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Research Article Araştırma Makalesi

Akdeniz Ülkelerinin Deniz Sağlığı Performanslarının Analizi: CRITIC Tabanlı MARCOS Yöntemi İle Bir Uygulama

Analysis of Marine Health Performances of Mediterranean Countries: An Application with CRITIC-based MARCOS Method

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Özet: Akdeniz'e kıyısı olan ülkelerin deniz sağlığı konusundaki faaliyetlerinin Akdeniz'in yarı kapalı havzası olması sebebiyle diğer Akdeniz ülkelerinin deniz sağlığı performanslarını etkileyebildiği için Akdeniz ülkelerinin deniz sağlığı performanslarının analizi büyük önem arz etmektedir. Bu kapsamda araştırmada, 19 Akdeniz ülkesinin 2021 yılı için Okyanus Sağlığı Endeksi (Ocean Health Index-OHI) bileşenlerine ait değerler üzerinden söz konusu ülkelerin deniz sağlığı performansları CRITIC tabanlı MARCOS çok kriterli karar verme (ÇKKV) yöntemi ile ölçülmüştür. Bulgulara göre, CTIRIC yöntemi kapsamında ülkelere göre en önemli deniz sağlığı bileşeninin ''Geçim Kaynakları ve Ekonomiler'' olduğu tespit edilmiştir. Ayrıca bulgular kapsamında CRITIC tabanlı MARCOS yöntemine göre deniz sağlığı performansı en fazla olan ilk üç ülkenin sırasıyla Slovenya, İspanya ve Fransa, en az olan ilk üç ülkenin ise Suriye, İsrail ve Libya olduğu gözlenmiştir. Araştırmada ayrıca CRITIC tabanlı MARCOS yöntemine göre ülkelerin ortalama deniz sağlığı performansı ölçülmüş ve ortalamadan düşük değerde olan ülkelerin Akdeniz'in deniz sağlığının daha iyi olması için deniz sağlığı performanslarını artırmaları gerektiği sonucuna ulaşılmıştır. Yöntem açısından ise OHI kapsamında ülkelerin deniz sağlığı performansları başta CRITIC tabanlı MARCOS yöntemi olmak üzere CRITIC tabanlı ARAS, EDAS, COPRAS ve TOPSIS ÇKKV yöntemleri ile ölçülebileceği değerlendirilmiştir.

Anahtar kelimeler
- Deniz Sağlığı
- Akdeniz Ülkeleri
- CRITIC
- CRITIC tabanlı MARCOS

Abstract: The analysis of the marine health performances of the Mediterranean countries is of great importance as the activities of the countries with a coast on the Mediterranean on marine health can affect the marine health performances of other Mediterranean countries due to the semi-enclosed basin of the Mediterranean. In this context, the marine health performances of 19 Mediterranean countries were measured by the CRITIC-based MARCOS multi-criteria decision-making (MCDM) method, over the values of the Ocean Health Index (OHI) components for 2021. According to the findings, it has been determined that the most important marine health component according to the countries within the scope of the CRITIC method is "Livelihoods and Economies". In addition, within the scope of the findings, it was observed that the first three countries with the highest marine health performance according to the CRITIC-based MARCOS method were Slovenia, Spain, and France, respectively, while the first three countries with the lowest performance were Syria, Israel, and Libya. In the study, the average marine health performance of the countries was also measured according to the CRITIC-based MARCOS method and it was concluded that the countries with a lower value than the average should increase their marine health performance to improve the marine health of the Mediterranean. In terms of method, it was evaluated that the marine health

Keywords
- Marine health
- Mediterranean countries
- CRITIC
- CRITIC based MARCOS

performances of the countries within the scope of OHI could be measured with the CRITIC-based MARCOS method and CRITIC-based ARAS, EDAS, COPRAS, and TOPSIS MCDM methods.

1. GİRİŞ

Ülkeler arası ilişkilerin yoğunlaşması ve bu yoğunlaşmanın getirdiği doğrudan ve dolaylı faaliyetler sebebiyle dünya üzerindeki denizlerin ve okyanusların sağlık düzeyleri önemli güncel ve küresel bir sorun niteliği kazanmıştır (Demir, 2014: 123; Gilmour, 2021: 2). Özellikle deniz ekosistemlerine zararlı maddelerin girmesi ve deniz sağlığının ihmal edilmesi deniz suyunun kalitesinde, iklimlerde ve atmosferde değişikliğe neden olmaktadır. Bu durum, denizlerden yararlanabilme seviyesini, çevre, can, mal güvenliğini ve deniz sağlığını tehlikeye düşürmektedir (Ilgar ve Güven, 2007: 119).

Dünyanın yaklaşık olarak %70'ini okyanuslar ve denizler oluşturmaktadır. Ülkeler açısından su ürünleri, turizm, su sporları, ulaşım ve taşımacılık alanları ile denizlerin oluşturduğu iklimsel özellikler açısından denizlerden pek çok olumlu getiri sağlanabilmektedir. Bunun yanında, içme ve kullanma suyunun tedarik ve kullanılmış suyun tahliye edilmesinde denizlerden faydalanılmaktadır (Peker, 2007: 4). Bu bağlamda ülkelerin denizlerden faydalanmasının önemi kapsamında sürekli olarak kendilerinin deniz sağlığı performanslarını analiz etmektedir. Böylelikle ülkeler, deniz sağlığı performansları hakkında kendilerinde farkındalık oluşturarak deniz sağlığı konusundaki eksikliklerini, yeterliliklerini ve üstünlüklerini belirleyebilmektedir. Böylelikle ülkeler, deniz sağlığı kapasitelerinin farkındalığıyla deniz sağlığı konusunda eksikliklerini gidermek, yeterliliklerini geliştirmek ve üstünlüklerinin sürdürebilirliğini oluşturmak için politikalar, stratejiler ve faaliyetler gerçekleştirebilmektedir. Bunun dışında ülkeler birbirlerinin deniz sağlığı performanslarını takip ederek deniz sağlığı konusunda iyi olan ülkeler ile işbirlikleri ve ortaklıklar oluşturabilmektedir. Buna göre, ülkelerin deniz sağlığı performanslarının ölçümü önem kazanmakta olup, ülkeler deniz sağlığı performanslarını ölçen ölçeklere ihtiyaç duymaktadır.

Ülkelerin deniz sağlığı performanslarını ölçen tek metrik Halpern vd., (2012) tarafından oluşturulan Okyanus Sağlığı Endeksi (Ocean Health Index – OHI)'dir. OHI, temel anlamda denize ve okyanusa kıyısı olan ülkelerin insanlarının sağlıklı denizlerden ve okyanuslardan bekledikleri gıda, kültürel, ekonomik ve sosyal değer getirilerinin faydalarının sürdürülebilirlik seviyesini ölçmektedir. Bu bağlamda OHI, okyanus ve deniz sağlığının korunmasına yönelik ülkelere farkındalık kazandırmaktadır. Bunun yanında OHI, deniz ve okyanusların kirlilikten korunmasına yönelik ülkelere planlar hazırlamasına katkı sunmaktadır. OHI 10 bileşen ve bu 10 bileşene bağlı 8 alt bileşenden oluşmaktadır. Metodolojik olarak alt bileşenlerin aritmetik ortalamaları ile bileşenler, bileşenlerin aritmetik ortalamaları ile ülkelerin deniz sağlığı performansları ölçülebilmektedir (Halpern vd., 2012; OHI, 2021). Söz konusu bileşenler ve alt bileşenlere ait açıklamalar Tablo 1'de gösterilmiştir.

Tablo 1. OHI bileşenleri ve alt bileşenleri.

No	Bileşenler ve Alt Bileşenler	Açıklamalar
1	Gıda Tedariki	Ülkelerin doğada yakalanan ve çiftlikte yetiştirilen deniz ürünlerinin sürdürülebilir kapasitesini ölçmektedir.
1.1	Balıkçılık	Ülkelerin doğa ortamındaki balıkçılık sürdürülebilirlik performansını ölçmektedir.
1.2	Deniz Kültürü	Ülkelerin çiftliklerde üretilen balıkçılığın sürdürülebilirlik performanslarını ölçmektedir.
2	Balıkçılık Fırsatları	Ülkelerde küçük ve yerel ölçekte balık tutması gereken kişilerin bu faaliyetlerini yapma fırsat seviyesini ölçmektedir.
3	Doğal Ürünler	Ülkelerin gıda dışı deniz kaynaklarının sürdürülebilir hasadının ne kadar iyi maksimize ettiğini ölçmektedir.
4	Karbon Kapasitesi	Ülkelerin sağladıkları karbon kapasitesini ölçmektedir.
5	Sahilleri Koruma	Ülkelerin sahil koruma seviyesini ölçmektedir.
6	Geçim Kaynakları ve Ekonomiler	Ülkelerin sürdürülebilir denizcilikle elde edilen işlerin performansını ve geliri ölçmektedir.
6.1	Geçim Kaynakları	Ülkelerin deniz ile ilgili işlerinin kalitesini ve niceliğini ölçmektedir.
6.2	Ekonomiler	Ülkelerin denizden sağladığı gelirin değerini ölçmektedir.
7	Turizm ve Eğlence	Ülkelerin sürdürülebilir turizm seviyesini ölçmektedir.
8	Hassaslık	Ülkelerin deniz ve sahilleri koruma seviyelerini ölçmektedir.
8.1	İkonik Türler	Ülkelerin önemli deniz ürünlerini koruma seviyelerini ölçmektedir.
8.2	Kalıcı Özel Yerler	Ülkelerin deniz ile ilgili kültürel yerleri koruma seviyelerini ölçmektedir.
9	Temiz Su	Ülkelerin temiz deniz sağlama performanslarını ölçmektedir
10	Biyolojik Çeşitlilik	Ülkelerin deniz yaşamı zenginliği ve çeşitliliğini koruma performanslarını ölçmektedir.
10.1	Deniz Habitatlarını Koruma	Ülkelerin deniz türlerinin doğadaki yaşam alanları koruma performanslarını ölçmektedir.
10.2	Deniz Türlerini Koruma	Ülkelerin deniz türlerini koruma performanslarını ölçmektedir.

Kaynak: OHI, 2021

Deniz sağlığını bozan en önemli etkenlerden bir tanesi deniz kirliliğidir (Halper vd., 2012). Literatür değerlendirildiğinde, deniz kirlenmesi ile ilgili olarak pek çok tanımlamaya rastlamak mümkündür. Söz konusu tanımlardan uluslararası anlamda geçerli olanı Birleşmiş Milletler Deniz Hukuku Sözleşmesi'nde belirtilen açıklamadır. Birleşmiş Milletler Deniz Hukuku Sözleşmesinin 1. Madde. 4. Fıkra'sına göre deniz kirliliği, denizdeki canlı kaynaklara ve deniz yaşamına zarar veren ve deniz yaşamı için zararlı olabilen maddelerin insan tarafından doğrudan ya da dolaylı olarak denizlere aktarılması sonucu deniz suyu kalitesinin bozulmasına, deniz faaliyetlerini kısıtlanmasına ve insan sağlığını bozulması kapsamında tehlikeye neden olan faaliyetler bütünü olarak tanımlanmıştır (Proelß, 2017: 26). Deniz kirliliği kısaca, denize ve denizin çevresine zarar verebilecek ve insan sağlığını tehlikeye düşürecek faaliyetleri içermektedir. Bu çerçevede deniz kirliliği farklı açılardan tasnif edilebilmektedir. Bunlar; kara ve atmosferik faaliyetler ile oluşan kirlilik, deniz yatağından kaynaklanan kirlilik, taşkınlar sebebiyle oluşan kirlilik ve deniz taşımacılığı ile deniz vasıtalarından kaynaklanan kirliliktir (Birkan, 2019: 27).

Akdeniz bölgesinde son dönemlerde özellikle ülkeler arasındaki enerji, ekonomi, çevre ve ticaret alanındaki ilişkiler yoğunlaşmıştır. Akdeniz'in konum açısından Avrupa, Asya ve Afrika ülkelerinin kesiştiği yerde olmasıyla ve buna bağlı olarak stratejik öneminin olması açısından uluslararası ulaşım faaliyetlerinde odak noktası hale gelmesiyle Akdeniz'in deniz sağlığı durumu dikkat çekici bir seviyeye ulaşmıştır (Kanlı ve Falcıoğlu, 2021). Bunun yanında, Akdeniz'in yarı kapalı yapısı, akıntı sisteminin özelliği ve sahil şeritlerinde nüfus yoğunluğunun artması Akdeniz'in deniz sağlığının tehlikeli altına girmesine neden olmuştur (Türkmen ve Aras, 2011: 3). Ayrıca Dünya genelindeki 344 türün 144'ü Akdeniz'de plastik atık yüzünden tehlike altınadır (Onay vd., 2021: 19). Bunların dışında, dünya petrol geçişlerinin yaklaşık olarak %28'i Akdeniz bölgesinde gerçekleşmesi açısından deniz kazaları sonucunda Akdeniz'de her yıl yaklaşık olarak 20.000 ton petrol sızıntısı olmaktadır (Günel, 2004'den akt. Özkan ve Sunar, 2008: 743). Son olarak Akdeniz'in kıyı bölgelerinden gelen atık su Akdeniz'in deniz sağlığının ciddi anlamda bozulmasına neden olmaktadır (AÇA, 2006: 16).

Akdeniz'in deniz sağlığına yönelik 1978 Barcelona Sözleşmesi, 1982 Cenova Anlaşması, 1995 Avrupa Akdeniz Ortaklığı, 1997 Helsinki Avrupa Akdeniz Çevre Bakanları Kısa ve Orta Vadeli Çevresel Eylem Planı, 2000 Avrupa Birliği Su Çevre Direktifi ve 2003 Biyolojik Çeşitliliğe Yönelik Stratejik Eylem Planı gibi önemli girişimlerde bulunulmuştur (AÇA, 2006: 68-72). Ayrıca Avrupa Birliği Antlaşması ve Avrupa Birliği'nin İşleyişi Hakkında Antlaşma'da deniz kaynaklarının korunması ile ilgili olarak çeşitli hükümler bulunmaktadır (Avrupa Birliği Genel Sekreterliği, 2011). Akdeniz'in yarı kapalı bir havzası olduğu için her hangi bir Akdeniz ülkesinin veya ülkelerinin deniz sağlığına yönelik faaliyetleri diğer Akdeniz ülkelerinin deniz sağlığı performanslarını etkileyebilmektedir. Dolayısıyla Akdeniz'de deniz sağlığının korunmasına yönelik özellikle Akdeniz'e kıyısı olan ülkelerin girişimlerinin kayda değer olduğu düşünülmektedir. Bu bağlamda, Akdeniz ülkelerinin deniz sağlığı performanslarının analizi büyük önem arz etmektedir (Carvalho ve Civili, 2001; Massoud vd., 2003; Gao ve Zhang, 2021, Ghorbal, vd., 2021).

Araştırmada Akdeniz ülkelerinin deniz sağlığı performanslarının belirlenmesinin önemi açısından 19 Akdeniz ülkesinin (Arnavutluk, Cezayir, Fas, Fransa, GKRK, Hırvatistan, İspanya, İsrail, İtalya, Karadağ, Libya, Lübnan, Malta, Mısır, Slovenya, Suriye, Tunus, Türkiye, Yunanistan) 2021 yılı için OHI bileşenlerine ait değerler üzerinden söz konusu ülkelerin deniz sağlığı performansı CRITIC tabanlı MARCOS yöntemi ile ölçülmüştür. Literatür incelendiğinde, pek çok araştırmada bileşenlerin önemlilik derecelerinin hesaplanması açısından CRITIC ve karar alternatiflerinin performans ölçümünde ve seçim probleminde ise MARCOS yöntemi tercih edildiği tespit edilmiştir. Bu durum, CRITIC ve MARCOS yönteminin güvenilir olduğunu göstermektedir. CRITIC yönteminde bileşenlerin göreli ağırlıklarının tespit edilmesi, öznelliğin azalması ve bileşen ağırlıkların dominant olmayan karakterlerinin dikkate alınmaması gibi avantajlar sağlamaktadır (Ulutaş ve Topal, 2020: 1). Ayrıca karar alternatiflerinin ölçülmesinde MARCOS yönteminin duyarlılık ve güvenirlik seviyesi yüksek olan bir çok kriterli karar verme (ÇKKV) yöntemidir (Boral vd., 2020). Dolayısıyla ülkelerin deniz sağlığı performanslarının ölçümünde CRITIC tabanlı MARCOS yöntemi tercih edilmiştir.

Literatürde CRITIC ve MARCOS yöntemlerinin birlikte kullanılmasına yönelik araştırmaların kısıtlı olması açısından bu araştırmanın literatürü zenginleştirdiği düşünülmüştür. Bunun dışında literatürde, Akdeniz veya bölge ülkelerin deniz sağlığı performanslarının herhangi bir ÇKKV yöntemi ile tespit edilen bir araştırmaya rastlanılmamış olmaması ve hangi Akdeniz ülkelerinin deniz sağlığı performanslarını artırması gerektiğinin analizinin nicel değerlere istinaden yapılması açısından bu araştırmanın literatüre katkı sağladığı düşünülmüştür. Buna göre araştırmanın bulgular ve tartışma kısmında ülkelerin deniz sağlık performansları ölçülmüş ve analiz edilmiştir. Sonuç kısmında ise bulgular ve tartışma kısmında elde edilen nicel değerlere istinaden çıkarımlar özetlenmiştir.

2. LİTERATÜR TARAMASI

Araştırmanın literatürü iki kısımdan oluşmaktadır. Bunlardan birincisinde deniz sağlığı ile ilgili araştırmalar açıklanmıştır. İkincisinde ise CRITIC ve MARCOS yöntemleri ile ilgili olan çalışmalar belirtilmiştir.

Literatür incelendiğinde, Akdeniz'in deniz sağlığı açısından ağırlıklı olarak deniz kirlenmesi konusu işlenmiştir. Bu kapsamda Compa vd., (2019), literatür verileri ve tür dağılım haritaları ile Akdeniz'deki plastik kirliliği girdilerinin deniz çeşitliliği üzerindeki etkisini incelemiştir. Araştırmada, Akdeniz'de mekânsal olarak plastik yoğunluğunun ve yaşam türlerinin plastik yutma riskinin okyanuslara göre daha fazla ve konum açısından deniz türlerinin yoğunluğunun plastik kirliliği yoğunluğuna yakın olduğu tespit edilmiştir. Liubartsevaa vd., (2019), 2019 yılı için sağladığı veriler ile İtalya'da çeşitli kıyı bölgelerindeki plastik durumunu incelemişlerdir. Araştırmada, söz konusu bölgelerdeki plastik akışın 2013-2017 yıl aralığındaki ortalama plastik akışından daha düşük bir seviyede olduğu gözlenmiştir. Ayrıca araştırmada, söz konusu bölgede oluşan plastiğin yarısından fazlasının deniz taşımacılığından kaynaklandığı sonucuna ulaşılmıştır. Guerranti vd., (2020), Akdeniz'e dökülen nehirlerin mikro plastik seviyelerini azaltmak için Akdeniz'in kıyı bölgelerin izlenmesi ve kontrol altına alınması gerektiğini ifade etmiştir. Yıldırım vd., (2020), Ortatoroslar Güzelyayla bölgesinde bulunan ve Akdeniz'e dökülen Deliçay'ın kaynağı ile boşalım noktası arasında belirlenen 11 farklı lokasyonda akarsu debisini inceleyerek su kimyasında meydana gelen değişimleri tespit etmek amacıyla fiziksel parametre ölçümleri yapmışlardır. Bulgulara göre, Deliçay'dan alınan su örneklerinin tarımsal sulama amaçlı kullanıma uygun olduğu tespit edilmiştir. Araştırmanın devamında, akarsu örneklerine ilişkin parametrelere göre kirlilik düzeyinin Deliçay'daki suyun çıkış noktasına (Akdeniz'e dökülme) doğru artış gözlendiği tespit edilmiştir. Araştırmacılar söz konusu bu kirlilik düzeyi artışının daha çok tarımda kullanılan kimyasal atıklar ile evsel ve endüstriyel atıkların kontrolsüz deşarz ile oluştuğunu belirlemişlerdir. Gregorietti vd., (2021), 2013-2019 yıl aralığındaki ilgili veriler ile Akdeniz'deki plastik durumunu araştırmışlardır. Araştırmada; Tunus, Palermo kıyı bölgeleri ile Castellammare körfezi ve Egadi adasının plastiğe maruz kalma seviyesinin yüksek olduğu tespit edilmiştir. Karadirek vd., (2019), bibliyometrik analiz yaparak 1970-2016 yıl aralığında Akdenizde'ki deniz kirliliği literatürünü Web of Sciences (WoS) ve Scobus veri tabanlarından analiz etmişlerdir. Çalışamada, 2000 yılından sonra Akdeniz'de deniz ve çevre kirliliği konusunda araştırmaların fazlalaştığı gözlenmiştir. Bunun yanında; Fransa, İspanya, İtalya, Yunanistan ve Türkiye'nin deniz kirliliği konusunda en çok adı geçen ülkeler olduğu belirlenmiştir. Sharma vd., (2021), 2021 yılı için literatürden elde ettiği veriler ile Akdeniz'deki mikro plastik kirliliği inceleyerek üç önemli bulgu elde etmişlerdir. Bunlardan birincisi, Akdeniz bölgesindeki mikro plastik konsantrasyonunun Pasifik okyanusundan fazla olmasıdır. İkincisi, Akdeniz bölgesinin yarı kapalı morfoloji durumu ve Akdeniz'de ülkelerden kaynaklanan faklı türden plastik üreten faaliyetler nedeniyle Akdeniz'in deniz sağlığı konusunda hassas bir yapıya ulaşmasıdır. Üçüncüsü ise Akdeniz'de farklı ve çeşitli plastik türlerinin fiziksel ve kimyasal özelliklerinin plastik partiküller ve su kütlesindeki organik maddeler arasında etkileşiminde önemli rol oynamasıdır. Soto-Navarro vd., (2021), 2020 yılında 3D modelleme yöntemi ile Akdeniz'de deniz kirliliği riskini incelemişlerdir. Bulgulara göre, Akdeniz'de deniz kirliliğin daha çok sıcak noktalardaki kıyı bölgelerinde yoğunlaştığı gözlenmiştir. Bu kapsamda araştırmacılar, Akdeniz'de deniz kirliliğin önlenmesi için özellikle kıyı bölgelerinin kontrol seviyelerinin artırılması gerektiğini vurgulamışlardır. Zilifli ve Tuncer (2021), 2019 yılında Doğu Akdeniz bölgesinde yer alan Dalyan-İztuzu sahilindeki mikro plastik kirliliğini 14 istasyondan örnekleme ile analiz etmişlerdir. Araştırma sonucuna göre, söz konusu bölgede mikro plastik yoğunluğunun ortalama 0, 148 $\pm$ 0,07 partikül /m^2 olarak tespit edilmiştir. Diğer bir bulguya göre, Dalyan-İztuzu bölgesinin Marmara ve diğer Akdeniz bölgesindeki çalışmalar ile kıyaslandığında mikro plastik kirliliğinin daha az olduğu bulgusuna ulaşılmıştır.

Yöntem açısından literatür incelendiğinde CRITIC ve MARCOS ile ilgili araştırmalar Tablo 2'de belirtilmiştir.

Tablo 2. CRITIC ve MARCOS literatürü.

Araştırmacı/ Araştırmacılar	Yöntem	Konu
Bouraima vd., (2021)	Entropi tabanlı MARCOS	Sahra altı Afrika demiryolu performansının ölçülmesi
Biswas vd., (2021)	Entropi tabanlı MARCOS	G7 ve BRICS ülkelerinin sosyoekonomik kalkınmasının ve COVID-19'a dayanıklılığın karşılaştırılması
Ali (2021)	CRITIC tabanlı MARCOS	CRITIC-MARCOS yöntemine dayalı yeni bir puanlama fonksiyonu
Dwivedi vd., (2021)	CRITIC tabanlı MARCOS	Çelik endüstrisinin performansını analizi
Gençtürk vd., (2021)	CRITIC tabanlı MARCOS	COVID-19 pandemisinin katılım bankaları üzerine etkilerinin incelenmesi
Çınaroğlu (2021)	CRITIC tabanlı MARCOS	Yenilikçi ve girişimci üniversite analizi
Arsu ve Ayçin (2021)	CRITIC tabanlı MARCOS	OECD ülkelerinin ekonomik, sosyal ve çevre açısından değerlendirilmesi
Badi ve Pamucar (2020)	Gri MARCOS	Çelik firması için kombine Gri-MARCOS yöntemleriyle tedarikçi seçimi
Stevic ve Brkovic (2020)	FUCOM tabanlı MARCOS	Bir taşımacılık şirketinde insan kaynaklarının değerlendirilmesi
Majidi vd., (2021)	SWARA tabanlı MARCOS ve COCOSO	İran limanlarının sürdürülebilirlik performansının ölçülmesi
Çelik ve Gül (2021)	BWM tabanlı MARCOS	Baraj inşaatı güvenliği için tehlike tanımlama, risk değerlendirmesi ve kontrolü
Stankovic vd., (2020)	Bulanık MARCOS	Karayolu trafik risk analizi
Korucuk (2021)	CRITIC	Ordu ve Giresun illerinde yapılan çalışmada lojistik performans unsurlarının önemliilk derecelerinin tespiti
Varghese ve Karande (2021)	AHP tabanlı MARCOS	Dişlileri ve kesme sıvılarını seçme problemi
Saraji vd., (2021)	Bulanık CRITIC tabanlı COPRAS	Sürdürülebilir bir dijital dönüşüm için endüstri 4.0'ın kabulüne yönelik zorlukların değerlendirilmesi
Memiş ve Korucuk (2022)	CRITIC tabanlı Gri İlişkisel Analiz	Giresun’da yemek işletmelerine yönelik pazarlama inovasyonu kriterlerinin önemlilik derecelerinin belirlenmesi ve en ideal firma seçimi

3. MATERYAL ve METOT

3.1. Araştırmanın veri seti, analizi ve kısıtı

Araştırmanın veri setini 2021 yılı için Akdeniz’e kıyısı olan 19 ülkenin OHI bileşenlerine ait değerler oluşturmaktadır. OHI raporunda Akdeniz’e kıyısı bulunana Bosna Hersek ve Monako ülkelerinin deniz sağlığına ilişkin olarak bazı bileşenlerinin değerleri olmadığından dolayı söz konusu ülkeler araştırmaya dâhil edilememiştir. Araştırma için sağlanan veriler açık kaynaktan sağlandığı ve herhangi bir deneye veya gözleme dayalı olmadan araştırma sonuçları elde edildiğinden dolayı araştırma için etik kuruluna başvurulmamıştır. Araştırmanın kısıtı açısından bu araştırmada OHI boyutları kapsamında Akdeniz ülkelerinin deniz performansı tespitinde sadece 2021 yılı için OHI raporunda yer alan verilerinden yararlanılmıştır. Araştırmada kolaylık sağlaması açısından OHI bileşenlerinin kısaltmaları Tablo 3'de sunulmuştur.

Tablo 3. OHI bileşenlerin kısaltmaları.

Bileşenler	Kısaltmalar
Gıda Tedariki	OHI1
Balıkçılık Fırsatları	OHI2
Doğal Ürünler	OHI3
Karbon Kapasitesi	OHI4
Sahilleri Koruma	OHI5
Geçim Kaynakları ve Ekonomiler	OHI6
Turizm ve Eğlence	OHI7
Hassaslık	OHI8
Temiz Su	OHI9
Biyolojik Çeşitlilik	OHI10

3.2. CRITIC yöntemi

CRITIC yöntemi, karar alternatiflerinin kriterlere ilişkin verilerine istinaden kriterlerin ağırlık katsayılarını veya önemlilik derecelerini objektif değerlendirmeyle ölçen bir tekniktir. CRITIC yönteminin diğer ağırlık katsayı hesaplama tekniklerinden ayıran en önemli özelliği, kriterlerin ağırlık katsayılarının uzman görüşler ile sağlanan öznel sonuçların değil, ağırlık katsayılarının standart sapma ve korelasyon analizi dikkate alınarak hesaplanmasıdır (Ayçin, 2019: 76). Bu bağlamda yöntemin uygulama aşamaları aşağıda açıklanmıştır (Dinçer, 2019, 42; Arslan, 2020, 120-122, Ecer, 2020, 87; Öztel ve Alp, 2020, 32-33).

A_i: i. karar alternatifi

C_j: j. değerlendirme kriteri

x_{ij}: j. değerlendirme kriterine göre i. alternatifin değeri

x_j^{mak}: j. kritere göre karar alternatiflerinin maksimum değeri

x_j^{min}: j. kritere göre karar alternatiflerinin minimum değeri

r_{ij}: j. değerlendirme kriterine göre i. alternatifinin aldığı değer

p_{jk}: herhangi bir j kriteri ile k kriteri arasındaki ilişki katsayıları

σ_j: j. kriterin standart sapma değeri (j= 1,2,…,n)

w_j: j. değerlendirme kriterinin ağırlığı (j= 1,2,…,n)

1. Aşama: Karar Matrisinin Sağlanması

$$X=\begin{matrix} A_1 \\ A_2 \\ \vdots \\ A_m \end{matrix}\begin{bmatrix} x_{11} & x_{12} & x_{1n} \\ x_{21} & x_{22} & x_{2n} \\ \vdots & \vdots & \vdots \\ x_{m1} & x_{m2} & x_{mn} \end{bmatrix} \quad (1)$$

2. Aşama: Karar Matrisinin Normalize İşlemi

Fayda Yönlü Kriterler İçin

$$r_{ij}\frac{x_{ij}-x_j^{min}}{x_j^{maks}-x_j^{min}} \text{……….j=1,2,….,n} \quad (2)$$

Maliyet Yönlü Kriterler İçin

$$r_{ij}\frac{x_j^{maks}-x_{ij}}{x_j^{maks}-x_j^{min}} \text{……….j=1,2,….,n} \quad (3)$$

3. Aşama: İlişki Katsayı Matrisinin Oluşturulması

$$p_{jk}=\frac{\sum_{i=1}^{m}(r_{ij}-\overline{r_j}).(r_{ik}-\overline{r_k})}{\sqrt{\sum_{i=1}^{m}(r_{ij}-\overline{r_j})^2.(r_{ik}-\overline{r_k})^2}} \text{ j, k=1,2,…,n} \quad (4)$$

4. Aşama: C_j değerlerinin Ölçülmesi

$$\sigma_j=\sqrt{\frac{\sum_{i=1}^{m}(r_{ij}-\overline{r_j})^2}{m-1}} \quad (5)$$

$$C_j=\sigma_j.\sum_{k=1}^{n}\left(1-p_j\right) j=1,2,\ldots,n \quad (6)$$

5. Aşama: Kriter Ağırlıkların (Önemlilik Derecelerinin) Ölçülmesi

$$w_j=\frac{C_j}{\sum_{k=1}^{n}C_j} \quad (7)$$

3.3. MARCOS yöntemi

MARCOS, alternatifleri ile referans değerleri olan ideal ve anti-ideal alternatiflerin ilişkisini belirleyerek karar alternatiflerin performanslarını ölçen bir ÇKKV tekniğidir. Söz konusu ilişkiler kapsamında karar alternatiflerinin fayda fonksiyonları ölçülür ve ideal ile anti-ideal hesaplarına göre uzlaşık sıralama oluşturulur. İdeal ve anti-ideal çözüm, fayda ve maliyet çözümlü kriterlere göre değişmektedir. Fayda kriterleri için ideal çözüm en büyük değere sahip olan karar alternatifi, buna karşın maliyet kriterleri için ideal çözüm ise en küçük değerdeki karar alternatifidir. Dolayısıyla anti-ideal çözümde fayda kriterleri için en küçük, maliyet kriterleri için ise en büyük değere sahip karar alternatifi aranmaktadır (Ecer, 2020: 338). Buna ilişkin olarak MARCOS yönteminin uygulama adımları aşağıda sunulmuştur (Chattopadhyay vd. 2020: 56-58; Ecer, 2020: 339-342).

1. Aşama: Karar Matrisinin Oluşturulması

$$X=\begin{bmatrix} x_{11} & x_{12}\cdots & x_{1n} \\ x_{21} & x_{22}\cdots & x_{2n} \\ \vdots & \vdots & \vdots \\ x_{m1} & x_{m2}\cdots & x_{mn} \end{bmatrix} \quad (8)$$

2. Aşama: Genişletilmiş Karar Matrisinin Oluşturulması

Genişletilmiş karar matrisi, karar matrisine ideal çözüm (Al) ve anti-ideal çözümün (AAl) eklenmesiyle sağlanır. Bu durum 9 da açıklanmıştır.

$$X=\begin{matrix} \\ A_1 \\ A_2 \\ \vdots \\ A_m \\ AAl \\ Al \end{matrix}\begin{matrix} C_1, C_2\ldots\ldots \quad C_n \\ \begin{bmatrix} x_{11} & x_{12}\cdots & x_{1n} \\ x_{21} & x_{22}\cdots & x_{2n} \\ \vdots & \vdots & \vdots \\ x_{m1} & x_{m2} & x_{mn} \\ x_{aa1} & x_{aa2}\cdots & x_{aan} \\ x_{ai1} & x_{ai2} & x_{ain} \end{bmatrix}\end{matrix} \quad (9)$$

AI ve AAI değerlerinin hesaplanması için fayda temelli kriterler için eşitlik 10, maliyet temelli kriterler için ise eşitlik 11'den faydalanılır.

$$\begin{cases} AI=min_i x_{ij} \text{ , fayda temelli kriter ise } (j\in B) \\ AAI=mak_i x_{ij} \text{ , fayda temelli kriter ise } (j\in B) \end{cases} \quad (10)$$

$$\begin{cases} AI=mak_i x_{ij} \text{ , maliyet temelli kriter ise } (j\in C) \\ AAI=min_i x_{ij} \text{ , maliyet temelli kriter ise } (j\in C) \end{cases} \quad (11)$$

3. Aşama: Genişletilmiş Karar Matrisinin Standartlaştırılması

Eşitlik 12 ile maliyet yönlü, eşitlik 13 ile fayda yönlü kriterler hesaplanarak eşitlik 14 ile genelleştirilmiş karar matrisinin standartlaştırılmış değerleri ölçülür.

$$n_{ij}=\frac{x_{ij}}{x_{ai}}, j \in B \quad (12)$$

$$n_{ij}=\frac{x_{aj}}{x_{ij}}, j \in C \quad (13)$$

$$N=\begin{bmatrix} n_{11} & n_{12}\dots & n_{1n} \\ n_{21} & n_{22}\dots & n_{2n} \\ \vdots & \vdots & \vdots \\ n_{m1} & n_{m2} & n_{mn} \\ n_{aa1} & n_{aa2}\dots & n_{aan} \\ n_{ai1} & n_{ai2} & n_{ain} \end{bmatrix} \quad (14)$$

4. Aşama: Ağırlıklı Matrisin Oluşturulması

$$v_{ij}=n_{ij}.w_j \quad (15)$$

$$V=\begin{bmatrix} v_{11} & v_{12}\dots & v_{1n} \\ v_{21} & v_{22}\dots & v_{2n} \\ \vdots & \vdots & \vdots \\ v_{m1} & v_{m2} & v_{mn} \\ v_{aa1} & v_{aa2}\dots & v_{aan} \\ v_{ai1} & v_{ai2} & v_{ain} \end{bmatrix} \quad (16)$$

5. Aşama: Karar Alternatiflerinin Fayda Derecelerinin Hesaplanması

Ağırlıklı matrisin elemanlarının toplamı:

$$S_i=\sum_{i=1}^{n} v_{ij} \quad (17)$$

İdeal çözüme göre fayda seviyesi:

$$K_i^+=\frac{S_i}{S_{ai}} \quad (18)$$

Anti-ideal çözüme göre fayda seviyesi:

$$K_i^-=\frac{S_i}{S_{aai}} \quad (19)$$

6. Aşama: Karar Alternatiflerinin Fayda Fonksiyonlarının Belirlenmesi

İdeal çözüme göre fayda fonksiyonu

$$\mathbf{f}\left(K_i^+\right)=\frac{K_i^-}{K_i^++K_i^-} \quad (20)$$

Anti-ideal çözüme göre fayda fonksiyonu

$$\mathbf{f}\left(K_i^-\right)=\frac{K_i^+}{K_i^++K_i^-} \quad (21)$$

7. Aşama: Alternatiflerin Fayda Fonksiyonların Belirlenmesi

$$f(K_i)=\frac{K_i^++K_i^-}{1+\frac{1-\mathbf{f}\left(K_i^+\right)}{\mathbf{f}\left(K_i^+\right)}+\frac{1-\mathbf{f}(K_i^-)}{\mathbf{f}(K_i^-)}} \quad (22)$$

4. BULGULAR ve TARTIŞMA

Bulgular kapsamında ilk olarak CRITIC yöntemi ile OHI bileşenlerinin önemlilik dereceleri tespit edilmiştir. Bu bağlamda CRITIC yönteminin birinci aşamasında açıklanan eşitlik 1 ile karar matrisi sağlanmıştır. Yöntemin ikinci aşamasında ise bileşenler fayda yönlü (maksimizasyon) oldukları eşitlik 2 ile karar matrisi değerlerinin normalizasyon değerleri ölçülmüştür. Buna göre, karar matrisi ve karar matrisinin normalize değerleri Tablo 4'de açıklanmıştır.

Tablo 4. Karar matrisi.

Karar Matrisi										
Ülkeler	**OHI**	**OHI 1**	**OHI 2**	**OHI 3**	**OHI 4**	**OHI 5**	**OHI 6**	**OHI 7**	**OHI 8**	**OHI 9**	**OHI 10**
Kriter Yönleri		**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**
Arnavutluk	72,1	18	83	96	73	73	100	59	66	78	75
Cezayir	66,1	59	73	89	66	99	100	18	37	46	74
Fas	69,7	15	75	68	75	100	100	60	68	57	79
Fransa	75,2	57	76	84	70	99	79	59	81	66	81
GKRK	65,7	53	73	77	71	72	63	57	57	63	71
Hırvatistan	75,4	38	78	98	65	66	79	100	81	73	76
İspanya	75,6	72	69	79	71	98	77	63	80	69	78
İsrail	56,7	31	94	19	71	75	100	16	38	47	76
İtalya	73,3	60	67	76	67	72	86	77	83	68	77
Karadağ	66	19	76	98	75	75	78	53	36	75	75
Libya	55,5	48	53	91	67	66	67	7	32	51	73
Lübnan	62,4	54	68	75	68	68	83	46	35	58	69
Malta	72,9	47	73	75	64	72	87	100	82	54	75
Mısır	69,5	54	68	80	70	90	98	38	64	52	81
Slovenya	76,8	81	69	86	73	99	100	38	60	80	82
Suriye	58,4	46	86	57	66	65	100	11	32	48	73
Tunus	60,7	29	64	74	66	66	82	60	49	55	62
Türkiye	67,9	55	82	99	75	70	100	19	33	71	75
Yunanistan	74,8	57	65	79	67	92	61	100	81	68	78

Karar Matrisinin Normalizasyonu ve Ağırlıklandırılması (rij)										
Ülkeler	**OHI 1**	**OHI 2**	**OHI 3**	**OHI 4**	**OHI 5**	**OHI 6**	**OHI 7**	**OHI 8**	**OHI 9**	**OHI1 0**
Kriter Yönleri	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**	**Mak.**
Arnavutluk	0,045	0,732	0,963	0,818	0,229	1	0,559	0,667	0,941	0,65
Cezayir	0,667	0,488	0,875	0,182	0,971	1	0,118	0,098	0	0,6
Fas	0	0,537	0,613	1	1	1	0,57	0,706	0,324	0,85
Fransa	0,636	0,561	0,813	0,545	0,971	0,462	0,559	0,961	0,588	0,95
GKRK	0,576	0,488	0,725	0,636	0,2	0,051	0,538	0,49	0,5	0,45
Hırvatistan	0,348	0,61	0,988	0,091	0,029	0,462	1	0,961	0,794	0,7
İspanya	0,864	0,39	0,75	0,636	0,943	0,41	0,602	0,941	0,676	0,8
İsrail	0,242	1	0	0,636	0,286	1	0,097	0,118	0,029	0,7
İtalya	0,682	0,341	0,713	0,273	0,2	0,641	0,753	1	0,647	0,75
Karadağ	0,061	0,561	0,988	1	0,286	0,436	0,495	0,078	0,853	0,65
Libya	0,5	0	0,9	0,273	0,029	0,154	0	0	0,147	0,55
Lübnan	0,591	0,366	0,7	0,364	0,086	0,564	0,419	0,059	0,353	0,35
Malta	0,485	0,488	0,7	0	0,2	0,667	1	0,98	0,235	0,65
Mısır	0,591	0,366	0,763	0,545	0,714	0,949	0,333	0,627	0,176	0,95
Slovenya	1	0,39	0,838	0,818	0,971	1	0,333	0,549	1	1
Suriye	0,47	0,805	0,475	0,182	0	1	0,043	0	0,059	0,55
Tunus	0,212	0,268	0,688	0,182	0,029	0,538	0,57	0,333	0,265	0
Türkiye	0,606	0,707	1	1	0,143	1	0,129	0,02	0,735	0,65
Yunanistan	0,636	0,293	0,75	0,273	0,771	0	1	0,961	0,647	0,8

Yöntemin üçüncü aşamasında eşitlik 4 ile ilişki katsayısı matrisi sağlanmıştır. Yöntemin devamında dördüncü aşamasında standart sapma değerleri eşitlik 5, C_j değerleri ise eşitlik 6 ile ölçülmüştür. En son aşamada ise bileşenlerin önemlilik dereceleri eşitlik 7 ile hesaplanmıştır. Buna göre; ilişki katsayısı matrisi, 1-p matrisi, C_j, standart sapma (σ), bileşenlerin önemlilik dereceleri (w_j) ile bileşenlerin önemlilik derecelerinin sıralaması Tablo 5'de gösterilmiştir.

Tablo 5. İlişki matrisi (p), 1-p, standart sapmalar, cj, önemlilik dereceleri ve sıralamalar.

İlişki Matrisinin Oluşturulması (p)										
Bileşenler	**OHI1**	**OHI2**	**OHI3**	**OHI4**	**OHI5**	**OHI6**	**OHI7**	**OHI8**	**OHI9**	**OHI10**
OHI1	1	-0,357	0,125	-0,21	0,349	-0,141	-0,063	0,178	0,101	0,315
OHI2	-0,357	1	-0,41	0,304	-0,1	0,557	-0,171	-0,15	0,005	0,138
OHI3	0,125	-0,413	1	0,108	0,04	-0,226	0,219	0,135	0,58	0,066
OHI4	-0,21	0,304	0,108	1	0,312	0,275	-0,262	-0,16	0,465	0,352
OHI5	0,349	-0,101	0,04	0,312	1	0,147	0,052	0,371	0,105	0,667
OHI6	-0,141	0,557	-0,23	0,275	0,147	1	-0,43	-0,24	-0,16	0,216
OHI7	-0,063	-0,171	0,219	-0,26	0,052	-0,43	1	0,834	0,431	0,102
OHI8	0,178	-0,152	0,135	-0,16	0,371	-0,245	0,834	1	0,397	0,473
OHI9	0,101	0,005	0,58	0,465	0,105	-0,159	0,431	0,397	1	0,322
OHI10	0,315	0,138	0,066	0,352	0,667	0,216	0,102	0,473	0,322	1
1-(p) Değerleri										
OHI1	0	1,357	0,875	1,21	0,651	1,141	1,063	0,822	0,899	0,685
OHI2	1,357	0	1,413	0,696	1,101	0,443	1,171	1,152	0,995	0,862
OHI3	0,875	1,413	0	0,892	0,96	1,226	0,781	0,865	0,42	0,934
OHI4	1,21	0,696	0,892	0	0,688	0,725	1,262	1,163	0,535	0,648
OHI5	0,651	1,101	0,96	0,688	0	0,853	0,948	0,629	0,895	0,333
OHI6	1,141	0,443	1,226	0,725	0,853	0	1,43	1,245	1,159	0,784
OHI7	1,063	1,171	0,781	1,262	0,948	1,43	0	0,166	0,569	0,898
OHI8	0,822	1,152	0,865	1,163	0,629	1,245	0,166	0	0,603	0,527
OHI9	0,899	0,995	0,42	0,535	0,895	1,159	0,569	0,603	0	0,678
OHI10	0,685	0,862	0,934	0,648	0,333	0,784	0,898	0,527	0,678	0
Standart Sapmalar, Cj ve wj Değerleri										
Standart Sapmalar	0,272	0,223	0,228	0,325	0,393	0,347	0,316	0,397	0,321	0,233
Cj	2,368	2,046	1,91	2,545	2,77	3,126	2,623	2,849	2,166	1,477
wj	**0,099**	**0,086**	**0,080**	**0,107**	**0,116**	**0,131**	**0,110**	**0,119**	**0,091**	**0,062**
Sıralama	**6**	**8**	**9**	**5**	**3**	**1**	**4**	**2**	**7**	**10**

Tablo 5'e göre bileşenlerin önemlilik dereceleri; OHI6 (w_{OHI6}=0,131), OHI8 (w_{OHI8}=0,119), OHI5 (w_{OHI5}=0,116), OHI7 (w_{OHI7}=0,110), OHI4 (w_{OHI4}=0,107), OHI1 (w_{OHI1}=0,099), OHI9 (w_{OHI9}=0,091), OHI2 (w_{OHI2}=0,086), OHI3 (w_{OHI6}=0,080) ve OHI10 (w_{OHI6}=0,062) olarak sıralanmıştır. Tablo 5 değerlendirildiğinde, OHI bileşenlerin önemlilik derecelerinin birbirinden farklı olduğu gözlenmiştir. Ayrıca OHI6 bileşeninin önemlilik derecesinin fazla olması kapsamında OHI6 bileşeninin diğer bileşenler arasında belirgin farklılıkları bulunmaktadır. Yine Tablo 5 incelendiğinde, OHI10 bileşeninin önemlilik derecesinin az olması kapsamında diğer bileşenler arasında belirgin farklılıklar mevcuttur.

MARCOS yönteminin ilk aşamasında tıpkı CRITIC yönteminde olduğu gibi karar matrisi oluşturulur. Söz konusu karar matrisi CRITIC yönteminde daha öncesinden Tablo 3'de belirtilmiştir. Yöntemin ikinci aşamasında eşitlik 10 ideal çözüm ve eşitlik 11 anti ideal çözüm değerleri belirlenerek genişletilmiş karar matrisi değerleri hesaplanmıştır. Söz konusu genişletilmiş karar matrisi değerleri Tablo 6'da belirtilmiştir.

Tablo 6. Genişletilmiş karar matrisi değerleri.

Bileşenler	OHI1	OHI2	OHI3	OHI4	OHI5	OHI6	OHI7	OHI8	OHI9	OHI10
İÇ	1	1	1	1	1	1	1	1	1	1
AİÇ	0,185	0,564	0,192	0,853	0,650	0,610	0,070	0,385	0,575	0,756

İÇ: İdeal Çözüm, **AİÇ:** Anti İdeal Çözüm

MARCOS yönteminin üçüncü aşamasında Tablo 6'da açıklanan genişletilmiş karar matrisi değerleri eşitlik 13 ve eşitlik 14 yardımıyla standartlaştırılmıştır. Bu bağlamda standartlaştırılmış karar matrisi Tablo 7'de açıklanmıştır.

Tablo 7. Standartlaştırılmış karar matrisi.

Ülkeler	OHI1	OHI2	OHI3	OHI4	OHI5	OHI6	OHI7	OHI8	OHI9	OHI10
Arnavutluk	0,222	0,883	0,97	0,973	0,73	1	0,59	0,795	0,975	0,915
Cezayir	0,728	0,777	0,899	0,88	0,99	1	0,18	0,446	0,575	0,902
Fas	0,185	0,798	0,687	1	1	1	0,6	0,819	0,713	0,963
Fransa	0,704	0,809	0,848	0,933	0,99	0,79	0,59	0,976	0,825	0,988
GKRK	0,654	0,777	0,778	0,947	0,72	0,63	0,57	0,687	0,788	0,866
Hırvatistan	0,469	0,83	0,99	0,867	0,66	0,79	1	0,976	0,913	0,927
İspanya	0,889	0,734	0,798	0,947	0,98	0,77	0,63	0,964	0,863	0,951
İsrail	0,383	1	0,192	0,947	0,75	1	0,16	0,458	0,588	0,927
İtalya	0,741	0,713	0,768	0,893	0,72	0,86	0,77	1	0,85	0,939
Karadağ	0,235	0,809	0,99	1	0,75	0,78	0,53	0,434	0,938	0,915
Libya	0,593	0,564	0,919	0,893	0,66	0,67	0,07	0,386	0,638	0,89
Lübnan	0,667	0,723	0,758	0,907	0,68	0,83	0,46	0,422	0,725	0,841
Malta	0,58	0,777	0,758	0,853	0,72	0,87	1	0,988	0,675	0,915
Mısır	0,667	0,723	0,808	0,933	0,9	0,98	0,38	0,771	0,65	0,988
Slovenya	1	0,734	0,869	0,973	0,99	1	0,38	0,723	1	1
Suriye	0,568	0,915	0,576	0,88	0,65	1	0,11	0,386	0,6	0,89
Tunus	0,358	0,681	0,747	0,88	0,66	0,82	0,6	0,59	0,688	0,756
Türkiye	0,679	0,872	1	1	0,7	1	0,19	0,398	0,888	0,915
Yunanistan	0,704	0,691	0,798	0,893	0,92	0,61	1	0,976	0,85	0,951
İÇ	1	1	1	1	1	1	1	1	1	1
AİÇ	0,185	0,564	0,192	0,853	0,65	0,61	0,07	0,386	0,575	0,756

Devamında 5'inci aşamada ülkelerin eşitlik 17 ile ağırlıklı toplam matrisinin elemanlarının toplamı (S_i), eşitlik 18 ile ideal çözüm fayda derecesi (K_i^+) ve eşitlik 19 ile anti ideal çözüm fayda derecesi (K_i^-) ölçülmüştür. Dolayısıyla ülkelere ait S_i, K_i^+ ve K_i^- değerleri Tablo 8'de sunulmuştur.

Tablo 8. Ülkelere ait S_i, K_i^+ ve K_i^- değerleri.

Ülkeler	**S_i**	**K_i^+**	**K_i^-**
Arnavutluk	2,013	0,805	1,664
Cezayir	1,844	0,738	1,524
Fas	1,941	0,777	1,604
Fransa	2,113	0,845	1,746
GKRK	1,854	0,742	1,532
Hırvatistan	2,105	0,842	1,739
İspanya	2,131	0,853	1,761
İsrail	1,601	0,64	1,323
İtalya	2,063	0,825	1,705
Karadağ	1,845	0,738	1,524
Libya	1,571	0,628	1,298
Lübnan	1,753	0,701	1,449
Malta	2,034	0,814	1,681
Mısır	1,95	0,78	1,611
Slovenya	2,167	0,867	1,791
Suriye	1,644	0,657	1,358
Tunus	1,695	0,678	1,401
Türkiye	1,91	0,764	1,578
Yunanistan	2,098	0,839	1,734
İÇ	2,500	1	2,066
AİÇ	1,210	0,484	1

Yöntemin 6'ıncı aşamasında ülkelerin ideal fayda fonksiyon değeri ($f(K_i^+)$) eşitlik 20, anti ideal fayda fonksiyon değeri ($f(K_i^-)$) ise eşitlik 21 ile hesaplanmıştır. Yöntemin son aşamasında ise eşitlik 22 ile ülkelerin deniz sağlığı koruma performans değerleri (ülkelerin uzlaşık çözümü=$f(K_i)$) belirlenmiştir. Buna göre tespit edilen değerler Tablo 9'da gösterilmiştir.

Tablo 9. Ülkelerin ($f(K_i^+)$), ($f(K_i^-)$) ve $f(K_i)$ değerleri.

Ülkeler	**$f(K_i^+)$**	**$f(K_i^-)$**	**$f(K_i)$**	**Sıralama**
Arnavutluk	0,674	0,326	0,695	8
Cezayir	0,674	0,326	0,637	14
Fas	0,674	0,326	0,671	10
Fransa	0,674	0,326	0,730	3
GKRK	0,674	0,326	0,640	12
Hırvatistan	0,674	0,326	0,727	4
İspanya	0,674	0,326	0,736	2
İsrail	0,674	0,326	0,553	18
İtalya	0,674	0,326	0,713	6
Karadağ	0,674	0,326	0,637	13
Libya	0,674	0,326	0,543	19
Lübnan	0,674	0,326	0,606	15
Malta	0,674	0,326	0,703	7
Mısır	0,674	0,326	0,674	9
Slovenya	0,674	0,326	0,749	1
Suriye	0,674	0,326	0,568	17
Tunus	0,674	0,326	0,586	16
Türkiye	0,674	0,326	0,660	11
Yunanistan	0,674	0,326	0,725	5
Ortalama			0,661	------

Tablo 9 incelendiğinde, ülkelerin deniz sağlığı performansları en fazla olan ilk üç ülkenin sırasıyla Slovenya, İspanya ve Fransa, en az olan ilk üç ülkenin ise Suriye, İsrail ve Libya olduğu tespit edilmiştir. Devamında ülkelerin ortalama deniz sağlığı koruma performansı 0,661 olarak hesaplanmıştır. Söz konusu ortalama değerin üstünde olan ülkelerin Slovenya, İspanya, Fransa, Hırvatistan, Yunanistan, İtalya, Malta, Arnavutluk, Mısır ve Fas, ortalamanın altında olan ülkelerin ise Türkiye, GKRK, Karadağ, Cezayir, Lübnan, Tunus, Suriye, İsrail ve Libya olduğu tespit edilmiştir.

Tablo 9'a göre; Slovenya'nın, İspanya'nın, Fransa'nın, Hırvatistan'ın, Yunanistan'ın, İtalya'nın ve Malta'nın deniz sağlığı performans değeri ortalama performans değerinden sırasıyla %11,8, %10,3, %9,51, %9,16, %8,87, %7,32 ve %5,98 değerinde fazla olması sebebiyle söz konusu ülkelerin diğer ülkeler arasında deniz sağlığı performansı açısından belirgin farklılıkları bulunmaktadır. Aynı şekilde; Libya'nın, İsrail'in, Suriye'nin, Tunus'un ve Lübnan'ın deniz sağlığı performans değeri ortalama performans değerinden sırasıyla %9,8 %21,8, %19,5, %16,3 ve %12,8 değerinde fazla olması kapsamında söz konusu ülkelerin diğer ülkeler arasında deniz sağlığı performansı açısından belirgin farklılıkları mevcuttur. Yine Tablo 9 değerlendirildiğinde, Türkiye'nin deniz sağlığı performans değeri ortalama performans değerine çok yakın olduğu gözlenmiş olup, Türkiye'nin deniz sağlığı performans değeri ortalama değerin sadece %0,11 kadar az değerdedir. Bunun dışında, ortalama değerin üstünde olması kapsamında Mısır ve Fas'ın okyanus sağlığı performans değerleri ortalama değerden sırasıyla %1,94 ve %1,49 fazla, ortalama değerin altında olması kapsamında ise Cezayir'in, GKRK'nin ve Karadağ'ın okyanus sağlığı performans değerleri ortalama değerden sırasıyla %3,66, %3,15 ve %3,69 oranında az olduğu gözlenmiştir. Sonuçlara göre, GKRK hariç deniz sağlığı performansın fazla olması çerçevesinde ilk 7 ülkenin sadece Avrupa Birliği ülkelerinin olması dikkat çekicidir. Dolayısıyla Avrupa Birliği Antlaşması ve Avrupa Birliği'nin İşleyişi Hakkında Antlaşma'da deniz kaynaklarının korunması ile ilgili hükümlerin deniz sağlığının korunmasında genel olarak etkin olduğu değerlendirilebilir.

Araştırmada yöntem açısından ülkelerin deniz sağlığı performans değerleri ayrıca CRITIC tabanlı ARAS, EDAS, COPRAS ve TOPSIS ÇKKV yöntemleri ile ölçülmüş ve ölçülen değerler ile ülkelerin OHI değerleri arasındaki ilişki nicelikleri tespit edilmiştir. Söz konusu değerler Tablo 10'da belirtilmiştir.

Tablo 10. Yöntemler arasındaki Pearson ilişki katsayısı değerleri

Yöntemler	OHI	MARCOS	ARAS	EDAS	COPRAS	TOPSIS
OHI	1					
MARCOS	0,999**	1				
ARAS	0,985**	0,985**	1			
EDAS	0,999**	0,984**	0,999**	1		
COPRAS	0,999**	0,984**	0,999**	0,999**	1	
TOPSIS	0,979**	0,945**	0,978**	0,977**	0,979**	1

$^{**}p<0,01$

Tablo 10'a göre, OHI değerleri ile CRITIC tabanlı MARCOS ile diğer CRITIC tabanlı ÇKKV yöntemleri arasındaki ilişkilerin hepsinin anlamlı (**p<0,01), pozitif yönlü ve çok yüksek seviyede olduğu tespit edilmiştir. Bu sonuca göre, OHI'nın CRITIC tabanlı MARCOS ve diğer CRITIC tabanlı ÇKKV yöntemleri ile açıklanabileceği değerlendirilmiştir.

5. SONUÇ

Akdeniz'de özellikle ülkeler arasında enerji, ekonomi, çevre ve ticaret alanında karşılıklı ilişkilerin yoğunlaşmasıyla Akdeniz'in deniz kirliliği sorunu önemli bir seviyeye ulaşmıştır. Ayrıca Akdeniz ülkelerinin deniz sağlığı konusundaki performanslarının farkında olması ve bu farkındalık ile deniz sağlığı konusundaki stratejilerinin, yöntemlerinin ve faaliyetlerinin Akdeniz'de deniz kirliliğinin önlemesinde ve deniz sağlığının korunmasında önemli olduğu değerlendirilmektedir. Bu kapsamda araştırmada, Akdeniz'e kıyısı olan 19 ülkenin 2021 yılı için en son ve güncel olan OHI bileşenlerine ait değerler üzerinden söz konusu ülkelerin deniz sağlığı performansları CRITIC tabanlı MARCOS yöntemi ile ölçülmüştür.

Bulgulara çerçevesinde CRITIC yöntemi kapsamında ülkelere göre en önemli OHI bileşenlerinin önemlilik dereceleri OHI6 (Ekonomi ve Geçim Kaynakları), OHI8 (Hassaslık), OHI5 (Geçim Kaynakları ve Ekonomiler), OHI7 (Turizm ve Eğlence), OHI4 (Karbon Kapasitesi), OHI1 (Gıda Tedariki), OHI9 (Temiz Su), OHI2 (Sanatsal Balıkçılık Fırsatları), OHI3 (Doğal Ürünler) ve OHI10 (Biyolojik Çeşitlilik) olarak sıralanmıştır. Başka bir bulguya göre, CRITIC tabanlı MARCOS yöntemi kullanılarak deniz sağlığı performansı açısından en fazla performansa sahip ilk üç ülkenin Slovenya, İspanya ve Fransa, son üç ülkenin ise Libya, İsrail ve Suriye olduğu tespit edilmiştir. Ülkelerin ayrıca ortalama deniz sağlığı performans ölçülmüş ve ortalamadan yüksek olan ülkelerin Slovenya, İspanya, Fransa, Hırvatistan, Yunanistan, İtalya, Malta, Arnavutluk Mısır ve Fas olduğu gözlenmiştir. Türkiye'nin ise deniz sağlığı performans değerinin ortalama değere çok yakın olduğu tespit edilmiştir. Bunun yanında, araştırmada GKRK dışında diğer Avrupa Birliği grubunda yer alan ülkelerin deniz sağlığı performansı açısından ilk 7 sırada olduğu belirlenmiştir. Bu sonuca istinaden, Avrupa Birliği İşleyişi Hakkında Antlaşma'da deniz kaynaklarının korunması ile ilgili hükümlerin deniz sağlığının korunmasına yönelik olarak genel anlamda belirleyici olduğu düşünülmüştür. Devamında araştırmada ülkelerin deniz sağlığı performans değerleri CRITIC tabanlı ARAS, EDAS, COPRAS ve TOPSIS yöntemleri ile ölçülerek ülkelerin OHI değerleri ile CRITIC tabanlı MARCOS ve diğer ÇKKV yöntemleri kapsamında tespit edilen değerler arasında ilişki değeri hesaplanmıştır. Bu sonuca göre, OHI ile CRITIC tabanlı MARCOS ve diğer ÇKKV değerleri arasındaki ilişki değerleri tümünün anlamlı, pozitif yönlü ve çok yüksek olduğundan dolayı OHI'nın başta CRITIC tabanlı MARCOS yöntemi olmak üzere diğer CRITIC tabanlı ÇKKV yöntemleri ile açıklanabileceği sonucuna ulaşılmıştır.

Literatür değerlendirildiğinde, Karadirek vd., (2019)'nin 1970-2016 yıl aralığında Akdeniz'de deniz kirliliği konusunda bibliyometrik araştırmasına istinaden en fazla adı geçen ülkelerin Fransa, İspanya, İtalya, Yunanistan ve Türkiye olduğunu belirtmişlerdir. Fakat bu araştırmada Türkiye hariç söz konusu ülkelerin 2021 deniz sağlığı performansı açısından 19 ülke içinde ilk 6 sırada olduğu ve ülkelerin deniz sağlığı performans değerlerinin ortalama deniz sağlığı performansından yüksek değerde olduğu tespit edilmiştir. Ayrıca araştırmada, Türkiye'nin ortalama deniz sağlığı performansının ortalama seviyeye en yakın olan ülke olduğu belirlenmiştir. Bu kapsamda söz konusu araştırmalar bütünsel olarak değerlendirildiğinde, 2021 yılı için 1970-2016 yıl aralığına göre Fransa, İspanya, İtalya, Yunanistan ve Türkiye'nin deniz sağlığı konusunda belirli bir performans sergilediği düşünülmüştür.

Literatür incelendiğinde, ülkelerin deniz sağlığı performanslarını herhangi bir ÇKKV yöntemi ile ölçen bir araştırmaya rastlanılmamış olması ve buna istinaden araştırmanın özgün bir nitelik taşıması açısından araştırmanın literatüre katkı sağladığı ve zenginleştirdiği düşünülmüştür. Ayrıca araştırmanın konusu açısından Akdeniz ülkelerinin deniz sağlığı konusunda yapacağı faaliyetler sadece bölgesel anlamda değil, tüm Akdeniz'in deniz sağlığını ilgilendireceği için araştırmada söz konusu Akdeniz ülkelerinin deniz sağlığı performanslarının analizinin sağlanması açısından bu araştırmanın literatüre olumlu etkisinin olduğu değerlendirilmiştir. Araştırmanın kısıtı açısından ise bu araştırmada OHI boyutları kapsamında Akdeniz ülkelerinin deniz performansı tespitinde sadece 2021 yılı için OHI raporunda yer alan verilerinden yararlanılmıştır. Gelecek çalışmalar için en güncel OHI raporunun yanında, ülkelerin deniz sağlığı performanslarını açıklayan tüm OHI raporlarına ait ilgili değerler dikkate alınarak OHI boyutları arasındaki ilişkilerin belirlenmesine yönelik çalışmaların yapılmasının daha uygun olacağı değerlendirilmiştir.

Akdeniz'de herhangi bir ülkenin deniz sağlığı performansı açısından yapmış olduğu faaliyetler tüm Akdeniz'e kıyısı olan ülkeleri etkileyebilmektedir. Buna göre öneriler kapsamında öncelikli olarak Akdeniz'in daha sağlıklı, elverişli, faydalanabilir olabilmesi için ortalama deniz sağlığı performans değerinden az performansa sahip olan Türkiye, GKRK, Karadağ, Cezayir, Lübnan, Tunus, Suriye, İsrail ve Libya'nın deniz sağlığı performanslarını artırıcı stratejiler ve faaliyetler gerçekleştirmelidir. Söz konusu ülkelerin deniz sağlığı konusundaki olumlu politikaları ve faaliyetleri ile Akdeniz'den ekonomik anlamda daha etkin, etkili ve verimli katma değer sağlanabilmesi kapsamında biyolojik çeşitliliğinin niteliğinin ve niceliğinin sürdürülebilirliği sağlanıp, deniz ürünlerinden daha anlamlı olarak istifade edilebilecektir. Bunların dışında, deniz sağlığının sağlanmasıyla Akdeniz bölgesine kıyısı olan ülkelerin rekabet faaliyetleri daha kaliteli olabilecek ve ülkeler kendi ekonomilerine ve küresel ekonomiye olan katkılarını daha fazla sağlayabileceklerdir. Dolayısıyla gelişen rekabet ile deniz ürünleri ve turizm konusunda hizmet kalitesi artabilecek ve buna bağlı olarak deniz ürünleri piyasasının ve turistlerin dünya çapında ilgi odağı Akdeniz'e kıyısı olan ülkeler olabilecektir. Ayrıca OHI bileşenlerinin birbirlerini tamamlaması açısından ülkeler söz konusu bileşenlerin birbirleri ilgilendirecek, ilişkilendirecek ve sağlayacak faaliyetler oluşturarak ülkeler deniz sağlığı performanslarını artırabilir. Bunların dışında, Akdeniz ülkeleri kendilerine özgü deniz kültürü doğrultusunda deniz sağlığı politikalarını geliştirerek deniz sağlığı konusunda sürdürülebilirlik sağlayabilecektir. Yöntem kapsamında ise gelecek çalışmalarda ülkelerin deniz sağlığı performansları farklı ÇKKV yöntemleri ile ölçülerek yöntemler sonucu elde edilen sonuçlar karşılaştırılabilir ve tartışılabilir. Ek olarak dünya üzerinde farklı bölgelerde mevcut ülkelerin deniz sağlığı performansları hesaplanabilir ve hesaplanan performans değerleri bölgeler bazında kıyaslanabilir. Aynı zamanda OHI kapsamında ülkelerin deniz sağlığı performanslarını ölçmesinin daha gerçekçi olması açısından ülkelere standart bileşenler yerine her ülkeye özgü OHI bileşenleri tespit edilebilir ya da bileşen sayısı artırılabilir.

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Research Article **Araştırma Makalesi**

Biofilm Formation and Prevention of Bacteria Isolated from Fish and Fish Stalls

Balık ve Balık Tezgahlarından İzole Edilen Bakterilerin Biyofilm Oluşumu ve Önlenmesi

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Abstract: Biofilms are structures formed by bacteria in the presence of convenient media. Bacteria protect themselves from chemicals such as ozone, heat, light, and chlorine with a biofilm structure. Fish is an important food item and it is an environment where bacteria can easily reproduce. Therefore, it is also appropriate for biofilm formation. Biofilm formation in fish and fish stalls is a threat to human health. This study aims to identify bacteria isolated from fish and fish stalls and to determine their biofilm-forming ability. In addition, it investigates the antibacterial effect of rock salt, lemon juice, and vinegar against biofilm-forming bacteria by the disk diffusion method. Forty-seven bacteria were isolated and identified from fish and fish stalls. The biofilm-forming abilities of the identified bacteria were determined by qualitative and quantitative analyzes. According to the analysis results, it was determined that 36 bacterial isolates formed a biofilm. Vinegar and lemon juice, which are natural products, have been shown to strongly inhibit the growth of biofilm-forming bacteria. However, no significant effect of rock salt was found against biofilm-forming bacteria.

Keywords
- Biofilm
- Natural products
- Antibacterial effect
- Fish

Özet: Biyofilm, bakterilerin uygun besiortamları varlığında oluşturdukları yapılardır. Biyofilm yapısı ile bakteriler kendilerini ozon, ısı, ışık, klor gibi kimyasal maddelerden korumaktadır. Balık önemli bir gıda maddesidir ve bakterilerin kolayca üreyebildiği bir ortamdır. Bu nedenle biyofilm oluşumu için de uygundur. Balık ve balık tezgahlarında biyofilm oluşumu insan sağlığını tehdit eden bir durumdur. Bu çalışma balık ve balık tezgahlarından izole edilen bakterileri tanılamayı ve onların biyofilm oluşturma kabiliyetlerini saptamayı amaçlar. Ayrıca kaya tuzu, limon suyu, sirkenin biyofilm oluşturan bakterilere karşı disk difüzyon metot ile antibakteriyal etkisini araştırır. Balık ve balık tezgahlarından 47 bakteri izole edilmiş ve tanılanmıştır. Tanılanan bakterilerin biyofilm oluşturma kabiliyetleri kalitatif ve kantitatif analizler ile saptanmıştır. Analiz sonuçlarına göre, 36 bakteri izolatının biyofilm oluşturduğu saptanmıştır. Doğal ürünler olan sirke ve limon suyunun, biyofilm oluşturan bakterilerin büyümesini güçlü bir şekilde engellediği görülmüştür. Ancak kaya tuzunun biyofilm oluşturan bakterilere karşı önemli bir etkisi bulunmamıştır.

Anahtar kelimeler
- Biyofilm
- Doğal ürünler
- Antibakteriyal etki
- Balık

1. INTRODUCTION

People should pay attention to nutritional elements to lead a healthy and quality life. However, sometimes undesirable situations may occur in foods due to physical, chemical, and biological reasons. Microbial developments that may occur on food or in the environment where food is present are biological factors. Microbial developments in foods appear as foodborne infections or food poisoning. Therefore, food safety has become an important issue in terms of public health all over the world at present. Microbial growth, biotoxins, mycotoxins, and chemical contaminants that can be seen in foods can become threatening to human health. For this reason, the emergence of foodborne diseases and their turn into epidemics affect society in terms of health, economic and social aspects (Erkmen, 2010).

The factor that promotes microbial spoilage in fish is microorganisms transmitted from the environment while the fish is alive or during processing. Environmental temperature accelerates the growth of microorganisms (Koutsomanis and Nychas, 2000). Bacteria multiply rapidly on the surface of the fish or on the stalls where the fish are stored, forming a biofilm as an extracellular structure. This structure formed by the clustering of microorganisms is a biofilm. Biofilm occurs when food hygiene is not provided and threatens human health. In cases where hygiene is poor, biofilm formation may occur on the surface of fish and in the storage stalls after the fish is caught (Nurcan and Kubilay, 2016). Many chemical preservatives are used on foods to prevent biofilm formation. However, they threaten human health since most of these chemical substances have a carcinogenic effect. For this reason, the use of natural products is preferred to prevent the formation of biofilms in foods (Boğa and Binokay, 2010).

In this study, bacteria that can be found on the surface of fish and fish stalls were identified and those that formed biofilms were determined. In addition, the effects of natural products rock salt, lemon salt, vinegar, and their mixture on biofilm formation were compared.

2. MATERIALS AND METHODS

2.1. Materials

Samples were taken from five different fish species as *Sparus aurata*, *Dicentrarchus labrax*, *Mullus barbatus*, *Sardina pilchardus*, *Engraulis encrasicolus,* and fish stalls.

2.2. Collection of samples

Samples were collected from Kuşadası, Güzelçamlı, Söke, and İncirliova districts of Aydın province in December 2016. Samples taken from fish and fish stall surfaces were taken into 0.85% physiological saline water (FTS) using a sterile swap and stored. The collected samples were brought to the Microbiology Laboratory of the Biology Department of Aydın Adnan Menderes University and stored at +4^{o}C.

2.3. Isolation and identification of bacterial strains

For enrichment, 1 mL of the samples in the FTS tubes was inoculated into Tryptic Soy Broth (TSB) medium tubes and incubated at 37^{o}C for 24 hours. At the end of the incubation, a serial dilution was made from 10^{-1} to 10^{-6}. 0.1 µL of 10^{-4}, 10^{-5}, 10^{-6} dilutions were taken and inoculated into Tryptic Soy Agar (TSA) media. All Petri dishes were incubated at 37^{o}C for 24 hours. Colony selection was made at the end of the incubation and the purification process was applied. Pure cultures were taken in 20% Skim Milk and stored at -20^{o}C (Törün et al., 2017; Tekin and Çoban, 2021; Çoban and Barışık, 2021).

For molecular identification, total genomic DNA was isolated from the broth culture of bacterial strains (De Boer and Ward, 1995). Amplification of 16S rDNA of bacterial strains was performed by using universal primers (Lane, 1991). PCR reactions were performed at 94ºC 3 min, 94ºC 40 sec, 60ºC 40 sec, 72ºC 40 sec with 35 cycles, respectively. Amplification products were sent GATC BioTech, Germany for sequence analysis. The phylogenetic tree was created using the Maximum Likelihood method (Tamura and Nei, 1993). The sequence samples were compared using BLASTn software in GENBank. ClustalW program (MEGA 7.0 software) was used for sequences (Kumar et al., 2016).

2.4. Analysis of biofilm-forming

The biofilm formation ability of bacterial isolates was been examined qualitatively and quantitatively. Qualitative biofilm formation was investigated on congo red agar media (Freeman et al., 1989). The bacteria strains have inoculated the plates and Petri dishes were incubated at 37^{o}C for 24 hours. As a result of incubation, strains that formed dry crystallized rough black colonies were evaluated as biofilm positive, while strains that formed red or pink smooth colonies were evaluated as biofilm negative (Melo et al., 2013; Shrestha et al., 2018).

Quantitative biofilm formation was examined in the Elisa Plate (Christensen et al., 1982). Bacterial strains were inoculated in TSB medium and incubated overnight at 37^{o}C. After, 150 µL of the activated cultures were added to the individual wells of 96-well Elisa Plate and incubated at 37^{o}C for 3 days. Later, samples from the Elisa Plate were poured and washed 3 times with sterile dH_2O and dried. And then, 150 µL of 0.1% crystal violet was put in the wells and left for 45 minutes, washed, and air-dried again. Next, 200 µL ethanol: acetic acid (v/v) was added to the wells and waited for 10 minutes. From here, 100 µL of it was taken into a new Elisa Plate and measured in a spectrophotometer at OD 570 nm (Mathur et al., 2006; Melo et al., 2013).

TSB medium was used as negative control and *Staphylococcus aureus* ATCC 25923 was used as a positive control. Strains with optical density values ≥ 0.240 were considered strong adhesion, strains with optical density values 0.120-0.240 were considered moderate adhesion, and strains ≤ 0.120 were considered weak/negative (Mathur et al., 2006; Demir and İnanç, 2015).

2.5. Preparation of natural substances

Rock salt, grape vinegar, and lemon juice used as natural products were purchased commercially from the market. Rock salt (10 mg/mL) was prepared with sterile water. The total acidity rate of the grape vinegar (pH 3.52) used is 4g/100 mL. The titration acidity value of the lemon juice (pH 4.5) used is 45g/L. Mixtures of rock salt, vinegar, and lemon juice 1:1:1 (v/v) were prepared and its effect was tested against biofilm-forming bacteria.

2.6. The effect of natural substances against biofilm-forming bacteria

Biofilm-forming bacteria were determined by qualitative and quantitative analysis. The effects of natural substances against biofilm-forming bacteria were determined using the agar well diffusion method (CLSI, 2015; EUCAST, 2019). The tested microorganisms were incubated at 30-37^{o}C for 24 h and then, cell number was regulated as 1×10^8 cells/mL according to 0.5 McFarland standard tube (Çoban et al., 2021; Şahin et al., 2021; Koseoglu et al., 2022). Mueller Hinton Agar media (MHA) (20 mL) were prepared in Petri dishes for analyzing the effect of natural substances. The microorganism cultures (100 µL) were inoculated on plates. Wells with a diameter of 6 mm were created on the medium with a sterile stick. After, the wells were filled with 50 µL of rock salt, vinegar, lemon juice, and their mixtures. Distilled water was used as a positive control. Next, all Petri dishes were incubated at 30-37°C for 24 h (Çoban et al., 2021; Şahin et al., 2021; Koseoglu et al., 2022).

2.7. Statistical

All the experiments were carried out in triplicate. The results were expressed as the mean value of three independent replicates ± the standard deviation using SPSS v22 program.

3. RESULT AND DISCUSSION

3.1. Isolated and identified strains

In total, 47 bacterial species were identified in this study. The DNA sequences were compared using BLASTn software in GENBank and molecular identification was performed. The bacteria species obtained are given in Table 1.

Table 1. Bacteria species isolated and identified from samples

Sample Location	Species	Accession No	% Similarity
Skin	*Enterobacter hormaechei strain FQP44*	MF144523.1	97
	Staphylococcus aureus strain K6	KX821633.1	97
	Staphylococcus aureus strain SSW20	KU922502.1	97
	Citrobacter freundii strain B25	FJ494899.1	99
	Staphylococcus aureus strain RCB1010	KT261222.1	99
	Proteus penneri strain wf-1	KT029130.1	97
	Staphylococcus aureus strain K2	KX821629.1	96
	Enterobacter cloacae strain 39	KX395979.1	98
	Staphylococcus aureus strain 5 BWI	KX456107.1	96
	Staphylococcus epidermidis strain P8	KF705248.1	98
	Lactococcus garvieae strain IMAU50143	FJ749537.1	96
	Staphylococcus aureus strain RCB1010	KT261222.1	98
	Lactococcus garvieae strain ZSJ5	KU324937.1	98
	Staphylococcus pasteuri strain AIMST.Pbst4	KM087104.1	98
	Enterococcus gallinarum	AB904770.1	95
	Staphylococcus pasteuri strain HN-35	KT003275.1	95
	Staphylococcus aureus strain SSW20	KU922502.1	99
Gill	*Serratia marcescens strain AR1*	KX343948.1	97
	Lactococcus lactis	KT633921.1	98
	Staphylococcus warneri strain STM81	KY393084.1	97
	Proteus vulgaris strain M20	KT792741.1	97
	Raoultella ornithinolytica strain JSM 05182054	KY352821.1	96
	Moellerella wisconsensis strain X	KP159747.1	98
	Proteus penneri strain ALK624	KC456589.1	96
	Staphylococcus pasteuri strain AIMST.Pbst4	KM087104.1	98
	Carnobacterium divergens strain LHICA_53_4	FJ656716.1	98
	Leuconostoc mesenteroides strain HL2	KY233187.1	95
	Proteus penneri strain NSPPN01	KT361197.1	95
	Proteus mirabilis strain T7	KJ626258.1	96
	Staphylococcus aureus strain K8	KX821635.1	99
Fish Stalls	*Citrobacter youngae strain NSKP2*	KY992522.1	95
	Staphylococcus aureus strain HN-5	KT003251.1	96
	Lactococcus garvieae strain ZSJ5	KU324937.1	98
	Leuconostoc mesenteroides strain	KF697659.1	95
	Leuconostoc mesenteroides strain 30-1	KJ477402.1	98
	Leuconostoc mesenteroides MFL24	LT853601.1	98
	Enterococcus casseliflavus	LC122272.1	96
	Enterococcus faecalis strain H50	KJ626240.1	98
	Proteus vulgaris strain BPGM7	KX156180.1	98
	Staphylococcus aureus strain K2	KX821629.1	98
	Lactococcus garvieae strain CMGB-L23	MF348235.1	98
	Staphylococcus aureus strain K5	KX821632.1	99
Collecting Plastic Pipe	*Staphylococcus capitis strain STM79*	KY393082.1	99
Boat Metal Surface	*Leuconostoc mesenteroides strain TUST005*	KC456619.1	95

Phylogenetic analysis was carried out using MEGA 6 software. Phylogenetic tree was showed in Figure 1.

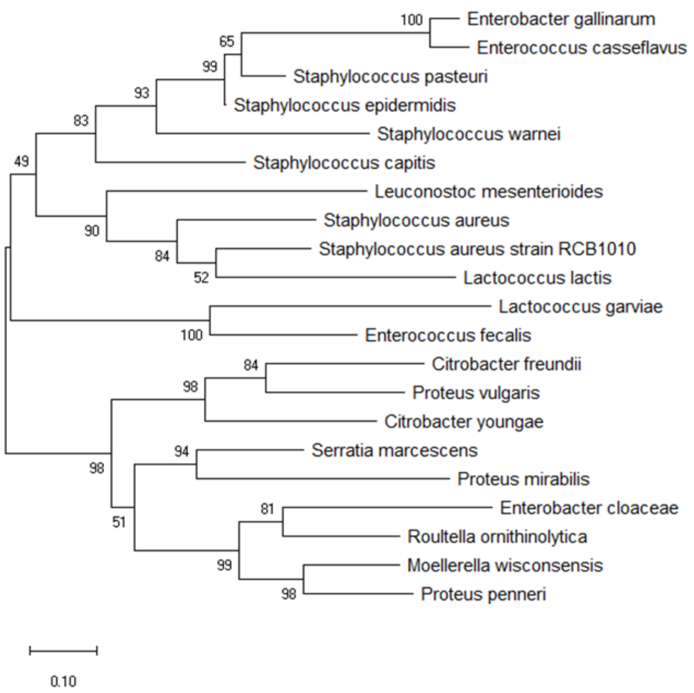

Figure 1. Phylogentic tree obtained by maximum likelihood method

3.2. Analysis of biofilm-forming

3.2.1. Qualitative determination of biofilm formation

Qualitative biofilm formation was obtained using congo red agar media. While black colony-forming bacteria are considered positive for biofilm, red-reddish colonies are considered biofilm negative on congo red agar medium (Figure 2a, b).

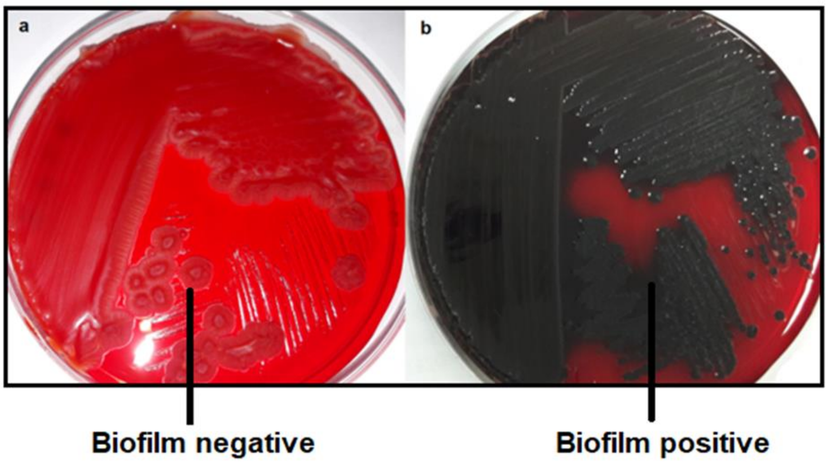

Figure 2.a. Biofilm negative in qualitative determination (*Bacillus vallismortis*) b. Biofilm positive in qualitative determination (*Staphylococcus aureus*)

3.2.2. Quantitative determination of biofilm formation

Spectrophotometer was used for the quantitative determination of biofilm formation. The results obtained in the microplate method used for the quantitative determination of biofilm formation are given in Table 2.

Table 2. Biofilm-forming bacterial species and their adherence values

	Optical Density Value			
Species	**Sample**	**Negative Control**	**Positive Control**	**Adherence**
L. mesenteroides strain MFL24	0,312	0,065	0,086	Strong
L. mesenteroides	0,459	0,065	0,086	Strong
P. penneri strain ALK624	0,399	0,065	0,086	Strong
L. garvieae strain ZSJ5	0,465	0,065	0,086	Strong
L. mesenteroides strain 30-1	0,459	0,065	0,086	Strong
S. capitis strain STM79	0,483	0,065	0,086	Strong
S. epidermidis strain P8	0,640	0,065	0,086	Strong
P. penneri strain NSPPN01	0,391	0,065	0,086	Strong
L.lactis	0,434	0,065	0,086	Strong
S. marcescens strain AR1	0,310	0,065	0,086	Strong
E. cloacae strain 39	0,123	0,065	0,086	Moderate
L. mesenteroides strain TUST005	0,123	0,065	0,086	Moderate
R. ornithinolytica strain JSM 05182054	0,160	0,065	0,086	Moderate
P.penneri strain wf-1	0,163	0,065	0,086	Moderate
S. aureus strain 5 BWI	0,225	0,065	0,086	Moderate
S. aureus strain K8	0,165	0,065	0,086	Moderate
S. pasteuri strain AIMST.Pbst4	0,175	0,065	0,086	Moderate
S. aureus strain K6	0,277	0,065	0,086	Moderate
P. mirabilis strain T7	0,286	0,065	0,086	Moderate
S. pasteuri strain HN-35	0,122	0,065	0,086	Moderate
C. youngae strain NSKP2	0,194	0,065	0,086	Moderate
P. vulgaris strain BPGM7	0,141	0,065	0,086	Moderate
S. aureus strain HN-5	0,135	0,065	0,086	Moderate
E. gallinarum	0,102	0,065	0,086	Weak
S. aureus strain SSW20	0,114	0,065	0,086	Weak
S. aureus strain K2	0,116	0,065	0,086	Weak
C. freundii strain B25	0,094	0,065	0,086	Weak
S. aureus strain RCB1010	0,054	0,065	0,086	Weak
S. warneri strain STM81	0,086	0,065	0,086	Weak
P. vulgaris strain M20	0,095	0,065	0,086	Weak
S. aureus strain K5	0,058	0,065	0,086	Weak
M. wisconsensis strain X	0,090	0,065	0,086	Weak
L. garvieae strain CMGB-L23	0,105	0,065	0,086	Weak
C. divergens strain LHICA_53_4	0,114	0,065	0,086	Weak
E. faecalis strain H50	0,081	0,065	0,086	Weak
E. casseliflavus	0,085	0,065	0,086	Weak

(Negative Control: Medium, Positive Control: *Staphylococcus aureus* ATCC 25923)
(Strong: ≥0.240, Moderate: 0.120-0.240, Week: ≤0.120)

3.3. Antibacterial activity of natural substances

Antibacterial activities of natural substances (rock salt, vinegar, lemon juice, and their mixtures) against biofilm-forming bacteria species were tested according to the Agar-well diffusion method (Table 3). There was no statistically significant difference in the rate of inhibition zones.

Table 3. The effect of natural substances (rock salt, vinegar, lemon juice and their mixtures) against biofilm forming bacteria species

Bacteria Species	**Inhibition zones (mm)**				
	1	**2**	**3**	**4**	**5**
L. lactis	12.33±0.57	11.00±1.00	_	12.33±0.5	_
L. mesenteroides	15.66±2.5	17.00±2.64	_	10.00±0.0	_
M. wisconsensis strain X	22.00±1.00	22.33±1.52	_	18.00±3.0	_
L. garvieae strain IMAU50143	17.66±0.57	13.33±1.15	10.00±1.0	10.33±1.5	_
P. mirabilis strain T7	_	_	_	_	_
S. aureus strain RCB1010	14.33±0.57	13.66±1.15	_	_	_
C. freundii strain B25	20.00±1.73	20.33±0.57	_	20.33±0.5	_
R. ornithinolytica strain JSM 05182054	11.33±1.52	15.33±2.51	_	14.33±0.5	_
S. aureus strain K2	12.66±0.57	15.33±2.3	9.33±0.57	17.66±2.5	_
P. penneri strain wf-1	15.33±2.51	21.33±0.57	_	18.66±2.0	_
L. mesenteroides strain 30-1	13.66±1.15	20.33±1.52	_	15.33±2.3	_
L. mesenteroides strain TUST005	13.66±1.15	18.00±2.64	_	15.33±2.5	_
P. penneri strain ALK624	_	_	_	_	_
S. pasteuri strain AIMST.Pbst4	18.00±1.73	20.00±2.64	_	18.66±1.1	_
C. divergens strain LHICA_53_4	17.66±0.57	14.33±1.15	_	_	_
E. cloacae strain 39	14.33±1.15	20.33±0.57	_	14.33±0.5	_
S. aureus strain HN-5	14.33±0.57	10.33±1.52	_	_	_
S. aureus strain K6	12.00±1.00	20.33±0.57	_	15.66±2.5	_
L. mesenteroides strain HL2	15.66±0.57	17.33±2.51	_	10.00±0.0	_
L. mesenteroides strain MFL2	_	_	_	_	_
P. penneri strain NSPPN01	18.33±1.15	21.66±2.08	_	16.00±1.7	_
E. casseliflavus	24.00±1.00	14.33±1.15	_	13.33±0.5	_
C. youngae strain NSKP2	_	20.00±1.00	_	15.00±2.0	_
E. gallinarum	21.66±2.08	12.33±0.57	_	_	_
S. pasteuri strain HN-35	13.33±0.57	13.33±0.57	_	10.33±0.5	_
L. garvieae strain ZSJ5	15.66±0.57	_	_	_	_
S. epidermidis strain P8	18.33±0.57	15.00±2.64	_	10.00±0.0	_
E. faecalis strain H50	14.33±0.57	18.33±1.15	_	13.33±0.5	_
S. capitis strain STM79	10.00±0.0	10.33±1.52	_	_	_
S. aureus strain SSW20	13.66±1.15	12.33±1.15	_	13.33±0.5	_
S. aureus strain 5 BWI	12.33±2.51	20.00±1.73	_	14.33±1.1	_
S. warneri strain STM81	10.66±1.15	15.66±0.57	_	_	_
P. vulgaris strain BPGM7	15.33±2.51	20.66±2.08	_	15.66±0.5	_
P. vulgaris strain M20	15.66±0.57	20.33±0.57	_	15.66±2.5	_
S. marcescens strain AR1	11.33±0.57	17.00±1.73	_	7.33±0.00	_
L. garvieae strain CMGB-L23	15.66±0.57	11.00±1.00	_	_	_
S. aureus strain SSW20	13.66±1.15	12.33±1.15	_	13.33±0.5	_
S. aureus strain K5	13.33±0.57	20.00±1.00	_	13.33±0.5	_

1: Lemon juice, 2: Vinegar, 3: Rock salt, 4: Mixture, 5: Distilled water (The results were expressed as the mean value of three independent replicates ± the standard deviation)

4. DISCUSSION

Diseases caused by foodborne pathogens are important for public health. Biofilm formation is of great importance in the occurrence of these diseases. The formation of biofilm by microorganisms depends on the environment and the bacteria itself. Biofilms form on moist surfaces such as foods and food processing equipment. A biofilm is made up of many types of bacteria. The effect of temperature and pH is also important in the adhesion of bacteria to the surface. (Zhao et al., 2017). The structure of the bacterial cell wall (surface charge, hydrophility, surface energy, and organelles) also affects biofilm formation (Chauhan et al., 2014).

In recent years, it has been observed that the effect of microbial biofilms on fish health is important. It has been observed that *Listeria*, *Salmonella*, *Shigella*, *Vibrio*, *Bacillus* and *Aeromonas* bacteria cause fish infections and biofilm formation (Mizan and Ha, 2015). Fish pathogens can form a strong biofilm on wood, metal, fiberglass, and glass. These materials are used a lot in aquaculture units. Biofilm formation in these materials is a threat to fish and human health. Biofilm is seen on the body of the fish and the surface of many materials such as metal surfaces, freezers, and fishing nets used in facilities (Pippo et al., 2018). Lactic acid bacteria (LAB) may cause biofilms and in this sense, it represents a concern for the food industry (Winkelströter et al., 2014). *Listeria*, *Pseudomonas*, *Stenotrophomonas*, *Brochothrix*, *Serratia*, *Acinetobacter*, *Rhodococcus,* and *Chryseobacterium* were isolated from food conveyor belts (Giaouris and Simoes, 2018).

In our work; samples were taken from fish looms (plastic, mica, wooden surface), the gill and skin of the fish, as well as the metal boat storage, and plastic collection pipe. As a result of the findings, thirty-six bacteria were capable of forming biofilms of the 47 bacteria identified from these surfaces. Among the bacteria identified, it was determined that *L. mesenteroides* strain MFL24, *L. mesenteroides*, *P. penneri* strain ALK624, *L. garvieae* strain ZSJ5, *L. mesenteroides* strain 30-1, *S. capitis* STM79, strain, *P. penneri* strain NSPPN01, *L. lactis*, *S. marcescens* strain AR1 formed strong adherence. In addition, *E. cloacae* strain 39, *L. mesenteroides* strain TUST005, *R. ornithinolytica* strain JSM 05182054, *P. penneri* strain wf-1, *S. aureus* strain 5 BWI, *S. aureus* strain K8, *S. pasteuri* strain AIMST.Pbst4, *S. aureus* strain K6, *P. mirabilis* strain T7, *S. pasteuri* strain HN-35, *C. youngae* strain NSKP2, *P. vulgaris* strain BPGM7, *S. aureus* strain HN-5 demostrated moderate adherence. However, *E. gallinarum*, *S. aureus* strain SSW20, *S. aureus* strain K2, *C. freundii* strain B25, *S. aureus* strain RCB1010, *S. warneri* strain STM81, *P. vulgaris* strain M20, *S. aureus* strain K5, *M. wisconsensis* strain X, *L. garvieae* strain CMGB-L23, *C. divergens* strain LHICA 534, *E. faecalis* strain H50, *E. casseliflavus* indicated weak adherence. Giaouris et al. (2020) showed that *S. aureus*, *S. enterica* and *L. monocytogenes* which cause spoilage in foods form biofilm.

ArunKumara (2019) isolated biofilm-forming *Vibrio* spp. from fish samples. *Vibrio* species formed biofilm as strong (10 strains), moderate (17 strains), and weak (23 strains). *Vibrio*, *Serratia*, *Rhodococcus*, *Carnobacterium*, *Micrococcus*, *Morganella*, *Yersinia*, *Lactobacillus* were found on fish and seafood processing surfaces (Møretrø et al., 2016; Langsrud et al., 2016; Møretrø and Langsrud, 2017). *Pseudoalteromonas* (7 strains), *Vibrio* (7 strains), and *Halomonas* (1 strain) were isolated from sediment in fish farms (Ijima et al., 2009).

Some biofilm-forming bacteria (*S. aureus*, *S. epidermidis*, *C. freundii*, *S. marcescens*, *E. faecalis*, *P. aeruginosa*, *L. garvieae*, *P. vulgaris*, *C. koseri*, *E. cloacae*, *P. mirabilis*, *E. gallinarum* CRL 1826) found in human skin, eyes and mucus can infect fish and fish stalls for sale in the absence of sanitation (Shin et al., 2013; Brandwein et al., 2016; Niederle et al., 2019; Raksha et al., 2019; Diriba et al., 2020). Biofilm-forming bacteria species we obtained in our study are compatible with the literature information. In this respect, contamination in fish is thought to be of human origin. Therefore, it is asserted that the employees do not act in accordance with the sanitation and hygiene rules.

The main purpose of stopping biofilm formation in the fish industry is to prevent the bacteria before they form the biofilm structure. Bacteria that cannot form a biofilm have low infectious power. It has been observed that the prevention of biofilm formation is important in the fight against fish diseases (Pippo et al., 2018). Malic acid, lysozyme, garlic oil, and oregano oil have been investigated for biofilm inhibition in the fish industry (Galie et al., 2018).

Foodborne pathogenic bacteria cause food poisoning and cause great economic losses. For this reason, it is necessary to give importance to hygiene in food processing equipment and food sales benches. Therefore, inhibition of these bacteria is important to maintain food safety and public health. In this respect, sanitation is an important rule in food and food contact surfaces (Laxmi and Sarita, 2018). Strategies for the control of biofilm formation in foods and on surfaces have been investigated by researchers (Galie et al., 2018; Bai et al., 2021; Dass and Wang, 2022). Depending on the structure of the biofilm, different methods can be used to prevent biofilms. These include mechanical cleaning, the use of antimicrobial agents, and the prevention of microbial adhesion to a surface with chemicals. To remove the biofilm, mechanical force must first be applied to the surface. Mechanical cleaning is very effective in preventing biofilm formation stages. Because cleaning with mechanical processes is more effective than gel cleaners or low-pressure cleaning systems. However, every system is not convenient for mechanical cleaning. There are hard-to-reach points in the systems. Chemical cleaning with appropriate mineral and organic acids should be applied immediately after mechanical cleaning. The system should be cleaned with corrosion inhibitors as the acids used may cause corrosion of the metals. In recent years, different methods such as electrical fields, catalyzed modified surfaces, ultrasound, enzymes, ammonia and formaldehyde, detergents, high-pressure cleaning systems have been used to prevent biofilm formation. Enzymes provide an effect in cleaning extracellular polymers formed in the biofilm matrix. Different enzymes such as protease, α-amylase, and β-glucanase are used to remove biofilm structures formed by various microorganisms (Kartal et al. 2021; Srinivasan et al., 2021).

Apart from enzymes, chemicals such as lactic acid, sodium hypochlorite, benzalkonium chloride, hydrogen peroxide, and citric acid are mostly used for sanitation. However, most of these substances are toxic and threaten human health (Lim et al., 2017; Carrascosa et al., 2021). For this reason, in our study, natural products (lemon juice, vinegar, rock salt) that are non-toxic, do not threaten human health, and have low cost have been tried for sanitation. Therefore, antibacterial tests of lemon juice, vinegar, and rock salt as natural products were carried out against biofilm-forming bacteria. When the antibacterial effects of the products used were compared, it was observed that the most effective natural products were vinegar and lemon juice (12-24 mm inhibition zones). Lemon juice and vinegar showed high activity against all tested bacteria except *P. mirabilis strain T7*, *P. penneri strain ALK624*, *L. mesenteroides strain MFL2* bacteria. While lemon juice was only effective against *L. garvieae strain ZSJ5* bacteria (15 mm inhibition zones), vinegar was only effective against *C. youngae strain NSKP2* bacteria (20 mm inhibition zones). While rock salt was only low effective against *L. garvieae strain IMAU50143* and *S. aureus strain K2* (9-10 mm inhibition zones), it was not effective against any of the other bacteria.

Vinegar is a natural product rich in organic acids such as acetic, succinic, malic, lactic, tartaric acid, and other fermentation products. Due to the organic acids, it contains, vinegar destroys the cell wall of bacteria, inhibits macromolecule synthesis, and disrupts the intracellular osmotic balance (Chen et al., 2016). Lemon juice is a widely consumed food for health due to its vitamin C (ascorbic acid) content. In addition, it contains citric acid, phenolic compounds, flavonoids, and essential oil. Therefore, the antibacterial effect of lemon juice is also known against pathogen bacteria (Aruoma et al., 2012).

Kahraman et al. (2022) researched antibacterial effect of home-made apple and grape vinegar against some food pathogenic bacteria such as *L. monocytogenes* RSK 472, *E. faecalis* ATCC 29212, *S. aureus* ATCC 43300, Methicillin-resistant *S. aureus* ATCC 25923, *B. cereus* ATCC 33019, *S. enteritidis* ATCC 13076, *S. typhimurium* ATCC 14088, *P. fluorescens* ATCC 13525, *E. coli* O157:H7 ATCC 35150. They showed that grape vinegar was more effective than apple vinegar. Kara et al. (2021) investigated the antibacterial activity of different vinegar samples against *S. aureus*, *K. pneumonia*, *E. coli* (ATB: 57), *E. coli* (ATB: 97), *P. aeruginosa* as pathogenic bacteria. While vinegar samples had a powerful effect against *S. aureus* and *P. aeruginosa* (15-32 mm inhibition zones), they had a moderate effect against *K. pneumonia*, *E. coli* (ATB: 57), *E. coli* (ATB: 97) (11-14 mm inhibition zones). In a similar study, the antibacterial effect of grape vinegar and apple vinegar was investigated against biofilm-forming bacteria. It was found that both vinegars showed high activity against *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 bacteria (21-22 mm inhibition zones) (Kahraman et al., 2021). Ousaaid et al. (2021) expressed that apple vinegar had considerable effect against *S. typhi*, *E. coli* O157:H7, *V. cholerae*, *C. albicans*, *C. tropicalis* (11-19 mm inhibition zones). Singh et al. (2020) tested the antibacterial activity of lemon juice as an antibacterial agent against *S. flexneri*, *S. epidermidis*, *Citrobacter spp*. and *Salmonella typhi*. It was shown that it had a respectable effect against *S. flexneri*, *S. epidermidis*, *Citrobacter spp*. (12-15 mm inhibition zones). Hamza et al. (2018) infered that apple, black raisin, garlic, and palm vinegar had a strong effect against *S. aureus*, *P. aeruginosa*, *Acinetobacter spp*., *E. faecalis*, *E. coli*, *K. pneumonia* (25-32 mm inhibition zones). Bakır et al. (2017) investigated the antibacterial effect of different vinegars against *S. aureus*, *S. typhimurium*, and *E. coli*. It was demonstrated that the highest effect was against *S. typhimurium* bacteria (16 mm inhibition zones). De et al. (2017) investigated the antimicrobial activity of raw lemon against human enteric pathogens such as *K. pneumonia*, *S. typhi*, *P. vulgaris*. While the lemon juice had a high effect against *K. pneumonia*, *S. typhi* (15-21 mm inhibition zones), it had no effect against *P. vulgaris*. Oikeh et al. (2016) evaluated the antimicrobial activity of different citrus juice such as

C. tangerine (tangerine), *C. paradisi* (grape), *C. limon* (lemon), and *C. aurantifolia* (lime) against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *Salmonella spp.*, *C. albicans*, *A. niger*, *Penicillum spp.* While the lemon juice had a strong effect (18-24 mm inhibition zones) against *S. aureus*, *P. aeruginosa*, *C. albicans*, it had a moderate effect (10-12 mm inhibition zones) against *E. faecalis*, *E. coli*, *Salmonella spp.* bacteria. On the other hand, it had a very low effect (9 mm inhibition zones) against *A. niger*, *Penicillum spp.* In another study, the antibacterial activity of acetic acid was examined against biofilm forming pathogens on burns patients. It was determined that different concentrations of acetic acid were found to be effective against *P. aeruginosa*, *A. baumannii*, *S. aureus*, *E. faecalis*, *E. coli*, *K.pneumoniae* (Halstead et al., 2015).

It is appropriate to use vinegar and lemon juice to prevent biofilm in fish processing equipment and sales benches. In this study, it has been determined that vinegar and lemon juice can be used within the scope of food safety.

5. CONCLUSION

It is important to protect food hygiene and safety from the production to consumption of many foods such as fish, meat, and dairy products. The unsuitable storage conditions of the foods and the failure to comply with the sanitation rules of the equipment used encourage the development of bacteria that cause food spoilage and poisoning. In addition, it is also possible to contaminate food through human contact or by breathing. As a result, bacteria that develop in foods threaten human health by forming a biofilm structure. The growth of biofilm-forming bacteria in foods is due to inadequate hygiene and sanitation. According to the results of our study, it was found that the bacteria that developed in fish and fish stalls were mostly of human origin. It can be understood that the people dealing with this work do not act in accordance with the sanitation rules. Many of the isolated bacteria also appear to have strong and moderate biofilm-forming abilities. Natural products were used to prevent the growth of these bacteria and the formation of biofilm. Vinegar and lemon juice was found to be effective among natural products for hygiene and sanitation. The results obtained have brought a new perspective to the biofilm control strategy. Wiping food surfaces with vinegar or lemon juice is a precaution against biofilm-forming bacteria for sanitation. Compared to chemical disinfectants, the use of vinegar and lemon juice is an attractive practice due to its safe and inexpensive cost.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR’S CONTRIBUTIONS

The contribution of the authors is equal.

ETHICAL STATEMENT

There are no ethical issues with the publication of this manuscript.

DATA AVAILABILITY STATEMENT

Data used in this study are available from the corresponding author upon reasonable request.

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Research Article **Araştırma Makalesi**

Effects of *Lactococcus lactis* and *Bacillus* sp. on Hatchery Performance of Rainbow Trout (*Oncorhynchus mykiss*) Eggs until Larval Stage

Lactococcus lactis ve *Bacillus* sp.'nin Yumurtadan Larval Döneme Kadar Olan Evrede Gökkuşağı Alabalığı (*Oncorhynchus mykiss*)'nın Kuluçka Performansına Etkileri

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Abstract: In this study, the effect of *Lactococcus lactis* and *Bacillus* sp. (Gram-positive bacteria) on survival rates of rainbow trout (*Oncorhynchus mykiss*) from egg to larval stage was investigated. In addition, the effects of bacteria on the blue sac syndrome in the fry stage of fish and basic water quality criteria were also noted. Bacteria were applied by immersion method to healthy-eyed eggs during the incubation period. Two bacteria and the control group were examined in a duplicated plan. The highest survival rate was 92.5% observed in the *Bacillus* sp. group (*L. lactis*; 70% and control; 45%). The lowest survival rates in all groups were observed during the alevin stage. It was found that the amount of nitrite in the water decreased in the bacteria-applied groups, and it was determined that the effects of blue sac syndrome (caused by the pathogen *A. hydrophila*) were suppressed in the *Bacillus* sp. group.

Keywords
- Bacteria
- Blue sac fry syndrome
- Hatchery
- Nitrate
- Rainbow trout

Özet: Bu çalışmada *Lactococcus lactis* ve *Bacillus* sp. (Gram-pozitif bakteri) uygulamasının gökkuşağı alabalığının (*Oncorhynchus mykiss*) yumurtadan larva dönemine kadar yaşama oranları araştırıldı. Ayrıca çalışmada balıkların yavru aşamasında mavi kese hastalığı üzerine bakterilerin etkileri ve temel su kalitesi kriterleri de not edilmiştir. Bakteriler kuluçka döneminde sağlıklı gözlü yumurtalara daldırma yöntemiyle uygulandı. İki bakteri ve kontrol grubu, iki tekerrürlü deneme planında incelendi. En yüksek hayatta kalma oranı *Bacillus* sp. grubunda (*L. lactis*; %70 ve kontrol; %45) %92.5 ile gözlendi. Tüm gruplarda en düşük hayatta kalma oranları alevin döneminde gözlendi. Bakteri uygulanan gruplarda sudaki nitrit miktarının azaldığı, *Bacillus* sp. grubunda ise mavi kese sendromunun (patojen etken *A. hydrophila*) etkilerinin baskılandığı belirlendi

Anahtar kelimeler
- Bakteri
- Mavi kese sendromu
- Kuluçkahane
- Nitrat
- Gökkuşağı alabalığı

1. INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is well-known fish species in aquaculture around the world. Broodstock management, nutritional needs, hatchery performances, water quality criteria, nutrient content, and diseases have been investigated in several studies (Atar, et al., 2009; Öztürk and Altinok, 2014). Recently, biotechnological applications such as hybridization, and female, or male stock options for fish have been used successfully in aquaculture systems (Rehman et al., 2022). Besides, there are several studies on health problems in rainbow trout (Öztürk and Altinok, 2014). Diseases caused by bacteria, viruses, and parasites are frequently reported by researchers, especially in intensive breeding systems (Woynarovich et al., 2011).

The highest mortality rates in trout hatcheries were observed in the egg and fry stages. Infertile eggs, water quality problems, and losses caused by pathogens, particularly fungus, have been reported in trout hatcheries (Kayış et al., 2017). Particularly, gas bubble and blue sac fry syndrome in the alevin stage of trout are commonly reported (Kayış et al., 2015; Balta and Dengiz, 2020). Various precautions and applications have been used to prevent these losses (Kayış 2019; Austin et al., 2022). Maintenance of hygiene is among the most important practices. However, the use of some chemicals for disinfection is quite common. For this purpose, formalin has been effectively used from the egg to the fry stage (Barnes et al., 2001). Iodine compounds are other disinfectants that have been recommended for rainbow trout eggs (Goldes and Mead, 1995). Apart from chemicals, other applications have also been used to improve the quality of hatchery water. Ozone and ultraviolet rays have been reported to reduce the pathogenic load of water (Forneris et al., 2003).

Recently, the general use of some probiotic organisms for the welfare of fish has been investigated. Probiotics are beneficial organisms that are known to suppress the harmful microbial flora in the living body and, recently, have been increasingly used in aquaculture due to the negative effects of disinfectants (Kayış, 2019; Austin et al., 2022). Probiotics are used to increase growth performance, improve water quality, suppress bacterial load, improve reproductive performance, and reduce stress, especially by participating in food diets (Cruz et al., 2012). Bacteria such as *Bacillus* sp., *Lactobacillus acidophilus*, and *Lactococcus lactis* are known to be useful in aquaculture, particularly in suppressing pathogens (Yilmaz et al., 2022).

Lactococcus lactis is a Gram-positive bacterium that is especially used for fermenting dairy products. It has been reported to be abundant in the intestinal microbiota of freshwater fish (Gatesoupe 2008). *Lactococcus* sp. and other lactic acid bacteria of the genus *Leuconostoc* have been reported to be frequently used as probiotics in aquaculture (Merrifield, 2010). Bacteria of the genus *Bacillus*, which are rod-shaped Gram-positive bacteria, are highly resistant to heat. Although they are usually found in soil, they can also be found in air, water, dust, and feces. The vast majority of *Bacillus* sp. do not have pathogenic potential (except for *B. cereus* and *B. anthracis*) and have important microbiological applications (antibiotics, enzymes, toxins, and bioplastics) (Kemmerly and Pankey, 1993; Ahmed et al., 1995).

Therefore, the present study investigated the question “could some bacteria have a positive effect in preventing losses in trout hatchery systems?”. For this purpose, *Lactococcus lactis* and *Bacillus* sp., isolated from trout hatchery systems, were applied to the eggs and the results were evaluated. Also, other two trials were designed to determine probiotic effectiveness against *Aeromonas hydrophila*, which is pathogenic to trout species and a causative agent of blue sac fry syndrome, and on some water quality parameters values such as nitrite, nitrate, ammonia, and phosphate.

2. MATERIAL AND METHODS

2.1. Eggs and potential probiotic bacteria

The rainbow trout (*Oncorhynchus mykiss*) eggs used in this study were obtained from a trout farm at Rize in the Black Sea region of Turkey. To eliminate possible bacterial and fungal pathogens that may have been present in these eggs, 1.65 mg/L formalin was applied to the eggs for 15 min (Barnes et al., 2001). A total of 120 eggs were used for each group of bacteria and control *Lactococcus lactis* (isolated from *Oncorhynchus mykiss*) and *Bacillus* sp. (isolated from *Salmo* sp.) were isolated from the hatcheries of some trout farms in the Eastern Black Sea Region of Turkey (Kayış et al., 2021). For the molecular identification of the bacteria their genomic DNA was obtained by a DNA extraction isolation kit (Qiagen). Specific primers to the 16S rRNA region of eubacteria (27 Fwd 5’-AGA GTT TGA TCC TGG CTC AG-3', 1492 Rev 5’-GTT TAC CTT GTT ACG ACT T-3') were used. PCR reaction is carried out using genomic DNA of bacteria and the primers (Model Px2 ThermoHybrid; Thermo Electron Inc., Waltham, MA, USA). The 1465-bp amplified products were purified with a NucleoSpin PCR purification kit (Macherey-Nagel) and sent for sequencing by double-sided reading (ABI PRISM 310 genetic analyzer, Applied Biosystems). Accession numbers of *Bacillus* sp. and *Lactococcus lactis* are MW295490 and MW295471 respectively in the National Center for Biotechnology Information. The bacteria were previously preserved at –80 °C at the Fish Diseases Laboratory, Faculty of Fisheries of Recep Tayyip Erdogan University. The experiment was designed as a static system, with three groups (control, *Lactococcus lactis*, and *Bacillus* sp.) in duplicates. The eggs were placed in glass containers (20 eggs/500 ml) and sufficient ventilation was provided using an air pump.

2.2. Experiment I (Survival rates of Eggs and Determination of Potential probiotic effects of Bacteria)

The immersion method was used to transmit the bacteria to the eggs. For this, purpose bacteria were transferred to Tryptic Soy Agar (TSA) (Merck 1.05458, Darmstadt, Germany) medium and incubated at 20±2 °C for 24 h. Bacterial colonies were then transferred to 50 ml Tryptic Soy Broth (TSB) (Merck 1.05459, Darmstadt, Germany) medium and the cultures were kept at 20±2 °C for 12 h. This culture was then centrifuged at 9950 g for 5 min and the medium was separated from the bacteria. Sterile Phosphate-Buffered Saline (PBS) (15 mL) was added to the bacteria and the mixture was homogenized using a vortex mixer. About 15 ml of this bacterial suspension was then added to each test container and the bacteria were allowed to contaminate the eggs. To determine the number of bacteria, 10^{-6} and 10^{-7} dilutions were spread on the surface of Plate Count Agar (PCA) (Merck 1.05463, Darmstadt, Germany), and colony counts were performed (APHA 1998; Kayış et al., 2017), and the bacterial count was determined as 4.6×10^{10} and 1.2×10^{10} CFU/ml for *Lactococcus lactis* and *Bacillus* sp., respectively. In this experiment, the temperature of the water was recorded as 14.5±0.6 °C and pH was 6.5±1.3. The eggs reached the stage of the alevin within eight days from the start of the experiment. The alevin phase continued for ten days. After this phase, the trial was conducted for one week. The fish were fed with commercial trout feed during this stage. During this time, the metabolic wastes in the tanks were cleaned and the same amount of fresh water was added instead of the decreased water.

2.3. Experiment II (Blue sac fry syndrome inhibition trial)

Kayış et al. (2015), stated that *Aeromonas hydrophila* infection is the cause of blue sac fry syndrome disease in trout alevins. To investigate the inhibitory effect of the bacteria against the fish pathogen *Aeromonas hydrophila*, four groups (control, *A. hydrophila, A. hydrophila- Lactococcus lactis and A. hydrophila- Bacillus* sp.) of eggs were designed in two repetitions as another independent experiment (Table 1), and the survival rate of the eggs until the fry stage was determined. For this purpose, the bacteria (*Bacillus* sp. and *Lactococcus lactis*) were added to water including eyed trout eggs which were infected with *Aeromonas hydrophila*. At the end of the experiment, it was observed to what extent the blue sac fry syndrome in the alevin was present.

Table 1. Groups in the inhibitory effect of the bacteria against *Aeromonas hydrophila* trial

Groups	Amount of Eggs	Amount of Bacteria
Control (C)	200	(-)
Aeromonas hydrophila (A)	200	(1.6×10^{10})
A. hydrophila- Lactococcus lactis (A-L)	200	(1.6×10^{10})-(4.6×10^{10})
A. hydrophila- Bacillus sp. (A-B)	200	(1.6×10^{10})-(1.2×10^{10})

2.4. Experiment III (Reduction of values of some water parameters)

Another experiment was conducted to observe the reduction of values on some water parameters (ammonia, nitrite, nitrate, and phosphate) by bacteria in water, including fry fish. Three groups; control (C), *Lactococcus lactis* (L), and *Bacillus* spp. (B) were used in this experiment. Fifty fish (average weight of 0.5 g) were placed in each group in duplicate (5-liter tanks). At the beginning of the experiment, the water parameters were measured. Fish were fed commercial trout feed once a day (in the morning, at 2% of body weight) and provided sufficient oxygen (The average amount of dissolved oxygen in the tanks was measured as 9.2±0.9). The bacteria were then added to the groups separately (*Lactococcus lactis* 1.2×10^{11} CFU/mL, *Bacillus* spp. 2.3×10^{10} CFU/mL). Then, on the 13^{th} day, the water parameters were measured again. In both this and the *Aeromonas hydrophila* inhibition trial, the water temperature was recorded as 16.5±0.3 °C and the pH was measured as 6.7±0.8. Water quality parameters were measured by spectrophotometer (Hach DR3900).

2.5. Statistical analysis

The statistical significance of the difference between the water quality values of the groups was carried out with the analysis of variance (ANOVA) in the SigmaPlot 12.0 program. Survival analysis of different groups was carried out with the Kaplan–Meier test.

3. RESULTS

The number of eggs that survive until the hatching stage is given in Figure 1. The group treated with *Bacillus* sp. was observed to have the highest survival rate (92.5%), followed by *Lactococcus lactis* (70%). The control group had the lowest survival rate (45%) (Figures 1, 2).

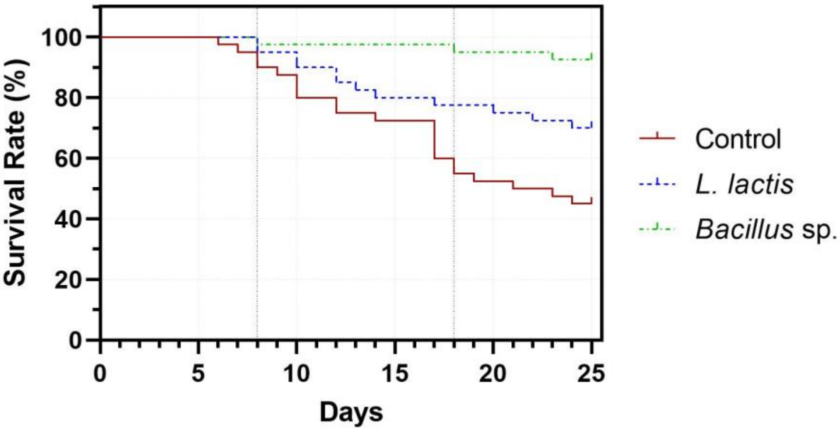

Figure 1. Survival rate of the groups

It was observed that the major losses in all the groups were in the alevin stage. After the alevin stage, the deaths were observed to decrease. No significant pathological finding was observed in any group. During the feeding phase with commercial trout feed, the group treated with *Bacillus* sp. showed more appetite than the other groups.

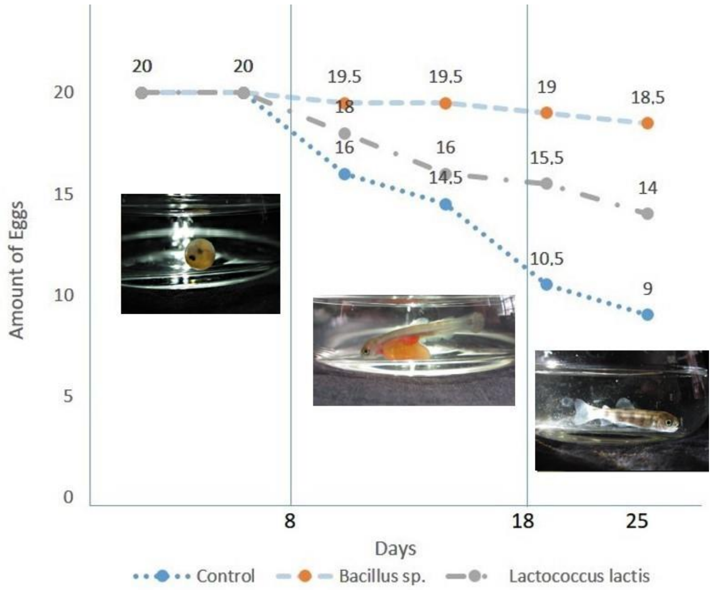

Figure 2. Mortalities of the fish during the experiment (I).

Regarding the inhibitory effect of bacteria against *Aeromonas hydrophila* (Blue sac fry syndrome), the survival rate was recorded as 80% in the control group (C), 79% in the *A. hydrophila* (A) group, 80% in the *A. hydrophila-Lactococcus lactis* (A-L) group, and 77% in the *A. hydrophila-Bacillus* sp. (A-B) group. Also, the Blue Sac Fry Syndrome was observed in 3% of trout in the (A) and (A-L) groups in the alevin stage. This symptom was not observed in groups (B) and (C) (Figure 3).

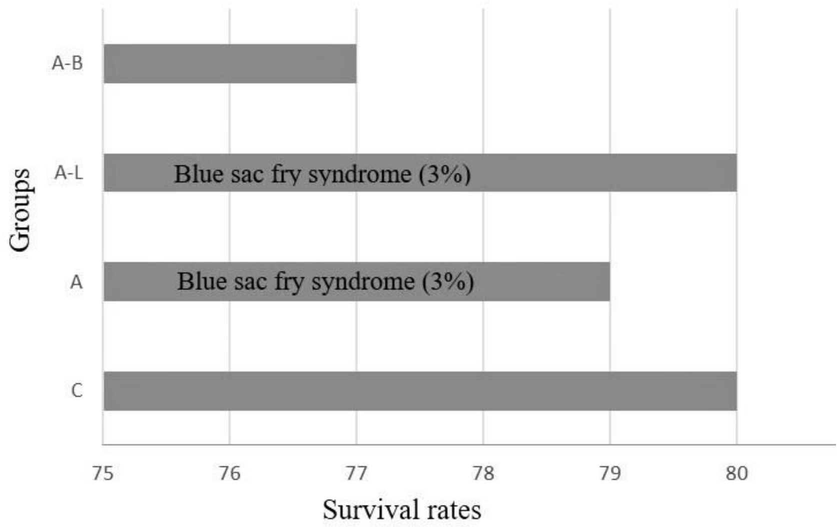

Figure 3. Result of *Aeromonas hydrophila*/Blue sac fry syndrome trial (Experiment II). Control (C), *A. hydrophila* (A), *A. hydrophila- Lactococcus lactis* (A-L), *A. hydrophila- Bacillus* spp. (A-B).

In the water criteria trial, both Gram-positive bacteria reduced the amount of nitrite in the water. Moreover, the nitrate content was observed to be lower in the *Bacillus* group compared to the other groups (Figure 4). In this trial, fish deaths started on the 11th day in the control and *Lactococcus lactis* groups. While all fish in the control group died by the 13th day, the percentage mortality in the *Lactococcus lactis* and *Bacillus* sp. groups was 52% and 68%, respectively (Figure 5).

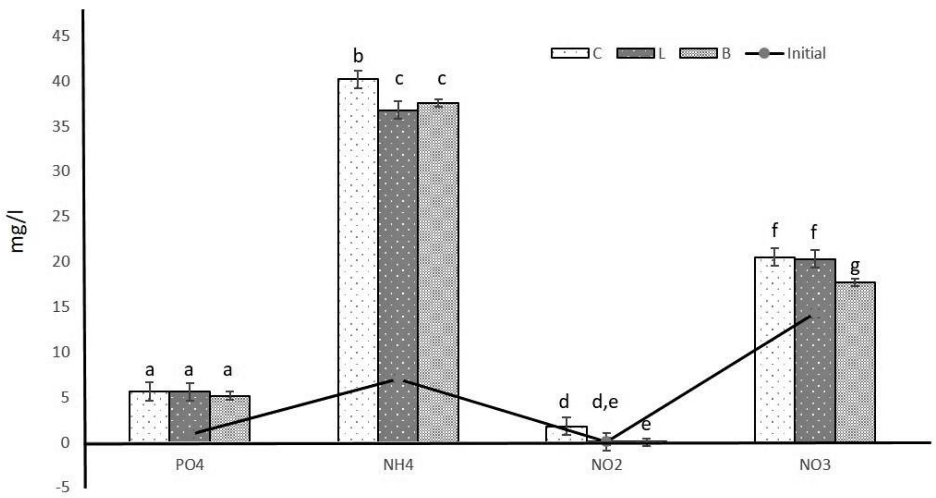

Figure 4. Some water criteria in reduction of some water parameters trial (Experiment III). Control (C), *Lactococcus lactis* (L) *Bacillus* sp. (B).

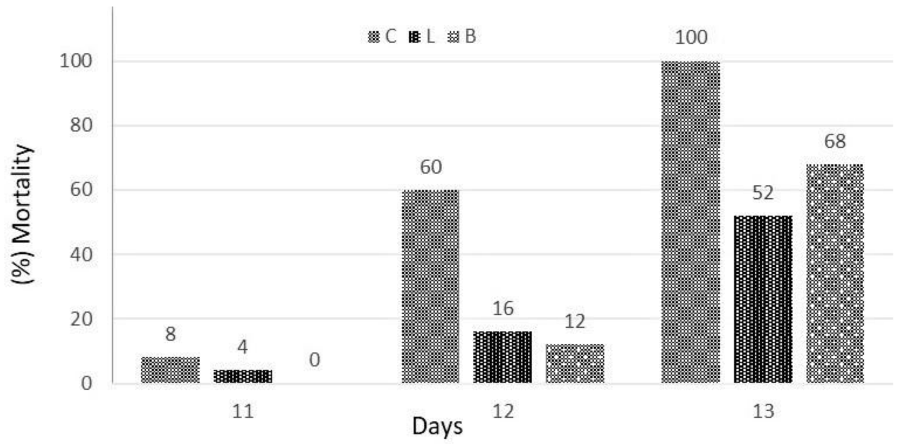

Figure 5. Fish mortalities (%) in the water criteria trial (Experiment III). Control (C), *Lactococcus lactis* (L) *Bacillus* sp. (B).

4. DISCUSSION

The use of probiotics stands out in all live production sectors in the world (Austin et al., 2022). For example, *Carnobacterium* spp. has been reported to be effective against some fish pathogens (*Aeromonas hydrophila, A. salmonicida, Flavobacterium psychrophilum, Photobacterium damselae*, and *Vibrio* spp.) in rainbow trout (Robertson et al., 2000). Probiotics some times are used to improve the water quality in fish farms and aquarium units. In this context, bacteria of the genus *Bacillus* are widely used. Therefore, the benefits of *Bacillus* sp. and *Lactococcus lactis* isolated from trout hatchery systems were investigated to the survival rate of trout eggs in the present study. The results indicated that *Bacillus* sp. and *L. lactis* had a positive effect on the survival rate of trout eggs.

Egg deaths in freshwater hatchery systems are reported because of fungal infestations (Thoen et al., 2011). However, some studies show that bacteria in trout hatchery systems can also cause the deaths of fish (Declercq et al., 2013; Yardımcı and Turgay, 2021). Bacterial contamination studies generally have been conducted on trout eggs to determine the negative effects of various bacteria. These studies have reported that egg mortality rates increased and reached high levels during the alevin phase (Kayış et al., 2017). Similarly, in this study, it was observed that mortality rates increased during the alevin phase. However, it was observed that these deaths were significantly prevented using *Bacillus* sp. in the aquatic systems. This is an important finding, and it may be advisable to use bacteria of the genus *Bacillus,* which have probiotic properties, during the egg stage.

In the present study, the difference in survival rates of fish in *Bacillus* sp. and *Lactococcus lactis* groups can be discussed. It is thought that especially water quality criteria may affect these rates. Water temperature and pH are important factors in the reproduction of bacteria in aquatic systems. Other factors such as adherence to the surface and organic load are also important. These criteria are necessary for the reproduction of bacteria as well as enzyme activities. The optimum temperature for the activation of enzymes of *Bacillus subtilis* is 37 °C, while the optimum pH is reported as 5.5 (Sneath, 1986). For *Lactococcus lactis*, the optimum temperature is 30-40°C (Chen at. al., 2015). These values are higher than those recorded in this experiment. For the incubation of trout eggs, the optimum water temperature is 12 °C or lower, although the temperatures in the experiment were tolerable for the eggs. The difference in survival rate between the use of *Bacillus* sp. and *Lactococcus lactis* group may be explained by the optimum temperature values of the bacteria.

The blue sac fry syndrome in fish is explained by different reasons. Among those, physicochemical parameters and chemical contamination of the water are well-known. Temperature, pH, nitrogen compounds and some dissolved gasses and xenobiotics (retene (7-isopropyl-1-methylphenanthrene) are the most important reasons that can cause blue sac disease on alevins (Noga, 2010; Brzuzan et al., 2007). However, recently, there have been reports that this symptom is caused by bacteria. It has been stated that *Aeromonas hydrophila* causes this symptom in the alevin stage of trout (Kayış et al., 2015). This study confirms the previous reports on the blue sac symptom. The blue sac syndrome was observed in all the fish groups infected with *A. hydrophila*, except for those given *Bacillus* sp. as a probiotic (Figure 2). This result revealed the preventive effect of *Bacillus* sp. against the blue sac fry syndrome in trout alevins. It is reported that *Bacillus* spp. produces some metabolites with antibiotic properties. Due to this feature, these bacteria are used against many pathogenic bacteria (Miljakovic et al., 2020). Details of the reasons and mechanism of this positive effect (inhibition of blue sac fry syndrome/*Aeromonas hydrophila*) can be investigated in future studies.

It is known that some bacteria improve some water quality criteria. It has also been reported that these bacteria suppress pathogens in fishponds. These positive effects are also tried to be evaluated in terms of aquaculture (Padmavathi et al., 2012). In a study, positive effects of *Bacillus subtilis* and *Lactobacillus acidophilus* on growth performance and pond water quality of Nile tilapia (*Oreochromis niloticus*) fingerlings were reported. (Khalafalla et al., 2020). Similarly, Hlordzi et al. (2020), stated that *Bacillu*s species showed great success in maintaining water quality at a low cost in aquaculture. The present study determined that the nitrite content in water was significantly decreased, especially in the fish groups treated with *Bacillus* sp. and *Lactococcus lactis*. This situation was found to support the study of Korenekova et al. (2004) and Usharani et al. (2017), on the reducing effect of *L. lactis* bacteria on nitrate levels in milk and *Bacillus* sp. in wastewater respectively. This finding is particularly important, as nitrite is known to cause brown blood disease caused by nitrite in fish. The survival rates of the fish in this trial support this finding (Figure 4).

5. CONCLUSION

The beneficial effects of *Bacillus* sp. and *Lactococcus lactis* have been demonstrated in the present study, especially in preventing deaths, blue sac fry syndrome of trout eggs and fry, and reducing the nitrite level of hatchery water. Based on these results, we can recommend that farmers use the bacteria in trout hatchery systems.

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CONFLICT OF INTEREST

Example: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Fiction: ŞK, EP; Literature: EP; ŞK; Methodology: ŞK, EP; Performing the experiment: ŞK, EP, ZZİ, EA, MM; Data analysis: EP, ŞK; Manuscript writing: ŞK; Supervision: ŞK. All authors approved the final draft.

ETHICAL STATEMENTS

This study was conducted with the approval of Animal Experiments Local Ethics Committee of Recep Tayyip Erdoğan University (Date: 27.01.2016, No: 2016/10).

DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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Research Article **Araştırma Makalesi**

The Effect of Anthropogenic Activities on the Fish Fauna of the Devrez Stream (Türkiye)

Devrez Çayı (Türkiye) Balık Faunası Üzerine Antropojenik Faaliyetlerin Etkisi

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Abstract: In this study, fish species diversity and protection criteria, habitat evaluation, and threat elements of the ichthyofauna of the Devrez Stream were examined monthly between April 2020 and March 2021. Eight fish taxa (7 natives, 1 non-native) belonging to four families (Cyprinidae, Gobionidae, Leuciscidae, Gobiidae) were identified. *Chondrostoma angorense* Elvira, 1987 (endemic) from the mentioned species was reported for the first time in the Devrez Stream. *Squalius* sp. (32.5%), *Chondrostoma angorense* (24.4%), *Capoeta tinca* (23.9%), and *Alburnoides freyhofi* (11.1%) were the most common and abundant species while *Pseudorasbora parva* (0.5%), *Barbus anatolicus* (1.2%), *Alburnus goekhani* (2.4%) and *Neogobius fluviatilis* (4%) were the most rarely encountered species in the stream. According to our observations, drought, agricultural irrigation, and Devrez Kızlaryolu Dam, which is under construction, were considered major threats to the fish fauna.

Keywords
- Ichthyofauna
- Endemism
- Turkiye inland fishes
- Devrez Kızlaryolu Dam

Özet: Bu çalışmada Nisan 2020 ile Mart 2021 tarihleri arasında Devrez Çayı'nın balık türü çeşitliliği ve koruma ölçütleri, habitat değerlendirmesi ve ihtiyofaunayı tehdit eden unsurlar aylık olarak incelenmiştir. Dört familyaya ait (Cyprinidae, Gobionidae, Leuciscidae, Gobiidae) sekiz balık taksonu (7'si yerli, 1'i yerli olmayan tür) tespit edilmiştir. Söz konusu türlerden *Chondrostoma angorense* Elvira, 1987 (endemik) Devrez Çayı'ndan ilk kez rapor edilmiştir. Çayda *Squalius sp.* (%32,5), *Chondrostoma angorense* (%24,4), *Capoeta tinca* (%23,9) ve *Alburnoides freyhofi* (%11,1) en yaygın türler iken, *Pseudorasbora parva* (%0,5), *Barbus anatolicus* (%1,2), *Alburnus goekhani* (%2,4) ve *Neogobius fluviatilis* (%4) en az rastlanan türlerdir. Gözlemlerimize göre kuraklık, tarımsal sulama ve yapımı devam eden Devrez Kızlaryolu Barajı balık faunası için başlıca tehditler olarak düşünülmektedir.

Anahtar kelimeler
- İhtiyofauna
- Endemizm
- Türkiye içsu balıkları
- Devrez Kızlaryolu Barajı

1. INTRODUCTION

The geographical location of Türkiye between the Asian and European continents, its location in the mid-latitude temperature and the subtropical climate zone (İyigün et al., 2013), and its different topographic structure have caused great differences in the local climate. Climatic variations affect hydrology (Burn & Hag Elnur, 2002), flora, and fauna including fish diversity (Giannetto & Innal, 2021). Türkiye has thirteen freshwater ecoregions (Abell et al., 2008) and a rich freshwater fish fauna with 409 species in inland waters, including 194 endemic species (Çiçek et al., 2018). However, Türkiye's freshwater fish fauna is threatened by various anthropogenic alterations, such as water extraction, habitat degradation, construction of dams and HEPPs (Hydroelectric Power Plant), sand extraction from the rivers, pollution and introduction of non-native species (Fricke et al., 2007; Freyhof et al., 2014; Küçük et al., 2018).

Studies on ichthyofauna, which were initiated by Abbot (1835) for the first time, have been continuing intensifying in recent years (Fricke et al., 2007; Güçlü et al., 2013; Kuru et al., 2014; Çiçek et al., 2018;

Turan et al., 2018). Although many freshwater fish faunas in Türkiye have been studied, different results might have been obtained even in studies conducted in the same place. It is thought that differences in the sampling methods and sampling times may cause these results. It is extremely important to update the fish fauna studies of water systems and take necessary precautions against the threats to protect fish biodiversity. However, few studies (İlhan & Balık, 2008; Yoğurtçuoğlu et al., 2020) have been conducted on fish fauna in the Devrez Stream. Unlike previous studies, this study is a longer-term study, and fish samples were captured regularly every month for one year. The present study aims to provide further information on the population structure of fishes, habitat evaluation, and threat elements of the Devrez Stream. The specific aims of the study were to: (i) provide taxonomic features and current assessment of fish species; (ii) identify habitat structure and threat elements and discuss protection measures; (iii) evaluate the possible effects of the Kızlaryolu Dam under construction on the river ecosystem. Investigation of the effects of human-induced factors on the fish fauna of the Devrez Stream contributes to a greater understanding of species conservation.

2. MATERIAL AND METHODS

2.1. Study area

The Devrez Stream is located in the northern part of Anatolia within the provincial borders of Çankırı, Kastamonu, and Çorum (Figure 1) in Türkiye. This stream is an important tributary of the Kızılırmak River with a length of 186 km, a basin area of 3364 km^{2}, and a flow rate of 4.081 m^3/s. The climate of the region transitions between the Black Sea and Central Anatolia land-type climates. According to 1929-2020 data, annual average temperatures vary between 9.8 ℃ and 11.3 ℃ (see https://www.mgm.gov.tr, accessed 28 January 2022). Annual total precipitation varies between 348 mm and 545 mm in the basin. The precipitation in the basin is below the Tükiye's average value. Most of the precipitation falls in the winter and spring months and the flow regime is irregular (DSI, 2014).

Flowing in the southwest-northeast direction, the Devrez Stream is located in a tectonically very active region. The North Anatolian fault line, which is Türkiye's most active fault zone, forms roughly the northern border of the Devrez Stream basin (Köle, 2016).

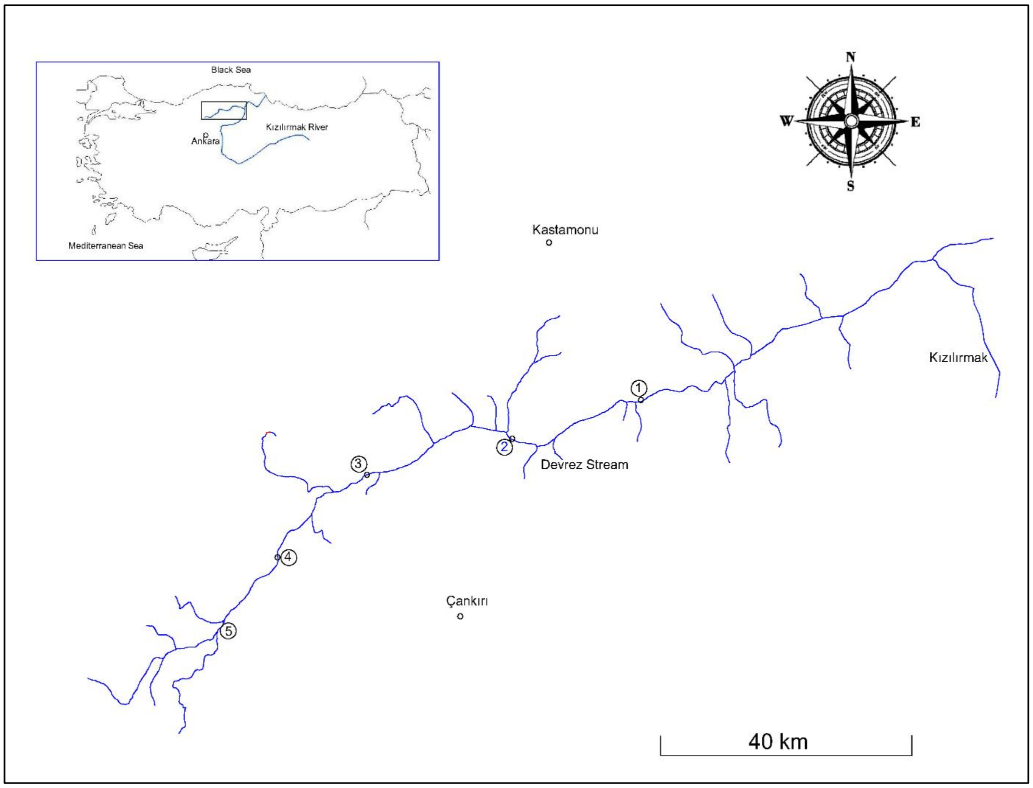

Figure 1. Devrez Stream map and sampling stations

The Devrez Stream (Figure 2), which has a stony and sandy ground, has a wide bed that creates deep ponds in places. The stream has significant importance for agricultural activities in the basin. Although Güldürcek Dam on İçin Creek, a branch of the Devrez Stream was put into operation in 1988 for drinking water and irrigation purposes, demands for irrigated agriculture in the region have been increasing. Therefore, the construction of Devrez Kızlaryolu Dam located 7 km southeast of Kurşunlu district of Çankırı province was initiated in 2017 and planned to be completed in 2023 as a second dam for purpose of water demand on both agricultural activities and energy. The height of the dam from the foundation is 91.5 meters with a reservoir volume of 114.82 hm^3, a reservoir area of 3759 km^2, and the total irrigation area of 1767 ha (DSI, 2014).

Figure 2. Devrez Stream (near Köpürlü Village)

2.2. Sampling

Sampling in the Devrez Stream was conducted from April 2020 to March 2021 with gill nets, cast nets, and fishing rods, and assistance was received from local fishermen. The fishes were fixed and preserved in 4% formaldehyde.

2.3. Analyses

Metric measurement was carried out with a 0.01 mm sensitive caliper and ruler. Meristic characters were counted under a stereomicroscope. The last two branched rays articulated on a single pterygiophore in dorsal and anal fins are counted as "1½ (Kottelat & Freyhof, 2007). Longitudinal scales were used since there is no linea lateral in Gobiidae family. Elvira (1997), Kottelat and Freyhof (2007), Turan et al. (2017, 2018), Özuluğ et al. (2018), Bayçelebi (2019) and Van der Laan (2021) were used for classification. The relative abundance of species was calculated via the equation of 1.

Relative abundance %= (ni/N) x100 (1)

where *ni* is the number of individuals of the species and *N* is the total number of individuals of fish caught in the river (Magurran, 1996).

The biotic and abiotic factors thought to threaten the fish were evaluated and the conservation status of fish species was provided from the IUCN Red List (International Union for Conservation of Nature and Natural Resources) (IUCN, 2022- version 2021-3).

3. RESULTS

3.1. Fish diversity and relative abundance

A total of 422 fish specimens were collected from the Devrez Stream during this study. Eight species belonging to four families Cyprinidae (2 taxa), Gobionidae (1 taxon), Leuciscidae (4 taxa), and Gobiidae (1 taxon) were identified. All species were recorded as rheophilic fishes except *Pseudorasbora parva* and *Neogobius fluviatilis* (Table 1, 2).

Table 1. Geographic information on sampling stations in the Devrez Stream

Station number	Locality	Coordinates	Altitude (m)
1	Near Aşağıdikmen Village	40°58′34″N/34°04′40″E	581
2	Near İnköyü Village	40°53′58″N/33°39′30″E	839
3	Near Kızılcaköyü Village	40°50′16″N/33°23′46″E	978
4	Near Köpürlü Village	40°46′37″N/33°17′05″E	1007
5	Near Yuva Village	40°37′02″N/33°02′08″E	1300

Table 2. Native status, IUCN, habitat guild and abundance categories of fishes sampled from the Devrez Stream (n: native; e: endemic; nn: non-native; NE: not evaluated; LC: least concern)

Species	# of fish	Station number	Native status	IUCN	Habitat guild	Abundance category
CYPRINIDAE						
Barbus anatolicus Turan, Kaya, Geiger and Freyhof, 2018	5	1,3,4	n, e	NE	Lotic	Unknown
Capoeta tinca (Heckel, 1843)	101	1,2,3,4,5	n, e	LC	Lotic	Decreasing
GOBIONIDAE						
Pseudorasbora parva (Temminck and Schlegel, 1846)	2	5	nn	LC	Lotic/ lentic	Unknown
LEUCISCIDAE						
Alburnoides freyhofi Turan, Kaya, Bayçelebi, Bektaş and Ekmekçi, 2017	47	2,3,4,5	n, e	NE	Lotic	Unknown
Alburnus goekhani Özuluğ, Geiger and Freyhof, 2018	10	2,4,5	n, e	NE	Lotic	Unknown
Squalius sp.	137	1,2,3,4,5	n	LC	Lotic	Unknown
Chondrostoma angorense Elvira, 1987	103	2,3,4,5	n, e	LC	Lotic	Decreasing
GOBIIDAE						
Neogobius fluviatilis (Pallas, 1814)	17	1,3,4,5	n	LC	Lotic/lentic/ marine	Unknown

The distribution of fish species in terms of percentage and number of fish caught during this study is presented in Figure 3. The most dominant family was Leuciscidae with four species. One non-native species (*P. parva*) and five endemic species (*B. anatolicus*, *C. tinca*, *A. freyhofi*, *A. goekhani* and *C. angorense*) were recorded. *Squalius* sp. (32.5%), *C. angorense* (24.4%), and *C. tinca* (23.9%) were the most common and abundant species in the Devrez Stream. The most rarely encountered species were *P. parva* (0.5%), *B. anatolicus* (1.2%), *A. goekhani* (2.4%), *N. fluviatilis* (4%) and *A. freyhofi* (11.1%). *P. parva* was caught from only one station while *C. tinca* and *Squalius* sp. specimens were caught from all sampling stations.

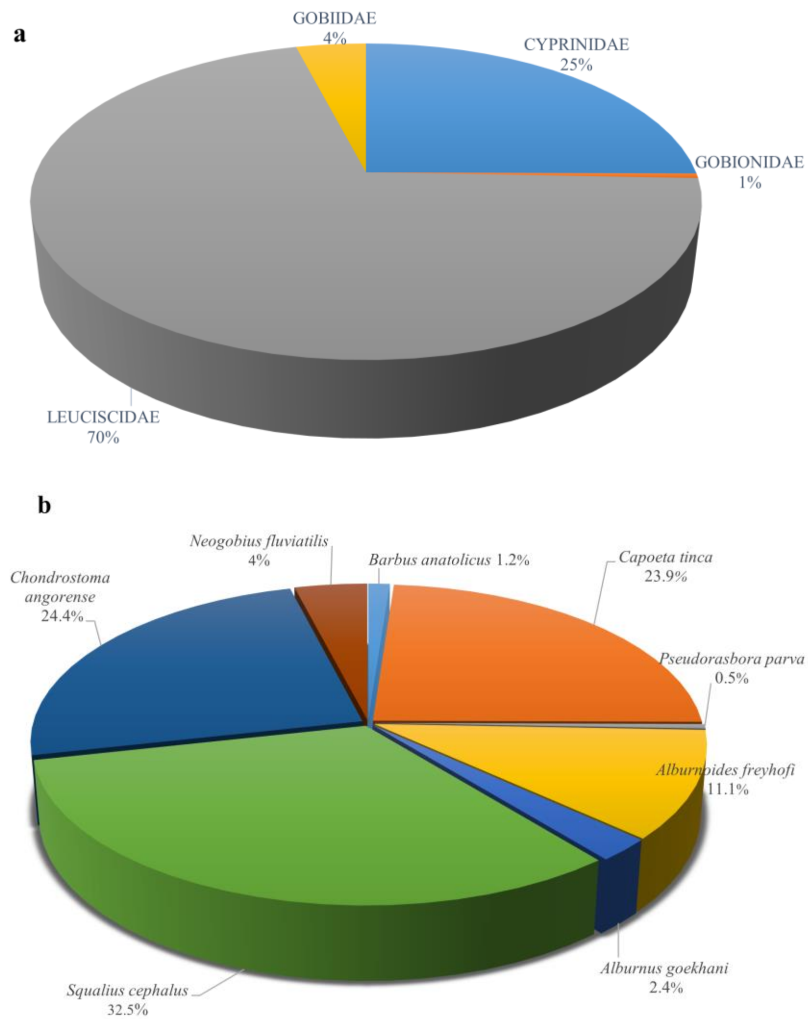

Figure 3. % distribution of families (a) and species (b)

The photos of *B. anatolicus*, *C. tinca*, *P. parva*, *A. freyhofi*, *A. goekhani*, *Squalius* sp., *C. angorense,* and *N. fluviatilis* samples are presented in Figures 4, 5. The number of species captured monthly is shown in Table 3.

Figure 4. Fishes of the Devrez Stream. **a,** *Barbus anatolicus* (SL: 147 mm) **b,** *Capoeta tinca* (SL: 165 mm) **c,** *Pseudorasbora parva* (SL: 77 mm) (fixed material) **d,** *Alburnoides freyhofi* (SL: 83 mm) **e,** *Alburnus goekhani* (SL: 100 mm). Fishes represented as “a, b, d, e” were captured from station 4, “c” was from station 5.

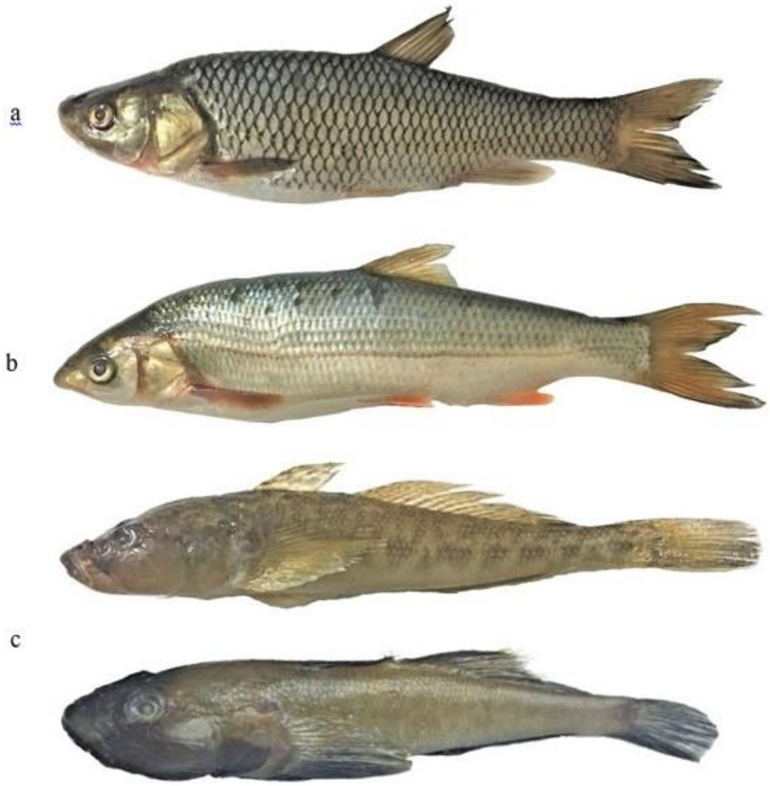

Figure 5. a, *Squalius* sp. (SL: 185 mm) **b,** *Chondrostoma angorense* (SL: 203 mm) **c,** *Neogobius fluviatilis* (SL: 106 mm) (above); a male individual with a black body in the breeding season (SL: 95 mm). Fishes represented as "a, b, c" were captured from station 4.

3.2. Vegetation

Devrez Stream was characterized by low turbidity, relatively shallow, and high abundance of emergent and submerged aquatic macrophytes e.g., *Potamogeton* sp., *Typha* sp., *Juncus* sp., *Scirpus* sp., *Ranunculus* sp. There were also *Mentha* sp., *Equisetum* sp., *Rubus* sp., *Fraxinus* sp., *Salix* sp., *Verbascum* sp., and *Urtica* sp. in the study area. Although sand and silt predominate in low-slope areas, gravel-cobble substrates form the stream bed in most areas (see Figure 2).

3.3. Human impacts

The construction of the Devrez Kızlaryolu Dam is considered the biggest threat to the stream ecosystem as it will change the stream structure and flow regime. In addition to this, there are villages and agricultural activities along the Devrez Stream basin. These parts of the stream are affected by domestic waste and agricultural aids such as pesticides and fertilizer. Agricultural irrigation together with drought causes a decrease in the amount of stream water in the summer months. Some local people engage in amateur fishing in the different parts of the stream, commonly by using gill nets and cast nets. The most preferred and caught fishes by inhabitants are *Squalius* sp., *C. tinca, C. angorense*, and *B. anatolicus*.

Table 3. Number of examined fishes and capture date in the Devrez Stream

	CYPRINIDAE		**GOBIONIDAE**	**LEUCISCIDAE**				**GOBIIDAE**	**Total number of samples**
Capture date and examined number of specimens	***B. anatolicus***	***C. tinca***	***P. parva***	***A. freyhofi***	***A. goekhani***	***Squalius* sp.**	***C. angorense***	***N. fluviatilis***	
April 2020	-	7	-	9	-	20	8	5	49
May 2020	1	6	2	17	7	12	7	1	53
June 2020	-	4	-	6	3	9	1	1	24
July 2020	1	8	-	-	-	1	11	2	23
August 2020	1	9	-	1	-	8	10	1	30
September 2020	2	12	-	7	-	13	9	3	46
October 2020	-	14	-	3	-	12	8	4	41
November 2020	-	11	-	-	-	15	10	-	36
December 2020	-	5	-	-	-	8	8	-	21
January 2021		8	-	-	-	15	10	-	33
February 2021	-	11	-	4	-	13	12	-	40
March 2021	-	6	-	-	-	11	9	-	26
SL (min-max) mm	**143-191**	**101-336**	**75-87**	**68-92**	**93-113**	**85-353**	**94-205**	**87-118**	**422**

4. DISCUSSION

Five out of eight fish species, *B. anatolicus, C. tinca, A. freyhofi, A. goekhani* and *C. angorense,* found in the Devrez Stream are endemic and the protection of these species is extremely important in terms of Türkiye's biodiversity. All the fishes caught in the Devrez Stream were native except *P. parva* which had spread rapidly in Türkiye's inland waters (Ekmekçi & Kırankaya, 2006). *P. parva* is considered a threat, especially for endemic species (İnnal, 2012) due to its high success in reproduction, growth, competition with other species, and transmission of parasites and new diseases that have not been seen before in the environment (Gozlan et al., 2005; Gürbüz, 2018; Özcan & Tarkan, 2019). Although its current population is low in the Devrez Stream according to our observations, *P. parva* might be a threat to endemic fishes in the future. Therefore, *P. parva* population should be monitored periodically for the sustainability of the stream ecosystem.

The most common and abundant species is *Squalius* sp. (32.5%) in the Devrez Stream. *Squalius* sp. distributed in the Kızılırmak and Yeşilırmak basins is considered to be a possible new species, because morphologically different from other *Squalius* species in the nearby basins (Bayçelebi, 2019). Therefore, further studies are needed to elucidate the *Squalius* sp. Although *C. tinca* and *C. angorense* are listed as the least concern (LC) in IUCN Red List (IUCN, 2022), their abundance category is decreasing. Fortunately, the relative abundance of these species compared to other species in the stream is not found low in the present study. As the native species, *Barbus anatolicus* (1.2%), *Alburnus goekhani* (2.4%) and *Neogobius fluviatilis* (4%) were the most rarely encountered species in the stream. Since they are recently described species (Turan et al., 2018, 2017; Özuluğ et al., 2018) conservation status evaluations for *B. anatolicus, A. freyhofi* and *A. goekhani* are lacking. Further detailed research is needed to indicate current population trends for fish species in the Devrez Stream.

İlhan and Balık (2008) identified 30 fish species and two subspecies in the Western Black Sea region. In their study, the Devrez Stream was one of the sampling locations in which seven fish species were identified as *Alburnoides bipunctatus*, *Barbus tauricus escherichii*, *Capoeta baliki*, *Capoeta sieboldii*, *Alburnus escherichii*, *Leuciscus cephalus*, *Nemacheilus angorae*. Freshwater fish diversity in the Anatolian Midwestern Black Sea basin was studied by Yoğurtçuoğlu et al. (2020). In their study, 13 species (*Rhodeus amarus, Barbus anatolicus, Capoeta tinca, Neogobius fluviatilis, Pseudorasbora parva, Alburnoides freyhofi, Alburnus derjugini, Alburnus escherichii, Alburnus goekhani, Squalius* sp.*, Oxynoemacheilus angorae, Oxynoemacheilus samanticus, Oxynoemacheilus seyhanensis)* were determined in the Devrez Stream. *A. bipunctatus*, previously reported by İlhan and Balık (2008), is understood to be the valid species as *A. freyhofi*.

İlhan and Balık (2008) reported *Barbus tauricus escherichii* (valid as *Luciobarbus escherichii)* in the Devrez Stream. It is thought that *L. escherichii* was not encountered in this study due to some restrictions such as using only gill nets, cast nets, and fishing rods in fishing. *Barbus anatolicus* was recognized in the present study, being recently recognised as a valid species (Turan et al., 2018). *B. anatolicus* inhabited only in Kızılırmak drainage while all records of *B. tauricus* were from small coastal rivers flowing into Black Sea (Turan et al., 2018; Yoğurtçuoğlu et al., 2020).

İlhan and Balık (2008) also found *Capoeta baliki* and *Capoeta sieboldi* in their study in the Devrez Stream. In this study, different from the findings of İlhan and Balık (2008), *Capoeta tinca* was determined. Meanwhile, recent studies show that *Capoeta baliki* was a junior synonym of *C. tinca* (Özdemir, 2015; Bektaş et al., 2019). Comparing the findings of present study with previous studies, *Chondrostoma angorense* was reported for the first time from the Devrez Stream. These differences might be due to differences in sampling locations, fishing tools, and low flow rates especially in summer months due to drought and agricultural irrigation.

Devrez Stream has significant importance for agricultural activities including rice farming, which requires much water in the region. According to the water samples taken from Devrez Stream, the irrigation water was determined as class C2S1. Devrez Stream has suitable irrigation conditions for all types of soil and plant species since it contains moderate salt and trace amounts of sodium having 450-600 micromhos/cm electrical conductivity and SAR (sodium adsorption rate) values between 0.33-1.63 (DSI, 2014). However, chemical fertilizers and pesticides used especially in rice farming and domestic wastes may leak into the soil and groundwater and cause toxic effects on fishes and other ecosystem elements in the basin in the long term.

The use of the Devrez Stream for irrigation purposes causes a decrease in the amount of water, especially in summer. Although dams and HEPPs change flow regimes (Fricke et al., 2007, Freyhof et al., 2014, Ablak-Gürbüz & Bonner, 2020) that play a primary role in riverine systems (Boavida et al., 2020), irrigated agriculture and domestic supply in the basin create water demand that requires the development of infrastructure such as dams (Zeiringer et al., 2018). Therefore, Devrez Kızlaryolu Dam construction was started in 2017 to support agricultural activities and energy production with a reservoir volume of 114.82 hm^3. While approximately half of this amount (61.67 hm^3) will be used for irrigation purposes, the amount reserved for aquatic organisms in Devrez Stream is only 8.07 hm^3 (DSI 2014). According to Acreman and Ferguson (2010), the critical abstraction level for rivers is 20%, except least sensitive rivers where 30% abstraction is allowed for the lowest level of protection. The abstraction in the Devrez Stream will be even more than 30%. Meanwhile, the planned environmental flow rate (0.26 m^3/s) of the Devrez Stream is far below the current flow rate (4.081 m^3/s).

Although the Devrez Stream might be classified as in good condition due to its ecological status (Acreman and Ferguson, 2010), changes in the flow regime disrupt river ecosystems through alteration of physical habitat and sediment supply. The flow regime is a significant factor that affects fish distribution and community structure (Osmundson et al., 2002). Even though Devrez Kızlaryolu Dam is thought to be economical as it will irrigate 15,989 ha of gross agricultural area and generate 5 MW of electricity per year (DSİ, 2014), the environmental flow rate to be released is crucial to protect aquatic life. Thus, once the Kızlaryolu Dam is operational, it should release the appropriate environmental flow to maintain the ecological status of the stream.

Dam construction also might be a threat, especially for fish species adapted to live in lotic systems since dams converted lotic environments to lentic habitats. In this study, all species were recorded as rheophilic fishes which prefer or live in swift and clear flowing water except *P. parva* and *N. fluviatilis*. Fortunately, despite being rheophilic, *C. tinca* and *Squalius cephalus* can also be found in some large lakes in Türkiye (Becer & Sarı, 2017). Yet, *S. cephalus* is potamodromous and needs to undertake spawning migrations to inflowing streams (Kottelat & Freyhof, 2007).

It is thought that when the Kızlaryolu Dam starts to hold water, these rheophilic fishes may lose their habitats. Many endemic fish species especially those that adapt to lotic systems in Anatolia are affected by similar situations (Fricke et al., 2007). According to Osmundson et al. (2002) Colorado pikeminnow populations endemic to the Colorado River Basin were extirpated in the lower sub-basin by the 1970s due to extensive dam construction during the 1930s to 1960s. Similarly, *Luciobarbus escherichii* which prefers flowing water is now less common due to the dams and HEPPs built on the Kızılırmak River (Çiçek et al., 2016). Although İlhan and Balık (2008) caught *L. escherichii* in the Devrez Stream at 40° 56' 27" N 33° 53' 52" E coordinates, it was not found in this study. Although the construction of the dam has not been completed yet, the fact that the species diversity is lower than in previous studies may be due to the geographical features and topographic structure of the river basin as well as the difference in the sampling method. The protection of the Devrez Stream, which has a high rate of endemic and native fishes, is important for the maintenance of its biodiversity.

Continuous turbidity occurring during the construction of the HEPP and dam, mixing of cement and other additives into the water, and structures that block the movement of aquatic organisms have also adversely affected aquatic life in the river. As a result of the decrease or even disappearance of the current, the benthic structure will also change, and the benthos adapted to the flowing water may largely disappear in the sections with stagnant water. Due to the drought, which is frequently seen in the summer, the water decrease in the Devrez Stream will become permanent with the construction of the dam.

5. CONCLUSION

Conservation of endemic species living in the Devrez Stream (62.5%) is of great importance in terms of Türkiye's fish biodiversity. Devrez Kızlaryolu Dam, which is under construction is thought to impose the greatest threat to fish in the Devrez Stream since the amount reserved volume of water for aquatic organisms is considered insufficient. It is crucial to determine the appropriate environmental flow rate for the stream structure to protect aquatic life. Moreover, as a non-native species, *P. parva* might be a threat to endemic fishes in the future, although its current population in the Devrez Stream is low. Therefore, the stream ecosystem should be monitored periodically for the sustainability of endemic and native fish species. This study will contribute to the monitoring and conservation strategies of the fish fauna of the Devrez Stream in the future.

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CONFLICT OF INTEREST

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

This article was written by a single author.

ETHICAL STATEMENTS

Ethics committee certificate was not requested since experimental animals were not used in the study and sampling was made in the form of dead fish from the fishermen.

DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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Research Article **Araştırma Makalesi**

Meiobenthic Assemblages of the Laspi Bay (Crimea, Black Sea): Taxonomic Diversity and Quantitative Development

Laspi Körfezi (Kırım, Karadeniz) Meiobentik Toplulukları: Taksonomik Çeşitlilik ve Kantitatif Büyüme

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Abstract: It is known that degassing processes are currently observed in the underwater part of the beach zone of Laspi Bay (the southern coast of Crimea). The site of gas emissions (methane) is confined to the coastal part of the bay and located at a distance of 15.0 – 40.0 m from the coastline. Data on the taxonomic richness and quantitative development of meiobenthos in Laspi Bay (2017) are presented for the first time in this study. The meiobenthic assemblages of the studied area were very diverse including representatives of 24 high taxa from Protozoa and Metazoa (phylum, class, order). The most numerous group at almost all stations was the free-living nematodes. The taxonomic richness by station did not reveal any noticeable variability, including 12 to 18 faunal groups, while an unevenness of spatial distribution in the density of meiobenthic settlements was clearly expressed. Minimum values of 63.2×10^3–72.9×10^3 ind./m^2 were noted in the seaward zone, and maximum values of 1368×10^3–2051×10^3 ind./m^2 in the eastern coast where localized methane seepage areas were observed.

Keywords
- Meiofauna
- Protozoa
- Metazoa
- Methane seeps
- Abundance

Özet: Laspi körfezi (Kırım'ın güney kıyıları)'nin sahil bölgesinde deniz tabanından gaz çıkışları olduğu bilinmektedir. Söz konusu gaz (metan) sızıntı alanları, körfezin kıyı kesimi ile sınırlı kalmaktadır ve kıydan 15.0-40.0 m mesafede yer almaktadır. Bu çalışmada, Laspi Körfezi meiobentosunun (2017) taksonomik zenginliği ve kantitatif büyümesi hakkında ilk veriler sunulmaktadır. Çalışma bölgesindeki meiobentik topluluklar çok çeşitlilik göstermiş ve Protozoa ve Metazoa'ya ait 24 yüksek takson (filum, sınıf, ordo) kaydedilmiştir. Neredeyse tüm istasyonlarda en bol bulunan organizma grubu serbest-yaşayan nematodlar olmuştur. İstasyonlardaki taksonomik zenginlik dikkate değer bir değişkenlik göstermemiş, her istasyonda 12-18 faunal grup tespit edilmiştir. Ancak meiobentik toplulukların yoğunluğunda düzensiz bir dağılım kaydedilmiştir. Doğu kıyısında, metan gazının çıktığı lokal alanlarda maksimum değerlere (1368×10^3–2051×10^3 birey/m^2) ulaşılırken, denize doğru ilerledikçe minimum değerler (63.2×10^3–72.9×10^3 birey/m^2) gözlenmiştir.

Anahtar kelimeler
- Meiofauna
- Protozoa
- Metazoa
- Metan sızıntı kaynakları
- Bolluk

1. INTRODUCTION

Laspi Bay is an open bay, located in the southwestern part of the Crimean Peninsula (the Black Sea, 44°024'44''– 44°025'20''N; 33°042'10''– 33°042'45''E); the length of its coastline, limited by the capes Sarych and Aya, is about 4 km. The hydrodynamic regime of its water area is a result of the influence of anticyclonic circulation systems, the inflow of deep waters into the surface layers due to surge phenomena and water exchange with the open sea, which contributes to the dynamic activity and aeration of waters (Atsikhovskaya and Chekmeneva, 2002).

Even during the Greek colonization of the peninsula, it received its name from the main and only village of Laspi that existed by the end of the 18th century (the saddle of the Ilya-Kaya and Machuk mountains). The literal translation of the Greek word "laspi" ("dirt, turbidity") reflects periodically repeated mudflows, floods, and active landslide activity in the territory of the Laspinskaya basin. The complex landslide slope outlining the Laspi Bay has a highly dissected relief and is indented by numerous erosion troughs, which are the channels of temporary streams. During precipitation, these bring muddy freshwater into the bay, with parameters very different from seawater, which in turn, affects the distribution of hydrological parameters in the coastal zone (Budnikov et al., 2019).

The first studies of the diversity and spatial distribution of benthic communities in Laspi Bay were carried out in 1983 at depths up to 25 m (Petuhov et al., 1990, 1991). As a result, 49 species of macrobenthic fauna were found, and based on the principle of species dominance at these depths, the authors identified two groups of communities as *Venus gallina* and *Gouldia minima-Pitar rudis*. Subsequently, a biodiversity assessment and a study of the status of macrozoobenthos in the zone of loose sediments of Laspi Bay was carried out in 1996, as a result of which 131 species of benthic fauna were identified. Particularly, Mollusca (44 species), Annelida (43), Crustacea (31), and 13 species of other groups were recorded (Revkov and Nikolaenko, 2002). At the studied site, the authors identified three independent biocoenotic complexes confined to the coastal and more open areas of the bay. The most significant differences in the complexes were associated with the development of certain dominant species. The authors concluded that the total number of species in the Laspi Bay, the number of species of macrozoobenthos inhabiting loose sediments of the Crimean coast of the Black Sea agreed with the long-term studies (Kiseleva, 1981).

The results obtained verify the high species richness of the zoobenthos in Laspi Bay, which allowed the authors to designate this area as one of the natural aquatic gene pool reserves of the region providing information about the relatively favorable condition of the bottom ecosystems of the studied area at the Southern coast of Crimea.

Meiobenthos, as an important component of the bottom communities of Laspi Bay, has not been previously studied, and therefore full assessment of the structure of benthic communities couldn't be possible in this area during the study periods. In recent years, the coastal zone of Laspi has been actively built up, and the water area of the bay is subjected to domestic pollution through a collector that discharges polluted water from a coastal hotel complex into the bay.

Sanitary-biological research in separate parts of the Laspi Bay showed that this coastal water area can be generally characterized as relatively safe at present based on certain parameters. However, several indicators (the content of petroleum hydrocarbons in water, specifically in the bottom layer and the relatively high concentration of heterotrophic bacteria in seawater and bottom sediments) show that this part of the water area, which was previously considered a standard clean zone, is experiencing a significant anthropogenic impact. The obtained results may be related to the active development of the Laspi area observed in recent years. Pollutants enter the aquatic environment of the coastal waters of the Cape Aya reserve through sewage and other waters, and the environmental parameters indicate a low level of oxidation and, accordingly, self-purification (Tikhonova et al., 2020).

2. MATERIAL AND METHODS

2. 1. Study Area

In recent decades, outgassing has been observed in the underwater part of the Laspi Bay beach area. Methane emission zones, occupying a certain bottom area in the bay, were discovered by Shik (2006) in the summer of 2004. Gas seeps were visually noted at a distance of 15.0–40.0 meters from the coastline. The composition of gas fluid bubbles is represented by methane, ethane, propane, and hydrogen sulphide, which indicates the deep origin of the bubbles (Lysenko & Shik, 2015).

Scientists who monitored gas releases in the summer period from 2004 to 2013 (Lysenko & Shik, 2014) found a change in the location and number of specific points of gas bubble escapes while noting that the degassing area itself remained approximately within the same boundaries. Its total area was about 500 square meters. During the studies conducted in different years about these gas releases in the Laspi Bay, 10 to 20 points of jet gas seeps were recorded. On average, a flow of 30 to 80 gas bubbles per minute was observed from the seeps, ranging in size from 5.0 to 15.0 mm (possibly smaller bubbles also existed). The authors observed periodicity in their emissions. Based on the chemical analysis of data, methane was the main component of seeps and it was concluded that there were no fundamental differences in the composition of hydrocarbons between the gasses released in Laspi Bay and the fluids of deep-sea seeps of the Black Sea (Lysenko, 2014; Lysenko & Shik, 2015). More than 20 individual points of bubble gas emissions were also hydroacoustically and visually recorded by other researchers in the bay (Malakhova et al., 2015).

Jet gas seeps can affect the hydrochemical composition of water, as well as the temperature distribution in the overlying water column due to bubbling properties. Using an algorithm based on the analyses of the recorded acoustic signals generated by the bubbles, Ivanova et al. (2021) calculated the gas release rate for three shallow-water seeps in Laspi Bay, derived from audio recordings obtained during the summer of 2019. The rates were 14–16 bubbles/s and gas flows were 21, 46, and 28 L/day, respectively. Budnikov et al. (2019) estimated the daily volume of gas from permanent bubble gas emissions as 69 – 106 L/day released from the bottom of the site at a depth of 2 m in the bay.

However, to date, there has been no systematic observation of the volumes of emissions and the composition of cold gas jets over the entire bottom area and the coast of the bay over a long time. Lysenko and Shik (2013) suggested that the current activity of gas seeps in Laspi Bay was associated with seismic processes in the Foros uplift. Gas jets were indicators of the state of the degassing subsoil. The connection of jet gas emissions with geological bottom structures was previously shown in several studies (Shnyukov et al., 2005; Artemov et al., 2007).

The impact of methane on benthic organisms of the Black Sea has been poorly studied (Sergeeva & Gulin, 2007; Polikarpov et al., 1998; Luth & Luth, 1998) with contradictory data. Inarguably, the fluctuating process of gas release from the bottom into the habitat of the benthic fauna, along with the anthropogenic factors, can influence the formation of the structure and quantitative development of the bottom communities inhabiting Laspi Bay.

It is known that the effect of various methane concentrations on bottom ecosystems is significantly different and much more complex than previously considered (Yanko et al., 2009; Shnyukov & Yanko, 2014). The relationship between the distribution of meiobenthos and hydrocarbon gas concentrations (mostly methane) has been studied in the sediments of the north-western part of the Black Sea (Yanko et al., 2014, 2017a, b). Based on the analysis of abiotic parameters (physical and chemical parameters of the water column, geochemical, lithological, and mineralogical properties of the sediment) and biotic characteristics (quantitative and taxonomic composition of foraminifers, nematodes, and ostracods), the authors proposed the possibility of using meiobenthos as indicators of gaseous hydrocarbons deposited under the seabed.

In this paper, the taxonomic composition and quantitative distribution of meiobenthos in the water area of Laspi Bay are investigated for the first time. Considering the above, it was of interest to study the taxonomic structure of the meiobenthos and its spatial distribution in this area where methane degassing points were described. It should be borne in mind that we can only give an objective assessment of the recent spatial distribution and development of the meiofauna at this stage without discussing the specific relationship of these indicators with the activity and localization of degassing or the distribution of organic pollution due to the lack of indicator factors. Comprehensive hydrobiological and geological studies are needed to identify the existing relationships between the development of various meiobenthic taxa and the above-mentioned environmental factors.

2. 2. Sampling and processing of samples

In the Laspi Bay, 19 benthic stations were studied in the depth range of 4 – 21 m (Figure 1, Table 1). Bottom sediments were sampled (as two replicates) using push cores (height: 5 cm, mouth area: 18.1 cm^2) at each station by Scuba diving to study the meiobenthos of the area, and a manual bottom grab was used to sample the macrobenthos. Samples of bottom sediments were individually fixed using 75% alcohol immediately after the sampling. In the laboratory of the IBSS RAS, each sample was carefully washed through a sieve set, the upper one with a mesh size of 1 mm, and the lower one of 63 μm. Bengal rose solution (0.1%) was added to the sediment fractions retrieved on sieves to stain the material, and the samples were studied under a binocular microscope using a Bogorov chamber. All organisms recorded in the sample were counted and identified to the higher taxon level. Subsequently, all calculated and recorded individuals were extracted and temporary slides (glycerine + pure water) were prepared for their identification to species or morphospecies/morphotype level. Morphological analysis of the fauna was performed using Olympus CX41 and Nikon E200 stereomicroscopes equipped with a digital camera connected to a PC.

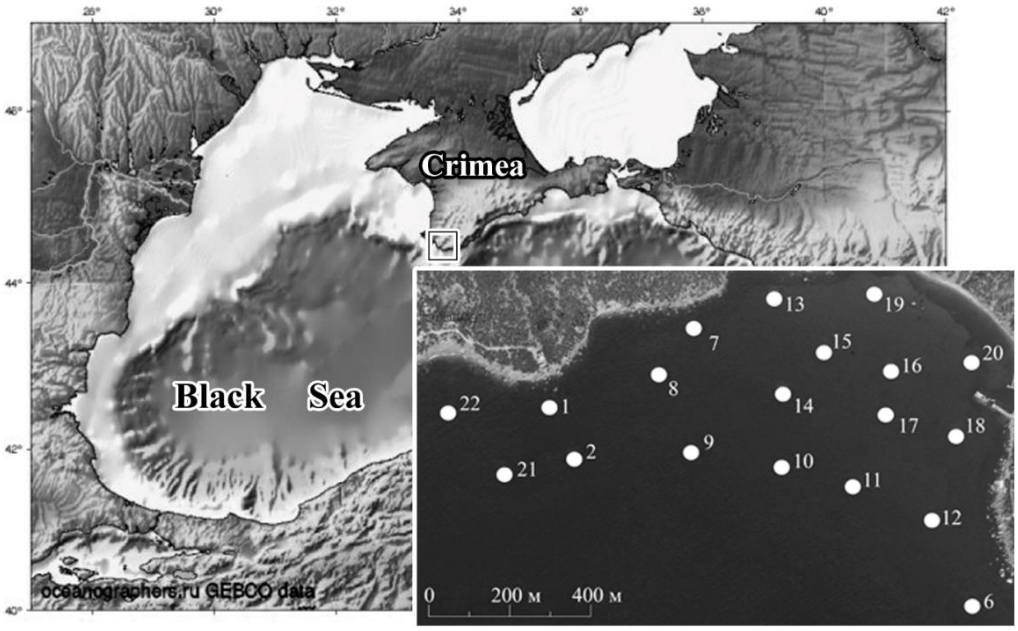

Figure 1. Sampling stations located in the Laspi Bay.

Concurrently, samples from the same stations were taken by the staff of the Marine Hydrophysical Institute of RAS for analysis of the geochemical composition of the bottom sediments (Table 1). Psammitic sediments were mainly characterized by fine-grained and coarse-grained fractions, with an admixture of stone and shell pebbles, as well as silty and pelitic material to varying degrees. According to the authors, the C_{org} (organic carbon) content in the bottom sediments of the bay varied within a range of 0.09 — 0.46%. The absence of Corg accumulation within the investigated depths was primarily due to the granulometric composition of the sediment, the hydrodynamic factor and the morphometric features of the coastal area of the bay (Orekhova & Ovsyany, 2020).

Carbonate structures, which were formed as a result of methane degassing from the sea bottom, were found at a depth of 1.5 – 2 m in the area of stations 13, 15, 19, and 20 (Lysenko & Shik, 2017). These structures with settled polychaetes and mollusks provided us with evidence of this process. The area of jet gas release is confined to the apex of the bay, where it somewhat protrudes to the coast (Station 19).

In addition, gas diffusions into beach sands and gravel stones were observed. Gaseous fluids may exist in the rest of the bay bottom (Budnikov et al., 2019). Undoubtedly, these processes enrich the bottom sediments of the coastal and central zones of the bay with methane bacteria, which are included in the trophic relationships of the benthic fauna. For a clear understanding of these phenomena, special comprehensive studies are required focusing on the benthic zone of Laspi Bay.

Table 1. Data of the stations in the Laspi Bay (2017) (by Orekhova and Ovsyany, 2020)

Station	Coordinates	Depth, m	Humidity, %	Sediment characteristics
1	44°25'05,5"N - 33°41'43"E	14.5	29.4	Dark-grey sand (91)* with alevrite (8) and gravel admixture (1)
2	44°25'01"N - 33°41'45"E	18	23.3	Coarse-grained sand (52) with gravel (47) and alevrite admixture (1)
6	44°24'50"N - 33°42'32"E	13	6.8	Stone gravel (99) with sand admixture (1)
7	44°25'12"N - 33°41'58"E	10	26.1	Dark-grey fine-grained sand (95) with alevrite (5)
8	44°25'08"N - 33°41'55"E	13	31.4	Dark-grey fine-grained sand (87) with alevrite (12) and shell gravel admixture (1)
9	44°25'02,5"N - 33°41'59"E	21	26.3	Dark-grey sand (57) with alevrite (42) and shell gravel admixture (1)
10	44°25'01,5"N - 33°42'10"E	14	27.6	Dark-grey sand (75) with alevrite (28) and shell gravel admixture (3)
11	44°25'00"N - 33°42'17"E	14	27.7	Dark-grey fine-grained sand (75) with alevrite (25)
12	44°24'57"N - 33°42'25"E	15	25.1	Dark-grey fine-grained sand (80) with alevrite (16) and shell gravel admixture (4)
13	44°25'14"N - 33°42'07,5"E	6	21.6	Sand (98) with gravel (1) and alevrite (1) admixture
14	44°25'07"N - 33°42'08"E	12	21.4	Sand (98) with gravel (1) and alevrite (1) admixture
15	44°25'10"N - 33°42'13"E	9	22.9	Dark-grey medium-grained sand (98) with alevrite admixture (2)
16	44°25'08"N - 33°42'20,5"E	8	22.5	Dark-grey fine-grained sand (86) with alevrite (13) and gravel admixture (1)
17	44°25'04"N - 33°42'22"E	10	27.4	Dark-grey fine-grained sand (64) with alevrite (35) and gravel admixture (1)
18	44°25'03,5"N - 33°42'28"E	9	21.2	Dark-grey coarse-grained sand (84) with gravel (15) and alevrite admixture (1)
19	44°25'14,5"N - 33°42'18,5"E	4	25.0	Dark-grey medium-grained sand (99) with alevrite admixture (1)
20	44°25'09"N - 33°42'29,5"E	5,5	23.1	Dark-grey medium-grained sand (92) with gravel (7) and alevrite admixture (1)
21	44°25'01"N - 33°41'37"E	17	19.0	Gravel (66) with sand (33) and alevrite admixture (1)
22	44°25'05"N - 33°41'35"E	11	-	Sand

* Percentage of the fractions is given in parenthesis.

Açıklama [DÜ1]: Is it possible to rearrange it on one line?

2. 3. Data Analysis

The meiobenthos composition was evaluated using multivariate statistics in the PRIMER 6 package (Cluster, SIMPER analyses) (Clarke, 1993; Clarke & Gorley, 2001). The presence/absence transformation of the number of taxa at the stations was used for the Cluster analysis. The measure of station similarity was based on the Bray-Curtis similarity coefficient. The determination of the leading taxa in the meiobenthos (SIMPER analysis) was carried out based on the assessment of their contribution to the intracomplex similarity based on the untransformed values of their abundance.

3. RESULTS and DISCUSSION

3. 1. Distribution of meiobenthic diversity and abundance

The meiobenthos composition of the studied area was very diverse with representatives of 24 high taxa both from Protozoa and Metazoa (phylum, class, order). The representation of meiobenthos at the high-level taxa showed no noticeable fluctuations, ranging from 12 to 18 faunal groups station by station, while a distinct variability is revealed in the distribution of the density of meiobenthos. The minimum values (63.2×10^3 – 72.9×10^3 ind./m^2) were noted in the seaward zone, and the maximum values (1368×10^3 – 2051×10^3 ind./m^2) were recorded on the eastern coast (Figures 2 and 3).

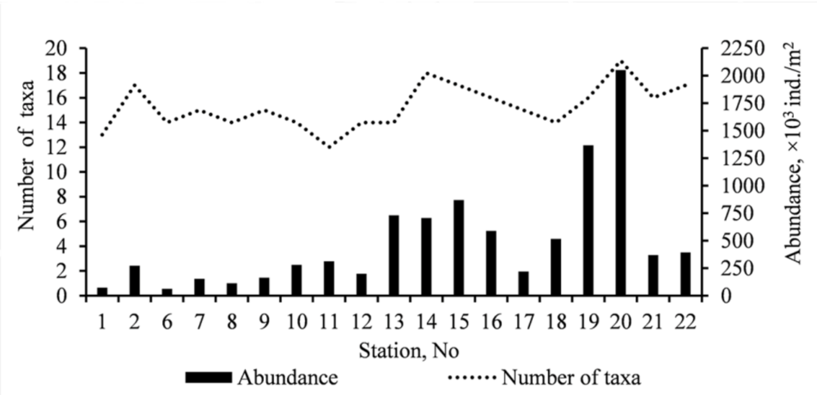

Figure 2. Abundance (10^3 ind./m^2) and diversity of meiobenthos in the Laspi Bay.

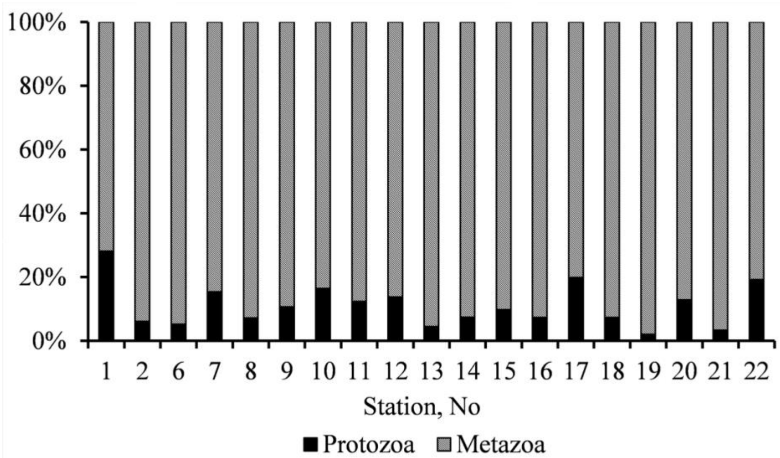

Figure 3. The share of Protozoa and Metazoa (based on densities) in the meiobenthos of the Laspi Bay.

In many publications focusing on the structure of benthic communities, the authors individually considered three size groups of benthos: macro-, meio- and microbenthos. It should be noted that the latter group is practically not taken into account in most of the studies when assessing the structure of benthic communities. Exclusively, multi-chambered hard-shelled foraminifers can be recognized among the protozoans as an exception, which has been included in benthic communities in just several works.

Based on our results on the Black Sea benthic communities, including the fauna of Protozoa and Metazoa, we cannot agree with this approach, since protozoans often exceed the linear dimensions of microbenthos in their size parameters, adopted in the classification of benthic groups (Mare, 1942). Representatives of Ciliophora, Gromiidea, and soft-walled Foraminifera may exceed the accepted sizes of micro- and meiobenthos by an order of magnitude or more in our collections. Moreover, in some cases, these representatives should be considered as part of the macrobenthos, not of meiobenthos and microbenthos based on their size (Sergeeva, 2019; Sergeeva and Anikeeva, 2018, 2020). Benthic protozoa are very numerous; they enter into trophic relationships with both microflora and meio- and macrofauna. They play an important role in the circulation of organic substances in the bottom ecosystems of water bodies, utilizing bacteria, fungi, diatoms, and bottom fauna. At the same time, they serve as a trophic link for higher benthic fauna (Sergeeva, 2019; Sergeeva and Anikeeva, 2018).

Given the information above, we also included the analysis of Protozoa to obtain a structure close to the real character of the meiobenthos of the Laspi Bay (macrobenthos will be evaluated in a different paper by other authors).

Protozoa. The population density of protozoa (Figure 3) in the meiobenthos of Laspi Bay accounted for a significant share (up to 20 – 26%) of the total meiobenthic structure. The share of protozoa in the composition of the meiobenthos was similar among the stations, with some exceptions (stations 13, 19, and 21). It should be considered that unaccounted factors are also available which unquestionably play a role in the density of protists and meiobenthos in general.

Ciliophora (Cl), Gromiidea (Gr), soft-walled (SWF, Monothalamea), and hard-shelled Foraminifera (HSF) were recorded in the studied area. Information about Protozoa of Laspi Bay is presented for the first time in this paper.

The share of individual groups of benthic unicellular organisms in the total number of Protozoa is presented in Figure 4, from which three dominant groups of unicellular organisms can be seen as Ciliophora, Gromiidea and soft-walled Foraminifera. These groups are permanent components in the taxocenoses of Protozoa of the Laspi Bay, while hard-shelled Foraminifera was exclusively recorded at several stations in small numbers. Soft-walled foraminifers (allogromiids) had the greatest quantitative development, however, gromiids, and ciliophorans also accounted for a significant proportion in the total abundance of Protozoa, while at some stations their share exceeded that of allogromiids. Since we cannot explain this situation in detail, specific studies are needed about the protozoans of the bay concerning environmental factors and the development of macrobenthos. It cannot be ruled out that bioturbation and trophic factors play important roles in the density of protozoa along with degassing and anthropogenic impact.

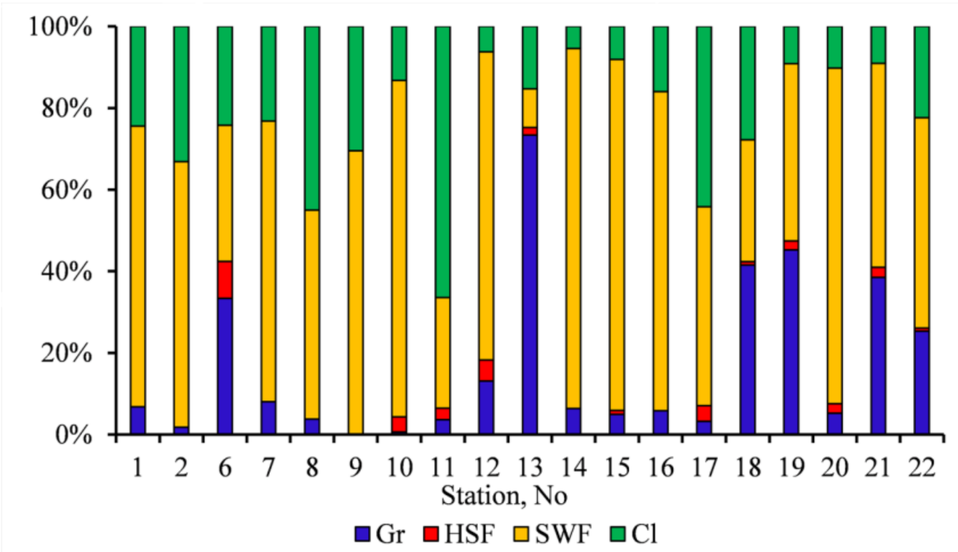

Figure 4. The share of benthic unicellular organisms in Protozoa of the Laspi Bay (Gr: Gromiidea, HSF: Hard-Shelled Foraminifera, SWF: Soft-Walled Foraminifera, Cl: Ciliophora).

Metazoa. 20 high-level taxa were recorded in the meiobenthic communities of the bay: Nematoda, Polychaeta, Oligochaeta, Platyhelminthes (Turbellaria), Nemertea, Kinorhyncha, Rotifera, Gastrotricha, Arthropoda (Harpacticoida, Ostracoda, Cumacea, Amphipoda, Decapoda, Diptera, Arachnida, Tardigrada), Mollusca (Bivalvia, Gastropoda, Polyplacophora) and Cnidaria. It is worth noting that a few representatives of unknown organisms were also found at some of the stations. These organisms, which were not identified to a class or order (incertae sedis) were referred to as "Miscellaneous". Characteristically, the taxonomic richness, including Protozoa, did not significantly vary throughout the studied area (12 – 18 in the rank of type or class) (Figure 2). Protozoa inhabited all the studied stations, and the taxonomic diversity of Metazoa varied within 8 – 14 faunal groups.

The multicellular meiobenthic composition of the Laspi Bay included the permanent meiofauna (eumeiobenthos) and the temporary meiofauna including the early stages of macrobenthos (pseudomeiobenthos). Tardigrada, Rotifera, and Gastrotricha were recorded for the first time in this area in the composition of the meiobenthic fauna. Representatives of two new genera of Gastrotricha— *Diplodasys* Remane, 1927 and *Tetranshyroderma* Remane, 1926— are the first records for the Black Sea (unpublished data). Gastrotrichs were distributed almost over the entire water area of the bay, and their population density varied between 2700 and 10500 ind./m^2. The greatest values were recorded in the central part of the bay (stations 13, 14, and 15). Tardigrades were also distributed over most of the bay with a population ranging from 300 to 43100 ind./m^2, with a maximum value at station 13.

In terms of abundance, the largest proportion of meiobenthos belonged to the free-living nematodes and the harpacticoids (Figure 5, Table 2). Quantitative indicators of the total density of meiobenthos and dominant taxa (nematodes and harpacticoids) decreased with depth (Fig. 6). Maximum numbers of nematodes (863 – 511×10^3 ind./m^2) and harpacticoids (774 – 671×10^3 ind./m^2) were recorded at the minimum depths of 4-5.5 m (stations 19 and 20).

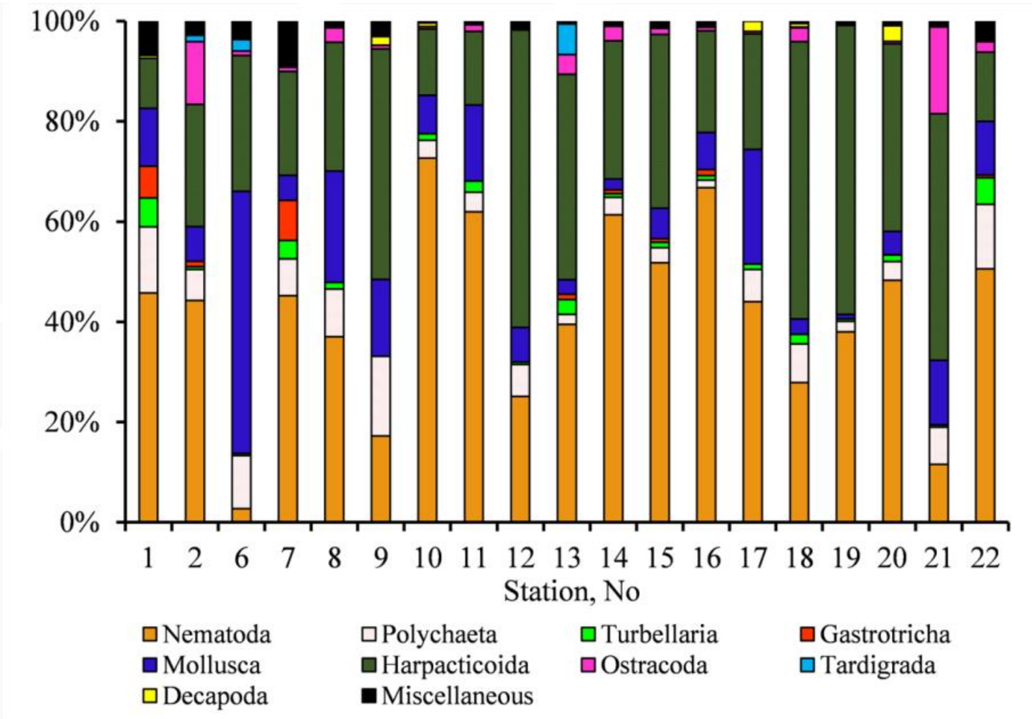

Figure 5. The share of the most characteristic Metazoan groups (based on densities) in the meiobenthos of the Laspi Bay.

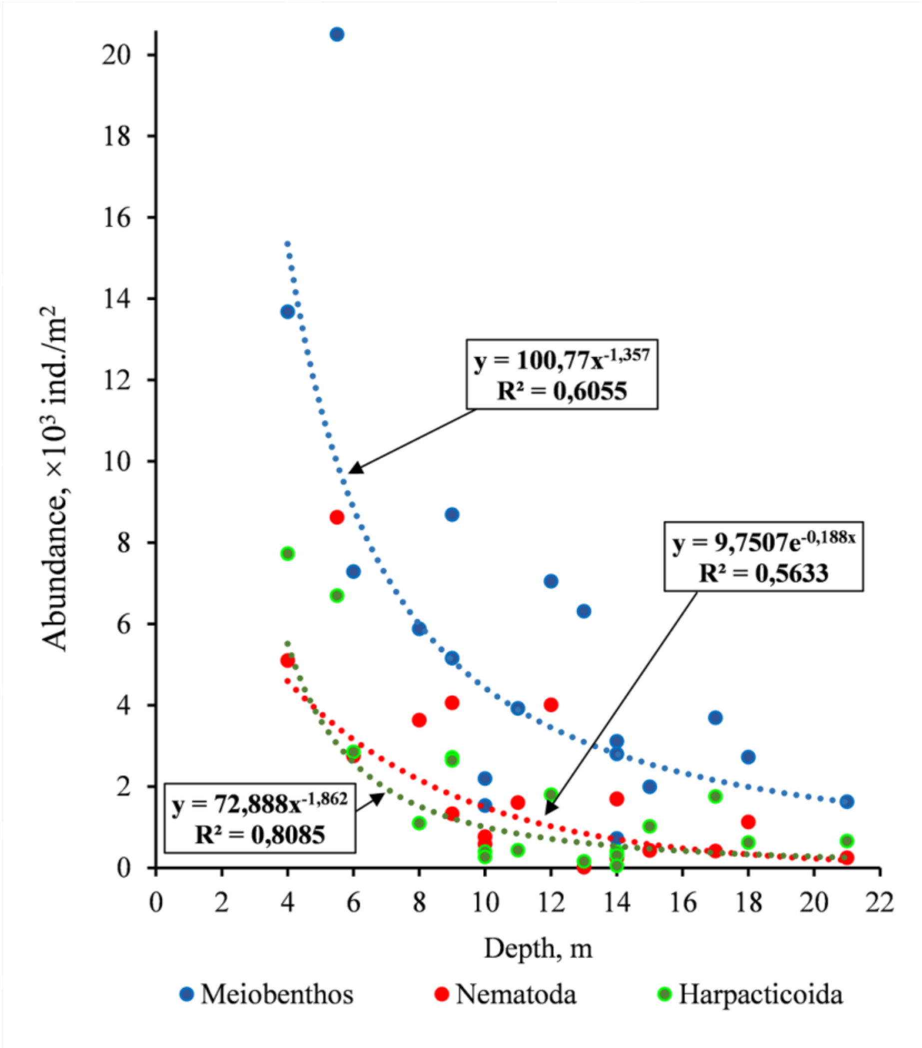

Figure 6. The abundance of meiobenthos and the dominant taxa along the depth gradient.

3. 2. Meiobenthos and the dominant taxa

Nematoda and Harpacticoida were the leading groups (Table 2), whose total contribution to intracomplex similarity, based on absolute abundance values was 67.19%. These two groups were followed by Bivalvia (10.4%), Polychaeta (7.1%), and soft-walled Foraminifera 6.7%). Multivariate analysis of the data using supraspecific taxonomic diagnostics did not allow for the identification of individual faunal complexes of meiobenthos in the Laspi Bay. The faunal similarity of all stations (Bray-Curtis similarity) of the area was relatively high and exceeded 75%.

Table 2. Main meiobenthic taxa based on their contribution to the interstation similarity, calculated using untransformed values of their average abundances (N, ind./m^2)

Taxa	N ± Standard	Minimum	Maximum	i	i / SE(i)	i%	Cum., i%
Nematoda	204092 ± 50673.1	1656	863052	17.47	1.39	38.74	38.74
Harpacticoida	168140 ± 49507.9	5244	773628	12.71	1.43	28.19	66.93
Bivalvia	26635 ± 4244.4	4968	80040	4.68	1.18	10.37	77.31
Polychaeta	)024 ± 3507.4	5348	;7068	20	.76	10	34.41
Foraminifera (soft-walled)	1013 ± 11101	1104	16384	03	.16	73	)1.14
Ciliophora	8293 ± 1823	828	:6772	16	.05	56	)3.70
Ostracoda	)415 ± 3550.5	0.00	;1824	66	ı.61	47	)5.17
Platyhelminthes	457 ± 1580.6	276	:4288	66	.29	46	)6.63
Other	3249 ± 713.9	552	.0935	48	ı.90	05	)7.69
Gromiidea	5852 ± 1676	0.00	:4012	42	ı.82	92	)8.61
Gastropoda	1249 ± 162.7	276	2484	26	.12	58	)9.19
Gastrotricha	2322 ± 733.1	0.00	.0443	15	ı.38	34	)9.53
Foraminifera (hard-shelled)	843 ± 317.6	0.00	5072	06	ı.54	13	)9.66
Arachnida	422 ± 114.4	0.00	1932	04	ı.58	09	)9.75
Amphipoda	552 ± 177.8	0.00	3036	04	ı.47	08	)9.83
Tardigrada	731 ± 2248.5	0.00	;3056	03	ı.30	06	)9.89
Cumacea	291 ± 95.5	0.00	1656	02	ı.44	05	)9.95
Rotifera	887 ± 429.7	0.00	5072	01	ı.23	02	)9.97
Oligochaeta	247 ± 159.2	0.00	3036	01	ı.27	01	)9.98
Nemertea	73 ± 41.4	0.00	552	00	ı.13	01	)9.99
Decapoda	022 ± 2826.7	0.00	;3820	00	ı.12	01	00.00
Diptera	44 ± 23.7	0.00	276	00	ı.13	00	00.00
Kinorhyncha	88 ± 73.3	0.00	1380	00	ı.08	00	00.00
Polyplacophora	15 ± 14.5	0.00	276	00	-	00	00.00
Cnidaria	73 ± 72.6	0.00	1380	00	-	00	00.00

Note: N is the average value of the number; i – absolute and i % – relative contributions of taxon “i” to the average Bray–Curtis similarity within the complex; SE - standard error

4. CONCLUSION

The results of the meiobenthos studies in Laspi Bay (2017) are presented for the first time in this paper. Meiobenthos of this water area is very diverse considering Protozoa and Metazoa, with a composition including 24 major taxa (type, class).

In the composition of benthic Protozoa throughout the entire area of the bay, Ciliophora, soft-walled Foraminifera, and Gromiidea presented with high densities, while hard-shelled Foraminifera was found exclusively at some of the stations and only in small quantities.

The diversity of higher taxa did not show significant fluctuations, ranging from 12 to 18 faunal groups in the area. However, a distinct variability was observed in the distribution of meiobenthos density as a whole. Its minimum values were 63.2×10^3 – 72.9×10^3 ind./m^2 in the open zone, and the maximums were 1368×10^3 – 2051×10^3 ind./m^2 in the inner eastern coastal part. The dominant groups were free-living nematodes and harpacticoids, and the role of these groups in the meiobenthic communities showed spatial variations. In general, the indicators of taxonomic richness and quantitative development of meiobenthic communities in the Laspi Bay are comparable to other water areas of the Crimean coastal zones (Sergeeva & Mazlumyan, 2006; Sergeeva et al. 2011; 2012). As various areas of Crimea were taken into consideration, the highest mean abundance value of meiobenthos was lower (596.2×10^3 ind. m^{-2}) in previous studies (Sergeeva, 2003).

Similar trends have been reported from the southern coast of the Black Sea (Sinop Bay, Türkiye) where 25 higher taxa were found with total meiobenthos abundances ranging from 18×10^3 – 935×10^3 ind./m^2 (Ürkmez et al., 2016a). A study on meiobenthos at the southwestern coasts (Igneada, Turkiye) of the Black Sea reported abundance values of 67–757 × 10^3 ind. m^{-2}, much lower than the documented values in the present study Sampling area in Igneada was represented with low human activity with limited anthropogenic impact (Ürkmez et al., 2016b).

It is assumed that the uneven quantitative distribution of meiobenthos may be due to the local (methane) degassing from the bottom of the Laspi Bay and the incoming domestic sewage. Future comprehensive geological, hydrochemical, and hydrobiological studies will allow us to describe the interaction of benthic communities with the above environmental characteristics of the studied bay.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Conceptualization: NGS; Methodology: NGS; Investigation: NGS; Formal analysis: TNR; Manuscript writing — original draft: NGS, TNR; Manuscript writing —review — editing: DU; Visualization: TNR; Discussion: DU; Translation: DU; Supervision: NGS; Finishing formalization: DU. All authors approved the final draft.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

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Research Article Araştırma Makalesi

Importance of Stability Analysis for Sustainable Fisheries in the Absence of Important Data

Önemli Verilerin Eksikliğinde Kararlılık Analizinin Sürdürülebilir Balıkçılıktaki Önemi

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Abstract: Assessment of fish stocks is especially important to avoid overfishing and obtain sustainable fishing policies, and there are many stock assessment methods such as XSA, VPA, BMS, CMSY, and MSVPA to analyze fish stocks. However, these assessment methods require an important amount of data for fish stocks such as diet data, natural mortality, fishing mortality, abundance index of species, predator ratio estimates, and so on. Unfortunately, we do not have such data for most of the fish stocks, and obtaining such data requires an important amount of money and time, but we still can predict important information about fish stocks such as biomass of fish stocks, the maximum sustainable yield, the biomass of fish lost or gained due to predator-prey relations, and even can track the effect of harvesting on predator-prey relations by building a mathematical model for fish populations and implementing a stability analysis. To obtain these outputs, we only need landing data and implement a parameter estimation constrained on stability conditions derived from the stability analysis of the mathematical model. Shortly, this study shows us how important stability analysis is to obtain important information about fish populations in the absence of important data.

Keywords

- Food chain
- Predator-prey relation
- Equilibrium
- Stability
- Sustainable fishery

Özet: Aşırı avcılığın önlenmesi ve sürdürülebilir balıkçılık politikalarının elde edilmesi için balık stoklarının yönetimi çok önemlidir ve balık stoklarını analiz etmek için XSA, VPA, BMS, CMSY ve MSVPA gibi birçok stok yönetim metotları vardır. Fakat bu stok yönetim metotlarını kullanmak için balık stoklarının beslenme verisi, doğal ölüm oranı, avlanan balık miktarı, balık stoku miktarı indeksi, avcı balık oranı gibi önemli dataların olması gerekir. Ne yazık ki, birçok balık stoku için bu tarz verilere sahip değiliz ve bu verileri elde etmek ekonomik olarak çok maliyetli ve zaman alıcı. Fakat bu veriler elimizde olmasa da matematiksel modeller ve kararlılık analizi yardımıyla balık stoku miktarı, maksimum sürdürülebilir avlanma miktarı, av-avcı ilişkisinin balık miktarına etkisi, avcılığın türler arası av-avcı ilişkisi üzerine etkisi gibi balık stokları ile alakalı birçok önemli bilgiye ulaşabiliriz. Bu önemli model çıktılarını elde edebilmemiz için, sadece avlanan balık miktarı verisi ve oluşturulan matematiksel modelin kararlılık analizine bağlı parametre tahmini yapmak yeterlidir. Kısacası bu çalışma, balık popülasyonları ile alakalı önemli veriler elimizde olmadığında, kararlılık analizinin balık popülasyonları ile alakalı önemli bilgileri elde etmedeki öneminden bahsetmektedir.

Anahtar kelimeler

- Besin zinciri
- Av-avcı ilişkisi
- Denge noktası
- Kararlılık analizi
- Sürdürülebilir balıkçılık

1. INTRODUCTION

Fish populations are very important members of aquatic systems since they play an important role in food webs from bottom-up or top-down in ecosystems. Thus, to sustain resilient and healthy ecosystems, the dynamics of fish populations are crucial in aquatic systems. Besides their importance in aquatic ecosystems, they are also important for humans worldwide as a source of food. However, fish populations have been facing overfishing worldwide due to wrong harvesting strategies or high exploitation/harvest rates applied to fish populations (Hilborn, 2012; Bardey, 2019). Thus, before applying any harvesting strategies, it is important to analyze the aquatic ecosystem in terms of the abundance of important species in the targeted fish population's food web.

There have been many conventional stock assessment methods for investigation on the abundance of fish populations such as XSA, VPA, BMS, CMSY, and MSVPA but most of such methods consider a single species model and require additional data in addition to the landing data. The most complete one among these assessment methods is the MSVPA method since this stock assessment method considers predator-prey relations by using multi-species models rather than a single-species model by including important predator and prey effects on a target fish stock. However, to perform MSVPA, detailed food-habit information is required. For example, the MSVPA assessment method requires data such as natural mortality, an estimate of fishing mortality from the previous year, an abundance index of species, suitability estimates, weight-at-age (or average weights), predator ratio estimates, and diet data. Unfortunately, we don't have these data for most of the fish populations that have been harvested. Almost similar data is needed for the other conventional stock assessment methods as well (Magnusson, 1995; Daskalov et al., 2020; Demirel et al., 2020). Thus, we cannot apply any of these stock assessment methods in the absence of the data mentioned above.

However, we still can investigate fish stocks and their current status to derive a sustainable fishery when we have only the landing data of fish stocks and the knowledge of important predators and prey that are significantly affecting the size of fish stocks. For example, the Atlantic bonito (*Sarda sarda*) and *Mnemiopsis leidyi* are the most significant predators has been affecting the size of the Black Sea anchovy in the Black Sea and zooplanktons are the main source of food for the Black Sea anchovy population. Thus, we need to include these predator-prey effects in the assessment of the Black Sea anchovy. One of the well-known tools helping to investigate the abundance or status of important fish stocks in food webs is the building of mathematical models for targeted fisheries. Using mathematical models for fisheries is a common method in fishery management when having limited data or just having landing data (Kot, 2001; Neubert, 2003; Demir and Lenhart, 2019). But solely using mathematical models is not enough to understand the dynamics of species and avoid overfishing. Therefore, it is also important to implement stability analyses to avoid overfishing and understand the dynamics of key species that are most influential as main predators or prey for fish stocks in food webs (Panja & Mondal 2015; Bentounsi et al., 2017; Agmour et al., 2018; Demir & Lenhart, 2019; Demir & Lenhart, 2021). Since fish populations depend on primary producers that are at the bottom of the food chain and consist of phytoplankton and zooplankton, it is essential to include them as important species in the investigation of fish stocks.

Therefore, considering food webs and implementing stability analysis for fishery models is essential in the management of fisheries (Kot, 2001; Bergland et al., 2018; Harun et al., 2019; Didiharyono et al., 2021). That is why, in this study, I used a food chain model and applied stability analysis to understand the dynamics and status of fish stocks before applying any harvesting strategies. The food chain model used in this study consisted of three species: two of them are fish populations and one of them is a zooplankton population.

2. MATERIAL AND METHODS

Even if this is not a data-driven study, I assume that we have only landing data and all the parameters are estimated depending on this landing data. A similar parameter estimation is proposed in the study of Demir and Lenhart, 2019 in the case study of the Black Sea anchovy assessment. Thus, in this study, parameter values were obtained depending on the stability conditions driven in this study (details are given at the end of subsection 2.4). Since the general goal of this study is to show how important stability analysis is when we only have landing data, I keep this study more theoretical rather than a specific case study. The fishery model was first introduced in the model formulation part (Eq. 1). Then, ensuring the existence of a solution for the fishery model, all the possible equilibrium points were found and investigated. After that, the stability analysis of important equilibrium points was numerically made. Finally, after capturing important values of harvest rates for a sustainable fishery, the predator-prey system was investigated under different levels of harvesting in the result section.

2.1 Model Formulation

I used a food chain model with three trophic levels to represent the behavior of the food web system, consisting of fish populations P_1 and P_2 as well as a zooplankton population, Z as prey of P_1 and P_2. In this food web, P_2 is also considered as a predator of P_1 in the food chain model given in Eq. (1). In this study, I consider the harvesting of P_1 with the harvest term, $h_1(t)P_1(t)$, which is proportional to the fish population $P_1(t)$and the harvest rate, $h_1(t)$, which represents the amount of fish taken from the system at time t. Similarly, I consider the harvesting of P_2 with the harvesting term, $h_2(t)P_2(t)$. Figure 1 shows the consumption of each compartment and Table 1 shows the description of the parameters given in the following food chain model

$$\begin{aligned}\frac{dP_1}{dt} &= r_1 P_1\left(1-\frac{P_1}{K_1}\right)+ m_0ZP_1 - m_1P_1P_2 - h_1P_1,\\ \frac{dP_2}{dt} &= r_2 P_2\left(1-\frac{P_2}{K_2}\right)+ m_2P_1P_2 + m_3ZP_2 - h_2P_2,\\ \frac{dZ}{dt} &= r_3 Z\left(1-\frac{Z}{K_3}\right)- m_4ZP_1 - m_5ZP_2\end{aligned} \tag{1}$$

with the initial conditions: $P_1(0) = P_{1,0}$, $P_2(0) = P_{2,0}$, and $Z(0) = Z_0$. The terms m_0ZP_1, $m_1P_1P_2$, $m_2P_1P_2$, m_3ZP_2, m_4ZP_1, and m_5ZP_2 represent interaction terms among species. For example, $m_1P_1P_2$, is a decay term for the fish population, P_1 due to the predation of P_1 by P_2 and $m_2P_1P_2$ is a growth term for the fish population, P_2 gained from the consumption of the fish population P_1. I also consider logistic growth rates for each population with intrinsic growth rates r_i and carrying capacities K_i for *i = 1, 2, 3*.

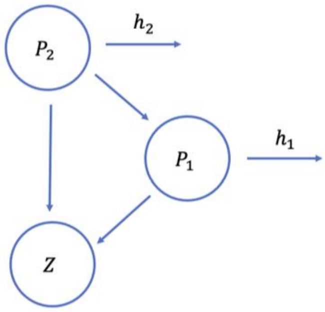


Figure 1. Flow diagram of the model illustrating the consumption among the compartments.

2.2. Positivity and Boundedness of the Model Outputs

In this part, the positivity and boundedness of the state variables in Eq. (1) were shown. For P_1, P_2, and Z with their initial conditions, there exists constants M_1, M_2, $M_3 > 0$ such that $0 < P_1(t) \leq M_1$, $0 < P_2(t) \leq M_2$, $0 < Z(t) \leq M_3$ for all $t \in [0, T]$. Here T denotes the final time. Firstly, I will show that $0 < Z(t) \leq M3$ for all $t \in [0,T]$. Then, the same technique will work for $0 < P_1(t) \leq M_1$ and $0 < P_2(t) \leq M_2$ for all $t \in [0, T]$.

$$\frac{dZ}{dt} = r_3 Z\left(1-\frac{Z}{K_3}\right)- m_4ZP_1 - m_5ZP_2$$

The integration factor technique will be used to show $Z(t) > 0$ for all $t \in [0,T]$, but firstly I substitute $Z = \frac{1}{\bar{Z}}$ into the above equation to obtain the following linear equation:

$$\frac{d\hat{Z}}{dt} = -(r_3 - m_4 P_1 - m_5 P_2)\hat{Z} + \frac{r_3}{K_3}$$

letting $\varphi(P_1, P_2) = -(r_3 - m_4 P_1 - m_5 P_2)$, we can write the above equation in the following linear form as:

$$\frac{d\hat{Z}}{dt} = \varphi(P_1, P_2)\hat{Z} + \frac{r_3}{K_3}$$

multiplying both sides of the equation by the integral factor $\mu = e^{\int_0^t \varphi(P_1,P_2)ds}$ and taking the integral over the interval $t \in [0, T]$, the following will be obtained as

$$\hat{Z}(t)e^{\int_0^t \varphi(P_1,P_2)ds} = \hat{Z}_0 e^{\int_0^t \varphi(P_1,P_2)ds} + \int_0^t e^{\int_0^t \varphi(P_1,P_2)ds} \frac{r_3}{K_3} > 0.$$

Since $\hat{Z}_0$, r_3, K_3, and the exponential function given in the above equation are positive, we can obtain $\hat{Z}(t)>0$. Thus, it follows that $Z(t)>0$ for all $t \in [0,T]$. With a similar approach, we can get $P_2 > 0$ and $P_1 > 0$ for all $t \in [0,T]$. Now, let us first show that $Z(t)$ has an upper bound over the interval *[0,T]*. Since all the coefficients are defined as positive and the states are positive, we can get the following inequality

$$\frac{dZ}{dt} = r_3 Z\left(1 - \frac{Z}{K_3}\right) - m_4 Z P_1 - m_5 Z P_2 \leq r_3 Z\left(1 - \frac{Z}{K_3}\right) \leq r_3 Z$$

arranging the above inequality and taking the integral from *0* to *t*, where $t \in [0,T]$ and T in $\mathbb{R}$, we will obtain

$$\int_0^t \frac{dZ}{Z} \leq \int_0^t r_3\, ds .$$

When we solve the integral, we obtain

$$Z \leq Z_0 e^{r_3 t} \text{ for all } t \in [0,T]$$

and for $M_3 = Z_0 e^{r_3 t}$, we can reach out the following result

$$0 < Z \leq M_3 \text{ for all } t \in [0,T].$$

Similarly, we can bound $P_1(t)$ by using $0 < Z \leq M_3$, and then bound $P_2(t)$ over the interval $t \in [0,T]$ as $0 < P_1(t) \leq M_1$ and $0 < P_2(t) \leq M_2$.

2.3. Existence of Equilibrium Points

Now I am going to examine the stability of the food chain model given in Eq. 1. First, let's set the time derivative parts equal to zero to obtain the equilibrium points of Eq.1 as

$$\begin{aligned} 0 &= r_1 P_1\left(1 - \frac{P_1}{K_1}\right) + m_0 Z P_1 - m_1 P_1 P_2 - h_1 P_1, \\ 0 &= r_2 P_2\left(1 - \frac{P_2}{K_2}\right) + m_2 P_1 P_2 + m_3 Z P_2 - h_2 P_2, \\ 0 &= r_3 Z\left(1 - \frac{Z}{K_3}\right) - m_4 Z P_1 - m_5 Z P_2 \end{aligned} \tag{2}$$

when we arrange Eq. 2, we will get the following

$$\begin{aligned} 0 &= P_1\left(r_1\left(1 - \frac{P_1}{K_1}\right) + m_0 Z - m_1 P_2 - h_1\right), \\ 0 &= P_2\left(r_2\left(1 - \frac{P_2}{K_2}\right) + m_2 P_1 + m_3 Z - h_2\right), \\ 0 &= Z\left(r_3\left(1 - \frac{Z}{K_3}\right) - m_4 P_1 - m_5 P_2\right). \end{aligned} \tag{3}$$

Now, let's get the nullclines of the above equations as

Nullclines for P_1 $P_1 = 0$ or $P_1 = \frac{K_1}{r_1}(r_1 + m_0 Z - m_1 P_2 - h_1)$

Nullclines for P_2 : $P_2 = 0$ or $P_2 = \frac{K_2}{r_2}(r_2 + m_2 P_1 + m_3 Z - h_2)$

Nullclines for Z : $Z = 0$ or $Z = \frac{K_3}{r_3}(r_3 - m_4 P_1 - m_5 P_2)$.

We then can get the equilibrium points of the food chain model in the order $E = (P_1^*, P_2^*, Z^*)$:

Case 1: Assume $P_1 = 0$, then we will obtain $P_2 = 0$ or $P_2 = \frac{K_2}{r_2}(r_2 + m_3 Z - h_2)$.

If $P_2 = 0$, we will then get $Z = 0$ or $Z = K_3$ which implies that $E_1 = (0,0,0)$ or $E_2 = (0,0,K_3)$.

If $P_2 = \frac{K_2}{r_2}(r_2 + m_3 Z - h_2)$, we have $Z = 0$ or $Z = \frac{K_3}{r_3}(r_3 - m_5 P_2)$ which follows that

$$E_3 = (0, K_2, 0) \text{ or } E_4 = \left(0, \frac{K_2}{r_2}(r_2 + m_3 Z - h_2), Z^*\right)$$

where $Z^* = \frac{1 + \frac{K_2}{r_3} m_5 \left(\frac{h_2}{r_2} - 1\right)}{\frac{1}{K_3} + \frac{K_2}{r_2 r_3} m_3 m_5}$. The equilibrium point E_4 is biologically feasible if $r_2 + m_3 Z > h_2$ and $\frac{r_3}{m_5 K_2} + \frac{h_2}{r_2} > 1$.

Case 2: Assume $P_1 = \frac{K_1}{r_1}(r_1 + m_0 Z - m_1 P_2 - h_1)$, then we have $P_2 = 0$ or $P_2 = \frac{K_2}{r_2}(r_2 + m_2 P_1 + m_3 Z - h_2)$. If $P_2 = 0$, then we have Z = 0 or $Z = \frac{K_3}{r_3}(r_3 - m_4 P_1)$, which implies that

$$E_5 = \left(\frac{K_1}{r_1}(r_1 - h_1), 0, 0\right) \text{ or } E_6 = \left(\frac{K_1}{r_1}(r_1 - h_1 + m_0 Z^*), 0, Z^*\right)$$

where $Z^* = \frac{1 + \frac{K_1}{r_3} m_4 \left(\frac{h_1}{r_1} - 1\right)}{\frac{1}{K_3} + \frac{K_1}{r_1 r_3} m_0 m_4}$.The equilibrium point E_5 is positive for $r_1 > h_1$, and so it is biologically feasible if $r_1 > h_1$. The equilibrium point E_6 is biologically feasible if $r_1 + m_0 Z^* > h_1$ and $\frac{r_3}{m_4 K_1} + \frac{h_1}{r_1} > 1$.

If $P_2 = \frac{K_2}{r_2}(r_2 + m_2 P_1 + m_3 Z - h_2)$, then we have Z = 0 or $Z = \frac{K_3}{r_3}(r_3 - m_4 P_1 - m_5 P_2)$. For Z = 0, the following equilibrium point is obtained

$$E_7 = \left(\frac{K_1}{r_1}(r_1 - h_1 - m_1 P_2^*), P_2^*, 0\right)$$

where $P_2^* = \frac{r_2 - h_2 + \frac{K_1}{r_1} m_2 (r_1 - h_1)}{\frac{r_2}{K_2} + \frac{K_1}{r_1} m_1 m_2}$. The equilibrium point E_7 is biologically feasible if $r_1 > h_1 + m_1 P_2^*$ and $r_2 + K_1 m_2 > h_2 + \frac{K_1}{r_1} m_2 h_1$.

For $Z = \frac{K_3}{r_3}(r_3 - m_4 P_1 - m_5 P_2)$, the coexisting equilibrium point is obtained as $E_8 = (P_1^*, P_2^*, Z^*)$, where

$$P_1^* = \frac{K_1}{r_1}(r_1 + m_0 Z^* - m_1 P_2^* - h_1)$$
$$P_2^* = \frac{K_2}{r_2}(r_2 + m_2 P_1^* + m_3 Z^* - h_2)$$
$$Z^* = \frac{K_3}{r_3}(r_3 - m_4 P_1^* - m_5 P_2^*)$$

By solving the above equations for the equilibrium point E_8, the following is obtained as

$$P_1^* = \frac{r_1 - h_1 + K_3 m_0 - (\frac{K_3}{r_3} m_0 m_5 + m_1)\left(\frac{r_2 - h_2 + K_3 m_3}{\frac{r_2}{K_2} + \frac{K_3}{r_3} m_3 m_5}\right)}{\frac{r_1}{K_1} + \frac{K_3}{r_3} m_0 m_4 + (\frac{K_3}{r_3} m_0 m_5 + m_1)\left(\frac{m_2 - \frac{K_3}{r_3} m_3 m_4}{\frac{r_2}{K_2} + \frac{K_3}{r_3} m_3 m_5}\right)}$$

$$P_2^* = \frac{r_2 - h_2 + K_3 m_3 - (m_2 - \frac{K_3}{r_3} m_3 m_4) P_1^*}{\frac{r_2}{K_2} + \frac{K_3}{r_3} m_3 m_5}$$

$$Z^* = \frac{K_3}{r_3} (r_3 - m_4 P_1^* - m_5 P_2^*)$$

and so, the equilibrium point E_8 is biologically feasible if $P_1^* > 0$, $P_2^* > 0$ and $r_3 > m_4 P_1^* + m_5 P_2^*$.

2.4. Stability Analysis of Coexisting Equilibrium Points

Now, I discuss the stability of equilibrium points in which at least two of the species coexist. Thus, the equilibrium points E_4, E_6, E_7, and E_8 will be investigated in this section. To investigate the stability of the food chain model at these equilibrium points, we first need to obtain the Jacobian (community) matrix of the food chain model. After that, the stability of these equilibrium points will be checked by using the Jacobian matrix at these equilibrium points. Note that when all the eigenvalues of this matrix are negative at an equilibrium point, this equilibrium point will be called a stable equilibrium. When at least one of the eigenvalues of this matrix at an equilibrium point is non-negative, then this equilibrium point is unstable.

Let us get the Jacobian matrix to check the stability of the equilibrium points. Assume that the functions *F*, *G*, and *H* equal the RHS of the food chain system (1) as follows:

$$\begin{aligned} \frac{dP_1}{dt} &= r_1 P_1 \left(1 - \frac{P_1}{K_1}\right) + m_0 Z P_1 - m_1 P_1 P_2 - h_1 P_1 = F(P_1, P_2, Z) \\ \frac{dP_2}{dt} &= r_2 P_2 \left(1 - \frac{P_2}{K_2}\right) + m_2 P_1 P_2 + m_3 Z P_2 - h_2 P_2 = G(P_1, P_2, Z) \\ \frac{dZ}{dt} &= r_3 Z \left(1 - \frac{Z}{K_3}\right) - m_4 Z P_1 - m_5 Z P_2 = H(P_1, P_2, Z) \end{aligned} \tag{4}$$

The Jacobian matrix will be in the form:

$$J = \begin{pmatrix} \frac{\partial F}{\partial P_1} & \frac{\partial F}{\partial P_2} & \frac{\partial F}{\partial Z} \\ \frac{\partial G}{\partial P_1} & \frac{\partial G}{\partial P_2} & \frac{\partial G}{\partial Z} \\ \frac{\partial H}{\partial P_1} & \frac{\partial H}{\partial P_2} & \frac{\partial H}{\partial Z} \end{pmatrix}$$

When we get the partial derivatives of the system (4), we obtain the following

$$J = \begin{pmatrix} r_1(1-\frac{2P_1^*}{K_1}) + m_0 Z^* - m_1 P_2^* - h_1 & -m_1 P_1^* & m_0 P_1^* \\ m_2 P_2^* & r_2(1-\frac{2P_2^*}{K_2}) + m_2 P_1^* + m_3 Z^* - h_2 & m_3 P_2^* \\ -m_4 Z^* & -m_5 Z^* & r_3(1-\frac{2Z^*}{K_3}) - m_4 P_1^* - m_5 P_2^* \end{pmatrix}$$

After obtaining the Jacobian matrix, the stability of equilibrium points is investigated by substituting the equilibrium points to the Jacobian matrix, J. Firstly, the stability of the coexisting equilibrium point, E_8 was investigated by using parameter values given in Table 1. Note that this study is not data-driven. If so, we could estimate these parameter values given in Table 1 fitting the model with landing data under the constraints of the stability of the predator-prey system when all the species coexist. Note that one directly can apply stability requirements for parameters during the parameter estimation, or first estimates parameters and then check the stability requirements with estimated parameters and arrange the upper and lower bound of the parameters' initial guesses until satisfying the stability requirements for the estimated parameters. One could fit the model below for a specific case study as:

$$\min\left(\frac{\sum_{k=1}^{n}\left(L_k - \hat{L}_k\right)^2}{\sum_{k=1}^{n}(L_k)^2} + \frac{\sum_{k=1}^{n}(L_k^* - \hat{L}_k^*)^2}{\sum_{k=1}^{n}(L_k^*)^2}\right)$$

where the letter n denotes the number of data points in the above formula, L_k is the landing data of species P_1, and $\hat{L}_k$ is the predicted landing that is obtained from the term $h_1 P_1$ of the model. Similarly, L_k^* is the landing data of species P_2, and $\hat{L}_k^*$ is the predicted landing that is obtained from the term $h_2 P_2$ of the model.

Table 1: Parameter descriptions and values used in Stability analysis of equilibrium points. Here e is a scientific notation in MATLAB and it is a shorthand for 10.

Parameters	**Descriptions**	**Unit**	**Value**	**Source**
$P_{1,0}$	Initial biomass of fish population, P_1	Tonnes	$9e^3$	Assumed
$P_{2,0}$	Initial biomass of fish population, P_2	Tonnes	$6e^2$	Assumed
Z_0	Initial biomass of zooplankton, Z	Tonnes	$3e^7$	Assumed
r_1	Intrinsic growth rate of fish population, P_1	$days^{-1}$	0.4	Assumed
r_2	Intrinsic growth rate of fish population, P_2	$days^{-1}$	0.3	Assumed
r_3	Intrinsic growth rate of zooplankton, Z	$days^{-1}$	0.5	Assumed
K_1	Carrying capacity of fish population, P_1	Tonnes	$1e^{+5}$	Assumed
K_2	Carrying capacity of fish population, P_2	Tonnes	$2e^{+3}$	Assumed
K_3	Carrying capacity of zooplankton, Z	Tonnes	$1e^{+7}$	Assumed
m_0	Growth rate of P_1 due to predation of Z	$(days \times Tonnes)^{-1}$	$3e^{-7}$	Assumed
m_1	Consumption rate of P_1 due to its predator P_2	$(days \times Tonnes)^{-1}$	$5e^{-5}$	Assumed
m_2	Growth rate of P_2 due to predation of P_1	$(days \times Tonnes)^{-1}$	$8e^{-6}$	Assumed
m_3	Growth rate of P_2 due to predation of Z	$(days \times Tonnes)^{-1}$	$4e^{-7}$	Assumed
m_4	Consumption rate of Z due to its predator P_1	$(days \times Tonnes)^{-1}$	$5e^{-5}$	Assumed
m_5	Consumption rate of Z due to its predator P_2	$(days \times Tonnes)^{-1}$	$4e^{-5}$	Assumed
h_1	Harvest rate of fish population P_1	$days^{-1}$	0.4	Assumed
h_2	Harvest rate of fish population P_2	$days^{-1}$	0.4	Assumed

To keep the study general, I did not fit the model with data instead the parameter values given in Table 1 are obtained by varying each parameter to reach a stable coexisting state for the three species. After obtaining these parameter values, I applied stability analysis to the other equilibrium points E_4, E_6, and E_7 as well by using the same parameter values given in Table 1. Then, the harvesting rates h_1 and h_2 are varied to see the effect of harvesting on the stability of the predator-prey system for the equilibrium points, E_4, E_6, E_7, and E_8. Also, note that the initial values of species given in Table 1 are chosen to be around the equilibrium point, E_8. Details are given in the result section.

3. RESULTS

In this part, firstly the stability of the equilibrium points, E_4, E_6, E_7, and E_8 was numerically investigated. Then, the status of the predator-prey dynamic of species was analyzed when different levels of harvesting were applied.

3.1. Stability Analysis of the Equilibrium point, E_8

Now, let's check the stability of the equilibrium point, $E_8 = \left(\frac{K_1}{r_1}(r_1 + m_0 Z^* - m_1 P_2^* - h_1), \frac{K_2}{r_2}(r_2 + m_2 P_1^* + m_3 Z^* - h_2), \frac{K_3}{r_3}(r_3 - m_4 P_1^* - m_5 P_2^*)\right)$. Here, the equilibrium point E_8 is biologically feasible if $P_1^* > 0$, $P_2^* > 0$, and $r_3 > m_4 P_1^* + m_5 P_2^*$. The Jacobian Matrix of E_8 is

$$J\Big|_{E_8} = \begin{pmatrix} r_1(1 - \frac{2P_1^*}{K_1}) + m_0 Z^* - m_1 P_2^* - h_1 & -m_1 P_1^* & m_0 P_1^* \\ m_2 P_2^* & r_2(1 - \frac{2P_2^*}{K_2}) + m_2 P_1^* + m_3 Z^* - h_2 & m_3 P_2^* \\ -m_4 Z^* & -m_5 Z^* & r_3(1 - \frac{2Z^*}{K_3}) - m_4 P_1^* - m_5 P_2^* \end{pmatrix}$$

where

$$P_1^* = \frac{r_1 - h_1 + K_3 m_0 - (\frac{K_3}{r_3} m_0 m_5 + m_1)\left(\frac{r_2 - h_2 + K_3 m_3}{\frac{r_2}{K_2} + \frac{K_3}{r_3} m_3 m_5}\right)}{\frac{r_1}{K_1} + \frac{K_3}{r_3} m_0 m_4 + (\frac{K_3}{r_3} m_0 m_5 + m_1)\left(\frac{m_2 - \frac{K_3}{r_3} m_3 m_4}{\frac{r_2}{K_2} + \frac{K_3}{r_3} m_3 m_5}\right)}$$

$$P_2^* = \frac{r_2 - h_2 + K_3 m_3 - (m_2 - \frac{K_3}{r_3} m_3 m_4) P_1^*}{\frac{r_2}{K_2} + \frac{K_3}{r_3} m_3 m_5}$$

$$Z^* = \frac{K_3}{r_3}(r_3 - m_4 P_1^* - m_5 P_2^*)$$

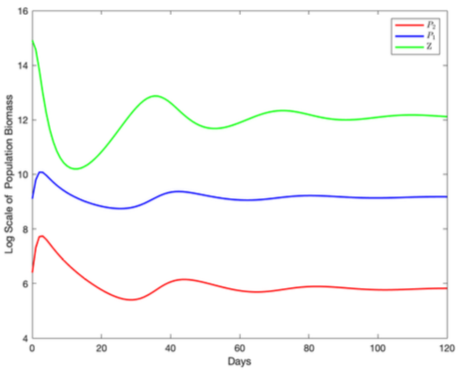
Now, I am going to obtain the characteristic polynomial of E_8 from the following matrix $J^* = |J_{|E_8} - \lambda I|$, where

$$J^* = \begin{vmatrix} r_1(1-\frac{2P_1^*}{K_1}) + m_0 Z^* - m_1 P_2^* - h_1 - \lambda & -m_1 P_1^* & m_0 P_1^* \\ m_2 P_2^* & r_2(1-\frac{2P_2^*}{K_2}) + m_2 P_1^* + m_3 Z^* - h_2 - \lambda & m_3 P_2^* \\ -m_4 Z^* & -m_5 Z^* & r_3(1-\frac{2Z^*}{K_3}) - m_4 P_1^* - m_5 P_2^* - \lambda \end{vmatrix}$$

such that

$$\begin{aligned} &P(\lambda) = \\ &\left(r_1(1-\frac{2P_1^*}{K_1}) + m_0 Z^* - m_1 P_2^* - h_1 - \lambda\right)\left[\left(r_2(1-\frac{2P_2^*}{K_2}) + m_2 P_1^* + m_3 Z^* - h_2 - \lambda\right)\right. \\ &\left.\left(r_3(1-\frac{2Z^*}{K_3}) - m_4 P_1^* - m_5 P_2^* - \lambda\right) + m_3 m_5 P_2^* Z^*\right] \\ &+ m_1 P_1^*\left[m_2 P_2^*\left(r_3(1-\frac{2Z^*}{K_3}) - m_4 P_1^* - m_5 P_2^* - \lambda\right) + m_3 m_4 P_2^* Z^*\right] \\ &+ m_0 P_1^*\left[m_4 Z^*\left(r_2(1-\frac{2P_2^*}{K_2}) + m_2 P_1^* + m_3 Z^* - h_2 - \lambda\right) - m_2 m_5 P_2^* Z^*\right] = 0 \end{aligned}$$

As can be seen, it is not easy to examine the stability analysis analytically. Therefore, the stability analysis was examined numerically by using parameter values given in Table 1. This stability analysis showed that the equilibrium point, E_8 is stable since the eigenvalues are obtained as $\lambda_1 = -0.08$, $\lambda_2 = -0.08$, and $\lambda_3 = -0.05$ by using parameter values from Table 1 (see the left-hand side plot in Figure 2). The equilibrium point, E_8 is obtained as (P_1^*, P_2^*, Z^*)= $(9e^3, 4.7e^2, 6e^6)$. After obtaining this stability result, I obtained the right-hand side plot in Figure 2 by changing h_2 from 0.4 to 0.58. This investigation shows that the fish population P_2 will collapse if the harvest rate h_2 equals 0.58 or above it. Besides this investigation, the harvest rate h_1 is varied to see the effect of harvesting on the dynamic of the fish population, P_1. When h_1 is taken as 0.5, then we obtain the solid blue curve, which is a different equilibrium state, given in Figure 3. If we set h_1 = 0.6, we will get the dashed blue curve that decays to zero. This analysis indicates that the coexistence equilibrium state of the system can change depending on the harvest rates h_1 and h_2.



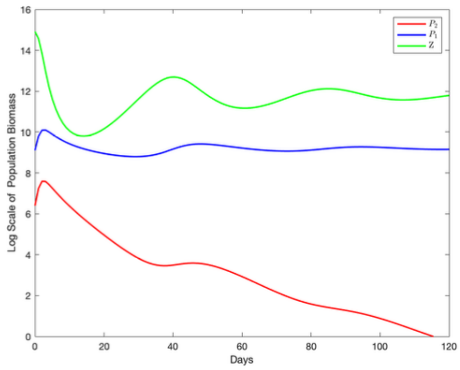


Figure 2. Dynamics of the species at the equilibrium point, E_8. The left plot is obtained by using parameter values given in Table 1. The right plots were obtained by using parameter values given in Table 1 but h_2 is taken as 0.58 instead of 0.4

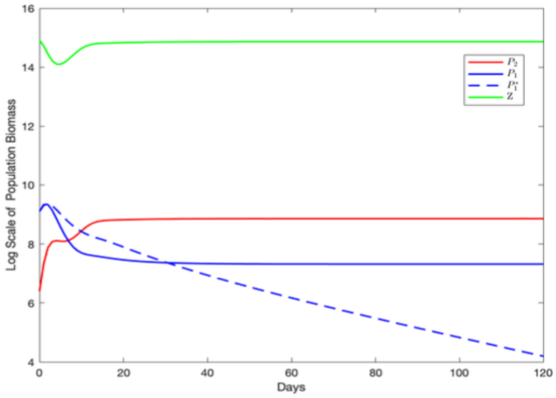


Figure 3. Dynamics of the species at the equilibrium point, E_8 by varying the harvest rate h1 and fixing the rest of the parameter values in Table 1. The solid blue curve is obtained for h_1 = 0.5, and the dashed blue curve is obtained for h_1 = 0.6

3.2. Stability Analysis of the Equilibrium point, E7

Let's investigate the stability of the equilibrium point $E_7 = \left(\frac{K_1}{r_1}(r_1 - h_1 - m_1 P_2^*), P_2^*, 0\right)$ which is the zooplankton-free equilibrium point and $P_2^* = \frac{r_2 - h_2 + \frac{K_1}{r_1} m_2 (r_1 - h_1)}{\frac{r_2}{K_2} + \frac{K_1}{r_1} m_1 m_2}$. The equilibrium point E_7 is biologically feasible if $r_1 > h_1 + m_1 P_2^*$ and $r_2 + K_1 m_2 > h_2 + \frac{K_1}{r_1} m_2 h_1$. The Jacobian matrix of E_7 is

$$J\Big|_{E_7} = \begin{pmatrix} r_1(1 - \frac{2P_1^*}{K_1}) - m_1 P_2^* - h_1 & -m_1 P_1^* & m_0 P_1^* \\ m_2 P_2^* & r_2(1 - \frac{2P_2^*}{K_2}) + m_2 P_1^* - h_2 & m_3 P_2^* \\ 0 & 0 & r_3 - m_4 P_1^* - m_5 P_2^* \end{pmatrix}$$

and the characteristic polynomial of E_7 from the following matrix

$$\left|J|_{E_7} - \lambda I\right| = \begin{vmatrix} r_1(1 - \frac{2P_1^*}{K_1}) - m_1 P_2^* - h_1 - \lambda & -m_1 P_1^* & m_0 P_1^* \\ m_2 P_2^* & r_2(1 - \frac{2P_2^*}{K_2}) + m_2 P_1^* - h_2 - \lambda & m_3 P_2^* \\ 0 & 0 & r_3 - m_4 P_1^* - m_5 P_2^* - \lambda \end{vmatrix}$$

obtained as

$$P(\lambda) = \left(r_3 - m_4 P_1^* - m_5 P_2^* - \lambda\right)\Big[(r_1(1 - \frac{2P_1^*}{K_1}) - m_1 P_2^* - h_1 - \lambda)$$
$$(r_2(1 - \frac{2P_2^*}{K_2}) + m_2 P_1^* - h_2 - \lambda) + m_1 m_2 P_1^* P_2^*\Big] = 0$$

Since the equilibrium point E_7 cannot be biologically feasible if the condition, $r_1 > h_1 + m_1 P_2^*$ does not hold, h_1 has to be less than $r_1 = 0.4$. Thus, when we investigate the system with $h_1 = 0.4$, we see that both P_1 and P_2 species will be collapsing, as shown in the left plot of Figure 4.

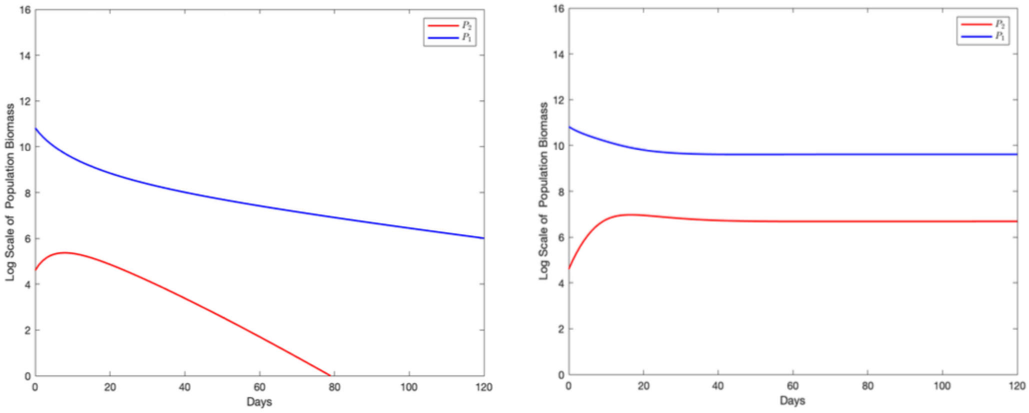


Figure 4. Dynamics of the species at Equilibrium points, E_7. The left plot was obtained by using parameter values from Table 1. The right plot was obtained using parameter values from Table 1 but $h_1 = h_2 = 0.35$ instead of $h_1 = h_2 = 0.4$

However, when we change both harvest rates h_1 and h_2 from 0.4 to 0.35, then we obtain a feasible and stable state for the equilibrium point, E_7 (see the right plot given in Figure 4). This stable equilibrium point is (P_1^*, P_2^*, Z^*)= $(1e^4, 2e^2, 0)$ with the eigenvalues $\lambda_1 = -0.04$, $\lambda_2 = -0.04$, and $\lambda_3 = -0.01$. As we decrease the harvest rates, the new stable state will be more abundant and resilient for the system in the absence of zooplankton. For instance, for the harvest rates $h_1 = h_2 = 0.3$, this equilibrium point will be (P_1^*, P_2^*, Z^*)= $(1.5e^4, 8e^2, 0)$. This simple investigation shows how important stability analysis is for fishery management.

3.3. Stability Analysis of the Equilibrium point, E_6

Let's examine the stability of the equilibria point $E_6 = (P_1^*, 0, Z^*)$ which is the top-predator-free equilibrium, and biologically feasible when $r_1 + m_0 Z^* > h_1$ and $\frac{r_3}{m_4 K_1} + \frac{h_1}{r_1} > 1$ since $P_1^* = \frac{K_1}{r_1}(r_1 - h_1 + m_0 Z^*)$ and $Z^* = \frac{1 + \frac{K_1}{r_3} m_4 (\frac{h_1}{r_1} - 1)}{\frac{1}{K_3} + \frac{K_1}{r_1 r_3} m_0 m_4}$.

The Jacobian matrix with the equilibrium point $E_6 = (P_1^*, 0, Z^*)$ is

$$J\Big|_{E_6} = \begin{pmatrix} r_1(1-\frac{2P_1^*}{K_1}) + m_0Z^* - h_1 & -P_1^*m_1 & m_0P_1^* \\ 0 & r_2 + m_2P_1^* + m_3Z^* - h_2 & 0 \\ -m_4Z^* & -m_5Z^* & r_3(1-\frac{2Z^*}{K_3}) - m_4P_1^* \end{pmatrix}.$$

Now, let us first get the Characteristic Polynomial, $P(\lambda) = det(|J - \lambda I|)$ to obtain eigenvalues of the J, where I is a 3x3 unit matrix.

$$\Big|J|_{E_6} - \lambda I\Big| = \begin{vmatrix} r_1(1-\frac{2P_1^*}{K_1}) + m_0Z^* - h_1 - \lambda & -P_1^*m_1 & m_0P_1^* \\ 0 & r_2 + m_2P_1^* + m_3Z^* - h_2 - \lambda & 0 \\ -m_4Z^* & -m_5Z^* & r_3(1-\frac{2Z^*}{K_3}) - m_4P_1^* - \lambda \end{vmatrix}$$

We now have the following characteristic polynomial as

$$P(\lambda) = \left(r_2 + m_2P_1^* + m_3Z^* - h_2 - \lambda\right)\Big[(r_1(1-\frac{2P_1^*}{K_1}) + m_0Z^* - h_1 - \lambda)(r_3(1-\frac{2Z^*}{K_3}) - m_4P_1^* - \lambda) + m_0m_4Z^*P_1^*\Big] = 0$$

where $P_1^* = \frac{K_1}{r_1}(r_1 - h_1 + m_0Z^*)$ and $Z^* = \frac{1+\frac{K_1}{r_3}m_4\,(\frac{h_1}{r_1}-1)}{\frac{1}{K_3}+\frac{K_1}{r_1 r_3}m_0\,m_4}$. Now, we need to find the eigenvalues of the characteristic polynomial given above.

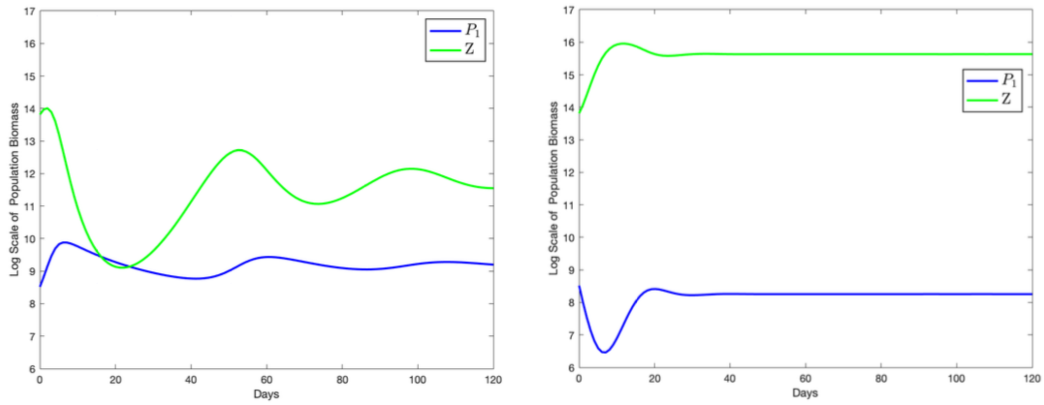


Figure 5. Dynamics of the species at Equilibrium points, E_6. The left plot was obtained by using parameter values from Table 1. The right plot was obtained using parameter values from Table 1 but $h_1 = 0.6$ instead of $h_1 = 0.6$

Since it is not easy to find the eigenvalues analytically, I will find them numerically for the given values of each parameter in Table 1. Since the eigenvalues are negative at the Equilibrium point E_6 as $\lambda_1 = -1.16$, $\lambda_2 = -0.43$, and $\lambda_3 = -0.04$, this equilibrium point is stable (see Figure 5 for different values of the harvest rate, h_1). This analysis indicates that increasing the harvest rate, h_1 causes a reduction in this fish population's abundance and causes increases in zooplankton's abundance in the food web.

3.4. Stability Analysis of the Equilibrium point, E_4

Now, let's investigate the last coexisting equilibrium point

$$E_4 = \left(0, \frac{K_2}{r_2}\,(r_2 + m_3 Z - h_2), Z^*\right),$$

where $Z^* = \frac{1+\frac{K_2}{r_3} m_5 \left(\frac{h_2}{r_2}-1\right)}{\frac{1}{K_3}+\frac{K_2}{r_2 r_3} m_3 m_5}$. The equilibrium point E_4 is biologically feasible if $r_2 + m_3 Z > h_2$ and $\frac{r_3}{m_5 K_2} + \frac{h_2}{r_2} > 1$.

$$J\Big|_{E_4} = \begin{pmatrix} r_1 + m_0 Z^* - m_1 P_2^* - h_1 & 0 & 0 \\ m_2 P_2^* & r_2(1 - \frac{2P_2^*}{K_2}) + m_3 Z^* - h_2 & m_3 P_2^* \\ -m_4 Z^* & -m_5 Z^* & r_3(1 - \frac{2Z^*}{K_3}) - m_5 P_2^* \end{pmatrix}$$

$$\Big|J|_{E_4} - \lambda I\Big| = \begin{vmatrix} r_1 + m_0 Z^* - m_1 P_2^* - h_1 - \lambda & 0 & 0 \\ m_2 P_2^* & r_2(1 - \frac{2P_2^*}{K_2}) + m_3 Z^* - h_2 - \lambda & m_3 P_2^* \\ -m_4 Z^* & -m_5 Z^* & r_3(1 - \frac{2Z^*}{K_3}) - m_5 P_2^* - \lambda \end{vmatrix}$$

$$P(\lambda) = \left(r_1 + m_0 Z^* - m_1 P_2^* - h_1 - \lambda\right)\Bigg[(r_2(1 - \frac{2P_2^*}{K_2}) + m_3 Z^* - h_2 - \lambda)$$
$$(r_3(1 - \frac{2Z^*}{K_3}) - m_5 P_2^* - \lambda) + m_3 m_5 Z^* P_2^*\Bigg] = 0$$

When we investigate the food chain system in the absence of the fish population, P_1, we see that both P_1 and Z species are increasing and reaching a stable state, as shown in the left plot of Figure 6. When we change the harvest rates h_1 from 0.4 to 0.6, we obtain a slightly different stable state (see the right plot given in Figure 6).

In this section, it is not discussed but one also can predict the amount of fish consumed by its predator by calculating the term $m_1 P_1 P_2$ in Eq.1 and predict the gain due to the consumption of its prey by calculating the term $m_0 Z P_1$ for the fish population P_1 and can do the same calculation for the other fish population P_2.

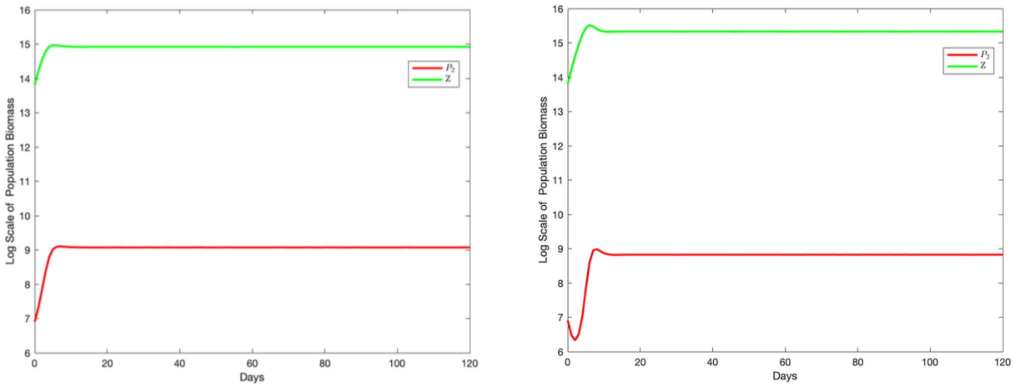


Figure 6. Dynamics of the species at Equilibrium points, E_4. The left plot was obtained by using parameter values from Table 1. The right plot was obtained using parameter values from Table 1 but $h_2 = 0.6$ instead of $h_2 = 0.4$

4. DISCUSSIONS

The fishery model used in the study was not fitted with any specific data to keep the study more general, but one could fit this fishery model or any fishery model with landing data to estimate species-specific parameter values conditional on the stability results of his/her fishery model. It is not hard to see that the results obtained in the study can hold in any case study. For example, in the absence of main food sources such as zooplankton, fish populations will be affected negatively and even can collapse in the absence of main food resources as shown in the study. Similarly, when we increase fishing efforts and pressure on fish populations, their size will be negatively affected. Thus, the results obtained in this study can hold in any case study.

This modeling method requires a few data as compared with the other fishery assessment methods such as XSA, VPA, BMS, CMSY, and MSVPA. In this method, having landing data is enough to capture important features of fish stocks such as biomass of fish stocks, the maximum sustainable yield, the biomass of fish lost or gained due to predator-prey relations, and the effect of harvesting on predator-prey relations as discussed in the result section. However, the other assessment methods require an important amount of data and estimates for fish stocks such as diet data, natural mortality, fishing mortality (landing data), abundance index of species, suitability estimates, weight-at-age (or average weights), predator ratio estimates, and so on. Obtaining such rich data requires an important amount of money and time.

Furthermore, most of such assessment methods consider single-species models instead of multi-species models in the investigation of fish stocks without including any predator-prey effects on fish stock dynamics. However, using single-species models often has overestimated sustainable harvest levels since the actual population levels are lowered due to food chain interactions, and too often traditional fishery management that uses single-species models has failed to take a precautionary approach to maintain and protect sustainable fisheries, biodiversity, and marine ecosystem function (Lauck et al.,1998; Foley, 2013; Demir & Lenhart, 2019). Therefore, this study was considered a multi-species fishery model in the investigation of fish stocks to eliminate any risk of overestimation of the maximum sustainable yield and collapses in fish stocks due to overfishing. This is another advantage of this modeling method besides the requirement of less data for the assessment of fish stocks.

This investigation also showed that stability analysis of fishery models is crucial in fishery management since it allows us to identify the upper or lower bounds of harvesting rates to reach a resilient and healthy food web where targeted fish populations are. For example, in this study, the stability analysis for coexistence equilibrium, E_8 shows that the harvest rates h_1 and h_2 are very important for the dynamic of fish populations and their fade in the food web since increases in these harvest rates may cause a reduction or even a collapse in fish populations (Figure 3) and analysis of this equilibrium point lets us figure out the critical upper bound of harvest rates for these fish populations that correspond to the maximum sustainable yield in literature. Thus, it is recommended to investigate the critical upper bounds of harvest rates for targeted fisheries by implementing stability analysis before applying any harvesting strategies.

The investigation of equilibrium points, especially the equilibrium point E_7 shows that the zooplankton population is very crucial for the fade of fish populations in the food web (Figure 4). In the absence of zooplankton, both fish populations are collapsing. Thus, this result indicates that any violation in the lower level of food webs directly affects upper levels.

Also, note that the estimated parameters in the study are conditional on the stability of the multi-species model used. It means that not only the targeted fish stocks but also the other species included in the study will be sustained in the long term if we apply the outputs of the study, especially the maximum sustainable (landing) yield. Note that even if this technique is applied to sustain mainly fish stocks, it also can be used to sustain any population and even an important proportion of a food web in an aquatic system if we have time series biomass or density data of the most important species in a food web. Therefore, to control and sustain our ecosystems we need to control and help our environment with a little touch as this study recommends.

In addition to the outputs covered in this study, one could estimate the optimal sustainable yield besides the maximum sustainable yield with no extra data but coupling the fishery model with optimal control tools (Neubert, 2003; Kelly et al., 2016) and could obtain optimal predator-prey dynamics among species (Demir & Lenhart, 2019). When catch per unit effort (CPUE) data is available for a fish population besides the landing data, one can also predict the optimal number of fishing fleets that need to be used in harvesting the optimal sustainable yield as proposed in the study Demir and Lenhart, 2019.

5. CONCLUSION

This study indicates that one can predict the status of fish stocks and obtain important outputs of fisheries such as the maximum sustainable yield, current biomass dynamics of fish stocks, and dynamics of their predators and prey thanks to the landing data and including the most influential predators and preys on fish stocks by using fishery models supported with stability analysis. This method requires only landing data as compared with other conventional assessment methods that require very rich data. Obtaining such data requires an important amount of money and time. Furthermore, most of these assessment methods consider single-species models when important outputs of fisheries are driven and this approach ignores and misses predator-prey effects on fishery management. Therefore, the method used in this study is more complete and requires less data as compared to other assessment methods to derive important outputs for fishery management.

This study also shows that it is essential to investigate the equilibrium points of species and their stability for a fishery model used in the investigation of fish populations to avoid overfishing and eliminate the risk of any collapse in fish populations due to overfishing (Figures 2, 3, and 4). It is also important to investigate the effect of fishing on the food web by including other important key species in fishery models besides the targeted fish populations as this study does. For instance, the zooplankton population is included in this study to see and track the effect of the fisheries on zooplankton abundance, and this also lets us examine the dynamics of the fish population in the absence of zooplankton.

One of the main recommendations of the study is that if the policymakers of fisheries consider the maximum sustainable (landing) yield as the maximum amount that can be harvested from the system, then they will be able to not only sustain fish stocks but also sustain the other species included in such an analysis. This modeling technique can also be used to investigate a food web in an aquatic system when time series of density (or abundance) data is available for the most influential species in the food web. Thus, this technique used in the study is also ecosystem friendly.

6. FUTURE WORK

The conventional stock assessment methods and the method used in this study lead us to investigate and obtain important outputs for fishery management, but the outputs come from deterministic models that provide rough predictions and do not consider variations affecting the birth rates of fish populations due to environmental changes. Also, these assessment methods do not consider measurement errors of data. Thus, to make the predicted outputs much better, one can use stochastic differential equations coupled with a measurement model as proposed by Marino et al., 2019. In this way, one can consider the birth rate variation and measurement errors in data.

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CONFLICT OF INTEREST

The author declares that no financial interests or personal relationships may affect this work.

AUTHOR CONTRIBUTIONS

Single author.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study

DATA AVAILABILITY STATEMENT

Research data is not shared.

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Author Guidelines

PAGE SIZE
Page should be A4 (21 cm x 29.7 cm) size.

MARGINS

Top: 2.5 cm Right: 2.5 cm Left: 2.5 cm Bottom: 2.5 cm Gutter: 0 cm

TEXT FORMATING

Font	: Times New Roman
Font size	: 12-point
Alignment	: Justified
Indent	: 1.25 cm
Line spacing	: 2
Line numbers	: Continuously throughout the manuscript
Page numbers	: Automatic numbered in the bottom center of the pages

TITLE PAGE
The title page should be uploaded to the system separately from the manuscript file. The title page should contain only the following information.

- Title
Title should be brief and informative reflecting the study. Abbreviations and formulae usage is not recommended.

- Running title
A short (running) title with a maximum of 75 characters should be given to reflect the title.

- Authors names
Name and surnames of the authors should be indicated clearly. Accuracy of the names spelling should be checked before submission.

- Institution
Format used: University/Institution, Faculty, Department, Province-COUNTRY
Example: Isparta University of Applied Sciences, Eğirdir Fisheries Faculty, Department of Aquaculture, Isparta-TURKEY

- Corresponding author
Please indicate the corresponding author who will be responsible for all the stages of publication, review, and post-publication. Contact information and mailing address of corresponding author should be given in the title page.
*Corresponding Author: Name Surname, e-mail: …

- ORCID's of the authors
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MANUSCRIPT FORMAT
Manuscripts in original articles, short communication, case report and reviews should be prepared in accordance with the format below*.

Original Article	**Short Communication**	**Case Report**	**Review Article**

Title

Short title

Authors

Institutions

Corresponding author e-mail

ORCID’s of the authors

Title

Abstract

Keywords

Turkish title*

Turkish abstract*

Turkish keywords*

1. Introduction

Original Article	Short Communication	Case Report	Review Article
2. Material and Methods 3. Results	2. FREE CONTENT	2. Case Report	2. FREE CONTENT
4. Discussion	3. Discussion	3. Discussion	3. Discussion
5. Conclusion	4. Conclusion	4. Conclusion	4. Conclusion

Acknowledgement

Funding

Conflict of Interest

Author Contributions

Ethical Statements

Data Availability Statement

References

* **Note**: Turkish title, abstract and keywords supports are provided for non-Turkish authors.

ABSTRACT

Abstract should concisely contain the purpose of the study, the methods used, the prominent findings, and its contribution to the literature. It should be written both in Turkish and English with a maximum of 300 words.

KEYWORDS

Keywords should be chosen from words that are not included in the title and reflect the study. At least 3 (three), maximum 5 (five) keywords should be specified. There should be a comma (,) between words and a dot (.) after the last word.

Keywords: CITES, aquaponics, production protocol, mortality, immunology.

DECIMAL NUMBERS

Comma “,” should be used in Turkish manuscripts and dot “.” should be used in English manuscripts.

Turkish: %10,25

English: 10.25%

SCIENTIFIC NAMES

The species name should be given without abbreviation (Cyprinus carpio) in the first place in the text, and then the genus name should be abbreviated (C. carpio).

TABLES

The table title should be positioned above the table and should be written concisely. Abbreviations used in the table should be explained below the table. The table must be in the form of a straight guide, with no special design applied. Authors are encouraged to convey the table contents to the reader in the table footer, independently of the article. Font size for footers should be 10 points. Tables should be cited in the text as Table 1, Table 2, etc. The tables should be given in the nearest place where it cited. Tables must be editable. Tables in screenshot or picture format are not accepted.

FIGURES

The figure title should be short and concise, centered at the bottom of the figure. Figures should have a minimum resolution of 300 DPI. Figures should be cited in the text as Figure 1, Figure 2, etc. The figures should be given in the nearest place where it cited.

ACKNOWLEDGEMENT

In this section, those who help to the conduct the study apart from financial support, are indicated.

Example: The authors thank Ahmet Taş (Isparta University of Applied Sciences, Turkey) for his helps during the laboratory part of the study.

FUNDING

In this section, institutions that provide financial support to the conduct of the study are indicated using the grant number.

Example-1: This study was supported by the Scientific Research Projects Coordination Unit of Isparta University of Applied Sciences grant 3241-E2-14.

Example-2: No financial support was received for the present study.

CONFLICT OF INTEREST

Conflicts of interest of the author(s), if any, are indicated in this section.

Example: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

The contributions of each author to the relevant stages of the study are indicated by using each work package and the first letters of the name and surname.

Example:
Fiction: IT; Literature: KL, TN; Methodology: CT, FU; Performing the experiment: FM, CT, FU; Data analysis: FU, TA; Manuscript writing: CT, FU, Supervision: CT. All authors approved the final draft.

ETHICAL APPROVAL STATEMENTS

The ethics committee approvals obtained for the study are indicated with information of institute, date, and number. Manuscripts that are not declare, although they require the Local Ethics Committee Approval in studies conducted with vertebrates, and the Approval for Ethics Committee Approval of Non-Interventional Investigates in survey/interview studies will not be considered for scientific evaluation.

Example-1: Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

Example-2: This study was conducted with the approval of Animal Experiments Local Ethics Committee of Isparta University of Applied Sciences (Date: 01.07.2010, No: 21438139-147).

DATA AVAILABILITY STATEMENT

In this section, data availability statement should be declared by the authors regarding the anonymous availability of the data used in the manuscript. Acta Aquatica Turcica encourages authors to share research data used.

Example-1: The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.11815566.v1

Example-2: The data used in the present study are available upon request from the corresponding author. Data is not available to the public due to privacy or ethical restrictions.

Example-3: Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

Example-4: Data sharing is not applicable for the present study as no new data was created or analyzed.

Example-5: Research data is not shared.

Example-6: Data supporting the findings of the present study are available in the supplementary material to this article.

CITATIONS

Citations are written in the following formats, in the order of the year, separated by a semicolon (;).
- Single author
(Author, Year)
-- It is thought to be … (Küçük, 2008; Güçlü, 2018a; Güçlü, 2018b).
-- According to Küçük (2008), …

- Two authors
(Author-1 and Author-2, Year)
-- They are among the important parameters (Küçük and Güçlü; 2001; Ekici and Koca, 2021a; Ekici and Koca, 2021b).
-- According to Ekici and Koca (2021b),…

- Three or more authors
(Author-1 et al., Year)
-- It can be repeated periodically (Yiğit et al., 2006a; Yiğit et al., 2006b; Boyacı et al., 2020).
-- According to Boyacı et al. (2020),…

REFERENCES LIST

References should be indented 1.25 cm from the second line and should prepared according to APA version 7. Ideally, the names of all authors should be provided. Usage of "et al" in long author lists (more than 10) will also be accepted. Except for special uses, only the first letter of the title of all references should be capitalized, and all words in the names of the sources (journal, publishing house and congress) should be written with a capital letter.

1-Journal articles

The name of the journal (italic) without shortening, volume (italic), issue, page numbers and DOI number having an active link should be specified.

Petrauskienė, L., Utevska, O., & Utevsky, S. (2009). Can different species of medicinal leeches (Hirudo spp.) interbreed? Invertebrate Biology, 128(4), 324-331. https://doi.org/10.1111/j.1744-7410.2009.00180.x

Wagenaar, D. A., Hamilton, M. S., Huang, T., Kristan, W. B., & French, K. A. (2010). A hormone-activated central pattern generator for courtship. Current Biology, 20(6), 487-495. https://doi.org/10.1016/j.cub.2010.02.027

2-Book

The title of book should be written in italic, and it should be followed with Publisher information.

Nesemann, H., & Neubert, E. (1999). Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea. Spektrum Akademischer Verlag.

Sawyer, R. T. (1986). Leech biology and behavior. Oxford University Press.

3-Book section

The title of the chapter should be normal, the title of the book should be in italic, the editor(s), the page numbers of the section, the publisher and the DOI number (if available) having active link should be included.

Le Couteur, D., Kendig, H., Naganathan, V., & McLachlan, A. (2010). The ethics of prescribing medications to older people. In S. Koch, F. M. Gloth, & R. Nay (Eds.), Medication management in older adults (pp. 29-42). Springer. https://doi.org/10.1007/978-1-60327-457-9_3

McCormack, B., McCance, T., & Maben, J. (2013). Outcome evaluation in the development of person-centred practice. In B. McCormack, K. Manley, & A. Titchen (Eds.), Practice development in nursing and healthcare (pp. 190-211). John Wiley & Sons.

4-Web pages / Online documents

The title of the page should be in italic, the name of the website and the active link to the page should be specified.

International Union for Conservation of Nature. (2010). Chondrostoma nasus. https://www.iucnredlist.org/species/4789/97800985

Wikipedia. (2021). Toxicology. https://en.wikipedia.org/wiki/Toxicology

5-Dissertations/Thesis

The title of the dissertation/thesis should be in italic, its type (Doctoral, Master's, Specialization in Medicine) and the name of the university should be specified.

Filik, N. (2020). Inhibition effect of phenolic compounds on the environmental sensing system of Aeromonas hydrophila strains isolated from cultured fish and determination of the clonal relationship between strains by pulsed field gel electrophoresis method. [Doctoral dissertation, Isparta University of Applied Sciences].

Ozdal, A. M. (2019). Effects on growth and coloration of red pepper supplementation as pigment sources to diets of jewel cichlid (Hemichromis guttatus). [Master's thesis, Isparta University of Applied Sciences].

6-Conference, symposium presentations

Event date, presentation title (italic), presentation type (Oral presentation, Poster presentation), event name, city and country should be given.

Ceylan, M., Çetinkaya, O. (2017, October 4 - 6). Assessment of population structure and size of medicinal leech Hirudo verbana, inhabiting some model wetlands of Turkey [Oral Presentation]. International Symposium on Limnology and Freshwater Fisheries, Isparta, Turkey.

Snoswell, C. (2016, October 31 - November 3). Models of care for store-and-forward teledermatology in Australia [Poster presentation]. 7th International Conference on Successes and Failures in Telehealth, Auckland, New Zealand.

NOTE: Manuscripts that are not prepared in accordance with the journal writing rules will not be considered for scientific evaluation.

Yazım Kuralları

SAYFA BOYUTU
Sayfa A4 (21 cm x 29,7 cm) formatında olmalıdır.

KENAR BOŞLUKLARI
Üst: 2,5 cm Sol: 2,5 cm Alt: 2,5 cm Sağ: 2,5 cm Cilt payı: 0 cm

YAZI STİLİ
Yazı karakteri : Times New Roman
Yazı karakteri büyüklüğü : 12 punto
Paragraf : İki yana yaslı
Paragraf girintisi : 1,25 cm
Satır aralığı : 2
Satır numarası : Metnin tümünde satır numarası atanmalıdır
Sayfa numarası : Sayfaların altına gelecek şekilde otomatik numaralanmış

BAŞLIK SAYFASI
Başlık sayfası, makale dosyasından ayrı olarak sisteme yüklenmelidir. Başlık sayfasında sadece aşağıdaki bilgiler yer almalıdır.

- Başlık
Başlık kısa, bilgilendirici ve çalışmayı net olarak yansıtmalıdır. Kısaltma ve formül kullanımı önerilmez.

- Kısa başlık
Başlığı yansıtacak şekilde maksimum 75 karakterde kısa bir başlık verilmelidir.

- Yazarlar
Yazarların ad ve soyadları kısaltılmadan açık olarak yazılmalıdır. Makale yüklenmeden önce yazar isimlerinin doğruluğu kontrol edilmelidir.

- Kurum bilgisi
Kullanılan düzen: Üniversite/Enstitü, Fakülte, Bölüm, İl-ÜLKE
Örnek: Isparta Uygulamalı Bilimler Üniversitesi, Eğirdir Su Ürünleri Fakültesi, Su Ürünleri Yetiştiriciliği Bölümü, Isparta-TÜRKİYE

- Sorumlu yazar
Makalenin tüm aşamalarından sorumlu olacak sorumlu yazar belirtilmelidir. Başlık sayfasında sorumlu yazarın iletişim bilgileri ve posta adresi verilmelidir.
*Sorumlu Yazar: Adı Soyadı, e-posta: …

- ORCID bilgileri
Tüm yazarların ORCID bilgileri belirtilmelidir. Lütfen ORCID tanımlaması yapmak için https://orcid.org adresini ziyaret ediniz.

MAKALE FORMATI
Araştırma makalesi, kısa makale, olgu sunumu ve derlemeler aşağıdaki formata uygun olarak hazırlanmalıdır.

<table>
<tr><th>Araştırma Makalesi</th><th>Kısa Makale</th><th>Olgu Sunumu</th><th>Derleme</th></tr>
<tr><td colspan="4">Başlık</td></tr>
<tr><td colspan="4">Kısa başlık</td></tr>
<tr><td colspan="4">Yazarlar</td></tr>
<tr><td colspan="4">Kurum bilgileri</td></tr>
<tr><td colspan="4">Sorumlu yazar e-posta adresi</td></tr>
<tr><td colspan="4">ORCID bilgileri</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="4">Başlık</td></tr>
<tr><td colspan="4">Özet</td></tr>
<tr><td colspan="4">Anahtar kelimeler</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="4">Title</td></tr>
<tr><td colspan="4">Abstract</td></tr>
<tr><td colspan="4">Keywords</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="4">1. Giriş</td></tr>
<tr><td>2. Materyal ve Metot</td><td rowspan="2">2. SERBEST İÇEREİK</td><td rowspan="2">2. Olgu Sunumu</td><td rowspan="2">2. SERBEST İÇEREİK</td></tr>
<tr><td>3. Bulgular</td></tr>
<tr><td>4. Tartışma</td><td colspan="3">3. Tartışma</td></tr>
<tr><td>5. Sonuç</td><td colspan="3">4. Sonuç</td></tr>
<tr><td colspan="4">Teşekkür</td></tr>
<tr><td colspan="4">Finans</td></tr>
<tr><td colspan="4">Çıkar Çatışması Beyanı</td></tr>
<tr><td colspan="4">Yazar Katkıları</td></tr>
<tr><td colspan="4">Etik Onay Beyanı</td></tr>
<tr><td colspan="4">Veri Kullanılabilirlik Beyanı</td></tr>
<tr><td colspan="4">Kaynaklar</td></tr>
</table>

ÖZET
Özet, çalışmanın amacını, kullanılan metotları, öne çıkan bulguları ve literatüre katkısını öz bir şekilde içermelidir. Hem Türkçe hem de İngilizce dillerinde maksimum 300 kelime olacak şekilde yazılmalıdır.
Not: Türk olmayan yazalar için Türkçe Özet desteği sağlanmaktadır.

ANAHTAR KELİMELER
Anahtar kelimeler başlıkta yer almayan, çalışmayı yansıtacak kelimelerden seçilmelidir. En az 3 (üç), en çok 5 (beş) kelime belirtilmeli; kelimeler aralarında virgül (,) son kelimeden sonra ise nokta (.) gelmelidir.
Anahtar kelimeler: CITES, akuaponik, üretim protokolü, mortalite, immünoloji.

ONDALIK GÖSTERİM
Türkçe makalelerde “,” (virgül) İngilizce makalelerde ise “.” (nokta) olmalıdır.
Türkçe: %10,25
İngilizce: 10.25%

LATİNCE GÖSTERİM
Tür ismi, metinde ilk geçtiği yerde kısaltılmadan (Cyprinus carpio), sonrasında ise cinsi ismi kısaltılarak (C. carpio) verilmelidir.

TABLOLAR
Tablo başlığı, tablonun üstüne gelecek şekilde kısa ve öz olmalıdır. Tabloda yer alan kısaltmalar tablonun altında açıklanmalıdır. Tablo özel bir tasarım uygulanmamış, düz kılavuz şeklinde olmalıdır. İhtiyaç bulunması halinde tablo içi metinde yazı karakteri büyüklüğü 10 puntoya kadar düşürülebilir. Tablolara metin içinde Tablo 1, Tablo 2, … şeklinde atıf yapılmalıdır. Tablolar, alıntılandıkları yere en yakın yerde verilmelidir.
Tablolar düzenlenebilir olmalıdır. Ekran görüntüsü veya resim formatındaki tablolar kabul edilmemektedir.

ŞEKİLLER
Şekil başlığı, şeklin altına ortalanmış olarak kısa ve öz olmalıdır. Şekiller minimum 300 DPI çözünürlükte olmalıdır. Şekillere metin içinde Şekil 1, Şekil 2, … şeklinde atıf yapılmalıdır. Şekiller, alıntılandıkları yere en yakın yerde verilmelidir.

TEŞEKKÜR
Bu bölümde finansal destek dışında çalışmanın yürütülmesine katkı sunanlar belirtilir.

Örnek: Yazarlar çalışmanın laboratuvar bölümünde yardım eden Ahmet Taş'a (Isparta Uygulamalı Bilimler Üniversitesi, Türkiye) teşekkür etmektedir.

FİNANS
Bu bölümde çalışmanın yürütülmesine finansal destek sağlayan kurumlar destek numarası kullanılarak belirtilir.

Örnek-1: Bu çalışma 3241-E2-14 proje numarası ile Isparta Uygulamalı Bilimler Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir.

Örnek-2: Bu çalışmanın yürütülmesinde herhangi bir finans desteği alınmamıştır.

ÇIKAR ÇATIŞMASI BEYANI
Bu bölümde yazarların varsa çıkar çatışmaları belirtilir.

Örnek: Yazarlar, bu çalışmayı etkileyebilecek finansal çıkarlar veya kişisel ilişkiler olmadığını beyan eder.

YAZAR KATKILARI
Bu bölümde isim ve soy ismin ilk harfleri kullanılarak yazarların çalışmanın ilgili aşamalarına yaptıkları katkılar belirtilir.

Örnek:
Kurgu: BT; Metodoloji: CT, FU; Deneyin gerçekleştirilmesi: FM, CT, FU; Veri analizi: FU, TA; Makale yazımı: CT, FU, Denetleme: CT. Tüm yazarlar nihai taslağı onaylamıştır.

ETİK ONAY BEYANI
Bu bölümde çalışmanın yürütülmesinde alınan etik kurul onayının alındığı kurum, tarih ve numarası belirtilir. Omurgalı hayvanlarla yürütülen çalışmalarda Yerel Etik Kurul Onayı, anket/mülakat çalışmalarında ise Girişimsel Olmayan Araştırmalar Etik Kurulu Onayı gerektirdiği halde beyan edilmeyen makaleler bilimsel değerlendirmeye alınmamaktadır.

Örnek-1: Bu çalışmada deney hayvanları kullanılmaması nedeniyle Yerel Etik Kurul Onayı alınmamıştır.

Örnek-2: Bu çalışma Isparta Uygulamalı Bilimler Üniversitesi Hayvan Deneyleri Yerel Etik Kurul onayı ile yürütülmüştür (Tarih: 01.07.2010, No: 21438139-147).

VERİ KULLANILABİLİRLİK BEYANI
Bu bölümde makalede kullanılan verilerin anonim kullanılabilirliğine ilişkin beyanda bulunulmalıdır. Acta Aquatica Turcica dergisi, yazarları araştırma verilerini paylaşmaya teşvik etmektedir.

Örnek-1: Bu çalışmada kullanılan veriler Figshare platformunda ttps://doi.org/10.6084/m9.figshare.11815566.v1 DOI adresi ile erişime açıktır.

Örnek-2: Bu çalışmada kullanılan verilere ilgili yazardan talep üzerine erişilebilir. Veriler, gizlilik veya etik kısıtlamalar nedeniyle kamuya açık değildir.

Örnek-3: Bu çalışmada kullanılan veriler makul talep üzerine ilgili yazardan temin edilebilir.

Örnek-4: Bu çalışmada yeni veri oluşturulmadığı veya analiz edilmediği için veri paylaşımı bu makale için geçerli değildir.

Örnek-5: Araştırma verileri paylaşılmaz.

Örnek-6: Bu çalışmada kullanılan veriler bu makalenin ekinde mevcuttur.

ATIFLAR
Atıflar yıl sırasına göre ve aralarında noktalı virgül (;) olacak şekilde aşağıdaki formatlarda yazılır:

- Tek yazar:
(Yazar, yıl)
-- … olduğu düşünülmektedir (Küçük, 2008; Güçlü, 2018a; Güçlü, 2018b).
-- Küçük (2008)'e göre …

- İki yazar:
(Yazar-1 ve Yazar-2, yıl)
-- … önemli parametreler arasında yer almaktadır (Küçük ve Güçlü; 2001; Ekici ve Koca, 2021a; Ekici ve Koca, 2021b).
-- Ekici ve Koca (2021b)'a göre …

- Üç ve daha çok yazar:
(Yazar vd., yıl)
-- … dönemsel olarak tekrarlayabilmektedir (Yiğit vd., 2006a; Yiğit vd., 2006b; Boyacı vd., 2020)
-- Boyacı vd. (2020)'e göre …

KAYNAKLAR
Kaynaklar APA 7. versiyona göre yazılmalıdır. Tüm yazarların isimleri verilmelidir, ancak 10. yazardan sonra "vd." kısaltması da kabul edilmektedir. Özel kullanımlar hariç olmak üzere tüm eser türlerinde eser isminin sadece ilk harfi büyük, eserin yayınlandığı veya sunulduğu dergi, yayınevi, kongre isimlerinde geçen tüm kelimeler büyük harfle başlanarak yazılmalıdır.

1-Makale
Dergi ismi kısaltılmadan (italik), cilt (italik), sayı, sayfa numaraları ve aktif link içerecek şekilde DOI numarasına yer verilmelidir:

Petrauskienė, L., Utevska, O., & Utevsky, S. (2009). Can different species of medicinal leeches (Hirudo spp.) interbreed? Invertebrate Biology, 128(4), 324-331. https://doi.org/10.1111/j.1744-7410.2009.00180.x

Wagenaar, D. A., Hamilton, M. S., Huang, T., Kristan, W. B., & French, K. A. (2010). A hormone-activated central pattern generator for courtship. Current Biology, 20(6), 487-495. https://doi.org/10.1016/j.cub.2010.02.027

2-Kitap
Kitap başlığı italik olacak şekilde ve yayın kuruluş ismi olacak şekilde verilmelidir.

Nesemann, H., & Neubert, E. (1999). Annelida, Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea. Spektrum Akademischer Verlag.

Sawyer, R. T. (1986). Leech biology and behavior. Oxford University Press.

3-Kitap bölümü
Bölüm başlığı normal, kitap başlığı italik olacak şekilde, editör(ler), bölümün sayfa numaraları, yayıncı kuruluş

ve varsa aktif link içerek şekilde DOI numarasına yer verilmelidir:

Le Couteur, D., Kendig, H., Naganathan, V., & McLachlan, A. (2010). The ethics of prescribing medications to older people. In S. Koch, F. M. Gloth, & R. Nay (Eds.), Medication management in older adults (pp. 29-42). Springer. https://doi.org/10.1007/978-1-60327-457-9_3

McCormack, B., McCance, T., & Maben, J. (2013). Outcome evaluation in the development of person-centred practice. In B. McCormack, K. Manley, & A. Titchen (Eds.), Practice development in nursing and healthcare (pp. 190-211). John Wiley & Sons.

4-Web sitesi
Sayfa başlığı italik, websitesinin ismi ve sayfanın aktif linki olacak şekilde verilmelidir.

International Union for Conservation of Nature. (2010). Chondrostoma nasus. https://www.iucnredlist.org/species/4789/97800985

Wikipedia. (2021). Toxicology. https://en.wikipedia.org/wiki/Toxicology

5- Tezler
Tez başlığı italik olacak şekilde, tez türü (Doktora, Yüksek lisans, Tıpta Uzmanlık) ve üniversite ismi belirtilmelidir.

Filik, N. (2020). Kültür balıklarından izole edilen Aeromonas hydrophila suşlarında fenolik bileşenlerin çevreyi algılama sistemi üzerine inhibisyon etkisi ve suşlar arasındaki klonal ilişkinin pulsed fıeld jel elektroforez yöntemiyle belirlenmesi [Doktora tezi, Isparta Uygulamalı Bilimler Üniversitesi].

Özdal, A. M. (2019). Effects on growth and coloration of red pepper suplementation as pigment sources to diets of jewel cichlid (Hemichromis guttatus) [Yüksek lisans tezi, Isparta Uygulamalı Bilimler Üniversitesi].

6- Konferans, sempozyum sunumları
Etkinlik tarihi, sunu başlığı (italik), sunum türü (Sözlü sunum, Poster sunum), etkinlik adı, şehir ve ülke verilmelidir.
Ceylan, M., Çetinkaya, O. (2017, Ekim 4 - 6). Assessment of population structure and size of medicinal leech Hirudo verbana, inhabiting some model wetlands of Turkey [Sözlü sunum]. International Symposium on Limnology and Freshwater Fisheries, Isparta, Türkiye.

Snoswell, C. (2016, Ekim 31 - Kasım 3). Models of care for store-and-forward teledermatology in Australia [Poster sunum]. 7th International Conference on Successes and Failures in Telehealth, Auckland, Yeni Zelanda.

NOT: Dergi yazım kurallarına uygun olarak hazırlanmayan makaleler değerlendirmeye