının yüksek basınçla jelleştirilmesi ve yüksek basınçla dondurulup-çözdürülmesi, su ürünlerinden fayda sağlamak için YHB' nin yoğun olarak araştırılmakta olan en önemli alanlarından biridir. Bu derlemede, YHB' nin su ürünlerinde kullanım alanları, avantajları ve dezavantajları hakkındaki yaklaşımlar değerlendirilmiştir.

Anahtar kelimeler: Yüksek basınç teknolojisi, balık, raf ömrü, mikrobiyal kalite, kimyasal kalite

MAKALE BİLGİSİ

DERLEME

Geliş : 26.09.2017

Düzeltilme : 19.01.2018

Kabul : 22.01.2018

Yayın : 27.04.2018



DOI:10.17216/LimnoFish.340039

* SORUMLU YAZAR

ilknurucak@ohu.edu.tr

Tel : +90 388 225 30 44

Utilization of High Hydrostatic Pressure in Seafood Processing and Preservation

Abstract: High hydrostatic pressure (HHP) processing of seafood has showed a great potential on improving the physicochemical, microbial and sensory quality of muscles. HHP at low temperatures can inactivate microorganisms with fewer changes to product freshness as compared to conventional preservation techniques and leads to an extension shelf life. Recent researches on the effects of HHP on seafood have gradually revealed the both benefits and defects of this novel processing technology. HHP has the advantage of ensuring reduction of spoilage in seafood, and preserving the organoleptic characteristics of the product compared to untreated muscle over storage time. However, the discolouration, cooked appearance and lipid oxidation are the drawbacks that could limit the application of high pressure on fish muscles. Furthermore, pressure-induced gelling and high pressure freezing-thawing of fish muscles are the main areas being investigated intensively to obtain the benefits of HHP seafood. Approaches in the utilization of HHP in seafood area, advantages and disadvantages of HHP were evaluated in this review.

Keywords: High pressure technology, fish, shelf life, microbiological quality, chemical quality

Alıntı

Uçak İ. 2018. Su Ürünleri İşleme ve Muhafazasında Yüksek Hidrostatik Basınç Kullanımı LimnoFish. 4(1): 47-57. doi: 10.17216/LimnoFish.340039

Giriş

Su ürünlerinin muhafazasında geleneksel işleme teknolojileri, uzun yıllardan beri yaygın olarak kullanılmaktadır. Geleneksel ısı işlem uygulamaları sırasında ürünün maruz kaldığı sıcaklığın istenmeyen kalite değişimlerine yol açması nedeni ile ısı olmayan ileri muhafaza tekniklerinin geleneksel yöntemlerle birlikte veya tamamen geleneksel yöntemlere alternatif olarak kullanılması son yıllarda yaygın hale gelmiştir. Su ürünlerinin muhafazasında genel olarak yapılması gereken iki temel

uygulamadan birincisi mikrobiyolojik aktivitenin durdurulmasıdır. Bu uygulama mikroorganizmaların tamamen uzaklaştırılması, gelişmelerinin engellenmesi veya öldürülmesi ile mümkün olmaktadır. Diğeri ise dokulardaki kimyasal olayların (enzimatik reaksiyonlar, su aktivitesi, oksidatif olaylar vb.) kontrol altına alınmasıdır. Bu kimyasal olaylar hem ürüne bulaşan mikroorganizmalardaki hem de ürünlerdeki tabii enzimler ve kimyasal reaksiyonlardan ileri gelmektedir. Bu noktada gıda teknolojisi açısından en önemli husus gıda

kalitesindeki ve besleyicilik değerindeki en az değişim ile gıda güvenliğinin en üst noktaya ulaştırılmasıdır (Arıcı 2006).

Yüksek hidrostatik basınç uygulamaları (YHB), termal yıkımları ortadan kaldırarak gıdaların 'tazelik' karakterinde minimum değişimler sağlamaktadır. Isıl işlemlere kıyasla, YHB uygulamaları gıdalarda daha taze lezzet, daha iyi görünüş, tekstür ve besin değeri ile sonuçlanmaktadır. Tüketici tarafından gıdanın kabul edilebilirliğinde, ürünün duyu özellikleri büyük bir rol oynamaktadır. YHB uygulamaları ortam ya da buzdolabı sıcaklıklarında gerçekleştirilebildiği için ısı işlemler sonucu ortaya çıkan istenmeyen kötü değişimleri elimine etmektedir.

Bu derlemede, yüksek hidrostatik basınç uygulamasının su ürünleri işleminde kullanım alanları, yüksek hidrostatik basıncın avantajları ve dezavantajlarının anlatılması amaçlanmıştır.

Yüksek hidrostatik basınç

Yüksek hidrostatik basınç, katı veya sıvı gıdaların ambalajlı veya ambalajsız olarak düşük sıcaklıklarda 100-1000 MPa basınca maruz bırakılmasıyla mikrobiyal inaktivasyonun sağlandığı bir teknolojidir (Cheftel ve Culioli 1997; Crehan vd. 2000). Gıdaların basınçla işlenmesi ABD'de Hite (1899) ve Hite vd. (1914) gibi araştırmacılar tarafından ilk olarak 19. yüzyılın sonlarında çalışılmıştır. Yüksek hidrostatik basıncın bakterileri inaktive ettiğine dair ilk rapor Roger tarafından 1895 yılında açıklanmış olmasına rağmen, gıda endüstrisinde yüksek hidrostatik basınç ile mikrobiyal inaktivasyonu açıklayan önemli çalışma Bert Hite'in Temmuz 1899'da yayınlanan makalesidir. Hite ilk çalışmasında oda sıcaklığında 1 saat 600 MPa'lık basınca maruz bırakılan çiğ sütün raf ömrünün 4 gün uzadığını göstermiştir. Bununla beraber, sütte asitlik artışını da 200 MPa'lık bir uygulama ile 24 saat geciktirmeyi başarmıştır. Hite vd. (1914), 400 ve 820 MPa arasında değişen basınç işlemine tabi tutulan çoğu meyve suyunun, işlemden sonra en az 5 yıl boyunca ticari olarak steril kaldıklarını göstermişlerdir.

Yüksek basınç işleminin oda sıcaklığında uygulanabilmesi, kovalent bağları ve küçük molekülleri fazla etkilememesinden dolayı; tat, aroma, vitaminler, pigmentler, fenoller ve diğer benzer bileşenler üzerinde önemli değişikliklere neden olmamaktadır. Kovalent ve kovalent olmayan bağları etkileyen ısı uygulamaların aksine, oda sıcaklığında YHB uygulamaları sadece nispeten zayıf kimyasal bağları (hidrojen, hidrofobik ve iyonik bağlar) etkilemektedir (Hendrickx vd. 1998). Bu nedenle gıdanın doğal görünümü ve lezzeti korunmuş olmaktadır. Yüksek basınç işlemi sırasında 1000 MPa'lık basıncın etkisini anlatmak için 10000 kg'lık

ağırlığın aynı anda 1 cm²'lik alana yaptığı etkiye eşit olmasıyla açıklanabilir (Özlü 2006) (1 atm=0.1 MPa=1 bar). Basınç uygulaması sırasında kullanılacak işlem sıcaklığı 0°C'nin altından başlayıp (adyabatik ısınmadan kaynaklanabilecek etkileri en aza indirmek için), 100°C'nin (gıdalarda sterilizasyon amacı ile) üzerine çıkabilir (Truong vd. 2015). Yüksek hidrostatik basınç uygulaması sırasında adyabatik ısınmadan dolayı sıcaklıkta küçük bir artış gerçekleşmektedir. Su için her 100 MPa'da 2-3°C sıcaklık artışı olmaktadır (Guerrero-Beltran vd. 2005; San Martin 2002). Suyun faz geçişleri de basınç tarafından etkilenmektedir. Bu nedenle su, -22°C'de 210 MPa'lık bir basınç altında sıvı halde kalmaktadır (Bridgman 1912). Ayrıca yüksek basınç destekli dondurma işlemi olarak adlandırılan işlem sırasında, süper soğuma nedeni ile küçük buz kristallerinin oluşması sağlanmaktadır (Cheftel ve Culioli 1997; Chevalier vd. 2000; Zhu vd. 2004). Yüksek basınç ile çözündürme sırasında ise, uygulanan basınç dondurulmuş ürün ile çevresi arasındaki sıcaklık farkını artırarak çözülme hızının da artmasını sağlamaktadır (Cheftel ve Culioli 1997; Zhu vd. 2004).

Yüksek hidrostatik basınç işlemi iki temel prensibe dayanmaktadır. Paskal izostatik prensibine göre; yüksek hidrostatik basınç ürünün hacmine, şekline ve büyüklüğüne bağlı olmaksızın ürünün her tarafına eşit şekilde etki gösterir. Le Chatelier prensibine göre de; ürünü çevreleyen sıvıya uygulanan kuvvetle sıvının sıkıştırılması ilkesine dayanmaktadır. Yüksek basıncın inaktivasyon mekanizması fiziksel olarak Le Chatelier prensibi ile açıklanabilir. Kuvvetten kaçış olarak bilinen yasaya göre, dengede olan bir fiziksel sisteme dışarıdan bir etki yapıldığında, sistem bu etkiyi en aza indirecek şekilde kendini değişikliğe uğratar. Basınç artarsa hacim azalır ve yoğunluk artar, dolayısıyla denge mol sayısı az olan tarafa kayar ve sistemin kimyasal dengesi değişir.

Tipik bir YHB sistemi basınç kabini, düşük basınç pompası, YHB pompası (intensifiyer), kapak, kapak aparatları, vanalar ve kontrol ünitesinden (basınç ve sıcaklık ölçümü) oluşmaktadır (Singh 2001). YHB sisteminde uygulanan basıncın gıdaya iletilmesinde basınçlama sıvısı olarak özel hidrolik yağlar, hidrokarbonlar veya su kullanılmaktadır (Cheftel ve Culioli 1997). Fakat pratikte hacim azalması, gazlara oranla daha az ve ucuz olmasından dolayı su en çok kullanılan sıvıdır. Basınçlama sıvısı olarak su kullanılmasının nedenleri; yüksek basınçlarda dahi hacim azalmasının ihmal edilebilir olması, gıdalar için saf, güvenilir ve ucuz olmasıdır. Su, işlem sırasında kaymayı sağlamak ve korozyonu önlemek amacıyla az miktarda yağ içerir.

Yüksek hidrostatik basıncın su ürünlerinde kullanım alanları

Yüksek hidrostatik basınç işlemi son zamanlarda su ürünlerinde oldukça geniş kapsamlı amaçlarla uygulanmaya başlamıştır. Özellikle istiridye, midye gibi kabuklu su ürünlerinin etlerinin çıkarılmasında (Moraru 2008) alternatif ve etkili bir yöntem olarak kullanılmakla beraber; surimi ve jel ürünlerinin tekstürel özelliklerinin iyileştirilmesinde (Suzuki ve Macfarlane 1984; Ikeuchi vd. 2006; Sikes vd. 2009), su ürünlerinin dondurulup çözündürülmesinde (Makita 1992; Zhao vd. 1998; Chevalier vd. 1999; Li ve Sun 2002), işlenmiş veya taze balıkların kalitesinin korunmasında kullanılmaktadır.

İstiridye, midye, deniztarağı, yengeç gibi kabuklu su ürünlerinin doğal koşullar altında açtırılması ticari olarak kullanılmaktadır. Geleneksel olarak, istiridyelerin addüktör kasları bir bıçak yardımı ile oldukça zaman alan bir işlem olarak elle kesilmektedir. Mikrodalgada ısıtma da kabukların açtırılmasında kullanılan bir yöntem olup, ısıl işlem olması nedeni ile duyuşal özelliklerde bazı değişimlere neden olmaktadır. *YHB*, soğuk bir işlem olarak uygulanabildiği ve normal ısıl işlemle kaslarda meydana gelen değişimlere yol açmadığı için kabukların açılmasını kolaylaştırmaktadır. İşlem sırasında ürün *YHB* ünitesine yerleştirilerek basınç 200-300 *MPa*'ya kadar çıkartılmaktadır. İşlem sonunda addüktör kas ayrılır ve kabuklu ürün kabuğundan ayrılarak kendiliğinden açılır. Kabuklu su ürünlerinde *YHB* kullanılması aynı zamanda istiridyelerde patojen bir bakteri olan *Vibrio türlerinin* de önemli ölçüde inaktive olmasını sağlamaktadır. Kural vd. (2008), yaptıkları bir çalışmada istiridyede bulunan *V. parahaemolyticus* hücrelerinin 2 dakikada 5 log azalması için basınç uygulamasının 350 *MPa*'dan fazla ve sıcaklığın 1-35°C arasında olması gerektiğini belirtmişlerdir. Bir başka çalışmada (Phuvasate ve Su 2015) 1,5°C sıcaklıkta 5 dk 250 *MPa* basınç uygulamasının istiridye homojenatlarında bulunan *V. parahaemolyticus*' un 6,4 log azalmasını sağladığı tespit edilmiştir.

Hepatit A, *HIV*, rotavirüs, poliovirüs'ün *YHB* ile inaktivasyonu üzerine çalışmalar vardır. Bazı çalışmalar yüksek basıncın su ürünlerinde hepatit A gibi bazı virüsleri inaktive etmede de etkili olduğunu göstermiştir (Calci vd. 2005). Virüsler, yüksek basınca olan hassasiyetlerine göre değişmekle birlikte yapısal olarak da farklılık gösteren canlı gruplarıdır (Murchie vd. 2005). Dan vd. (2009), istiridyelerde bulunan murine norovirus-1 (*MNV-1*) üzerinde yaptıkları çalışmada 0°C'de 5 dk 400 *MPa* yüksek basınç uygulamasının virüsü tamamen inaktive ettiğini tespit etmişlerdir.

Suyun yüksek basınç altında faz geçişi ile ilgili yapılan çalışmalar, suyun faz değişim sıcaklığının 0,1 *MPa*'da 0°C'den 210 *MPa* basınçta -21°C'ye düştüğünü göstermektedir. Ancak basınç 210 *MPa*'nın üzerine çıktığında sıcaklık tekrar yükselmektedir. Bu olay, su içeriği yüksek olan gıdalarda hızlı dondurma ve hızlı çözündürme sağlamaktadır (Le Bail vd. 2002). Tironi vd. (2007) levrek filetosunun yüksek basınç yardımı ile dondurulması ile elde ettikleri sonuçlardan yola çıkarak, yüksek basınç destekli dondurma işlemi sırasında dondurarak depolama boyunca raf ömrünün arttığını rapor etmişlerdir. Yüksek basınç yardımı ile dondurma balık kasında bazı yapısal değişimlere (renk, protein denaturasyonu vb.) neden olmakla beraber küçük buz kristalleri oluşmasını sağlayarak daha uzun bir raf ömrü ile sonuçlanmaktadır (Tironi vd. 2007). Ticari dondurma yöntemleri ile kıyaslandığında yüksek basınç yardımı ile dondurma işlemi balık kasında çözündürme süresini kısaltmakta ve çözündürme sırasında damlama kaybını da azaltmakta ancak konu ile ilgili yeterli sayıda çalışma bulunmamaktadır.

Yüksek hidrostatik basıncın su ürünlerinde mikrobiyolojik kalite üzerine etkileri

Yüksek hidrostatik basıncın etkisi öncelikli olarak uygulanan basınca ve uygulanan süreye bağlıdır. Basınç uygulaması boyunca mikroorganizma sayısındaki azalma, hücrelerdeki yaralanmaların kombinasyonu sonucu meydana gelmektedir (Rendules vd. 2011). Basınç artışıyla beraber önemli olan hücre fonksiyonları tehlike altına girer ve mikroorganizmaların bu zor koşullara dayanması gittikçe imkânsız hale gelir (Mota vd. 2013). Yüksek hidrostatik basıncın mikroorganizmalar üzerindeki yıkıcı etkisi; enzimlerin inaktivasyonu, *DNA*, *RNA* ve ribozomda meydana gelen hasar, hücre zarı ve hücre duvarının bozulmasına dayanmaktadır. Özellikle hücre zarının, *YHB* uygulamalarında bakteri inaktivasyonunun temel hedefi olduğu düşünülmektedir (Smelt 1998).

Mikroorganizmaların yüksek basınca direnci, mikroorganizmanın türü, gıdanın içeriği ve pH'ya bağlı olarak değişim göstermektedir. Düşük pH'da mikroorganizmaların basınca karşı direnci, daha yüksek pH'larda uygulanan basınca olan dirence göre daha zayıftır. Aynı mikroorganizma cinsinin farklı türleri yüksek basınca karşı aynı direnci göstermeyebilir. Isıya dirençli mikroorganizmalar, genellikle ısıya duyarlı olan mikroorganizmalardan daha yüksek oranda basınca dirençlidir. Mayalar ve küflerin vejetatif formları 200-300 *MPa* basınç uygulaması ile inaktive edilebilmektedirler. Gram negatif bakteriler, gram pozitif bakterilere oranla basınca ve ısıya karşı daha hassastırlar. Yüksek

hidrostatik basınca karşı en büyük direnci bakteri sporları göstermektedir. Bakteri sporları 1200 MPa basınca kadar direnç gösterebilirler. Virüslerin ise basınca karşı dirençleri oldukça değişkendir (Metrick vd. 1989; Smelt 1998; Heinz ve Knorr 1998; Hugas vd. 2002; Garriga vd. 2004).

Mikroorganizmaların yüksek basınç altındaki inaktivasyon kinetikleri; basınç seviyesi, uygulama zamanı, sıcaklık, pH, su aktivitesi ve gıda bileşenleri gibi birçok faktöre bağlıdır. YHB' nin mikroorganizmalar üzerindeki etkisi türlere göre de önemli farklılıklar göstermektedir. Sporlar oldukça dirençli olmakla birlikte, gram pozitif vejetatif bakteriler gram negatif vejetatif bakterilerden daha dirençlidirler. Durgunluk evresindeki bakteri hücreleri yüksek hidrostatik basınca logaritmik evredeki bakteri hücrelerinden daha dirençlidirler. Yüksek basınç uygulaması ile birlikte, hücre zarı geçirgenliği artmakta, hücre içi bileşenler parçalanmakta, hücrede enerji üreten reaksiyonlar inhibe olmakta, hücre büyümesi için gerekli olan enzimler inaktive olmakta ve büyüme için gerekli olan pH aralığı azalmaktadır (Hugas vd. 2002).

Kim vd. (2013) *Morganella morganii* ve *Photobacterium phosphoreum* inoküle ettikleri uskumruda (*Scomber japonicus*) yüksek hidrostatik basınç bakterilerin gelişimi ve biyogen amin oluşumu üzerine etkilerini incelemişlerdir. 10^4 kob/g yoğunluğunda bakteri inokülasyonu yapılan balığa 100, 200, 300 ve 400 MPa (3 dk) yüksek hidrostatik basınç uygulayarak 5°C'de (*P. phosphoreum* ile inoküle edilen gruplar) ve 12°C'de (*M. morganii* ile inoküle edilen gruplar) depolamışlardır. *Morganella morganii* inokülasyonundan sonra kontrol grubu ve 100 MPa basınç uygulanan grupta depolama sonunda (168 saat) bakteri hücre sayısı 10^8 kob/g olmuştur. 200 MPa ve üzerinde basınç uygulamasının *M. morganii* inaktivasyonunda etkin olduğu gözlenmiştir. Bunun yanında 24. saate kadar histamin içeriği 5 mg/kg'dan daha düşük miktarlarda bulunmuştur. Ayrıca kontrol ve 100 MPa basınç uygulanan gruplarda histamin içeriği 120. saatte hızla artış göstererek sırasıyla 3072 ve 2635 mg/kg olmuştur. 200 MPa ve üzerinde yüksek basınç uygulanması histamin oluşumu baskılanmış ve histamin içeriği 168. saatten sonra FDA'nın önerdiği limit olan 50 mg/kg'ı (FDA 2011) geçmemiştir. *P. phosphoreum* ile inoküle edilen kontrol grubu ve 100 MPa basınç uygulanan grupta depolama sonunda (360 saat) bakteri hücre sayısı 10^7 kob/g'a ulaşmıştır. 300 MPa ve 400 MPa basınç uygulanan gruplarda *P. phosphoreum* hücre sayısı 10^4 kob/g'ın altında bulunmuştur. *P. phosphoreum* inoküle edildikten sonra depolamanın 120. saatine kadar histamin içeriğinin 2 mg/kg'dan daha düşük olduğu gözlenmiştir. Depolama sonunda en yüksek histamin

değeri (270 mg/kg) kontrol grubunda bulunmuş ve 300 MPa ve üzerinde basınç uygulanan gruplarda bu değer 1 mg/kg altında kalmıştır. Teixeira vd. (2014), levrek filetolarına (*Dicentrarchus labrax*) farklı basınç ve süre parametrelerinin etkisini incelemek amacıyla 100, 250 ve 400 MPa ve 5, 15 ve 30 dk yüksek hidrostatik basınç uygulamışlardır. Filetolarda başlangıçtaki toplam canlı sayısı 10^5 kob/g olarak bulunmuş ve 100 MPa basınç uygulaması mikroorganizma sayısında önemli değişimlere neden olmamıştır. 250 MPa ve 400 MPa basınç uygulanan gruplarda toplam canlı sayısında azalmalar gözlenirken, 400 MPa 30 dk basınç uygulamasının diğer gruplara göre en etkili olduğu ve mikroorganizma sayısında 2 log azalma sağladığı bulunmuştur. Ramirez-Suarez vd. (2006), kıyılmış tuna balığına (*Thunnus alalunga*) 275 MPa, 310 MPa ve 2, 4, 6 dk yüksek hidrostatik basınç uygulayarak 4°C ve -20°C'de depolamışlar ve kalite parametrelerindeki değişimleri incelemişlerdir. Depolama sonunda (22 gün) yüksek basınç uygulanan gruplarda toplam psikrofil canlı sayısı kontrol grubundan ve 10^6 kob/g limit değerinden daha düşük bulunmuştur. Mikroorganizma yükünü azaltmada en etkili basınç uygulamasının 310 MPa ve 6 dk olduğu gözlenmiştir. Erkan vd. (2010), farklı basınç, sıcaklık ve sürelerde yüksek hidrostatik basınç uyguladıkları barbun (*Mullus surmelutus*) balıklarının 4°C'de depolanması süresince kalitesindeki değişimleri gözlemlemişlerdir. Toplam psikrotrof canlı sayısı kontrol grubunda 11. günde, 220 MPa 25°C 5 dk yüksek basınç uygulanan grupta 15. günde ve 330 MPa 3°C 5 dk yüksek basınç uygulanan grupta 17. günde limit değere ulaşmıştır. Montiel vd. (2012), vakum paketlenmiş dumanlanmış morina balığına yüksek hidrostatik basınç etkilerini araştırmışlardır. Bu amaçla balığa 400, 500 ve 600 MPa ve 5, 10 dk yüksek hidrostatik basınç uygulayarak 5°C'de 60 gün depolamışlardır. Depolamanın başında kontrol grubunda toplam canlı mikroorganizma sayısı 2,34 log cfu/g olarak bulunmuş ve yüksek basınç uygulamasıyla mikroorganizma sayısı azalmış ve depolama sonuna kadar limit değere ulaşmamıştır.

Yağız vd. (2009), somon balıklarının kalitesi üzerine yüksek hidrostatik basınç ve pişirmenin etkilerini araştırmışlardır. Bu amaçla somon filetolarını vakum pakitleyerek 150 ve 300 MPa 15 dk basınç uygulamışlardır. Yüksek basınç işleminin ardından vakum paketlenmiş filetoları sıcak su banyosunda pişirmişlerdir. Filetoların sıcaklığı 72°C'ye ulaşınca su banyosundan çıkarmışlar ve 4°C'de 6 gün boyunca depolamışlardır. Başlangıçta kontrol grubunun toplam canlı mikroorganizma sayısı 3,56 log kob/g olarak bulunmuş, depolama sonunda 6,16 log kob/g'a ulaşmıştır. 150 MPa basınç

uygulaması depolamanın ilk gününde 3 log azalmaya neden olurken depolama sonunda mikroorganizma sayısında 2 log azalma olmuştur. 300 MPa basınç uygulanan örneklerde depolama boyunca mikrobiyal gelişim gözlenmemiştir. Picouet vd. (2011), sous-vide teknolojisi ile pişirilmiş alabalık filetolarına 210, 310, 400 MPa ve 5, 10 dk yüksek hidrostatik basınç uygulayarak 4°C'de 13 gün depolamışlardır. Sous-vide pişirilmiş ve yüksek basınç uygulanmamış grupta başlangıçtaki toplam canlı sayısı 4,5 log kob/g olarak bulunurken, basınç uygulanan gruplarda artan basınçla beraber mikroorganizma sayısında azalma gözlenmiştir. Yüksek hidrostatik basınç uygulaması, kontrol grubuna göre mikroorganizma gelişiminde üstün bir durgunluk evresi (lag fazı) sağlamıştır. 310 MPa ve 400 MPa basınç uygulanan gruplarda toplam mikroorganizma sayısı 13. güne kadar limit değere ulaşmazken, kontrol grubu ve 210 MPa basınç uygulanan grupta 11. günde limit değere ulaşmıştır. Çalışma sonunda elde edilen sonuçlar doğrultusunda 310 MPa ve üzerindeki basınç uygulamalarının sous-vide teknolojisi ile üretilmiş salmón filetolarında kontrol grubuna göre 6 günlük bir raf ömrü artışı sağladığı bulunmuştur.

Yüksek hidrostatik basıncın su ürünlerinde fizikokimyasal kalite üzerine etkileri

Basınç uygulanmış balıkta meydana gelen kimyasal değişimler, tüketici tarafından ürünün kabul ve red edilme kriteri olan tazelik ve duyu kaliteyi de önemli ölçüde etkileyebilmektedir (Truong vd. 2015). Balık raf ömrü kısa ve bozulmaya en hassas gıdalardan biridir (Dalgaard 2003). Balık kasındaki bozulma çoğunlukla balıkta doğal olarak bulunan enzim aktiviteleri, mikrobiyal aktiviteler ve doymamış yağ asitlerinin otoksidasyonu nedeni ile meydana gelmektedir (Arias 2009). Sonuç olarak, balıkta gelişen kötü koku ve kötü tat, bahsedilen bu aktiviteler sonucunda meydana gelen kimyasal bileşiklerin oluşumu ve birikimi ile ilgilidir. Balık eti mikrobiyal aktivite sonucu uçucu bazik azot ve trimetilamine (TMA-N) dönüşen serbest amino asit ve trimetilamin oksit (TMA-O) bakımından zengindir. Bu bileşikler "balıksı" kokuyu oluşturan ve balığın bozulmasından sorumlu olup, tüketici tarafından ürünün red edilmesine yol açabilmektedir. TMA-N, mikroorganizmaların faaliyetleri sonucu TMN-O' in yıkılması sonucu oluşmakta iken, TVB-N balık etinin bozulması sonucu ortaya çıkan tüm uçucu bazik bileşikler (TMA-N, amonyum, dimetilamin, diğer bazik nitrojenli bileşikler) oluşturmaktadır.

Yapılan çalışmalar, yüksek basınç uygulamasının TMA-N ve TVB-N oluşumunu önemli derecede geciktirdiğini göstermiştir. Karim vd. (2011) yaptıkları çalışmada ringa (*Clupea harengus*) ve mezgit (*Melanogrammus aeglefinus*) filetolarını

vakum paketleyerek 200, 250 ve 300 MPa (1 ve 3 dk) YHB uygulayarak buz içerisinde 2°C'de 14 gün boyunca depolamışlardır. 250 MPa (3 dk) ve 300 MPa (1-3 dk) basınç uygulanan ringa filetolarında TVB-N değerlerinin depolama boyunca sınır değerinin altında kaldığını gözlemlemişlerdir. Mezgit filetolarında da yüksek basınç uygulaması TMA-N ve TVB-N değerlerinin limit değerlere ulaşmasını engellemiş, kontrol grubunda TMA-N değeri depolamanın 6. gününde 15,34 mg/kg, TVB-N değeri ise 10. günde 49,95 mg/kg olarak sınır değerlerinin üzerine çıkmıştır. Briones-Labarca vd. (2012) yaptıkları bir çalışmada, farklı sürelerde (3, 5, 8 dk) 500 ve 550 MPa basınç uyguladıkları kırmızı istiridyede TVB-N değerlerinin kontrol grubuna göre oldukça düşük düzeylerde kaldığını ve depolama sonunda (60 gün) limit değerin (35 mg N/100 g) altında olduğunu bildirmişlerdir. Aynı şekilde YHB uygulanan gruplarda TMA-N değerlerinin depolama sonunda kontrol grubundan düşük seviyelerde olduğu gözlenmiştir. 220, 250 ve 330 MPa (5, 10dk) yüksek basınç uyguladıkları istavrit filetolarını -30°C'de 2-3 gün muhafaza ettikten sonra TMA-N değerlerini inceleyen Erkan vd. (2011), basınç uygulanmış grupların TMA-N değerlerinin kontrol grubuna göre daha düşük olduğunu bildirmişlerdir.

Protein bakımından önemli bir besin kaynağı olan balık ve balık ürünleri, hasat aşamasından tüketim noktasına kadar uygun koşullarda muhafaza edilmediği takdirde insan sağlığı için tehlikeli bir gıda haline gelmektedir (Özogul vd. 2004). Su ürünleri başta olmak üzere, özellikle protein içeriği yüksek fermente gıdalarda bulunabilen ve depolama ile miktarı artan biyojen aminler, alerjik bünyeli bireylerde olduğu kadar sağlıklı bireyler için de sorun yaratabilmektedir. Biyojen aminler arasında histamin, potansiyel olarak tehlikeli görülmekte ve histamin zehirlenmesine yol açmaktadır. Balıkların yapısında bulunan aminoasitlerin enzimatik dekarboksilasyonu ile amin bileşikler oluşmaktadır. Dekarboksilaz enzimi için gerekli olan substrat serbest aminoasitlerdir. Bu nedenle balığın bozulması veya ayrışması süresince, bakteriyel üretim, aminoasit dekarboksilasyon faaliyeti ve proteoliz aktivitesinden dolayı amino asitler serbest kalmakta ve biyojenik amin üretilmektedir (Eitenmiller ve De Souza 1984). Su ürünlerinde yüksek basıncın biyojen amin oluşumu üzerine etkileri ile ilgili sınırlı sayıda çalışma mevcuttur. Ancak yapılan çalışmalar yüksek basıncın su ürünlerinde biyojen amin oluşumunu önemli derecede etkilediğini göstermektedir.

Matejkova vd. (2013), gökkuşağı alabalığı (*Oncorhynchus mykiss*) filetolarını vakum paketledikten sonra 300 MPa ve 500 MPa 10 dk yüksek basınç uygulayarak 3,5°C ve 12°C'de

depolayarak biyogen amin oluşumunu gözlemlemiştir. Alabalık filetosunda putresin, kadaverin ve tiraminin baskın biyogen aminler olduğu bulunmuştur. Yüksek basınç uygulanmamış filetolarda raf ömrünün 3,5°C'de 5-6 gün olduğu, yüksek basınç uygulamasıyla bu sürenin 21-28 güne kadar uzayabildiği gözlenmiştir. 12°C'de depolanan örneklerde yüksek basınç uygulamasının 3,5°C'de depolanan örneklerle göre daha az etkili olduğu bulunmuştur. Turna balığını (*Esox lucius*) vakum paketlenip yüksek hidrostatik basınç uygulayarak 3,5°C ve 12°C'de 70 gün depolayan Krizek vd. (2014) biyogen amin oluşumunu incelemiştir. Depolama boyunca histamin oluşumu önemli düzeylere ulaşmamış, 3,5°C'de depolanan örneklerde ise histamine rastlanmamıştır. Bunun yanında tiramin miktarı depolama boyunca sürekli artış göstermiştir. 300 MPa ve 500 MPa basınç uygulanarak 3,5°C'de depolanan örneklerde tiramin miktarı sırasıyla 55,8 mg/kg (28. gün) ve 82,1 mg/kg (70. gün) olarak bulunurken, 12°C'de depolanan örneklerde tiramin miktarı sırasıyla 51,5 mg/kg (14. gün) ve 110 mg/kg (21. gün) olarak gözlenmiştir. Depolama süresince 3,5°C'deki tüm filetolarda putresin ve kadaverin miktarları limit değeri geçmemiş, yüksek basınç uygulanan gruplarda bu miktarlar kontrol grubuna göre daha düşük değerlerde bulunmuştur. 12°C'de kadaverin miktarı kontrol grubunda 14. günde 118 mg/kg'a ulaşırken yüksek basınç uygulanan gruplarda 21. günde sınır değere ulaşmıştır. Çalışma sonunda elde edilen bulgular yüksek hidrostatik basınç uygulamasının biyogen amin oluşumunu önemli ölçüde azalttığını gösterirken, basınç uygulamasının düşük sıcaklıklarda depolamada daha etkin olduğu bulunmuştur. Kim vd. (2013), *M. morganii* ile inoküle ettikleri uskumru etinde 200 MPa'ya kadar yüksek basınç uygulamasının histamin oluşumunu önemli derecede etkilemediğini, ancak 300 ve 400 MPa basınç uygulamasının histamin oluşumunu önemli ölçüde baskıladığını belirtmişlerdir. Depolamanın 5. gününde yüksek basınç uygulanmamış grupta histamin miktarı 3073 mg/kg, 100 MPa 3 dk basınç uygulanmış grupta ise 2636 mg/kg olarak bulunmuştur. Bunun yanında 300 ve 400 MPa basınç uygulanan gruplarda histamin miktarı depolama boyunca yok denecek kadar az miktarlarda tespit edilmiş ve sınır değere ulaşmamıştır. Benzer şekilde, yapmış olduğumuz çalışmada da uygulanan yüksek basınç seviyesinin histamin oluşumunu önemli derecede etkilediği ve en iyi basınç ve süre uygulamasının 500 MPa 10 dk olduğu bulunmuştur. Soğuk dumanlanmış morina balığına yüksek basınç uygulayarak 5°C'de 60 gün depolayan Montiel vd. (2012), depolama süresince düşük miktarlarda sadece triptamin ve spermin tespit etmişlerdir.

Balık kasının pH'sı ölüm sonrası değişiklikleri, özellikle mikrobiyal bozulma ve enzimatik aktiviteyi önemli derecede etkilemektedir (Truong vd. 2015). Balık etine YHB uygulanması, bazı protein yapılarının denaturasyonuna ve böylece mevcut asidik grupların azalmasına neden olabilmektedir (Angsupanich ve Ledward 1998). Buna ek olarak, bu asidik grupların basınç yardımı ile iyonizasyonu da tercih edilmektedir (Ramirez-Suarez vd. 2006; Yamamoto vd. 1994); Erkan vd. (2010), farklı basınç ve sıcaklıklarda yüksek basınç uyguladıkları çipura filetolarını 19 gün boyunca 4°C'de depolayarak kalitede meydana gelen değişimleri incelemişler ve basınç uygulanan gruplarda pH değerlerinin kontrol grubuna göre daha düşük olduğunu bulmuşlardır. Depolamanın başında kontrol, 250 MPa 5 dk 3°C basınç uygulanan grup ve 250 MPa 5 dk 15°C basınç uygulanan gruplarda pH değerleri sırası ile 5,8 5,1 ve 5,2 olarak bulunurken depolama sonunda bu değerler 6,8 6,1 ve 6,4'e yükselmiştir.

Proteinler yapılarında kovalent bağlar, disülfid bağları, hidrojen bağları ve diğer interaksiyonları içerirler. Bu bağların birçoğunun kırılması sonucu proteinler modifiye olmakta ve özelliklerinde önemli değişimler meydana gelmektedir (Cheret vd. 2006). YHB uygulaması kovalent bağları kıramamasına rağmen, hidrojen bağları ve diğer interaksiyonları etkileyebilmektedir. Genel olarak, kuaternar yapı daha çok hidrofobik interaksiyonlara sahip olması nedeniyle yüksek hidrostatik basınç uygulamasına karşı daha hassastır. Proteinlerin sekonder yapıları üzerindeki değişimler ise ancak çok yüksek basınçlarda, heliksel yapının oluşumunu sağlayan hidrojen bağlarının kırılması sonucu ortaya çıkmaktadır. Sekonder yapıdaki bu değişim genellikle geri dönüşümsüzdür. Balık kası miyofibriller (tuzda çözünen), sarkoplazmik (suda çözünen) ve stroma (kollajen) proteinlerinden oluşmaktadır (Shahidi 1994). Miyofibriller proteinler balık kasında bulunan toplam ham proteinin %40-60 kadarını oluştururken, sarkoplazmik proteinler %30 kadarını oluşturmaktadır. Geri kalan kas proteinleri ise stroma proteinlerinden oluşmaktadır (Ohshima vd. 1993; Shahidi 1994; Shimidzu vd. 1996). Yüksek basınçın proteinler gibi gıda biyopolimerleri üzerindeki etkileri, bir denge sisteminin bozulmasına neden olan herhangi bir dış faktörün etkisini en aza indirme eğiliminde olduğu Le Chatelier prensibine dayanmaktadır (Mozhaev vd. 1996). Yüksek basınç uygulanmış balık kasında yapılan protein çözünürlüğü testleri, miyofibriller proteinlerin basınca sarkoplazmik proteinlerden daha duyarlı olduğunu göstermiştir. Yüksek basınç uygulanmış tilapia kasında miyofibriller proteinlerin çözünürlüğünün 200 MPa basınçtan sonra hızla azaldığı gözlenmiştir (Ko vd. 2006). Protein

çözünürlüğü ile ilgili yapılan çalışmalarda elektron mikroskobu incelemeleri sonucunda miyofibriller proteinlerin yüksek basınçtan en çok etkilendiği görülmüştür. Yüksek basınç uygulamasında ince filamentler kalın flamanetlerden daha çok denature olurken, ısı işlem uygulamaları hem ince hem de kalın filamentlerde denaturasyon ile sonuçlanmaktadır (Yoshioka ve Yamamoto 1998).

Yüksek hidrostatik basıncın su ürünlerinde lipit oksidasyonu üzerine etkileri

Su ürünlerinde muhafaza süresini sınırlayan en önemli faktörlerden biri yağlarda meydana gelen oksidasyondur. Bilindiği gibi balık eti özellikle *EPA* ve *DHA* gibi omega-3 çoklu doymamış yağ asitleri bakımından (*PUFA*) oldukça zengindir. Ancak *PUFA*'lar oksidasyona ve depolama süresince meydana gelen oksidatif değişimlere karşı oldukça hassastır. Yüksek basınç uygulanmış balık kasında, yapılarındaki metal iyonları nedeni ile hemoglobin ve myoglobin gibi heme proteinlerinin lipit oksidasyonunu yükselten en önemli endojen tetikleyiciler olduğu bildirilmektedir (Maqsood vd. 2012). Denaturasyona uğramış heme peroteinlerinden metal iyonlarının serbest kalması, balıkta bulunan doymamış yağ asitlerinin lipit oksidasyonunu hızlandırabilmektedir. Yüksek basıncın ortam koşulları sıcaklığında ve hatta daha düşük sıcaklıklarda uygulanabilmesi, gıdanın şekline, büyüklüğüne içeriğine bağlı olmadan her yere eşit şekilde uygulanabilmesi en önemli avantajlarından 300 *MPa*'nın altında kalan yüksek hidrostatik basınç uygulamalarının lipid oksidasyonu üzerindeki etkileri çok küçüktür. Fakat 300-400 *MPa*'nın üzerindeki basınçlar, gıdada belirgin değişimlere neden olabilmektedir (Medina-Meza vd. 2014).

Montiel vd. (2012), vakum paketlenmiş dumanlanmış morina balığına yüksek hidrostatik basıncın etkilerini araştırmışlardır. Bu amaçla balığa 400, 500 ve 600 *MPa* ve 5, 10 dk yüksek hidrostatik basınç uygulayarak 5°C'de 60 gün depolamışlardır. *TBARS* değerleri kontrol ve basınç uygulanan gruplar arasında önemli bir farklılık göstermemiş ve depolama sonuna kadar 1-2 mg MDA/kg olan limit değere ulaşmamıştır. Chevalier vd. (2001), kalkan balığına 100-200 *MPa* arasında 4°C'de 15 ve 30 dk yüksek hidrostatik basınç uygulamışlardır. Basınç uygulamasından sonra *TBA* değerlerinin artan basınç ve süre parametreleriyle artış gösterdiğini gözlemlemişlerdir. Yapılan çalışma sonunda, kalkan balığı için en uygun basınç uygulamasının 140 *MPa* olduğu bulunmuştur. Yağız vd. (2009), somon balıklarının kalitesi üzerine yüksek hidrostatik basınç ve pişirmenin etkilerini araştırmışlardır. Bu amaçla somon filetolarını vakum paketlenerek 150 ve 300

MPa 15 dk basınç uygulamışlardır. Yüksek basınç işleminin ardından vakum paketli filetoları sıcak su banyosunda pişirmişlerdir. Filetoların sıcaklığı 72°C'ye ulaşınca su banyosundan çıkarmışlar ve 4°C'de 6 gün boyunca depolamışlardır. İkincil oksidasyon ürünü olan *TBARS* değerleri tüm gruplarda depolama boyunca önemli derecede artış göstermiştir. Çalışma sonunda *YHB* uygulaması ve pişirme kombinasyonunun somon filetolarında kaliteyi koruyucu etkiye sahip olduğu bulunmuştur.

Yüksek basınç uygulamasının lipit oksidasyonuna etkileri üzerine yapılan çalışmalar oldukça sınırlı sayıda olmakla beraber bazı araştırmalar yüksek basıncın lipit oksidasyonunu tetiklediğini, ayrıca yüksek basıncın balık kasında lipit oksidasyonuna etkilerinin uygulanan basıncın seviyesi ve süresine, balığın türüne ve balıkentinin yapısına (beyaz kas, koyu renk kas) bağlı olarak değişiklik gösterdiğini belirtmişlerdir. Örneğin morina balığına oda sıcaklığında 20 dk uygulanan 400 *MPa*'nın altındaki yüksek basıncın lipit oksidasyonu üzerinde basınç uygulanmamış gruplara göre çok az etkisinin olduğu görülmüştür (Angsupanich ve Ledward 1998). Buna karşın 4°C'de 30 dk 100 *MPa* basınç uygulanan sazan balığının ve 4°C'de 15 dk 100 *MPa* basınç uygulanan kalkan balığının yüksek basınç uygulandıktan sonra lipit oksidasyonuna karşı daha hassas olduğu gözlenmiştir (Sequeira-Munoz vd. 2006). Bir başka çalışmada somon balığına uygulanan 300 *MPa* (oda sıcaklığında 15 dk) yüksek basınç lipit oksidasyonu düzeyinin basınç uygulanmamış gruplara göre önemli derecede azalmasını sağlamıştır (Ojagh vd. 2011).

Yüksek hidrostatik basıncın su ürünlerinde duyu kalite üzerine etkileri

Tüketici algısı için, balık etinin kabul edilebilirliğinin belirlenmesinde renk en önemli duyu özelliklerinden biridir (Liu vd. 2013). Muhtemelen, yüksek basıncın balık kasındaki en önemli zararlı duyu etkilerinden biri, basınç uygulanmış balıkentinin artan beyazlığından kaynaklanan pişmiş görünümdür (Matser vd. 2000). Balık etinin rengi genellikle *L* (aydınlık), *a* (kırmızılık), *b* (sarılık) renk parametreleri ile ölçülmektedir. Basınç uygulamasından sonra balık kasının *L*-değerinde artan basınçla ve artan basınç muamele süresi ile birlikte artış gözlenmiştir. Örneğin sazan balığında, oda sıcaklığında 300 *MPa* 10 dk yüksek basınç uygulaması *L*-değerinin artmasına ve 500 *MPa* basınçtan sonra balıkentinin pişmiş görünüm almasına neden olurken, 4°C'de 100 *MPa* 30 dk ve 4°C'de 140 *MPa* 15 dk yüksek basınç uygulanan sazan etinde *L*-değeri basınç uygulamasından sonra önemli derecede artmıştır

(Yoshioka ve Yamamoto 1998). Ayrıca oda sıcaklığında 200 MPa 20 dk'dan daha yüksek basınç seviyelerinde morina kasi kırmızılığını kaybetmiştir (Angsupanich ve Ledward 1998). Benzer şekilde, 7°C'de 400 MPa 5 dk'lık yüksek basınç uygulaması ile ringa etinin beyazlığı önemli düzeyde artmış ve pişmiş görünüm meydana gelmiştir (Hurtado vd. 2000). Genellikle soğutma ve dondurma işlemlerinden sonra yüksek basınç uygulanmamış balık kasi ile kıyaslandığında, basınç uygulanmış örneklerde daha iyi bir duyu kalite gözlenmektedir. Yüksek basınç biyojen amin üretimini baskılayabilmekte ve bu da depolama boyunca lezzet ve aromanın gelişmesini sağlamaktadır. Kamalakanth vd. (2011) ton balığının duyu kalitesini araştırmak için 4 puan üzerinin kabul sınırı olduğu 9 puanlı hedonik skala kullanmışlardır. Yüksek basınç uygulanmamış ve 20 güne kadar kabul edilebilir olan kontrol grupları ile kıyaslandığında oda sıcaklığında 100 MPa 5 dk basınç uygulanmış ve 2°C'de depolanan ton balığının 25 güne kadar kabul edilebilir, 200-300 MPa 5 dk basınç uygulanmış ton balığının ise 30 güne kadar kabul edilebilir olduğunu belirtmişlerdir.

Ülkemizde Su Ürünleri ve Yüksek Hidrostatik Basınç Uygulaması

Ticari olarak su ürünlerinde yüksek hidrostatik basınç kullanımı dünyanın birçok ülkesinde yaygın olarak kullanılmakta ve piyasaya sürülen ürünler HPP (high pressure processed) etiketi ile satılmaktadır. Son yıllarda ülkemizde de yüksek hidrostatik basınç uygulamasının su ürünlerinde kullanımı üzerine yapılan birçok bilimsel çalışma mevcuttur. Sipahioğlu (2013) tarafından yapılan çalışmada kitosan esaslı yenilebilir filmle kaplanmış gökkuşuğu alabalığına 220 MPa 5 dk yüksek basınç uygulanmıştır. Çalışma sonunda, yüksek basınç ve kitosan esaslı filmle kaplamanın kalite üzerinde olumlu etkiye sahip olduğu, bununla birlikte her iki yöntemin birlikte uygulanması sonucunda mükemmel bir sonuç elde edildiği tespit edilmiştir. YHB uygulanmış balıkların soğukta depolanması sırasında dayanım ömrünün olumlu etkilendiği gözlemleyen Üretener (2009), buzdolabı koşullarında muhafaza edilen tekir balıklarının duyu ve mikrobiyolojik parametrelere göre 10 gün tüketilebilir kaliteyi koruduğunu gözlemlemiştir. Ayrıca YHB uygulanmamış kontrol grubu çipuraların buzdolabında 10 gün raf ömrüne sahip olduğu bulunurken 3°C/5 dk/250 MPa YHB uygulanmış çipura örneklerinin kontrolden 6 gün daha fazla raf ömrüne sahip olduğu gözlemlenmiştir. Uçak ve Gokoğlu (2017), YHB uygulamasının ringa marinatinin duyu kalitesi üzerine etkilerini inceledikleri çalışmada, 100 ve 300 MPa basınç

uygulanan grupların panelistler tarafından en çok tercih edilen gruplar olduğunu tespit etmişlerdir. Akhan (2012), 200 MPa/5°C/5 dk basınç uygulanan hamsi örnekleri buzdolabında muhafaza edildiğinde 9 gün kadar raf ömrünün uzadığını gözlemlemiştir. Mezgit örnekleri için ise; örnekler sırasıyla 200 MPa/5°C/5 dk ve 400 MPa/15°C/5 dk basınç uygulandıklarında, raf ömürleri 13 ve 15 gün kadar uzatılırken; her iki balık türü için kontrol örnekleri ise 3 gün içerisinde tüketilemez duruma gelmiştir. Yapılan çalışmalar yüksek basıncın su ürünlerinde raf ömrünü arttırdığını ve tüketici tarafından kabul düzeyi yüksek ürünler elde edildiğini göstermektedir.

Sonuç

Yüksek hidrostatik basınç su ürünlerinin türüne, kullanılan basınç koşullarına (süre, sıcaklık, basınç seviyesi) ve birlikte kullanıldığı diğer işleme teknolojilerine bağlı olarak hem faydalı hem de tersi etkilerle sonuçlanabilmektedir. Yüksek basıncın etkisi uygulanan basınca, basınç süresine ve sıcaklığa bağlı olarak değişmekle birlikte, çok düşük sıcaklıklarda bile bozulmaya neden olan mikroorganizmaları inaktive edebilmekte, ayrıca ürünün tazelik parametrelerinde minimum değişimlerle beraber soğutulmuş ve dondurulmuş depolama süresince ürünün raf ömrünü uzatabilmektedir. Tüketiciler tarafından ürünün kabul edilebilirliğinde duyu özellikler oldukça önemli sayılmakta ve yüksek basınç işlemi ısı işlemlere kıyasla daha iyi tekstür, görünüş ve lezzet ile sonuçlanmaktadır. Bu nedenle optimum basınç, süre ve sıcaklık koşulları altında kullanıldığı takdirde, yüksek basınç işlemi ürünün kalitesinin geliştirilmesine ve güvenilirliğinin artmasına yardımcı olmaktadır. Yüksek basınç uygulaması çeşitli ülkelerde özellikle kabuklu su ürünlerinden et çıkarma işlemlerinde ve diğer bazı balık ürünlerinin işlenmesinde ticari olarak kullanılmaktadır. Ülkemizde, yüksek basıncın su ürünlerinde kullanımı ile ilgili çeşitli bilimsel çalışmalar mevcut olup ticari olarak kullanımı bulunmamaktadır. İleriki çalışmaların planlanmasında sanayi iş birliği de düşünülerek işlenmiş su ürünleri üreten ticari firmaların yüksek basınç sistemi hakkında bilgilendirilerek konu ile ilgili farklı yaklaşımlarla beraber su ürünlerinde yüksek basınç kullanımının sağlanabileceği düşünülmektedir.

Kaynaklar

Akhan 2012. Yüksek hidrostatik basınç uygulaması ile mezgit (*Gadus merlangus euxinus*) ve hamsinin (*Engraulis encrasicolus*) muhafazasının ve raf ömrünün uzatılması. [Yüksek Lisans Tezi]. Orta Doğu Teknik Üniversitesi. 96 s.

- Angsupanich K, Ledward DA. 1998. High pressure treatment effects on cod (*Gadus morhua*) muscle. Food Chem. 63(1):39-50.
- Arıcı M. 2006. Gıda muhafazasında yüksek hidrostatik basıncın mikroorganizmalar üzerine etkisi. Journal of Tekirdag Agricultural Faculty (JOTAF). 3(1):1-9.
- Arias C. 2009. Chilled and frozen raw fish. In: Fernandes R, editor. Microbiology handbook fish and seafood. Cambridge: Leatherhead Publishing. p. 1-25.
- Briones-Labarca V, Perez-Won M, Zamarca M, Aguilera-Radic JM, TabiloMunizaga G. 2012. Effects of high hydrostatic pressure on microstructure, texture, colour and biochemical changes of red abalone (*Haliotis rufecens*) during cold storage time. Innov Food Sci Emerg. 13:42-50.
doi: 10.1016/j.ifset.2011.09.002
- Bridgman P. 1912. Water in the liquid and five solid forms under pressure. Proc Am Acad Arts Sci. 47:441-558.
- Calci KR, Meade GK, Tezloff RC, Kingsley DH. 2005. High-Pressure Inactivation of Hepatitis A Virus within Oysters. Appl Environ Microbiol. 71(1):339-43.
doi: 10.1128/AEM.71.1.339-343.2005
- Cheftel JC, Culioli J. 1997. Effects of high pressure on meat: A review. Meat Sci. 46(3):211-236.
- Cheret R, Hernandez-Andres A, Delbarre-Ladrat C, De Lamballerie M, Verrez-Bagnis V. 2006. Proteins and proteolytic activity changes during refrigerated storage in sea bass (*Dicentrarchus labrax* L.) muscle after high-pressure treatment. Eur Food Res Technol. 222(5-6):527-535.
doi: 10.1007/s00217-005-0158-z
- Chevalier D, Le Bail A, Ghoul M. 2001. Effect of high pressure treatment (100-200 MPa) at low temperature on turbot (*Scophthalmus maximus*) muscle. Food Res Int. 34:425-429.
- Chevalier D, Sequeira-Munoz A, Le Bail A, Simpson B, Ghoul M. 2000. Effect of pressure shift freezing, air-blast freezing and storage on some biochemical and physical properties of turbot (*Scophthalmus maximus*). Lebensmittel-Wissen-schaft und-Technologie, 33, 570-577.
doi: 10.1006/fstl.2000.0721
- Chevalier D, Le Bail A, Chourot JM, Chantreau P. 1999. High pressure thawing of fish (whiting): Influence of the process parameters on drip losses. LWT. 32:25-31.
- Crehan CM, Troy DJ, Buckley D J. 2000. Effects of salt level and high hydrostatic pressure processing on frankfurters formulated with 1.5 and 2.5% salt. Meat Sci. 55(1):123-130.
- Dalgaard P. 2003. Fish spoilage of seafood. In: Benjamin C, editor. Encyclopedia of food sciences and nutrition. 2nd edn. Oxford: Academic Press. p. 2462-2471.
- Dan L, Qingjuan T, Jingfeng W, Yuming W, Qin Z, Changhu X. 2009. Effects of high-pressure processing on murine norovirus-1 in oysters (*Crassostrea gigas*) in situ. Food Control. 20(11):992-996.
doi: 10.1016/j.foodcont.2008.11.012
- Eitenmiller RR, Desouza SC. 1984. Enzymatic mechanisms for amine formation in fish. Acs Symposium Series. 262:431-442.
doi: 10.1021/bk-1984-0262.ch036
- Erkan N, Üretener G, Alpas H. 2010. Effect of high pressure (HP) on the quality and shelf life of red mullet (*Mullus surmelutus*). Innov Food Sci Emerg. 11(2):259-264.
doi: 10.1016/j.ifset.2010.01.001
- Erkan N, Üretener G, Alpas H, Selçuk A, Özden Ö, Buzrul S. 2011. Effect of High Hydrostatic Pressure (HHP) Treatment on Physicochemical Properties of Horse Mackerel (*Trachurus trachurus*). Food and Bioprocess Tech. 4(7):1322-1329.
doi: 10.1007/s11947-010-0415-4
- FDA 2011. Food and Drug Administration. [Erişim tarihi: 04 Haziran 2017]. Erişim adresi: <https://www.fda.gov/downloads/Food/GuidanceRegulation/UCM251970.pdf>
- Garriga M, Grebol N, Aymerich MT, Monfort JM, Hugas M. 2004. Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. Innov Food Sci Emerg. 5:451-457.
doi:10.1016/j.ifset.2004.07.001
- Guerrero-Beltran J, Barbosa-Canovas G, Swanson B. 2005. High hydrostatic pressure processing of fruit and vegetable products. Food Rev Int. 21(4):411-425.
doi: 10.1080/87559120500224827
- Heinz V, Knorr D. 1998. High pressure germination and inactivation kinetics of bacterial spores. In: High Pressure Food Science, Bioscience and Chemistry. Isaacs NS, editor. Cambridge: The Royal Society Chemistry. p. 436-441.
- Hendrickx M, Ludikhuyze L, Van Den Broeck I, Weemaes C. 1998. Effects of High Pressure on Enzymes Related to Food Quality. Trends Food Sci Tech. 9(5):197-203.
doi: 10.1016/S0924-2244(98)00039-9
- Hite BH, Giddings NJ, CE. 1914. The effects of pressure on certain microorganisms encountered in the preservation of fruits and vegetables. In: Farkas and Hoover. Morgantown. Bull WV Univ. Agric. Exp. Sta. Morgantown 146:1-67.
- Hite BH. 1899. The effect of pressure in the preservation of milk. Bull W.V. Univ. Agric. Exp. Sta., Morgantown, 58:15-35.
- Hugas M, Garriga M, Monfort JM. 2002. New mild technologies in meat processing: High pressure as a model technology. Meat Sci. 62(3):359-371.
doi: 10.1016/S0309-1740(02)00122-5
- Hurtado JL, Montero P, Borderias AJ. 2000. Extension of shelf life of chilled hake (*Merluccius capensis*) by high pressure. Food Sci Technol Int. 6(3):243-249.
- Ikeuchi Y, Yoshioka K, Suzuki A. 2006. Recent Advanced Topics on Application of High Pressure Technology to Meat Processing. The Review of High Pressure Science and Technology. 16:17-25.
doi: 10.4131/jshpreview.16.17
- Kamalakanth CK, Ginson J, Bindu J, Venkateswarlu R, Das S, Chauhan OP, Gopal TKS. 2011. Effect of high pressure on K-value, microbial and sensory characteristics of yellowfin tuna (*Thunnus albacares*) chunks in EVOH films during chill storage. Innov Food Sci Emerg. 12(4):451-455.
doi: 10.1016/j.ifset.2011.06.001

- Karim NU, Kennedy T, Linton M, Watson S, Gault N, Patterson MF. 2011. Effect of high pressure processing on the quality of herring (*Clupea harengus*) and haddock (*Melanogrammus aeglefinus*) stored in ice. Food Control. 22(3-4):476-484.
doi: 10.1016/j.foodcont.2010.09.030
- Kim DH, Kim KBW, Ahn DH. 2013. Inhibitory effects of high-hydrostatic-pressure treatments on histamine production in mackerel (*Scomber japonicus*) muscle inoculated with *Morganella morganii* and *Photobacterium phosphoreum*. Food Control. 34(2):307-311.
doi: 10.1016/j.foodcont.2013.04.032
- Ko WC, Jao CL, Hwang JS, Hsu KC. 2006. Effect of high pressure treatment on processing quality of tilapia meat fillets. J Food Eng. 77(4):1007-1011.
doi: 10.1016/j.jfoodeng.2005.08.029
- Krizek M, Matejkova K, Vacha F, Dadakova E. 2014. Biogenic amines formation in high-pressure processed pike flesh (*Esox lucius*) during storage. Food Chem. 151:466-471.
doi: 10.1016/j.foodchem.2013.11.094
- Kural AG, Shearer AEH, Kingsley DH, Chen H. 2008. Conditions for high pressure inactivation of *Vibrio parahaemolyticus* in oysters. Int J Food Microbiol. 127(1-2):1-5.
doi: 10.1016/j.ijfoodmicro.2008.05.003
- LeBail A, Chevalier D, Mussa DM, Ghoul M. 2002. High pressure freezing and thawing of foods: a review. Int J Refrig. 25(5):504-513.
doi: 10.1016/S0140-7007(01)00030-5
- Li B, Sun DW. 2002. Novel methods for rapid freezing and thawing of foods-a review J Food Eng. 52:175-182.
- Liu D, Zeng XA, Sun DW. 2013. NIR spectroscopy and imaging techniques for evaluation of fish quality-a review. Appl Spectrosc Rev. 48(8):609-628.
doi: 10.1080/05704928.2013.775579
- Makita T. 1992. Application of high pressure and thermophysical properties of water to biotechnology. Fluid Phase Equilib. 76:87-95.
- Maqsood S, Benjakul S, Kamal-Eldin A. 2012. Haemoglobin-mediated lipid oxidation in the fish muscle: A review. Trends Food Sci Technol. 28(1):33-43.
doi: 10.1016/j.tifs.2012.06.009
- Matejkova K, Krizek M, Vacha F, Dadakova E. 2013. Effect of high-pressure treatment on biogenic amines formation in vacuum-packed trout flesh (*Oncorhynchus mykiss*). Food Chem. 137(1-4):31-36.
doi: 10.1016/j.foodchem.2012.10.011
- Matser AM, Stegeman D, Kals J, Bartels PV. 2000. Effects of high pressure on colour and texture of fish. High Press Res. 19(1-6):109-115.
doi: 10.1080/08957950008202543
- Medina-Meza IG, Barnaba C, Barbosa-Canovas GV. 2014. Effects of high pressure processing on lipid oxidation: A review. Innov Food Sci Emerg. 22:1-10.
doi: 10.1016/j.ifset.2013.10.012
- Metrick C, Hoover DG, Farkas DF. 1989. Effects of high hydrostatic pressure on heat-resistant and heat-sensitive strains of *Salmonella*. J Food Sci. 54(6):1547-1564.
doi: 10.1111/j.1365-2621.1989.tb05156.x
- Montiel R, Alba MD, Bravo D, Gaya P, Medina M. 2012. Effect of high pressure treatments on smoked cod quality during refrigerated storage. Food Control. 23(2):429-436.
doi: 10.1016/j.foodcont.2011.08.011
- Moraru C. 2008. 21 Annual Cornell Conference on Dairy Markets and Product Research, Cornell University, Syracuse.
- Mota MJ, Lopes RP, Delgadillo I, Saraiva JA. 2013. Microorganisms under high pressure-Adaptation, Growth And Biotechnological Potential. Biotechnol Adv. 31(8):1493-1494.
doi: 10.1016/j.biotechadv.2013.06.007
- Mozhaev VV, Heremans K, Frank J, Masson P, Balny C. 1996. High pressure effects on protein structure and function. Proteins Struct Funct Genet. 24(1):81-91.
- Murchie LW, Cruz-Romero M, Kerry JP, Linton M, Patterson MF, Smiddy M, Kelly AL. 2005. High pressure processing of shellfish: A review of microbiological and other quality aspects. Innov Food Sci Emerg. 6(3):257-270.
doi: 10.1016/j.ifset.2005.04.001
- Ohshima T, Ushio H, Koizumi C. 1993. High-pressure processing of fish and fish muscles. Trends Food Sci Technol. 4(11):370-375.
- Ojagh SM, Nunez-Flores R, Lopez-Caballero ME, Montero MP, Gomez-Guillen MC. 2011. Lessening of high-pressure-induced changes in Atlantic salmon muscle by the combined use of a fish gelatin-lignin film. Food Chem. 125(2):595-606.
doi: 10.1016/j.foodchem.2010.08.072
- Özlü H. 2006. Et Teknolojisinde Yüksek Basınç Uygulamaları. Atatürk Üniversitesi, Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, Yüksek Lisans Tezi. 71 p.
- Özoğul F, Kuley, E, Özoğul Y. 2004. Balık ve Balık Ürünlerinde Oluşan Biyojenik Aminler. E.Ü. Su Ürünleri Dergisi. 21(3-4):375-381.
- Phuvasate S, Su YC. 2015. Efficacy of low-temperature high hydrostatic pressure processing in inactivating *Vibrio parahaemolyticus* in culture suspension and oyster homogenate. Int J Food Microbiol. 196:11-15.
doi: 10.1016/j.ijfoodmicro.2014.11.018
- Picouet PA, Cofan-Carbo S, Vilaseca H, Carbone Ballbe L, Castells P. 2011. Stability of sous-vide cooked salmon loins processed by high pressure. Innov Food Sci Emerg. 12(1):26-31.
doi: 10.1016/j.ifset.2010.12.002
- Ramirez-Suarez JC, Morrissey MT. 2006. Effect of high pressure processing (HPP) on shelf life of albacore tuna (*Thunnus alalunga*) minced muscle. Innov Food Sci Emerg. 7:19-27.
doi: 10.1016/j.ifset.2005.08.004
- Rendueles E, Omer MK, Alvseike O, Alonso-Calleja C, Capita R, Prieto M. 2011. Microbiological food safety assessment of high hydrostatic pressure processing: A review. LWT-Food Sci Technol. 44(5):251-1260.
doi: 10.1016/j.lwt.2010.11.001

- San Martin MF, Barbosa-Canovas GV, Swanson BG. 2002. Food processing by high hydrostatic pressure. *Crit Rev Food Sci.* 46(6):627-645.
doi: [10.1080/20024091054274](https://doi.org/10.1080/20024091054274)
- Sequeira-Munoz A, Chevalier D, LeBail A, Ramaswamy HS, Simpson BK. 2006. Physicochemical changes induced in carp (*Cyprinus carpio*) fillets by high pressure processing at low temperature. *Innov Food Sci Emerg.* 7(1-2):13-18.
doi: [10.1016/j.ifset.2005.06.006](https://doi.org/10.1016/j.ifset.2005.06.006)
- Shahidi F. 1994. Seafood proteins and preparation of protein concentrates. In: Shahidi F, Botta JR, editors. *Seafoods: chemistry, processing technology and quality*. New York: Blackie Academic and Professional. p. 3-10.
- Shimidzu N, Goto M, Miki W. 1996. Carotenoids as singlet oxygen quenchers in marine organisms. *Fish Sci.* 62(1):134-137.
- Sikes LA, Tobin BA, Tume KR. 2009. Use of High Pressure to Reduce Cook and Improve Texture of LowSalt Beef Sausage Batters. *Innov Food Sci Emerg.* 10: 405-412.
doi: [10.1016/j.ifset.2009.02.007](https://doi.org/10.1016/j.ifset.2009.02.007)
- Singh PR, Yousef AE. 2001. Technical Elements of new and emerging non-thermal food technologies. <http://www.fao.org/ag/agsi/Nonthermal/nonthermal>
- Sipahioğlu S. 2013. Kitosan Esaslı Yenilebilir Filmle Kaplanmış Gökkuşağı Alabalığı (*Oncorhynchus mykiss walbaum*) Filetolarının Raf Ömrü ve Kalitesi Üzerine Yüksek Hidrostatik Basınç İşleminin Etkisi (Yüksek Lisans Tezi). Süleyman Demirel Üniversitesi. 84 s.
- Smelt JPPM. 1998. Recent advances in the microbiology of high pressure processing. *Trends Food Sci Tech.* 9(4):152-158.
doi: [10.1016/S0924-2244\(98\)00030-2](https://doi.org/10.1016/S0924-2244(98)00030-2)
- Suzuki T, Macfarlane JJ. 1984. Modification of the Heat-Setting Characteristics of Myosin by Pressure Treatment. *Meat Sci.* 11:263-274.
- Teixeira B, Fidalgo L, Mendes R, Costa G, Cordeiro C, Marques A, Saraiva JA, Nunes ML. 2014. Effect of high pressure processing in the quality of sea bass (*Dicentrarchus labrax*) fillets: Pressurization rate, pressure level and holding time. *Innov Food Sci Emerg.* 22:31-39.
doi: [10.1016/j.ifset.2013.12.005](https://doi.org/10.1016/j.ifset.2013.12.005)
- Tironi V, LeBail A, De Lamballerie M. 2007. Effects of pressure-shift freezing and pressure-assisted thawing on sea bass (*Dicentrarchus labrax*) quality. *J Food Sci.* 72(7):381-387.
doi: [10.1111/j.1750-3841.2007.00472.x](https://doi.org/10.1111/j.1750-3841.2007.00472.x)
- Truong BQ, Buckow R, Stathopoulos CE, Nguyen MH. 2015. Advances in High-Pressure Processing of Fish Muscles. *Food Eng Rev.* 7(2):109-129.
doi: [10.1007/s12393-014-9084-9](https://doi.org/10.1007/s12393-014-9084-9)
- Ucak ve Gokoğlu. 2017. Effect of High Hydrostatic Pressure on Sensory Quality of Marinated Herring (*Clupea harengus*). *J Food Process Preserv.* 41(2)
doi: [10.1111/jfpp.12784](https://doi.org/10.1111/jfpp.12784)
- Üretener G. 2009. Yüksek Hidrostatik Basınç Uygulamasının Balık Kalitesi ve Raf Ömrü Üzerine Etkisi (Yüksek Lisans Tezi). İstanbul Üniversitesi. 111 s.
- Yağız Y, Kristinsson HG, Balaban MO, Welt BA, Ralat M, Marshall MR. 2009. Effect of high pressure processing and cooking treatment on the quality of Atlantic salmon. *Food Chem.* 116(4):828-835.
doi: [10.1016/j.foodchem.2009.03.029](https://doi.org/10.1016/j.foodchem.2009.03.029)
- Yamamoto K, Yoshida Y, Morita J, Yasui T. 1994. Morphological and physicochemical changes in the myosin molecules induced by hydrostatic pressure. *J Biochem.* 116(1):215-220.
- Yoshioka K, Yamamoto T. 1998. Changes of ultrastructure and the physical properties of carp muscle by high pressurization. *Fish Sci.* 64(1):89-94.
- Zhao YY, Fores RA, Olson DG. 1998. High hydrostatic pressure effects on rapid thawing of frozen beef. *J Food Sci.* 63(2):272-275.
- Zhu S, Le Bail A, Ramaswamy H, Chapleau N. 2004. Characterization of ice crystals in pork muscle formed by pressure-shift freezing as compared with classical freezing methods. *J Food Sci.* 69(4): 190-197.



Importance of Determining Genetic Code In Crayfish and Methods Used

Zübeyde HANOL BEKTAŞ 

Fisheries Research Institute, 32500, Eğirdir, Isparta, Turkey

ABSTRACT

Crayfish are one of many freshwater organisms that are subject to biodiversity changes. The crayfish stocks in the world and Turkey have been damaged due to the crayfish plague caused by *Aphanomyces astaci*, overfishing water pollution and other causes. For the continuation of endangered stocks of these species, it is necessary to protect the populations and restocking into appropriate environment. Environmental factors have an vital role in the genetic structure of a population. So the genetic diversity between population is vital because the information of the genetic construction of population is curical due to the ensuring sustainability and conservation of these species when evaluated with other factors but, unfortunately, still restricted. With this review, the genetic study conducted so far will be discussed to try getting important information for preservation and management of crayfish, because preservation of genetic diversity is very important for survival or persistence of these species such a long time.

Keywords: Genetic diversity, fisheries, protection, biodiversity, molecular identification

ARTICLE INFO

REVIEW

Received : 24.08.2017

Revised : 20.03.2018

Accepted : 03.04.2018

Published : 27.04.2018



DOI:10.17216/LimnoFish.335941

* CORRESPONDING AUTHOR

zubeydehanol17@gmail.com

Tel : +90 246 313 34 60

Kerevitlerde Genetik Şifre Belirlemenin Önemi ve Kullanılan Yöntemler

Öz: Kerevitler biyoçeşitlilik değişimlerine maruz kalan birçok tatlı su organizmalarından biridir. Dünyada ve Türkiye’de kerevit stokları *Aphanomyces astaci* fungusunun neden olduğu hastalık, aşırı avcılık, su kirliliği ve diğer nedenlerden tükenme tehlikesiyle karşı karşıya kalmıştır. Stokları tehlikede olan bu canlıların devamı için popülasyonların korunması ve uygun çevrelere yeniden stoklanması gerekmektedir. Çevresel faktörler bir popülasyonun genetik kompozisyonunda çok önemli rol oynar. Bu yüzden popülasyonlar arasındaki genetik çeşitlilik büyük öneme sahiptir çünkü popülasyonların genetik yapılarının bilinmesi bu türün devamının sağlanması ve korunması açısından hayati bir faktördür fakat ne yazık ki bu konuda yapılan çalışmalar hala sınırlıdır. Bu derlemeyle şimdiye kadar yapılan genetik çalışmalar tartışılarak kerevitlerin korunması ve yönetimi için önemli bilgiler elde edilmeye çalışılacaktır çünkü genetik çeşitliliğin korunması bu türlerin uzun süre yaşaması ya da varlığını sürdürmesi için çok önemlidir.

Anahtar kelimeler: Genetik çeşitlilik, su ürünleri, koruma, biyoçeşitlilik, moleküler tanımlama

How To Cite

Hanol Bektaş Z. 2018. Importance of Determining Genetic Code in Crayfish and Methods Used. LimnoFish. 4(1): 58-66. doi: 10.17216/LimnoFish.335941

Introduction

Importance of crayfish

In this review, the situation of the freshwater crayfish group, a water organism found in every continent, is discussed, 59% of these species found in North America, 23% of in Oceania (Southeast Asia Islands), 10% in South America, 5% in Europe and 1.1% in Asia with only 7 species. Also 1.4% in Madagascar with 9 species. But the other phylogenetic studies argues that there are only 4 species in Asia (Koizumi et al. 2012) and are widely regarded as keystones of habitats which they found. There are more than 600 crayfish species belong to

the three family that have different distribution over the world. In Turkey the crayfish species which have natural distribution in natural lakes, reservoirs, dam lakes, ponds and rivers is *Astacus leptodactylus* Esch., 1823. It is reported that this species represented by a single and two sub-species in Turkey. While *A. leptodactylus leptodactylus* Esch., 1823 have distribution in the Black Sea, North Marmara and Thrace Regions; Iznik and Terkos Lake with the Maritsa and the Danube River and Gelemen stream *A. leptodactylus salinus* Nordman, 1842 in South Marmara, Aegean and Central Anatolia Regions; Manyas, Eğirdir, Beyşehir, Ulubat,

Akşehir, Eber, Gölcük and Miliç lakes (Geldiay and Kocataş 1970; Harlioğlu and Harlioğlu 2006). Also Harlioğlu and Güner (2007) had reported *Austropotamobius torrentium* (Shrank, 1803) presence in Madara Brook.

Crayfish are valuable animals economic and ecologically. They have a significant connection in the aquatic food chain in many streams and lakes. (Helfrich and DiStefano 2009). Crayfish have an important environmental role in many freshwater habitats and play an important economic and cultural role in many communities. Unfortunately, in many parts of the world, some crayfish species in freshwater ecosystems are under pressure and efforts should be made to protect them. Owen et al. (2015) reported that crayfish is a highly endangered component of these freshwater ecosystems and that more than 30% of the world's species are at risk of extinction.

The factors threatening the crayfish populations

People have been changing the dispersion of species over the years, they not only causing extinctions, but also causing species to migrate through biogeographic obstacles and into new environments regardless of the geographic and genetic resources of stock material (Clavero et al. 2015). The population is declining and the extinction of species takes place at an unprecedented rate all over the world (May 2010). There are more than 79,800 species on The IUCN Red List, and more than 23,000 are danger of extinction, including 41% of amphibians, 34% of conifers, 33% of reef building corals, 25% of mammals and 13% of birds. The extinction occurs in marine, freshwater and also in terrestrial systems. Numbers of described threatened species by big groups of organisms were given in Table 1. (Cook et al. 2008).

Table 1. Numbers of threatened species by big groups of organisms (IUCN Red List version 2017-1)

	Estimated Number of described species	Number of species evaluated by 2017
VERTEBRATES		
Mammals	5,560	5,560
Bird	11,121	11,121
Reptiles	10,450	5,473
Amphibians	7,635	6,533
Fishes	33,500	16,134
INVERTEBRATES		
Insects	1000000	6,912
Molluscs	85.000	7.276
Crustaceans	47.000	3.177
Corals	2.175	864
Arachnids	102.248	249
Velvet Worms	165	11
Horseshoe Crabs	4	4
PLANTS		
Mosses	16.236	102
Ferns and Allies	12.000	417
Gymnosperms	1.051	1.011
Flowering Plants	268.000	20.725
Green Algae	6.050	13
Red Algae	7.104	58
FUNGI AND PROTISTS		
Lichens	17.000	8
Mushrooms	31.496	25
Brown algae	3.784	15

Researches shows that freshwater environments occupy only 0.8% of the Earth, with about 6% of species identified and under pressure from water pollution, flow modification, habitat fragmentation and exotic species. These habitat breaks cause high susceptibility to speciation, habitat destruction and limited disintegration capacity. The combination of

these factors increases the rate of rapid disappearance. So, freshwater ecosystems are vital in accordance to biodiversity, but are under severe pressure and therefore are in need of conservation efforts (Ricciardi and Rasmussen 1999; Owen et al. 2015). The freshwater species that in particular risk in many part of world are crayfish.

In the 19th century the natural crayfish have been depleted dramatically rapidly due to the emergence of crayfish plague and led to the extinction of natural species in many parts of Europe. All domestic European crayfish species have experienced strong declines over the last century, mainly due to the outbreak of crayfish plague, a disease caused by oomycete *Aphanomyces astaci* (Clavero et al. 2015). It is clear that the population of the world has decreased and the extinctions of species have occurred in an unprecedented proportion. Despite the fact that extinction is a natural phenomenon, evidence has shown that the rates of extinction at present are greater than in the past and possibly attributable to human actions. Since we can not fully understand the current and past biodiversity levels, it is difficult to predict the rates of extinction accurately (Furse et al. 2012). In Turkey, the crayfish population are also affect from crayfish plague. Crayfish plague disease problem is true for Turkish population. Harlioğlu (2004) had reported that after 1985, the yield of *A. leptodactylus* decreased tragically in many Turkish populations as a result of crayfish plague disease. In 1991, the harvest was only 320 tonnes.

In 2010, freshwater crayfish were evaluated in the IUCN Red-Threatened Species List and evaluating 528 of them, indicating that crayfish were among the 5 most threatened groups of animals in the world.

The crayfish species under risk of extinction are *Austopotamobius pallipes* (Lereboullet), *Astacus astacus* (Linnaeus), *A. leptodactylus* (Eschscholtz), *Austopotamobius torrentium* (Shrank) (Fetzner 2011). Recent threats include global climate change results increased environmental temperature (Furse et al. 2012), and rising density and compactness of variable weather events.

Low capacity in the natural distribution of crayfish and inhibition of gene flow through habitat alteration results strong/severe fragmentation of remaining populations. Therefore to prevent destruction of natural stocks, it is required to take measures such as protection of natural habitats and stocking of appropriate water resources.

The other major causes in the endangered of crayfish are environmental factors, low population numbers, small geographic limits and habitat loss.

Our objective in this review is to examine, present and discuss the applications of existing molecular genetic methods to protect economically valuable freshwater crayfish. Examples will be given of how these techniques are applied in freshwater crayfish, particularly in determining the levels of genetic diversity and how the obtained data can be used to maintain these very important species. Because the first thing to consider in order to protect

species is to know the genetic structure or diversity. That is, higher genetic diversity means more survival, and more successful results can be obtained if protection methods are taken in this direction.

Genetic Methods Used To Determining The Genetic Differences of Crayfish

Many national and international organisations and states make investment for the protection of this important aquatic organism. Although this organism studied many years, in genetic sence the obtained data from study which has started recently may have an critical role for the protection of crayfish (Gouin et al. 2006; McKniff 2012).

Molecular methods have been used to determine the levels of genetic diversity in a species, data requirements in stocking studies, estimation of effective population sizes, and determination of disease susceptibility (DeSalle and Amato 2004; Furse et al. 2012). Potential uses of molecular methods encompasses a wide range of applications, and the methods used to determine the genetic diversity of crayfish have been discussed under the following headings.

Since the mid of 1980s, the scientist have tried to determine the genetic structue of crayfish. Different methods have been used for this. The first methods were depend on protein electrophoresis that revealed a low variation between European crayfish (Fevolden et al. 1994). In recent years, the molecular methods have been applied in crayfish successfully and revealed high diversity.

For determining genetic differentiation of populations, there are several studies such as mitochondrial DNA (in genetic analysis the mitochondrial genomes are perfect targets because they have no introns, reduce the recombination and in the haploid mode of inheretence), ISSR, ITS1(Largiader et al. 2000; Li et al. 2012; Matallanas et al. 2012) RAPD PCR (Schulz 2000) and microsatellites (Gouin et al. 2002).

Mitochondrial DNA markers

Because of the maternal mode of heredity and lack of recombination, mtDNA was a commonly used genetic methods for investigation of population structure (Wilson et al. 1985; Avise et al. 1987).

Koizumi et al. (2012) investigated the genetic structure of Japon crayfish. They determined that the most of populations composed of 16S mtDNA haplotypes and showed important genetic divergence ($F_{st}=0,96$) and also the nuclear DNA sequences showed deep seperation between strains. However the mtDNA has advantages in population genetic structure study, it has some disadvantages also. The

first represents only one locus, and the deduced gene trees may be incompatible with organism phylogeny (Avice 1994). Resistance to a single genetic locus greatly reduces the significant spatial or temporal detection power. Second, mtDNA allows only reconstruction of maternal lines. Thus, in species-based populations, the population structure of maternal hereditary mtDNA may be different from the biparental heritable nuclear energy.

Inter simple sequence repeats (ISSR)

From the used genetic methods the inter-simple sequence repeats (ISSRs) are a new molecular marker of PCR amplification of DNA by a single primer consisting of a repeating sequence fixed by 2-4 arbitrary nucleotides at the 3' or 5' end, and have been used successfully many in populations genetic studies. Schulz et al. (2004) have been investigated 8 *A. astacus* stock using ISSR-PCR in Germany and Poland. They have been search 22 ambiguous and polymorphic markers using statistical analysis. They found that the polymorphic loci in a population ranged from 4-19. The determined relative genetic diversity between population ranged from 0.6-0.1 by Shannon index. For this reason, it has proven that ISSR markers are appropriate for assessing DNA variations between and within the population and for producing a significant distinction in most of the stocks.

ITS1 fragment analysis

ITS1 fragment analysis, one of the other molecular methods, is a relatively simple tool to study genetically distinct crayfish populations and to determine the origin of these populations. Despite that, the ITS1 fragment variation can be used to investigate differences between populations, as documented in a study carried out by Liu et al. (2013) investigated the population genetic structure of *Procambarus clarkii* in China using ITS1. They reported that diversity within population (95.26%) was higher than diversity between population (4.74%) using AMOVA. Genetic differences between Taiwan and the environment ($F_{ST} = 0.160$) was medium while difference between China and America populations ($F_{ST} = 0.682$ and 0.977) were significantly higher. Gene flow between China and American populations ($N_m = 0.006$ and 0.117 respectively) were significantly lower than China and the environment (1.536). In another study, Edsman et al. (2002) investigated 15 *A. astacus* population with ITS1 markers and they found that different population had different form of division.

RAPD (Random amplified polymorphic DNA) PCR

RAPD-PCR has some advantages over other techniques used for population analysis. (RFLPs and microsatellites). For example, RAPD-PCR is relatively faster, simpler and cheaper to conduct. Besides, it requires a small amount of DNA to form and provide a large number of polymorphic loci; Both are particularly valuable when working with populations of species that are danger of extinction or low genetic diversity RAPD-PCR has many advantages as well as some disadvantages. Of course, the most important shortcoming of RAPD-PCR is the dominant nature of the bands created by this method. In relation to this, Zhang et al. (2015) indicated that it is impossible to distinguish a heterozygous locus from a dominant RAPD marker in a DNA segment amplified from a homozygous locus (Zhang et al. 2005). Schulz (2000) examined the genetic structure of 5 *A. astacus* stock using RAPD-PCR. Although geographic proximity of these 5 stock less than 20 km the genetic structure of all of them were different.

Cytochrome oxidase subunit I (COI)

The cytochrome oxidase subunit I (COI), one of the maternal mitochondrial genes, has been used extensively to investigate the population structure (Schrimpf et al. 2011; Matallanas et al. 2012), phylogenetics, phylogeny and systematics of different crayfish species or groups.

Li et al. (2012) investigated distribution model after establishment and population genetic structure of *P. clarkii* in China. In this study they analyzed genetic structure and diversity of populations from 37 sampling region area using COI 16S rRNA mitochondrial gene sequences and 12 nuclear microsatellite. They reported that from the phylogenetic analysis, bayesian study and isolation between distance, the population located in same area have similar genetic composition and the population in China were high genetic diversity but they didn't show expansion. Their study revealed that the COI 16S rRNA mitochondrial gene sequences is good tool to evaluating population genetic structure and genetic diversity of *P. clarkii*. In another study, Schrimpf et al. (2011) investigated the haplotype diversity of *A. astacus* by analyzing the partial sequences of cytochrome oxidase subunit I of 416 sample from 92 crayfish stock in Black Sea, North Sea and Baltic Sea. From the defined 22 haplotype the one of them was common in whole working area. The high haplotype diversity ($HD = 0.94 \pm 0.24$) was found in Balkans while the low haplotype diversity ($HD = 0.299 \pm 0.038$ and $HD = 0.163 \pm 0.058$) were found in Central Asia. And also this study show to us the COI is useful in the investigation genetic diversity of *A. astacus*.

Schrimpf et al. (2014) studied a portion of the mitochondrial cytochrome oxidase subunit and 16S rRNA from 540 noble crayfish samples taken from 156 sites spread around five sea basins in Europe. Also, they carried out a microsatellite analysis of 289 individuals from 22 sites. Both mitochondrial and nuclear markers have been implicated in anthropogenic translocations in central Europe, resulting in genetically relatively homogeneous populations. Whereas, some areas showed a distinct genetic structure with endemic haplotypes and private alleles indicating that these areas were refugia for *A. astacus* in central Europe and that these populations have not been subject to anthropogenic translocations. Furthermore, researchers have found the highest genetic diversity in the Black Sea basin and especially in the Western Balkans and other Black Sea populations. And also, Akhan et al. (2014) investigated the genetic differentiation among Turkish populations of the narrow-clawed crayfish using a partial sequence of cytochrome oxidase subunit I gene (585 bp) of 183 specimens from 17 different crayfish populations. From this study they disclosed a strong haplotype structure with three prominent clades diverged by a range between 20 and 50 mutations and substantial inter-group pairwise sequence divergence (5.19–6.95 %). They determined the high level of genetic variability ($H_d = 95.8\%$, $p = 4.17\%$) and numerous private haplotypes. Helms et al. (2015) examined the genetic structure and morphology between three populations of 3 catchments of the drainage using mitochondrial COI gene sequences and geometric morphometry, and multiple populations of *Cambarus englishi* Hobbs and Halland *Cambarus halli* both of them endemic to the Tallapoosa River.

Bernini et al. (2016) described the genetic structure of *A. italicus* populations in northern Italy (Lombardy Alpine foothills and northern Apennines) by using the cytochrome c oxidase subunit I, to assess the present evolutionary diversity and phylogenetic history from a conservation perspective. As a result they proposed to consider these two clades as distinct molecular operational taxonomic units for the conservation of this endangered crayfish.

Microsatellites

The another genetic method microsatellites or variable number of tandem repeats (VNTRs) are a class of nuclear DNA with repeating units of 1-6 nucleotides, usually formed in a cluster called a locus. A microsatellite locus typically varies between 5 and 40 repeats; the most common forms of repeats are dinucleotide, trinucleotide, and tetranucleotide repeats. Two desirable features of microsatellites are its easy sample preparation and high information

content. In terms of preparation, microsatellite studies use small tissue samples that can be preserved for later use, e.g. freshwater crayfish samples in 95% ethanol, due to the stability of the DNA as compared to enzymes that degrade over time. This also allows the microsatellites to still be amplified during the process of the polymerase chain reaction (PCR). Because small samples are used, microsatellites are optimal for their use of fast and cheap DNA extractions. In addition, since microsatellites are species-specific markers, there is less likelihood that cross-contamination by non-target organisms is a problem (Selkoe and Toone 2006). In terms of high information content, microsatellites as single-locus, co-dominant markers can efficiently be combined in the genotyping process, which allows for fast and inexpensive replication (Selkoe and Toone 2006). Microsatellite markers are utilized in population genetics studies interested in population structure and the relatedness of individuals, migration (gene flow) rates, changes in the past ten to one hundred generations, and present-day demography or connectivity patterns (Selkoe and Toone 2006). Due to the relatively high mutation rate, microsatellites are useful for their large amount of genetic variability and rapid evolution rate for detecting more recent changes in population structure.

There are also several drawbacks of microsatellites. First, microsatellites require species-specific isolation (Selkoe and Toone 2006). Because the DNA sequences of the primers are highly conserved within a particular species, one has to make new primers for each experiment when working with different species. It is difficult to isolate new primers and the failure rate is high. Yue et al. (2008) studied 4 clone of *P. clarkii* with 5 microsatellite in 120 individuals. They reported that each clone contained identical individual as genetic and found as heterozygous in majority of the microsatellites and it was confirmed that all of them were belonged to *P. clarkii*. In another study, Yue et al. (2010) studied genetic diversity and population structure of *P. clarkii* using 9 polymorphic microsatellites. A significant heterozygote deficiency was observed in the studied population. And also, Ahn et al. (2011) studied population analysis of *Cambaroides similis* in Korea and developed and identified 8 microsatellite locus from 49 sample in 4 location (one population from Mt. Bukhan (BH), 3 of from Mt. Gwanak GA). The locus identified per locus was ranged between 2-12. Investigated heterozygosity and expected heterozygosity were found as 0.00-0.33 and 0.25-0.43 respectively. The genetic difference between GA and BH population was 0.789 and within GA population was 0.454. It was thought this high difference to be related from

geographic distance. Gross et al. (2013) developed 10 new microsatellite markers for reveal geographic structuring of *A. astacus*. It is a first large scale study for this species. They studied with 633 species and 18 locations. They identified two highly differentiated population groups along Baltic and Black Sea respectively. The Baltic Sea populations had lower genetic variations and private allele numbers than Black Sea. These studies show us the utility of microsatellites for determining genetic difference of crayfish. Also, Coleman et al (2013), Vorburger et al (2014), Blaha et al (2016), have studied with microsatellite markers in different species and demonstrated that the genetic markers so powerfull and useful to define and manage populations of threatened species based on the notion that populations with unique lineages of mtDNA.

Next generation sequencing method

The another and new genetic method which is used in genetic analysis is next generation sequencing method. Miller et al. (2013) were made genetic analysis of *Euastacus bispinosus* using Next generation sequencing method. In this study 10 out of 15 identified polymorphic loci were characterized using 22 individual in the Glenelg River. Number of locus per alleles and expected heterozygosity was determined as 2,80 and 0,36 respectively. These results are considered to be low for genetic diversity. Also in Crawford River 10 locus were genotyped and genetic diversity and population structure was investigated. As a result of analysis high genetic diversity were identified between species ($F_{ST}=0,49$). The all of the methods which mentioned above are useful for the determination of genetic analysis and diversity of crayfish species. For the sustainable of crayfish in the future these genetic methods and conservation strategies must be evaluated together.

Results

From the mt DNA markers, ISSR-PCR, COI, ITS1, RAPD-PCR, next generation sequencing and microsatellites techniques the Next generation sequencing and microsatellites have been introduced recently but give good results. Each methods have advantages and disadvantages but the important one is which is the most useful. For understanding of that we need to compare these method with each other or have to use different gene sequences in the mitochondria. But we can say that the molecular techniques used so far for investigation the population structure of crayfish species are useful. But these studies is not enough alone for the protection of these species in the strict sense. For the sustainability of these species in the future years firstly the genetic structures of all populations must

be reveal and than aquaculture studies must be conducted according to the results of these molecular studies. Otherwise the futurity of these species will be in threat.

Discussion

Conservation strategies can be achieved by understanding the genetic make-up because the endangered species are spatially fragmented. When there is a effort involving restocking and reintroduction we need a detailed information about population genetics of related species (Bernini et al. 2016). However, the information of genetic difference within and between remaining populations is a prerequisite for the continuation of species by many authorities (Avisé 1994; Riffel and Schreiber 1995; Haig 1998). During stock measurement, if these factors are ignored, a hazard such as neglected geographic variation of species which have evolutionary importance is occurs (Schulz et al. 2004). For this reason, Moritz (1994) put forward that animal populations have significant genetic diversity could be considered as separate inventories and managed as different units.

The management of genetic diversity and, ultimately, the survival of a population or an entire species depends on several factors. In the short term, it can be said that the genetic variation is insignificant, but in the long run, genetic diversity is required for the ability of the population to adapt to changing environmental conditions. For this reason, a follow-up management should include protection of genetic diversity. Choosing appropriate donor populations is easier and healthier when the estimated stock size and the quality of the disease quality together are assessed. Strong populations which have high polimorfizm or genetic diversity are preferred for determine the minimum genetic diversity, so that Moritz (1994) reported that the populations have important genetic difference might be managed as sepearate stocks and different protection units (Fevolden et al. 1994).

Cryptic variety can be seen in many freshwater species. Although species with similar morphology may vary from one another understanding the level of these cryptic diversity has vital prospects for the continuation of biological diversity of freshwater species, for the identification of potential resistant and vulnerable populations, and the conservation of species diversity. If these factors are ignored, the management plans will not be effective and probably will be results with species losses (McKniff 2012; Miller et al. 2013).

The heterogeneity of species may be reflect the genetic adaptation of them to the specific climate or environmental condition. Crayfish have a low genetic

diversity tendency and artificial production conditions can worsen this situation by selecting storage strategies or superior broodstocks. So the genetic diversity of crayfish in culture conditions is necessary with the management and periodic monitoring of aquaculture programs (Fetzner et al. 1997). The knowledge acquired from molecular genetic studies should be used in the sustainable management of crayfish to protect it from extinction.

The estimation of genetic diversity will help to identify potential resistance and sensitive stocks and will be a pointer in the protection of resistance stock and taking precautions for the monitoring of sensitive stock. In stocking studies genetic structure within and between population is ignored and this situation leads to serious decline in stocks and contamination of natural species. Furthermore a recovery of population can perform with healthy and sufficient population magnitude to sustain genetic polymorphism in order to adapt climatic change. So the genetic diversity must be considered in governing activities.

References

- Ahn DH, Park MIH, Jung JH, Oh MJ, Kim S, Jung J, Min G-S. 2011. Isolation and characterization of microsatellite loci in the Korean crayfish, *Cambaroides similis* and application to natural population analysis. *Anim Cells Syst.* 15(1): 37-43.
- Akhan S, Bektaş Y, Berber S, Kalayci G. 2014. Population structure and genetic analysis of narrow-clawed crayfish (*Astacus leptodactylus*) populations in Turkey. *Genetica.* 142(5):381-395.
doi: 10.1007/s10709-014-9782-5
- Avisé JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC, 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu Rev Ecol Syst.* 18: 489-522.
doi: 10.1146/annurev.es.18.110187.002421
- Avisé JC. 1994. Molecular markers, Natural History and Evolution. Chapman and Hall (eds.) New York 511p.
- Bernini G, Bellati A, Pellegrino I, Negri A, Ghia D, Fea G, Galeotti P. 2016. Complexity of biogeographic pattern in the endangered crayfish *Austropotamobius italicus* in northern Italy: molecular insights of conservation concern. *Conserv Genet.* 17(1):141-154.
doi:10.1007/s10592-015-0767-4
- Blaha M, Žurovcová M, Kouba A, Polícar T, Kozák P. 2016. Founder event and its effect on genetic variation in translocated populations of noble crayfish (*Astacus astacus*). *J Appl Genetics.* 57(1):99-106.
doi: 10.1007/s13353-015-0296-3
- Clavero M, Nores C, Kubersky-Piredda S, Centeno-Cuadros A. 2016. Interdisciplinarity to reconstruct historical introductions: solving the status of cryptogenic crayfish. *Biol Rev.* 91(4):1036-1049.
doi: 10.1111/brev.12205
- Coleman RA, Weeks AR, Hoffmann AA. 2013. Balancing genetic uniqueness and genetic variation in determining conservation and translocation strategies: a comprehensive case study of threatened dwarf galaxias, *Galaxiella pusilla* (Mack) (Pisces: Galaxiidae). *Mol. Ecol.* 22(7): 1820-1835.
doi: 10.1111/mec.12227
- Cook BD, Page TJ Hughes JM. 2008. Importance of cryptic species for identifying 'representative' units of biodiversity for freshwater conservation. *Biol Conser.* 141(11): 2821-2831.
doi:10.1016/j.biocon.2008.08.018
- DeSalle R, Amato G. 2004. The expansion of conservation genetics. *Nature Rev.* 5(9): 702-712.
doi:10.1038/nrg1425
- Miller AD, Rooyen AV, Sweeney OF, Whiterod S, Weeks A R. 2013. The development of 10 novel polymorphic markers through next generation sequencing and a preliminary population genetic analysis for the endangered Glenelg spiny crayfish, *Eustacus bispinosus*. *Mol Biol Rep.* 40(7):4415-9.
doi: 10.1007/s11033-013-2531-5
- Edsman L, Farris JS, Kallersjö M, Prestegård T. 2002. Genetic differentiation between noble crayfish, *Astacus astacus* (L.), populations detected by microsatellite length variation in the rDNA ITS1 region. *Bull. Fr. Pêche Piscic.* 367 (2002): 691-706.
doi: 10.1051/kmae:2002082
- Fetzner Jr J M, Sheehan R J, Seeb Wl. 1997. Genetic implication of broodstock selection for crayfish aquaculture in the Midwestern United States. *Aquaculture.* 154(1): 39-55.
doi: 10.1016/S0044-8486(97)00026-4
- Fetzner Jr J W. 2011. The crayfish and lobster taxonomy browser. Available online via: <http://iz.carnegiemnh.org/crayfish/NewAstacidea/infraorder.asp?io=Astacidea>
- Fevolden S E, Taugbøl T, Skurda L J. 1994. Allozymic variation among populations of noble crayfish, *Astacus astacus* L., in southern Norway: implications for management. *Aquacult Fish Manage.* 25(9): 927-935.
doi:10.1111/j.1365-2109.1994.tb01354.x
- Furse J M, Bone J W P, Appleton S D, Leland J C, Coughran J. 2012. Conservation of Imperiled Crayfish-Euastacus bindal (Decapoda: Parastacidae), a Highland Crayfish from Far North Queensland, Australia. *J Crustacean BioL.* 32(4):677-683.
doi:10.1163/193724012X633405
- Geldiay R, Kocataş A. 1970. Türkiye Astacus (Decapoda) Populasyonlarının Dağılışı ve Taksonomik Tespiti, Ege Üni. Fen Fak. Gen. Zool. Kürsüsü, İlmi Raporlar serisi, No:94, Biologie 63, İzmir, 11p.[in Turkish].
- Gouin N, Souty-Grosset C, Ropiquet A, Grandjean F. 2002. High dispersal ability of *Austropotamobius pallipes* revealed by microsatellite markers in French brook. *Bulletin Français de la Pêche et de la Pisciculture.* 367(2002):681-689.
doi:10.1051/kmae:2002059
- Gouin N, Grandjean F, Souty-Grosset C. 2006. Population genetic structure of the endangered crayfish *Austropotamobius pallipes* in France based on microsatellite variation: biogeographical inferences

- and conservation implications. *Freshwater Biol.* 51(7):1369-1387.
doi: [10.1111/j.1365-2427.2006.01570.x](https://doi.org/10.1111/j.1365-2427.2006.01570.x)
- Gross R, Palm S, Kõiv K, Prestegard T, Jussila J, Paaver T, Geist J, Kokko H, Karjalainen A, Edsman L. 2013. Microsatellite markers reveal clear geographic structuring among threatened noble crayfish (*Astacus astacus*) populations in Northern and Central Europe, *Conserv Genet.* 14 (4):809-821.
doi: [10.1007/s10592-013-0476-9](https://doi.org/10.1007/s10592-013-0476-9)
- Haig S. 1998. Molecular contributions to conservation. *Ecology.* 79 (2): 413-425.
- Harhoğlu M. M 2004. The present situation of freshwater crayfish, *Astacus leptodactylus* (Eschscholtz, 1823) in Turkey. *Aquaculture.* 230 (1-4):181-187
- Harhoğlu M M, Harhoğlu A G 2006. Threat of non-native crayfish species introductions into Turkey: global lessons. *Rev Fish Biol Fish* 16(2):171-181.
- Harhoğlu M M, Güner U. 2007. Studies on the recently discovered crayfish, *Austropotamobius torrentium* (Shrank, 1803), in Turkey: morphological analysis and meat yield. *Aquaculture Res.* 37(5): 538- 542.
doi: [10.1111/j.1365-2109.2006.01451.x](https://doi.org/10.1111/j.1365-2109.2006.01451.x)
- Helfrich LA, DiStefano RJ. 2009. Sustaining America's Aquatic Biodiversity Crayfish Biodiversity and Conservation. Virginia Tech. 420-524.
- Helms B, Vaught RC, Suciú SK, Santos SR. 2015. Cryptic diversity within two endemic crayfish species of the Southeastern US revealed by molecular genetics and geometric morphometrics. *Hydrobiologia.* 755(1): 283-298.
doi: [10.1007/s10750-015-2311-4](https://doi.org/10.1007/s10750-015-2311-4)
- Koizumi I, Usio N, Kawai T, Azuma N, Masuda R. 2012. Loss of genetic diversity means loss of geological information: The endangered Japanese crayfish exhibits remarkable historical footprints. *Plos One.* 7(3): e33986.
doi: [10.1371/journal.pone.0033986](https://doi.org/10.1371/journal.pone.0033986)
- McKniff J. 2012. Investigation of the population genetics of crayfish (*Orconectes virilis*) using AFLP markers, Degree of Bachelor of Science. p: 1-39.
- Li Y, Guo X, Deng W, Luo W, Wang W. 2012. Population genetic structure and post-establishment dispersal patterns of the red swamp crayfish *Procambarus clarkii* in China. *Plos One.* 7(7):e40652
doi: [10.1371/journal.pone.0040652](https://doi.org/10.1371/journal.pone.0040652)
- Liu G, Zhou L, Li X, Lu D. 2013. Population genetic structure of the invasive red swamp crayfish in China revealed by ITS1 variations. *Biochem Genet.* 51(11-12): 841-852.
doi: [10.1007/s10528-013-9611-z](https://doi.org/10.1007/s10528-013-9611-z)
- Largiader C R, Herger F, Lörtscher M, Scholl A. 2000. Assessment of natural and artificial propagation of the white-clawed crayfish (*Austropotamobius pallipes* species complex) in the Alpine region with nuclear and mitochondrial markers. *Molecular Ecology.* 9 (1):25-37.
doi: [10.1046/j.1365-294x.2000.00830.x](https://doi.org/10.1046/j.1365-294x.2000.00830.x)
- Matallanas B, Callejas C, Ochando M D. 2012. A genetic approach to Spanish population of the threatened *Austropotamobius italicus* located at three different scenarios, *The Scientific World Journal.* 2012 Article ID 975930.
doi: [10.1100/2012/975930](https://doi.org/10.1100/2012/975930)
- May RM. 2010. Ecological science and tomorrow's world. *Philosophical Transactions of the Royal Society B.* 365:(1537): 41-47.
doi: [10.1098/rstb.2009.0164](https://doi.org/10.1098/rstb.2009.0164)
- Moritz C. 1994. Defining evolutionarily significant units for the conservation. *Trends Ecol Evol.* 9(10):373-375.
doi: [10.1016/0169-5347\(94\)90057-4](https://doi.org/10.1016/0169-5347(94)90057-4)
- Owen CL, Bracken-Grissom H, Stern D, Crandall KA. 2015 A synthetic phylogeny of freshwater crayfish: insights for conservation. *Philosophical Transactions Royal Society B.* 370(1662): 20140009.
doi: [10.1098/rstb.2014.0009](https://doi.org/10.1098/rstb.2014.0009)
- Ricciardi A, Rasmussen JB. 1999. Extinction rates in North American freshwater fauna. *Conserv Biol.* 13(5): 1220-1222.
doi: [10.1046/j.1523-1739.1999.98380.x](https://doi.org/10.1046/j.1523-1739.1999.98380.x)
- Riffel M, Schreiber A. 1995. Coarse-grained population structure in Central European sculpin (*Cottus gobio* L.): secondary contact or ongoing genetic drift? *J Zool Syst Evol Res.* 33(3-4):173-184.
doi: [10.1111/j.1439-0469.1995.tb00970.x](https://doi.org/10.1111/j.1439-0469.1995.tb00970.x)
- Schrimpf A, Schulz HK, Theissinger K, Parvulescu L, Schulz R. 2011. The first large-scale genetic analysis of the vulnerable noble crayfish *Astacus astacus* reveals low haplotype diversity in central European populations. *Knowl Manag Aquat Ec.* 401:35
doi: [10.1051/kmae/2011065](https://doi.org/10.1051/kmae/2011065)
- Schrimpf A, Theissinger K, Dahlem Jr, Maguire I, Pârvescu L, Schulz H K, Schulz R. 2014. Phylogeography of noble crayfish (*Astacus astacus*) reveals multiple refugia. *Freshwater Biol.* 59(4):761-776.
doi: [10.1111/fwb.12302](https://doi.org/10.1111/fwb.12302)
- Schulz HK, Smietana P, Schulz R. 2004. Assessment of DNA variations of the noble crayfish (*Astacus astacus* L.) in Germany and Poland using inter-simple sequence repeats (issrs) *Bull Fr Peche Piscic.* 372-373(2004):387-399.
doi: [10.1051/kmae:2004012](https://doi.org/10.1051/kmae:2004012)
- Schulz R. 2000. Status of the noble crayfish *Astacus astacus* (L.) in Germany: monitoring protocol and the use of RAPD markers to assess the genetic structure of populations. *Bull. Fr. Pêche Piscic.* 356(2000): 123-138.
doi: [10.1051/kmae:2000007](https://doi.org/10.1051/kmae:2000007)
- Selkoe KA, Toone RT. 2006. Microsatellites for ecologists: a practical guide to using and evaluating. *Ecol Lett.* 9 (5): 615-629.
doi: [10.1111/j.14610248.2006.00889.x](https://doi.org/10.1111/j.14610248.2006.00889.x)
- Vorburger C, Rhyner N, Hartikainen H, Jokela J. 2014. A set of new and cross-amplifying microsatellite loci for conservation genetics of the endangered stone crayfish (*Austropotamobius torrentium*). *Conservation Genet Resour.* 6(3):629-63.
doi: [10.1007/s12686-014-0157-0](https://doi.org/10.1007/s12686-014-0157-0)
- Wilson AC, Cann RL, Carr MG, Gyllensten UB, Helm-Bychowski M, Higushi R G, Palumbi SR, Prager EM, Sage RD, Stoneking M, 1985. Mitochondrial DNA

- and two perspectives on evolutionary genetics. *Biol J Linn Soc.* 26(4): 375-400.
[doi: 10.1111/j.1095-8312.1985.tb02048.x](https://doi.org/10.1111/j.1095-8312.1985.tb02048.x)
- Yue GH, Wang G L, Zhu B Q, Wang C M, Zhu Z Y, Lo L C. 2008. Discovery of four natural clones in a crayfish species *Procambarus clarkii*. *Int J Biol Sci.* 4(5): 279-282
- Yue GH, Li J, Bai Z, Wang CM, Feng F. 2010. Genetic diversity and population structure of the invasive alien red swamp crayfish. *Biol Invasions.* 12(8): 2697-2706.
[doi: 10.1007/s10530-009-9675-1](https://doi.org/10.1007/s10530-009-9675-1)
- Zhang YJ, Rao GY. 2005. Genetic diversity of an amphicarpic species, *Amphicarpaea edgeworthii* Benth. (Leguminosae) based on RAPD markers. *Biochem Syst Ecol.* 33(12): 1246-1257.
[doi:10.1016/j.bse.2005.07.009](https://doi.org/10.1016/j.bse.2005.07.009)